# PROCEEDINGS

of the

# PENNSYLVANIA ACADEMY OF SCIENCE

•

VOLUME 43

1969

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*Founded On April 18, 1924*

**PROCEEDINGS OF THE PENNSYLVANIA ACADEMY OF SCIENCE**

JOHN D. SINK, *Editor*
GLENN D. BENGTSON, *Associate Editor* CLAIRE H. SINK, *Associate Editor*

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The Pennsylvania State University
University Park, Pennsylvania 16802

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*MANUSCRIPTS.* All papers submitted for publication will normally have been presented at the spring meeting of the Academy. Manuscripts in completed form must be submitted to the Secretary for transmittal to the Editor at the time of presentation of papers. See "Suggestions to Authors" in this issue of the *Proceedings*.

*REPRINTS.* Reprints of papers in the *Proceedings* are obtainable only from the authors or their institutions.

*NEWS AND NOTES.* Announcements about meetings, personnel changes and other items of interest to the Academy should be sent directly to the Editor of the Newsletter, Phyllis C. Martin, Point Park College, Pittsburgh, Pennsylvania 15222.

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# PRESIDENT

# PENNSYLVANIA ACADEMY OF SCIENCE

## 1968-1970



Dr. Robert S. Chase, Jr., President, Pennsylvania Academy of Science; A.B., Haverford College; M.S., University of Arkansas; Ph.D., Bryn Mawr College. Joined the faculty of Lafayette College 1958. Currently Associate Professor of Biology and Dean of Studies. Fellow of the American Association for the Advancement for Science; member American Society of Zoologists, Sigma Xi; secretary-treasurer Pennsylvania Academy of Science 1964-65.



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# THE PENNSYLVANIA ACADEMY OF SCIENCE

**45TH ANNUAL MEETING — APRIL 17 - 19, 1969**

held at

**Franklin and Marshall College Lancaster, Pennsylvania**

★

**Local Committee**

Arthur W. Shively, *Chairman* ..... Franklin and Marshall College
Harry K. Lane ..... Franklin and Marshall College
John McDermott ..... Franklin and Marshall College
Joseph J. Donahue ..... Matheson Scientific, Inc.

## —PROGRAM—

### THURSDAY, APRIL 17, 1969

Executive Council Dinner ..... 6:30 P.M.
Stock Yard Inn

Executive Council Dinner ..... K100
President-Elect William A. Uricchio, *Carlow College,* Presiding

### FRIDAY, APRIL 18, 1969

Registration — 8:30 A.M. - 4:00 P.M. ..... Science Complex Lobby
Commercial Exhibits — 8:30 A.M. - 6:30 P.M. ..... P220
General Session — 9:00 A.M. - 10:30 A.M. ..... K200
Biology Session — 9:00 A.M. - 11:45 A.M. ..... F214
Geology Session — 9:00 A.M. - 11:45 A.M. ..... P121
Luncheon — 12:00 Noon - 1:15 P.M. ..... Kieffer Dining Hall
Annual Business Meeting — 1:30 P.M. - 2:30 P.M. ..... K200
President-Elect William A. Uricchio, Presiding. Report of the Executive Council; Election of Officers; Old and New Business
Biology Session — 3:30 P.M. - 5:30 P.M. ..... F214
Geography Session — 3:00 P.M. - 4:00 P.M. ..... K204
Reception for Academy Members and Friends — 5:00 P.M.
Annual Banquet — 6:30 P.M.
Lecture — 8:30 P.M.

**"Peripatetic Parasitologists"**

DR. LAWSON SOULSBY
*School of Veterinary Medicine, University of Pennsylvania*

### SATURDAY, APRIL 19, 1969

Biology Session — 9:00 A.M. - 11:00 A.M. ..... F214
Physiology Session — 9:00 A.M. - 11:45 A.M. ..... P121
Parasitology Symposium — 9:00 A.M. - 11:15 A.M. ..... K200



# ACADEMY BUSINESS

# 1969

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## MINUTES OF THE EXECUTIVE COUNCIL MEETING

### April 17, 1969

The Executive Council meeting of the Pennsylvania Academy of Science was called to order at 8:45 p.m. on Thursday, April 17, 1969, in Room K100 of the Science Complex Building at Franklin & Marshall College, Lancaster, Pa. President-elect William Uricchio was in the chair and the following officers and committee chairmen were present: Carolyn Gibson, Sister Gabrielle, Thomas Knepp, Bernard Fried, Arthur Shively, John Sink, Phyllis Martin, Charles Rutschky, Harry Lane, Kenneth Hoover.

The following reports were read and accepted (see reports): a.)—Treasurer's Report - Charles Rutschky, b.)—Auditor's Report - Adam Anthony, c.)—Secretary's Report - Bernard Fried, d.)—*Proceedings* Report - John Sink, e.)—PAS *Newsletter* Report - Phyllis Martin, f.)—Research Grants Committee - Kenneth Rockwell, g.)—Annual Science Talent Search - Sister Gabrielle, h.)—Junior Academy - Carolyn Gibsson.

The Council established a Visiting Lecture Committee to investigate promoting the Pennsylvania Academy of Science. Chairman John Sink, Bernard Fried, Carolyn Gibson and Sister Gabrielle were appointed to the committee which will use funds from the U.S. Steel bequest of $1,000 to promote the Academy. The committee recommended that dynamic speakers be selected from Academy members but, in the absence of qualified speakers from the ranks of the Academy, non-members be selected. The committee recommended the appointment of one speaker from the eastern part of the state and another from the west who will address mixed audiences on general topics in the sciences and will be paid an honorarium of $200 plus expenses. Details of how speakers will be appointed, the nature of topics, how many talks, etc. will be worked out by the committee. The final selection of speakers will be determined by the Executive Council.

The Council approved the recommendations of Editor Sink relative to the *Proceedings* as follows: a.)—Except under extraordinary circumstances papers to be considered for publication in the *Proceedings* by Academy members must be submitted at the time of their oral presentation at the annual spring meeting. b.)—The Academy will pay publication costs of a paper accepted for publication if the author is unable to pay. However, the author must obtain verification of his inability to pay from the chief fiscal officer of his institution.

The Council recommended that the Secretary write a letter of appreciation to President Arthur M. Blum, Point Park College, for his generous support of the PAS *Newsletter.*

The Council recommended the establishment of the Life Membership Category (*see* Volume 27, #2, March 1969, PAS *Newsletter)* upon payment of $200 to be voted on at the business meeting on April 18, 1969.

The Council approved of the following meeting places for future spring meetings: a.)—April 11-12, 1970 — Juniata College, b.)—1971 — Carlow College (formerly Mount Mercy College), c.)—1972 — Dickinson College.

The Council approved of the establishment of budgets for the officers of the Academy. Budgets are to be submitted to the Treasurer and must be accounted for.

The Council recommended that members who retire from active professional life and resign from the Academy be considered on a yearly basis for Honorary or Emeritus Membership in the Academy. The details of Emeritus Membership will be considered at a future meeting.

The Council approved the recommendation that starting in 1971 officers and committee chairmen present at the Executive Council dinner meeting will be reimbursed from the Academy for their dinner costs.

The Council approved the recommendation that Phyllis Martin present $500 to Point Park College for their assistance with the PAS *Newsletter* instead of the previous yearly amount of $300.

The Council approved the recommendation that participants in the parasitology symposium of April 19, 1969 be allowed to publish 3 pages without charge in the *Proceedings* and will also receive 100 free reprints.

The Council approved Kenneth Rockwell's recommendation relative to funding the grant request of W. J. Yurkiewicz.

The Executive Council meeting adjourned at 10:45 p.m.

## MINUTES OF THE ANNUAL BUSINESS MEETING

### April 18, 1969

President-elect William Uricchio called the business meeting to order at 1:30 p.m. Thirty-five Academy members were in attendance. The reports of officers and committee chairmen at the Executive Council meeting were read and accepted.

The membership voted to approve the recommended amendment to the bylaws relative to "Life Membership upon payment of $200.00" (see PAS *Newsletter* 27, #2, March 1969).

The report of the Resolutions Committee was read and accepted.

The Nominating Committee chaired by Phyllis Martin announced that only the office of Treasurer was open for election this year and nominated the incumbent, Charles Rutschky. A motion that nominations for this office be closed was approved and Mr. Uricchio instructed the Secretary to cast a ballot for Mr. Rutschky.

The following items not considered at the Executive Council meeting were discussed: a.) *Legislative Committee* — Mr. Gevers felt that if this committee can only be used as an information committee its usefulness was limited. The purpose of this committee was to obtain information about legislation relative to scientific matters in Harrisburg. The exact status of the effectiveness of this committee is uncertain. b.) *Membership Committee* — Mr. Miller informed the membership that they are on this committee-at-large and should try to recruit members into PAS.

The Secretary discussed the actions and recommendations of the Executive Council *(see* Minutes of the Executive Council meeting).

The business meeting was adjourned at 2:30 p.m.

# REPORTS OF OFFICERS AND COMMITTEES

## I — REPORT OF THE SECRETARY — Bernard Fried

The Secretary's office continues the task of updating membership, weeding out members who have not paid their dues for two or more years, sending out dues' notices, supplying mailing labels for Academy activities and answering the numerous queries relative to the Academy.

The Secretary helped arrange the program for the 45th Annual Spring Meeting with the assistance of the local committee at Franklin & Marshall College and the Editor of the *Newsletter*. The Secretary's office is preparing an updated membership listing (name, address, area of specialty) which will be published in Volume 43 of the *Proceedings*.

The Secretary is grateful to Mrs. Doris Lowe, Secretary of the Biology Department, Lafayette College and Mr. Joe Vicari, IBM operator, Lafayette College for their invaluable services.

The following is an itemized membership list as of April 2, 1969:

| | |
|---|---|
| Paid members | 442 |
| Unpaid members | 256 |
| Total members | 698 |
| New members (1968) | 89 |
| New members (1969, to date) | 32 |
| New members | 121 |
| Members lost | 23 |
| Net gain | 98 |

## II — REPORT OF THE TREASURER — Charles W. Rutschky, III

| ASSETS | 12/31/67 | 12/31/68 |
|---|---|---|
| CASH IN BANK | | |
| I. Checking Account | $ 45.44 | $ 1,194.23 |
| II. Publications Endowment Fund | 242.28 | 251.97 |
| III. General Savings Account | 4,889.88 | 234.12 |
| IV. Darbaker Account | 4,429.08 | 140.96 |
| Savings Certificate (5%) Darbaker Account | 0 | 4,000.00 |



| | | |
|---|---|---|
| ACCOUNTS RECEIVABLE | | |
| I. Excess page charges, Proceedings | 532.50 | 687.50 |
| II. Reprint orders | 0 | 92.45 |
| III. Library subscriptions | 92.50 | 77.50 |
| TOTAL ASSETS | $10,231.68 | $ 6,678.73 |
| **LIABILITIES** | | |
| Accounts payable | $ —0— | $ —0— |
| Uncollectable accounts | | 532.50 |
| Designated Funds | | |
| Uncashed checks | —0— | —0— |
| Publications Endowment Fund | 242.28 | 251.97 |
| Darbaker Account | 4,429.08 | 4,140.96 |
| Prepaid memberships | 40.00 | 35.00 |
| Surplus cash | 5,520.32 | 1,718.30 |
| TOTAL LIABILITIES | $10,231.68 | $ 6,678.73 |

**EXHIBIT "A"**

| | | |
|---|---|---|
| **RECEIPTS** | | |
| Membership dues — 1967 | $ 210.00 | |
| 1968 | 2,906.00 | |
| 1969 | 35.00 | |
| Total membership dues | | $ 3,151.00 |
| Library subscriptions | | |
| 1967 | 309.50 | |
| 1968 | 92.50 | |
| 1969 | 80.00 | |
| Back issues | 388.50 | |
| Newsletter | 1.00 | |
| Total library subscriptions | | $ 871.50 |
| Grants | | |
| L. K. Darbaker Estate | $ 647.72 | |
| A. A. A. S. Research Fund | 664.00 | |
| Total grants | | 1,311.72 |
| Interest and Dividends | | |
| General Savings Account | $ 147.25 | |
| Publications Endowment Fund | 9.69 | |
| Darbaker Account | | |
| Savings | 63.11 | |
| Certificate | 111.67 | |
| Total interest and dividends | | $ 331.72 |
| Annual Meeting, 1968 | | $ 329.11 |
| Page charges | | |
| Volume 41 (1967) | $ 1,769.50 | |
| Volume 42 (1968) | 1,001.50 | |
| Total page charges | | $ 2,771.00 |
| Transferred from savings | | $ 5,475.09 |
| TOTAL RECEIPTS | | $14,241.14 |
| **DISBURSEMENTS** | | |
| Printing | | |
| Volume 40 (1966) | $ 2,994.86 | |
| Volume 41 (1967) | 6,025.00 | |
| Reprints, guest paper | 9.50 | |
| Newsletter | 500.00 | |
| Editorial expenses | 22.00 | |
| Stationery and postage | 411.96 | |
| Total printing costs | | $ 9,963.32 |
| A. A. A. S. Grants | | 664.00 |
| Darbaker Prize Award | | 250.00 |
| Junior Academy | | 323.86 |
| N. S. F. Refund (Grant GE-5222) | | 554.72 |
| Wages | | 145.25 |

| | | |
|---|---|---|
| Annual meeting | | 56.89 |
| Academy conference | | 27.64 |
| Treasurer's bond | | 13.00 |
| A. A. A. S. dues | | 10.00 |
| Science Talent Search | | 100.00 |
| TOTAL DISBURSEMENTS | | $12,208.68 |
| Excess of Receipts over disbursements | $ 1,499.86 | |

**EXHIBIT "B" — Accounts of the Pennsylvania Academy of Science, Incorporated, for the period from January 1, 1968 to December 31, 1968.**

| | | | |
|---|---|---|---|
| I. | CHECKING ACCOUNT | | |
| | Balance in checking account December 31, 1967 | | $ 45.44 |
| | Deposits | $13,259.12 | |
| | Disbursements | | |
| | Bills paid | 11,120.82 | |
| | Junior Academy | 323.86 | |
| | Grants for research | 664.00 | |
| | Total disbursements | $12,108.68 | |
| | Charges against account | 1.65 | |
| | Balance in checking account December 31, 1968 | | $ 1,194.23 |
| II. | Publication Endowment Fund | | |
| | Balance on hand December 31, 1967 | | $ 242.28 |
| | Receipts | | |
| | Interest earned | $ 9.69 | |
| | Disbursements | —0— | |
| | Balance on hand December 31, 1968 | | $ 251.97 |
| III. | General Savings Account | | |
| | Balance on hand December 31, 1967 | | $ 4,889.88 |
| | Receipts | | |
| | Interest | $ 147.25 | |
| | Disbursements | | |
| | Transferred to checking | 4,800.00 | |
| | Balance on hand December 31, 1968 | | $ 234.12 |
| IV. | Darbaker Account | | |
| | Balance on hand December 31, 1967 | | $ 4,429.08 |
| | Receipts | | |
| | L. K. Darbaker Estate | $ 323.86 | |
| | Interest on savings | 63.11 | |
| | Interest on certificate | 111.67 | |
| | Total receipts | $ 498.64 | |
| | Disbursements | | |
| | Transferred to checking | | |
| | Earned interest | $ 175.09 | |
| | 1968 Prize | 250.00 | |
| | 1967 Prize | 250.00 | |
| | Certificate interest | 111.67 | |
| | Purchase of Savings Certificate | 4,000.00 | |
| | Total disbursements | $ 4,786.76 | |
| | Balance on hand December 31, 1968 | | |
| | Savings account | $ 140.96 | |
| | Certificate | 4,000.00 | |
| | Total on hand | | $ 4,140.96 |

## III — REPORT OF THE AUDITING COMMITTEE — Adam Anthony and Paul Simkins

We have examined the fiscal records of the Pennsylvania Academy of Science as maintained by the Treasurer. We have found the records in accord with standard accounting procedures. The balances shown in the Treasurer's report are in agreement with the statements of the banks holding the funds of the Academy.



## IV — REPORT OF THE EDITOR OF THE PROCEEDINGS — John D. Sink

Volume 42 of the *Proceedings* was published this year, complete in one issue (248 pages), following a revised format and policy approved by the Editorial Board. An abbreviated form of the annual meeting program was published in the *Proceedings* as approved by the Executive Council last year. The publishing delay was attributed to a few authors as well as the printer and the engraver.

The Editor expresses his appreciation to (a) the Editorial Board for carefully reviewing the manuscripts, (b) the Associate Editors for editing the manuscripts and reading the page proofs, (c) the authors for their cooperation and promptness, and (d) The Pennsylvania State University for underwriting the many expenses (secretarial, telephone, mailing, etc.) incurred by the Editor's Office.

The Editors and Editorial Board recommend that the Executive Council of the Academy approve publishing in the *Proceedings* manuscripts submitted by authors without a source of funds. The Academy would pay the page charges but the authors would be responsible for the reprint costs.

Another recommendation to the Executive Council by the Editors and the Editorial Board is the continuation of the policy of making presentation of a paper at the Annual Meeting a requirement for it to be published in the *Proceedings*. However, an exception to this should be invited reviews. Further, it is recommended that the President of the Academy be the only individual authorized to invite such review papers.

## V — REPORT OF THE EDITOR OF THE NEWSLETTER — Phyllis C. Martin

Six issues of the *Newsletter* were published and distributed in 1968-1969.

The financial account follows:

| | |
|---|---|
| Carried over from April 1, 1968 | $ 163.97 |
| Academy subsidy | 500.00 |
| Advertising | 795.00 |
| Total | $1,458.97 |
| DISBURSEMENTS | |
| D. H. Lucas Company for varityping | $ 208.00 |
| Citation for Dr. Guyton's honorary membership | 12.50 |
| Xerox — invoices and rate sheets | 5.60 |
| Post Office bulk mailing permit | 30.00 |
| Post Office advance deposit | 50.00 |
| Christmas gifts to Point Park College Print Shop and Computer Center Staff | 20.00 |
| Point Park College toward expenses involved | 300.00 |
| Photographic work — advertising | 15.00 |
| Office supplies | 5.17 |
| Postage miscellaneous | 14.83 |
| Telephone | 2.85 |
| Clerical Assistance | 93.50 |
| Total | $ 757.45 |
| Balance on hand at Craig Street Branch of Pittsburgh National Bank | $ 701.52 |

The Point Park Print Shop prints, folds and staples the *Newsletter*. The editor and assistants attach stickers, bundle according to Zip Code and mail it. The Computer Center prepares the stickers. Labor and supplies other than those listed under Disbursements are charged to the account of the President of Point Park College. For 1968-1969, Print Shop charges are $595.59; Computer Center charges are $54.00.

The editor is grateful to members for news items and to the advertisers for their continued support. They are American Optical Company, Burrell Corporation, Carolina Biological Supply Company, Keystone Microscope Company, and Williams, Brown and Earle, Inc. We are happy to recommend them to our members and friends.

The editor requests that the Academy express its appreciation to Mr. Arthur M. Blum, President of Point Park College for the generosity of the College in assisting with the production of the *Newsletter*.

## VI — REPORT OF THE PENNSYLVANIA JUNIOR ACADEMY OF SCIENCE — Carolyn A. Gibson

The Pennsylvania Science and Engineering Foundation of the Commonwealth of Pennsylvania granted the sum of $14,550 to the Pennsylvania Junior Academy of Science for the following purposes: (1) to stimulate participants towards increased participation in science oriented activities, (2) to identify and honor the outstanding examples of ability and aptitude, and (3) to provide an interface between the potentially science-oriented student and the scientific community.

To accomplish these aims, students throughout the state are encouraged to participate in extra-curricular science projects of a scientific nature which should motivate the students towards an enriched program in science beyond the scope of normal classroom activity. They are then given the opportunity to orally present their scientific papers in a series of competitive meetings from local through regional to state meetings.

In order to make the PJAS more efficient, the regions were changed by vote of the regional directors from 14 regions to 10. Some of the regions have been inactive in past years, but all 10 regions conducted a regional meeting during March and April of 1969. There were several counties that were not represented this year, but it is our aim to present programs in each region next year that will interest more students and teachers in the activity.

The State Meeting will be held May 1, 2, 3, 1969 at The University of Pennsylvania, Philadelphia. The Warwick Hotel will be the Headquarters of the State Meeting. Mr. Stanley Tracton, Regional Director of Region I is the Chairman for this year's meeting. The costs are high for the meeting, and if the seed money had not been available this year, other arrangements would have been made as to location and offerings of the 1969 State Meeting. An experiment is being tried for the 1970 State Meeting. Butler High School will host the State Meeting; Mr. Ivan Riemold will be Chairman. Mr. Riemold is a committee member of Region 9, Mr. Homer Christie is Regional Director. Plans will be made at the Spring Meeting of the Regional Directors for future meetings. Because of the increase in interest in PJAS throughout the state, sites for state meetings are a problem. From advance registrations, it is expected that 400 students and 100 sponsors will attend the 1969 State Meeting.

For several years, Region 7, Sister Gabrielle, O.S.F., Regional Director, has sponsored a series of lectures in the fall of the year for both junior and senior high school students. A certificate of attendance is presented to those students who are present for a stated number of these meetings. These lectures are very worthwhile and are very popular. The cooperation of the Pittsburgh colleges and universities is appreciated in their permission to use facilities and for the services of their scientific staff as lecturers. Lecturers are also recruited from the industries and hospitals in the area.

Region 9, Homer Christie, Regional Director, stimulates interest in science activities by conducting a workshop in research procedures for teachers and students, with demonstrations and lectures by scientists, and workshops by high school and college students. Slippery Rock State College is active in this capacity, and Grove City College assists with the Regional Meeting. Region 9 also sponsors a lecture each year in January or February to maintain interest in scientific activity. The lecture in 1969 was held at New Castle High School, and was on the topic of Drug Traffic Today.

Region 6, Joan Ruck, Regional Director, sponsored a breakfast for the judges prior to the Regional Meeting which was very effective. The week before the Regional meeting in her area, a symposium was held at Altoona High School that was organized by a student with the cooperation of the teachers there. Michael Stitt was the student who attended the AAAS meeting at Dallas in December as the alternate representative of PJAS at the national meeting. Through his efforts the junior academy activities at Altoona are resuming a position of leadership in the state.

The State PJAS co-sponsored with the Botanical Society of Western Pennsylvania, Department of Forests and Waters, Commonwealth of Pennsylvania, Junior Division of Audubon Society, Pennsylvania Game Commission, Sweadner Entomological Society, The University of Pittsburgh High School Science Research Program, and the Western Pennsylvania Conservancy, a Forest Ecology Symposium and Workshop at Hampton High School in Allegheny County, on November 16, 1968. Representatives from four regions of PJAS attended.

It is believed that the lectures, workshops, and symposia serve a worthwhile function in interesting young people and teachers in participating in investigative procedures. It is hoped that more regions will sponsor such activities, and that the State PJAS will be able to expand these activities.

*Committees.* The judging committee, Mr. Joseph Gennaula, Region 7, Chairman has conducted several meetings of his committee. The aim is to improve the quality of the judging at the regional and state meetings, and this committee will report on their work at the Regional Directors Meeting in Philadelphia. It is planned that a file of available judges will be compiled and maintained for the use of the regional directors.



A committee will interview and select two male students (12th) to attend the National Science Camp in West Virginia this summer. They will function at the State Meeting.

A committee from the Women's Auxiliary of Pennsylvania Veterinarians Association will judge and select three awards winners at the State Meeting in Biology.

The Westinghouse Science Talent Search and the Pennsylvania Science Talent Search, under the direction of Sister Gabrielle, O.S.F. was effective in Pennsylvania this year, and there were several honors winners and trip winners. Sister Gabrielle will present her report.

The young people in PJAS who are award winners at the State level have much to offer to the Senior Academy and to teachers in the State. The Pennsylvania Science Teachers have invited two students to present papers at their summer meeting. We would welcome an invitation to have similar presentations at the PAS meetings. We also invite you to attend the State Meeting of PJAS.

*Finances.* It is expected that PSEF will support PJAS to some extent for the next two years. This money is 'seed' money, and is being used as described above. In addition, a proposal to develop a ten year budget from other sources is being written, and will be presented to these sources either this spring or next fall. The total plan will be sent to the President and President-elect when completed for their advice and approval. It is hoped that a plan for an executive secretary can be achieved. We are asking for letters of endorsement from scientists, educators, and from industry as part of this proposal.

*Other PJAS Business.* At the spring meeting of the Regional Directors, the PJAS constitution was discussed, and certain changes proposed. A copy of the proposed revision is available at this meeting. The items that concern PAS are discussed briefly: (1) The President and President-elect will be members of the administrative personnel of PJAS, and will serve on the Advisory Committee, (2) Adult members of the educational, scientific, and industrial community may become patron members of PJAS, and (3) Approval of potential revisions.

## VII — REPORT OF 22nd ANNUAL PENNSYLVANIA SCIENCE TALENT SEARCH — Sister M. Gabrielle

The Science Talent Search competition is held in cooperation with the Westinghouse Educational Foundation to determine the most prominent scientists in American high schools. Awards were made to the 40 National Winners and 300 National Honors members. The 40 National Winners received an all expense paid trip to Washington, D.C. to attend the Science Talent Institute where further critical judging is conducted to determine the recipients of the 10 highest awards. This year and last year the number receiving scholarships has been increased. The top winner's award is $10,000. The two second place awards bring $8,000 each followed by three awards of $6,000 and four awards at $4,000. Finalists not receiving scholarships are awarded $250 each. The Board of judges select an Honor Group — roughly the 10 percent of contestants or 300 — to be specially recommended for scholarships to leading colleges and universities.

This year 1363 entree forms were requested by the high schools and only 181 students from Pennsylvania completed entries in the National Science Talent Search. This was an increase of 16 over last year's number. Of this number there were 3 National Winners and 25 made the Honors Group. Last year we had 12 in the Honors Group and this year it doubled to 25.

Over the past years Pennsylvania has had 58 National Winners and 403 Honors awards to place us again fourth in the Nation following New York, Illinois, and California. All winners in the national competitions are considered automatic winners in the 22nd Annual Pennsylvania Science Talent Search. To enter the national competitions the student must be in his senior year in public, private, or parochial school, take the science aptitude examination conducted by Science Clubs of America and write a report describing his own science project.

The school Science Committee of Pennsylvania Academy of Science, under the direction of the State Director, is responsible for arranging regional meetings throughout the State to further determine additional winners in the State Science Talent Search. To participate in this, a student must have taken the science aptitude examination, he must present a research paper at one of the regional meetings and be interviewed by a committee selected by the chairman of

the Talent Search. The names of the winners will be published in the forthcoming issue of the Newsletter of the Pennsylvania Academy of Science. In addition, these students will receive a certificate from the Pennsylvania Academy of Science, they will be recommended for scholarships and financial assistance to local and national colleges and universities. To insure better chances of receiving scholarships — this is the second year since we are interviewing the junior class students and submitting their names to colleges of their choice. By next year, we hope to have some statistics as to how well this project is working out.

## VIII — REPORT OF THE NOMINATING COMMITTEE — Phyllis C. Martin

The Nominating Committee of the Pennsylvania Academy of Science offers the following slate of nominees for presentation at the Annual Business Meeting of the Academy in April 1969:

*Treasurer:* CHARLES W. RUTSCHKY III (term: Spring 1969 to Spring 1971)

## IX — REPORT OF THE RESEARCH GRANTS COMMITTEE — Kenneth Rockwell

Two proposals have been submitted to the Research Grants Committee during the past year. The first of these, titled "Effect of sex on metabolism of triglyceride, glucose, and amino acids during the pupal period in the Indian-meal moth, *Plodia interpunctella*," by Dr. William Yurkiewicz, was reviewed, while the second was received too short a time before the spring meeting to permit review.

The committee recommends that support in the amount of three hundred and thirty ($330.00) dollars be awarded to Dr. William J. Yurkiewicz, Department of Biology, Millersville State College, Millersville, Pennsylvania. Announcement of this award will follow acceptance of this report.

The committee recognizes with concern that the above recommendation departs from the desire of AAAS to have these grants used for the encouragement of research among high school students, although the use of the funds is not restricted to such students.

## X — REPORT OF THE RESOLUTIONS COMMITTEE — Kenneth B. Hoover

BE IT RESOLVED that the Pennsylvania Academy of Science expresses sincere appreciation to:

1. Franklin and Marshall College for its willingness to open its facilities for the 45th Annual Spring Meeting.

2. The Committee on Local Arrangements — Arthur W. Shively, Harry K. Lane, John McDermott, and Joseph P. Donahue — for their planning and discernment of the needs that made this meeting a success.

3. The Exhibitors for providing excellent scientific equipment for our observation, and their courteous answering of questions.

BE IT FURTHER RESOLVED that the Academy commend and does in this manner indicate appreciation of:

1. Phyllis Martin for her efforts in producing the *Newsletter,* and to Point Park College for providing services in connection with the *Newsletter.*

2. The United States Steel Foundation for its continuing interest and support of the Academy programs.

BE IT RESOLVED that the Academy expresses regrets that President Chase could not attend the meeting due to the recent loss of his father.

BE IT FURTHER RESOLVED that the Academy record with sorrow the passing of Aura M. S. Booth, E. M. Gress, Robert Pearson, Stanley Reimann, George W. Richards, Jennie S. Siemienski and Ira J. Williams.



# ABSTRACTS

COMPARATIVE CHANGES IN THE LOCALIZATION OF MANUFACTURING IN THE UNITED STATES. E. W. Miller, *The Pennsylvania State University, University Park.*

The purpose of this study is to measure and interpret comparative changes in the localization of manufacturing in 70 selected Standard Metropolitan Statistical Areas of the United States between 1954 and 1963. Change is measured by a comparative change technique. Of the 70 SMSA's 32 experienced a comparative growth and 38 a comparative decline. To study the structure of the SMSA's a refined diversification index was prepared. It was concluded that a comparative growth or decline was not related to the degree of diversification of a SMSA. The growth or decline of a particular SMSA is due to a complexity of factors including the size and age of the manufacturing center, dominance of modern versus traditional industries, geographic location, and the influence of local economic conditions.

INCORPORATION OF GLUCOSE-U-$C^{14}$ INTO LIPIDS OF THE BLOW FLY DURING FLIGHT. Carolyn F. Mathur and W. J. Yurkiewicz, *Millersville State College, Millersville.*

Incorporation of the label from glucose-U-$C^{14}$ into lipids of the blow fly, *Phaenicia sericata,* was studied during flight and at rest. After three hours of continuous flight following injection of the glucose, only 0.5% of the radioactivity was found in lipids. One % was recovered from flies at rest for three hours. About 30% of the recovered label was in phospholipids and more than 40% was in the hydrocarbon plus sterol ester fraction in both flown and rested flies. Most of the remaining label was in glycerides. No differences caused by sex were found in the conversion of glucose to lipid.

ORIGINS OF WESTERN CIVILIZATION AND ABORIGINAL CAUCASUS. A. Grigolia, *Eastern Baptist Theological Seminary, Philadelphia.*

Aboriginal Caucasus is a remaining part of the ancient non-Semitic and non-Indo-European culture world of pre-Greco-Roman period. It circled the Mediterranean and Black seas including the Near East, and was composed from groups with common culture patterns. These patterns are manifested in the mythology and religious beliefs of the people, in technology, in socio-political structure and functions, in equality and respect of individual in social group, love of freedom and in orderly, almost ritualistic feasting, love of dancing, music, and elegance of attire and behavior. It was this culture group that gave Neolithic civilization to the peoples of the Mediterranean and Near East, creating using V. Gordon Childe's expression "the Neolithic Revolution" leading humanity from a food gathering economy to food producing economy, creating village and communal life, with the domestication of plants and animals, and leisure time for new discoveries and progress. There is not only academic value in the study of its culture history, but also strategic and political importance now as in ancient Greco-Roman period. It is of paramount importance in understanding and properly evaluating the meaning and origin of the present civilization of the Western World. Such studies are conducted from last century in the universities of Europe and are non existing in the United States.

MEETINGS OF THE PENNSYLVANIA ACADEMY OF SCIENCE IN RETROSPECT, 1959-1963. T. H. Knepp, *Stroudsburg Area High School, Stroudsburg.*

A series of slides taken at various spring and summer meetings of the Academy, showing members of the Academy and their families engaged in Academy activities — summer 1950, spring 1951, summer 1951, spring 1952, summer 1952, spring 1953, summer 1953, spring 1954, summer 1954, summer 1955, spring 1956, summer 1956, summer 1957, spring 1958, spring 1959, summer 1960, spring 1963.

RELATION OF THE RINGING EFFECT AND STRESS RELAXATION IN RINGING DIABASE BOULDERS. S. Schlossman and J. Gibbons, *Lafayette College, Easton.*

The relaxation of internal stress in ringing diabase boulders was investigated. The outer weathered shell was sawed from the boulder and a prismatic core cut from its center. Mechanical and electrical strain gauges were used to record the relaxation of the core, which was on the order of .003 in./in. There was a direct relationship between the dimunition of the ringing effect and the relaxation of the core. After the core had been fully relaxed it was placed in a uniaxial testing machine and restressed. Upon restressing the ringing could be reinduced, and then stopped when stress was relieved. The ringing effect was shown to be related to the stored elastic strain energy

which was the result of mineral expansion upon hydration of the outer shell of the rock.

PESTICIDE RESIDUE ANALYSIS BY NEUTRON ACTIVATION AND GAMMA SPECTROSCOPY. J. D. Sink, J. C. Miller, G. W. Sheritt and J. H. Ziegler, *The Pennsylvania State University, University Park.*

The analysis of pesticides has involved long and laborious procedures. A study was conducted to monitor the present chemical method and to explore a more sensitive and rapid analytical procedure for the determination of organochlorines in adipose tissue. The results suggest inaccuracies in the present chemical method and indicate neutron activation and gamma spectroscopy techniques would have potential as a rapid, reliable method of determining the organochlorine pesticide content in animal tissues using the $Cl^{37}$ (n, $\gamma$) $Cl^{38}$ reaction.

ANALYSIS OF THE IMMUNE RESPONSE IN THE MOUSE SPLEEN BY MEASUREMENTS OF METABOLIC ACTIVITY. R. S. Ranck, Jr., J. K. Fleming and D. D. Pearson, *Bucknell University, Lewisburg.*

A new technique is described for measuring the activity of immunocompetent cells during the early phases of the immune response. Litters of inbred mice were injected with ox red blood cells. Measurement of metabolic activity of spleen homogenates of injected and non-injected mice after 4 days was made using an oxygen electrode. Results appear to show a demonstrable increase in metabolic activity of the spleens of injected mice compared with the control group. The advantages of the technique are its applicability to all antigen-stimulated responses and its potential for quantitation.

THE AGE OF THE HIGHEST COALS OF THE SOUTHERN ANTHRACITE FIELD. W. C. Darrah, *Gettysburg College, Gettysburg.*

The geological age of the highest coals of the southern anthracite coal field of Schuylkill County is uncertain and several conflicting opinions have been advanced. Older views considered these coals to belong to the upper-most Pennsylvanian whereas more recent opinions incline toward a lower position. This recognition of ten species of fossil plants, hitherto restricted to the Monongahela and Washington equivalents, in the Tunnel, Gate, Faust and Brewery coals reopens the question of their correlation. New data give substantial support to the view that these coals are of Monongahela age, with the possibility of extension into the Washington.

MARGINAL MARINE DEPOSITS IN UPPER DEVONIAN CATSKILL FORMATION, NORTHEAST PENNSYLVANIA. J. D. Glaeser, *Pennsylvania Geological Survey, Harrisburg.*

The Flagstaff Mountain Member of the Catskill Formation at the Lehigh River is 98% sandstone and conglomeratic sandstone, is dominantly medium and coarser sand sizes, and has two-thirds of all its sandstones within two phi classes. Primary sedimentary structures in this 904-ft thick unit include parallel laminations, parting lineations, bimodal cross stratification, megaripples, scour megaripples, current crescents, and rib and furrow structures. Similar thick build-ups of moderately well-sorted sands containing the same assemblage of wave and current generated sedimentary structures occur today in marginal marine environments encompassing tidal deltas, beaches and off-shore bars.

OXYGEN UTILIZATION IN *SPONGILLA FRAGILIS* LEIDY WITH AND WITHOUT SYMBIOTIC ALGAE. T. E. Smigel and F. J. Little, Jr., *Bucknell University, Lewisburg.*

A study was undertaken to determine the respiratory rates of various preparations of the fresh water sponge *Spongilla fragillis* Leidy. Values of $\mu$M $O_2$ consumed/hr/gm of whole sponge, whole cell suspension, and cellular homogenate were achieved, as was an endogenous rate of respiration of sponge gemmules. Excepting the gemmules, each was done using both light and dark adapted material, with and without symbiotic algae, at 25 ± 0.1°C. Oxygen concentration was measured with a Clark oxygen electrode. The data have established an endogenous rate of oxygen consumption for sponge tissue and a comparison of whole sponge, cellular suspension and cellular homogenate respiration. They also suggest a whole organism effect.

ON ACHIEVING STABLE PREDATOR-PREY INTERACTIONS BETWEEN *HYDRA OLIGACTIS* (PALLAS) AND SELECTED CLADOCERANS. R. A. Bouchard and F. J. Little, Jr., *Bucknell University, Lewisburg.*

Triplicate or quadruplicate open 5-liter systems of various shapes and dimensions were used to achieve stable pre-predator interactions between *Hydra oligactis* (Pallas) and either *Daphnia pulex* (de Geer) or a local wild-type cladoceran, *Simocephalus vetulus* (Schodler). Observations showed *Daphnia* to orient to different parts of the tank as light conditions varied. Vertical illumination plus mirrored



tank sides achieved by covering the exterior with aluminum foil overcomes this tendency. *Daphnia* orientation and distribution then appears random. An increase in the ratio of open water to tank side should decrease *Hydra* efficiency vs. the free-swimming *Daphnia,* facilitating a stable interaction. Conversely, with *Simocephalus,* an increase in the ratio of open water to side should render *Hydra* more efficient because of crowding. This is because *Simocephalus* is less motile, has a thick shell, and spends a large proportion of its time at rest on the side of the tank. An interaction demonstrating a stabilizing trend was achieved. Others showed promising oscillations in both predator and prey populations but most tanks became contaminated with three species of very small ubiquitous cladocerans and small thick-shelled ostracod. Ostracods escaped predation in over 90% of all encounters and two species interactions with *Hydra* terminate rapidly, the *Hydra* becoming extinct. The contaminating cladocerans do appear to affect results. Modified with "closed" tanks, the method offers promise for testing various hypotheses and models concerning predator-prey interactions.

MYCORRHIZAE OF RED MAPLE *(ACER RUBRUM L.)*. R. J. Medve, *Slippery Rock State College, Slippery Rock.*

Mycorrhizae, beaded rootlets, and root hairs of red maple *(Acer rubrum L.)* were studied on both indigenous seedlings and seedlings grown under greenhouse conditions. Not all seedlings were found to possess mycorrhizae. Although the terms beaded rootlets and mycorrhizae have been used synonymously, the beaded rootlets were not always mycorrhizal. Beaded rootlets were terminal, intercalary, or lateral to non-woody roots and were first evident on 4-wk seedlings. The greatest development of beads occurred on first order non-woody roots. The endotrophic fungus was limited to the cortical parenchyma and did not penetrate the stele.

ARTICULAR MEMBRANE PROPRIOCEPTORS IN *LIMULUS CHELICERAE*. H. R. Maresh and S. B. Barber, *Lehigh University, Bethlehem.*

Primary sensory neurons which are located at the medial articular membrane between the coxa and tarsus of the *Limulus* chelicera signal relative movement and position of the joint segments. A histological study of neuron distribution and general characteristics of the proprioceptor area is given. Electrophysiological data concerning the presence of tonic and phasic units, their activity and relative distribution, are presented as well as a comparison with the activity of the medial membrane receptors of the trochanter-femur joint in the *Limulus* walking leg.

ATTEMPTED CULTIVATION OF CELLS FROM THE HONEYBEE. R. D. Luff and M. Levi, *Lehigh University, Bethlehem.*

Two insect balanced salt solutions, Trager's and Bishops, plus hemolymph form *Limulus polyphems* constituted the basis for research into honeybee cell culture medium. Both standard solutions were found to be hypotonic with honeybee hemolymph when assayed with a vapor pressure and a freezing point depression osmometer allowed close correlation between a final medium and the tonicity of bee hemolymph. Cells from larvae were disaggregated by forcing through a 320 mesh screen and planted into isosmotic medium. The cells attached to the culture bottles, showing neither lyses nor crenation; however, they did not multiply.

CERTAIN VISUAL RESPONSES OF ADULT APPLE MAGGOT, *RHAGOLETIS POMONELLA* (Walsh). G. E. Stanton, *University of Maine, Orono.*

Laboratory investigations on the responses of adult apple maggot indicated that colors of higher reflective characteristics and the hues yellow or yellow-green were most attractive. Black, blue, and red were among the ineffective hues. Trials using sticky boards as attractant surfaces indicated similar behavior in the field; however, very different responses were observed when using spheres coated with an adhesive. Both sexes and all age groups responded similarly to color. Response to spheres appeared more related to shape, size, and brightness than to hue, i.e., a positive response was elicited from sexually mature flies by "dark", appled-shaped objects.

AN ULTRASONIC MOTION DETECTOR FOR RECORDING ANIMAL ACTIVITY. J. L. Baird, Jr., B. D. Miller and P. G. Miller, *Lafayette College, Easton.*

A motion detector (Alton Electronics Company) designed to measure animal activity by utilization of ultrasonics is described. The detector emits a low intensity 40 kHz sound wave into an enclosure which has sound retaining properties. A transducer receives these sound waves as they are reflected from the enclosure walls, subject(s), and objects within the enclosure. Any movement on the part of the subject(s) disturbs the sound curtain and results in pulse output from the detector cir-

cuitry. The size of the moving object and the amount of movement can be interpreted from the pulse waveform. The activity patterns of several species (insects and rodent) are being investigated using this technique, which permits studying locomotor behavior without disturbing the test subjects.

STUDIES ON THE LIFE HISTORY OF *MOSESIA TREMAGLANDIS* (HALL, 1960). R. R. Williams, *Waynesburg College, Waynesburg.*

Experimental work was conducted on virgulate xiphidiocercariae being shed by *Pleurocera acuta* collected from the Tippecanoe River near Battleground, Indiana. *Cercaria tremaglandis* Hall, 1960 was demonstrated to be a species of *Mosesia,* the adult and metacercaria of which were not known prior to this study. The stonefly, *Acroneuria arida,* proved to be a good second intermediate host. Exposures of stoneflies to cercariae yielded infective encysted metacercariae within 15 days at 21-22°C. Metacercariae given orally to mice matured in the small intestines of these mice within 5 days. Relationships to closely related species are discussed.

STORIES THAT PARASITES TELL. C. E. Price, *Millersville State College, Millersville.*

Parasites can sometimes furnish information concerning the origin, distribution and/or taxonomic relationships of their hosts. This information was referred to as "parascript" by Dr. Harold Manter of the University of Nebraska. In the present study, we attempt to use monogenetic trematode parasites as indicators of past occurrences and present relationships existing among groups of fishes. Among the specific subtopics treated are: (a) proof for the marine origin of the whitefishes of the British Isles, (b) the theoretical origin and distribution of the cyprinid fishes, and (c) parasitological evidence for concluding that the catostomid fishes arose from cyprinid progenitors in North America. Several lesser points are discussed briefly.



# THE DARBAKER PRIZE PAPER

## DNA, RNA – CYTOPHOTOMETRY AND PROTEIN INTERFEROMETRY OF ERYTHROID CELLS FROM HYPOXIA EXPOSED RATS

R. B. MITCHELL AND ADAM ANTHONY
*Department of Biology, Physiology Section*
*The Pennsylvania State University*
*University Park, Pennsylvania 16802*

### ABSTRACT

Feulgen-DNA and Azure B-RNA cytophotometric analyses plus interferometric nuclear dry mass determinations were made on erythroid cell populations of adult rats exposed to 380 mm Hg for 2 and 7 days. DNA profiles of erythrocyte precursor cells indicated an increase in the rate of mitosis with 2 days exposure and a return toward control levels after 7 days. Increase in total RNA content of early erythroblast cells paralleled changes in DNA synthesis. Increases in dry mass content of normoblast nuclei at 2 days exposure suggest an increase in rate of release of immature red cells into the circulation.

### INTRODUCTION

Recent reviews on the physiology of hypoxia acclimation indicate that exposure to reduced barometric pressure is associated with a complex of bodily responses which aid animals in adapting to oxygen lack (1,2). It is generally conceded that metabolic events involved in augmentation of hemopoiesis are especially critical during the first week of exposure (3,4, 5). However, there is no information on the precise cytochemistry of hypoxia-induced hemopoietic changes known to be associated with marrow hyperplasia and peripheral polycythemia.

Present understanding of the complicated kinetics of erythroid differentiation and proliferation is far from complete. It is known that a reduction in tissue oxygenation causes release of a humoral substance called erythropoietin which stimulates bone marrow to produce erythrocytes (6,7,8). On the other hand, it is not known whether hypoxia exposure causes an increased production and release of preformed elements such as late erythroid cells (9) or primarily initiates differentiation of stem cell populations (10). Recognition that the latter mechanism of erythropoietin action would necessitate an increase in nucleoprotein metabolism and rate of stem cell transformation formed the basis for this study.

The aim of the present study is to attempt to clarify the hypoxic response of bone marrow by examining changes in nucleic acid and protein content using combined techniques of quantitative microspectrophotometry and interference microscopy. Specifically, the study was designed to determine whether hypoxia exposure causes any alterations in erythroblast mitotic activity, proerythroblast RNA metabolism or normoblast protein mass.

### MATERIALS AND METHODS

Twenty-four male albino rats (Holtzman) weighing 150-200 g were utilized. The decompression chamber used had a volume of 42 cubic ft, air turnover of 15 cubic ft per min at 380 mm Hg, constant temperature of 25° ± 2°C, and a 12 hr photoperiod. Hypoxia was induced in two groups of rats by exposure to a simulated altitude of 18,000 ft (380 mm Hg) for periods of 2 and 7 days, respectively. Controls were maintained at ambient pressure (725 mm Hg), a temperature of 23° ± 2°C, and a 12 hr photoperiod. At the end of each experimental period, rats were sacrificed and marrow samples taken by pipet from the distal end of a split femur. Marrow was diluted 3:1 with serum and thin marrow smears made from each animal. Smears were fixed for 5 min in absolute methanol and separate slides from each animal stained by the Feulgen procedure for DNA (11) and Azure B for RNA (12). Control and experimental smears from each experimental period were stained simultaneously to minimize error. Since Azure B stains both RNA and DNA, these slides were pretreated with electrophoretically purified, RNAase-free, bovine pancreas DNAase (Worthington Biochemical Corp.). Unstained

fixed slides were used for dry mass determinations.

Identification of specific erythropoietic cells was based on nuclear morphology, nuclear area and cytoplasmic tinctorial properties. All cytophotometric measurements were made with a single-beam microspectrophotometer (Precision Development Corp., Figure 1) and dry mass

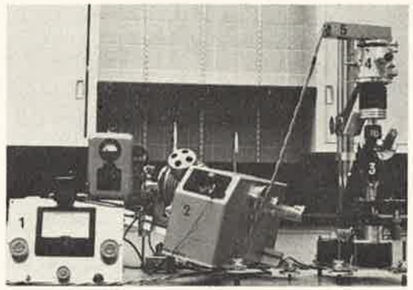


**Figure 1. Microspectrophotometer assembly showing (1) Photovolt power supply and meter (2) Monochromator and tungsten light source (3) Microscope with 20X, 0.4 N.A. condenser and 95X objective lens (4) Photometer head with viewing telescope and fixed apertures (5) Photodetector RCA 1P21 photomultiplier.**

determinations with a Leitz Mach-Zehnder double-beam interference microscope (Figure 2). Since these two quantitative methods are rather recent developments in the field of microscopic science, a brief statement of the principles involved may be in order. Microspectrophotometry, also referred to as absorption cytophotometry, identifies and measures specifically stained cellular substances by measuring intensity of light before and after its passage through a specified area of the cell (*ca* $0.5\mu^2$). It differs from conventional colorimetry in that the microscope replaces the cuvet containing a solution, and photometry is performed with a photodetector on a projected cell image. The basic formula used to determine changes in relative mass (m) of Feulgen-DNA or Azure B-RNA can be expressed as follows:

$$mk = (-\log T)A = O.D. \quad (A)$$

where k is the absorptivity and is a constant, A is the area of the specimen field, and the value for optical density (O.D.) is calculated from the measured transmittance (T) which is the ratio of the incident or reference light intensity ($I_0$) to the light intensity after passage through the substance to be measured (I).

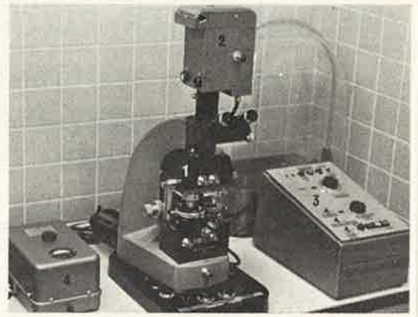


**Figure 2. Interference microscope with Orthomat automated camera. (1) Leitz double-beam interference microscope for transmitted light with 50X/0.85/0.17 objectives. (2) Orthomat camera (3) Orthomat control unit (4) Light source transformer.**

The present study employed the two wavelength method of microspectrophotometry as described by Patau and Ornstein (13,14). This method corrects for any errors caused by heterogeneous distribution of stained material. For determination of Feulgen-DNA content the wavelengths used were 555 m$\mu$ and 502 m$\mu$. For RNA determinations following Azure B staining the wavelengths were 595 m$\mu$ and 515 m$\mu$. In each case the larger wavelength corresponds to the absorption maximum of the dye complex.

With microscopic interferometry the directly measured quantity is the difference in optical path (Ø) between the light passing through the specimen and the reference light beam. This optical path difference, or retardation, is measured in millimicrons with the interference microscope. Total dry mass in unstained (fixed or fresh) cell preparations can be calculated from the following expression:

$$m = \frac{Ø\,A}{100\alpha}\,(g) \cdot 10^{-12} = \text{picograms}$$

where m is the mass, $\alpha$ is the specific refractive increment (0.18 $cm^3/g$ for cellular proteins) and Ø is the optical path difference. Thus the instrument actually serves as an ultrasensitive optical balance with a measuring accuracy of $10^{-14}$ grams. In the present study Ø was measured microdensitometrically from interference contrast photomicrographs of marrow smears at a final magnification of 19,000X (15).

## RESULTS

Microspectrophotometric data on the DNA content of erythroid cells in control, 2 day exposed and 7 day exposed rats are summarized in the form of histograms following the convention adopted by most workers in this area. Such DNA profiles facilitate detection of changes in mitotic activity patterns in cell populations simply from visual inspection of the graphs. More important, expressing DNA contents of nuclear populations as mean values would obscure the expected bimodal distribution patterns of dividing cells and thus lead to erroneous conclusions regarding relative DNA contents of cells in different stages of erythroid maturation.

*DNA Cytophotometry.* The data summarized in Figure 3 are based on a total of 400 individual photometric analyses of nuclear




**Figure 3. DNA distribution patterns for erythroid cells of the bone marrow from rats exposed to 380 mm Hg.**



DNA content. In all instances determinations were made on a randomly selected population that included all major stages in the erythroid developmental sequence (i.e. early and late erythroblasts, early and late normoblasts). It is immediately evident that the total population of cells exhibits a bimodal distribution into 2C and 4C classes (where C is defined as the amount of DNA in a spermatid); several interclass values also appear which are characteristically found in any proliferating tissue. Using morphological criteria, it was established that the normoblast cells were always in the 2C class (diploid DNA level) indicating an absence of DNA synthesis and mitotic activity.

It was found that erythroblasts could fall into either the 2C, interclass or 4C mode depending upon the presence or absence of premitotic DNA synthesis. With this in mind it is apparent that 2 day exposed rats have a greater number of cells containing 4C amounts of DNA. Thus, the data clearly show that after 2 days of hypoxia exposure there is a considerable increase in early and late erythroblasts exhibiting active DNA synthesis. In contrast, the DNA profile of 7 day exposed animals is shifted in the opposite direction with more cells in 2C and interclass categories. This indicates that after one week of exposure there is an abatement in the overall level of DNA synthesis, even below that of untreated controls.

*RNA Cytophotometry.* It was reasoned that if 2 day exposed rats exhibit a marked increase in mitotic activity among erythroid elements relative to control animals, this should also be reflected in differences in RNA content of early erythroblasts. This proved to be the case as shown in Figure 4 which summarizes data obtained from a total of 200 photometric analyses of early erythroblasts following Azure B staining. It is noteworthy that the metabolic

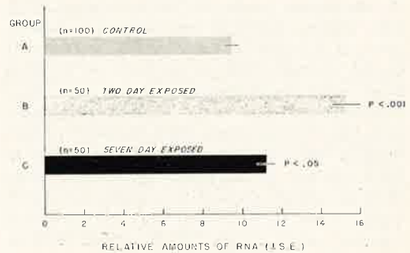



**Figure 4. Mean amounts of RNA in early erythroblast cells from rats exposed to 380 mm Hg.**

response pattern of RNA closely parallels that of DNA. Erythroblasts after 2 days of hypoxia show a highly significant increase in total cellular RNA whereas cells from 7 day exposed rats contain only a moderate increase in RNA relative to controls.

*Nuclear Dry Mass Measures.* The last aspect of this study dealt with the possible role of normoblast cells in the hypoxia induced activation of marrow erythropoiesis. Microinterferometric measures of dry mass were made on a total of 330 randomly selected late normoblast nuclei. The data in Figure 5 summarize the changes in nuclear size, mass per unit area, and total nuclear mass. Briefly these data show that after 2 and 7 days hypoxia exposure there is a slight increase in nuclear diameter of normoblast cells relative to control cells. It is also evident that only in the 2 day exposed group is there an increase in both mass per unit area and total nuclear mass.

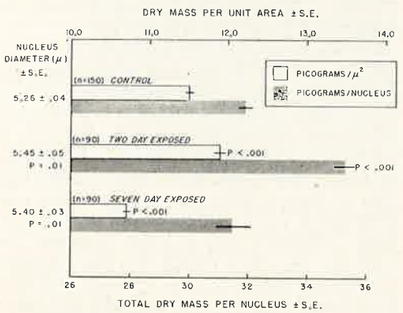



**Figure 5. Effect of hypoxia exposure (330 mm Hg) on dry mass of normoblast nuclei. Mass per unit area and total mass expressed in arbitrary units.**

Since it is known that the protein content of interphase nuclei is approximately 85% (16), nuclear dry mass can serve as a reliable index of changes in total protein content. It should be added that normoblast nuclei from these same experimental groups were also analysed for Feulgen-DNA content (cytophotometrically) to ascertain that observed protein changes were not attributable to changes in DNA content. As expected, normoblasts from all three groups showed no variation in DNA content from the 2C level, thus verifying that observed mass changes reflect protein changes.

## DISCUSSION

A major contribution of the present work is that it elucidates some of the adaptive cellular metabolic adjustments which trigger the erythropoietic response observed during early stages of acclimation to oxygen lack. Of equal importance is the demonstration that quantitative determination of patterns of DNA, RNA and protein changes in specific cells can be used to assess the precise functional role of various cellular elements in the regulation of erythropoiesis. It should be emphasized that the morphological identity of the bone marrow stem cell has never been established. As a consequence, there is much speculation as to its potentialities and role in population kinetics. For example, Lajtha (17) proposes the hypothesis that there may be two pathways for erythropoiesis. One pathway, omitting the last cell division, results in an early emergence of reticulocytes, whereas the alternative pathway having one more cell division results in a larger number of red cells. Such a model suggests that erythropoiesis might be stimulated by either increasing the number of stem cells feeding into the total marrow red cell population, often designated the "erythron", or by affecting the number of mitotic divisions within the erythron. The hypoxic rat was considered ideally suited for testing this hypothesis, since one of the primary actions of hypoxia is to augment the overall rate of marrow erythropoiesis.

Quantitative analysis of Feulgen-DNA profiles of cell populations comprising the marrow erythron revealed that with 2 days of hypoxia exposure there appear to be more erythroblasts in the DNA synthesis phase of the mitotic cycle. This increase in number of dividing cells reflects an apparent stimulation of stem cell differentiation toward the erythroid line. By day 7 of exposure, as the animal becomes acclimated to hypoxia, the histogram suggests the marrow may be returning to normalcy with respect to the relative number of dividing cells, i.e. reestablishing a homeostatic level.

As previously stated, early erythroblasts are identifiable on the basis of size and cytological appearance of cytoplasm and nuclear chromatin. It can be presumed that a population of such cells would include growing cells undergoing considerable protein synthesis as well as cells exhibiting premitotic DNA synthesis. Previous studies have shown that the bulk of RNA synthesis takes place in the earliest erythroblast stages (18). This restriction of RNA synthesis to earlier cell types indicates that the RNA responsible for subsequent hemoglobin formation is largely produced in the most immature cell types. It has been postulated that erythropoietin may induce differentiation of red cells from stem cells, which would be dependent upon DNA-directed RNA formation for the synthesis of new proteins (19). It is also believed that cytoplasmic RNA increases in amount during the growth of a cell preceding mitosis (20). In light of the above, one would predict an increase in RNA content of cells comprising the early stages of the erythron if one of the main actions of hypoxia is to stimulate stem cell differentiation along the erythroid line. The RNA data demonstrated this to be the case.

Data obtained from quantitative interferometric measurements of nuclear dry mass in marrow normoblast cells demonstrated that another important effect of hypoxia relates to the enhanced release of immature red cells into the circulation. Normoblast maturation ordinarily entails a progressive shrinkage of the nucleus until it becomes pyknotic and is eventually lost by extrusion. The increase in nuclear mass of marrow normoblasts in 2 day exposed rats supports the concept that one of the immediate effects of hypoxia is a rapid release of marrow cells into the circulation. Apparently the marrow is depleted of most cells in which nuclear dissolution has been initiated. This explains the well documented observation that reticulocytosis is a consistent feature of the early stages of exposure to oxygen lack. This 2 day increase in nuclear mass also supports the suggestion that erythropoietic stimulation may involve skipped divisions thus leading to an initial macrocytosis of reticulocytes (21).

The combined data from the present study demonstrate that at least two different regulatory mechanisms are involved in hypoxia mediated stimulation of marrow erythropoiesis. One involves an increased stem cell differentiation which leads to the presence of greater numbers of mitotic cells, notably the early erythroblasts. The other mechanism is concerned with facilitating the release of late normoblasts which have not yet completed their maturation process. Together, these effectively ensure a functional increase in that component of the marrow erythron delegated with the responsibility for blood cell proliferation.

## ACKNOWLEDGMENT

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## REFERENCES

1. Van Liere, E. J. and J. C. Stickney. 1963. *Hypoxia.* University of Chicago Press, Chicago.
2. Weihe, W. H. (ed.) 1964. *The Physiological Effects of High Altitude.* Macmillan Co., New York.
3. Tribukait, B. 1963. Der einfluss chronischer hypoxia entsprechend 1,000 - 8,000 m Hohe auf die erythropoiese der ratte. *Acta. Physiol. Scand.* 57:1.
4. Yoffey, J. M., N. C. W. Smith, and R. S. Wilson. 1966. Studies on hypoxia. IV The differential haemopoietic response to moderate and severe hypoxia. *Scand. J. Haemat.* 3:186.
5. Grant, W. C., and W. G. Root. 1952. Fundamental stimulus for erythropoiesis. *Phisiol. Rev.* 32:449.
6. Harris, J. W. 1963. *The Red Cell.* Harvard University Press, Cambridge. p. 131.
7. Jacobson, L. O. and M. Doyle (ed.) 1962. *Erythropoiesis.* Grune and Stratton, New York. p: 140.
8. Stohlman, F., S. Ebbe, B. Morse, D. Howard, and J. Donovan. 1966. Regulation of erythropoiesis. XX Kinetics of red cell production. *N. Y. Acad. Sci.* 149:156.
9. Fisher, J. W., L. G. Lajtha, A. S. Buttoo, and D. D. Porteous. 1965. Direct effects of erythropoietin on the bone marrow of the isolated perfused hind limbs of rabbits. *Brit. J. Haem.* 11:342.
10. Filamanowicz, E. and C. W. Gurney. 1961. Studies on erythropoiesis. XVI Response to a single dose of erythropoietin in polycythemic mouse. *J. Lab. Clin. Med.* 57:65.
11. Humason, G. L. 1967. *Animal Tissue Techniques* (2nd ed.) W. H. Freeman and Co., San Francisco. p. 309.
12. Flax, M. H. and M. H. Himes. 1952. Microspectrophotometric analysis of metachromatic staining of nucleic acids. *Physiol. Zool.* 25:297.
13. Patau, K. 1952. Absorption microphotometry of irregular shaped objects. *Chromosoma.* 5:341.
14. Ornstein, L. 1952. The distributional error in microspectrophotometry. *Lab. Invest.* 1:250.
15. Bartels, P. H. 1963. Personal communication.
16. Allfrey, V. G., A. E. Mirsky and H. Stern. 1955. The chemistry of the cell nucleus. *Advanc. Enzymol.* 16: 411.
17. Lajtha, L. G., R. Oliver and C. W. Garney. 1962. Kinetic model of a bone marrow stem cell population. *Brit. J. Haemat.* 8:442.
18. Grasso, J. A. and J. W. Woodard. 1967. Cytochemical studies of nucleic acids and proteins in erythrocytic development. *J. Cell Biol.* 33:645.
19. Krantz, S. B., and E. Goldwasser. 1965. On the mechanism of erythropoietin-induced differentiation. II The effect on RNA synthesis. *Biochem. Biophys. Acta.* 103:325.
20. De Robertis, E. D. P., V. W. Nowinski, and F. A. Saez. 1965. *Cell Biology.* (4th ed.) W. B. Saunders Co., Philadelphia. p. 308.
21. Brecher, G., and F. Stohlman, Jr. 1963. The macrocytic response to erythropoietic stimulation. In *Erythropoiesis.* (L. O. Jacobson and M. Doyle, ed.) Grune Stratton, Inc., New York. p. 216.

# A SIMPLIFIED EXPERIMENTAL DESIGN FOR THE SIMULTANEOUS TESTING OF SEVERAL INDEPENDENT VARIABLES

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## ABSTRACT

It is often desirable to design experiments which will provide meaningful data while avoiding numerous successive tests of each variable. Many experimenters, especially in the natural sciences, find most reference works on experimental design require a strong background in mathematics, particularly statistics.

There is a method designed by Box and Wilson for the simultaneous testing of several independent variables which reduces drastically the number of experiments normally utilized. The author has greatly simplified their method so that their geometrical models (which represent the variables under test) can be applied without recourse to any mathematics beyond simple arithmetic.

## INTRODUCTION

Scientific experimentation requires a consideration of all possible conditions, an accurate evaluation of all the effects produced by these conditions, and a logical selection of the best conditions—based on reasoning and evaluation of test data. With man's experiences throughout life in weighing two or more possibilities in terms of greater effectiveness or desirability, it seems logical that experimentation based upon planned testing methods should be instinctive with everyone. This does not seem to be true, however, and a wealth of technical papers and texts on design of experiments attests to this. There is a certain point in scientific investigation where intuition and personal feelings must be replaced by the impersonal logic of cause-and-effect relationships. Technical articles and other sources describe many methods of planning and conducting research in which well-organized methods of investigation are utilized. It is unfortunate that in most cases a rather extensive knowledge of mathematics, particularly in statistics, is needed in order to utilize a given method.

It is the purpose of this paper to provide certain assistance to the new experimenter who is well trained in his professional discipline but who has little or no experience in the design of scientific experiments. A selected list of references which require only limited mathematics ability is included as an appendix to this paper.

The following discussion is presented in simple mathematical terms in order to suggest one system of planning and evaluating a testing program. Undoubtedly, not all research projects are adaptable to this plan, but it is quite versatile and applicable to many circumstances. For clarity, the discussion has been organized into two sections; the first describes the evaluation of three independent variables by the Box-Wilson system (1) and the second discusses selection of the test variables and evaluation of the results.

## EVALUATION OF THREE INDEPENDENT VARIABLES

The method for the selection of specific experiments which is described in this section has been based on a paper by Box and Wilson (1). The basic tenet of the plan of selection of experimental points is based upon the establishment of a central point or origin and consideration that all test parameters will be referred to this one central point. A system of three test parameters was selected to demonstrate the general scheme although the Box-Wilson plan can be used for any number. Using three parameters as an illustration of the method, the construction shown in Figure 1 can be prepared.

A cube is constructed, using the conventional Cartesian coordinate system of three mutually perpendicular axes which are labeled X, Y, Z. The point O, O, O, which is the origin of the Cartesian system, has been placed at the center of the cube. A sphere can be considered to be drawn about the center of the same origin, of such radius that it touches the cube at each of the eight corners, producing a figure consisting of a cube with circumscribed sphere. The eight solid dots (Fig. 1 and Fig. 2) represent points at the corners of the cube which are also common to the sphere, and the circles of Figure 2 represent points on the sphere which are also of interest.

The sphere has a valuable property in this construction—all points on the sphere represent the same distance from the origin. Unfortunately only eight points of the inscribed cube have this property, and any other points on the surface of the cube represent a lesser distance from the origin.



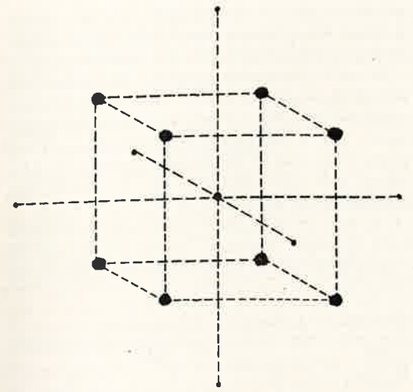


**Figure 1.**

It would be useful to relate test parameters to the Cartesian coordinate system, which simply describes the cube and at the same time achieves the advantage of the spherical construction in which all points are equidistant from the origin. The reader who is familiar with spherical polar coordinates will immediately recognize that the conversion from Cartesian to Polar coordinates will introduce three new variables (a polar radius and two angles of rotation), so this conversion will not provide any assistance.

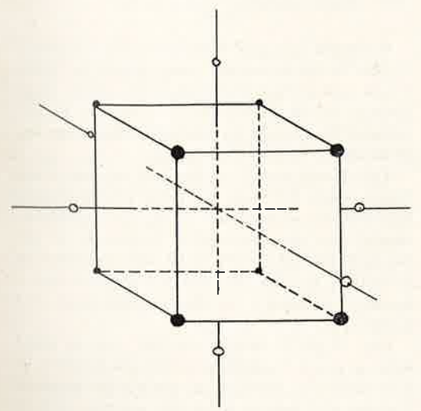


**Figure 2.**

A useful selection of points can be made, however, in the cubic plan, by using the cube corners and six additional points on the sphere which have a unique characteristic. Since the cube has been oriented so that each of the axes passes through a cube face (either in positive or negative sense in regard to the origin), then the center of each cube face represents the singular variation of the test parameter. For example, at the cube face where the +X axis emerges, the Y and Z values are O, O. Furthermore, at the surface of the sphere the value of each coordinate is that of the sphere radius which is constant, of course, for the complete sphere. The open circles of Figure 2 show the six additional points which can then be used. These points provide for separate testing of the positive and negative variation of each of the parameters, whereas the eight points of the cube corners represent combination values of the increments. Beginning with the origin there are fifteen possible selections. These have been listed in Table 1.

TABLE 1. *The 15 Selected Test Points.*

| Test | Axis | | |
|---|---|---|---|
| Number | X | Y | Z |
| 1 | X | Y | Z |
| 2 | X | Y | −Z |
| 3 | X | −Y | Z |
| 4 | X | −Y | −Z |
| 5 | −X | Y | Z |
| 6 | −X | Y | −Z |
| 7 | −X | −Y | Z |
| 8 | −X | −Y | −Z |
| 9 | $\alpha$ | O | O |
| 10 | $-\alpha$ | O | O |
| 11 | O | $\alpha$ | O |
| 12 | O | $-\alpha$ | O |
| 13 | O | O | $\alpha$ |
| 14 | O | O | $-\alpha$ |
| 15 | O | O | O |

It should be noted that the first eight test points represent the eight corners of the construction circle shown in Figure 1. The six following test points (Nos. 9 through 14) represent the locations on the construction sphere which are intersected by the three Cartesian axes. Finally, the 15th point represents the origin for both cube and sphere.

The terms X, Y, Z and their corresponding negative counterparts (for a cube) represent the same distance from the origin to any cube face. The term $\alpha$ is equal to one-half of the length of the body diagonal of the cube. A simple construction will show that for a cube

with sides 2 units in length, that $\alpha$ is equal to $\sqrt{3}$. In all cases, of course, $\alpha$ is equal to $\sqrt{3}$ times one-half of the length of the cube edge. The selection of values for X, Y, and Z in terms of test variables must now be made in order to relate this geometric construction to an experimental problem.

## SELECTION OF TEST VARIABLES

The 15 experiments listed in Table 1 can be listed in terms of variable parameters if we can determine the identity and size of unit distance along the X, Y, and Z axes. In other words, it is necessary to establish a unit of variation for each of the parameters. These variations will be made in both the positive and negative directions from the origin. It is obvious, of course, that the origin must also be established.

It is to be assumed, of course, that a person planning to design an experimental program is knowledgeable in his subject matter and that choice of test parameters presents no problem. These parameters may be a chemist's study of concentration, temperature, and pressure on a chemical reaction; or an educator's study of the effectiveness of elapsed time, experience, and performance level in knowledge retention; or a mathematician investigating the value of homework, frequency of testing, and use of programmed material in the learning of calculus. There are a myriad of possibilities which can be considered in regard to several basic elements, the most important being the assumption that the experimenter will be able to select the appropriate parameters which are to be tested.

The determination of the origin (which will be the mid-point for the variable parameters) may be made by a reasonable intuitive estimation of a logical starting point or may be the result of preliminary experiments. Similarly, the incremental steps for the testing of each parameter may be intuitive or they may have to be determined by experimentation. Several examples can be given here: if one wishes to study the effects of classroom temperature on some learning process, a logical nominal value would be a generally accepted (or average) temperature, such as 70°F and reasonable variations above and below could be 2° to 3° steps. If it is desired to evaluate the amount of pigment content in a house paint it might be well to establish the most often used concentration in commercial products, say 35%, as the nominal value and test in 5% steps both above and below this. In another instance it might be desirable to study composition changes in which a certain constituent may be varied through the complete range from 0% to 100%. Here a nominal value of 50% could be used.

A set of parameters used by the author for preparation of test firings of a new television phosphor will demonstrate the general arrangement. Let X be phosphor composition, Y be firing time and Z be firing temperature. The origin and unit values of X, Y, and Z are selected to be X = 50% ± 5%, Y = 800°C ± 50°C, and Z = 3 hours ± 0.5 hours. Test values such as those shown in Table 1 can now be determined for the 15 experiments as shown in Table 2.

TABLE 2. *A Phosphor Preparation.*

| Test Number | Axis X | Y | Z |
|---|---|---|---|
| 1 | 55% | 850°C | 3.5 hr |
| 2 | 55% | 850°C | 2.5 hr |
| 3 | 55% | 750°C | 3.5 hr |
| 4 | 55% | 750°C | 2.5 hr |
| 5 | 45% | 850°C | 3.5 hr |
| 6 | 45% | 850°C | 2.5 hr |
| 7 | 45% | 750°C | 3.5 hr |
| 8 | 45% | 750°C | 2.5 hr |
| 9 | 58.5%* | 800°C | 3.0 hr |
| 10 | 41.5%* | 800°C | 3.0 hr |
| 11 | 50% | 885°C** | 3.0 hr |
| 12 | 50% | 715°C** | 3.0 hr |
| 13 | 50% | 800°C | 3.85 hr*** |
| 14 | 50% | 800°C | 2.15 hr*** |
| 15 | 50% | 800°C | 3.0 hr |

* These are 50% ± $\sqrt{3}$ (5%)

** These are 800 ± $\sqrt{3}$ (50) °C

*** These are 3.0 ± $\sqrt{3}$ (.5) hr

It should be noted that the careful selection of the 15 experimental tests shown in Table 2 has considered five values for three parameters. If all combinations were to be examined, this could be calculated from the expression $X^n$ or $5^3$ which is 125.

The determination of the best sample of the fifteen must be made by some predetermined standard of evaluation. In the phosphor example, the measurement of brightness of the finished phosphor was used. The method of evaluation must be reliable and accurate but is actually unrelated to the experimental test design.

The 15-point testing method can be used with as much confidence as the preparation of tests and the method of evaluation permits. Success will depend upon the wisdom used in selecting the starting or nominal values and



the size of the test increments. Considerable mathematical simplification has been used in order to present an uncomplicated discussion. The interested reader is referred to the original paper for discussion of confidence limits, second order effects, equation fit, and so on.

## DISCUSSION

A method described by Box and Wilson (1) for the simultaneous testing of independent variables using a minimum number of experiments has been discussed. A plan for the testing of three parameters has been described in detail. Although the discussion described the use of only 15 tests the experimenter is not restricted to this number. A second set of 15 points could be utilized exactly as was shown for one set in which the second set is located at twice the distance from the origin. Alternately, an additional array of 15 test points could be planned about the most successful single test which emerged from a previous set of 15 tests. And finally, additional arrays of tests with various origins and differing variations of test parameters are capable of being utilized—in accordance with the desires and imagination of the investigator.

An experimental design which is more complex than the three component plan discussed here can also utilize the Box-Wilson method. The method consists of determining the minimum number of variables to be tested and then applying the same technique as that described in this paper but for n independent variables whereas this paper has considered the testing of three independent variables. Any number of parameters can be evaluated even though a geometric design cannot be drawn for all of the combinations.

## REFERENCE

1. Box, G. E. P. and K. B. Wilson. 1951. On the experimental attainment of optimum conditions. *J. Roy. Stat. Soc.* 13:1.

## APPENDIX

Anderson, R. L. and T. A. Bancroft. 1952. *Statistical Theory in Research*. McGraw Hill Book Co., New York.

Anderson, T. W. 1958. *An Introduction to Multivariant Statistical Analysis*. John Wiley & Sons, New York.

Boulding, K. E. and W. A. Spivey. 1960. *Linear Programming*. MacMillan, New York.

Cochran, W. G. and G. M. Cox. 1957. *Experimental Designs* (2nd Ed.). John Wiley & Sons, New York.

Cox, D. R. 1961. *Planning of Experiments* (2nd Ed.). John Wiley & Sons, New York.

Deming, W. E. 1950. *Some Theory of Sampling*. John Wiley & Sons, New York.

Dwyer, P. S. 1951. *Linear Computations*. John Wiley & Sons, New York.

Finney, D. J. 1955. *Experimental Design and its Statistical Basis*. Univ. Chicago Press, Chicago.

Fisher, R. A. 1953. *The Design of Experiments*, (6th Ed.). Oliver and Boyd, London.

Kempthorne, O. 1952. *The Design and Analysis of Experiments*. John Wiley & Sons, New York.

Mann, H. B. 1949. *Analysis and Design of Experiments*. Dover Publications, New York.

# LOCATIONAL PATTERNS OF MANUFACTURING IN THE BUFFALO METROPOLITAN AREA IN THE POST WORLD WAR II PERIOD

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## ABSTRACT

Manufacturing has located in three types of areas in the Buffalo Metropolitan Area since World War II. The inner area includes the city of Buffalo, where land use has been the most intensive, and until recently, where the most accessible locations in the BMA have been found. This accessibility however, has declined with increasing congestion and with less effective space remaining for the development of manufacturing. As a result, this has become the main area of manufacturing decline during the last 30 years.

The second and more outlying area includes much of the inner ring of suburbs surrounding the central city of Buffalo. Since World War II this belt has become one of the most accessible parts of the BMA with improvements of roads and services, and space has been available at relatively cheap rates for large sprawling industries. As a result, this zone has reflected the greatest growth of manufacturing of any part of the BMA during the last 30 years.

The most outlying area occupies the remaining two thirds of the BMA. The landscape here is still largely rural in character and is lacking in great accessibility. The sparse road net, lack of population and of services bears out this appraisal of the area. Accordingly, manufacturing has largely avoided this most outlying part of the BMA. Rather rugged terrain in the southern part of the metropolitan ring, and poor drainage in the eastern part of the ring have also prevented any major developments by manufacturing.

## INTRODUCTION

Three significant trends of manufacturing have occurred in the Buffalo Metropolitan Area (Fig. 1) (1) since the end of World War II. First, a notable outward shift of manufacturing employment has been observed. Second, a continued great density of manufacturing in the central city has continued. Third, a very uneven distribution of manufacturing throughout the BMA has become apparent. In fact, it appears that only a few key areas have attracted a great number of plants or have been favorable for the development of large plants with many employees. Rather, many sections have remained empty of manufacturing and many other parts of the area have actually declined in such significance. A study of the total environment of the BMA will reveal some of the reasons for the existence of these three trends. The main purpose of this paper, in fact, will be to analyze how and why various distributional patterns of manufacturing in the BMA have been changing during this post World War II period.

In previous studies on the distribution and development of manufacturing in various SMA's, the treatment of a large area as a single unit often has led to overgeneralizations. Commonly, only such large political units as the county, city, or metropolitan ring were studied. Thus, internal differences and changes were not recognized or were neglected. One of the earliest studies of changes within parts of a larger area was done by Reinemann on Chicago (2). He analyzed along a southeast-northwest traverse, the nature of manufacturing as well as changes during the post World War II period through 1954. Significant differences and changes in the distribution and development of manufacturing were found in the 4 zones he recognized. Unfortunately, only one traverse was taken from the core to the outermost area of the metropolitan region. Two, and better yet, 3 such traverses would have provided better insight into the changing locational patterns of manufacturing within smaller areas.

In 1959-1960, results of 3 studies on parts of the New York Metropolitan Area were published (3). In each, a careful analysis was made of the types of manufacturing as well as their distribution in the inner parts and



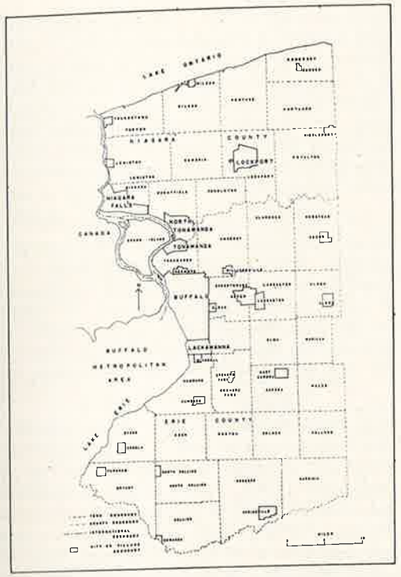


Figure 1.

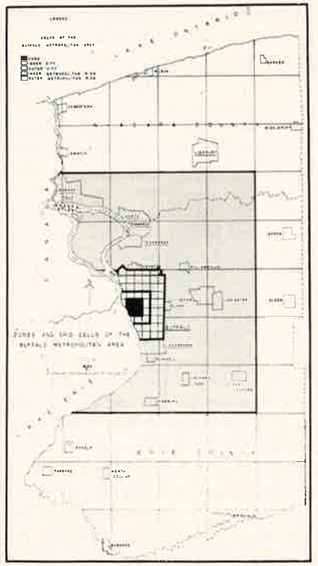


Figure 2.

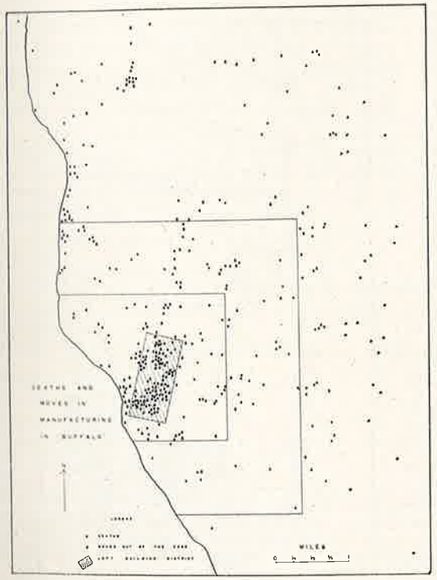


Figure 3.

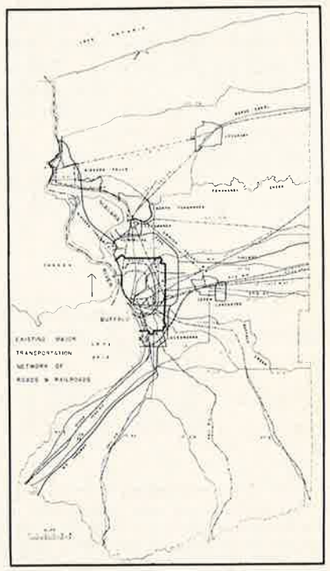


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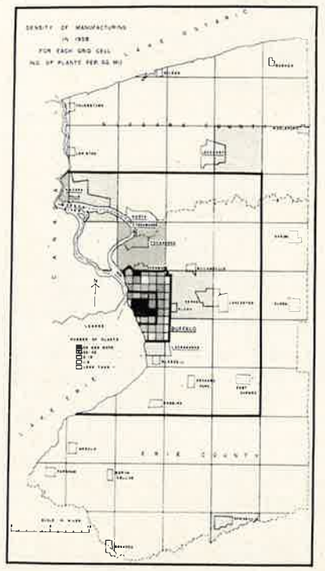



Figure 5.

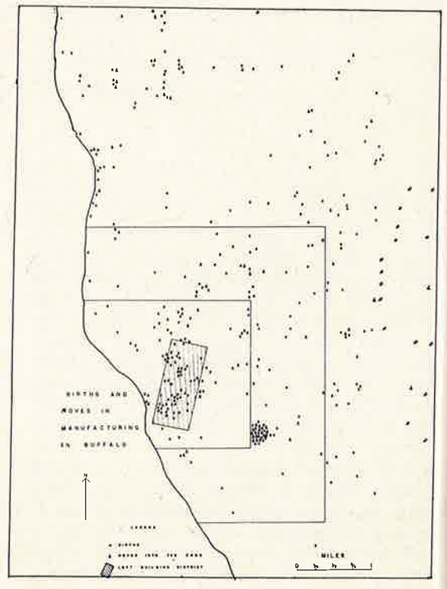



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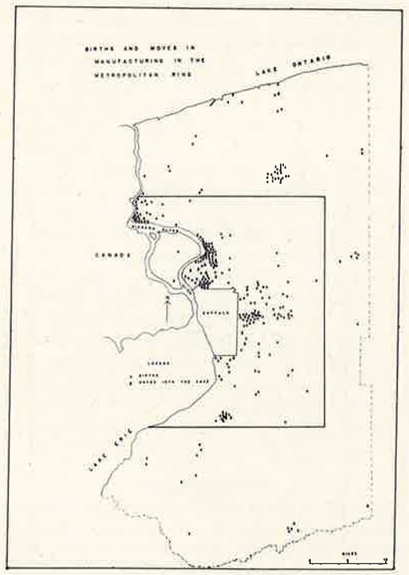



Figure 7.

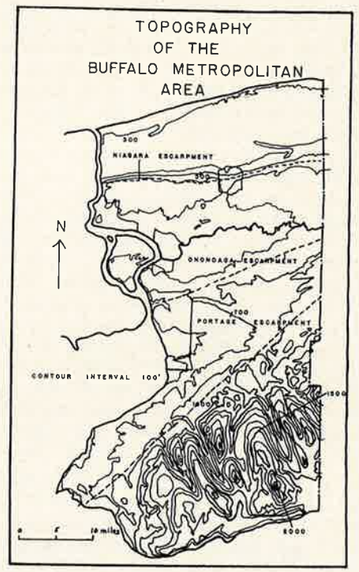



Figure 8.



more specifically, the core of the city. None of these publications however, provided a complete picture of the distribution and development of manufacturing in the entire New York Metropolitan Area, since many important small areas were omitted. Nevertheless, these 3 contributions did much to further knowledge of manufacturing development and distribution within certain small areas of major urban centers.

A year later, some 6 manufacturing districts in various part of Chicago were analyzed (especially for site factors) in a planning study (4). The examination of the nature of manufacturing in the districts was limited however, and the ring of areas surrounding the central city was omitted entirely from the study.

In this paper, the small geographical units will be examined, a part of the city and part of the metropolitan ring (Fig. 2) (5). With this type of analysis, many of the overgeneralizations of past studies should be avoided. Also, new patterns of distribution may be discovered, patterns and trends that were disguised and unnoticed when larger units only were under observation.

At least two types of raw data have indicated post World War II trends, namely, manufacturing employment and numbers of manufacturing concerns (6). In most instances, data from other criteria for measuring manufacturing, such as value of raw materials, value of finished products, value added, amount of capital invested, amount of power consumed, and physical output, were either difficult to obtain, or became distorted as the value of money changed from year to year.

## THE AREA OF DECLINING MANUFACTURING SIGNIFICANCE

The entire city of Buffalo has lost much manufacturing since the end of World War II (Fig. 3). The core zone in particular has had many deaths and some plant relocations out of the zone. This most central area, in fact, had more such losses than any other part of the BMA (Tables 1 and 2). Furthermore, of the various plants in the zone in 1949, some 43% of these had either died or moved out by 1958. The number of plant losses was greatest in the Central Business District where many loft structures were located. The inner and outer city zones also showed many deaths, though they had slightly more births than deaths. The inner and outer city zones reflected large employment losses. This trend occurred in part because of plant deaths and relocations, but mainly because of an actual decline in employment of many large and medium sized plants.

This general decline of manufacturing significance of the 3 zones of Buffalo suggests that the environment of that area was becoming less favorable for manufacturing development since the end of World War II. One evident trend was the growing problem of traffic congestion, lack of parking, and lack of loading facilities (7). The fact that a greater number of cars is funneling into the core has contributed to this congestion. Wilber Smith and associates (8) state that in most larger metropolitan areas ". . . automobiles carry more than half of all people who enter downtown areas . . ." Double parking of trucks that must park in the street because of lack of off-street parking of many older manufacturing firms in the central part of the city has fur-

TABLE 1. *Number of Births, Deaths, and Moves*[1]

| Zone | No. of Births | No. of Deaths | Difference | No. of Moves into Zone | No. of Moves out of Zone | Difference |
|---|---|---|---|---|---|---|
| Core | 100 | 144 | − 44 | 0 | 49 | −43 |
| Inner City | 70 | 63 | + 7 | 25 | 18 | + 7 |
| Outer City | 139 | 113 | + 26 | 34 | 24 | +10 |
| Inner Ring | 197 | 83 | +114 | 20 | 8 | +12 |
| Outer Ring | 58 | 49 | + 9 | 4 | 4 | 0 |
| Total | 564 | 452 | 112 | | | |

[1] The raw data on changes in number of plants between 1949 and 1958 were obtained from the New York State Department of Commerce (9). The number of births refers to the number of plants in 1958 that had not yet become established in 1949. The number of deaths refers to the number of plants in 1949 that had gone out of existence by 1958. The number of moves into a zone refers to the number of plants in that zone in 1958 that had a location outside of the zone in 1949. The number of moves out of a zone refers to the number of plants in that zone in 1949 that had moved out of the zone by 1958.

TABLE 2. *Changes in Manufacturing Significance within the SMA*[1]

| Zone | 1949 | Percent | 1958 | Percent | Changes |
|---|---|---|---|---|---|
| | Number of Employees | | | | |
| Core | 24,104 | 12.1 | 21,539 | 9.8 | −2565 |
| Inner City | 21,483 | 10.8 | 18,794 | 8.6 | −2689 |
| Outer City | 45,521 | 22.6 | 43,431 | 19.8 | −2090 |
| Inner Ring | 99,218 | 49.9 | 120,773 | 55.2 | +21,555 |
| Outer Ring | 9,227 | 4.6 | 14,625 | 6.7 | +5398 |
| SMA | 199,553 | 100.0 | 219,162 | 100.0 | 19,609 |
| | Number of Establishments | | | | |
| Core | 445 | 28.7 | 354 | 21.5 | −91 |
| Inner City | 223 | 14.4 | 242 | 14.7 | +19 |
| Outer City | 390 | 25.1 | 425 | 25.7 | +35 |
| Inner Ring | 343 | 22.1 | 468 | 28.4 | +125 |
| Outer Ring | 149 | 9.7 | 159 | 9.7 | +10 |
| SMA | 1,550 | 100.0 | 1,648 | 100.0 | +98 |

[1] The raw data on number of plants and on number of employees was obtained from the New York State Department of Commerce (10).

ther increased serious traffic bottlenecks. These above problems in traffic have in turn caused many parts of the city to no longer be the most accessible area in the SMA.

These above problems of transportation have caused the original compact packet of functions in the center of Buffalo to break up, and many of these city-oriented functions have been moving out to other parts of the metropolitan area. Hoover and Vernon in *Anatomy of a Metropolis* (11), indicate that these changes are occurring in large metropolitan centers of the nation. The survey indicates that ". . . eventually small plants will have almost as wide a geographic choice of locations as large plants and as the handicaps of distance have been overcome, the differences between areas—differences in transportation facilities, labor, market characteristics, and external economies (services and convenience)—have been reduced. The changes in transportation and in travel efficiency within the core and inner city zones since World War II have encouraged an increasing number of suppliers to relocate to more outlying parts of the BMA, especially where new roads and other services have been provided. Then, with the increased use of the automobile many skilled and semi-skilled operators, particularly many female workers, became more mobile than formerly. Instead of using public transportation facilities, many workers either have their own cars or are in car pools. Finally, industries needing to consult frequently with their customers on a given stage of manufacturing need not locate in the city heart today, with the increased use of the car, the telephone, and the truck, for rapid communication between manufacturer and customer is possible over much greater distances.

Not only have the variety of services been declining for manufacturing in the city, but changes in the use of power and in the use of machinery have been occurring which has meant less demand for hand labor. The most significant change in the use of power has been the changeover from the use of steam-power to the use of electricity. Though this change in power first began in the 1920's, many older plants in the city of Buffalo have only made this basic change since World War II. Once a plant changes over to electricity, the horizontal floor layout becomes more efficient, than the multi-story building where numerous belts and shafts had to be used in connecting the various pieces of machinery. Most of the plants in Buffalo could not relocate in modern one or two floor layouts because of the lack of land space. Raymond Vernon (12) points out that:

> By and large, the possibility of carving out a new site or greatly enlarging an old site in the central city become increasingly difficult with the pasage of time. Zoning regulations were a part of the problem; these regulations, which first appeared in American cities to any extent in the 1920's often inhibited the expansion of manufacturing in neighborhoods where some manufacturing already existed. To be sure, such restraints ordinarily did not apply to plants in existence prior to the adoption of the zoning requirements. But they did operate to discourage the radical expansion or total replacement of plants in many city areas.
>
> Even where zoning ordinances played no role, however, the assembly of a city site was a formidable operation. As the city developed, most of its land was cut up in small parcels and covered with durable structures of one kind or another. The problem of assembling these sites, in the absence of some type of condemnation power, required a planning horizon of many years, and a willingness to risk the possibility of price gouging by the last holdout. Moreover, once a site was acquired, razing costs alone could easily run on the order of $50,000 an acre in current dollar terms. All told, the value of the site could amount to 20 or 30 times more than that of an equivalent area in a developed surburban location. In these circumstances, it was small wonder that many manufacturing establishments chose a surburban location in replacing their obsolescent structures.



TABLE 3. *Plant Space Ratios (these are median figures for each zone).*[1]

| Zone | Amount of Indoor Floor Space per Employee | Ration or Indoor Floor Space and Outdoor Land Space |
|---|---|---|
| Core | 500 sq. ft. | 1 = 0.07 |
| Inner City | 571 | 1 = 0.80 |
| Outer City | 684 | 1 = 0.33 |
| Inner Ring | 685 | 1 = 3.7 |
| Outer Ring | 424 | 1 = 4.2 |

[1] The data was obtained from answers of written questionnaires mailed out in the summer of 1961 (13).

Furthermore, many of these crowded, mixed neighborhoods have deteriorated into blighted areas and even slums, and therefore, are dangerous, especially for women workers who often have to walk several blocks to the bus stop.

Probably just as important as the need for more plant space is the need for greater efficiency of the space layout. Many of the old buildings in Buffalo have excessive pillars, narrow and odd shaped bays, poor lighting, and insufficient elevator facilities. The old materials make a fire hazard, and high fire insurance rates must often be paid. Table 3 shows the average floor space per employee in the three zones of Buffalo. These ratios, while not inadequate for plants manufacturing mainly unstandarized products and using mainly hand labor, are very inadequate for plants producing more standardized products and needing more space for machinery. This trend toward greater mechanization by many plants in the city center has increased space needs, and therefore, has made many loft structures obsolete. The desire for more space was indicated in Table 4.

The numerous road building and urban renewal projects in Buffalo have caused the mortality rate of manufacturing plants to be high in the area (13). After the completion of the New York State Thruway in 1956, two major feeder routes were constructed from the Thruway into the Central Business District, one east and one south from Buffalo. In addition, the Niagara Thruway, a limited access highway parallel to the Niagara River, was constructed between the western edge of the Central Business District of Buffalo and the city of Niagara Falls, some 15 miles to the northwest (Fig. 4). These developments, along with the building of several public housing projects in the inner and outer city zones and in parts of the core, have resulted in the tearing down of numerous loft structures and the displacement of much industry (14).

## THE AREA OF CONTINUED GREAT CONCENTRATION OF MANUFACTURING

The core, the inner city, and the outer city zones continued to have a greater density of

TABLE 4. *Future Plant Space Needs (percentage of plants answering questionnaire in a given zone).*[1]

| Zone | Needs are Adequate | Needs are Inadequate | | |
|---|---|---|---|---|
| | | Will try and Acquire Adjacent Space | Will Need to Move to New Location | Total of Inadequate Needs |
| Core | 64% | 18% | 18% | 36% |
| Inner City | 50 | 25 | 25 | 50 |
| Outer City | 73 | 15 | 12 | 27 |
| Inner Ring | 80 | 12 | 8 | 20 |
| Outer Ring | 78 | 11 | 11 | 22 |

[1] The data was obtained from answers of written questionnaires mailed out in the summer of 1961 (13).

manufacturing plants than any other part of the BMA (Fig. 5 and Table 5). The core in particular was still an "incubator area" for many new plants, and this zone had more births per square mile than any other part of the BMA (Fig. 6). The inner city zone had more births than deaths, and had more plants moving into the zone than any other part of the BMA excepting the outer city zone (Table 1). Most of the plant births in Buffalo were small concerns of 1-9 employees.

TABLE 5. *Density Changes of Manufacturing Plants in the Zones—(Number of plants per square mile).*[1]

| Zone | 1949 | 1958 | Changes |
|---|---|---|---|
| Core | 114.1 | 90.8 | −23.3 |
| Inner City | 24.8 | 26.9 | +2.1 |
| Outer City | 14.4 | 15.7 | +1.3 |
| Inner Ring | 0.6 | 0.9 | +0.3 |
| Outer Ring | 0.1 | 0.1 | 0.0 |

[1] The raw data on number of plants in each zone was obtained from the New York State, Department of Commerce (10).

Many of these new plants as well as older ones in the city, especially in the core, desired small rental space (Table 6). Schmidt claims that ". . . small plants often use lighter materials and equipment, have less emphasis on straight-line production, etc., so that multistory plants more nearly suit their needs than those of mass producers" (17). The cluster of plant births in the southeast part of the inner city zone can be explained by the fact that several former warehouses were converted into loft space for manufacturing after World War II (Fig. 6) (18). According to Vernon (19), though land costs are characteristically much higher in city centers than in their metropolitan rings, ". . . prevailing rental rates for manufacturing space in the congested central city is scarcely higher than that in suburbs."

TABLE 6. *Building Ownership Status of Manufacturing Concerns — (Percentage of plants answering written questionnaire in a given zone)*[1]

| Zone | Rental Space | Owning Space |
|---|---|---|
| Core | 58% | 42% |
| Inner City | 9 | 91 |
| Outer City | 25 | 75 |
| Inner Ring | 11 | 89 |
| Outer Ring | 36 | 64 |

[1] The data was obtained from answers to a written questionnaire mailed out in the summer of 1961 (15).

Many of Buffalo's plants manufacture unstandardized products, and therefore, use largely hand labor. The fact that labor costs were the leading cost in operating a plant in the three zones of Buffalo indicates this orientation to hand labor (Table 7). Also, the small floor space per employee of manufacturing in the city indicates no great degree of mechanization for many remaining plants (Table 3). Food and apparel concerns still use primarily female labor, in part because of their manual dexterity. This type of labor still tends to use public transportation to a great degree, and the best location for this type of transportation still tends to be in the city (20). Thus, these various service advantages of the city are still significant in most of the larger cities of the nation according to Hoover and Vernon (21).

TABLE 7. *Plant Operating Costs for Each Zone—(Percentage figures represent percent of total costs for plants; of these various costs, median figures are used of the plants, for each zone)*[1]

| Zone | Labor | Raw Material |
|---|---|---|
| Core | 44% | 25% |
| Inner City | 50% | 25% |
| Outer City | 50% | 30% |
| Inner Ring | 31% | 41% |
| Outer Ring | 22% | 50% |

[1] The data was obtained from answers to a written questionnaire mailed out in the summer of 1961 (16).

Many small industries have remained in Buffalo because of inertia (22). To move to a new location would be too expensive for many plants, particularly those having a low margin of profit. The only alternative for many plants to an actual move is either to maintain a limited business or to close operations entirely. The great number of plant deaths in these zones suggests that many other plants are probably not faring well either and are just barely maintaining themselves.

The factor of inertia also appears to be present in maintaining large plants in the city. The inner city zone has several large and medium sized grain mills along the mouth of the Buffalo River and along the Ohio Basin. The large Republic Steel plant and National Analine Chemical Dye plant have also remained along the Buffalo River further upstream. The outer city area, especially in northwest Buffalo, has numerous old large plants, especially in electrical machinery, non-electrical machinery, and fabricated metals, and in transportation equipment. The largest manufacturing plant in Buffalo however, is in the northern part of the core zone, the Trico plant with more than 4000 employees, a manufacturer of windshield wiper blades.

Apparently such plants are finding that even though local site conditions are not what they could be, the large amounts of capital invested in buildings, land and equipment, makes a move prohibitively expensive. Therefore, these plants continue operating and attempt to improve their efficiency in ways that will counteract the effects of a rather poor site. Many of these large plants in the city are not particularly affected by the local factors, but rather are more influenced by situation factors (23). Only when the site factors become extremely poor will a plant consider a relocation. In such cases, a plant will often begin a branch plant in another area, outside of the core zone and even outside of the BMA, and then over a period of years move parts of the equipment from the old plant to the new location.

## THE AREA OF GREAT MANUFACTURING GROWTH

The inner metropolitan ring showed greater growth in manufacturing than did any other zone between 1949 and 1958 (Table 2). Net employment increases totaled slightly above 21,000 employees. This growth reflected in part numerous plant births and relocations into the zone, particularly of jobber plants in fabricated metals and non-electrical machinery, but also of small concerns in stone-clay-glass, and printing (Table 1). Other factors influencing employment growth were the expansion of large existing industries, and the birth of several large plants in the zone. These basic types of manufacturing included transportation and equipment, stone-clay-glass, chemicals, and fabricated metals. Transportation alone contributed to 25% of this net employment growth of the zone.

Three areas in the inner metropolitan ring in particular reflected enormous growth: the town of Tonawanda and the Tonawandas, Niagara Falls, and the town of Cheektowaga (Figs. 1 and 7). The town of Tonawanda and the Tonawandas appear to have many advantages for manufacturing development. There are a variety of railroad facilities in the Tonawandas, and in the area along the Niagara River in the town of Tonawanda. As a result, many rail sidings have been built in these areas. The Niagara River bordering the north and west part of the region has made inexpensive water transportation possible for the carrying of bulky materials to basic manufacturing concerns in the zone. Furthermore, the town of Tonawanda and the Tonawandas, now has the densest road network of any part of the entire metropolitan ring. Then, there are several main thorofares that have greatly increased the mobility of labor. Most significant of all has been the completion of the Niagara Thruway from downtown Buffalo to Niagara Falls, for this important expressway runs through the western part of the zone.

Because of the fine transportation facilities here, other features have developed and have made the area favorable for manufacturing development. A dense population has located in the town of Tonawanda and in the Tonawandas, so that by 1958 the area had a sizeable portion of the labor supply of the entire metropolitan ring. The improved road net has also made this area well located in regard to the local markets of the entire SMA. Transportation facilities have also served to attract public water and sewerage systems in many parts of the area. Then, too, zoning ordinances have remained attractive to the development of manufacturing. Certain parts of the area have even been specifically zoned for heavy manufacturing, especially the western part of the town of Tonawanda along the Niagara River. Many other parts of the town have ordinances attractive to manufacturing development and expansion. As a result of all these factors, many large blocks of land have been made available for manufacturing, particularly since the land rates, until relatively recently, have been fairly low.

Water from the Niagara River is available for the various manufacturing processes. This resource has attracted several basic manufacturing plants to the town. The Spaulding Company in Tonawanda is an example of such a concern. This plant, uses, on an average, 30 million gallons of water a day (15). A final factor not to be overlooked in attracting manufacturing to the area has been the development and expansion of the Charles R. Huntley steam generating plant on River Road. The expansion program of the plant in 1958 added $101,548,000 worth of electric and gas facilities so that this unit now has a capacity of 1,186,000 kwh (24).

Niagara Falls attracted more expansion of manufacturing employment than any other part of the entire inner metropolitan ring between 1949 and 1958 (Fig. 7). More than a

third of the employment growth of the zone took place in Niagara Falls alone. This growth has been primarily in basic types of manufacturing, particularly in chemicals, stone-clay-glass, electrical machinery, and transportation equipment. The dense road and rail net, and the location of the Niagara Falls Air Base for testing of aircraft products have been attractive features. Most significant of all, however, has been hydroelectric power. Several plants, including du Pont, and General Abrasives Co. indicated that power was of primary importance in attracting and maintaining manufacturing in the city (15). According to Renner (25) "Whenever power costs constitute the major percentage of the total cost of the finished product, the manufactural plant drives as close to the focus of power generation as possible." A rockslide in 1956 damaged the Schoellkolf station on the American side of the Niagara River but use of Canadian power by American Industries for the next few years relieved most of this shortage. Soon after the rockslide, the construction of a new power project was begun. By 1963 part of these facilities were opened, and within a few years it is estimated by the Niagara Mohawk Power Co., that the plant will have a capacity of 2,190,000 kilowatts (24). This power expansion program should do much to continue attracting manufacturing with heavy power demands to the Niagara Falls area.

A factor nearly as significant as power in attracting manufacturing to Niagara Falls has been the proliferation of plants using each other's products. This has been a significant locational factor in the chemical industry. To site just a few examples: du Pont uses much chlorine from Olin Mathieson Co. for pulp and paper bleaching. Several of the paper companies in Niagara Falls also use this local chlorine. The Pure Carbonic Co. (division of Air Reduction) obtains carbon gas from the Chemicals Division of Union Carbide for the manufacture of $CO_2$. The Goodrich Company obtains chlorine from Hooker Chemical Company (26).

The town of Cheektowaga during the period 1949-1958 had a greater increase in new plants than any other part of the Buffalo Metropolitan Area excepting the town of Tonawanda and the Tonawandas (Figs. 1 and 7). Such industries as stone-clay-glass, fabricated metals, non-electrical machinery, and miscellaneous all reflected significant growth. Many of the new plants were attracted by the market outlets of the nearby basic type of manufacturing (27). The Buffalo Airport also attracted some of the new plants, especially those manufacturing electronics parts and using the air freight facilities. The fine rail and road network, as well as the dense market of the area also attracted many types of manufacturing to the area. The completion of the New York State Thruway has attracted many truck oriented activities. Again, zoning laws in the towns of Cheektowaga and Lancaster have been fairly liberal and permissive for manufacturing (28). Many of these advantages of the Cheektowaga-Lancaster area for manufacturing were pointed out by Smith of American Machine and Foundry Company (29). Future growth of manufacturing will undoubtedly be attracted by the completion of the huge modern electronic railroad yards of the New York Central System in the town of Cheektowaga in 1957. The yard now handles 2600 cars a day, and will eventually handle up to 3200 cars a day (30).

The Lackawanna area is the fourth area of major growth of manufacturing in the zone. The Bethlehem Steel plant, which expanded to 16,000 workers by 1949, is easily the largest single manufacturing concern in the SMA (10). The concern formerly chose the Lackawanna site because of its location on the Outer Harbor near the mouth of the Buffalo River. Here, not only do lake vessels carrying bulky materials dock at the plant, but there are large supplies of pure water available for use in the various processes of iron and steel making. The Ford Stamping plant in the nearby town of Hamburg came into the area soon after 1949. By 1958 it had expanded to around 4000 workers.

The great concentration of manufacturing in the Lockport area of the outer metropolitan ring is in great contrast to the rest of the zone (Fig. 5). The greater density of roads and railroad facilities has attracted much of the population, and therefore, the labor supply and markets of the zone. Accordingly, various services have also been attracted to the city where the demands of a greater population are more varied. The factor of inertia has also maintained high manufacturing densities in Lockport. Many plants in past decades were attracted to the area because of cheap water power and transportation provided by the Barge Canal. In recent years the canal has lost much of its former significance, but much industry has remained, and now uses truck or rail transportation.



## THE AREA OF CONTINUED LOW MANUFACTURING DENSITY AND UNEVEN DISTRIBUTION

The density of manufacturing plants was considerably lower in the metropolitan ring than in the more central part of the SMA (Buffalo proper) (Fig. 5). The inner ring especially had just under one plant per square mile, even though great manufacturing growth took place there. Furthermore, more than two thirds of the inner ring had under one plant per 36 square miles (31). The great growth of manufacturing in the zone was largely restricted to Niagara Falls, the Tonawandas and the town of Tonawanda, the town of Cheektowaga and a part of the town of Lancaster, and the city of Lackawanna. The outer ring only had significant manufacturing concentration in the Lockport area.

The lack of adequate transportation facilities in many parts of the metropolitan ring is perhaps the single most important reason why so much of the area has shown only minor development in manufacturing. Even though many roads have been constructed including the three expressways, the overall density of the road network in the area remains considerably less than in the city of Buffalo itself. Outside of the rural-urban fringe, in many areas in the east and south parts of the inner ring roads are spaced several miles apart. The rather rugged terrain of part of southern Erie County and the poor drainage in the eastern part of the zone have made extensive road-building expensive and impractical (Fig. 8).

Because the road network is sparse in many parts of the ring, the population density has remained low, again particularly in the areas outside of the rural-urban fringe. A lack of labor supply, therefore, is a deterrent to manufacturing development in many areas. The local market of the zone has remained limited and this fact has prevented plants from locating in many parts of the area especially those requiring frequent consultation between plant and its market outlet. The lack of labor mobility of many parts of the outer metropolitan ring and even parts of the inner ring has prevented many basic durables plants from locating in such areas, for these plants hire much labor.

Many services essential for manufacturing are lacking in parts of the inner and outer metropolitan ring. Public sewerage and water facilities are particularly limited. Proper installation of a single well, for example, requires as much as $50,000 to $60,000 (32). Moreover, unless certain state specifications regarding water, sewerage, and fire protection are met, the insurance rates for a plant rise abruptly (33). Repair services and suppliers have located in only a few locations. Likewise, rental space, especially in loft buildings, is not of common occurrence. Low cost semi-skilled female labor is rather scarce in many parts of the area, most of this supply still being mainly in the inner zones of Buffalo. To train female labor for various sewing operations in the apparel industry, for example, is expensive (34).

The inner metropolitan ring in particular is becoming more of a "bedroom" area for the SMA, and therefore manufacturing has had an increasingly difficult time in expanding. The towns of Amherst and Orchard Park, in particular, have favored the development of residential structures to the exclusion of most other uses. Several other towns have strict ordinances against the development of manufacturing. As a result, significant development of manufacturing is possible in only a few towns in the entire inner metropolitan ring. Only Tonawanda, Cheektowaga, and Lackawanna, provide tolerant atmospheres, and even in certain districts of these communities expansion is limited by zoning restrictions and other problems (35).

## REFERENCES AND NOTES


1. Hereafter the phrase Buffalo Metropolitan Area will be abbreviated to the BMA.
2. Reinemann, M. W. 1955. *The Localization and the Relocation of Manufacturing within the Chicago Urban Area* (unpublished PhD. dissertation). Geography Dept., Northwestern University.
3. Hall, Max, (Ed.). 1959. *Made in New York*. Harvard University Press, Cambridge, Mass.; Hoover, E. M., and Raymond Vernon. 1959. *Anatomy of a Metropolis*. Harvard University Press, Cambridge, Mass.; Lichtenberg, R. M. 1960. *One-Tenth of a Nation*. Harvard University Press, Cambridge, Mass.
4. Department of Planning, *Local Patterns of Major Manufacturing Industries in the City of Chicago*. 1960.
5. Five rather concentric zones were studied and set up, beginning with the core or heart of Buffalo proper, and extending outward to the most outlying part of the SMA. Three of these zones are located in the city of Buffalo and two are in the surrounding ring. Each of these five zones were set up by the use of two criteria: density of manufacturing distribution in 1958 (the areal unit measuring density used in the city of Buffalo was one square mile, whereas in the metropolitan ring the unit was 36 square miles or the approximate size of one town or township), and growth and decline in number of establishments and in employment between 1949 and 1958.
6. New York State Department of Commerce, *Industrial Directories of New York State for 1949 and 1958*.
7. Reimann and Georger, Inc., a manufacturing concern, moved from the core to the town of Cheektowaga in 1956. The problem of the previous location was lack of space for "truck pick ups and deliveries". This in-

formation was received as one of the returning written questionnaires mailed out in the summer of 1961.

8. Wilber Smith and Associates. 1961. *Future Highways and Urban Growth.*
9. The raw data on changes in number of plants between 1949 and 1958 were obtained from the New York State Department of Commerce, *loc. cit.*
10. The raw data on number of plants and on number of employees was obtained from the New York State Department of Commerce, *loc. cit.*
11. Hoover, E. M., and Raymond Vernon, *loc. cit.*, pp. 54-55, 61.
12. Vernon, R. 1959. *The Changing Economic Function of the Central City.* Committee for Economic Development, New York Metropolitan Regional Study, Graduate School of Public Administration of Harvard University for the Regional Plan Association, Inc., p. 53.
13. Numerous plants indicated that a recent move or a pending move was because of the factor of some urban renewal project. This information was obtained from answers of written questionnaires mailed out in the summer of 1961.
14. Ibid.
15. The data was obtained from answers of written questionnaires mailed out in the summer of 1961.
16. Ibid.
17. Schmidt, R. N. 1954. *A Consideration of Some Aspects of Business Growth.* (PhD Dissertation). University of Michigan. p. 223.
18. A particularly good example of this trend is the building at 710 E. Seneca Street in the area east of the Central Business District of Buffalo.
19. Vernon, R., *loc. cit.* p. 18.
20. This conclusion was obtained from answers of written questionnaires to numerous food processing industries that were mailed out in the summer of 1961.
21. Hoover, E. M., and Raymond Vernon, *loc. cit.*, p. 53.
22. Inertia refers to those forces which tend to perpetuate manufacturing in a given place once it has become established, even though the original favorable factors that once attracted it there are in part no longer present. Hoover discusses the problem of inertia, although he does not label it as such. He says, "The fact that some new location may have greater income potentialities than the one now occupied does not mean that the person or firm concerned will immediately move to the new location or even that he ought to. There are many obstacles to the mobility of persons and production of equipment." The quoted material is from Hoover, E. M. *The Location of Economic Activity.* McGraw-Hill Book Company, Inc., New York, 1963. p. 9.
23. The situation factors refer to that part of the environment outside of the local site, which affect the plant. Three levels are most often considered here: 1) The relations between the plant with the environment of the zone and of the city of Buffalo, 2) The relations between the plant with the environment of the SMA, and 3) The relations between the plant with the environment of the larger region (state of New York, the American Manufacturing Belt, or of the entire nation.)
24. Niagara Mohawk Power Corporation. 1959. *Niagara Frontier.* p. 21.
25. Renner, G. T. 1947. Geography of industrial location. *Econo. Geog.* 42:178.
26. Oral communication with Baker of Olin-Mathieson, McGrath of Pure Carbonic, and Balverson of Goodrich, July 2, 1961.
27. Basic activities are manufacturing concerns that earn most of their income outside of the local Buffalo area. American Optical Company attracts many jobbers (establishments that perform subsidiary functions, including manufacturing, related to the operations of larger establishments in the same industry. The larger aircraft plants, for example, tend to "farm-out" many manufacturing operations to the small plant so that the former can specialize on the mass production phases) that do machinery work, and that make electronics parts for American Optical, said Moor of the American Optical Company, June 10, 1961.
28. This information was obtained from answers of written questionnaires and personal interviews of plants during the spring and summer of 1961.
29. Smith of American Machine and Foundry Co. April 15, 1961.
30. *Buffalo Evening News,* January, 1963.
31. The areas unit of 36 square miles is the approximate size of a town or a township.
32. Ervin Gibbs, Industrial realtor for one of the real estate companies in Middletown, Conn. Oral communication was in April of 1965.
33. Muncy states in a report on metropolitan areas that: "Many industries found that these — communities lacked the experience, the funds, and the desire to provide the standard of services expected in an urban environment. Rural tax rates soon caught up with city rates." She further observed that: "These experiences have resulted in a trend for many market-oriented and labor-oriented industries to locate at the edge of built-up areas, but emphatically inside city limits." Muncy, D. A. March 1954. Land for Industry —A Neglected Problem. *Harvard Business Review.* 32:51.
34. It takes 6 months to 1 year to train a person to sew women's apparel (stylized) from $800 to $1000. Oral communication with Thom of Barmon Bros. March 12, 1961.
35. This information was obtained from answers of written questionnaires mailed out the summer of 1961. Also, many industrially zoned areas throughout the country became a "catch-all" where all other land uses are permitted. "Choice sites along the new highways are usurped by gasoline stations, motels, and outdoor movies." Maryland State Planning Commission, Baltimore Regional Planning Council, *Industrial Land Development,* Technical Report Number 2, 1959, pp. 59-60.



# LABOR FORCE CHARACTERISTICS OF LOW-INCOME HOUSEHOLDS: A CASE STUDY OF WESTMORELAND COUNTY, PENNSYLVANIA[1]

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## ABSTRACT

Current programming to assist low income people places strong emphasis on policies to increase employment and earnings. Yet little is known about the labor force behavior and employment potential of the low income population or the extent to which increased employment can alleviate the problem. This paper explores the labor force participation of 809 low income households and 1610 non-low income households and relates labor force participation to individual and household characteristics. Implications for programming to aid low income people through labor market mechanisms are drawn.

## INTRODUCTION

The National Advisory Committee on Rural Poverty in its recent report to the President entitled *The People Left Behind* (2) called attention to a severe and persistent, though little noticed, social problem. At about the same time a study published by the U.S. Department of Agriculture (1) showed that there were more rural poor in Pennsylvania than in any state outside the South. To some extent before the appearance of these reports and to a greater extent since then, programming to ameliorate the rural-low income problem has proceeded apace. The emphasis of much of this programming has been on increasing employment opportunities in rural areas. However, the rural low income population has been the subject of little systematic study. Thus, programming has proceeded in many cases with little knowledge of existing conditions.

The objective of this paper is to examine the extent to which there are differences in labor force participation among low income and other households in a predominantly rural area and to determine factors related to these differences. These findings are essential in assessing the contribution of employment problems to low income status and the potential for alleviating poverty through labor force mechanisms.

## SITE OF THE STUDY

The site of this study was Westmoreland County, Pennsylvania. The County is located in southwestern Pennsylvania and is bordered on the west by Allegheny County, which includes the city of Pittsburgh. It is not an area of particular affluence or poverty. Median family income in 1960 was $5597 compared to $5719 for Pennsylvania and $5660 for the United States. (4) In 1960 17.7 percent of the County's families had incomes less than $3000 dollars compared to 21.4 percent nationally and 16.8 percent for the State. The Pittsburgh SMSA, which includes Westmoreland County is not an area of unusually high concentration of low paying occupations nor do wage rates in comparable occupations in the area differ much from those in other areas of the state and nation. Thus, the county represents a typical problem, considerable numbers of low income rural households scattered among families of average or better means.

## PROCEDURE OF THE STUDY

A survey of households in Westmoreland County was conducted in July and August of 1966 by the Westmoreland County Community Action Agency assisted by the Department of Agricultural Economics and Rural Sociology of The Pennsylvania State University. A geographical random sample of households in 34 census tracts in the County with 20 percent or more of households with incomes under $3000 in the 1960 Census was drawn. Tracts were sampled at a rate of approximately 2 percent of their 1960 population. A total of 809 useable low income household schedules and 1610 useable non-low income household schedules were obtained by personal interview with an adult household member, usually the household

[1] The study on which this paper is based is presented in detail in the co-author's M.S. thesis (3).

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head or spouse. Questions about household size and composition, demographic characteristics of members and household income in 1965 were asked of all households. Those identified as low income according to the official Office of Economic Opportunity income, household size and residence criteria then in use were asked a wide variety of additional questions with particular emphasis on employment.*

## FINDINGS OF THE STUDY

*Demographic Characteristics.* Mean household size of the low and non-low income populations were virtually identical, 3.69 and 3.78 persons, respectively. However, the low income population was composed of a disproportionately large number of very small and very large households, table 1. Part of the reason for the proportionately greater representation of large households, of course, resulted from the definition of low income used, which required higher income levels to escape poverty as family size increased.

TABLE 1. *Size of Low Income and Non-Low Income Households.*

| Household Size | Low Income Household Number | Low Income Household Percent | Non-Low Income Households Number | Non-Low Income Households Percent |
|---|---|---|---|---|
| 1 | 170 | 21.3 | 75 | 4.7 |
| 2 | 201 | 24.8 | 342 | 21.2 |
| 3 | 83 | 10.3 | 350 | 21.7 |
| 4 | 83 | 10.3 | 371 | 23.0 |
| 5 | 86 | 10.6 | 241 | 15.0 |
| 6 | 54 | 6.7 | 123 | 7.6 |
| 7 | 59 | 7.3 | 62 | 3.9 |
| 8 | 27 | 3.3 | 23 | 1.4 |
| 9 | 19 | 2.3 | 13 | 0.8 |
| 10 | 8 | 1.0 | 5 | 0.3 |
| 11 | 9 | 1.1 | 3 | 0.2 |
| 12 or more | 8 | 1.0 | 2 | 0.1 |
| Total | 809 | 100.0 | 1,610 | 99.9 |

Low income households contained a higher proportion of children under 14 than did non-low income households, and nearly twice the proportion of persons 65 or older. On the other hand there were fewer persons in the low income population in the working force ages of 14 through 64 years, 39.3 percent compared to 52.4 percent in the non-low income population.

Only 57.8 percent of the low income households had a male and female head present compared to 86.2 percent of the non-low income households. This resulted in part from the larger number of single member households in the low income population, but even among households of two or more members low income households were more frequently headed by only one head. In most cases this was a woman.

Low income household heads were older and had considerably less formal education than heads of other households. Only 58.4 percent of low income household heads were in the 25 through 65 age range compared to 82.0 percent of the heads of other households. Most low income heads had completed eight grades or less, while fewer than 20 percent were high school graduates. On the other hand, high school graduates made up over a third of the non-low income households and there were proportionately many fewer who had not completed at least eighth grade. The difference in educational attainment among the low and non-low income groups was partially a reflection of age differences, but also prevailed within age groups.

A substantially smaller proportion of low income household heads than of non-low income household heads were employed. Among male heads employment was 43.9 percent and 84.2 percent comparing low and non-low income heads; the corresponding percentages among female heads were 8.8 and 15.3 percent respectively. Unemployment was substantially higher among low income households. The low employment level combined with the large number of households headed by a female led to a substantial difference in the distribution of households by number of employed persons. More than half (52.2 percent) of low income households had no member employed while only 12.7 percent of other households had no employed member.

*Labor Force Participation.* Because of these striking differences in employment among the two groups the labor force participation of household members was examined further to determine the extent to which these differences were accounted for by differences in household composition of the two groups. All household members 14 years old or older and not enrolled in school were included in this analysis and were considered to constitute the potential labor force in the two groups. These groups included 1641 low income household members and 3637 non-low income household members. Persons who were employed or unemployed but wanter work were considered labor force participants.* Those not working and not wanting work were considered non-participants.

* The poverty income level for non-farm households was $1500 for one person plus $500 for each additional household member. For a farm household it was $1050 for one person plus $350 for each additional household member. "Income" for this purpose did not include public assistance payments, unemployment compensation payments or charitable contributions.



TABLE 2. *Composition of the Potential and Actual Force in Low Income Households in Westmoreland County.*

| Component Groups | Percent of Potential Labor Force[1] | Percent Participating In Labor Force[2] | Percent of Labor Force Non-Participants | Percent of Labor Force Non-Participants | Unemployment Rate |
|---|---|---|---|---|---|
| Persons 65 and over | 28.3 | 7.3 | 42.8 | 5.3 | 32.4 |
| Males | (13.6) | (8.1) | (20.4) | (2.8) | (33.3) |
| Females | (14.7) | (6.7) | (22.4) | (2.5) | (31.3) |
| Persons 64 and under | | | | | |
| Male heads | 20.7 | 78.8 | 7.2 | 41.9 | 13.9 |
| Other males | 11.2 | 77.7 | 4.1 | 22.4 | 25.2 |
| Female heads[3] | 8.2 | 44.8 | 7.4 | 9.5 | 50.0 |
| with dependent children[4] | (3.7) | (39.3) | (3.7) | (3.8) | (62.5) |
| without dependent children[4] | (4.5) | (49.3) | (3.7) | (5.7) | (41.7) |
| Female spouses[5] | 22.2 | 12.6 | 31.8 | 7.2 | 50.0 |
| with dependent children[4] | (15.3) | (12.0) | (22.1) | (4.7) | (46.7) |
| without dependent children[4] | (6.9) | (14.2) | (9.7) | (2.5) | (56.3) |
| Other females | 8.2 | 59.7 | 5.2 | 12.6 | 46.3 |
| Unknown | 1.2 | 36.8 | 1.2 | 1.1 | 0.0 |
| Total or Average | 100.0 | 38.9 | 99.9 | 100.0 | 27.2 |

[1] All persons 14 years old or older and not enrolled in school.
[2] Percent who were employed or unemployed but wanted work.
[3] In households with no male head.
[4] Dependent children under 14 years of age living at home.
[5] Female spouses in households with male heads.

Tables 2 and 3 clearly indicate the effect of the age difference on employment in the two populations. More than a quarter of the potential labor force in the low income population was 65 or older, while only 12 percent of the others were in this age category. The labor force participation rate of 7 percent was the same among these older persons in the two income groups. Nearly half (42.8 percent) of all labor force non-participants in low income households were 65 or older.

Among males under 65 years of age labor force participation was high in both groups, about 79 percent in the low income population and 97 percent in the non-low income population. However, males under 65 years old constitute only 32 percent of the low income population compared to 43 percent of the non-low income population. Males under 65 constituted only 11 percent of labor force non-participants in low income households.

After the elderly the next largest group of labor force non-participants were females with dependents under 14 years of age living at home. These constituted approximately 20 percent of the potential labor force of both populations. A higher proportion of those in the low income population than in the non-low income population had no spouse. Labor force participation rates among low income females with dependents was somewhat lower than among their counterparts in non-low income households, 12 percent compared with 19.8 percent, but the difference was not great.

Females without dependents under 14 at home constituted 11.4 percent of the low income potential labor force and 15.7 percent of the non-low income potential labor force. Again, labor force participation of those in the non-low income group is somewhat, but not appreciably, higher than in the low income group.

Unemployment, calculated by dividing the percentage unemployed but wanting work by the percentage of labor force participants, was substantially higher in the low income population than in the non-low income group. This could provide a partial explanation for the

* In this study the term unemployed indicated that the respondent was not working but "wanted work." This differs from the usual definition of an unemployed person as someone who is not working but "looking for work." It is likely that the use of the later definition would have reduced the proportion of persons, especially women, in the unemployed category since many of them had not recently actively searched for work and because of family responsibilities would have been quite restricted in the amount and kind of employment they could accept.

TABLE 3. *Composition of the Potential and Actual Labor Force in Non-Low Income Households in Westmoreland County.*

| Component Groups | Percent of Potential Labor Force[1] | Percent Participating In Labor Force[2] | Percent of Labor Force Non-Participants | Percent of Labor Force Participants | Unemployment Rate |
|---|---|---|---|---|---|
| Persons 65 and over | 12.0 | 7.1 | 25.4 | 1.5 | 16.1 |
| Males | (6.1) | (10.9) | (12.4) | (1.2) | (8.3) |
| Females | (5.0) | (3.3) | (13.0) | (0.3) | (42.9) |
| Persons 64 and under | | | | | |
| Male heads | 34.6 | 96.7 | 2.6 | 59.7 | 1.0 |
| Other males | 8.7 | 91.1 | 1.8 | 14.1 | 9.8 |
| Female heads[3] | 2.0 | 54.1 | 2.0 | 2.0 | 10.0 |
| with dependent children[4] | (0.4) | (53.3) | (0.4) | (0.4) | (12.5) |
| without dependent children[4] | (1.6) | (55.2) | (1.6) | (1.6) | (9.4) |
| Female spouses[5] | 35.0 | 21.0 | 63.1 | 13.2 | 31.8 |
| with dependent children[4] | (20.9) | (19.8) | (39.3) | (7.4) | (32.7) |
| without dependent children[4] | (14.1) | (22.9) | (24.8) | (5.8) | (22.2) |
| Other females | 7.4 | 71.4 | 4.8 | 9.4 | 22.1 |
| Unknown | 0.2 | 44.4 | 0.3 | 0.2 | 25.0 |
| Total or Average | 100.2 | 56.1 | 100.0 | 100.1 | 8.2 |

[1] All persons 14 years old or older and not enrolled in school.
[2] Percent who were employed or unemployed but wanted work.
[3] In households with no male head.
[4] Dependent children under 14 years of age living at home.
[5] Female spouses in households with male heads.

lower rate of labor force participation in the low income groups, since the incentive for seeking employment would hardly be great with so many already unemployed. Unemployment among females was particularly high in both income groups.

In spite of high unemployment, however, low income labor force non-participants did not give discouragement with job hunting as a major reason for not wanting work, table 4. Less than 3 percent of the non-participants indicated they were discouraged with job hunting. Most of the males were either elderly or answered that they had handicaps or illness which prevented employment. Among females, age, illness, and family responsibilities all were important reasons for not seeking work.

Data on age, sex, dependency status and health of low income labor force non-participants are summarized in table 5. Of the 30 percent who were male, one-third had health problems or disabilities and most of the remainder were 65 or older. Another third of the non-participants were women without dependents. Again more than half were 65 or older and many of the rest had health problems or disabilities.

TABLE 4. *Reasons Low Income Labor Force Non-Participants Stated for Not Wanting Employment, by Age.*

| Reasons | Number of Persons by Age | | | | | Total | |
|---|---|---|---|---|---|---|---|
| | 24 or under | 25-44 | 45-64 | 65 or over | Age Unknown | Number | Percent[1] |
| Retired — too old | 0 | 2 | 66 | 366 | 1 | 435 | 43.5 |
| Care of children | 37 | 167 | 53 | 12 | 2 | 271 | 27.1 |
| Care of ill or disabled adult | 0 | 4 | 22 | 8 | 0 | 34 | 3.4 |
| Keeping house | 20 | 84 | 69 | 28 | 2 | 203 | 20.3 |
| Ill health, disabled | 8 | 26 | 106 | 68 | 0 | 208 | 20.8 |
| Tired of looking for work | 0 | 0 | 2 | 3 | 0 | 5 | 0.5 |
| Just don't want work | 4 | 6 | 6 | 7 | 0 | 23 | 2.3 |
| Other | 9 | 5 | 5 | 0 | 0 | 19 | 1.9 |
| No information | 12 | 12 | 16 | 8 | 0 | 48 | 4.8 |

[1] In this table percentages are of total respondents. Since many non-participants in the labor force gave more than one response, the percentages add to more than 100.



TABLE 5. *Percent of Low Income Labor Force Non-Participants by Age, Health, and Family Position.*

| Age | Family Position | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female With Children[1] | | Female Without Children | | | |
| | Health Problem | No Health Problem | Health Problem | No Health Problem | Health Problem | No Health Problem | No Information | Total |
| 24 or less | 0.3 | 0.5 | 0.0 | 3.2 | 0.5 | 1.6 | 1.1 | 7.2 |
| 25 to 44 | 1.4 | 0.4 | 0.7 | 16.7 | 0.5 | 2.0 | 0.4 | 22.1 |
| 45 to 64 | 5.7 | 2.1 | 1.2 | 4.7 | 3.6 | 9.2 | 0.6 | 27.1 |
| 65 or over | 2.8 | 17.5 | 0.4 | 1.1 | 3.6 | 17.1 | 0.6 | 43.1 |
| Unknown | 0.0 | 0.1 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 | 0.4 |
| Total | 10.2 | 20.6 | 2.3 | 26.0 | 8.2 | 29.9 | 2.7 | 99.9 |

[1] Dependent children under 14 years of age living at home.

## CONCLUSIONS

In improving the incomes of poor families in Westmoreland County through employment-related programs, low labor force participation is a major obstacle. More than 60 percent of the low income potential labor force was not in the active labor force, and more than half the low income households had no employed persons. The two largest groups of non-participants, persons 65 or older and females with dependent children comprised nearly three-quarters of the non-participants. For these persons the potential for increased employment appears marginal.

Some elderly people are willing and able to work and experience of enumerators during the survey indicated a strong desire among many of the elderly for financial independence. However, the low income elderly were already participating in the labor force to about the same extent as were the elderly in other households. Increasing employment among this group, especially in the private sector, would encounter severe obstacles. Productivity, health, and skills of these persons are poor and hiring costs, at least under conventional terms, would be considerable. Some recent experiments with public employment of the physically fit elderly in neighborhood and highway beautification projects, in children's day-care centers, and in skill exchanges between elderly persons suggests the kinds of employment that may be appropriate. But such programs will undoubtedly require substantial public expenditures and will probably be most useful as supplements to existing welfare or retirement incomes. The very substantial proportion of elderly among the rural low income population compels particular attention to their problems.

Women with dependent children at home could be freed for additional employment by the provision of day-care facilities for children. However, given the educational and skill levels of many of these women this may not be an economical course of action since many women possessing these characteristics were already unemployed. The social wisdom of removing dependent children to public care centers while mothers work has also been questioned. However, this could at the same time provide an opportunity for remedial educational programs for low income children that might help to eliminate recurrence of the problem.

Among one-half of the potential labor force members who were neither elderly nor caring for dependent children, labor force participation was also low. Most of the effort at stimulating improved labor force participation should probably be concentrated here. Many of these persons were males reporting health problems or disability as the reason they did not want work. Although their range of activities might be limited some of these persons undoubtedly could perform useful work if properly trained and if jobs were available. Training and job development for women is also needed.

While training and job development are potentially useful solutions to the problems posed by some of those not in the labor force — as well as some of those in the labor force — it is important that the potential contribution of such programs not be overestimated. There are substantial numbers of the low income population who are not likely to be helped by such programs and they certainly should not be viewed as a cure-all. To do so could undermine long run support for the program and disillusion the low income people.

## REFERENCES


1. Bird, A. R. and J. L. McCoy. 1967. *White Americans In Rural Poverty*. Agricultural Economics Report No. 124. U.S. Government Printing Office, Washington, D. C.
2. President's National Commission on Rural Poverty. 1967. *The People Left Behind*. U.S. Government Printing Office, Washington, D. C.
3. Sauders, Kenneth D. 1968. *Employment-Related Characteristics of Low-Income Households In Westmoreland County, Pennsylvania*. Unpublished M.S. thesis, Department of Agricultural Economics and Rural Sociology, The Pennsylvania State University.
4. U.S. Bureau of the Census. *Census of Population: 1960. Detailed Characteristics, Pennsylvania*. U.S. Government Printing Office, Washington, D. C. 1962.




# LIPID COMPOSITION OF THE MONARCH BUTTERFLY, DANAUS PLEXIPPUS


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## ABSTRACT

Lipids in the monarch butterfly, *Danaus plexippus* (L.) were examined by thin layer and gas chromatography. There appears to be a net synthesis of lipid from non-lipid components during the pupal period. As in other Lepidoptera, the newly emerged adult female contains less lipid than the male.

Neutral lipids were separated into triglyceride, mono- and diglyceride, free fatty acid, sterol ester, sterol, and hydrocarbon fractions. Triglyceride was the major fraction and accounted for over 80% of the neutral lipids in both larvae and adults.

The main fatty acids in all lipid fractions were palmitate, palmitoleate, stearate, oleate, linoleate, and linolenate. The fatty acid composition was similar to that of the host milkweed, *Asclepias incarnata*.


## INTRODUCTION

The monarch butterfly, *Danaus plexippus* (L.), is a continental migrant that uses lipid to support flight (1). Very little is known about lipid metabolism in this insect except that prior to migration the adult lays down a large supply of fat (2). This paper reports on a study of the neutral lipid content and composition of *D. plexippus* in an attempt to elucidate the mechanisms by which lipid is accumulated.

## MATERIALS AND METHODS

*D. plexippus* larvae were collected in the field on the milkweed, *Asclepias incarnata*, and brought into the laboratory. The larvae were maintained on milkweed and all insects were held at 23 ± 2°C for the duration of the experiment. Mature larvae just prior to pupation and newly emerged adults (0 to 12 hr after emergence) were used for lipid analysis.

Lipids were extracted from individual insects with chloroform-methanol, 2:1 (v/v) and purified by the method of Folch *et al.* (3) as described previously (4). Neutral lipids were fractionated by thin layer chromatography on silica gel plates using a solvent system of petroleum ether-diethyl ether-glacial acetic acid, 85:15:1 (v/v/v) and quantitated by the colorimetric method of Marsh and Weinstein (5) as reported earlier (6).

For gas chromatographic analysis each lipid fraction was saponified with 10% ethanolic KOH and fatty acid methyl esters were prepared using the boron trifluoride technique (7). Standard gas chromatographic procedures (F and M Scientific Methods Bulletin No. 117), including the use of 6% diethylene glycol succinate columns and a hydrogen flame detector, were employed in fatty acid identification and quantitation.

## RESULTS AND DISCUSSION

The total lipid content of larval and adult *D. plexippus* is shown in Table 1. In all insects about 90% of the lipid was neutral lipid and most of the remainder was phospholipid. Larvae, though considerably heavier than adults, contain less lipid per gram live weight and per individual insect. Because the adults were newly emerged and had not fed, the data indicate that lipid synthesis from non-lipid components must be occurring during the pupal period. The lower amount of lipid in the female is in accord with the findings for other Lepidoptera (1) but is in contrast with the reasoning of Williams (8) that migratory moths (and

TABLE 1. *Average weight and neutral lipid content of larvae and adults of the monarch butterfly,* Danaus plexippus.

| | Larva (8)* | Adult ♂ (6) | Adult ♀ (6) |
|---|---|---|---|
| Live weight (g) | 1.51 | 0.94 | 1.04 |
| Lipid-free dry matter (g) | 0.13 | 0.14 | 0.13 |
| Neutral lipid (mg) | 65 | 85 | 74 |
| Neutral lipid per gram live weight (mg/g) | 43 | 90 | 71 |

* Numbers in parentheses indicate the number of insects examined.

TABLE 2. *Percent composition of neutral lipid fractions in larvae and adults of the monarch butterfly,* Danaus plexippus.

| Insect | N | Percent composition of neutral lipids | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tri-glyceride | Mono- and diglyceride | Free Fatty Acid | Sterol | Sterol Ester | Hydro-carbon |
| Larva | 6 | 86.2 | 2.1 | 3.5 | 4.5 | 2.4 | 1.4 |
| Adult ♂ | 6 | 87.3 | 2.0 | 1.9 | 3.5 | 1.5 | 3.8 |
| Adult ♀ | 6 | 85.7 | 2.2 | 2.6 | 3.9 | 1.6 | 4.1 |

presumably butterflies) have no sexual dimorphism in lipid content since both sexes migrate and probably use equal amounts of lipid for flight. Beall (2) found that both sexes of *D. plexippus* in migration do have essentially the same lipid content. Also, he reports that following adult emergence and prior to migration *D. plexippus* accumulates considerable amounts of lipid, apparently from conversion of carbohydrates in nectar to lipid (1). Thus, it appears that *D. plexippus* is similar to other Lepidoptera in that it emerges from the pupal period with a lower lipid content in the female, but because it is migratory it differs in that both sexes accumulate lipid rapidly resulting in equal lipid contents at the time of migration. The lower original lipid content in the adult female suggests that the rate of lipid synthesis might be higher in the female than in the male.

The per cent composition of the various neutral lipid fractions is shown in Table 2. This composition is very similar to that of other Lepidoptera (1). There are apparently no differences caused by sex and the only difference between adults and larvae is that adults contain two to three times as much hydrocarbon. This is thought to be caused by the lipid soluble components which are associated with the bright coloration and the greater surface area of the adult (9). The sterol fraction has been further studied by gas chromatography (10) and was shown to be a mixture of cholesterol, campesterol, ß-sitosterol, and perhaps their stereoisomers.

Fatty acids in the various lipid fractions of adult *D. plexippus* are shown in Table 3. The results are mean values based on three separate determinations. Fatty acids with 16 and 18 carbons constitute over 97% of the fatty acids and are the only ones reported here. Essentially no differences related to sex were apparent. Linolenate (C-18:3) was the major fatty acid in triglyceride, sterol ester, and phospholipid fractions; while palmitate (C-16) predominated in free fatty acid and mono- and diglyceride. Linolenate is not usually the most abundant fatty acid in insects, but several species within different orders (1,11) exhibit a similar composition. The percent composition of the fatty acids within the total lipids of the host plant was determined to be as follows: palmitate (C-16) 41.1, palmitoleate (C-16:1) 4.2, stearate (C-18) 4.7, oleate (C-18:1) 3.7, linoleate (C-18:2) 7.4, and linolenate (C-18:3) 39.5. Thus, *D. plexippus* is similar to its host plant in that both contain much palmitate and linolenate; however, the insect has considerably

TABLE 3. *Fatty acids in lipids of the monarch butterfly,* Danaus plexippus.

| Lipid fraction | Sex | Percent composition of fatty acids | | | | | |
|---|---|---|---|---|---|---|---|
| | | 16 | 16:1 | 18 | 18:1 | 18:2 | 18:3 |
| Triglyceride | ♂ | 30.1 | 1.4 | 4.9 | 18.3 | 7.9 | 36.0 |
| | ♀ | 31.2 | 2.7 | 4.5 | 22.0 | 9.0 | 30.9 |
| Mono- and | ♂ | 24.8 | 0.9 | 18.5 | 20.8 | 10.9 | 23.9 |
| Diglyceride | ♀ | 30.0 | 1.7 | 14.0 | 21.3 | 11.1 | 21.1 |
| Free Fatty | ♂ | 35.9 | 4.3 | 13.3 | 26.2 | 11.6 | 8.6 |
| Acid | ♀ | 30.2 | 4.1 | 12.5 | 29.3 | 15.6 | 8.4 |
| Sterol Ester | ♂ | 17.7 | 10.5 | 5.9 | 22.9 | 10.0 | 34.1 |
| | ♀ | 16.4 | 6.8 | 4.3 | 19.1 | 10.0 | 42.7 |
| Phospholipid | ♂ | 10.5 | t* | 13.2 | 14.6 | 15.6 | 46.6 |
| | ♀ | 12.0 | t | 12.5 | 14.9 | 16.5 | 44.6 |

* Trace amount, less than 0.5 percent.



more stearate, oleate, and linoleate. There are several explanations to account for this difference. *D. plexippus* may selectively retain certain fatty acids and thus increase their concentration. Another possibility is that shorter chain fatty acids such as palmitate could be lengthened to 18 carbons (1). Finally, saturation of linolenate and linoleate by the insect could account for the higher levels of stearate, oleate, and linoleate. Experiments are now in progress to determine the pathways by which *D. plexippus* modifies its dietary fatty acids.

## REFERENCES


1. Gilbert, L. I. 1967. Lipid metabolism and function in insects. In *Advances in Insect Physiology* (J. W. L. Beament *et al.*, eds.) Volume 4. Academic Press, Inc., New York. p. 69.
2. Beall, G. 1948. The fat content of a butterfly, *Danaus plexippus* Linn., as affected by migration. *Ecology* 29:80.
3. Folch, J., M. Lees and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* 226: 497.
4. Yurkiewicz, W. J. 1967. Phospholipid metabolism in the embryo of the Indian meal moth, *Plodia interpunctella* (Hubner). *Proc. Penna. Acad. Sci.* 41:27.
5. Marsh, J. B. and D. B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.* 7:574.
6. Yurkiewicz, W. J. and K. E. Whelchel. 1969. Lipids in the melanic mutant of the large milkweed bug, *Oncopeltus fasciatus*. *Comp Biochem. Physiol.* 29:879.
7. Metcalfe, L. D. and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* 33:363.
8. Williams, C. B. 1945. Notes on the fat content of two British migrant moths (Lepidoptera). *Proc. R. ent. Soc. Lond.* 20:6.
9. Kolattukudy, P. E. 1968. Biosynthesis of surface lipids. *Science* 159:498.
10. Reichstein, T., J. von Euw, J. A. Parsons and M. Rothschild. 1968. Heart poisons in the monarch butterfly. *Science* 161:861.
11. Barlow, J. S. 1964. Fatty acids in some insects and spider fats. *Can. J. Biochem.* 42:1365.

# FATTY ACID COMPOSITION OF THE MELANIC MUTANT LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS

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## ABSTRACT

Fatty acids in the lipid fractions of Abbott melanic mutant and normal wild strains and of the Penn State strain of the large milkweed bug, *Oncopeltus fasciatus,* were studied by gas-liquid chromatography. The major fatty acids in all strains were palmitic (C-16), palmitoleic (C-16:1), stearic (C-18), oleic (C-18:1), and linoleic (C-18:2). The melanic mutant differs from both other strains in that it possesses considerably more C-18:2 in free fatty acid, triglyceride, and sterol ester fractions. Also, the mutant contains less C-18:1 in triglyceride and sterol ester fractions. Sex appears to have little influence on the fatty acid composition of all strains.

## INTRODUCTION

Recently, the melanic mutant (1) of the large milkweed bug, *Oncopeltus fasciatus* (Dallas), was examined for alterations in lipid metabolism associated with inherited melanism (2). No quantitative or qualitative differences in neutral lipids or phospholipids were found between melanic mutant and normal adults. However, recent work (3) on the bronze mutant mosquito, *Aedes aegypti,* (4) suggests that fatty acid composition within the lipid fractions might vary with differences in melanin content or metabolism. This paper reports on a study of fatty acids within the various lipid fractions of (a) Abbott melanic mutant milkweed bugs, (b) Abbott normal milkweed bugs from the mutant-producing colony and (c) normal, wild milkweed bugs from a Penn State strain.

## MATERIALS AND METHODS

Large milkweed bugs, *Oncopeltus fasciatus,* were reared in the laboratory at 23±2°C. on a diet of milkweed seeds, *Asclepias syriaca,* and water. Newly emerged adults within 72 hr of eclosion were used for analysis.

Lipids were extracted and fractionated by thin layer chromatography as described previously (2). Each lipid fraction was saponified with 10% ethanolic KOH and fatty acid methyl esters were prepared using the boron trifluoride technique (5). Standard gas chromatographic procedures (F and M Scientific Bulletin No. 117) were employed in fatty acid identification and quantitation. The mean values from 2 to 4 separate determinations are presented in the Tables.

TABLE 1. Fatty acids in the Abbott melanic mutant strain of the large milkweed bug, *Oncopeltus fasciatus.*

| Lipid fraction | Sex | Percent composition of fatty acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 14 | 16 | 16:1 | 17 | 18 | 18:1 | 18:2 | 18:3 |
| Triglyceride | ♂ | t* | 17.0 | 7.0 | t | 2.5 | 24.0 | 61.5 | t |
| | ♀ | t | 5.0 | 4.0 | t | 1.0 | 26.0 | 63.0 | t |
| Mono- and Diglyceride | ♂ | t | 17.0 | 7.0 | t | 7.0 | 27.5 | 42.0 | t |
| | ♀ | t | 15.0 | 5.0 | t | 14.0 | 25.0 | 40.0 | t |
| Free Fatty Acid | ♂ | 3.5 | 19.0 | 9.0 | t | 7.0 | 28.0 | 32.5 | t |
| | ♀ | 2.0 | 22.5 | 5.5 | t | 8.5 | 25.5 | 37.0 | t |
| Sterol Ester | ♂ | t | 7.0 | 13.0 | 2.0 | 3.0 | 10.0 | 60.5 | t |
| | ♀ | t | 7.0 | 10.5 | 2.0 | 2.5 | 12.5 | 66.5 | t |
| Phospholipid | ♂ | t | 8.5 | 2.5 | t | 12.5 | 8.5 | 62.0 | t |
| | ♀ | t | 10.0 | 1.5 | t | 12.5 | 14.0 | 61.0 | t |

* trace amount, less than 0.5 per cent



TABLE 2. Fatty acids in the Abbott normal strain of the large milkweed bug, *Oncopeltus fasciatus.*

| Lipid fraction | Sex | Percent composition of fatty acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 14 | 16 | 16:1 | 17 | 18 | 18:1 | 18:2 | 18:3 |
| Triglyceride | ♂ | t* | 8.0 | 4.0 | t | 4.0 | 32.0 | 49.0 | t |
| | ♀ | t | 10.0 | 4.5 | t | 4.0 | 33.5 | 48.0 | t |
| Mono- and Diglyceride | ♂ | t | 17.5 | 9.0 | t | 6.0 | 27.0 | 40.0 | t |
| | ♀ | t | 11.0 | 6.0 | t | 8.5 | 34.0 | 39.5 | t |
| Free Fatty Acid | ♂ | 4.5 | 25.0 | 8.0 | t | 7.0 | 35.0 | 21.0 | t |
| | ♀ | 8.0 | 20.0 | 16.5 | t | 9.0 | 27.5 | 17.0 | t |
| Sterol Ester | ♂ | t | 11.5 | 13.0 | t | 4.0 | 19.0 | 52.5 | t |
| | ♀ | t | 13.0 | 10.5 | t | 3.5 | 16.0 | 57.0 | t |
| Phospholipid | ♂ | t | 10.0 | 3.0 | t | 15.5 | 14.0 | 58.0 | t |
| | ♀ | t | 7.5 | 2.0 | t | 13.0 | 15.5 | 63.0 | t |

* trace amount, less than 0.5 per cent

## RESULTS AND DISCUSSION

Fatty acids of the lipid fractions of Abbott melanic mutant, Abbott normal, and Penn State normal *O. fasciatus* are shown in Tables 1, 2, and 3 respectively. The major fatty acids in all strains are palmitic (C-16), palmitoleic (C-16:1), stearic (C-18), oleic (C-18:1), and linoleic (C-18:2). The melanic mutant contains more linoleic (C-18:2) in the free fatty acid, triglyceride, and sterol ester fractions and less oleic (C-18:1) in the triglyceride and sterol ester fractions than the normal wild type. No differences in fatty acid composition between sexes were observed for all strains even though females contain considerably more neutral lipid than males (2). High percentages of unsaturated acids have been noted previously in normal wild type *O. fasciatus* (6) and can be attributed to the milkweed diet where we have observed C-16 = 6%, C-16:1 = 8%, C-18 = 1%, C-18:1 = 26%, and C-18:2 = 55%. Since all strains were fed from the same seed supply, the fatty acid composition differences observed may be due to retention, catabolism, or anabolism of certain fatty acids. Why fatty acid metabolism should be altered by differences in melanin content is not clear. Hackman and Goldberg (7) found that larvae of the melanic mutant blowfly, *Lucilia cuprina,* have a phenoloxidase system with a high activity on the substrates from which melanin is formed, while such activity is not present in larvae of the normal wild strain.

It seems unlikely that fatty acids would be involved in melanin formation since tyrosine, which is converted into melanin, is formed

TABLE 3. Fatty acids in the Penn State strain of the large milkweed bug, *Oncopeltus fasciatus.*

| Lipid fraction | Sex | Percent composition of fatty acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 14 | 16 | 16:1 | 17 | 18 | 18:1 | 18:2 | 18:3 |
| Triglyceride | ♂ | t* | 7.5 | 3.0 | t | 3.5 | 35.0 | 51.0 | t |
| | ♀ | t | 8.0 | 5.5 | t | 4.0 | 34.0 | 46.5 | t |
| Mono- and Diglyceride | ♂ | t | 9.0 | t | t | 8.5 | 33.0 | 42.0 | t |
| | ♀ | t | 14.0 | 7.5 | t | 8.5 | 30.5 | 40.0 | t |
| Free Fatty Acid | ♂ | 7.0 | 22.0 | 6.0 | t | 23.0 | 23.0 | 21.0 | t |
| | ♀ | 5.0 | 33.0 | 10.0 | t | 8.5 | 22.5 | 19.0 | t |
| Sterol Ester | ♂ | 1.5 | 14.0 | 8.0 | t | 9.5 | 23.0 | 42.0 | t |
| | ♀ | t | 16.5 | 10.0 | t | 13.0 | 16.5 | 40.0 | t |
| Phospholipid | ♂ | t | 8.0 | 2.0 | t | 13.5 | 15.5 | 61.0 | t |
| | ♀ | t | 7.5 | 2.5 | t | 23.0 | 16.0 | 60.0 | t |

* trace amount, less than 0.5 per cent

from the essential amino acid phenylalanine. Phenylalanine, however, is considered to be an essential amino acid for most but not necessarily all insects (8) so the possibility of phenylalanine synthesis directly or indirectly from fatty acids cannot be completely discounted. Experiments are now being devised to examine phenylalanine biosynthesis from labeled fatty acids in the milkweed bug.

## REFERENCES


1. Abbott, C. E. 1968. Inherited melanism in the large milkweed bug, *Oncopeltus fasciatus* (Heteroptera: Lygaeidae). *Ann. ent. Soc. Am.* 61:542.
2. Yurkiewicz, W. J. and K. E. Whelchel. 1969. Lipids in the melanic mutant of the large milkweed bug, *Oncopeltus fasciatus*. *Comp Biochem. Physiol.* 29:879.
3. Yurkiewicz, W. J. and S. C. Bhalla. Unpublished information.
4. Bhalla, S. C. and G. B. Craig, Jr. 1967. Bronze, a female-sterile mutant of *Aedes aegypti*. *J. Med. Ent.* 4:467.
5. Metcalfe, L. D. and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* 33:363.
6. Kinsella, J. E. 1966. Observations on the changing lipid patterns during the life cycle of *Oncopeltus fasciatus*. Comp. Biochem. Physiol. 17:1209.
7. Hackman, R. H. and M. Goldberg, 1968. A study of a melanic mutant of the blowfly *Lucilia cuprina*. *J. Insect Physiol.* 14:765.
8. Chefurka, W. 1965. Intermediary metabolism of nitrofenous and lipid compounds in insects. In *The Physiology of Insecta* (M. Rockstein, ed., Volume 2. Academic Press, New York. p. 669.




# CYTOCHEMICAL ANALYSIS OF LIVER HISTONES IN ACID-EXPOSED BROOK TROUT

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## ABSTRACT

Cytochemical analyses were made of the nuclear histone composition of hepatic tissue from acid-exposed brook trout. Liver sections were stained for histones by the eosin-fast green method which has been used for detecting histone changes in other vertebrate species. Three spectrally distinct cell types were determined on the basis of differential affinities for eosin and fast green.

Exposure of fish to low pH for 4 days did not affect the relative proportions of the three cell types indicating that this aspect of liver function is not influenced by short exposure to low pH.


## INTRODUCTION

It has long been suspected that some species of fish are capable of adapting to water of acidic pH through metabolic adjustments at the cellular level. However, in spite of many articles on the effects of low pH on fish respiration (1,2,3,4) one is impressed by the absence of information on the role of the liver in regulation of tissue metabolism during acclimation to acid pH waters. This is due, in part, to a lack of information on the precise nature of cytochemical and histopathological changes which may enable one to assess functional changes in liver tissue.

Recently, Bloch (5) suggested that cytochemical staining for nuclear histones might be profitably employed for the detection of metabolic alterations in liver parenchymal cells. This technique involves the use of an eosin-fast green stain mixture which differentiated lysine-rich and arginine-rich nuclear histones. He demonstrated that eosin binds preferentially to lysine-rich histones. Such histones have been shown to be strong inhibitors of DNA-dependent RNA synthesis (6). Recently, this staining reaction has also been successfully employed to detect functional metabolic changes in liver cells of rats exposed to oxygen lack for 1, 2 and 7 days (7). The knowledge that acid-exposed fish are also subject to oxygen lack stemming from impaired gill function (1,2) formed the basis for the present study.

This report deals with the eosin-fast green staining response of liver parenchymal cells in fish exposed to water of acid pH. An important goal of this investigation is to evaluate the use of the eosin-fast green staining technique in the early diagnosis of functional metabolic changes in fish liver. Specific aims of this study can be stated as follows: (a) to characterize specific hepatic cell types on the basis of spectral analyses of nuclei following histochemical staining with eosin-fast green, (b) to determine whether exposure to water of acid pH results in changes in relative proportions of different cell types and (c) to assess the utility of the eosin-fast green stain in detecting changes in the metabolic state of parenchymal cells.

## MATERIALS AND METHODS

Brook trout, *Salvelinus fontinalis,* were obtained from the Research Station of The Pennsylvania Fish Commission, Benner Springs. The fish weighed 50 to 75 g and measured 15 to 17 cm in length.

Fifteen control and fifteen experimental fish were maintained in separate tanks of dechlorinated water at 48-52 C. Tanks were well aerated and filtered at all times. The pH of water in the experimental tank was decreased by gradual addition of concentrated hydrochloric acid over a four day period until fish exhibited loss of equilibrium and began to swim erratically. These symptoms generally occurred at about pH 3.9. On the morning of day five fish were killed by electrical shock and dissected.

Slices of liver about 2 mm thick were fixed in phosphate-buffered 4% formalin (pH 7) for 24 hr, processed in an Autotechnicon, embedded in Paraplast (56-57° C) and sectioned at 6 micra.

Control slides were treated with 0.2N HCl for 4 hr to remove histones before staining. All slides were stained according to the eosin-fast green procedure outlined by Bloch (5). Stained sections were dehydrated in an ethanol series, cleared in xylene and mounted in Permount. Preliminary microscopic observations were

made of stained liver sections from acid-exposed and normal fish to detect any gross differences in the staining pattern of the liver cords.

Supplemental data concerning histological features of fish liver were obtained from tissue processed as above but stained with Harris hematoxylin and eosin (8).

An absorption spectrum for a dilute solution of eosin-fast green was obtained with a Beckman DB spectrophotometer. For ease of comparison, spectral data for dyes in solution were converted from percent transmittance to optical density. A Leitz microspectrophotometer was employed for spectral analyses of pink, green and purple stained nuclei. Absorption measurements were made at 5 mμ intervals in the visible range (450-700 mμ) on six cells of each nuclear type. Mean optical densities were calculated and plotted against wavelength to obtain an absorption curve for each nuclear type.

## RESULTS

*Spectral analyses of differentially stained liver nuclei.* In solution, the eosin-fast green stain mixture has two absorption peaks, one at 515 mμ and another at 615 mμ. A solution of eosin alone exhibits maximal absorption at 515mμ and fast green has one major peak at 615 mμ. Spectral data obtained using a Bechman DB recording spectrophotometer, illustrate that the dyes do not interact in solution (Figure 1 A).

When liver tissue is stained with the eosin-fast green dye mixture, three types of parenchymal cells can be visually differentiated: cells with pink nuclei, cells with green nuclei and cells with purple nuclei. The pink and green nuclei stain uniformly with respect to both stain intensity and hue. That is, to the naked eye pink nuclei appear to have an affinity for eosin and green nuclei an affinity for fast green. On the other hand, purple nuclei exhibit gradations of intensity and coloration ranging from light lavender to dark purple, suggesting some absorption of both dyes in this group of nuclei.

Microspectrophotometric analyses of the three cell types showed that all nuclei absorb both dyes. For instance, in this study as well as an earlier study with eosin-fast green stained liver tissue from the rat (7) it was found that pink nuclei have an absorption maximum at about 535 mμ with a secondary absorption peak at 635 mμ; green nuclei have a major absorption peak at 635 mμ with a minor peak at 535 mμ; and purple nuclei exhibit a considerable

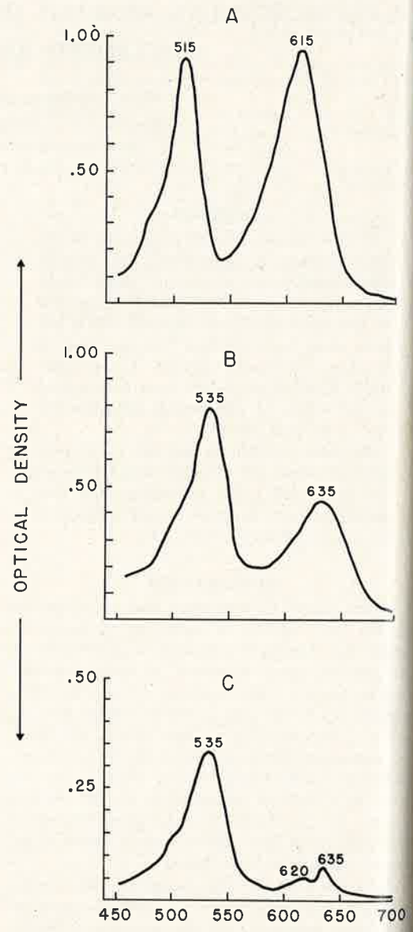



**Figure 1. (A)—Spectral absorption curve of eosin-fast green stain in solution, (B)—Spectral absorption curve of rat liver nucleus stained by the eosin-fast green method, (C)—Spectral absorption curve of trout liver nucleus stained by the eosin-fast green method.**

amount of absorption in both these regions. However, nuclei of fish liver exhibit a third minor absorption peak at 620 mμ which is not observed in rat liver nuclei (Figure 1 B and C).



It can further be seen that eosin and fast green absorption peaks *in situ* do not correspond exactly with absorption maxima of the two dyes in solution. There is a shift in absorption maxima of about 20 mμ toward the higher wavelength of the visible spectrum indicating a chemical alteration has occurred in the formation of the dye complex.

*Effect of acid-exposure on relative abundance of pink-stained, green-stained and purple-stained parenchymal nuclei.* Microscopic analysis of stained liver sections from acid-exposed and normal fish revealed no apparent differences in the pattern of staining in liver cords. Liver tissue from both acid-exposed and normal fish exhibited somewhat random staining with a relatively equal abundance of both pink and green nuclei. Only a small proportion of the nuclei stained purple. No particular areas of the tissue displayed concentrations of either pink or green nuclei.

*Histology of fish liver.* Microscopic examination of sections of fish liver stained with hematoxylin and eosin revealed the absence of any definite lobulation as is typical in most mammalian livers. It was likewise noted that the liver cords, which are somewhat radially arranged around the central veins, are composed of two or three layers of cells in contrast to the single layer of cells in mammals. Bile ducts, portal veins and hepatic arteries rarely form portal triads but are most commonly separate and randomly dispersed throughout the tissue. The hepatic sinusoids are lined with flattened reticular cells as well as von Kupffer cells. Very fine bile canaliculi originate between the two cells comprising a liver cord and pass peripherally into the bile ducts. Trout liver nuclei are generally 3-5 μ in diameter as compared to the 8-10 μ diameter of rat liver nuclei.

## DISCUSSION

Microspectrophotometric analyses of fish liver tissue stained by the eosin-fast green technique revealed that individual nuclei displayed different affinities toward these two dyes as evidenced by their different absorption spectra. Visibly one can easily distinguish three main cell types on the basis of nuclear coloration; cells with green nuclei which exhibit a strong affinity for fast green, cells with pink nuclei which exhibit relatively strong affinity for eosin and purple nuclei which exhibit a strong affinity for both dyes. The spectral data indicate that no nuclei bind one dye to the exclusion of the other. In 1966, Bloch suggested that the selectivity of such staining in various stages of grasshopper spermatogenesis was caused by exclusive binding of nuclear proteins to either eosin or fast green. In a subsequent paper (9), however, Bloch presented spectral data showing that nuclear differences in grasshopper gonads are due to differences in relative amounts of both dyes rather than selective binding for either dye. This study adds support to the latter interpretation.

Little is known about the precise chemical nature of this staining reaction. However, there is considerable direct and indirect evidence which suggests that eosin-fast green staining specificity is due to differences in relative amounts of lysine-rich and arginine-rich histone (5, 9). It is also fairly well documented that an increase in the eosinophilic component is directly correlated with cessation or inhibition of RNA synthesis (5). This has been established using the combined techniques of autoradiography (with $^{3}$H-uridine) and eosin-fast green staining in studies of snail, grasshopper and mole crab spermatogenesis; HeLa cells in log phase growth; erythrocytosis in *Xenopus;* and mouse liver parenchymal cells (5,9). Thus, one can conclude that the differentially stained cells are not only chemically, but functionally, distinct.

The utility of the eosin-fast green staining technique as a parameter for evaluating functional metabolic changes in liver was established in a previous study in our laboratory (7). It was found that liver nuclei of rats exposed to moderate hypoxia exhibit an increase eosinophilia. A similar response was not found in fish. The absence of a definite lobulation in fish liver as was noted in this study and the presence of the two-celled liver cord confirm the earlier observation of Elias and Bengelsdorf (10). These histological features indicate that fish liver does not possess the type of functional zonation occurring in mammals. This would explain the random distribution of nuclei which exhibit preferential binding for eosin and fast green in fish liver.

The presence of a third, but minor peak, in the spectral absorption curve of fish liver nuclei may reflect a real differences in histone composition between fish liver and rat liver or this may reflect a particular response of fish liver histones to the staining reaction. Studies employing polyacrylamide gel electrophoresis, which have been successfully used for histone analysis (11), are under way in an effort to resolve this question. It is hoped that such studies will elucidate the nature of the minor absorption peak at 620 mμ.

## SUMMARY

The eosin-fast green staining response of liver parenchymal nuclei was analyzed in brook trout exposed to water of acid pH for 4 days. In any given tissue, three visibly and spectrally distinct cell types could be differentiated. Neither control nor acid-exposed trout exhibited any differences with respect to the relative proportions of the three cell types. It was tentatively concluded that short term exposure to acid pH does not materially affect this aspect of liver function. Spectral absorption curves of the fish liver nuclei reveal a minor absorption peak not previously reported in mammalian liver nuclei. Microscopic analyses of trout liver show that it differs from mammalian liver in the absence of lobulation and the presence of two-cell rather than single cell liver cords.



## ACKNOWLEDGMENT

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## REFERENCES


1. Westfall, B. A. 1945. Coagulation film anoxia in fishes. *Ecol.* 26:283.
2. Ellis, M. M. 1937. Detection and measurement of stream pollution. *Bull. Bureau Fisheries* 48:365.
3. Doudoroff, P. and M. Katz. 1953. Critical review of the literature on the toxicity of industrial wastes and their components to fish. *Sewage and Industrial Wastes* 22:1432-1458.
4. Floyd, R. and D. H. M. Jordon. 1964. Some factors affecting the resistance of rainbow trout to acid waters. *Int. J. Air Water Poll.* 8:393-409.
5. Bloch, D. P. 1966. Histone differentiation and nuclear activity. *Chromosoma* (Berl.) 19:317-339.
6. Huang, R. C. and J. Bonner. 1962. Histone, a suppressor of chromosomal RNA synthesis. *Proc. Nat. Acad. Sci.* (USA) 48:1216-1222.
7. Crissman, H. 1968. Eosin-fast green staining of nuclear histones in liver cells of normal and hypoxic rats. Master's Thesis. The Pennsylvania State University.
8. Humason, G. L. 1967. *Animal Tissue Techniques.* W. H. Freeman and Co., San Francisco.
9. Bloch, D. P. and C. J. Claypool. 1967. Synthesis of ribonucleic acid and histone changes during spermatogenesis in grasshopper *Chortophaga viridifasciata. Nature* 215:966-967.
10. Elias, H. and H. Bengelsdorf. 1952. The structure of the liver of vertebrates. *Acta Anatomica* 14:297-337.
11. Gurley, L. R. and J. M. Hardin. 1968. The metabolism of histones. *Arch. Biochem. Biophys.* 128:285-292.




# MUCOPOLYSACCHARIDE HISTOCHEMISTRY OF GILL EPITHELIAL SECRETIONS IN BROOK TROUT EXPOSED TO ACID pH


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## ABSTRACT

Histochemical analyses were made of gills from trout exposed to acid pH. An important aspect of this work was an examination of specific stains (e.g. colloidal iron, mucicarmine, and alcian blue) with respect to their utility in detecting changes in mucin content and distribution in acid-exposed fish. It was established that colloidal iron is superior to other stains in terms of contrast and discreteness of staining. Gills of trout exposed to acid water showed an increase in mucin content and a proliferation of cells between gill lamellae such that individual lamellae are indistinguishable.


## INTRODUCTION

It is generally accepted that acid mine drainage is responsible for a progressive decimation of wild fish populations in many Pennsylvania streams. Although there has been considerable speculation regarding causes of fish death, the precise physiological basis of fish mortality remains unknown. Most workers currently favor the hypothesis that fish mortality is due largely to decreased oxygen availability to bodily tissues (1). It has been suggested that anoxia probably results from a markedly lowered transport of oxygen across gill filaments (2). This belief stems from observations that dying fish usually have a thick mucus-like film covering gill filaments.

Significantly, few studies have been concerned with histopathological changes in gills of fish exposed to acidic water. Furthermore, no reports were found which employed histochemical staining for the analysis of cellular changes in gill filaments.

The aim of the present study is to investigate histological and histochemical changes in gill filament tissue following short-term exposure of brook trout *(Salvelinus fontinalis)* to lethal concentrations of acid. Three mucopolysaccharide-specific stains were used in an attempt to gain more insight concerning the process of mucus secretion and formation of a coagulation film in gill filaments.

## MATERIALS AND METHODS

Hatchery reared brook trout *(Salvelinus fontinalis)* were obtained from the Research Station of the Pennsylvania Fish Commission, Benner Springs, Pennsylvania, for use in this study. The fish weighed 50-75 g and varied in length from 15-17 cm. Twelve control and twelve experimental fish were kept in separate tanks of dechlorinated water at 7-10°C and maintained under constant filtration. Concentrated HCl was added gradually to the experimental tank over a period of four days until the fish exhibited loss of equilibrium. For example, at about pH 3.9 the fish began to swim erratically, turn over, and secrete copious amounts of mucin, especially in the gill area.

On the fifth day, the fish were sacrificed by electric shock. Gills were removed, fixed overnight in Perenyi's fixative (3), washed in 50% ethanol for 24 hr, dehydrated, cleared, and infiltrated with paraffin. All gill tissue was sectioned at 6$\mu$ and representative sections were stained with the following dye combinations: (a) alcian blue counterstained with chlorantine red (4), (b) mucicarmine and Groat's hematoxylin (5), and (c) colloidal iron and nuclear fast red (4). Sections were also routinely stained with hematoxylin and triosin for comparative purposes.

## RESULTS

Microscopic examination of gill specimens in brook trout revealed that the gross morphology of gill filaments, as well as the histological makeup of the branchial epithelium are the same as described for other teleosts (6,7). Basically, a gill arch suspends a number of gill filaments, which is covered on both sides by transverse, leaf-like structures designated as lamellae. Since the entire surface of a lamella is covered with simple squamous respiratory epithelial cells, the lamella can be considered the functional unit of the gill.

All three mucopolysaccharide-specific stains used in this study proved superior to routine hematoxylin-triosin staining in identification and localization of mucous containing cells in lamellae. Colloidal iron was especially useful for detecting cells in early stages of mucous

droplet formation; this stain also facilitated differentiation of respiratory epithelial cells from disintegrating mucous cells. Although alcian blue and mucicarmine were useful in differentiating between mucous and non-mucous elements, neither of these stains was judged as good as colloidal iron for detection of changes in mucus secretion. For example, the intensity of mucin staining with mucicarmine was so faint that it was often overshadowed by the basophilic cytoplasm resulting from the hematoxylin counterstain. The alcian blue-chlorantine red combination also led to varying degrees of non-specific staining of mucous cells again resulting in poor contrast due to inadequate stain localization. As a consequence, detailed microscopic analyses of gill filaments from treated and untreated fish were restricted to colloidal iron-nuclear fast red stained specimens with the other stains used simply for confirmatory checks of major findings.

Before summarizing the histopathological changes observed in acid-exposed fish, it is desirable to briefly state some of the salient cytological features of control gill lamellae. Distal portions of the gill filament are devoid of lamellae and characteristically exhibit abundant numbers of mucous cells. Frequently, several isolated mucous cells occur at the lamellar base (Figure 1A). Non-secreting mucous cells are readily identified on the basis of their large, vesicular, centrally located nucleus. In contrast, squamous cells have densely stained flattened nuclei situated close to the basement membrane. The lamellar core contains supporting cells (modified connective tissue), blood vessel endothelium, and connective tissue. There is little evidence of either mitotic activity or pycnosis in any cellular elements. The lamellae are well separated with no mucous cells or debris in interlamellar spaces.

In acid-exposed fish (Figure 1B) there are several obvious differences. The amount of mucous is increased at the base of the lamella. More important, following exposure to acid conditions, isolated mucous cells commonly occur along the entire length of each lamella. Since this was never observed in untreated fish, one is forced to conclude that one of the histopathological changes associated with acid exposure is the appearance of mucous cells in lamellae. No cytological evidence was found which indicated the presence of mucous cells along lamellae was the result of cell migration or relocation of mucous cells from basal areas of lamellae. It was observed that acid exposure

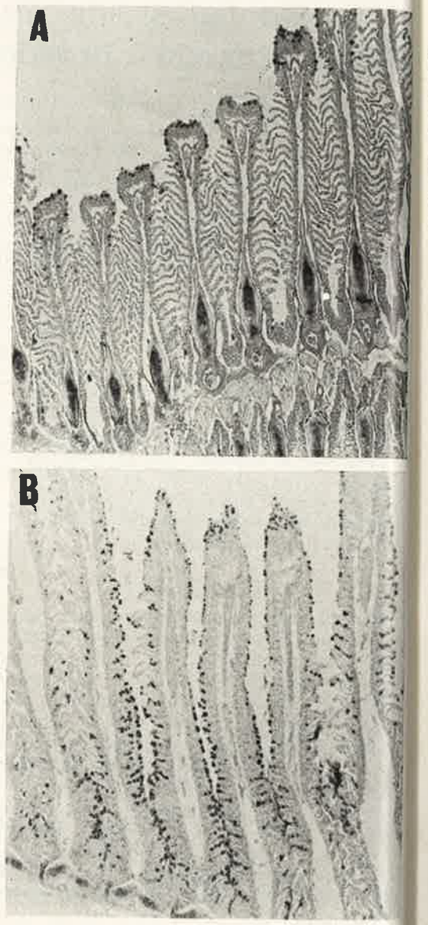



**Figure 1. (A) Untreated brook trout gill stained with colloidal iron-nuclear fast red, showing mucin at filament tips (X37). (B) Brook trout gill exposed to acid water demonstrating increased mucin between gill lamellae (X37).**

appears to facilitate disintegration of mucous cells which seems to be related to the extrusion process. When the whole cell extrudes its contents the integrity of contiguous squamous cells is disrupted, resulting in an increased folding of lamellae or, often interlocking of lamellae with those on adjacent filaments.



Another important histopathological change associated with acid exposure is the appearance of densely staining nuclei in the interlamellar spaces. The nuclei, together with the extruded mucus, compose the coagulation film. These nuclei are characteristically found in clumps which vary in size from ten to several hundred. In the latter case the interlamellar space is entirely obliterated, and the lamellae are no longer distinct structures.

## DISCUSSION

One contribution of the present study was the assessment of the utility of selected mucopolysaccharide stains for analysis of changes in gill mucin after exposure to acid conditions. It was concluded that colloidal iron was superior to alcian blue and mucicarmine for mucin detection in fish gills. Mowry (4) and Belanger (8) have used mucopolysaccharide stains to study mammalian and avian tissue respectively, and have obtained similar results.

The second finding of this study was that histopathological changes in fish gills are not limited to increased release of mucin. The process referred to as "gill coagulation" (2) or "gill clubbing" (9) is more complex. Histochemical data revealed that at least two types of alterations are involved in the formation of a coagulum of gill filaments. One is the increased synthesis of mucin, coupled with a tendency for disruption of epithelial integrity due to destruction of large numbers of mucous cells. Thus, the mucous film consists of nuclei and cellular debris within a mucus matrix, which may explain why it adheres so well to the gill lamellae. Nuclei within the film remain intact and clumps of nuclei adhere to respiratory epithelial cells.

On the basis of cell numbers, nuclei filling an interlamellar space approximate the total number of cells composing several lamellae. The source of these nuclei is uncertain. It is unlikely that the nuclei are produced by disintegrating mucous cells in the lamella, and similarly there is no cytological evidence which indicates that the coagulum is the result of extravasation from gill capillaries. The most probable explanation of the gill coagulum is that it is largely composed of cells from disrupted lamellae. This argument is supported by the observation that the integrity of the lamella is destroyed with the disintegration of mucous cells.

It should be added that active cell proliferation cannot be completely discounted since it is difficult to identify cells in fish tissue undergoing mitotic activity. Though metaphase figures are distinguishable in mammals, they are no so distinct in fish tissue. A preferable index of mitotic activity in fish would be DNA labeling studies or measurement of DNA content using microspectrophotometric techniques.

## ACKNOWLEDGMENT

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## REFERENCES

1. Ellis, M. M. 1937. Detection and measurement of stream pollution. *Bull. Bureau Fisheries.* 48:365.
2. Westfall, B. A. 1945. Coagulation film anoxia in fishes. *Ecol.* 26:283.
3. Galligher, A. E. 1934. *The Essentials of Practical Microtechnique.* Privately Published. Cited in: Humason, G. E. 1967. *Animal Tissue Techniques.* W. H. Freeman and Co., San Francisco. p. 22.
4. Mowry, R. W. 1963. The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue G8X and their combination with periodic acid Schiff reaction. *Ann. N. Y. Acad. Sci.* 106:402.
5. Humason, G. E. 1967. *Animal Tissue Techniques.* W. H. Freeman and Co., San Francisco. p. 319.
6. Bevelander, G. 1935. A comparative study of the branchial epithelium in fishes with special reference to extra-renal excretion. *J. of Morph.* 57:335.
7. Keys, A. and E. N. Willner. 1932. Chloride secreting cells in the gills of fishes with special references to the common eel. *J. of Physiol.* 78:368.
8. Belanger, L. F. 1963. Comparisons between different histochemical and histophysiological techniques as applied to mucous secreting cells. *Ann. N. Y. Acad. Sci.* 106:364.
9. Wood, E. 1957. Gill diseases. *Prog. Fish Cult.* 19:7.

# EFFECTS OF SALT AND NEUTRAL RED SOLUTIONS ON MOVEMENTS OF INVASIVE ONCOSPHERES FROM THE TAPEWORM HYMENOLEPIS DIMINUTA

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## ABSTRACT

Hexacanth embryos released from shells were observed in 0.05-0.5 N solutions of NaCl, KCl, $CaCl_2$, $MgCl_2$. Rates of movement and survival were recorded. Temperatures were used in the range of 22-28°C. Rates increased as the temperatures increased. In sodium and potassium, rates decreased as the concentration increased. In $CaCl_2$ rates increased up to 0.15 N, then decreased gradually as normality increased. In calcium rates were higher and oncospheres survived longer. The percent of active oncospheres decreased as normality was increased. Activation of movements occurred in distilled water or salts 30-60 seconds after removal of shell. This delay increased to 8 minutes in 0.3 N. Activation was related to fluid uptake. Addition of neutral red as 0.1-0.001% in 0.1 N KCl or mammalian Ringer solution greatly reduced the rate of movement and survival. Visible localization was in the protoplasmic meshwork of the giant AA′ cells. It is suggested that these cells may be important to sustained body movements.

## INTRODUCTION

The oncosphere is an invasive tapeworm embryo characterized by six hooks and a contractile body. Once released from the confinement of protective membranes, the oncosphere begins a rhythmic movement. This activity is a measure of its viability. The early investigations of Isobe (1) tested a wide variety of chemical solutions for their effect on activity. He realized the importance of suitable chemical media to the viability and activity of oncospheres. The present studies were initiated some years ago by the writer (2) in order to explore variables that needed control during study of living oncospheres. Various substances such as neutral red were then added to media and tested for effects. Progress made to date demonstrates that movement and survival of living oncospheres depends upon a favorable fluid medium. A suitable medium is also essential for prolonged examination of normal living morphology, motility, and response to specific substances in solution.

## MATERIALS AND METHODS

Eggs were obtained from the stools of laboratory infected rats (3). Moist pellets were crushed in water, centrifuged several times to remove soluble materials, and the clear fluid was discarded. The sediment of eggs and debris was mixed with 3N NaCl, then centrifuged briefly to separate debris from the eggs floated to the surface. The eggs were removed with a dropper and refrigerated in dilute salt solution until needed. Under these conditions eggs survived as long as 6 months with no apparent effect upon the rate of movement.

Slides for the examination of *partially hatched oncospheres* were prepared as follows. Eggs were removed from storage fluids with a micropipet, passed through several changes of distilled water in a series of slide depressions, then transferred into the test solution. Next, 8-10 eggs were transferred to a fresh drop of test media, placed on a microscope slide, covered with a vaseline-sealed coverglass, observed by the stereo microscope, and the egg shells cracked by gentle pressure on the coverglass. The shell separated from the colloidal envelope and movements began within the minute. Throughout observations the embryo remained enclosed by the colloidal envelope. The prepared slide was placed in the stage incubator containing a ruled England Finder slide. The temperature and position of each oncosphere was recorded. The rate of movement was determined by use of a time-o-lite laboratory timer. Each oncosphere was counted several times during a 20-min period and the average for all movements was used. For survival the number of oncospheres active at the end of 20 min was recorded and percentages calculated. Total survival time required observing activities from activation to death.

Slides of *hatched oncospheres* free of all membranes were prepared from washed eggs transferred into an artificial hatching solution used previously (3). When hatched, oncospheres were transferred through two changes of the test solution with a micropipet, placed on a slide in fresh media, and covered with a vaseline-sealed coverglass. The number of extensions per minute were then counted and the length of life determined. For the neutral red experiments 8-10 oncospheres were examined separately for each test solution, which restricted the number of tests made each day. In this way the time of staining and its localization could be noted. In these studies the average rate of movement was the numerical average based on summary of movements for oncospheres counted at a given time interval after hatching.

## RESULTS FOR SALT SOLUTIONS

These observations were made largely on oncospheres enclosed by their colloidal envelope but removed from the shell. Following removal of the shell there was a short latent period or delay in initiation of movements covering 30-60 sec. However, as the concentration of the medium increased, this period lengthened by 3-8 min in 0.3 N media. The initial rate of movement was variable, sometimes above, sometimes below the average for the first 20 min of activity. In some cases the rate tended to increase to a maximum during the first minutes and then decline gradually. In other cases the initial rate was highest and was followed by a progressive decline. There was also variation in the rhythm of a given oncosphere. Usually an even sustained rate occurred; however, in some oncospheres there was an erratic slowing down, then a speeding up, or a brief pause, followed by continued activity.

The rate of movement was proportional to the temperature (Figure 1) in each concentration studied. It was evident that in $CaCl_2$ solutions the average rate of movement was greater at 26°-28°C than at 22°-23°C. The pH was controlled, although in the range of pH 5-8 it did not appear to have injurious effects on the rate of movement or survival. Good movements occurred in the range of pH 3-11. However, at pH 2.0 (0.1N KCl or 0.1N $CaCl_2$ acidified with HCl) or lower, movements ceased within 15 minutes or never started. At pH of 12 there was a very long latent period, poor movements, and poor survival.

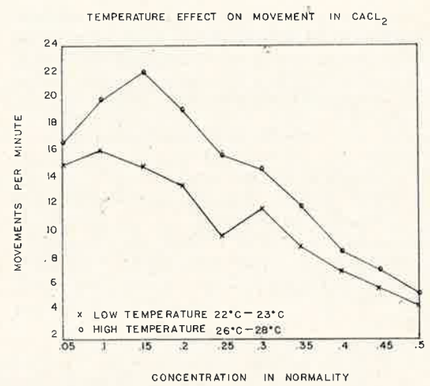



**Figure 1. Temperature effect on movement in $CaCl_2$. Rates were consistenly higher at the higher temperatures.**

The highest initial rates were often in the hypotonic media below 0.15N. As the concentration of medium was increased, there was a proportional decrease in the average rate (Figure 2). In NaCl, KCl, and $MgCl_2$ the rates decreased as the concentration increased. In $CaCl_2$ there was an increase up to 0.15N, followed by a gradual decrease in higher concentrations. The oncospheres in $CaCl_2$ maintained higher rates with better survival in all concentrations as compared to other salts tested. As the concentrations increased, the range of movements narrowed with the majority being slow. As more concentrated media

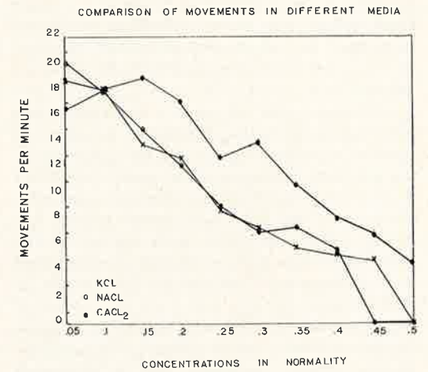



**Figure 2. Comparison of movements in different media. Rate of movement and survival decreased in higher concentrations.**

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[2] Contribution #28 to cytology and development of oncospheres.

were used, there was a progressive increase in the number of non-motile, dead oncospheres. The percentages of active oncospheres remaining after 20 min of observations showed differences related to the salts used (Figure 3). Cal-

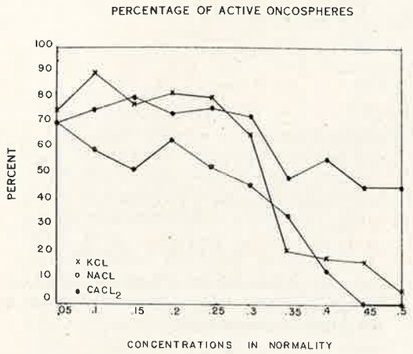



**Figure 3. Percentage of active oncospheres. Survival, as measured by decrease in percent of active oncospheres after 20-min observations, was reduced in increased normalities.**

cium solutions consistently showed the greatest numbers of active oncospheres in the higher concentrations.

There was a difference in the posture of the body at death. In sodium, potassium, or magnesium solutions the oncosphere died in extension with hooks thrust laterally. In calcium, on the other hand, the oncosphere remained in retraction and holding. In some preparations the body protoplasm appeared more fluid in sodium, and potassium, whereas it was more viscous and gel in the calcium chloride.

Activation after the shell was removed did not require particular solutions. It occurred readily in distilled water, salt solutions, hatching solutions. Initiation of movements appeared to be related to mechanical factors arising by breakage of the shell and osmotic factors due to the uptake of fluid. Prolongation of the latent period in high concentrations suggested the importance of fluid uptake prerequisite to activation.

Longer survival was obtained in a mixture of salts provided by a modified Mammalian Ringer solution. However, rate of movement and survival were not entirely satisfactory basis for comparison. It was observed that in most solutions protoplasmic swellings (blebs) and various clear vesicles appeared. The blebs appeared within 20 min in the stock Ringer 0.1542N (0.3 mg $CaCl_2$). This had a refractive index of 1.3340 and was considered nearly isotonic. The calcium concentration of the stock Ringer was progressively changed with the result that the period without bleb formation was greatly increased to an optimum mixture containing 0.45mg/ml. Stock Ringer, 0.1614N (0.4mg $CaCl_2$/ml), blebs after 43 min; Stock Ringer, 0.1623N (0.45 mg $CaCl_2$/ml), blebs *only after 64 min;* Stock Ringer, 0.1632N (0.5 mg $CaCl_2$/ml), blebs in 44 min; Stock Ringer, 0.1650N (0.6 mg $CaCl_2$/ml), blebs in 28 min; Eagle Basal Salt Medium blebs in 40 min. The composition of the Ringer with the more suitable mixture was: NaCl, 8.5 g/L; KCl, 0.25 g/L; $CaCl_2$, 0.45 g/L, and phosphate buffers added to adjust the pH. This solution had a refractive index of 1.3342. The blebs appeared about the time there was a decrease in body diameter after swelling in dilute or non-isotonic media and were interpreted as artifacts. Swelling was complete in 5 min; blebs formed in 15-30 min.

## NEUTRAL RED EXPERIMENTATION

Neutral red has long been used as a vital dye and with certain organisms does not appear to have toxic effects. However, oncospheres in dilute solutions quickly became stained and appeared to live short lives. The previous results with single salts provided control media for testing the idea that the dye was toxic. The embryos were released from their shells but remained enclosed by a colloidal envelope. Controls in 0.1N KCl survived an average of 91 min and in 0.1N $CaCl_2$, a more favorable medium, survived 116 min. Experimentals in a medium of 0.01% neutral red in 0.1N KCL, survived 72 min and in 0.01% neutral red in 0.1N $CaCl_2$, survived an average of 51 min. The average rates of movement were also lower in the neutral red preparations. Thus, in those conditions the activity and survival were reduced when the dye was present.

Hatched oncospheres, free of all membranes, were next tested. The Mammalian Ringer solution was employed and the pH was adjusted in the acidic range of pH 5-6, which limited the visible localization of the stain to the protoplasm of the giant cells. Hatched oncospheres placed in 0.01% neutral red in mammalian Ringer stained readily at alkaline pH. However, coloration did not occur at values below pH 4.5 in a hypotonic Ringer. At pH 5.7 only the protoplasmic meshwork of the mid-



proximal area of the giant cells was stained. Moreover, in a series of dilutions, 0.01, 0.001, 0.0001, 0.00001% in Ringer at pH 5.7, the giant cell protoplasm was visibly stained only in dilutions of 0.01 and 0.001%. Other cells in the body were not visibly stained. The neutral red localized in the meshwork of the giant cell.

The rate of movement and survival times for oncospheres were recorded in each of the above dilutions. The total survival time was determined from the initial movements to final movements. Control preparations consisted of oncospheres in Mammalian Ringer lacking dye. The graph (Figure 4) showed that controls

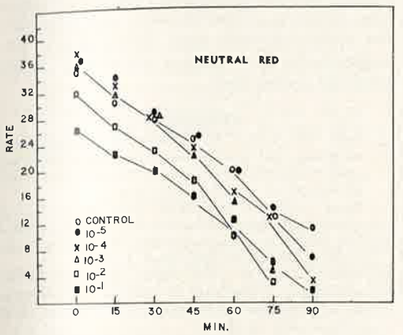



**Figure 4. Neutral red effect on movement. Rate of movement and survival were decreased in high concentration of the dye. Specimens were visibly stained in concentrations of $10^{-3}$, $10^{-2}$, $10^{-1}$.**

and dilutions $10^{-4}$ and $\times$ $10^{-5}$ were initially very similar. Initial movements were at a high rate of 35-42/min. and there was no visible staining. During the period of sustained rhythmic activity, the rate gradually declined until death after 80-100 min. The lowest rates were, however present, after 60 min in the solutions with dye. In the other dye concentrations, $\times$ $10^{-1}$, $\times$ $10^{-2}$, $\times$ $10^{-3}$%, specimens showed coloration of the giant cells in a few seconds and their initial rates of 26-32/min. were lower than controls and the other dilutions. The rates declined more quickly after the first 60 min. The total survival time from hatching to death varied within a certain range for each dilution and have been grouped for convenience. For controls the average survival time was 156 min (range 132-168). For dilutions of dye $10^{-4}$ and $10^{-5}$, the average survival was 121 min (range 108-139). For those dilutions in which visible staining occurred ($10^{-1}$, $10^{-2}$, $10^{-3}$), the average survival time was 106 min (range 83-138).

## TESTS WITH ACRIFLAVINE

Some experiments were made with acriflavine dye in Mammalian Ringer. The giant cell protoplasm was the primary unit visibly stained. The rates of movement at the end of ten minutes were: controls, 26/min; 0.0001% acriflavine, 22/min; and 0.001%, 18/min. The survival times were: controls, 134 min; 0.0001%, 60 min; and 0.001%, 37 min. The decrease in activity was associated with greater amount of dye in solution.

## DISCUSSION

The reasons for the gradual decline in activity during the time of survival in all media tested (Figure 4) are unknown. One possibility is that oxygen and carbon dioxide tensions needed to be controlled. Another reason may have to do with the exhaustion of reserve energy, or that the movement is programmed to last only a finite period. The type of salt used and its concentration are important for the sustained activity. In these tests the sodium, potassium, and magnesium ions are less favorable for sustained activity. The calcium solution, on the other hand, is more favorable for optimum motility and survival. When the mixture of salts is used as a modified Mammalian Ringer, the addition of excess calcium was beneficial. The rate of activity and length of survival are adversely affected when the concentration is increased beyond 0.2N. The sustained retraction of oncospheres in higher concentrations of calcium suggest that the ability of the musculature to relax is impaired. It may also relate to a more viscous consistency observed in these oncospheres. In hypertonic solutions fluid is removed from the body by osmotic conditions. In the study of living oncospheres the use of optimal media is essential to observation of the normal protoplasmic condition, morphology, and motility.

The initiation of activity in this oncosphere following removal of the shell, or removal of the young oncospheres from the tapeworm, may not require the uptake of a special chemical. Rather, it may require uptake of water by the colloidal systems. This activation can occur in distilled water, but is greatly delayed in concentrations of salts above 0.2N. Fluid uptake may signal release of an enzyme system.

These results, dealing with effects of salts on motility, are illumined by those on living amoebae summarized by Chambers and

Chambers (4). Immersion of amoebae in sodium or potassium solutions stops streaming protoplasmic movements and cause early death. Immersion in calcium solutions have little adverse affect and amoebae survive for long periods. The monovalent ions of sodium and potassium actually enter the cell causing liquefaction. This is supported by injection of ions into cytoplasm where they produce liquefaction. Calcium, on the other hand, does not appear to enter the cell under immersion conditions, since when introduced by injection, it produces rapid coagulation of the protoplasm, reaction unlike the immersion tests (4). Caution must be used in interpreting observed changes in immersion experiments, since the effects on protoplasm can only be indirectly attributed to the cations that penetrate (5).

In the neutral red experiments reduction in motility and survival is greatest in the more concentrated solutions. The increased impairment is also greatest in those oncospheres showing visible staining. In these cases the visible red coloration is limited to the contractile protoplasmic meshwork of the giant AA′ cells in the cortex in 0.001% dye, pH 5.7. However, it is not certain that other cells or systems having to do with movement, even though not visibly stained, are unaffected. The reduction of body movements is correlated with injury to the giant cells after their uptake of neutral red. This suggests the general theory that these cells are important for sustained body movements. This viewpoint has further support if it can be correctly assumed that the cells most seriously injured are those that contain the greatest amount of visible dye in their protoplasm. The giant cells are the only visible stained protoplasms, and therefore are the most seriously injured. The capacity for sustained body movements appears to depend on the continued viability of the giant cells. Further testing of this view is planned.

In the study of amoeboid movement, neutral red has been used in a variety of ways. The dye appears to bind to the protein in actively moving cells (6). Additional studies suggest that visible staining is associated with injury (7,8,9). Moreover, Prescott (9) showed that the dye is taken up by actively streaming protoplasm, since under high hydrostatic pressures streaming is inhibited and amoebae do not take up neutral red. Goldacre (10) commenting on Prescott's report, believed the uptake of neutral red by *Amoeba proteus* from acidic solutions pH 4-5, was an active uptake into the protein and not passive by diffusion. The usual penetration of neutral red is from an alkaline medium into the more acidic protoplasm where the dye forms complexs with protein (11). Injection of neutral red described by Chambers and Chambers (12) directly into the protoplasm causes coagulation of proteins. It has been suggested by Noland (13), and Jahn and Bovee (14), that the dye inhibits solation of the protein, and the stained plasmagel, unable to solate, cannot continue contractile movements. These findings seem to support the studies on the oncosphere. The dye accumulates in active contractile cells and when they are visibly stained, toxic effects occur. The embryos die in extension since they cannot complete contractions.

## REFERENCES


1. Isobe, M. 1926. The significance, physicochemical conditions, and pharmacological studies on the movement of the hexacanth embryo. *Acta Sch. Med. Univ. Kioto* 8:519.
2. Ogren, R. E. and J. J. Ogren. 1964. The effect of increased salt concentrations on the rate of movement and survival of oncospheres from the tapeworm, *Hymenolepis diminuta*. *Amer. Zool.* 4:291.
3. Ogren, R. E. 1967. The cellular pattern in invasive oncispheres of Hymenolepis diminuta as revealed by an enzyme-acetic acid-orcein method. *Trans. Amer. Microsc. Soc.* 86:250.
4. Chambers, R. and Chambers, L. 1961. *Exploration into the Nature of the Living Cell*. Harvard Univ. Press, Cambridge, Mass., pp. 127-135.
5. *Ibid.*, p. 130.
6. Goldacre, R. J. and Lorch, I. J. 1950. Folding of protein molecules in relation to cytoplasmic streaming, amoeboid movement, and osmotic work. *Nature* 166: 497.
7. Goldacre, R. J. 1952. The folding and unfolding of protein molecules as a basis of osmotic work. *Int. Rev. Cytol.* 1:135.
8. Andresen, N. 1944. Cytoplasmic components in the amoeba *Choas choas* Linne. *Compt. rend. trav. lab. Carlsberg Ser. Chim.* 24(7):139.
9. Prescott, D. M. 1953. Relation between dye uptake and cytoplasmic streaming in *Amoeba proteus*. *Nature* 172:593.
10. Goldacre, R. J. 1953. *Nature* 172:593. Note: remarks on Prescott (1953).
11. Chambers, R. and Chambers, L. 1961. *Ibid.*, pp. 177-178.
12. *Ibid.*, p. 182.
13. Noland, L. E. 1957. Protoplasmic streaming: A perennial puzzle. *J. Protozool.* 4:1.
14. Jahn, T. L. and Bovee, E. C. 1967. Motile behavior of Protozoa. In *Research in Protozoology* (T. Chen, ed.) Volume 1. Pergamon Press Inc., N. Y., p. 94.




# SIMPLE TECHNIQUE FOR DEMONSTRATING CHROMOSOMAL COILS USING TRADESCANTIA POLLEN MOTHER CELLS

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## ABSTRACT

*Tradescantia* L. is one of the few organisms in which chromosomal coils can be seen. The classic technique employed to demonstrate these coils is presented and contrasted with a new technique developed by the author. The new technique is over ten times faster and much simpler than the classic method and is equally reliable. Photomicrographs of *Tradescantia* meiotic chromosomes illustrate the results obtained by means of the new technique.


## INTRODUCTION

It is well known that chromosomes shorten then lengthen as they proceed through the stages of mitosis and meiosis. The mechanism by which this change in length occurs is not well understood. A currently accepted hypothesis is that the chromosome is a coiled structure which shortens by a despiralization process in which the coils or gyres become larger in diameter and fewer in number (1-3). The coiled structure of *Tradescantia* and *Trillium* meiotic chromosomes supports this hypothesis.

*Tradescantia* has been the object of several genetic and cytotaxonomic studies (2,3) and has become a useful instructional tool in the cytogenetics laboratory. Aside from the fact that it illustrates chromosomal coiling, *Tradescantia* is desirable in that it has a small number (N=6) of relatively large chromosomes and polyploid species which are useful in the study of meiosis. Translocation rings etc. are relatively common.

Sax and Humphrey (3) demonstrated chromosomal coils in *Tradescantia* pollen mother cells by employing a rather laborious technique which includes pretreatment of smeared anther material in 20% ethanol, fixation in 2% osmic, 1% chromic acid, and staining with crystal violet in accord with the elaborate Newton procedure (4). Various pretreatment solutions, including warm water have been employed by different workers (5). The author and his collegues at Vanderbilt University have used the above method and have found it to be as inconsistent as it is lengthy.

## MATERIALS AND METHODS

To fill the need for a simple and reliable technique, a method was developed by which the coils of *Tradescantia* meiotic chromosomes are quickly relaxed. The material can be fixed, stained, mounted, and ready for observation in minutes. An anther, at the proper stage of development, is torn open in a drop of water on a slide and the contents mixed well in the water. The slide is heated *slowly* over a flask of gently boiling water until the volume of the drop decreases about one-half. Aceto-orcein or a similar fixative-stain is then mixed into the water drop. A cover slip is added and excess stain removed with a tissue. While a preliminary examination may now be made, it is desirable to flatten the cells. I usually do this by wrapping the slide in a paper tissue, placing a rubber stopper over the coverslip, holding the stopper in place with the two middle fingers, and tapping these fingers gently with my other hand. A permanent mount is easily made *via* the dry ice method.

## RESULTS AND DISCUSSION

Amphidiploid material was used to prepare the accompanying illustrations. Figure 1a is an example of prophase one, diplotene-diakinesis stage, in pollen mother cells of *Tradescantia* and illustrates the chromosomes as they appear before the coils are relaxed. Figure 1b presents the same material after it has been pretreated to relax the chromosomal coils. While not every cell on a slide reacts to the same degree, many will present the satisfactory results seen in Figure 1b. While Figure 1c contains chromosomes with relaxed coils, it also illustrates another phenomenon. The arrow points to a region where the chromatids, while still in prophase one, are easily seen.

Figure 1d represents a group of anaphase two chromosomes. These have not been treated for coiling and the black spot is an artifact. The coils discussed earlier, called primary coils, have now disappeared by despiralization. These former chromatids, when carefully examined,

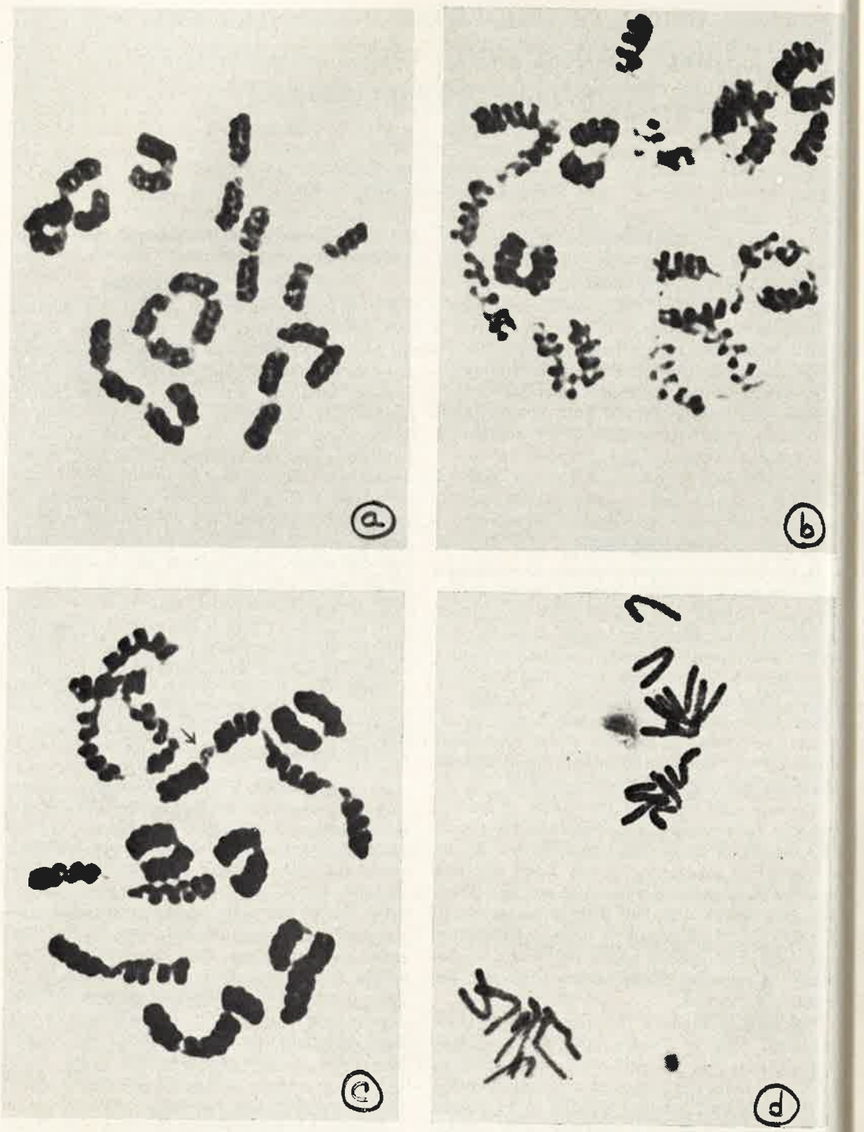



**Figure 1.** Tradescantia **pollen mother cell chromosomes. All figures X 1000. (a) Amphidiploid material at diakinesis, untreated. (b) Amphidiploid material treated to demonstrate chromosomal coils. (c) As (b), note chromatids at arrow. (d) Untreated amphidiploid material, anaphase two, showing secondary coils.**



also exhibit a coiled structure. These are the secondary coils and presumably were present in the earlier stages also. For a discussion of chromosomal coiling *per. se.* the reader should consult Swanson (1,2).


## SUMMARY

A new technique is presented by which the chromosomal coils of *Tradescantia* pollen mother cells can be prepared for quick examination. This simple procedure, found to be as reliable as the longer and more complex techniques employed by earlier workers, makes use of direct pretreatment with warm water and a fixative-stain such as aceto-orcein. Photographs illustrate the appearance of amphidiploid meiotic chromosomes as they appear before and after treatment.



## ACKNOWLEDGMENT

Studies for this paper were conducted in 1966 at Vanderbilt University where the author was an N.D.E.A. fellow. The work was done in the laboratory of Dr. William Clement and consisted of a special study in cytogenetics.


## REFERENCES


1. Swanson, C. P. 1957. *Cytology and Cytogenetics.* Prentice Hall, Englewood Cliffs. pp. 196-206.
2. Swanson, C. P. 1942. Meiotic coiling in *Tradescantia* L. *Bot. Gaz.* 103:457.
3. Sax, K. and L. M. Humhprey. 1934. Structure of meiotic chromosomes in microsporogenesis of *Tradescantia* L. *Bot. Gaz.* 96:353.
4. Baker, J. R. 1950. *Cytological Technique.* John Wiley and Sons, Inc., New York.
5. Sharma, A. K. and A. Sharma. 1965. *Chromosome Techniques.* Butterworths, London. p. 194.

# QUANTITATIVE DIFFERENCES IN THE IMMUNE CROSS-REACTIONS OF HETEROLOGOUS ERYTHROCYTES

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## ABSTRACT

The sharing of specific antigenic sites is considered to be the cause of cross-reactions among related antigens. Using the plaque technique the number of immunocompetent cells contributing to both the homologous and cross-reacting systems can be counted. That part of the primary response that involves IgM hemolytic antibody was followed, two through seven days following stimulation. Both homologous and cross-reacting systems were measured. The cross-reaction follows the same general pattern but consistently differs quantitatively from the homologous reaction. The cross-reaction was then measured in depth at a four day response, and results indicate that 12% of the antigenic sites on ox and sheep erythrocytes are the same. The use of this method for enumerating or mapping the antigenic sites on an erythrocyte antigen is discussed.

## INTRODUCTION

Red blood cells have long been used in investigations concerned with the different aspects of the immune response, and there have been several studies to determine the characteristics of the red blood cell as an antigen (1-3). That which is known about the erythrocyte stems primarily from these studies and those concerned with the hemagglutinating systems. It is the intent of this presentation to elucidate some of the antigenic characteristics of sheep and ox erythrocytes with experimental data obtained by studying their cross-reactions with antibodies produced early in the response.

The surface of one erythrocyte may have 90,000 to 600,000 antigenic sites, many of which are similar or have exactly the same molecular composition. (4) One lesion in the red blood cell membrane, caused by the reaction of one of these sites with its specifically-complementary antibody and complement, is all that is necessary for lysis of the cell (5,6). According to some investigators, one antibody producing cell can produce specific immunoglobulin (7-10), although others have refuted these data (11,12). The evidence that one antibody producing cell can produce more than one specific antibody at any one time, however, has been either non-reproducible (7) or attributed to contaminants in the antigen preparations (8). When Biozzi *et al.* (13) found rosettes bearing both pigeon and sheep RBC's, using lymphoid cells from mice stimulated with only one of these antigens, they interpreted this as an indication of single-cellular production of more than one antibody. It is just as likely, however, that the rosette-forming cell was producing an antibody which was complementary to a specific antigenic site common to both types of erythrocytes, and the rosette formation involving both antigens was due to one antibody.

Cross-reactions are generally considered to be due to the sharing of specific antigenic sites to which one specific antibody is produced. Assuming, therefore, that (a) one cell produces just one antibody of one specificity and (b) this one antibody is all that is required for the lysis of any red blood cell which bears the complementary antigen of that antibody, it is then possible to determine the extent to which antigenic sites are shared by heterologous erythrocytes. On a cellular level, this can be done with the agar plaque technique, first proposed by Jerne *et al.* (14) and later improved by Dresser and Wortis (15). With this technique, the number of lymphoid cells participating in a primary response to a specific erythrocyte antigen can be counted. By subjecting these homogeneous spleen cells to a different erythrocyte, the number of cells producing an antibody which is effective against a "shared" site can be counted. The extent of this cross-reaction is therefore indicative of the extent to which red cell surface sites are shared. In this study the kinetics of the primary response are investigated using two cross-reacting antigens. The response to both erythrocytes is studied in depth at four days to determine the extent to which they "share" sites.



## MATERIALS AND METHODS

*Mice.* Inbred female mice of the NIH strain were used in all of these experiments. Litters of six mice each were used for the kinetics studies. They were housed in the laboratory, fed Purina Lab Chow, and given water *ad libidum*.

*Antigens.* Both the sheep and ox erythrocytes were obtained from the Baltimore Biological Laboratories. They were used as fully prepared reagents, having been washed, pooled, and stabilized. A fresh batch of each antigen was used for each series and none of the preparations was kept for more than two weeks.

*Agar Plaque Technique.* The plating technique is essentially the same as that suggested by Dresser and Wortis (15) which utilized IBF agarose (free of anti-complementarity), Dulbecco's phosphate buffered saline, and guinea pig serum (complement). The spleens were teased into single cell suspensions and diluted in cold Basal Eagle's Medium (Earle's Base without glutamine). This medium was also used to prepare the top layer of 0.6% agarose and to dilute the complement. Approximately $10^8$ of the appropriate erythrocytes were introduced into the top layer with 0.1 ml of a known dilution of spleen cells. Plates were incubated for 2 hr before addition of complement and for 45 min afterward, at which time the plaques were counted.

*Experimental Design.* Two litters of mice were used for each of three series, one at 4 weeks, another at 7 weeks, and another at 10 weeks of age. All the mice were intraperitoneally injected with 2 x $10^8$ erythrocytes on the same day, each of the two litters being subjected to a different antigen. Two through seven days following the injections, one mouse from each litter was sacrificed and appropriate dilutions of spleen cells were plated—4 plates for the homologous reaction and 3 plates for the cross-reaction. Following the appropriate incubation period and complement additions, the plaques were counted with the aid of a bacteria colony counter. Using a Spencer counting chamber, cell counts were done on each spleen cell suspension at a dilution of 1:500, counting only nucleated cells.

To investigate the extent of the "sharing" of antigenic sites by sheep and ox erythrocytes, two groups of 11 mice each were used. One group was sensitized with sheep RBC's and the other with ox RBC's. The homologous responses as well as the cross-reactions for each mouse were measured by the plaque technique at day 4. Spleen cell counts and plaque assays were done exactly as described above. Data were recorded for each mouse as percent-cross-reaction, and the mean and standard deviation for each group of mice were calculated.

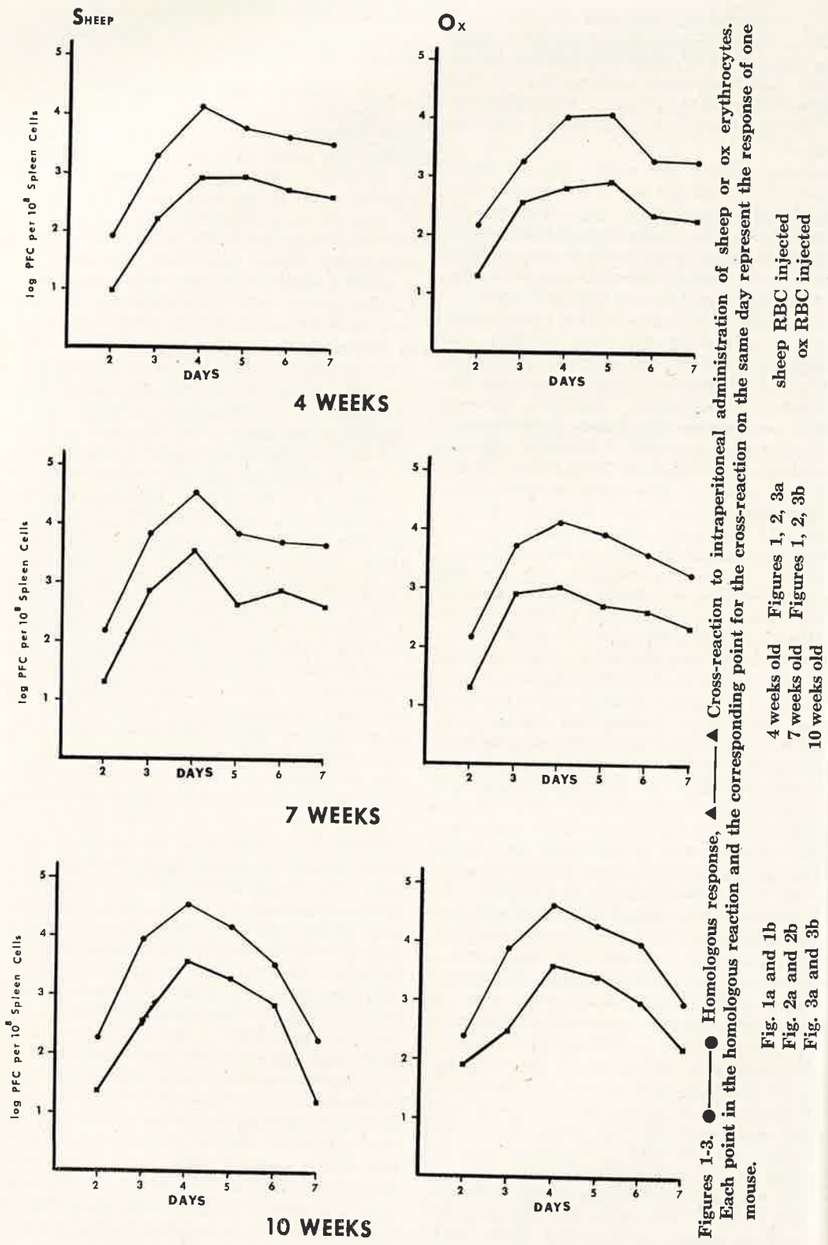
## RESULTS AND DISCUSSION

We chose to use highly inbred mice for these investigations because, being highly syngeneic, they would respond with a lesser degree of variance. Comparative studies done by Friedman (16) on the responses of four strains of mice to an intravenous administration of erythrocytes indicate that NIH mice respond consistently better than mice of $C_3H$, $C_{57}$, or AKR strains, at least with regard to plaque-forming spleen cells. This indicates that the mice used in this investigation were well suited to its purpose.

The studies of the kinetics of the response were done using mice at three different ages (Figures 1-3) to determine if there was a substantial difference in the response of these mice with regard to the cross-reaction as well as the homologous reaction. Radovich *et al* (17) found no significant differences in the homologous responses of mice 8 to 20 weeks old with intraperitoneal injections of sheep RBC's. Takeya and Nomoto (18), using the plaque technique to study the responses of mice 3 to 25 days of age, found that in young mice the number of PFC is small and that each PFC has a smaller capacity than those found in adult mice. Our 4 week old mice displayed a response (Figure 1) somewhat lower than the similar responses for the 7 and 10 week old mice (Figures 2a-3b). This lower response is most likely indicative of the immaturity of mice at 4 weeks of age. Consequently, mice 7 weeks of age were used for the percent-cross-reaction study.

Winn (19) found that the response of a large number of inbred strains of mice to a single injection of sheep RBC's, was confined almost exclusively to the elaboration of antibody of the IgM class. Furthermore, there is a great deal of evidence to indicate that the technique used in this investigation detects only those cells producing IgM hemolytic antibody (4,15,20-25). These figures, then, represent the rise and fall in the number of spleen cells which are producing IgM hemolytic antibody two through seven days after sensitization by sheep or ox erythrocytes, and the number of cells which are producing a cross-reacting IgM hemolytic antibody. These data for the homologous reaction with sheep concur with those of Radovich *et al.* (17).

It is generally evident from these figures that the type of immunoglobulin involved in




**Figures 1-3.** ● Homologous response, ▲ Cross-reaction to intraperitoneal administration of sheep or ox erythrocytes. Each point in the homologous reaction and the corresponding point for the cross-reaction on the same day represent the response of one mouse.

| | | |
|---|---|---|
| Fig. 1a and 1b | 4 weeks old | Figures 1, 2, 3a |
| Fig. 2a and 2b | 7 weeks old | Figures 1, 2, 3b |
| Fig. 3a and 3b | 10 weeks old | |

| | | |
|---|---|---|
| | | sheep RBC injected |
| | | ox RBC injected |



the cross-reactions is the same as that involved in the homologous reaction. Clones of cross-reacting antibody producing cells remain in the system, at least in this early response, and these observations indicate that cells responsible for the cross-reactions are merely a specific proportion of those which participate in the homologous reaction. Based on the previously mentioned assumptions, therefore, the cross-reactions here observed are due to specific antigenic sites shared by both antigens.

If these propositions are correct, the degree of cross-reactivity should remain relatively the same from mouse to mouse for each antigen. To study this, the spleens of mice receiving equal injections of sheep or ox erythrocytes were assayed for homologous PFC and cross-reacting PFC on day 4. The percent-cross-reaction was then calculated for each mouse and the data were compiled (Table 1). Spleens of

TABLE 1. *The percent-cross-reactions of antibodies produced by spleen cells from mice sensitized with either ox or sheep erythrocytes.*

| Number of Mice | Sensitizing Antigen | Percent Homologous Reaction | Percent Cross-Reaction |
|---|---|---|---|
| 11 | Sheep RBC's | 100 | 12.89 |
| 1 | Ox RBC's | 100 | 11.51 |

11 mice sensitized with an intraperitoneal injection of ox erythrocytes exhibited a cross-reactivity of 12.89% ± 2.90%. Spleens of 11 mice sensitized in the same manner with sheep erythrocytes exhibited a cross-reactivity of 11.51% ± 9.47%. The high degree of variance noted in the sheep erythrocyte sensitized spleens is primarily due to the extreme data from two mice, and these alone are most likely due to an error in sampling. We are confident that a greater number of observations would indicate that the percent-cross-reaction for each antigen is approximately 12%.

On the basis of these data it is concluded that 12% of all antigenic sites found on the surface of either ox or sheep erythrocytes are exactly the same. It is further suggested that similar studies involving this use of antigen cross-reactivity can be effective in enumerating the antigenic sites shared by two or more cross-reacting erythrocytes and, coupled with similar investigations, can lead to the actual "mapping" of these sites on the surface of the red blood cell.

## REFERENCES

1. Ingraham, J. S., A. A. Biegel, M. R. Watanabe and C. W. Todd. 1967. Effect of anti-allotype sera on hemolytic plaque formation by single rabbit spleen cells. *J. Immunol.* 99:1023.
2. Santos-Buch, C. A. and J. R. Guzman-Rivero. 1968. Isolation of a protein antigen following extraction of lipids from sheep RBC stroma. I. Hemolytic IgM 1° response and hemolytic IgG 2° response in rabbits. *J. Immunol.* 100:475.
3. Walsh, P., P. H. Maurer and M. Egan. 1968. Detection of immune response against synthetic polymers of amino acids employing the PFC system. II. Cross-reactions of normal sheep RBC's with antibody against GLU, Lys, Ala, and Try Terpolymers. *J. Immunol.* 100:266.
4. Kabat, E. A. 1968. *Structural Concepts in Immunology and Immunochemistry* (1st ed.) Holt, Rinehart and Winston, Inc., New York. p. 239.
5. Boros, T. and H. J. Rapp. 1965. Hemolysin titration based on fixation of the activated first component of complement: Evidence that one molecule of hemolysin suffices to sensitize an erythrocyte. *J. Immunol.* 95: 559.
6. Humphrey, J. H. and R. R. Dourmashkin. 1965. In *CIBA Foundation Symposium on Complement* (G. E. W. Wolstenholme and J. Knight, eds.) Little, Brown, and Company, Boston. p. 175.
7. Gershon, H., S. Bauminger, M. Sela and M. Feldman. 1968. Studies on the competence of single cells to produce antibodies of two specificities. *J. Exptl. Med.* 128:223.
8. Green, I., P. Vassalli, V. Nussenzweig and B. Benacerraf. 1967. Specificity of the antibodies produced by single cells following immunization with antigens bearing two types of antigenic determinants. *J. Exptl. Med.* 125:511.
9. Pernis, B., G. Chiappino, A. S. Kelus and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. *J. Explt. Med.* 122:853.
10. Playfair, J. H. L., B. W. Papermaster and L. S. Cole. 1965. Focal antibody production by transferred spleen cells in irradiated mice. *Science* 149:998.
11. Attardi, G., M. Cohn, K. Horibata and E. S. Lennox. 1964. Antibody formation by rabbit lymph node cells. I. Single cell responses to several antigens. *J. Immunol.* 92:335.
12. Hiramoto, R. N. and M. S. Hamlin. 1965. Detection of two antibodies in single plasma cells by the paired flourescence technique. *Immunology* 95:214.
13. Biozzi, G., C. Stiffel, D. Mouton, M. Liacopaulos-Briot, C. Decreusefond and Y. Bouthillier. 1966. Studies on the immunocyto-adherence phenomenon in the course of immunization. *Ann. Inst. Pasteur* 110:7.
14. Jerne, N. K., A. A. Nordin and C. Henry. 1963. In *Cell-Bound Antibodies* (B. Amos and H. Koprowski, eds.) Wistar Institute Press, Philadelphia. p. 109.
15. Dresser, D. W. and H. H. Wortis. 1967. In *Experimental Immunology* (D. M. Weir, ed.) F. A. Davis, Philadelphia. p. 1054.
16. Friedman, H. 1964. Distribution of antibody-plaque-forming cells in various tissues of several strains of mice injected with sheep RBC's. *Proc. Soc. Exp. Biol. Med.* 117:526.
17. Radovich, J., H. Hemingsen and D. W. Talmage. 1969. The immunologic memory of LAF$_1$ mice following a single injection of sheep RBC's. *J. Immunol.* 102:288.
18. Takeya, K. and K. Nomoto. 1967. Characteristics of antibody response in young or thymectomized mice. *J. Immunol.* 99:831.
19. Winn, H. J. 1965. In *CIBA Foundation Symposium on Complement* (G. E. W. Wolstenholme and J.

Knight, eds.) Little, Brown, and Company, Boston. p. 133.

20. Adler, F. J. 1965. Studies on mouse antibodies. The response to sheep RBC's. *J. Immunol.* 95:26.
21. Hege, J. S. and L. J. Cole. 1966. Antibody-plaque-forming cells: kinetics of 1° and 2° responses. *J. Immunol.* 96:559.
22. Plotz, P. H., H. Colten and N. Talal. 1968. Mouse macroglobulin antibody to sheep erythrocytes: a non-complement fixing type. *J. Immunol.* 100:752.
23. Plotz, P. H., N. Talal and R. Asofsky. 1968. Assignment of direct and facilitated hemolytic plaques in mice to specific immunoglogulin classes. *J. Immunol.* 100:744.
24. Sterzl, J. and I. Riha. 1965. Detection of cells producing 7s antibodies by the plaque technique. *Nature (London)* 208:858.
25. Zaalberg, O. B., V. A. Van Der Meul and M. J. Van Twisk. 1968. Antibody production by isolated spleen cells: a study of the cluster and the plaque techniques. *J. Immunol.* 100:451.



# COMPARISON OF ESTERASE AND CHOLINESTERASE ELECTROPHORETIC PATTERNS IN HUMAN SERUM PROTEINS

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## ABSTRACT

Human sera samples were subjected to acrylamide gel electrophoresis and enzymatic staining for esterase and cholinesterase. The resulting patterns were analyzed for the number of bands per sample and for the percentage of reoccurrence of the bands in the group. Cholinesterase gave the possibility of 47 bands while esterase only displayed 44. The predicted major bands appeared frequently. There is some question as to the grouping of the two least mobile of the major bands. It appears that the bands in the cholinesterase electrophoretic pattern are the same as the bands in the esterase patterns. The reverse is not true.

## INTRODUCTION

The electrophoretic patterns of esterase and cholinesterase in acrylamide gel appear to be similar when examined visually. The question under investigation is whether both patterns are due to the same enzyme activity. It is believed that the esterases are a family of enzymes whose members have overlapping, but distinct, patterns of substrate specificity (1). Hence, the electrophoretic pattern of esterase possibly does not represent a specific enzyme in a multimolecular form, but a group of enzymes each with a broad substrate specificity. Cholinesterase is thought to represent this multimolecular form and is referred to as an isoenzyme (2). The isoenzyme cholinesterase from human serum is usually referred to as pseudocholinesterase as opposed to acetylcholinesterase ("true" cholinesterase) (2). Its systematic name is Acylcholine acyl-hydrolase and the Enzyme Commission (EC) Number is 3.1.1.8 (3).

The number of bands appearing in the electrophoretic patterns of esterase is greater when acrylamide gel is used as opposed to starch gel (4). Allen *et al.* (5) report that they discovered 17 to 19 bands of esterase activity in acryamide gel compared to 10 bands in starch gel. Cholinesterase activity usually produces 5 or more bands in starch gel (6,7,8). The five bands are referred to as C-1 through C-5 (6). The band (isoenzyme) with the greatest (anodic) mobility is designated C-1 (2).

The proposed paper investigates the question of whether both patterns are due to the same enzyme activity by measuring the Rf values of the bands in each pattern and by comparing the mean Rf values and the frequency of appearance of the bands.

## MATERIALS AND METHODS

*Electrophoresis.* Fifty-six selected serum samples were subjected to vertical gel electrophoresis (9,10) in a 7%, 3mm, polyacrylamide gel (Cyanogum-41). The 20 $\mu$l samples were separated at pH 8.4 with a Tris-$Na_2$EDTA-boric acid buffer (11). A constant voltage of 300 volts was used. The coolant in the cooling plates was maintained at 11°-15°C. The period of electrophoresis continued for approximately 2 hr.

*Incubation-Staining Solution. Esterases* — A tris-maleate buffer, pH 7, was employed (12, 13). The substrate was alpha-naphythyl acetate (Sigma Chemical Co., St. Louis). Fast Blue RR was the diazonium salt. The gels were incubated 5 to 12 hr. (13,14,15). *Cholinesterase* — The chemicals and procedure are the same as those for the esterases except that the substrate 6-bromo-2naphthyl-carbonaphthoxy choline iodide (Sigma Chemical Co.) was substituted for the substrate alpha-naphythyl acetate (1,13,14). After removing the gel from the incubation solution, the surface of the gel was washed and then cleared for 3 hr. in a 5% glycerine solution.

*Analysis.* The gels were read in a Densicord model 542 densitometer (Photovolt) using the linear response setting. The peaks on the graphs were measured for their distance from the origin and Rf values were calculated. Approximately 5 major peaks were found in all graphs. One graph was used where the major peak with the least mobility was selected as Rf equals 1.00. From this the Rfs of the 4 other peaks were calculated. These 5 points

were used as a model and an Rf equals 1.00 on the other graphs was chosen so as to make the majority of the 5 peaks agree in Rf value with the standard (model). From these Rf values, ranges were set up for the bands found in the patterns. The mean and 95% confidence interval of each band were calculated.

## RESULTS AND DISCUSSION

Figures 1 and 2 show the distribution of bands per sample. According to Fig. 1, the mean number of the esterase bands per sample is 15.6. The range is from 7 to 24 bands. The mean number of cholinesterase bands per sample is 18.61. The number of bands per sample ranges from 8 to 29. The cholinesterase has a larger number of bands per sample than does the esterase. In examining the individual results it was found that one gel (which contained 8 samples) in the esterase series had only 7 or 8 bands per sample causing the graph to be skewed in one direction. It appears that this gel did not stain sufficiently to give valid results. Removing this gel from the series causes the curve to shift from the left and yields a new mean for the esterase band at 16.91. This agrees with the mean of 18.61 for the cholinesterase. This agrees more with the results of Allen *et al.* (5) who reported the presence of 17 to 19 bands of esterase activity in acrylamide gel. No references have been found that reported this many cholinesterase bands. Figures 3 and 4 show the frequency distribution of the bands vs the mean Rf values. Examination of Fig. 4 shows the 5 major cholinesterase bands. These stand out because they are found more frequently than the others. All of them are found at least 75% of the time and 2 of them are found 100% of the time. The major bands in Fig. 4 (cholinesterase) correspond for the most part with the major bands in Fig. 3 (esterase). The Rf values of these major peaks, C-4 and C-5, seem to be deviated far enough from each other to make their comparison between the enzyme tests questionable. It is possible that they are not the same. This partially agrees with Brody (16) who incubated a gel in physostigmine (a cholinesterase inhibitor) before staining it for esterase activity. He found that bands C-1, C-2, and C-3 were inhibited and C-4 and C-5 were comparatively weakened. Thus, bands C-4 and C-5 are possibly not the same as their counterparts in the esterase pattern. The counterparts are possibly a composite of the C-4 or C-5 and one or more bands which are brought in by the broad specificity of the esterase (1).

TABLE 1. *Comparison of 95% Confidence Intervals of the Esterase Bands and Cholinesterase Bands*

| Band No. | Esterase | Cholinesterase | Band No. | Esterase | Cholinesterase |
|---|---|---|---|---|---|
| 1 | 6.04±0.022 | 6.05±0.035 | 23 | 3.15±0.017 | 3.14±0.015 |
| 2 | 5.87±0.022 | 5.86±0.019 | 24 | 3.04±0.018 | 3.05±0.013 |
| 3 | 5.74±0.042 | 5.71±0.016 | 25 | 2.96±0.013 | 2.96±0.010 |
| 4 | 5.56±0.062 | 5.57±0.021 | 26 | 2.88±0.018 | 2.87±0.021 |
| 5 | 5.37±0.024 | 5.38±0.029 | [27] | 2.71±0.020 | 2.63±0.026 |
| [6] | 5.23±0.005 | 5.23±0.008 | 28 | 2.65±0.075* | 2.64±0.076* |
| 6A | | 5.05±0.020 | 29 | 2.55±0.018 | 2.55±0.026* |
| 7 | 4.92±0.035 | 4.91±0.018 | 30 | 2.43±0.017 | 2.40±0.033 |
| 8 | 4.76±0.015 | 4.77±0.014 | 31 | 2.30±0.013 | 2.26±0.027 |
| 9 | 4.67±0.013 | 4.67±0.014 | [32] | 2.08±0.015 | 2.02±0.019 |
| 10 | 4.55±0.011 | 4.54±0.012 | 33 | 2.01±0.057* | 1.98±0.049* |
| 11 | 4.44±0.010 | 4.45±0.010 | 34 | 1.90±0.024 | 1.87±0.016 |
| 12 | 4.36±0.012 | 4.36±0.017 | 35 | 1.76±0.015 | 1.77±0.019 |
| 13 | 4.29±0.007 | 4.29±0.009 | 36 | 1.63±0.018 | 1.64±0.015 |
| 14 | 4.21±0.010 | 4.21±0.010 | 37 | 1.53±0.012 | 1.54±0.017 |
| 15 | 4.10±0.013 | 4.10±0.013 | 38 | 1.44±0.018 | 1.44±0.013 |
| 15A | | 4.10±0.000* | 39 | 1.26±0.022 | 1.26±0.024 |
| 16 | 3.99±0.014 | 3.98±0.013 | 39A | | 1.09±0.075 |
| 17 | 3.88±0.010 | 3.88±0.011 | [40] | 0.93±0.026 | 0.80±0.022 |
| 18 | 3.77±0.019 | 3.78±0.013 | 41 | 0.56±0.036 | 0.60±0.081 |
| 19 | 3.65±0.019 | 3.66±0.013 | 42 | 0.30±0.083 | 0.37±0.044 |
| 20 | 3.51±0.034 | 3.49±0.026 | 43 | 0.15±0.033 | |
| [21] | 3.33±0.025 | 3.34±0.023 | 44 | 0.07±0.012 | 0.04±0.022 |
| 22 | 3.26±0.029 | 3.23±0.015 | | | |

* The 95% Confidence Intervals of this band overlaps the 95% Confidence Intervals of the preceding band.
[] indicates a Major Band.



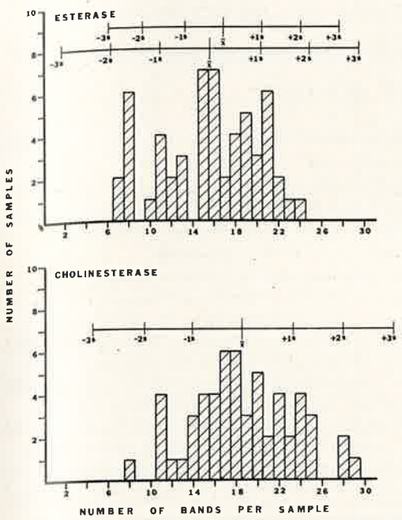



**Figure 1. (upper). Distribution of Esterase Bands per Sample. (The upper set of mean and standard deviations is adjusted to remove biased samples.)**

**Figure 2. (lower). Distribution of Cholinesterase Bands per Sample.**

In Table 1, it will be found that an overlapping in the confidence intervals of the C-4 band and the one following it in the cholinesterase and esterase columns indicates the possible need to break C-4 up into two different bands.

The C-5 band should also probably be divided into new bands. The results of the investigation show the C-5 band present 100% of the time. This does not agree with the literature (6,7). The problem appears to be the variable intensity of the C-5 band (6). Because of this variability in the intensity, it is difficult to separate the C-5 band from the surrounding bands.

The band closest to the origin is a collection of the heavier serum components that have difficulty migrating into the gel.

Table 3 shows the number of bands occurring at different percentages of time. It is a partial summary of Figures 3 and 4. The data show that there are in all but one case, more points visible in the cholinesterase than in the esterase. Why is this if esterase is supposed to be a family that includes the cholinesterases? The broad specificity of the esterase substrate could be the answer. The bands in the esterase were not as clearly defined as were the bands in the cholinesterase as evidenced from the densitometer readings. Some esterase bands were possibly hidden by larger bands or concealed in background stain.

Comparison of the mean of the Rfs and observation of the differences between the esterase and cholinesterase in Table 1 leads us to the opinion that the bands in the cholinesterase electrophoretic pattern are the same as the bands in the esterase pattern. The reverse is not true.

TABLE 2. *The Rf Values* of the Major Bands in Figures III and IV.*

| Esterase | Cholinesterase |
|---|---|
| 5.23±0.005 | 5.23±0.008 |
| 3.33±0.025 | 3.34±0.023 |
| 2.71±0.020 | 2.63±0.026 |
| 2.08±0.015 | 2.02±0.019 |
| 0.93±0.026 | 0.80±0.022 |

* The Values given are in the form of the mean and the standard deviation.

TABLE 3. *The Number of Bands in Figures III and IV occurring at Different Percentages of Time.*

| Percentages | Esterase | Cholinesterase |
|---|---|---|
| 100 | 4 | 2 |
| 75 | 5 | 6 |
| 50 | 7 | 10 |
| 25 | 27 | 36 |
| 10 | 42 | 44 |
| Total Possible | 44 | 47 |

## REFERENCES

1. Markert, C. L. and F. Moller. 1959. Multiple forms of enzymes: Tissue ontogenetic, and species specific patterns. *Proc. Nat. Acad. Sci.* 45 :753.
2. Wilkinson, J. H. 1965. *Isoenzymes*. J. B. Lippincott, Philadelphia. p. 5.
3. International Union of Biochemistry. 1961. Report of the Commission on Enzymes. Pergamon Press, Oxford, Eng.
4. Hunter, R. L., J. T. Rocha, A. R. Pfrender and D. C. DeJong. 1964. The impact of gel electrophoresis upon our understanding of the esterases. *Ann. N.Y. Acad. Sci.* 121:532.
5. Allen, R. C., R. A. Popp and D. J. Moore. 1965. Separation and relative quantitation of mouse plasma esterase with disc electrophoresis. *J. Histochem. Cytochem.* 13:249.

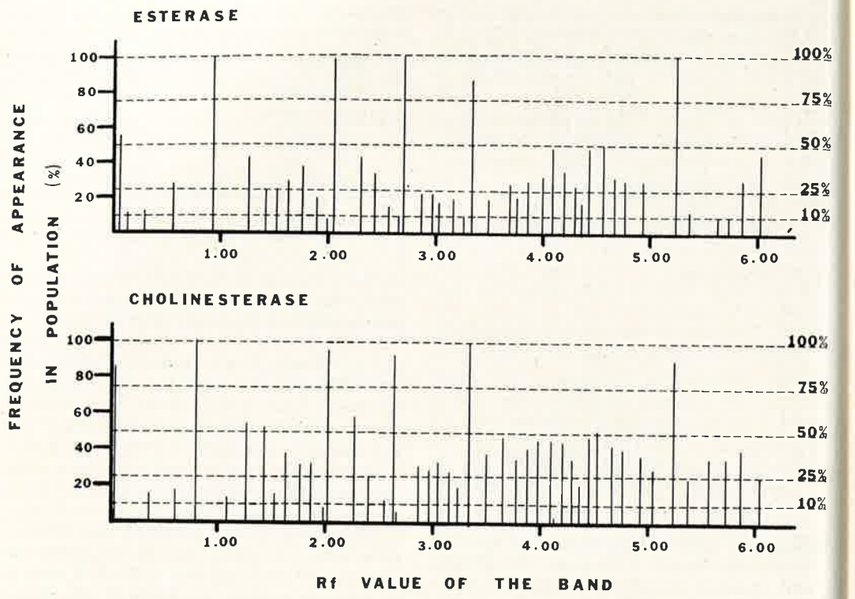



**Figure 3. (Upper). Frequency Distribution of Esterase Bands vs Rf Values**

**Figure 4. (Lower). Frequency Distribution of Cholinesterase Bands vs Rf Values**


6. Harris, H., D. A. Hopkinson, E. B. Robson and M. Whittaker. 1963. Genetical studies on a new variant of serum cholinesterase detected by electrophoresis. *Ann. Hum. Genet.* 26:359.
7. Ashton, G. C. and N. E. Simpson. 1966. C5 types of serum cholinesterase in a brazilian population. *Amer. J. Human Genet.* 18:438.
8. Van Ros, G. and R. Druet. 1966. Uncommon electrophoretic patterns of serum cholinesterase. *Nature.* 212: 543.
9. E-C Apparatus Corporation. Vertical gel electrophoresis: assembly and operation — Technical Bulletin 128. E-C Apparatus Corporation, Philadelphia, Pa.
10. Raymond, S. 1964. Acrylamide gel electrophoresis. *Ann. N.Y. Acad. Sci.* 121:350.
11. Peacock, A. C., S. L. Bunting and K. G. Queen. 1965. Serum protein electrophoresis in acrylamide gel: Patterns from normal human subjects. *Science.* 147:1451.
12. Gomori, G. 1955. Preparation of buffers for use in enzyme studies. In *Methods in Enzymology* (S. Colwick and N. Kaplan, eds.) Volume 1. Academic Press, Inc., New York. p. 138.
13. Burstone, M. S. 1962. *Enzyme Histochemistry.* Academic Press, New York.
14. Lawrence, S. H., P. J. Melnick and H. E. Weimer. 1960. A species comparison of serum proteins and enzymes by starch gel electrophoresis. *Proc. Soc. Exp'tl. Biol. and Med.* 105:572.
15. de Grouchy, J. 1958. Repartition des activities esterasiques et phosphatasiques du serum humain par rapport aux sero-proteines actuellement connues. *Rev. Franc. Etudes Clin. et Biol.* 3:881.
16. Brody, I. A., J. S. Resnick and W. K. Engel. 1965. Detection of atypical cholinesterase by electrophoresis. *Arch. Neurol.* 13:126.




# MACROINVERTEBRATE SPECIES DIVERSITY AS AN INDEX TO WATER POLLUTION IN SHAMOKIN CREEK AND ITS TRIBUTARIES

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## ABSTRACT

An attempt was made to relate changes in macroinvertebrate species diversity in 10 m sections of tributaries upstream from a mine acid polluted creek. Stations were established and bottom samples collected using a Surber sampler. Attempt was made to collate the biological analysis with measured physical and chemical conditions of the areas investigated. The effect of back-pollution was determined using changes in species diversity.

## INTRODUCTION

Numerous investigations have been made of the effects of organic pollution downstream from the point of entry to recovery. These studies have concentrated on defining the various pollution zones and the organisms associated with them. Some of the best have been Bartsch (1); Bartsch and Ingram (2); Brinley (3); Campbell (4); Gaufin and Tarzwell (5); Kolkwitz and Marrson (6,7); Patrick (8). However, no one has studied back-pollution at tributaries. Such areas contain interfaces and offer steep gradients. Therefore, they should be excellent environments for intensive analysis because they exhibit, in a few meters, the complete diversity which would be expected in several miles of a polluted stream. If true, these small areas should provide ideal for manipulation and controlled experiments toward pollution control.

This study was undertaken during the summer and fall of 1968 to test the first hypothesis. Specifically: Is back pollution occurring? To what extent does it affect macroorganisms (9) living there and how do various levels of mine acid pollution relate to species diversity and/or populations of these areas?

Shamokin Creek (Fig. 1) is a permanent stream located in a transition zone of hard coal region to rolling farmland. It originates near Centralia, Pennsylvania, flows southwesterly for 35 km through Northumberland County, and empties into the Susquehanna River one mile south of Sunbury, Pennsylvania. Stream depth ranges from a few cm on riffles to more than 1.5 m in a few pools. Width exceeds 15 m at the mouth and the annual mean flow is 1.66 cms near the mouth (10). Cover along the stream course varies from open, unshaded areas to fairly dense tree-lined banks.

The tributaries selected for this study, Stonington Run and Buddys Run, are nearly 7 m wide at their mouths, and depth rarely exceeds 0.5 m. Both are located west of the anthracite coal regions, draining primarily farm areas. Current velocity during summer months in the study areas was less than 0.1 m/sec. Depth at all stations investigated did not exceed 22 cm. Bottom debris consisted of loose pebbles to fine sand (11).

Shamokin Creek receives raw sewage from approximately 50,000 residents living above the study area. In addition to domestic wastes, the stream receives large quantities of acid mine drainage from stripping and deep coal mine operations, and large loads of suspended coal particles from several washing stations located on the stream. Table 1 provides a summary of physicochemical analyses of the stream from 1963 to 1967 (10).

Stonington Run receives small amounts of domestic sewage from residents along the stream and some barnyard seepage. Buddys Run, on the other hand, serves as a waste disposal for a large poultry processing plant located approximately 4.8 km upstream from the mouth.

## PROCEDURES

*Biological.* The interface between the tributaries and Shamokin Creek was established by observing its migration distance for two weeks and then selecting the mean difference from the highest and lowest point of migration. This served as a base line for placing stations every 2.5 m for a distance of 10 m up Stonington and Buddys Run. Stations in Shamokin Creek were established from the upstream bank of the tributary at right angles to the interface line. Five bottom samples were collected, at random, at each station in Stonington Run

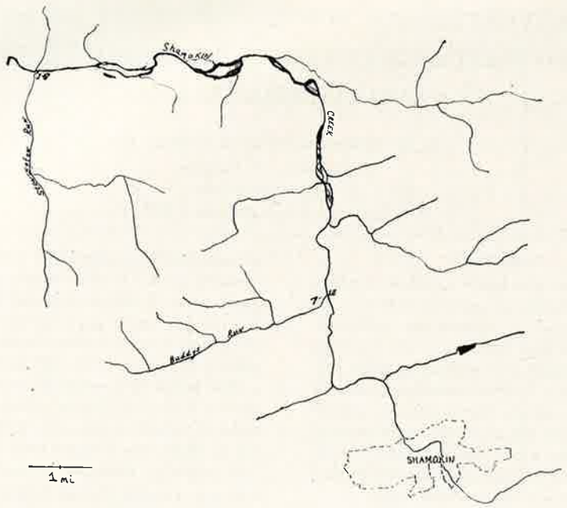



**Figure 1. Map showing Shamokin Creek, Stonington Run, and Buddys Run and the collection stations in each.**

and Shamokin Creek, while three were taken from Buddys Run. A modified 0.25 $m^2$ Surber sampler was used for the collecting (12). Each sample was taken by placing the lower edge of the sample quadrat along the station line. Data thus indicate organisms living to 0.25 m above the established station.

The procedure used for collecting organisms in Stonington Run was that outlined by Surber (12). Contents of the net were placed on a white oilcloth and sorted. All specimens were preserved in 70% ethanol and stored in 3 dram bottles for later classification.

*Tubifex* worms were collected in Buddys Run using the modified Surber sampler. The net contents were placed in a pail and the organisms separated by sugar flotation (13).

In the laboratory, *Tubifex* specimens were counted using a Folsom Plankton Splitter (14). Invertebrates from Stonington Run were counted and identified to genus where possible, otherwise to order or family. Weights were determined after drying for 48 hours at 90° C.

*Physicochemical.* Water samples were taken during the study period at each station in Shamokin Creek and at the interface, 5-m, and 10-m stations on the tributaries. Originally, samples were collected at each 2.5 m interval but this procedure was abandoned when it was found that the quality of the water did not vary greatly at such intervals. Measurements were made of temperature, pH, dissolved oxygen, depth and current velocity. Water samples were collected from approximately the same location as midstream bottom samples. Stream velocity was measured at the same time using styrofoam floats.

Water temperature was measured at each station with a mercury thermometer. A Photovolt Model 126A portable meter was used to measure pH. Dissolved oxygen samples were taken at each station and fixed by the Alsterberg (Azide) modification of the Winkler method (15). Samples were collected using a shallow water sampler consisting of a 26 inch piece of plastic pipe fitted at each end with No. 8 rubber stoppers, drilled and fitted with capillary tubing (upstream) and rubber tubing for transference to collection bottles (16). Samples were placed in 126-ml bottles and the entire sample titrated with .016N sodium thiosulfate. Diurnal oxygen, pH, and temperature measurements were made during the investigation.

## RESULTS AND DISCUSSION

The physical and chemical conditions in Shamokin Creek (Tables 1 and 2) make it very unfit for a diversified, or even a limited number of macroinvertebrate organisms. A combination of low dissolved oxygen, high



TABLE 1. *Principle parameters, range and average, for Shamokin Creek from 1963 to 1967. Values given in mg/l unless otherwise stated.*[1]

| Chemical Parameters | 1963 | 1964 | 1965 | 1966 | 1967 |
|---|---|---|---|---|---|
| Dissolved oxygen (ppm) | 1.6-3.7<br>2.4 | 2.8-6.5<br>4.6 | 0.4-4.0<br>2.4 | 2.8-10.8<br>5.9 | 2.4-5.2<br>3.9 |
| pH Units | 3.2-4.8<br>4.3 | 3.6-4.1<br>3.8 | 3.8-4.2<br>4.0 | 2.8-4.0<br>4.1 | 3.6-3.9<br>3.8 |
| Turbidity Units | 200-450<br>290 | 120-900<br>442 | 300-600<br>415 | 50-240<br>155 | 50-750<br>406 |
| Sulfate | 215-870<br>671 | 360-640<br>549 | 675-1200<br>923 | 176-675<br>513 | 525-620<br>576 |
| Suspended Solids (Total) | 370-680<br>505 | 160-924<br>641 | 335-1220<br>677 | 176-260<br>217 | 15-828<br>475 |
| Iron (Total) | 25-125<br>63 | 11.2-70<br>39 | 56-100<br>69 | .2-100<br>55.1 | 20-100<br>61.3 |
| Chloride | 5-13<br>32.2 | 4-80<br>9.8 | 12-27<br>18.3 | 1-20<br>10.5 | 0-20<br>6.5 |
| BOD | 20-72<br>49.8 | 16-60<br>45 | 36-48<br>42.3 | 20-59<br>33.8 | 4-27<br>12.1 |

[1] Courtesy of Richard M. Boardman, Chief, Water Quality Section of Pennsylvania Dept. of Health, Harrisburg, Pennsylvania.

sedimentation, and high hydrogen ion concentration (low pH) are all definite, obvious limiting factors for aquatic organisms; the primary limiting conditions here being low dissolved oxygen and pH.

However, there is a *Tendipes* (=Chironomus) population of 2/.25 $m^2$ present where heavy sedimentation occurs along quiet marginal areas. These apparently anomalous findings agree with those of Harp and Campbell (17). They found *T. plumosus* survival occurred at pH's below 6.0 and measurable free acidity only when sufficient leaf litter was present to lessen the acidiy of the overyling water. But no macroinvertebrates were collected from any stations in Shamokin Creek. All quadrats exhibited lack of adequate sedimentation to provide a less acid substrate for colonization. Also, the large amount of iron carried by Shamokin Creek has fused the bottom rubble into a nearly solid mass, further limiting formation of an adequate habitat for large macroinvertebrates.

Keup, Ingram, and Mackenthun (18), state that as a given pollutant increases, species are eliminated in order of their sensitivity. In the extreme, pollution may eliminate all organisms in the area. Thus, Shamokin Creek may be compared to the septic zones of Bartsch (1); Bartsch and Ingram (2); and Campbell (4). This combination of factors: low dissolved oxygen and pH, high suspended solids, sulfates and BOD's has led to the elimination of all large forms of benthic organisms.

Back-pollution obviously will influence species diversity in tributaries at interfaces with the main stream. It most frequently occurs during periods of high water and, as has been observed, during two of four diurnal oxygen sampling perods, rises for unaccountable reasons; daily in each the level of Shamokin Creek rose six inches or more. Although these exposures are usually brief, the results here nicely reflect the statement by Keup *et al.* (18) that extremes in conditions, not the average, have the greatest effect upon biological communities.

Beginning at Station 2, which represents the interface of Shamokin Creek and Stonington Run, both diversity and biomass are limited (Fig. 2-2). The invertebrate population here is composed largely of *Tendipid* larvae which are pollution tolerant organisms. A small portion of the macroorganisms collected there are considered clean water indicators (5,12). The presence of these organisms at the most pol-

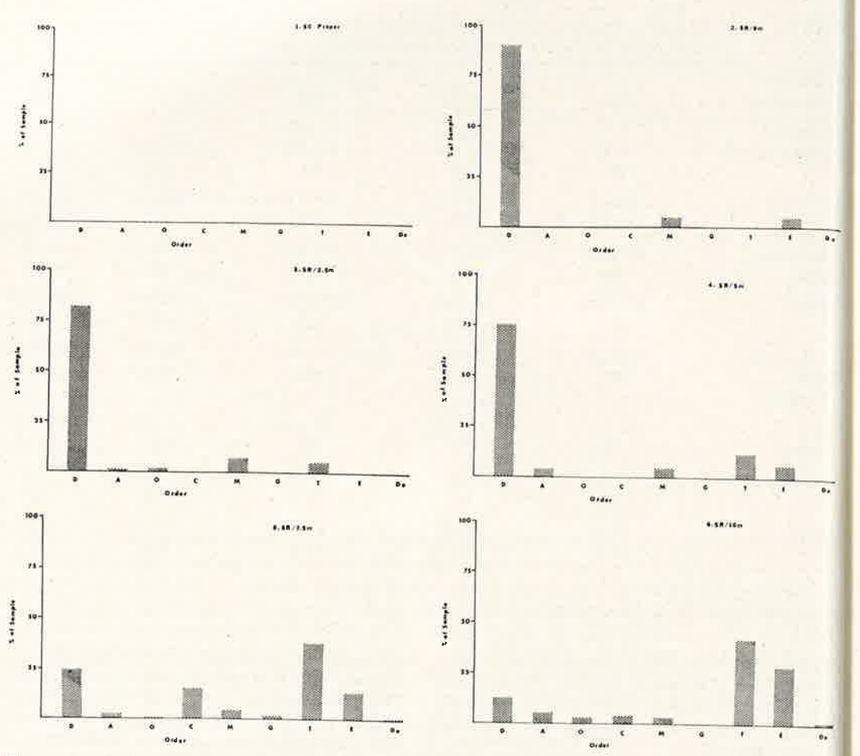


**Figure 2. Orders of macroinvertebrates collected at Shamokin Creek (SC)—Stonington Run (SR) stations. Diptera (D), Annelida (A), Odonata (O), Coleoptera (C), Megaloptera (M), Gastropoda (G), Trichoptera (T), Ephemeroptera (E), Decapoda (D). Meters indicate distance upstream from the main stream.**

luted portion of the tributary can be accounted for through migration during periods in which back-pollution was not occurring. This is because no mayfly or alder fly larvae were collected at Station 3 (Fig. 2-3) and it is doubtful that pollution would eliminate these organisms at a higher level on the stream and spare those closest to the point of pollution.

As the distance increases upstream, new orders of invertebrates appear in the samples (Fig. 2-3, 4, 5, and 6). In addition to new species, this also reflects a shift from dominance of pollution tolerant organisms to one of clean water indicators. For example, Station 2 has 90.4% tolerant organisms and 9.6% clean water indicators. Station 6, on the other hand, has 70% Ephemeroptera and Trichoptera, the level of Dipterans being reduced to 12.6%. The stations between these points indicate a cline in diversity and shift in indicator organisms. This gradient peaks at Stations 5 and 6. Station 5 is believed to be representative of most of the macroinvertebrates living in Stonington Run and can be considered to constitute a nonpolluted environment.

The values obtained for Buddys Run (Fig. 3-8, 9, 10, 11, and 12) are of little value in demonstrating back-pollution. As noted above, large amounts of organic wastes are continually entering the stream from above the study area resulting in a species reduction downstream. The only macroinvertebrate occurring in the study area was *T. tubifex,* a pollutant tolerant organism indicative of septic zones. The level of these organisms reached it greatest concentration, 30,272/m$^2$, at the interface station indicating both that they are resistant to the periodic influx of Shamokin Creek water and that they utilize the organic material which are deposited during these periods. Fig. 4 further indicates that there is little agreement with increasing distance upstream and the drop in biomass of these sludgeworms. Variation in the bottom type at each quadrat can account for a lack in uniformity—*Tubifex* appeared in higher numbers in muddy bottom areas of the stations sampled.

It has been difficult to account for the organisms found in Buddys Run on the basis of the chemical parameters which were measured. A similar problem has been encountered by Patrick (8) in the Connestoga Basin Study. Dissolved oxygen, pH, and temperature values (Table 2) indicate a clean stream while the biological analysis points to a very polluted condition. Since the chemical analysis was made during early October, it is obvious that it does not present a true picture of the stream's physicochemical condition. If analysis were made during a period of low stream flow and high temperatures, better agreement would result. This emphasizes the importance of "continuous" vs. intermittent physicochemical sampling.

As would be expected, the import of high levels of organic nutrients results in Buddys Run supporting a larger biomass per unit area than Stonington Run (Fig. 4). But at the same time, this creates a near anaerobic environment in which only the most resistant organisms can survive. *Tubifex* worms are well suited for such an environment, for as Dausend



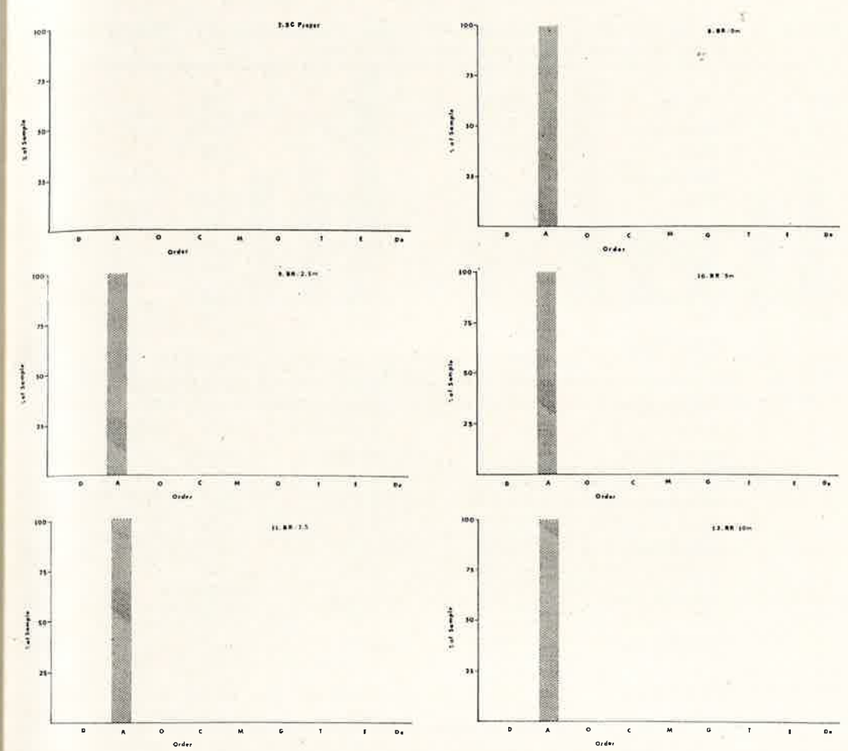


**Figure 3. Orders of macroinvertebrates collected at Shamokin Creek (SC)—Buddys Run (BR) stations. Diptera (D), Annelida (A), Odonata (O), Coleoptera (C), Megaloptera (M), Gastropoda (G), Trichoptera (T), Ephemeroptera (E), Decapoda (D). Meters indicate distance upstream from the main stream.**

TABLE 2. *Diurnal physicochemical means and ranges of Stonington Run, Buddys Run, and Shamokin Creek during summer and fall of 1968.*

| Date | Stream | Station | DO (ppm) | pH | Water temp °C |
|---|---|---|---|---|---|
| 7-29 to 7-30 | Stonington Run | 2 | 4.98-8.15<br>7.57 | 3.3-7.9<br>6.8 | 17-21.2<br>19.1 |
| | Stonington Run | 3 | 7.55-8.45<br>8.03 | 6.6-8.1<br>7.2 | 17-21<br>19.1 |
| | Shamokin Creek | 1 | 4.68-6.95<br>5.16 | 3.2-3.4<br>3.3 | 9.5-25.5<br>19.6 |
| 8-25 to 8-26 | Stonington Run | 2 | 5.0-5.7<br>5.52 | 6.8-7.4<br>7.2 | 20-25.1<br>23.0 |
| | Stonington Run | 3 | 5.0-5.92<br>5.39 | 7.2-7.9<br>7.4 | 21-25.2<br>23.2 |
| | Stonington Run | 4 | 4.54-5.75<br>5.23 | 7.2-7.6<br>7.4 | 21-25.2<br>23.2 |
| | Stonington Run | 5 | 5.22-5.97<br>5.57 | 7.2-7.6<br>7.4 | 21-25.2<br>23.2 |
| | Stonington Run | 6 | 5.0-5.78<br>5.34 | 7.3-7.5<br>7.4 | 21-25.2<br>23.2 |
| | Shamokin Creek | 1 | 2.03-3.27<br>2.54 | 3.2-3.6<br>3.4 | 19.1-27.0<br>22.9 |
| 10-9 to 10-10 | Buddys Run | 8 | 6.02-8.83<br>7.28 | 6.5-6.9<br>6.7 | 11.5-17.0<br>13.8 |
| | Buddys Run | 10 | 6.74-9.67<br>7.76 | 6.8-7.2<br>7.0 | 11.5-17.0<br>13.8 |
| | Shamokin Creek | 7 | 1.02-3.85<br>2.44 | 4.0-3.6<br>3.8 | 8.0-19.4<br>11.6 |

(19) points out, they are capable of surviving in anaerobic conditions for forty-eight days at 0-2°C and at progressively shorter periods of time at higher temperatures. It is improbable that any stream, under natural conditions, ever is devoid of measurable free oxygen for extended periods.

As shown above, macroinvertebrates reliably and differentially reflect net condition while intermittent measurement of physical and chemical parameters may fail to do so. Only "continuous" monitoring of physicochemical conditions can resolve this paradox. Tributary interfaces demonstrably constitute natural microcosms which accurately mirror the large system of which they are a part. Being small they are amenable to experimental manipulation, control, and "continuous" monitoring, in replicate. As such they can provide means to achieve a better understanding of the interactions between physical and chemical parameters and organisms. In short, which parameters are important in "pollution," how do they interact, how long must they do so, and what are the critical levels for each under varying conditions?

## SUMMARY

The results of this study indicate that backpollution does occur and has a definite influence upon the macroinvertebrates of tributary streams. These periodic influxes of polluted water, in one case demonstrated, remove organisms in the order of their sensitivity, thus making them excellent indicators.

It is also evident that to properly correlate biological analysis with measured chemical and physical parameters, will require "continuous" monitoring. In one case, Buddys Run, the physicochemical conditions indicate a clean water stream while the high density of pollution tolerant *Tubifex* worms collected there point to an entirely different situation.

The clinal change in species diversity in a few meters provides a new tool for pollution studies. These areas represent the diversity of



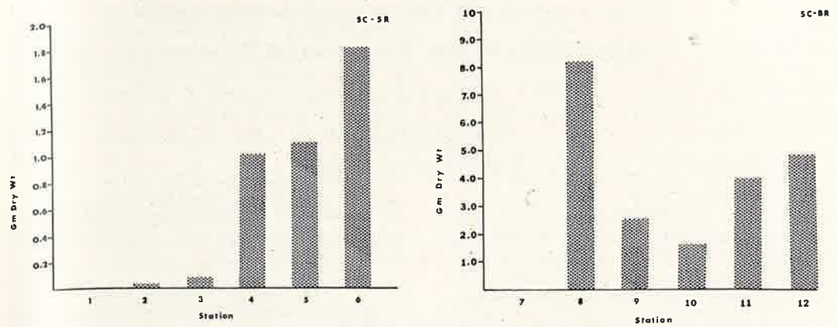



**Figure 4. Dry weight of macroinvertebrates collected from Shamokin Creek (SC)—Stonington Run (SR) and Shamokin Creek (SC)—Buddys Run (BR) stations. Stations 1&7=SC; 2&8 =interface; 3&9=2.5 m upstream; 4&10=5 m upstream; 5&11=7.5 m upstream; and 6&12 =10 m upstream.**

streams which previously would be found only with several miles of sampling. Because these areas are small, they can be manipulated for a better understanding of the processes involved with stream pollution.

## REFERENCES

1. Bartsch, A. F. 1948. Biological aspects of stream pollution. *Sewage Works Jour.* 20:292-302.
2. Bartsch, A. F. and W. M. Ingram. 1959. Stream life and the pollution environment. *Public Works* 90:104-110.
3. Brinley, F. J. 1943. Sewage, algae and fish. *Sewage Works Jour.* 15:78-83.
4. Campbell, M. S. A. 1939. Biological indicators of intensity of stream pollution. *Sewage Works Jour.* 11(1):123-127.
5. Gaufiin, A. R. and C. M. Trazwell. 1956. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. *Sewage and Indust. Wastes* 28(7):906-923.
6. Kolkwitz, R. and M. Marrson. 1908. Oekologie der pflanzlichen Caprobien. *Berichte der Deutschen Botanischen Gesellschaft* 26a:505-519.
7. Kolkwitz, R. and M. Marrson. 1909. Oekologie der tierischen Saprobien. *Internationale Revue der gesamten Hydrobiologie and Hydrographie* 2:126-152.
8. Patrick, Ruth. 1950. Biological measure of stream conditions. *Sewage and Indust. Wastes* 22(7):926-938.
9. Macroorganisms are defined as those retained by a No. 30 sieve (openings of 0.0232 inch and formed of wire 0.0114 to 0.0165 inch in diameter). Keup, Ingram, and Mackenthun (1936).
10. Boardman, R. M. 1969. Personal communication. Department of Health, Commonwealth of Pennsylvania, Harrisburg.
11. Welch, P. S. 1948. *Limnological Methods.* McGraw-Hill Book Company, Inc., New York. p. 353.
12. Surber, E. W. 1953. Biological effects of pollution in Michigan waters. *Sewage and Indust. Wastes* 25(1): 70-86.
13. Anderson, R. O. 1959. A modified flotation technique for sorting bottom fauna samples. *Limnol. and Oceanogr.* 4:223-225.
14 CM², Menlo Park, California 94025.
15. American Public Health Association. 1955. *Standard Methods for the Examination of Water, Sewage, and Industrial Wastes,* 10th ed. New York.
16. Sheaffer, K. K. and F. J. Little, Jr. In preparation.
17. Harp, R. L. and R. S. Campbell. 1967. The distribution of *Tendipes plumosus* (Linne ') in mineral acid water. *Limnol. and Oceanog.* 12(2):260-263.
18. Keup, L. E., W. M. Ingram and K. M. Mackenthun. 1966. The role of bottom-dwelling macrofauna in water pollution investigations. U.S. Dept. of Health, Ed., and Welfare, Pub. Health Service, Div. of Water Supply and Pollution Control. 23 pp.
19. Dausend, K. 1931. Uber die atmung der tubifiziden. *Zeitsche. vergl. Physiol.* 14:557-608.

# STUDIES ON BACTERIOPHAGES FOR THE THERMOPHILE, BACILLUS COAGULANS[1]

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## ABSTRACT

Ten bacteriophages were isolated for thermophilic bacilli belonging to the *Bacillus coagulans* group of Allen (1). All of the phages were isolated from materials taken from environments common to thermophilic bacteria. The phages were placed in three groups on the basis of the character of lysis on a propagating host, plaque morphology and host range studies.

Characterization studies of the phages included single step growth cycles, patterns of heat inactivation at 85°C, rates of adsorption at 65°C, 50°C and 37°C, and the effect of urea and the chelating agent, sodium citrate, on the phages. The minimal latent periods ranged from 30 min to 75 min and the average burst size from 23 to 170. The heat inactivation patterns varied but the phages demonstrated activity after 90 min exposure to 85°C. Adsorption of the phages followed a first order reaction. The phages were not inactivated by urea but were inactivated by the chelating agent, sodium citrate.

## INTRODUCTION

Several reports have been made on phage cell systems in thermophiles. The first mention of a bacteriophage for thermophilic microorganisms was made at the twenty-eighth Annual meeting of the Society of American Bacteriologists (9). In 1928 two French bacteriologists published a report on bacteriophages for thermophilic bacilli (2).

More recent reports have included studies on lytic phage cell systems in thermophiles (5,8,10,14), and also on the presence of temperate phages in the thermophilic microorganism *Bacillus stearothermophilus.* (11,13).

The general purpose of this study was to investigate phage host interaction in the thermophile, *Bacillus coagulans.* The present report deals with the isolation and characterization of bacteriophages active on strains of this species.

## MATERIALS AND METHODS

*Bacterial Strains.* The four strains of *Bacillus coagulans* used in this study were isolated from leaf and compost (manure) samples and were designated strains 3, 5, 8 and 17, respectively.

*Media.* The liquid medium used for growing host cultures was similar to the S broth described by Betz and Anderson (4).

It is referred to as modified S broth and contained polypeptone (B.B.L.) 5.0 g; proteose peptone (Difco), 5.0 g.; NaCl, 5 g.; glucose, 3 g.; yeast extract (Difco), 3 g.; $MgSO_4$, 2.5 g.; $Ca(PO_4)_2$, trace; distilled water, 1 liter. The pH was adjusted to 7.4 with NaOH before autoclaving.

Brain Heart Infusion (B.H.I.) (Difco) agar was used for double layer phage plating (1). Hard and soft agar contained 2.0 and 0.7 percent agar, respectively.

*Incubation.* In all experiments the temperature of incubation was 65 C. Plates prepared for phage assay required six hours incubation at 65 C for plaque observation.

*Phage Assay.* Phages were assayed by the agar layer technique (1). Serial dilutions of the phage containing materials were made through half strength B.H.I. broth blanks and 0.1 and 1.0 ml. portions of the appropriate dilutions were plated with 0.1 ml. of a log phase host culture (1 x $10^8$ cells per ml.) as a lawn in 3 ml. of top agar.

*Phage Isolation.* Five to ten grams of a soil, leaf or compost sample were mixed with a modified S broth culture of one of the *B. coagulans* strains. This mixture was incubated at 65 C for 12 to 15 hours. After incubation an aliquot of the mixture was cleared by centrifugation and Milipore filtration. The filtrate was spotted on seed layers of the culture originally used as the presumptive host.

Following isolation of the phages three successive single plaque isolations were performed to insure purity of the phage stock suspension.

*Host Range.* In order to determine the host ranges of the phages approximately $10^7$ particles of each phage were spotted on seed layers of the four isolated strains of *B. coagulans* and *B. coagulans* ATCC strain 8038 and also on isolated strains of *B. stearothermophilus, B. subtilis,* and *B. sphaericus.* Plates were incubated as described and examined for lysed areas.

*Viral Growth Cycles.* A modification of the method of Ellis and Delbrück (6) was used for the viral growth experiments.

Exponentially growing host cells and phage particles in a multiplicity of about one phage particle per cell were incubated in modified S broth in a shake flask at 65 C.

A five minute adsorption period was followed by centrifugation at 5000 R.P.M.'s for five minutes. The supernatant was decanted and the pellet of cells resuspended in broth. The mixture was incubated at 65 C and thereafter samples were taken at five minute intervals and after proper dilution assayed for plaque forming units.

*Adsorption.* In order to observe the adsorption patterns of the phages to host cells at 65 C, cells in the log phase of growth and phage particles were mixed in S broth at a ratio of 1 phage particle per 100 cells. Samples were withdrawn at minute intervals and diluted in a mixture of nutrient broth and chloroform chilled to 4 C. The mixture was shaken immediately in order to emulsify the chloroform with the broth and disrupt the host cells. At the completion of the adsorption phase of the experiment the supernatant of the chloroformed samples was assayed for free phage particles.

This procedure was repeated with the adsorption mixture incubated at 50 C. and 37 C.

*Thermal Inactivation.* Thermal inactivation patterns at 85 C in modified S broth were determined for eight of the phages by incubating a dilution of each phage containing $10^7$ to $10^8$ plaque forming units per ml. at 85 C and sampling at intervals for assay of survivors by plaque count.

*Effects of Urea and Potassium Citrate.* One hundred fold dilutions of phage were exposed for 15 minutes to 2.5 M urea in modified S broth at room temperature. A loop of the dilution was spotted on a plate seeded with the host strains and the zone of lysis compared with that of a control in which the urea was omitted.

One of the phages was exposed in a similar manner to 0.04 M citrate in modified S broth for 15 minutes at 65 C and the relative number of surviving phages determined.

[1] Part of this work is a portion of a Ph.D. thesis submitted by the author to the graduate school of St. Bonaventure University. A preliminary report of this work was made (7).



## RESULTS

*Phage Isolation.* Ten phages active on the four strains of *B. coagulans* described above, were isolated from various leaf and compost samples. Each phage received its designation from its host strain (phage 3 had as its host, *B. coagulans* strain 3). Phages with the same host strain were designated with the strain number and a subscript. The two phages for *B. coagulans* strain 8 were phages $8_1$ and $8_2$. The phages as a group were relatively stable and could be stored for many months in the refrigerator without appreciable loss of titer. Chloroform had no effect on these phages.

*Plaque Morphology.* Three different types of plaques were observed, Phages 3, $8_1$ and $8_2$ produced similar appearing plaques with sparsely turbid centers and irregular margins. The average diameter was between 0.5 and 0.8 mm (Fig. 1).

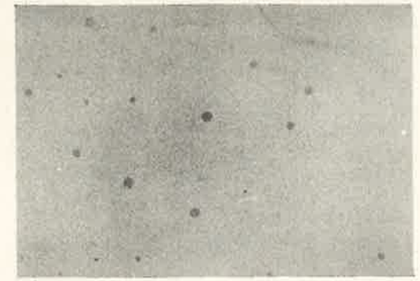


**Figure 1. Plaques of phage 3 on** B. coagulans **strain 3. Magnification 5X.**

A very small and turbid plaque with an average diameter between 0.2 and 0.3 mm was observed for phage 5 (Fig. 2).

Phages 17, $17_1$, $17_2$, $17_3$, $17_4$, $17_5$ produced a third type of plaque with turbid centers and



**Figure 2. Plaques of phage 5 on** B. coagulans **strain 5. (Magnification 5X.)**

TABLE 1. *Host ranges of the ten phages on five strains of Bacillus coagulans*

| Host Strain | Phages | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | $8_1$ | $8_2$ | 5 | 17 | $17_1$ | $17_2$ | $17_3$ | $17_4$ | $17_5$ |
| 3 | + | + | + | − | − | − | − | − | − | + |
| 5 | − | − | − | + | − | − | − | − | − | − |
| 8 | + | + | + | − | − | − | − | − | − | + |
| 17 | + | + | + | − | + | + | + | + | + | + |
| ATCC* | + | + | + | + | + | + | + | + | + | + |

+ (lysis)
− (no lysis)

Spots were made with phage filtrate containing approximately $10^7$ particles/ml.
**Bacillus coagulans* ATCC strain 8038.

well defined margins. The diameter ranged from 0.7 to 0.9 mm (Fig. 3).

*Host Range Studies.* Table 1 shows the results of the host range studies of the phages on five strains of *B. coagulans*, the strains isolated by the author, and *B. coagulans* ATCC strain 8038. Phages 3, $8_1$, $8_2$ and $17_5$ had identical host ranges. Phage 5 demonstrated activity only on *B. coagulans* strain 5 and the ATCC strain. Phages 17, $17_1$, $17_2$, $17_3$ and $17_4$ demonstrated only on *B. coagulans* strain 17 and the ATCC strain. When strains of *B. stearothermophilus, B. subtilis* and *B. sphaericus* were screened for phage susceptibility the results were negative.

*Viral Growth Experiments.* The results of the viral growth experiments are shown in Table 2. There was considerable variation in latent periods and burst sizes among the phages. The shortest latent period was approximately 30 minutes for phage $17_1$ and the longest about 75 minutes for phages $17_2$ and $17_3$. Burst sizes varied from 23.8 for phage 3 to 170.9 plaque forming units per infected cell for phage $8_2$.

*Adsorption.* In all instances, adsorption of the phages followed a first order reaction (Table 3). At 65 C, 93 to 95 percent of the particles adsorbed to the host cells within 15 minutes of incubation. The rate of adsorption was altered when the temperature of incubation was reduced. At 50 C only 81 to 89 percent of the particles adsorbed to the host cells within 15 minutes. At 37 C only 79 to 83 percent of the particles adsorbed to the host cells in the same period of time.

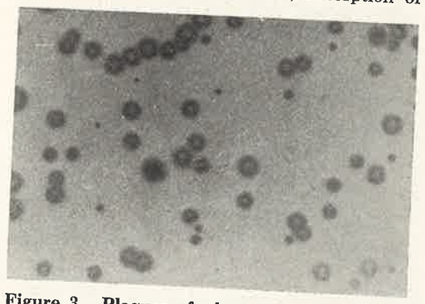


**Figure 3. Plaques of phage 17 on** B. coagulans **strain 17. (Magnification 5X).**

*Thermal Inactivation.* The phages varied somewhat in their patterns of inactivation at 85 C. The thermal inactivation patterns for four of the phages are shown in Fig. 4.

Phage 3 demonstrated the greatest heat resistances among the eight considered with only 88.2% of the phages inactivated after 90 minutes exposure to 85 C. Phage 17 and $17_1$ showed the least resistance with 95.5 and 94.6% inactivated respectively after 90 minutes exposure to the same conditions.

The other phages considered ($8_2$, $17_1$, $17_3$, $17_4$, $17_5$) were approximately 90% (±1) inactivated by exposure to 85 C for 90 minutes.

TABLE 2. *One-step growth characteristics of the Bacillus coagulans phages*

| Phage | Latent Period | Average burst size |
|---|---|---|
| | min. | |
| 3 | 40-45 | 24 |
| 5 | 35-40 | 136 |
| $8_1$ | 35-40 | 143 |
| $8_2$ | 35-40 | 170 |
| 17 | 40-45 | 49 |
| $17_1$ | 30-35 | 53 |
| $17_2$ | 70-75 | 50 |
| $17_3$ | 70-75 | 38 |
| $17_4$ | 40-50 | 32 |
| $17_5$ | 50-55 | 22 |



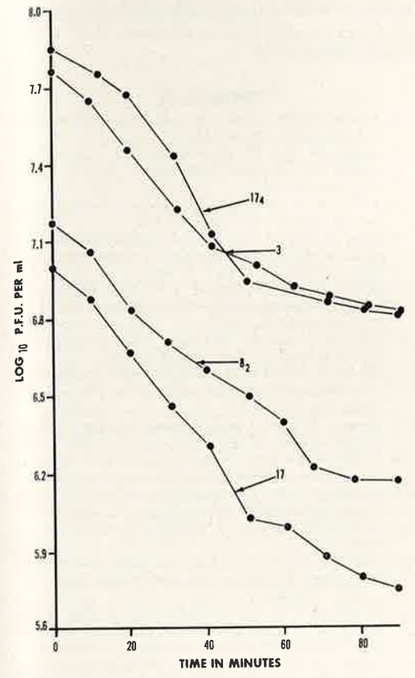



**Figure 4. Patterns of thermal inactivation of four phages active on** B. coagulans **incubated in S broth at 85°C.**

TABLE 3. *The effect of temperature on the rate of adsorption of the Bacillus coagulans phages*

| Phage | Percent adsorption in 15 minutes | | |
|---|---|---|---|
| | 65 C | 50 C | 37 C |
| 3 | 95 | 84 | 81 |
| 5 | 95 | 86 | 81 |
| $8_1$ | 99 | 89 | 79 |
| $8_2$ | 98.5 | 89 | 80 |
| 17 | 99 | 84 | 82 |
| $17_1$ | 98 | 81 | 79 |
| $17_2$ | 99 | 83 | 83 |
| $17_3$ | 96 | 83 | 78 |
| $17_4$ | 97 | 87 | 80 |
| $17_5$ | 93 | 84 | 79 |

## DISCUSSION

Ten thermophilic bacteriophages active on *B. coagulans* were isolated from leaf and compost samples, by a relatively simple procedure.

The isolated phages were tentatively placed in three groups on the basis of the character of lysis on a propagating host (as observed in a phage infected broth culture), plaque morphology and host range studies. This is shown in Table 4.

Phages 3, $8_1$ and $8_2$ were placed in Group I. These phages were similar in many ways. They were isolated from three different sources but appear very closely related.

Phage 5 differed from the other phages and was placed in a separate group. Its character of lysis, host range and plaque morphology

TABLE 4. *Classifications of 10 phages for Bacillus coagulans*

| Criteria for Classification | Groups | | | |
|---|---|---|---|---|
| | I<br>3, $8_1$, $8_2$ | II<br>5 | IIIA<br>17, $17_1$, $17_2$<br>$17_3$, $17_4$ | IIIB<br>$17_5$ |
| Character of lysis on propogating host (broth culture)* | complete clearing | little clearing | moderate clearing | moderate clearing |
| Plaque type | medium size clear | small, cloudy | Large, cloudy center | Large, cloudy center |
| Host range: ability to lyse strains** | | | | |
| 3 | + | − | − | + |
| 5 | − | + | − | − |
| 8 | + | − | − | + |
| 17 | + | − | + | + |

*Turbidmetric assay made with a photoelectric colorimeter.
**+ (lysis)
− (no lysis)

indicated a separate host phage system.

The third group included those phages active on *B. coagulans* strain 17. All of these phages demonstrated a similar pattern of lysis and a similar plaque morphology. The group was divided into two sub groups, A and B on the basis of host ranges.

Group A included the four phages active only on *B. coagulans* strain 17. Phage $17_5$ with its broader host range was placed in group B.

The data from the single step growth experiments correlated with the grouping of the phages except in the case of phage 3. The burst size of this phage, a member of Group I, varied considerably from the burst size of the other two members of the group. Although the growth experiment for phage 3 was repeated several times the data remained consistent.

The ability of these phages to withstand high temperatures for long periods indicated unusually stable proteins. The resistance to inactivation by urea appeared to confirm this fact.

Although the exact nature of the inactivating effect of citrate was not ascertained, it may be postulated from the work of Thompson and Shafia (12) on a thermophilic phage system, that the citrate affects the concentration of calcium or some other divalent cation and that the ion affected was essential for either adsorption of the phages or their synthesis, or both.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Adams, M. H. 1959. *Bacteriophages*. Interscience Publishers, Inc., New York.
2. Adant, M. and R. Bruynoghe. 1928. Les bacteriophages des microbes thermophiles. *Compt. Rendus Soc. Biol.* 99:1244.
3. Allen, M. G. 1953. The thermophilic aerobic sporeforming bacteria. *Bact. Rev.* 17:125.
4. Betz, J. and K. E. Anderson. 1964. Isolation and characterization of bacteriophages active on *Clostridium Sporogenes*. *J. Bact.* 87:408.
5. Egbert, L. and H. Mitchell. 1967. Characterization of TØ3, a bacteriophage for *Bacillus stearothermophilus*. *J. Virology* 1:610.
6. Ellis, E. L. and M. Dellbruck. 1939. The growth of a bacteriophage. *J. Gen. Physiol.* 22:365.
7. Gammon, R. and K. E. Anderson. 1965. Isolation and characterization of bacteriophage for thermophilic Bacilli. *Bact. Proc.* 65:116.
8. Herano, J. 1961. A bacteriophage infecting a thermophilic bacterium. *J. Gen. Appl. Microb.* 7:10.
9. Koser, A. A. 1927. Bacteriophage active against a thermophilic Bacillus. *J. Bact.* 13:14.
10. Onodera, N. 1961. On some characteristics of a newly isolated thermophilic bacteriophage and consideration of its thermostability. *J. Electromicropscopy* (Tokyo) 10:91.
11. Shafia, F. and T. L. Thompson. 1964. Isolation and preliminary characterization of bacteriophage phi u-4. *J. Bact.* 87:999.
12. Thompson, T. L. and F. Shafia. 1962. Energy requirements for adsorption of bacteriophage phi u-4. *Biochem. Biophys. Res. Comm.* 8:467.
13. Welker, N. and L. Campbell. 1964. Induction of a bacteriophage in *Bacillus stearothermophilus*. *Bact. Proc.* 63:136.
14. White, R., C. E. Georgi and W. E. Militzer. 1955. Characteristics of a thermophilic bacteriophage. *Proc. Soc. Exptl. Biol. Med.* 88:373.



# MINERAL COMPOSITION CHANGES IN PORCINE MUSCLE DURING GROWTH

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## ABSTRACT

Forty animals *(Sus domesticus)* representing two breeds (Hampshire and Chester White) were used in this study. Equal numbers of each breed were randomly assigned to each of four chronological age groups (75, 125, 175, and 225 days). These animals were reared on the same diet and in a controlled temperature (15°C) environment. An approximate 25-g sample of *M. longissimus dorsi* was excised immediately after exsanguination. The muscle sample was frozen in liquid nitrogen and subsequently powdered with a Waring Blendor. The elemental analysis was performed using a Jarrell-Ash direct-reading atomic absorption spectrometer. As the data indicated, the major mineral components (calcium, phosphorus, potassium, magnesium) generally decreased and concomitantly two important minor mineral constituents (zinc, iron) increased with advancing animal age. Aimal breed appears to influence the muscle content of two major (phosphorus, sodium) and two minor (zinc, iron) mineral elements.

## INTRODUCTION

The chemical composition of animal tissues is not fixed and constant but changes throughout life (1). Skeletal muscle is generally considered one of the most important animal tissues. Although investigations on the chemical composition of muscle were initiated in the nineteenth century (2), very little is yet known about the chemistry of muscle during growth and development (3). While comprising only about 5 percent of the body weight of animals, mineral elements play a disproportionately large part in the metabolic processes (4) by influencing enzymes and their reactions. This paper reports the results of chronological age and animal breed effects on the underlying compositional changes in the mineral constituents that occur during the growth and development of skeletal muscle.

## MATERIALS AND METHODS

Forty animals *(Sus domesticus)* representing two breeds (Hampshire and Chester White) were used in this study. Equal numbers of each breed were randomly assigned to each of four chronological age groups (75, 125, 175, and 225 days). These animals were reared on the same diet and in a controlled temperature (15°C.) environment.

An approximate 25-g sample of skeletal muscle *(M. longissimus dorsi)* was excised immediately after exsanguination. This muscle sample was immediately frozen in liquid nitrogen and subsequently powdered with a Waring Blendor. A Jarrell-Ash direct-reading atomic absorption spectrometer was used in the elemental analysis.

The F-Test was used to test for significant differences among the means of the various groups. Between mean differences from group to group were tested using Tukey's method as outlined in Snedecor (5).

## RESULTS AND DISCUSSION

In Table 1 the data are summarized for the major or macro mineral elements. These are usually found in higher concentration and are associated with preserving the physical integrity and structure of cells and tissues (6). The data indicate that the amount of each major mineral found in muscle is greatly influenced by animal age. Generally, the content of each mineral element was higher ($P < .05$) in the muscles of younger (75-d) animals than in those older than 125-days of age. Dickerson and Widdowson (7), reporting on a wet tissue basis, found increases in potassium phosphorus and magnesium; and decreases in sodium and calcium with muscle development. No statistically significant ($P > .05$) age effects on two important muscle mineral ratios (8) were observed. The major mineral composition of muscle tissues is not greatly affected by animal breed. However, Hampshire muscle had a higher ($P < .01$) potassium/magnesium ratio than did that of Chester Whites. When the data were summed over all ages, significantly ($P < .05$) greater amounts of phosphorus and sodium were found in Hampshire than in the

TABLE 1. *Major mineral composition changes in M. Longissimus dorsi during growth and development (fat-free, dry weight basis)*

| | Major Minerals (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Age/Breed | Calcium | Phosphorus | Potassium | Sodium | Magnesium | Sodium/ Calcium | Potassium/ Magnesium |
| 75-days | | | | | | | |
| Hampshires | 0.042 | 1.38 | 3.21 | 0.29 | 0.13 | 6.93 | 24.89 |
| Chester Whites | 0.037 | 1.35 | 3.02 | 0.25 | 0.14 | 6.65 | 21.58 |
| | 0.005 | 0.03 | 0.19 | 0.04 | 0.01 | 0.28 | 3.31** |
| 125-days | | | | | | | |
| Hampshires | 0.033 | 1.29 | 2.82 | 0.20 | 0.12 | 6.20 | 22.90 |
| Chester Whites | 0.032 | 1.20 | 2.78 | 0.19 | 0.12 | 6.36 | 22.95 |
| | 0.001 | 0.09 | 0.04 | 0.01 | 0.00 | 0.16 | 0.05 |
| 175-days | | | | | | | |
| Hampshires | 0.035 | 1.33 | 2.90 | 0.23 | 0.13 | 6.51 | 21.64 |
| Chester Whites | 0.035 | 1.30 | 2.94 | 0.21 | 0.13 | 6.03 | 21.82 |
| | 0.000 | 0.03 | 0.04 | 0.02 | 0.00 | 0.48 | 0.18 |
| 225-days | | | | | | | |
| Hampshires | 0.034 | 1.34 | 2.80 | 0.19 | 0.14 | 5.61 | 20.31 |
| Chester Whites | 0.031 | 1.19 | 2.60 | 0.18 | 0.13 | 5.86 | 20.49 |
| | 0.003 | 0.15 | 0.20 | 0.01 | 0.01 | 0.25 | 0.18 |
| All ages | | | | | | | |
| Hampshires | 0.036 | 1.33 | 2.93 | 0.23 | 0.13 | 6.31 | 22.44 |
| Chester Whites | 0.034 | 1.26 | 2.83 | 0.21 | 0.13 | 6.22 | 21.71 |
| | 0.002 | 0.07* | 0.10 | 0.02* | 0.00 | 0.09 | 0.73 |
| All breeds | ** | * | ** | ** | * | | |
| 75-days | 0.040 | 1.36 | 3.12 | 0.27 | 0.13 | 6.79 | 23.24 |
| 125-days | 0.033 | 1.24 | 2.80 | 0.19 | 0.12 | 6.28 | 22.92 |
| 175-days | 0.035 | 1.31 | 2.92 | 0.22 | 0.13 | 6.28 | 21.73 |
| 225-days | 0.033 | 1.26 | 2.70 | 0.19 | 0.13 | 5.73 | 20.40 |
| $LSD_{.05}$ | 0.003 | 0.06 | 0.14 | 0.02 | 0.01 | - | - |

* $P < .05$ ** $P < .01$

Chester White *M. longissimus dorsi.*

A data summary of the minor or micro mineral composition of *M. longissimus dorsi* during growth and development is presented in Table 2. Not much is known concerning the role of these elements; although they are usually associated with oxidation-reduction enzyme systems (8). A significantly ($P < .05$) greater amount of muscle nickel was found in younger (75-d) than in animals older than 125 days. On the other hand, muscle tissue of older (225-d) animals contained more ($P < .01$) iron and zinc than did that from the younger (75-d) animals. Animal breed was shown to greatly influence the muscle content of iron. Hampshire muscle always contained more iron than the Chester White muscle at every age studied. The amount of copper in older animals (225-d) and the amount of zinc for all ages was also greater in Hampshire muscle.

In addition to the major and minor minerals discussed, trace amounts (< 1.00 ppm) of aluminum, manganese, barium and strontium were found in the *M. longissimus dorsi.*

The studies described in this paper quantitatively demonstrate the effects of chronological age and of animal breed on the mineral composition of *M. longissimus dorsi (Sus domesticus)* during growth and development. As the data indicated, the major mineral components (calcium, phosphorus, potassium, magnesium) generally decreased and concomitantly two important minor mineral constitutents (zinc, iron) increased with advancing animal age. Perhaps this suggests changes in cellular integrity and structure and reflects subtle changes in muscle oxidative-reductive enzyme systems. Animal breed appears to influence the muscle content of two major (phosphorus, sodium) and two minor (zinc, iron) mineral elements.



TABLE 2. *Minor mineral composition changes in M. Longissimus dorsi during growth and Deveieopment (fat-free, dry weight basis)*

| | Minor Minerals (ppm) | | | |
|---|---|---|---|---|
| Age/Breed | Zinc | Iron | Nickel | Copper |
| 75-days | | | | |
| Hampshire | 140.00 | 33.76 | 6.11 | 4.52 |
| Chester Whites | 137.80 | 24.66 | 6.15 | 4.06 |
| | 2.20 | 9.10* | 0.04 | 0.46 |
| 125-days | | | | |
| Hampshire | 110.40 | 29.68 | 5.64 | 2.53 |
| Chester Whites | 104.40 | 19.92 | 5.49 | 2.82 |
| | 6.00 | 9.76* | 0.15 | 0.29 |
| 175-days | | | | |
| Hampshire | 151.20 | 32.50 | 5.29 | 3.09 |
| Chester Whites | 137.60 | 23.06 | 5.41 | 1.95 |
| | 13.60 | 9.44** | 0.12 | 0.14 |
| 225-days | | | | |
| Hampshire | 181.20 | 41.18 | 5.98 | 2.58 |
| Chester Whites | 158.00 | 30.44 | 5.12 | 1.97 |
| | 23.20 | 10.74* | 0.86 | 0.61* |
| All ages | | | | |
| Hampshire | 145.70 | 34.28 | 5.75 | 2.93 |
| Chester Whites | 134.45 | 24.52 | 5.54 | 2.70 |
| | 11.25 | 9.76** | 0.21 | 0.23 |
| All breeds | ** | ** | * | |
| 75-days | 138.90 | 29.21 | 6.31 | 4.29 |
| 125-days | 107.40 | 24.80 | 5.56 | 2.68 |
| 175-days | 144.40 | 27.78 | 5.35 | 2.02 |
| 225-days | 169.60 | 35.81 | 5.55 | 2.28 |
| $LSD_{.05}$ | 6.89 | 3.15 | 0.37 | - |

* $P < .05$ ** $P < .01$

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## REFERENCES

1. Spray, C. M. and E. M. Widdowson. 1950. The effect of growth and development on the composition of mammals. *Brit. J. Nutr.* 4:332.
2. Lawes, J. B. and J. H. Gilbert. 1859. Experimental inquiry into the composition of some of the animals fed and slaughtered as human food. *Phil. Trans. Roy. Soc. London* 149:493.
3. Widdowson, E. M. and J. W. T. Dickerson. 1964. Chemical composition of the body. In *Mineral Metabolism* (C. L. Comar and F. Bronner, eds.) Volume 2A. Academic Press, Inc. New York. p. 2.
4. White, A., P. Handler, E. L. Smith and D. Stetten, Jr. 1959. *Principles of Biochemistry*. McGraw-Hill Book Co., Inc. New York. p. 223.
5. Snedecor, G. W. 1956. *Statistical Methods* (5th ed.). Iowa State College Press, Ames. p. 251.
6. Fruton, J. S. and S. Simmons. 1958. *General Biochemistry* (2nd ed.). John Wiley and Sons, Inc., New York. p. 907.
7. Dickerson, J. W. T. and E. M. Widdowson. 1960. Chemical changes in skeletal muscle during development. *Biochem. J.* 74:247.
8. Heilbrunn, L. V. 1952. *An Outline of General Physiology* (3rd ed.). W. B. Saunders Co., Philadelphia. p. 177.

# THE BIG SHELLBARK HICKORY IN CENTRAL PENNSYLVANIA

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## ABSTRACT

*Carya laciniosa*, the big shellbark hickory, occurs along the whole length of the Chillisquaque Creek floodplain, south and east of Milton, in Northumberland and Montour Counties, Pa. The trees are very large; they are difficult to distinguish from accompanying shagbark hickories. The shellbark is absent along nearby tributaries of the Susquehanna such as Penns Creek. This is the northermost location in the state. Features distinguishing this hickory from the shagbark and the mockernut are given.

## INTRODUCTION

The big shellbark, shellbark, big-leaf shagbark or kingnut hickory, *Carya laciniosa*, is a tree similar to the shagbark hickory, *Carya ovata*.

It is a tree primarily of west of the Alleghenies, in the Ohio and Mississippi River drainages, from Ohio, West Virginia and Tennessee to eastern Kansas, though it occurs sporadically in some eastern states such as New York and Alabama.

The tree was early reported in southern Pennsylvania by Wood (1) and others. Many of the distribution records are inaccurate due to incorrectly identified specimens. The shellbark is easily confused with the shagbark hickory and the mockernut *(Carya tomentosa,* formerly named *C. alba);* the bark and nuts are similar to those of the shagbark and the leaves are similar to those of the mockernut.

For positive identification hickories require bark of the trunk (or notes on the bark), twigs with good true terminal buds (lacking on fruiting twigs), preferably in the fall condition, good leaves (especially showing the lower leaflet surfaces) and good mature fruits; in some cases, as in the bitternut hickory, bud-scale scars are extremely helpful. Most of the hickories can be named with difficulty with only one or two of these features present. Strangely pistillate and staminate flowers are of little help in identification. Thus the big shellbark is characterized by shaggy bark, by elongated terminal buds whose outer bud-scales are thick, dark brown, and often sharp-pointed, by heavy, orange or buff, usually puberulent twigs, by large leaves whose rachises and lower surfaces of the seven to nine leaflets are finely fascicled-hairy, and by very large fruits which have thick husks and very large, pale brown, angled, thick-shelled nuts which are pointed at both ends. Shagbark is similar but has mostly five, very rarely seven, leaflets, dark greenish-brown twigs, smooth or very hairy leaves and twigs, medium-sized fruits with thick husks and white, medium-sized, angled, thin-shelled nuts pointed at only one end. One critical feature of shagbark is the presence of one or two dense tufts of hairs associated with the apex of each serration on the leaflets (2); in the big shellbark the leaflet margins are usually rather uniformly ciliate. In mockernut hickory the bark is tight; the outer dark bud-scales have fallen off by autumn on the subglobose grayish-silky terminal buds; the lower surfaces of the seven to nine leaflets, the rachises and the twigs are usually, though not always, coarsely fascicled-hairy; the husk is thinner and the nut is smaller than in the shellbark. Most herbarium specimens are incomplete, some consisting of only a single leaf, with no notes, and the identification is difficult or impossible. The identification of many of the specimens of *Carya* in the Pennsylvania herbaria have been checked and many of the misidentifications corrected, but there are still uncertainties.

Most published maps and most descriptions of the distributions indicate that the kingnut hickory occurs over most of the southern part of Pennsylvania but more commonly in the southeast, a rather surprising distribution. The trees occur almost exclusively in rich bottomlands of streams, hence the distribution may be very local. On some maps (3), the tree is incorrectly indicated as occurring in the whole western part of the state. According to various authors (4-6) this hickory occurs in these counties: Bucks, Berks, Philadelphia, Montgomery, Delaware, Northampton, Lancaster, York, Franklin, Huntingdon, and Allegheny; however, there is no full agreement.

## RESULTS AND DISCUSSION

Within the past fifteen years this hickory has been discovered by the author along the Chillisquaque Creek southeast and east of Milton in Northumberland and Montour counties (also Columbia?). The trees are comparatively numerous along the whole length of the valley, some of the trees being extremely large. This valley is rather narrow, is often flooded in spring, and has deep rich soil. Shagbark also occurs in the valley and it is impossible to distinguish the two species without close observation. The trees often have nuts without embryos, and there is a possibility that the trees are not reproducing well and may eventually die out (if they are not cut down first). It is rather strange that other nearby tributaries of the Susquehanna River, such as Buffalo Creek, Penns Creek, Little Fishing Creek, appear to lack the big shellbark completely.

This distribution in Central Pennsylvania, representing apparently the northernmost area in Pennsylvania, is not indicated in any of the older maps or most distribution records, though Noll (7) in his overlooked Flora of Pennsylvania states noted the treee is common along the Chillisquaque Creek in Northumberland county. This location, based on my collections in herbaria at University of Pennsylvania and Penn State, is shown in Fowells (8), whose maps by Little are probably the most detailed and accurate for trees of the United States. On the latest, but unpublished, maps made by Doctors Wherry, Fogg, and Wahl, located at the University of Pennsylvania, Northumberland and Montour counties are shown as well as Bucks, Berks, Montgomery, Dauphin, Cumberland and Bedford counties.

The nearest record to that of the Chillisquaque Creek is in Dauphin County and is based on a specimen collected October 4, 1868 by Isaac Burk "along Northern Central [now Penn Central] R. R. above Harrisburg." On this specimen the terminal bud is gone and the fruit is small so the determination is uncertain. The trees of this locality probably no longer exist.

It is very possible that future collections along some of the small streams of southern Pennsylvania may turn up other records.

## REFERENCES

1. Wood, A. 1868. *Classbook of Botany*. Barnes & Co., New York.
2. Braun, E. L. 1961. *The Woody Plants of Ohio*. Ohio State University Press, Columbus.
3. Munns, E. N. 1938. *The Distribution of Important Forest Trees of the United States*. U.S.D.A. Miscellaneous Publication 287, Washington, D. C.
4. Porter, T. C. 1903. *Flora of Pennsylvania*. Ginn and Co., Boston.
5. Illick, J. S. 1923. *Pennsylvania Trees*. Pennsylvania Department of Forestry, Harrisburg.
6. Taylor, N. 1915. Flora of Vicnity of New York. *Memoirs New York Bot. Gard.* 5.
7. Noll, H. R. 1852. *The Botanical Classbook and Flora of Pennsylvania*. Lewisburg, Pa.
8. Fowells, H. A. 1965. *Silvics of Forest Trees of the United States*. Agriculture Handbook 271. U.S.D.A. Forest Service, Washington, D. C. Map, p. 132, prepared by E. L. Little, Jr.

# RELATIONSHIP OF HYDROMEDUSAE TO WATER CHARACTERISTICS AND UPWELLING IN YAQUINA BAY, OREGON

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## ABSTRACT

Plankton tows taken at regular intervals from April 1966 to November 1967 at four stations in Yaquina Bay, Oregon, encompassed a region of the estuary from essentially marine conditions to nearly fresh water. Abundance of hydromedusae reached a maximum of over 300/m$^3$ near the oceanic end of the bay. Numbers generally were lower in samples taken in fresher water. The abundance of medusae appeared to be related to the intrusion of salty, cool marine water into the bay. Some of the largest numbers of medusae observed appeared in water whose temperature and salinity characteristics tended to indicate upwelling.

## INTRODUCTION

Hydromedusae are the free-swimming, sexual stage of the Orders Hydroida and Trachylina of the Class Hydrozoa, Phylum Cnidaria. They usually have a fixed polypoid stage which liberates medusae asexually. In the Trachylina the polyp stage may be reduced or absent.

There have been few major ecological works on Hydromedusae. Hydrographic data have been collected with biological samples on many oceanographic expeditions (1-7). Russell (8,9) proposed the use of a number of zooplankters as indicators of hydrographic and biological conditions. Kramp (5) found no evidence of temperature effects on the vegetative phase of the medusae that he found. Werner (10), however, noted that reproduction was affected by temperature conditions with temperature influencing whether a young medusae will develop into a sexually or asexually reproducing adult.

This study deals with the relationship between the distribution of hydromedusae and the hydrographic conditions in the Yaquina Bay estuary, Oregon. The paper is based on part of the author's Ph.D. thesis (11) presented at Oregon State University. In addition to hydrographic relationships the thesis contained results of studies of relationships of hydromedusae to other zooplankton, especially trophic relationships.

## MATERIALS AND METHODS

Yaquina Bay is an estuary opening into the Pacific Ocean near 44°37′ N on the Oregon coast. This study was confined to the part of the bay from the Newport Bridge (station "Bridge") to Buoy 45 ("Buoy-45"), a channel distance of about 8.7 nautical miles upstream (16.5 km) (Fig. 1).

In the lower bay the channel is dredged annually to a depth of about eight meters as far upstream as McLean Point (Fig. 1), above this the channel is maintained at a minimum of three to four meters as far as Toledo (12). Above McLean Point there are shallow tidal flats bordering the channel for about 1.2 nautical miles (2.0 km). The bay is considerably narrower above this area.

Quantitative samples of plankton have been taken with a No. 6 mesh (0.239 mm apparture) nylon net and a Clarke-Bumpus plankton sampler (13) at each of four stations in Yaquina Bay (14) at approximately weekly intervals from April 1966 to November 1967. Two 24-hr studies (at Buoy 21), two six-station spatial surveys (from the Newport Bridge to Buoy 45), and two daily studies were made in September 1966 and March 1967.

Samples were taken by an oblique, step-tow at one meter above the bottom, at mid-depth (except in water less than 3 m), and just under the surface of the water. Plankton samples were immediately preserved in ten percent formalin in sea water. Temperature and salinity data were determined from samples taken in conjunction with all plankton tows. After collection, the samples were analyzed in the laboratory for composition of zooplankton.

These plankton samples constitute part of a long series taken regularly from Yaquina Bay by Frolander and his research group (15).

## RESULTS AND DISCUSSION

Figures 2 and 3 show the abundances of the various species of hydromedusae found in Yaquina Bay on the days indicated, at points corresponding to the average temperature and salinity found at the station when the sample was taken. The data plotted include the six-station spatial surveys of September 13, 1966 and March 23, 1967, and the four-station spatial surveys of December 2, 1966, and June 17, 1967. These are represented by diagrams



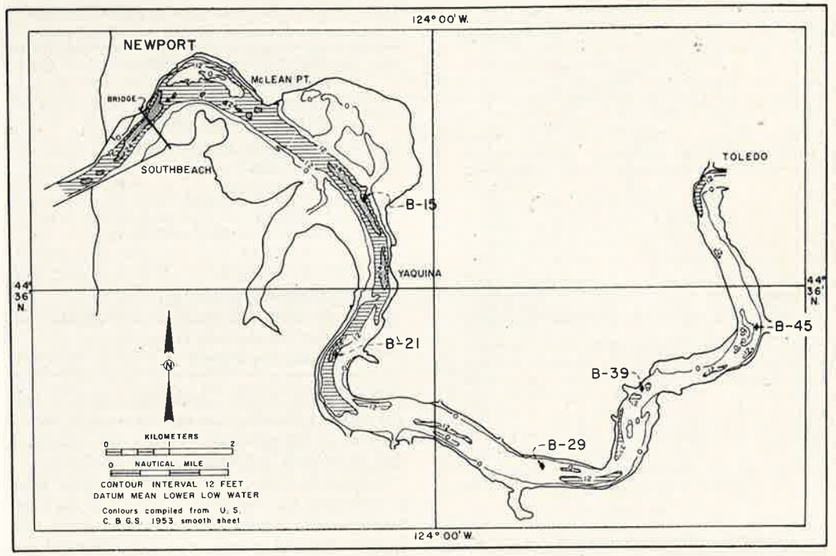



**Figure 1. Map of Yaquina Bay, Oregon, indicating sampling stations (diamonds). (From Frolander, 1964).**

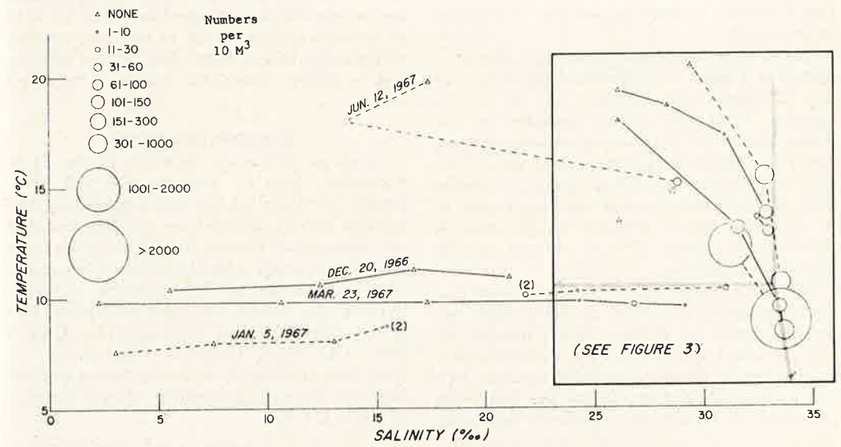



**Figure 2. Relationship of hydromedusae to Yaquina Bay water characteristics (average between surface and bottom temperatures and salinities). Numbers in or near circles refer to medusa species (see Table 1).**

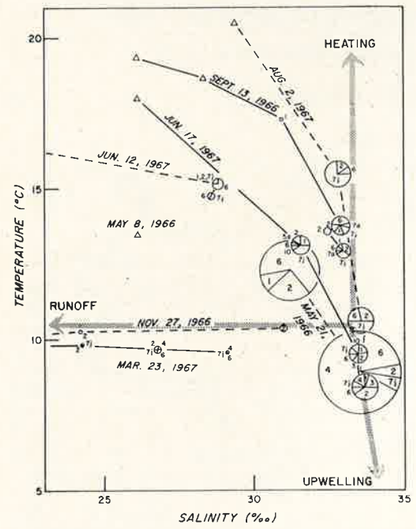



**Figure 3. Detail of part of Figure 2.**

connected by solid lines. These four surveys are equally spaced temporally. In addition, the spatial surveys of May 8 and 21, and November 12, 1966, January 5, June 12 and October 2, 1967, are plotted to provide representation of the extremes of abundance of hydromedusae, and a variety of temperature and salinity values. A process vector diagram (16) is included on the graph.

The temperature-salinity-medusa points presented in Figure 2 are quite representative of the distribution of medusae in all of the samples. From the weekly samples it was found that 83% of the hydromedusae were found in average temperatures less than 15°C, 88% were found at average salinities greater than 20°/oo. *Sarsia eximia* was found once at an average (top to bottom) temperature of 20.34°C and salinity of 26.25°/oo and once at 8.54°C and 9.66°/oo.

The central point of the process vector diagram represents the type of water most frequently found at Oregon shore stations by Pattullo and Denner (16). The upwelling vector represents deeper, generally nutrient rich (17), water originating outside the bay. Also presented are vectors for fresh water and heating.

These data support the concept that the hydromedusae are associated with the more oceanic water types, represented by more saline, colder water. Upstream water is usually characterized by decreasing salinity and/or increasing temperature (during the summer), and decreasing numbers of hydromedusae.

TABLE 1. *List of species graphed in Figures 2 and 3 on the following pages.*

1. *Dipurena ophiogaster*
2. *Sarsia eximia*
3. *Sarsia prolifera*
4. *Rathkea octopunctata*
5. *Polyorchis pencillatus*
   a. adult
   j. juvenile
6. *Obelia* sp.
7. *Phialiduim gregarium*
   a. adult
   j. juvenile
8. *Aequorea* aequorea
9. Family: Moerisiidae (moerisiid)
10. *Proboscidactyla* sp.
11. *Aglantha digitale*

Upwelled water from off the Oregon coast is indicated by the large numbers of medusae found in the coldest, most saline water. The species found include the abundant *Sarsia eximia* and *Phialidium gregarium. Rathkea octopunctata* may be an indicator of water thus upwelled and consequently moved into the bay.

Since upwelled water is generally rich in nutrients needed for production of organic matter, any organism that may indicate such rich water would have value in production forecasting. Some hydromedusae may be used as indicators. More work is needed, however, to properly assess their distribution offshore and in deeper water, the source of upwelled water.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Bigelow, H. B. 1917. Explorations of the coast water between Cape Cod and Halifax in 1914 and 1915, by the U. S. Fisheries schooner "Grampus", Oceanography and plankton. *Bull. Mus Comp. Zool.* Harvard Univ. 61:163.
2. Bigelow, H. B. 1919. Hydromedusae, siphonophores and ctenophores of the "Albatross" Philippine expedition. *Bull. No. 100,* 1(5):279.
3. Kramp, P. L. 1919. *Medusae.* Part I. Copenhagen, Luno. 111 p. (Danish Ingolf-Expedition, 1895-1896. Vol. 5, no. 8)
4. Kramp, P. L. 1926. *Medusae.* Part II. Luno, Copenhagen. 102 p. (Danish Ingolf-Expedition, 1895-1896. Vol. 5, no. 10)
5. Kramp, P. L. 1942. *Medusae.* Reitzel, Copenhagen. 168 p. (Kommissionen for Videnskabelige Uneersogelser I Gronland. The Godthaab Expedition, 1928. Medd. Gronland 81(1):1-168.
6. Kramp, P. L. 1959. *The Hydromedusae of the Atlantic Ocean and adjacent waters.* Copenhagen, Carlsberg Foundation. 283 p. (The Carlsberg Foundation's Oceanographical Expedition Round the World, 1928-1930. Dana-Report No. 46)
7. Haeckel, E. 1879. *Das System der Medusen.* Gustav Fischer, Jena. 3 vols. + atlas.
8. Russell, F. S. 1935. On the value of certain plankton animals as indicator of water movements in the English Channel and North Sea. *J. Bio. Ass.* 20:309.
9. Russell, F. S. 1939. Hydrographical and biological conditions in the North Sea as indicated by plankton organisms. *J. J. Cons. Int. Explor. Mer.* Sheets 2, 28, 29, 51, 54 and 99-102.



10. Werner, Bernhard. 1963. Effect of some environmental factors on differentiation and determination in marine Hydrozoa, with a note on their evolutionary significance. *Ann. Acad. Sci.* 105(8):461.
11. McCormick, J. M. 1969. Hydrographic and trophic relationships of hydromedusae in Yaquina Bay, Oregon. Ph.D. thesis. Oregon State University, Corvallis.
12. Manske, D. C. 1968. Distribution of recent Foraminifera in relation to estuarine hydrography, Yaquina Bay, Oregon. Ph.D. thesis Oregon State University, Corvallis.
13. Clarke, G. L. and D. F. Bumpus. 1950. *The plankton sampler—an instrument for quantitative plankton investigations.* Baltimore, Maryland 8 p. (American Society of Limnology and Oceanography. Special Publication No. 5)
14. Frolander, H. F. 1964. Biological and chemical features of tidal estuaries. *J. Water Pollut. Contr. Fed.* 36:1037.
15. Frolander, H. F. 1968. Unpublished research data on physical, chemical and biological characteristics of Yaquina Bay, Oregon. Oregon State University, Corvallis. Department of Oceanography.
16. Pattullo, J. G. and Warren Denner. 1965. Processes affecting sea water characteristics along the Oregon coast. *Limnol. Oceanogr.* 10:443.
17. Raymont, J. E. G. 1963. *Plankton and Productivity of the Oceans.* MacMillan. 660 p.

# SURVEY OF THE VASCULAR AQUATIC PLANTS OF THE LOWER NONTIDAL DELAWARE RIVER

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## ABSTRACT

Areas of vascular aquatic plant growth in the lower nontidal Delaware River were mapped and the density of the growth classified as light, medium, or heavy. Quadrat samples from each type of growth density were used to calculate the weight of the standing crop of plants in the study area. Samples of plant material were dried to determine the water content. Dried samples were ashed to estimate the organic content of the dried material. Flow studies indicated that the $4.6 \times 10^6$ kg (wet weight) of plant material in the river could be carried, under low flow conditions, through the entire study area (45 miles) to Zone II of the Delaware Estuary in 31 hr. The decay of this plant material may contribute to the existing heavy Biochemical Oxygen Demand of the bottom sediments in the estuary.

## INTRODUCTION

*Purpose.* The purpose of this study was to determine the extent, amount, and types of aquatic vascular plants in the Delaware River from Raubsville, Pennsylvania (Delaware River Mile [D.R.M.](1) 178.5) to Trenton, New Jersey (D.R.M. 133.4) as recommended in the *Lehigh River Biological Investigation* (2). Past studies (3) indicate the presence of a dissolved oxygen sag in Zone II (D.R.M. 108.4 to 133.3) of the Delaware Estuary. It is hoped that this study will produce quantitative data on the aquatic plants in the Delaware River which could be used in further evaluation of the Zone II oxygen sag.

*Background.* Aquatic vascular plants, although they may occur under natural conditions, are believed to be increasing in response to nutrients due to man-made activities. The increased amount of plant material in many rivers causes changes in the ecology, becomes a nuisance to recreational activities, and in general, impairs water quality. This study is a result of the *Lehigh River Biological Investigation* (2) which notes large amounts of aquatic plants in the lower nontidal portion of the Delaware River and recommended that this area of heavy plant growth be further studied.

## METHODS

*Description of the Study Area.* The study area was limited to the Delaware River between Raubsville, Pennsylvania (D.R.M. 178.5) and Trenton, New Jersey (D.R.M. 133.4). This section constitutes the lower part of the Delaware River Basin above tidewater. The average width of the river in this area is about 700 ft. Most plants were found in water 1 to 6 ft in depth, therefore study was usually concentrated in such areas.

*Survey.* The first step in the study involved locating and mapping the areas of aquatic vascular plant growth in the study area. This involved a four-day float trip (October 17, 18, 22, and 23) in a canoe which was equipped with a three horsepower motor. The areas of plant growth were recorded on topographic maps as heavy, medium, and light. Areas covered by each type of growth were calculated by a grid transparency method.

*Quantitative Work.* Quantitative estimates by weight of plants in each type of area were determined by sampling quadrats of each area. In each of the different type areas it was planned to sample a 10 by 10 meter square (100 $m^2$). However, in the heavy and medium areas it was necessary to reduce the sampling plot to a 5 meter square (25 $m^2$) and in the light area a 5 by 10 rectangle (50 $m^2$) was sampled. Plants were removed by hand with care to retain as much of the root as possible. The plants were rinsed in the river to remove silt and then placed in plastic bags in which holes had been punched to allow the adherent water to drain. Samples were allowed to drain for approximately 10-15 min and then weighed on a spring scale to determine wet weight. Representative samples of the wet weights of each area were then air dried and this weight was recorded as the air dried weight. A small sample of air dried material was ground with a mortar and pestle and then dried at 105°C for 24 hr. The weight after oven drying was recorded as oven dried weight and represents the weight of the sample with almost no water. Oven dried samples were ashed in a muffle furnace at 550°C (4) and the resultant weight loss recorded.



*Qualitative Work. A Manual of Aquatic Plants* by Fasset (5) was used to identify the plant specimens collected in this study.

*Flow Study.* During the float trip, an attempt was made to obtain estimates of the flow velocity of the river. Oranges were deposited at the starting point of the float trip. They floated downstream as we made preparations for the trip. Later in the day, the oranges were found in the river downstream from the point they were deposited. Their final position and the elapsed time was recorded and later examined to estimate the flow in that particular stretch of the river.

## RESULTS

TABLE 1. *Samples from each area yielded the following data:*

| | Wet weight/ 25 $m^2$ | Kg/ha | Wet weight/ $m^2$ | Oven dried weight/$m^2$ |
|---|---|---|---|---|
| Heavy area | 78.8 Kg | 31,520 | 3.15 Kg | 482 g |
| Medium area | 10.8 " | 4,320 | .43 " | 66 " |
| Light area | 3.2 " | 1,280 | .13 " | 20 " |

*Quantitative (Biomass).* When wet plants were air dried, they lost 83.3% of their weight. Oven drying produced an additional weight loss of 1.4%. The total weight loss from wet weight to oven dried weight was 84.7%. When oven dried plants were ashed, they lost an average of 69.1% of their weight.

*Qualitative (Species).* The most abundant species were *Myriophyllum spicatum, Potamogeton pectinatus, Heteranthera dubia,* and *Elodea canadensis. Potamogeton crispus* and *Vallisneria americana* were less abundant but constituted an important amount of the species noted in this study. The other species listed in Table 3 were present but not very abundant.

TABLE 2. *Estimated wet weight of plants in the study area:*

| | Total area covered ($m^2$) | Wet weight Kg/$m^2$ | Total weight of plants (Kg) |
|---|---|---|---|
| Heavy | 1,323,000 | 3.15 | 4,168,000 |
| Medium | 942,000 | .43 | 405,000 |
| Light | 806,000 | .13 | 105,000 |
| Total | | | 4,678,000 |

*Delaware River Flow.* The results of the flow data (Table 4), using oranges, indicated that flow time between Raubsville (D.R.M. 178.5) and Trenton (D.R.M. 134.3) to be approximately thirty hours under low flow conditions.

## DISCUSSION

*Plant Distribution.* The distribution of plants often followed predictable patterns. Most plants occurred at a depth of 1 to 6 ft and in relatively slow flowing water. Plant beds often followed the inside bends of the river or occurred behind islands. Areas protected from the main current were often the site of extensive plant growth.

At times, areas below industrial and munici-

TABLE 3. *A check list of the different species of submerged vascular aquatic plants in the study area. (6)*

| Sampling Station Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Delaware River Mile | 134 | 140 | 143 | 148 | 154 | 158 | 166 | 170 | 174 | 179 |
| *Zosteraceae* (pondweed) | | | | | | | | | | |
| *Potamogeton pectinatus* (L.) | X | X | X | X | X | X | X | X | X | X |
| *Potamogeton crispus* (L.) | X | X | X | X | X | X | X | X | X | X |
| *Potamogeton foliosus* (Raf.) | | X | X | | | | | X | | |
| *Potamogeton nodosus* (Poir.) | | | X | | X | X | X | X | | X |
| *Potamogeton hybrid* | | | X | | X | X | X | | X | X |
| *Potamogeton illinoensis* (Morong.) | | X | | | X | | | | | |
| *Hydrocharitaceae* (waterweed) | | | | | | | | | | |
| *Elodea canadensis* (Michx.) | X | | | | X | X | | | X | X |
| *Elodea nuttallii* (Planch.) | | X | X | | | X | | | | |
| *Gramineae* (eel grass) | | | | | | | | | | |
| *Vallisneria americana* (Michx.) | X | X | X | | X | X | X | X | X | |
| *Pontederiaceae* (mud plantain) | | | | | | | | | | |
| *Heteranthera dubia* (Jacq.) | X | X | X | X | X | X | X | X | X | X |
| *Haloragaceae* (water-milfoil) | | | | | | | | | | |
| *Myriophyllum spicatum* (Fern.) | X | X | | X | X | X | X | X | X | X |

TABLE 4. *Flow information—using oranges—October 17, 18, 22, 23, 1968*

| Date | 10/17/68 | 10/18/68 | 10/22/68 | 10/23/68 |
|---|---|---|---|---|
| Location Deposited | Raubsville opposite hotel | Frenchtown bridge | Lambertville New Hope Bridge | Scudders Falls Bridge |
| D.R.M. | 178.5 | 164.3 | 148.7 | 139.0 |
| Flow at Trenton (c.f.s.) | 3,490 | 3,420 | 4,490 | 3,420 |
| Oranges in/out | 24/13 | 24/23 | 12/9 | 9/4 |
| Time in | 10:30 AM | 9:30 AM | 9:37 AM | 12:40 PM |
| out | 1:00 PM | 10:15 AM | 1:50 PM | 2:30 PM |
| Location where oranges were recovered (D.R.M.) | 175.79 | 163.17 | 142.48 | 136.64 |
| Time traveled (hours) | 2.5 | .75 | 4.22 | 1.83 |
| Distance (miles) | 2.71 | 1.13 | 6.22 | 2.36 |
| Flow (mph) | 1.0 | 1.6 | 1.8 | 1.4 |

pal waste effluents indicated considerable plant growth. In this study, however, no effort was made to determine accurately the effects of these effluents on plant growth and distribution.

*Quantitative Data.* Although other studies have used more refined techniques (7), much of their data is in general agreement with the data collected in this study. Other investigations of dry weight (4) and standing crop as $g/m^2$ (7) found results similar to the results found in this study with only a slight variance. This variance is expected because productivity varies with the amount of light, water, and nutrients available (8). Furthermore, water content varies with the plant species (our samples contained a mixture), seasonal stages of development, and the environment (9).

The calculated standing crop of plants may have been a low estimate due to two factors. First, the sampling was done in October and the maximum density of these plants usually occurs in August or early September. During the survey we noted that many plants had already detached and were floating downstream. The second factor involves flow conditions. The higher than normal flows and increased turbidity during late spring and early summer may have inhibited the growth of rooted aquatics in 1968. Residents along the river claim that plant growth was less in 1968 than in previous years, especially in comparison to 1965 and 1966.

*Flow Study.* A U.S. Public Health Service study (10) calculated the flow of the Delaware River in our study area to be 0.97 mph (2,450 cfs, Trenton). Flow estimates during this study were calculated to be slightly less than 1.5 mph (3,705 cfs, Trenton). The difference was due primarily to variability of flow at Trenton. This information is important in determining the approximate rate of amounts of aquatic plants entering Zone II of the Delaware Estuary.

## ACKNOWLEDGMENTS

This study was coordinated with programs of the Delaware River Basin Commission and we would like to thank them for the use of their equipment and facilities. We would also like to express special appreciation to Dr. Francis J. Trembley and Dr. Basil W. Parker, both professors at Lehigh University, for their service and assistance.

## REFERENCES

1. D.R.M.—Delaware River Mile. A mileage system in which mile zero is located at the intersection of the centerline of the navigation channel and a line between the Cape May Light and the tip of Cape Henlopen. Distances from mile zero are measured along the central navigation channel to Trenton, New Jersey and from there north along the state boundaries to the junction of the East and West Branches of the Delaware River. Delaware River Basin Commission. 1964. *Channel and River Mileage Delaware Bay and River*. Staff Paper 105. p. 19.
2. Craighead, W. M. and D. P. Pollison. 1968. *Lehigh River Biological Investigation*. A joint release of the Delaware River Basin Commission, Pennsylvania Department of Health, and Pennsylvania Fish Commission. p. 59.
3. Department of the Interior and Federal Water Pollution Control Administration. 1966. *Delaware River Comprehensive Study*, (Preliminary Report and Findings). Philadelphia, Pennsylvania. p. 36.
4. Westlake, D. F. 1965. Some basic data for investigations of the productivity of aquatic macrophytes. In *Primary Productivity in Aquatic Environments* (C. R. Goldman, ed.). Mem. 1st. Ital. Idrobiol., 18 Suppl. University of California Press, Berkeley. p. 235.
5. Fasset, N. C. 1957. *A Manual of Aquatic Plants*. University of Wisconsin Press, Madison.
6. Craighead, W. M. and D. P. Pollison. 1968. *Lehigh River Biological Investigation*. A joint release of the Delaware River Basin Commission, Pennsylvania Department of Health, and Pennsylvania Fish Commission. p. 40.
7. Edwards, R. W. and M. Owens. 1960. The effects of plants on river conditions I. Summer crops and estimates of net productivity of macrophytes in a chalk stream. *J. Ecol.* 48:151.
8. Penfound, W. T. 1956. Primary production of vascular aquatic plants. *Limnol. Oceanog.* 1:92.
9. Wetzel, R. G. 1965. Techniques and problems of primary productivity measurements in higher aquatic plants and periphyton. In *Primary Productivity in Aquatic Environments* (C. R. Goldman, ed.). Mem. 1st. Ital. Idrobiol., 18 Suppl. University of California Press, Berkeley. p. 252.
10. *Delaware River Basin, New York, New Jersey, Pennsylvania, and Delaware*. 1962. (Vol. III). Appendix C. U.S. Public Health Service, House Document No. 522. 87th Congress, 2nd Session. p. 122.

# TEMPERATURE PROFILES AND HEAT FLOW IN SEDIMENTS OF NUANGOLA

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## ABSTRACT

Thermal profiles, taken at 15-m intervals from original shore of glacial lake Nuangola across bog mat and at two stations on lake, 30 m and 60 m from edge of mat, reveal nearly isothermal condition of 9.5°-10.0° at 7 m below bog surface, 10.6°-11.8°C at 7 m below edge of mat, and 11.8°-12.2° C at 5 m in bottom sediments under 2 m of water 30 m from mat. Temperatures show more annual difference in shallower bog sediments but less thermal lag than deeper bog sediments. Heat apparently moves into bog sediments from lake water and lake sediments as well as from bog surface.

## INTRODUCTION

Nuangola Lake in Nuangola Borough of Luzerne County, Pennsylvania, lies in a glacially-gouged basin just south of Penobscot Mountain. Approximately one-third of the lake is now bordered by typical decodon-chamaedaphne-cranberry-sphagnum mat. Lake Nuangola was initially 12 m to 16 m deep but now is 6 m at its deepest spot. The sediments are highly organic, to a thickness of 13 m at the edge of the mat where this study was made, and rest on a layer (1 m thick) of bluish clay. Approximately one third of the initial lake's area is now occupied by bog mat, which in the successionally more advanced areas has black spruce and tamarack stands.

Temperatures were taken thirteen times, at approximately monthly intervals from 1 April 68 through 31 March 69 at nine stations, including seven on the bog and two on the lake. At each station on the bog, temperatures were taken vertically at 1-m intervals either down to the original lake bottom or to 7 m where original bottom was deeper. At two lake stations, where water was 1.5 m to 2 m deep, the maximum depth to which the thermistor could be forced into the sediments was 5 m.

## METHODS

A thermistor (YSI #T2630) mounted in a metal probe was forced into sediments at each station by means of a sectionalized rod, on the dates shown in Table Two. A cable connected the thermistor with the read-out box. The thermistor was permitted to adjust for several minutes in each position.

Station I was 50 m from the shore of the initial lake's basin and the others (II through VII) in a straight line, were at 15-m intervals out to the edge of the mat. Stations VIII and IX were on the lake, 30 and 60 m from the edge of the mat respectively. The data for the stations here presented show, (a) two thermal profiles of the bog and portion of the lake, one profile at the thermal maximum of the top layer of the bog in August and the other at the thermal minimum of the surface layer in February, and (b), the annual record of temperatures of the seven levels at station VI located 15 m from the edge of the mat.

Temperatures at the various positions for the dates 12 August 68 and 5 February 69 are shown in Table 1, which is set to represent a profile of the bog and adjacent lake portion, showing the several levels for stations I through IX, with reference to the original bottom and the present lake bottom.

Table 2 contains the temperatures at the seven levels of station VI from 1 April 68 through 31 March 69 as well as the mean monthly surface air temperatures for the same period, and the means for the same months for the years 1931-1968.

## RESULTS AND DISCUSSION

The warming and cooling of the sediments in Nuangola Bog during the period of this study show a typical annual trend with a seasonal lag. The amount of variation is less in the deeper sediments so that at the level of 7 m the total variation during the year is less than 1° C. Thus the condition at 7 m is almost isothermal and so comparable to the conditions in Stewart's Dark Lake as reported by Johnson and Likens (1). The mean annual temperature of the sediment at 7 m for station VI was 9.7° and the mean annual surface air temperature for the same period was 9.7°.

Absorption of heat through insolation is more readily accomplished by the lake water than by the bog sediments with their low vegetational cover. The sublimnetic sediments



TABLE 1. *Two profiles of Nuangola Bog and adjacent portion of Lake Nuangola.*

| | Temperatures (Centigrade) in sediments on August 12, 1968 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Stations | I | II | III | IV | V | VI | VII | VIII | IX | |
| Depths | | | | | | Bog | | Lake | | |
| 1 m | 13.9 | 13.3 | 13.3 | 14.4 | 16.7 | 18.3 | 20.0 | 27.0 | 26.7 | Lake |
| 2 m | | 12.2 | 11.1 | 11.7 | 12.8 | 14.4 | 15.6 | 25.1 | 25.6 | |
| 3 m | | 11.1 | 9.5 | 10.0 | 10.6 | 12.2 | 12.8 | 15.3 | 15.6 | Sediments |
| 4 m | | | 8.9 | 9.1 | 10.0 | 11.1 | 11.7 | 12.1 | 12.2 | |
| 5 m | | | 8.3 | 8.9 | 9.4 | 10.0 | 11.1 | 12.2 | 12.2 | |
| 6 m | | | | 8.9 | 9.4 | 10.0 | 11.1 | 12.2 | 12.2 | |
| 7 m | | | | | 9.4 | 10.0 | 10.6 | 12.2 | 12.2 | |
| | Temperatures (Centigrade) in sediments on February 5, 1969 | | | | | | | | | |
| Stations | I | II | III | IV | V | VI | VII | VIII | IX | |
| Depths | | | | | | Bog | | Lake | | |
| 1 m | 2.8 | 2.8 | 3.9 | 1.7 | 3.4 | 4.5 | 6.7 | 2.7 | 3.0 | Lake |
| 2 m | | 5.0 | 6.1 | 4.5 | 5.6 | 6.7 | 8.4 | 3.0 | 4.4 | |
| 3 m | | 7.2 | 7.2 | 6.7 | 7.2 | 8.4 | 10.0 | 10.6 | 9.8 | Sediments |
| 4 m | | | 8.3 | 7.8 | 8.9 | 8.9 | 10.6 | 11.7 | 10.9 | |
| 5 m | | | 8.9 | 8.5 | 8.9 | 9.5 | 10.6 | 12.0 | 11.9 | |
| 6 m | | | | 8.9 | 8.9 | 9.5 | 10.6 | 11.8 | 11.8 | |
| 7 m | | | | | 9.3 | 9.5 | 10.6 | 11.8 | 11.8 | |

TABLE 2. *Temperatures (°C) in sediments of Nuangola Bog at station VI.*

| | Dates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Depths | 4/1 | 5/9 | 6/11 | 7/16 | 8/12 | 9/13 | 10/11 | 11/11 | 12/9 | 1/8 | 2/5 | 3/5 | 3/31 |
| 1 m | 4.5 | 8.5 | 13.0 | 18.0 | 18.3 | 17.0 | 14.5 | 11.0 | 8.0 | 5.5 | 4.5 | 5.5 | 5.7 |
| 2 m | 5.5 | 8.0 | 10.0 | 15.5 | 14.7 | 14.5 | 13.5 | 11.5 | 9.5 | 7.5 | 6.7 | 5.5 | 6.2 |
| 3 m | 7.5 | 8.0 | 9.0 | 12.5 | 12.2 | 12.0 | 11.5 | 11.0 | 10.0 | 9.5 | 8.4 | 6.5 | 7.4 |
| 4 m | 8.3 | 8.3 | 9.0 | 11.0 | 11.1 | 10.5 | 10.5 | 10.5 | 10.0 | 10.0 | 8.9 | 8.5 | 8.6 |
| 5 m | 8.5 | 8.5 | 9.5 | 10.5 | 10.0 | 10.0 | 10.0 | 10.0 | 10.5 | 10.0 | 9.5 | 9.0 | 9.1 |
| 6 m | 9.5 | 9.5 | 9.5 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 9.5 | 9.5 | 9.5 | 9.5 | 9.5 |
| 7 m | 9.5 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 9.5 | 9.5 | 9.5 | 9.5 | 9.5 |
| 1968-1969* | 11.5 | 13.5 | 19.5 | 23.0 | 21.5 | 18.0 | 12.0 | 5.0 | -2.5 | -3.3 | -2.8 | | |
| 1931-1969* | 8.9 | 15.0 | 19.4 | 22.2 | 21.0 | 17.5 | 11.3 | 5.0 | -2.5 | -3.2 | -3.4 | | |

*Monthly mean air temperature

at various depths in the lake bottom were warmer than the bog sediments of comparable depths on comparable dates. The mean annual temperature at the depth of 7 m beneath the surface of the lake, i.e., 5 m into the bottom, at station IX was 2.3° C warmer than the sediment at the same level, 7 m below the bog surface, at station VI. Thus the heat apparently flows from the limnetic sediments into the bog sediments.

## REFERENCE

1. Johnson, N. M. and G. E. Likens. 1967. Steady-state thermal gradient in the sediments of a meromictic lake. *Jour. Geophys. Res.*, 72:3049.



# EARLY SUCCESSION ON ABANDONED CORNFIELDS IN SOUTHERN CHESTER COUNTY, PENNSYLVANIA

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## ABSTRACT

In developing field sampling techniques to aid students studying ecology at West Chester State College in understanding succession, some interesting quantitative data was obtained from abandoned corn fields. This data together with personal observations and records from additional sites in Southern Chester County suggest several trends and raise certain questions that may be useful to field biologists for further research.

## INTRODUCTION

It is possible to combine teaching in field laboratory courses with the accumulation of useful data. This philosophy has been used in designing and implementing field activities in the basic ecology course for biology students at West Chester State College.

As a result students have helped to make a contribution to the understanding of certain basic aspects of ecology in Chester County. In addition to data presented in this paper, the students also have developed quantitative materials on the forest vegetation of Chester County, and on ecological studies in the urban environment.

## METHODS

The quantitative data was obtained primarily by undergraduate classes in ecology at West Chester State College from 1964 to 1968. All species identifications and plot vegetation estimates were carefully checked. The total number of $1m^2$ plots in any particular year reported was limited by the size of the class. All plots were located at random in the field to be studied, either through the use of a grid or coordinates established by random numbers. The fields studied were all in the vicnity of West Chester State College in Southern Chester County, Pennsylvania.

Though it is realized that the data is very limited due to the small size of the samples, it is felt that certain trends were evident and useful in interpreting early succession on abandoned fields.

## RESULTS

*Species Composition the First Growing Season Following Abandonment.* Three different abandoned cornfields were studied as to their vegetative composition the first year after abandonment (Table 1).

Each field appeared to be relatively unique in the percent composition of dominant species of plant cover. However, a pattern is suggested by the presence of certain basic dominants in varying percentages on each of the sites, namely, common ragweed *(Ambrosia artemisiifolia* L.), and foxtail grass *(Setaria faberii* Herrm.).

A variety of additional sites in Southern

TABLE 1. *Percent of total plant cover on three abandoned cornfields, the first growing season after abandonment, southern Chester County, Pennsylvania, all sites on Glenelg Channery Silt Loam (1).*

| Site | South Campus Cornfield Site | | Tigue Road Cornfield Site | | South New Street Cornfield Site | |
|---|---|---|---|---|---|---|
| Number of $1m^2$ plots | 10 plots, Nov. 1964 | | 6 plots, Nov. 1967 | | 9 plots, Nov. 1968 | |
| Percent of plant cover by species | Common Ragweed | 51 | Foxtail Grass | 90 | Foxtail Grass | 26 |
| | Foxtail Grass | 8 | Common Ragweed | 2.5 | Dandelion | 18 |
| | Horseweed | 7 | Horseweed | 2 | Horseweed | 9 |
| | Dandelion | 0 | Dandelion | 0 | Pigweed | 8 |
| | Pigweed | 0 | Pigweed | 0 | Common Ragweed | 4 |

TABLE 2. *Four selected species as indicators of vegetation change following abandonment on two sites in Southern Chester County, Pa. Data indicates frequency/number of 1x1 meter square plots in study.*

| Species | South Campus Cornfield Site | | | | | Tigue Road Cornfield Site | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dates | Nov 64 | Apr 65 | Nov 65 | Apr 66 | Nov 66 | July 67 | Nov 67 | Apr 68 | Nov 68 |
| Common Ragweed | 9/10 | 4/8 | 0/2 | 0/6 | 0/7 | 6/8 | 3/5 | 0/7 | 19/36 |
| Horseweed | 4/10 | 0/8 | 0/2 | 0/6 | 0/7 | 2/8 | 2/5 | 1/7 | 3/36 |
| Oxalis sp. | 6/10 | 4/8 | 0/2 | 2/6 | 0/7 | 3/8 | 0/5 | 1/7 | 2/36 |
| Heath Aster | 6/10 | 1/8 | 2/2 | 6/6 | 7/7 | 0/8 | 0/5 | 0/7 | 1/36 |
| Crow Garlic | 1/10 | 2/8 | 1/2 | 0/6 | 3/7 | 5/8 | 0/5 | 5/7 | 29/36 |

Chester County were carefully observed by the author the first growing season following abandonment. These observations are summarized in Table 4.

These observations tend to support the view that the three basic dominants recorded on the three cornfield sites are also basic common denominators on a variety of sites in Southern Chester County the first year following abandonment.

*Species Composition Change Following Abandonment.* The data from two abandoned cornfields have been compared for change in species composition following abandonment (Table 2). The South Campus site was studied for three growing seasons until it was plowed in the Spring of 1967. The Tigue Road site has been studied for two growing seasons.

Four common species were selected from the data obtained as indicators of species change: two annuals, common ragweed and horseweed *(Erigeron canadensis* L.), one annual-perennial *Oxalis* sp, and two perennials, heath aster *(Aster pilosus* Willd.), Crow Garlic *(Allium vineale* L.).

Though the number of randomly located plots is quite limited, it is felt that the data does give some indication of the rate of change of these annual and perennial species during early succession.

Species composition change in early plant succession can also be considered as basically a change in growth form; that is from a large percentage of plants with annual growth form to these with perennial growth form (Table 3).

The change in relationship of growth form from a large perecntage of annuals the first growing season to predominately perennials the following seasons was fairly rapid on the South Campus site. However, it has been relatively slow on the Tigue Road site.

It is felt that this was due to a thick mat of foxtail grass litter that developed at the end of the first growing season on the Tigue Road site. As a result, new species have been slow in becoming established on the area. In contrast, on the South Campus site, the litter layer developed more slowly allowing new species to become quickly established.

The data on percent of bare ground showing

TABLE 3. *Growth form as an indicator of species composition change following abandonment of two cornfield sites in Southern Chester County, Pa. Authority for designation of species growth form was Fernald (2) and Stone (3).*

| | South Campus Cornfield Site | | | | | Tigue Road Cornfield Site | | | |
|---|---|---|---|---|---|---|---|---|---|
| Year, Season | Fall 64 | Spring 65 | Fall 65 | Spring 66 | Fall 66 | July 67 | Fall 67 | Spring 68 | Fall 68 |
| Number of 1x1 meter square plots | 10 | 8 | 2 | 6 | 7 | 8 | 5 | 7 | 36 |
| Number, annual growth form | 7 | 3 | 0 | 0 | 1 | 5 | 3 | 2 | 4 |
| Number, perennial growth form | 8 | 13(1)* | 8 | 14 | 13(3)* | 3 | 1 | 5 | 9 |
| Number, intermediate growth form | 6 | 5 | 5 | 4 | 4 | 7 | 2 | 3 | 7 |
| Total species recorded on plots | 21 | 21 | 13 | 18 | 18 | 15 | 6 | 10 | 20 |

*Number in parenthesis indicate the number of woody perennial species present.



TABLE 4. *Observations of growth form and dominant species composition on various sites in Southern Chester County, Pa., Fall 1968. All sites are either first or second growing season following abandonment.*

| Description of Site | Number annuals | Number perennial | Number intermediate | Dominent Species Composition |
|---|---|---|---|---|
| 1st growing season, 1968<br>Abandoned cornfield, wet in spring, Brandywine Creek, near Chadd's Ford, Rocky Hill Rd., Worsham Silt Loam | 3 | 5(0)* | 2 | Common Ragweed, 60% of plant cover |
| 1st growing season, 1968; corn four consecutive years<br>Abandoned cornfield, near Brandywine Creek, Shaw's Bridge, Congaree Silt Loam and Glenelg Channery Silt Loam | 6 | 8(0) | 4 | Foxtail Grass, over 95% of plant cover |
| 1st growing season, 1968<br>Abandoned cornfield, near Brandywine Creek, Shaw's Bridge, Glenelg Channery Silt Loam | 8 | 5(0) | 3 | Foxtail Grass, over 90% of plant cover |
| 1st growing season, 1968<br>Abandoned cornfield, near Brandywine Creek, Shaw's Bridge, Congaree Silt Loam | 4 | 6(0) | 1 | Foxtail Grass and Pennsylvania Knotweed, equally 85% of plant cover |
| 1st growing season, 1968<br>Former alfalfa-orchard grass pasture plowed and abandoned, Shaw's Bridge near Brandywine Creek | 6 | 10(0) | 8 | Common Ragweed and Foxtail Grass, dominant species |
| 1st growing season, 1968, corn two consecutive years including 1966. Used 2,4-D while in corn. Put to winter wheat fall 1966, no herbicide, abandoned. Manor Loam, Folchman Road site | 4 | 5(0) | 5 | Foxtail Grass, Pennsylvania Knotweed and Canada thistle dominants |
| 1st growing season, 1968, formerly in barley including 1966. Planted to winter wheat fall, 1966, harvested, abandoned. No herbicides used. Adjacent to the above field. Manor Loam Folchman Road site | 5 | 20(6) | 8 | Common Ragweed, Canadian thistle, Yellow Rocket, Wild Carrot |
| 1st growing season, 1968<br>Abandoned cornfield, South New Street site, Glenelg Channery Silt Loam | 12 | 16(3) | 5 | Pigweed, 8% of plant cover,<br>Foxtail Grass, 26%<br>Common Chickweed, 20% |
| 1st growing season, 1968<br>Exposed soil, new Science Building, Campus West Chester State College, bulldozed site, mixed weathered soil | 8 | 2(0) | 1 | Pigweed (dominant)<br>Foxtail Grass<br>Common Ragweed<br>Rough Pigweed<br>Barnyard Grass |
| 1st growing season, 1968<br>Exposed soil dump, ridge of soil 100'x20'x12' high. New Dormitory site, Campus, Wester Chester State College, top soil, loam | 11 | 9(0) | 5 | Pigweed—over 50% of plant cover. 9 species were frequent |

| | | | | |
|---|---|---|---|---|
| 1st growing season, 1968<br>Exposed weathered serpentine soil, bulldozed roadside area, Brinton Quarry, Chrome Gravelly Silty Clay Loam. All organic horizons were removed | 12 | 1(0) | 5 | Pigweed (dominant)<br>3 species were frequent |
| 2nd growing season, 1968<br>Abandoned cornfield, Mill Road site, Manor Loam | 3 | 4(0) | 7 | Crow Garlic<br>Canada thistle<br>Foxtail Grass<br>Brome Grass |
| 2nd growing season, 1968<br>Abandoned cornfield (Wet-spring site) Brandywine Creek, near Chadd's Ford, Weymouth Farm site. Worsham Silt Loam | 6 | 18(2) | 8 | Common Ragweed (dominant)<br>Willowherb<br>Sedge Cyperus sp.<br>Wild Carrot<br>Indian Tobacco<br>Hypericum<br>Foxtail Grass |

*Number in parenthesis indicates the number of woody perennial species present.

on the sample plot shows this difference between the two sites very graphically. On the South Campus site, the average percent bare ground was as follows: 1964 (10 plots)—45%, 1965 (2 plots)—15%, 1966 (7 plots)—6%. The average for the Tigue Road site was 1967 (5 plots)—2%, 1968 (9 plots)—1%. The first full growing season for the South Campus site was 1964, and for the Tigue Road site, 1967.

For comparison, to these studies, species structure was carefully observed and recorded on a number of other sites in early succession in Southern Chester County (Table 4).

Even though a variety of soil and site conditions are represented, they all appear to suggest the same pattern as that found on the two cornfields: an abundance of annuals the first growing season shifting gradually at various rates of change to dominance by perennial growth form.

Woody perennials are slow to enter the fields, though may be present in numbers (see Folchman Road site and South New Street site (Table 4) where selective pressures from herbicides and intensive cultivation have not been too great (eliminating residual plants) and there is a good seed source nearby.

*Some Related Problems of Site and Micro-Climate During Early Succession in Southern Chester County.* It was found that at the beginning of the second growing season, plants of yellow rocket *(Barbarea vulgaris* R.Br.) on the Tigue Road site on a west facing slope on Glenelg Channery Silt Loam with a heavy foxtail grass litter cover were only about ¼ the height of plants on a first year site, South New Street (also on Glenelg Channery Silt Loam). Along the edge of the Tigue Road site where the soil had been recently disturbed and exposed (no litter present) the plants were of normal height. The nearby Mill Road site (about 1½ miles north) also in the second growing season had a litter cover similar in composition (abundance of foxtail grass, *Setaria faberii.)* though not quite so thick. It was on a south facing slope, and better drained (Manor Loam). The Yellow Rocket plants there were of normal height growth.

Later in the season, it was noted that the foxtail grass *(Setaria faberii)* on the Tigue Road site formed flowering heads about a month later (mid-August) than, the same species on the first year South New Street site (Mid-July) which was nearly adjacent to it. Both were on Glenelg Channery Silt Loam.

On the Weymouth Farm site along the Brandywine, a wet portion of a cornfield was abandoned in Spring 1967. It was found that during the first growing season approximately 70% of the vegetation cover was common ragweed. Within the same abandoned field, populations of ragweed plants at the end of the first growing season varied from approximately 10 inches in height to over 36 inches in height. This gave a very distinctive rolling appearance to the rag weed dominated vegetation.

The general moisture condition of the field suggests a micro climate factor possibly related to soil aeration as a major factor in these plant population height differences. It also suggests that ragweed is very sensitive to site conditions.

During the second growing season in 1968, it was found that common ragweed and foxtail grass *(Setaria faberii)* on the Weymouth Farm site were about one-half the height of common ragweed and foxtail grass *(Setaria faberii)* on the Tigue Road site also in its second growing season. The Weymouth site was on a flood plain soil, Worshan Silt Loam. It is felt that these observable differences may be due to the micro climate differences between the two sites related to moisture conditions resulting from the soil type.

These observations of differences related to litter, drainage, and soil type suggest that a complex of moisture-temperature-aeration, interactions are major factors in species reaction to site changes during early succession.

*Serpentine Soil.* In early summer 1967 a special problem was completed by a student testing growth characteristics of two species of plants on the Brinton Quarry Serpentine Barren, near West Chester State College. For the study, a 10 x 3 ft plot was established in the middle of the barren and the sod cover removed exposing the top of the black Al horizon. After the completion of the study, test vegetation was removed and the revegetation of the site carefully observed and photographed.

By Fall 1968, only a few scattered plants of about 6 species were established on the site.

In contrast, a nearby serpentine site also at Brinton Quarry was bulldozed to the deeper weathered soil. The vegetation on this site followed that of the average disturbed site in Southern Chester County. The species appeared to be entirely different on the two nearby sites. The bulldozed site was along a road which suggests a difference in seed source and dissimination between the two sites.

It might be useful to add that an isolated abandoned cornfield in a heavy forested area in Northern Michigan observed in 1968, showed a similar lack of pioneer species.

## SUMMARY

The evidence in this study suggests that selective influences resulting from the kind of crop, the length of time of continual croping on a site, degree and type of cultivation, and use of herbicides, interacting with the basic qualities of the site (soil type, slope, exposure) result in a diversity of vegetation composition in early succession.

It also suggests, however, that these variations generally result in changes in abundance of a few major dominant species, namely, common ragweed, pigweed, and foxtail grass. These basic species are referred to as common denominators of early succession in Southern Chester County.

The study also suggests that the type of early cover resulting from selective influences operating before and during the first growing season following abandonment, may result in a litter cover that allows for rapid or slow early succession.

## REFERENCES

1. Soil Survey, Chester and Delaware Counties, Pennsylvania, 1963. U.S.D.A., Series 1959, No. 19.
2. Fernald, M. L. 1950. *Gray's Manual of Botany,* American Book Company, N. Y.
3. Stone, H. E. 1945. *A Flora of Chester County, Pennsylvania,* The Academy of Natural Sciences, Philadelphia.

## APPENDIX

Species List (2)

*In order of reference in the study.*
Common Ragweed *Ambrosia artemisiifolia* L.
Pigweed *Chenopodium album* L.
Foxtail Grass *Setaria faberii* Herrm.
Horseweed *Erigeron canadensis* L.
Oxalis *Oxalis europaea* Jord., *Oxalis stricta* L.
Heath Aster *Aster pilosus* Willd.
Crow Garlic *Allium vineale* L.
Yellow Rocket *Barbarea vulgaris* R.Br.
*Species referred to in tables but not identified in text.*
Pennsylvania Knotweed *Polygonum pensylvanicum* L.
Canada Thistle *Cirsium arvense* (L.) Scop.
Wild Carrot *Daucus carota* L.
Common Chickweed *Stellaria media* (L.) Cyrillo
Rough Pigweed *Amaranthus hybridus* L.
Barnyard Grass *Echinochloa crusgalli* (L.) Beauv.
Brome Grass *Bromus tectorum* L.
Willowherb *Epilobium coloratum* Bichler
Sedge *Juncus tenuis* Willd.
Indian Tobacco *Lobelia inflata* L.
Hypericum *Hypericum mutilum* L.
Dandelion *Taraxacum officinale* Weber.

# SUMMARY OF FOUR YEARS OF OBSERVATIONS OF BIRD POPULATIONS ALONG BRANDYWINE CREEK, CHADDS FORD, PENNSYLVANIA

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## ABSTRACT

During the last four years (January 1965-February 1969) an attempt has been made to record bird population changes by species along a 5½ mile transect bordering Brandywine Creek near Chadds Ford, Pennsylvania. The populations were sampled at least once each month and in many cases, several times a month. Certain field note-taking techniques were developed to record particular types of information.

## INTRODUCTION

In January 1965 a 5½ mile transect was established along the Brandywine Creek near Chadds Ford, Pennsylvania on which bird populations were observed and recorded by species. All of the observations and identifications (1-3) were made by the two co-authors from January 1965 to March 1969.

The habitat along the transect is diversified mesic hardwoods (tulip tree, red oak, white ash) xeric hardwoods, (black oak, white oak, hickory) and flood plain hardwoods (red maple, silver maple, sycamore, box elder), make up over 50% of the dominant plant cover. The remainder consists primarily of cornfields, moist meadows and pasture, and Reading Railroad right-of-way. In addition to the aquatic habitat of the Brandywine Creek, is one old river channel which has water in portions the year around, two small seasonal ponds, and two small marshes.

## METHODS

In over four years of sampling, 95 field trips of from 4½ to 6 hours duration each were taken. As much as possible all months of the year were sampled. The trips usually finished about 2:00 P.M. A particular effort was made to sample following severe environmental conditions such as deep snow, during nesting season in June, and during Spring migration in May. All months of the year were sampled except July in 1965 and August in 1967.

The following information was recorded for each sample (each field trip was considered a sample): total number of birds present by species; whether a bird was singing or calling; whether a bird was on the transect or flying over the transect; nesting records; birds feeding or traveling with young; feeding habits; foraging groups (size, species composition, and numbers).

Additional notes were made on observed mammals (number of grey squirrels in particular), and the phenology of plants—both woody and herbaceous.

## RESULTS AND DISCUSSION

*Nesting Species.* Fifty-eight species of birds commonly nested along the transect from 1965-1968 (Table 1). An additional 4 species nested 3 out of 4 years; Warbling Vireo (nest), Veery (territorial singing), Eastern Bluebird (nest), and Hairy Woodpecker (regular presence). Three species were found 2 out of 4 years; Sparrow Hawk (regular presence), American Redstart (territorial singing), and Ruby-throated Hummingbird (nest). One nesting record in 4 years of observation was obtained for the following species, Least Flycatcher (territorial singing), Killdeer (regular presence), Barred Owl (adult with young), Prairie Warbler (territorial singing), Blue Grosbeak (singing male), Broad-winged Hawk (nest).

The total number of species thought to have nested along the Brandywine transect during 4 years, from 1965 to 1968 was 71 species of which 13 species or 18% were transient nesters. This would indicate a relatively stable nesting species population from year to year.

*Relative Stability of Summer Resident Bird Populations.* Forty-seven of the nesting species are classified as summer residents in the transect area. From this group, 28 species were selected as indicators of summer population changes from year to year.

Species that had an average population change over 100% from year to year were classified as unstable (Table 2).

Fifteen species are listed as having relatively stable populations over the four-year study period. Thirteen species appear to have unstable summer populations over the four-year period of study, as defined above.



TABLE 1. *Common nesting species of birds along a 5½ mile transect, Brandywine Creek, Chadd's Ford, Pennsylvania; 1965-1968*

| | | |
|---|---|---|
| Green Heron | Rough-winged Swallow* | Eastern Meadowlark** |
| Canada Goose* | Barn Swallow* | Red-winged Blackbird** |
| Mallard* | Purple Martin* | Baltimore Oriole* |
| Wood Duck* | Blue Jay* | Common Grackle* |
| Turkey Vulture | Crow* | Brown-headed Cowbird* |
| Bobwhite* | Carolina Chickadee* | Scarlet Tanager** |
| Ring-necked Pheasant* | Tufted Titmouse* | Cardinal* |
| Rock Dove | White-breasted Nuthatch* | Indigo Bunting** |
| Mourning Dove** | House Wren* | American Goldfinch* |
| Black-billed Cuckoo** | Carolina Wren | Rufous-sided Towhee* |
| Chimney Swift | Mockingbird* | Chipping Sparrow* |
| Belted Kingfisher | Catbird** | Field Sparrow* |
| Yellow-shafted Flicker* | Brown Thrasher** | Song Sparrow* |
| Red-bellied Woodpecker* | Robin* | |
| Downy Woodpecker (drumming)** | Wood Thrush* | |
| Eastern Kingbird** | Starling* | |
| Crested Flycatcher* | Yellow-throated Vireo** | |
| Eastern Phoebe** | Red-eyed Vireo* | |
| Acadian Flycatcher** | Yellow Warbler** | |
| Wood Pewee** | Ovenbird** | |
| | Louisiana Waterthrush** | |
| | Kentucky Warbler** | |
| | Yellowthroat* | |
| | Yellow-breasted Chat** | |
| | House Sparrow* | |

*nest or young observed
**territorial singing observed. Regular presence during nesting season (not starred).

Data on several species suggest cyclic population tendencies, namely, House Wren, Catbird, Woodthrush, Red-eyed Vireo, Ovenbird, Kentucky Warbler, Yellowthroat, Red-wing Blackbird, and Indigo Bunting.

It will take many more years of records to judge whether these trends and judgements are real, the result of sampling error or random events that happened in an apparent pattern, as a matter of chance.

*Relative Stability of Permanent Resident Bird Populations.* Of 71 probable nesting species on a 5½ mile transect, 24 are listed as permanent residents. From this list, 16 common species were chosen as indicators of population changes. The data was grouped to indicate winter population changes from November to, and including March; and summer population changes from April to, and including August.

Each species was classified as relatively stable or unstable as to average population size from year to year. The winter populations and summer populations were considered separately (Table 3).

It was found that of the winter populations of 16 permanent resident species, 8 were relatively stable as to population size from year to year during 5 years of observations; Turkey Vulture, Yellow-shafted Flicker, Red-bellied Woodpecker, Downy Woodpecker, Chickadee, Tufted Titmouse, Mockingbird, and Cardinal. Three species were relatively stable for four winters, until the 1968-1969 season; Bluejay (low population), Crow (high population), White breasted Nuthatch (low population). Five species had unstable winter populations, Ring-necked Pheasant, Starling, American Goldfinch, Field Sparrow, and Song Sparrow.

*Relative Stability of Winter Resident Bird Populations.* Eleven species of winter resident birds were selected as indicator species of population stability from year to year. They were classified as relatively stable or unstable as to average population size on the transect during five years of observations (Table 4).

Eight of the 11 indicator species were listed as unstable; American Merganser, Red-breasted Nuthatch, Winter Wren, Golden-crowned Kinglet, Purple Finch, Tree Sparrow, White-throated Sparrow, and Fox Sparrow. The Red-tailed Hawk and State-colored Junco were listed as relatively stable. The Brown Creepers were relatively stable until the 1968-69 season

TABLE 2. *Average Summer Population size on a 5½ mile transect, Brandywine Creek, Chadd's Ford, Pennsylvania, based on two samples each June, 1965-1968.*

| Species | 1965 | 1966 | 1967 | 1968 | Population Stability |
|---|---|---|---|---|---|
| Green Heron | 1 | 1 | 2 | 1 | relatively stable |
| Chimney Swift | 5 | 5 | 6 | 3 | relatively stable |
| Eastern Kingbird | 2 | 4 | 5 | 4 | unstable |
| Crested Flycatcher | 7 | 8 | 6 | 5 | relatively stable |
| Eastern Phoebe | 2 | 3 | 2 | 3 | relatively stable |
| Acadian Flycatcher | 13 | 12 | 11 | 10 | relatively stable |
| Wood Pewee | 6 | 5 | 3 | 9 | unstable |
| Rough-winged Swallow | 1 | 3 | 1 | 2 | relatively stable |
| Barn Swallow | 8 | 2 | 9 | 11 | unstable |
| House Wren | 11 | 6 | 9 | 6 | relatively stable |
| Catbird | 9 | 13 | 12 | 8 | relatively stable |
| Robin | 17 | 22 | 28 | 28 | relatively stable |
| Wood Thrush | 16 | 6 | 6 | 10 | unstable |
| Red-eyed Vireo | 20 | 30 | 37 | 33 | relatively stable |
| Yellow Warbler | 4 | 4 | 7 | 2 | unstable |
| Ovenbird | 5 | 4 | 3 | 2 | unstable |
| Kentucky Warbler | 4 | 3 | 3 | 2 | relatively stable |
| Yellowthroat | 6 | 3 | 10 | 9 | unstable |
| Yellow-breasted Chat | 1 | 3 | 4 | 3 | unstable |
| Eastern Meadowlark | 7 | 7 | 5 | 9 | relatively stable |
| Red-winged Blackbird | 9 | 12 | 18 | 23 | unstable |
| Baltimore Oriole | 2 | 5 | 8 | 5 | unstable |
| Common Grackle | 25 | 57 | 54 | 54 | unstable |
| Brown-headed Cowbird | 4 | 6 | 5 | 11 | unstable |
| Scarlet Tanager | 1 | 2 | 2 | 2 | relatively stable |
| Indigo Bunting | 15 | 19 | 26 | 19 | relatively stable |
| Rufous-sided Towhee | 5 | 3 | 6 | 4 | relatively stable |
| Chipping Sparrow | 4 | 3 | 1 | 1 | unstable |

when there was an unusually high population.

*Stability of Winter Bird Populations—Discussion.* The causes for population instability from year to year among certain less common winter resident species is not clear (Golden-crowned Kinglet, Brown Creeper, Fox Sparrow, Winter Wren, and Purple Finch). Mild or severe winters do not result in larger or smaller wintering population of these species. In the case of the Brown Creeper, the field data suggest a gradual build up of wintering populations. This may result from a recovery following the use of DDT on Elms, one of the preferred feeding habitats of the Brown Creeper. The sudden appearance of Purple Finches and Red-breasted Nuthatchs on certain years suggests a possible cyclic cause.

Among the ground feeding species such as sparrows (Song, Tree, White-throated) and the Junco, population stability from year to year appears to be related to snow cover. In the three winters in which there was at least a 10 inch snowfall[1] during either late December or January (1964-65, 1965-66, 1966-67) and with over 10 inches in February in 1966 and 1967 there was a considerable reduction in populations of wintering sparrows and to some extent Juncos. Song Sparrows appear particularly sensitive to snow cover. The mild winters of 1967-68 and 1968-69 resulted in a marked increase in wintering populations of these species (Table 6).

The mast crop appears to have a considerable effect on the winter populations of Bluejay. There appeared to be a virtual crash in the Bluejay population during the 1968-69 mild winter in which there was a poor mast crop. Grey squirrels also virtually disappeared on the transect. The populations of White-breasted Nuthatch and to some extent the Tufted Titmouse appeared to be affected by the poor mast crop. In the case of the Tufted Titmouse, it resulted in very variable wintering populations on the transect from week to week during the winter of 1968-69. Probably

[1] Weather data obtained from U.S. Weather Bureau Records, Philadelphia, Pa.



TABLE 3. *Classification of population stability of 16 permanent resident species of birds along a 5½ mile transect, Brandywine Creek, Chadd's Ford, Pennsylvania 1965-1969.*

| Species | Relative Stability as judged by average population size from year to year: Winter Populations November-March 1965-69 | Summer Populations April-August 1965-68 |
|---|---|---|
| Turkey Vulture | relatively stable | relatively stable |
| Ring-necked Pheasant | unstable | unstable 65 poor<br>66 good<br>67 poor<br>68 good |
| Yellow-shafted Flicker | relatively stable | relatively stable |
| Red-bellied Woodpecker | relatively stable | relatively stable |
| Downy Woodpecker | relatively stable | relatively stable |
| Bluejay | relatively stable until 68-69 (low) | relatively stable |
| Crow | relatively stable until 68-69 (high) | relatively stable |
| Chickadee | relatively stable | relatively stable |
| Tufted Titmouse | relatively stable | relatively stable |
| White-breasted Nuthatch | relatively stable until 68-69 (low) | relatively stable |
| Mockingbird | relatively stable | relatively stable |
| Starling | unstable | unstable |
| Cardinal | relatively stable | relatively stable |
| American Goldfinch | unstable | relatively stable |
| Field Sparrow | unstable | relatively stable |
| Song Sparrow | unstable | relatively stable |

indicating considerable movement among the population from the surrounding area. Previous to the 1968-69 season, the wintering populations of titmice appeared to be relatively stable from week to week, and month to month.

In the case of certain species that tended to travel in winter flocks, it was not possible to judge the winter population, because of sampling variability. This included such species as Ring-necked Pheasant, Starling, Field Sparrow and American Goldfinch.

The American Goldfinch offers an interesting problem. It is present in considerable numbers some winters, virtually absent in others. In the 1968-69 season it was present in numbers until the 1st of February and then disappeared for the rest of the winter (7 weeks). During the 1967-68 winter, another mild season, it was present in numbers all winter.

Strong winds tended to build up temporary populations of certain species along the Brandywine during the winter. This was particularly evident in early February 1968 when after continuous strong winds there was considerable population build-up of Cardinals and Chickadees. Within a week, the populations were back to normal. No other species appeared to be affected.

As far as can be determined from the data from 1965 to 1969, the instability of winter

TABLE 4. *Relative stability of 11 species of winter resident birds as to average population size from year to year during five years of observations along a 5½ mile transect, Brandywine Creek, Chadd's Ford, Pennsylvania. January 1965-March 1969.*

| Species | Relative Stability |
|---|---|
| American Merganser | unstable |
| Red-tailed Hawk | relatively stable |
| Red-breasted Nuthatch | unstable |
| Brown Creeper | relatively stable until 68-69 (high) |
| Winter Wren | unstable |
| Golden-crowned Kinglet | unstable |
| Purple Finch | unstable |
| Slate-colored Junco | relatively stable |
| Tree Sparrow | unstable |
| White-throated Sparrow | unstable |
| Fox Sparrow | unstable |

TABLE 5. *Number of foraging groups observed and percent of 16 indicator species (see text) observed that were in foraging groups on a 5½ mile transect along Brandywine Creek, Chadd's Ford, Pennsylvania, 1965-1969.*

| Month, | | 1965-66 | | 1966-67 | | 1967-68 | | 1968-69 | |
|---|---|---|---|---|---|---|---|---|---|
| Time of Month | | Number Groups | Percent in Groups | Number Groups | Percent in Groups | Number Groups | Percent in Groups | Number Groups | Percent in Groups |
| September | 1-15 | | | | | 3 | 17 | 2 | 6 |
| | 16-30 | | | | | 3 | 18 | 6 | 20 |
| October | 1-15 | | | | | 3 | 10 | 3 | 21 |
| | 16-31 | | | | | 3 | 6 | 11 | 38 |
| November | 1-15 | | | | | 5 | 35 | 9 | 21 |
| | | | | | | | | 11 | 43 |
| | 15-30 | | | 6 | 27 | | | 12 | 60 |
| December | 1-15 | | | 7 | 15 | 5 | 38 | 7 | 34 |
| | | | | | | 9 | 60 | | |
| | 16-31 | | | | | 6 | 23 | | |
| January | 1-15 | | | 5 | 35 | 14 | 48 | 4 | 33 |
| | | | | 11 | 66 | 13 | 43 | 10 | 78 |
| | 16-31 | | | | | 7 | 45 | 8 | 57 |
| February | 1-15 | 7 | 36 | 8 | 58 | 15 | 45 | 7 | 42 |
| | | | | | | 11 | 68 | 11 | 38 |
| | 16-28 | 7 | 49 | 7 | 65 | 17 | 78 | 5 | 30 |
| March | 1-15 | 7 | 30 | 3 | 16 | | | 14 | 69 |
| | | | | | | | | 13 | 53 |
| | | | | | | | | 7 | 43 |
| | 15-31 | | | | | 10 | 53 | 7 | 41 |
| | | | | | | 14 | 43 | 9 | 60 |
| April | 1-15 | 4 | 19 | 1 | 2 | 6 | 39 | 3 | 7 |
| | 16-30 | 1 | 3 | 1 | 4 | 0 | 0 | | |

populations of certain permanent residents on the transect (Goldfinch, Field Sparrow, Song Sparrow) does not appear to effect the stability of their summer population size on the transect. It will be interesting to see if the poor mast crop of the 1968-69 season will effect the size of summer populations of Bluejays, Tufted Titmice, and White-breasted Nuthatches on the transect in 1969.

If any generalization can be drawn from these summaries of population variability from year to year, it is that probably all of these species are unstable at some time or another. That is, if they are observed over a long enough period of time, say 20 years. However, considering the problems of accurate field sampling, the variability of individual winter seasons and other major environmental differences from year to year (moisture, temperature) the populations generally show a remarkable stability.

*Foraging Groups.* It is quickly evident to a careful field observer that birds are not found at random in the winter, but generally in feeding or traveling groups. These groups, designated here as foraging groups are particularly evident along the Brandywine Creek from September to early April.

In reviewing the species composition of the winter foraging groups over the four years of the study, it was found that they were composed primarily of certain species, in varying numbers, and relationships during the course of the season. These species, 16 in number were used as indicators of foraging group activity on the 5½ mile transect.

The indicator species were as follows: Tufted Titmouse, Chickadee, Downy Woodpecker, Hairy Woodpecker, White-breasted Nuthatch, Brown Creeper, Golden-crowned Kinglet, Carolina Wren, Field Sparrow, Tree Sparrow, White-throated Sparrow, Song Sparrow, Junco, Purple Finch, Goldfinch, and Cardinal.



TABLE 6. *Total number of individuals of 16 indicator species that could form foraging groups, as an indicator of winter population density on a 5½ mile transect along Brandywine Creek, Chadd's Ford, Pa, 1965-1969. Samples are arranged in order of time of sampling in months with more than one sample.*

| Month | 1964-65 | 1965-66 | 1966-67 | 1967-68 | 1968-69 |
|---|---|---|---|---|---|
| September | | 145 | 86 | 59 | 91 |
| | | | 69 | 82 | 116 |
| October | | 168 | 250 | 186 | 328 |
| | | | | 187 | 389 |
| November | | 242 | 179 | 370 | 402 |
| | | | | | 489 |
| | | | | | 382 |
| December | | 241 | 246 | 345 | 282 |
| | | | | 378 | |
| | | | | 307 | |
| January | 94 | 130 | 87 | 288 | 173 |
| | | 218 | 105 | 190 | 273 |
| | | | | 260 | 227 |
| February | 119 | 157 | 93 | 283 | 284 |
| | | 140 | 66 | 327 | 290 |
| | | | | 314 | 233 |
| March | 129 | 159 | 129 | 316 | 264 |
| | | | | 196 | 173 |
| | | | | | 218 |
| | | | | | 175 |
| | | | | | 221 |
| April | 114 | 117 | 123 | 188 | 136 |
| | 105 | 119 | 142 | 143 | |

The results of the observations of foraging groups and their composition on a 5½ mile transect are presented in a series of tables.

A summary of the number of foraging groups observed and the percent of the 16 indicator species that were in foraging groups is presented in Table 5.

Table 6 is a summary of the total number of individuals of the 16 indicator species that were present on the transect. This is used as a measure of winter population density on the transect.

Table 7 is a summary of the frequency of the foraging groups by the number of species of which they were composed. It is intended to measure species diversity of foraging groups through the winter months.

The size of foraging groups through the winter season is summarized in Table 8.

Finally, Table 9 is a measurement of the tendency for individual species to occur in foraging groups during the winter season.

Several generalizations are suggested by the data in the tables:

(a) That the number of foraging groups recorded in a given winter is related to the size of the wintering population. For example, the number of foraging groups recorded on the transect during the mild winters of 1967-68 and 1968-69 is generally higher than during the more severe winters with considerable snow cover, 1965-66 and 1966-67. The larger size of wintering populations of birds tending to form foraging groups during these mild winters is very evident in Table 6 (see particularly December through March).

(b) The species diversity of the foraging groups appears to be reduced in October, January, February, March and April (Table 7). In mid and late winter this may be due to a gradual reduction in the diversity and quality of the food or supply available in the various habitats. The tendency for larger species di-

TABLE 7. *Frequency of the number of species composing individual foraging groups by month, on a 5½ mile transect along Brandywine Creek, Chadd's Ford, Pa., 1965-1969.*

| Number of Species in Foraging Group | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. |
|---|---|---|---|---|---|---|---|---|
| 2 | 5 | 7 | 14 | 11 | 30 | 34 | 39 | 9 |
| 3 | 1 | 4 | 6 | 4 | 17 | 22 | 21 | 4 |
| 4 | 2 | 5 | 9 | 6 | 9 | 20 | 11 | 1 |
| 5 | 2 | 3 | 3 | 5 | 7 | 8 | 6 | 2 |
| 6 | 1 | | 3 | 5 | 4 | 5 | 1 | |
| 7 | 1 | | 4 | 2 | 2 | 2 | 3 | |
| 8 | 1 | 1 | 1 | | | 2 | 3 | |
| 9 | | | 2 | | 1 | 2 | | |
| 10 | | | 1 | 1 | 1 | | | |
| 11 | 1 | | | | | | | |
| 12 | | | | | | | | |
| 13 | | | | | 1 | | | |
| Total Number of Foraging Groups | 14 | 20 | 43 | 34 | 72 | 95 | 84 | 16 |
| Number of Samples (trips/month) | 4 | 4 | 5 | 5 | 8 | 10 | 9 | 6 |
| Average Number of Foraging Groups Per Sample | 3.5 | 5 | 8.6 | 6.8 | 9.0 | 9.5 | 9.3 | 2.7 |
| Percent of Foraging Groups with four or less species | 57 | 80 | 67 | 62 | 78 | 80 | 85 | 87 |

versity in foraging groups in November and December may be related to the general abundant and diversified food supply available. The September foraging groups are mixed with migrating species resulting in considerable species diversity. If the figures from October are accepted, (based on 4 samples), they may indicate an interim period between the passage of migrants and the stabilization of the wintering populations on the area.

(c) The size of the foraging groups tends to become smaller in January, February and March as compared with November and December (Table 8). Surprisingly, the size of the foraging groups in September is small though they are diversified as to species. Foraging groups in April also tend to be smaller than the winter and fall months.

(d) By measuring the tendency of individual species to join foraging groups, a type of index to species' "sociability" can be developed (Table 9). The index indicates a species' "sociability" through the winter season, accompanying the general build up in the num-

TABLE 8. *Size of foraging groups by month, expressed as a percent of groups in a size class. Observations on a 5½ mile transect along Brandywine Creek, Chadd's Ford, Pa., 1965-1969.*

| Size of Foraging Groups | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. |
|---|---|---|---|---|---|---|---|---|
| Percent of foraging groups 5 or less individuals | 43 | 30 | 16 | 35 | 36 | 44 | 39 | 50 |
| Percent of foraging groups 10 or less individuals | 79 | 65 | 37 | 50 | 65 | 68 | 67 | 88 |
| Percent of foraging groups 20 or less individuals | 93 | 85 | 74 | 71 | 83 | 85 | 83 | 94 |
| Total number of foraging groups observed | 14 | 20 | 43 | 34 | 72 | 95 | 84 | 16 |



TABLE 9. *Percent of total number of birds observed by species that were in foraging groups of two or more species on a 5½ mile transect along Brandywine Creek, Chadd's Ford, Pa., 1965-1969.*

| Species | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. |
|---|---|---|---|---|---|---|---|---|
| Tufted Titmouse | 34 | 29 | 63 | 50 | 72 | 71 | 39 | 13 |
| White-breasted Nuthatch | 17 | 27 | 63 | 20 | 52 | 72 | 31 | 0 |
| Chickadee | 31 | 35 | 58 | 55 | 65 | 82 | 37 | 0 |
| Downy Woodpecker | 15 | 30 | 33 | 19 | 43 | 58 | 8 | 11 |
| Cardinal | 5 | 16 | 18 | 21 | 23 | 40 | 7 | 0 |
| White-throated Sparrow | — | 21 | 48 | 43 | 58 | 72 | 62 | 93 |
| Junco | — | 89 | 36 | 22 | 59 | 78 | 70 | 68 |
| Tree Sparrow | — | — | 39 | 14 | 80 | 72 | 50 | — |
| Song Sparrow | 0 | 9 | 19 | 19 | 7 | 38 | 29 | 28 |

ber of foraging groups in January, February and November. It is interesting to note the sharp reduction in participation in foraging groups in March by species that are establishing early nesting territories, for example: Tufted Titmouse, White-breasted Nuthatch, Chickadee, Downy Woodpecker, and Cardinal. Also, the continued high participation in foraging groups by winter residents such as White-throated Sparrow, Junco, and Tree Sparrow. The high participation of the Song Sparrow in late February and March is due to a large percentage of migratory Song Sparrows moving through the transect area, resulting in a masking of local birds which are establishing territories.

(e) Formation of foraging groups. From observations of several hundred foraging groups over 5 winter seasons, the following two hypotheses as to their development are offered; (a) That generally they build from a nucleus of species with closely related feeding habits such as Chickadee, Titmouse, Downy Woodpecker relationship; or a White-throated Sparrow, Junco, Cardinal relationship, into quite diverse groups. This build-up of complexity appears to begin in the morning and reach a peak between about 10 A.M. and 2:00 P.M. (b) That generally foraging groups develop from either of these two nuclei in a more or less random fashion, birds being attracted by activity of other birds.

## REFERENCES

1. Annotated List of Chester County Birds. 1962. West Chester Bird Club, West Chester, Pennsylvania.
2. Arnett, John H. Jr. 1954. A Field List of Birds of the Philadelphia Region, Delaware Valley Ornithological Club, Academy of National Sciences, Philadelphia.
3. Peterson, R. T. 1947. *A Field Guide to the Birds* Houghton Mifflin Company, Boston.

# ODONATA OF THE POTOMAC RIVER WATERSHED IN PENNSYLVANIA

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## ABSTRACT

Odonata were collected from two Pennsylvania counties (Fulton and Franklin) in the Potomac drainage during 1968. Sites chosen varied from an artificial lake at 1480-ft elevation to streams at about 700-ft elevation. Previous records listed only 10 species of dragonflies from these counties to which 36 more are herein added. These species are enumerated and certain ones, particularly *Enallagma basidens* Calvert, discussed in more detail with respect to distribution.

## INTRODUCTION

Dragonflies were collected from two Pennsylvania counties (Fulton and Franklin) in the Potomac drainage area of the state during the summer of 1968 (Fig. 1). It was hoped that records from this area, lying between the well collected areas of central Pennsylvania and lower Potomac drainage near Washington, D. C., would aid in determining factors influencing Odonata distribution and add to our knowledge of Pennsylvania insect fauna.

At the outset of this study the records of Odonata were as follows for the Potomac drainage counties in Pennsylvania: Franklin Co. on Conococheague Creek, *Calopteryx angustipennis* Selys, *Calopteryx maculata* Beauvois, *Lestes rectangularis* Say, *Hagenius brevistylus* Selys and *Libellula lydia* Drury; Fulton Co. at Cowans Gap State Park, *Enallagma exsulans* Hagen, *Anax junius* Drury, *Gomphus exilis* Selys, *Epitheca cynosura* Say, *Libellula lydia* Drury and *Erythemis simplicicollis* Say; Adams Co. at York Springs, *Nehalennia irene* Hagen; Bedford Co., no known records (1).

## RESULTS

Collecting results are presented in Table 1. The species are listed in the order presented in the critical check list of Pennsylvania Odonata (2). The locations at which collections were made are described below.

(a). West Branch Conococheague Creek at Fannettsburg, Franklin Co. (elevation 740 ft). This moderately large stream is dammed to form a lake just south of the town. We collected both on the lake and on the stream above the lake. The lake is quite eutrophic and the stream bottom very silty, presumably from agriculture pollution. The edges of the lake are choked with elodea and filamentous algae and the shores with cattails. The stream exits from deciduous woods about 100 yd above the upper end of the lake. *Basiaeschna janata* Say, *Dromogomphus spinosus* Selys, *Gomphus lividus* Selys, *Macromia illinoiensis* Walsh, *Didymops transversa* Say, *Calopteryx augustipennis* Selys, and *Argia sedula* Hagen were taken on the stream. *Calopteryx maculata* Beauvois, *Epitheca cynosura* Say and *Argia violacea* Hagen frequented both the stream and lake. The other species recorded were found on the lake. Collecting dates for this location were May 25, 26: June 15, 29; August 11; and September 8.

(b). Tonoloway Creek, South of Sipes Mill, Fulton Co. (elevation 700 ft). The stream is shallow and fairly swift with a bottom alternating from stony in shallow spots to silt and sand in the deeper, slower portions. At the collecting place, the stream lies partly in a narrow, wooded gorge. Hemlock and raspberries are common along the streamside. There are farms in the area drained by the creek. Collecting dates were June 1, 30; July 4; and August 18.

(c). Meadow Ground Lake, West of McConnellsburg, Fulton Co. (elevation 1480 ft). This is an artificial lake constructed in 1963 on a very small stream, Roaring Run, which drains into Licking Creek. The upper end of the lake is quite boggy and has sphagnum, blueberries and jack-in-the-pulpit among its vegetation. Most collecting was done there. *Argia moesta* Hagen was collected on the stone dam, *Dromogomphus spinosus* Selys was quite frequent in the vicinity of the dam and perched on the gravel road to the dam. Collecting dates were June 29; August 18, 24; and September 28.

(d). Cowans Gap State Park, Fulton Co. (elevation 1200 ft). Collecting was done on the Little Aughwick Creek in the woodlands below the lake. This is a clear, fast mountain stream flowing through deciduous woods. There is rhododendron and dogwood was in bloom at the time of collection. The stream lies in the



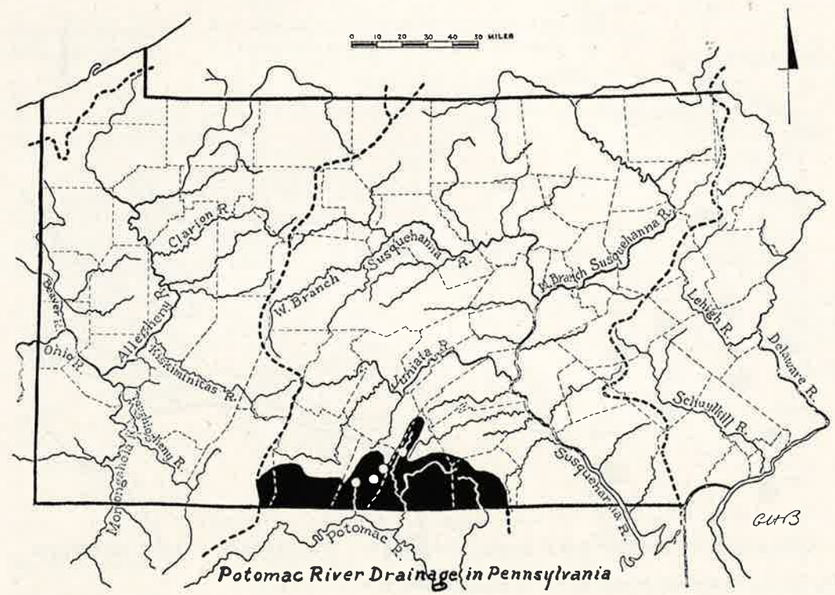



**Figure 1. Potomac River Drainage in Pennsylvania. Collecting locations are marked by white circles. From West to East these are Tonoloway Creek, Meadow Ground Lake, Cowans Gap, and Conococheague Creek.**

valley immediately to the north-west of the West Branch Conococheague, but is in the Juniata River drainage. We include this site to augment our Fulton Co. records. Collecting was done May 25 only.

## DISCUSSION

Fulton and Franklin counties lie in the south-east portion of the Appalachian Mountains in Pennsylvania, and as would be expected their dragonfly fauna bears a close resemblance to that of central Pennsylvania (3, 4,5). This is indicated by the species which we collected in these counties but which are not recorded by Donnelly (6) for the Piedmont near Washington, D. C. These were *Gomphus rogersi* Gloyd, *Gomphus villosipes* Selys, *Leucorrhinia intacta* Hagen, *Calopteryx angustipennis* Selys, and *Enallagma hageni* Walsh. Though found on coastal plains streams, *Didymops transversa* Say is not found in the Piedmont, and *Gomphus lividus* Selys, which we found to be frequent in Fulton and Franklin counties, is rare in the Washington area. Interestingly, since Donnelly lists these as common in the Piedmont, we found *Basiaeschna janata* Say, *Dromogomphus spinosus* Selys and *Macromia illinoiensis* Walsh to be quite frequent in Fulton and Franklin counties.

*Enallagma basidens* Calvert has not been recorded previously from the Appalachians in Pennsylvania though it is wide-spread in western Pennsylvania (7,8) and is known from the Delaware Water Gap (8) (Fig. 2).

This species was described from Texas in 1902 (9). The first record for *Enallagma basidens* Calvert outside of Texas was from Kansas in 1917 (10). It was discovered in Indiana in 1929 (11), and by 1942 had been found as far East as Ohio and the Carolinas (11). By 1950 it was recorded from the Washington, D. C. area (6), and for the first time in western Pennsylvania in 1959 (7). This appears to be a true, rapid extension of the species range as many of the sites where it is now common were regularly visited by experienced collectors for many years before it suddenly appeared (11).

*Enallagma basidens* Calvert appears from its present distribution in Pennsylvania (3, 7,8) to have entered the state along the Ohio.

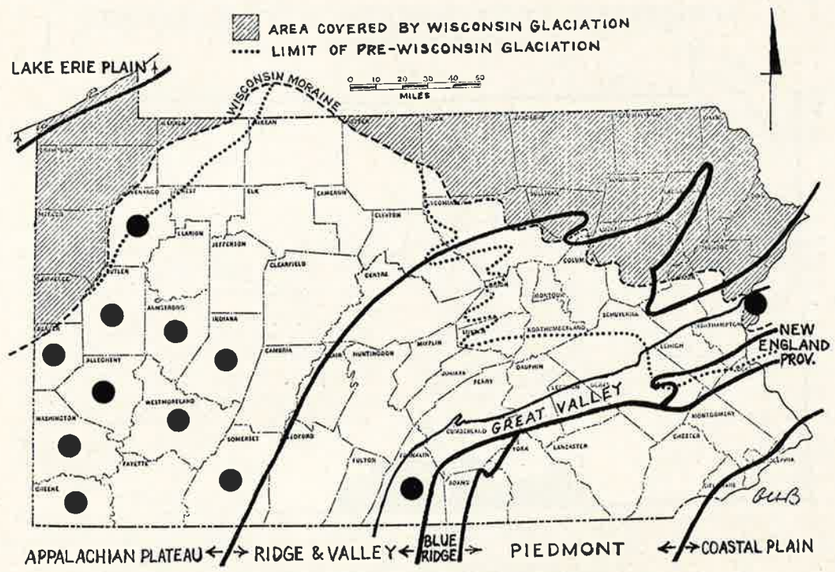



**Figure 2. Collection locations for** Enallagma basidens.

Delaware and Potomac river drainages. Thus possible dispersal routes for vagile species of Odonata entering the state from the south exist in the major river systems of the state.

## REFERENCES

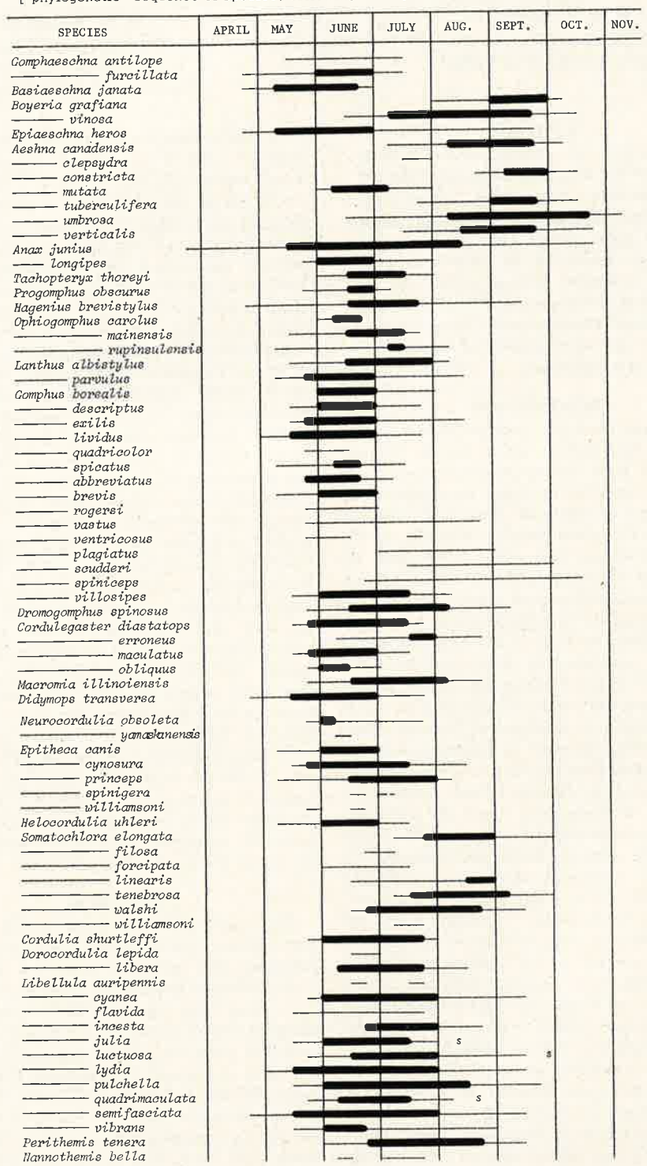

1. Beatty, G. H. and A. F., Unpublished, compiled records.
2. Beatty, G. H. and A. F. 1968. Check List and Bibliography of Pennsylvania Odonata, *Pa. Acad. Sci. Proc.* 42:120.
3. Beatty, G. H. and A. F., and C. N. Shiffer. 1969. A survey of the Odonata of central Pennsylvania. *Pa. Acad. Sci. Proc.* 43:127.
4. Beatty, G. H. and A. F. 1968. Origins and biogeographic affinities of the Odonata fauna of Pennsylvania. *Pa. Acad. Sci. Proc.* 42:110.
5. Beatty, G. H. and A. F. 1969. Edaphic factors in the distribution of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 43:137.
6. Donnelly, T. W. 1961. The Odonata of Washington, D. C. and vicinity. *Ent. Soc. Wash. Proc.* 63:1.
7. Raff, R. A. 1960. Two species of Odonata not previously recorded from Pennsylvania. *Ent. News* 71: 262.
8. Ahrens, C., and G. H. and A. F. Beatty. 1968. A survey of the Odonata of western Pennsylvania. *Pa. Acad. Sci. Proc.* 42:103.
9. Calvert, P. P. 1902. *Biologia Centrali-Americana,* Vol. 50, *Neuroptera.* p. 114.
10. Kennedy, C. H. 1917. The dragonflies of Kansas. *Univ. Kans. Bull.*, Biol. Series, 18:135.
11. Montgomery, B. E. 1942. The distribution and relative seasonal abundance of the Indiana species of *Enallagma. Ind. Acad. Sci. Proc.* 51:277.


TABLE 1. *Odonata collected or observed in Fulton and Franklin Counties 1968.*

| SPECIES | LOC* | TIME OF OBSERVATION OR COLLECTION months divided into quarters | | | | | | | | | | | | | | | | NOTES ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | May | | June | | | July | | | | August | | | | September | | | (a) | (b) |
| | | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | |
| *Basiaeschna janata* | C | x | | x | | | | | | | | | | | | | | 2 | |
| | C | | | | | | | | | | | | | | | | | 3 | |
| | T | | x | | | | | | | | | | | | | | | P | E |
| *Boyeria grafiana* | T | | | | | x | | | | | | | | | | | | 2 | |
| *B. vinosa* | T | | | | | | | | | | | x | | | | | | P | SR |
| *Epiaeschna heros* | T | | | | | x | | | | | | | | | | | | 2 | |
| *Aeshna umbrosa* | MG | | | | | | | | | | | | | x | | | x | 2 | |
| *Anax junius* | C | x | | x | | | | | | | | | | | | | x | 3 | SR |
| | MG | | | | | | | | | | | | | | | | | 3 | |
| *Hagenius brevistylus* | MG | | | | | | | | | | | | | | | | | P | |
| *Gomphus lividus* | C | | | | x | | | | | | | | | | | | | 2 | |
| | T | | | x | | | | | | | | | | | | | | 2 | |
| *G. rogersi* | CG | x | | | | | | | | | | | | | | | | P | |
| *G. villosipes* | C | | | | x | | | | | | | | | | | | | 2 | |
| *Dromogomphus spinosus* | C | | | | | x | | | | | | | | | | | x | 2 | |
| | T | | | | | x | x | | | | | | | | | | | 2 | |
| | MG | | | | | | | | | | | | | | | x | x | 2 | |
| *Maromia illinoiensis* | C | | | | x | | | | | | | | | | | | x | 2 | |
| | T | | | | | x | | | | | | | | | | | | 2 | |
| *Didymops transversa* | C | x | | | | | | | | | | | | | | | | 2 | |
| *Epitheca cynosura* | C | x | | | x | | | | | | | | | | | | | 3 | |
| | MG | | | | | x | | | | | | | | | | | | 3 | |
| *E. princeps* | C | | | | x | | | | | | | | | | | | | 3 | SR |
| | MG | | | | | x | | | | | | | | | | | | 3 | SR |
| *Libellula cyanea* | C | | | | x | | | | | | | | | | | | | 2 | |
| | MG | | | | | | | | | | | | | | | | | P | SR |
| *L. luctuosa* | C | | | | x | | x | | | | | | | | | | x | 3 | |
| | MG | | | | | | | | | | | | | | | x | x | 3 | |
| *L. lydia* | C | x | | | x | | | | | | | | | | | | | 3 | |
| | T | | | x | | | | | | x | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | 3 | |
| *L. pulchella* | C | | | | x | | | | | | | | | | | | | 3 | |
| | T | | | | | x | | | | | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | 2 | |
| *Perithemis tenera* | C | | | | | | | | | | | | | | | | | 2 | |
| | MG | | | | | | | | | | | | | | | | | 2 | |
| *Erythemis simplicicollis* | C | | | | x | | x | | | | | | | | | | | 2 | |
| *Sympetrum rubicundulum* | C | | | | | | | | | | | | | | | | | 3 | |
| | MG | | | | | | | | | | | | | | | | | 3 | |
| *S. semicinctum* | C | | | | | | | | | | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | 1 | |
| *S. vicinum* | C | | | | | | | | | | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | P | |
| *Pachydiplax longipennis* | C | | | | | | | | | | | | | | | | | 2 | |
| | MG | | | | | | | | | | | | | | | | | P | |
| *Leucorrhinia intacta* | C | | | | x | | | | | | | | | | | | | 2 | |
| | MG | | | | | | | | | | | | | | | | | 2 | |
| *Tramea lacerata* | C | | | | | | | | | | | | | | | | | P | |
| *Calopteryx angustipennis* | C | | | | x | | | | | | | | | | | | | P | |
| *C. maculata* | C | | | | x | | | | | | | | | | | | | 3 | |
| | T | | | | | | | | | | | | | | | | | 3 | |
| | MG | | | | | | | | | | | | | | | | | 3 | |
| *Lestes inequalis* | C | | | | | | | | | | | | | | | | | P | |
| *L. rectangularis* | C | | | | x | | | | | | | | | | | | | 1 | |
| | MG | | | | | | | | | | | | | | | | | 3 | |
| *Argia moesta* | T | | | | | | | | | | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | 2 | |
| *A. sedula* | C | | | | | | | | | | | | | | | | | 3 | |
| *A. violacea* | C | | | | | | | | | | | | | | | | | 3 | |
| | T | | | | | | | | | | | | | | | | | 3 | |
| *Chromagrion conditum* | C | x | | | x | | | | | | | | | | | | | 3 | |
| *Enallagma aspersum* | T | | | | | | | | | | | | | | | | | P | |
| *E. basidens* | C | | | | | | | | | | | | | | | | | P | |
| *E. carunculatum* | C | | | | | | | | | | | | | | | | | P | |
| *E. civile* | C | | | | | | | | | | | | | | | | | P | |
| | MG | | | | | | | | | | | | | | | | | 3 | |

| | | |

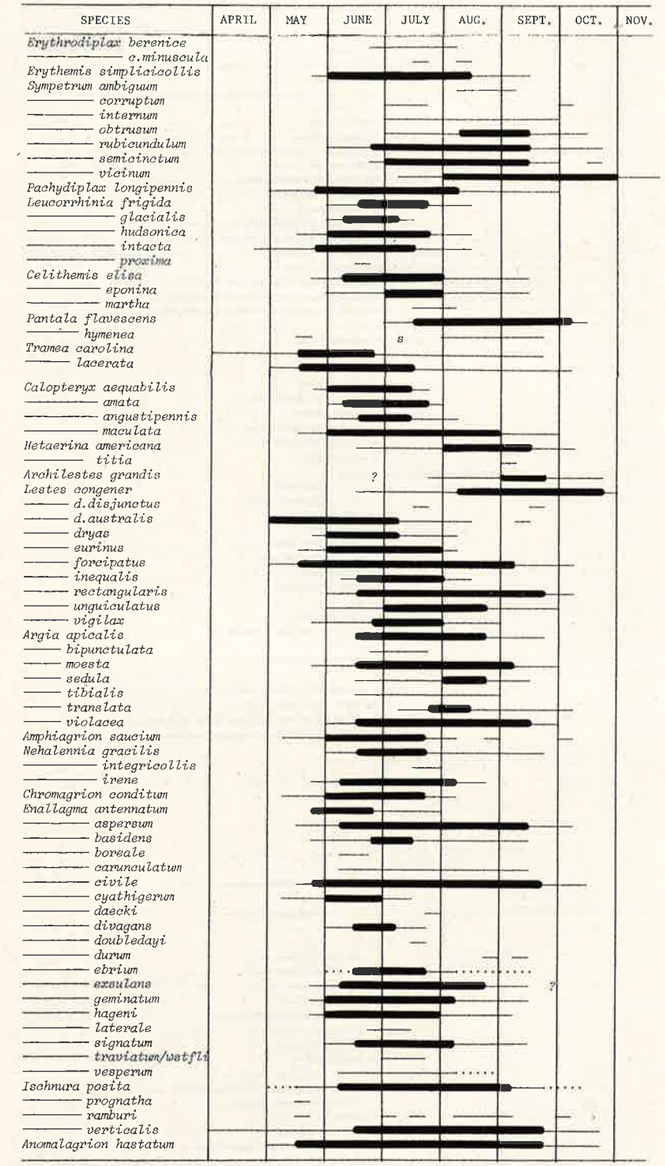
Table 1. SEASONAL DISTRIBUTION OF IMAGOS OF PENNSYLVANIA ODONATA . ["phylogenetic" sequence of species, following Pennsylvania Check List, 1968 (9)][1]




(continued on next page)

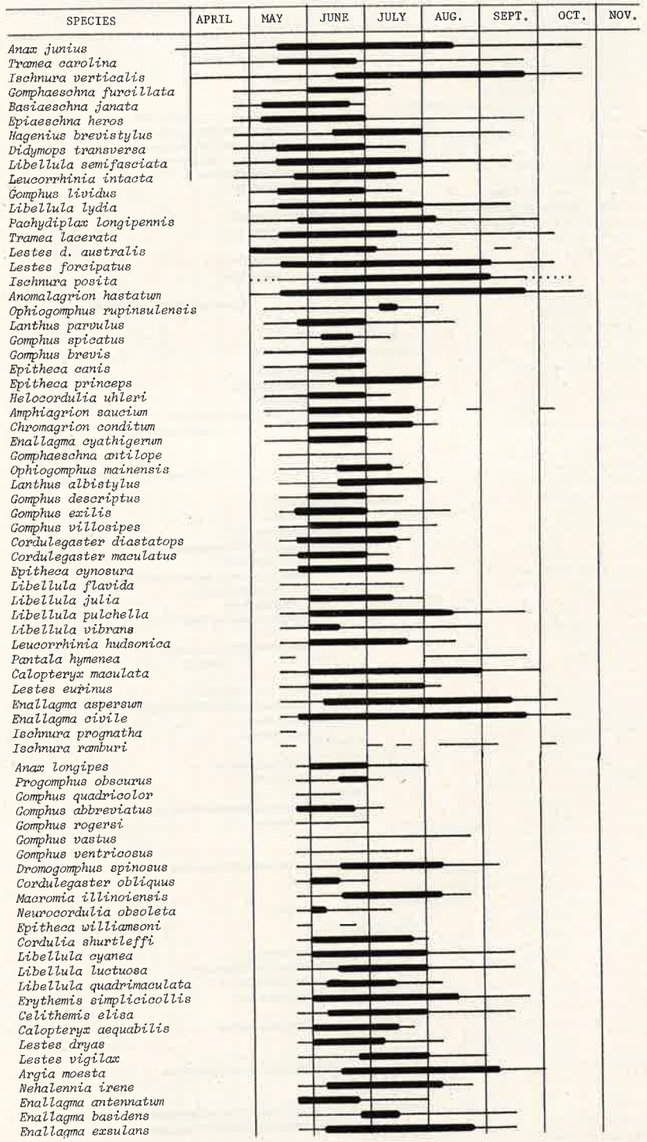


Table 1 (continued)




[1]Earliest and latest dates for specimens are connected by a thin line. Thickened line represents period of maximum abundance in Pennsylvania in general. Nearly all quarter-months along the lines are represented by specimen records.

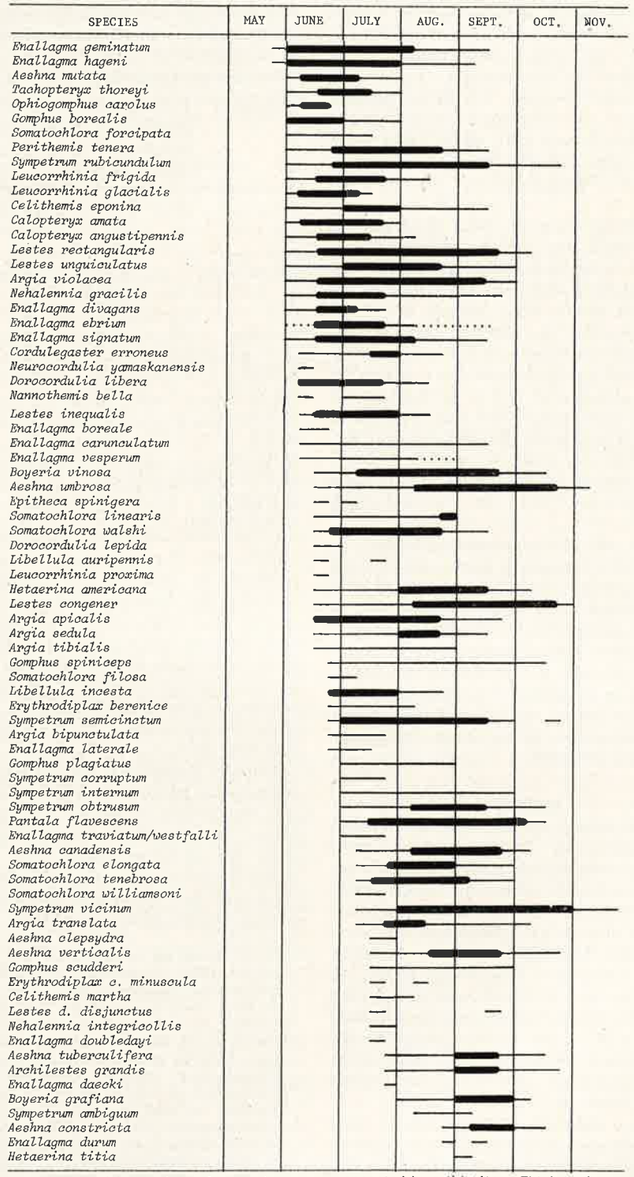
Table 2. SEASONAL DISTRIBUTION OF FLIGHT PERIODS OF PENNSYLVANIA ODONATA. (sequence of species in order of emergence)[1]




(continued on next page)



Table 2. Continued.




[1]Earliest and latest dates for specimens are connected by a thin line. Thickened line represents period of maximum abundance in Pennsylvania in general.

Pennsylvania Check List (11) which purports to be phylogenetically arranged, while in Table 2 the order proceeds from the earliest species to emerge in the spring, *Anax junius,* to the last to appear in the summer. Table 2 is especially useful because it can be directly compared with such existing tables as those of Bick (17) and Robert (18), while Table 1 can likewise be compared with those of Schiemenz (1), Valle (2), Wesenberg-Lund (3) and Portmann (4).

In tabulating seasonal data displayed on Tables 1 and 2, the number of records per quarter-month determined the "period of maximum abundance" shown by thickening of the bar for each species with enough records to compare frequency of collection *intra se.* Because of large variation in number of records-per-species-per-quarter-month, the criterion for "maximum abundance" had to be flexible; collecting over these many years was not done on a planned survey basis and therefore no uniform quantification could be applied to determine the period of maximum adult flight (which might be called the "normal flight season").

Inasmuch as the information-display on Tables 1 and 2 integrates many years of collecting, the precise annual phenology of most species is bound to be obscured, especially in the case of species wherein emergence appears to be correlated with climatological variables. Even more variable is the date of emergence of collectors in the spring. Therefore the tables must be interpreted as showing the average normal flight season, with all-time earliest and latest records indicated.

## RESULTS

The flight season for Odonata in Pennsylvania, as shown on Table 2, extends for 8 months, from late March until late November, while the emergence season is for 6 months from late March to late September. There are few records of October emergences, and none for November. The tables, and the foregoing statements, integrate information from the entire state; in the northern part of the state, and at higher elevations, emergences are later and flight seasons are shorter, than for the state in general. In the southern part of the state and in regions with a warmer climate (25,27) the converse is true.

By 1 May about 10 Odonata species have emerged somewhere in Pennsylvania, and by 1 June roughly 77 species are on the wing. By 1 July 124 species of Pennsylvania Odonata have appeared, leaving only 28 out of the 152 tabulated species (data for the remaining 18[1] (11) were not tabulated) to emerge between 1 July and the end of August, after which time no further species appear.

Table 3 compares monthly emergence summations for Pennsylvania with similar data presented by Bick (17) for Louisiana, where, however, the Odonata flight-season is 12 months long. Differences in percentages of the total Odonata fauna collected as adults during given months are more illustrative of the great climatic difference between Pennsylvania and Louisiana, than of differences in composition of the Odonata faunas, which, incidentally, are also very great.

TABLE 3. *Percentage of total Odonata fauna flying each month, April-November, with Pennsylvania and Louisiana compared.*

| | NO. OF SPECIES FLYING (OUT OF 152) | PERCENTAGE OF TOTAL FAUNA (152) | PERCENTAGE OF TOTAL FAUNA OF LOUISIANA BICK, 1957 (10) |
|---|---|---|---|
| APRIL | 10 | 6% | 80% |
| MAY | 77 | 51 | 80 |
| JUNE | 120 | 79 | 92 |
| JULY | 133 | 87 | 94 |
| AUGUST | 95 | 62 | 92 |
| SEPTEMBER | 89 | 58 | 86 |
| OCTOBER | 43 | 28 | 52 |
| NOVEMBER | 2 | 2 | 36 |

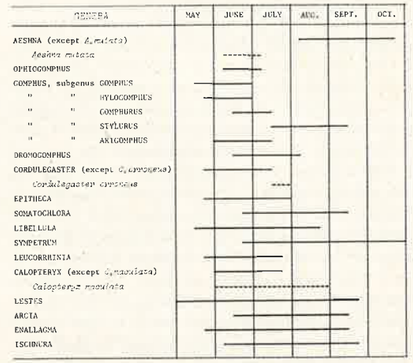
Climatic (latitudinal and altitudinal) differences in the phenology of Odonata faunas are also emphasized in Valle's diagram (2) comparing data for Finland with those for Denmark (3) and Switzerland (4). Comparison of this information display with that on Table 3 indcates that the integrated pattern of flight seasons in Pennsylvania is more like that of Denmark, with a July peak, than those of Finland or Switzerland where peak numbers of species fly in August. Louisiana Odonata, with a continuous "plateau" from April to September, show a semi-tropical phenological pattern reflecting thermal data given by Bick (17), a correlation to be dealt with in another paper (27).

[1] The 18 species not tabulated, and their seasonal data, are as follows: *Nasiaeschna pentacantha* (1 larva), *Ophiogomphus edmundo* (3 June), *Ophiogomphus howei* (20 May), *Gomphus viridifrons* (3 May; 25,28 June), *Gomphus fraternus* ("May"; 23,28 June), *Gomphus lineatifrons* (17 June), *Gomphus amnicola* (26 June-14 July), *Gomphus notatus* (6 August), *Gomphus furcifer* (3 larvae only), *Macromia alleghaniensis* (24-26 June; 8 September), *Macromia georgina* (11 June), *Macromia taeniolata* (no data), *Libellula axilena* (21 June), *Libellula deplanata* (no data), *Libellula exusta* (20 June), *Libellula needhami* (?) (7 August), *Calopteryx apicalis* (no data), *Enallagma piscinarium* (16 July). Except for *Gomphus viridifrons, G. fraternus, G. amnicola* and *Macromia alleghaniensis,* these are all single-specimen or one-time occurrences.



Montgomery's (12,14,15) suggestion that species with long flight-seasons also have wide geographical distribution is borne out by data displayed on Table 1 which show especially long flight seasons for such species as *Anax junius, Sympetrum rubicundulum* and *vicinum, Pachydiplax longipennis, Tramea carolina* and *lacerata, Enallagma aspersum* and *civile, Ischnura verticalis* and *Anomalagrion hastatum,* which are also very widely distributed. The converse of this is harder to demonstrate because species appearing from available data to have very short flight seasons may be characterized by small population-size or narrow habitat requirements, thereby reducing probability of their being collected, even though they may be present over quite a long season.

TABLE 4. *Seasonal Distribution in Pennsylvania of Main Flight Periods*[1] *of Some Major Genera of Odonata.*




[1] "period of maximum abundance" on Tables 1 & 2
Broken line indicates a species, rather than a genus.

## DISCUSSION

In a provocative paper on bioenergetics (31), Kennedy proposes correlations between evolutionary level and seasonal distribution of Odonata, and gives as evidence seasonal distribution tables for Indiana Odonata-Anisoptera. One of his theses is that primitive groups (families, genera) of Odonata have their main flight season at times of lower total environmental energy than do more advanced, or modern, groups. Kennedy's tables (31) show GOMPHIDAE, PETALURIDAE and CORDULEGASTRIDAE to follow this plan by having an early flight season, and AESHNIDAE to comply by flying mainly in late summer and autumn when environmental energy-levels are again lower. Most CORDULIINAE, which Kennedy recognized as primitive LIBELLULIDAE, are also shown to have an early, low energy, season, while other LIBELLULIDAE have their main flight season in mid-summer when environmental energy is at a peak.

Table 4 summarizes Tables 1 and 2 of the present paper for comparison with Kennedy's (31) results. The first 5 genera, *Aeshna* through *Cordulegaster,* comply generally with Kennedy's evidence, though two groups, the subgenus *Stylurus* and the genus *Dromogomphus,* appear to be seasonally disjunctive. One species of *Aeshna* and one of *Cordulegaster* can be seen to be discordant; they give rise to speculations on evolution (32) outside the range of this paper.

The genera *Epitheca* and *Somatochlora,* of the subfamily CORDULIINAE of the LIBELLULIDAE (11) can be seen in Table 4 to have longer normal flight season than any of the foregoing groups except *Aeshna* and *Cordulegaster,* and although Kennedy (31) did not suggest separately that more primitive Odonata have shorter flight seasons, consideration of information displayed on Tables 1 and 2 leads to this proposition, which is in harmony with Kennedy's thesis on thermal regulation since the period between April-May emergence and the mid-June beginning of highest-level environmental energy values is shorter than the highest energy-level period from mid-June to late August. *Libellula* and *Sympetrum* have even longer flight seasons than any of the CORDULIINAE, in keeping with their evolutionary modernity and evident adaptive success, but *Leucorrhinia* stands out as more primitive by having a shorter, earlier, flight season which is also in accord with Kennedy's (31) theory of latitudinal distribution since this northern Holarctic genus is quite specialized in its habitat requirements.

Kennedy (31), unfortunately, did not consider any Zygoptera in his stimulating study. The present tabulation of seasonal information on Pennsylvania Zygoptera set forth on Tables 1 and 4 indicates the modern evolutionary status of many genera at least, if Kennedy's theory is correct that species and higher groups flying chiefly in summer are more

advanced than those which fly in the spring and late summer-autumn period. The genera *Lestes, Argia, Enallagma* and *Ischnura* all have a flight period in Pennsylvania similar to that of *Libellula,* an acknowledged modern group, while one genus, *Calopteryx,* has a shorter flight period like that of the more primitive *Leucorrhinia.* One species of *Calopteryx,* however, the familiar, black-winged *C. maculata,* demonstrates its higher evolutionary level (inferred from Kennedy's standards) by having a flight season five weeks longer than that of the other 3 species (from which it also shows considerable structural discontinuity). On this basis it appears that the Zygoptera in general may be no less modern in their evolutionary level than are the LIBELLULIDAE. The common assumption (33) that Anisoptera are descended from a Zygoptera-like ancestor is not borne out by this seasonal information. Indeed, the probably polyphyletic origin of most Odonata families is well demonstrated by information displayed on Tables 1 and 4, unless Kennedy's energy theory is untenable. In regions with less temperature variation, such as Louisiana, where Bick (17) failed to find the same correlations brought out by Kennedy (31) for Indiana, it will be necessary to modify Kennedy's criteria for relative evolutionary position of the same taxa of which their status farther north, on Kennedy's terms, is much more clear. Considering only families and genera that occur in Pennsylvania, it appears that PETALURIDAE, GOMPHIDAE, CORDULEGASTRIDAE and LIBELLULIDAE, ascending in that order, are increasingly modern, while the AESHNIDAE represent another phyletic line, and the Zygoptera another.

## REFERENCES


1. Schiemenz, H. 1957. *Die Libellen Unserer Heimat.* Stuttgart (Germany), Kosmos. 154 p.
2. Valle, K. J. 1938. Zur Okologie der finnischen Odonaten. *Ann. Univ. Turku* (A) 6:1-76.
3. Wesenberg-Lund, C. 1913. Odonaten-Studien. *Int. Rev. ges. Hydrobiol. u. Hydrogr.* 6:155-228; 373-422.
4. Portmann, A. 1921. Die Odonaten der Umgebung von Basel. Inaugural Dissertation. Lorrach. 101 p.
5. Borror, D. J. 1944. An annotated list of the Odonata of Maine. *Can. Ent.* 76:134.
6. Borror, D. J. 1951. New records of Maine dragonflies. *Ent. News* 62:209.
7. Bick, G. H. 1957. The Odonata of Oklahoma. *Southwestern Naturalist* 2:1.
8. Lawrence, V. M. 1967. The distribution of Odonata in Indiana and Ohio. *N. Centr. Br. Entom. Soc. Amer. Proc.* 22:117.
9. Walker, E. M. 1941. List of the Odonata of Ontario, with distributional and seasonal data. *Roy. Can. Inst. Trans.* 23:201.
10. Kormondy, E. J. 1958. Catalogue of the Odonata of Michigan. Univ. Mich. Mus. Zool. Misc. Publ. 104. 43 p.
11. Beatty, G. H. & A. F. 1968. Check list and bibliography of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 42:120.
12. Montgomery, B. E. 1942. The distribution and relative seasonal abundance of the Indiana species of Enallagma. *Ind. Acad. Sci. Proc.* 51:273.
13. Montgomery, B. E. 1944. The distribution and relative seasonal abundance of the Indiana species of Agrionidae. *Ind. Acad. Sci. Proc.* 53:179.
14. Montgomery, B. E. 1945. The distribution and relative seasonal abundance of the Indiana species of Corduliidae and Libellulidae. *Ind. Acad. Sci. Proc.* 54:217.
15. Montgomery, B. E. 1947. The distribution and relative seasonal abundance of Indiana species of five families of dragonflies. *Ind. Acad. Sci. Proc.* 56:163.
16. Montgomery, B. E. 1948. The distribution and relative seasonal abundance of the Indiana species of Lestidae. *Ind. Acad. Sci. Proc.* 57:113.
17. Bick, G. H. 1957. The Odonata of Louisiana. *Tulane Studies in Zoology* 5:71.
18. Robert, A. 1953. Les Odonates du Parc du Mont Tremblant. *Can. Ent.* 85:316.
19. Valle, K. J. 1926. Turun Ymparistojen Sudenkorennoiset (The Odonata of the vicinity of Turku—in Finnish). *Ann. Univ. Turku* (A) 2:1-35.
20. Valle, K. J. 1928. Jaasken Sudenkorennoiset (The Odonata of the Parish of Jaaski—in Finnish). *Ann. Univ. Aboe* (A) 2:1-42.
21. Hiekkanen, A. I., and K. J. Valle. 1937. Hankasalmen Sudenkorennot (The Odonata of the Parish of Hankasalmi—in Finnish). *Ann. Zool. Soc. Zool.-Bot. Fenn. Vanamo* 5:1-35.
22. Valle, K. J. 1945. Sudenkorentoelaimistosta Viitasaaren pitajan pohjoisosassa (on the Odonata fauna of nish). *Suomen Hyonteistieteellinen Aikakauskirja* 11: the northern part of the Parish of Viitasaari—in Fin-Wales, Sydney; Handbook 12. 133 p.
23. White, H. B., III. 1963. Seasonal distribution and abundance of Odonata at a large pond in central Pennsylvania. *N.Centr.Br.Entom.Soc.Amer.Proc.* 18: 120.
24. White, H. B., III, and G. H. & A. F. Beatty. 1968. The Odonata fauna of Bear Meadows, a boreal bog in central Pennsylvania. *Pa. Acad. Sci. Proc.* 42:130.
25. Beatty, G. H. & A. F. 1968. Origins and biogeographic affinities of the Odonata fauna of Pennsylvania. *Pa. Acad. Sci. Proc.* 42:110.
26. Beatty, G. H. & A. F. 1969. Edaphic factors in the distribution of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 43:137.
27. Beatty, G. H. & A. F. Climatic factors in the distribution of Pennsylvania Odonata. *In preparation.*
28. Ahrens, C., and G. H. & A. F. Beatty. 1968. A survey of the Odonata of western Pennsylvania. *Pa. Acad. Sci. Proc.* 42:103.
29. Beatty, A. H. & A. F., and C. N. Shiffer. 1969. A survey of the Odonata of central Pennsylvania. *Pa. Acad. Sci. Proc.* 43:127.
30. Beatty, G. H. & A. F., and C. N. Shiffer. A survey of the Odonata of eastern Pennsylvania. *In preparation.*
31. Kennedy, C. H. 1928. Evolutionary level in relation to geographic, seasonal and diurnal distribution of insects. *Ecology* 9:367.
32. Beatty, G. H. & A. F. 1969. Evolution and speciation in the subgenus Schizuraeschna, with observations on *Aeshna (Schizuraeschna) mutata* Hagen. *Pa. Acad. Sci. Proc.* 43:147.
33. Fraser, F. C. 1957. A reclassification of the Order Odonata. Royal Zoological Society of New South 67-100.




# A SURVEY OF THE ODONATA OF CENTRAL PENNSYLVANIA


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## ABSTRACT

Eight-hundred seventy county records of 138 species of Odonata were tabulated for the 32 central Pennsylvania counties comprising the watersheds of the Susquehanna and Potomac Rivers. In this area of 21,362 square miles dragonflies have been collected at 221 locations from 1892 to 1968. The majority of the records are based on over 26,000 specimens collected since 1944 by G. H. Beatty and A. F. Beatty and their associates. Further sources of data are enumerated and documented, and noteworthy species are discussed.


## INTRODUCTION

In 1968 results were presented of a survey of the Odonata fauna of western Pennsylvania (1) wherein 127 species were recorded as occurring in the 22 western Pennsylvania counties included in the watershed of the Ohio River (Fig. 1), for a total of 791 county records

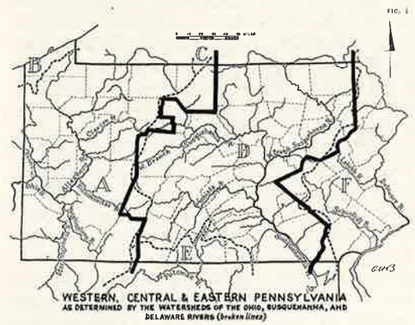



**Figure 1**

as such are defined in that publication and used in the present paper. Herein are recorded by counties the 138 species of Odonata now known to occur in the 32 counties of central Pennsylvania belonging to the watersheds of the Susquehanna and Potomac Rivers (Fig. 1). The Susquehanna drains the largest watershed of any U.S. river debouching on the Atlantic Seaboard and therefore it is of special interest in a current inquiry into the significance of river systems in the distribution of Odonata (2). This watershed is by far the largest such natural division of Pennsylvania, with about 21,000 square miles, in contrast to the roughly 16,000 square miles of the Ohio basin in western Pennsylvania and 6,000 square miles of the Delaware watershed in eastern Pennsylvania. The Odonata fauna of the latter region will be similarly reported on in the near future.

The idea of dividing Pennsylvania into 3 zones, the Odonata fauna of each of which is separately surveyed and reported on, may strike some workers as questionable, especially when it is assumed that those interested in Odonata faunistics of Pennsylvania as a whole will have to study 3 tables in order to learn the all-state distribution of any species. After a separate survey of one of these divisions had already been made (1) it appeared that such data should be presented in relatively brief form; in an all-state survey lists of county data for each species would be too extensive to be presented in tabular form. Further to justify this procedure the writers point out that Pennsylvania is unique in the eastern United States in being divisible into 3 major river basins which approximate North-South bands (Fig. 1). Taking these river basins as survey units is a step in the direction of analyzing distribution by natural, rather than political, boundaries (2). Insufficient information on adjoining states is an obstacle to immediate fulfillment of this aim. Those interested in an all-state list of Odonata records by counties will be




**Figure 5.** See Table 1.

served by a summary to be published following the forthcoming survey of the Odonata of eastern Pennsylvania.

The physiographic diversity of Pennsylvania (3-5) undoubtedly contributes to the richness of its Odonata fauna of about 170 species (6), and this edaphic diversity in the 32 counties of the Susquehanna basin may well be more significantly responsible for the large number of species now known than is the extensive geographical area involved. (Climatic diversity in this part of Pennsylvania has already been discussed (5), and this, and biotic features, will be further treated in forthcoming papers.) The Susquehanna basin (Fig. 2) includes glaciated and unglaciated Appalachian Plateau Province, glaciated and unglaciated Ridge and Valley Province (with its interesting subdivision, the Great Valley), a generous portion of the Piedmont Province, and all of the Blue Ridge Province that occurs in Pennsylvania (3,4,7).

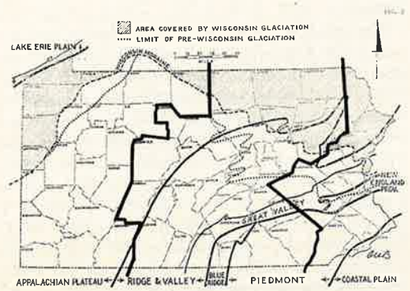



**Figure 2**

In contrast, western Pennsylvania, already surveyed for Odonata (1), lies almost entirely within the Appalachian Plateau Province, part of which was covered by Wisconsin glaciation (Fig. 2). In western Pennsylvania have been found 127 species of Odonata (1), 12 of which are not known to occur in central Pennsylvania, but in this central Pennsylvania region 138 species are now known to occur, and 23 of these are not on the list (1) of western Pennsylvania Odonata. Thus the Ohio and Susquehanna basins together are now known to support 150 Odonata species in Pennsylvania, of which they have 115 in common. The 35 species not common to both watersheds may prove to be the most meaningful ones to investigate in attempting to elucidate the significance of river systems and watersheds in the geographical distribution of Odonata. Comparison of the present list with that already published (1) for western Pennsylvania will reveal the identity of these species, which will also be listed in a future summary paper. At a later date, when composition of the Odonata fauna of eastern Pennsylvania has been reported on, further analysis of these elements will be undertaken.

## PROCEDURE

Inasmuch as this survey summarizes the gradual accumulation of data on Odonata occurrences for more than 90 years, it cannot be constructed precisely parallel to the previously published (1) western Pennsylvania survey wherein comparison was made between already existing knowledge, both published and otherwise, and the results of an intensive 1-year fieldwork project undertaken by one of the authors. Therefore the present procedure is to give the summary of data on the occurrence of Odonata in the Susquehanna basin in central Pennsylvania in the same form as that used for western Pennsylvania (1) so that the two can readily be compared or combined.

Pennsylvania collecting locations for Odonata have been indicated on the map (Fig. 3) as fully as possible, but in some counties one spot has to represent two or more collecting sites because of space limitations. Odonata have been collected in about 650 locations in Pennsylvania, which are represented by about 520 spots on the map. Approximately 221 of these 650 collection locations are in the 32 central Pennsylvania counties allocated to the Susquehanna-Potomac basins. It is impossible to be strictly accurate about this since watershed lines and county lines do not often coincide, and collecting locations can be subdivided or fused, depending on personal opinion. So many collectors have been involved in this work that uniformity in this instance is impossible.

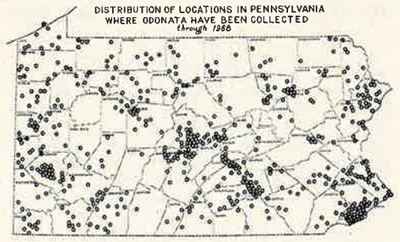



**Figure 3**



The nature of work at these central Pennsylvania locations varies greatly. Many have been visited only once and only one or a few species collected. Several dozen, at least, have been visited repeatedly, and two of these have been the subject of long-term studies that have resulted in published reports (8,9) on Ten Acre Pond and Bear Meadows in Centre County. Except at these two sites no program of continuing observations have been pursued at any central Pennsylvania Odonata habitat.

Number of known species per county is shown on a map (Fig. 4) and also on Table 1.

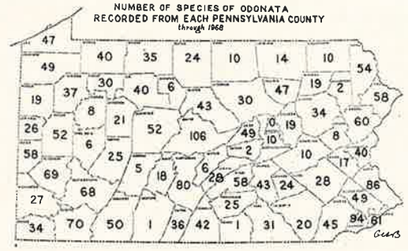



**Figure 4**

This map, and Figure 3, make it possible to determine the extent of fieldwork in each county. Since it appears that at least 100 species of Odonata could be collected in any central Pennsylvania county (Centre County now has 106), the total of 870 county records from the Susquehanna-Potomac basins, out of a "possible" 3,200, would show present results to be about 27% "complete," in contrast to results from western Pennsylvania which are looked upon as about 38% "complete" (1). These percentages are merely suggestive; a main purpose of this paper is to promote more informed and better-planned fieldwork on central Pennsylvania Odonata.

While the maps (Figs. 3,4) indicate the activity of Odonata collectors in central Pennsylvania, it is necessary to refer to Table 2 for data on the Odonata *per se*. While numbers following species-names refer to specific counties, and can serve as exact data on the county level, one of the purposes of Table 2 is to show graphically the relative incidence of the various species of Odonata now known to occur in central Pennsylvania. Species the names of which are followed by many county numbers are "common" or "widespread" roughly in proportion to the number of counties indicated. Species with few county numbers are for the same reason "rare" or hard to collect or both. It is these species that must be carefully studied if distributional factors are to be better understood.

TABLE 1. *Counties of "Central" Pennsylvania (Susquehanna-Potomac Watershed).* See Fig. 5.

| COUNTY NO. (see Table 2) | | NO. OF SPECIES RECORDED | NO. OF COLLECTING LOCATIONS |
|---|---|---|---|
| 23 | ADAMS | 1 | 1 |
| 24 | BEDFORD | 1 | 1 |
| 25 | BLAIR | 18 | 5 |
| 26 | BRADFORD | 14 | 4 |
| 27 | CAMBRIA | 5 | 1 |
| 28 | CAMERON | 6 | 4 |
| 29 | CENTRE | 106 | 40 |
| 30 | CLEARFIELD | 52 | 12 |
| 31 | CLINTON | 43 | 5 |
| 32 | COLUMBIA | 19 | 3 |
| 33 | CUMBERLAND | 25 | 13 |
| 34 | DAUPHIN | 43 | 14 |
| 35 | FRANKLIN | 42 | 8 |
| 36 | FULTON | 36 | 3 |
| 37 | HUNTINGDON | 80 | 22 |
| 38 | JUNIATA | 28 | 7 |
| 39 | LACKAWANNA | 2 | 2 |
| 40 | LANCASTER | 20 | 6 |
| 41 | LEBANON | 24 | 5 |
| 42 | LUZERNE | 34 | 6 |
| 43 | LYCOMING | 30 | 7 |
| 44 | MIFFLIN | 6 | 2 |
| 45 | MONTOUR | 0 | 0 |
| 46 | NORTHUMBERLAND | 10 | 2 |
| 47 | PERRY | 58 | 13 |
| 48 | SNYDER | 2 | 1 |
| 49 | SULLIVAN | 47 | 10 |
| 50 | SUSQUEHANNA | 10 | 4 |
| 51 | TIOGA | 10 | 6 |
| 52 | UNION | 49 | 5 |
| 53 | WYOMING | 19 | 3 |
| 54 | YORK | 31 | 6 |
| | | 870 | 221 |

## SOURCES OF DATA

To explain the origin of the data entered on Table 2 it is necessary to review the history of Odonata investigations in central Pennsylvania. Although the Odonata fauna of southeastern Pennsylvania was reported on by Calvert in 1893 (10), and that of western

Table 2. ODONATA OF "CENTRAL" PENNSYLVANIA (Susquehanna-Potomac Watershed).

| | No.[1] | Species | County Number[2] |
|---|---|---|---|
| | 2 | *Gomphaeschna furcillata* | 29,31,49 |
| | 3 | *Basiaeschna janata* | 29,32,34,35,36,37,38,43,47,52 |
| # | 4 | *Boyeria grafiana* | 28,29,30,36,*37*,43,47 |
| | 5 | ——— *vinosa* | 29,30,32,33,36,37,40,41,43,47,50,54 |
| | 7 | *Epiaeschna heros* | 29,30,*31*,34,*36*,*37*,40,47 |
| | 8 | *Aeshna canadensis* | 26,*27*,29,30,37,38,42,47 |
| * | 9 | ——— *clepsydra* | 49 |
| | 10 | ——— *constricta* | 29 |
| # | 11 | ——— *mutata* | 29,33,37 |
| | 12 | ——— *tuberculifera* | 29,30,37,42,52 |
| | 13 | ——— *umbrosa* | 29,30,31,32,33,34,36,37,40,*42*,43,46,47,50,53 |
| | 14 | ——— *verticalis* | *27*,29,30,33,37,42,47 |
| | 15 | *Anax junius* | 25,26,29,30,31,32,33,34,35,36,37,38,40,41,42,*43*, 47,49,54 |
| | 16 | —— *longipes* | 29,*37*,*54* |
| | 17 | *Tachopteryx thoreyi* | 29,*33*,34,37 |
| # | 18 | *Progomphus obscurus* | 34,38,47 |
| | 19 | *Hagenius brevistylus* | 29,32,33,34,35,36,*37*,38,43,44,47,52 |
| * | 20 | *Ophiogomphus carolus* | 29,31,32,43,49,50 |
| *# | 21 | ——————— *edmundo* | 34 |
| *# | 22 | ——————— *howei* | 33 |
| | 23 | ——————— *mainensis* | 30,31,37,43,47,49 |
| | 24 | ——————— *rupinsulensis* | 33,34,47,53 |
| | 25 | *Lanthus albistylus* | 29,43,46,47,52,54 |
| | 26 | ——— *parvulus* | 29,30,31,37,41,43,47,49,51,52,54 |
| | 27 | *Gomphus borealis* | 29,30,31,37,49,51,52 |
| | 28 | ——— *descriptus* | 29,30,37,43,49,52 |
| | 29 | ——— *exilis* | 25,29,32,34,36,37,38,41,42,43,47,49,50,52 |
| | 30 | ——— *lividus* | 29,30,33,34,35,36,37,38,40,47,52 |
| | 31 | ——— *quadricolor* | 34,38,43 |
| | 32 | ——— *spicatus* | 29,49,53 |
| * | 33 | ——— *abbreviatus* | 37,47,52 |
| | 34 | ——— *brevis* | 28,31,34,43,49,52 |
| # | 36 | ——— *fraternus* | 37 |
| *# | 37 | ——— *lineatifrons* | 47 |
| # | 38 | ——— *rogersi* | 29,30,36,37 |
| | 39 | ——— *vastus* | 33,34,38,47,54 |
| *# | 40 | ——— *ventricosus* | 34,54 |
| *# | 41 | ——— *amnicola* | 34,47 |
| # | 44 | ——— *scudderi* | 30 |
| | 45 | ——— *spiniceps* | 33,47 |
| | 47 | ——— *villosipes* | 29,30,31,32,33,34,35,37,*47*,49,52,*53*,54 |
| | 48 | *Dromogomphus spinosus* | 33,34,35,36,38,43,46,47,52,54 |
| | 49 | *Cordulegaster diastatops* | 29,30,31,37,42,47,49,52 |
| | 50 | ——————— *erroneus* | 28,29,34,37,47 |
| | 51 | ——————— *maculatus* | 29,30,31,37,44,47,49,52 |
| | 52 | ——————— *obliquus* | 29,37,47 |
| | 55 | *Macromia illinoiensis* | 28,29,*31*,34,35,36,37,*38*,43,46,47,52 |
| | 57 | *Didymops transversa* | 29,31,33,34,35,37,38,40,42,47,49,52 |
| * | 58 | *Neurocordulia obsoleta* | 34 |
| *# | 59 | ——————— *yamaskanensis* | 29,47 |
| | 60 | *Epitheca canis* | 29,31,32,37,42,49 |
| | 61 | ——— *cynosura* | 25,29,30,31,32,34,35,36,37,38,42,47,49,52 |
| | 62 | ——— *princeps* | 24,*29*,34,*35*,*36*,*38*,42,43,47,49,53,*54* |
| *# | 63 | ——— *spinigera* | 26,43 |
| | 65 | *Helocordulia uhleri* | 29,30,31,37,43 |
| # | 66 | *Somatochlora elongata* | 29,30,52 |
| * | 68 | ——————— *forcipata* | 31,51 |
| | 69 | ——————— *linearis* | 29 |
| | 70 | ——————— *tenebrosa* | 27,29,30,37,40,41,42 |
| # | 71 | ——————— *walshi* | 29,31,42 |
| | 73 | *Cordulia shurtleffi* | 29,30,31,37,42,43,47,49,53 |
| * | 74 | *Dorocordulia lepida* | 29 |
| | 75 | ——————— *libera* | 29,31,37,42,49,52 |
| * | 76 | *Libellula auripennis* | 29,54 |
| *# | 77 | ——————— *axilena* | 29 |
| | 78 | ——————— *cyanea* | 29,32,35,*36*,37,38,41,47,52,*54* |
| | 81 | ——————— *flavida* | 34 |
| | 82 | ——————— *incesta* | 49 |
| | 83 | ——————— *julia* | *26*,29,30,31,32,37,*42*,*43*,49,52,53 |
| | 84 | ——————— *luctuosa* | 25,29,30,31,32,34,35,36,37,*38*,40,41,42,46, 47,52,*53*,*54* |
| | 85 | ——————— *lydia* | *25*,26,27,29,30,31,32,34,35,36,37,40,41,42, 43,47,48,49,*50*,51,*52*,54 |
| | 87 | ——————— *pulchella* | 25,26,29,30,31,32,33,34,35,36,37,38,40,41, 42,43,47,49,51,52,*53*,*54* |

(continues on next page)



Table 2. Continued.

| | No. | Species | County Number |
|---|---|---|---|
| | 88 | ——————— *quadrimaculata* | 29,31,37,42 |
| | 89 | ——————— *semifasciata* | 29,30,31,35,37,38,42 |
| * | 90 | ——————— *vibrans* | 29,47 |
| | 91 | *Perithemis tenera* | 29,30,32,33,34,35,36,37,41,42,46,47,53,54 |
| | 95 | *Erythemis simplicicollis* | *25*,29,30,31,32,35,36,37,38,41,*47*,52,*53*,54 |
| | 98 | *Sympetrum internum* | 29,31 |
| | 99 | ——————— *obtrusum* | 29,30,31 |
| | 100 | ——————— *rubicundulum* | 29,30,31,35,36,37,40,41,47,49,51,52,*53* |
| | 101 | ——————— *semicinctum* | 29,30,35,36,37,43,47 |
| | 102 | ——————— *vicinum* | 27,29,30,31,32,35,36,37,41,47,53,54 |
| | 103 | *Pachydiplax longipennis* | 26,29,30,35,36,37,38,41,42,46,47,49,52,54 |
| | 104 | *Leucorrhinia frigida* | 29,30,31,37,42,49,50 |
| *# | 105 | ——————— *glacialis* | 29,30 |
| # | 106 | ——————— *hudsonica* | 29,31,37,49 |
| | 107 | ——————— *intacta* | 25,29,30,31,32,35,36,37,38,41,42,*47*,49,52 |
| *# | 108 | ——————— *proxima* | 42 |
| | 109 | *Celithemis elisa* | 29,30,35,40,41,49,*53*,54 |
| | 110 | ——————— *eponina* | 39,41,52,54 |
| | 112 | *Pantala flavescens* | 29,30,*33*,37,40,47,*53*,54 |
| | 113 | ——— *hymenea* | 29 |
| | 114 | *Tramea carolina* | 29,*35*,*37*,41 |
| | 115 | ——— *lacerata* | *25*,29,34,35,37,*41*,47,54 |
| * | 116 | *Calopteryx aequabilis* | 29,44,52 |
| | 117 | ——————— *amata* | 28,29,30,37,42,49,50,51,52 |
| # | 118 | ——————— *angustipennis* | 28,29,35,52 |
| * | 119 | ——————— *apicalis* | 40 |
| | 120 | ——————— *maculata* | 25,29,*30*,31,33,35,36,37,40,41,44,47,48,49, 50,51,52 |
| | 121 | *Hetaerina americana* | 38,40,43,47,54 |
| | 122 | ——————— *titia* | 33 |
| | 123 | *Archilestes grandis* | 29 |
| | 124 | *Lestes congener* | 29,30 |
| | 126 | ——— *d. australis* | 29,30,37 |
| | 127 | ——— *dryas* | 29,42,49 |
| | 128 | ——— *eurinus* | 29,30,31 |
| | 129 | ——— *forcipatus* | 29,31,37 |
| | 130 | ——— *inequalis* | 29,35,37,41,49,52 |
| | 131 | ——— *rectangularis* | 29,30,34,35,36,37,40,46,47,52 |
| | 132 | ——— *unguiculatus* | 29,37 |
| | 133 | ——— *vigilax* | 25,26,29,37,49,52 |
| | 134 | *Argia apicalis* | 34,37,38,47,54 |
| | 136 | ——— *moesta* | 25,26,29,34,36,37,38,40,43,44,47,52,54 |
| | 137 | ——— *sedula* | 34,35,37,47 |
| | 139 | ——— *translata* | 37,47,54 |
| | 140 | ——— *violacea* | 25,29,30,33,34,35,36,37,40,47,49,50,52 |
| | 141 | *Amphiagrion saucium* | 25,29,30,34,37,46,49,52,54 |
| * | 142 | *Nehalennia gracilis* | 29,37,52 |
| | 144 | ——————— *irene* | 23,29,31,34,37,42,49,51,52,53 |
| | 145 | *Chromagrion conditum* | 25,29,31,35,37,41,42,43,49,52 |
| # | 146 | *Enallagma antennatum* | 29 |
| | 147 | ——————— *aspersum* | 29,33,36,37 |
| | 148 | ——————— *basidens* | 35 |
| *# | 149 | ——————— *boreale* | 29 |
| | 150 | ——————— *carunculatum* | 29,35 |
| | 151 | ——————— *civile* | 25,29,30,35,36,37,38,42,47 |
| | 152 | ——————— *cyathigerum* | 29,31,49 |
| | 154 | ——————— *divagans* | 29,34,35,36,37 |
| | 157 | ——————— *ebrium* | 26,29,42,49,52,53 |
| | 158 | ——————— *exsulans* | 26,29,33,34,35,36,37,38,43,44,47,49,52,54 |
| | 159 | ——————— *geminatum* | 29,35,38,52 |
| | 160 | ——————— *hageni* | 25,26,29,30,31,33,34,35,36,37,41,42,43,49,52 |
| *# | 161 | ——————— *laterale* | 49 |
| | 163 | ——————— *signatum* | 29,30,35,36,37,53,54 |
| | 165 | ——————— *vesperum* | 26,29,49 |
| | 166 | *Ischnura posita* | 29,34,35,37,49,52 |
| | 169 | ——————— *verticalis* | 25,26,29,30,31,33,34,35,36,37,38,39,40,41,42 43,46,47,49,50,51,52,53,54 |
| | 170 | *Anomalagrion hastatum* | 29,34,37 |

*not known from western Pa. #not known from eastern Pa.

1Species numbers as found in Pa. Check List, 1968 (6). 2County numbers following species refer to Table 1. County numbers in italics refer to "sight records."

Pennsylvania by Williamson in 1902 (11), the Odonata of the vast region lying between these two reported areas has never been the subject of a comprehensive study (6).

First specific mention of any Odonata from the region to which this survey is limited was by Hagen in 1875 (12) and 1878 (13), who recorded *Lanthus parvulus* Selys and described *Lanthus albistylus* Hagen (under the name *Gomphus naevius)* from "Berks Co., and York Co., Pennsylvania". Hagen's role in reporting on the Odonata fauna of central Pennsylvania was fortuitous, and it is possible that the two names above refer to the same single specimen.

In 1898 C. W. Johnson, later a famous New England dipterist and malacologist, made a collection of Odonata at North Mountain, Pennsylvania, which was reported on by P. P. Calvert (14). In manuscript notes made available to the authors (6) Calvert states that North Mountain is in Wyoming County. The present village of North Mountain is, however, in Lycoming County. Calvert's paper reports *Ophiogomphus rupinsulensis* Walsh, *Gomphus spicatus* Hagen and *Cordulia shurtleffi* Scudder, all for the first time in Pennsylvania. From 1909 to 1924 Calvert reported (15,16,17, 18,19,20,21,22) on much interesting Odonata material from central Pennsylvania, most of which was collected by the staff of the Division of Zoology of the Pennsylvania Department of Agriculture, Harrisburg, under Professors H. A. Surface and J. G. Sanders. Much of this Odonata material was collected by or at the behest of V. A. E. Daecke, who had previously been associated with Calvert at Philadelphia. Names later well known in entomology, such as W. R. Walton, J. N. Knull, H. P. Bailey, H. B. Kirk, A. B. Champlain, and often that of Daecke himself, are on the labels of specimens from Charter Oak (Huntingdon County), Marysville, Camp Hill, Lemoyne, Inglenook, Dauphin, Heckton Mills, Weaver, Perdix, West Fairview, Linglestown, etc., all except Charter Oak being in the vicinity of Harrisburg. Many of the first Pennsylvania records of rare species (such as nos. 11, 22, 31, 37, 40, 41, 59, 81 in Table 2) are found in Calvert's reports (15-22) on material collected by his friend Daecke or as the result of Daecke's enthusiasm for increasing knowledge of these insects. Daecke's death in 1918 and the departure of most of his Odonata-collecting colleagues terminated this period of enthusiastic work on central Pennsylvania Odonata. Daecke was immortalized by Calvert in the name of the exquisite *Enallagma daecki* Calvert, a curious, attenuated damselfly found along the Atlantic Coastal Plain, and once in Pennsylvania (6).

State College, Centre County; and The Pennsylvania State University, have long been a headquarters for work on Odonata of central Pennsylvania. In the 1930's Professor S. W. Frost began a collection of insects which has since become the Frost Entomological Museum at The Pennsylvania State University, Department of Entomology. From its inception this collection has contained much important Odonata material, most of which is from central Pennsylvania. Beginning in the 1940's, when John Gillespie and G. H. Beatty began to take special interest in the Odonata material in this collection, Professor Frost increased his efforts to build up this part of the collection, and on many fields trips he went out of his way to secure Odonata material. This material, collected by Frost himself and by numerous colleagues and students, is a most important resource in the present survey of the Odonata fauna of central Pennsylvania. Among the collectors of central Pennsylvania Odonata specimens in the Frost Entomological Museum are J. O. Pepper, H. M. Tietz, B. F. Coon, C. W. Rutschky, V. R. Haber, L. Stannard, I. C. Aurelio, C. J. Hillson, M. Goodrich, R. L. Campbell, R. R. Brown, and many others to be enumerated below who collected primarily for G. H. & A. F. Beatty.

In the 1940's John Gillespie did important fieldwork on Odonata in central Pennsylvania, records of which are here included, and it was he who inspired G. H. Beatty to commence in this region an Odonata study (23) that has continued for 24 years. Twenty six thousand Odonata specimens, collected by or for G. H. & A. F. Beatty in central Pennsylvania during this 24-yr period form the nucleus of the present survey. A number of students at The Pennsylvania State University, and others, have gone out of their way to help the present authors with fieldwork and collecting in central Pennsylvania. Notable among these have been J. A. Chemsak, G. A. Bryan, Jr., W. R. Holt, C. Ahrens, E. M. Craighead, E. N. Brown, R. R. Brown, T. Birtley, J. O. Schmidt, J. M. Runner, M. H. Runner, R. L. Campbell, J. Hvizdash, Jr., J. Ellenberger, L. D'Achille, R. Divany, D. Kauch, S. Couch, and K. C. Schuyler, Jr. The Odonata collection of G. H. & A. F. Beatty, which contains the collections of J. Gillespie and R. A. Raff and material collected by most of the foregoing contributors, stands as the foremost collection of central Pennsylvania Odonata. Much of this material has been shared with the Frost Entomological Museum and many other collections.



Of immense importance in increasing knowledge of the Odonata fauna of central Pennsylvania has been the recent work of H. B. White, III, R. A. Raff, and C. N. Shiffer. Much of White's work is summarized in recent publications (8,9), but hundreds of his other records are essential components of the present survey. Raff's work in central Pennsylvania (his equally important results in western Pennsylvania have already been reported (1)) and that of C. N. Shiffer (24) are reported in the present paper and in a current contribution on Odonata of the Potomac drainage in Pennsylvania (25). Collections made by C. Cook in 1951 and by T. W. Donnelly in 1953 and 1959 have contributed important data to the present survey. Miscellaneous records contained in publications by Hostetter and Derickson (26), E. M. Walker (27,28) and Westfall and Trogdon (29) have also been included in the present survey.

## NOTEWORTHY ODONATA SPECIES

A considerable number of the species listed herein (Table 2) as comprising the known Odonata fauna of the Susquehanna-Potomac watersheds in Pennsylvania are especially noteworthy for a variety of reasons. Some appear to be exceedingly rare throughout their ranges, which may be quite limited. *Ophiogomphus edmundo* Needham is known only from the male holotype from North Carolina and the female allotype from Inglenook, Dauphin County, Pennsylvania. In the original description (33) Needham states that these specimens are in many ways similar to *O. aspersus* Morse, but he satisfied himself that they are specifically distinct and both of the same undescribed species. *Ophiogomphus howei* Bromley is another excessively rare species known from the holotype female from Massachusetts and an allotype male from Lemoyne, Cumberland County, Pennsylvania, described by Calvert in 1924 (22) without any proof that it was conspecific with the Massachusetts holotype. The writers have heard news of the recent rediscovery of this species in Pennsylvania. *Gomphus quadricolor* Walsh is more abundant in other parts of its range, which extends from New Hampshire as far west as Wisconsin and south to Tennessee and Alabama, but it has proved so uncommon in Pennsylvania that only 5 specimens have been collected, and at widely scattered locations: in Dauphin (18), Juniata and Lycoming counties in central Pennsylvania, and in Bucks and Forest counties in eastern and western Pennsylvania, respectively; and only one specimen at each location. *Gomphus abbreviatus* Hagen is another species seldom collected, though the writers have found it emerging by hundreds at one location in eastern Pennsylvania. In central Pennsylvania it has been collected at several locations in Union and Perry counties, usually as larvae which were subsequently reared. *Gomphus lineatifrons* Calvert, like *G. quadricolor,* is better known outside Pennsylvania, but over a smaller range from Michigan, Ohio and Illinois, south to Tennessee and Alabama. The only Pennsylvania specimens are two females in the original type series from Weaver, Perry County, collected by Daecke in 1917 (21). It has been searched for many times since, but never found. *Gomphus rogersi* Gloyd has a peculiar history. Originally described from North Carolina, the name of this species fell into synonymy, and it was not until the true *Gomphus consanguis* Selys, with which it had been confused, was identified in 1962 (29) that the name *rogersi* was restored to its rightful place. Meanwhile *rogersi* was discovered in Pennsylvania and is now known from five counties in western Pennsylvania (1) and from Clearfield, Huntingdon and Fulton counties in central Pennsylvania. The description of the larva of *rogersi* (29) is based on this central Pennsylvania material. Known only from Pennsylvania, North Carolina and Tennessee, *Gomphus rogersi* appears to have its metropolis in Pennsylvania, where it is known from at least 10 locations. *Gomphus ventricosus* Walsh has been found in scattered locations from Nova Scotia to Wisconsin but not farther south than Ohio and Indiana. It is uncommon everywhere in its range and is seldom encountered by collectors. The only Pennsylvania records are from Dauphin and York counties, where Daecke collected all but one of the 9 specimens between 1912 and 1916 (19). *Gomphus amnicola* Walsh is chiefly a mid-western species, extending into Massachusetts and southern Canada, but its occurrences are scattered and its numbers usually small. The only Pennsylvania collections of *amnicola* date back to 1909-1912, when 7 specimens were collected along the Susquehanna River near Harrisburg by Daecke and Kirk (18,19). *Gomphus scudderi* Selys has a limited distribution north and east of the Great Lakes, and in the southern Appalachians. In Pennsylvania *scudderi* has been found by Carsten Ahrens (1) at several locations in the Ohio River basin. In central Pennsylvania one specimen was collected at Shawville, Clearfield County, by John Bauer

of the Carnegie Museum, who has also collected many other important Odonata specimens at this and other Pennsylvania locations. *Neurocordulia yamaskanensis* Provancher, like other species of this mysterious genus, is seldom met with by Odonata collectors. In southeastern Canada it frequents the shores of lakes, but it is a river species farther south. In central Pennsylvania 3 specimens were taken by Daecke at Perdix, Perry County, 1910 and 1913 (16). The present authors collected an exuviae identified as this species on Penn Creek, Centre County, 1957. *Enallagma laterale* Morse is something of a rarity in its range along the Atlantic seaboard from Long Island north to Maine, except for occasional local abundance on Cape Cod, Massachusetts (34). Its disjunctive occurrence at a glacial pond in Sullivan County, Pennsylvania, was therefore surprising when it was discovered in 1956, and 24 specimens collected on 29 June and 8 July. Regrettably, this location has not been revisited.

Another type of noteworthy occurrence of Odonata in central Pennsylvania concerns species that are common enough in their ranges to the North, but which are so peripheral in Pennsylvania that they are found only rarely and in very specialized habitats (5): *Aeshna clepsydra* Say (1 specimen from Sullivan Co.); *Epitheca spinigera* Selys (2 specimens from Bradford Co., through Carl Cook; 2 from Lycoming Co. by H. B. White, III); *Somatochlora forcipata* Scudder (7 specimens from Tamarack, Clinton Co., by Frost, Raff and White; 1 from Tioga Co. by Beatty; 1 specimen from Monroe Co., through Carl Cook); *Leucorrhinia glacialis* Hagen (common at one location in Centre Co. (8); otherwise found in Pennsylvania only once, at one location in Clearfield Co.); *Leucorrhinia proxima* Calvert (only a single male specimen known from Pennsylvania, collected by G. H. & A. F. Beatty at Red Rock, Luzerne Co., 17 June, 1957); *Enallagma antennatum* Say (an abundant species in an area surrounding the Great Lakes and including western Pennsylvania; included here, however, because it is known from only one location in central Pennsylvania); *Enallagma boreale* Selys (one central Pennsylvania specimen, from Centre County, collected by G. H. Beatty, 16 June, 1955; also known from 5 specimens collected in Monroe Co. in 1906 and 1907 and in the Calvert Collection).

Several species often thought of as being more northern or boreal in their affinities are quite at home in specialized habitats in central Pennsylvania. *Gomphus borealis* Needham, described from the Adirondack Mountains, New York, appears to have an affinity for nonnatural ponds in central Pennsylvania, including artificial impoundments and beaver-dam ponds. It has been found in abundance at many of these in Centre, Clearfield, Clinton, Huntingdon, Union, Sullivan and other counties. *Somatochlora walshi* Scudder has a parallel affinity for sphagnum bogs and is often quite abundant at such habitats in Centre and Clinton counties. At Bear Meadows in Centre County it appears to be as abundant as it is anywhere in its northward-reaching range (9). *Calopteryx aequabilis* Say, with extensive distribution north and west of Pennsylvania, has been found in Pennsylvania at only two locations, though it is abundant year after year at both. One location, a medium-sized stream, the Unami Creek, is in southeastern Pennsylvania, while the other, in the Susquehanna basin presently under discussion, is a small river, Penn Creek in Centre, Mifflin and Union counties. At both of these locations *aequabilis* has been found repeatedly in substantial numbers, appearing as thoroughly at home as anywhere in its range. *Enallagma basidens* Calvert is a species originating in southwestern United States which has only recently been found in Pennsylvania, where it is now known from all watershed divisions of the state. A more detailed discussion of this phenomenon is being presented elsewhere (25). Another southern species that has appeared in Pennsylvania, though only once, is *Libellula axilena* Westwood, of which a single specimen was collected at Bear Meadows, Centre County, by G. H. Beatty in 1946 (9).

Adventive species from the Coastal Plain are few in central Pennsylvania, but more numerous in the Delaware River basin in the eastern part of the state (2). *Libellula auripennis* Burmeister, *Dorocordulia lepida* Hagen and *Calopteryx apicalis* Burmeister are Coastal Plain species each of which has been taken once or twice in central Pennsylvania (9,15).

Finally, in discussing some of the noteworthy species in the Odonata fauna of central Pennsylvania, it is necessary to refer to two species reported to be uncommon throughout their ranges, which have been found at one central Pennsylvania location in remarkable abundance. One of these, *Aeshna mutata* Hagen, is of such great interest that it is the subject of a separate paper (30), though its presence at Ten Acre Pond, Scotia, Pennsylvania, has already been reported (8). The other species, *Anax longipes* Hagen has been reported from this same location (8) but the remarkable features of this occurrence were not commented on. *Anax longipes* is known to occur over a very wide range in North, Central and South America, but verified large populations and established colonies are not documented in the many reports (31,32). At Ten Acre Pond, Scotia, Centre County, Pennsylvania, a substantial population of *longipes* has been under observation from 1959 through 1968. In 1960 a minimum number of emergences was established by 40 exuviae collected by H. B. White, III, and G. H. Beatty between 8 June and 6 August. Since exhaustive collecting was not attempted, the total number of adult *longipes* at this location must have been much greater than 40, even taking into account the mishaps that emerging Odonata are heir to. It might be added that possibly half of the specimens of *Anax longipes* in the world have been collected at this central Pennsylvania pond.



## ACKNOWLEDGMENTS

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## REFERENCES

1. Ahrens, C., and G. H. & A. F. Beatty. 1968. A survey of the Odonata of western Pennsylvania. *Pa. Acad. Sci. Proc.* 42:103.
2. Beatty, G. H. & A. F. 1969. Edaphic factors in the distribution of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 43:137.
3. Fenneman, N. M. 1938. *Physiography of Eastern United States*. McGraw-Hill Book Co., New York. 714 p.
4. Ashley, G. H. 1933. The Scenery of Pennsylvania. Penna. Geol. Surv. (4th series) Bull. G 6. 91 p.
5. Beatty, G. H. & A. F. 1968. Origins and biogeographic affinities of the Odonata fauna of Pennsylvania. *Pa. Acad. Sci. Proc.* 42:110.
6. Beatty, G. H. & A. F. 1968. Check list and bibliography of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 42:120.
7. Leverett, F. 1934. Glacial Deposits in Pennsylvania. Penna. Geol. Surv. (4th series) Bull. G 7. 123 p.
8. White, H. B., III. 1963. Seasonal distribution and abundance of Odonata at a large pond in central Pennsylvania. *N. Centr. Br., Entom. Soc. Amer., Proc.* 18:120.
9. White, H. B., III, and G. H. & A. F. Beatty. 1968. The Odonata fauna of Bear Meadows, a boreal bog in central Pennsylvania. *Pa. Acad. Sci. Proc.* 42:130.
10. Calvert, P. P. 1893. Catalogue of the Odonata (dragonflies) of the vicinity of Philadelphia, with an introduction to the study of this group of insects. *Amer. Ent. Soc. Trans.* 20:152a-272.
11. Williamson, E. B. 1902. A list of the dragonflies observed in western Pennsylvania. *Ent. News* 13:65,108.
12. Hagen, H. A. 1875. Synopsis of the Odonata of America. *Boston Soc. Nat. Hist. Proc.* 18:20.
13. Hagen, H. A. 1878. 4th additions to Synopsis des Gomphines (by E. de Selys Longchamps). *Acad. Belg. Bull.* (2) 46:459.
14. Calvert, P. P. 1898. [on some dragonflies from North Mtn., Penna.]. *Ent. News* 9:184.
15. Calvert, P. P. 1909. [new record of *Calopteryx apicalis* Burm. for Penna.]. *Ent. News* 20:186.
16. Calvert, P. P. 1910. [*Neurocordulia yamaskanensis* Provancher new for Penna.]. *Ent. News* 21:429.
17. Calvert, P. P. 1911. [*Aeshna mutata* Hagen new for Penna.]. *Ent. News* 22:336.
18. Calvert, P. P. 1916. [on *Gomphus amnicola* and *quadricolor* from Penna.]. *Ent. News* 27:380.
19. Calvert, P. P. 1918a. [exhibit of Odonata from central Penna.]. *Ent. News* 29:198.
20. Calvert, P. P. 1918b. [exhibit of Odonata from central Penna.]. *Ent. News* 29:278.
21. Calvert, P. P. 1921. *Gomphus dilatatus, vastus* and a new species, *lineatifrons*. *Amer. Ent. Soc. Trans.* 47:221.
22. Calvert, P. P. 1924. The supposed male of *Ophiogomphus howei* Bromley. *Ent. News* 35:265.
23. Beatty, G. H., III. 1946. Dragonflies (Odonata) collected in Pennsylvania and New Jersey in 1945. *Ent. News* 57:1,50,76,104.
24. Shiffer, C. N. 1968. Homeochromatic females of the dragonfly *Perithemis tenera*. *Pa. Acad. Sci. Proc.* 42:138.
25. Raff, R. A. & E. C., and G. H. & A. F. Beatty. 1969. Odonata of the Potomac River drainage in Pennsylvania. *Pa. Acad. Sci. Proc.* 43:114.
26. Hostetter, D. R., and S. H. Derickson. 1933. Preliminary biological survey of Lake Conewago, Mount Gretna, Pennsylvania. *Pa. Acad. Sci. Proc.* 7:8.
27. Walker, E. M. 1912. The North American dragonflies of the genus *Aeshna*. Univ. Toronto Studies, Biol. Ser. No. 11. 213 p.
28. Walker, E. M. 1925. The North American dragonflies

of the genus *Somatochlora*. Univ. Toronto Studies, Biol. Ser. No. 26. 202 p.
29. Westfall, M. J., Jr., and R. P. Trogdon. The true *Gomphus consanguis* Selys. *Florida Entomologist* 45: 29.
30. Beatty, G. H. & A. F. 1969. Evolution and speciation in the subgenus *Schizuraeschna*, with observations on *Aeshna (Schizuraeschna) mutata* Hagen. *Pa. Acad. Sci. Proc.* 43:147.
31. Bick, G. H. 1953. The occurrence of *Anax longipes* Hagen in Mississippi. *Ent. News* 64:230.
32. Rise, M. D., and W. Cruden. 1966. New records for *Anax longipes*. *Ent. News* 77:187.
33. Needham, J. G. 1951. A new species of *Ophiogomphus*. *Ent. News* 62:41.
34. Gibbs, R. H., and S. P. Gibbs. 1954. The Odonata of Cape Cod, Massachusetts. *N. Y. Ent. Soc. Jour.* 42: 167.



# EDAPHIC FACTORS IN THE DISTRIBUTION OF PENNSYLVANIA ODONATA

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## ABSTRACT

Pennsylvania is examined as a habitat for Odonata from the standpoint of physiography, glacial characteristics, and watersheds of river systems, all being features distinct from climate. Known distributions of most of the 170 species comprising the Pennsylvania Odonata fauna were plotted with respect to these features, and the results are summarized and discussed.

## INTRODUCTION

One has only to look at Higbee's exhaustive stream map (1) of Pennsylvania to be wonderstruck by the enormous number of aquatic habitats that reticulate the Pennsylvania landscape. Thousands of named streams and ponds, lakes, swamps and bogs in Pennsylvania all have their populations of dragonflies. One can only wish that it were possible systematically to survey a comprehensive selection of these habitats in a lifetime—just in Pennsylvania. To what extent, then, can we hope to have an accurate faunistic knowledge of the Odonata of this state?

Since the state of Pennsylvania as a whole has no natural boundaries except the Delaware River on the East and a bit of Lake Erie shore in the Northwest, the list (2) of 170 species of Odonata now known to occur in Pennsylvania has too little geographical significance. It might be just as well to add to it the 43 additional species known from adjoining states (2) and call the total list of 213 species the Odonata fauna of the "Pennsylvania region". Within the political borders of Pennsylvania this region can be subdivided in at least 4 ways: by physiography; by watersheds; by climate; and by life zones, biomes or forest regions. First, it can be zoned edaphically, on the basis of physiography. Edaphic is used here to mean pertaining to all physical factors that are not climatic. With its wide variety of physiographic provinces (Fig. 1), Pennsylvania tempts the biogeographer to consider biotic differences between the Appalachian Plateau and the Appalachian Valley; or Ridge and Valley Province; and the Piedmont; with due regard for the small segments of Atlantic Coastal Plain, New England Province, Blue Ridge, and Lake Erie Plain that give Pennsylvania a total of 7 major physiographic divisions (3,4). Both the Appalachian Plateau and the Ridge and Valley Province have glaciated and unglaciated divisions (5), so there are no less than 9 zones in Pennsylvania (Fig. 1) the Odonata faunas of which might be studied and compared. Extensions of these zones into other adjoining

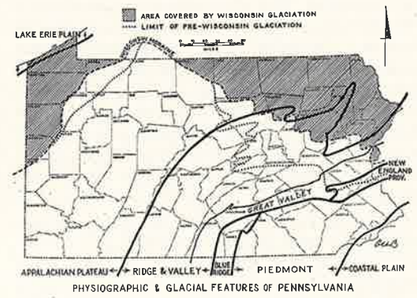



**Figure 1. Physiographic and Glacial Features of Pennsylvania**

states must be considered also, since the political boundaries of the state are biologically, or biogeographically, meaningless, and therefore it is desirable to view Pennsylvania more as an arbitrary sampling area within a larger context rather than as a rectangle of geography suspended in space. To know what species of Odonata occur in each of the 9 zones outside of Pennsylvania is rather difficult since none of the adjoining states has been surveyed as thoroughly as Pennsylvania, and though much has been published on the Odonata of New York (6), New Jersey (7), Maryland (8,9), West Virginia (10,11) and Ohio (12,13), the data thus available yield but a fragmentary picture of the Odonata faunistics of this greater Pennsylvania region.

Watersheds or drainage basins form another edaphic basis for subdividing Pennsylvania into natural areas for faunal investigations of Odonata distribution. In this case there are 3 major and 3 minor divisions (Fig 2). The

Susquehanna River, with the largest drainage basin of any U.S. river entering the Atlantic Ocean, drains 21,000 of Pennsylvania's 45,000




**Figure 2. Drainage Basins of Pennsylvania.**

square miles. The Ohio River basin occupies 15,600 square miles in western Pennsylvania, and the Delaware River drains 6422 square miles of eastern Pennsylvania. Remaining are about 500 square miles draining into Lake Erie, 96 square miles draining into the Atlantic Ocean through the Genessee and St. Lawrence Rivers, and 1571 square miles of the Potomac River basin (14) in the southern part of the state.

The significance of the two foregoing edaphic bases for biogeographical subdivision of the state rests on two factors: first, the distribution of different types of habitats is dependent upon physiographic factors. It is the distribution of *habitat-types* which needs primarily to be investigated, and then Odonata-distribution might be rationally predicted by deduction. But complete, organized information of this nature is not available, and too little is known about the actual habitat requirements of even the commonest species. Natural lakes, for example, are found only in the glaciated sectors of the state, and this is usually true of bogs.

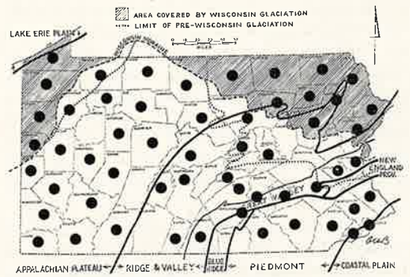



**Figure 3. Distribution in Pennsylvania, by counties, of** Ischnura verticalis.

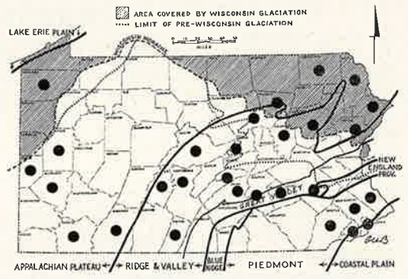



**Figure 4. Distribution in Pennsylvania, by counties, of** Gomphus exilis.

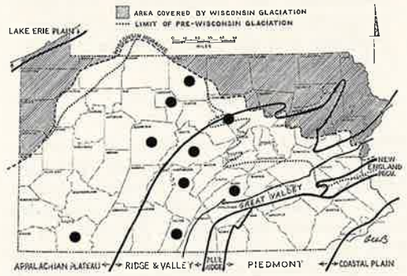



**Figure 5. Distribution in Pennsylvania, by counties, of** Boyeria grafiana.

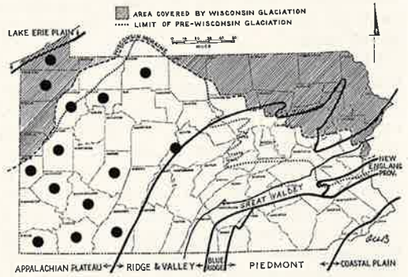



**Figure 6. Distribution in Pennsylvania, by counties, of** Enallagma antennatum.

The distribution of lake- and bog-dwelling species of Odonata might be expected to be more or less limited to glaciated areas, but man has sprinkled the state with bodies of water of his own making, thereby complicating the analysis. Data brought together on Table 4 show that only 4 rarely-collected species are apparently restricted to glaciated territory. The "ecological distribution" of each species, dependent on substrate characteristics, water quality, rheology (and, of course, climate) is critical in



determining what species are permanently present at any site, and in any region. Second, river system to an unknown extent, undoubtedly play some role in dynamic range expansions of some species of Odonata. This was referred to by the writers (15) in connection with such species as *Enallagma basidens* (14) and *Archilestes grandis*, but many other species may be involved in a less conspicuous way.

A third way in which Pennsylvania can be subdivided in attempting to put Odonata distribution-investigations on a more rational footing is not edaphic, and therfore it will be the subject of a future paper (18). Climate and climatic factors *vis a vis* Odonata were introduced in some detail in an earlier paper (15), but it is obvious that too little is known of the physiological requirements (thermal aspects of diapause, lethal temperature thresholds, etc.) of any considerable number of species for it to be possible to relate species distributions to climatic zones with any degree of validity. Too little is known of the actual geographical distribution of even the most commonplace species for it to be possible to determine inductively whether isotherms or isopleths are really meaningful in delimiting distribution areas.

Finally, life zones, biomes and the like can be utilized in attempting to work out the faunistics of Pennsylvania Odonata. This, too, will be dealt with in a forthcoming paper.

## PROCEDURE

An approach to the problem of assaying gross edaphic factors such as physiography and river systems for their influence on Odonata distribution is to try to find species that are confined to any of these divisions. On the basis of present knowledge what species, if any, are largely or totally restricted to the Appalachian Plateau, or either or both of its glaciated (Wisconsin) sectors? Or what species are confined to the Piedmont or the Atlantic Coastal Plain? Answers to questions like these may lie in the extensive compilations of data (16,17) that are in the process of being worked out by the present writers.

In attempting, in a general way, to throw light on distributional problems, though not to answer the questions definitively, the presently known distributions of most of the 170 species on the Check List of Pennsylvania Odonata (2) were plotted by counties on maps (Fig. 1) whereon the chief physiographic and glacial features were also outlined. The undesirable aspects of plotting distribution only by counties can be appreciated by considering Lycoming County, which can be seen to contain four physiographic divisions (or even more of the older, pre-Wisconsin, glaciation is considered). The basis of the present analysis is, however, a compilation of county records only, as contained in recent and current reports (16,17), and therefore this kind of flaw is inevitable.

After known distributions by county were plotted on what has been adopted as a standard base map (Fig. 3-6) it was possible to tabulate results displayed on Table 4. First, however, it was necessary to evolve a list of species for which this information would be especially meaningful. Quite a few species are so widely distributed in Pennsylvania that attempts at a geographical breakdown within the state would yield only the information that they occur everywhere. By tabulating the 170 Pennsylvania Odonata species (2) by percentage of the 67 Pennsylvania counties from which they have been recorded (16-18) it has been possible to obtain a list of 50 Pennsylvania species of most widespread incidence (Table 1). Some of these species, however, have a low incidence in some major part of the state, and some of these may be absent or little-known in extensive areas adjoining Pennsylvania. For this reason the total ranges of all Pennsylvania species were mapped out and a further criterion was established for exclusion from the final list of species for geographical analysis. Species were excluded only if they were both widespread in Pennsylvania and recorded from the surrounding states to the North, South, East and West (Table 2). A shorter list of species about which insufficient information is available (Table 3) was then added to the list displayed as Table 2, and by excluding the 73 species on these combined lists the final list was produced of the 97 species of which the Pennsylvania distribution is to be analyzed according to the proposed edaphic factors (Table 4).

In addition to tabulating the physiographic provinces in which each species on Table 4 is known to occur, the writers placed a transparent overlay (Fig. 2) over each of the species spot-maps, and the resulting information on river basins was entered on the table. Thus all edaphic affinities considered in this study are displayed on Table 4.

## RESULTS

Table 4 makes it possible to determine which of the 97 species under consideration are confined to definite edaphic divisions of Pennsylvania. Forty-four species are tabulated from 3 or 4 of the major provinces, and therefore will not be considered further. Data on occur-

Table 1. FIFTY MOST-WIDESPREAD ODONATA SPECIES OF PENNSYLVANIA, BASED ON INCIDENCE OVER ENITRE STATE. RANKED IN ORDER OF PERCENTAGE INCIDENCE BY COUNTIES.

| RANK | | PERCENTAGE INCIDENCE |
|---|---|---|
| 1. | *Ischnura verticalis* | 84% |
| 2. | *Libellula pulchella* | 77 |
| 3. | *Libellula lydia* | 77 |
| 4. | *Calopteryx maculata* | 70 |
| 5. | *Anax junius* | 67 |
| 6. | *Libellula luctuosa* | 67 |
| 7. | *Enallagma exsulans* | 61 |
| 8. | *Argia violacea* | 60 |
| 9. | *Sympetrum rubicundulum* | 58 |
| 10. | *Aeshna umbrosa* | 58 |
| 11. | *Epitheca cynosura* | 57 |
| 12. | *Sympetrum vicinum* | 57 |
| 13. | *Leucorrhinia intacta* | 55 |
| 14. | *Pachydiplax longipennis* | 54 |
| 15. | *Argia moesta* | 52 |
| 16. | *Erythemis simplicicollis* | 49 |
| 17. | *Perithemis tenera* | 48 |
| 18. | *Enallagma hageni* | 48 |
| 19. | *Chromagrion conditum* | 46 |
| 20. | *Gomphus villosipes* | 45 |
| 21. | *Lestes rectangularis* | 45 |
| 22. | *Enallagma signatum* | 45 |
| 23. | *Boyeria vinosa* | 43 |
| 24. | *Gomphus exilis* | 43 |
| 25. | *Enallagma civile* | 43 |
| 26. | *Amphiagrion saucium* | 42 |
| 27. | *Nagenius brevistylus* | 40 |
| 28. | *Epitheca princeps* | 40 |
| 29. | *Sympetrum semicinctum* | 40 |
| 30. | *Nehalennia irene* | 40 |
| 31. | *Ischnura posita* | 40 |
| 32. | *Lanthus parvulus* | 39 |
| 33. | *Celithemis elisa* | 39 |
| 34. | *Gomphus lividus* | 37 |
| 35. | *Enallagma aspersum* | 37 |
| 36. | *Enallagma geminatum* | 37 |
| 37. | *Cordulegaster diastatops* | 36 |
| 38. | *Didymops transversa* | 36 |
| 39. | *Macromia illinoiensis* | 34 |
| 40. | *Libellula semifasciata* | 34 |
| 41. | *Dromogomphus spinosus* | 33 |
| 42. | *Hetaerina americana* | 33 |
| 43. | *Libellula cyanea* | 31 |
| 44. | *Libellula julia* | 31 |
| 45. | *Tramea lacerata* | 30 |
| 46. | *Lanthus albistylus* | 28 |
| 47. | *Somatochlora tenebrosa* | 27 |
| 48. | *Sympetrum obtrusum* | 27 |
| 49. | *Pantala flavescens* | 27 |
| 50. | *Lestes congener* | 27 |

rences in the Lake Erie Plain are insufficient to serve as bases for conclusions, though 2 species, *Gomphus spicatus* and *Enallagma antennatum,* have been found in Pennsylvania only in the Appalachian Plateau Province and the Lake Erie Plain. Another species, *Sympetrum corruptum,* has been collected only in the Atlantic Coastal Plain and the Lake Erie Plain in Pennsylvania, and a fourth species, *Aeshna mutata,* otherwise known from the Ridge and Valley Province, was collected once (16) at Presque Isle on Lake Erie. This latter occurrence of a single individual is, however, in the "adventive" category. All other species tabulated from the Lake Erie Plain are known to occur in two or more other major edaphic divisions of the state. The Lake Erie Plain was included only because records from Presque Isle were entered separately on the county-record spot-maps. Similar tabulation of species from the Blue Ridge and New England physiographic provinces was impossible because these divisions occupy small fractions of several counties, and Odonata records, being plotted for the most part at the county level only, are therefore not separable according to location within a county; what was simple to do for Presque Isle and the Lake Erie Plain was impossible for the other minor divisions, except the Atlantic Coastal Plain, for which an individual tabulation had already been made because of special interest in this region. The small extension of New England Province into Pennsylvania likewise deserves a special study; there are enough records from northern Bucks county to make this possible in the future.



Table 2. EXCLUDED SPECIES (1). PENNSYLVANIA ODONATA SPECIES THE DISTRIBUTION OF WHICH IS KNOWN TO BE STATEWIDE, AND THE RANGES OF WHICH EXTEND NORTH, SOUTH, EAST, AND WEST OF PENNSYLVANIA, AND WHICH ARE THEREFORE EXCLUDED FROM INTRA-STATE DISCUSSION OF EDAPHIC DISTRIBUTION FACTORS.

| SPECIES NO., PA. CHECKLIST (2) | | INCIDENCE RANK (TABLE 1) |
|---|---|---|
| 5. | *Boyeria vinosa* | 23 |
| 13. | *Aeshna umbrosa* | 10 |
| 15. | *Anax junius* | 5 |
| 26. | *Lanthus parvulus* | 32 |
| 29. | *Gomphus exilis* | 24 |
| 30. | *Gomphus lividus* | 34 |
| 47. | *Gomphus villosipes* | 20 |
| 48. | *Dromogomphus spinosus* | 41 |
| 49. | *Cordulegaster diastatops* | 37 |
| 51. | *Cordulegaster maculatus* | — |
| 55. | *Macromia illinoiensis* | 39 |
| 61. | *Epitheca cynosura* | 11 |
| 62. | *Epitheca princeps* | 28 |
| 70. | *Somatochlora tenebrosa* | 47 |
| 78. | *Libellula cyanea* | 43 |
| 84. | *Libellula luctuosa* | 6 |
| 85. | *Libellula lydia* | 3 |
| 87. | *Libellula pulchella* | 2 |
| 89. | *Libellula semifasciata* | 40 |
| 91. | *Perithemis tenera* | 17 |
| 95. | *Erythemis simplicicollis* | 16 |
| 100. | *Sympetrum rubicundulum* | 9 |
| 101. | *Sympetrum semicinctum* | 29 |
| 102. | *Sympetrum vicinum* | 12 |
| 103. | *Pachydiplax longipennis* | 14 |
| 107. | *Leucorrhinia intacta* | 13 |
| 109. | *Celithemis elisa* | 33 |
| 112. | *Pantala flavescens* | 49 |
| 115. | *Tramea lacerata* | 45 |
| 120. | *Calopteryx maculata* | 4 |
| 121. | *Hetaerina americana* | 42 |
| 129. | *Lestes forcipatus* | — |
| 131. | *Lestes rectangularis* | 21 |
| 136. | *Argia moesta* | 15 |
| 140. | *Argia violacea* | 8 |
| 141. | *Amphiagrion saucium* | 26 |
| 144. | *Nehalennia irene* | 30 |
| 145. | *Chromagrion conditum* | 19 |
| 147. | *Enallagma aspersum* | 35. |
| 151. | *Enallagma civile* | 25 |
| 158. | *Enallagma exsulans* | 7 |
| 159. | *Enallagma geminatum* | 36 |
| 160. | *Enallagma hageni* | 18 |
| 163. | *Enallagma signatum* | 22 |
| 166. | *Ischnura posita* | 31 |
| 169. | *Ischnura verticalis* | 1 |

Of the 35 species of Odonata shown on Table 4 to be restricted to 2 out of the 4 major physiographic divisions, 26 (Nos. 2,4,18,20,23,24,27, 28,34,36,38,60,63,65,66,71,73,75,88,106,117,118, 122,127,148,157) are recorded from both the Appalachian Plateau and the Ridge and Valley provinces in Pennsylvania. These species might best be described as apparently not occurring in the Piedmont and Coastal Plain provinces. Therefore they might be thought to be edaphically restricted by differences between the provinces where they do occur and those where they do not, except for the fact, not part of the present study, that the Piedmont and Coastal Plain are in general the warmest part of the state. Most of these 25 species *(P. obscurus, C. angustipennis* and *E. basidens* being the only exceptions) are definitely northern in their general ranges, and it is logical to expect that their absence from the Piedmont and Coastal Plain has thermal, rather than edaphic, correlations. This will be dealt with in a future paper (19). *P. obscurus* is a widespread species from Texas and Florida north

Table 3. EXCLUDED SPECIES (2). ODONATA SPECIES RECORDED FROM PENNSYLVANIA, THE STATUS OF WHICH IS TOO UNCERTAIN FOR THEIR DISTRIBUTION TO BE ANALYZED.

| SPECIES NO. PA. CHECKLIST (2) | | FOOTNOTE # |
|---|---|---|
| 6. | *Basiaeschna pentacantha* | 1,2 |
| 21. | *Ophiogomphus edmundo* | 2,3 |
| 46. | *Gomphus furcifer* | 1 |
| 54. | *Macromia georgina* | 2,3 |
| 56. | *Macromia taeniolata* | 8 |
| 64. | *Epitheca williamsoni* | 4 |
| 67. | *Somatochlora filosa* | 3,4 |
| 72. | *Somatochlora williamsoni* | 3 |
| 74. | *Dorocordulia lepida* | 4 |
| 77. | *Libellula axilena* | 2,3,5 |
| 79. | *Libellula deplanata* | 2,3,7 |
| 80. | *Libellula exusta* | 2,3,7 |
| 86. | *Libellula needhami* | 2,3,4 |
| 93. | *Erythrodiplax berenice* | 4 |
| 94. | *Erythrodiplax c. minuscula* | 4,5 |
| 96. | *Sympetrum ambiguum* | 5 |
| 111. | *Celithemis martha* | 4 |
| 119. | *Calopteryx apicalis* | 4,5 |
| 143. | *Nehalennia integricollis* | 3,4,5 |
| 149. | *Enallagma boreale* | 6 |
| 153. | *Enallagma daecki* | 3,4 |
| 155. | *Enallagma doubledayi* | 3,4 |
| 156. | *Enallagma durum* | 3,4 |
| 161. | *Enallagma laterale* | 3,4,6 |
| 162. | *Enallagma pissinarium* | 2,3,7 |
| 167. | *Ischnura prognatha* | 2,3,5 |
| 168. | *Ischnura ramburi* | 4 |

1. Larval record only ( 2) from one location on Ohio border.
2. Known only from one specimen.
3. Known only from one location.
4. Adventive from Coastal Plain.
5. Adventive from South.
6. Adventive from North.
7. Taxonomic status uncertain.
8. Not found in last 100 years.

to New England that is especially abundant in the Coastal Plain. Its absence from the Piedmont and Coastal Plain in Pennsylvania is unaccountable, unless it is due to absence of suitable sandy-bottom streams. *C. angustipennis* is a rare species of very limited southern Appalachian distribution. Its absence from the Piedmont and Coastal Plain in Pennsylvania might be edaphically influenced, but its occurrences everywhere are too few to permit generalizations of this nature. *E. basidens* has dispersed into Pennsylvania from southwestern U.S. and is continuing to establish new stations (14). Since it has been found in the Piedmont and Coastal Plain near Washington, D.C. (20) it will probably be found soon in these provinces in Pennsylvania.

The remaining 9 species,

| | | |
|---|---|---|
| *Gomphaeschna antilope* | AP | CP |
| *Gomphus abbrevatus* | RV | P |
| *G. ventricosus* | RV | P |
| *Libellula auripennis* | RV | P |
| *Sympetrum corruptum* | LE | CP |
| *Calopteryx aequabilis* | RV | P |
| *Nehalennia gracilis* | RV | P |
| *Enallagma traviatum/wstfli* | AP | P |

, known to occur in 2 of Pennsylvania's 4 main physiographic provinces other than the foregoing combination of Appalachian Plateau and Ridge and Valley provinces (which therefore might be called the NON-Piedmont-Coastal Plain group), have various peculiarities. *Gomphaeschna antilope* is usually a coastal plain species, but its occurrence also in western Pennsylvania illuminates only its mysterious character. Its propensity for entering buildings and appearing in other unexpected places, but never being found purposefully, is a subject for further study. *Gomphus abbreviatus* and *Calopteryx aequabilis* have both been found in well established colonies, often together on the same large streams, in both Piedmont and Ridge and Valley provinces in Pennsylvania (17). An edaphic correlation seems likely here, and it will be noted (Table 4) that both of these characteristically lotic species are found only in the basins of the Delaware and Susquehanna rivers, which debouch close together into the Atlantic Ocean. *Gomphus abbreviatus* is recorded (12) in very small numbers from Ohio, and *Calopteryx aequabilis* is abundant in the upper Mississippi basin, so their absence from western Pennsylvania is a mystery. *Gomphus ventricosus*, which has not been found in Pennsylvania for over 50 years (17) also fits into this category, though it appears to have abandoned both of its former stations in Pennsylvania. *Argia tibialis* has been collected very sparingly in Pennsylvania on the Appalachian Plateau and in the Piedmont, and probably has a relation to edaphic factors similar to that of *G. abbreviatus, G. ventricosus* and *C. aequabilis*. Unlike the latter 3, however, *tibialis* is abundant on the Coastal Plain in New Jersey and southward. *L. auripennis, S. corruptum, N. gracilis* and *E. traviatum/westfalli* are all lentic species and evidently have no close relation with river systems, in contrast to *E. basidens* which appears to be dispersing along major river valleys. *L. auripennis* in Pennsylvania seems accidental and not established in any of the 3 locations at each of which one specimen was collected (17,18). *Sympetrum corruptum* was collected



Table 4. ANALYSIS OF DISTRIBUTION OF 97 SPECIES OF PENNSYLVANIA ODONATA WITH RESPECT TO EDAPHIC FACTORS OF PHYSIOGRAPHY AND RIVER BASINS.[1]

| SPECIES # ON PA. CHECKLIST (2) | | LOTIC (F), or LENTIC (S) | PHYSIOGRAPHIC PROVINCES | | | | | RIVER BASINS | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | LAKE ERIE PLAIN | APPALACHIAN PLATEAU | RIDGE AND VALLEY | PIEDMONT | COASTAL PLAIN | OHIO R. BASIN | SUSQUEHANNA R. BASIN | DELAWARE R. BASIN |
| 1. | *Gomphaeschna antilope* | S/F | | AP | | | CP | O | | D |
| 2. | ——— *furcillata* | S/F | | AP+ | RV | | (CP) | O | S | (D) |
| 3. | *Basiaeschna janata* | F | | AP+ | RV | P | CP | O | S | D |
| 4. | *Boyeria grafiana* | F | | AP | RV | | | O | S | |
| 7. | *Epiaeschna heros* | S/F | | AP+ | RV | P | CP | O | S | D |
| 8. | *Aeshna canadensis* | S | | AP+ | RV+ | P | | O | S | D |
| 9. | ——— *clepsydra* | S | | AP | | | | | S | D |
| 10. | ——— *constricta* | S | | AP+ | (RV) | | CP | O | S | D |
| 11 | ——— *mutata* | S | LE | | RV | | | (LE) | S | |
| 12. | ——— *tuberculifera* | S | | AP | RV+ | | CP | O | S | D |
| 14. | ——— *verticalis* | S | | AP+ | RV+ | | CP | O | S | D |
| 16. | *Anax longipes* | S | | AP+ | RV | P | CP | O | S | D |
| 17. | *Tachopteryx thoreyi* | S | | AP+ | RV | P | | O | S | D |
| 18. | *Progomphus obscurus* | F | | AP | RV | | | O | S | |
| 19. | *Hagenius brevistylus* | F | LE | AP+ | RV | P | CP | O | S | D |
| 20. | *Ophiogomphus carolus* | F | | AP+ | RV+ | | | | S | D |
| 22. | ——— *howei* | F | | | RV | | | | S | |
| 23. | ——— *mainensis* | F | | AP+ | RV | | | O | S | D |
| 24. | ——— *rupinsulensis* | F | | AP+ | RV | | | O | S | D |
| 25. | *Lanthus albistylus* | F | | AP+ | RV | P | | O | S | D |
| 27. | *Gomphus borealis* | S | | AP+ | RV | | | O | S | D |
| 28. | ——— *descriptus* | F | | AP+ | RV+ | | | O | S | D |
| 31. | ——— *quadricolor* | F | | AP | RV | P | | O | S | D |
| 32. | ——— *spicatus* | S | LE | AP+ | | | | O | S | D |
| 33. | ——— *abbreviatus* | F | | | RV | P | | | S | D |
| 34. | ——— *brevis* | F | | AP+ | RV+ | | | O | S | D |
| 35. | ——— *viridifrons* | F | | AP | | | | O | | |
| 36. | ——— *fraternus* | F | | AP | (RV) | | | O | (S) | |
| 37. | ——— *lineatifrons* | F | | | RV | | | | S | |
| 38. | ——— *rogersi* | F | | AP | RV | | | O | S | |
| 39. | ——— *vastus* | F | | AP | RV | P | | O | S | D |
| 40. | ——— *ventricosus* | F | | | RV | P | | | S | |
| 41. | ——— *amnicola* | F | | | RV | | | | S | |
| 42. | ——— *notatus* | F | | AP | | | | O | | |
| 43. | ——— *plagiatus* | F | | | | (P or CP) | | | | D |
| 44. | ——— *scudderi* | F | | AP | | | | O | S | |
| 45. | ——— *spiniceps* | F | | AP | RV | P | CP | O | S | D |
| 50. | *Cordulegaster erroneus* | F | | AP+ | RV | P | | O | S | D |
| 52. | ——— ——— *obliquus* | F | | AP | RV | P | | O | S | D |
| 53. | *Macromia alleghaniensis* | F | | AP | | (P) | | O | | |
| 57. | *Didymops transversa* | F | | AP+ | RV+ | P | CP | O | S | D |
| 58. | *Neurocordulia obsoleta* | F | | (AP) | (RV) | P | | | S | D |
| 59. | *Neurocordulia yamaskanensis* | F | | | RV | | | | S | |
| 60. | *Epitheca canis* | S | | AP+ | RV+ | | | O | S | D |
| 63. | ——— *spinigera* | S | | AP | RV | | | | S | |
| 65. | *Helocordulia uhleri* | F | | AP+ | RV | | | O | S | D |
| 66. | *Somatochlora elongata* | F | | AP | RV | | | O | S | |
| 68. | ——— ——— *forcipata* | S | | AP+ | | | | | S | D |
| 69. | ——— ——— *linearis* | F | | AP | RV | P | | O | S | D |
| 71. | ——— ——— *walshi* | (F) | | AP | RV+ | | | O | S | |
| 73. | *Cordulia shurtleffi* | (F) | | AP+ | RV+ | | | O | S | D |
| 75. | *Dorocordulia libera* | (F) | | AP+ | RV+ | | | O | S | D |
| 76. | *Libellula auripennis* | S | | | RV | P | | | S | D |

(continues on next page)

Table 4. Continued.

| SPECIES # PA.CHECKLIST (2) | | LOTIC (F), or LENTIC (S) | PHYSIOGRAPHIC PROVINCES: LAKE ERIE PLAIN | APPALACHIAN PLATEAU | RIDGE AND VALLEY | PIEDMONT | COASTAL PLAIN | RIVER BASINS: OHIO RIVER BASIN | SUSQUEHANNA R. BASIN | DELAWARE R. BASIN |
|---|---|---|---|---|---|---|---|---|---|---|
| 81. | ——— *flavida* | S | | AP | RV | P | | O | S | D |
| 82. | ——— *incesta* | S | LE | AP | (RV) | P | CP | O | S | D |
| 83. | ——— *julia* | S | | AP+ | RV+ | P | (CP) | O | S | D |
| 88. | ——— *quadrimaculata* | (S) | | AP+ | RV+ | | | O | S | D |
| 90. | ——— *vibrans* | (S) | | | RV | P | CP | | S | D |
| 92. | *Nannothemis bella* | S | | AP | RV | | (CP) | | | D |
| 97. | *Sympetrum corruptum* | S | LE | | | | CP | (LE) | | D |
| 98. | ——— *internum* | S | | AP+ | RV | | CP | O | S | D |
| 99. | ——— *obtrusum* | S | LE | AP+ | RV | P | CP | O | S | D |
| 104. | *Leucorrhinia frigida* | S | | AP+ | RV | P | (CP) | O | S | D |
| 105. | ——— *glacialis* | S | | (AP) | RV | | | | S | |
| 106. | ——— *hudsonica* | S | | AP+ | RV+ | | | (O) | S | |
| 108. | ——— *proxima* | S | | | RV | | | | S | |
| 110. | *Celithemis eponina* | S | LE | AP+ | RV+ | P | CP | O | S | D |
| 113. | *Pantala hymenea* | S | LE | AP | RV | P | CP | O | S | D |
| 114. | *Tramea carolina* | S | LE | AP | RV | P | CP | O | S | D |
| 116. | *Calopteryx aequabilis* | F | | (AP) | RV | P | | | S | D |
| 117. | ——— *amata* | F | | AP+ | RV | | | O | S | D |
| 118. | ——— *angustipennis* | F | | AP | RV | | | O | S | |
| 122. | *Hetaerina titia* | F | | AP | RV | | (CP) | O | | (D) |
| 123. | *Archilestes grandis* | (F) | | AP | RV | | CP | O | S | D |
| 124. | *Lestes congener* | S | | AP+ | RV | P | CP | O | S | D |
| 125. | ——— *d.disjunctus* | S | | AP+ | | | | O | | D |
| 126. | ——— *d.australis* | S | LE | AP+ | RV | P | CP | O | S | D |
| 127. | ——— *dryas* | S | LE | AP+ | RV+ | | | O | S | D |
| 128. | ——— *eurinus* | S | | AP | RV | P | CP | O | S | D |
| 130. | ——— *inequalis* | S | LE | AP+ | RV | P | CP | O | S | D |
| 132. | ——— *unguiculatus* | S | | AP+ | RV | P | CP | O | S | D |
| 133. | ——— *vigilax* | S | LE | AP+ | RV | P | CP | O | S | D |
| 134. | *Argia apicalis* | F | | AP+ | RV | P | | O | S | D |
| 135. | ——— *bipunctulata* | S | | AP | | | | | | D |
| 137. | ——— *sedula* | F | | (AP) | RV | P | | O | S | D |
| 138. | ——— *tibialis* | F | | AP+ | | P | | O | | D |
| 139. | ——— *translata* | F | | AP | RV | P | | O | S | D |
| 142. | *Nehalennia gracilis* | S | | | RV | P | | | S | D |
| 146. | *Enallagma antennatum* | S/F | LE | AP+ | | | | O | S | |
| 148. | *Enallagma basidens* | (S) | | AP | RV | | | O | S | D |
| 150. | ——— *carunculatum* | (F) | LE | AP+ | RV | P | | O | S | D |
| 152. | ——— *cyathigerum* | S | | AP+ | RV | | (CP) | O | S | (D) |
| 154. | ——— *divagans* | F | | AP | RV | P | CP | O | S | D |
| 157. | ——— *ebrium* | S | LE | AP+ | RV+ | | | O | S | D |
| 164. | ——— *traviatum/westfalli* | S | | AP+ | | P | | O | | D |
| 165. | ——— *vesperum* | S | | AP+ | RV | P | | O | S | D |
| 170. | *Anomalagrion hastatum* | S | | AP+ | RV | P | CP | O | S | D |

[1]Symbols: AP+ = found in both glaciated and unglaciated sections
AP = found only in glaciated sections
(AP) = doubtful or single-specimen records
S/F = found in both lentic and lotic habitats
(S) = status of larval habitats in Pennsylvania uncertain

once near Philadelphia in 1889 (18) and more recently at Presque Isle, Lake Erie (16), in large enough numbers to indicate at least an intermittent population. Occurrences east of the Alleghanies of *corruptum* are very rare, and therefore its distribution in Pennsylvania, like that of *Enallagma antennatum*, can be considered edaphically controlled until a better explanation is forthcoming. *Nehalennia gracilis* is possibly edaphically influenced, though in the opposite compass-direction. Though it is found sparingly to the West, and commonly to the North, of Pennsylvania, it appears to be absent from the Appalachian Plateau and the Ohio River basin in Pennsylvania. *Enallagma traviatum* presents a unique problem because this name may cover two entities in Pennsylvania (2,16). Until the taxonomic problem is solved its geographical relations will remain obscure, since there may prove to be one eastern species in the Piedmont (18), and another to the West, on the Appalachian Plateau (16). Between these extremes *traviatum* is unknown.

The 18 species remaining (out of the 97 on Table 4) after elimination of 44 known from 3 or more physiographic provinces, and the 34 species discussed above, are each known from



a single physiographic province in Pennsylvania. Nine of these 18 (Nos. 22,35,37,41,42,53, 59,108,135) can now be elimiated from the present discussion on the same grounds given on Table 3 for excluding other species. They are one-specimen, or one-time, or one-place occurrences that do not yield enough data for biogeographic purposes. Incidentally, Nos. 54, 64,67,86,93,94,111,143,153,156,167 and 168 on Table 3 are all Coastal Plain species that have been collected one or two times at one or two peripheral places in the Coastal Plain Province in Pennsylvania, and can therefore be considered either edaphically, or, more likely, climatically (19) influenced in their Pennsylvania distribution, while Nos. 22,35,37,41,42,53,59,108 and 135 are known only from the more interior Ridge and Valley and Appalachian Plateau provinces and have northern or western affinities. Documentation and discussion of these records may be found elsewhere (2,16,17,18).

The remaining 9 species,

| | |
|---|---|
| *Aeshna clepsydra* | *AP* (17,18) |
| *Aeshna mutata* | RV (16,17,21) |
| *Gomphus spicatus* | AP+ (16,17,18) |
| *G. plagiatus* | P &/or CP (18) |
| *G. scudderi* | AP (16,17) |
| *Somatochlora forcipata* | AP+ (17) |
| *Leucorrhinia glacialis* | RV (17) |
| *Lestes d. disjunctus* | AP+ (16,18) |
| *Enallagma antennatum* | AP+ (16,17), each |

known from a single physiographic province in Pennsylvania, do not help much in assaying the influence of edaphic factors on dragonfly distribution. *A. clepsydra, G. spicatus, S. forcipata* and *L. d. disjunctus* appear to be merely "northern" in Pennsylvania, and their restriction to an edaphic division of the state probably is fortuitous (outside of the fact that most of the northern and colder parts of the state coincide with the Appalachian Plateau Province). *A. mutata* and *L. glacialis,* both collected in considerable numbers at just one pond in central Pennsylvania (21) are undoubtedly restricted by some factor, but consideration of all that is known about their distributions suggests that they are not limited mainly by physiographic factors so much as by local habitat characteristics. *E. antennatum* (Fig. 6) is one of the very few Pennsylvania Odonata species that evidently is physiographically controlled, for it occurs all over the western part of the Appalachian Plateau, right up to the edge in Centre County, but not elsewhere in the state. Too little is known of *Gomphus plagiatus* and *scudderi* in Pennsylvania to permit their discussion beyond the information given above.

Finally it should be mentioned that a number of northern species, such as Nos. 8,10,12,14, 83,98,104 and 152, which have been taken in the Piedmont and Coastal Plain near Philadelphia as the result of particularly intensive collecting (18), were probably not permanent residents, but rather members of temporary colonies such as those discussed by Walker (22), which would sooner or later succumb to unfavorable conditions.

## CONCLUSION

Adult Odonata are among the most vagile of insects (20,22), and this makes it exceedingly difficult to study factors governing their distribution. While it is quite true that species do have their ranges to which they are in the long run restricted (15), the exigencies of typical collecting activity yield data that are insufficient to determine the actual extent of the long-term or average ranges of species. A spot on a map (Figs. 3-6) may indicate collecting a species once or 100 times, or finding one individual or many. Few locations are subject to continuing observations (17). Only these can yield data that are not subject to suspicion of including too many temporary or accidental short-term occurrences.

Analysis of available data on known distribution of Odonata in Pennsylvania has produced few clues to possible separate influence of gross physiography on dragonfly distribution. It is clear that influence of physiography on local habitat types should be separately investigated, and, even more important, the ecological requirements and characteristics of each species must be determined. Then these results could be rationally correlated. Induction, from what little is now known about Odonata occurrences, to generalities on edaphic controls of distribution, has proved fruitless. Only a few species, such as *Gomphus abbreviatus, Gomphus spicatus. Sympetrum corruptum, Colopteryx aequabilis* and *Enallagma antennatum,* show rather clear edaphic correlations, which in every case indicate only part of a congeries of factors (climate, local habitat characteristics, etc.) complexly interrelated in determining the actual distribution of species. River systems prove to be even less influential in restricting ranges, though it remains probable that river valleys are an important avenue of dispersal. Of the 97 species on Table 4, 57 are known from all 3 of the major river basins in Pennsylvania, and only 12 are known from a single watershed, which usually proves to be that of the Susquehanna River, the largest in the state.

Glacial characteristics of Pennsylvania phys-

iography could not be found to be clearly influential on Odonata distribution since only 4 species, *Aeshna clepsydra, Epitheca spinigera, Leucorrhinia proxima* and *Argia bipunctulata,* out of the 97 on Table 4 are known to occur only in regions covered by Wisconsin glaciation, and these are all one-time or two-time occurrences in the state and therefore in the "adventive" category.

If geographical factors governing the ranges of Odonata species are to be understood, climatic influences will have to be thoroughly investigated (19) and ecological physiology of each species will have to be worked out in detail.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Higbee, H. W. 1965. Stream map of Pennsylvania. Pa. Agr. Exp. Sta., University Park, Penna.
2. Beatty, G. H. & A. F. 1968. Check list and bibliography of Pennsylvania Odonata. *Pa. Acad. Sci. Proc.* 42:120.
3. Fenneman, N. M. 1938. *Physiography of Eastern United States.* McGraw-Hill Book Co., New York. 714 p.
4. Ashley, G. H. 1933. The Scenery of Pennsylvania. Penna. Geol. Surv. (4th series) Bull. G 6. 91 p.
5. Leverett, F. 1934. Glacial Deposits in Pennsylvania. Penna. Geol. Surv. (4th series) Bull. G 7. 123 p.
6. Leonard, M. D. (Ed.). 1928. A list of the insects of New York. Cornell Univ. Agr. Exp. Sta. Memoir 101. p. 45.
7. Calvert, P. P. 1910. Odonata, in J. B. Smith's Report on the Insects of New Jersey. N. J. State Museum, Ann. Rept. 1909. p. 73.
8. Fisher, E. G. 1940. A list of Maryland Odonata. *Ent. News* 51:37.
9. Moorefield, H. 1941. Addenda to the Odonata of Maryland. *Ent. News* 52:133.
10. Cruden, R. W. 1962. A preliminary survey of West Virginia Odonata. *Ent. News* 73:156.
11. Ahrens, C. 1968. A list of Odonata taken in the Cranesville Swamp in 1966. *Arboretum Newsletter* (W.Va.Univ.) 17:6.
12. Alrutz, R. W. 1961. Notes and records of Ohio dragonflies and damselflies. *Ohio J. Sci.* 61:13.
13. Cruden, R. W., and N. L. Currie. 1961. Additions to the state and county records of Ohio dragonflies. *Ohio J. Sci.* 61:189.
14. Raff, R. A. & E. C., and G. H. & A. F. Beatty. 1969. Odonata of the Potomac River drainage in Pennsylvania. *Pa. Acad. Sci. Proc.* 43:114.
15. Beatty, G. H. & A. F. 1968. Origins and biogeographic affinities of the Odonata fauna of Pennsylvania. *Pa. Acad. Sci. Proc.* 42:110.
16. Ahrens, C., and G. H. & A. F. Beatty. 1968. A survey of the Odonata of western Pennsylvania. *Pa. Acad. Sci. Proc.* 42:103.
17. Beatty, G. H. & A. F., and C. N. Shiffer. 1969. A survey of the Odonata of central Pennsylvania. *Pa. Acad. Sci. Proc.* 43:127.
18. Beatty, G. H. & A. F., and C.N. Shiffer. A survey of the Odonata of eastern Pennsylvania. *In preparation.*
19. Beatty, G. H. & A. F. Climatic factors in the distribution of Pennsylvania Odonata. *In preparation.*
20. Donnelly, T. W. 1961. The Odonata of Washington, D.C., and vicinity. *Ent. Soc. Wash. Proc.* 63:1.
21. Beatty, G. H. & A. F. 1969. Evolution and speciation in the subgenus *Schizuraeschna,* with observations on *Aeshna (Schizuraeschna) mutata* Hagen (Odonata). *Pa. Acad. Sci. Proc.* 43:147.
22. Walker, E. M. 1957. Changes in the insect fauna of Ontario. *In Changes in the Fauna of Ontario,* edited by F. A. Urquhart. Univ. of Toronto Press, Toronto, published for Royal Ontario Museum. 75 p.



# EVOLUTION AND SPECIATION IN THE SUBGENUS SCHIZURAESCHNA, WITH OBSERVATIONS ON AESHNA (SCHIZURAESCHNA) MUTATA HAGEN (ODONATA).

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## ABSTRACT

Diagnostic characters of *Schizuraeschna* Calvert and its 4 species are given and illustrated. Geographical distribution is outlined and its bearing on evolution and species-formation is discussed. Distributional history, habitats and phenology of *Aeshna (Schizuraeschna) mutata* Hagen, endemic in northeastern U.S., are discussed in the context of parallel aspects of other species of *Schizuraeschna* in western North America, including Mexico.

## INTRODUCTION

The subgenus *Schizuraeschna* was founded by Calvert in 1952 (1) to contain four species that differ from all other species of the genus *Aeshna, sensu lato,* (2) in only one character, i.e., the terminal abdominal appendages of the adult male dragonfly (1). These appendages (Fig. 1) in the four species of *Schizuraeschna (multicolor* Hagen, *mutata* Hagen, *jalapensis* Williamson and *dugesi* Calvert) are distinctive in the "bifid apex of each male superior appendage" (1) (Fig. 1). Other characters cited by Calvert (1,2) separate *Schizuraeschna* from some of the other subgenera of *Aeshna, sensu lato,* but no character other than that of the male appendages separates these four species from all others.

Nevertheless, to one who has had field experience with all of the *Schizuraeschna* species (and one of the writers was surprised to find that he is unique in that respect) and with numerous other species of *Aeshna, sensu lato,* this group is indeed a distinctive one, and its members are readily identified as such, especially the males, and these even while in flight. Among large dragonflies they are certainly the bluest, having bright cerulean blue eyes and face, and a conspicuously blue-spotted thorax and abdomen. When male specimens are collected the unusual form of the terminal appendages (Fig. 1) is at once evident, and this apical bifurcation is the basis of Calvert's subgeneric name.

## DISCUSSION

The geographical distribution of the four entities included in *Schizuraeschna* (Fig. 2) is most suggestive of the evolutionary history of the group. *Aeshna multicolor,* the postulated ancestor of the other 3 entities, is a very well-known and widespread western species ranging along the Cordillera from 19° to 53° N. Lat. (2,5) and spreading eastward in the U.S. as far as Kansas, Nebraska and Missouri (4). It appears that these easternmost occurrences are ephemeral, as is the one at Marthas Vineyard, Massachusetts, documented by a male specimen in the writers' collection that was collected by John Gillespie, 20 August, 1943. Comparison of the latter specimen with series of *A. multicolor* from California, Nevada and Arizona, and with many *A. mutata* from Pennsylvania, reveals that the Marthas Vineyard specimen is clearly *multicolor* and not *mutata* (which is also known from Massachusetts (3)). The Marthas Vineyard specimen was also studied by E. M. Walker in 1955, and he concurred in the decision that it is unquestionably *multicolor.*

Examination of the sporadic records of *Aeshna multicolor* in eastern parts of its range (Fig. 2), and consideration of the Massachusetts specimen, leads to the conclusion that here is a widely distributed species with strong dispersal potential and a demonstrated tendency to push out to the Northeast, where the habitat requirements of a characteristically Cordilleran species are unlikely to be met, at least with respect to foundation of permanent populations. The individual which, by a stroke of luck, was collected on the coast of Massachusetts may have been one of a long succession of pioneers that tried to colonize the Northeast only to meet with ecological or physiological defeat. If this is so, is it too rash to speculate on the possibility of a mutant strain of *Aeshna multicolor* also pushing northeastward, but endowed with a physiological difference that enabled it to succeed where others had failed? And if this ecologically better-adapted pioneer happened to have, or

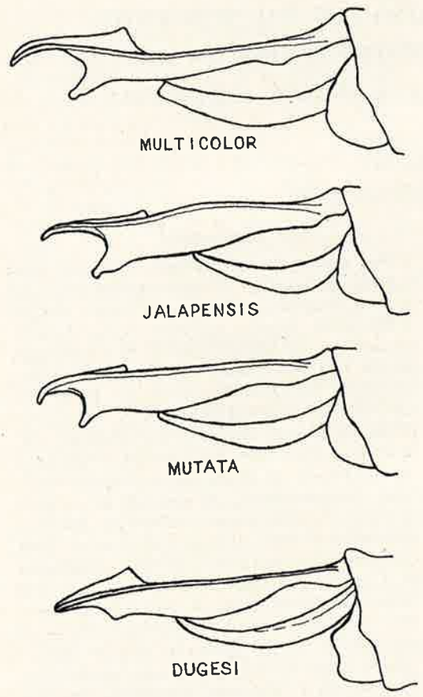



**Figure 1. Male appendages of the 4 species of** Schizuraeschna. **Drawings of** multicolor, jalapensis **and** mutata **after Williamson (11); dugesi original to same scale by G. H. Beatty.**

later develop, structural differences now found in *mutata,* then all requirements for establishment of *Aeshna mutata* as a separate entity would have been met. In the genetic isolation of the disjunctive northeastern colony, selection, inbreeding and drift could easily result in the differences of structure and proportions pointed out by Walker (3,5), as well as reduced vagility from that of the ancestral *multicolor* stock, tending to confine *mutata* to a comparatively small geographical range. Walker (5) suggests that *mutata* is "possibly only a subspecies of *multicolor*." That *Aeshna mutata* arose by mutation could hardly have occurred to Hagen, who described it in 1861 (6) from a female specimen with the locality "North America" in the same publication wherein he also described *Aeshna multicolor* from Texas and Mexico.

The form of the male appendages of *Aeshna mutata* is very similar to that of *multicolor* (Fig. 1), but if populations of the two entities are geographically isolated there is less need for the structural discontinuity of male appendages which in many groups of Odonata is thought of as an isolating device (3, p. 38). A parallel situation prevails in the case of a third species of *Schizuraeschna, A. (S.) jalapensis,* from Mexico and Central America, which Walker declared (3) to be "still more closely related to. . . . .*mutata*" (than to *multicolor).* It appears that *jalapensis* has resulted from a southward colonization by ancestral *multicolor* stock which had already reached a relatively stable southern limit of its normal range in central Mexico. Coming from the same original stock as *mutata,* and evolving under similar conditions of geographical isolation, it might be expected that *jalapensis* would resemble *mutata* more than *multicolor,* and indeed it does (Fig. 1). The range of *Aeshna jalapensis* (Fig. 2) from Jalapa, Mexico, to Chiriqui, Panama, including Guatemala and Costa Rica (2) is not much more extensive than that of *mutata* (Figs. 2,3), which is known from Massachu-




**Figure 2. Known geographic distribution of the subgenus** Schizuraeschna **and its 4 species.**



setts and southern Ontario, south to Kentucky and west to Indiana and Michigan (4,5). Neither occupies a very large area compared to the extensive range of the ancestral *multicolor* (2,5) and both of these offspring species appear, compared with *multicolor,* to be uncommon over most of their known ranges. Exceptions in the case of *mutata* will be pointed out.

*Aeshna dugesi,* the fourth species of *Schizuraeschna,* shows the greatest structural discontinuity of all (Fig. 1). This species is known from but seven locations: two in Arizona (7), one of which is represented by unpublished material in the writers' collection; two in Texas (8), including an unpublished record from Balmorhea, Reeves County; one in Baja California, Mexico (9); and two in central Mexico, one of which is the type locality (10) and the other of which was discovered by the writers in 1958 (fig. 2). Everywhere that *dugesi* has been collected *multicolor* also occurs. Therefore if this entity differed from *multicolor* only to the extent that *mutata* and *jalapensis,* in their geographical isolation, differ, it probably could never maintain its genetic distinctiveness in the face of continuing opportunities to interbreed with typical *multicolor.* It appears that *dugesi* arose from the ancestral stock of *multicolor* with the advent of a mutant strain that had no special ecological adaptations but was characterized by male appendages (Fig. 1) that somehow reduced the probability of successful mating (3, p. 38) with the females of *multicolor* that were all around. With no geographical discontinuity to enforce isolation, *dugesi* could be expected to show greater structural discontinuity from the ancestral stock, while *mutata* and *jalapensis,* in their ecologically enforced geographical isolation, both from the parental *multicolor* stock and from each other, had no need for great structural discontinuity, which can be seen to be lacking in the male appendages (Fig. 1).

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## AESHNA MUTATA

*Aeshna mutata* was a species scarcely known at all until E. B. Williamson discovered it almost in his backyard in Indiana in 1907 and immortalized it in several detailed papers (11, 12) dealing with the Vanemon Swamp in Wells County, Indiana. As few people could, Williamson appreciated this exquisite creature, prized it for its rarity and beauty, and ruefully predicted the destruction of its habitat and its possible extinction from the earth. "And in that day," wrote Williamson (12), "when the swamp will have become a cornfield will *Aeshna mutata* also have disappeared from the earth?" In 1954 G. H. Beatty went with Mary Williamson to the site of the Vanemon Swamp. It had indeed become a cornfield and pitiful remnants of spatterdock were here and there among the corn rows. *Aeshna mutata* was gone. In 1955 he went with S. W. Frost to the "Ten Acre Pond" (13) at Scotia, Pennsylvania (Figs. 3,4). One of the first dragonflies he saw there was one so exquisitely blue that it seemed unreal. From 16 June until 19 July, 1955, *mutata* was observed and collected. In succeeding years, but not every year, up until 1968, *mututa* has been seen and collected at this pond by G. H. & A. F. Beatty, H. B. White, III (13), R. A. Raff, T. W. Donnelly, C. N. Shiffer and undoubtedly others. No other reported colony of *mutata* has been so prolific of specimens and data on this remarkable dragonfly.

## BIONOMICS

At Ten Acre Pond (Fig. 3) larvae and exuviae of *mutata* have been collected on several occasions, and two of these exuviae are the basis of the larval description in E. M. Walker's admirable book (5) on Canadian Odonata. Even on occasions when many imagos were found at the pond, immatures and exuviae were exceedingly difficult to find, leading to the suspicion that Ten Acre Pond may not be the main larval habitat of *mutata.* There are other, less accessible, ponds, pools and ore pits nearby, the Odonata faunas of which have not been investigated. In view of the fact that Ten Acre Pond has dried up completely in



**Figure 3. "Ten Acre Pond," Scotia, Pennsylvania. View toward South. Photograph by G.H. Beatty, September, 1956.**

recent years, and that *Aeshna mutata* has continued to occur there as imagos, it is natural to conclude that there are other habitats nearby where *mutata* completes its life-history even when Ten Acre Pond has dried up completely.

Oviposition by *Aeshna mutata* has been observed at Ten Acre Pond by G. H. Beatty and C. N. Shiffer on several occasions. A relationship with spatterdock, *Nuphar advena* (Fig. 4),



**Figure 4. "Ten Acre Pond," looking northeast from extreme southwest corner, across extensive beds of spatterdock. Photograph by S.W. Frost.**

parallels Williamson's observations in Indiana (11,12), and this is the only oviposition site that has been observed. Numerous females of *mutata* perched on top of spatterdock flowers several inches above the water and inserted the eggs below the water line. Drying up of the pond has greatly reduced the amount of spatterdock since oviposition was first observed in 1955, but oviposition has been observed by C. N. Shiffer as recently as 1968, showing that the entire life cycle of *mutata* may be passed at Ten Acre Pond if it does not dry up completely during the larval period.

In contrast to the observations of Williamson (11) and Price (14) to the effect that *Aeshna mutata* flies over its aquatic habitat only in the morning, the writers and their associates have observed *mutata* on numerous occasions flying over Ten Acre Pond in the afternoon and evening. In 1955 it was observed that *mutata* often increased in numbers over the pond in the afternoon; where there had been only a few in the morning there were sometimes dozens in the afternoon. Males flew in and out among the spatterdocks (Fig 4) at about the level of the leaves, which were often a foot or two above the water, until darkness overtook them and the collector. For mid-afternoon of 28 June, 1955, Beatty's field notes observed "hundreds of *mutata* must have been present; the air over the pond was filled with the flashing of sunlight on their wings."

The exact ecological requirements of *Aeshna mutata* are not entirely clear. The locations where it has been collected (Fig. 5) in Indiana,

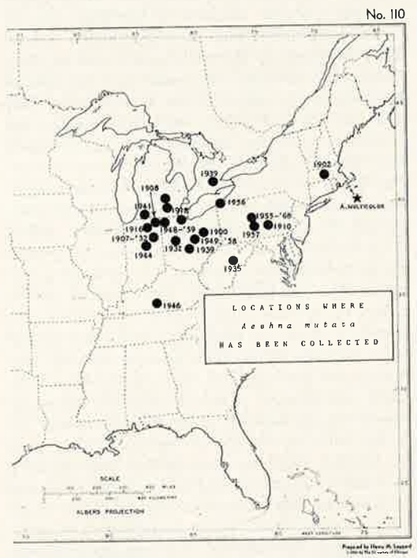



**Figure 5. Known geographical distribution of** Aeshna mutata, **showing years when this species was recorded from each location. Literature references in text.**

Michigan and parts of Ohio were covered by Wisconsin glaciation; those in Kentucky, West Virginia, Pennsylvania and other parts of Ohio are unglaciated. One is hard put to define an *Aeshna mutata* habitat; certainly not all have spatterdock, and some, like the lamented Vanemon Swamp, had little open water. Ohio lakes where Price (14,15) and Harwood (16) collected *mutata* are glacial, and in Price's case it was necessary to pursue *mutata* with a boat.

Elevations at which *mutata* has been found are the lowest, in general, for any species of *Schizuraeschna,* ranging from 600 ft. in Kentucky (17), 700-800 ft. in Ohio (14,15,16,18,19), and 900 ft. in Michigan (20), to 1200 ft. in West Virginia (21) and 1350 ft. in Pennsylvania (13,22,23,28). *Aeshna multicolor,* it is true, occurs at sea level in British Columbia and California (5), but most of its stations are much higher, ranging up to 8600 ft. in Mexico (2). *Aeshna jalapensis* is recorded from 4400 to 6000 ft. in Central America and Mexico (2), and the writers have collected it in Mexico from 4000 to 8000 ft. Calvert (2) records *Aeshna dugesi* from elevations of from 4900 to 6750 feet in Mexico, while the writers have it from 2900 ft. in Texas to 7600 ft. in Mexico. Perhaps all that is shown by these data is that in lower latitudes the same sort of organisms are usually found at higher elevations.



The seasonal distribution of *Aeshna mutata* is noteworthy. While most species of *Aeshna* fly in late summer in the U.S., and all do in Pennsylvania except *mutata* (24), this species emerges as early as 5 June (13) and has usually disappeared by mid-July. It would appear that *mutata* has seldom had an opportunity to meet other species of *Aeshna.* This temporal isolation from its congeners may well have affected its genetic makeup. Other species of *Schizuraeschna* are known to fly over much longer periods, but here again the element of latitude makes comparison of dates practically meaningless. *Aeshna multicolor* is known (2) to fly somewhere in its range (Fig. 2) from May to September, while *A. jalapensis* has been collected from May to October in Mexico, Guatemala and Costa Rica (2). *Aeshna dugesi* is recorded from 25 June-6 July in Texas (8), and 6-7 October in Baja California, Mexico (9), while the writers have specimens taken on 1 September (Arizona), 14 September (Texas) and from 13 July to 30 August in central Mexico near the type locality.

While habitats of *Aeshna mutata* are not easily defined, and those of the adaptable *multicolor* run the gamut of many types of lakes and ponds (5), *jalapensis* and *dugesi* have more definable habitat-types. *Aeshna jalapensis,* in Oaxaca and Chiapas, Mexico, frequents small mountain tarns in pine forest regions, while at one place in the state of Veracruz the habitat is a large pool made by the outflow of a gigantic spring at an elevation of 4000 feet. *Aeshna dugesi,* on the other hand, inhabits intermittent arid-country canyon streams (8). This ecological specialization might be an isolating factor if it were not for the fact that such streams are almost the only aquatic habitats in thousands of square miles of Mexico and southwestern U.S., and for want of its preferred habitats *Aeshna multicolor* often occurs in such locations (8,9) right along with *dugesi.*

Because of its reputation for rarity it is desirable to review *Aeshna mutata's* known distribution in space and time (Fig. 5). In his great 1912 monograph (3) of the genus *Aeshna,* E. M. Walker accounts for *mutata's* known distribution in Massachusetts, Ohio, and Indiana, where Williamson continued to collect it in 9 of the 15 years from 1907 to 1921 (29). After being absent, or at least not found, at the Vanemon Swamp from 1921 to 1931, *Aeshna mutata* reappeared there in 1932 and was one of the last dragonflies collected by Williamson before his death in 1933 (25). It was not seen again in Indiana until 6 June, 1944, when Ross Scott, a beekeeper, took three specimens by hand on a cold morning from fence posts, bee hives and tree trunks in Grant County (26). In Michigan a dozen or more specimens of *mutata* were collected in 1941 by the amazing R. R. Dreisbach (27), and 2 other Michigan occurrences, in the vicinity of Ann Arbor, have been reported (20). Various Ohio records of *mutata* have been published (3,14,15,16,18, 19) and Homer Price (26) has furnished others. The one Kentucky specimen reported by Cook (17) and two females from Elkins, West Virginia (21) collected by Carsten Ahrens, account for all of the remaining specimens except those from Pennsylvania (13,22,23,28) and a single Canadian record from southern Ontario (5).

Most of the foregoing were one-time occurrences; only Williamson's observations in Indiana (11,12,15,25), those of Price (14,15,26) and Harwood (16) in Ohio, and of Beatty et al. (13,22,23) in Pennsylvania qualify as continuing observations. So little is known, even now, about the requirements of *Aeshna mutata* that much more work on its ecological and life-history aspects requires to be done.

## ACKNOWLEDGMENTS

The writers are especially indebted to John Gillespie for the amazing specimen of *Aeshna multicolor* from Marthas Vineyard, and to E. M. Walker for his painstaking study of it, and for his description of their larval material of *mutata* in his superb book. Thanks are also extended to M. J. Westfall, Jr., E. J. Kormondy, Homer Price, Paul D. Harwood and Carsten Ahrens for unpublished records, and to Mary Williamson and S. W. Frost for introduction to Williamson's classic locality, and for introduction to *Aeshna mutata* itself.

## REFERENCES


1. Calvert, P. P. 1952. New taxonomic entities in Neotropical Aeshnas. *Ent. News* 63:253.
2. Calvert, P. P. 1956. The Neotropical species of the "subgenus Aeshna". Amer. Ent. Soc. Memoirs No. 15. v + 251 + iv p.
3. Walker, E. M. 1912. The North American dragonflies of the genus *Aeshna*. Univ. Toronto Studies, Biol. Ser. No. 11. 213 p.
4. Needham, J. G. and M. J. Westfall, Jr. 1955. *A Manual of the Dragonflies of North America (Anisoptera)*. Univ. Calif. Press, Berkeley. 615 p.
5. Walker, E. M. 1958. *The Odonata of Canada and Alaska*, Vol. 2. Univ. of Toronto Press, Toronto. 318 p.
6. Hagen, H. A. 1861. *Synopsis of the Neuroptera of North America*. Smithsonian Institution, Washington. 347 p.
7. Westfall, M. J., Jr. *Personal communication.*
8. Gloyd, L. K. 1958. The dragonfly fauna of the Big Bend Region of Trans-Pecos Texas. Univ. Mich. Mus. Zool. Occ. Papers No. 593. 23 p.
9. Williamson, E. B. and J. H. Williamson. 1930. Five new Mexican dragonflies. Univ. Mich. Mus. Zool. Occ. Papers No. 216. 34 p.
10. Calvert, P. P. 1905. Odonata. *In* Biologia Centrali-Americana, Vol. 50, Neuroptera. London.
11. Williamson, E. B. 1908. Three related American species of Aeshna. *Ent. News* 19:264.
12. Williamson, E. B. 1921. Two days with Indiana Odonata. *Ent. News* 32:19.
13. White, H. B., III. 1963. Seasonal distribution and abundance of Odonata at a large pond in central Pennsylvania. *N.Centr.Br.Entom.Soc.Amer.Proc.* 18:120.
14. Price, H. F. 1958. Additional notes on the dragonflies of Ohio. *Ohio J. Sci.* 58:50.
15. Price, H. F. 1950. Notes on the dragonflies of northwestern Ohio. *Ohio J. Sci.* 50:71.
16. Harwood, P. D. 1960. Additional notes on the Odonata of Ohio. *Ohio J. Sci.* 60:341.
17. Cook, C. 1951. Some new dragonfly records for Kentucky. *Ent. News* 62:181.
18. Borror, D. J. 1937. An annotated list of the dragonflies of Ohio. *Ohio J. Sci.* 37:185.
19. Kormondy, E. J. 1956. *Macromia alleghaniensis* Williamson in Ohio with new records of other Odonata in Ohio. *Ohio J. Sci.* 56:13.
20. Kormondy, E. J. 1958. Catalogue of the Odonata of Michigan. Univ. Mich. Mus. Zool. Misc. Publ. 104. 43 p.
21. Ahrens, C. *Personal communication.*
22. Ahrens, C., and G. H. & A. F. Beatty. 1968. A survey of the Odonata of western Pennsylvania. *Pa. Acad. Sci. Proc.* 42:103.
23. Beatty, G. H. & A. F., and C. N. Shiffer. 1969. A survey of the Odonata of central Pennsylvania. *Pa. Acad. Sci. Proc.* 43:127.
24. Beatty, G. H. & A. F., and H. B. White, III. 1969. Seasonal distribution of Pennsylvania Odonata. *Pa. Pa. Acad. Sci. Proc.* 43:119.
25. Montgomery, B. E. 1934. Records of Indiana dragonflies-VII. *Ind. Acad. Sci. Proc.* 43:211.
26. Price, H. F. *Personal communication.*
27. Kormondy, E. J. *Personal communication.*
28. Calvert, P. P. 1911. [*Aeshna mutata* Hagen new for Penna.] *Ent. News* 22:336.
29. Montgomery, B. E. 1947. The distribution and relative seasonal abundance of Indiana species of five families of dragonflies. *Ind. Acad. Sci. Proc.* 56:163.




# GEOGRAPHIC ASSOCIATION BETWEEN MIGRATION AND SELECTED ASPECTS OF AGE STRUCTURE

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## ABSTRACT

Despite the rather close geographic association between net migration and trends in median age, evidence is presented to support the argmuent that persons under 18 and those 65 and over respond differently to the repellent and attractive qualities of various communities, factors so often associated with the decision to migrate. In counties of Pennsylvania which recorded significant out-migration during the 1950 decade, and where the number of inhabitants under-18 actually decreased as expected, there were gains in the population 65 and over of sufficient magnitude to suggest more growth than is explainable by a residual, elderly population enjoying an increasing life expectancy. Most of the counties which lost great numbers via migration attracted aged migrants and thereby established disparate flows of an age-selective nature.


## INTRODUCTION

The effects of migration upon age structure have received a great deal of attention (1), and the conclusions drawn from such study vary little in that most, if not all, agree that the age-selective nature of migration (i.e., young adults display the greatest propensity to migrate, and the tendency wanes with age) leaves an older population in places where out-migration dominates and, where the influx of new residents exceeds out-migration, there is a more youthful populace. The validity of this generalization, utilizing the counties of Pennsylvania as the spatial-statistical base and the 1950 to 1960 decade as the time period is examined here. Three aspects of age (median age, population under 18, and population 65 and over) are studied as their trends relate, in the geographic sense, to net migration.

## RESULTS AND DISCUSSION

The degree of geographic association between net migration and trends in age structure was analyzed using regression-correlation techniques. The following discussion is focused upon the results of the analysis.

The relationship between migration and median age (Fig. 1) indicates that the counties which lost 15 percent or more of their 1950 population via migration are anomalous in the sense that their populations aged over the period, as measured by the median, far more than expected. Nevertheless, it is evident that the generalization presented at the outset—that in-migration usually fosters a reduction in median age and out-migration an increase—is acceptable in the case of the migrational behavior of the inhabitants of Pennsylvania's counties during the 1950 decade. Indeed, as the coefficients of correlation (r) shown on each scattergram (Fig. 1, 2, 3) indicate, the relationships between migration and the selected age variables are well within the range of statistical significance (2).

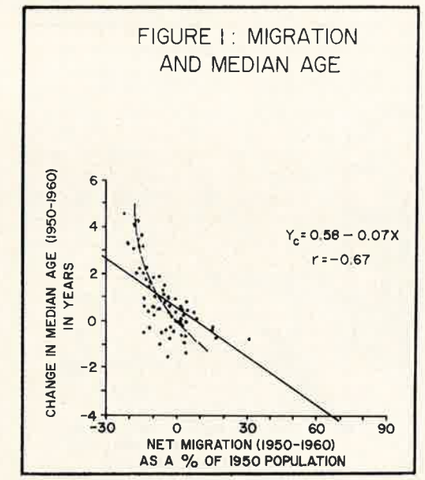



**Figure 1. Migration and Median Age. Although a prediction line illustrating a linear-negative relationship has been fitted, the curve denotes that, for counties registering a net loss due to out-migration of sufficient magnitude, the relationship is considerably weaker, as reflected by the nearly vertical slope at that end of the curve.**

TABLE 1. *Net Migration and Trends in Age Structure, 1950-1960 for Counties with Net Migrations of 15% or more of their 1950 Populations.*

| County | Net Migration as a % of 1950 Population | Change in Median Age in Years | % Change in Population: Under 18 | % Change in Population: 65 and over |
|---|---|---|---|---|
| Bucks | 81.7 | −3.3 | 182.4 | 58.1 |
| Montgomery | 30.9 | −0.7 | 80.5 | 50.7 |
| Chester | 16.5 | −0.6 | 59.7 | 33.6 |
| Cumberland | 15.6 | −0.2 | 57.4 | 32.5 |
| Delaware | 15.4 | −0.3 | 62.3 | 56.9 |
| Luzerne | −17.7 | 4.4 | −7.1 | 27.8 |
| Northumberland | −18.7 | 3.2 | −4.5 | 18.7 |
| Schuylkill | −19.2 | 4.3 | −9.1 | 21.1 |
| Fayette | −21.4 | 3.4 | −6.0 | 23.9 |
| Greene | −23.3 | 4.7 | −12.9 | 23.1 |

Turning to a more specific aspect of age, trends in numbers of persons under 18 are related to net migration on Figure 2. Suffice it to say that the degree of relationship is very high, indicating when its regional growth patterns were related to migration, that this age group performed as expected. It is worth noting, as well, that the counties for which significant in-migration was reported had rather high fertility rates when compared to counties within which out-migration prevailed (3). It could be argued, therefore, that in-migration contributes to a reduction of median age in two ways: first, because of the relative youth

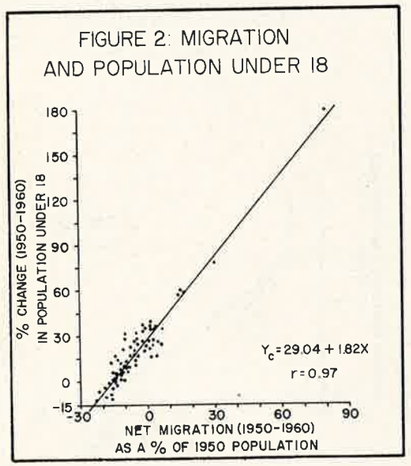



**Figure 2. Migration and Population Under 18. This scattergram depicts an array of dots which are linear and highly positive. This reflects the age-selective nature of migration in the sense that the under-18 cohort does not normally migrate independently of the young adult and middle age groups, their (the youth's) parents and families.**

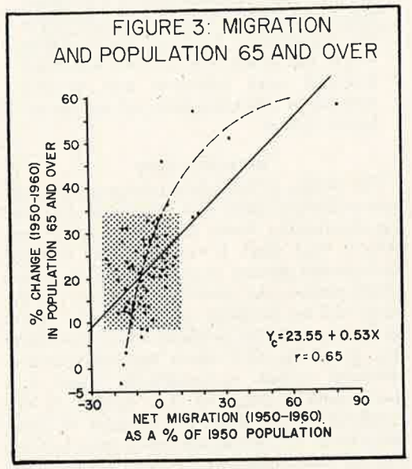



**Figure 3. Migration and Population 65 and Over. This diagram suggests that, despite the linear plot, the curvilinear is far more appropriate. Observe that for counties which either lost population or gained slight to moderate numbers due to net migration (enclosed by the superimposed, rectangular pattern), their array is quite random, signifying little relationship within a limited range of variation in migration but a positive relationship over the total range.**



of the migrants, *per se*, and second, because of the transferral of the migrants' greater fertility, a correlate of their youth, to the county of destination.

Despite the relatively high coefficient of correlation for the geographic association between net migration and percentage change in the population 65 and over during the 1950 decade (Fig. 3), it is obvious that the randomness of the array at the lower end of the migrational scale introduces some doubt concerning the relationship. The differences between the group of counties which gained considerably via migration and those which lost population because of migration, are not as great for the population 65 and over as one might expect (Table 1).

By applying the age-specific mortality rates to the cohort 55 and over in 1950 in the ten counties listed on Table 1, it was possible to estimate (4) crudely the movements of the elderly during the 1950 decade. Such estimation (Table 2), reveals that, of the five counties where out-migration reached 15% or more, four actually received a net gain of migrants at the advanced ages. Even admitting the probability of a wide margin of error in these estimates, there is, nevertheless, the suggestion of a migrational eddy—elderly persons moving into counties within which the general flow was outward, and thereby operating independently of the young adults and middle-age groups upon whom the migratory behavior of the population under 18 is so highly dependent.

Concerning the error in the age-specific migration estimates, the death rates for the five counties in question (those with net migration of −15.0% or more) averaged 11.1 percent, compared to 9.0 for the five counties which gained a significant percentage of migrants. Thus, if the age-specific mortality rates for the Commonwealth were adjusted according to the crude death rates of each county, the results would suggest that age-specific death estimates used to compute the migration figures on Table 2 are *understated* for the five counties which lost inhabitants and overstated for the five which gained. This implies that, since the original adjustments were based upon age-specific mortality, the under-estimation of death would create an *error on the low side* of migration into the counties of Greene, Fayette, Schuylkill, Northumberland, and Luzerne. The migrational counter-current, so called, indicates an absolute increase in the elderly inhabitants of the selected counties. The evidence of such absolute growth of the aged cohort thereby negates the notion that their increase in median age is only a relative one, predicated on the out-flow of the younger inhabitants and the resultant inflation of the proportion of aged in the residual population.

It remains to further substantiate these findings and to sort out the reasons why these counties, which lost heavily through out-migration and whose economic stagnation during the period is a matter of record, attracted elderly migrants during a period of out-migration of the other age groups. One could speculate that this might represent a return to former communities of residence, from which these now elderly persons were repelled at an earlier time because of depressed economic conditions. This line of reasoning might be worth following, considering the importance of previous knowledge concerning the place of destination as a factor in the decision to migrate, as well as the comparative freedom afforded retirees by company retirement plans and social security benefits. Freedom, that is, from the very economic stagnation which might have weighed heavily in their initial decision to move out. Depressed areas offer certain positive features to such persons—less expensive and readily available housing, low property taxes, and a generally low cost of living—strong attractions to those attempting to subsist on fixed incomes.

## REFERENCES AND NOTES

1. Simkins, P.D., and G.A. Schnell. 1964. Recent Population Changes in the Anthracite Belt. *Proc. Penna. Acad. Sci.* 87:220. Simkins, P.D. 1965. Distribution of the Aged in Pennsylvania. *Proc. Penna. Acad. Sci.* 38: 183. Bogue, D.J. 1969. *Principles of Demography*. J. Wiley and Sons, New York.
2. Primary data for maps and tables used throughout this article are from U.S. Census of Population and the Pennsylvania Department of Health.

TABLE 2. *Estimated Net Migration, 1950-1960, of the Population 55 and Over in 1950.*

| County | Number | % (5) | County | Number | % (5) |
|---|---|---|---|---|---|
| Bucks | 6,451 | 25.8 | Luzerne | −539 | −0.8 |
| Montgomery | 13,644 | 20.9 | Northumberland | 929 | 4.4 |
| Chester | 4,690 | 16.9 | Schuylkill | 323 | 0.9 |
| Cumberland | 2,757 | 17.1 | Fayette | 1,670 | 5.2 |
| Delaware | 8,686 | 13.3 | Greene | 988 | 12.7 |

3. The five "growth" counties had an average crude birth rate of 24.4, whereas the five with heavy losses averaged just under 19.0. The range in crude birth rate for all 67 counties was from 16.0 to 28.0.
4. The estimating procedure involved the reduction of the population 55 or over for selected counties by the age-specific death rates for the Commonwealth, 1950. The elderly cohort so reduced represents the "expected" size of the population 65 or over in each county in 1960. By comparing the "expected" population 65 or over to the enumerated figure, the difference is assumed to be net migration.
5. These percentages represent net migration, 1950 to 1960, as a percentage of the population 55 and over in 1950.



# GEOPOMOLOGICAL ORDER IN PENNSYLVANIA

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## ABSTRACT

Cluster analysis indicates that similar patterns of fruit growing regions occur in Pennsylvania, whether or not a spatial contiguity constraint is imposed. The resulting pomological regions are largely a function of climate with large centers of commercial fruit production distorting broader trends.

## INTRODUCTION

In studies of the distributions of fruit crops and their relationships with the physical environment, it is often necessary to treat each crop individually unless two or more crops have essentially similar ecological demands. However, when confronted with an assemblage of fruit crops whose physical constraints are similar in nature but quantitatively different, it can be hypothesized that spatial variations in mix of these various crops will be influenced by the spatial variations of relevant physical factors. The purpose of this study is to synthesize pomological regions within Pennsylvania using numerical taxonomy (1) and to identify some physical factors influencing the resulting regional patterns.

## INDEX OF RELATIVE IMPORTANCE

The crops used in this study are apples, peaches, pears, grapes, plums and prunes, sour cherries, and sweet cherries. These are the fruits for which the 1964 *U. S. Census of Agriculture* for Pennsylvania provides county data.

In computing the index, numbers of trees or vines of all ages rather than acreages were used, since the former is likely to be a more precise measure for counties with very little fruit production. To smooth variations due to possible severe local weather conditions, average values were taken from 1959 and 1964 census data.

In order to maintain a focus on the mix (rather than on intensity) and to avoid logical problems arising, for example, from comparing numbers of apple trees with numbers of grape vines, a significance index was obtained for each fruit crop for each county. This index considers: a) the importance of the crop in the county relative to production of that crop within the entire state, and b) the importance of the crop within the county relative to other fruit crops grown there.

The index was obtained as follows. First, a crude index was computed by dividing the average numbers of trees or vines for each crop for each county by the total number of trees or vines of that crop within the Commonwealth. Intensity was then eliminated by scaling these crude indices so that, within a county, the final index for each crop summed to 1. As a result, this standardization also eliminates problems inherent in comparing subdivisions of unequal area.

The formula of this *index of relative importance* is

$$I_{ij} = \frac{\left[\dfrac{T_{ij}}{\sum_{i=1}^{m} T_{ij}}\right]}{\sum_{j=1}^{n}\left[\dfrac{T_{ij}}{\sum_{i=1}^{m} T_{ij}}\right]}$$

where $I_{ij}$ and $T_{ij}$ are the index of relative importance and the average number of trees or vines for county $i$ and crop $j$, and $m$ and $n$ are the numbers of counties and crops involved.

## TAXONOMIC GROUPING

An iterative numerical taxonomy computer program, TAXON (2), was used in grouping the 67 counties into pomological regions. These regions or clusters were based on simple linear taxonomic distance in 7-dimensional space. The formula for $D_{ij}$, the taxonomic distance between a pair of counties or regional clusters $i$ and $j$, is

A pairing of two operational taxonomic units (OTU's), which could be either a single county or a previously formed cluster, occurred when the taxonomic distance between the two OTU's

$$D_{ij} = \left[ \sum_{k=1}^{7} (I_{ik} - I_{jk})^2 \right]^{1/2}$$

was below the current critical taxonomic distance in use during an iteration. A special option in the program provides for pairing only when the two OTU's are spatially contiguous as indicated in a spatial contiguity matrix of l's and O's submitted with the data. When a pairing occurred, moreover, weighted mean values of the seven indices were computed for the two OTU's. These means were weighted according to the numbers of counties previously clustered into each current OTU. After pairing, the two OTU's were treated as a single OTU with a weight equal to the number of counties now included in the cluster. After an iteration the critical taxonomic distance was incremented by a fixed amount, and another iteration was begun until all counties had been joined into a single cluster.

Regions were formed by constructing and examining a taxonomic dendogram. The sample dendogram (Fig. 1) illustrates this procedure. Two stems are joined into a single stalk at the taxonomic distance, measured along the vertical axis, where the two OTU's, were paired. Regions can be defined by cutting off the taxonomic tree at a specific taxonomic distance. This is done subjectively. Those counties that are still linked together belong to the same region.

Two sets of regions were obtained from the data, one with and the other without the spatial contiguity constraint enforced. The dendrograms were inspected and cutoff values were chosen at taxonomic distances just before the remaining OTU's merged into very large and seemingly meaningless agglomerations. Figure 2 shows the regions formed with the

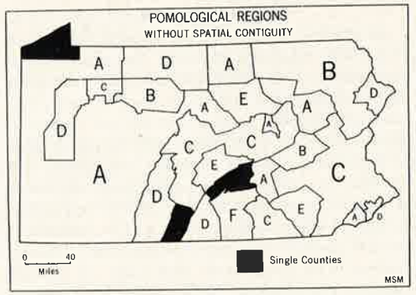



**Figure 2.**

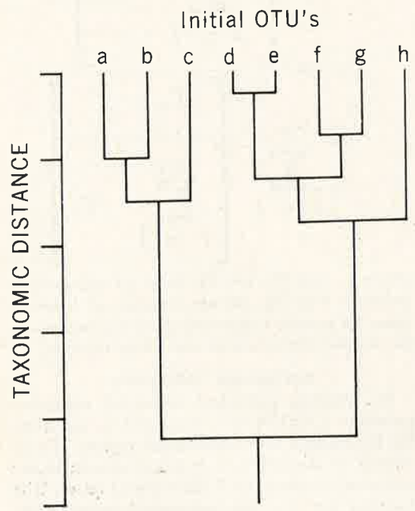



**Figure 1. Simple taxonomic dendogram. At the 0.60 level of similarity two clusters would be evident: a-b-c and d-e-f-g. At the 0:95 level of similarity only one cluster, d-e, would be evident, the remaining OTU's being single.**

contiguity constraint imposed. The cutoff distance for this classification was 0.190. If complete similarity (1.00) is said to occur when two OTU's have the same index values for all seven crops, and if complete dissimilarity (0.00) is said to occur when two OTU's have only one but not the same crop, this threshold value represents groupings formed at the 0.87 level of similarity. Figure 3 shows the regions formed without the spatial contiguity con-

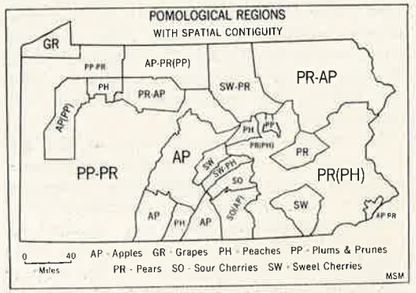



**Figure 3.**



straint. For these groupings, the critical distance was 0.236 or the 0.83 level of similarity.

The coincidence of most of the boundaries (Fig. 2, 3) indicates that contiguous regions tend to occur naturally. This fact adds reliability to the various broad trends evident in Figure 2.

## INTERPRETATION OF THE REGIONS

The pomological regions shown in Figure 2 were identified by showing on the map those crops whose averaged indices were well above the expected value of 1/7 that would have occurred if each county had the same index of importance for each of the seven crops. In addition, more stringent thresholds were employed for the single counties, since the grouped counties had the indices for their prominent crops diluted when the weighted means were computed. Only crops with indices greater than 2/7 were used for identifying the single counties. Furthermore, with larger clusters two classes of identifiers were used. The first shows those crops for which the index is 1/4 or greater. The second identifier, shown in brackets where used in Figure 2, indicates less important crops with indices between 1/4 and 1/5. In all cases, the crop with the higher index is shown in the first position.

The use of the stringent index of relative importance permits the larger patterns of Figure 2 to show a greater and more meaningful relation to climate than regionalizations based on simple percentages within each county (3). Pomological order exists and is a function of the physical environment. Except for random noise, such as a high relative importance of peaches in Forest County (where orchards are insignificant), the pomological regions are largely consistent with Jannick's (4) ranking of fruit crops from greatest to least winter hardiness: apples, plums, pears, cherries, peaches, and grapes.

Climatic influences on pomological order are also supported by the Pennsylvania Crop Reporting Service's geographic data on the probability of late spring frosts, which are a major destroyer of fruit (5). Apples are relatively important in the commercial fruit regions of south central and central Pennsylvania and in the frost-hazardous northern and northeastern parts of the Commonwealth. Plums and prunes are of major importance on the Applaachian Plateau, where the frost danger is serious just west of the Allegheny Front in central Pennsylvania. Pears are of significance in the minor commercial fruit region of southeastern Pennsylvania and in the more severe northeastern and western regions. Cherries are largely important in south central Pennsylvania on the South Mountain Foothills and in Lancaster County. They are commercially significant in both of these areas. Peaches are of importance in the relatively mild southeast. Grapes dominate in Erie County where the lake moderates temperatures during the critical spring and fall periods.

Worthy of note is the fact that the major fruit area, south central Pennsylvania, does not show up as an agglomeration of several counties. There is less agreement across county boundaries here than in less commercially important areas on the Plateau and in the northeast and the southeast. Is this due to the incidence of many large commercial growers in south central Pennsylvania or to local variations in the physical environment? This and other, possibly cultural phenomena, such as the concentration of the relative importance of plums in the west, are yet to be explained.

## REFERENCES

1. Sokal, R. R., and P. H. A Sneath. 1963. *Principles of Numerical Taxonomy*. W. H. Freeman Co., San Francisco.
2. TAXON was written by Conrad Strack of the Department of Geography, The Pennsylvania State University.
3. See, for an example of the simple percentage method, Olmstead, C. W. 1956. American orchard and vineyard regions. *Econ. Geog.* 32:189.
4. Janick, J. 1963. *Horticultural Science*. W. H. Freeman Co., San Francisco. p. 400.
5. Kauffman, N. M., and R. G. Butler, 1961. Late spring and early fall freezes in Pennsylvania. *Crop Reporting Service Bulletin*. n. 15.

# THE DISTINCTIVENESS OF ATMOSPHERIC PRESSURE SYSTEMS

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## ABSTRACT

Cyclones and anticyclones are often described according to their surface characteristics. However, the third demension, height, needs to be investigated before valid statements concerning these pressure systems can be made. In this paper, two surface "highs" and two surface "lows" are located on weather maps. Upper air charts are used to determine barometric pressure at the 500 mb level. It becomes evident that the systems aloft may, or may not, be similar to those at the surface of the earth.

## INTRODUCTION

The terms cyclone and anticyclone, which indicate low and high atmospheric pressure respectively, generally refer only to relative barometric pressure variations at the surface of the earth. However, these terms are often used as though all "lows" are similar and as though one "high" is similar to all others. The fallacy in this assumption is that low and high refer only to barometric pressure and usually at some definite horizontal level such as sea level.

Generalized statements are often made as though there was but one type of each; particularly is this misconception common in geographic literature. The reason for these generalizations is that analysis is often based exclusively on data from surface weather charts.

The purpose of this paper is to show that there are two distinct types of both cyclones and anticyclones; however, it is difficult if not impossible to distinguish the various types using only surface weather data. The only way that the vertical component of pressure systems can be analyzed is through the use of constant level or constant pressure charts.

The United States Weather Bureau issues, along with the surface chart, a 500 mb constant pressure chart which shows conditions approximately half way through the atmosphere. Contrasts and comparisons between atmospheric data for the surface of the earth and conditions aloft can be made by analyzing these two charts. It is information from these levels that will be used in this paper. Obviously, in weather forecasting, data from various levels are analyzed.

## TYPES OF PRESSURE SYSTEMS

Figure 1 reveals a low pressure area over the southwestern section of the United States. This zone of convergence might lead one to hurriedly and incorrectly conclude that precipitation is occurring or likely to soon occur in this region. In fact, precipitation is not evident or likely to soon occur since the dew point is approximately 30° to 40° below the temperature; also, cloud cover is light or nonexistent.

Investigation of conditions aloft on the 500 mb chart indicates that high pressure is evident at that level. This warm core or thermal "low" is, therefore, only a "low" at or near the surface of the earth and is produced by intense surface heating. This system is likely

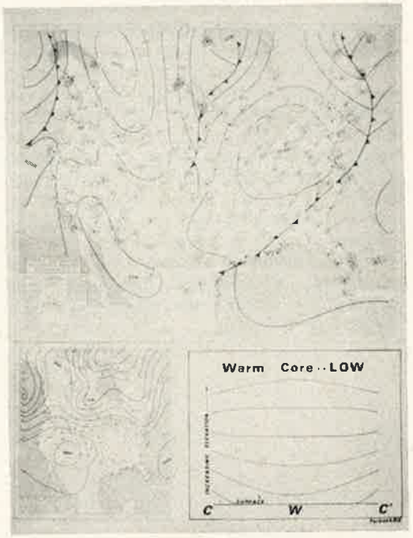



**Figure 1**



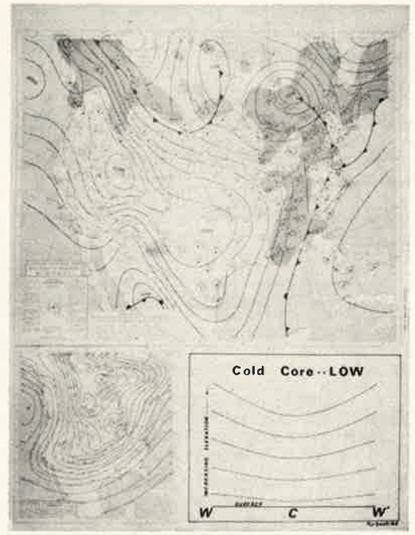



**Figure 2**

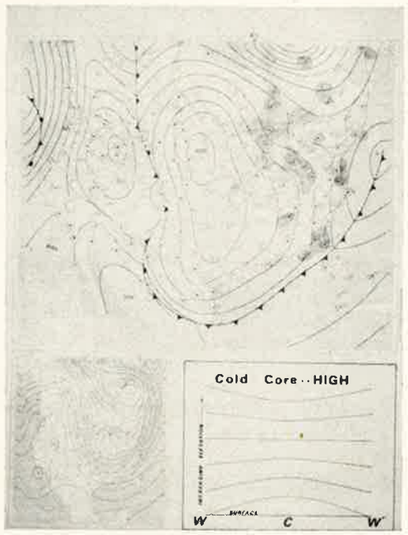



**Figure 3**

to pulsate within its source region and not move in an easterly direction as one would expect the typical cyclone to do. This type of "low" fits nicely into the now outdated convectional theory of cyclonic development.

Figure 2 shows a "low" with strong cyclonic circulation lying just north of the Great Lakes region. An occluded front extends southeastward across eastern Pennsylvania with rain evident in the Maritime Polar and Maritime Tropical air masses from the St. Lawrence River to the Gulf of Mexico. This cyclone represents the type which is most often used as an example when discussing low pressure systems.

An anlysis of atmospheric conditions aloft reveals that enclosed contours are evident to at least three miles. Therefore, it is quite obvious why one would expect the probability for widespread precipitation to be great in this cold core "low." This dynamically produced system undoubtedly originated to the west or south of its present position and then migrated in the prevailing westerly wind belt.

Figure 3 reveals the counterpart of the warm core "low" portrayed by Figure 1. Here the eastern two-thirds of the United States is covered by cold dense Continental Artic air producing a high pressure system. Radiational cooling within the source region of Canada produced this anticyclone which spilled southward to the Gulf of Mexico. Negative temperature values are evident equatorward of 40° north and snow is falling in southern Georgia.

However, the upper air chart indicates that this cold core "high" is actually capped by enclosed low pressure contours at the 500 mb level. Therefore, one may safely conclude that this is only a high pressure system at or near the surface of the earth. This system represents the type which is most often used as an example of the typical anticyclone.

Figure 4 shows a high pressure system is evident over the southeastern portion of the United States. Although Maritime Tropical air covers much of this region, neither rain nor heavy cloud cover is evident. Aloft, high pressure exists at least to the mid level of the atmosphere.

This warm core "high" is really an extension inland of the Bermuda High. It is from this type of system that subsidence aloft often produces inversions and heat waves or Indian Summer. Also, it is likely to pulsate but not become a moving cell of high pressure.

## SUMMARY AND CONCLUSIONS

It is apparent that making generalized statements concerning cyclones and anticyclones and categorizing each of them into single classification systems is both incomplete and

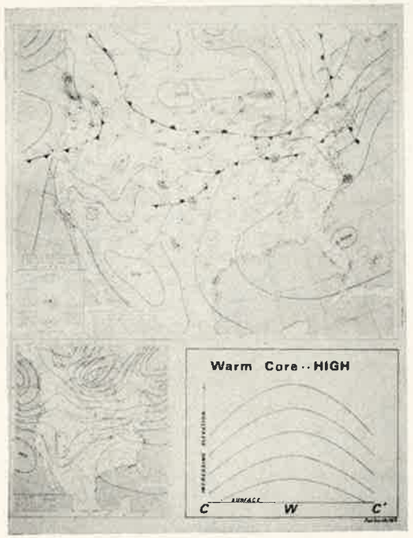



**Figure 4**

incorrect. The third dimension, vertical development, must be considered before one can draw valid conclusions pertaining to their characteristics.

There is not one but two distinct types of each system and any attempt to explain their development and resultant weather will be erroneous without some knowledge of the vertical component.

## REFERENCES

1. Blair, T. and R. Fite. 1965. *Weather Elements* (5), Prentice-Hall, Englewood Cliffs, New Jersey. pp. 152-167.
2. Day, J. 1966. *The Science of Weather*. Addison-Wesley Publishing Co., Reading, Mass. pp. 99-108.
3. Donn, W. L. 1965. *Meteorology*. McGraw-Hill, New York. pp. 222-224.
4. Malone, T. F. (ed.) 1951. *Compendium of Meteorology*. American Meteorological Society, Boston, Mass.
5. Riehl, H. 1965. *Introduction to the Atmosphere*. McGraw-Hill, New York. pp. 147-170.
6. Strahler, A. 1963. *The Earth Sciences*. John Wiley & Sons, Inc., New York. pp. 266-277.
7. Taylor, G. F. 1963. *Elementary Meteorology*. Prentice-Hall, Englewood Cliffs, New Jersey. pp. 113-119.
8. Trewartha, G. 1968. *An Introduction to Climate* (4). McGraw-Hill, New York. pp. 195-220.
9. Environmental Sciences Service Administration, U. S. Weather Bureau. *Daily Weather Maps*. Washington, D. C.



# SYLLABI, SIGNIFICANCE AND THEORY IN GEOGRAPHY: A PROBLEM DEFINED

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My concern is with loss of meaning in the classroom. Does what happens in geography classrooms signify anything in particular? Or is "geography" merely a convenient noise standing for what certain people named geographers do?

Many of us who have had experience in the college classroom with freshmen students are acutely aware of the difficulty of communicating with them. The problem, I submit is our's no less than their's, because what we communicate is information and an approach to the world. Facts to be sure, but not without interpretation. And it is in the intepretation that the meaning, and our problem lies; for students have already acquired a good bit of information and many explanations. They have sensed the world directly and have heard, read, and seen it through the media of mass communication. They are not without data, but data is not all that they bring with them to class.

In addition to views acquired from the general intellectual climate and their own sense data they bring the views of other disciplines. Several other "sciences" concern themselves with the lateral extent of their particular set of phenomena, the mappable distributions of whatever objects or processes they study. The college student has formed a kind of personal geography for himself without any previous formal instruction, and all this cultural baggage contributes to the erosion of significance in our communication with him.

Not all geographers are aware of this problem, but many have identified it and determined to do something about it. At the University of Minnesota for three summers, Institutes for trainers of teachers have been funded under the National Defense Education Acts, Title XI by the U.S. Department of Health, Education and Welfare. These Institutes were conceived, organized and, for the first two years, directed by Professor Fred Lukermann. Lukermann stated his purpose in an introductory talk to the twenty-five participants of the 1966 Institute, and thereby expanded upon the statements contained in the initial publicity. His purpose was to incorporate new knowledge about the world into all levels of teaching without completely overturning the traditional curriculum. The old themes and objectives, development of a sense of distance and direction, were to be combined with new emphases in such a way as to "retain the conceptual structure of geography" so that it is recognizable as geography whether the student encounters it in grade one, on television or at graduate school. Lukermann called not for more content, but more meaningfulness; "no new geography but a different conceptualization."

This concern for meaning interests me because I am a Christain geographer. Geography's conceptual structure and Christain doctrine are merely different forms of theoretic thinking and I cannot compartmentalize my thought indefinitely. I am a whole man.

Others have attempted to trace the links between loss of meaning, and the metaphysical contentions implicit in artforms by an historical method (1). The best known work on geographical methodology in English, Hartshorne's *The Nature of Geography* (2), uses the same method to establish the connections between geography and philosophy. But to make the history of ideas the principle of explanation for other branches of science is an aberration of true science. Historicism is simply a kind of substitute for religion when it pretends to explain the totality of reality (3). The struggle to integrate old and new knowledge goes on. Lukermann called for a summary from participants in the Institute program including a ten page course outline, and a statement of what the institute was trying to do. This paper is part of my response.

At Kutztown State College where I taught for three years, every student is obliged to take one geography course in fullfillment of the general education requirement. This introductory course entitled "Elements of Geography" is offered every semester, summers included. I taught it seven times. About three-fifths of all my students were in that course.

Most of the State Colleges have a similar course and comparable requirements.

Because it is a multiple section course taught by every member of the geography faculty a common course outline is a necessity. Dr. C. Josephine Moyer, now deceased, set the approach for the outline used at Kutztown until her retirement, in June 1965. Dr. Moyer did more than many academic geographers to prepare teachers for the public schools. Like Lukermann she attempted the ambitious objective of introducing the whole scholarly discipline, not just her speciality. She was a climatic determinist deeply interested in culture and economy. The sheer volume of material which she packed into that syllabus was more than most faculty could handle in three semester-hours.

The outline is entirely factual, but its sequence of topics is suited to the interpretation which Dr. Moyer gave it; Earth-sun relationships, Latitude and Longitude, Maps and Map Reading, the Atmosphere, Climates, Vegetation, Soils, Landforms, Minerals, Land Use and Population. In this topical approach each set of phenomena are studied as mapped distributions e.g. atmospheric temperature and rainfall, covering the entire earth. When classified as complexes the physical phenomena then become concrete objects e.g. climates. Dr. Moyer, a consistant determinist, went one step further and made the environmental complexes principles of explanation of the land use patterns and population distribution, although this interpretation was not made explicit in the course outline. Other topical approaches are, of course available, and they will select and arrange their facts quite differently. This environmentalist approach is not unusual in college geography courses, although its emphases vary from campus to campus and even from instructor to instructor, in accordance with which set of phenomena is considered causal and determinative.

New factual knowledge can be incorporated into this kind of outline (solar system-ubiquitous physical phenomena-descrete human distributions) easily enough; but the result is simply an increase in course content without a new conceptualization. Given the limitations of time and student retention selection of facts is unavoidable. Scientists usually have a proprietary interest in one particular set of concrete objects or processes which he considers "the" facts to be selected for explanation. If geography claims such a proprietary interest its loss of meaning is apodictic; it has abandoned the classic geographic interest in space order and necessarily loses its distinctive significance. No recognizable discipline remains. A new conceptualization requires new theory, not a new foundation (4).

The syllabus at Kutztown provided no place for discussion of geographical theory, only of facts and phenomena. Dr. Moyer and others read into it her environmental determinism and thus provided connections among the ideas by attributing casuality to one set of phenomena (the atmospheric). Relativisms and determinisms of any kind provide a meaning. They are not, emphatically not nonsense, but I found the omission of all reference to the thought of geographers before Ratzal (5) especially difficult to live with. So I provided a lecture or two at the beginning of the course discussing Strabo and the classical heritage and the quest for classifications, conceptual connections and significance in geography (6). In sum I made explicit an approach to the study of the world congruent with the scholarship of the Greeks and, by implication, with all previous generations of classic geographers in America and elsewhere.

I found a warm response from students to this attempt to state the approach before exemplifying it. It may be that it attracted the intellectually gifted students who concentrated in geography and were good enough to be admitted to the honor society, Gamma Theta Upsilon. At the very least "my students" dominated the Gamma Psi Chapter numerically after two years although I taught only one fourth to one sixth of the students enrolled in the introductory courses. I interpret this response as an interest in meaning on the part of students and a rejection of data for their own sake. Students are human beings who respond to a professor's communication with them as persons.

Philosopher's and theologians will recognize what I was doing; I was perpetuating a worldview. I am aware that not all geographers are conscious of doing the same; nor was I aware of what I was doing until the question was raised by a physicist teaching an introductory physics course at the University of Michigan, Flint Campus. Like most geographers I have not been trained to examine the logical possibilities of different conceptual structures, much less to generate by speculation new explanatory constructs. Most of us, I suggest, have been perpetuating the worldview of some authority, like Ratzal, without considering its implications or accepting in the rest of our lives what that view entails. We are unaware of our lack of conceptual consistency though



it is often obvious to our students. At times we come very close to talking nonsense.

If we are to avoid meaninglessness we must make our own personal worldview explicit some time during the course comparing it to the view presupposed by the body of knowledge which comprises the course content. There are two dangers in such a procedure: compromising the university's official neutrality and, second, violating the student's freedom to choose his own worldview. But the only alternative is an implicit denial that geographers make a choice among the small number of worldviews available and thereby structure their conceptions into a coherent geography. Such a denial is not merely irrational, it is anti-rational. We may choose to found our geography on a major logical discontinuity, but we cannot choose the consequence of the next earthquake in metaphysics. Let's call nonsense by its name.

Geographers are truly free to choose where they start, but having got on the train, to change the metaphor, we may not choose our destination except by getting off it. DeGraef, a physicist, attempts to be consistent in his introductory course by teaching it in the textbook fashion emphasizing its mechanistic worldview. Not until the last lecture does he explicitly contrast his own Christian worldview with that implicit in his descipline and point out the way its ideas are linked together into a line of thought. He gets off the train and invites his students to do the same. His Dean knows of DeGraef's approach and has given his approval (7). DeGraef does not attempt to reconstruct physics but he does try to protect his students from the destructive power of the mechanistic worldview by identifying it for what it is, a metaphysical contention.

From the viewpoint of physics this may not be satisfactory, but at least it is constructive, it does not completely overthrow the traditional curriculum. Lukermann has shown that the classical French School of geography was unified by the necessary logical connections among its major themes and by their links, in turn, to a theory, the objective frequency theory of probability (8). Recent contributions to theory in geography tend in the direction of unity of view (9) and tend to confirm Lukermann's assertion that analysis and explanation in geography are possible only within such a framework. Geography like physics, is mechanistic; it can no longer account for persons unless viewed as things, as objects.

## REFERENCES


1. Schaeffer, F. A. 1968. *The God Who Is There*. Inter-Varsity Press, Chicago, Ill. p. 30.
2. Hartshorne, R. 1939. The nature of geography. *Ann. Assoc. Am. Geo.* 24 3,4:482 p.
3. Burki, H. 1968. Christianity and culture. *Crux*, 52:9.
4. Young, W. 1967. *Foundation of Theory*. Craig Press, Nutley, N. J. p. 92.
5. Ratzal, F. 1882. *Anthropogeographie*. Stuttgart, Germany.
6. Somers, L. M. and C. L. Vinge. 1957. Geographers quest. *Cent. Rev.* 14:
7. DeGraef. Donald. 1965. Personal communication.
8. Lukermann, F. 1965. The calculdes probabilities & ecole francaise de geographie. *Ann. Assoc. Am. Geog.* 55:629.
9. Harvey, P. ed. 1967. Contributions to geography theory. *J. Reg. Sci.* 72-Supplement.

# ORIENTATION OF PHENOCLASTS IN A CONGLOMERATE DEPOSIT FROM THE NORTH BORDER OF THE NEWARK-GETTYSBURG BASIN

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## ABSTRACT

Frequency distributions of the dip magnitude of the long axes of various sized pebbles in Triassic border conglomerates indicate a mudflow origin of these deposits. Comparison of these distributions to theoretical and actual fabric diagrams of mudflow deposits confirms this hypothesis.

## INTRODUCTION

The border conglomerates of the Triassic Newark-Gettysburg Basin are considered fanglomerates by McLaughlin (1), Glaeser (2), and others. These conglomerates are similar to deposits of alluvial fan origin in the Connecticut Triassic Basin. The purpose of this paper is to demonstrate the alluvial fan origin and the depositional mechanism of these diamictites from their sedimentologic character.

The study area is a conglomerate and mudstone sequence two miles south of Riegelsville, N.J. (Rieglesville, Pa.-N.J., U.S. G.S. 7.5 Minute Topographic Quadrangle and U.S.G.S. 7.5 Minute Geologic Quadrangle Map GQ-593). A considerable stratigraphic thickness of mudstones and conglomerates is exposed in bluffs and road cuts along the road between Riegelsville and Milford. The outcrop locality is known locally as Gravel Hill.

## SAMPLING AND ANALYSIS

Pebble outlines, taken from vertical faces approximately normal to the strike of the beds, were traced on a 40 x 50 cm clear acetate sheet oriented parallel to the bed. This method allowed a greater amount of data to be collected than would have been possible by direct measurement. Thirteen conglomerate beds from five outcrops were sampled. The number of beds sampled at each outcrop depended on the size of the outcrop, accessibility, and state of weathering.

The sheet was rotated to the horizontal before measurement of pebble orientation to correct for the present inclination of the bed. The dip magnitude of the long axis of the pebble was measured using the procedure described by Dreimanis (3). 858 pebbles were measured, averaging 66 pebbles at each sample point. Frequency distributions were plotted for the entire sample and each conglomerate bed.

## PALEOCURRENTS

No directional sedimentary structures were found in the study area. The standard methods used to determine current direction from particle orientation could not be used because of the degree of induration of the conglomerate. No exposed bedding planes were found.

The pebbles were removed from three boulder size samples using a hammer and chisel. As they were removed, they were marked with a line parallel to the dip direction. In this manner, the orientation of the long axis of 96 pebbles relative to the strike of the beds was determined (Table 1). Long axes approximately 45° to the strike were disregarded because they could not be classified either parallel or perpendicular to the strike. 64.5% of the long axes were perpendicular to the strike of the beds, with the remainder lying parallel to the strike. One pebble was disregarded.

TABLE 1. *Orientation of phenoclast long axes.*

| Shape | Parallel to Strike | Perpendicular to Strike |
|---|---|---|
| Blade | 47.9% | 31.3% |
| Disk | 10.4% | 2.1% |
| Spheroid | 0.0% | 0.0% |
| Roller | 6.2% | 2.1% |
| | 64.5% | 35.5% |

Sample size: 96 phenoclasts

Glen *et al.* (4), and Bhattacharyya (5) indicate that a larger proportion of the long axes of the particles in a mudflow will be more nearly parallel to the flow direction than normal to it. Applying this to the data of the report, flow direction appears to have been roughly perpendicular to the north-south strike, from west to east presumably off a fault scarp.



Actual current direction could be as much as 30° to the north or south of this east-west direction. For pebbles dipping from 0° to 30°, the difference between true and actual dip would be slight and would not affect the frequency distributions to any great degree. Sixty-six percent of the pebbles dip from 0° to 30°.

## FREQUENCY DISTRIBUTIONS

White (6) shows that the frequency distribution of a Keweenawan conglomerate bed is normal with the mode at 8° downstream. Similarly, Krumbein's (7) frequency distribution of a flood gravel is also normal with the mode at 13.5° downstream. These two distributions are characteristic of imbricated stream gravels.

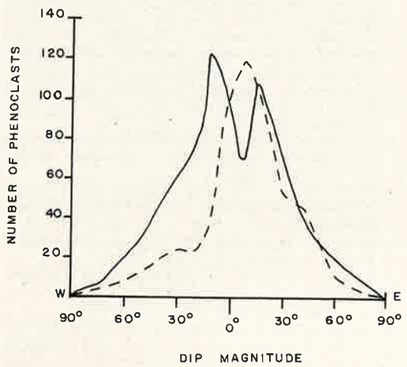



**Figure 1. The solid line is the frequency distribution of the Triassic border conglomerates. The dashed line is the frequency distribution of a Keweenawan conglomerate bed adapted from White (6). The Keweenawan distribution is characteristic of imbricated stream gravels. Note the single mode at 8° downstream. The triassic distribution is bimodal, with modes in the 10-20° west (upstream) and 10-20° east (downstream) class intervals.**

On the other hand, the frequency distribution of the entire Triassic conglomerate sample is bimodal with peaks in the 10-20° west (upstream) and 10-20° east (downstream) class intervals (Fig. 1), indicating that a fluvial mechanism may not have been the main depositonal mechanism. Two dip modes are seen, one dipping upstream, the other downstream. This implies a unidirectional laminar flow which oriented the long axes of the pebbles in the direction of and parallel to the surface of flow. The red mudstone matrix, poor sorting and bimodality of shallow dipping pebbles are characteristic of mudflow deposits.

A comparison of the frequency distributions of the conglomerates with theoretical and actual mudflow fabric diagrams confirms their origin as mudflow deposits. According to Lindsay (8), mudflow fabrics are periodic in nature, beginning as a weak girdle dipping upstream. Initially several weak modes develop along the girdle, but in time they consolidate to form a single mode which gradually increases in strength as its plunge decreases. Maximum fabric strength develops when the mode is horizontal. From this point onward the fabric begins to degenerate, first to a single mode dipping downstream, then to a weak girdle, and finally to a random state similar to the initial fabric. The fabric preserved depends upon the instant in the cycle the mudflow was arrested.

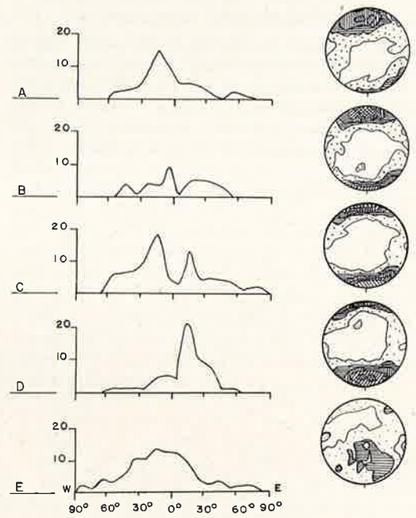



**Figure 2. Dip magnitude is plotted on the abscissa, frequency on the ordinate. The distributions A through E are characteristic of the thirteen conglomerate beds sampled. The modes of dip magnitude are comparable to the modes in the mudflow fabric diagrams after Lindsay (8). These fabric diagrams represent mudflows at different stages of development. Tick marks on the fabric diagrams indicate flow direction.**

Figure 2 shows representative frequency distributions from the thirteen conglomerate beds sampled. The mudflow fabric diagram next to each frequency distribution has a comparable dip mode distribution. Distribution A has a single mode dipping upstream, characteristic of a mudflow travelling a short distance in laminar flow. In distribution B, the single mode dipping upstream is beginning to decay into two modes—one dipping upstream, the other downstream. The upstream and downstream dipping modes are well developed in C. In distribution D, only a single mode dipping downstream is present. Random orientation of the pebbles seen in distribution E is either the result of turbulent flow or the degeneration of a fabric to its original state.

The modes of dip magnitude in the frequency distributions are comparable to those of the mudflow fabric diagrams. The frequency distributions represent mudflows at different stages of development.

## SUMMARY AND CONCLUSIONS

In summary, the modes of dip magnitude in frequency distributions of Triassic conglomerates are similar to those of mudflow deposits. On the basis of this similarity, Triassic border conglomerates are shown to represent different stages of mudflow development. These mudflows are part of the sediment deposited as an alluvial fan along the fault scarp bordering the Newark-Gettysburg Basin.

TABLE 2. *Gravel size distribution.*

| | |
|---|---|
| Small Cobbles | 22.9% |
| Very Coarse Pebbles | 54.2% |
| Coarse Pebbles | 22.9% |
| | 100.0% |

## ACKNOWLEDMENTS

The writer is indebted to Dr. R. W. Faas, Department of Geology, Lafayette College for encouragement and guidance during research and critically reviewing the manuscript.

## REFERENCES

1. McLaughlin, D. B. and B. Willard. 1949. Triassic Facies in the Delaware Valley. *Pa. Acad. Sci.* 23:34.
2. Glaeser, J. D. 1966. Provenance, dispersal and depositional environments of Triassic sediments in the Newark-Gettysburg Basin. Pennsylvania Topographic and Geologic Survey, Harrisburg.
3. Dreimanis, A. 1959. Rapid macroscopic fabric studies in drill cores and hand specimens of till and tillite. *J. Sed. Pet.* 29:459.
4. Glen, J. W., J. J. Donner and R. G. West. 1957. On the mechanism by which stones in till become oriented. *Am. J. Sci.* 255:194.
5. Bhattacharyya, D. S. 1966. Orientation of mineral lineation along the flow direction in rocks. *Tectonophysics* 3:29.
7. Krumbein, W. C. 1940. Flood gravels of San Gabriel Canyon, California. *G.S.A. Bull.* 51:639.
8. Lindsay, J. F. 1968. The development of clast fabric in mudflows. *J. Sed. Pet.* 38:1242.



# GEOCHEMICAL AND GEOPHYSICAL STUDY OF MAFIC AND ULTRAMAFIC ROCKS IN MINE RIDGE, SOUTHEASTERN PENNSYLVANIA

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## ABSTRACT

The metagabbro of the Gap nickel deposit, in Mine Ridge, Lancaster County, Pennsylvania occurs as a lens in Precambrian rocks in the core of Mine Ridge anticline. It has been assumed that the metagabbro and nickel mineralization record a unique event in the area. The present investigation seeks to determine the validity of this assumption. Lenses of other ultramafic and mafic rocks in Mine Ridge have been remapped. Thin and polished section petrographic, geochemical, magnetic and gravimetric studies are underway. The geochemical study of several of these lenses showed nothing above background. One gravimetric and magnetic traverse shows anomalies along the margins of one ultramafic lens.

## INTRODUCTION AND METHODS

The present investigation was largely confined to the Precambrian core of the Mine Ridge anticline in the Gap 7½′ quadrangle, Lancaster County, Pennsylvania. It was our purpose to learn the relationships among the various rock types of the area and whether or not there might be metallic ore deposits associated with any of the mafic or ultramafic rocks. To this end, the distribution of the rocks was mapped, a thin- and polished-section study was made of the mafic and ultramafic rocks, soil samples were collected and analyzed for their trace element content, several whole-rock trace-element analyses were made, and one gravimeter and several magnetometer traverses were made over three ultramafic bodies.

## RESULTS AND DISCUSSION

*Petrography and Petrology.* Five distinct rock types of presumed Precambrian age were recognized in the area: gneiss (the Baltimore Gneiss), amphibolite gneiss, and a biotite-rich amphibolite gneiss, amphibolite, and serpentinite. The distribution of the mafic and ultramafic rocks is shown in Figure 1.

The Baltimore Gneiss is extremely variable in composition and is the most abundant rock in the area. The gneiss is locally intruded by pegmatite. Where these two rocks occur together in outcrop, the contact is usually sharp.

The pegmatite is generally very coarse-grained and of simple mineralogy; its major mineral constituents are quartz, K-feldspar, and plagioclase, with minor amounts of biotite, muscovite, and black tourmaline. The pegmatite is granitic in composition and follows the Baltimore Gneiss in abundance.

The amphibolite gneiss is closely associated with pegmatite, but is quantitatively of less importance. The gneiss may tend toward a schist as the proportion of amphibole to plagioclase increases. The area mapped as amphibolite gneiss in Figure 1 corresponds only to the area of greatest concentration of amphibolite gneiss float, which is not limited to this area alone.

The amphibolite gneiss is medium-grained and has as its major mineral constituents hornblende and plagioclase, with minor biotite, sphene, apatite, and epidote (sulfides were noted, but not identified). Garnet and quartz are distinctly absent. This gneiss is distinguished from more mafic layers of Baltimore Gneiss by its lack of quartz and K-feldspar, either or both of which may be present in the Baltimore Gneiss.

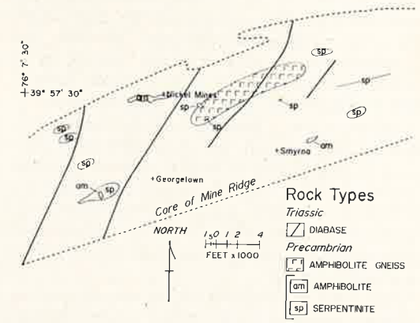



**Figure 1.**

A rock similar in mineralogy to the amphibolite gneiss, but containing as much biotite as hornblende and more plagioclase, was also noted. This rock does not have the strong foliation of the gneiss and its lacks both the coarse grain size and K-feldspar of the pegmatite. In one outcrop along U. S. Route 30 west of Coatesville, Pennsylvania, pods of strongly foliated amphibolite gneiss up to several feet in length occur isolated within this more leucocratic, biotitic rock. More than 75% of the outcrop, however, is pegmatite.

The amphibolite, which is ultramafic and not so obviously foliated as the amphibolite gneiss, consists almost entirely of coarser-grained hornblende, with minor plagioclase, biotite, chlorite, and tremolite-actinolite. The Gap nickel intrusion at the village of Nickel Mines contains, in addition, pyrrhotite, chalcopyrite, pentlandite, pyrite and millerite, which were not observed in the other two amphibolite occurrences.

The Gap amphibolite is associated with pegmatite and the amphibolite body near Smyrna (Figure 1) is also associated with pegmatite, which in this particular instance contains beautiful radial crystal groups of tremolite-actinolite. Also associated with both of these amphibolites is a peripheral zone of mica schist, which in the case of the Smyrna amphibolite body contains large (up to ½″) crystals of pyrite.

The serpentinite, with its highly variable mineralogy, contains varying amounts of serpentine (variety antigorite), talc, tremolite-actinolite, chlorite, carbonate, magnetite-ilmenite, as well as pyrrhotite, chalcopyrite, pyrite, and bornite. One serpentinite body completely encloses an amphibolite core.

The tremolite-actinolite occurs as large crystals or as small incipient crystals in serpentine. The chlorite occurs in close association with tremolite-actinolite and is generally surrounded by a rim of magnetite-ilmenite. The talc occurs primarily as large irregular patches or as small patches in serpentine or tremolite-actinolite and is closely associated with carbonate.

*Geochemistry.* Analysis of one sulfide-rich serpentinite yielded as Ni-content of 0.15% and a Cu-content of 0.05% (Rose, *personal communication);* but this is even less than the 0.2% Ni generally found in magnesian serpentines by Deer *et al.* (1). Three sulfide-poor serpentinite samples contained similar concentrations of Ni.

The Ni/Co ratios of three serpentinite samples averaged 0.1097, which is very close to the ratio for each sample. The amphibolite body near Smyrna has a similar Ni/Co ratio of 9.8244. The ratio for the Gap amphibolite, however, was 4.5151 and for the amphibolite enclosed within serpentinite 1.9401. The Ni/Co ratios indicate a common parentage for at least the Smyrna amphibolite body and the two serpentinite bodies investigated. Fifty soil samples were analyzed for Ni, Co and Cu. All ran uniformly low in the elements.

*Geophysics.* In Figure 2 are shown the results of a magnetometer and gravimeter traverse over a serpentinite body. There is a fairly close correlation between the local magnetic and gravimetric anomalies along this traverse. A need does exist for detailed magnetic susceptibility and density determinations on the minerals of the serpentinite, so that the more important correlations between the anomalies and the minerals producing them can be made. The local magnetic anomalies, when mapped for the same body, Figure 3, show no consistent orientation and vary considerably in magnitude. The striking anomalies may indicate concentrations of either magnetite or pyrrhotite or both.

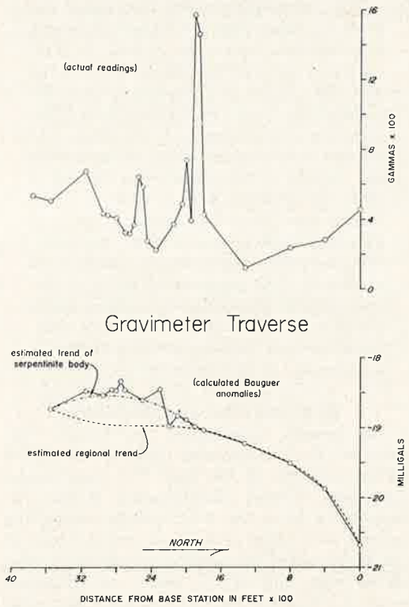



**Figure 2.**



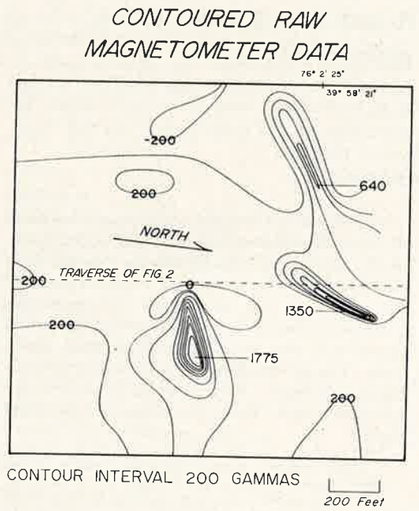



**Figure 3.**

Three magnetometer traverses were taken across the amphibolite body near Smyrna, with the result that there were no apparent local anomalies associated with the body. This same result was obtained by four traverses over the serpentinite-amphibolite body near Georgetown.

## CONCLUSIONS

The most important conclusion to be drawn from the present investigation is that there are probably no important concentrations of Ni in the area studied. This is evident both from the geophysical and geochemical data presented above. Further, the boundaries of areas mapped as metagabbro by Knopf and Jonas (2), but mapped here as amphibolite gneiss, are not accurate. Float of amphibolite gneiss was found widely distributed outside of these areas. Serpentinite, too, is much more abundant than Knopf and Jonas indicated.

## REFERENCES

1. Deer, W. A., R. A. Howie and J. Zussman. 1962. *Rock Forming Minerals,* Vol. 3, Sheet Silicates, John Wiley and Sons, Inc., New York.
2. Knopf, E. B. and A. I. Jonas. 1929. *Geology of the McCalls Ferry-Quarryville District, Pennsylvania.* Bull. 799, U. S. Geol. Survey, Washington, D. C.

# OLENELLUS FAUNA OF THE KINZERS FORMATION, SOUTHEASTERN PENNSYLVANIA

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## ABSTRACT

The Kinzers Formation of Early and Middle Cambrian age contains numerous complex lithofacies and at least three faunal zones. The lowermost, or *Bonnia-Olenellus* zone, is described here.

The *Bonnia-Olenellus* flora and fauna of the Kinzers Formation contain seven phyla and numerous genera and species. Fourteen previously described species belonging to the genus *Olenellus* are considered to be invalid and to represent forms produced by deformation, injury, or growth stage. The fauna occurs in shale, weathered sandstone, and shaly limestone. Horizontal and vertical lithofacies changes cannot physically be traced any significant distance because of structural complexities.

A strain ellipse determination was made on the basis of six deformed trilobites occurring together on a bedding surface. There is 18% shortening in one direction and approximately the same amount of lengthening perpendicular to that direction, with proportional amounts of distortion between these principal directions.

## INTRODUCTION

The Kinzers Formation of Southeastern Pennsylvania crops out in a band running through central Berks, Lancaster, and York counties. The formation consists of complex carbonate, sandstone, and shale lithofacies of Early and Middle Cambrian age. Previous stratigraphic and interpretive work was done by Walcott (1) and by Stose and Jonas (2,3,4). The faunas were principally described by Walcott (1) and Resser and Howell (5).

The Kinzers Formation thickens from some 25 ft in the Chester Valley of Berks County to nearly 500 ft near Thomasville in western York County. Carbonate facies are more common to the northwest and terrigenous silts and clays increase to the south and east as the formation grades into the deeper water Conestoga Limestone. The general Kinzers section described by Stose and Stose (4) is essentially correct for Lancaster and York Counties, and is here quoted.

> "Upper member: Earthy limestone; weather to soft buff tripoli and fine sandstone; generally fossiliferous, and interbedded with dark argillaceous thin-bedded limestone. 125-180 feet
>
> "Middle member: Thick white to mottled or spotted marble and granular limestone; some coarsely conglomeratic, containing Archaeocyathid reefs and minor amounts of dark thin-bedded argillaceous limestone; earthy generally fossiliferous limestone at base, weathers to buff tripoli. 100-175 feet
>
> "Lower member: Dark shale, with earthy limestone which weathers to buff tripoli at the base. (Shale is highly fossiliferous in Lancaster area.) 100-150 feet" (Total thickness 325-505 feet)

In the Kinzers, a highly complex structural pattern of folds, faults, and thrusts is superimposed on an already complex set of facies. Therefore, biostratigraphic studies are imperative for the accurate interpretation and placement of the many partial sections exposed.

## BIOSTRATIGRAPHY

The bio-zonation of the Lower Cambrian has been much debated with no complete consensus at present. The writers concur with Rasetti (6) in recognizing a single Lower Cambrian zone characterized by species of *Olenellus* and *Bonnia* and designated by him the *Bonnia-Olenellus* zone. Consequently, the title might better have read "*Bonnia-Olenellus* Fauna." In the Kinzers Formation, the *Bonnia-Olenellus* zone includes all rocks of the lower and middle members plus the lowermost bed of the upper Kinzers member. The remaining upper member beds contain Middle Cambrian faunas which will be described in a subsequent publication.

Six sub-faunas or faunules are present within the *Bonnia-Olenellus* zone and are here described in ascending order.

### BONNIA FAUNULE

*Bonnia* sp. (cc)
*Olenellus* sp. (c)
undetermined ptychoparid sp. (rr)
*Salterella* sp. (cc)
*Yorkia* (?) sp. (rr)
*Hyolithes* sp. (r)

(cc = very common; c = common; r = rare; rr = very rare)

The *Bonnia* faunule occurs in the basal weathered tripoli of the Lower member of the Kinzers.



### WANNERIA WALCOTTANA FAUNULE

Trilobites:
*Wanneria walcottana* (Wanner) (c)
*Eoagnostus roddyi* Resser and Howell (r)
*Olenellus thompsoni* (Hall) (c)
*Olenellus nodosus* Resser and Howell (rr)
*Bonnia* sp.
*Boniella yorkensis* Resser and Howell (rr)
*Kootenia pricei* Resser (r)
*Lancastria roddyi* (Walcott) (rr)

Other Arthropods:
*Tuzoia dunbari* Resser (rr)
*Tuzoia getzi* Resser (rr)
*Tuzoia nitida* Resser (rr)
*Tuzoia* sp. (rr)
*Anomalocaris pennsylvanica* Resser (rr)
*Protocaris marshi* Walcott (rr)
*Sidneyia* sp. (rr)
*Roddyia typa* Resser (rr)
*Hymenocaris dubis* Resser and Howell (rr)
*Isoxys* (?) sp. (rr)

Miscellaneous:
*Morania sparsa* Resser and Howell (r)
*Marpolia filosa* Resser and Howell (r)
*Dalyia pennsylvanica* Resser and Howell (r)
undetermined algae
*Leptomitus walcotti* Resser and Howell (r)
*Lepidocystis wanneri* Foerste (rr)
*Camptostroma roddyi* Ruedemann (r)
*Paterina roddyi* Resser and Howell
*Nisusia festinata* (Billings) (rr)
*Margaretia ramosa* Resser and Howell (r)
*Margaretia stosei* Resser and Howell (r)
*Helcionella pricei* Resser and Howell (r)
*Pelagiella exigua* Resser and Howell
*Salterella acervulosa* Resser and Howell (cc)
*Hyolithes americana* Billings (rr)
*Hyolithes communis* Billings (r)
*Tubulella pennsylvanica* (Resser and Howell) (r)
undescribed annelids 2 species (rr)

(Species of this excedingly rich faunule are found in the carbonate and terrigenous shales of the lower member.)

### YORKIA WANNERI FAUNULE

Brachiopods:
*Yorkia wanneri* Walcott (cc)
*Nisusia festinata* (Billings) (cc)
*Kutorgina cingulata* (?) Billings (rr)
*Paterina bella* (Billings) (r)

Mollusca ?:
*Hyolithes americana* Billings (r)
*Hyolithes communis* Billings (r)
*Salterella* sp. (r)

Trilobites:
*Olenellus thompsoni* (Hall) (c)
*Bonnia capito* (Walcott) (c)
*Bonnia tumifrons* Resser (r)
*Bonnia wanneri* Resser (cc)

(The *Yorkia wanneri* faunule occurs in the basal weathered carbonate sandstones of the middle member of the Kinzers.)

### ACROTHELE DECIPIENS FAUNULE

*Olenellus* sp. (rr)
*Chancia* (?) sp. (rr)
undetermined phyllopod (rr)
*Acrrothele decipiens* (r)
*Acrotreta* sp. (r)
*Tubulella* sp. (r)

The *Acrothele decipiens* faunule is known from only a single locality whose exact stratigraphic position within the middle Kinzers is uncertain. It occurs in fissile grey carbonate shale immediately below a bed of dolomitic limestone conglomerate locally known as "leopard rock" because of its weathering character.

### ARCHAEOCYATHUS FAUNULE

*Archaeocyathus* sp. (rr)
(*Archaeocyathus* sp. is rare in the purer limestones of the upper part of the Kinzers middle member.)

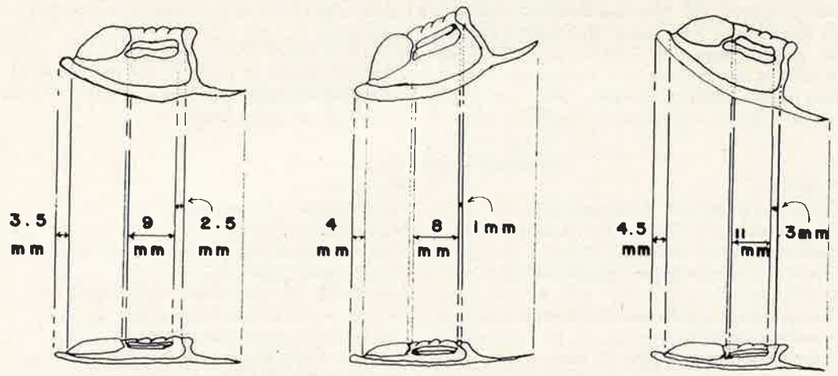



**Figure 1. Projected effect of 60% sedimentary compaction on** Olenellus gilberti **Meek (1874).**

KOOTENIA MARCOUI FAUNULE

*Kootenia marcoui* (whitfield) (r)
*Bonnia capito* (Walcott) (c)
*Periommella yorkensis* Resser (r)
*Ptychoparella* sp. (r)

The *Kootenia marcoui* faunule is the uppermost *Bonnia-Olenellus* zone faunule recognized in the Kinzers Formation. It is found in the basal beds of the upper Kinzers. The latter two species were found in float and may have been mixed in from a higher bed.

## FOSSIL DEFORMATION

Fossil deformation has resulted from at least four processes: (a) initial environmental forces; (b) sedimentary compaction; (c) rock flowage; and (d) offsetting by cleavage. Because of deformation the following characteristics used by others to determine Kinzers "species" of *Olenellus* are believed to be invalid.

(a) Scars and missing parts—used to distinguish *O. peculiaris* Resser and Howell.

(b) Relative lengths of eyes, rim, glabella, and hypostoma—changes in these features can be produced by sedimentary compaction (Fig. 1), or rock flowage (Fig. 2).

(c) Length-width ratios—changes in relative width and length is usually produced by rock flowage.

(d) Genal angles and angles of glabellar furrows—used to distinguish *O. getzi* Dunbar (Fig. 2).

Two non-deformational factors likewise thought to be invalid descriptive criteria are:

(e) Geographical differences.

(f) Growth stage—used to distinguish the genus "Paedeumias".

Figure 3 shows the results of a study of the statistical variability in *Olenellus thompsoni*, in which the holotypes and paratypes of Resser and Howell's "species" were compared with 24 specimens of Dunbar's *"O. getzi"*. With the exception of rim width, all features examined fell within two standard deviations of the mean. Rim width in *"O. wanneri"* exceeded two standard deviations, but this is not thought to be significant because in *"O. latilimbatus"* one specimen likewise exceeded two deviations above the mean but a second specimen fell below the mean (Fig. 3). Also, *"O. jonasae"* left cheek exceeded two standard deviations, but the right cheek fell almost exactly upon the mean.

Consequently, *Olenellus thompsoni* (Hall) and *Olenellus nodosus* Resser and Howell are the only two species of *Olenellus* that can presently be recognized in the Kinzers Formation.

Deformed fossils can also be used in determining strain ellipses on rock surfaces and thus the amount of lengthening and shortening that has occurred in fossils found on a given plane (Fig 4).

A slab of Kinzers Formation containing six nearly complete and several partial specimens was analyzed according to the method proposed by Wellman (7). Long axes (axes of symmetry) and another axis known to have been perpendicular to this (such as the straight posterior edge of the cephalon) can be used to determine the strain ellipse on that surface. This ellipse had developed from a circle of the same area and can therefore be used to determine the amount of shortening (and lengthening) in any direction.



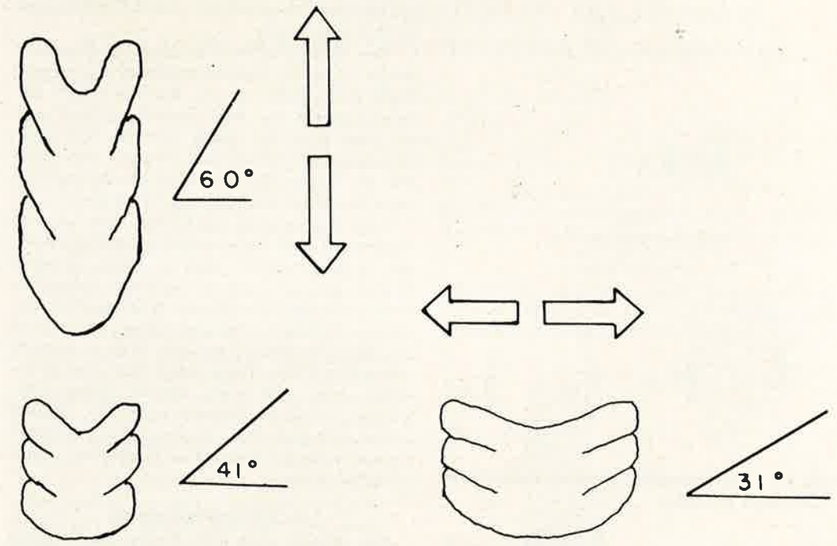



**Figure 2. Angle change of lateral glabella furrows resulting from rock deformation.**

| | # specimens measured | Eye Shortness f/c | Cephalon Breadth e/c | Eye Spacing g/e | Glabella Proportions h/i | Glabella Length h/c |
|---|---|---|---|---|---|---|
| *O. getzi* | 24 | 22 0.38 .54 | 14 2.2 3.0 | 27 0.41 .55 | 16 0.96 1.76 | 28 0.44 .60 |
| standard deviation | | 0.08 | 0.4 | 0.07 | 0.4 | 0.08 |
| *O. thompsoni* | 4 | 0.48 | 2.1 | 0.44 | 0.90 | 0.45 |
| *O. alius* | 1 | 0.37 | 2.0 | 0.44 | 1.05 | 0.40 |
| *O. jonasae* | 2 | 0.48 | 2.2 | 0.45 | 0.78 | 0.36 |
| *O. latilimbatus* | 2 | 0.42 | 1.8 | 0.48 | 0.91 | 0.39 |
| *O. roddyi* | 1 | 0.43 | 1.7 | 0.48 | 1.14 | 0.50 |
| *O. similaris* | 1 | 0.36 | 1.8 | 0.46 | 1.14 | 0.36 |
| *O. nitidus* | 1 | 0.56 | 2.6 | 0.49 | 0.88 | 0.44 |
| *O. wannari* | 1 | 0.61 | 2.3 | 0.51 | 0.67 | 0.26 |

| | # specimens measured | Glabella Width i/e | Rim Width 2xa/e | Rim Length b/c | Rim Proportions a/b |
|---|---|---|---|---|---|
| *O. getzi* | 24 | .16 0.22 .28 | .07 0.13 .19 | .03 0.07 .11 | 1.1 2.3 3.5 |
| standard deviation | | 0.03 | 0.03 | 0.02 | 0.6 |
| *O. thompsoni* | 4 | 0.24 | 0.11 | 0.06 | 2.0 |
| *O. alius* | 1 | 0.20 | 0.14 | 0.06 | 2.2 |
| *O. jonasae* | 2 | 0.20 | 0.18 .21 .14 | 0.05 | 3.0 |
| *O. latilimbatus* | 2 | 0.24 | 0.15 .21 .09 | 0.08 | 1.7 |
| *O. roddyi* | 1 | 0.26 | 0.07 | 0.03 | 2.0 |
| *O. similaris* | 1 | 0.24 | 0.09 | 0.06 | 1.2 |
| *O. nitidus* | 1 | 0.19 | 0.12 | 0.06 | 2.5 |
| *O. wannari* | 1 | 0.17 | 0.23 | 0.13 | 2.0 |

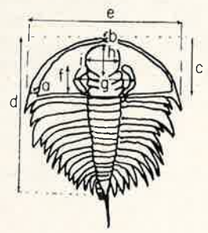



**Figure 3. Statistical comparison of** Glenellus **species from the Kinzers Formation with O.** getzi **Dunbar (1925). Small numbers flanking O.** getzi **data represent ± two standard deviations.**

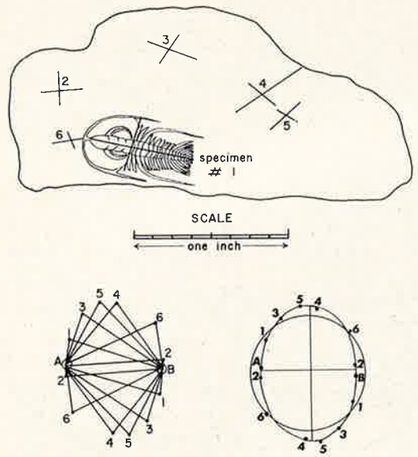



**Figure 4. Determination of strain ellipse from deformed trilobites.**

This is illustrated in the diagram where specimen number one is redrawn with the axis of symmetry and the posterior edge of the cephalon traced as lines. Only these two lines are reproduced for the other five specimens. Two arbitrary points "A" and "B" were spotted in such a way that the line joining them was not parallel to any of the lines drawn for each fossil. Each pair of lines for each fossil was then transferred to this sketch and formed the sides of parallelograms, two corners of which were the points "A" and "B", and the other two corners provided points for the strain ellipse. The number of points available were the two arbitrary ones "A" and "B" and two for each trilobite, giving fourteen in all. An ellipse was carefully drawn as closely as possible through these points. Wellman (7) suggests downward projecting of a circle of appropriate radius drawn on a sheet of glass, manipulated in such a way as to have the projected circle pass through the arbitrary points and fit most closely to the points formed from the distorted fossils.

"The theory of the method is extremely simple. Prior to rock deformation each angle was a right angle, and the fourteen points defined a circle, the two arbitrary points and the pairs from the fossils marking diameters. Homogeneous deformation changed the circle into an ellipse, the arbitrary points and the pairs becoming diameters of the ellipse." (7).

With the example illustrated here, it was determined that the shortening (and lengthening) ratio was 0.82; that is, those elements located along the axis of maximum elongation were originally only 82% as large as they now appear. Similarly elements along the axis of maximum shortening are only 82% as large as originally. Those lying along the axes of no strain have not been altered appreciably. (These represent diameters of the original circle). Elements lying between these principal axes have been shortened or lengthened a proportional amount.


## ACKNOWLEDGEMENTS

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## REFERENCES


1. Walcott, C. D. 1896. The Cambrian rocks of Pennsylvania. *U. S. Geol. Survey Bull.*, No. 134, pp. 1-43.
2. Jonas, A. I., and Stose, G. W. 1930. Atlas of Pennsylvania No. 168, Lancaster Quadrangle, *Pa. Geol. Survey, 4th* series, pp. 26-29.
3. Stose, G. W., and Jonas, A. I. 1939. Geology and mineral resources of York County, Pennsylvania. *Pa. Geol. Survey, 4th Series, Bull.* C 67, pp. 55-57.
4. Stose, G. W., and Stose, A. I. 1944. Geology of the Hanover-York District, Pennsylvania. *U. S. Geol. Survey Prof. Paper*. 204, pp. 18-33.
5. Resser, C. E., and Howell, B. V. 1938. Lower Cambrian *Olenellus* Zone of the Appalachians. *Geol. Soc. American Bull.* 49:195.
6. Rasetti, F. 1951. Middle Cambrian stratigraphy and faunas of the Canadian Rocky Mountains. *Smithsonian Miscellaneous Collections*, v. 116, n. 5, 277pp.
7. Wellman, H. M. 1962. A graphic method for analysing distortion caused by tectonic deformation. *Geol. Mag.*, 99:348.




# STRUCTURAL INTERPRETATION OF THE GEOLOGY NEAR MORGANTOWN, SOUTHEASTERN PENNSYLVANIA


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## ABSTRACT

Possible recumbent folding has been interpreted for the first time in the Morgantown region of southern Berks County, near its common corner with Lancaster and Chester Counties. The southeastern quarter of the Morgantown Quadrangle is underlain by north-dipping Cambrian quartzites and carbonates. This gentle, fairly uniformly dipping structure changes significantly in the vicinity of the village of Morgantown. Axial plane cleavage in a section of rocks exposed just south of the village limits indicates folds approaching recumbency. Overturned bedding and horizontal cleavage to the north may represent remnants of overturned isoclinal folds.


## INTRODUCTION

No detailed structural mapping has heretofore been done in the immediate vicinity of Morgantown, Pennsylvania. Previous workers, Bascom and Stose (1), and Sidler and MacClintock (2) mapped the various lithologies and recognized the large scale monoclinal character of the bedrock. This paper proposes that the structure is more complex than previously stated and offers a cursory look at the possibility of near recumbent folding of the Cambrian carbonates.

## DESCRIPTION OF THE AREA

Morgantown lies in the southeastern quarter of the Morgantown 7.5 min quadrangle, Welsh Mountain lies at the southern boundary of the quadrangle, and its crest follows the regional strike of N70-80E. Northward, the Antietam Quartzite forms several small outliers in the valley underlain by the Vintage and Ledger Dolomites. The northern boundary of the valley is marked by the sandy basal member of the "Morgantown Formation". The name "Morgantown Formation" is used by the authors to identify a specific lithologic sequence and is not meant to represent a new formation. The "Morgantown Formation" upholds the low ridge that divides this area into the Twin Valleys; north of the ridge, the remaining limy and dolomitic units of the "Morgantown Formation" underlie the northern valley.

## STRATIGRAPHY

The following formations are seen from south of Welsh Mountain to the northern edge of the northern part of Twin Valleys (Fig. 1):

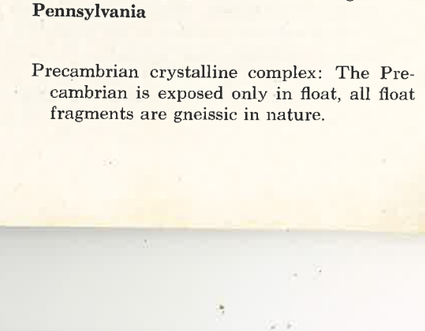



**Figure 1. Stratigraphic column: Morgantown Pennsylvania**

Precambrian crystalline complex: The Precambrian is exposed only in float, all float fragments are gneissic in nature.

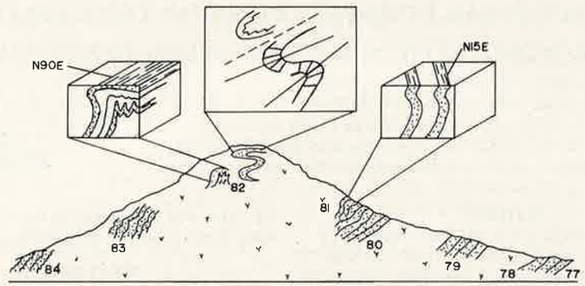
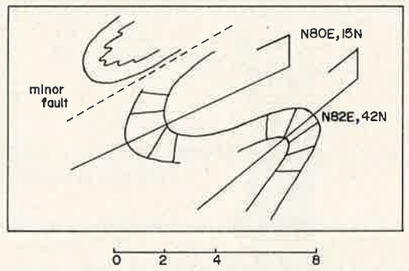



**Figure 2. Outcrop south of Morgantown. See text for description.**




**insert from Figure 2.**

Chickie Quartzite: The Chickies Formation atop Welsh Mountain is a pure white to vitreous grey-blue quartz sandstone with some weathered feldspar grains. Occasional sericitic partings are seen throughout the section.

Antietam Quartzite: The Antietam Formation on the outliers is a ferruginous fine-grained light grey to dark brown quartz sandstone.

Vintage Dolomite: The Vintage Formation, where exposed, is a light grey to buff dolomite which weathers to a knarled, pitted surface.

Ledger Dolomite: The Ledger Formation is a white to light blue-grey dolomite and light to dark blue-grey impure limestone sequence.

"Morgantown Formation": The "Morgantown Formation" is a multicolored impure limestone and dolomite sequence with a red to brown sandy basal member. Occasional stromatolitic horizons and ripple marked surfaces are seen in the formation.

Triassic complex: The overlying Triassic rocks are not differentiated but consist of red sandstones, conglomerates, and breccias.

## STRUCTURAL GEOLOGY

Folding in the area can be divided into two groups; flexural folding of the Vintage and Ledger Formations, and passive folding, after Donath and Parker (3), of the "Morgantown Formation". This paper deals exclusively with the "Morgantown Formation".

North and south of Morgantown, the "Morgantown Formation" is intensely folded and fractured. Overturned beds, horizontal cleavage planes, and small exposed recumbent folds are seen throughout the formation in the immediate vicinity of the town. The major deformation is seen above the sandy basal member.

South of Morgantown, exposures show a fold or set of folds (Fig. 2) similar to that described by Wise (4). The rocks are tilted to the north with no major deformation apparent at the south end of the outcrop. To the north, and stratigraphically upward, the amount of

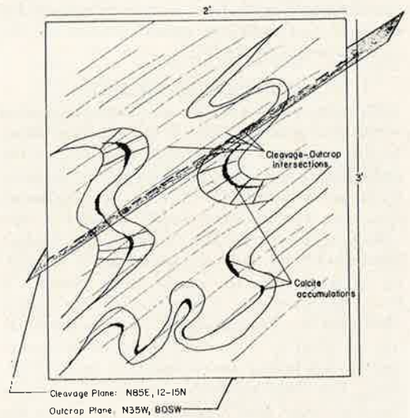



**Figure 3.**



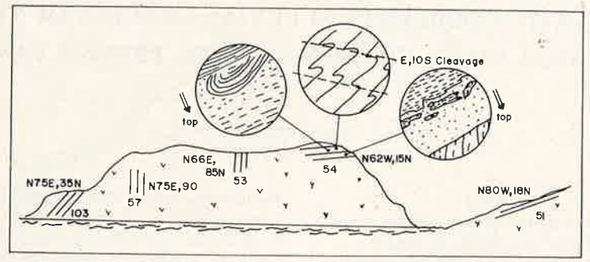



**Figure 4. Overturned section of Morgantown Formation northeast of town.**

deformation increases. At station 81, small drag folds are seen and slickensided planes parallel to bedding indicate a north-south force couple. At station 82, small scale tightly compressed folds are seen, one in particular aproaches recumbency. Vertical beds with digitations are also seen at this station. Station 84 shows tightly folded laminations, the axial planes of which strike N55E, and dip 25S.

In a recently opened sewage ditch in Morgantown, well developed cleavage was seen. Cleavage attitude was approximately N50W, 15NE.

Figure 4 shows a portion of an outcrop south-southeast of Morgantown along the Conestoga Creek. The outcrop shows tight passive folds on the order of 2 to 3 feet. Axial plane cleavage is clearly seen here. Its attitude, N85E, 12-15N, indicates the axial plane of the fold is approaching recumbency.

About one-half mile north-northwest of Morgantown along the Conestoga Creek, several outcrops show a section of overturned "Morgantown Formation". Figure 4 shows the spatial relationships of these outcrops; the inserts show several top indicators.

Figure 5 is a plot of the poles to bedding in the area. The open circles are poles to overturned beds. Virtually all poles plot in a restricted zone south of the origin, indicating schematically the north-dipping monoclinal nature of the area.

## SUMMARY

The structure of the "Morgantown Formation" near Morgantown, Pennsylvania, is vastly more complex than previously thought. This paper points out the presence of small recumbent folds and overturned sections. More detailed structural work needs to be done in the area to determine the exact nature of the deformation and its relation to the Honeybrook Uplift.

## ACKNOWLEDGMENTS

The authors note with thanks the financial assistance of the National Science Foundation to the Masters' Program at Franklin and Marshall College.

## REFERENCES

1. Bascom, F. and C. W. Stose. 1938. Geology and mineral resources of the Honeybrook and Phoenixville Quadrangles, Pennsylvania. *U.S.G.S. Bull. 891.*
2. Sidler, A. and C. MacClintock. 1954. Report of progress of the Morgantown-Churchtown Area. Unpublished senior project. Franklin and Marshall College, Lancaster, Pennsylvania.
3. Donath, F. A. and R. B. Parker. 1961. Folds and folding. *Geol. Soc. Amer. Bull.* 75:45.
4. Wise, D. U. 1958. An example of recumbent folding south of the Great Valley of Pennsylvania. *Pa. Acad. Sci.* 32:172.

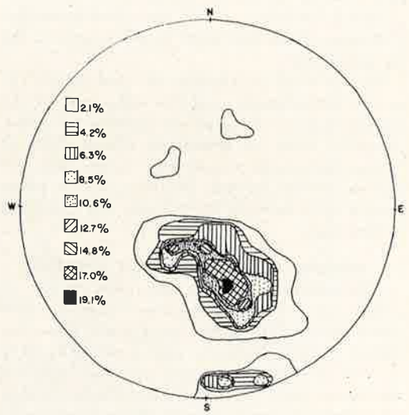



**Figure 5. Poles to 47 Bedding Planes**

# ERRATIC COBBLES IN ALLUVIAL GRAVELS OF THE LEHIGH RIVER, CARBON COUNTY, PENNSYLVANIA

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## ABSTRACT

Igneous and metamorphic cobbles occur in Lehigh River floodplain gravels as far south as Lehighton, 22 miles downstream from the Wisconsin terminal moraine at Hickory Run. The cobbles were brought into Pennsylvania from the Canadian Shield and/or the western Adirondack Mountains by the Wisconsin glaciation and subsequently eroded from glacial drift in post-Wisconsin time. The position of these cobbles downstream from the Wisconsin terminal moraine is a clue to the maximum distance of load transport which has occured in the Lehigh River since Wisconsin glaciation in the Lehigh River drainage basin.

## INTRODUCTION

This paper gives the first detailed account of igneous and metamorphic erratics in the glaciated part of northeastern Pennsylvania. Other reports of erratics in northeastern Pennsylvania are rare. Lewis (1) noted a few igneous boulders about 15 miles east of the Lehigh River in Monroe County, while White (2) observed "no granitic or metamorphic rocks" during his field work in Pike, Monroe and Carbon Counties. No erratics were reported by Gardner (3) in the Brodheadsville-Pohopoco Mountain area 10 miles east of the Lehigh River.

Recent work by Epstein (4) and Bucek (5) in the Stroudsburg area (25 miles east of the Lehigh River) revealed no igneous or metamorphic rocks in Wisconsin glacial drift or recent sediments. Crowl (6) found only two gneiss pebbles in the Delaware River valley glacial drift between Port Jervis and Delaware Water Gap.

## IGNEOUS AND METAMORPHIC ERRATICS

The following igneous and metamorphic erratics were collected by the writer in 1968 from gravel bars along the Lehigh River between Lehigh Tannery and Lehighton (Fig. 1) and identified from thin sections and hand specimens by T. Saylor, Pennsylvania Geological Survey: flaser granite, three varieties of granite gneiss, hornblende diorite, feldspathic-micaceous quartz schist, magnetite-rich hornblende diorite, biotite granite and biotite-hornblende (granitic) schist. The distribution of these erratics is shown in Figure 1. Thin sections show that in all of the erratics the feldspar is partly to completely kaolinized or seriticized and the quartz is highly strained and sometimes fractured. However, all of the erratic cobbles are very hard and appear unweathered in hand specimen.

These cobbles range in size from 2 inches to over 1 foot in diameter. They generally have low roundness and sphericity values and sometimes have flat sides with only slightly rounded corners. A cursory examination of erratic-cobble distribution was carried out by examining 100-200 square yards of each selected gravel bar and noting lithology, size and relative abundance of erratics. This examination indicates that the quantity of erratics is variable and generally small except at locality 4, which has the greatest concentration of erratic cobbles, and at locality 8 (Fig. 1). The size of erratics is variable, but noticeably smaller at localities 9 and 10 (Fig. 1) where the cobbles are generally less than 2½ inches in diameter. No pebble-size erratics have been observed at any of the sample localities. No erratic cobbles occur downstream from Lehighton (locality 10, Fig. 1).

## GEOMORPHIC CHARACTER

Between Lehigh Tannery and Lehighton (Fig. 1) the Lehigh River occupies a gorge cut into folded Upper Devonian and Mississippian sedimentary rocks, mainly sandstones. The valley walls in this gorge are steep to precipitous and the valley bottom is narrow. Between Lehighton and Palmerton the valley bottom is broader and the surrounding topography more subdued. The trace of the Wisconsin terminal moraine crosses the Lehigh River valley near Hickory Run and extensive kame deposits occur in the river valley immediately upstream from the moraine (near locality 1, Fig. 1).

Discontinuous gravel bars occur along the river either on the inside of river bends or on the margins of long straight reaches. These bars are generally less than 100 feet wide and



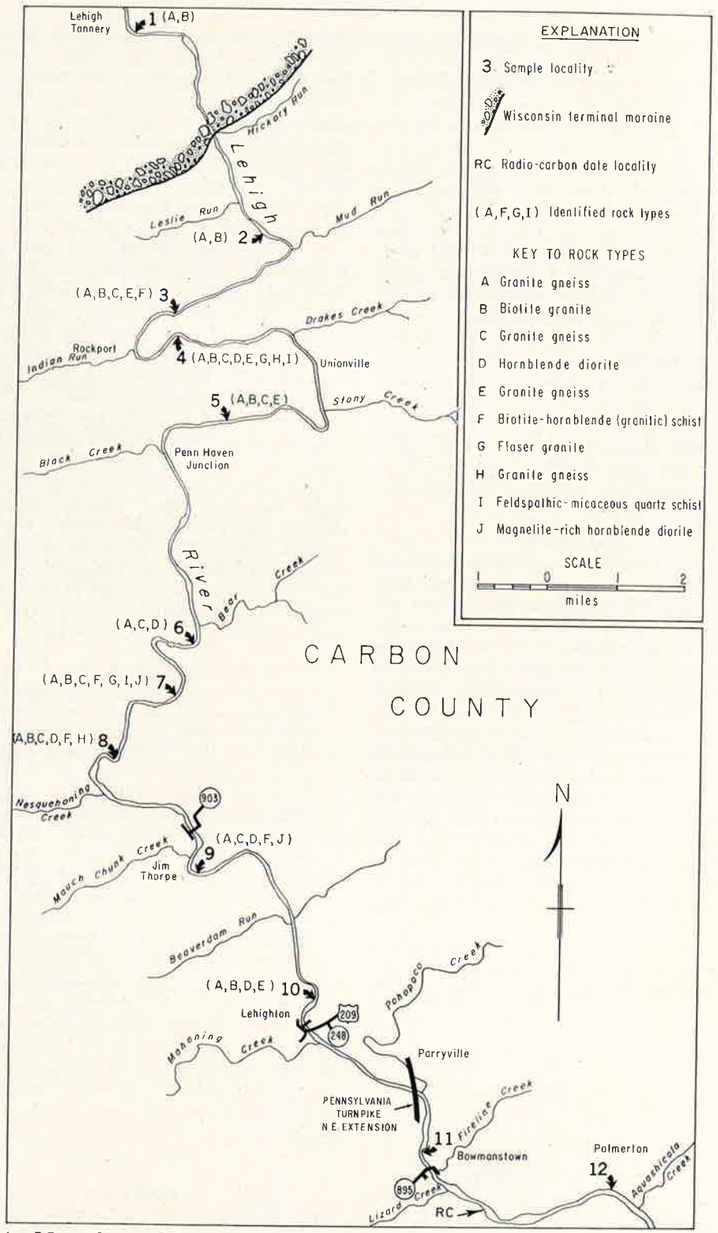



**Figure 1. Map of gravel bar sample localities along the Lehigh River between Lehigh Tannery and Palmerton. Erratic rock types at each locality are indicated.**

1500 feet long. North of Lehighton the river sometimes flows on bedrock, but alluvial material generally covers the river bed. Between Lehighton and Palmerton the gravel bars are larger and the river flows entirely on alluvial material.

The gravel bars are, in some places, recent accumulations a few feet thick (e.g., localities 3 and 5, Fig. 1) and, elsewhere, older deposits (e.g., localities 9 and 12, Fig. 1). The older deposits comprise up to 8 feet of sand overlying gravel of unknown thickness. The gravel is a mixture of sand and rounded to angular rock fragments, generally less than 1 foot in diameter. The rock fragments are mainly sandstones derived from the Catskill, Pocono and Mauch Chunk Formations. The overlying sand has a sharp contact with the gravel and charcoal collected near Lizard Creek (RC, Fig. 1) from some sandy clay at this contact yielded a radio-carbon date of 1800 ± 100 years B. P. In some places the uppermost foot of sand contains coal and ash.

## DISCUSSION

All of the igneous and metamorphic erratics discussed above apparently were derived from the Canadian Shield and/or the western Adirondack Mountains of New York. The occurrence of erratic cobbles in Wisconsin glacial drift in the Lehigh River region indicates transport of erratics to northeastern Pennsylvania by the Wisconsin glacier. Orientation data for Wisconsin glacial striae and constructional topography near the Lehigh River imply derivation of drift material from a northerly source. Illinoian (?) glacial drift is not considered a source for these erratic cobbles because no igneous nor metamorphic rocks have been found in the Illinoian (?) deposits of northeastern Pennsylvania and rock materials in the Illinoian (?) deposits are deeply weathered.

The history of the Lehigh River valley is only partly known, but some events are relatively certain. The position of Illinoian (?) outwash sands and gravels in the Mahoning and Pohopoco Creek valleys (Fig. 1) suggests that drainage channels in the Lehigh River drainage basin had nearly the same base level prior to Illinoian (?) glaciation as they have today. High level remnants of outwash gravels near localities 2 and 8 (Fig. 1) indicate that extensive alluviation occurred in the Lehigh River valley during Illinoian (?) deglaciation, but nearly all of this material was eroded prior to Wisconsin glaciation in northeastern Pennsylvania. Total alluviation in the Lehigh River valley during and after Wisconsin glaciation probably did not exceed the maximum amount of sand and gravel now present (20-25 feet) and the upper sand sequence in river bars probably represent flood-plain deposits formed during the last 1800 years.

The Lehigh River is currently down-cutting and apparently most of the cobble-size and larger materials being moved by the river are being eroded from older (Wisconsin) deposits within the river valley. Thus the absence of igneous and metamorphic cobbles below Lehighton indicates that 22 miles is the maximum distance of downstream transport of cobble-size material in the Lehigh River since the time of maximum Wisconsin glaciation in the area (probably over 12,000 years ago).

## REFERENCES


1. Lewis, H. C. 1884. Report on the terminal moraine in Pennsylvania and western New York. Pennsylvania Geol. Survey, 2nd ser., Bull. Z, p. 67-91.
2. White, I. C. 1882. The geology of Pike and Monroe Counties. Pennsylvania Geol. Survey, 2nd. ser., Bull. G 6, p. 42.
3. Gardner, L. R. 1966. The Pleistocene geology of the Brodheadsville and Pohopoco Mountain (7½′) quadrangles, Pennsylvania. The Pennsylvania State University, Unpublished M.S. thesis, 99 p.
4. Epstein, J. B. 1969. Surficial geology of the Stroudsburg quadrangle, Pennsylvania-New Jersey. Pennsylvania Geol. Survey, 4th ser., Bull. G. 57.
5. Bucek, M. F. *in press*. Surficial geology of the East Stroudsburg 7½′ quadrangle, Monroe County, Pennsylvania. Pennsylvania Geol. Survey, 4th ser.
6. Crowl, G. H. Ohio Wesleyan University, Delaware, Ohio. Personal communication, 1968.




# THE LARGE AMOUNT OF COLLUVIUM IN VALLEY DEPOSITS NEAR LANCASTER, PENNSYLVANIA

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## ABSTRACT

Contrary to general belief that stream valleys are filled with alluvium the valley of Little Conestoga Creek where it cuts through a ridge of Antietam quartzite, 3 miles north of Lancaster, is floored by a fill of sand and gravel size colluvium at least 14 ft thick in contrast to stream deposits ranging from 3 to 4 ft thick. The alluvium consists of channel gravels, a grey gravelly silt, a well sorted bar silt, and uppermost a brown silt believed to be deposited during overbank flooding. The colluvium units consist of yellow silt and an angular quartzite breccia with blocks up to 1½ ft in long diameter. The colluvium grades into the rock rubble mantling the valley sides. It is all derived from the Antietam quartzite ridge, but extends ⅜ mile downstream from the ridge. The colluvium is believed to have been deposited during a period of extreme periglacial frost action. A similar basal colluvial unit is present at other localities in the area.


## INTRODUCTION

The purpose of this paper is to describe the deposits in a narrow portion of the Little Conestoga Creek valley where it cuts through a ridge of Antietam quartzite three miles north of Lancaster and to show the large percentage of colluvium comprising these deposits.

The section of the Little Conestoga Creek valley described is approximately 200 ft wide with a relief from the valley floor to the tops of the bordering valley walls of about 75 ft. The stream, about 20 ft wide at normal flow, now meanders in the center of a floodplain which widens markedly downstream where the stream issues onto an area underlain by carbonate rocks. Former channel deposits show that at one time it followed a more easterly course in this part of the valley.

## METHODS

To study the floodplain, a vertical cross section was constructed by use of auger holes, a short trench, and a hammer seismograph study. The investigation revealed that the valley deposits are comprised largely of colluvium overlain by rather thin alluvial deposits (Fig. 1).

## RESULTS AND DISCUSSION

The alluvial deposits consist of channel gravels, a grey gravelly silt, a well sorted bar silt and uppermost a brown silt. The stream gravels are typically poorly sorted channel deposits composed of 35-45% well-rounded gravel and 35-40% sand. None of the stream gravels contained more than 8% silt and clay. The grey silt which also contains rounded gravel and is poorly sorted, is continuous laterally with the channel gravel layers, though not contemporaneous, extending 20 feet away from the stream beneath the floodplain and a few feet beneath the main channel gravel layers. It is composed of 35 to 50% silt, 20-30% rounded gravel and less than 10% clay size sediment. This deposit is believed to represent a former path of the stream during a period of lateral migration and downcutting to its present channel position.

The bar silts which overlie both the grey silts and the channel gravel are upwards of 90% silt and very well sorted. This unit is identifiable as the intermediate ripple marked silt of a point bar. Toward the bottom, this silt interfingers with the stream gravels due to a shift in the stream course. The lower silts were apparently stable against such an influx of gravel only because they were vegetated.

The uppermost unit of the alluvial series is a poorly sorted brown silt containing about 65% silt and 30% clay. This deposit is believed to have been deposited on the floodplain sur-

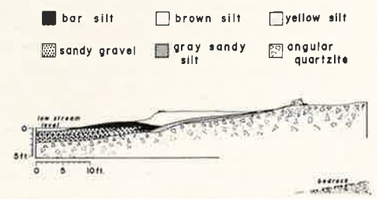



**Figure 1.**

face during overbank flooding.

In contrast to the alluvial beds, which average only 3 to 4 ft in total thickness, the remaining valley deposits are colluvium determined by a hammer seismography to be at least 14 ft thick near the eastern margin of the valley bottom.

The colluvial units of the valley deposits are a yellow silt and an angular quartzite breccia. Although the cross section (Fig. 1) gives a limited view of the colluvial silt, it reaches a thickness of 2½ ft in the northeastern end of the floodplain and is not to be underestimated in volume. Toward the stream the unit increases from 60 to 80% silt sizes with a corresponding decrease in sizes coarser than sand. The silt has obviously been derived from the interstitial material of the breccia.

The breccia itself is 87% larger than sand sizes, with some of the wholly unrounded blocks up to 1½ ft long diameter. This unit is about 13 ft thick and, in the outer portion of the floodplain, extends as a horizontal surface to the foot of the valley wall. The valley wall itself is covered by a similar angular breccia. The yellow silt and breccia units between them comprise about 85% of the material filling the valley.

A seismic check of the area shows that the shallow dipping bedrock surface is carpeted by a thick layer of angular breccia, and this breccia sheet is known to extend ⅜ of a mile downstream beyond the limits of the ridge. All of the material in the sheet is locally derived from the Antietam Ridge. It is characterized by a steep internal inclination and poor sorting. The most probable method of its emplacement would seem to have been extreme periglacial frost action.

The cross section presented here is by no means limited to this one station, but is known to occur at other localities throughout the length of the Little Conestoga Creek. Of the other sites studied in lesser detail, most were found to contain a distinct basal unit of locally derived colluvial material. The extent to which this unit predominates at any locality is controlled by the relief of the valley walls.

## CONCLUSION

The surprising fact revealed by this study is the large amount of colluvial material comprising the valley deposits at the locations studied and the thin surficial veneer of the alluvial deposits.

The importance of colluvium in the valley deposits of narrow stretches of the Little Conestoga Creek valley supports the findings of Lattman (1) and Adler and Lattman (2).

## ACKNOWLEDGMENTS

This project was completed with financial assistance provided by an Undergraduate Research Participation program grant to the Department of Geology at Franklin and Marshall by the National Science Foundation.

## REFERENCES

1. Lattman, L. H. 1960. Cross section of a floodplain in a moist region of moderate relief, *J. Sediment. Petrology* 30:275.
2. Adler, A. A. and L. H. Lattman. 1961. Floodplain sediments of Halfmoon Creek, Pennsylvania, *Bull. Mineral Ind. Exp. Sta.* 77:1.



# FLOOD PLAIN SEDIMENTS AND THEIR MOVEMENT DURING A HIGH DISCHARGE, NEAR MANHEIM, PENNSYLVANIA

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## ABSTRACT

Five distinctive types of deposit comprise the flood plain of Rife Run, 1.5 miles north of Manheim, Pennsylvania. They are as follows: main channel lag gravels, lateral accretion sands and silts on pointbars, vertical accretion overbank silt deposits, small former channel fills, and quartz and shale colluvium. During normal flow, coarse angular vein quartz and shale fragments from the channel banks augment the channel lag deposits. During a 1968 flood, painted channel lag gravels were transported toward a point-bar 150 ft downstream. Mantling this point-bar were similar gravels producing an unusual type of point-bar deposit unrelated to lateral cutting.

## INTRODUCTION

The grass and shrub covered flood plain is located on Rife Run, 1.5 miles north of Manheim, Pennsylvania. Its downstream surface has a gentle gradient of 0.5 ft per 100 ft. The flood plain is comprised of sediment ranging in size from gravel to clay, having been transported to the flood plain by a combination of two processes: downslope gravity movements from the valley walls and downstream transportation of sediments.

## DESCRIPTION OF SEDIMENTS

These two processes have produced five types of deposit on the flood plain. (a) A coarse *channel lag deposit,* with a median size of 15.2 mm and a sorting coefficient of 1.42, is continuous beneath the flood plain. Fine sediments have been removed from the layer by the winnowing action of the stream prior to a shift in the channel. This layer is exposed in the creek at C in Figure 1. (b) Material is deposited on point-bars as a *lateral accretion deposit,* and fine sediments are generally on the top, grading to coarse gravels at the bed load. The point-bar at A, however, is comprised almost entirely of large angular fragments of quartz over the entire surface. The angular quartz is being transported from the bed load and the channel lag deposit at C to the point-bar at A. (c) A fine *overbank deposit* is left by vertical accretion during a period of high discharge, when the stream exceeds bank-full stage. Common overbank deposits include swale-fills, levees, crevasse splays, and flood basins or backswamp deposits. Only two of these deposits are found on this flood plain, levees and flood basins. (d) *Colluvium* is brought from the valley walls and forms a poorly sorted wedge shaped lens that interfingers with the alluvial deposits of the flood plain. Angular fragments of quartz are abundant, derived from veins in the Cocalico Shale bedrock. The example of this zone is located at B. (e) A *channel fill deposit,* located at D, is a remnant of an abandoned stream channel. This is considered a transitional deposit because it generally includes both bed and suspended load sediments. There are two sections to this lens, a fine zone in the upper half with a median size of 4 mm and a sorting coefficient of 1.92, and a coarse zone beneath, with a median size of 8.2 mm and a sorting coefficient of 2.65.

## STREAM COMPETENCY

In an attempt to determine the effects of a flood, angular grains of quartz were painted and placed on the surface of a point-bar and in the stream. A swath, 7 inches wide and 4.6 ft long, was painted on the top of the point-bar to cover all of the grains. This path was positioned so that it was perpendicular to the flow of the water when it went across the point-bar. In the stream channel 25 pebbles, with intermediate axes ranging from 2 to 100 mm, were placed at C perpendicular to the flow. These preparations were completed prior to a 5-in rainfall in Lancaster County, on September 10, 1968, which flooded virtually the entire valley.

Upon checking the areas after the rain, movement had occurred. Most of the grains on the point-bar had been moved, but only 38 could be found, with the smallest 18 ft away. The transported grains ranged in size from 4 to 94 mm, with a median size of 17.5 mm. The entire area of point-bar was carefully searched and it was estimated that the re-

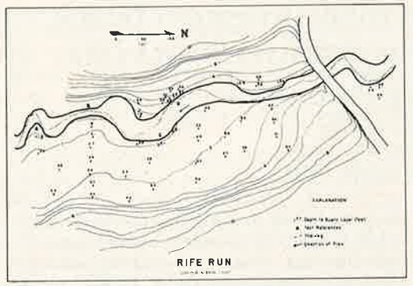



**Figure 1. Plane table map of Rife Run flood plain.**

covered grains accounted for only 30% of the total. The remainder probably was carried downstream and deposited elsewhere. This resulting point-bar was comprised mostly of coarse gravels with the fine materials having been winnowed out and carried further downstream. The result, therefore, was a thick lens of quartz.

Of the 25 pebbles placed into the stream at C, only 3 could be located. These had intermediate axes of 46, 68, and 89 mm. The smallest pebble had been transported the greatest distance, 12.0 ft. The remaining 22 pebbles may have had any of three fates: (a) They may have been transported downstream further than expected; (b) They may have been covered with a layer of silt deposited during the waning stages of the flood; (c) The paint may have been removed, thereby avoiding detection.

## CONCLUSION

For all recovered particles the finer sediments were transported the greater distance. Although the exact discharge required to move these particles was unknown, flood conditions prevailed. The stream was able to move large particles both on the bed load and across the point-bar. Material was constantly being added to and removed from the point-bar. The fine sediments were more easily carried, permitting the channel lag to become better sorted.

This point-bar deposit is an unusual accumulation of bed load material. Instead of a fine grained deposit remaining after a future shift in the channel, there will be a thick zone of large angular quartz. This, therefore, suggests that another type of point-bar deposit, coarse, well sorted, and angular, may be present within a flood plain. Without knowledge of the present stream the examination of a cross section of this deposit would not lead one to interpret it as a point-bar.

## ACKNOWLEDGMENTS

Special appreciation is expressed to the National Science Foundation sponsored Special Master's Program in Geology and the faculty at Franklin and Marshall College, who made this study possible.

## SELECTED BIBLIOGRAPHY

Adler, A. A. and L. H. Lattman. 1961. Flood plain sediments of Halfmoon Creek, Pennsylvania. *Bull. Mineral Ind. Exp. Sta. Penn. State Univ.* 77:1.

Allen, J. R. L. 1965. A review of the origin and characteristics of recent alluvial sediments. *Sedimentology* 5:89.

Brush, L. M. 1964. Sediment sorting in alluvial channels. *Am. Assoc. Petrol. Geologists Bull.* 48:519.

Happ, S. C., G. Rittenhouse and G. C. Dobson. 1940. Some principles of accelerated stream and valley sedimentation. *U. S. Dep. Agr. Tech. Bull.* 695:1.

Harms, J. C. and R. K. Fahnestock. 1965. Stratification, bed forms and flow phenomena. *Soc. Econ. Paleontologists and Mineralogists Spec. Publ.* 12:84.

Lattman, L. H. 1960. Cross section of a flood plain in a moist region of moderate relief. *J. Sediment. Petrology* 30:275.

Mackin, J. H. 1948. Concept of the graded river. *Bull. Geol. Soc. Am.* 59:453.

Wolman, M. G. 1954. A method of sampling coarse river-bed material. *Am. Geophys. Union Trans.* 35:951.

Wolman, M. G. 1955. The natural channel of Brandywine Creek, Pennsylvania. *U. S. Geol. Surv. Profess. Papers* 271:1.

Wolman, M. G. and L. B. Leopold. 1957. River flood plains: some observations on their formation. *U. S. Geol. Surv. Profess. Papers* 282-C:87.



# CLASTIC DIKES IN THE CATSKILL FORMATION NEAR MILLERSTOWN, PENNSYLVANIA[1]

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## ABSTRACT

Several clastic dikes are exposed in the Duncannon Member of the Upper Devonian Catskill Formation near Millerstown, Pennsylvania. They occur as subvertical wedges of grayish red sandy siltstone cutting finer beds of argillaceous siltstone and extend downward from thin, discontinuous beds of similar color and lithology. These dikes are low energy structures which were filled from above. The dikes and their overlying associated siltstone lenses may be part of crevasse splay deposits which flowed over and filled incipient slump crevices in overbank alluvial sediments on the floodplain of the Catskill delta.

## INTRODUCTION

The term "sandstone dike" or "clastic dike" has been used to describe any tabular body of sand, silt, or conglomerate which cuts across the enclosing rock unit in the manner of an igneous dike or hydrothermal vein, but which is obviously of sedimentary origin (1). There are two broad categories of clastic dikes. The first, or high energy type, are those which have been injected into fissures of tectonic origin, and the second, or low energy type, are those which have filled fractures or crevices which opened in soft or semi-consolidated sediment as a result of dessication, incipient slumping, or hillside creep (2). The first, or injected type, are generally of larger magnitude, tend to be more truly tabular in shape, may have preserved flow structures, and usually have a pattern which is related to the tectonic history of the area (3,4). The latter, or low energy type, are smaller, ordinarily lack internal structure except for an occasional transverse stratification, may have a less regular pattern and tapering cross section, and reflect syndepositional conditions (5,6).

The dikes discussed here are of the low energy type, which were emplaced prior to the solidification of the enclosing strata.

[1] Published with permission of the Director of the Pennsylvania Geological Survey.

## LOCATION: GEOGRAPHIC AND STRATIGRAPHIC

These dikes occur in the Duncannon Member of the Upper Devonian Catskill Formation (7) in an excellent new exposure where U. S. Highway 22-322 crosses the western end of the Buffalo-Berry Mountain syncline four miles south of Millerstown in Perry County, Pennsylvania (Fig. 1). This area was mapped by Faill and Wells in the preparation of a forthcoming report on the geology of the Millerstown Quadrangle (8).

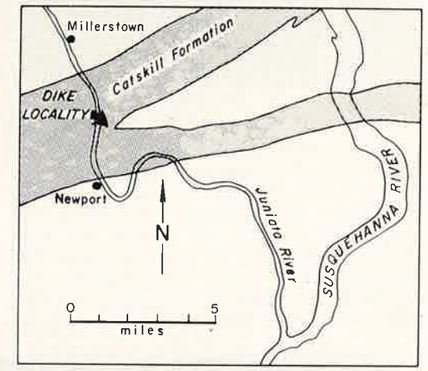



**Figure 1. Location of clastic dikes near Millerstown, Pennsylvania.**

The Duncannon Member of the Catskill Formation consists of cyclic deposits of gray sandstone and grayish red sandstone, siltstone, and silty claystone which exhibit a pronounced upward decrease in grain size within each cycle.[1] There are at least 6 major cycle present at this locality, ranging in thickness from 30 to 136 ft, and averaging 66 ft thick. Each cycle has a basal non-red (medium gray, light gray, or light olive gray) locally conglomeratic, medium to fine grained sandstone which oc-

[1] The term *cycle* is preferred over *cyclothem* because in the original definition, which is retained in the most recent A.G.I. Glossary, the term *cyclothem* is restricted to a particular group of cyclic sedimentary rocks. Allen and Friend (1968) apply the term *cyclothem* to the cyclic deposits in the Catskill Formation in this area.

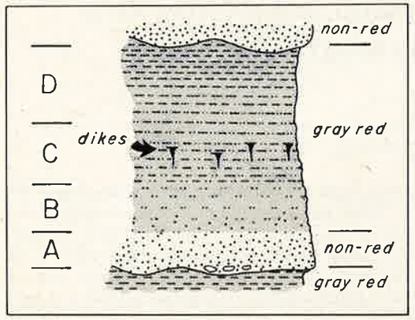



**Figure 2. Idealized Duncannon Member fining upward cycle. Part A is medium gray or light olive gray cross bedded sandstone. Part B is reddish gray sandy siltstone. Part C is reddish gray argillaceous siltstone. Part D is grayish red silty claystone, which is commonly light greenish gray in the upper few inches. No scale is intended, since the thickness of each member of a cycle is quite variable.**

cupies a channel eroded into the top of the underlying cycle. This sandstone becomes finer upward, and is succeeded by grayish red, very fine grained silty sandstone, light grayish red argillaceous siltstone, and grayish red silty claystone. Commonly the contacts between the different parts of a cycle are gradational and indistinct. In several of the cycles, the red siltstone and silty claystone members are repeated in a sequence of nested sub-cycles. An idealized Duncannon Member cycle is shown in Figure 2.

The sandstone dikes occur in the grayish red argillaceous siltstone member of cycle number three in the Buffalo Mountain West section.

The structural position of these dikes is on the gently dipping north limb of the syncline, within 1,000 ft of the trace of the axial plane of the fold.



**Figure 3. Clastic dike showing angular inclusions of host rock.**

## DESCRIPTION

These dikes are composed of medium to coarse grained silt which locally grades into very fine grained sandstone (Fig. 3). The color is dark grayish red to light grayish red (5 R 3/2 to 5/2) or greenish gray (5 GY 6/1). The dike material is argillaceous, micaceous, siliceous, slightly hematitic, moderately well to poorly sorted, and has little porosity or permeability.

The host rock is a fine grained argillaceous siltstone, grayish red to dark grayish red (5 R 3/2 to 4/2), which in some instances grades upward into silty claystone. The dikes penetrate downward into the host rock from an overlying bed of sandy siltstone, usually 2 to 3 inches thick, of the same color and lithology as the dikes themselves. These beds are minor interruptions of the general fining upward pattern, and are of limited lateral extent. The dikes are connected to the overlying beds and apparently were formed from the same sediment. Angular fragments of the host rock sometimes occur in the upper parts of the dikes and the associated overlying beds (Fig. 4).






**Figure 4. Clastic dikes in the Duncannon Member.**

The geometric form of these dikes is usually that of a slightly tapering sub-vertical wedge, and the dike walls are sharp. The dikes may be straight, slightly irregular, or have a zig-zag pattern in cross section.

Six clastic dikes were measured in detail and range in height from four to twelve inches, and taper from a width of ⅜ to one inch at the top to a width of ½ to 1/16 inch or less at the base. At least one of the dikes formed from the downward coalescing of two smaller dikes and has been truncated by erosion. The four dikes on which a strike could be determined trend from 115° to 150°, and dip from 50° southwest to 67° northeast.

There are no apparent flow structures in these dikes. The only internal structure is a faint transverse lamination resulting from the orientation of mica flakes in the settling position, which is seen only rarely (Fig. 5).

These dikes are restricted to a lateral distance of 35 ft, at two stratigraphic levels three feet apart, which is a relatively small area of an exposure nearly 4,000 ft long and 100 ft high.



**Figure 5. This dike, the largest exposed at this locality, exhibits a faint transverse stratification. Scale is 12 inches long.**

## ORIGIN AND SIGNIFICANCE

According to Shepps and others (9), Allen and Friend (10), and McCave (11), the Catskill Formation was formed on an alluvial coastal plain or delta complex traversed by several rivers. The clastic dikes observed in the Duncannon Member are interpreted as part of the overbank alluvial material deposited in small fissures in floodplain or flood basin deposits. These fissures or crevasses probably originated through hillside creep or slumping of semi-consolidated mud toward local depressions or undercut banks. The sediment comprising the dikes and the overlying associated siltstone lenses may be part of a crevasse-splay deposit (12) which spread out over the floodplain through a break in a natural levee. Such an origin is suggested by the coarser grain size of the sediment, the lobate cross section of the overlying siltstone beds, and their occurrence beyond the edge of the channel.

The small number of clastic dikes present here is a reflection of the fact that their formation first required the opening up of cracks or small crevasses into which the sediment could settle. These openings developed only when a certain fortuitous combination of conditions of sediment character and downslope movement occurred, which was probably a rare event.

An alternative origin is suggested by the

similarity of these dikes to those described by Judd and Heron (13), from the Atlantic coastal plain of North Carolina and South Carolina. Both groups of dikes are composed of silt, taper downward from a maximum width of less than six inches, were formed from an overlying source of sediment, and are oriented in only one or two directions within any single outcrop. Judd and Heron (13) conclude that the Carolina dikes were formed by "translocation of fine material from within the "A" soil horizon on the coastal plain, and are of pedological origin".

Clastic dike formation by injection of quicksand upward into joints or dessication cracks in overlying sediment is rejected for the Duncannon Member dikes because of the lack of flow structures, the weak horizontal bedding in the dikes, and the absence of other dessication structures. It is possible that the overlying connected siltstone lenses are injected sedimentary sills, but compactional wrinkles are not present in the enclosing strata and internal evidence of forceful injection is lacking.

These structures would be useful for determining tops of beds in areas of extensive deformation, since they are known to penetrate from above and generally pinch out downward.

## REFERENCES


1. Potter, P. E. and F. J. Pettijohn. 1963. *Paleocurrents and Basin Analysis*. Academic Press, New York, 296 pp.
2. Shrock, R. R. 1948. *Sequence in Layered Rocks*. McGraw-Hill Book Co., New York, 507 pp.
3. Diller, J. S. 1890. Sandstone dikes. *Geol. Soc. Amer. Bull.* 1:411.
4. Harms, J. C. 1965. Sandstone dikes in relation to Laramide faults and stress distribution in the Southern Front Range, Colorado. *Geol. Soc. Amer. Bull.* 76:981.
5. Reimnitz, E. and N. F. Marshall. 1965. Effects of the Alaska earthquake and tsunami on recent deltaic sediments. *J. Geoph. Res.* 70:2363.
6. Oomkens, E. 1966. Environmental significance of sand dikes. *Sedimentology* 7:145.
7. Dyson, J. 1967. Geology and mineral resources of the southern half of the New Bloomfield Quadrangle, Pennsylvania: Penn. Geol. Survey, 4th Series, Atlas 137 cd, 86 pp.
8. Faill, R. T. and R. B. Wells. Geology and mineral resources of the Millerstown 15 minute Quadrangle, Pennsylvania: Penn. Geol. Survey, 4th Series, Atlas 136. (in preparation).
9. Shepps, V. C., *ed.* 1963. Symposium on Middle and Upper Devonian stratigraphy of Pennsylvania and adjacent states: Penn. Geol. Survey, 4th Series, Report G-39, 301 pp.
10. Allen, J. R. L. and P. F. Friend. 1968. Deposition of the Catskill facies, Appalachian region: with notes on some other Old Red Sandstone basins. *In* Late Paleozoic and Mesozoic Continental Sedimentation, Northeastern North America: Geol. Soc. Amer. Special Paper 106, p. 21.
11. McCave, I. N. 1969. Correlation of marine and nonmarine strata with example from the Devonian of New York State. *Am. Assoc. Petroleum Geol. Bull.* 53:155.
12. Allen, J. R. L. 1965. A review of the origin and characteristics of recent alluvial sediments: *Sedimentology* 5:89.
13. Judd, J. B. and S. D. Heron. 1969. Clastic dikes in the North Carolina and South Carolina coastal plain. *Geol. Soc. Amer. Abstracts* part 4, p. 41.




# CYTOPHOTOMETRIC STUDIES OF DNA IN CIRCULATING ERYTHROCYTES OF BROOK TROUT EXPOSED TO ACID pH


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## ABSTRACT

Trout exposed to low pH (3.5) for four days accumulate excessive P.A.S.-positive mucin notably in the gill region. This is known to interfere with oxygen diffusion across gill filaments leading to hypoxemia and death. To determine whether any augmentation of hemopoiesis occurs in such trout, quantitative analyses were made of nuclear Fuelgen-DNA profiles of circulating populations of erythrocytes using the two-wavelength method of microspectrophotometry. No evidence was found of either enhanced hemopoiesis or increased rate of red cell destruction in acid-water exposed fish. These data suggest that one of the reasons why trout die in acid water is that compensatory erythropoiesis does not occur or is not as rapidly initiated in fish as in homeotherms in response to oxygen lack.


## INTRODUCTION

Studies in this laboratory and elsewhere have shown that short-term exposure to acid pollutants can prove lethal to fresh water fish and it has been postulated that the primary locus of injury is in the gill region. For example, Westfall (1) and Plonka (2) observed that excessive mucin secretion by epithelial cells of gill filaments is grossly evidenced in acid-exposed fish. Carpenter (3) reported that coagulation of such mucin on gill filaments leads to impaired efficiency of respiratory gas exchange, thus resulting in hypoxemia and eventual suffocation from oxygen lack.

This study was designed to investigate the extent to which exposure to acid water affects certain aspects of hemopoiesis in brook trout. The approaches used involved quantitative cytophotometric analyses of Feulgen-DNA in circulating erythrocytes and microscopic analysis of cytomorphological changes in leucocytes and erythroid cells.

## MATERIALS AND METHODS

A total of 40 hatchery-reared brook trout (Research Station of the Pennsylvania Fish Commission, Benner Springs, Pennsylvania) weighing 50-75 gms. and 15-17 cm. in length was used in this study. Control fish were maintained in dechlorinated water at pH 7.0. Experimental fish were exposed to acid water over a 4 day period. Small amounts of concentrated HCl were added to the water every 8 hours resulting in a final pH of about 3.5 on day 4. The following morning all fish were sacrificed by electric shock.

Blood was withdrawn from the anterior kidney head with heparinized syringes and smears were made and fixed in absolute methanol for 2 minutes. Areas of the gill arch were dissected free, fixed in Perenyi's, washed in 50% alcohol, embedded in paraffin and sectioned at 6$\mu$. Both gill sections containing red blood cells and the blood smears were stained with Feulgen for DNA (4). Quantitative cytophotometric analyses of RBC nuclei provided a measure of possible changes in rate of cell degeneration. Measurements were made with a single beam microspectrophotometer by the two wavelength method as described by Patau and Ornstein (5,6). In addition to cytophotometry, red blood cells stained with Giesma (4) were analyzed for morphological evidence of cellular damage.

To determine if exposure to acid water caused an augmentation of RBC production, blood was stained vitally with brilliant cresyl blue (4) and reticulocytes plus young erythrocytes were counted. Supplemental data involved measures of hematocrits in treated and untreated fish. In addition, differential counts on the Giemsa stained blood provided data on possible alterations in the blast cell population as well as the leucocyte response. White cells were identified on the basis of size, nuclear shape and staining affinity using a classification scheme similar to that proposed by Jakowska and Catton (7,8).

## RESULTS

Two indices were used to assess the extent of red blood cell destruction: (a) changes in relative proportions of cells showing slightly decreased DNA levels in a population of circulating erythrocytes and (b) cytological evidence of nuclear degeneration as reflected

in increased intensity of staining (pycnosis) or dissolution of the nuclear membrane associated with karyorrhexis. Neither index revealed any appreciable change in the rate of red blood cell destruction. Data obtained from cytophotometric analysis of Feulgen-DNA contents are conventionally presented in the form of histograms (Fig. 1) since such plots facilitate the detection of possible changes in DNA content between comparable cell populations from control and experimental fish. From Figure 1, it is readily apparent that the DNA profiles of blood from fish maintained in acid water are the same as those of controls. This is true whether one analyzes blood smears or erythrocytes in sections of gill filament vessels.

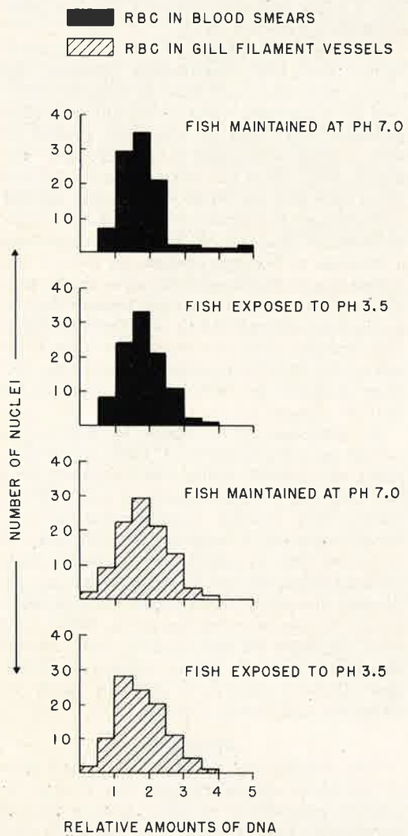



**Figure 1. Feulgen-DNA profiles of RBC nuclei in blood smears and gill filament vessels.**

In other words, there is no increase in relative numbers of nuclei exhibiting lower amounts of DNA which one would anticipate if exposure to acid appreciably increased the rate of red blood cell destruction.

Cytological studies of erythrocytes stained with Giemsa also supported the above finding that low pH exposure does not result in an increased rate of cell destruction. For example, there was no indication of an increase in the proportion of cells exhibiting pycnosis and related signs of incipient cell breakdown.

During the course of this study data were also obtained which indicated that exposure of fish to acid does not appear to materially enhance the rate of red blood cell production. Evidence for this was based on hematocrit determinations and microscopic analyses of blood smears stained with brilliant cresyl blue (Fig. 2A) or Giemsa. For example, no significant change was observed in the hematocrit or in the percentage of reticulocytes (including young erythrocytes) between the two groups. The reticulocyte count in controls was 11 ±2% and that of fish exposed to acidic pH was 11 ±3%. In addition, counts of all erythroid cells in peripheral blood showed no change in relative number of blast cells when fish were exposed to acid water. Figure 2B shows typical blast cells in the peripheral blood.

The white blood cell population also appeared unaltered after acid exposure (Fig. 2C). The data showed no significant change in the proportion of non-granular to granular leucocytes which occurred in approximately a 5:1 ratio. Furthermore, there were no consistent changes in the cytological appearance or in relative percentages of lymphocytes (27%), monocytes (less than 1%) or neutrophils (55) which could be attributed to acid exposure. Similarly, there were no changes in either the numbers or staining quality of giant cells and degenerating cells.

## DISCUSSION

It has been shown that when fish are exposed to acid pollutants heavy mucoid material in the gills accumulates in interlamellar spaces and hinders movement of individual filaments. This condition apparently impairs circulation and results in a stasis of blood in gill capillaries (9). Because of the close proximity between red blood cells in these capillaries and environmental water, one might expect prolonged acid exposure would increase the rate of red blood cell degeneration in this immediate area. However, this did not prove to be the case. Histological examination of erythrocytes from both the gill region and kidney head showed no morphological evidence of nuclear pycnosis or degeneration. Cytophotometric analysis of nuclear DNA content was therefore employed in an attempt to determine whether exposure to acid caused more subtle changes in the nucleoprotein makeup of a significant number of circulating RBC. Since this method involves measuring the nuclear Feulgen-DNA content on an individual cell basis, increased degeneration of RBC would result in a shift to the left in the DNA histogram of a given population of cells. Figure 1 showed no shift in DNA profiles, thereby signifying no measurable change in nuclear DNA content.



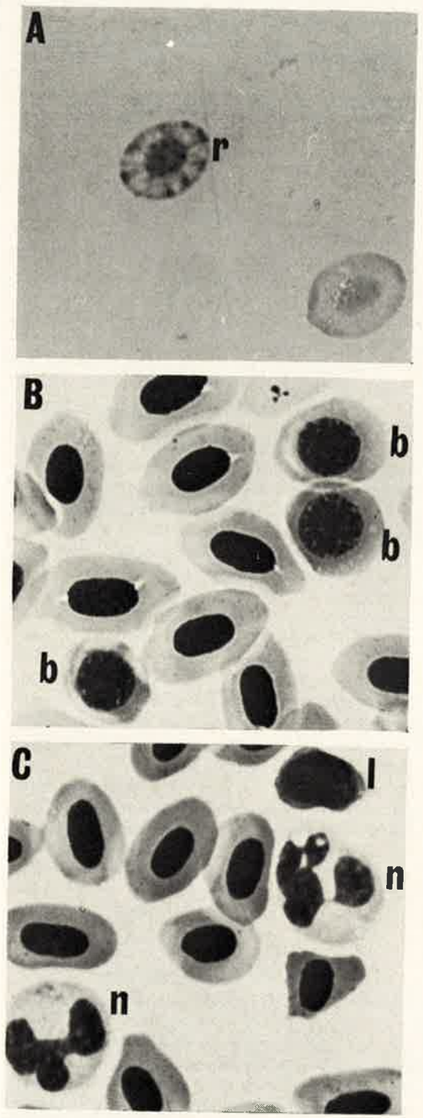



**Figure 2. Blood smears from anterior kidney head of brook trout exposed to acid pH. (1480x). A. Stained vitally with brilliant cresyl blue, R - reticuloycte; B. Stained with Giemsa, B - blast cells; C. Stained with Giemsa, L - lymphocyte, N - neutrophil.**

A second aspect of this study investigated whether acid-induced anoxia increased the rate of RBC production. In homeotherms, anoxia causes an increased erythropoiesis within hours after exposure. It is believed that tissue anoxia causes release of an erythropoietic stimulating factor which in turn increases the number of stem cells and blast cells feeding into the total erythron. Increased hematocrit as well as increased number of reticulocytes appearing in the peripheral blood have been found to be reliable indices of enhanced erythropoiesis in warm blooded animals (10). In the present study, however, acid exposed fish did not exhibit these types of hemal responses. Either compensatory erythropoiesis did not occur or it was not as rapidly initiated as in homeotherms.

There were also no cytological changes observed in circulating leucocytes which could be attributed to acid exposure indicating the absence of an "inflammatory-type" response in these fish.

Thus, on the basis of the overall data it is concluded that exposure of brook trout to acid water is not associated with either an increased rate of red blood cell destruction or an augmentation of erythropoiesis.

## ACKNOWLEDGMENT

This study was aided in part by the Federal Water Pollution Control Administration Grant DXJ1805.

## REFERENCES

1. Westfall, B. A. 1945. Coagulation film anoxia in fishes. *Ecology*. 26:283.
2. Plonka, A. C. and W. H. Neff. Personal communication.
3. Carpenter, K. E. 1927. The lethal action of soluble metallic salts on fishes. *Brit. Jour. Exper. Biol.* 4: 378.
4. Humason, G. E. 1967. *Animal Tissue Techniques*. (2nd ed.) W. H. Freeman and Co., San Francisco, p. 242.

5. Patau, K. 1952. Absorption microphotometry of irregular-shaped objects. *Chromosoma.* 5:341.
6. Ornstein, L. 1952. The distributional error in microspectrophotometry. *Lab. Invest.* 1:250.
7. Jakowska, S. 1956. Morphologie et nomenclature des cellules du sang des teleosteens. *Revue d'Hematologie.* 11:519.
8. Catton, W. T. 1951. Blood cell formation in certain teleost fishes. *Blood, Jour. Hematol.* 6:39.
9. Ellis, M. M. 1937. Detection and measurement of stream pollution. *Bulletin of the Bureau of Fisheries.* 48:365.
10. Jacobson, L. O. and M. Doyle, (ed.). 1962. *Erythropoiesis.* Grune and Stratton, New York.



# HEPATIC TUMOR INDUCTION AND HORMONAL ENVIRONMENT

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## ABSTRACT

A modified hormonal environment induced by androgen administration 5 days after birth resulted in enhanced liver growth in response to the carcinogen, dimethylaminoazobenzene. Liver growth was associated with an increase in protein and nucleic acids. Neonatal androgen depressed ovarian growth but concomitant DAB had no further effect.

## INTRODUCTION

Hormonal environment can have a definite influence on the incidence of hepatic tumors induced by carcinogens as indicated by the marked sex difference in responsiveness. In the rat, 0.03% 2-acetylaminofluorene fed for six months induced liver tumors in 90% of male and 10% of female rats (1). An insight into the endocrine-carcinogen interaction might also be gained by invoking a permanent endocrine imbalance experimentally. This can be accomplished by administering a single injection of androgen or estrogen at 5 days of age (2,3). After 50 days of age the androgenized animal exhibits constant estrus (4) and as an adult is permanently sterile. The ovary of the androgen sterile rat exhibits enhanced androgen and estrogen synthesis (5). Recently the androgen sterile rat has been shown to exhibit an enhanced response to a carcinogen, N-hydroxy-N-2-fluorenylacetamide (6). The present study seeks to examine the response of the androgen sterile rat to another carcinogen, dimethylaminoazobenzene.

## MATERIALS AND METHODS

Pregnant Sprague-Dawley rats were obtained from Camm Research Institute, Wayne, N. J. The rats were housed individually and fed D & G Laboratory diet ad libitum. Three days after birth the litters were reduced to 8 and sexed in favor of females. On day 5, half of the females in a litter were injected subcutaneously with 1.25 mg of testosterone propionate. The litter was weaned at day 21 and on day 30 the rats were placed on experiment. Androgenized and control rats were fed a diet containing 0.06% dimethylaminoazobenzene (DAB) for 120 days. Androgenized and control rats fed a normal diet served as controls.

On the morning of autopsy the rats were rapidly decapitated, blood collected from the severed neck vessels and the sera obtained for analysis. Serum and adrenal corticosterone was determined by a modification of the method of Hilf *et al.* (7). Liver nucleic acids were determined by the method of Hutchison and Munro (8) and tissue nitrogen by the Kjeldahl method of Pregl and Roth (9).

## RESULTS

Neonatal androgen treatment induced vaginal opening prematurely and a subsequent constant estrus. In this altered hormonal environment, body weight gain, slightly but definitely, exceeded that of the control. Concomitant feeding of carcinogen negated the androgen effect on body weight gain (Table 1).

Ovarian weight was subnormal following neonatal testosterone propionate administration and the ovary contained only follicles in contrast to the normal ovary which contained many corpora lutea. Carcinogen feeding did not alter ovarian weight gain significantly in normal or androgen sterile rats.

Liver weight responded to testosterone propionate being 8.6 grams as compared to 7.3 grams in control rats. Furthermore, the larger liver contained more protein and more RNA. Carcinogen feeding increased liver weight to 9.9 grams and despite the decrease in protein concentration the greater mass resulted in a total liver protein increase which simulated that obtained with androgen. The carcinogen increased liver cell numbers (DNA) without a cellular hypertrophy (protein/DNA). Total liver RNA increased. The carcinogen fed androgen sterile rat exhibited the largest liver and represented an increase of 3.3 grams in comparison with a normal animal. The total protein, RNA and DNA reflected the increase in organ size. Thus the abnormal hormonal environment enhanced the influence of a carcinogen on the liver.

Adrenal and serum corticosterone were not influenced by the experimental manipulations.

TABLE 1. *Effect of Neonatal Androgen Sterilization and a Carcinogen on Rat Liver and Ovary*

| Treatment | No. Rats | Body wt. gm | Liver wt. gm | Liver Protein gm | RNA Total mg | RNA mg/gm | DNA Total mg | DNA mg/gm | Ovary wt. mg |
|---|---|---|---|---|---|---|---|---|---|
| NONE | 10 | 238 | 7.3±0.2 | 1.03±0.06 | 74±2.0 | 10±0.2 | 27±1.0 | 3.6±0.2 | 28±2 |
| DAB | 10 | 216 | 9.9±0.3 | 1.18±0.05 | 83±3.1 | 9±0.3 | 34±1.3 | 3.7±0.3 | 34±3 |
| $T_p$ | 11 | 271 | 8.6±0.2 | 1.21±0.03 | 87±3.3 | 10±0.3 | 31±1.4 | 3.7±0.2 | 61±5 |
| DAB + $T_p$ | 13 | 247 | 10.6±0.3 | 1.47±0.06 | 92±3.9 | 9±0.3 | 39±1.3 | 3.7±0.2 | 60±7 |

DAB = dimethylaminoazobenzene
$T_p$ = testosterone propionate

## DISCUSSION

Neonatal androgen to female rats followed by the feeding of N-hydroxy-N-2-fluorenylacetamide resulted in the appearance of liver cancer at a time when cancer was not present in carcinogen fed normal rats (6). Furthermore, the metabolic pattern of the carcinogen could not fully account for the alteration in biologic effect suggesting more complex interactions between the hormones and carcinogens. The modified hormonal environment permitted an enhanced response of liver weight to the feeding of a carcinogen. Similar results were obtained with a different carcinogen, dimethylaminoazobenzene. With DAB, liver mass was greater in the androgen sterile rat. The larger liver was associated with an increase in protein, nucleic acids, and numbers of cells.

Following testosterone propionate at 5 days of age, the ovary becomes polyfollicular and fails to form corpora lutea. Neonatal androgen negates the diurnal cholesterol rhythms in the ovary and increases cholesterol content to a concentration exceeding that of diestrus (10). These ovaries continue to produce steroids but may well lack the cyclic phases of the normal. No influence of DAB was noted on ovarian weight and an influence on steroidogenesis has not been reported.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Leathem, J. H. 1955. 2-acetylamino-fluorene liver tumor induction as related to nutrition and hormones. *Proc. Cancer Res.* 2:30.
2. Barraclough, C. A. and J. H. Leathem. 1954. Infertility induced in mice by a single injection of testosterone propionate. *Proc. Soc. Exptl. Biol. Med.* 85: 673-674.
3. Leathem, J. H. 1956. The influence of steroids on prepuberal animals. *Proc. Third International Cong. Animal Reprod.* p. 13-14.
4. Doeg, L. H. and J. H. Leathem. 1958. Estrous cycles in mice following prepuberal steroid administration. *Proc. Penna. Acad. Sci.* 32:202-210.
5. Weisz, J. and C. W. Lloyd. 1965. Estrogen and androgen production in vitro from 7-$H^3$-progesterone by normal and polycystic rat ovaries. *Endocrinology* 77:735-744.
6. Weisburger, E. K., R. S. Yamamoto, R. M. Glass, P. H. Grantham and J. H. Weisburger. 1968. Effect of neonatal androgen and estrogen injection on liver tumor induction by N-hydroxy-N-2-fluorenylacetamide and on the metabolism of the carcinogen in rats. *Endocrinology* 82:685-692.
7. Hilf, R., F. F. Burnett and A. Borman. 1960. The effect of Sarcoma 180 and other stressing agents upon adrenal and plasma corticosterone in mice. *Cancer Res.* 20:1389-1393.
8. Hutchison, W. C. and H. N. Munro. 1961. The determination of nucleic acids in biological materials: a review. *The Analyst* 86:768.
9. Pregl, F. and H. Roth. 1935. *Die Quantitative Organische Microanalyse*. Springer Verlag: Berlin.
10. Clark, J. H. and M. X. Zarrow. 1967. A circadian rhythm in ovarian cholesterol. *Acta Endocrinol.* 56: 445-452.



# EFFECTS OF THYROXINE ON TISSUE RESPIRATION IN SQUALUS ACANTHIAS

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## ABSTRACT

A group of dogfish *(Squalus acanthias)* was injected daily for six days with thyroxine. After treatment, the animals were killed and the kidneys, liver, and a portion of the longitudinal bundle of the hypaxial musculature were excised and homogenized. Respiration rates were determined polarographically in the presence of (a) excess substrate (succinate, beta-hydroxybutyrate, and alpha-ketoglutarate, separately, (b) excess ATP in the presence of excess substrate, and (c) excess ADP in the presence of excess substrate. Generally, the levels of thyroxine used produced no differences between respiratory rates of control or thyroxine treated liver, kidney and muscle homogenates.

## INTRODUCTION

At present, most investigations concerning the effects of thyroid hormone on cellular metabolism involve the use of mammals and birds. Thyroxine has been shown in these animals to have a controlling effect on metabolism through its action on oxygen consumption and heat production. In most poikilotherms, this relationship has not been conclusively demonstrated; in fact, some cold-blooded vertebrates synthesize little or no thyroxine or synthesize it very slowly (1). In certain poikilotherms, such as lizards, thyroxine is effective in increasing oxygen consumption only when the animal is kept near its temperature optimum (2). Early workers were unable to demonstrate any effect of thyroxine treatment on adult *Squalus* tissue respiration as measured with a Warburg apparatus. More recent studies, however, have shown an increased oxygen consumption in thyroxine treated shark embryos but oxygen consumption returned to normal after prolonged treatment (3).

This investigation is intended to elucidate the effect of thyroxine on oxygen consumption at the cellular level by examining the respiration of liver, muscle and kidney homogenates from thyroxine treated *Squalus*. We wish to re-examine some of the earlier data which showed no demonstrable effect of thyroxine, using an oxygen electrode system which has a much faster response time than the Warburg apparatus used in earlier experiments.

## MATERIALS AND METHODS

Ten male spiny dogfish *(Squalus acanthias)* were captured off the coast of Bar Harbor, Maine, and stored in wooden live cars. The ten animals were randomly assigned to two groups. One group received daily 1 ml injections of $10^{-9}$ gm/ml 1-thyroxine, I.P., for six days while the other group served as saline injected controls. On the sixth day of treatment, the animals were dispatched and portions of the liver, kidney and hypaxial musculature were removed and homogenized in an isolation medium consisting of 75mM sucrose, 225 mM mannitol and 10$\mu$M EDTA, pH 7.1±0.1. After homogenizing, 0.2 ml was placed in 3 ml of reaction medium consisting of 15 mM sucrose, 45 mM mannitol, 40 mM KCl, 20 mM $MgCl_2$, 2 $\mu$M EDTA and 20 mM phosphate buffer ($K_2HPO_4$, $KH_2PO_4$), pH 7.4±0.1. Substrates used while making respiratory measurements included 1 M succinate, 1 M beta-hydroxybutyrate, 1M alpha-ketoglutarate, 0.1 M ATP and 0.05 M ADP. Respiratory measurements were made polarographically using a Clark oxygen electrode (4,5).

After endogenous respiration was established, the substrates were added: 32 $\mu$l of either succinate, beta-hydroxybutyrate, or alpha-ketoglutarate, followed by 20 $\mu$l of ATP, and finally, 10 $\mu$l of ADP. Addition of each substrate caused a change in the rate of oxygen consumption which was allowed to stabilize before the next substrate was injected.

The respiration rates of different homogenates were standardized for comparison by dividing by the nitrogen content of the homogenate (micro-Kjeldahl nitrogen analysis) thus enabling the expression of rates in terms of $\mu MO_2 \cdot sec^{-1} \cdot mg\ N^{-1}$.

## RESULTS AND DISCUSSION

Liver homogenate respiration rates of the experimental dogfish were statistically indistinguishable from the controls (Fig. 1). However, the following trends are evident: endogenous and substrate rates were increased in the experimental animals when beta-hydroxybutyrate and alpha-ketoglutarate were used as substrates while the substrate rate induced by succinate was reduced in the experimental animals. The substrate-ATP and substrate-ADP rates of the control animals were greater compared to experimental animals respiring in the presence of excess succinate and beta-hydroxybutyrate. Respiration was only slightly stimulated by the addition of ATP and little or no stimulation occurred after addition of ADP to the system.

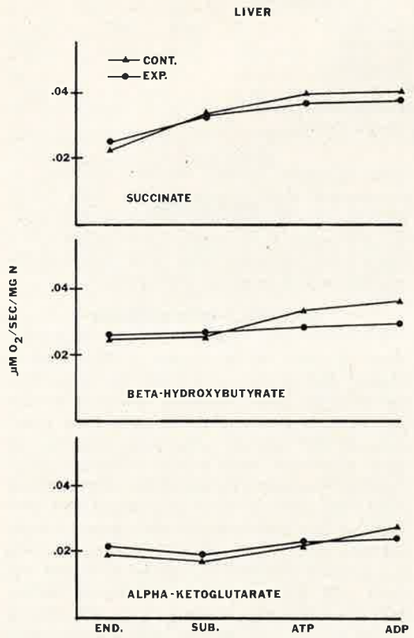



**Figure 1. Respiratory rates of liver homogenates from** Squalus acanthias.

Muscle homogenate respiration rates of the experimental animals and the controls were also statistically indistinguishable (Fig. 2). All of the respiration rates of the experimental animals tended to be reduced compared to the controls. Respiration was stimulated upon addition of ATP but no effect was found upon addition of ADP.

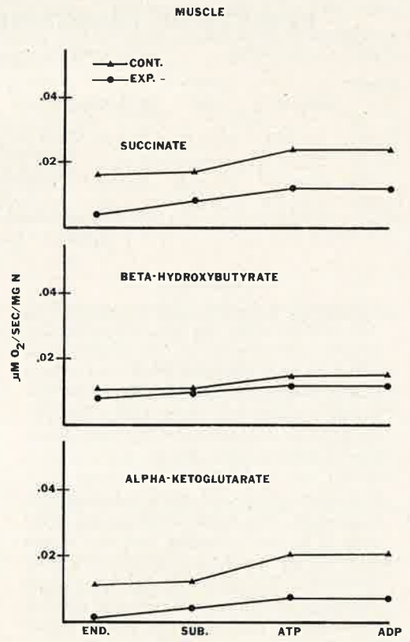



**Figure 2. Respiratory rates of muscle homogenates from** Squalus acanthias.

Respiration rates in kidney homogenates showed an opposite trend compared to that of muscle (Fig. 3). Although statistically indistinguishable, all respiratory rates of experimental animals tended to be higher than controls. Addition of succinate to the respiring system caused a considerable increase in the rate of oxygen utilization while the addition of alpha-ketoglutarate caused only a slight stimulation. Stimulation with beta-hydroxybutyrate was intermediate between the latter two. Respiration was slightly stimulated upon addition of ATP and again upon addition of ADP.

Succinate, beta-hydroxybutyrate, and alpha-ketoglutarate increase oxygen consumption when added to the respiring system, indicating a low endogenous level of the particular substrate in the homogenate. By adding excess substrate to the system, we have created a saturated dehydrogenase enzyme system thus shifting the rate-limiting factor of oxygen consumption to the endogenous concentration of ADP.



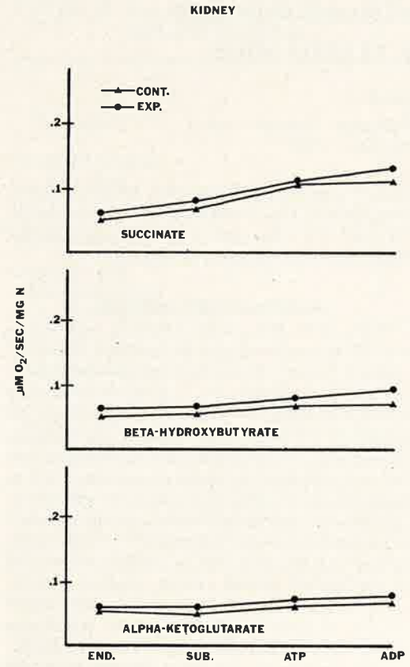



**Figure 3. Respiratory rates of kidney homogenates from** Squalus acanthias.

Stimulation of respiration upon addition of ATP was observed in all homogenates. This stimulation was caused by the presence of ATPase which caused the breakdown of ATP to ADP. The subsequent rephosphorylation of ADP to ATP was accompanied by an increased oxygen consumption. The failure of exogenous ADP to stimulate respiration in liver and muscle indicates that enough ADP was produced by ATPase activity to saturate the cytochrome system. Respiratory stimulation in kidney due to ADP indicates that respiratory rates may be controlled in an organ by the rate at which ADP is made available to the respiring system.

Increased oxygen consumption in thyroxine treated homeotherms may be explained by the fact that thyroxine is known to uncouple oxidative phosphorylation and hence lower the P:O ratio. A low P:O ratio is accompanied by an increase in oxygen consumption since more oxygen is required to produce the specific level of ATP used to satisfy basic energy requirements. Increase in oxygen consumption with a low output of ATP results in a higher net production of heat which is important in maintaining constant body temperature in homeotherms.

In teleosts, it has been demonstrated that thyroidectomy and administration of 1-thyroxine does not decrease or increase oxygen consumption (6). Since thyroxine did not produce an increase in oxygen consumption in *Squalus,* it is probable that the same thing holds true for adult elasmobranchs.

In conclusion, we have shown through the sophisticated and rather advanced techniques employed in this study that thyroxine does not have a controlling effect on oxygen consumption in *Squalus acanthias.* However, in light of previous work done with lizards, it is hoped that this study may prove instrumental in stimulating research concerning the effects of thyroxine on oxygen consumption in warm water sharks.

## REFERENCES

1. Berg, O. A. and A. Gorbman. 1954. Normal and altered thyroidal function in the domesticated goldfish, *Carassius auratus. Proc. Soc. Exptl. Biol. and Med.* 86:156.
2. Maher, M. J., and B. H. Levedahl. 1959. The effect of the thyroid gland on the oxidative metabolism in the lizard, *Anolis carolinensis. J. Exptl. Zool.* 140:169.
3. Gorbman, A. and H. A. Bern. 1962. *A Textbook of Comparative Endocrinology.* John Wiley and Sons, Inc., New York. p. 163.
4. Strickland, E. H., F. D. Ziegler and A. Anthony. 1961. Oxygen electrode for measurement of tissue slice respiration. *Nature* 191:969.
5. Munro, D. W. and A. Anthony. 1966. Respiration and phosphorylation in liver homogenates from cold-exposed chipmunks, hamsters, and rats. Research report. Physiology Laboratories, Penna. State Univ.
6. Matty, A. J. 1957. Thyroidectomy and its effect upon oxygen consumption of a teleost fish, *Pseudoscarus guacamadia. J. Endocrinol.* 15:1.

# PREVENTION OF CORTISONE-INDUCED CLEFT PALATE IN ALBINO MICE

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## ABSTRACT

The effect of riboflavin and pyridoxine on cortisone-induced cleft palate was studied in gestating Swiss ICR albino mice. The cortisone was administered intramuscularly (10 mg) on day 10 while the riboflavin and pyridoxine were given either by injection on day 10 or in the diet from day 1 through day 17. No significant reduction in the incidence of cortisone-induced cleft palate was observed when the animals received a single intramuscular injection of either riboflavin (1, 10 or 30 mg) or pyridoxine (1 or 10 mg). In contrast, the animals that received either riboflavin (6.3 to 7.9 mg/day) or pyridoxine (6.6 to 8.4 mg/day) supplemented diets exhibited a significant reduction ($P < 0.001$) of cortisone-induced cleft palate.

## INTRODUCTION

The incidence of cleft palate and harelip is about 1 in every 770 live births in the United States (1, 2). Clefts have been explained as an initial failure of segments of primary tissue to unite properly to form the face and palate. It also has been suggested that primary embryonic tissues actually unite, but that mesodermal tissues, which later penetrate the areas to form the permanent structures, do not progress far enough to close the structure when the primary tissues are resorbed (2).

Cleft palate, hamartoma of the palate and harelip have been observed in animals following administration of therapeutic agents such as ACTH (3, 4), cortisone (5, 6), triamcinolone (7), thalidomide (8, 9), insulin (10), carbutamide (11), hypervitaminosis A (12, 13), actinomycin D (14), and caffeine (15). There is also strong evidence that cortisone may induce cleft palate in man (16, 17).

Although the amount of data on drug-induced cleft palate is voluminous there is, however, a paucity of data concerning its prevention. Strean (1) observed that pyridoxine caused some reduction of the incidence of cleft palate in his cortisone-treated mice while Larsson (18) reported that pyridoxine reduced the incidence of embryonic resorption but not that of cortisone-induced cleft palate. Larsson also showed that co-administration of vitamin $B_{12}$ did not offer any protection against the effect of cortisone in A/Jax mice (18).

## MATERIALS AND METHODS

Swiss ICR albino mice weighing between 25 and 35 grams were bred in breeding boxes using a 2:1 ratio. Females were examined daily for the appearance of a vaginal mucous plug which was taken as evidence of copulation. All pregnant females were housed individually throughout the remainder of the gestating period and delivered by cesarean section prior to term on day 17. Females in the test groups received a 10 milligram intramuscular injection of cortisone acetate (calculated as base compound) on the 10th day of gestation. Females in the treated-control groups received equivolumetric (0.5 ml) injections of the vehicle (0.25% methocel). Riboflavin and pyridoxine were administered either by intramuscular injection on day 10 (1, 10 or 30 mg) or in the diet (food-drug trituration) from day 1 through day 17. Supplemented diets contained an additional one milligram of riboflavin or pyridoxine per gram of powdered diet (0.1%). The average oral daily dose of riboflavin was 6.3 milligrams between days 1 and 10 and 7.9 milligrams between days 10 and 17. The average daily dose of pyridoxine was 6.6 and 8.4 milligrams respectively. Data were evaluated statistically using a Chi-square test with Yates' Correction.

## RESULTS AND DISCUSSION

*Dietary supplementation.* Animals that received either riboflavin or pyridoxine supplemented diets throughout the gestation period exhibited a significant reduction ($P < 0.001$) of cortisone-induced cleft palate (Table 1). All three groups that received cortisone showed a significantly higher ($P < 0.001$) incidence of cleft palate, dead embryos and resorptions when compared with the treated-control group. There was no significant difference between the incidence of embryos and resorptions in the cortisone group that received only the control diet and the cortisone-treated groups whose



TABLE 1. *The effect of riboflavin and pyridoxine on cortisone-induced cleft palate, dead embryos and resorptions.*

| Treatment | Litters | Implants | Fetuses: Normal | Fetuses: Cleft Palate | Embryos and Resorptions |
|---|---|---|---|---|---|
| Treated Controls (control diet) | 10 | 111 | 104 | 0 | 7 |
| Cortisone I.M. (control diet) | 10 | 119 | 23 | 59* | 37* |
| Cortisone I.M. (Riboflavin added to control diet) | 10 | 101 | 58 | 12** | 31* |
| Cortisone I.M. (Pyridoxine added to control diet) | 9 | 98 | 38 | 28** | 32* |

Cortisone (10 mg., I.M.) was administered on day 10. The treated controls received an equivolumetric injection (0.5 ml) of the vehicle (0.25% methocel). The average oral doses of the vitamins were as follows: Riboflavin - 6.3 mg/day between days 1 and 10 and 7.9 mg/day between days 10 and 17; Pyridoxine - 6.6 and 8.4 mg/day respectively.

* $P < 0.001$ compared with treated control group.

** $P < 0.001$ compared with the cortisone treated group that received only the control diet.

There was no significant difference between the incidence of dead embryos and resorptions in the three groups that received a cortisone injection. One fetus was born dead in the cortisone treated group that received only the control diet.

diets were supplemented with riboflavin or pyridoxine (Table 1).

The average body weights of the females in the three groups that received cortisone were essentially similar on day 17 but were between 6.4 and 7.8 grams below the average of the treated-control group (Table 2). The average body weights of the live fetuses in the treated-control group and the cortisone-riboflavin the cortisone and cortisone-pyridoxine groups were approximately 0.1 grams below the average group were essentially similar while those of age of the treated-control group (Table 2).

The average daily food consumptions of all four groups were essentially similar (Table 2).

*Intramuscular administration.* There was no significant reduction in the incidence of cortisone-induced cleft palate when the gestating

TABLE 2. *Average daily food consumption and body weights.*

| Treatment* | Average Daily Food Consumption (gm/day): Days 1-10 | Average Daily Food Consumption (gm/day): Days 10-17 | Average Body Weight (grams): Maternal Day 1 | Maternal Day 10 | Maternal Day 17 | Live Fetuses |
|---|---|---|---|---|---|---|
| Treated Controls (control diet) | 6.06 | 7.68 | 30.5 | 33.3 | 45.9 | 0.69 |
| Cortisone I.M. (control diet) | 6.77 | 8.23 | 29.4 | 33.0 | 38.8 | 0.56 |
| Cortisone I.M. (Riboflavin added to control diet) | 6.28 | 7.92 | 30.1 | 33.8 | 39.5 | 0.63 |
| Cortisone I.M. (Pyridoxine added to control diet) | 6.56 | 8.44 | 30.3 | 32.4 | 38.1 | 0.57 |

* See footnotes listed under Table 1.

TABLE 3. *The effect of riboflavin and pyridoxine on cortisone-induced cleft palate, dead embryos and resorptions.*

| Treatment | Dose I.M. (mg) | Litters | Implants | Fetuses | | Embryos and Resorptions |
|---|---|---|---|---|---|---|
| | | | | Normal | Cleft Palate | |
| | | Test 1 | | | | |
| Treated Controls | Vehicle | 11 | 109 | 105 | 1 | 3 |
| Cortisone | 10 | 11 | 113 | 46 | 30* | 37* |
| Cortisone + Riboflavin | 10<br>1 | 11 | 135 | 80 | 32 | 23*** |
| Cortisone + Pyridoxine | 10<br>1 | 10 | 117 | 34 | 18 | 65** |
| | | Test 2 | | | | |
| Treated Controls | Vehicle | 15 | 143 | 128 | 3 | 12 |
| Cortisone | 10 | 10 | 97 | 30 | 13* | 54* |
| Cortisone + Riboflavin | 10<br>10 | 10 | 97 | 35 | 12 | 50* |
| Cortisone + Pyridoxine | 10<br>10 | 9 | 81 | 30 | 18 | 33* |
| | | Test 3 | | | | |
| Treated Controls | Vehicle | 10 | 111 | 108 | 0 | 3 |
| Cortisone | 10 | 10 | 111 | 62 | 38* | 11 |
| Cortisone + Riboflavin | 10<br>30 | 10 | 119 | 36 | 35 | 48** |
| Riboflavin | 30 | 5 | 54 | 52 | 0 | 2 |

* $P < 0.001$ compared with the treated control group.
** $P < 0.001$ compared with the cortisone treated group.
*** $P < 0.01$ compared with the cortisone treated group.

There was no significant difference between the incidence of cleft palate that was observed in the cortisone treated groups and the groups that received cortisone plus riboflavin or cortisone plus pyridoxine.

Dead fetuses: Test 1 - cortisone (3), cortisone plus riboflavin (1); Test 2 - control group (1); Test 3 - riboflavin group (2).

females received a single intramuscular injection of either riboflavin (1, 10 or 30 mg) or pyridoxine (1 or 10 mg) on day 10 (Table 3). The effect of riboflavin and pyridoxine on the incidence of cortisone-induced dead embryos and resorptions was variable and neither vitamin exhibited a consistent significant effect or any clear-cut dose-response relationship.

The average body weights of the females in the test groups were essentially similar to those of the treated controls on days 1 and 10. The averages ranged between 29.1 and 32.8 grams on day 1 and 32.5 and 36.7 grams on day 10. The average body weights of the females in the eight test groups that received cortisone injections were similar on day 17 (36.3 to 46.3 grams) but were between 5.4 and 12.3 grams below the averages of the treated-control groups (48.0 to 51.7 grams). The average of the group that received only a 30 mg injection of riboflavin on day 10 was similar (49.9 grams) to the average of the corresponding treated-control group (50.4 grams).

The average body weights of the live fetuses in the eight test groups that received a cortisone injection were essentially similar but were



between 0.1 and 0.3 grams below the averages of the treated-controls (0.75 to 0.80 grams). The average of the group that received only a 30 mg injection of riboflavin on day 10 was similar (0.72 grams) to the average of the corresponding treated-control group (0.75 grams).

The fact that cortisone has a wide spectrum of activity on carbohydrate, fat and protein metabolism raises the possibility that its teratogenic effect may be due to an alteration in metabolism rather than a direct action of the drug *per se*. Glucocorticoids like cortisone and hydrocortisone not only decrease glucose utilization and protein synthesis by the cells but also stimulate gluconeogensis by the liver, accelerate both mobilization of fatty acids and lipogenesis, and increase protein catabolism in the cells.

Little is known about the basic mechanism by which glucocorticoids mobilize proteins from the tissue cells. This may be due to: (a) an increase in permeability of the cells to amino acids, (b) a decrease in the active transport of amino acids into the cells, (c) an inactivation of an enzyme system that is responsible for intracellular trapping of amino acids, or (d) alterations in cellular enzyme systems which would lead to increased protein catabolism and decreased protein synthesis by the cells.

Investigators have already considered exogenous as well as endogenous causes for the appearance of cleft palate and harelip. Accidents, illness, nutrition, maternal age, vaginal bleeding, menstrual history, types of medication ingested by the gestating female, and emotional stress were considered. None of these studies really explain why these deformities occur (2). Warkany's experiments suggest that the cause may be exogenous (19, 20). For example, Kalter and Warkany (20) showed that riboflavin deficiency in rats during gestation produced skeletal defects in the young: shortening of the mandible, radius, ulna, tibia, and fibula, and various forms of syndactylism and cleft palate. Strean (1) also observed that pyridoxine caused some reduction of the incidence of cleft palate in his cortisone-treated mice while Larsson (18) reported that pyridoxine reduced the incidence of resorption but not that of cortisone induced cleft palate. Others working with thalidomide showed that all six metabolites of thalidomide identified in the urine of the dog were derivatives of glutamic acid (21). On the basis of these findings Faigle et al. (21) suggested that the teratogenic effects of thalidomide were probably a reflection of vitamin antagonism or antimetabolites, affecting glutamic acid metabolism in particular. Finally, Staples and Holtkamp (22) showed that riboflavin supplementation had some protective effects against the long bone changes induced in Dutch-Belted rabbits by thalidomide.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Strean, L. P. 1958. *The Birth of Normal Babies.* Twayne Publishers, New York. p. 30 and p. 39.
2. Westlake, H., and D. Rutherford. 1966. *Cleft Palate.* Prentice-Hall, Inc., Englewood Cliffs, N. J. pp. 4-6.
3. Fraser, F. C., H. Kalter, B. E. Walker and T. D. Fainstat. 1954. Experimental production of cleft palate with cortisone and other hormones. *J. Cell. & Comp. Physiol.* 43: Supp. 1, 237.
4. Jost, A. 1951. Sur le role de la vasopressine et de la corticostimuline dans la production experimental de lesions des extremites foetales. *Compt. rend. Soc. biol.* 145: 1805.
5. Fraser, F. C., and T. D. Fainstat. 1951. Production of congenital defects in the offspring of pregnant mice treated with cortisone. *Pediatrics* 8: 527.
6. Warburton, D., D. G. Trasler, A. Naylor, J. R. Miller and F. C. Fraser. 1962. Pitfalls in tests for teratogenicity. *Lancet* 2: 1116.
7. Walker, B. E. 1965. Cleft palate produced in mice by human-equivalent dosage with Triamcinolone. *Science* 149: 862.
8. Giroud, A., H. Tuchmann-Duplessis and L. Mercier-Parot. 1962. Thalidomide and congenital abnormalities. *Lancet* 2: 298.
9. King, C. T. G., and F. J. Kendrick. 1962. Teratogenic effects of thalidomide in Sprague-Dawley rat. *Lancet* 2: 1116.
10. Brinsmade, A., F. Buchner and H. Rubsaamen. 1956. Missbildungen an kaninchenembryo durch insulininjektion beim muttertier. *Naturwiss.* 43: 259.
11. Meyer, de R., and M. Isaac-Mathy. 1958. A propos de l'action teratogene d'un sulfamide hypoglycemiant (N-sulfanityl, N-butyluree: Bz 55). *Ann. endocrinol.* 19: 167.
12. Giroud, A., and M. Martinet. 1959. Teratogenese par hypervitaminose A chez le rat, la souris et le lapin. *Arch. franc. Pediat.* 16: 1.
13. Roux, Ch. 1961. Malformations urinaires experimentales et interpretation des ureterohydronephroses congenitales sans obstacles anatomiques. Le Francois, Paris.
14. Tuchmann-Duplessis, H., and L. Mercier-Parot. 1959. A propos de l'action teratogene de l'actinomycine. *Compt. rend. Soc. biol.* 153: 1697.
15. Nishimura, H., and K. Nakai. 1960. Congenital malformations in offspring of mice treated by caffeine. *Proc. Soc. Exper. Biol. & Med.* 104: 140.
16. Doig, R. K., and O. McK. Coltmann. 1956. Cleft palate following cortisone therapy in early pregnancy. *Lancet* 2: 730.
17. Harris, J. W., and I. P. Ross. 1956. Cortisone therapy in early pregnancy. Relation to cleft palate. *Lancet* 1: 1045.
18. Larsson, K. S. 1962. Studies on the closure of the secondary palate. V. Attempts to study the teratogenic action of cortisone in mice. *Acta. odont. scand.* 20: 1.

19. Warkany, J., and E. Schraffenberger. 1946. Congenital malformations induced in rats by maternal vitamin A deficiency. Arch. Opthalmol. 35: 150.
20. Kalter, H., and J. Warkany. 1957. Congenital malformations in inbred strains of mice induced by riboflavin deficient, galactoflavin containing diets. *J. Exp. Zool.* 136: 531.
21. Faigle, J. W., H. Keberle, W. Riess and K. Schmid. 1962. The metabolic fate of thalidomide. *Experientia* 18: 389.
22. Staples, R. E., and D. E. Holtkamp. 1963. Effects of parental thalidomide treatment on gestation and fetal development. *Expt. & Molecular Pathol.* Supp. 2: 81.



# IN VITRO METABOLISM OF AVIAN BONE OBTAINED DURING DIFFERENT STAGES OF THE EGG CYCLE[1]

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## ABSTRACT

Laying hens were fed a ration marginal in calcium (1.9% Ca) to maximize calcium resorption from bone during shell formation and calcium accretion when no shell was being formed. Oxygen uptake, lactate release and citrate release were determined on viable and heat-inactivated rib sections obtained during periods of shell and no shell formation. The only significant difference between the two periods consisted in a higher lactate release by bone cells during shell formation. Qualitatively, the *in vitro* metabolism of avian bone was similar to that of human bone. Quantitatively, avian bone was 4 to 5 times more active, although cellularity was approximately the same.

## INTRODUCTION

For many years it has been apparent that in the physiology of normal mammalian bone there is a dynamic equilibrium between two separate and opposing processes — bone resorption and bone accretion. These processes are probably mediated by cellular metabolism and its end products.

Many investigators have studied mammalian skeletal physiology by the method of short-term incubation of bone fragments for two to five hours. This method offers precise control of the medium and enables viable tissue to perform many complex metabolic functions as described by Peck and Dirksen (1). Although less durable bone cells die soon after isolation, Borle *et al.* (2) have shown that the rate of oxygen uptake by mammalian bone slices is constant up to five to six hours after the tissue has been removed. Similar results were obtained in our laboratory for avian bone slices.

Borle *et al.* (3) used pretreatment with parathyroid extract or estradiol-benzoate to induce either bone resorption or bone accretion in mice. Short term incubation of metaphyseal slices from these animals showed that lactate production was markedly increased after parathyroid extract. Citrate production was small in comparison to lactate. Differences in citrate production and oxygen utilization between resorption and accretion were not significant.

The present experiment was designed to study the *in vitro* metabolism of sliced rib samples from the laying hen obtained during different stages of the egg cycle. The laying hen was chosen as the experimental animal because it shows discrete patterns of calcium mobilization from bone during egg shell formation and calcium apposition in bone when no egg shell is being formed (4).

## MATERIALS AND METHODS

A total of 144 Single Comb White Leghorn hens were maintained on tap water and fed the ration described by Mueller *et al.* (5) beginning 2.5 to 3 weeks prior to surgery. Based on earlier work (5) it was expected that this diet, which contained 1.9% calcium, would maximize calcium turnover in bone while calcium balance was maintained. Throughout the experiment, which was carried out in the summer of 1967, artificial light was provided from 5 A.M. to 7 P.M. each day.

From the 144 hens, 14 were selected for surgery on the basis of regular egg laying patterns. Rib samples were obtained by biopsy from seven birds after an egg had been in shell gland for 13 to 17 hours (resorption period) and from seven other birds on a day when no egg was being formed (accretion period). The hens resumed a normal egg laying pattern within 4 to 8 days after the operation. They were then sacrificed during the opposite part of the egg cycle and a homologous rib sample was obtained from the other side of the bird.

The following surgical technique was used to obtain the first rib sample: The birds were anesthetized by injecting 25 mg to 75 mg of Nembutal into the right wing vein. The area around the fifth and sixth rib was cleansed, an incision was made and the two ribs were cut at the tubercules in the spinal region and

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at the interchondral articulations. The two ribs were placed immediately into chilled physiological saline. The muscle and skin layers were then sutured and the bird returned to her cage.

After connective tissue had been removed from the ribs, they were split longitudinally and the marrow was flushed out of the medullary bone trabeculae with 5 to 6 streams of cold saline from a syringe. Histological examination showed that this procedure removed about 90 per cent of the marrow from the rib samples. The ribs were then cut into approximately 1 mm thick slices. The slices were placed into cold saline, mixed, removed and blotted on filter paper. Two 50 mg (wet weight) aliquots were analyzed for DNA content by the modified method of Giles and Myers (6). Two 50 mg aliquots were heat-inactivated in double-sidearm Warburg flasks by boiling for one minute in 0.1 ml buffer. Two other aliquots received no treatment and were placed in Warburg flasks and designated as viable samples. To each of the four flasks was added 2.0 ml of calcium-free Krebs-Ringer medium, buffered with bicarbonate and a gas mixture of 5% $CO_2$ and 95% $O_2$ (pH 7.4), and 0.5 ml of a glucose solution calculated to give a final glucose concentration of 2 mg/ml. To one sidearm of each flask was added 0.5 ml 3.4 mM $HgCl_2$, to the other 0.3 ml saturated $KMnO_4$, and to the center well 0.3 ml 2M $K_4Fe(CN)_6$. After the flasks had been attached to the manometers, the air was evacuated from the system and replaced with a gas mixture containing 95% $O_2$ and 5% $CO_2$. This procedure was repeated 5 times. Incubations were carried out at 37.5°C. for two hours.

$O_2$ uptake was determined according to Cheng (7) after the tissue had been killed with $HgCl_2$ and $CO_2$ had been absorbed by the method of Dixon (8). The medium was then removed from the Warburg flasks and centrifuged at 3000 rpm. A 2.5 ml aliquot of the supernatant was added to 1.0 ml of a 20 percent trichloracetic acid solution. After centrifugation at 4500 rpm the supernatants were decanted from the precipitated protein. Citrate content was determined according to Lindenhayn and Hirthe (9), lactate content with the method of Barker and Summerson (10).

The cellular component of lactate and citrate release was calculated by subtracting the quantity released by heat-inactivated slices from the quantity released by the corresponding viable samples. The data were analyzed by paired t-test.

## RESULTS

There were no significant differences in DNA content, oxygen uptake and citrate release between periods of shell and no shell formation (Table 1). Viable samples released only about 5 percent more citrate into the medium than heat-inactivated samples. Thus, it is likely that cellular metabolism led to little accumulation of this metabolite in either period. In the case of lactate, release by viable samples was over two times greater than release by inactivated samples, indicating that a major part of this release was due to cellular activity.

On a DNA basis, bone slices obtained during shell formation released 85 times more lactate than citrate into the medium; for periods of no shell formation the ratio was 63 (Table 1). The difference in lactate production between the two parts of the egg cycle was significant at the 5% level. The last column of Table 1 shows the correlation coefficients between the values for the two different rib samples from the same bird. With the exception of DNA content and cellular citrate release, the correlation coefficients were highly significant (1% level).

TABLE 1. *Cellularity and Metabolic Activity of Bone Tissue Obtained during Periods of Shell and No Shell Formation.*

| Parameter | Shell Formation | No Shell Formation | Correlation Coefficient[1] |
|---|---|---|---|
| DNA, mg/100 mg wet bone | 0.121±0.010[2] | 0.117±0.010 | 0.504 |
| $O_2$ Uptake, $\mu$M/hr/mg DNA | 1.98 ±0.17 | 2.10 ±0.20 | 0.937[3] |
| *Citrate Release* | | | |
| Total, $\mu$M/hr/100 mg wet bone | 0.057±0.007 | 0.058±0.008 | 0.815[3] |
| Inactivated, $\mu$M/hr/100 mg wet bone | 0.055±0.006 | 0.055±0.009 | 0.655[3] |
| Cellular, $\mu$M/hr/mg DNA | 0.027±0.010 | 0.029±0.011 | 0.435 |
| *Lactate Release* | | | |
| Total, $\mu$M/hr/100 mg wet bone | 0.479±0.057 | 0.440±0.053 | 0.886[3] |
| Inactivated, $\mu$M/hr/100 mg wet bone | 0.194±0.022 | 0.202±0.030 | 0.802[3] |
| Cellular, $\mu$M/hr/mg DNA | 2.30 ±0.24 [4] | 1.84 ±0.22 | 0.815[3] |

[1] Between values obtained from the same bird during periods of shell and no shell formation.
[2] Mean ± standard error.
[3] Significant at 1% level.
[4] Significantly different (1% level) from period of no shell formation.



## CONCLUSIONS

The generally good agreement between the *in vitro* metabolism of the two rib samples, which were obtained at different times from the same bird, shows that the method developed for this experiment should be useful in studies of skeletal metabolism. The relatively low correlation for cellular citrate release may be due to the fact that average cellular citrate release was only about three times larger than the respective standard error. The comparatively low correlation for DNA content is difficult to explain.

This experiment shows that the *in vitro* metabolism of avian and mammalian bone is similar. Flanagan and Nichols (11) reported oxygen consumption rates of 0.47 and 1.98 micromoles per mg DNA per hour for bone from adult humans and 55 day old rats, respectively. Furthermore, avian like mammalian bone releases much more lactate than citrate into the medium during incubation.

Egg shell formation, like the addition of parathyroid hormone to mammalian bone cultures, increased lactate production. This may indicate that parathyroid hormone plays an important role in the mobilization of calcium from bone for egg shell formation. However, additional work is needed before the regulation of skeletal calcium mobilization and apposition in relation to the egg cycle is completely understood.

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## REFERENCES

1. Peck, W. A. and T. R. Dirkson. 1966. The metabolism of bone fragments *in vitro*. *Clin. Orthop.* 40:243.
2. Borle, A. B., N. Nichols and G. Nichols. 1960. Metabolic studies of bone *in vitro*. I. Normal Bone. *J. Biol. Chem.* 235: 1206.
3. Borle, A. B., N. Nichols and G. Nichols, Jr. 1960. Metabolic studies of bone *in vitro*. II. The metabolic patterns of accretion and resorption. *J. Biol. Chem.* 235: 1211.
4. Simkiss, K. 1967. *Calcium in Reproductive Physiology*. Reinhold Publishing Co., New York.
5. Mueller, W. J., R. Schraer and H. Schraer. 1964. Calcium metabolism and skeletal dynamics of laying pullets. *J. Nutrition* 84: 20.
6. Giles, K. W. and A. Myers. 1965. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 206: 93.
7. Cheng, P. Y. 1952. A correction in the derivation of Vogler's method for extending the reading of a Warburg manometer. *Arch. Biochem. Biophys.* 36: 489.
8. Dixon, M. 1951. *Manometric Methods*. (3rd ed.). University Press, Cambridge, England. p. 161.
9. Lindenhayn, K. and D. Hirthe. 1967. Citric acid in biological material, especially bones. *Clin. Chem. Acta.* 17: 149.
10. Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* 138: 535.
11. Flanagan, B. and G. Nichols, Jr. 1965. Metabolic studies of human bone *in vitro*. *J. Clin. Invest.* 44: 1788.

# RESPONSE OF THE INTESTINE TO A STATE OF MAGNESIUM DEFICIENCY

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## ABSTRACT

Magnesium is an essential body fluid constituent and is one of the major mineral components of the body. Deficiency of this vital electrolyte produces profound results, with the gastrointestinal tract, as an area with one of the highest mitotic activities in the body, among the first areas affected; there is a rapid decline in the activity of vital enzyme systems (e.g. ß-glycerophosphatase), blockade of mitotic activity in the villus epithelium and nutritive failure followed by decline or death of the organism. Neural hyperexcitability develops to the point of convulsions and there is the possible induction of certain brain lesions. Magnesium deficiency in man may be a clinical problem which has received insufficient attention.

## INTRODUCTION

The normal adult has approximately 20-25 grams of magnesium, 98% of which is in the cells and skeleton. The predominant intracellular position of this cation has physiological significance due to the requirement of many enzyme systems for magnesium in order to function optimally. Cardiac and skeletal muscle, as well as nervous tissue, depend on proper ionic balance for normal function. Furthermore, magnesium has been shown to antagonize the action of thyroxine in the uncoupling of oxidative phosphorylation (1-4).

The villus epithelium of the rat intestinal mucosa is a very dynamic tissue, and its cells are replaced approximately every two days. Therefore, it seems quite likely that any condition, such as an inadequacy of magnesium, which would deprive this system of its normal metabolic activity, would be a major contributing factor in the disruption of the structure and function of the gastrointestinal tract. Protection of this vital system would conversely do much to prevent the disintegration of the tract, which would ultimately result in aiding the survival of the total organism.

Previous studies in this laboratory (5) have shown that a state of magnesium deficiency results in a rapid and profound disruption of intestinal metabolism and cellular renewal capabilities of this tissue.

The present study will examine, in detail, the consequences of a condition of magnesium deficiency: to the animal; to the metabolic state of the intestine, as evidenced by specific enzyme activities; and to the morphological state of the intestine, as evidenced by structural appearance and mitotic activity. Evidence will also be presented which suggests that magnesium deficiency in man may be a substantial clinical problem, which has long been overlooked.

## METHODS

Male Wistar rats, in the weight range of 125-175 gm, were utilized for this study. All animals were housed in individual cages. Control animals were maintained on a commercial rat food (Purina Laboratory Chow). Experimental animals were maintained, for varying periods, on a magnesium deficient test diet (magnesium content $< 5$ ppm: Nutritional Biochemicals Corporation, Cleveland), or a magnesium deficient test diet which was reconstituted with magnesium. Unless otherwise stated, all animals were supplied food and water *ad libitum*. Certain animals were maintained on a fluid regimen of distilled water. Food and water intake were measured daily. Electrocardiographic readings were obtained utilizing standard limb leads and a portable EKG machine.

At predetermined time intervals, representatives of each series were selected at random and sacrificed. The proximal portion (15 cm) of the intestine was rapidly removed, thoroughly washed with cold water and stored in an ice bath. From this freshly removed portion of the duodenum, a segment about 2 cm long was immediately seperated from the rest of the tissue, fixed in formalin, to be used for histological study. A 3 cm segment was separated, prepared as previously described (5) and utilized in the determination of the % water content of the mucosa.

The remainder of the excised duodenum (10 cm) was then separated into mucosal and muscular segments as previously described (5), and the mucosal samples were homogenized



and values for alkaline phosphatase (ß-glycerophosphatase) activity were determined by the amino naphthol sulfonic acid method of Bodansky (6-8). Magnesium determinations were performed utilizing the Titan Yellow method of Spare (9).

The portion of the duodenum which had been fixed in formalin was sectioned at about approximately 5 $\mu$ and stained with hematoxylin and eosin. Sections were retained for the study of mitotic activity only if the crypts and villi were cut throughout their length.

Several animals were examined for cerebral and brain stem pathology by utilizing standard histological techniques. At the time of the sacrifice, the brains were removed, fixed in 10% formalin, frozen for serial sectioning according to the method of Marshall (10), and stained for cells with cresyl echt violet. The terminology employed was according to the atlas of König and Klippel (11).

With the aid of a statistician, the statistical significance of the data was determined utilizing either the "t" test or the analysis of variance.

## RESULTS

Chronic depletion of magnesium via a deficient test diet resulted in increased irritability of the organism, to the point of occasional convulsive seizures. Electrocardiographic readings revealed an increased irritability in cardiac tissue as evidenced by a decreased PR segment and an increased Q wave. Food intake was depressed and after two weeks, a chronic depression in growth commenced, so that the animals on the deficient diet for two months were 30-35% below the weight of the control animals (Fig. 1).

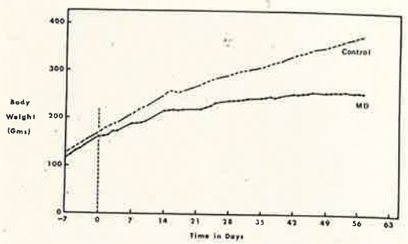



**Figure 1. Effect of Magnesium Deficiency on the Growth Patterns of Young Male Wistar Rats.**

After remaining on the deficient diet for 5-10 days, there arose a distinctive redness about the ears, around the eyes and the back of the head. By 2-3 weeks, there developed a characteristic shagginess of the hair, loss of hair, and development of lesions on the back of the head, in the area of the eyes, ears and neck.

Within 24 hours after the start of the magnesium deficient regimen (m.d.r.), serum magnesium levels fell in excess of 50% (2 mg% to 0.9 mg%) and remained depressed throughout the three month test period. Mucosal magnesium levels exhibited a similar, though less drastic decline (30%: 0.5 mg% to 0.35 mg%).

There was an almost immediate decline in the activity of intestinal ß-glycerophosphatase which persisted for up to three months (40%: 9.5 mg% $P_i$ to 6 mg% $P_i$). From the second day of the m.d.r., the activity of the serum ß-glycerophosphatase was depressed and remained at a lower level throughout the three

TABLE 1. *The Effect of Magnesium Deficiency on the Mitotic Activity of the Rat Duodenum.*

| | No. of Animals | | % Prophase | | % Metaphase | | % Anaphase | | % Telophase | | Total % Mitosis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | MD | C | MD | C | MD | C | MD | C | MD | C | MD |
| Day 1 | 8 | 18 | 3.7 | 1.3 | 5.8 | 3.7 | 1.7 | 1.1 | 0.6 | 0.1 | 11.8 | 6.2 |
| Day 2 | 7 | 8 | 3.7 | 1.9 | 5.7 | 3.9 | 1.5 | 1.4 | 0.7 | 0.1 | 11.7 | 7.2 |
| Day 3 | 7 | 9 | 2.8 | 0.9 | 4.8 | 3.6 | 1.9 | 1.6 | 0.6 | 0.2 | 10.2 | 6.4 |
| Day 4 | 6 | 7 | 2.6 | 2.0 | 5.8 | 5.0 | 2.2 | 1.7 | 0.8 | 0.3 | 11.3 | 9.2 |
| Day 7 | 6 | 7 | 2.8 | 1.3 | 5.5 | 4.2 | 2.0 | 2.2 | 1.0 | 0.4 | 11.2 | 8.3 |
| Day 10 | 6 | 7 | 1.9 | 0.8 | 5.4 | 4.0 | 2.5 | 1.2 | 0.6 | 0.3 | 10.2 | 6.3 |
| Day 14 | 9 | 7 | 2.3 | 1.1 | 6.4 | 4.3 | 1.8 | 1.1 | 0.6 | 0.1 | 11.1 | 6.6 |
| Day 21 | 4 | 6 | 3.0 | 1.3 | 5.0 | 3.9 | 2.2 | 1.6 | 0.7 | 0.2 | 10.7 | 7.0 |
| Day 29 | 9 | 9 | 2.1 | 0.9 | 6.5 | 4.4 | 2.0 | 1.5 | 0.6 | 0.2 | 11.0 | 7.0 |
| Day 49-77 | 7 | 6 | 2.0 | 0.9 | 6.0 | 4.0 | 1.8 | 1.0 | 0.5 | 0.1 | 10.3 | 6.0 |

C = control series; MD = magnesium deficient series.

Analysis of variance shows that magnesium deficiency is accompanied by a highly significant ($P < 0.001$) reduction in all phases of the mitotic cycle.

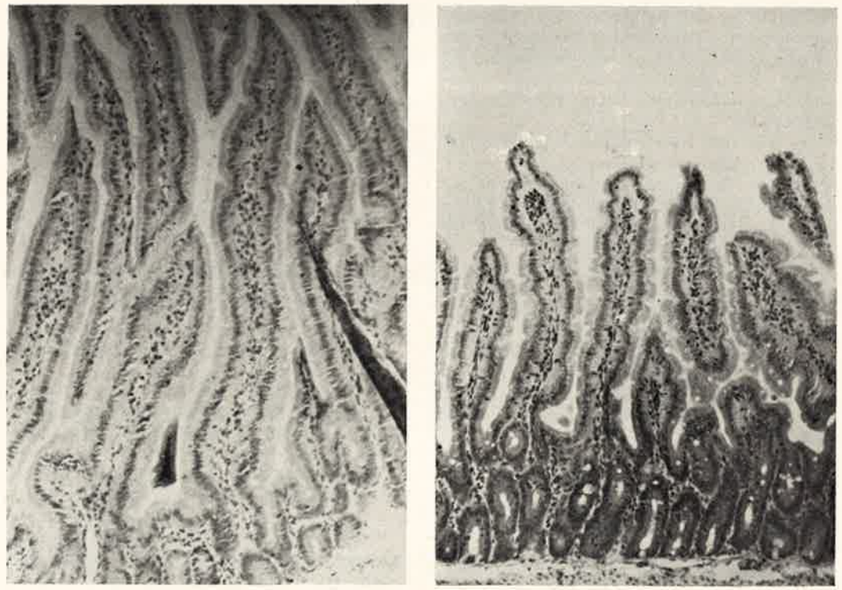


**Figure 2. Effect of Magnesium Deficiency on Villus Height of the Rat Duodenum. Left: control series; right: magnesium deficient series. Both stained with hematoxylin and eosin. Magnification: 150X.**

month test period. However, this depression was of borderline significance.

There was a highly significant reduction in the percentage of mucosal cells exhibiting mitotic activity as early as the first day of the m.d.r. (Table 1). Similar reductions were seen in all phases of the mitotic cycle. This low level of mitotic activity was maintained throughout the three month test period. In keeping with the reduced mitotic activity of the tissue, there was a concomitant reduction in the size of the individual villi of the small bowel (Fig. 2), as well as in the number of cells found in each villus (Table 2).

None of the external, biochemical or morphological signs of a state of magnesium deficiency was seen in animals which were placed on a magnesium deficient test diet which had previously been reconstituted with magnesium to the level of standard chow (0.25%). Similarly, magnesium deficient animals immediately lost all signs of the deficient state when placed on the reconstituted diet.

When animals were maintained on a fluid regimen of distilled water — rather than tap water — the signs of a state of magnesium deficiency were identical, with the exception that the depressions in intestinal ß-glycerophosphatase activity and in cellular renewal of the intestinal mucosa averaged about 10% greater in severity. However, whereas maintenance of a state of magnesium deficiency for a period of three months produced rare fatalities, deficient animals which were also supplied with distilled water, frequently expired as early as the first week of the m.d.r. (12).

In collaboration with Dr. Eugene J. Zawoiski, Assistant Professor of Physiology, Jefferson Medical College, several animals were examined in a preliminary search for cerebral and brain stem pathology. None of the examined animals (three) which were on the magnesium deficient diet and tap water showed any central pathology, even though some exhibited convulsions and a loss of the righting reflex. Conversely, all of the examined animals (three) which were on the deficient diet and distilled water showed hemorrhagic loci. All of these animals had experienced violent convulsive seizures. Areas of involvement included the recessus pinealis, the nuclei of the lateral and medial habenule, pretectalis nucleus, nucleus tractus optici, the stratum griseum superficale, colliculi superioris, formatio reticularis, tractus tectospinalis and the nucleus ventralis tegmenti.



TABLE 2. *The Effect of Magnesium Deficiency on the Height and Cell Number of the Villi of the Rat Duodenum.*

| | No. of Animals | | Villus Height(μ) | | Cells per Villus | |
|---|---|---|---|---|---|---|
| | C | MD | C | MD | C | MD |
| Day 1 | 8 | 18 | 5.6 | 5.0 | 156 | 135 |
| Day 2 | 7 | 8 | 5.4 | 4.8 | 123 | 117 |
| Day 3 | 7 | 9 | 5.6 | 4.8 | 132 | 120 |
| Day 4 | 6 | 7 | 5.7 | 4.8 | 136 | 115 |
| Day 7 | 6 | 7 | 5.6 | 4.7 | 134 | 117 |
| Day 10 | 6 | 7 | 5.9 | 4.9 | 147 | 124 |
| Day 14 | 9 | 7 | 5.4 | 4.4 | 130 | 114 |
| Day 21 | 4 | 6 | 5.1 | 4.4 | 117 | 120 |
| Day 29 | 9 | 9 | 5.4 | 4.3 | 135 | 100 |
| Day 49-77 | 7 | 6 | 5.0 | 4.5 | 126 | 109 |

C = control series; MD = magnesium deficient series.

## DISCUSSION

We have shown that chronic restriction of dietary magnesium results in two distinct manifestations. One is a nutritional disturbance which results in a cessation of growth in young adult rats which had been in a dynamic growth phase. This manifestation develops after maintenance of the magnesium deficient regimen (m.d.r.) for a period of two weeks. The second mainfestation is neural, and is characterized by vasodilation, hyperexcitability, and if of sufficient severity, convulsions. This dual nature of the signs of a state of magnesium deficiency was seen as early as 1932 by Kruse, Orient and McCollum (13).

It is of extreme interest to note the rapidity with which manifestations of a magnesium deficiency appear. Whereas our data show a highly significant decline in serum magnesium within 24 hours (to below 1 mg%), Tufts and Greenberg (14) report that it took up to 7 days for their 40-100 gram rats to fall to this low level of magnesium. Furthermore, it took at least two weeks for the magnesium level to approach 1 mg% in the chronic dogs of Kruse *et al.* (13); 1 mg% magnesium was a point below which the dogs could not survive. A large number of our deficient rats had a serum magnesium content of below 1 mg% (0.7 to 0.9 mg%) throughout the three month test period, with rare fatalities. While the level of serum magnesium dropped precipitously within the first 24 hours of the m.d.r., magnesium was maintained at these low levels, and did not progressively decline. The magnesium content of the intestinal mucosa was significantly reduced throughout the three month test period.

Concomitant with the rapid fall in the mucosal magnesium, there was a similar decline in the activity of intestinal ß-glycerophosphatase. The parallelism between the level of mucosal magnesium and intestinal phosphatase activity can be explained by the fact that magnesium is a vital cofactor which is required for the adequate activity of this enzyme. Reduction in magnesium content therefore negates part of the optimum condition for enzyme function, thereby resulting in lowered activity. Intestinal phosphatase activity remained depressed for up to three months, as did the level of mucosal magnesium.

The activity of serum ß-glycerophosphatase did not exhibit the dramatic alterations in activity which were seen in the intestinal enzyme. Serum phosphatase levels were consistently lower than their corresponding control values, but rarely were these results shown to be significant. This may merely reflect the fact that there is a certain minimal level of magnesium which is required for adequate phosphatase activity. Since the intestinal mucosa contains one-fourth the concentration of magnesium which is found in the serum (0.5 mg% vs. 2.0 mg%), it may therefore follow that the intestinal system will respond much more readily to a deficiency state.

The ability of the intestinal mucosa to maintain itself via its normal rate of mitosis was also severly affected. These highly significant declines in the mitotic activity of the mucosa closely paralleled alterations in tissue magnsium content and phosphatase activity. As a further consequence of the state of magnesium deficiency and of the sharply reduced mitotic

activity of the intestinal mucosa, the size of the intestinal villi was markedly reduced, as was the average number of cells found in each villus. Villus height was significantly shortened by the third day of the m.d.r. and remained so. The reduction in villus size, plus loss of phosphatase activity must certainly have hindered the absorptive capacity of the small intestine, thereby contributing to the onset of the nutritive failure seen in magnesium deficiency.

Cerebral tissue was examined in order to ascertain whether cerebral and/or brain stem pathology could be a possible explanation for the appearance of convulsive states during a period of magnesium deficiency — an additional explanation to the currently accepted theory of neural hyperexcitability. Preliminary examination of a limited number of brains suggested that there may have been an induction of central lesions when deficient animals were also given distilled water. Final clarification of this point must await additional study.

It would appear that our supply of tap water contains sufficient magnesium (ca. 0.4 mg%) to just keep the animals alive and to prevent the possible induction of central lesions, but an insufficient amount of magnesium to prevent the described external, biochemical and histological signs of a state of magnesium deficiency.

The results herein presented should not be viewed as isolated data in a non-human system. There is a growing body of information which strongly points to magnesium as a significant entity in numerous clinical conditions in man. Caddell (15) has made extensive studies of protein — calorie malnutrition in childhood (kwashiorkor) and suggests the urgent need for magnesium as a therapeutic agent. The role of magnesium in enzyme systems such as alkaline phosphatase and in vitamin B coenzyme synthesis are given as possible explanations of many of the manifestations of kwashiorkor.

Wacker *et al.* (16, 17) have reviewed many neuromuscular disorders in man which have the features of experimental magnesium deficiency as seen in animals. These include idiopathic epilepsy and eclampsia, as well as conditions such as pregnancy, chronic nephritis and acute pancreatitis, in which either muscular twitching or convulsions are present. Wacker (18) has also described a normocalcemic magnesium deficiency tetany syndrome in man while Ertel *et al.* (19) have recently described a patient with hypomagnesemia in neonatal tetany associated with maternal hyperparathyrodism. As detection techniques continue to improve, the importance of magnesium in many nutritional and neural imbalances will certainly be recognized.

These data support the belief, which we have previously proposed (20-23, 5) and which is supported by such current studies as those of Ferguson *et al.* (24) and Poka *et al.* (25), that the intestinal mucosa may serve as a biological dosimeter to monitor the body's response to various deleterious conditions.

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## REFERENCES

1. Bain, J. A. 1954. The effect of magnesium upon thyroxine inhibition of phosphorylation. *J. Pharmacology.* 100:2.
2. Tapley, D. F. and C. Cooper. 1956. The effect of thyroxine and related compounds on oxidative phosphorylation. *J. Biol. Chem.* 222:341.
3. Kleiber, M., M. D. D. Boulter and D. M. Greenberg. 1941. Fasting catabolism and food utilization of magnesium deficient rats. *J. Nutrition.* 21:363.
4. Corradino, R. A. and H. E. Parker. 1962. Magnesium and thyroid function in the rat. *J. Nutrition.* 77:455.
5. Rosenfeld, L. M. 1968. Response of intestinal metabolism and cellular renewal systems to varying experimental conditions. *Proc. Pa. Acad. Sci.* 42:206.
6. Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66:375.
7. Bodansky, A. 1933. Phosphatase studies, II. Determination of serum of phosphatase. Factors influencing the accuracy of the determination. *J. Biol. Chem.* 101:93.
8. Kaplan, S. A. and F. T. del Carman. 1956. Quantitative ultramicroanalysis for the clinical laboratory. *Pediatrics.* 14:857.
9. Spare, P. D. 1962. A study of the titan yellow dye lake methods for the estimation of serum magnesium. *Tech. Bull. Reg. Med. Tech.* 32:14.
10. Marshall, W. H. 1940. An application of the frozen sectioning techniques for cutting serial sections through the brain. *Stain Technol.* 15:133.
11. Konig, J. F. R. and R. A. Klippel. 1963. *The Rat Brain.* A stereotaxic atlas of the forebrain and lower parts of the brain stem. The Williams and Wilkins Co., Baltimore.
12. Rosenfeld, L. M. in preparation.
13. Kruse, H. D., E. R. Orient and E. B. McCollum. 1932. Studies on magnesium deficiency in animals, I. Symptomatology resulting from magnesium deprivation. *J. Biol. Chem.* 96:519.
14. Tufts, E. V. and D. M. Greenberg. 1937. The biochemistry of magnesium deficiency, I. Chemical changes resulting from magnesium deprivation. *J. Biol. Chem.* 122:693.
15. Caddell, J. L. 1965. Magnesium in the therapy of protein-caloric malnutrition of childhood. *J. of Pediatrics.* 66:392.
16. Wacker, W. E. C. and B. L. Vallee. 1958. Magnesium metabolism. *New Eng. J. of Med.* 259:431, 475.
17. Wacker, W. E. C. and A. F. Parisi. 1968. Magnesium metabolism. *New Eng. J. of Med.* 278:658, 712, 772.
18. Wacker, W. E. C., F. D. Moore, D. D. Ulmer and B. L. Vallee. 1962. Normocalcemic magnesium deficient tetany. *J. A. M. A.* 180:161.
19. Ertel, N. H., J. S. Reiss and G. Spergel. 1969. Hypomagnesemia in neonatal tetany with maternal hyperparathyroid. *New Eng. J. of Med.* 280:260.
20. Rosenfeld, L. M. 1963. Influence of total body radiation in systemic and intestinal alkaline phosphatase levels in the rat. *Amer. J. Med. Sci.* 246:115.



21. Rosenfeld, L. M., M. H. F. Friedman, and S. Kramer. 1963. Influence of total body radiation on alkaline phosphatase levels in the rat. *The Physiologist.* 6:266.
22. Rosenfeld, L. M. and M. H. F. Friedman. 1964. Intestinal and serum alkaline phosphatase levels in the rat following ionizing radiation. *The Physiologist.* 7:238.
23. Rosenfeld, L. M. 1967. Radiation and the cell: A Review. *Proc. Pa. Acad. Sci.* 41:166.
24. Ferguson, A., W. C. Watson, J. D. Maxwell and G. S. Fell. 1968. Alkaline phosphatase levels in normal and diseased small bowel. *Gut.* 9:96.
25. Poka, L., G. Czirbusz, E. Foldi, A. Farkas, J. Kerner and I. Bartek. Changes in structure and enzyme activity in malabsorption during gastrointestinal paresis. 1968. *Digestion.* 1:33.

# TREMATODES AND AQUATIC INSECTS: MEANS OF ASSESSING PARASITE INVASION AND HOST REACTION

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## ABSTRACT

In comparison with gross dissection of experimentally infected arthropods, study of serial sections of insects fixed at various time intervals after infection permits more-accurate recovery data and allows for precise observation of routes of trematode entry and effects of hemocytes on the parasites. Giemsa's stain is used for general morphological observations; histochemical tests (PAS, Alcian blue, ferrous iron retention) are applied to alternate slides and facilitate study of the deposition of parasite secretions and the extent of the melanizing response.

The host reaction within a given insect can be expressed quantitatively by grading depths of encapsulating hemocyte layers and the extent of the melanization reaction about each parasite and arriving at indices of hemocyte activity or melanization which reflect total antiparasite activity within an individual host. This permits statistical evaluation of the effects of blocking agents and of superinfection on the host response.

Hemocyte-counting techniques and electrophoretic study of hemolymph proteins are useful in assessing the capacity of insects to deal with trematode parasites and in studying the effects of infection on the physiological state of the insect.

## INTRODUCTION

Members of at least twelve families of digenetic trematodes utilize arthropods as second intermediate hosts (1,2,3,4). Salt (5) included trematodes in his discussion of host reactions of insects to metazoan parasites, and showed that the encysted metacercariae became encapsulated by insect hemocytes and that melanin may be deposited within the host capsule. Burns (6), Hall and Groves (7), and Hall et al. (8) reported on penetration of aquatic insects by trematode larvae and on mechanisms of the insect defense. The last authors compared tissue damage and host reactions elicited by trematodes in normal versus unusual insect intermediate hosts.

When relying extensively on gross dissection of experimentally infected insects, information pertaining to route of entry and development of the host reaction is frequently overlooked. Methods of preparing serial sections and of recording and analyzing data are reviewed in the present paper. Techniques for counting insect blood cells and for electrophoretic study of hemolymph protein fractions in infected and uninfected insects are also discussed.

## METHODS, RESULTS, AND DISCUSSION

1. *Exposure of Insects.* Cercariae used in infection experiments were obtained by shedding from infections in river snails *(Nitocris dilatatus)* which had been collected from natural habitats in the Cheat River system of West Virginia. Most of the experiments have been performed with two types of stylet cercariae: virgulate xiphidiocercariae of several species (7); or a microcotylocercous cercaria tentatively identified as the larva of *Plagioporus lepomis* (4). Larval and nymphal insects were taken from smaller streams which were not infested with stylet cercariae of these types.

Insects were placed individually in Stender dishes containing 50 to 450 cercariae, depending on cercarial type and the size of the insect. In preliminary experiments, arthropods were exposed to different cercarial species and were examined by gross dissection and teasing 3 to 72 hours after initial exposure to determine the extent of penetration and the presence or absence of metacercarial cyst membranes (7,9). In cases where cercariae entered arthropods consistently and in moderately large numbers, infection experiments were repeated, and insects were fixed in Bouin's or Carnoy's fluid at 55 to 70°C at intervals ranging from 10 minutes to 384 days post-infection. Fixation was allowed to proceed for 24 to 48 hours. In recent work, the cuticle of large insects has been punctured with Minuten pins immediately after killing, and the first 60 minutes of the fixation process has been performed *in vacuo* at 60°C (8). In the case of longer time periods, some insects were killed and examined by gross dissection or by teasing. Exposure of insects was performed at 15-17°C or at room temperature (22°C). Insects killed after 15 hours were held at 12 to 22°C.



2. *Histological Techniques.* Bouin's-fixed material was washed in 50% ethanol and stored in 70% ethanol; Carnoy's-fixed material was stored in 70% ethanol. Prior to further processing, larger insects were cut transversely into two sections (i.e., headthorax and abdomen) (8), and those exhibiting heavy sclerotization were immersed in a solution of 4% phenol in 80% ethanol for 24 to 48 hours prior to dehydration (10). Dehydration was accomplished in a butanol-ethanol series (11). Whole or cut insects were embedded in Fisher Tissue Mat or in Spectrum Paraplast, and transverse or sagittal sections were cut at 8 to 10 $\mu$ with an American Optical model 820 microtome. Originally slides were stained with Giemsa's stain (12), thionin (13), or by the periodic acid-Schiff's (PAS) procedure (14). Later, alternate slides were stained in Giemsa's stain, Alcian blue or toluidine blue 0 (15), PAS, PAS with diastase digestion, or with Lillie's ferrous iron technique for melanin (16) in order to obtain further information regarding deposition of formed parasite secretions, and melanization of the parasite by the encapsulating hemocytes.

3. *Observations and Tabulation of Data.* Sections of the insects were searched thoroughly. In many instances, parasites were fixed in process of entry, permitting precise observation of route and means of penetration (7,8). In addition, data pertaining to attachment, position of parasite within host, damage to host tissues, host reaction to the parasite and its effect on parasite morphology, parasite secretion, host reaction to parasite secretion, and formation and repair of entry wounds was recorded for each parasite and tabulated for each insect. This permitted study of the distribution of the trematode larvae at various times after infection, and made it possible to determine the extent of the damage caused by the parasites and the extent of the host reaction (8).

A listing of host-parasite combinations studied to date is given in Table 1. Figures for recovery of trematodes were considerably higher than those obtained by gross dissection techniques, even though insects were infected and examined or fixed for sectioning at the same time (8). The numbers of parasites completing entry and the extent of the host reaction varied considerably with the host-parasite combination (Table 1). Entry was usually completed by 8 hours after exposure, and in those host-parasite systems where host responses were common, the hemocyte encapsulating process was evident at this time.

In host-parasite systems where large numbers of worms entered and the host response was seen about relatively small numbers of metacercariae, there was usually growth of the parasites. This seems to represent a normal host-parasite relationship. These conditions were observed in combinations involving *Cercaria stenodorya* in *Acroneuria* naiads; *C. tremaglandis* in two species of *Isonychia* naiads; and *C. polypyreta* in *Hexagenia* naiads (Table 1). Combinations where relatively few parasites entered, or where large numbers of parasites were killed by the host encapsulating and melanizing mechanisms were indicative of abnormal parasite-host relationships and are represented by *C. tremaglandis-Hexagenia* and *P. lepomis-Tendipes* systems (Table 1, Figs. 2-4). The host-parasite relationships involving *C. stenodorya* in *Isogenus* naiads, and *C. tremaglandis* in *Acroneuria* naiads, represent intermediate situations. In these last two instances, the trematodes completed entry in moderate numbers; some of them were trapped in the cuticle or epidermis, or were killed by the host response, while others remained viable and exhibited growth and/or varying degrees of internal differentiation.

Damage caused by the parasites was usually slight, and limited to formation of entry wounds and to displacement or lysis of fat body tissue (8). However, in the *C. polypyreta-Hexagenia* system, the gonadal and muscle tissues were frequently damaged by the parasites (Fig. 5,6), and there was evidence for increased insect mortality in heavy infections.

4. *Quantitative Expression of the Host Reaction.* In two experiments involving *C. tremaglandis* in *Hexagenia naiads,* the antiparasite (hemocyte and melanizing) activity was expressed semiquantitatively in order to obtain indices which would permit statistical evaluation of differences in host response following injection of phenylthiourea, a tyrosinase inhibitor, or varying with the intensity of the worm burden. The reaction about each parasite within an insect was studied and the following information was recorded and tabulated: **1.** thickness of the host capsule, including range and mean values in microns; **2.** general constituency of the capsule—i.e., relative amounts of hemocytes, fiber-like elements, and melanin were expressed semi-quantitatively using a scale which ranged from — to 4+; **3.** maximum thickness of the melanin layer in mi-

TABLE 1. *Histological Studies of Trematode Infections in Aquatic Insects*

| Parasite | Host | No. of Insects Examined | Dose | Time Range Post-infection | Worms recovered after 8 hr | Percent of worms with host response after 8 hr | Nature of Host Response | Remarks |
|---|---|---|---|---|---|---|---|---|
| Virgulate cerceriae | | | | | | | | |
| *Cercaria stenodorya* | *Acroneuria carolinensis* (Plecoptera) | 18 | 300-400 | 1 hr to 384 days | 52-305 (avg = 202.9) | 0.7 | Some hemocyte accumulation; occas. melanization of trapped worms. | Worm remains unencysted; exhibits growth and development. |
| *C. stenodorya* | *Isogenus hastatus* (Plecoptera) | 18 | 300-400 | 1 hr to 23 days | 3-47 (avg = 22.6) | 12.0 | Some hemocyte accumulation. | Worm remains unencysted; exhibits some differentiation. |
| *C. tremaglandis* | *Isonychia* sp a (*I. sericata?*) (Ephemeroptera) | 8 | 280-354 | 1 hr to 24 hr | 120-144 (avg = 134.0) | 1.0 | Slight hemocyte accumulation. | Worm encysts. |
| *C. tremaglandis* | *Isonychia* sp b (*I. annulata?*) | 26 | 200-302 | 1 hr to 10 days | 11-120 (avg = 39.6) | 0.8 | Hemocyte encapsulation rarely observed. | Worm encysts; exhibits growth and development. |
| *C. tremaglandis* | *Hexagenia recurvata* (Ephemeroptera) | 24 | 300-369 | 1 hr to 60 days | 0-205 (avg = 52.6) | 73.0 | Hemocyte encapsulation and melanization very common. | Worm encysts; is killed by host response. |
| *C. tremaglandis* | *Acroneuria carolinensis* | 12 | 300-400 | 1 hr to 42 days | 1-65 (avg = 16.8) | 33.6 | Encapsulation & melanization occurs about worms in hemocoel. | Worm encysts; those in hemocoel are killed; worms in fat body develop. |
| *C. polypyreta* | *Hexagenia recurvata* | 15 | 150-350 | 1 hr to 15 days | 29-170 (avg = 91) | 5.0 | Hemocyte accumulation & melanization after 7 days. | Worm encysts; some growth, development. |
| Microcotylocercous cercariae | | | | | | | | |
| Cercaria of *Plagioporus lepomis* | *Tendipes attenuatus* (Diptera) | 35 | 20-100 | 10 min to 25 days | 2-34 (avg = 11) | 57.6 | Hemolymph coagulation, melanization very common. | Worm encysts; no development; most parasites are killed. |



crons; **4.** condition of the parasite—i.e., living or dead, based on morphological integrity and staining reaction; and **5.** extent to which the parasite was infiltrated with melanin. Numerical values of 0.5, 1.0, 2.0, 3.0 or 4.0 were assigned to each cyst to express the relative numbers of encapsulating hemocytes, and similar values were used to express the depth of the melanin layer. Separate indices of hemocyte activity and melanization were then obtained for each insect by 1.) multiplying the total number of observations in each numerical category (0.5 to 4.0) by the respective numerical value; 2.) adding the products obtained in step 1; and 3.) dividing the sum of the products by the total number of metacercarie within the insects.. *Hexagenia* naiads used in these two experiments were exposed to 50 to 450 cercariae each and were fixed 48 to 52 hours after the initial time of exposure. Intensity of infection ranged from 2 to 202 worms per insect. Melanization indices ranged from 0.00 to 2.77 and indices of hemocyte activity from 0.50 to 2.50.

In one of the experiments, 9 experimental naiads 15 to 20 mm long were injected with phenylthiourea crystals 1 to 4 hours prior to exposure to 300-400 cercariae. Four of the nine control naiads were left untreated, and five were sham-injected with saline prior to individual exposure to similar numbers of cercariae. There was no evidence of inhibitory effect of phenylthiourea on the melanization of encapsulated parasites at 48 hours. However, the extent of the host reaction was observed to vary inversely with the numbers of parasites which had entered the insects. This negative correlation was statistically significant ($p < .05$) using either hemocyte activity or melanization indices to express the intensity of the host reaction. Figure 1 shows the inverse relationship between the melanization index and the number of metacercariae within a mayfly naiad.

In an attempt to verify this relationship, a second experiment was performed without phenylthiourea treatment of the *Hexagenia* naiads. Nineteen insects were exposed to graded doses of cercariae (50, 150, 300, or 450 cercariae per insect) and were fixed 50 to 52 hours after initial time of exposure. In this group, the numbers of parasites within an insect showed a slight negative correlation with the melanization index; this relationship was not significant at the 5-percent level. However, the negative correlation between numbers of parasites and the index of hemocyte activity was significant ($p < .02$).

These results are similar to results obtained

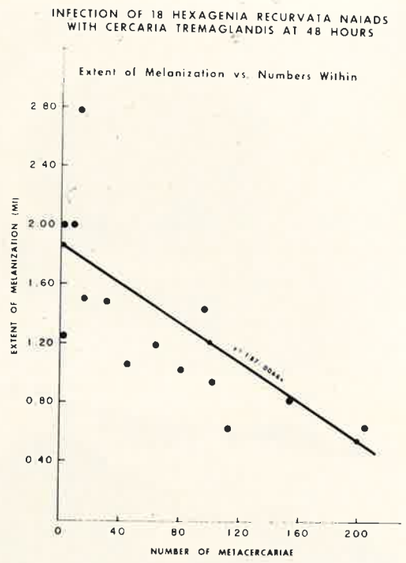



**Figure 1.**

by others who have investigated the response of mosquito larvae to rhabditoid nematodes (17), and suggest that the ability of an insect to respond to helminth parasites is limited.

5. *Hemocyte Studies.* Since quantitation of host response data indicated that the ability of the host to react to the parasites was limited, numbers of circulating hemocytes were studied in two host-parasite combinations: *Cercaria tremaglandis* in *Acroneuria carolinensis* naiads, and *C. tremaglandis* in naiads of *Hexagenia recurvata*. In each instance, naiads were exposed to 300 to 400 cercariae each for periods of 3 to 6 hours and were bled 6 or 24 hours after exposure. Uninfected (control) naiads were subjected to similar conditions and were bled, and the hemolymph was processed in an identical manner. Insects were removed from the water, dried, and bled through an incision at the base of one of the thoracic legs. Hemolymph was collected in a white blood cell pipette designed for use with small mammals,[2] diluted 1:19 with 2% disodium ethylenediamine tetra-acetic acid (disodium EDTA), and the cells were counted using standard proce-

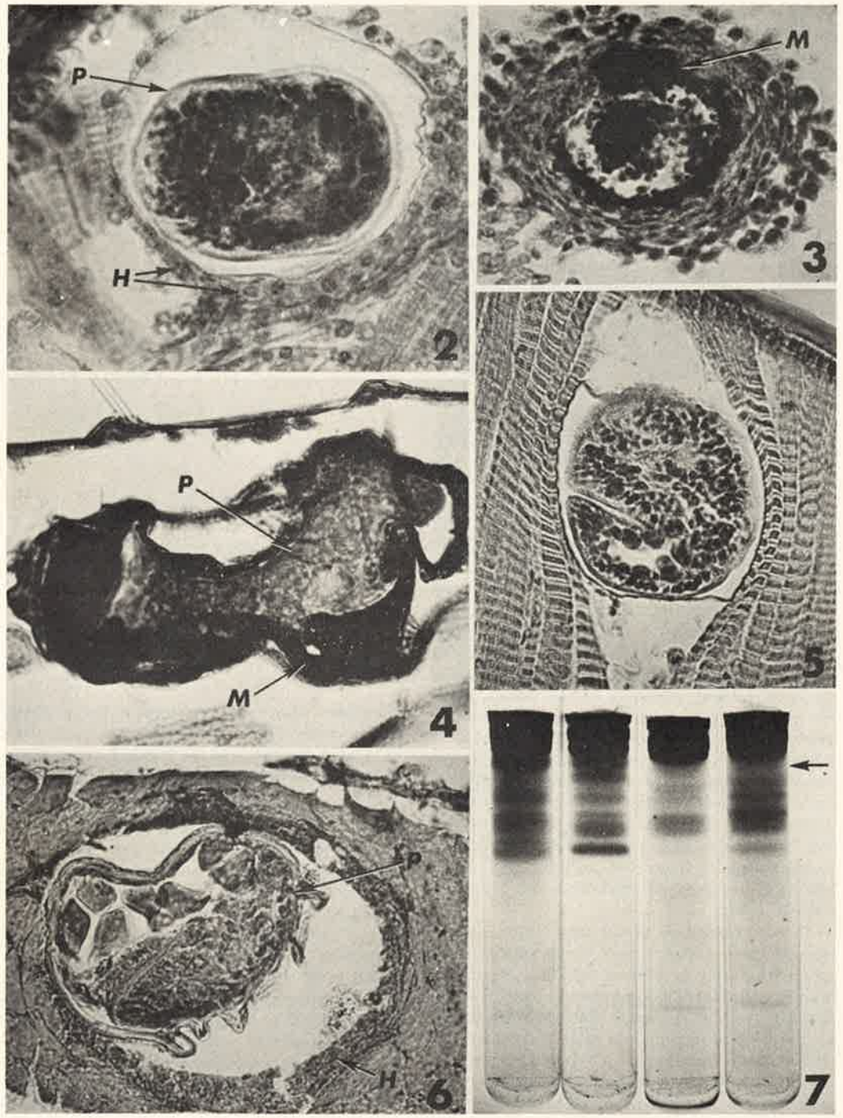



**Figure 2. Infection of** Hexagenia recurvata **naiad with** Cercaria tremaglandis; **early stage of host response, 15 hours post-infection. Note encapsulation of metacercaria (P) with hemocytes (H). Giemsa's stain, 700 X.**

**Figure 3. Infection of** H. recurvata **naiad with** C. tremaglandis; **later stage of host response, 48 hours post-infection. A layer of melanin (M) is seen beneath the encapsulating hemocytes and has begun to infiltrate the dead parasite. Giemsa's stain, 550 X.**



**Figure 4. Infection of** Tendipes attenuatus **with larvae of** Plagioporus lepomis **20 days after exposure. The dead parasite (P) is surrounded by a layer of melanin (M). PAS reaction with light green counterstain, 450 X.**

**Figure 5. Infection of** H. recurvata **with** *C. polypreta* **48 hours after exposure. Note displacement and atrophy of muscle fibers adjacent to the encysted parasite. Alcian blue with Van Giesan's counterstain, 450 X.**

**Figure 6. Infection of** H. recurvata **with** C. polypyreta **15 days after exposure. Note displacement of muscle fibers by parasite (P) and encapsulating hemocytes (H). Alcian blue with Van Giesan's counterstain, 450 X.**

**Figure 7. Acrylamide gel electrophoresis of** Sialis **larval hemolymph 24 hours after exposure to** P. lepomis **cercariae. Hemolymph of uninfected naiads is seen in the two columns at the left; Hemolymph from insects containing 8 to 94 (avg = 54) metacercariae is fractionated in the two columns at the right. The arrow points to fractions which consistently contain less protein in infected insects. Proteins are stained with buffalo blue-black B.**

dures (18). Total hemocyte counts for 19 *Acroneuria* naiads bled at 6 hours, and for 10 naiads bled for 24 hours post-infection ranged from 8,700 to 32,600 cells per cubic mm of hemolymph and did not differ significantly from counts for 29 control naiads (10,000 to 25,300 cells/mm$^3$; $p > .05$) (8). However, counts obtained with 30 *Hexagenia* naiads were depressed at 6 and 24 hours after infection and differed significantly from those of control naiads. Counts for 20 experimental naiads, 24 hours after infection ranged from 2,100 to 23,200 (avg = 9,900) cells/mm$^3$; counts for 20 control naiads were 3,000 to 34,000 (avg = 21,900) cells/mm$^3$; $p < 0.001$.

6. *Electrophoretic Analysis of Hemolymph Proteins.* Three host-parasite systems have been investigated to determine the effect of infection on the levels of specific hemolymph proteins. Disc electrophoresis was performed in polyacrylamide gel, using the Canalco Model 12 apparatus and a Beckman RD-2 power supply, with techniques modified from those described by Davis (19). These host-parasite systems include one representing a normal parasite-host relationship, *C. stenodorya-A. carolinensis;* one representing an abnormal parasite-host combination, *C. tremaglandis-H. recurvata;* and one system which is currently being studied histologically, namely, *P. lepomis* cercariae in larvae of an unidentified alderfly, *Sialis* sp. In the last case, the cercariae enter in relatively large numbers and are met by a host reaction, but the ulimate fate of the metacercariae has not been determined.

Insects were infected by individual exposure to cercariae, bled in groups of 3 to 10 at 1 to 14 days after infection, and examined by dissection to determine infection intensities. Uninfected control insects, collected at the same time and place and handled in a manner identical to that used with infected insects, were bled and the hemolymph processed concurrently with the samples from infected insects. Hemolymph from infected and uninfected control insects was pooled separately in capillary pipettes, centrifuged to remove the hemocytes, and separated into aliquots for use in preparing the sample gels. Samples of 12 $\mu$l of *Hexagenia* hemolymph were applied to graded 7%-10% gel columns (20); 4-5 $\mu$l samples of *Acroneuria* and *Sialis* hemolymph were separated in standard 7% lower gel. Constant current was applied at 3-4 ma per gel column for 60 to 80 minutes. Hemolymph proteins were separated into 15 to 21 fractions, depending on the species of insect. *C. stenodorya-A. carolinensis* and *C. tremaglandis-H. recurvata* systems were investigated repeatedly at 18 to 24 hours after infection; in neither case was there evidence for alteration in hemolymph protein pattern, despite the presence of large numbers of metacercariae in some instances. The *Sialis* larvae which had been exposed to cotylocercous cercariae were bled at 1, 2, 3, 7, and 14 days. Infection was associated with a decrease in protein in two relatively minor fractions which migrated one-fifth of the distance to the tracking dye (Fig. 7). This decrease in protein was seen at 1 to 14 days and appears to be significant. It was particularly evident in heavily infected larvae (25 to 80 worms per insect). Attempts are currently being made, using disc electrophoresis, to investigate hemolymph levels of enzymes such as

tyrosinase and leucine aminopeptidase which could play a role in melanin formation.

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## REFERENCES

1. Hall, M. C. 1929. Arthropods as intermediate hosts of helminths. *Smithsonian Misc. Coll.* 81:1.
2. Timon-David, J. 1958. Role des insectes comme hotes intermediares dans les cycles des trematodes digenetiques. *Proc. 10th Internat. Cong. Entomol.* 3:657.
3. Etges, F. J. 1953. Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella amnicolae* n. sp. (Trematoda: Microphallidae). *J. Parasitol.* 39:643.
4. Dobrovolny, C. G. 1939. The life history of *Plagioporus lepomis*, a new trematode from fishes. *J. Parasitol.* 25:461.
5. Salt, G. 1963. The defence reactions of insects to metazoan parasites. *Parasitology* 53:527.
6. Burns, W. C. 1961. Penetration and development of *Allassogonoporus vespertilionis* and *Acanthatrium oregonense* (Trematoda: Lecithodendriidae) cercariae in caddis fly larvae. *J. Parasitol.* 47:927.
7. Hall, J. E., and A. E. Groves. 1963. Virgulate xiphidiocercariae from *Nitocris dilatatus* Conrad. *J. Parasitol.* 49:249.
8. Hall, J. E., J. E. Weaver, and B. Gomez-Miranda. 1969. Histopathology of lecithodendriid trematode infections in stonefly naiads. *J. Inv. Pathol.* 13:91.
9. Hall, J. E. 1960. Virgulate xiphidiocercariae from Indiana and Michigan. *Am. Midland Naturalist* 63:226.
10. Slifer, E. H., and R. L. King. 1933. Grasshopper eggs and the paraffin method. *Science* 78:366.
11. Cable, R. M. 1958. *An Illustrated Laboratory Manual of Parasitology*. (4th ed.). Burgess Publishing Co., Minneapolis. p. 143.
12. Armed Forces Institute of Pathology. 1960. *Manual of Histologic and Special Staining Technics*. (2nd ed.). McGraw-Hill, New York. p. 109.
13. Lillie, R. D. 1954. *Histopathological Technic and Practical Histochemistry*. (2nd ed.). McGraw-Hill, New York. p. 284.
14. Armed Forces Institute of Pathology. 1960. *Manual of Histologic and Special Staining Technics* (2nd ed.). McGraw-Hill, New York. p. 132.
15. _____. pp. 140, 147.
16. Barka, T., and P. J. Anderson. 1963. *Histochemistry: Theory, Practice, and Bibliography*. Harper and Row, New York. p. 185.
17. Welch, H. E., and J. F. Bronskill. 1962. Parasitism of mosquito larvae by the nematode, DD136 (Neoaplectanidae). *Can. J. Zool.* 40:1263.
18. Jones, J. C. 1962. Current concepts concerning insect hemocytes. *Am. Zoologist.* 2:209.
19. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann N. Y. Acad. Sci.* 121:404.
20. Wright, G. L. Jr., and W. L. Mallmann. 1966. Differential disc electrophoresis of serum proteins. *Proc. Soc. Exp. Biol. Med.* 123:22.



# STRONGYLOIDES RATTI (NEMATODA) IN NEWBORN OFFSPRING OF INOCULATED RATS

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## ABSTRACT

Infections were found in offspring of mother rats inoculated at various times in the course of their pregnancy or lacatation. Infections also occurred in pups of an uninoculated rat nursed by an inoculated foster dam and in pups of dams whose skin and fur and environment were sterilized at appropriate intervals. Larvae were isolated from mammary tissues but none were found in stained tissue sections. Worms were found in the intestines of the newborn as early as 14 hours postpartum when the mother was inoculated two days prepartum. Oviposition took place in the young hosts as early as the third day postpartum.

## INTRODUCTION

In the general life cycle of nematodes of the genus *Strongyloides*, the infective larvae invade the host percutaneously and are carried via the lymphatics and circulatory system to the lungs. They penetrate the capillary walls and migrate up the trachea and down into the alimentary tract, establishing themselves in the small intestine.

Nematodes and other helminths with extra-intestinal migration in their life cycles are capable of invading fetuses, as reported for *Schistosoma japonicum* in man and dogs, hookworms in man, dogs, and pigs, ascarids in cattle and dogs, lungworms in sheep, and filarial worms in dogs (1,2). The transmission of the swine kidney worm to fetuses has been recently demonstrated (3). Both experimental and natural prenatal infections with *Stronglyoides* have been reported (4,5,6,7,8,9,10). Moreover, nematodes with a visceral migration phase can be transferred in the mother's milk. This has been shown for the hookworms, *Uncinaria lucasi* in seals (11,12) and *Ancylostoma caninum* in dogs (13,14), *Trichinella spiralis* in mice (15), and *Strongyloides ransomi* in pigs (16).

The scope of prenatal, perinatal, and neonatal infections is of increasing interest and more needs to be known of the precise mechanisms involved. It was thought that *S. ratti*, in view of its over-all life cycle, where there is both homogenesis and heterogenesis, might serve as a convenient instrument for laboratory studies on these phenomena. This paper is a report of the accomplishments to date.

My work on *Strongyloides* infections in the newborn host was initially concerned with inoculating female rats with relatively small numbers (approximately 150-200) of *S. ratti* during nonpregnant periods and, after mating the rats, following the course of the infection and the possible transmission of this parasite to the rat offspring. However, such experiments did not result in infected pups. Since then, experiments have been carried out with 200 to 24,000 larvae inoculated during the course of pregancy or lactation. These procedures have produced infected offspring. Two such experiments have been reported (17) in brief. These and additional investigations suggest possible different and interesting modes of transmission. In view of this, what has been reported by others and the practical importance of *Strongyloides* infections in the newborn, it is of value to present my findings in detail.

## MATERIALS AND METHODS

The infective larvae were isolated from week-old charcoal cultures of feces of stock-infected laboratory rats. The strain (G-60) of *S. ratti* was originally obtained in 1960 from a wild rat in the Philadelphia, Pa., area. All rats were inoculated subcutaneously, usually in the midline of the abdomen (MA).

Except where indicated, rats used in this work were Sprague-Dawley descendants, commercially-bred (Charles River or Carworth Farms) or born and raised in our laboratory. The Long-Evans strain was raised in our laboratory. Rats were kept individually in plastic cages with wood shavings as bedding, and transferred to sterilized cages with new bedding at least every two days. Purina Laboratory Chow® and tap water were available to the rats at all times.

Rat pups were examined for worms prior to parturition by sacrifice of the dam or as soon after birth as possible and at intervals thereafter. The lungs and the intestines were pressed between glass slides and examined directly under the compound microscope. Worms were isolated from crushed or minced tissues under

the stereomicroscope or in a modified Baermann apparatus utilizing a cotton-gauze filter supported by bottomless drinking cups in a Pilsner glass. Usually, two pups were used for each time interval, the tissues of one being employed primarily for direct examination and the tissues of the other being primarily for fixation and histological processing. Samples of worms for particular intervals were heat-killed and measured with an optical micrometer.

## RESULTS

*Multiple inoculations of dams*

Pregnant rats were inoculated subcutaneously 2, 3, or 4 times with infective larvae from the 2nd to the 20th day prior to parturition (Table 1). Three different sites of inoculation were used. Of 13 dams (identified as A in Table 1) inoculated MA, four had litters infected with *S. ratti*. Twenty-nine of the 38 offspring of these four rats were examined directly from the day of birth to the 4th day following parturition and were found to be infected. Positive offspring were also obtained from rat H inoculated twice in the interscapular region (IS) but no parasites were found in the pups of rat L inoculated twice by gavage.

*Single inoculations of dams*

Infected pups were obtained from dams inoculated as much as six days prior to parturition (Table 1, dam C). Worms were found in the intestines of the pups as early as 14 hours (E) and eggs were deposited in the intestinal mucosa as early as three days (D) post-partum. Eleven of the 13 pups of dam K inoculated in the tail two days prepartum were infected, larvae being found in the duodenum of the offspring as early as 18 hours following parturition and oviposition beginning on the 3rd day.

Dam G was inoculated four days after parturition in the course of her nursing her litter. The dam was separated from the litter for one hour following the inoculation to reduce the possibility of the pups coming in contact with material which might have oozed out of the site of inoculation. Larvae resembling the infective forms were found in the pups on the 2nd day post-inoculation and eggs were in the intestinal mucosa on the 5th day.

In two different experiments, involving the transfer of litters of three inoculated (MA) and three uninoculated dams, only one litter was found to be infected. Dam N was inoculated three days prior to parturition. Her offspring were removed from the cage immediately after birth to be nursed by dam M whose litter, born the day before, was then given to dam N. Seven of the nine pups born of the uninfected natural mother but nursed by the inoculated foster mother were found to be infected, larvae being present as early as 18 hours after the exchange and eggs appearing in the intestines on the 4th day. None of the offspring exchanged between dams O, P, Q, and R were found to contain worms or eggs.

*Worms in mammary tissue*

Two pregnant rats were each inoculated SM two days before parturition, dam S receiving 10,000 and T receiving 20,000 larvae. Both rats were killed immediately after parturition. The mammary glands were removed and most of them were processed for recovery of larvae in modified Baermanns as described above. The remainder was fixed in Bouin's and processed for histological examination. No worms were recovered from dam S, but several larvae were isolated from the glands of T. No worms were found in hematoxylin-eosin stained slides of mammary tissues of either rat.

*Disinfection of fur and skin*

Since an infected dam's external surfaces may become contaminated with feces and larvae, an experiment was carried out to remove this possible source of infection for the pups. Two pregnant rats were each inoculated prior to parturition with 24,000 larvae MA. Four hours after the inoculation, each was lightly anaesthetized with ether and immersed completely, except for the head, into a solution of Wescodyne® in 95 percent ethanol.

Rat U gave birth to 10 pups 22 hours after the inoculation and was dipped again at that time prior to the onset of nursing. Excess disinfectant on the animal was wiped off with paper towelling. The dam was allowed to dry by herself and was then placed in a clean cage with fresh bedding with her pups. This process of dipping and change of bedding was repeated at 24 and 48 hours after parturition; a change of cage and bedding was also made at 36 hours. Three living pups were sacrificed and examined and seven pups which had died were examined at these intervals. One pup was alive and one was dead at 54 hours post-parturition; the dead one was positive for worms, these being like the infective forms and were found only in the intestines.

In addition to the first, rat V was dipped and put into a clean cage with clean bedding at seven hours prior to, immediately after, and 18 hours after parturition. The dam and her pups were also placed in a clean cage with new bedding at 24 hours after parturition. Of the



TABLE 1. *Establishment of* Strongyloides ratti *in newborn rats through single and multiple inoculations of pregnant and lactating dams.*

| DAMS | | INOCULATIONS | | | EXAMINATION OF OFFSPRING** | | |
|---|---|---|---|---|---|---|---|
| *Identity* | *No.* | *Route** | *Days from partum* | *No. Worms* | *Days Postpartum* | *No. Inoc.* | *No. Positive for worms* | *Days Positive* |
| A | 13*** | MA | −20 to −2 | 200 to 7000 | −1 to 12 | 2 to 4 | 99 | 0 to 4 |
| B | 2 | MA | −7 | 9300 to 12,000 | 0 to 7 | 1 | 15 | – |
| C | 1 | MA | −6 | 11,000 | 1 to 3 | 1 | 7 | 1 to 3 |
| D | 1 | MA | −4 | 5,400 | 0,3,4 | 1 | 9 | 3,4 |
| E | 1 | MA | −2 | 6,400 | 0,1,2,4 | 1 | 7 | 0 (14 hours) 1,2 |
| F | 1 | MA | −1 | 20,000 | 0 to 4 | 1 | 13 | 1 to 4 |
| G | 1 | MA | +4 | 10,000 | 5 to 9,12 | 1 | 7 | 6 to 9,12 |
| H | 1 | IS | −8, −5 | 4600 & 6900 | 0 to 2 | 2 | 7 | 0 to 2 |
| I | 1 | IS | −8 | 9,300 | 0,1 | 1 | 3 | – |
| J | 1 | Tail | −10 | 6,400 | 0 to 3 | 1 | 7 | – |
| K | 1 | Tail | −2 | 6,400 | 0 to 3,5 | 1 | 13 | 0 (18 hours) 1 to 3,5 |
| L | 1 | Oral | −7, −4 | 9300 & 14,000 | 0,7 | 2 | 9 | – |

*MA = subcutaneous, midline of abdomen: IS = subcutaneous, interscapular region

**Worms present only in pressed tissue sections of intestines

***5 Long-Evans strain and 8 Sprague-Dawley descendants

10 pups born, all died in the course of the experiment and eight were examined. Only one of five at 42 hours had worms, these also being similar to the infective forms and found in the intestines.

The dams were killed when the last of their pups were examined, that is, at approximately 76 (U) and 70 (V) hours post-inoculation. Each had larval worms in the lungs and duodenum. Some worms in the duodenum had a developing vulva but in no case were eggs found.

*Summary of findings in infected new born*

Worms were found as early as 14 hours after parturition. In the second and third days postpartum, there was an overlapping of developmental stages, both infective-like larvae and juveniles with a developing vulva being found. Ten of the former had a mean measurement of 0.677 mm, which is slightly more than the mean (0.637 mm) but in the range of the infective forms. Eight of the juveniles measured 1.30 mm. Mature, gravid worms and oviposition were evident as early as the third day. The mean length of six adults on the fourth day, the more-frequent prepatent period, was 1.69 mm. To date, in no case have worms been observed or recovered from organs other than the small and large intestines.

## DISCUSSION

While it has been demonstrated that *Strongyloides ratti* may be in the intestines of rats in as little as 14 hours postpartum, the means by which the worms were acquired is not clear. There are three possibilities for the transmission of this nematode, any or all of which may have taken place in this study. A contaminated environment may have been the source. However, in view of the results with dams D, K, U, and V, it is of interest to consider the above findings with respect to prenatal and transmammary transmission, both of which have been reported for *Strongyloides* (4,5,6,7,8,9,10, 16).

Certain parts of the data suggest intrauterine transmission or acquisition at the time of birth. The finding of eggs in the newborn as early as three days (litters of dams D and K) postpartum suggests this. In my experience with *S. ratti* in rats of at least five weeks of age, a prepatent period, determined by coprocultures, of three days has been extremely rare but it has occurred. In recent work in my laboratory (18), the earliest recovery of eggs by zinc sulphate flotation or larvae by culture was four days. Other prepatent periods have been reported and these might be reflections of the inoculum size. With singly established worms, positive cultures of 24-hour fecal collections were obtained on the fifth day after applying the infective larva to the skin (19), but not the fourth. The mode in this work was the sixth day. A prepatent period, based on cultures, of three days in rats inoculated with 1000 or 2000 larvae, four days with 500, and six days with another group which received 1000 has been reported (20). Considering these results and those from other work it was concluded (20) that the average prepatent period was 3.8 days. Thus, there is variation in the pre-oviposition interval but the earliest time of eggs appearing in feces is three days. This along with the failure to recover larvae from the lungs serve as arguments against the acquisition of the worms prior to the onset of nursing. However, in these arguments, it is assumed that there would be a lung migration or that such could be detected by the methods employed. It should be noted here that lung migration may not be required by *S. ransomi* in pigs since a prepatent period of three days (21) rather than the usual six (7) has been found.

The experiments with rats G, M, N, and particularly U and V, indicate a strong possibility of transmission through the inoculated dam's milk. The treatment of U and V was rather severe; it appeared that the dams were adversely affected by it and may actually have become inebriated. It has been reported (22) that part of the rat's maternal behavior includes the licking of her body during pregnancy and after parturition. Thus, the Wescodyne® and alcohol could have been ingested and thereby the nursing procedures disrupted. Nursing was observed and milk was found in the stomachs and intestines of autopsied pups. Since an unusually large part of the litters died, it would seem that the pups were not provided with sufficient opportunity to nurse, that the affected mother did not properly attend in other ways to her young, or that the disinfectant was ingested by the young, perhaps in the milk. Nevertheless, finding an infected pup in each litter where such sanitary measures were employed and where neither parent yielded egg-producing worms lends support to the possibility of transmammary transmission.

Prenatal or transmammary transmission of nematodes may be associated with variations in the extraintestinal migratory pattern of the larvae due, at least in part, to the physiological status of the host. Differences in the migration



of *S. ratti* and other species of *Strongyloides* appear in the literature. While in one study *S. ratti* larvae were recovered from various non-intestinal tissues, including lung, as much as 13 days after inoculation (23), in another such worms were found only in the lungs and never after 96 hours (24). The opinion has been expressed (25) that age resistance in cattle more than two years old prevents larvae of *S. papillosus* from completing their normal lung-to-intestine migration and these larvae then migrate in other parts of the body until they die or, perhaps, gain access to a fetus.

Recently, the possibility of changes in *S. ransomi* larvae which would affect their recovery from tissue has been discussed and reference made to a "quiescent state" which occurred at 24 hours after percutaneous infection of pigs (26). This may account for the very small number of *S. venezuelensis* larvae recovered from various organs and tissues of rats at intervals in the first two days post-inoculation and prior to invasion of the intestine (20). Moreover, it may have a bearing on the results reported above.

From the foregoing, it is evident that further investigations to obtain a better understanding of the physiology and morphology of the histotropic stages of *Strongyloides* are desirable. Such studies are in progress. In addition to furthering our knowledge of the biology of this nematode, it is hoped that such experiments will contribute to elucidating the mechanisms of transmissions of *Strongyloides* to the newborn.

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## REFERENCES

1. Cort, W. M. 1921. Prenatal infestation with parasitic worms. *J. Am. Med. Assoc.* 76:170.
2. Flamm, H. 1959. *Die pranatalen Infektionen des Menschen*. Georg Thieme Verlag, Stuttgart. p. 92.
3. Batte, E. G., D. J. Moncol, and C. W. Barber. 1966. Prenatal infection with the swine kidney worm *(Stephanurus dentatus)* and associated lesions. *J. Am. Vet. Med. Assoc.* 149:758.
4. Enigk, K. 1952. Zur biologie von Strong loides. *Z. Tropenmed. Parasitol.* 3:358.
5. Pfeiffer, H. 1962. Die pranatale Invasion von *Strongyloides papillosus* beim Rind. *Z. Parasitenk.* 22:104.
6. Supperer, R., and H. Pfeiffer. 1962. Untersuchungen uber die Gattung *Strongyloides* II. Die praenatale Invasion beim Rinde, sowie Beobachtungen uber allergische Hautreaktionen nach perkutaner Invasion. *Berl. Munch. Tierarztl. Wchnschr.* 75:344.
7. Stewart, T. B., W. N. Smith, and D. J. Jones. 1963. Prenatal infection of pigs with the intestinal threadworm *Strongyloides ransomi*. J. Parasitol. 49(Suppl.): 45.
8. Stone, W. M. 1964. *Strongyloides ransomi* prenatal infection in swine. *J. Parasitol.* 50:568.
9. Neto, M. P., R. P. de Mello, R. V. do Nascimento and A. C. de Souza Moreira. 1965. Ocorrencia de larvas de *Strongyloides ransomi* Schwartz and Alicata, 1930, em figado de suino natimorto no Municipio de Itaguai. *Atas. Soc. Biol. Rio de J.* 9:40. Abstr. in *Helminthol. Abstr.*, 1968 37:103.
10. Supperer, R. 1965. Untersuchungen uber die Gattung Strongyloides. VI. Die pranatale Invasion. *Berl. Munch. Tierarztl. Wchnschr.* 78:108.
11. Olsen, O. W., and E. T. Lyons. 1962. Life cycle of the hookworm *Uncinaria lucasi* Stiles, of northern fur seals, *Callorhinus ursinus*, on the Pribilof Islands in the Bering Sea. *J. Parasitol.* 48(Suppl.):42.
12. Olsen, O. W., and E. T. Lyons. 1965. Life cycle of *Uncinaria lucasi* Stiles, 1901 (Nematoda: Ancylostomatidae) of fur seals, *Callorhinus ursinus Linn.*, on the Pribilof Islands, Alaska. *J. Parasitol.* 51:689.
13. Stone, W. M., and M. H. Girardeau. 1966. *Ancylostoma caninum* larvae present in the colostrum of a bitch. *Vet. Rec.* 79:773.
14. Stone, W. M., and M. Girardeau. 1968. Transmammary passage of *Ancylostoma caninum* larvae in dogs. *J. Parasitol.* 54:426.
15. Denham, D. A. 1966. Infections with *Trichinella spiralis* passing from mother to filial mice pre- and postnatally. *J. Helminthol.* 40:291.
16. Moncol, D. J., and E. G. Batte. 1966. Transcolostral infection of newborn pigs with *Strongyloides ransomi*. *Vet. Med. Small Animal Clin.* 61:583.
17. Katz, F. F. 1964. A preliminary report on the nematode, *Strongyloides ratti*, in newborn offspring of infected dams. *Bull. N. J. Acad. Sci.* 9:41.
18. Petriello, R. P. 1966. *Observations on the life cycles of a homogenetic strain of Strongyloides venezuelensis and a homo/heterogenetic strain of S. ratti (Nematoda, Rhabditoidea) in different host species.* Master of Science Thesis, Seton Hall University.
19. Graham, G. L. 1936. Studies on Strongyloides. I. *S. ratti* in parasitic series, each generation in the rat established with a single homogonic larva. *Am. J. Hyg.* 24:71.
20. Sheldon, A. J. 1937. Some experimental studies on *Strongyloides ratti*. *Am. J. Hyg.* 25:39.
21. Frickers, J. 1953. Strongyloidosis bij het varken. *Tijdschr. Diergeneesk.* 78:279-303. Abstr. in *J. Am. Vet. Med. Assoc.*, 1953, 123:145.
22. Roth, L. 1967. Maternal behavior in the rat. *Bull. N. J. Acad. Sci.* 12:41.
23. Spindler, L. A. 1958. The occurrence of the intestinal threadworms, *Strongyloides ratti*, in the tissues of rats, following experimental percutaneous infection. *Proc. Helminthol. Soc. Wash. D.C.* 25:106.
24. Abadie, S. H. 1963. The life cycle of *Strongyloides ratti*. *J. Parasitol.* 49:241.
25. Supperer, R., and H. Pfeiffer. 1964. Untersuchungen uber die Gattung Strongyloides. III. Resistenz. *Zbl. Veterinarmed.* 11:143.
26. Stone, W. M., and C. F. Simpson. 1967. Larval distribution and histopathology of experimental *Strongyloides ransomi* infection in young swine. *Can. J. Comp. Med. Vet. Sci.* 31:197.

# THE EFFECTS OF TREMATODE LARVAE ON THE LOCOMOTION OF NATURALLY INFECTED NASSARIUS OBSOLETUS (GASTROPODA)

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## ABSTRACT

The rates of locomotion in normal and parasitized mud snails, *Nassarius obsoletus,* were determined under laboratory conditions. Four seasonal groups of animals (late summer, two in the winter and early spring, 1968-1969), totaling 239 normal and 228 parasitized individuals, were used in the comparison. Forty-seven of the nonparasitized and 72 of the parasitized snails did not move during the prescribed time period for each trial. The rates of movement were calculated from the remainder in each group. Infected animals moved at mean rates which were 54, 80, 73, and 91% of the normal rates for each of the four successive collections. In all but the spring group, the differences were statistically significant. A variety of seasonal factors are related to these differences. The lower mean rate of locomotion in infected snails appears to amplify the ecological observations of others that there is a differential in the migratory activity in infected and normal *N. obsoletus.*

## INTRODUCTION

A few reports in the literature deal with the effects of larval trematode infections on the locomotion of gastropods. Most of these are based on cursory observations or indirect evidence, with no attempt to determine precisely the differential in the rates of locomotion between parasitized and nonparasitized snails. Some investigators reported that, under laboratory conditions, infected snails were more sluggish than the uninfected, and that the degree of inactivity was a function of the length of time that snails had been parasitized (1,2,3). However, laboratory observations by others indicated no obvious differences in locomotor activity (4,5).

Ecological studies on the seasonal incidence of larval trematodes in snail populations have led some to infer that infections tend to immobilize snail hosts so that they do not undertake the normal seasonal migrations. This was suggested for the marine gastropods *Nassarius obsoletus* (Say) (6,7) and *Littorina littorea* (L.) (8). Gambino (9), however, failed to detect evidence which would indicate differential migrations in *N. obsoletus.*

Numerous reports on the pathology and depletion of food reserves in infected snails recently have been summarized by Cheng (10). In addition, it has been shown that certain larval trematodes cause deleterious effects to the musculature and connective tissues of the host's foot (2,3,11). All of this suggests that parasitization may lead to the impairment of locomotor ability.

The purpose of the present study was to determine in the laboratory the rates of locomotion in parasitized and normal mud snails *Nassarius obsoletus.* Copeland (12) appears to be the only person who has published on the locomotory rate in this species. Unfortunately, he used only small numbers of animals and did not distinguish between parasitized and nonparasitized individuals. The reasons for selecting *N. obsoletus* were: 1) its trematode fauna are well known; 2) it is easily collected and maintained; 3) to shed light on Sindermann's (7) ecological inferences concerning the differential rates of locomotion.

## MATERIALS AND METHODS

Collections of *Nassarius obsoletus* were made in the intertidal area of Delaware Bay, south of Pierces Point, N.J. (lat. 39° 04.5′ north; long. 74° 54.9′ west) on the following dates: 9/7/68, 12/8/68, 2/16/69, and 4/3/69. Almost all the animals were gathered within 75 meters of the high-tide level.

Upon return to the laboratory, the normal and infected animals were separated by means of conventional isolation techniques, with cercarial release being the indicator of parsistism. The possibility that some parasitized animals were not releasing cercariae and, therefore, would be classified as uninfected, was overcome by a labelling and examination technique. This involved marking each snail with nail polish in a coded manner. At the conclusion of each series of tests, snails in the uninfected group were sacrificed and examined under a binocular microscope for traces of parasitism. If such evidence was found, the experimental data for the animal was transferred to the infected group. All of the infected snails were examined similarly, and the species of larvae identified.

A rectangular Pyrex dish (28 x 16 x 4.5 cm) served as the testing apparatus. The dish was placed on a brown background and illuminated from above with fluorescent light, the intensity of which fluctuated within a range of 34 to 48 foot-candles. At the start of each test, the dish was filled with filtered sea water to a depth of 0.5 cm. The temperature of the water varied from 17 to 20° C. Two snails were then placed approximately 3 cm apart in the center of this container. When the gastropod attached its foot to the substratum and raised its shell from the bottom of the dish, a stop watch was started. The animal was allowed to move about in the dish for 90 seconds and was then removed. If the snail did not immediately attach its foot and begin to move, a period of 5 minutes was allowed for the animal to do so. However, if at the end of 5 minutes the gastropod was still withdrawn in its shell, it was removed from the apparatus and its negative locomotion was recorded. After the snail had moved about in the dish, the water was decanted and replaced with a solution of India ink in filtered sea water. In the process of settling, carbon particles in the ink solution became entrapped in the mucilaginous trail left by the snail. After a few minutes the ink solution was decanted, and the mucous trail was clearly delineated on the bottom of the dish. The container was inverted over illumination from beneath, and the trail traced onto a piece of paper. A line was drawn along the center of the trail, and the length of this line was then measured with either a map measurer or dividers (0.5 cm between points of the dividers). In most cases the dividers were used because they were easier to manipulate. The length of the trail was then converted to distance travelled in cm/sec. The aperture size of the snail (i.e., the distance from the most-anterior point of the siphonal canal to the most-posterior portion of the aperture opening) was measured with a millimeter ruler. This measurement was the criterion by which the snails were grouped into size classes. In preparation for the next test, the dish was thoroughly washed and dried. The same filtered sea water was used for approximately 10 trials, and then was replaced with fresh filtered sea water. All locomotor tests were usually completed within 3 weeks after the snails had been collected, and during this period none were fed. The data were statistically treated by analysis of variance (13).

## OBSERVATIONS AND RESULTS

The incidence of larval trematode infections in the various collections of *N. obsoletus* and the distribution of trematode species within each collection are listed in Table 1. The percentage of infected snails gradually decreased from September to April. While a large number of factors may be involved in this decline, a greater mortality among infected snails during the winter months may be an important cause. *Himasthla quissetensis* and *Zoogonus lasius* were the dominant species, the former predominating in the September collection and the latter being dominant thereafter. In almost

TABLE 1. Incidence of larval trematode infections and species distribution in *Nassarius obsoletus* from Delaware Bay, New Jersey.

| Dates of collection | No. of snails | No infected[1] | % infected | Larval species[2] and No. of infections | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D | E | F |
| 9/7/68 | 485 | 101 | 24.9 | 83 | 11 | 2 | 2 | 2 | 1 |
| 12/8/68 | 317 | 47 | 14.8 | 4 | 35 | 8 | | | |
| 2/16/69 | 1612 | 51 | 3.2 | 10 | 29 | 7 | 2 | 2 | 1 |
| 4/3/69 | 1899 | 54 | 2.8 | 11 | 38 | 3 | | 2 | |

1. Based on cercarial emergence.
2. Species of trematodes: A = ***Himasthla quissetensis*** (Miller and Northrup, 1926) Stunkard, 1938; B = *Zoogonus lasius* (Leidy, 1891), Stunkard, 1940; C = ***Austrobilharzia variglandis*** (Miller and Northrup, 1926) Penner, 1953; D = *Cercaria nassa* Martin, 1945; E = ***Lepocreadium seiferoides*** (Miller and Northrup, 1926) Martin, 1938; F = *Cercaria dipterocerca* Miller and Northrup, 1926—probably *Stephanostomum dentatum* (Linton, 1900) Manter, 1931 (14).

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all collections, *Austrobilharzia variglandis* was the third-most-numerous trematode.

Infected snails in the four consecutive collections moved at mean rates which were 54, 80, 73, and 91% of the normal rates (Table 2). The mean rates of locomotion in the parasitized animals were significantly less than those in the uninfected groups in all but the last collection. In conjunction with this fact, it can be seen that the mean rates for the infected animals increased from fall to spring. On the other hand, no general trend in the mean rates of movement was noted in the normal groups.

A great deal of individual variability within a population of snails is indicated by the observed ranges in the rates of locomotion (Table 2). Many factors are probably involved in this variation. One that immediately comes to mind is the effect of the gastropod's size on its performance during testing. Snails in the infected and uninfected groups were all adults, as confirmed by determining mean shell lengths within the aperture length size classes (0.9, 1.0, 1.1, and 1.2 cm) and comparing them with the shell length data in Scheltema's (15) growth study of *N. obsoletus*. There were no significant differences in the mean rates between size classes, and similar individual variability occurred within each of the size classes. A typical example of this is the uninfected group in the 9/7/68 collection. There were sufficient numbers of animals in only the 1.0-, 1.1-, and 1.2-cm size classes to run a statistical comparison. The mean rates for the three size classes were 0.14, 0.12, and 0.13 cm/sec, respectively, and the ranges were 0.07 to 0.26, 0.02 to 0.20, and 0.02 to 0.21 cm/sec.

Some of the individual variations in the parasitized groups may have been due to differences in the length of time that snails had been infected. Since we were utilizing naturally infected animals, it was not possible to determine how long each snail had been parasitized. It may also be that there is a difference in the overall effects on the host caused by various species of larvae. These snails were parasitized by a variety of trematode species. Some, such as *H. quissetensis,* go through redial stages which are capable of considerable tissue destruction. Others, such as *Z. lasius,* produce sporocysts which appear to be less capable of causing tissue damage.

Variation in uninfected groups could be due partially to the fact that some of the nonparasitized snails may have recovered from previous infections and, therefore, may be less active than snails never infected. This factor was not investigated and could not be adequately studied without examining serial sections of the snails. The range in locomotor rates may merely reflect the variation to be expected among individuals of any natural population.

The percentage of the animals tested which did not move is an additional indicator of the difference in the activity in the normal and parasitized groups. The percentages of normal animals which did not move compared to the infected snails which behaved similarly during the trials were: 9/7/68, 3.1 versus 30.4%; 12/8/68, 18.7 versus 47.7%; 2/16/69, 17.8 versus 25.0%; and 4/3/69, 45.3 versus 26.0%, respectively (Table 2, columns 2 and 3). A possible explanation for the reversal of the general effect in the last collection will be discussed later.

TABLE 2. Mean rates of locomotion in normal and naturally infected *N. obsoletus* in four collections from Delaware Bay, N.J., including the range in the rates of locomotion and the number active and inactive during the tests.

| Dates of collections and groups | No. tested | No. not moving | No. moving[3] | Rate cm/sec Range | Mean | P value |
|---|---|---|---|---|---|---|
| 9/7/68 | | | | | | |
| N[1] | 65 | 2 | 63 | .02-.26 | .13 | |
| P[2] | 82 | 25 | 57 | .02-.18 | .07 | <.01 |
| 12/8/68 | | | | | | |
| N | 48 | 9 | 39 | .02-.19 | .10 | |
| P | 44 | 21 | 23 | .04-.14 | .08 | <.05 |
| 2/16/69 | | | | | | |
| N | 73 | 12 | 61 | .02-.19 | .11 | |
| P | 52 | 13 | 39 | .03-.19 | .08 | <.01 |
| 4/3/69 | | | | | | |
| N | 53 | 24 | 29 | .02-.20 | .11 | |
| P | 50 | 13 | 37 | .02-.20 | .10 | N.S.[4] |

1. N = Normal snails.
2. P = Parasitized snails.
3. Rate of locomotion calculated from these snails.
4. Not significant.



An attempt was made to determine how closely the average rates of locomotion of a group of normal or parasitized gastropods would correspond, if the group was tested several times. Such a determination was made on the animals collected on 9/7/68 and to a greater extent with snails from the collection of 2/16/69. In this latter case, normal snails were tested twice while infected snails were run three times. Mean rates of locomotion for the first trial versus the second in the uninfected group were 0.11 and 0.11 cm/sec (P value much greater than 0.05). Average rates for the infected snails for the first, second and third trials were 0.08, 0.08, and 0.07 cm/sec (P value much greater than 0.05). Thus, there were no significant differences in the mean rates of movement for the uninfected or infected groups when they were tested repeatedly. This would indicate that the average rates of locomotion for the various groups (calculated from data obtained during first tests, Table 2), are reasonably accurate values of locomotor activity under the testing conditions.

## DISCUSSION

There was no indication of migration of parasites into the foot tissues in *N. obsoletus;* thus this aspect of parasitism must be discarded as a factor in limiting the host's locomotor ability. The primary cause, therefore, for the observed differential in locomotion must be assumed to be the extensive damage to other tissues.

As mentioned earlier, Copeland (12) studied the locomotion in *N. obsoletus*. He calculated rates of movement by recording the length of time required for individual snails to move 5 mm. In one series of tests, 25 trials on each of two individual snails were conducted, and the mean rates for the two animals were 0.21 and 0.17 cm/sec. He also ran 10 trials on each of six gastropods and calculated a mean rate of 0.17 cm/sec. Copeland's results correspond generally with our values (Table 2), even though he used only eight snails, did not record their size, and made no attempt to determine if any were parasitized.

A significant differential in the rate of movement, under laboratory conditions, between normal and parasitized snails would seem to have certain ecological implications. It has been reported that *N. obsoletus* displays schooling behavior and that massive numbers of these animals have been observed to traverse distances in excess of 180 ft in 13 days (16, 17). Our data show that not only are such migrations definitely within the capabilities of *N. obsoletus,* but that such distances could be traversed in less than one day. Of course, movements of snails in the natural environment are very gradual and circuitous. It appears that the migratory and schooling potential of the parasitized snails would be below that of the nonparasitized individuals.

The gradual migration of mud snails from the intertidal area into deeper water during the late fall or early winter is a well-known phenonmenon (18, 6, 7, 15). Scheltema's (15) observations in New Jersey were made at the same location in Delaware Bay where the snails for the present study were collected. We have witnessed this migration in the collecting area and elsewhere in New Jersey (19). In the more northern latitudes it occurs earlier than in New Jersey, and it appears that the decreasing air and water temperatures are the primary stimuli. The survival value of this migration appears obvious, and is exhibited by a variety of intertidal invertebrates. In deeper waters they are protected from the severe winter conditions of the intertidal zone in the northern latitudes, i.e., freezing temperatures, heavy accumulation of ice, and anaerobic conditions under the ice.

Monthly changes in the incidence of trematodes and the movement of marked infected and uninfected *N. obsoletus* in Maine suggest that parasitization by *Austrobilharzia variglandis* and certain other species *(Zoogonus lasius* and *Himasthla quissetensis* especially) retarded the normal offshore migration of the host in autumn (7). This contributed to a relatively heavy incidence of parasitized snails in the inshore area at this time of year. Unfortunately, Sindermann did not present data on the rates of movement in the marked snails, so it is impossible to compare the differential in locomotory rates determined in the laboratory with his field observations. In New Hampshire it was observed that "healthy" *N. obsoletus* migrated to deep water in the fall while "feebler" individuals were unable to reach deeper water (6). Other studies indicated that

infections in *Littorina littorea* with the larvae of *Cryptocotyle lingua* tend to immobilize the host to the extent that it may not participate in normal offshore migrations in the late autumn (8).

It has been observed that the incidence of trematode infections in *N. obsoletus* increases through the summer months and reaches a peak in the autumn. Sindermann (7) suggests that the simultaneous maturation of infections with *A. variglandis* acquired by *N. obsoletus* from migrating birds during the spring and the differential in migratory movements of normal and parasitized snails provides a plausible basis for the occurrence of a peak of infection in autumn. Moreover, due to this differential in activity, the infected gastropods would be left in the unfavorable environment of the intertidal zone in winter. He also reported that the number of infections in the upper intertidal zone tend to decrease during the winter in proportion to the severity of the weather conditions. Miller and Northrup (20) have similarly proposed that the death of a greater percentage of infected snails during the winter months might be one possible factor related to the seasonal variation in the incidence of larvae. It should be noted that we observed the highest rates of infection in the September collection and that the incidence gradually declined thereafter. This decrease may be due to a combination of two factors. In the 9/7/68 collection the greatest differential between normal and parasitized *N. obsoletus* was observed, indicating that at this time of year one should expect to find the greatest number of weak infected animals. Thus, during the autumn offshore migrations many of the weakest infected snails may remain behind in the intertidal zone. The hostile environment of this zone in winter might cause the death of many of these weakened animals. Thus, a gradual mortality among the parasitized snails due to weather conditions might account for the decline in the incidence of infection. Support of this hypothesis is found in the results of a cumulative mortality record for the normal and infected animals kept for the September collection. At the end of 17 weeks in the laboratory, 1% of the normal animals and 73% of the infected group had died. Conditions provided for these snails in the laboratory were poor, with infrequent water change and very infrequent feeding. This high mortality in the latter group is indicative of the added stress imposed by the trematode burden. It may also be indicative of the differential in mortality between normal and infected snails which may occur under stress conditions in the natural environment and especially under the severe winter weather conditions.

In the 4/3/69 collection, a large number of uninfected animals (45.3%) did not move when they were tested. This indeed seems to be in contradiction to the results for normal snails not moving in the previous collections. In the collection of 12/8/68, 9 normal animals (4 females) did not move when tested, and in the 2/16/69 group of uninfected snails which were tested, 12 (6 females) did not move. On the other hand, in the 4/3/69 collection, 24 normal animals did not move when tested, and 20 of these were females. Containers in which the normal snails of the April collection were maintained had large numbers of egg cases along the sides and animals were often observed copulating. Neither copulation nor large numbers of egg cases were observed when the other collections of snails were being maintained. Thus, it is possible that the large percentage of animals in the last collection that did not move when tested may be due, in part, to some physiological or behavioral change related to reproductive activity of the uninfected females.

From September to April there was a gradual increase in the mean rates of movement in the infected groups (Table 2). This increase could be due to the death of weaker infected snails during the winter months (Table 1 shows the decline in the percentage of infected animals). Thus, as the snails were randomly gathered on the intertidal flat on each successive collection, it would become more and more probable to collect stronger infected snails (the survivors), accounting for the increased mean rates of movement. These stronger parasitized snails may have been parasitized for a shorter length of time and consequently not as adversely affected (this does not eliminate the possibility that some snails with older infections are not as adversely affected as others). These circumstances may account for the fact that no significant difference was observed between the normal and parasitized snails in the last collection.

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## REFERENCES

1. Chernin, E. 1967. Behavior of *Biomphalaria glabrata* and of other snails in a thermal gradient. *J. Parasit.* 53:1233.
2. Hurst, C. T. 1927. Structural and functional changes produced in the gastropod mollusk, *Physa occidentalis*, in the case of parasitism by the larvae of *Echinostoma revolutum*. *Univ. Calif. Publ. Zool.* 29:321.
3. Pan, C. T. 1965. Studies on the host-parasite relationship between *Schistosoma mansoni* and the snail *Australorbis glabratus*. *Am. J. Trop. Med. Hyg.* 14:931.
4. Mathies, A. W., and W. W. Cort. 1956. Larval trematode infections in snails of different sizes. *J. Parasit.* 42:429.
5. Vernberg, W. B., and F. J. Vernberg. 1967. Interrelationships between parasites and their hosts. III. Effect of larval trematodes on the thermal metabolic response of their molluscan host. *Exp. Parasit.* 20:225.
6. Batchelder, C. H. 1915. Migration of *Illyanassa obsoleta, Littorina littorea,* and *Littorina rudis*. *Nautilus* 29:43.
7. Sindermann, C. J. 1960. Ecological studies of marine dermatitis-producing schistosome larvae in northern New England. *Ecology* 41:678.
8. Sindermann, C. J., and A. E. Farrin. 1962. Ecological studies of *Cryptocotyle lingua* (Trematoda: Heterophyidae) whose larvae cause "pigment spots" of marine fish. *Ecology* 43:69.
9. Gambino, J. J. 1959. The seasonal incidence of infection of the snail *Nassarius obsoletus* (Say) with larval trematodes. *J. Parasit.* 45:440.
10. Cheng, T. C. 1967. Marine molluscs as hosts for symbioses. With a review of known parasites of commercially important species. In *Advances in Marine Biology* Vol. 5, (F. S. Russell, ed).
11. Cheng, T. C., and J. S. Cooperman. 1964. Studies on host-parasite relationships between larval trematodes and their hosts. V. The invasion of the reproductive system of *Helisoma trivolvis* by the sporocysts and cercariae of *Glypthalmins pennsylvaniensis*. *Trans. Amer. Microsc. Soc.* 83:12.
12. Copeland, M. 1919. Locomotion in two species of the gastropod genus *Alectrion* with observations on the behavior of pedal cilia. *Biol. Bull.* 37:126.
13. Hays, W. L. 1963. *Statistics for Psycologists*. Holt, Rinehart, and Winston, Inc., New York.
14. Stunkard, H. W. 1961. *Cercaria dipterocerca* Miller and Northrup, 1926, and *Stephanosotomum dentatum* (Linton, 1900) Manter, 1931. *Biol. Bull.* 120:221.
15. Scheltema, R. S. 1964. Feeding habits and growth in the mud-snail *Nassarius obsoletus*. *Chesapeake Sci.* 5:161.
16. Jenner, C. E. 1956. A striking behavioral change leading to the formation of extensive aggregation in a population of *Nassarius obsoletus*. *Biol. Bull.* 111: 291.
17. Jenner, C. E. 1959. Aggregation and schooling in the marine snail *Nassarius obsoletus*. In *Int. Oceanogr. Congr.* (M. Sears, ed.).
18. Morrison, E. I., and J. C. Medcof. 1943. Studies on the shore snail, *Nassarius obsoleta* (Say) in Bideford River, P. E. I. *Res. Bd. Canada, Mss. Repts. of the Biol. Sta.* No. 390 p. 43.
19. McDermott, J. J. 1951. Larval trematode infection in *Nassa obsoleta* Say, from New Jersey waters. Masters Thesis, Rutgers University, New Brunswick, N.J.
20. Miller, H. M., and F. E. Northrup. 1926. The seasonal infestation of *Nassa obsoleta* (Say) with larval trematodes. *Biol. Bull.* 50:490.

# TRANSPLANTATION OF TREMATODES TO THE CHICK CHORIOALLANTOIS

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## ABSTRACT

Work on the relationship of the avian embryo and helminths is reviewed with emphasis on studies of trematodes maintained on the chorioallantoic membrane of the domestic chick. The use of the chick chorioallantois to study intraspecific variation, feeding and digestion, cercarial and metacercarial development, behavior, regeneration and repair of damage, chemotherapy, surface interrelationships, and temperature tolerance of trematodes is discussed.

## INTRODUCTION

The avian embryo has been used extensively in embryology, virology, and protozoology (1,2, 3,4,5). The few studies available on its use in helminthology include the growth of *Trichinella spiralis* in the embryo of the domestic chick and an unsuccessful attempt to obtain development of avian schistosomes in chick eggs (6,7). The occasional reports of the oviduct fluke, *Prosthogonimus macrorchis,* and nematodes of the genera *Ascaridia* and *Heterakis* in hens' eggs indicate that helminths can live in this site (8). The chorioallantoic membrane of the domestic chick has been used to study survival, development, and growth of monogenetic and digenetic trematodes (9,10,11,12,13). The precautions and techniques involved in avian embryo studies have been discussed in numerous works (1,2,3,4,9,14,15).

This paper reviews the literature on trematodes maintained on the chorioallantoic membrane of the domestic chick and discusses studies for which the chorioallantois may provide a tool to gain information on the biology and physiology of the Trematoda.

## DISCUSSION

*Intraspecific Variation.* The chorioallantoic membrane can be used to study intraspecific variation of trematodes that survive there (9, 10). The extent of variation of most trematodes is not known and many flukes described as new are probably morphological variants of previously described species. Trematodes of the same species vary considerably in body size, organ shape, and sexual maturity, depending upon their immediate host environment. Studies of the type provided by Beaver (16) on the intraspecific variation of *Echinostoma revolutum* are needed to determine what a trematode species really is.

Intraspecific variation of *Philophthalmus hegeneri* grown on the chorioallantois and in chicks' eyes has been reported (9,17). Studies on *Polystomoides* sp. from the oral mucosa of turtles and from the chick chorioallantois indicated that the shape of certain organs varied (depending on the nutritional and physicochemical environment of the site), suggesting that *Polystomoides oris* is a probable synonym of *P. coronatum* (10).

*Cercarial and Metacercarial Development.* Cercariae and metacercariae may develop into adult flukes on the chick chorioallantois (9, 11, 18), which may prove useful when the definitive host of a larval trematode is unknown or difficult to maintain in the laboratory. When testing larval trematodes for possible development on the chorioallantois, the incubation temperature of the eggs should be varied since flukes of cold-blooded hosts are killed at 37 C (10,12,13).

The cercariaeum of *Zoogonus lasius* from *Nassarius obsoletus* snails developed on the chorioallantois without forming a metacercaria (18). Free metacercariae of *Leucochloridiomorpha constantiae* dissected from the oviduct of *Campeloma decisum* snails developed to ovigerous adults on the chick chorioallantois (19). Normally this fluke develops to sexual maturity in the bursa of Fabricius of birds. Detailed work on the growth and development of this fluke on the chorioallantois and in the domestic chick, including comparative histochemical studies, is in progress (19). Mechanically excysted metacercariae of *Philophthalmus hegeneri* have been grown on the chick chorioallantois (9). Suboptimal development of in vivo excysted metacercariae of *Echinostoma revolutum* has also been reported (11).

Encysted metacercariae should be excysted prior to their implantation on the chick chorioallantois. Requirements for excystation of trematode metacercariae vary considerably from one species to another. In some forms, simple chemical procedures, heat, or mechanical methods may be satisfactory, whereas other trematodes are more fastidious in their excystation needs and require considerable experimentation prior to their successful excystation.



*Feeding and Digestion.* Precise information on feeding and digestion for most trematodes is lacking. Trematodes feed on mucus, cell debris, tissues, and blood. Some trematodes are more specific in their feeding habits than others. Feeding and digestion can be studied in flukes that survive on the chick chorioallantois. Although details are lacking, trematodes ingest and utlize blood from the vascular surface of the chorioallantoic membrane (9,10,11). Studies are in progress on feeding and digestion of *Echinostoma revolutum* on the chick chorioallantois and in the intestine of the domestic chick (20) in which echinostomes are removed from chicks' intestines, starved for up to 2 days in non-nutrient Tyrode's, then implanted on the chorioallantois or reimplanted into chicks' intestines. Methods for cultivating *E. revolutum* on the chick chorioallantois and for its implantation into normal and surgically altered chicks have been described (11,21).

*Behavior.* Behavior of trematodes reared in vivo is difficult to study and the limited work on in vitro cultivation of trematodes has not been concerned with this problem. If a large window is made in the egg and precautions taken against the condensation of moisture of the window by the use of an auroscope (14), observations can be made on locomotion, migration, mating, and oviposition of trematodes on the chorioallantois.

*Leucochloridiomorpha constantiae* occur singly on the chorioallantois on certain days and in clusters on other days, a type of behavior probably related to sexual phenomena (19). In chorioallantois-grown *E. revolutum* the cirrus is often protruded (11). *E. revolutum* often tears a hole through the membrane with its oral collar of spines, moves through the opening and relocates on the undersurface of the chorioallantois (22). Turtle blood flukes of the genus *Spirorchis* oviposit at a rapid rate on the surface of the chorioallantoic membrane (13).

*Regeneration and Repair of Damage.* Precise information on regeneration and repair of damage in trematodes is lacking and most studies are incidental observations on the ability of trematodes to repair their wounds following accidental or experimental damage (23,24,25). Although the details are not available, adult *Polystomoides* sp. and *E. revolutum* experimentally damaged and implanted on the chorioallantois were able to repair their wounds (26). Assuming a trematode is able to survive on the membrane, experiments could be designed to determine its capabilities to regenerate missing parts or to heal wounded tissues.

*Chemotherapy.* The chorioallantoic membrane could provide an interesting test site for drug chemotherapy against trematodes of medical and veterinary significance. To my knowledge there are no published chemotherapy studies using worms maintained in hens' eggs. The convenience of handling hens' eggs and their relative inexpensiveness should be an impetus to initiate such work.

*Surface Interrelationships.* The tegument of a trematode forms a unique relationship with the surface of the chorioallantoic membrane. There is no information available on this relationship at either the level of the light microscope or electron microscope. The structure of the trematode tegument was reviewed recently (27). Information on the structure of the chick chorioallantois at the level of the light microscope and electron microscope is also available (28). The relationship of a trematode and the chorioallantois may be different from that of the fluke and its immediate site in a normal definitive host.

*Temperature Tolerance.* Information on temperature ranges that adult flukes can tolerate are difficult to obtain from trematodes reared in normal sites, and in vitro cultivation experiments have not been concerned with this problem. The chorioallantoic membranes of 8- to 12-day old chick embryos can be maintained for approximately one week at 30 to 39 C (29), and can be used to determine the temperature range at which a trematode can survive or develop. Unpublished studies on *E. revolutum* indicate that this fluke can survive on chorioallantoic membranes maintained at 30 to 39 C (29).

The aforementioned studies illustrate ways in which the chick chorioallantois can be used to solve problems in trematode biology and physiology. It is my hope that parasitolgists will use, modify, or addend some of these suggestions to implement their teaching and research programs.

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## REFERENCES


1. Hamburger, V. 1960. *A Manual of Experimental Embryology* (Rev. Ed.), University of Chicago Press, Chicago, Ill.
2. Rugh, R. 1962. *Experimental Embryology* (3rd Ed.). Burgess, Minneapolis, Minn.
3. Rivers, T. M. 1952. *Viral and Rickettsial Infections of Man* (2nd Ed.). J. B. Lippincott and Co., Philadelphia, Pa.
4. Pipkin, A. C., and D. V. Jensen. 1958. *Exper. Parasit.* 7:491.
5. Pipkin, A. C. 1960. *Exper. Parasit.* 9:167.
6. McCoy, O. R. 1936. *J. Parasit.* 22:54.
7. Brackett, S., and A. J. Beckman. 1942. *Amer. J. Hyg.* 36:216.
8. Romanoff, A. L., and A. J. Romanoff. 1949. *The Avian Egg.* John Wiley and Sons, Inc., New York.
9. Fried, B. 1962. *J. Parasit.* 48:545.
10. Fried, B. 1965. *J. Parasit.* 51:983.
11. Fried, B., L. J. Weaver, and M. D. Kramer. 1968. *J. Parasit.* 54:939.
12. Fried, B., and L. J. Weaver. 1969. *Turtox News.* 47:39.
13. Fried, B., and R. E. Tornwall. 1969. *Proc. Helm. Soc. Wash.* 36:86.
14. Paul, J. 1960. *Cell and Tissue Culture* (2nd Ed.). Williams and Wilkens Co., Baltimore, Md.
15. Arora, K. L. 1969. *Iowa Sci. Teachers' J.* 6:14.
16. Beaver, P. C. 1937. *Ill. Biol. Monog.* 15:3.
17. Fried, B. 1962. *J. Parasit.* 48:395.
18. Fried, B. 1964. Unpublished studies.
19. Bacha, W. J. Jr. 1969. Unpublished studies.
20. Fried, B., and J. H. Caruso. 1960. Unpublished studies.
21. Fried, B., and W. Vonroth, Jr. 1968. *Exper. Parasit.* 22:107.
22. Fried, B. 1969. Unpublished studies.
23. Sinitsin, D. F. 1932. *Biol. Zentralb.* 52:117.
24. Beaver, P. C. 1937. *J. Parasit.* 23:423.
25. Fried, B., and L. R. Penner. 1964. *J. Parasit.* 50:266.
26. Fried, B. 1968. Unpublished studies.
27. Lee, D. L. 1966. In *Advances in Parasitology, IV* (B. Dawes, ed.), Academic Press, N.Y.
28. Ganote, C. E., D. L. Beaver, and H. L. Moses. 1964. *Lab. Investig.* 13:1575.
29. Fried, B., and D. A. Foley. 1969. Unpublished studies.




# HISTOCHEMICAL IDENTIFICATION OF MUCOSUBSTANCES IN THE SCOLEX OF LACISTORHYNCHUS TENUIS (CESTODA: TRYPANORHYNCHIDA)

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## ABSTRACT

The scolex of *Lacistorhynchus tenuis*, from the spiral valve of the smooth dogfish, was studied with a variety of histochemical techniques. PAS positive areas, persisting after diastase treatment, were found in the region of the apex of the scolex and in the bothridia. These areas showed a metachromatic reaction with Toluidine Blue; a positive alcianophilia with Alcian Blue at pH 1.0 and 2.5, and in 0.2M and 0.5M $MgCl_2$. Histochemical results indicate the presence of acidic, sulfated mucosubstances present in these two areas of the scolex. Moving posteriorly in the scolex, PAS positive mucostances were present in subcuticular areas but these were negative when stained with Alcian Blue under all conditions. The bothridial surfaces and the apex of the scolex are in intimate contact with the tissues of the host. The bothridia can function in locomotion of the worm. Possible functions of the acid mucosubstances may be concerned with a buffering, protective actain against the secretions and contents of the spiral valve. The presence of these materials may also contribute to the locomotor activity of the bothridia.


## INTRODUCTION

The general morphology of the scolex of Trypanorhynchid Cestodes has been the subject of a considerable amount of investigation.

The abundant literature on these morphological details of the Order were reviewed in detail by Dolfuss (2) in 1942. He cited the reports of various authors of the apparent histological structure of glands and secretory cells in the scolex and pointed out that no chemical evidence was available about the nature of any of this secretory material. Rees (8-10) has described the functional morphology of the bothridia, tentacles, and nervous system for several different species. Lumsden (6) has recently reported in the ultrastructure of the integument and parenchyma of the scolex of *Lacistorhynchus tenuis* and compared it to other species of cestodes.

Evidence for glandular structures in the scolex region has been presented for several species of tapeworms. Much of this work has already been summarized by Smyth (12) in his report on the cytochemistry of secretory cells in the rostellum of *Echinococcus granulosus*.

While observing the movement of *Lacistorhynchus tenuis*, we noted the streaming of material from the anterior tip and the bothridial surfaces of the scolex. Over a period of an hour or so of observation, a viscous adhesive residue would be left on a clean glass slide. We undertook a histochemical examination of the scolex in an attempt to identify this material.

## MATERIALS AND METHODS

Mature specimens of *Lacistorhynchus tenuis* were recovered from the spiral valve of the smooth dogfish *Mustellis canis*. The fish were collected from the pound nets maintained by the Sunrise Fish Company of Islip, N.Y. off the western tip of Fire Island. The worms were removed within 2 hours of the capture of the fish. After recovery, the worms were washed briefly in Ringer solution and then fixed in neutral buffered formalin. Serial sections, from 3 to 10$\mu$ in thickness, were prepared in all planes of the scolex. Masson's Trichrome Stain was employed to study the general morphology of the scolex. Himes and Moriber Triple Stain was used to screen the chemical composition of the scolex. A variety of staining techniques were used for the identification of mucosubstances. These included the following:

1. Periodic Acid—Schiff Reaction (PAS)
2. Alcian Blue (AB)—pH 1.0 and 2.5 (Spicer (13) 1960)
3. Alcian Blue with 0.1M, and 0.2M, 0.5M, 1.0M, MgCl (Scott, Darling and Quintarelli (11) 1964)
4. Azure A at pH 1.0, and 4.0 (Spicer (13) 1960).
5. Toluidine Blue O-

6. Diastase digestion prior to PAS Staining was used in control slides.

## RESULTS

PAS and Himes and Moriber staining revealed an abundance of Magenta and Red staining material in the scolex area. PAS positive reactions persisted in the apical region of the scolex and on the bothridial surface after diastase digestion to eliminate glycogen. Positive Alcianophilia occurred in these same regions with Alcian Blue at pH 1.0 and 2.5. A strong metachromatic reaction was indicated with Toluidine Blue - Azure A at pH 1.0 and 4.0 resulted in a metachromasia that was interpreted as an indication of acidic mucosubstances. This reaction was not as consistent and reliable in its results as were the other techniques employed. No specific reaction was observed in sections stained with Alcian Blue plus 0.1M $MgCl_2$. Positive alcianophilia was present in sections stained in MgCl concentrations of 0.2 and 0.5M. Negative results were found with 1.0M salt solutions. Scott, Darling, and Quintarelli reported that sulfated mucopolysaccharides stain selectively at concentrations of 0.2 M MgCl or greater. Chondroitin sulfates stain up to 0.5M concentrate while other rare sulfomucosubstances stain at 1.0M $MgCl_2$.

All of the histochemical tests point towards the occurrence of sulfated mucosubstances present in the scolex region. These are concentrated in the apical regions and in clusters of cells along the bothridial surface. In most sections, no apparent accumulation of mucosubstances on the external surface was observed and we assumed that the necessary saline washing of the worms after removel from the host eliminated any deposits of surface material. Proceeding posteriorly along the peduncle of the scolex, while PAS positive material was detected in the subcuticular region, the incidence of particularly Alcianophilic sulfated mucosubstances diminished rapidly.

## DISCUSSION

The bothridial surfaces and the apical regions of the scolex of *Lacistorhynchus tenuis* are two of the anatomical regions of the tapeworm that are apt to be in intimate contact with the tissues of the host. The third structure, the tentacles, are retractable and in addition penetrate beyond the mucosa to serve as the primary organs of attachment. The bothridia may contribute to the attachment but they are also of importance in the movement of the worm over the surface of the spiral valve. By alternating the action of the two bothridia, the tapeworm is capable of good degree of locomotion. The histochemical localization of the acidic sulfated mucosubstances in these two regions does not explain their function.

The role of mucins in invertebrates has not been investigated in detail. Jakowaska (4) has pointed out the small amount of information and the need for further research in this area. Among the Platyhelminthis, the occurrence of mucosubstances has been reported particularly among the various stages of the life cycle of digenetic trematodes. Dixon (1) and Rees (10) have described the importance of both acid and neutral mucosubstances in the formation of the metacercarial cysts of *Fasciola hepatica* and *Parorchis acanthus*. James and Bower (5) obtained positive results for the presence of mucoproteins and mucopolysaccharides in the developing cercaria and in the daughter of sporocysts of *Cercaria bucephalopsis haemaena*. The evidence for the mucoidal secretions of cercariae has been presented by a wide variety of authors. Among other invertebrates, the production of mucosubstances by Mollusca has been studied extensively. Here, as stated by Hillman (3), "The variety of substances described and their similarities with glycasaminoglycans of known function in higher organisms indicates that these materials may have quite sophisticated roles in the physiology of the mollusks."

In considering the role of the acidic mucosubstances in the bothridial and apical regions of the scolex of *Lacistorhynchus tenuis,* two distinct possibilities should be kept in mind. First of all, the secretion of these materials in two areas that are in intimate contact with the host tissue may well serve as a protective device by a buffering action against the secretions and contents of the spiral valve. Secondly, the mucosubstances might well contribute to the locomotor function of the bothridia and apex of the scolex by its viscous, somewhat adhesive nature. The secretion could facilitate both the adhesion of the bothridia to the host tissue as well as providing some degree of lubrication for movement.

## REFERENCES

1. Dixon, K. E. 1965. The structure and histochemistry of the cyst wall of the metachercaria of *Fasciola hepatica* L. *Parasitology* 55:215.
2. Dolfuss, R. 1942. Etude critiques sur les Tetrarhynques. *Arch. Mus. Paris.* 19:1.
3. Hillman, R. E. 1968. Histochemistry of mucosubstances in the mantle of the clam, *Mercenaria mercenaria*. 1. A glycosaminoglycan in the first marginal fold. *Trans. Amer. Micros. Soc.* 87:361.
4. Jakowska, S. 1965. The role of the zoologists in the modern research on mucous secretions. *Ann. N. Y. Acad. Sci.* 118:903
5. James, B. L., and E. A. Bower. 1967. Histochemical observations on the occurrence of carbohydrates, lipids, and enzymes in the daughter sporocyst of *Cercaria bucephalopsis haimanea*. La-Caze-Duthiers, 1854. (Digenea: Bucephalidae). *Parasitology* 57:79.
6. Lumsden, R. 1966. Ultrastructure of the medullary parenchymal cells of a Trypanorhynchid cestode *Lacistorhynchus tenuis* (v. Beneden, 1858) with emphasis on specialization for glycogen metabolism. *J. Parasitol.* 52:417.
7. Rees, G. 1941. The scolex of *Aporhynchus norvegicus*. *Parasitology* 33:433.
8. Rees, G. 1944. A new cestode of the genus *Grillotia* from a shark. *Parasitology* 35:180.



9. Rees, G. 1950. The pleroceroid larva of *Grillotia heptanchi*. *Parasitology* 40:265.
10. Rees, G. 1967. The histochemistry of the cystogenous gland cells and cyst wall of *Parorchis acanthus*, Nicoll and some details of the morphology and fine structure of the cercaria. *Parasitology* 57:87.
11. Scott, J. E., J. Dorling, and G. Quintarelli. 1964. Differential staining of acid glycosaminoglycans by Alcian Blue in salt solution. *Biochem. J.* 90:4.
12. Smyth, J. D. 1964. Observations on the scolex of Echinococcus granulosus with special reference to the occurrence and cytochemistry of secretory cells in the rostellum. *Parasitology* 54:515.
13. Spicer, S. S. 1960. A correlative study of the histochemical properties of roden acid mucopolysaccharides. *J. Histochem. Cytochem.* 8:18.

# ONCOSPHERE MOVEMENT: AN INTERESTING FEATURE OF INVASIVE EMBRYOS FROM THE TAPEWORM HYMENOLEPIS DIMINUTA

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## ABSTRACT

The rhythmic movements were coordinated and responsive to environmental factors. Each cycle, from extension through retraction, was divisible into time intervals. A 3-second cycle had extension of 1.0 sec., holding 1.2 sec., retraction 0.70, relaxation 0.1 sec. As movements slowed, holding increased. Motility required cortical musculature, various prominent cells, hook musculature, medullary retractor system, and a possible coordination system. Activation before hatching was observed. Laser-beam irradiation impaired motility when applied to central body. Motility was affected by chemicals, temperature, and mechanical stimuli.

## INTRODUCTION

The tapeworm hexacanth is an invasive embryonic stage capable of introducing the genetic program for a completely new stage into an intermediate host. It has generally been understood that the primary means for doing this depends on strong body movements. These movements are characterized by: 1) rhythymicity; 2) strong hook and body action; 3) coordination; 4) responsiveness to environmental factors; 5) temporary state of activity, since oncospheres become inactive during metamorphosis; and 6) orientation of the oncosphere body with the anterior hook-bearing end pointed in the direction of progression. The oncosphere has its own special organization: anterior-posterior axis passing between the hooks, a marked degree of bilateral symmetry, the functional embryonic anterior body (somatophore) which contains the hooks and their musculature, the posterior body (mesophore) which contains large embryonic cells. The uppermost dorsal aspect bears the lateral hooks; the ventral aspect faces the substrate below and bears the medial hooks.

The purpose of the present summary is to provide a general look at the features that characterize oncosphere movement. Perhaps this will aid in directing research toward the areas of promise.

Historical survey indicates a number of brief descriptions but no major papers devoted to movements or musculature. The early descriptions were made of oncospheres from fish parasites. Meggitt (1) examining an oncosphere from *Proteocephalus filicollis* said:

> "It slowly contracts antero-posteriorly, stretching out its hooks as far as possible . . . with a quick jerk it elongates . . . at the same time striking downwards with its hooks until they lie flat against the body. It then contracts as before." (p. 132)

Another account was given by Essex (2) for oncospheres from the cestode *Corallobothrium fimbriatum:*

> "After watching their movements for hours, it became quite evident that the hooks were incapable of independent action; that their extension only occurred when the body of the oncosphere was elongated . . .; and that their return to a position of rest occurred only when the oncosphere again assumed a spherical form, i.e., the elongation of the oncosphere extended the hooks and its contraction withdrew them. The presence of muscle fibers attached to the hooks is extremely doubtful. . . ." (p. 35)

Brief observations were also made for *Crepidobothrium* (3), *Archigetes* (4), *Ophiotaenia* (5), and *Proteocephalus* (6, 7). In the latter case some musculature was described moving the hooks and body. Early reports by Janicki and Rosen (8) for *Diphyllobothrium latum* noted movements but did not see musculature to accomplish it. Classic papers by Vogel (9, 10) described morphology and movements of coracidium and the hexacanth embryo of *D. latum.* The first detailed description of musculature was given by Michajlow (11) for *Trianeophorus nodulosus* oncospheres with a more brief account of motility. A brief description was given by Clarke (12) for movements of oncospheres from another pseudophyllidean. Observing coracidia of *Schistocephalus solidus,* he confirmed that in retractions, hooks were pulled in; during elongation, hooks were thrust forward toward the direction of coracidial movement, and that constrictions appeared and disappeared behind the hooks.



Descriptions of motility in cyclophyllidean oncospheres were made in relation to invasion of intermediate hosts, activation, and viability. A new vivid description was provided by Stunkard (13) for protraction and retraction of the small anoplocephalid embryos of *Cittotaenia.* Viability and motility of oncospheres from species of *Taenia* have been given some attention (14, 15, 16). The more advanced studies of Silverman (17, 18) included viability, activation, hatching, hook musculature, and actions. He also reported (19) the in-vitro effect of anti-serum on oncosphere motility.

Movements of oncospheres were diagrammed by Witenberg (20) for *Mesocestoides* but musculature was not observed. Observations by Ogren (6, 21) added details to the movements and revealed musculature in living and stained specimens. Motility in *Dipylidium caninum* oncospheres has been observed (22, 23) and Venard's account is one of the more detailed. Studies on *Raillietina cesticillus* include accounts of viability, temperature effect, and activation (24, 25, 26). New features for musculature and movement were provided by Ogren for *Oochoristica symmetrica* and *Dilepis undula* (27, 28).

Oncospheres of *Hymenolepis* have provided good material for investigation. Isobe (29) classified movements into rapid and slow, then tested effects of diverse substances in one of the first papers devoted to physiology of motility and activation. Kiribayashi (30) employed *H. nana* and correlated oncosphere movements with temperature. Reid, Allaman, and Fitch (31) showed how hook movements aided escape from membranes in hatching. Electron microscopic studies of muscle and cell organization were recently given by Collin (32, 33) for *H. citelli.* The following studies all employed *H. diminuta.* Movements were redescribed in some detail by Ogren (34), with photomicrographs showing enlargements of the posterior body as a regular feature of retraction. Contractile movements of giant AA′ cells correlated with body actions were described (35). Oncosphere morphology providing location of somatic and embryonic cells, some of which are associated with musculature (36, 37), was given. Relative times required for actions and holding were reported (38) and expanded in the present paper. Oncosphere musculature, fibers, and cells were illustrated with special attention being given to extensor and retractor systems (39). The effects of increased salt concentrations on movements and survival were reported (40). Most recently, dilute neutral red solutions have been shown to reduce motility associated with selective staining of giant cell protoplasm (41).

Most published accounts merely report the rhythmic extension and retraction of hooks without regard to related body changes, except for occasional mention of a constriction behind the hooks and changes from elongated to spherical shapes. There are also inconsistencies as to the order of hook movements. Three patterns are mentioned: 1) lateral and medial hooks protrude together; 2) medial hooks protrude first (10, 23); 3) lateral hooks protrude first, followed by the medial pair (13, 21, 27, 34).

The first view may result from failure to differentiate the separate actions. The second and third views appear to suggest the existence of two distinctly opposite patterns. However, it is reasonable that only one pattern exists and the differences are due to interpretation and difficulties in separating the actions. The action described here for *Hymenolepis* fits the *third pattern.* Extension of the body coincides with protrusion of lateral hooks, whereas protrusion of the medial hooks depends on lateral hook action and correlates with enlargement of the anterior body. Thus, in the *third view,* the only one mechanically possible, the medial hooks cannot be protruded without previous action of the laterals.

## METHODS

A variety of techniques have been used (36, 37) as reported elsewhere in this volume (41). Oncospheres, free of shell and of all membranes, were observed in a suitable physiological solution. The rates of movement were counted visually with the aid of a laboratory timer. Records were also made by simple manual signal and ink marked kymograph or physiograph. Short strips of cine film were taken with a 16-mm motion picture camera. Time factors were obtained from this record by counting the number of frames required for given movements and records of activities. Additional records were made by 35-mm photomicrographs. Temperature was controlled when desired by use of a simple stage incubator.

## MORPHOLOGY OF MOVEMENTS, TIME FACTORS, AND RATES

A sequence of changes in body shape, elongated to spherical (2, 34), characterized the rhythmic activity (Fig. 1-5). During *extension,* the lateral hooks were protruded first, then abducted backward, while a constriction developed behind the hooks; medial hooks were next protracted, while the anterior body ex-

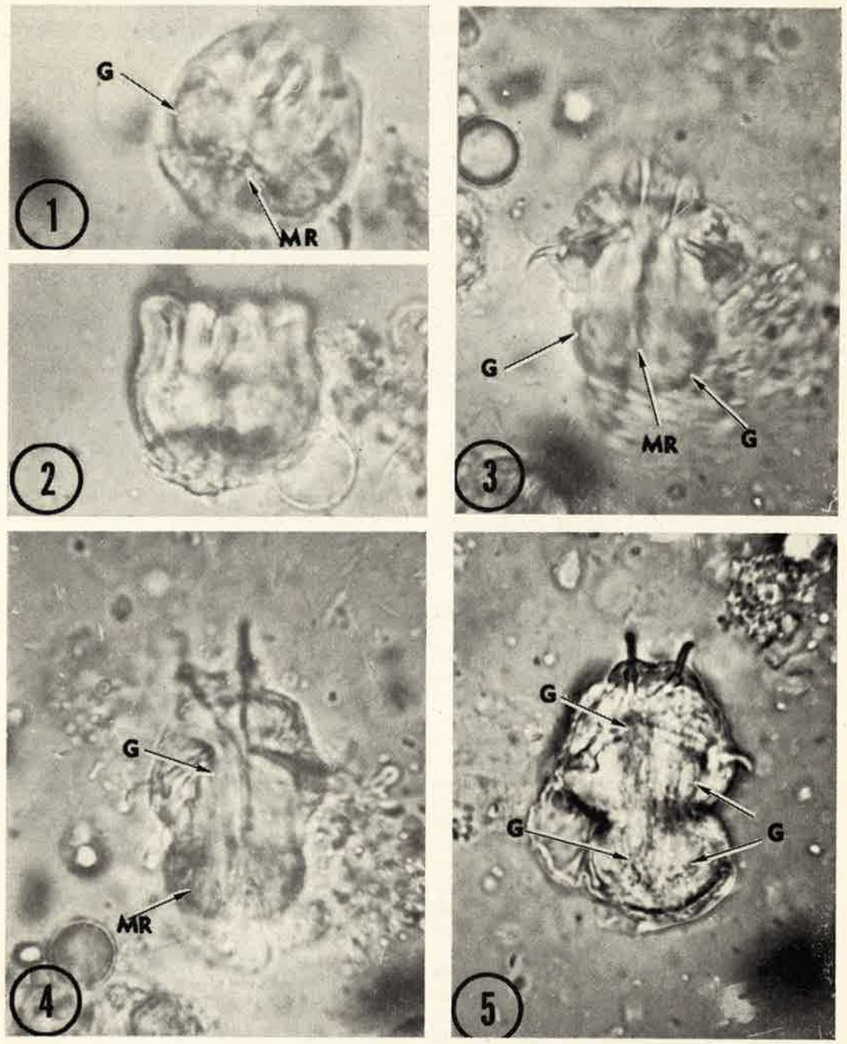



**Figure 1-5. A sequence of postures shown by oncospheres during one movement cycle. These postures were assembled from separate 35 mm frames taken during prolonged activity of oncospheres in artificial hatching solution. The giant AA′ cells (G) are evident in the posterior region. In Fig. 1, 3, 4, the shadow of the retractor system is visible (MR).**
**1) Retraction; 2) Beginning of protraction for lateral hooks; 3) Abduction or back-stroke of lateral hooks, beginning of medial hook protraction and body constriction; 4) Elongation, medial hook abduction; 5) Holding, deep constriction.**



panded and the posterior body narrowed as the contraction was completed. The lateral hooks made a rowing motion and the medial hooks performed a clawing action toward the substrate. The final morphology was a constricted peanut or vase-shape. *Retraction* occurred quickly, pulling the hooks into the shortened body; the anterior body contracted, narrowed, and folded inwards forming a pocket around the hooks, the posterior body enlarged and became rounded. A pyriform shape resulted, with the largest volume behind the hooks. The following events took place: 1) lateral hook protraction, 2) anterior enlargement and mid-body constriction, 3) medial hook protraction, 4) holding, 5) retraction, and 6) posterior enlargement.

The above sequence occurred within definite time intervals. The normal rate of movement varied, depending on conditions, from 15 to 35 extensions per minute. Observations and analysis of motion picture frames demonstrated that each cycle at a rate of 20/min required 3 sec. In this cycle each extension and retraction had an initial action period and a holding or resting period (Fig. 6). Representative time intervals

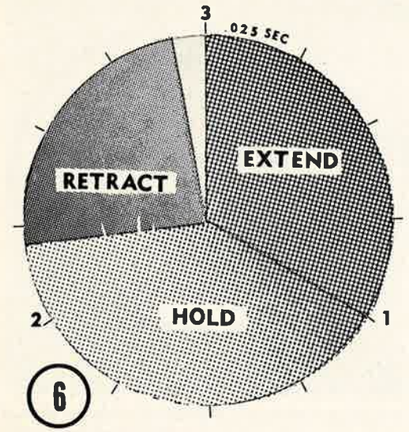



**Figure 6. Time allocation of one movement cycle.**

for the 3-sec cycle were: extension 1.0 sec., holding 1.20 sec; retraction 0.70 sec, relaxation 0.1 sec. It was during the period of holding that forward progression occurred due to the powerful thrusting of the hooks. Records of movements during visual observation were made by ink recorder through a physiograph. The recorded cycle for a specimen active at 30/min required 1.8 to 2.0 sec. Extension required 1.5 sec. The lateral hooks protracted 0.5 sec in advance of the medial hooks. There occurred a 1.0 sec holding period. The lateral hooks moved for 1.5 sec to complete their cycle of protraction through abduction. Retraction required 0.5 sec and followed quickly after completed body constriction.

There were variations in the duration of the cycle (Fig. 7). At the highest rate recorded

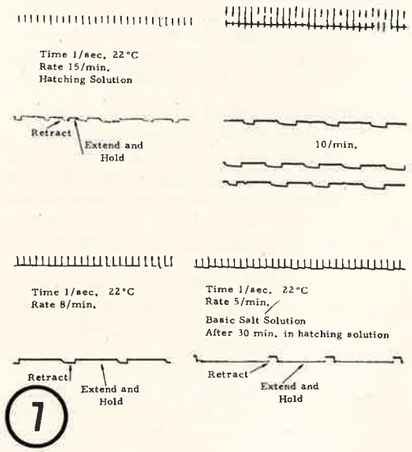



**Figure 7. Ink tracings made with a signal magnet during visual observation of oncosphere activity. The observer closed the circuit as the body retracted, thus inscribing an ink mark on the moving kymograph drum.**

(40/min.), each cycle required only 1.5 sec. On the other hand, as the rate decreased to less than 15/min, the duration of each cycle increased from 4.0 to 7.50 sec. The individual phases varied, with extension and holding requiring the longest periods, and retraction being shorter and more rapid. When the rate slowed, all phases were longer, but holding had the greatest duration. In rapid movements, the holding phase was reduced. In very-rapid activity the hooks were protracted, then quickly pulled back only to be protracted again. This rapid rhythm had very little pause between events. More moderate activity was characterized by holding pauses of variable duration. Prolonged observation of single oncospheres revealed that pauses varied in a random man-

ner. A long pause would be followed by more regular movements.

Isobe, (29), in studies on the activities of oncospheres from *Hymenolepis diminuta,* recognized two types of movement: 1) type A, quite vigorous movements; and 2) type B, very dull sluggish movements. The time factors for these were not given. Similar motions were observed in our studies. The differences were complex and involved rate, strength of contractions, frequency, and relative holding periods. Type-B motion was characteristic of dying or injured oncospheres. Type-A motion, on the other hand, was typical of normal embryos and could be subdivided into 1) very rapid rhythm with little holding and 2) moderate rhythm with distinct holding within each cycle.

## CONTRACTILE SYSTEMS INVOLVED IN BODY AND HOOK MOVEMENTS

The entire body was a muscular bag of contractile elements. Muscle fiber contractions in live preparations were observed in bright field, phase contrast, interference contrast microscopy. The contracting fibers showed marked birefringence with crossed Nicol prisms in polarizing microscopy. The following systems were involved: 1) sub-cuticular sheets of muscle beneath the covering, largely oblique and circular directions; 2) giant AA′ cells in the body cortex with ameboid-like contractions related to body movements; 3) musculature moving the hooks based on cortical attachments; and 4) medullary musculature of fibers and cells responsible for retraction. Although not clearly identified at the present time, a primitive coordination center may exist.

### THE SUBCUTICULAR MUSCLE SHEET

Beneath the thin body coat, a layer of muscle was laid down during oncosphere morphogenesis. This layer, especially thin in the immature Stage II condition, became progressively thicker and reached its maximum by invasive Stage IV. Electron micrographs by Collin (32, 33) permitted a detailed view of these fibers. However, they were seen by interference microscopy and with crossed Nicol prisms, in live oncospheres. Circular, longitudinal, and oblique fibers were observed. Their birefringence appeared momentarily during contractions. These fibers appeared to be under regular tonus contracting down on the more fluid body. Longitudinal contractions rounded the body, whereas circular and oblique contractions served as constricting waves compressing the front, the middle, or the rear of the body. The subcuticular sheet along with the confined body fluids and cells constituted a type of hydraulic skeleton capable of dynamic changes. There were a few somatic nuclei associated with this musculature situated largely at the rear of the body. The margins of the giant AA′ cells also appear to be specifically associated with this sheet of fibers in stained preparations (36) and in living specimens.

## PROTOPLASMIC MOVEMENTS OF CORTICAL GIANT AA′ CELLS

In oncospheres of *Hymenolepis,* these granular-appearing cells had extensive protoplasm spread out beneath large areas of the subcuticular muscle. It was originally assumed that changes in shape of these cells were due to compression of overlying muscles (Reid, 42). However, my observations (34, 35) revealed that each giant cell had its own contractile

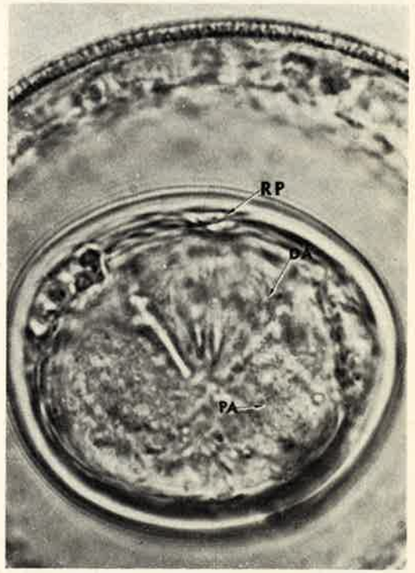



**Figure 8. Invasive hexacanth embryo (Stage IV) enclosed in outer shell and protective membranes. Oncosphere is in a retracted, quiescent posture. The retraction pocket (RP) is formed by retraction of the medial hooks. The giant cells are the granulated regions to the right and left behind the hooks. Each giant AA′ cell has a distal protoplasm (DA) and a broad proximal protoplasm (PA). Enlargements in (Fig. 9, 11) are from the right and left areas of this figure. Leitz, 100X Plano APO oil immersion objective.**



properties very much like ameboid protoplasm. A distinct protoplasmic mesh appeared to contract in the cell. In development, these cells manifested an early capacity for movement and, when activation occurred during hatching, they showed the first signs of contractile activity. The pair formed a prominent U-shaped area, each lateral cell with its own nucleus, distinct proximal and distal portions, and strong protoplasmic activity (Fig. 8).

## INACTIVE CONDITION WITHIN PROTECTIVE SHELL (Stage IV)

During oncosphere morphogenesis, musculature and the capacity for movement developed. However, in the invasive condition, the oncosphere was confined within a protective shell and envelopes, and its movements were suppressed.

In order to examine the inactive condition of the oncosphere, yellowed eggs from the stools were washed, placed in mammalian Ringer solution, and covered with a vasoline-sealed cover glass, without compression. The Leitz Plano 100x APO oil immersion objective gave the best resolution. In this stage the oncosphere was motionless, in a retracted posture with the hooks pulled inward nearly parallel. A small depression or pocket was produced in front of the body between the hooks as the result of their retraction. The lateral hooks were pulled inwards toward the midline, being held in this position until initiation of body movements. The oncosphere remained in this quiescent condition through long periods of observation, and even slow oscillations could not be verified.

During the quiescent period, a type of activity was observed in the protoplasm of the giant cells. From a dorsal view the giant AA′ cells appeared as somewhat C-shaped, granulated, or stippled gel areas. The *proximal,* or main protoplasm, was expanded behind the hooks and followed the body contour from dorsal to part of the ventral cortex. The *distal* protoplasm, between the hooks, formed lobed and narrow dactyliform processes, curved ventrad toward the midline along the frontal pocket. They terminated as dense rounded endings or as protoplasmic fibers. The moderate-sized nucleus with its nucleolus, was usually peripheral at the rear sides of the proximal protoplasm and was at times hard to locate, since easily confused with adjacent nuclei.

The viscous cytoplasm of the giant cells contained a distinct meshwork somewhat more gel than the surrounding fluid cytoplasm. Agitation in the gel meshwork in some specimens was evident due to Brownian movement in the semifluid surroundings. Close inspection of the meshwork for long periods of time revealed that it had a three-dimensional structure and was in a constant state of very slow change resulting in gradual breakdown of one mosaic and reformation of another. The meshwork strands showed slow periodic changes, expansions, fusions, constrictions to form a beaded condition or dissolution. Along the hyaline margins of the cell, in continuity with the protoplasmic muscle sheet, there were also changes, with slow appearance of a mesh and slight enlargement of the border, then a change in meshwork leaving more homogeneous protoplasm. The meshwork could appear and disappear in presumably homogenous protoplasm. Four arrangements were common: 1) parallel strands, beaded or wavy; 2) ladder units of parallel strands joined by short cross bridges; 3) radial strands leading to a central circular strand, or an open ring; 4) unoriented meshwork (Fig. 9-12).

## ACTIVATION AND HATCHING

In the case of *Hymenolepis diminuta,* eggs dissected from gravid proglottids were enclosed by shell and membranes in varying degrees of completion, depending on the level of morphogenesis. In these cases simply placing the eggs in a favorable solution resulted in activation of the oncosphere, as was shown by Isobe (29). For our studies we used yellow, tanned, eggs recovered from the stools. These were relatively impermeable to aqueous media. Oncospheres so protected remained inactive for several weeks in distilled water or brine solutions. However, cracking the shell by a microprobe or pressure on the cover glass admitted fluid and released the embryo enclosed only by colloidal envelope.

There was a latent period of 45 to 75 seconds before movements began. During the initial 30 seconds, fluid appeared to be diffusing inwards. The oncosphere was motionless. Then suddenly the giant AA′ cell protoplasm and meshwork of the proximal region expanded, then relaxed. There was a slight increase in fluidity of the protoplasm as granules became agitated. Next, there was a slight increase in movement of the meshwork, followed by a second short contraction. In a few seconds the lateral hooks moved, then the entire body moved, until the oncosphere was thrashing about at a fast rhythm. Areas of homogeneous protoplasm beneath the cortex developed meshwork, then became homogeneous as contractions occurred. The giant cell meshwork itself contracted. On relaxation

there was an expansion, the borders spread out; but on contraction the borders were distinct and compact. Activation showed some uncoordinated movements but these were soon lost. The initial rate of body movements was often the highest, but could be suppressed, depending on the media employed. In other cases, the rate began moderately, rose to a maximum, and declined during the first 20 minutes. These observations suggested that the giant AA′ cells served some role in activation and motility (41).

### CORTICAL HOOK MUSCULATURE

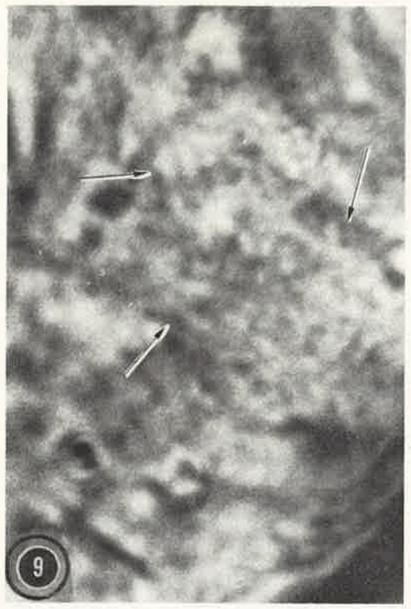
The body musculature served primarily to move the hooks and thus accomplish invasion of intermediate host. Cortical fibers attached to the hooks served to protract and pull the hooks backwards, while a medullary fiber system was active in retraction (39). Only a brief account will be given here. Each pair of hooks had cortical muscle fibers for protraction, abduction, and adduction. All actions were aided by contraction of the subcuticular muscle sheet. Hook movements had two fundamental purposes: 1) tearing through a covering or surface; and 2) migration by forward progression.

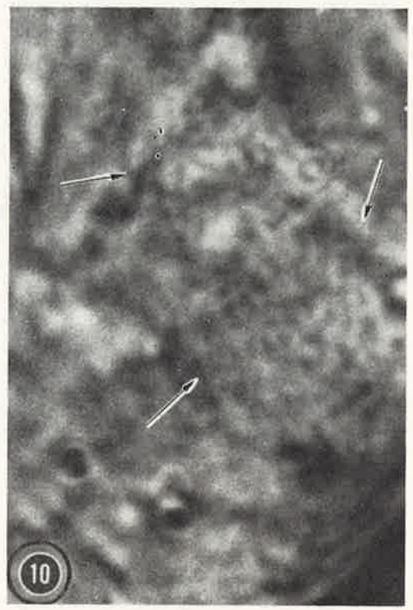
### MEDULLARY RETRACTOR SYSTEMS

The specialized aspects of cells and musculature responsible for hook retraction required further detailed study, although some observations have been published (27, 28, 36, 38, 39). Three subdivisions were recognized: 1) the medullary center; 2) an axial longitudinal system; and 3) the large embryonic cells (Class II, BB′, EE′).

### CHANGES IN COORDINATION SHOWN BY LASER-BEAM IRRADIATION

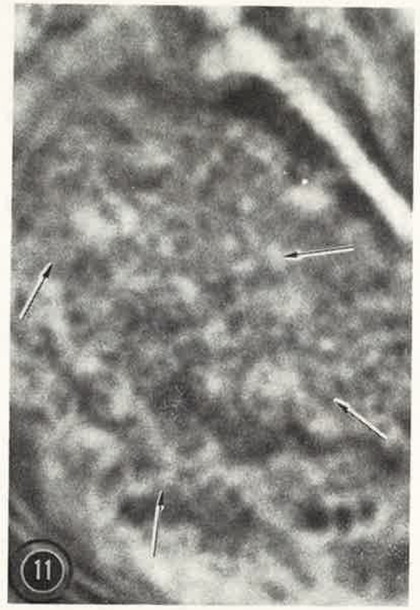
Normal movements at moderate-to-fast rates showed a high degree of coordination. Hooks were protracted and retracted. Constriction of the posterior was coordinated with the enlargement of the anterior, pulling the body forward. As movements slowed, however, the regularity was impaired and uncoordinated actions occurred. Spasms were observed of one group of muscles, resulting in local hook movements, prolonged holding or local subcuticular contractions. The center for coordinated action was not identified with certainty. It was postulated that the medullary center, as well as other cells, may be involved.

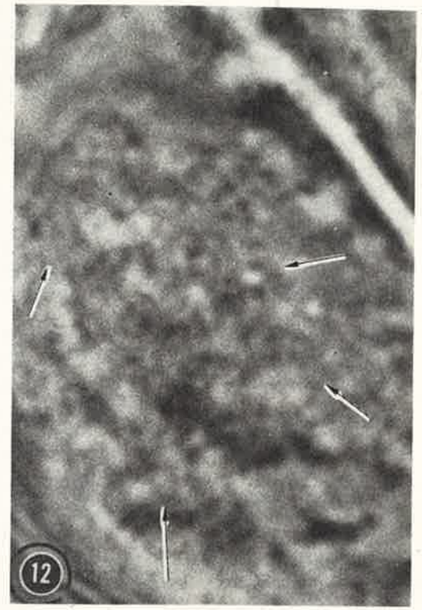



**Figures 9, 10. Comparison of right giant A cell taken at two different time intervals. Enlargements made from quiescent condition (Fig. 8). Giant cell meshwork shows definite slow changes when two pictures taken several minutes apart are compared. Changes in pattern can be seen distinctly near and between the arrows. Objective used was Leitz 100X APO Plano. Exposure was 10-second duration.**






**Figures 11, 12. Comparison of left giant A cell with an exposure taken several minutes later. Changes in the meshwork can be seen near and between the arrows. Some of the meshwork can be seen stretched over the lateral hook. The protoplasmic margin of the cells is distinct in these prints.**

Evidence for a coordination center was supplied by studies with a biolaser. Through the courtesy of Mr. R. W. Forestieri, Sales Engineer, TRG Incorporated, exploratory studies were conducted with their Model 513 Biolaser System. A beam of approximately 20 microns diameter and a pulse output of approximately 180 millijoules was used. Two sites with known contractile activity were treated.

1) A pulse applied to the medullary center between the lateral hooks produced complete inactivation of the hook movements. However, although the hooks were inactive, the protoplasm of the giant AA′ cells continued to show regular contractile movements, especially in the proximal region around the nucleus.

2) A pulse was applied over the proximal area of one giant AA′ cell, just in front of its nucleus, and all body movements ceased. After about a minute, hook movements returned. However, the injured cell showed no activity, manifested an injured hyaline area near its nucleus, and was enlarged when compared to the uninjured control cell. The uninjured control cell on the opposite side of the body continued to be contractile. Next, a second pulse was given to the medullary center between the hooks. The regular body movements ceased. After a few minutes some regular contractions returned to the injured tissue over the proximal region of the control cell.

Four conclusions were made from these preliminary trials: 1) the protoplasm of the giant cells contracted independently of the main musculature; 2) one giant cell could contract independently while the other was inactivated; 3) the giant cells have some importance for movements; 4) the medullary center may be important for coordinated hook movements.

## RESPONSE TO MECHANICAL STIMULUS

Isobe (29), studying eggs of *Hymenolepis diminuta* from dissected proglottids, was one of the first to discover that an increase in motility resulted from mechanical stimulus, arising through taps on the cover glass.

We have confirmed this by more direct methods. Individual hatched oncospheres, free of membranes, were placed in basic salt solution (BSS) or mammalian Ringer solution in depression slides and their rate of movement determined. They were next transferred by micropipet to a fresh drop of media. The rate of movements increased after each transfer, then slowly returned to near original value. Free oncospheres in Ringer solution, when probed with a micromanipulator needle, responded with increased activity.

## RESPONSE TO CHANGES IN TEMPERATURE

Unhatched oncospheres were shown by Isobe (29) to increase their activity with an increase in temperature and to decrease activity in low temperature. Kiribayshi (30) correlated movements with temperature for oncospheres of *H. nana.* Using *Raillietina cesticillus,* it has been shown that storage at low temperatures promoted greater degree of activation by eggs removed from proglottids, stored, then tested for motility at room temperature (24, 26, 43). We performed a series of experiments with oncospheres of *Hymenolepis diminuta* enclosed only by colloidal envelope or free of all membranes (40, 41). Their rate of movement declined at temperatures below 21°C and increased at higher temperatures. Up to 26°C the increased rate was generally proportional to rise in temperature, about one cycle for each 2 degrees. Above 27°C the rate tended to decline. The influence of temperature on activity and hatching was investigated by Berntzen and Voge (44) for hymenolepidid cestodes. A temperature of 25°C was optimum for *H. diminuta;* temperatures of 37 to 39°C were unfavorable with immobilization or decreased activity.

## RESPONSE TO CHEMICALS IN SOLUTION

The degree of motility was increased or decreased in various inorganic and organic media. This was shown by Isobe in his studies on embryos from dissected proglottids (29). He tested a wide variety of substances for their ability to produce rapid or slow motility. Activation and favorable movements occurred in dilute inorganic salt solutions: NaCl, KCl, $CaCl_2$, $Na_2CO_3$, $NaHCO_3$, NaOH, KOH, $K_2SO_4$, $Na_2SO_4$. Solutions of grape sugar were also favorable. He further tested a variety of organic substances and found that motility was adversely affected by the following: alcohol, chloral hydrate, morphine, cocaine, atropine, tetrodotoxin (Sankyo), quinine, emetin, adrenaline. Observable effects were not produced by curare. The investigations of Berntzen and Voge (44) on the effect of chemical factors and enzymes on hatching of *Hymenolepis* reflect their favorable or unfavorable influence on motility as one factor in hatching.

During the past few years students and I have made exploratory studies, developing a technique for studies on motility. Many attempts have been inconclusive, due to difficulties in controlling variables. Published studies showed that increase salt concentrations or dilute concentrations of neutral red or acroflavine decreased rate of movements and survival time (40, 41).

## DISCUSSION

The above survey indicates a beginning in the physiology of oncosphere movement. This is a complex motility, dependent on most cell systems of the hexacanth. Certain features stand out as important to the acts of penetration and invasion of the host tissues. *First,* the morphology of the hooks, their special actions and musculature, are well-designed for this task. *Second,* response of the oncosphere to mechanical stimuli is an aid in hatching and penetration. Movements of the foregut during ingestion and peristalsis could readily stimulate the oncosphere to stronger, more-rapid actions. *Third,* response of the oncosphere to chemical stimuli in the alimentary tract and tissues may cause more rapid and more vigorous movements to aid in migration. Moreover, chemical substances could also decrease motility and prevent penetration. A type of primitive chemotaxis may also be taking place if the oncosphere is able to move into a chemical gradient that increases motility.

The time factors of movements require further examination with aid of an electronic system for recording activity. Are some of the contractile systems slow, e.g., extensor; and others fast, e.g., retractor? Is there a coordination center? How does the embryo maintain its rhythm? Is it myogenic? Does it depend on a primitive neuromuscular system?

The studies of activation made to date suggest differences among the groups of tapeworms. Reviews (17, 43, 44) suggest that species of *Taenia* require different conditions than those of *Hymenolepis.* In our own experience with *H. diminuta,* only two factors were needed for activation: 1) removal or cracking of the shell, and 2) uptake of fluid.



A mechanism to explain the long quiescent period before hatching is needed. It is possible that medullary musculature upon completing its differentiation, retracts, holding the body in this one posture. Another possibility is the presence of an enzyme inhibitory system suppressing the systems responsible for motility. Activation would be the release from this restraint.

## ACKNOWLEDGMENTS

This contribution #29 to the cytology and differentiation of oncospheres was supported by research grants from the National Science Foundation (Gl 2905, GB 1480, Gb 4328), The Pennsylvania Academy of Science, and Wilkes College. The studies were aided by the following students: David R. Rilling, Juanita Skarnulis, Ann Wellington, Kathleen Dennis, Maria Supko Ross, Maureen Flanely, and Rita Budnar.

## LITERATURE CITED

1. Meggitt, F. J. 1914. The structure and life history of a tapeworm *(Ichthyotaenia filicollis* Rud.) parasitic in the stickleback. *Proc. Zool. Soc. London* 1:113.
2. Essex, H. E. 1927. The structure and development of *Corallobothrium. Ill. Biol. Monographs* XI(3): pp. 1-75.
3. Magath, T. B. 1929. The early life history of *Crepidobothrium testudo. Ann. Trop. Med. and Parasitol.* 23:121.
4. Wisniewski, L. W. 1930. Das Genus *Archigetes* R. Leuk. Eine Studie zur Anatomie, Histogenese, systematik und Biologie. *Mem. Acad. Polon.* III. B2: 1-60 pp.
5. Thomas, L. J. 1941. The life cycle of *Ophiotaneia perspicua* La Rue, a cestode of snakes. *Rev. med. trop. y parasitol. bact. clin. y lab.* 7:74.
6. Ogren, R. E. 1953. *Concepts of Early Tapeworm Development Derived from Comparative Embryology of Oncospheres.* Thesis: Univ. of Ill. Grad. School.
7. Ogren, R. E. Studies on the embryonic development of a proteocephalid tapeworm. (Abstract). *J. Parasitol.* 43: (5, 2):31.
8. Janicki, C., and F. Rosen. 1917. Le Cycle evolutif du *Dibothriocephalus latus. Bull. Soc. Neuchatel. Sci. Nat.* 42:19.
9. Vogel, H. 1929. Studien zur Entwicklung von *Diphyllobothrium.* I. Teil: Die Wimperlarvae von *Diphyllobothrium latum. Z. Parasitenk.* 2:213.
10. Vogel, H. 1930. Studien uber Entwicklung von *Diphyllobothrium.* II. Teil: Die Entwicklung des Proceroids von *Diphyllobothrium latum. Z. Parasitenk.* 2:629.
11. Michajlow, W. 1933. Les stades larvaires de *Triaenophorus nodulosus* (Pall.) I. Le Coracidium. *Ann. Parasitol. Humaine et Comp.* 11:339.
12. Clarke, A. S. 1954. Studies on the life cycle of the pseudophyllidean cestode *Schistocephalus solidus. Proc. Zool. Soc. London* 124:257.
13. Stunkard, H. W. 1934. Studies on the life history of anoplocephaline cestodes. *Z. Parasitenk.* 6:481.
14. Yoshino, K. 1933. Studies on the post-embryonal development of *Taenia solium.* Pt. I. On the hatching of the eggs of *Taenia solium.* (In Japanese) *Taiwan Igakki Zasshi* 32:1392.
15. Penfold, W. J., H. B. Penfold, and M. Phillips. 1937a. The criteria of life and viability of mature *Taenia saginata* ova. *Med. J. Australia,* yr. 24, v 2:1.
16. Penfold, W. J., H. B. Penfold, and M. Phillips. 1937b. Artificial hatching of *Taenia saginata* ova. *Med. J. Australia,* yr. 24 v. 2:1039.
17. Silverman, P. H. 1954a. Studies on the biology of some tapeworms of the genus *Taenia.* I. Factors affecting hatching and activation of taeniid ova, and some criteria of their viability. *Ann. Trop. Med. and Parasitol.* 48:207.
18. Silverman, P. H. 1954b. Studies on the biology of some tapeworms of the genus *Taenia.* II. The morphology and development of the taeniid hexacanth embryo, with some notes on the state of development and propagation of gravid segments. *Ann. Trop. Med. and Parasitol.* 48:356.
19. Silverman, P. H. 1955. A technique for studying the *in vitro* effect of serum on activated taeniid hexacanth embryos. *Nature* 176:598.
20. Witenberg, G. 1934. Studies on the cestode genus *Mesocestoides. Arch. Zool. Italiano* 20:467.
21. Ogren, R. E. 1956. Development and morphology of the oncosphere of *Mesocestoides corti,* a tapeworm of mammals. *J. Parasitol.* 42:414.
22. Chen, H. T. 1934. Reactions of *Ctenophalides felis* to *Dipylidium caninum. Z. Parasitenk.* 6:603.
23. Venard, C. E. 1938. Morphology, bionomics, and taxonomy of the cestode *Dipylidium caninum. Ann. N.Y. Acad. Sci.* 37, Art. 5:273.
24. Nice, S. 1940. Improved methods of activating tapeworm embryos. *Bios* 20:128.
25. Reid, W. M., J. E. Ackert, and A. A. Case. 1938. Studies on the life history and biology of the fowl tapeworm *Raillietina cesticillus* (Molin). *Trans. Amer. Microsc. Soc.* 57:65.
26. Reid, W. M., S. J. Nice, and R. C. McIntyre. 1949. Certain factors which influence activation of the hexacanth embryo of the fowl tapeworm, *Raillietina cesticillus* (Molin). *Trans. Ill. Acad. Sci.* 42:165.
27. Ogren, R. E. 1957. Morphology and development of oncospheres of the cestode *Oochoristica symmetrica* Baylis, 1927. *J. Parasitol.* 43:505.
28. Ogren, R. E. 1958. The hexacanth embryo of a dilepidid tapeworm I. The development of hooks and contractile parenchyma. *J. Parasitol.* 44:477.
29. Isobe, M. 1926. The significance, physiochemical conditions, and pharmacological studies on the movement of the hexacanth embryo. *Acta. Sch. Med. Uni. Kioto* 8:519.
30. Kiribayashi, S. 1933. Studies on the growth of *Hymenolepis nana,* with special reference to the possibility of differentiation of *H. nana* var. *fraterna* (Stiles). *Taiwan Iggakkai Zasshi, Taihoku* 32:117, (Eng. Suppl.).
31. Reid, W. M., L. Allaman, and F. Fitch. 1951. Some factors involved in the hatching of *Hymenolepis diminuta* oncospheres. (Abstract). *J. Parasitol.* 37(5-2):24.
32. Collin, W. K. 1968. Electron microscope studies of the muscle and hook systems of hatched oncospheres of *Hymenolepis citelli* McLeod, 1933 (Cestoda: Cyclophyllidea). *J. Parasit.* 54:74.
33. Collin, W. K. 1969. The cellular organization of hatched oncospheres of *Hymenolepis citelli* (Cestoda, Cyclophyllidea). *J. Parasitol.* 55:149.
34. Ogren, R. E. 1961. The mature oncosphere of *Hymenolepis diminuta. J. Parasit.* 47:197.
35. Ogren, R. E. 1967a. Observations on the protoplasmic component of the epidermal gland in living oncospheres of *Hymenolepis diminuta. Trans. Amer. Microsc. Soc.* 86:68 (Abstract).
36. Ogren, R. E. 1967b. The cellular pattern in invasive

oncospheres of *Hymenolepis diminuta* as revealed by an enzyme-acetic acid-orcein method. *Trans. Amer. Microsc. Soc.* 86:250.

37. Ogren, R. E. 1968. Characteristics for two classes of embryonic cells in oncospheres of *Hymenolepis diminuta* stained for cytoplasmic substances. *Trans. Amer. Microsc. Soc.* 87:82.
38. Ogren, R. E., D. R. Rilling, and C. A. Wellington. 1962. Observations on movements of infective oncospheres from the tapeworm, *Hymenolepis diminuta*. *Amer. Zool.* 2:545 (Abstract).
39. Ogren, R. E., and R. M. Magill. 1963. Demonstration of protein in the protective envelopes and embryonic muscle in oncospheres of *Hymenolepis diminuta*, a tapeworm of mammals. *Proc. Penna. Acad. Sci.* 36:160.
40. Ogren, R. E., and J. J. Ogren. 1964. The effect of increased salt concentrations on the rate of movement and survival of oncospheres from the tapeworm, *Hymenolepis diminuta*. *Amer. Zool.* 4:291 (Abstract).
41. Ogren, R. E., J. J. Ogren, and J. J. Skarnulis. 1969. Effects of salt and neutral red solutions on movements of invasive oncospheres from the tapeworm *Hymenolepis diminuta*. *Proc. Penna. Acad. Sci.* 43: In Press.
42. Reid, W. M. 1948. Penetration glands in cyclophyllidean oncospheres. *Trans. Amer. Microsc. Soc.* 67:177.
43. Read, C. P., and J. E. Simmons. 1963. Biochemistry and physiology of tapeworms. *Physiological Reviews* 43:263.
44. Berntzen, A. K., and M. Voge. 1965. *In vitro* hatching of oncospheres of four Hymenolepidid cestodes. *J. Parasit.* 51:235.



# FINE STRUCTURE OF THE CEPHALIC GLAND CELLS OF THE CERCARIA OF CRYPTOCOTYLE LINGUA

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## ABSTRACT

Membrane-bounded secretory granules or droplets, produced by cephalic (penetration) gland cells of *Cryptocotyle lingua* cercariae, were studied by electron microscopy. Rough endoplasmic reticulum and Golgi bodies appear to take part in granule production by way of a sequence of morphologically discernible stages. Electron-lucent Golgi vacuoles may condense into presumptive granules containing an eccentric dense core in a moderately dense matrix. In later developmental stages, dense spheroids appear to be emitted from the secretory granules. Microtubules, located along the inner margins of the plasma membrane of both gland cells and ducts, are arranged parallel to the direction of flow in ducts. The possible involvement of these structures during the parasite's invasion of the host is discussed.

## INTRODUCTION

Secretions produced by cephalic or penetration gland cells of many trematode cercariae are thought to aid the parasite during its invasive maneuvers into an intermediate or definitive host. According to studies on *Cryptocotyle lingua* (1), *Schistosoma mansoni* (2,3,4), and *Posthodiplostomum minimum* (5), among others, the secretions were reported to have one or more of the following functions: lysis, adhesion, lubrication, protection, enzyme-guidance, keratin dissolution, and production of the primary cyst of the metacercaria. The techniques used in these investigations included histochemistry, intravital and histologic staining at the level of light microscopy, and biochemistry.

Stunkard (1) found 18 cephalic gland cells centrally clustered in the cercarial body of *C. lingua*, each cell opening anteriorly by way of a duct in the vicinity of the mouth. He reported that the contents of the cells are extruded during cercarial penetration into the fish host and subsequent encystment. The aim of the present work is to extend these observations by describing the fine structure of these unicellular gland cells and their secretory products. Some results presented here appeared in preliminary form in the Proceedings of the 26th annual meeting of the Electron Microscopy Society of America (6).

## MATERIALS AND METHODS

Cercariae were obtained directly from snail tissues, prior to their emergence as free-swimming larvae. Some were intraredial cercariae developing within brood chambers of rediae; others were tissue or intramolluscan cercariae developing within snail tissue following their emission from rediae.

Snails *(Littorina littorea)* infected with *C. lingua* were collected from Sheep Pen Cove, Nonamessett Island, near Woods Hole, Mass. Shells of infected snails were crushed. Infected gonadal and digestive gland body parts were diced on a chilled wax plate covered with ice cold 3% glutaraldehyde in 0.1 M Millonig's phosphate buffer pH 7.3 to which had been added 2 mM $CaCl_2$. After 1½ to 2 hours in this fixative, the tissues were washed, postfixed in 1% osmium in sea water, and prepared for embedment in Epon (7). Sections were cut on glass knives on either a Porter-Blum MT-1 or MT-2 ultramicrotome. Semithin sections (about 1.0 $\mu$) were mounted on microslides, stained with methylene blue-azure II (8), and studied with the light microscope. Thin sections were mounted on specimen grids, stained with aqueous uranyl acetate (9) and lead citrate (10), and examined with Philips EM 300 or Hitachi 11C electron microscopes.

## OBSERVATIONS

Production of cephalic gland secretory products begins within early intraredial stages of cercariae, so that by the time the cercariae are emitted from rediae, cephalic gland cells are charged with numerous granules. Figure 1 is a photomicrograph of a semithin section of a cercaria after its emergence into snail tissue from the redia. The perinuclear regions of cephalic gland cells *(cg)* are in the middle of of the body of the cercaria, half-way between




**Figures 1 and 2.** Cryptocotyle lingua **cercariae. 1. Photomicrograph of a cercaria among snail tissue after emerging from redia. (cg) unicellular cephalic glands; (os) oral sucker; (ev) excretory vesicle. 1,200X. 2. Electron micrograph of a portion of a cercaria. Note parts of at least four cephalic gland cells (cg) with numerous granules, many containing a dense core (dc). Apical secretory cells (sc) produce granules for transport to the tegument (T) via internuncial processes (not illustrated). (N) nucleus of cephalic gland cell. 11,400X.**



the oral sucker *(os)* and the excretory vesicle *(ev)*.

In cercariae sectioned for electron microscopy (Fig. 2), cephalic gland cells *(cg)* are found mediad to the cytons of other subsurface secretory cells *(sc)*. The latter, more-apical glandular cells are tegumental cells connected by internuncial processes (not illustrated) to the cytoplasmic anucleated surface layer, the tegument *(T)*. Some or all of these tegumental cells may correspond to the cystogenous glands of light microscopy described by Stunkard (1) as unicellular cells scattered irregularly over the surface of the *C. lingua* cercarial body.

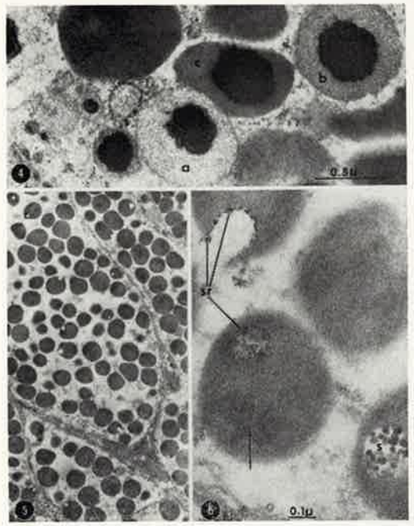
Rough-surfaced endoplasmic reticulum *(er)* and Golgi complexes *(G)* are encountered only in the basal portion of each cephalic gland cell (Fig. 3). Cisternae of the endoplasmic reticulum may appear as numerous peripheral channels running parallel to the plasma membrane (not illustrated), or as ovoid or elongated sacs (Fig. 3). A moderately dense material is found in the cisternae of the endoplasmic reticulum. Golgi complexes, usually encountered in close association with endoplasmic reticulum, appear either as aggregates of vesicles of various densities, or as long, narrow, parallel stacks of saccules containing a dense substance (Fig. 3, *G)*. A few small mitochondria are scattered in the peripheral cytoplasm of the glands. The nucleus of the gland cell has scattered dense aggregations of chromatin (Fig. 2, *N)*.




**Figure 3. Secretory granule production in the basal portion of a cephalic gland cell. Presumptive granules may begin as electron lucent vacuoles (v) near Golgi complexes (G) and endoplasmic reticulum (er). A dense core (dc) is found in vacuoles and in granules undergoing condensation. The Golgi apparatus in the upper left consists of a few parallel saccules. (mt) microtubules. 10.-000X.**

Secretory granules or droplets of cephalic glands undergo several morphologically discernible stages of development, beginning at the site of formation in the basal portion of the glandular cell, and continuing apically as they move toward the gland ducts. Electron lucent vacuoles of various sizes are found in or near Golgi zones (Fig. 3, *V)*. They may represent early forms of secretion granules, much like the "condensing vacuoles" which have been reported to lead to the formation of zymogen granules in pancreatic exocrine cells (11). Thus, concentration of secretory material within Golgi vacuoles may give rise to cephalic gland granules. Presumptive granules, apparently derived from the Golgi vacuoles, contain an eccentric dense core *(dc)* in a translucent intragranular area (Fig. 3). Other early stages of granule maturation have a dense core surrounded by a matrix of variable density (Figs. 2,3,4). During early condensation, the granular matrix around the dense core presumably becomes increasingly more dense until a moderate density is attained (Fig. 4, *a,b,c)*. Another stage in early granule formation, devoid of a dense core and reminiscent of one found in certain secretory cells of the *Fasciola hepatica* cercaria (12), has a space between its enclosing membrane and matrix (Fig. 3, *arrow)*.

The dense core appears as a tightly wound network of electron opaque fibers (Fig. 3,4). It is absent from granules in transit toward more-apical portions of cephalic gland ducts (Fig. 5-9). At a certain stage in their development, secretion granules contain an eccentrically placed electron-lucent area with dense spheroids *(s)* about 200 Å in diameter (Fig. 5,6). The relationship between the dense core and the intragranular spheroids is unclear. Presumed remnants of the intragranular spheroids, however, appear to burst from secretory granules in the form of a dense grainy material (Fig. 6, *sr)*. An amorphous patchlike structure, of slightly greater density than the rest of the matrix, occurs in secretion granules at this stage of their development (Fig. 6,7). Granules




**Figures 4, 5, and 6. Cephalic gland granules. 4. Granules (a, b, c) illustrating a possible sequence of changes in density of the matrix of granules undergoing condensation. Note the opaque, fibrous-like dense core in these granules. 25,000X. 5. Granules located more apically in cephalic gland apparatus than those shown in previous figures. Note that none has a dense core, and some show an eccentric lucent area or chamber. 4,800X. 6. Granules of the type in Figure 5, with dense spheroids (s) or their remnants (sr) in an eccentric intragraular lucent chamber. A chamber (upper left) appears to release spheroidal remnants. Note amorphous patch-like substructure at arrow. 48,000X.**

awaiting discharge from the anterior portion of ducts are aligned behind each other in rows (Fig. 9). The limiting membranes of the granules are closely apposed to each other in this part of the cephalic gland apparatus (Fig. 9, *arrows*).

Microtubules are aligned beneath the inner surface of the plasma membrane of each cephalic gland cell and its ductlike extension (Fig. 3,7,8,9, *mt*). There is no definite microtubular orientation in the more basal regions of gland cells (Fig. 7), but apically, the tubules are oriented more or less parallel to the longitudinal axis of the ducts (Fig. 8,9). Muscle cells *(Mu)* containing mitochondria *(m)* and glycogen particles *(g)* are found in interglandular spaces (Fig. 7).

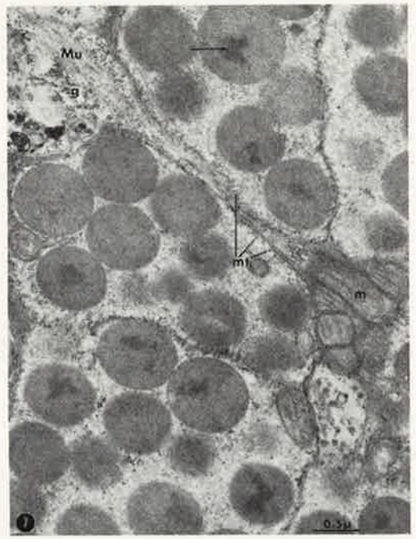


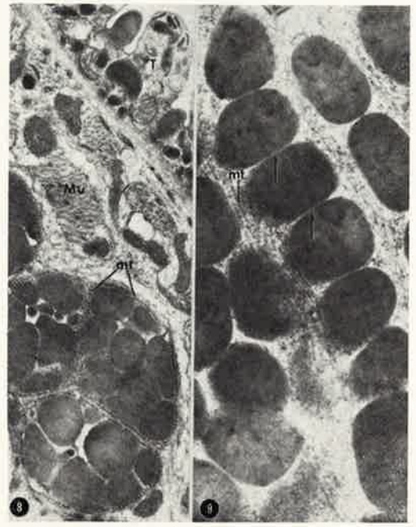

**Figure 7. Well-formed granules with dense substructure (arrow). Microtubules (mt) are aligned beneath the plasma membrane. A portion of a muscle cell (Mu) containing glycogen particles (g) and mitochondria (m) is found between secretory cells. 16,000X.**

## DISCUSSION

Our electron micrographs suggest that ribosomes and cisternae of the rough endoplasmic reticulum in conjunction with Golgi complexes are important in producing cephalic gland secretion granules in the cercaria of *C. lingua*. The protein moeity destined to be incorporated into the secretion product probably originates in the ribosomes of the endoplasmic reticulum and is funneled to the Golgi region in a manner similar to that reported for other secretory cells (11), (reviewed by Beams and Kessler, 13). In earlier work, the Golgi zone was interpreted as a site where protein incorporation into the secretory product occurs. But in light of recent ultrastructural studies by Peterson and Leblond (14), Rambourg, et al. (15), and others, the Golgi complex has also emerged as an important cell organelle for the fabrication of mucopolysaccharides and glycoproteins. Peterson and Leblond (14) interpreted their results to mean that within the Golgi complex, monosaccharides link to each other and to proteins in manufacturing glycoproteins. They also suggested that sulfation of complex carbohydrates to make mucopolysaccharides also occurred in the Golgi zone. In histochemical work with the light microscope on cercariae of *P. minimum*, Bogitsh (5) found that this parasite's penetration gland cells produce two types of secretions: a carbohydrate-protein complex, probably a glycoprotein, and an acid mucopolysaccharide. Although additional histochemical work combined with electron microscopy will be needed to determine whether Golgi bodies in cercarial penetration glands are involved in the formation of these and other substances, the production of a complex heterogenous secretory product in the gland cells of *C. lingua* is suggested by the presence of a dense core during early condensation of granules and by other focally perceptible intragranular substructures.

Microtubules have been reported to function in developing and maintaining asymmetry in cells, in channeling solated parts of the cytoplasm, and in providing the motive force for cytoplasmic streaming (reviewed by Porter, 16). Halton and Dermott (17) found microtubules in rows within the plasma membrane of certain secretory cells and their ducts in two species of adult trematodes. They ascribed a purely supportive function to the microtubules, since normal contractions of body musculature could conceivably effect extrusion of secretion granules. In the cephalic gland apparatus of *C. lingua*, it also seems likely that microtubules serve as cytoskeletal and channeling devices, especially in the ducts where they are oriented parallel to the direction of flow. We presume that subtegumental body musculature and interglandular myofilaments, on receiving appropriate stimuli at the time of host penetration, help to extrude the glandular contents. Close apposition of limiting membranes of granules awaiting export from the anterior ends of cephalic gland ducts of *C. linuga* is reminiscent of the fusion of zymogen granule membranes observed in the mammalian pancreas by Ichikawa (18). He suggested that such coalescence may permit a rapid extrusion of secretory product through a limited surface area. A similar mechanism may obtain for penetration gland granules of cercariae where rapid discharge of granules is needed during the parasite's invasion of the host.






**Figures 8 and 9. Cephalic gland ducts. 8. Microtubules (mt) encompass transversely-sectioned cephalic gland ducts. (Mu) muscle; (T) tegument. 12,500X. 9. Ducts sectioned longitudinally. Note antero-posterior orientation of microtubules (mt) and close apposition of limiting membranes of granules (arrows). 21,000X.**

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## REFERENCES

1. Stunkard, H. W. 1930. The life history of *Cryptocotyle lingua* (Creplin), with notes on the physiology of the metacercaria. *J. Morph. and Physiol.* 50:143-191.
2. Lewert, R. M. and C. L. Lee. 1956. Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and the larvae of *Strongyloides ratti*. *J. Inf. Dis.* 99:1-14.
3. Stirewalt, M. A., and F. J. Kruidenier. 1961. Activity of the acetabular secretory apparatus of *Schistosoma mansoni* under experimental conditions. *Exptl. Parasit.* 11:191-211.
4. Stirewalt, M. A., and W. A. Fregeau. 1966. An invasive enzyme system present in cercariae but absent in schistosomules of *Schistosoma mansoni*. *Exptl. Parasit.* 19:206-215.

5. Bogitsh, B. J. 1963. Histochemical observations on the cercariae of *Posthodiplostomum minimum*. *Exptl. Parasit.* 14:193-202.
6. Krupa, P. L., A. K. Bal, and G. H. Cousineau. 1968. Some secretory products of the invasive stage of a digenetic trematode. *Proc. Electron Microscp. Soc. Am.* p. 156-157.
7. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* 9:409-414.
8. Richardson, K., L. Jarett, and E. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Tech.* 35:313-323.
9. Watson, M. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. biophys. biochem. Cytol.* 4:475-478.
10. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17:208-212.
11. Caro, L. G., and G. E. Palade. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. An autoradiographic study. *J. Cell Biol.* 20:473-495.
12. Mercer, E. H., and K. E. Dixon. 1967. The fine structure of the cystogenic cells of the cercaria of *Fasciola hepatica* L. *Z. Zellforsch.* 77:331-334.
13. Beams, H. W., and R. G. Kessler. 1968. The Golgi apparatus: Structure and function. *In:* G. H. Bourne, J. F. Danielli, and K. W. Jeon (eds.), *Intern. Rev. Cytol.* 23:209-276.
14. Peterson, M., and C. P. Leblond. 1964. Synthesis of complex carbohydrates in the Golgi region, as shown by radioautography after injection of labelled glucose. *J. Cell Biol.* 21:143-148.
15. Rambourg, A., W. Hernandez, and C. P. Leblond. 1969. Detection of complex carbohydrates in the Golgi apparatus of rat cells. *J. Cell Biol.* 40:395-414.
16. Porter, K. R. 1966. Cytoplasmic microtubules and their functions. *In:* G. E. W. Wolstenholme and M. O'Connor (eds), Ciba Foundation Symposium on Principles of Biomolecular Organization, p. 308-345. Little, Brown, and Company, Boston.
17. Halton, D. W., and E. Dermott. 1967. Electron microscopy of certain gland cells of two digenetic trematodes. *J. Parasit.* 53:1186-1191.
18. Ichikawa, I. 1965. Fine structural changes in response to hormonal stimulation of the perfused canine pancreas. *J. Cell Biol.* 24:369-385.



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| 1937 | T. D. Cope | 1952 | W. S. Lapp | 1968-70 | R. S. Chase, Jr. |
| 1938 | G. H. Ashley | 1953 | E. P. Claus | | |
| 1939 | L. K. Darbaker | 1954 | E. C. Herber | | |

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