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University Park, Pennsylvania

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INFORMATION RETRIEVAL AND THE GOLDEN HAMSTER

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ABSTRACT

Three styles of punched cards have been adapted for the discovery and recovery of information on the golden hamster, particularly, in respect to its uses as a laboratory animal. These cards have been identified as author card, bibliography card, and journal card. Their use has facilitated the preparation of an extensive bibliography on the golden hamster.

INTRODUCTION

At the same time that a colony of golden hamsters, *Mesocricetus auratus* (Waterhouse, 1839), was established at Bucknell University in 1946, work was begun on the compilation of a comprehensive bibliography. At that time very little was known concerning the characteristics or requirements of this species as it had been introduced as a laboratory animal only fifteen years before (Adler, 1948). The very fact that so little was known concerning the animal contributed to its desirability for investigations by beginning students at either undergraduate or graduate levels since there was much basic work to be done. As the bibliography grew in both size and scope its usefulness was increased by the use of edge notched bibliography cards, which have been described previously (Magalhaes, Meister, and Kunkel 1953; Magalhaes 1954). The biblography has proved useful not only for students and faculty at our university but also for other investigators including Whitney (1963).

A "Ditto" edition of the bibliography was finally typed in 1959 but unfortunately it was comprehensive only for 1931 through 1953 with scattered references from 1954 through 1958. Several hundred copies of this bibliography were distributed and the edition was soon exhausted. In response to many requests for additional information the present study was undertaken during the academic year 1963-1964 when the combination of a sabbatical year and a contract from the National Medical Library of the National Institutes of Health provided the time and necessary secretarial help. Grateful acknowledgment is made of the use of books at the libraries of Harvard Medical School, Princeton University, the College of Physicians in Philadelphia, New York Medical Library, Tulane University, and Geisinger Memorial Hospital in Danville, Pennsylvania. Finally, without the cooperation of many authors, students and librarians the bibliography would never have reached its present state.

PROCEDURE

The first task of the present study was to convert the form of literature citation from the style adopted by the Conference of Biological Editors of the American Institute of Biological Scences (1960) to that used by Index Medicus. The major change was in the journal abbreviations, but the position of the date, the form for name(s) of author(s), and punctuation were modified. In addition to these changes in the literature citations themselves, additional subject categories were needed. For the last ten years have seen not only an increase in numbers of articles published, but also an increase in the fields of biological and medical sciences. Biology has become molecular as well as organismal and man and experimental animals have moved out into space thus increasing the complexity of the environment as well. With all of this in mind three styles of punched cards were designed to assist in the extension and analysis of the hamster bibliography.

JOURNAL CARD—As shown in Figure 1, the journal card provides a handy reference to the abbreviations used for the titles of journals. The style is that adopted by Index Medicus, which lists both the abbreviations and the full titles and place of publication of all journals abstracted. A new list appears each year in the January issue. For journals not included in Index Medicus, abbreviations were devised by consulting Rogers and Charen (1962). Information on the libraries in which copies of the journal were found, the journal code number, the total number of references found in the journal, the types of articles published, the major subjects covered, abstracting journals which include the journal in their coverage and finally the years of the publication that were checked can be stored in the holes at the edge of the card by notching appropriate places. Indirect or numerical codes were used for the journal code number, number of references, years checked, and libraries consulted. Other items were coded directly with each hole representing a single subject or abstracting journal. In preparation of the bibliography, when five or more references were found in a specific journal, a careful search was made of indices, tables of contents, summaries or conclusions, material and method sections of individual papers and finally author indices were checked for familiar names. In this way many references were uncovered even when the titles themselves did not give a clue to the fact that hamsters had been used. Finally, the journal cards were used to record the year, volumes, and issues scanned for references and the names of authors of hamster articles and the total number of hamster article per volume consulted.

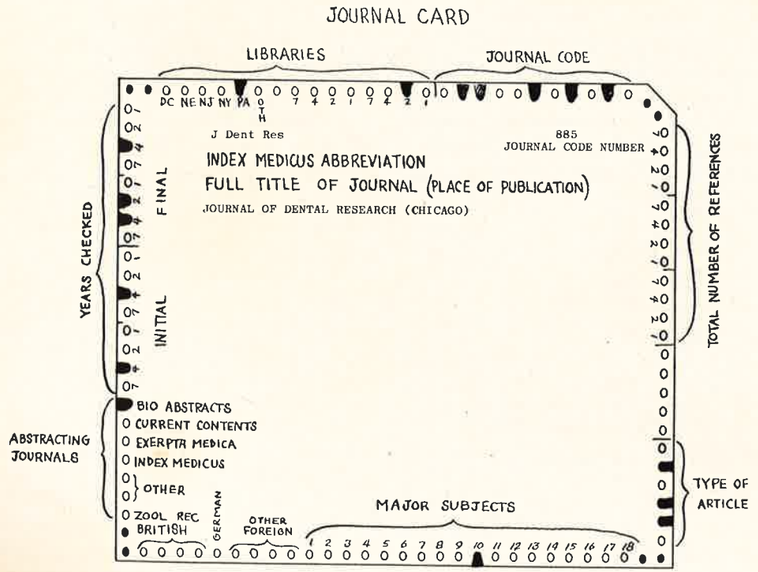

Figure 1

BIBLIOGRAPHY CARD—This card represents a revision of the card previously used. A double row of holes at the edges make it possible to store more information than was possible with the single row of holes on the cards previously used. As is shown in Figure 2 the literature citation is typed directly on the face of the card with the author, title, journal name and volume, pages, and date. The author's name is coded in the field across the top of the card using the deep holes for the initial letter, the group of vowels or "other letter" for the second letter, and the outer holes for the third letter of the author's name. This system of indexing author's names was devised by Hyslop and Wassenberg (1958) for a filing system for metallurgical literature. The number of authors is recorded next. For a single author the card is not punched; for two authors the outer hole (author no. 2) is punched, and for more than two the deep hole is punched. The type of article is shown by punching the appropriate regions of the next group of six holes; shallow punches indicate title only, note or letter, or comparative study in which hamsters were used incidentally, or in small numbers; deep punches signify abstract, original research with hamsters the principal subject or in large numbers, or review article, and finally, intermediate punches indicate the use of hamster serum or antibodies, hamster cells cultures, or hamsters as hosts for pathogenic organism or transplants.

A notch in copy or index holes shows that a reprint or photocopy is available in our collection and that it has been indexed. The time of publication and journal code numbers are indicated in their fields by numerical double row

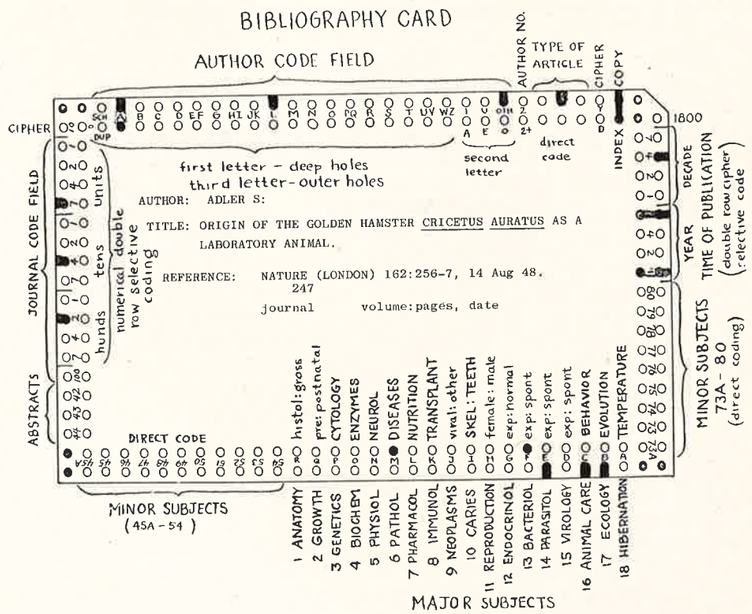

Figure 2.

selective coding as explained in the Royal McBee Corporation Instruction Manual. The seven holes in the lower left portion of the card are used to show the location of abstracts in standard abstracting journals. Major subject categories are recorded at the bottom of the card, while the minor subjects are indicated in areas at the right and bottom left of the card.

AUTHOR CARD: Figure 3 is a copy of a typical author card with the name of author and his current address, co-authors, and record of letters sent and reply received. The author code, and major subpects are the same as those previously described for the bibliography card. The geographical location of the author is recorded along the bottom of the card. A code book was prepared of the numbers assigned to continents, states or countries, cities, and laboratories.

RESULTS

JOURNAL CARDS: By March 1964, 446 journal cards were prepared giving abbreviations and code number for the journals in which hamster articles had been found. In 1953 only 204 titles were included in the code list for journal titles. Thus, in the last ten years the number of journals in which hamster articles have appeared has more than doubled. The journal cards have been most useful as a means of checking abbreviations for journal titles. It is expected, however, that the record of journals searched and the summaries of authors included in different volumes will help to avoid duplication of search and aid in the recovery of information once the cards are completed.

BIBLIOGRAPHY CARDS: All of the citations included in the original bibliography of 1953 and twice as many more references from 1953 through 1963 have been typed onto the cards of the style described for a total of over 3,500 references. The advantage of the bibliography cards is that they provide a means for recording and recovering much more information than was possible in previously used cards or on cards of the traditional type. It is hoped that these cards will also speed the preparation of an extentive index for the bibliography.

AUTHOR CARDS: The author cards have proved their value in making possible a systematic survey of research workers who have made use of hamsters in their investigations. Authors have been particularly helpful in locating articles published in symposia, international

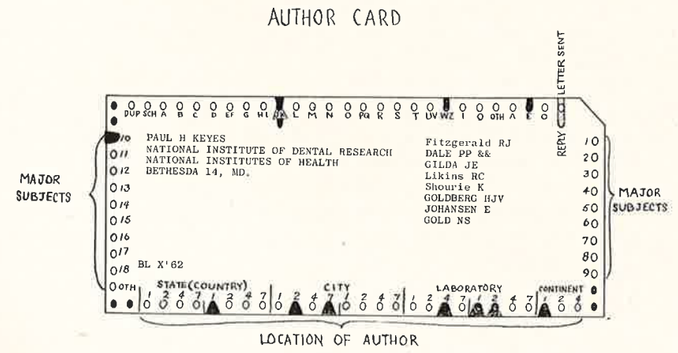

Figure 3.

congresses, book chapters, theses, and other irregularly published, but nevertheless important materials. Even in journal scanning knowledge of active authors is essential if new material is to be located.

CONCLUSION

Punched cards have been found to be useful not only as bibliography cards, but also for the maintenance of author and journal files.

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A MICROSCOPIC STUDY OF LETHAL ELECTROTROPISM IN PLANTS

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ABSTRACT

Plant response to an electrostatic field is defined as electrotropism, and characterized as stimulative, destructive, or lethal. The growth response or destructive mechanism which is apparently activated in plants by the action of an electrostatic field is discussed in terms of micro-chemical analyses and microscopic data. It is shown that increases in active element concentration of iron, aluminum, and zinc are 110, 230, and 220 per cent respectively as compared with control plants when orchard grass is exposed to an electric potential gradient of 75 kV/m magnitude. The absence of polarized salts in the damaged plant tips strongly suggests the presence of polarized molecular complexes such as proteins or metallo-enzymes.

INTRODUCTION

Several examples of severe plant damage resulting from growth in an electrostatic field-environment have been reported by the author (5) (6), and several examples of plant growth stimulation have also been reported in earlier work by Blackman and Legg (1), and Musso (8). It cannot be stated with complete certainty that plant growth response in an electric field is impeded in all cases, but all work reported by this author certainly supports this opinion. However, it will be conceded that the possibility of plant growth stimulation in a field whose properties are defined in a particular manner does exist, both from the standpoint that numerous investigators have indicated this trend, and from the standpoint that such a phenomenon is certainly physically possible.

In any event, it has been established by Murr (5, 6) that plant growth is definitely impeded by electrostatic fields of sufficient magnitude and defined in such manner that the natural electrical environment is duplicated. This phenomenon has been observed in several of the common grasses. Since it has been previously shown by Murr (7) that the mechanism of growth impedance involves more than a mere ionic transfer within the plant tissue, the response must involve more than a simple migration of charged particles by the action of the applied field. Such a response is termed electrotaxis. A more proper description of the responses observed in the experiments to be described seems best defined as electrotropism. In this respect, electrotropism will be taken to mean any physiological or chemcal response of the plant or cell systems to an electrostatic field.

Considering the nature of the plant damage already observed (5, 6, 7), and the possibility of beneficial responses being induced by the action of an electrostatic field, electrotropism might be conveniently classed as three types or degrees of field action, i.e.; (1) Stimulative electrotropism, (2) Destructive or degenerative electrotropism, (3) Lethal electrotropism. This paper will deal primarily with the last category of electrotropism.

In a previous paper dealing generally with the destruction of plants in an electrostatic field, it was suggested by this author (5) that the mechanism of destruction consisted of a simple physiological-physical response as a result of the field action. However, a detailed micro-chemical analysis in later research completely refuted the simplicity of the original mechanism (7). On the basis

of this new experimental data, it was proposed that damage results from a disturbance of the normal metabolic enzyme functions in the plant and most likely an increase in the availability of certain enzymes which, in view of the experimental data, appear to be associated with the respiratory process in cells.

The present paper will attempt to construct, using spectroscopic and microscopic data, a suitable model for the explanation of all phases of electrotropism; with special emphasis being given to lethal electrotropism. It will also be shown that through the use of certain experimental-mathematical approximations in conjunction with analytical data, the nature of crop damage may be estimated when it occurs in the natural environment as a result of destructive electrotropism.

MATERIALS AND METHODS

The procedure employed in experimental work to be reported in this paper was similar to that employed in previous research reported by Murr (5, 6, 7). Experiments were performed at four electrostatic potential gradient reference levels (as defined by Murr (4)), namely; 30 40, 50, and 75 kilovolts per meter. The plant material in the 30, 50, and 75 kV/m experiments was used for spectrometric analysis, while that at 40 kV/m was employed primarily for microscopic investigations. Standard quantitometer methods were employed in the plant spectrometric analyses of both active and control plant leaf-tip samples. The results were computer corrected by calibration standards and reference to standard sample data.

The microscopic investigations consisted of sectioning both active and control leaf specimens and safranin-fast green staining of these sections using techniques previously reported by Murr (6). Of particular interest in this investigation was the observation of microscopic detail at comparatively high magnifications. This also included an accurate microscopic counting of chloroplast densities in both the active and control leaf sections representing the entire tip damage range, and a comparison of average differences observed.

RESULTS

Figure 1 shows an important phase of the spectrometric analysis of the orchard grass samples subjected to destructive field action compared with control samples. The data here is presented as a ratio $\overline{\S}$ as defined by

$$\overline{\S} = \left(\frac{M_{\alpha}}{M_{\gamma}} - 1\right) \qquad (1)$$

where M α and M γ are the active and control sample concentrations of the elements represented in ppm. It has been previously established by the presentation of the results of similar spectrometric analyses (7) that there is no occurrence of major elements representative of inorganic salt radicals concentrated in the damaged tips as proposed in an earlier theoretical damage mechanism (6). In this same analysis, however, it was also shown that extremely significant differences did exist in the concentrations of zinc and iron in the damaged tips of plants exposed to continuous electric field action. The analysis presented in Fig. 1 is nearly identical to previous results obtained by Murr (7), and attests to the reproducibility of the results.

Looking at these micro-analytical results with the possible thought that they might indicate cell damage of a more internal nature than originally conceived, the microscopic analysis was intended to reveal possible internal cell damage. The sequence of photomicrographs shown in Fig. 2 illustrates rather strikingly the tip

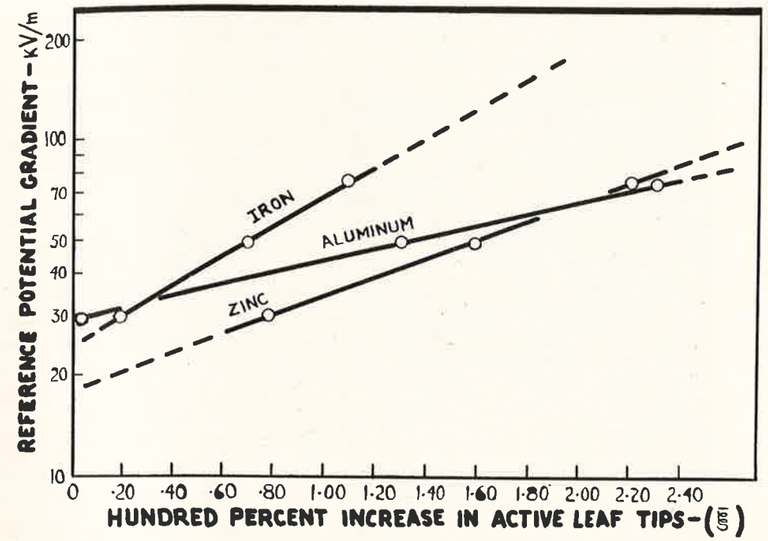

Fig. 1. Spectrometric analysis for minor elements in the leaf tips of active and control orchard grass plants. (tips here refers to leaf segments approx. 2 inches in length.)

voiding produced in the orchard grass plants exposed to relative potential gradients of 40 kV/m. Figure 2 (A) shows a transverse leaf section in a damaged tip. The voids, seemingly caused by the absence of internal cell structure and regular arrangements of chloroplasts, occur with this same unmistakable regularity in all leaf sections in the damaged zones.

In Fig. 3 is shown the results of a microscopic analysis of chloroplast density in the 10 micron thick sections microtomed from representative tip segments of both active and control plant leaves. The leaf areas shown in the leaf schematic, A, B, C; correspond to the brown damage zone, the dark green transition zone, and the undamaged active leaf portions respectively, as previously outlined by Murr (6). In this analysis, both active and control leaves were examined at approximately the same structure stations as indicated in Fig. 3. Three separate studies were performed on section areas excluding the main stem area (i.e., the central vascular system was not included in the density count), and the histograms of Fig. 3 represent the average chloroplast density for these observations. The chloroplast density itself, it should be pointed out, is a relative magnitude devised merely to illustrate the differences which occurred.

Although Fig. 3 illustrates an unmistakable concentration of the chloroplast density in the dark green transition zone (B) of the active plants, the density is still less than the normal density in the control leaf sections. It seems unlikely, therefore, that this density rise could explain the color change in this transition zone, but it might shed some light on the mechanism of leaf tip damage.

While the microscopic analysis of chloroplast density properties presented in Fig. 3 has neglected the central vascular system, the sequence of photomicro-

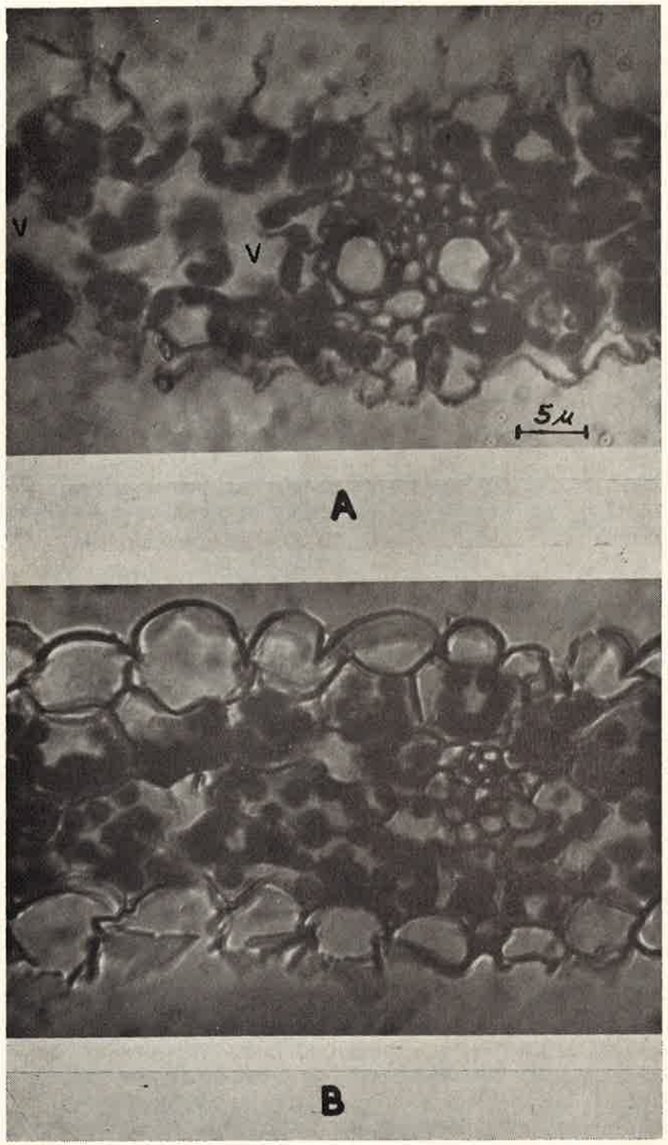

Fig. 2. Outer portions of active (A) and control (B) sections of orchard grass continuously exposed to a relative potential gradient of 40 kV/m for 2 weeks. (V) indicates void formations. Section thickness—10 microns.

graphs in Fig. 4 illustrates similar damage transitions in the central vascular area of a typical orchard grass leaf which has been subjected to continuous action potential gradients of 40 kV/m for several weeks. Figure 4 illustrates two very obvious properties of the damaged leaf. First, chloroplast and supporting cell structure is not definable in the damaged zone, and the stain density of the active sections is possibly indicative of some field induced reaction, and second; epidermal protection in the tip regions is completely absent.

DISCUSSION AND THEORETICAL ANALYSIS

Considering the photomicrographic evidence presented in Figs. 2 and 4, and with reference to the spectrometric data presented in Fig. 1, it would seem logical to conclude that the damage mechanism which is activated during destructive electrotropism in plants has an internal origin rather than an external one (external here refers to the leaf epidermis, etc.). That is, in the original investigation of this problem by Murr (5, 6), it was concluded that the cell damage resulted from

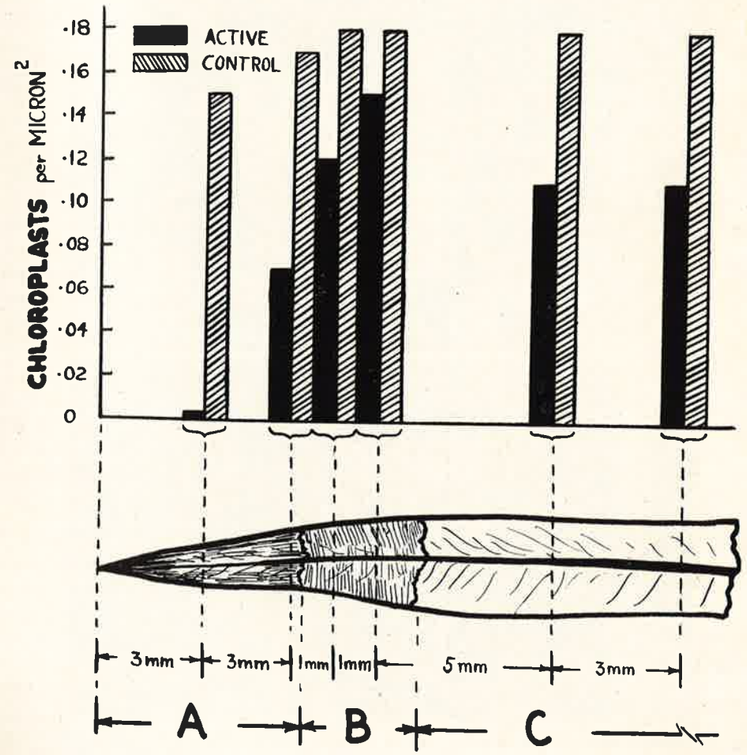

Fig. 3. Chloroplast densities in orchard grass sections exposed at 40 kV/m. Schematic leaf section shows (A) brown damage zone, (B) dark green transition zone, (C) undamaged active leaf tip zone. Section thickness—10 microns.

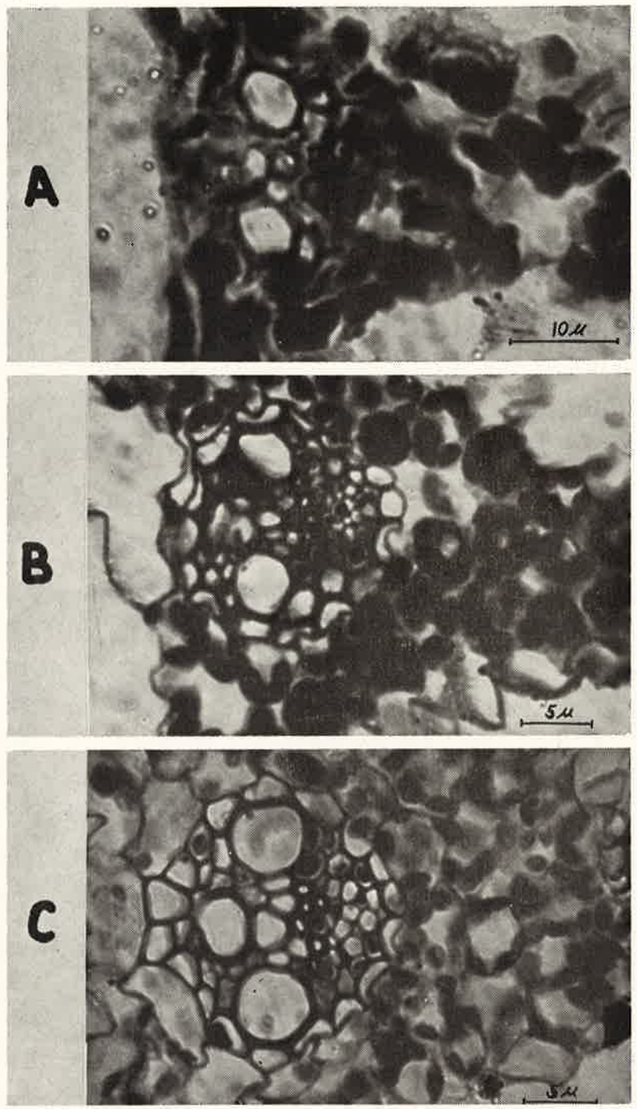

Fig. 4. Lethal electrotropism in the central vascular system in orchard grass exposed concontinuously for 2 weeks at 40 kV/m relative potential gradient. (A) brown damage zone, (B) undamaged active leaf tip zone just below the dark green transition zone. (C) unexposed (control) leaf section in approximately the same position as the undamaged active leaf portion of (B).

the bursting of epidermal cells which allowed protoplasmic dehydration to take place-resulting in the destruction of the leaf-tip areas. Thus, destruction was assumed to be triggered with the bursting of the epidermis. However, in view of the information presented in this paper, it would be conceivable to assume that the respiratory or related metabolisms of the cells are considerably accelerated by the field action (Fig. 1). The immediate result of this metabolic acceleration seems to be the production of an increased number of chloroplasts (as evidenced by the histograms of Fig. 3) which precedes the lethal tissue damage; and which essentially forms the dark green color zone (Fig. 3 (B)), not by virtue of the chloroplast density, but rather by a chemical-enzyme change which takes place in this zone. As this enzyme action becomes rapidly accelerated, it appears as though a general tissue deterioration develops. This development then continues to accelerate, while the epidermis, disrupted in a similar manner, allows dehydration to commence. The catastrophic cell disruption by the enzyme concentrations, combined with accelerated protoplasmic dehydration, eventually causes the chloroplasts and tissue systems to agglomerate into seemingly dried, unidentifiable bodies as illustrated in Fig. 4 (A).

If one assumes that the concentrations of metal ions in the active plants as representative of enzyme activity, then it would seem logical to assume that the action causing the enzyme acceleratons would also accelerate mineral variations in the soil as the plant strives to meet the demands of the distorted metabolism. A careful emission spectroscopic study was undertaken to clarify this point. Figure 5 shows the results of this study for aluminum. The aluminum analysis is here presented as concentration of the Al_2O_3 group. The histograms shown definitely establish the contention that the soil surface or upper soil strata is depleted of its aluminum reserve as the potential gradient is increased, and as the metabolism becomes increasingly demanding through the electrotropic action. The demand here seems to be met by root action or some similar physiological response as opposed to a charged particle transfer process such as electrophoresis and related types of electrotaxis.

The assumption that these enzyme complexes are representative of the respiratory metabolism is based in part on the studies of metallo-enzyme systems by Schulke (9) and Vannotti (11), in which the role of iron and zinc appear to be well documented. In the case of aluminum, however, it is not certain what role it might play in plant cell metabolisms. Horecker, et al (2) have shown that certain Cytochrome C reducing enzymes may be activated by aluminum ions, while Szucs (10) contends that aluminum ions in excess cause protoplasmic

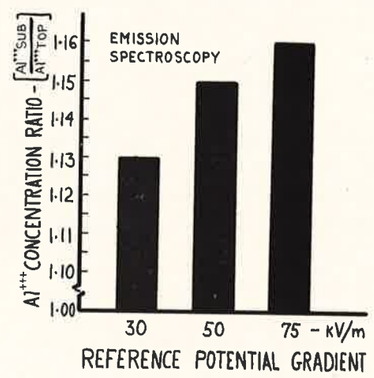

Fig. 5. Emission spectroscopy soil analysis for aluminum in the active plots after continuous exposure experiments using relative potential gradients of 30, 50, and 75 kV/m. Control plot soil concentration remained approximately constant and is not shown.

hardening. Now in the case of enzyme stimulation as presented in the preceding paragraphs, either of the previously mentioned roles of aluminum could be incorporated into this scheme, and adequately interpreted in terms of the microscopic studies showing what might well be cell hardening along with the apparent agglomeration of structure.

It may be well to discuss the importance of electrotropism with reference to the natural environment. Actually, there has never been any direct evidence (to this author's knowledge) which relates plant or crop damage of any kind to an electrostatic field action. However, if one now considers the evidence presented in this paper and others (5, 6, 7), it will be realized that certain crop damage occurring in the natural environment may be the direct result of electrotropism. Miller (3) refers to the occurrence of cereal damage in the Great Plains during the dust storms and similar charge phenomena.

From the graph of Fig. 1 and by varying the form of eq. (1) it will be observed that

$$\left(\frac{M_\alpha}{M_\gamma}\right) = \left(\bar{\xi}_S + 1\right) \qquad (2)$$

where M_α and M_γ are the element concentrations as previously defined. The equations for the curves shown in Fig. 1, having been obtained in the following forms:

$$\left\{\begin{array}{l} \log_{10} E = .43\,\bar{\xi}_{(Fe)} + 1.40 \\ \log_{10} E = .17\,\bar{\xi}_{(Al)} + 1.47 \\ \log_{10} E = .27\,\bar{\xi}_{(Zn)} + 1.25 \end{array}\right\} \qquad (3)$$

can then be transformed, by use of eq. (2) into the interesting relationships;

Equations (4), although technically only good approximations, might be extremely helpful as an analytical "measuring stick" of the degree of electrotropism, if any, experienced by a damaged leaf tip in the natural environment under certain defined conditions. Theoretically speaking, the relative potential gradient might be approximated if a spectrometric analysis were performed on the damaged plant leaves, and comparing such an analysis with a control sample analysis. Thus, finding the ratio M_α / M_γ in ppm concentrations by spectroscopic analysis would reveal the approximate relative potential gradient magnitude (E) in kilovolts per meter causing the leaf damage, or operating on the plant system.

$$\left\{\begin{array}{l} \left(\frac{M_\alpha}{M_\gamma}\right)_{Fe} = 2.3\left(\log_{10} E - .97\right) \\ \left(\frac{M_\alpha}{M_\gamma}\right)_{Al} = 5.9\left(\log_{10} E - 1.30\right) \\ \left(\frac{M_\alpha}{M_\gamma}\right)_{Zn} = 3.7\left(\log_{10} E - .98\right) \end{array}\right\} \qquad (4)$$

It must be observed that when the conditions exist where there are no measurable differences in element concentratios, the left-had terms in eq (4) are equal to unity. In this case, the value of E for each element will still have some finite value (as shown in Fig. 1), which will be defined as the threshold gradient. Although the threshold gradient has little physical significance, it should be understood that the values of E which exceed these quantities represent, essentially, the reference potential gradient inflicting destructive or lethal electrotropism in the plants under study.

SUMMARY AND CONCLUSIONS

The results presented in this paper tend to strongly support the hypothesis that destructive and lethal electrotropism in plants results from some still undefined enzyme acceleration. Destructive electrotropism may be defined, with reference to the microscopic data presented, as the damage of plant tissue which does not necessarily involve the total immediate death of the plant. Lethal electrotropism is the complete destruction of plant life by the electric field action. Destructive electrotropism may be a recoverable phenomenon when it occurs following the short duration exposure of plants to an electrostatic field. Actually, the degree of electrotropic damage is dependent upon the degree of enzyme acceleration and concentration stimulated by the electric field action.

It is possible, perhaps, to stimulate enzyme activity to a level which would enhance plant growth without damaging normal metabolic functions. Such conditions may have resulted in the experiments of Blackman and Legg (1) and Musso (8) who reported plant growth increases following the short period exposure to an electric field. Electrotropism of this nature would then be classified as stimulative.

Based on the experimental information introduced in this investigation, the following conclusions may be drawn:

(1) Leaf damage in orchard grass which results from prolonged exposure to an electrostatic field is associated with a significant increase of minor element concentration, presumably in a combined form.

(2) Since the electrical arrangement of the system will not support an explanation of spectroscopically detected minor element concentrations in the active leaf tips by a simple electrotaxis mechanism or some related electrophoretic action, it is concluded that the elements detected, namely iron, zinc, and aluminum, are representative of some respiratory protein or metallo-enzyme complex. Such a molecular complex could conceivably polarize under the influence of the electrostatic field.

(3) The assumption that a molecular polarization action is activated by the electric field action could be established to some degree by relieving the field action on the plants at an early stage in their growth. In such instances, field relaxation will necessitate relaxation of dipolar molecules. The relaxation of these dipolar molecules in turn should allow plant growth recovery.

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POLLEN ANALYSIS OF THE BEAR MEADOWS BOG OF CENTRAL PENNSYLVANIA

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ABSTRACT

Samples from a core 285 cm (9½ feet) deep taken in the Bear Meadows bog of central Pennsylvania have been analyzed for their pollen content. The pollen profile indicates a comparatively diversified forest from the very bottom. The spruce maximum is absent, instead pine predominates with some admixture of oak, birch and fir. Since the age of the lowermost organic level at 213 cm (7 feet) is known to be 10,320 years, this means that during the Valders readvance central Pennsylvania harbored already a somewhat complex forest cover. This would seem to confirm Braun's hypthesis of forest refugia during Wisconsin time within Allegheny Plateau and Allegheny Mountains.

INTRODUCTION

The Bear Meadows Bog, a Pennsylvania State Monument, is located in the Ridge and Valley Section of the Appalachian system of Central Pennsylvania. It lies about six miles southeast of State College and is easily accessible by car.

Its altitude is 1820 feet above sea level. It is surrounded almost entirely by rather steep ridges, some of which reach an elevation of 2400 feet. (Fig. 1). Only to the east-north-east is there a narrow valley through which the drainage takes place. The outlet is known as "Sinking Creek".

The geology of the area is complicated, as is usual in this region of strongly folded strata. In general, the high ridges consist of resistant Tuscarora sandstone of Silurian age, while the bog itself rests on Ordovician rocks of the Juniata formation which consists of sandstone interbedded with shale.

The present vegetation of the area is, with the exception of that of the bog itself, typical of this section of Pennsylvania and has been described by Westerfeld (41, 42).

Bear Meadows lies well south of the Wisconsin glacial border and, as far as is known, has never been reached by any of the preceding glaciations. Its formation, then, is not due to any direct glacial action, but to the special topographic features of the site and to the damming of the outlet either by landslides or by animal (beaver) activities.

Sears (37) considered Bear Meadows an "apparently beaver-made Lake." Braun (1) refers to it as "an unusual feature of the Ridge and Valley Section." While bogs are common in the glaciated northeastern and northwestern corners of Pennsylvania, they are rare in this part of the Commonwealth. Thus while a great deal is known about the history of vegetation in glaciated areas, where many bogs have been investigated, our knowledge of the post-glacial vegetational development in nonglaciated areas is rather spotty. This study, it is hoped will help to fill the gap at least in part.

METHODS OF INVESTIGATION

Bear Meadows is a rather shallow bog. Sears (37) has given a somewhat condensed 7-foot profile of this bog as a part of a general survey of North-American bogs. Dr. Potzger took a 7-foot core in 1951 which was eventually analyzed for pollen by Trotter (39). He analyzed every 6 inches but counted only 100 sporomorphs for each level. As he himself admits, his was a rather hurried investigation. Several sporomorphs, such as *Nyssa* pollen, were overlooked. In the present study up to 22 percent of *Nyssa* pollen was found at certain levels.

Since there are reports of 13 or even 24 feet of peat in Bear Meadows, a special effort was made to locate these deeper deposits. The search was, however, fruitless. The profile obtained for the purpose of this study is 9½ feet (285 cm) deep. The first 7 feet (213 cm) consist of peat and the last 2½ feet (72 cm) of very fine bluish-gray clay. Since the clay was very compact and there was danger that the sampling end of the borer would be lost, it was decided to stop at the indicated depth. The thickness of the clay layer remains, therefore, unknown although it is suspected that some of the greater depths of peat reported above may include part, at least, of the clay layer.

It should be noted that the deeper part of the bog, as reported in this paper, was not found in the open part but rather in the spruce-covered section to the north. (Fig. 1).

Sampling was done with a Hiller peat borer provided with a removable sleeve. Since the sleeve is 25 cm long, each sample was divided into five sections, each 5 cm long. Only the central part of each section was taken and stored in a vial.

In the laboratory the samples were boiled in KOH, acetolyzed (12), stained with an aqueous solution of safranin and stored away in cellosolve for eventual

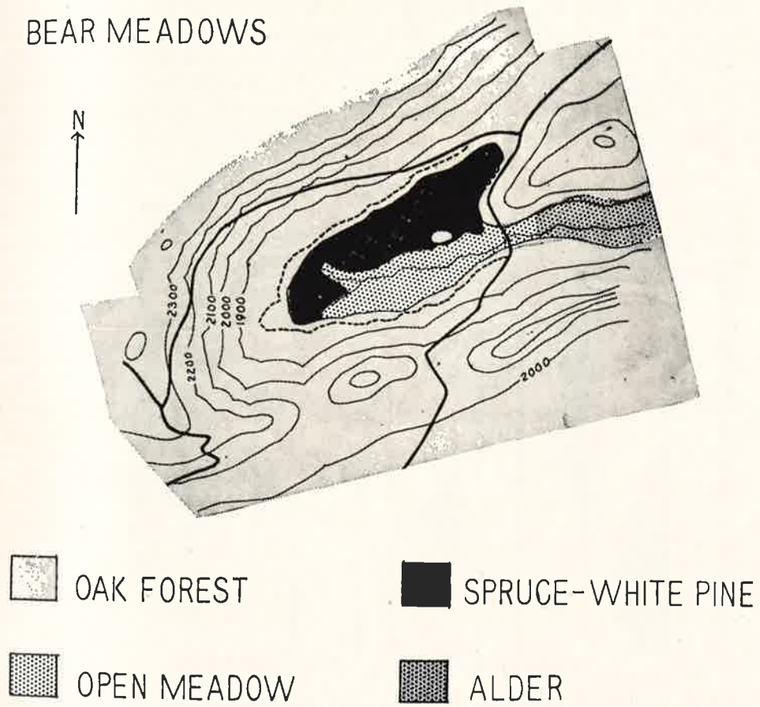

Figure 1. Map of the Bear Meadows area.

study. When clay was found admixed, the sample, after boiling it in KOH, was boiled in HF and then acetolyzed and stained.

Pollen and spore counting was carried out with an AO Microstar microscope at a magnification of 430x. For each level counting was continued until at least 200 grains of arboreal pollen (AP) were counted. In many instances, when a more representative picture was deemed necessary, many more than 200 AP were counted.

The pollen of the following genera was considered as arboreal pollen: *Picea, Abies, Larix, Pinus, Tsuga, Fagus, Castanea, Betula, Ostrya, Carpinus, Acer, Juglans, Carya, Tilia, Fraxinus, Nyssa, Liriodendron,* and *Ulmus*. Pollen of *Alnus* and *Salix* was not included in the AP count, since these genera, under the prevailing conditions of central Pennsylvania, are localized and restricted to valley streams and to the few scattered swamps and as such are not part of the larger regional vegetational picture.

The percentage representation of the pollen of shrubs (including *Alnus* and *Salix*), herbaceous plants, of spores of ferns and fern allies, and mosses was calculated in relation to the total arboreal pollen.

The results of the pollen analysis are presented in fig. 2 and Table I. Since a pollen diagram containing the percentages of all sporomorphs found during the analysis would have been too unwieldy, only the more important taxa have been included in the diagram in fig. 2, while Table I gives the percentages of sporomorphs omitted from the diagram as well as the unknowns.

Very useful in sporomorph identification were the publication of Erdtman (12, 13), Faegri (14), Wodehouse (43), Sears (35), Dombrovskaya, Koreneva and Tiuremnov (10), and a small

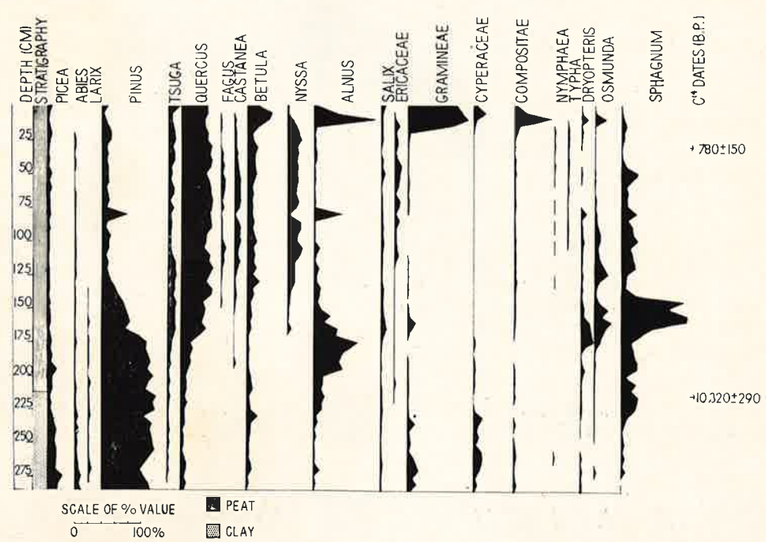

Figure 2. The pollen diagram of Bear Meadows Bog.

but representative collection of slides of modern pollen and spores.

The inability to attain specific identifications in certain genera is disappointing. It limits considerably the ecological and phytogeographical applications of the pollen spectrum. In western and central Europe generic identifications are

Depth (cm)	Ostrya Carpinus	Acer	Juglans	Carya	Tilia	Fraxinus	Liriodendron	Ulmus	Rhus	Aeulifoliaceae	Corylus	Polygonum	Chenopodium	Undetermined	No. Sporomorphs Counted
0	T	4	1			2	2				T			2	707
5		1	5			3	2						4	3	1230
10		1				1		3					5	2	950
15	T	1	T		1		1		3	1	1	T	T	2	776
20							1			1				3	691
25		T	T		T		T			2				2	717
30		1		T				2		1				1	742
35			T					1						1	675
40		1	T	T				1		2				2	625
45		1	T		1		1	T		2	1			3	619
50	T	2	T	2	1			T	1	2	T			5	804
55				1	T	T		T	1	2	1			2	735
60			1			T	T	T	1	1	1			1	635
65	T		T	T		1	T	T	T	1	1			1	883
70			T	2		T	1	T		2	3		T	3	874
75	2	T	T	1		T	T	T		1	1		T	2	793
80	1		T	1		1		T		1	T			2	841
85	2	T	T	2	T	1	T	T	T	2	T			3	891
90		1	T	1	T		T	T		4	2	T		3	967
95	2	3		2	2	T				2	1		T	4	946
100	2	2	T	1	1	T	T	T	1	4	1	T	T	3	994
105	1	2	T	1	1	1	T	T		4	1	T		3	954
110	2	2	T	1	1	T	T	T	2	2	1	T		2	902
115	3	2	1	1	1	T	1	1		2	T	T	T	2	942
120	3	2	T	1			T	T	1	1	1		T	3	826
125	1	4		1	T		T		1	1	T			3	914
130	2	1	1	T					2	2	1			3	884
135	2	1	T	T	T				1	2	T			3	740
140	1	4	T	1				T	T	3	T			4	538
145	3	4		T				1	T	2	T			2	763
150	T	4			T			1	1	1	1			3	639
155	2	1						T	T	1	1	T		2	649
160	T	4			T			1		T	1			2	968
165	3	T			T			T		1	T			2	729
170	1	1			T									3	635
175	1	2								1	T	T		3	789
180	2	1												4	656
185	2	2												3	684
190	1	1												3	637
195	3	T			T			T	T					2	660
200	1	T												3	692
205	1	1								T	1			4	719
210	2										1			4	646
215	T	1									1			5	637
220	T													4	716
225	1													3	603
230	1	1												3	732
235	1										T			3	696
240		T											T	1	643
245	T					T		1						1	739
250	T												T	2	652
255														3	718
260		T				T								3	739
265	1		T			T								2	655
270	T				T									2	633
275														1	774
280														2	741
285														4	677

Table 1. Percentage representation of sporomorphs not included in the pollen diagram, of the unknown sporomorphs, and the total number of sporomorphs counted for each level. (T indicates less than 1 percent).

in most cases quite satisfactory but in North America where the flora is much richer, a more precise pollen identification would be highly desirable.

It is customary among American palynologists to separate the recovered *Pinus* pollen into two classes based on size; the small-sized pollen being generally considered as belonging to *P. banksiana* and the large-sized pollen being generally lumped together as *Pinus* spp. During the course of this study differences in the size of pine pollen were noticed, but it was felt that very little from the ecological point of view would be achieved by including the small pine pollen counts in the pollen spectrum for several reasons.

First of all, there is a lack of conformity in taking the measurements. Some palynologists measure the entire pollen grain from wingtip to wingtip. Others, like Martin (24), have presented data based on wing length measurements. Still others have measured only the body lengths.

In second place, it is by no means certain what species of pine are actually represented by the various size classes of pine pollen. As already mentioned, some palynologists assign the small-sized pollen to *P. banksiana* and the large-sized pollen to *P.* spp. Others, like Martin (24) find the three size classes represented. Martin assigns these provisionally to *P. banksiana, P. resinosa,* and *P. strobus.*

Finally, the small-sized pollen grains were observed to occur throughout the entire pollen profile including the uppermost levels. Since it is very improbable that this small pollen, at least at the upper levels and in Bear Meadows, could belong either to *P. banksiana* or *P. resinosa,* it appears obvious that its presence must be explained in some other way.

Zaklinskaya (44) in her work on gymnospermous pollen grains of the Cenozoic sediments of Kazakhstan has adopted a morphological approach in identifying pine pollen grains. During the course of the present work an attempt has been made to bring her descriptions under some sort of a key in order to apply her results to our pine pollen. The attempt has been unsuccessful.

At the 15 and 20 cm levels many fine clay particles were observed. They did not reappear again until the 210 cm level. From this level on, the samples consisted entirely of clay. The presence of clay particles in the upper levels is probably due to floodings.

At several levels particles of charcoal were also observed. These levels are: 20, 70, 75, 80, 100, 115, 175, and 260 cm. The presence of charcoal may indicate forest fires.

Certain levels are rather poor in pollen content and the pollen itself appears corroded. This was observed at the 110, 120, 175, and 210 cm levels. Whether this can be correlated with the peat oxidation mentioned by Westerfeld (42) remains to be seen.

In the coarse fraction from the 80 cm level fragments of a microsporangiate *Alnus* catkin and a fern sporangium were seen. This probably accounts for the sudden icrease of *Alnus* pollen and *Dryopteris* spores at this level. Pine pollen also increases suddenly at the same level but no evidence of a *Pinus* microsporangium was found. It should be noted that Sears' (3) profile shows a similar increase in pine pollen percentage at the 4 foot level and Trotter's profile another at the 3 foot level. The fact that the 80 cm level is one of those in which charcoal was observed may be important. In this region there is always an increase of pine after forest fires and, perhaps, such an occurrence may be indicated here.

The question of percentage representation of pollen of the various species as related to the actual vegetation of a region, has been discussed by Erdtman (12), Davis and Goodlett (7), and Walker and Hartman (40). The dispersal of pollen is discussed by Erdtman (12), Rowley (32), Fedorova (15, 16), Monoszon (26), and the vertical migration of the various pollen types within the peat by Rowley and Rowley (33). Since no detailed phytosociological study of the Bear Meadows is available, it is not possible to make any comparisons for this bog. In general, it may be assumed that wind disseminated pollen is over-represented and the insect borne pollen under-represented in the present profile. It is also probable that the pollen of plants growing within the bog is better represented than that of plants growing on the surrounding ridges.

In this connection mention must be made of the small amount of *Acer* pollen recovered from the Bear Meadows samples. Trotter (39) reports that in his analysis a total of only 6 pollen grains, possibly of *Acer,* were counted in the entire profile. Sears (37) does not report this pollen at all. In this study Acer pollen was found, but in quantities much smaller than one would expect, since *Acer rubrum* is rather common in the bog and on the surrounding ridges. Except for the fact that *Acer* is entomophilous, it is difficult to explain the near absence of its pollen from the profile. Otto (29), Richards (31), Cox (4), Walker and Hartman (40) and others report fair percentages of *Acer* pollen from bogs studied by them. It appears that profiles of bogs in glaciated areas show higher *Acer* pollen percentages than those from bogs from unglaciated areas.

In general, the pollen profile given here is similar to those of Sears (37) and Trotter (39), except that more taxa are included and the profile is 2½ feet deeper.

Westerfeld (42) gives two radiocarbon dates for Bear Meadows. The age of the lowermost organic level at 7 feet (213 cm) is 10,320± 290 yrs B. P. and that of peat at the 12 inch (31 cm) level has been determined as being 780± 150 yrs B. B. If the first date is correct then the age of the 7 foot level is about identical with that of the Mankato substage of the Wisconsin glaciation (38).

The spruce maximum, which is found in most bogs located in the formerly glaciated regions, is absent here. Schrock's Glade bog, although from an unglaciated area also shows it and so does Martin's (24) profile from southeastern Pennsylvania. Thus it would seem that the Bear Meadows profile, as given here, is truncated, especially since the greatest part of the clay sediment was not examined. The gradual increase in *Picea* pollen toward the bottom indicates a possible spruce maximum below the reach of the present sampling.

The above notwithstanding, the present spectrum is useful in reconstructing the vegetational history of central Pennsylvania at least since Valders glacial readvance.

Flint (18) and Hough (21) give a series of maps tracing the development of the Great Lakes and showing the position of the glacial front at the various stages. Moore (27) correlates these stages with radiocarbon dates. According to Moore 10,500 years ago, which is about the age of the lowermost organic level of Bear Meadows, the glacial margin was only about 170 miles directly north of this location. It is interesting that, when the glacial front was still so close, the vegetation of Bear Meadows, as indicated by the pollen assemblage, re-

sembled the extant vegetation of the Great Lakes region, known to ecologists as the Lakes Forest.

Except for relatively short summaries, such as those of Sears (36, 37), Deevey (8), Leopold (23), and in part Braun (1, 2), no comprehensive studies of postglacial vegetational development, comparable to those of Godwin (20) for Great Britain, Firbas (17) for Central Europe, and Neishtadt (28) for the USSR, have been published in North America. Thus it is somewhat difficult to project the Bear Meadows picture onto the broader regional background.

The region immediately west of Pennsylvania was studied by Potzger and his students. For the New York State the profiles published by McCulloch (25), Cox (4), and Durkee (11) are available. New Jersey was studied by Potzger and Otto (30). Deevey (8), Leopold (22), and Davis (5, 6) report on profiles from New England. Cocke, Lewis and Patrick (3) studied Dismal Swamp of Virginia and Frey (19) the Singletary Lake of North Carolina. In Pennsylvania, Schrock (34), Martin (24), and Walker and Hartman (40) have published pollen profiles of bogs in various locations. Only four of those mentioned above, namely those of Dismal Swamp, Singletary Lake, Schrock's Glade Bog, and Martin's Marsh Creek, show profiles from unglaciated areas. But again, Dismal Swamp and Singletary Lake are a little too far south to be comparable with Bear Meadows. The profile from Marsh Creek is taken to indicate the presence of a tundra flora; the "uncorrected" profile, however, is in substantial agreement with the Bear Meadows profile, except for its greater age. Schrock's profile is also similar. The minor differences may, perhaps, be explained by differences in elevation of the three bogs.

The xerothermic period or hypsithermal interval (9) is fairly well indicated in the present profile by the increase in oak pollen and the appearance of *Castanea* and *Nyssa* pollen. Undoubtedly, the hypsithermal interval could be even more clearly indicated if more exact specific determinations of pollen, especially in the genera *Pinus* and *Quercus,* were possible.

The sharp increases in pollen percentages of *Alnus, Salix,* Gramineae, Compositae, *Dryopterfis,* and *Osmunda* in the uppermost levels are apparently due to recent floodings of the bog and the consequent regression of tree cover.

It is difficult to delimit in the present profile the various zones established by Deevey for the New England bogs. Only zones B and C seem clearly indicated. It should be pointed out, however, that these zones are based on profiles from glaciated areas which is not the case in Bear Meadows.

Every pollen profile, apart from indicating the climatic trends of the region in which the bog is located, reflects also conditions which are peculiar to each bog. Bear Meadows is now without a body of open water. That this was not always so, is shown by the occurrence of nymphaeaceous pollen at various levels. Small amounts of this pollen occur at the 260 cm level. It reappears at intervals up to the 20 cm level. Since the age of peat at the 1 foot level (31 cm) is about 780 years, it seems probable that until recently there must have existed in Bear Meadows an open body of water, if not continuously then at least periodically. *Typha* is absent from the extant vegetation; its pollen, however is present in the profile and seems to correlate fairly well with nymphaeaceous pollen.

It appears, then, that the water level must have varied considerably in the past. Even during the present century there have two rises in water level as indicated by the dead trees which now stand in the open part of the bog. These historic rises were due to beaver activities. Whether the prehistoric changes in water level were due to the same cause or to some other agents, cannot be determined on the basis of knowledge now available.

Thus, contrary to some opinions, the Bear Meadows bog is of a relatively great antiquity and as such can be useful in the study of the development of the postglacial vegetation in Pennsylvania.

The presence of a comparatively diversified forest, such as is indicated by the pollen assemblage at the 7 Foot (213 cm), during the Valders readvance, seems to lend support to Braun's hypothesis of forest refugia during Wisconsin time within the Allegheny Plateau and Allegheny Mountains. The age of 10,320±290 yrs. B. B. and the vegetation inferred from the pollen assemblage for the 210 cm level seem to be in close agreement with date of 9310 yrs. B. P. for the pine period of the Hartstown bog in northwestern Pennsylvania.

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ANTIMETABOLITES IN LICHENS
III. ADDITIONAL CHARACTERIZATIONS OF THE EXTRACTS[1]

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ABSTRACT

It was previously reported that extracts of the lichen *Umbilicaria papulosa* contain phytocidal and fungicidal substances. This paper reports additional characterizations of the extracts. Samples of lichens collected from three areas in Pennsylvania and one in Virginia all showed similar antimetabolic activity. Although the original aqueous extracts showed little if any fugicidal activity, concentrating the extracts five times increased fungicidal potency without markedly changing phytocidal activity. Phytocidal activity was not reduced by adding indoleacetic acid to the medium. Neutralizing the extracts did not reduce antimetabolic potency.

INTRODUCTION

In a previous paper (1) it was reported that extracts of the lichen *Umbilicaria papulosa* contained two types of inhibitors. One type is phytocidal and is soluble in water. The other type, which is fungicidal, is soluble in ether. This paper reports the results of aditional studies on the character of the extracts.

All studies during the first year were conducted with samples of *U. papulosa* which had been collected on Laurel Hill of the Allegheny Mountains near Rector, Pennsylvania. During the current year collections were made in three additional areas, two in Pennsylvania and one in Virginia. It seemed desirable to test the antimetabolic potency of extracts of the lichen collected in several different localities. The methods off bioassay were described in the earlier paper.

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COMPARISON OF SAMPLES FROM DIFFERENT AREAS

Relative phytocidal potency of lichen extracts from the four areas is presented in table 1. The results indicate that samples of this lichen which were collected in four different regions possess similar antimetabolic activity. A ten per cent solution of the crude extract inhibited growth of cucumber roots in the range of 68.7 to 79.5%. In regard to antifungal activity (table 2), ten ml of extract per 15 ml of agar produced 100% inhibition of the growth of both test fungi. This concentration of extract is apparently well above the optimum for preventing growth, especially for *Molinia fructicola*. A better idea of relative po-

TABLE 1

Relative Phytocidal Potency of Extracts of *U. papulosa* from Four Areas

(Per Cent Inhibition)

Area	Ml. Extract Per 100 ml. H_2O				
	2	4	6	8	10
Cooksburg, Pa.	21.8	47.5	59.2	58.6	68.7
Highfield, Pa.	20.6	46.9	62.8	71.0	75.6
Rector, Pa.	32.7	59.9	63.5	68.2	75.3
Panorama, Va.	28.0	60.4	65.2	69.7	79.5

TABLE 2

Relative Fungicidal Potency of Extracts of *U. papulosa* from Four Areas

(Per Cent Inhibition)

Area	Fungus	Ml. of Extract C		
		1	5	10
Cooksburg, Pa.	G. cing.	0	80	100
Highfield, Pa.	G. cing.	14.1	72	100
Rector, Pa.	G. cing.	5.1	93.5	100
Panorama, Va.	G. cing.	0	91.3	100
Cooksburg, Pa.	M. fruct.	51.6	100	100
Highfield, Pa.	M. fruct.	58.0	100	100
Rector, Pa.	M. fruct.	60.0	100	100
Panorama, Va.	M. fruct.	43.4	100	100

tency can be gained from observing the effect of only one ml of the extract on M. fructicola. Inhibition caused by this concentration ranged from 43.4% to 60%. These slight fluctuations in degree of inhibition of growth can be attributed to variation in rate of growth of the test organisms.

EFFECTS OF CONCENTRATING EXTRACTS

It was stated in the first paper (1) that the crude extract was separated into an aqueous phrase and an ether phase, showing phytocidal and fungicidal activity, respectively. In many of the experiments it appeared that a very slight amount of phytocidal activity was possessed by the ether phase and that the aqueous fraction might have a little fungicidal activity. To test this more extensively, we concentrated the two fractions five times. The results of these tests appear on table 3. Concentrating the ether phase served to increase *phytocidal* activity from 28.1 to 41.2%. Concentrating the water phase increased the *fungicidal* effect on Monilinia fructicola from 18.4 to 62.5%. The fungicidal effect on *Glomerella cingulata* was increased only from 13.6 to 16.4%. This can be explained by the fact that *M. fructicola* is much more sensitive to the action of the lichen extract than is *G. cingulata*. This "double activity" of the two extracts could be due to impurities.

TABLE 3

Effect of Concentration on Antimetabolic Activity

	Phytocidal Activity (Per Cent Inhibition)	
	Single Strength	Concentrated 5 Times
Water Phase	77.2	88.7
Ether Phase	28.1	41.2

		Fungicidal Activity (Per Cent Inhibition)	
		Single Strength	Concentrated 5 Times
Water Phase	G. cing.	13.6	16.4
Water Phase	M. fruct.	18.4	62.5
Ether Phase	G. cing.	69.5	100.0
Ether Phase	M. fruct.	100.0	100.0

TABLE 4

Effect of I.A.A. on Inhibtion by Lichen Extract

	Phytocidal Activity (Growth of Roots; Per Cent of Control) Molar Concentration of I.A.A.			
	10^{-12}	10^{-10}	10^{-8}	10^{-6}
I.A.A. Alone	99.3	96.4	92.7	65.9
I.A.A. + 1 ml Lichen Extract	36.0	36.8	46.9	30.1

COMBINATION OF INDOLEACETIC ACID AND LICHEN EXTRACT

Since the lichen extract prevents growth of cucumber roots, it was suggested that the active principle in the extract may affect production of indoleacetic acid. Experiments were set up to test this theory by adding various concentrations of IAA to the lichen extract before testing it on cucumber seeds. First, solutions of IAA alone were employed to compensate for the effect of IAA itself. Then inhibitory concentrations of lichen extract plus a range of concentrations of IAA were added to petri dishes containing the cucumber seeds. In table 4 it will be observed that IAA in concentration of 10^{-6} Molar is definitely inhibitory and that this compound must be diluted down to 10^{-12} before it loses its inhibitory effect. Concentrations of 10^{-6}, 10^{-8}, 10^{-10}, and 10^{-12} M were all used with the lichen extract just in case that quantities of IAA which were inhibitory alone might still offset the effect of lichen extract when the two were combined. It will be noted that IAA in any of the above-mentioned concentrations had no mollifying effect on the lichen extract.

EFFECT OF CHANGING THE pH ON ANTIMETABOLIC ACTIVITY

All of the lichen extracts were acid in reaction. It is possible, therefore, that inhibition of growth in these experiments was due to the acidity of the extract. To investigate this point some of the crude lichen extract was treated with

TABLE 5

Effect of Changing pH on Antimetabolic Potency

Series	Fungicidal Activity (Per Cent Inhibition)	Phytocidal Activity (Per Cent Inhibition)
Original Extract (pH 4.4)	93.8	51.2
Original Extract Corrected to pH 6.5	91.4	50.7

NaOH until the pH was equal to 6.5 (the pH of the solution was 4.4). The pH of 6.5 was selected because it is optimum for growth of most plants.

The results of these experiments are presented in table 5. It will be observed that changing the pH of the extract from 4.4 to 6.5 did not affect the phytocidal or the fungicidal activity.

SUMMARY

Extracts of the lichen Umbilicaria papulosa were previously reported to contain phytocidal and fungicidal substances.

Additional characterizations of the extracts revealed the following information:

a. Samples collected from four different regions showed similar antimetabolic activity.

b. Concentrating the fractions increased phytocidal activity of the ether extract and the fungicidal activity of the aqueous fraction.

c. The addition of indoleacetic acid to the extract did not counteract the phytocidal effect on cucumber seedlings.

d. Changing the pH of the crude extract from 4.4 to 6.5 did not affect antimetabolic potency.

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OCCURRENCE OF THE PINE FALSE WEBWORM IN PENNSYLVANIA (*HYMENOPTERA, PAMPHILIIDAE*)

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ABSTRACT

This European pine sawfly is reported from Penfield, Clearfield County; Lake Ariel, Wayne County; and Berwyn, Chester County. Collections were made in pine nurseries by state inspectors. There were only two records for the Commonwealth prior to 1958. The webworm seems now to be entrenched in Pennsylvania. Of immediate concern is its presence in the Valley Forge Park Area. The report on this insect enemy of pine was done in cooperation with the Department of Plant Industry, Harrisburg.

The sawfly considered in this paper was first reported in Pennsylvania in 1925. Middlekauff (1958) found two records for the Commonwealth. The first was the capture of two males flying around an Austrian pine at Chestnut Hill. The label read V-7-25 (F. F. Smith, U.S.N.M.). The second was a female from Philadelphia, labeled IV-22-39 (D. Poe, U.S.N.M.). This European sawfly is reported now from these additional stations: P e n f i e l d, Clearfield County; Berwyn, Chester County; and Lake Ariel, Wayne County. Investigations and collections made by state nursery inspectors led to these new records. I am indebted to the Commonwealth of Pennsylvania, Bureau of Plant Industry, Harrisburg, for making samples available for study and determination.

The pine false webworm, Acantholyda erythrocephala (Linnaeus), has a geographic range from England, through central and northern Europe to Lapland, Caucasus, western Siberia and Korea. The North American records are from Connecticut, New Jersey, New York, and Pennsylvania. It seems to be rather widespread in New Jersey with specimens taken in various localities. But the first record in the United States was from Pennsylvania.

Johnston's Nursery at Penfield, Clearfield County, was the scene of a serious sawfly infestation in 1944. Young red pines (Pinus resinosa Ait.) were under attack and being defoliated by webspinning larvae. N. C. Farr, state nursery inspector, investigated the occurrence and collected material from the nests of pellets and frass on the pines. Specific identification of the collections at first was inconclusive because so little larval material was available for comparison and study. With the Yuasa key (1922), the specimens ran to the family Pamphiliidae and to the genus Acantholyda, A. Costa. Additional collections at other stations in the Commonwealth provided material which confirmed the identification as the pine false webworm.

On April 1, 1954, Mr. George B. Sleesman collected a number of adults from around a large white pine (Pinus Strobus L.) at a Christmas tree nursery near Berwyn, Chester County. Specimens were found in great numbers which later stripped the needles from the tree, leaving only buds on the naked frame of the white pine. From this collection, 16 ♂'s and 4 ♀'s are in the possession of the Bureau of Plant Industry, Harrisburg. Periodic annual visits to the station show that at intervals the white pine is completely defoliated when hundreds boil out of the ground from their earthen cells and attack the tree. It is believed that the Valley Forge Park area is widely infested with this sawfly.

The latest record we have occurred this past summer. Larvae were taken on red pine (Pinus resinosa Ait.) at Pine Grove Nursery, R. D. #1, Lake Ariel, Wayne County, by Mr. Andrew Andreychik, state nursery inspector for that region. There is only one generation per year and collection was made at the most destructive phase of the feeding stages.

The pine false sawfly is a striking and robust species which may be easily identified. The male is almost entirely steel blue with minor light colored areas. The female may be recognized by the orange head and steel blue body.

The webworms tend to be gregarious but do not feed openly on the plant host. They are found concealed in silken webs at the base of the leaves and may be collected from the accumulation of pellets and debris of the nests. Specimens taken from the webbing show dark yellow heads with the thorax and abdomen greenish white. The bodies were slender, somewhat cylindrical, and flattened on the ventral side. The heads were well defined, exposed, and globular. Body segments were distinct and numbered thirteen. The cephalic three are thoracic and the caudal ten abdominal.

The anterior aspect of the webworm reveals a globose to circular head which is well exposed beyond the body. Pigmentation is very deep, but the surface is smooth and polished. The vertex of the head is separated in the middle by the epicranial suture which divides into two arms, producing a figure like an inverted Y. The area enclosed by the two branches of the epicranial suture is the front and is heavily pigmented. Ventral to the front is the clypeus which appears as an indistinct sclerite with light pigmentation. The labrum is entire and is attached to the ventral margin of the clypeus. Rudimentary setae are present as slight protuberances on the lateral edges of the labrum. The mandibles articulate with the lateral edges of the clypeus and the inner margin of each mandible fits under the edge of the clypeus and labrum. The single ocellus on each side is ventral and lateral to the antennae and is hidden in the anterior view. Antennae composed of seven segments close the frontal aspect of the head.

The lateral view of the webworm shows a thoracic region composed of the three anterior segments of the body. Each segment bears prominent spiracles on the lateral edges. Three pairs of thoracic legs occur. These are conspicuous, straight, sharply pointed, and five segmented. The ten abdominal segments are well defined and much alike in structure except for the last. The dorsal region is subdivided into three or four annulets and several spiracles are scattered on the ventrolateral side. Prolegs are absent. The last segment is convex on the lateral area and bears a pair of subanal processes with ventral setae. Annulets are indistinct or missing on the dorsal surface of this segment.

From the new records reported in this paper it would appear that the pine false webworm is firmly established in Pennsylvania. The species is capable of causing local defoliation of various pines. Light infestations seem to be the rule on red pines while severe defoliation occurs on white pines. The most serious problem for the Commonwealth is at Valley Forge. State inspectors believe that the infestation is entrenched in the Park area.

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A PRELIMINARY SURVEY OF THE AQUATIC GASTROPODS OF CUMBERLAND COUNTY, PENNSYLVANIA AND INCIDENCE OF THEIR TREMATODE LARVAE

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ABSTRACT

Collections of aquatic gastropods were made at forty arbitrarily selected stations throughout the County from mid-September through early November of 1963. Species of gastropods were examined for trematode larvae and approximate incidences calculated. Species of gastropods found were *Campeloma decisum* (Say), *Fontigens attenuata* Haldeman, *Goniobasis virginica* Gmelin, *Mudalia carinata* (Brug.), *Stagnicola palustris elodes* (Say), *Fossaria obrussa* (Say), *Pseudosuccinea columella* (Say), *Helisoma anceps* Menke, *Gyraulus* (*Torquis*) *parvus* (Say), *Ferrissia rivularis* Say, *Physa gyrina* Say, *Physa heterostropha* Say. Cercarial types found were monostome, stylet, strigeid, echinostome, and species of *Notocotylus* and *Quinquiserialis*.

INTRODUCTION

This paper consists of a checklist of the snails found in Cumberland County thus far (Figure 1). The checklist is annotated in the following manner:

A species followed by a list of numbers which refer to Figure 1; an approximate incidence percentage of trematoda larvae followed by a number in parenthases which also refers to Figure 1; and any other pertinent information.

Phylum **MOLLUSCA**
Class *GASTROPODA*
Order **Ctenobranchiata** Schweigger
Family VIVIPARIDAE (Gray) Gill

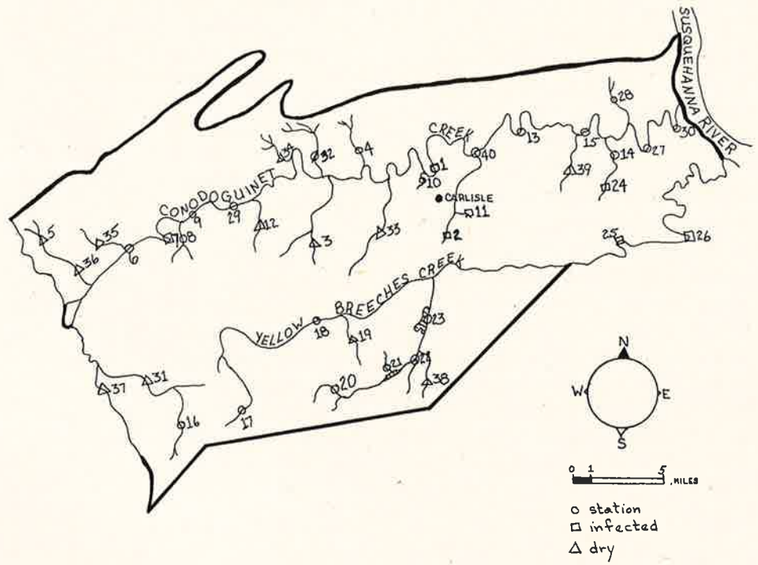

Figure 1. Cumberland County, Pa., collecting sites.

Campeloma decisum (Say): 1; no infections

Family AMNICOLIDAE (Tyron) Gill

Fontigens attenuata Haldeman: 2; 10% (2) monostome and 3% (2) stylet; very abundant

Family PLEUROCERIDAE Fisher

Goniobasis virginica Gmelin: 1, 9, 13, 15, 27, 30; no infections; found with heavy growths of algae

Mudalia carinata (Brug.): 1, 6, 7, 8, 9, 13, 22, 29, 30; no infections; found in shallow rapid water with a filamentous alga growing from spire.

Order **Pulmonata** Cuvier

Family LYMNAEIDAE (Broderip) Baker

Stagnicola palustris elodes (Say): 1, 2, 7, 14, 18, 24, 25, 29; no infections; more stunted than pond form.

Fossaria obrussa (Say): 2,; no infections; found with *S.p. elodes*.

Pseudosuccinea columella (Say): 41 (pond north of Carlisle); not collected by author

Family PLANORBIDAE H. and A. Adams

Helisoma anceps Menke: 8, 13, 18, 23; no infections

Gyraulus (*Torquis*) *parvus* (Say): 11, 8; 7% (11) *Quinquiserialis*

Family ANCYCLIDAE Menke

Ferrisia rivularis (Say): 1, 7, 13; no infections

Family PHYSIDAE Dall

Physa gyrina Say: 7, 8, 9, 10, 11, 15, 18, 22, 24, 25, 26, 27, 29; 2.5% (7) strigeid, 5% (25) echinostome, 10% (26) *Notocotylus*.

Physa heterostropha Say: 2, 8, 11, 13, 14, 22, 24, 29; 3% (24) echinostome

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ADRENAL-LIKE FUNCTIONS OF GONADOTROPHIN STIMULATED OVARIES

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ABSTRACT

Treatment of immature female rats with pregnant mare serum gonadotrophin will extend survival following adrenalectomy. The reduction in serum albumin synthesis associated with adrenal cortical insufficiency is delayed by gonadotrophin treatment.

INTRODUCTION

The extended survival of various mammals subjected to adrenalectomy during pregnancy or pseudopregnancy has implicated the ovary as a potential substitute. Stimulation of the ovary to develop lutein tissue and to extend the life span following adrenalectomy has provided both favorable and unfavorable results (Emery and Schwabe, 1936). However, pregnant mare serum gonadotrophin (PMS) did improve survival despite evidence that the ovary was less sensitive to PMS after adrenalectomy (Mandl, 1954). The usual interpretation of such experiments is that progesterone is the agent responsible for extending life but the possibility cannot be excluded that the ovary may also secrete corticoids (Zuckerman, 1953). Furthermore, normal levels of corticosterone have been noted in the serum of adrenalectomized-PMS treated rats and the concentrations of serum NPN and of liver and muscle glycogen support the idea of an adrenal-like function of the ovary (Leathem and Anilane, 1964).

Protein metabolism is adversely influenced by adrenal insufficiency as evidenced by a decreased synthesis of serum albumin (Levin and Leathem, 1942). In fact, a subnormal serum albumin concentration was noted in adrenalectomized rats maintained on NaCl (Aschkenasy, 1960) but not on cortisone acetate (Leathem, 1962). The serum proteins provided another end point for assessing the PMS-maintained adrenalectomized rat.

MATERIALS AND METHODS

Immature female rats were purchased from Charles River Breeding Laboratories. The rats were brought to 60-65 gm body weight on Purina Chow; thereafter they were fed a semi-purified diet containing 20% casein and 25% fat (Wolf and Leathem, 1955). Pregnant mare serum gonadotrophin (PMS, Cutter) was injected subcutaneously once daily in 10 I. U. dosages for five days. The rats were then adrenalectomized and the hormone continued for five or ten days. Normal animals with and without PMS treatment but fed the casein diet served as controls. In addition, untreated adrenalectomized rats were examined. Since survival averaged only 6.8 days in this group, autopsy was performed on day five.

Total serum protein was estimated by the biuret technic (Gornall, Bardawell, and David, 1948). Electrophoretic separations of serum components were accomplished using the Spinco model R paper electrophoresis for twenty hours. The paper strips were stained in bromphenol blue dye and read on the analytrol.

RESULTS AND DISCUSSION

Feeding a semi-purified diet for ten days improved the nutritional state of the immature female rat. Total serum protein increased accompanied by an increase in serum albumin from 2.65 to 3.05 gm/100 ml. Simultaneous casein feeding and administration of PMS improved both total serum protein and serum albumin over that of the dietary control. Steroid release from the ovary may have caused the improvement in the serum proteins (Levin and Leathem, 1942).

To provide a nutritional state comparable to control animals, the rats used for adrenalectomy were prefed the casein diet for 5 days. Adrenalectomy was performed and 5 days later the sera were obtained. The adrenalectomized rat exhibited a decrease in serum albumin despite a decrease in plasma volume. Hemoconcentration prevented, at least in part, a change in total serum protein. (Table I).

Immature rats treated with PMS for 5 days to iduce ovarian stimulation, then adrenalectomized and continued on PMS therapy will survive for 18 days (Leathem and Anilane, 1964). Similar animals examined at 5 days after adrenalectomy exhibited serum albumin levels approximating normal concentrations. However, at 10 days after adrenalectomy and despite PMS therapy, serum albumin was clearly subnormal and serum globulin was elevated. Continued ovarian stimulation may with the dosage of PMS used exhaust the ovarian function. Nevertheless an adrenal-like function of the ovary is indicated.

Table I

Serum Proteins of Adrenalectomized PMS Treated Rats

Treatment	Days	No. Rats	Total Protein gm/100 ml	Albumin	Globulin			
					α-1	α-2	β	γ
NONE	--	11	5.23	2.65	0.61	0.41	0.85	0.51
FED CASEIN	10	7	5.47	3.05	0.56	0.39	0.70	0.61
CASEIN + PMS	10	23	6.06	3.32	0.72	0.40	0.81	0.69
ADX	5	15	5.81	2.15	0.71	0.45	0.93	0.85
PMS x 5; ADX								
+ PMS	5	9	5.66	2.72	0.74	0.49	0.99	0.56
+ PMS	10	5	5.72	2.12	0.98	0.61	1.04	1.04

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KIDNEY ENZYMES AND ANABOLIC STEROIDS

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ABSTRACT

Alkaline phosphatase and Beta glucuronidase activities in the mouse kidney are influenced by androgens. Androgenicity can be detected by a decrease in alkaline phosphatase and a protein anabolic effect may be indicated by an increase in Beta glucuronidase. The enzymes permit an estimation of longevity of steroid action.

INTRODUCTION

It is well known that androgens will stimulate growth and protein metabolism in the prostate and seminal vesicle. Androgens will also cause nitrogen retention, body weight gain and an increase in muscle mass. Reduction in nitrogen and creatine excretion can be obtained with testosterone in castrated humans of both sexes, human eunuchoids, postmenopausal women and prepuberal children. Testosterone induced nitrogen retention is of value in panhypopituitarism, in breast cancer and in the recovery from debilitating disease. However, the androgenic effect is an undesirable "side effect" especially in females, thus prompting a search for methods to estimate anabolic steroids. In this regard, the levator ani/seminal vesicle weight ratio has been used to relate anabolic to androgenic activity but the results must be interpreted with reservation (Ahren, et al., 1962).

Fishman (1961) has called attention to renal Beta glucuronidase as being sensitive to slight changes in the steroid molecule with a view towards measuring anabolic effects. Testosterone propionate is known to increase Beta glucuronidase, while reducing alkaline phosphatase in the mouse kidney (Leathem, 1962) suggesting an anabolic and androgenic activity. Another steroid, Δ^1-17α methyl testosterone (Dianabol) which is anabolic in man but only a weak androgen exhibited an effect on only renal Beta glucuroidase activity when tested in mice (Leathem, 1962). Further studies on renal enzymes are presented.

MATERIAL AND METHODS

The Fishman procedure involves the use of adult male mice and the injection of 1 mg of steroid on alternate days for 7 injections. On day 14 the mice were autopsied, fresh kidney weight obtained and the organ examined for Beta glucuronidase activity (Fishman, et al., 1948). In the current studies, kidney alkaline phosphatase activity was also estimated (Huggins and Talalay, 1945). To determine whether a change in renal alkaline phosphatase was dose related, adult male Swiss mice were studied after 0.7, 1.4, 2.8 and 5.6 mg total dosages of testosterone propionate (Perandren, Ciba). In addition, the renal enzyme activity were measured in Swiss mice following 7 mg of testosterone propionate, $\Delta^1$17α methyl testosterone (Dianabol, Ciba, in oil and in suspension) and Δ^1 testosterone undecylenate (Ciba). The reaction of adult male C_3H mice to 7 mg of testosterone or Dianabol was examined in animals fed Rockland mouse pellets or a semi-purified diet containing 24% casein and 25% fat.

Longevity of hormone action was studied in Swiss mice castrated when 8-10 grams body weight. After a two week postoperative period a single subcutaneous injection of 19 nortestosterone deconate (Organon) was administered. Mice

were examined at 5, 10, 15 and 20 days after injection of 0.25, 0.5 and 5.0 mg dosages.

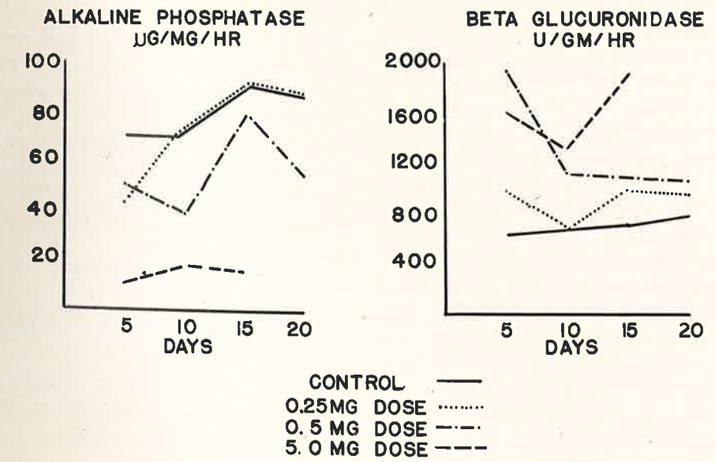
RESULTS

Adult Swiss mice were given testosterone propionate in 7 doses of 0.1, 0.2, 0.4 and 0.8 mg each on alternate days. Kidney weight per 100 gms body weight increased from 1417 in untreated controls to 1757 in mice given 5.6 mg. Only the lowest dosage did not increase kidney weight. Nevertheless, the 0.1 mg dosage decreased renal alkaline phosphatase activity significantly from 86 μg/mg/hr to 55 μg/mg/hr. Renal enzyme activity was 48, 35 and 23 μg/mg/hr following 0.2, 0.4 and 0.8 mg daily dosages respectively.

Testosterone propionate, 1 mg x 7, caused a significant decrease in alkaline phosphatase and a significant increase in Beta glucuronidase in the mouse kidney. Dianabol in suspension did not influence renal alkaline phosphatase despite the 7 mg total dose but significantly increased renal Beta glucuronidase. Dianabol in oil had no effect. Δ^1 testosterone undecylenate in a 7 mg total dosage over the 14 day period decreased alkaline phosphatase activity from 34 to 14 μg/mg/hr and increased Beta glucuronidase from 707 to 2005 u/gm/hr.

The response of adult male C_3H mice were examined in response to testosterone propionate and to Dianabol. The renal enzyme changes simulated those obtained with Swiss mice. Testosterone propionate in 1 mg dosages given on alternate days for 7 injections reduced renal alkaline phosphatase and increased Beta glucuronidase. Dianabol at the 7 mg dosage was primarily effective on renal Beta glucuronidase. Diet influenced the enzyme activities of untreated mice. C_3H mice fed the semi-purified 24% casein diet for 14 days exhibited renal alkaline phosphatase activities of 46 μg/mg/hr as compared with 86 μg/mg/hr

KIDNEY ENZYMES IN CASTRATED SWISS MICE FOLLOWING 19-NORTESTOSTERONE DECANOATE



in pellet fed mice. However, Beta glucuronidase activity was 284 μ/gm/hr following the casein diet and 94 μ/gm/hr following the commercial diet.

The effect of a single injection of a long acting steroid, 19 nortestosterone deconate was tested in Swiss mice castrated when immature. Treated control mice were compared at each of the 5, 10, 15 and 20 day post-injection periods. Following 0.25 mg and 0.5 mg renal alkaline phosphatase was not influence but the 5.0 mg dose depressed enzyme activity in 5 days and sustained this action. Renal Beta glucuronidase was not altered by 0.25 mg of steroid but 0.5 mg increased enzyme activity in 5 days and sustained the effect for at least 10 more days (Figure 1). Following a 5.0 mg single injection of steroid renal Beta glucuronidase rose 1000 units of activity/gm/hr above control and remained at this level for the duration of the experiment.

DISCUSSION

The kidneys of adult and immature rodents exhibit a renotrophic response to testosterone propionate (Kochakian, et al., 1954; Kochakian and Harrison, 1962). The increased kidney size is accompanied by an increase in kidney protein (Kochakian, et al., 1950; Korner and Young, 1955; Granitsas and Leathem, 1962) but may also involve an increase in water (Pfeiffer, et al., 1940). Testosterone will stimulate the incorporation of C^{14} glycine into renal protein, but the effect returns to normal in 2-4 days (Freiden, et al., 1961). Using RNA: DNA ratios to measure protein synthesis, Kassenaar, et al., (1962) characterized the androgen response of the kidney as due to an increase in cell size as opposed to the seminal vesicles which undergoes both hypertrophy and hyperplasia.

The kidney is well known to contain enzymes which respond to androgens. In this regard d-amino actid oxidase, arginase, glutamic-pyruvate transaminase, acid phosphatase, alkaline phosphatase and Beta glucuronidase have been examined. Kochakian (1959) noted an increase in enzyme activity when a kidney weight response was noted. However, alkaline phosphatase exhibited a dose related response in which this renal enzyme increased following a low dose of androgen but decreased following a large dose. Kochakian (1959) considered the decrease in enzyme as representing an overstimulation. In the present experiments a decrease in alkaline phosphatase was noted in the absence of a kidney weight change. Kochakian (1959) indicated that arginase activity was more sensitive to 17 methylated steroids and Fishman (1961) observed that renal Beta glucuronidase was remarkably sensitive to slight changes in the steroid molecule.

Previous investigators have called attention to the influence of diet on the renal enzyme response to androgens (Kochakian, 1945; Fishman, et al., 1955). An influence of diet was noted in the present studies, but the response to androgen was not modified. Furthermore, sex and strain of mice have been noted as factors to be considered (Fishman and Farmelant, 1953). Current investigations reveal that Swiss and C_3H mice responded well and that castration does not modify the response but castration will minimize variations in the assessment of longevity of steroid action.

ACKNOWLEDGMENTS

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PROTEIN METABOLISM, PROTEIN NUTRITION AND HYPOPHYSECTOMY

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ABSTRACT

The influence of hypophysectomy and protein nutrition was studied in adult male rats following a month of protein-free feeding. Hypophysectomy slowed the rate of protein repletion, induced organ weight changes and prevented synthesis of serum albumin. Lactalbumin, caesin and wheat gluten failed to modify the effects of pituitary ablation. Wheat gluten restricted the repletion of normal animals.

INTRODUCTION

Feeding a low level of protein, despite adequate caloric intake, will deplete the body protein stores. As the proteins of the body are expended in protein depletion, organ function begins to change, the digestive tract is altered, and hormone production is reduced. The tissues soon begin to conserve nitrogen, and protein catabolism is reduced. The refeeding of protein reveals an enhanced tendency for tissue protein anabolism. Data are needed to clarify whether or not hormones will aid adequate nutrition in the refilling of body protein stores (Leathem, 1962).

It is well known that protein anabolism is reduced by hypophysectomy and restored by growth hormone (Russell, 1957). However, at least part of the physiological change ascribed to pituitary ablation does not occur if protein stores of the body are depleted prior to the operation. Thus if adult male rats are hypophysectomized at the end of one month of protein free feeding and then refed protein, a body weight gain and nitrogen retention are observed (Leathem, 1958). However, body weight gain is subnormal and kidney mass clearly decreases.

Hypophysectomy is known to alter serum protein concentrations by decreasing albumin and increasing globulin (Levin and Leathem, 1942). If rats are fed a protein-free diet for 30 days, total serum protein decreases primarily because of a loss of serum albumin. Refeeding 18% casein resulted in a return of total serum protein to normal concentrations despite hypophysectomy owing to an increase in globulin; serum albumin failed to improve. In contrast, the normal rat exhibited an increase of total serum protein levels during refeeding because of an increase in albumin.

Despite the many physiological studies relating protein metabolism to the pituitary, the precise nutritional requirements of the hypophysectomized rat are not known (Meites and Nelson, 1960). The present investigation is concerned with an examination of dietary protein quality on several aspects of protein metabolism during the repletion of hypophysectomized rats.

MATERIALS AND METHODS

Adult male Long-Evans strain rats were used. These animals were fed a semi-purified agar based diet (Glasser, 1954) devoid of protein for 34-45 day periods to deplete the protein stores. Hypophysectomy was then performed and for the next 20 days, diets containing 18% casein, lactalbumin or wheat gluten were fed ad libitum. Paired fed unoperated rats served as controls for each dietary group. Normal rats fed 18% casein for 30 days or fed a protein free diet for 37 or 70 days provided additional control data.

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At autopsy, blood serum was obtained and total protein was estimated by the biuret method (Gornall, Bardawell and David, 1948). Serum was separated electrophoretically using the Spinco model R paper electrophoresis system. For serum protein analysis, strips were stained in bromphenol blue dye and read on the Spinco Analytrol. In addition the fresh weights of liver, kidney, testes, heart and gastrocnemius muscle were obtained.

RESULTS

A diet lacking protein induced a sharp decrease in body weight. Associated with the body weight loss was a significant decrease in liver, kidney, heart, testis and gastrocnemius muscle weight. Testis weight was reduced only 12%, however. Relating organ weight to body weight revealed that, in general, organ weight decreases paralleled the loss in body weight. Extending the protein depleting period to 70 days did not substantially reduce liver or kidney weights beyond that noted after 37 days. In fact, liver weight relative to body weight was greater in long term protein depleted rats than in fullfed rats (3.6 vs 2.5 gm/100 gm body weight). However, the more prolonged depletion period did reduce heart, muscle and testis weight.

As anticipated, protein depletion decreased total serum protein due to a reduction in serum albumin. The degree of change was related to the duration of the depletion period.

After one month of protein depletion, the refeeding of 18% casein induces a rapid gain in body weight. However, the part played by the endocrine system in the repletion process is not clear. Therefore, adult male rats were hypophysectomized before refeeding the 18% casein. Contrary to the loss in body weight usually associated with pituitary ablation, these rats gained 50 gm during 20 days despite the lack of growth hormone. The hormone deficiency was apparent, nevertheless, as the paired fed normal rat gained 102 gm. The greater body weight gain by the normal rat was reflected in enhanced kidney and heart weight increases in comparison with the hypophysectomized rat. In fact, the kidney failed to participate in the anabolic process in hypophysectomized rats. This organ not only failed to increase in actual mass but became smaller in relationship to body weight. Testis atrophy induced by hypophysectomy was not influenced by available dietary protein (Table 1).

Table 1

Hypophysectomy, Protein Metabolism and Protein Nutrition

	Protein Free Diet	35 - 45 days					
		18% Casein		18% Lactalbumin		18% Wheat Gluten	
		Hypox	Control	Hypox	Control	Hypox	Control
No. of rats	9	8	8	6	6	14	14
Body Wt. Change gm	-62	+50 ± 22	+102 ± 29	+21 ± 30	+77 ± 29	+12 ± 15	+10 ± 14
Organ Wt. gm							
Liver	10.27 ± 1.42	9.48 ± 1.38	10.76 ± 1.33	8.41 ± 1.29	10.48 ± 1.39	9.49 ± 1.79	10.33 ± 2.43
Kidney	1.76 ± 0.27	1.70 ± 0.15	2.31 ± 0.35	1.55 ± 0.18	2.40 ± 0.21	1.58 ± 0.22	2.03 ± 0.30
Testis	2.73 ± 0.21	0.94 ± 0.20	3.00 ± 0.24	1.02 ± 0.12	2.85 ± 0.35	1.10 ± 0.16	2.79 ± 0.34
Heart	0.83 ± 0.11	0.79 ± 0.08	1.05 ± 0.15	0.67 ± 0.11	0.98 ± 0.10	0.70 ± 0.10	0.85 ± 0.11
Muscle	1.22 ± 0.14	1.33 ± 0.10	1.53 ± 0.20	1.17 ± 0.13	1.42 ± 0.20	1.16 ± 0.16	1.17 ± 0.18
Organ Wt. gm/100 gm B.W.							
Liver	3.03 ± 0.31	2.69 ± 0.42	2.56 ± 0.13	2.54 ± 0.28	2.67 ± 0.16	2.89 ± 0.27	3.16 ± 0.38
Kidney	0.52 ± 0.05	0.47 ± 0.03	0.55 ± 0.04	0.47 ± 0.04	0.62 ± 0.05	0.48 ± 0.03	0.63 ± 0.05
Testis	0.81 ± 0.10	0.26 ± 0.06	0.73 ± 0.13	0.31 ± 0.04	0.73 ± 0.05	0.34 ± 0.05	0.87 ± 0.10
Heart	0.25 ± 0.04	0.22 ± 0.02	0.25 ± 0.02	0.20 ± 0.03	0.25 ± 0.02	0.21 ± 0.02	0.27 ± 0.02
Muscle	0.36 ± 0.04	0.36 ± 0.03	0.36 ± 0.03	0.35 ± 0.02	0.36 ± 0.03	0.36 ± 0.04	0.36 ± 0.02
Serum Protein							
Total gm/100 ML	4.92 ± 0.33	6.21 ± 0.30	5.75 ± 0.28	5.74 ± 0.33	5.42 ± 0.28	5.86 ± 0.41	5.30 ± 0.76
Albumin	1.95 ± 0.30	2.07 ± 0.20	2.55 ± 0.12	1.89 ± 0.36	2.48 ± 0.31	1.93 ± 0.28	2.20 ± 0.47
Globulin	2.97 ± 0.29	4.14 ± 0.25	3.20 ± 0.31	3.85 ± 0.54	2.94 ± 0.31	3.93 ± 0.37	3.10 ± 0.42
A/G ratio	0.67 ± 0.13	0.50 ± 0.06	0.81 ± 0.10	0.51 ± 0.15	0.86 ± 0.17	0.50 ± 0.10	0.71 ± 0.14

± S.D. = standard deviation

Refeeding of casein was followed by an increase in total serum protein which was greater in the hypophysectomized than in the normal rat. In the absence of the hypophysis, however, serum albumin failed to improve whereas serum globulin increased resulting in no improvement in the albumin/globulin ratio. In the normal rat being refed 18% casein, a significant increase in serum albumin was noted with a lesser increase in globulin (Table 1).

Proteins differ in nutritive value when tested in normal animals, but few data are available on hypophysectomized rats. Therefore, 18% lactalbumin and 18% wheat gluten were substituted for casein during the repletion of normal and hypophysectomized rats prior protein depleted. Hypophysectomized rats gained 21 gm in body weight compared with a 77 gm body weight gain exhibited by normal rats fed lactalbumin for 20 days. Organ weight and serum protein changes simulated those seen when casein was fed. Total serum protein increased in rats with and without pituitary glands but unlike casein refeeding, the feeding of lactalbumin did not differentiate between the two physiological states. The failure to synthesis albumin did, however, characterize the pituitary deficiency.

Prior protein depleted hypophysectomized rats gained only 12 gm when fed 18% wheat gluten, but the normal rat failed to outgain the operated animal. Organ weights revealed that kidney and heart weights were less in hypophysectomized rats and that muscle failed to increase in normal animals. Furthermore, liver weight to body weight ratios remained abnormally high in wheat gluten fed rats. Total serum protein increased significantly in hypophysectomized rats fed wheat gluten but not in normal rats. In both groups of rats serum albumin was not improved but serum globulin did increase (Table 1).

DISCUSSION

Hypophysectomy is followed by a loss in body weight, a decrease in nitrogen retained and a reduced uptake of amino acids into liver and muscle (Manchester and Young, 1959; Korner, 1961). Thus protein anabolism is reduced by hypophysectomy. However, at least part of the physiological changes ascribed to pituitary ablation do not occur if the protein stores of the body are depleted prior to the operation. Hypophysectomy does not negate enhanced anabolism of repletion as determined by amino acid incorporation into the diaphragm (Wool, 1960) or by nitrogen balance (Leathem, 1958). However, body weight gain is subnormal, kidney mass decreases, and serum albumin concentration fails to improve. The feeding of lactalbumin, a protein superior to casein in nutritive value, was not beneficial (Leathem, 1962). In the present studies, a protein of lesser biological value (wheat gluten) revealed that a normal rat pair fed to the hypophysectomized animal did not exhibit a greater body weight gain, nor an improved serum albumin synthesis. Perhaps pituitary hormone synthesis is less efficient in wheat gluten fed animals. The data emphasize that hormones control rates of reaction and that protein metabolism in a hormone deficiency circumstance may be markedly influenced by the state of the body-protein reserves.

ACKNOWLEDGMENTS

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PLANT GROWTH RESPONSE FOLLOWING EXPOSURE TO A SHORT DURATION ELECTROSTATIC FIELD

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ABSTRACT

Crop yield on a dry weight basis is less for grain sorgum seedlings grown under the influence of a short period electrostatic field of 40 kilovolts per meter reference potential gradient as compared with an equal number of control plants under identical growth conditions with no field applied. Normal crop yield returns following successive harvests after the removal of the electric field, which appears to be indicative of some polarization phenomena associated with plant metabolisms.

INTRODUCTION

In a study of plant growth under the influence of an electrostatic field conducted by Briggs, (1926) some 40 years ago, it was shown that over a ten year period that plants treated in the field and in the greenhouse showed no response to charged networks suspended above them. Several other investigators, e.g.; Jorgensen and Priestley, (1914), Jorgensen and Stiles, (1917), Shibasawa and Shibata, (1927) about the same time reported plant growth increases for cereals and grass crops grown under the influence of a short duration electric field. More recently, however; Murr, (1963) has shown that grass crops grown under continuously simulated environmental electrostatic fields of sufficient magnitude produce less growth, and are observed to develop a leaf damage which, under proper electric field magnitudes, can completely destroy the plants. This leaf damage mechanism is not completely understood, but existing experimental evidence seems to suggest a polarization phenomenon which may effect enzyme constituents of the respiratory metabolism (Murr, 1963).

Since exposure of plants to an electrostatic field for long periods of time tended to cause leaf burning and general growth damage (Murr, 1963), it seemed only logical to assume that contrary to experimental findings of the past several decades, that plants grown under short period electric fields might respond to these conditions by developing subnormal crop yields. It was further proposed that growth normalcy would return following the elimination of the electric field, and the consequent relaxation of dipole stresses.

EXPERIMENTAL PROCEDURE

Two identical plots were each planted with thirty germinated seeds of grain sorgum in the greenhouse (mean temperature of 80°F) as in previous experiments by the author, (1963). A reference potential gradient of 40 kilovolts per meter was established across one plot (Murr, 1962), while the other plot remained uncharged in order to establish an experimental control. Three days after the initial planting and the application of the electrostatic field, the excitation voltage for the field was turned off; and the plants in both the activated and the control plots allowed to grow normally.

Following the initial saturation of the soil, the moisture level in each plot was maintained at approximately 16% by weight (75% field capacity for the sand-lime-soil mixture used). All other environmental factors, e.g., light, temperature, etc. associated with the plots in the greenhouse were maintained as nearly identical as possible for active and control plants.

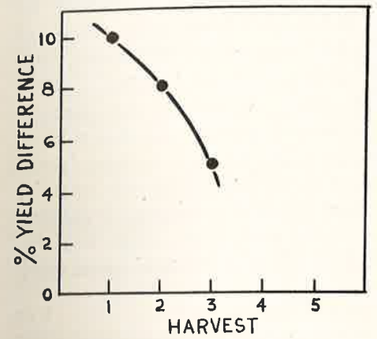

Fig. 1. Activated plant growth decrease expressed as a percentage of the control plant yield on a dry weight basis. Three harvests are shown.

The plant heights in each were measured twice daily throughout the course of the experiments, and the average plant height per day for both the activated and the control plots computed. Units of inches were used to make the measurements since it was desired to show only apparent differences in plant height of the active and control plots, and not a low correlation-statistical average of micro-measurements. The plants in each plot were harvested at intervals of approximately one week to a height of 1 inch above the soil. The harvested dry weights were then compared on a weight per plant basis.

RESULTS

Figure 1 shows the results of the comparison of the dry weights of harvested plants. These results appear to be in

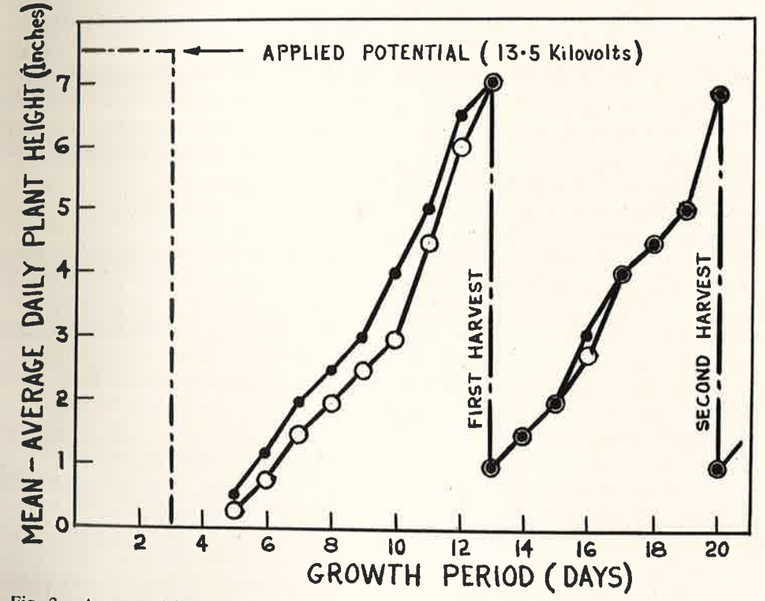

Fig. 2. Average daily growth height response of grain sorgum following exposure to an electrostatic field as compared with control plant averages. Following the second harvest, the response was the same for activated and control plant height averages. Open circles represent active plant data; solid circles represent control plant data.

complete agreement with the proposed theory and the previously reported investigation of electric field polarization by Murr, (1963). The results appear to be quite contrary to the earlier results of Blackman and Legg, (1924) and others, e.g., Jorgensen and Priestley, (1914) and Jorgensen and Stiles, (1917). The present concept is further supported by the plant height response curves illustrated in Figure 2. Similar response curves have also been obtained for orchard grass started from seedlings. It should be pointed out that in the short period exposure experiments presented here, no plant or leaf damage was observed.

DISCUSSION AND CONCLUSIONS

The results of the present investigation clearly indicate that short period electrostatic potential gradients of sufficient magnitude can cause a decrease in plant growth and subsequent crop yield. If this phenomenon is extended to include crop yields in a general sense, it may be that the mechanism involved would give a possible explanation for crop damage following large storms and similar localized charge density phenomena as reported by Miller, (1938). The return of the activated plants to normal crop yield as a function of growth and increased harvests shown in Figure 1 seems to be in line with what one would expect in considering a polarization-relaxation concept, which would allow for a gradual return of normal metabolic responses.

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THYROIDAL UPTAKE AND TURNOVER OF I-131 IN RATS EXPOSED TO REDUCED BAROMETRIC PRESSURE*

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ABSTRACT

Albino rats exposed to a simulated altitude of 20,000 to 23,000 feet (350-308 mm Hg) exhibit transient alterations in thyroid function as well as changes in body weight, water balance and food consumption. These changes are most marked during the first few days of exposure.

INTRODUCTION

For several years our group has been concerned with the mechanisms of adaptation of animals to lowered oxygen tension. Much of our previous work dealt with compensatory adjustments which occur in the cardiovascular system and in respiratory enzymes of rats exposed to a simulated altitude of about 20,000 feet (1-7). Briefly, the main findings of these earlier studies are as follows. One of the most striking changes noticed in altitude exposed animals is a marked increase in the volume of blood vessels. For example, Kreider observed that the total blood volume increases in rats exposed to altitude while the blood plasma volume decreases (1). It was further shown that there is an increase in the volume of larger blood vessels as well as capillaries during altitude acclimation (1, 2). A separate study showed that an increase in the concentration of cardiac and skeletal myoglobin constitutes another feature of adaptation (3, 4, 5). Cellular metabolism, however, seems to be unaffected by altitude exposure (6, 7).

During the course of this earlier work we were continually impressed with the observation that the adaptive changes in the cardiovascular system are initiated very shortly after the introduction of animals into the altitude chamber. Also, these changes follow in the wake of an immediate and marked drop in body weight of animals. In view of the cardinal role of the thyroid in the regulation of metabolism, the present study was designed to investigate the role of the thyroid gland during the initial period of altitude acclimatization. The specific aim was to determine if measurable changes occur in thyroid function during the onset of altitude exposure.

PROCEDURE

Fifty-four mature, female Sprague-Dawley rats, weighing about 200 grams were used during the course of the present study. Twelve of these were used for data on body weight changes, water and food consumption and urinary volume for 8 days prior to decompression and 6 successive days following introduction into a decompression chamber maintained at 308 mm Hg pressure. Forty-two rats were used for radiometric analyses of thyroid function. In all cases control rats were maintained at the State College altitude of 1200 feet (*ca* 725 mm Hg). The altitude-exposed groups were housed at a pressure of 308 mm Hg which simulates an altitude of about 23,000 feet.

In vivo measurements of radioactivity in the thyroid region of the neck were made using a DS-101 scintillation detector and a model 181-A decade scaler (Nuclear Chicago). Briefly, the procedure was as follows. Rats were intraperitoneally injected with 5 microcuries of

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I^{131} (Tracervial, Abbott) upon introduction into the decompression chamber and the radioactivity was counted using a lead collimator positioned over the thyroid area of immobilized, unanaesthetized rats. Corrections were made for the radioactivity in extrathyroidal tissues and for isotopic decay. Radiometric data were taken during three time intervals within the first one hour after I^{131} injection to determine the maximal count in the neck region and subsequently at 24 hour intervals for 6 successive days without removing the rats from the decompression chamber. Five groups of rats were used: (a) controls kept at ambient pressure (725 mm Hg); (b) rats given I^{131} at the onset of altitude exposure (308 mm Hg); (c) rats given I^{131} after 15 days of previous exposure and kept in the chamber for 6 additional days (308 mm Hg); (d) thiouracil fed (0.09% in drinking water) controls; and (e) thiouracil treated rats given I^{131} at the onset of altitude exposure (308 mm Hg). For convenience, the data are presented as semilog plots of average counts per minute against time intervals after I^{131} injection. Straight lines are obtained for the comparison of raidoiodine release rates. In all cases, average neck counts were made on the same 5 groups of rats during the first hour and at daily intervals for 6 days using 7-11 rats per group. The standard error of the mean radioactive counts ranged from 5 to 8 percent. The radiometric data were analyzed using Student's *t* test.

RESULTS

Before summarizing the thyroid data it is pertinent to briefly describe the immediate behavioral response of rats subjected to an altitude of approximately 23,000 feet above sea level. One first notices that the rats are very lethargic, they do not move about the cage, consume no food and drink very little water during the first 24 hours after introduction into the altitude chamber. The consequence of this is a marked drop in weight, which reaches its lowest value at the end of the second day of exposure, and begins to increase slowly thereafter. The growth rate continues to be slower than in the control animals, so that they normally do not regain their pre-exposure weight until about four weeks of confinement in the altitude chamber.

A typical set of data on a group of 12 rats is shown in Figure 1, which summarizes the weight changes for a week before altitude exposure and for six days during altitude exposure. The data on food and water consumption and urinary excretion are also presented. It is seen that the precipitous weight loss which occurs during the first day of exposure can be largely attributed to the decrease in the amount of water and food consumed. It may also be noted that although there is a drop in urine volume, the magnitude of this drop is not as great as the decrease in water intake.

Radiometric analyses of thyroid function showed that the onset of exposure to reduced pressure is also characterized by transient alterations in thyroid activity. Specifically, it was observed that a higher percentage of radioactivity remains in the thyroid gland of altitude exposed rats relative to control rats at the end of 24 hours following I^{131} injection. However, there was no appreciable difference in the *rate of release* of radioiodine of hypoxic rats as compared to controls after the first day of exposure to reduced pressure. The data supporting these findings are summarized in Figures 2 and 3.

The rate of disappearance of radioactivity from the thyroid region during the first 24 hours after I^{131} injection is shown in Figure 2 for control rats, acclimated rats, unacclimated rats and two thiouracil treated groups. These data

show that maximal counts were obtained in all groups within the first hour after injection of radioiodide. At this time, the counts in the thyroid region proved to be significantly lower ($p<.01$) in unacclimated rats exposed to hypoxia than those of controls or of rats which had acclimated for 15 days prior to injection. The higher neck counts observed in unacclimated rats exposed to hypoxia for

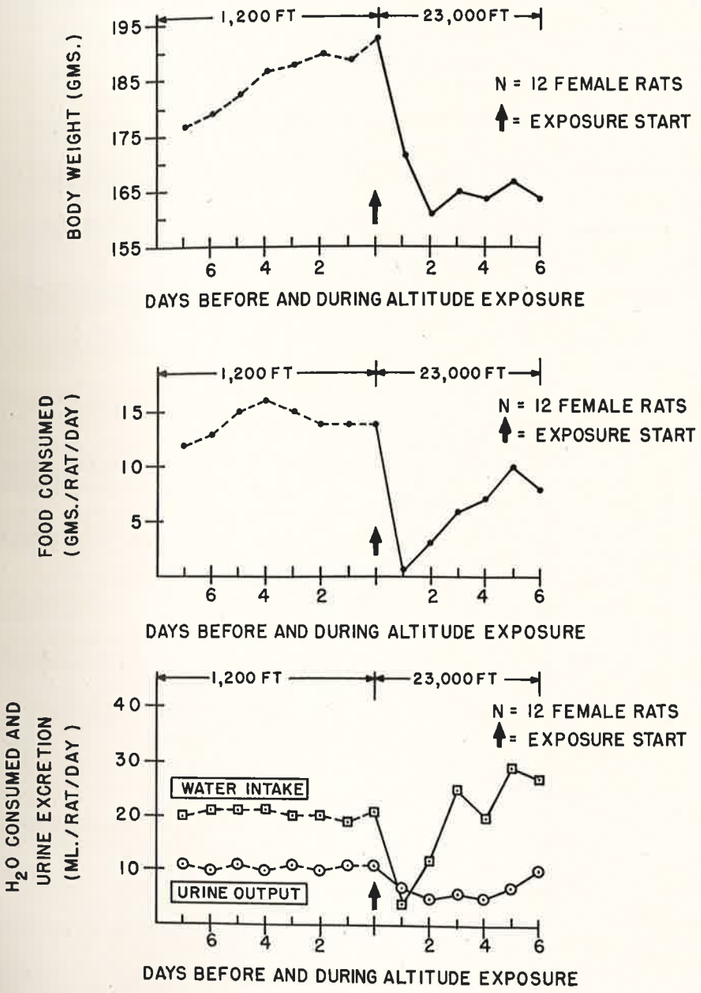


FIG. 1 METABOLIC RESPONSE OF RATS TO 23,000 FEET SIMULATED ALTITUDE. VALUES EXPRESSED AS MEANS.

24 hours does suggest that the onset of hypoxia is associated with a greater retention of labeled thyroid hormone at the end of the first day of exposure.

In Figure 3, the slopes of the lines drawn through the average counts reflect the rate of release of thyroid hormone in the same groups of rats beyond the 24

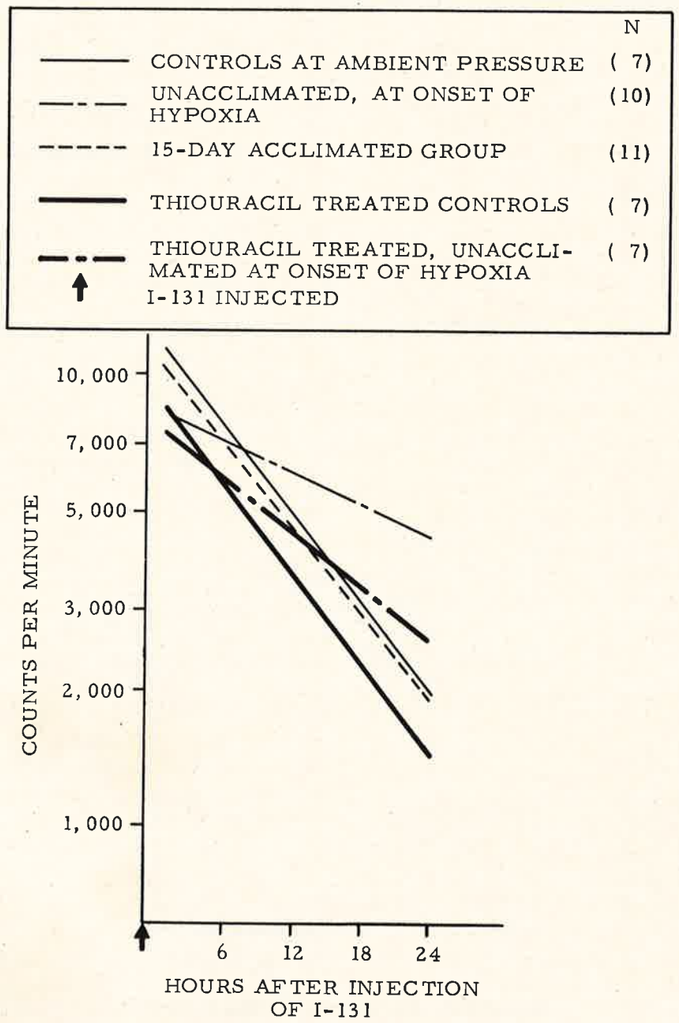
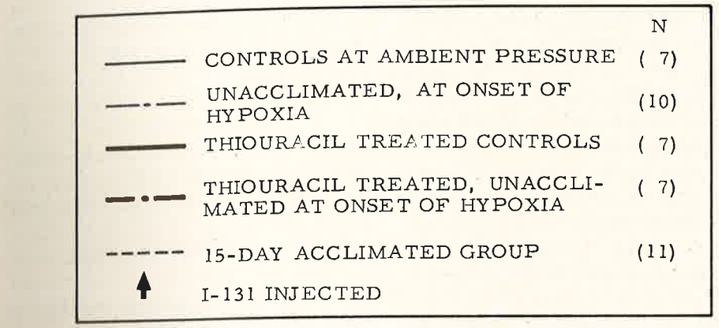


Fig. 2 Maximal radioactivity and 24-hour thyroidal uptake of I-131 during first day of exposure to reduced barometric pressure (308 mm Hg).

hour point with continued exposure. It is apparent that after the first day there are no appreciable differences in the turnover of I^{131} of control rats as compared to acclimated or unacclimated rats for the next 5 days. Thiouracil fed rats, as expected, had lower counts than the corresponding control and hypoxic groups



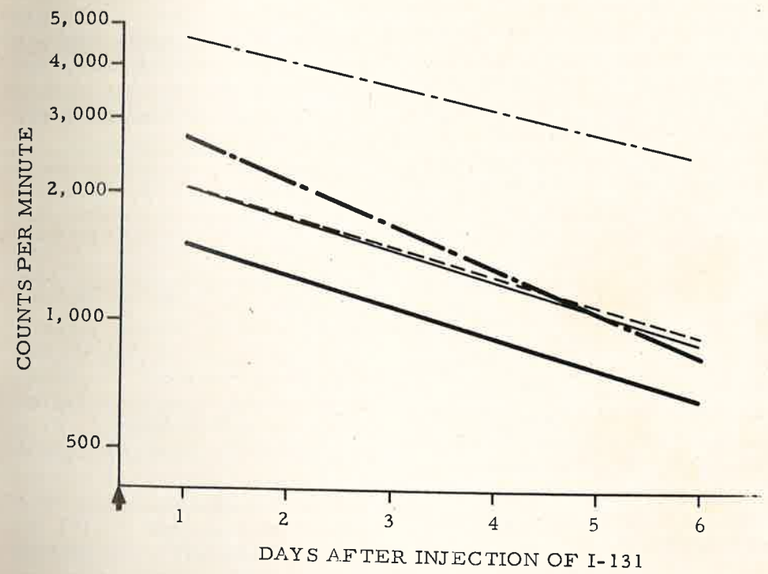


Fig. 3 Effect of exposure to 23,000 feet simulated altitude on the turnover of I-131 by the rat thyroid for 1 to 6 days after injection of I-131.

which were not fed thiouracil. It was of interest, however, that thiouracil appeared to be less effective in blocking recycling of radioiodide in the hypoxic rats than in the controls during both the first 24 hour interval as well as during the next 5 day period.

To recapitulate, the above radiometric data indicate that: (1) unacclimated rats exhibit a transient hypothyroidism during the first day of exposure to hypoxia, as reflected in an increased retention of labeled thyroid hormone at the 24 hour point but no difference in the rate of release of hormone beyond day one, and (2) rats which have acclimated for 15 days appear to be euthyroid with respect to both the 24 hour uptake of radioiodide and the rate of release of thyroid hormone during 6 additional days of continued exposure.

An indirect measure of the relative amounts of thyroid stimulating hormone (TSH) being released from the pituitary can be obtained from a comparison of the weights of the thyroids of rats exposed to altitude and their controls. This was done from representative animals from both the thiouracil treated groups and the rats which received no thiouracil. It was found that the mean weight ($\pm$ SE) of the thyroid glands of the altitude exposed rats (7 $\pm$ 0.4 mgm.) decreased to about half the weight of the controls (15 $\pm$ 1.6 mgm.). The thiouracil treated control thyroids were also significantly heavier (24 $\pm$ 2.8 mgm.) than the thiouracil treated altitude exposed rats (13 $\pm$ 0.7 mgm.). Histologic and histochemical analysis of these thyroids supported the interpretation that hypoxia resulted in reduction of TSH output and consequently a lowered thyroid function.

DISCUSSION

The decrease in water consumption and volume of urine excreted by altitude exposed rats agrees with the results of Mefferd and Hale (9) who studied the effects of thermal conditioning on the metabolic response of rats to altitude exposure. Similar results were also obtained by Anthony who observed no appreciable drop in urine volume with a highly significant drop in water intake during the first day of exposure of rats to a simulated altitude of about 23,000 feet (10). It is probable that one of the factors which accounts for the rapid increase in hematocrit, which is a well documented feature of altitude acclimatization, is a negative water balance at the onset of exposure. The increased hematocrit is most marked during the first day of altitude exposure and occurs too rapidly to be a consequence of erythropoiesis.

Stickney and Van Liere (11) reported that growth and body weight drop in rats exposed to altitude. The present finding that altitude exposure induces a lose of body weight agrees with the findings of Mefferd and Hale (9). Mefferd *et al.* observed an increased fasting weight loss in altitude acclimated rats, but concluded that this was not due to water imbalance, since the water:urine ratio was not altered.

There can be no doubt that the metabolic adjustments such as lethargy, transient anorexia, and weight loss which reflect the immediate response of rats to hypoxic conditions are tied in with changes in thyroid function. It was suggested by Verzar *et al.* (12) that the thyroid may play an important part in the metabolic adjustment of animals to altitude. They found that rats subjected to 18,000 feet simulated altitude had a decreased thyroidal uptake of I-131, but at lesser altitudes only a light decrease was noted. It was further noted that after several days of exposure to altitude I-131 was again taken up with normal

velocity, so that presumably the changes in thyroid function were only temporary. Their data agree with the present findings of decreased thyroid function during the initial phase of altitude acclimatization and of a restoration of thyroid function after 15 days of exposure.

These thyroid changes are presumably the consequence of a decreased output of thyroid stimulating hormone by the pituitary, as evidenced by decreased thyroid weight and histological and histochemical changes in the thyroids from the animals used in this experiment. Many kinds of environmental stimuli affect the release of thyroid hormone. It is well known that systemic stressors (13) usually induce a prompt and prolonged inhibition of thyroid secretion, presumably a consequence of decreased release of TSH.

It is possible that the initial response of rats to altitude (i.e. loss of body weight, drop in food and water consumed and urine produced, lowered thyroid function) reflects a reduction in the metabolic rate of these animals. It is reasonable that such a response could be beneficial during hypoxia. For example, lowered metabolism and conservation of energy is known to be associated with many stressful situations. Inactivity and lowered metabolism of rats immediately upon exposure to hypoxia could reduce their oxygen consumption until permanent physiological changes, such as increased hemoglobin in the blood and cardiovascular enlargement, restore an adequate oxygen supply to the various tissues and organs.

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GENETICS OF *GRIZZLE-BELLY* (Sl^{gb}) IN THE MOUSE*

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ABSTRACT

Matings of Grizzle-belly mice with both Steel and Steel-Dickie mice produce anemic progeny. The interactions of these genes indicate that they are alleles. Additional evidence of the allelic relationship is provided by linkage tests of Steel-Dickie and Ames-waltzer. The recombination value of 16.9 ± 3.0% is in agreement with the value of 16.0% reported between Grizzle-belly and Ames-waltzer. On the basis of these results both the Steel and *dystrophia muscularis* loci are considered to be part of linkage group IV.

Hematological investigations revealed that Grizzle-belly heterozygotes possess a macrocytic anemia through adulthood.

INTRODUCTION

The mutant Grizzle-belly in the house mouse was first described by Schaible (1960) apparently having arisen spontaneously from an untreated non-agouti female of genetic constitution (*aa bt bt* $Mi^{wh}/+$). The mutant behaves as a dominant producing one-half grizzle-belly progeny in outcrosses of Grizzle-belly to normal mates. Matings of heterozygotes ($Sl^{gb}/+$) produce approximately one-fourth anemic progeny and three-fourths normal progeny when classified at birth or shortly thereafter. The anemics apparently represent homozygous Grizzle-belly (Sl^{gb}/Sl^{gb}) and usually die during the first postnatal week of life. Grizzle-belly heterozygotes exhibit a normal or near normal viability and fertility. Grizzle-belly was reported by Schaible (1963a) to be in Linkage group IV on the basis of testcross results with Ames-waltzer (av). There was reported a value of 16% recombination between the two loci. Additional three-point testcrosses which included silver (si) reported by Schaible (1963b) indicate that the gene order is silver, Grizzle-belly, Ames-waltzer (si-Sl^{gb}-av.).

Grizzle-belly with its effects on pigmentation and hematopoetic organs closely resembles alleles at the steel locus (Russell, 1963). Several alleles have been described for this locus including Steel (Sl) and Steel-Dickie (Sl^{d}), and some 30 other "steeloid" mutants have been discovered, many of which have been considered to be allelic to Steel. Recombination tests between Steel and *dystrophia muscularis* (dy) and Steel-Dickie and dy yielded a recombination value of 31.9 ± 2.2% (Wolfe, 1963).

Since Grizzle-belly and Steel were so similar in their effects, it was felt of interest to examine the allelic relationship of the genes. The present investigation is concerned with the interactions of Grizzle-belly with Steel and Steel-Dickie, establishment of the Steel locus in Linkage Group IV of the mouse, and a hematological study of Grizzle-belly.

ALLELIC TESTS OF GRIZZLE-BELLY WITH STEEL AND STEEL-DICKIE

Steel and Steel-Dickie mice were obtained through the courtesy of Dr. H. Glenn Wolfe of the Jackson Laboratory, Bar Harbor, Maine. Both mutants were on a C57BL/6J background, having been backcrossed to this strain for 5 and 7 generations respectively at the start of these investigations. Grizzle-belly was originally obtained from Dr. Robert Schaible and was subsequently backcrossed into the C57BL/6 strain maintained at Rutgers University.

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Table 1. Results of Crosses of Grizzle-belly (Sl^{gb}) with Steel (Sl) and Steel-Dickie (Sl^{d})

Mating	Progeny		
	Anemic (Sl/Sl^{gb})	"Steeloid" ($Sl/+$ or $Sl^{gb}/+$)	Normal (+/+)
$Sl/+$ x $Sl^{gb}/+$	6	20	6
	Anemic (Sl^{d}/Sl^{gb})	"Steeloid" $Sl^{d}/+$ or $Sl^{gb}/+$)	Normal (+/+)
$Sl^{d}/+$ x $Sl^{gb}/+$	12	45	15

Results of crosses of Grizzle-belly with Steel and Steel-Dickie are given in Table 1. It may be seen that in both sets of crosses anemic progeny were produced. These progeny apparently represent the compound heterozygotes Sl/Sl^{gb} and Sl^{gb}/Sl^{d}. Most of the anemics died during the second week of life although one individual of genetic constitution Sl^{gb}/Sl^{d} survived to adulthood. The mouse, a male, was a black-eyed white and was anemic at all stages, having a red blood cell count of about 3.04 x 10^{6} per mm^{3} at 6 months of age compared to a 10.25 x 10^{6} for normal mice. Although it was mated to females of known fertility, no progeny were produced. The individual was dissected at seven months of age. The testes were seen to be atrophic and there was no sign of spermatogenesis in microscopic sections.

Although the number of progeny in each cross is small, the observed numbers of anemics, steeloids, and normal are close to what is expected. There is a slight deficiency of anemic progeny, which, if real, might indicate that some of the compound heterozygotes die in utero.

LINKAGE OF STEEL-DICKIE AND AMES-WALTZER

Further evidence of the allelism of Steel alleles and Grizzle-belly was provided by testcrosses involving Steel-Dickie and Ames-waltzer in repulsion heterozygotes (Sl^{d}+/+av x +av/+av). Results are shown in Table 2. Linkage is established between Steel-Dickie and Ames-waltzer. The recombination value of 16.9% is in agreement with the value of 16% reported by Schaible for grizzle-belly and Ames-waltzer. Results indicate that the Steel-locus is located with Linkage Group IV of the mouse. The position of *dystrophia muscularis* (dy) in Group IV remains to be elucidated.

HEMATOLOGY OF GRIZZLE-BELLY

A detailed hematological investigation of Grizzle-belly was undertaken, and the complete results will be presented elsewhere. Some of the preliminary data are presented in Table 3. Standard

Table 2. Testcross Results of Steel-Dickie with Ames-Waltzer

Mating	No. Litters	No. born	Progeny (classified 3-4 weeks of age)			
			Sl^{d}	av	+	Sl^{d}av
Sl^{d}+/+ av x + av/+ av.	19	178	72	61	16	11

$$\text{Recombination} = \frac{27}{160} = 16.9 \pm 3.0$$

Table 3. Mean values with standard errors for red blood cell counts (RBC), per cent hematocrit (H%) and cell volumes (MCV) for adult males and females of grizzle-belly and normal mice.

Genotype	Sex	n	RBC/mm^3 x 10^6	(H %)	MCV (u^3)
$Sl^{gb}/+$	♀	10	8.51 ± .27	48.82 ± .72	57.75 ± 4.24
+ / +	♀	10	10.81 ± .24	49.74 ± .43	45.80 ± 0.99
$Sl^{gb}/+$	♂	10	8.77 ± .21	45.90 ± .85	52.30 ± 1.33
+ / +	♂	10	10.25 ± .32	48.10 ± .60	47.40 ± 1.89

hematological methods as applied to the Unopette (Freundlich and Gerarde, 1963) were used to determine the red blood cell count per cubic mm., the hemoglobin determination per 100 cc. of blood, and the percentage of packed red cells (hematocrit) in Grizzle-belly heterozygotes and their normal litter-mates in adults of both sexes. It may be seen from Table 3 that Grizzle-belly heterozygotes appear to possess a slight macrocytic anemia. Red blood cells showed a decrease in numbers of about 21 per cent and 14 per cent in the female and male heterozygotes respectively. However, Grizzle-belly heterozygotes exhibited a greater mean cell volume.

DISCUSSION

Until recently Linkage Group IV was difficult to test against newly arisen mutants due to the unsuitable nature of the known markers in the group including rodless retina (r), pigmy (pg) and silver (si). The establishment of Ames-waltzer in this linkage group and the subsequent discovery of linkage of Grizzle-belly with Ames-waltzer has provided some valuable markers for testing this linkage group. The results reported in this paper indicate that Grizzle-belly is an allele of the Steel locus and also places *dystrophia muscularis* in Linkage Group IV. As of the time of this report, 19 of the minimum possible 20 linkage groups have been discovered. Since some of the groups are based on only a few markers and in some cases could not be adequately tested against Linkage Group IV, it is possible some of these other groups are in fact part of Group IV.

Based on the hematological results the macrocytic anemia of Grizzle-belly heterozygotes persists through the adult life of the animal. The degree of red blood cell decrease in number is comparable to that reported for Steel. In the initial description of Steel, Sarvella and Russell (1956) noted that red cell counts were 20-30% lower in Steels than in their wild-type litter-mates when tested between 7 and 13 days postnatally. Bennett (1956) in a more comprehensive hematological study of steel observed red blood cell counts throughout life of Steel heterozygotes and normal mice. Red bleed cell counts in Steels reached a low of 62.6% of the normal counts at 1 week of age, climbed to 78.9% at 2 weeks of age and remained around that value through adulthood (2-5 months of age). The number of red blood cells in Steel was of the order 7.70 x 10^6/mm^3 at 2-5 months. Results for Grizzle-belly of comparable age give a mean red blood cell count of about 1 million more cells. This difference may be due in part to differences in genetic background and in part to differences between the two alleles. One of the principal differences among the three alleles at the Steel locus

that have been studied in some detail is the difference in viabilities of the homozygous types. For example, almost all Sl/Sl homozygotes die in utero whereas nearly all Sl^{gb}/Sl^{gb} types survive to birth, but die shortly thereafter. This difference in viability appears to hold regardless of genetic background. In general, the Grizzle-belly allele appears to be similar to Steel and Steel-Dickie in the nature of its pleiotrophic effects on pigmentation and hematopoiesis.

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TWO UNUSUAL NEW CERCARIAE FROM CRAIG COUNTY, VIRIGINIA[1]

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ABSTRACT

Two new species of cercariae are described from the gastropod *Anculosa subglobosa* (Say) collected in John's Creek, Craig County, Virginia. Characteristics by which both of these cercariae can be distinguished from morphologically similar species are given.

INTRODUCTION

During the months of June through August, 1963, the authors conducted a survey of larval trematodes infecting freshwater molluscs in the proximity of Mountain Lake Biological Station, University of Virginia, Mountain Lake, Giles County, Virginia. The purpose of this survey was to determine locations where there existed a high percentage of infection among molluscs by larval trematodes so that subsequent embryological studies on larval trematodes and physiological studies on host-parasite relationships between these helminths and their molluscan hosts could be carried out. The results of such studies are being reported elsewhere. During the course of this survey, a number of new cercariae were encountered. In this paper is reported two new species of cercariae isolated from *Anculosa subglobosa* (Say) collected in Craig County.

MATERIALS AND METHODS

A total of 140 specimens of *Anculosa subglobosa* were collected from that segment of John's Creek in Craig County approximately one mile from the Giles and Craig county line. The snails were brought into the laboratory, individually isolated in distilled water in finger bowls, and fed lettuce leaves. Daily examination of the snails revealed that 48 or 34.2 per cent emitted a monostomate opthalmocercaria while 2 or 1.4 per cent emitted a relatively large distomate cercaria. Most of the snails began emitting cercariae within 48 hours after being isolated; however, a few did not commence releasing cercariae until after 72 hours. All the snails were maintained for 14 days before they were discarded. No instances of dual infections were found.

Specimens of both species of cercariae were studied in the living state by use of a phase-contrast microscope. The cercariae were slowed down by the weight of a No. 1, 18 mm. square cover-glass. In some instances staining with neutral red was employed to facilitate the study of internal morphology.

The shells of a number of the infected snails were subsequently removed and the redial generations of both species were studied in smear preparations of the hepatopancreatic tissue.

All the measurements given below were made with an ocular micrometer and given in millimeters.

Cercaria multiglandulosa n. sp.
(Figures 1, 2)

This unusual monostomate cercaria resembles the members of the "Bioculate" group of Faust (1917) since there are two eye-spots present. In comparing this cercaria with the morphologically similar ones, it was decided that it should be considered as representative of a new species.

The specific name, *multiglandulosa,* is derived from the Latin words *multus,* meaning many, and *gladulosus,* meaning glandular, to describe the many penetration glands present in this species.

Description of cercaria (based on 10 specimens): Opthalmomonostomate cercaria with fin tail. Body elongate, 0.170 (0.152-0.192) long by 0.089 (0.072-0.104) wide; cuticle completely spinous except for that covering tail; three prominent papillae present at anterior end, one medial, two mediolateral; oral sucker subterminal, 0.033 (0.025-0.041) by 0.029 (0.025-0.032); esophagus long, bifurcating in middle third of body to form short intestinal ceca; no pharynx observed; intestinal ceca short, terminating at midlength of body; two irregularly shaped eye-spots, one on each side of midlength of esophagus, composed of brownish-black pigment granules; parenchyma includes light brownish pigment granules; 12 large penetration glands, roughly arranged in two rows, situated immediately posterior to cecal bifurcation; ducts from penetration glands directed anteriorly along midline of body, empty to exterior anteriorly in area of papillae; excretory vesicle large, V-shaped, containing refractile granules; tail 0.321 (0.280-0.360) long by 0.032 (0.031-0.032) wide, pointed terminally, with two transparent membranous fins; tail fins extend from anterior one-third of tail length to posterior extremity.

Host: *Anculosa subglobosa* (Say)

Type locality: John's Creek, Craig Co., Virginia.

Cercaria multiglandulosa is highly motile. It swims actively by thrashing its powerful tail. It will settle to the bottom of the dish periodically where it is not as efficient at crawling as many distomate cercariae. When attached to the substratum, its body will extend and contract. With each contraction, the size of the excretory vesicle is greatly diminished. Continuous observation of *C. multiglandulosa* at the bottom of finger bowls and on glass slides did not reveal any symptoms of encystment. It is acutely positively phototrophic. When placed in a bowl half covered with black paper, the cercariae will congregate in the exposed portion within 15 to 20 minutes.

Cercaria multiglandulosa develops in rediae which are situated between the hepatopancreatic tubules in the molluscan host. When teased out of the host's tissues, the rediae are highly motile, their movement is made possible by the extension and contraction of the body wall musculature and by flexual movement. The older rediae examined (Fig. 2) include from 23-30 fully developed cercariae. No naked cell aggregates, germ balls, or developing cercariae are present. In younger specimens, germ balls and developing cercariae, as well as fully developed cercariae, are present in the brood chambers.

Description of redia (based on 8 specimens): Body elongate, with blunt anterior and posterior ends, averaging 1.300 long by 0.276 (0.264-0.280) wide, may be as long as 1.664 when extended, and 1.000 when contracted; brownish in appearance due to pigment granules in cercarial parenchyma; pharynx at anterior end, 0.043 long by 0.039 wide; intestinal cecum unbranched, simple, 0.138-0.180 long by 0.043 wide, terminating in anterior one-fifth of body; fully developed cercariae motile in brood chamber; no birth pore observed.

As stated above, *Cercaria multiglandulosa* is most similar to the members of the "Bioculate" group of Faust (1917) since like the latter, it is pigmented, without a ventral sucker and a pharynx, with two eye-spots, and develops in

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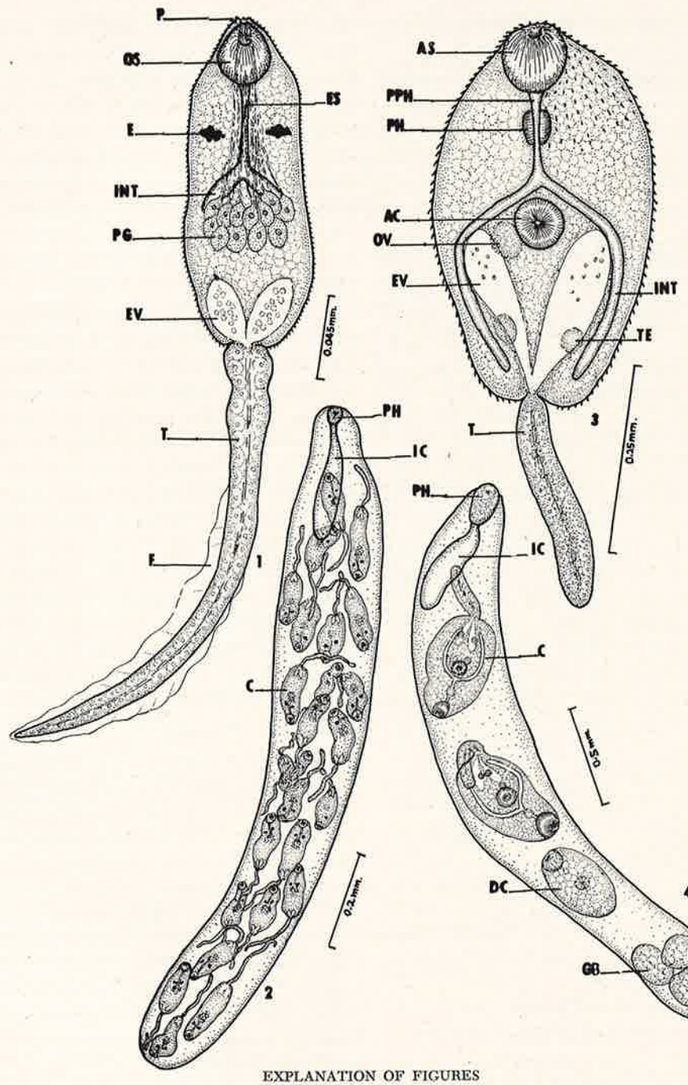

EXPLANATION OF FIGURES

AC = acetabulum; AS = anterior sucker; C = fully developed cercaria; DC = developing cercaria; AC = acetabulum; AS = anterior sucker; C = fully developed cercaria; DC = developing cercaria; E = eye-spot; ES = esophagus; EV = excretory vesicles; F = fin-fold; GB = germ ball; IC = redial intestinal cecum; INT = cercarial intestinal cecum; OS = oral sucker; OV = developing ovary; P = papilla; PG = penetration gland; PH = pharynx; PPH = prepharynx; T = tail; TE = developing testes.

Fig. 1. *Cercaria multiglandulosa* n; sp.; drawn from living specimen. (Ventral view.)
Fig. 2. Redia of *C. multiglandulosa;* drawn from living specimen.
Fig. 3. *Cercaria magnivesica* n. sp.; drawn from living specimen. Cystogenous glands not shown. (Ventral view.)
Fig. 4. Redia of *C. magnivesica;* drawn from living specimen.

rediae. However, there are some striking differences in that posterolateral "adhesive" or "locomotor" pockets are absent in *C. multiglandulosa,* prominent penetration glands are present, and there is a fin-tail. In comparing *C. multiglandulosa* with the morphologically similar species, it was found that it is most similar to *Cercaria aurita* Faust, 1918 from *Goniobasis pulchella* in Illinois, *C. hemispheroides* Faust, 1924 from *Planorbis saigonensis* in Soochow, China, and *C. yenchingensis* Faust, 1930 from *Bithynia fuchsiana* in Peking, China. The new species differs from these three species primarily by the presence of longitudinal fin-folds on its tail and by the presence of prominent penetration glands. In these morphologically similar species, such structures are absent. It can be further distinguished from *C. aurita* by the absence of posterolateral "locomotor" pockets and lappet processes situated just lateral to the eye-spots. Furthermore, the short intestinal ceca of *C. multiglandulosa* differs it from *C. aurita* since in the latter the ceca are club-shaped and reach to near the posterior end of the body.

C. multiglandulosa can be further distinguished from *C. hemispheroides* by its body shape which is not rotund on the dorsal surface and somewhat discoid and concave on the ventral surface as it is in the latter. Furthermore, the shape of the eye-spots in the two species differ, being elongate oval in *C. hemispheroides,* and the body dimensions of *C. multiglandulosa* (0.170 mm. by 0.089 mm.) are smaller than that of *C. hemispheroides* (0.20 mm. by 0.18 mm.) Like the new species, *C. hemispheroides* lacks posterolateral pockets.

The new species can be further distinguished from *C. yenchingensis* by the length of its intestinal ceca since in the latter the ceca terminate near the posterior end of the body. Furthermore, the body dimensions of *C. yenchingensis,* being 0.006 mm. by 0.046 mm., is considerably smaller than that of the new species.

Cercaria magnivesica n. sp.
(Figures 3, 4)

Of the 140 specimens of *Anculosa subglobosa* examined, only 2 were infected by a globose, leptocercous cercaria which is being described herein as representing a new species.

The specific name, *magnivesica,* is derived from the Latin words *magnus,* meaning great or large, and *vesica,* meaning vesicle, to describe the large, conspicuous excretory vesicle found in this cercaria.

Description of cercaria (based on 10 specimens): Distomate, leptocercous cercaria. Body pear-shaped, slightly dorsoventrally flattened, 0.538 (0.020-0.540) long by 0.322 (0.300-0.340) wide; slightly wider in anterior half; cuticle covering body beset with large, conspicuous spines arranged in symmetrical rows, that covering tail is aspinous; anterior sucker terminal, 0.066 (0.062-0.68) long by 0.068 (0.062-0.070) wide; acetabulum, 0.042 (0.038-0.044) long by 0.063 (0.060-0.064) wide, smaller than anterior sucker, situated in middle of body; prepharynx, 0.024 (0.019-0.026) long, pharynx, 0.045 (0.041-0.045) long by 0.043 (0.041-0.044) wide, and short esophagus, 0.054 (0.049-0.056) long, present; cecal bifurcation immediately anterior to acetabulum; intestinal ceca long, reaching near posterior end of body; developing ovary present, ovoid, situated to right and slightly posterior to acetabulum; developing testes, two in number, ovoid, situated intercecally in posterior one-third of body; excretory vesicle V-shaped, large and conspicuous, containing refractile granules, arms extending to mid-

length of body; tail, 0.330 (0.320-0.338) long by 0.040 (0.038-0.042) wide, shorter than body, blunt posteriorly. Subcuticular cystogenous glands present in body proper.

Host: *Anculosa subglobosa* (Say)

Type locality: John's Creek, Craig Co., Virginia.

Cercaria magnivesica is a sluggish swimmer, spending most of the time crawling along the bottom of the finger bowl by utilizing its suckers. Its body is capable of a certain degree of contraction and extension although not dramatically. By far the majority of the cercariae emitted from infected snails are found at the bottoms of finger bowls. Continuous observation of *C. magnivesica* revealed that after swimming and crawling for from 10-45 minutes, it undergoes preparation for encystment by losing its tail and its body contracts so that it is practically round.

C. magnivesica develops in rediae which are embedded between the gastropod host's hepatopancreatic tubules. Unlike in the case of the rediae of *C. multiglandulosa,* relatively few rediae of *C. magnivesica* are present in each infected snail. Approximately 10-25 rediae per snail are present. Furthermore, each redia only enclosed from 2-4 fully developed cercariae in its brood chamber. This redia is very sluggish.

Description of redia (based on 5 specimens): Body elongate, posterior end more rounded than anterior end, 3.080 (3.020-3.152) long by 0.484 (0.463-0.492) wide; pharynx at anterior terminal, 0.210 (0.207-0.216) long by 0.124 (0.116-0.128) wide; intestinal cecum unbranched, simple, 0.488 (0.484-0.490) long, terminating in anterior one-fourth of body; germ balls, 3-6 in number, situated in posterior portion of brood chamber; fully developed cercariae, 2-4 in number, sluggishly motile, situated more anteriorly, developing cercariae may be present; no birth pore observed.

Since the morphological features of *Cercaria magnivesica,* except for the presence of the large excretory vesicle and the gonadal anlagen, is common to most distomate leptocercous cercariae, it is difficult to state which of the known species it is most similar to. However, a search of the literature revealed that this cercaria resembles *Cercaria complicata* Faust, 1930, to a certain degree but differs from it since it lacks the 6 pairs of prominent penetration glands found in *C. complicata.* It is surprisingly similar to the cercariae of *Fasciola* spp. in possessing a pear-shaped body, no penetration glands, and apparently does not require a second intermediate host. However, its body dimensions are larger; its tail is shorter than the body; its excretory vesicle is conspicuously larger; there is a prepharynx, and the morphology of the redial stage is different from that of *Fasciola* spp. in that no ambulatory buds are present.

DISCUSSION

It is of interest to note that monostomate cercariae of both the "Bioculate" and "Trioculate" groups of Faust (1917), which roughly correspond with the "Urbanensis" and "Ephemera" groups of Sewell (1922) respectively, do not require a second intermediate host but will encyst on stones, aquatic vegetation, etc. and the encysted metacercariae are ingested by the definitive host. In this respect, *Cercaria multiglandulosa* differs biologically from the known cercariae of these groups since it apparently does not encyst outside of a host. The utilization of a second intermediate host, or possibly direct penetration into the definitive host, is suggested by its possession of prominent penetration glands since the function of such glands is known to be associated with the process of penetration.

The fact that the older rediae of *C. multiglandulosa* only include fully developed cercariae and no birth pore is present may be suggestive that the cercariae escape by rupturing the redial wall thus destroying the rediae in the process. In addition, the synchronized development of cercariae in rediae, i.e., the cercariae develop at approximately the same rate, is apparent since all the cercariae observed in the older rediae are fully developed. Even in younger rediae, where germ balls exist in addition to fully developed cercariae, the former have all commenced to elongate thus indicating that the process of differentiating into cercariae has begun (see Cheng and James, 1960; Cheng, 1961). No young germ balls, i.e., those composed of a few cells enveloped within a membrane, or naked cell aggregates are present.

In the case of *Cercaria magnivesica,* although only a few cercariae and germ balls are present in the brood chambers of the rediae, the relatively early stage of differentiation and growth of the germ balls observed in the same rediae in which fully developed cercariae are also found suggest that synchronized development, as observed in the rediae of *C. multiglandulosa,* does not occur.

The absence of penetration glands in *C. magnivesica* and its preparative behavior for encystment, while attached to the bottom of finger bowls, both suggest that this cercaria does not require a second intermediate host.

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FOOD HABITS OF THE TOADFISH, *OPSANUS TAU* (*L.*), IN NEW JERSEY WATERS

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ABSTRACT

The digestive tract contents of 136 toadfish collected from 1952 to 1957 were analyzed. One hundred seventeen fish were obtained in Delaware Bay and its tributaries while the remainder came from the Mullica River. The fish ranged from 5.0 to 33.1 centimeters in total length. There was a 2:1 ratio of males to females, and no female was over 28 centimeters in length. Food organisms were found in 106 (78%) of the animals. Crustaceans were found in over 98% of the fish with food. Xanthid crabs, hermit crabs and prawns were the dominant food items among the thirteen identifiable species of crustaceans. *Neopanope texana sayi, Pagurus longicarpus* and *Crangon septemspinosa were* the dominant species. Although thirteen species of molluscs were identified, their incidence and numbers were insignificant in comparison to the crustaceans. The incidence of fish in the stomach contents was also low. There is a positive correlation between the availability of various crustacean species in the environment and their incidence as toadfsh food.

INTRODUCTION

The toadfish is a common permanent inhabitant of the oyster grounds of New Jersey and elsewhere along the Atlantic coast. Suitable shelter and an abundance of food organisms for this voracious feeder are provided in the oyster-bed community. Although the toadfish is somewhat of an indiscriminate feeder, all of the available evidence indicates that its diet consists principally of crustaceans (Linton, 1905; McDermott and Flower, 1952; Chrobot, 1951; Schwartz and Dutcher, 1963). Reid (1954) reported a similar predominance of crustaceans in the stomachs of the related species *Opsanus beta* Goode and Bean from the Gulf of Mexico.

Aside from the above mentioned studies there is relatively little quantitative information available on the food habits of *O. tau*. Previous observations on the food of this species in New Jersey waters is limited to the short accounts of Verrill (1871), Stauber (1943) and McDermott and Flower (op. cit.). It is the purpose of this paper to present the food analyses of over one hundred fish collected primarily in Delaware Bay and its tributaries, and also in the Mullica River, the principal tributary of Great Bay located along the southern Atlantic coast of the state. The food habits in relation to the available bottom fauna will be discussed.

COLLECTIONS AND METHODS OF ANALYSIS

The majority of the 135 toadfish examined from 1952 to 1957 (Table I) were collected in oyster dredges, while the remainder were obtained with hook-and-line or from the compartments in cement blocks used as weights for oyster spat collectors. One hundred and seventeen fish were collected from the New Jersey side of Delaware Bay. Most of these were captured on the main oyster-producing areas of the Bay (Maurice River Cove), while several were collected in the adjacent tributaries. The other 18 fish came from the oyster beds of the Mullica River.

The total lengths of most of the fish were measured to the nearest 1/16 inch and later converted to centimeters, while others were measured to the nearest 0.1 centimeter (Table I). All but 12 of the 135 specimens were sexed. Of those not sexed, 7 were in the 5-10 cm. category.

TABLE I

Toadfish collections

Location and year	Number, mean total length and range (cm.)							
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Delaware Bay								
1952			9 20.4 8.0-27.0	1 8.2	13 21.4 5.0-32.0	14 21.9 5.2-30.0		
1953			29 24.5 16.8-31.1					
1954								1 20.9
1955		24 25.4 16.0-33.1		25 21.5 13.1-26.7				
1957							1 30.8	
Mullica River								
1955			3 20.5 15.3-31.0	7 21.7 14.8-26.0	2 17.8 14.7-20.0			
1956	6 20.9 5.3-24.8							

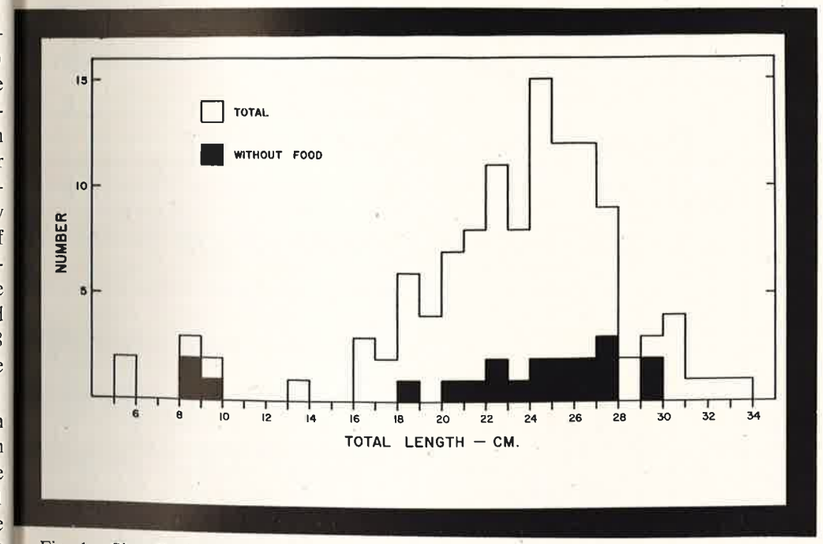

Fig. 1. Size distribution of toadfish in one-centimeter intervals collected in Delaware Bay from 1952-1957.

TABLE II

Analysis of the digestive tracts of toadfish collected in Delaware Bay

Food item	1953 % Frequency	1953 Number	1955 % Frequency	1955 Number	Total % Frequency	Total Number	% of fish food
Crustacea							
Neopanope texana sayi	70.7	57	61.4	78	66.4	222	40.8
Eurypanopeus depressus	30.0	9	34.1	36	25.3	48	8.8
Rhithropanopeus harrisi	10.0	2	2.3	3	5.3	14	2.1
Panopeus herbsti	10.0	2	13.6	7	9.5	10	1.8
Xanthids — unidentified			4.5	3	2.1	3	0.6
Callinectes sapidus					3.2	1	0.2
Ovalipes ocellatus			2.3	1	1.1	1	0.2
Libinia sp.					3.2	3	0.6
Pagurus longicarpus	70.0	43	43.2	55	49.5	146	26.8
Pagurus pollicaris					1.1	1	0.2
Upogebia affinis			2.3	1	1.1	1	0.2
Crangon septemspinosa	35.0	8	43.2	57	28.4	66	12.1
Palaemonetes sp.			2.3	1	2.1	2	0.4
Small crustaceans*					3.2		
Mollusca							
Crepidula fornicata			9.1	4	4.2	4	0.7
Crepidula convexa	10.0	2			3.2	4	0.7
Crepidula plana					2.1	2	0.4
Polinices duplicatus					1.1	1	0.2
Anachis avara			4.5	3	4.2	5	0.9
Mitrella lunata			2.3	1	1.1	1	0.2
Nucula proxima			2.3	1	1.1	1	0.2
Crassostrea virginica					1.1	1	0.2
Mercenaria mercenaria			2.3	1	1.1	1	0.2
Mulinia lateralis					1.1		
Ensis directus	5.0	P1	11.4	P5	6.3	6**	1.1
Mya arenaria	93.2 (27/29)		73.5 (36/49)		80.1 (92/115)		
Pisces — unidentified	29		49		115		
Nematodes	20		44		95		
Number of fish examined							
Number with food							

Food item	1952 % Frequency	1952 Number
Crustacea		
Neopanope texana sayi	71.0	87
Eurypanopeus depressus	9.7	3
Rhithropanopeus harrisi	6.5	9
Panopeus herbsti	3.2	1
Xanthids — unidentified		
Callinectes sapidus	3.2	1
Ovalipes ocellatus		
Libinia sp.	9.7	3
Pagurus longicarpus	45.2	48
Pagurus pollicaris	3.2	1
Upogebia affinis		
Crangon septemspinosa	3.2	1
Palaemonetes sp.	3.2	1
Small crustaceans*	9.7	P3
Mollusca		
Crepidula fornicata		
Crepidula convexa	3.2	2
Crepidula plana	6.5	2
Polinices duplicatus	3.2	1
Anachis avara	6.5	2
Mitrella lunata		
Nucula proxima		
Crassostrea virginica	3.2	P1
Mercenaria mercenaria		
Mulinia lateralis	3.2	1
Ensis directus	3.2	P1
Mya arenaria	3.2	P1
Pisces — unidentified		
Nematodes	78.4 (29/37)	
Number of fish examined	37	
Number with food	31	

P Present; superscript indicates number of fish
* Mostly amphipods (gammarids and *Unicola irrorata* identified); also, the megalops of a crab found on one occasion.
** Arbitrary approximation

The fish examined ranged from 5.0 to 33.1 cm. in total length. The length distribution of the 117 fish from Delaware Bay may be seen in Figure 1. One hundred five of these were sexed—68 males and 37 females. Males ranged from 13.1 to 33.1 cm. and averaged 25.4 cm., while the females were 16.0 to 27.6 cm. and averaged 21.5 cm. in total length. This 2:1 sex ratio, and the fact that fish larger than 28 cm. were males, corresponds very closely with the recent data of Schwartz and Dutcher (1963) for the toadfish in Chesapeake Bay around Solomons, Maryland, collected in the summer of 1961.

Ninety-seven of the 117 fish (82.9%) from Delaware Bay had food in their stomachs and/or intestines. Nine of the 18 fish (50.0%) from the Mullica River had food, and all of these were from the 1955 collection. The quantitative data presented below are based only on the total number of fish with food in their digestive tracts (Tables II and III).

All except the Mullica River fish were examined soon after capture in an unpreserved condition. The stomachs and intestines of 12 of the 18 fish from the Mullica were removed on the date of collection and preserved in formalin for later analyses. No distinction was made between the contents of the stomach and the intestine. The food organisms were identified and counted. Volumetric analyses were not attempted, but the numerical data on the individual items will serve the same purpose in the discussion of the food habits.

Crustaceans, other than the prawns, were usually easily identified. Very often the whole animals in various stages of digestion were wrapped in sheaths of mucus. Carapaces of the prawns could be identified and counted. Figures for the latter may be considered as minimal because some may have been mutilated beyond recognition. All univalve shells were carefully examined for either the remains of hermit crabs or the soft parts of the snail. Several empty snail shells, which were obviously old and eroded, were no doubt ingested with living food species and are not included in the data. Bivalve shells were examined for evidence of adhering soft parts, and obvious old, blackened fragments are also not included. Ingested fish were nearly always in an advanced state of digestion and highly fragmented. Specific identifications could not be made (except for one case among the Mullica River fish) and consequently numerical data comparable to those obtained for the invertebrate food species are not available. Aquatic vegetation and debris were seldom found.

RESULTS

Crustaceans predominated in the fish from the Delaware Bay region (Table II) as well as in the limited sample of fish

TABLE III

Analysis of the digestive tracts of toadfish collected in the Mullica River, 1955.

Food item	% Frequency	Number	% of fish food
Crustacea			
Neopanope texana sayi	55.6	14	45.2
Eurypanopeus depressus	11.1	3	9.7
Rhithropanopeus harrisi	22.2	4	12.9
Panopeus herbsti	11.1	1	3.2
Callinectes sapidus	11.1	1	3.2
Pagurus longicarpus	11.1	1	3.2
Upogebia affinis	33.3	3	9.7
Mollusca			
Urosalpinx cinera	11.1	1	3.2
Pisces			
Hippocampus hudsonius	11.1	1	3.2
Unidentified	22.2	P2	6.5°
Nematodes	66.7 (8/12)		
Number of fish examined		12	
Number with food		9	

P Present; supercript indicates number of fish
° Arbitrary approximation assuming 1 fish in each of the two stomachs

from the Mullica River (Table III). Data presented in Table II (last column) reveal that crustaceans account for 94.8% of the toadfish food. The two fish collected in November, 1954, and October, 1957 (Table I), were both feeding on crustaceans. The former contained 2 *Eurypanopeus* and 1 *Panopeus,* while the latter had 1 large *Pagurus pollicaris* with *Crepidula fornicata* and *C. plana* attached to its shell.

Xanthid crabs comprised 54.1% of the fish food, with *Neopanope* the dominant species. As many as 12 of the latter were found in a single fish. Since the bulk of the fish were collected in Delaware Bay proper, where *Neopanope* is the most common xanthid (McDermott and Flower, 1952; McDermott, 1960), there is a positive correlation between the availability of this crab and its incidence in the toadfish. *Rhithropanopeus* was found in five fish from the Delaware Bay collections. These fish were captured in the tributaries of the Bay. This crab is the dominant species in the lower salinity areas. Twelve of the 14 crabs came from toadfish collected in the Maurice River and Nantuxent Creek. This species also appeared in 2 of the 9 fish from the Mullica River. It would appear that there is no preference for certain xanthid species but the availability of the species determines their incidence as toadfish food. Schwartz and Dutcher (1963) found only *Rhithropanopeus* and *Eurypanopeus,* of the five species of xanthids occurring in Chesapeake Bay, in the large number of toadfish which they examined. This correlates very well with the known distribution of these two species in the headwaters of the Bay (Ryan, 1956).

Pagurus longicarpus is the next most common food item in Delaware Bay. As many as 12 were found in a single stomach. In Delaware Bay it is the dominant hermit crab on the oyster grounds, whereas the larger *P. pollicaris* occurs predominantly in the lower high-salinity regions, but even here it is subordinate to the other species. There appeared to be no seasonal differences in the incidence of these pagurids or the xanthids mentioned previously.

The prawn, *Crangon septemspinosa,* was far more prevalent in the digestive tracts than *Palaemonetes* sp. (probably *vulgaris*). The annual and seasonal incidence of *Crangon* was rather erratic. In 1952, one prawn was found in the 9 fish with food collected from June 5-28, while none was found in the fish obtained in August and September. Of the 29 fish collected in June of 1953, 7 had fed on this species. In 1955, 19 of the 24 fish collected in May had *Crangon,* with up to 10 in a single stomach. However, in the July collection of the same year prawns were absent. These observations indicate that *Crangon* may be one of the more important food items in the diet of the toadfish during the spring.

Callinectes and *Libinia* are common residents of the oyster beds, but they were rarely found in the toadfish. *Ovalipes,* a more stenohaline species and not a frequent inhabitant of the oyster beds of Delaware Bay, would not be expected to be a significant item in the diet. Its only occurrence was in May, 1955, in a fish from the lower part of the Bay along the Bay Shore Channel.

Upogebia affinis, the mud lobster, was found in one fish from Delaware Bay and in three from the Mullica River. This animal is the only truly burrowing species of all the crustaceans listed. As it seldom if ever leaves its burrow once it becomes established (Pearse, 1945), one would not expect it to be a very common food item.

Although 13 species of molluscs were identified in the intestinal contents of the toadfish, there appears to be no question that they were incidental items in the diet. Their low frequency of occurrence and very small numbers (Tables II and III) illustrate this point. Approximately 4% of the toadfish food consisted of molluscs but about half of this figure consisted of the various *Crepidula* species which were always attached to or associated with hermit crabs in the same stomach. These limpets are just as incidental as the hydroid *Hydractinia* and the tubiculous annelid *Sabellaria* which were also found on the hermit crab shells. The other four species of gastropods were always associated with large numbers of crustaceans except in one case where one *Anachis* was the only food item present.

The clams *Mya* and *Ensis* found in a single stomach in June, 1952, were probably of the 1952 year-class, and were thus very small nad fragile. The same fish had 2 *Neopanope* and 5 *P. longicarpus* in its stomach. In a fish collected in September, 1952, the fragments of approximately 2 oyster spat were found with 5 *Neopanope* and 5 *P. longicarpus.* The other small bivalves were also always associated with many crustaceans.

It appears that the ingestion of molluscs by the toadfish is only a chance occurrence, that is, they are taken up incidentally by the toadfish in its quest for the more conspicuous surface dwelling crustaceans. Stauber (1943), who was interested in the predators of oyster drills in Delaware Bay, drew the same conclusion concerning *Urosalpinx cinerea.* He examined an undetermined number of toadfish, and on only one occasion did he find that a living *Urosalpinx* had been ingested. Any other drill shells were usually inhabited by hermit crabs.

Fish are probably a more important item in the diet than are the molluscs. The remains of fish were found in 6 of the 97 toadfish from Delaware Bay, and 3 of the 12 from the Mullica River. If volumetric data were obtained there is no doubt that their value as toadfish food would be considerably greater than the molluscs. Schwartz and Dutcher's (1963) observations suggest the same conclusion. The only fish identified in the present study was a seahorse from a Mullica River specimen (Table III).

There were no apparent seasonal food species preferences in the toadfish except for the one suggested above. Furthermore, there did not appear to be any differences in the food habits between the sexes or in various size groups within each sex. Even the very young fish (under 10 cm. in length) fed primarily on crustaceans such as small mudcrabs, prawns and amphipods.

The nematode *Contracaecum habena* (Linton) was found in the digestive tracts of 80.1% of the toadfish collected in Delaware Bay (Table II) and in 66.7% of the small sample from the Mullica River (Table III). These data confirm the generally high incidence of this parasite as reported for other areas by Linton (1905), Gudger (1908), Chrobot (1951), and Schwartz and Dutcher (1963).

DISCUSSION

Linton (1905) examined the digestive tracts of 136 toadfish from Beaufort, North Carolina. As his primary concern was the degree of parasitic infection, he did not present quantitative data which can be compared easily with those of the present study. Nevertheless, his observations showed that crustaceans comprised the bulk of the diet. In 34 (90%) of the 38 collections of toadfish with food (1 to 11 fish per collection) crustaceans of various species were found. Hermit crabs, shrimps and a variety of other decapods were the dominant forms.

Reid (1954) examined 26 *Opsanus beta* collected at Cedar Key, Florida. Twenty-two contained food. Various brachyurans occurred in 82% of the stomachs, and penaeid and crangonid shrimps were found in 32%. Hermit crabs, while present, were not important food items.

Verrill's (1871) observations are of special interest with regard to the Mullica River information. He examined toadfish collected in the Great Egg Harbor area of New Jersey, and the Mullica River is a part of this estuarine system. *Callinectes, Crangon* and *Palaemonetes* were the principal food species, but he also "often" found the mud snail *Nassarius obsoletus* and small fishes including the pipefish *Syngnathus*. While he apparently did not find *Upogebia* in the toadfish, he did record this burrowing species from the flounder, *Lophopsetta maculata,* collected in the same region.

The present study confirms the observations of previous investigators (op. cit.), that *Opsanus tau* feeds primarily on crustaceans. Only two of the 106 fish with food (less than 2%) lacked some species of crustacean in their digestive tracts. The importance of the individual species in the diet is probably related to their availability or abundance in the particular environment. The uniformity of the per cent frequency of *Neopanope* in toadfish stomachs collected in Delaware Bay during 1952, 1953 and 1955 is one illustration of this point (Table II). The dominance of *Rhithropanopeus* in toadfish from low salinity areas is another example, and this was also shown quite clearly in the data of Schwartz and Dutcher (1963).

Nearly all of the crustaceans listed in Table II are surface dwelling or epifaunal species and consequently would be more subject to capture than some of the burrowing crustaceans, bivalves, gastropods and polychaetes. The conspicuous activity of the crustaceans may attract the toadfish whereas, for example, the sluggish activity of some of the surface dwelling gastropods may not. Toadfish collected in areas where the snail *Nassarius obsoletus* was very abundant apparently did not consume this species. In the same areas, sprightly hermit crabs inhabiting *Nassarius* shells were frequently found in the intestinal contents.

Toadfish collected in the dredges of the commercial oystermen of Delaware Bay are often killed with the oyster culling tools and thrown overboard. This practice would seem inadvisable in light of the fact that the toadfish is a predator of the xanthid crabs which in turn are known predators of oysters and other commercial bivalves (McDermott, 1960). The predatory activity of the toadfish would appear to be an important element in the population dynamics of the crustaceans comprising the oyster-bed community.

ACKNOWLEDGMENTS

These studies were carried out while the author was stationed at the Oyster Research Laboratory, Rutgers University, at Bivalve, New Jersey. I am indebted for the use of these facilities and for the encouragement of the Director of the Laboratory, Dr. Harold H. Haskin. I am especially indebted to Mr. Franklin B. Flower, Mr. William A. Richards, local oyster growers, and Dr. David Dean for their help in securing toadfish.

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MICROSPECTROPHOTOMETRY OF THE ANTERIOR PITUITARY OF THE PIG

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ABSTRACT

Absorption spectra, obtained with a simplified microspectrophotometer from 2 micron² cytoplasmic areas of PAS-positive cells of the porcine anterior pituitary, permit the differentiation of two types of basophils. Such studies may aid in the standardization of techniques used to demonstrate specific cell types and the quantification of intracellular hormones.

INTRODUCTION

Our present concepts of the cytophysiology of the anterior pituitary are far from complete (8, 10). Six hormone fractions are known to be secreted by the adenohypophysis (4), and many studies have attempted to link these hormones to histologically distinct cell types. Classical staining techniques differentiate only 3 cell types within the gland (11) while modern histochemical procedures permit further differentiation (1, 7, 9, 13).

Photometric analysis of tissue sections has facilitated the quantification of nuclear desoxyribonucleic acid in pituitary cells (6) and the characterization of cytoplasmic constituents (2). Such studies support the belief that microspectrophotometry may provide the histologist with the microbioassay tool necessary for identification and quantification of intracellular hormones in endocrine tissues (14).

A simplified microspectrophotometer patterened closely after an instrument described by Strother and Wolken (12) has been constructed in this laboratory. It is being used to study the basophil cells of the anterior pituitary. We wish to present the results of some pilot studies done on periodic acid-Schiff stained, porcine, anterior pituitaries and describe the major details of this instrument and its operation.

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MATERIALS AND METHODS

Tissues:

Whole pituitary glands were dissected from the heads of 5 immature female and 6 castrate male pigs (average live body wt. = 204 ± 5 lbs.) within 10 minutes of the time of death. Subsequent observation of the glands showed no differences in the cell types present, and all animals were treated as a single group. Freshly removed glands were placed in isotonic saline at 0° C. for return to the laboratory. One half of each of the glands was fixed in 10% formalin, rinsed, dehydrated in dioxane (2 changes, 2 hours each), and embedded in paraffin; blocks were sectioned at 6 μ. Mounted sections of each gland were hydrated, oxidized for 5 minutes in 1.0% aqueous periodic acid, and immersed in Schiff's reagent (5) for 15 minutes. After rinsing in bisulfite solutions and tap water, the sections were stained in Harris' hematoxylin to bring out nuclear details, dehydrated in alcohol, cleared in xylol, and mounted in Permount.

Microspectrophotometer:

The microspectrophotometer used in this study differs little from the simplified microspectrophotometer (M-1) described by Wolken and Strother (14). Construction of the present instrument was carried out, in part, with the assistance of Dr. G. K. Strother. The major components of the instrument are shown in Fig. 1. Absorption spectra are obtained using the 90X, oil immerision ob-

jective of the microscope with individual readings being taken at intervals of 10mμ. throughout the wavelength range of 400 to 700 mμ. At this magnification the area from which the spectrum is obtained does not exceed 2 μ². Calculation of the percent absorption for any given wavelength requires that two readings be made as close to simultaneously as possible. The first of these is taken with the photoconductive cell in place over the specimen, while the second is obtained from an adjacent area of the slide. Accordingly, specimens for study are selected, in part, on the basis of their proximity to blank areas of the slide. Raw data are corrected for uneven illumination of the microscope field by obtaining readings from the photoconductive cell in both "on" and "off" positions without any specimen in place. From these readings a correction factor is calculated. Percent absorption, so determined, is plotted against wavelength to

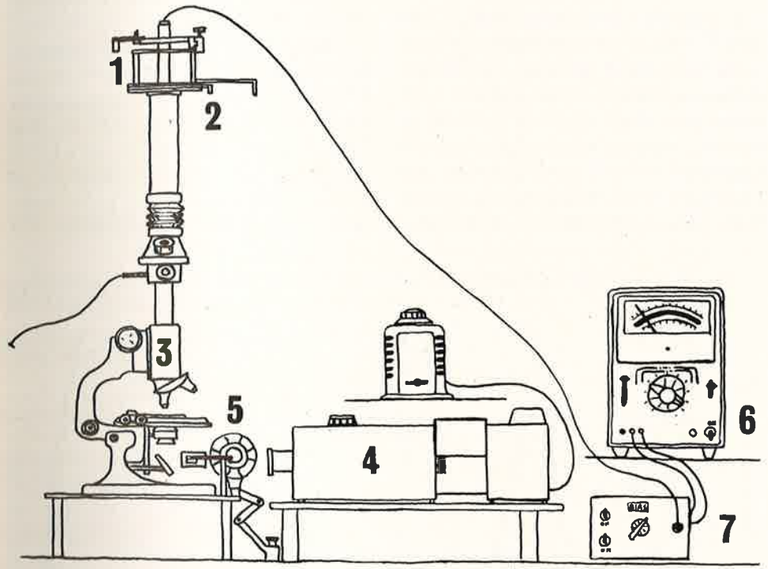

Figure 1: Drawing of Microspectrophotometer - major components

1. Housing for CdSe photoconductive cell (Clairex Corp., N. Y.).
2. Sliding shutter with reflective underside mounted above a viewing ocular used to locate the photoconductive cell over exact portion of specimen to be examined.
3. AO Spencer research microscope, apochromatic objectives, revolving, calibrated, mechanical stage, and substage condenser (N.A. = 1.3).
4. B. & L. (model 33-86-02) diffraction grating monochromator, with tungsten filament lamp.
5. B. & L. Nicol illuminator, used as source of white light.
6. Hewlett-Packard (model 412 A) DC vacuum-tube voltmeter, used to display the output of photoconductive cell.
7. Bias Voltage Control, wired between the photoconductive cell and the voltmeter and used to balance the darkfield output of the photoconductive cell and bring the voltmeter to zero before taking a reading.

give a spectral curve characteristic of the specimen. The results presented are based on spectral data obtained from a total of 60 individual cell determinations. Replicate determinations were made initially on the same cell. However, since the spectral curves were reproducible even on adjacent sections of the same cell, this was not adopted as routine procedure.

RESULTS

To check the performance characteristics of the above instrument, an absorption spectrum was obtained using a Corning CS 1-60 didynium glass filter in place of the usual tissue specimen. Comparison of the spectrum so obtained with a spectrum for the filter, supplied by the manufacturer, shows that they are in close agreement (Fig. 2) and indicates that the sensitivity of the instrument is satisfactory for the investigation of biological materials.

The major results, based on spectral analyses of two visually distinct, PAS-positive, basophil cell types, can be summarized as follows: (1) both the PAS-red and PAS-purple cells have absorption spectra of the same general shape with an absorption maximum in the wavelength range from 480 to 620 mμ. (2) the two cells, however, can be differentiated on the basis of the magnitude of the absorptive index (percent absorption) in the violet region of the spectrum (400 to 440 mμ.). Since all of the spectral data obtained for the two types of basophils studied gave the same results, only one typical series of spectral runs, based on three individual cells of each type, is shown in Fig. 3. For visual orientation, the *in vitro* spectrum of Schiff's reagent reacted with formaldehyde (obtained with a Beckman DB

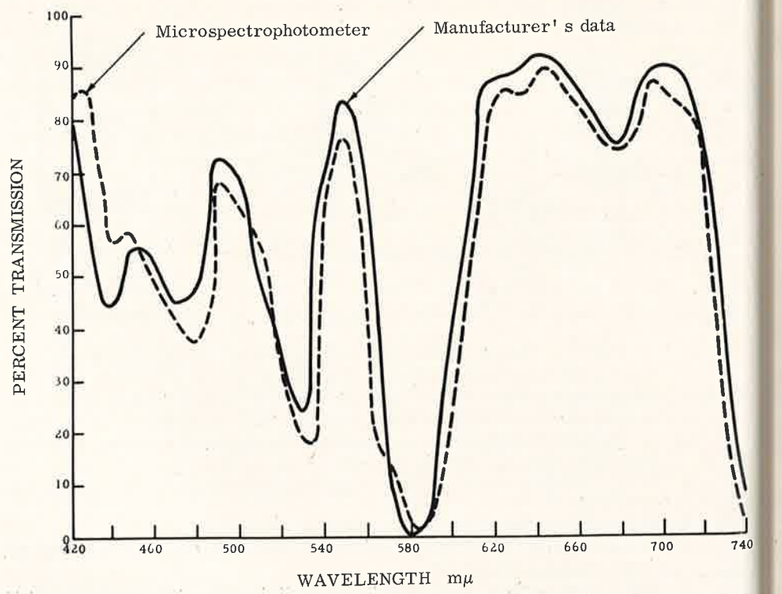

Fig. 2. Absorption spectrum of Corning CS 1-60 Didymium Filter using microspectrophotometer compared with absorption spectrum provided by manufacturer.

spectrophotometer) is superimposed on the mean spectral curves of the PAS-red and PAS-purple basophils.

The absorption spectra for the two types of basophils are observed to be in close agreement except at wavelengths shorter than 450 mμ. At these shorter wavelengths, PAS-red cells show greater absorption than PAS-purple cells. This is precisely the difference which would be predicted from visual identification of the two cell types. The close agreement of the greater portion of the mean absorption spectra for both PAS-positive cell types with the *in vitro* absorption spectrum of Schiff's reagent after adding formaldehyde can be taken as positive evidence that the color seen in the tissue sections is due to the occurrence of the Schiff-aldehyde reaction at appropriate cellular sites.

DISCUSSION

Halmi (3) was among the first to suggest that pituitary basophils could be subdivided into at least two different structural and functional types. Purves and Griesbach (9) subsequently demonstrated that similar differentiation of basophil cell types was possible in pituitaries stained with the periodic acid-Schiff technique alone. The porcine anterior pituitary gland displays at least two distinct PAS-positive cell types based upon slight differences in the color of the stain and upon the general structure of the cells. The first of these types consists of large, round cells with vesicular nuclei and cytoplasm homogeneously filled with small, red, PAS-positive granules. These were designated PAS-positive, red cells. The second type consists of smaller, more irregular cells with smaller nuclei and cytoplasm packed with dense, PAS-positive material staining deep magenta. These were designated PAS-positive, purple cells. No attempt has been made to determine the endocrine functions of these two types. However, on the basis of differences in cyto-

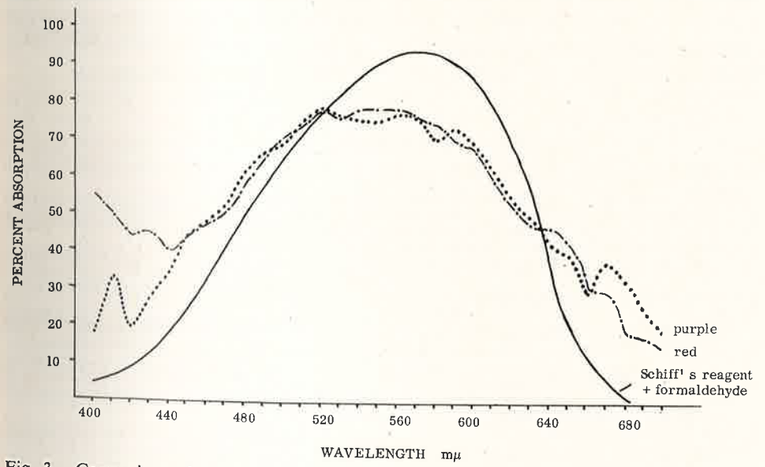

Fig. 3. Comparison of mean absorption spectra of PAS positive red and purple cells within vitro absorption of schiff's reagent plus formaldehyde.
Anterior Pituitary:
Hog # 00-7863 red
Hog # 02-7863 purple

morphology, staining affinity with PAS and spectral characteristics it is reasonable to conclude that they are functionally distinct. Further analysis of the specific functions of these cell types would be desirable.

Fand and Thorell (2) recently have demonstrated that the intensity of the color developed in the PAS reaction is proportional to the amount of the given glycoprotein hormone present, although the chromogenicity per biological unit varies from one hormone to another. The possibility thus exists for utilizing this reaction to quantify the amount of intracellular hormone under varying physiological conditions. To obtain such measurements, the exact thickness of the tissue section must be known. Studies are presently underway in this laboratory to discover a simple, reliable method for obtaining such data which does not require the use of interference microscopy.

We are persuaded that microspectrophotometric analysis of endocrine organs such as the anterior pituitary can yield important qualitative and quantitative information concerning the specific function of individual cells within intact organisms. Responses of individual cells, in terms of hormone output, to changing physiological conditions could be followed in routinely prepared microscopic tissue sections, a procedure not previously possible.

SUMMARY

A simple microspectrophotometer has been constructed in this laboratory and is being used to characterize the periodic acid-Schiff positive cells (basophils) of the porcine anterior pituitary. Preliminary studies have shown that this instrument is capable of detecting qualitative differences in cells judged to be of different types on the basis of appearance in pituitary sections. Specifically, it was found that PAS-purple basophils have a lower absorptive index than PAS-red basophils in the 400 to 450 mμ. region of the visible spectrum, although the maximum absorptive band is the same (560 mμ.). Studies are presently underway to further characterize these cell types spectrophotometrically and to develop possible methods for quantification of intracellular hormone content.

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PROBLEMS IN PRODUCING CHANGES IN CHROMOSOME BEHAVIOR

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ABSTRACT

Approaches to experimental formation of polytene chromosomes are discussed. Speculations are advanced that a chromosome may be viewed stoichiometrically.

It should be possible soon to evoke the more gross behavioral patterns of chromosomes by manipulating their environment. There is a growing effort (7) to apply to cell division processes the insight we have from the DNA, messenger RNA, ribosome protein factory model. Flying in the face of today's too neat and tidy nucleic acid dogma, studies with excesssively irradiated wheat seeds (2) have shown that correlative growth and differentiation into seedlings is possible without DNA synthesis or cell division going on. In addition there remains the possible uncoupling of nuclear division and cytokinesis in many forms, both normal and abnormal, even though generally chromosomes are equally distributed to two daughter cells.

Much of the chromosome behavior one is interested in involves the movements in the mitotic and meiotic dances. We should be attempting the production of these movements in isolated subcellular systems. And once the normal process has been elucidated, there are the weird variations yet to explain. Metz (5) has described in *Sciera* a monopolar condition in which some of the chromosomes move opposite to the direction in which their spindle fibers pull them. This animal also provides us with instances of special chromosomes limited only to the germ line, precocious behavior of sex chromosomes moving before the others from the metaphase plate, and finally nuclear membrane formation which is completed before all the chromosomes have reached the newly forming nuclei.

But perhaps most fascinating is the problem of formation of the giants. We are certainly still full of questions concerning development of the very large specialized chromosomes (1) as the ovarian lampbrush chromosomes and the highly polytene forms in certain dipteran cells. The familiar giant polytene chomosomes of the salivary gland of *Drosophila* more than match the nuclear duplication in the polyploid salivary gland cells of the snail, *Helix* (8). Yet as far as interpreting the initiation of either, we have no satisfactory mechanism.

For modification of chromosomes along the path to polyteney, there are four approaches open to the researcher: 1) to produce the proper induction to become polytene by the fortunate addition of the effective chemical or chemicals at the right position, concentration, and time. 2) to interfere with the process in the normal development of polyteney and hope to explain the effect of the inhibiting agent employed. 3) to discover natural biological variations in ploytene production within or between species in which there are also seen correlative chemical differences. 4) to move chromosomes from cells in which they do not become polytene to cellular environments in which chromosomes normally become polytene.

Some transplantation experiments (3) of giant chromosomes have been undertaken to observe the puffing reaction of the specific bands of the chromosome to specific cytoplasmic situations. Other transplants (4) have been accomplished

to introduce radioactive forms of native nuclear proteins and nucleic acids in order to follow autoradiographically the movement of macromolecules into recipient cytoplasm or nucleus. Attempts should be made to introduce a normal diploid cell into a salivary gland cell. As yet unavailable is a culture medium in which to maintain isolated salivary glands while chromosome differentiation proceeds, or a suitable medium in which to move nuclei about. To date one either pipettes a donor nucleus within a blob of its original cytoplasm or micro-probes the nucleus through two plasma membranes when donor and recipient cells are tightly opposed. Beside the basic importance of such proposed studies to chromosome physiology, the possibility of making giant chromosomes in any organism, including a human, offer intriguing cytogenetic possibilities.

Most chromosome cytologists have studied, for basic or applied science reasons, the behavior of chromosomes following irradiation (6). This work will continue to furnish clues and more difficulties for tomorrow's physiology of the chromosome.

In pursuing studies of chomosomes it it well to remember there is a realm of stoichiometry in living systems and there is a realm of chemical mixtures. Although we no longer dream of a biogen molecule of protoplasm, yet our view of the cell has developed with more and more definite molecules found within the cell. These are definite chemical entities. We talk of a definite protein although it may change its three dimensional configuration, its binding to different counter ions at its charged sites, or its combination with substrate and regulator molecules. Currently viruses are viewed in the main as macro-molecular complexes for which one should be able to write structural formulas good to the last carbon atoms. By the time we reach the level of bacaeria, though, we have left the realm of the law of definite atomic proportion. However, might the chomosome, except for its skin, be a specific macromolecular complex precise to the last atom? Perhaps even the polytene chromosome will be ultimately susceptible to synthesis by an artful organic chemist.

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ELECTROPHORETIC, SOLUBILITY, AND CRYSTALLINE STUDIES OF GALLINACEOUS HEMOGLOBINS

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ABSTRACT

Hemoglobin of three species of gallinaceous birds were examined as to electrophoretic, solubility, and crystalline differences. These species were: domestic fowl (*Gallus domesticus*), Japanese quail (*Coturnix coturnix*), and bob white quail (*Colinus virginianus*). Each hemoglobin exhibited two electrophoretic components with identical mobilities; however, the three hemoglobins differed on the basis of solubility and crystalline formation. Intra-specific hemoglobin differences were not found.

INTRODUCTION

Within the past few years rapid developments have occurred in the study of hemoglobins. This paper is involved with three aspects of Gallinaceous hemoglobins: electrophoretic, solubility, and crystalline. The species of Gallinaceous birds involved in this study were: domestic fowl (*Gallus domesticus*), bob white quail (*Colinus virginianus*), and Japanese quail (*Coturnix coturnix*). This experiment was begun as an attempt to find interspecific as well as intraspecific hemoglobin differences that could be used as appropriate genetic markers.

Electrophoretic: The electrophoretic analysis of bird hemoglobin is a relatively unexplored field. Johnson and Dunlap (1955), Misra and Choudhury (1960), and Fraser (1961) worked with chicken hemoglobin and found two distinct fractions with paper electrophoresis. Fraser (1961) found a complete reversal of the concentrations of hemoglobin with chick development. The slower moving fraction of the embryonic hemoglobin contained 84% of the protein, while at the time of hatching this fraction contained only 28% of the protein; and this situation remained through adult life. D'Amelio and Sulvo (1959), working with starch, found three fractions for chicken hemoglobin. Agar plate double diffusion tests indicated that two of the fractions exhibited serological specificity. The third and slowest moving fraction had specific serological characteristics.

More extensive work has been done in human and other mammalian hemoglobins. Popp and Cosgrgove (1960) found different electrophoretic patterns for different strains of inbred mice. Much of the work in hemoglobin electrophoresis in humans has been summarized by Ingram (1963).

Solubility and Crystalline: Popp and Cosgrove (1960) have found that genetically different mouse hemoglobins differ in solubility and in crystal formation. Using a different technique of crystallization, Drabkin and Wise (1962) were able to separate artificial hybrid hemoglobins of dogs and albino rats. Consequently, there appears to be a sound basis for the use of this technique as a means of determining hybridization and genetically pure lines.

Hemoglobins have also been artificially crystallized by Pick (1962) using polarized light and by Ellfolk (1963) with the use of chromatographic paper and ammonium sulfate. Adding strength to the concept of using solubility and crystalization as means of separating hemoglobins is the recent work of Rumen and Love (1963). By observing minor

1 A contribution from the Laboratory of Immunogenetics, Jenks Biological Laboratories, Lafayette College, made possible by a Research Grant from the Advanced Studies and Research Fund of Lafayette College. The author is grateful to Mr. Jack Carty, Lafayette College for photographic assistance.

differences in crystals and in solubility in strong salt solutions, they were able to separate lamprey hemoglobin into 6 fractions.

MATERIALS AND METHODS

Electrophoresis: The following procedure, adopted from Block, (Durram and Zweig (1958) was followed in preparing the hemoglobins: The blood was collected by cardiac puncture with sodium citrate as the anti-coagulant. The erythrocytes were separated from the serum by centrifugation. The serum was discarded, and the cells washed three times with 0.75% sodium chloride to remove serum proteins. After the third washing an equal volume of distilled water was added to the packed erythrocytes. To this, 0.4% of the total volume of toluene was added and agitated 30 minutes to remove the lipid content. This mixture was centrifuged, and the layer containing the hemoglobin was removed with a disposable pipette. The resulting hemoglobin was used directly or stored at -20° C.

The electrophoretic epuipment employed was a Beckman Durrum type electrophoresis migration chamber (Model R, Series D) supplied with current by a Spinco Duostat. Two buffers were used with the first Veronal buffer maintained at a pH 8.6, and the second, Sodium and Potassium Phosphates, maintained at a pH of 5.2. 0.01 ml samples of hemoglobin containing 3 grams of proteins per 100 grams were used. All migrations were for 18 hours at starting and terminal sources of 5 milliamperes and 52 volts. The electrophoretic strips (Whatman No. 1 paper) were stained in the customary manner with alcoholic bromophenol blue, then treated with ammonia fumes.

pared following the method outlined by Popp and Cosgrove (1960). Erythrocytes were obtained by heart puncture and treated as previously described in washing erythrocytes for electrophoretic studies.

Varying molarities of buffer were prepared by mixing the phosphate buffer with distilled water in appropriate predetermined amounts. Preparations contained 0.1 ml of HbCO in a total volume of 5.0 ml distilled water added as necessary to change the molarity of the phosphate buffer. Each reduction in the buffer by 0.1 cc and a corresponding increase in the amount of water reduces the molarity of the final buffer solution by 0.07. Washed erythrocytes (0.1 cc) were added to the tubes with an equal amount of distilled water to hemolyze the cells. This addition of blood also reduces the molarity of the buffer solution by 0.07.

Carbon monoxide was bubbled through the hemoglobin solution for one minute to convert the oxyhemoglobin (HbO_2) to carbon monozyhemoglobin (HbCO), a procedure that reduces the rate of hemoglobin deterioration. The buffer solution was added to the carbon monoxyhemoglobin and shaken vigorously to produce a foam which slows the transfer of gases which, in turn, slows the rate of hemoglobin deterioration. The tubes were incubated at 30° C. for twenty hours and filtered. The optical density of the filtrate was recorded with a Coleman Junior Spectrophotometer with the wavelength at 575 millimicrons. The optical density was plotted as a function of buffer molarity. Precipitate formations were examined under a microscope at 100 X for characteristic shape and size.

ginianus, and 4 samples of *Gallus domesticus* hemoglobin were analyzed individually with each showing comparable electrophoretic patterns. In all cases, hemoglobin examinations involved adult birds representing both sexes.

Two electrophoretic fractions, designated A and B in order of their electrophoretic mobility measured in mm from point of application, were present in the 3 different hemoglobins with fraction B representing the major hemoglobin fraction in each of the three species. The mobilities of A and B were identical, with 50 mm for fraction A and 69 mm for fraction B for each of the three species of birds when examined with the sodium and potassium phosphate buffer maintained at a pH of 5.2.

Solubility and Crystaline: Figure 1 shows the salting-out curves for HbCO as prepared from erythrocytes of the three species of birds.

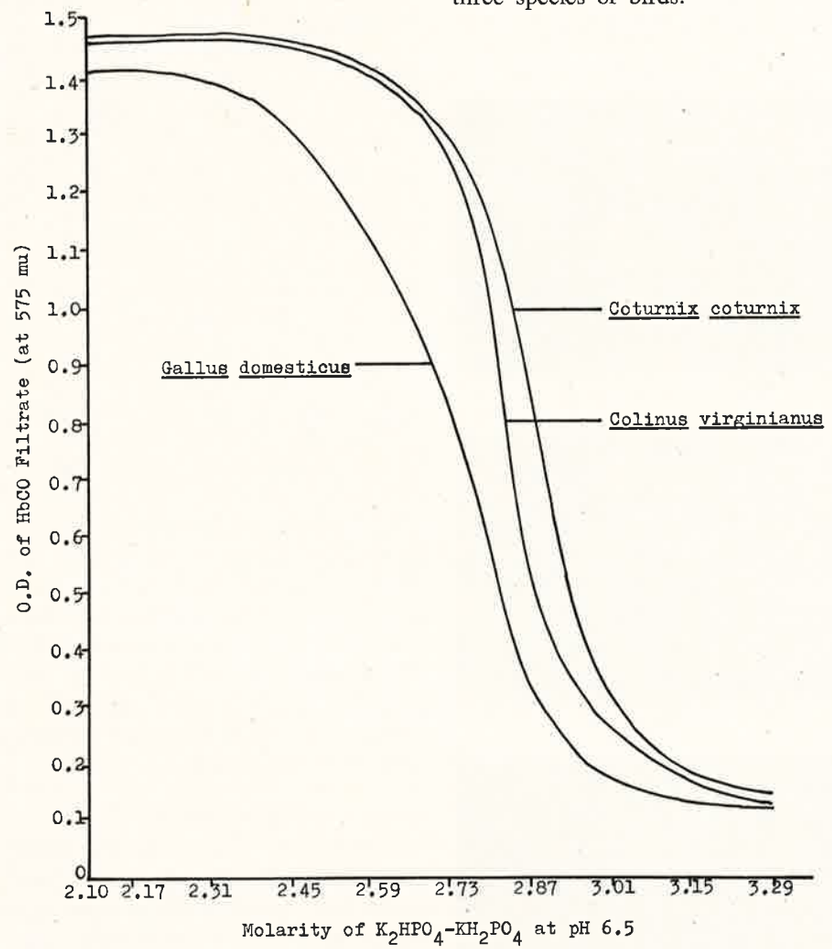
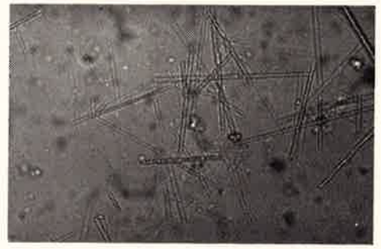


Table 1. Comparison of hemoglobins of three Gallinaceous birds as to electrophoretic, solubility, and crystalline properties.

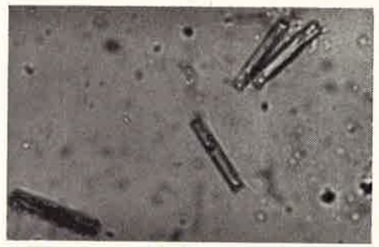
Genus and species	Electrophoretic Patterns (No. fractions)	(Mobilities mm)	Solubility (Optical Density)	Crystal formation (Type) (Size in mm)
Gallus domesticus	2	50—69	1.0-1.3	Needle 0.3100 x 0.00775
Colinus virginianus	2	50—69	0.8-1.1	Needle 0.1550 x 0.0310
Coturnix coturnix	2	50—69	0.6-0.8	Needle 0.0620 x 0.0155

The individuality of a particular hemoglobin is determined by observing the optical density at a determined molarity.

Fig. 2—Crystalline Formations



A. Gallus domesticus



B. Coturnix coturnix

C. Colinus virginianus

The saltingout curve for *Gallus* is distinct; however, the curves for *Colinus* and *Coturnix* are quite similar.

A comparison of the electrophoretic fractions, optical density, and crystalline properties are depicted in Table 1.

The electrophoretic patterns are identical; however, the solubility and crystalline properties are characteristic for the particular species. The greatest amount of distinct crystals with *Gallus* hemoglobin was observed between 2.59-2.94 molarities. Molarities below 2.59 contained few crystals; whereas, molarities above 2.94 gave few distinct crystals with much amorphous precipitation. *Colinus* and *Coturnix* hemoglobin exhibited characteristic crystals between molarities 2.80-2.94. Molarities below 2.80 exhibited very few crystals and those above 2.94 exhibited few crystals with much amorphous precipitation. Figure 2 depicts the characteristic crystalline formation. Crystals were formed in 2.80 M phosphate for *Gallus,* 2.73 M phosphate for *Coturnix* and *Colinus* — the molarity at which size and definition seem to be maximum.

DISCUSSION

Comparative studies as to electrophoretic fractions and mobilities of hemoglobin from *Gallus, Colinus,* and *Coturnix* failed to reveal hemoglobin differences. Hemoglobin from the three species of birds contained two fractions with fraction B being larger. Also, the mobilities were identical with fraction A migrating 50 mm and fraction B migrating 69 mm, respectively.

However, additional studies involving solubility and crystalline properties of hemoglobins clearly demonstrated that the three hemoglobins were distinct.

The optical density of HbCO filtrate is an expression of the solubility of HbCO at a specific pH and salt concentration. Since K_2HPO_4-KH_2PO_4 at pH 6.5 has a large buffering capacity, the pH of the solution remains essentially constant as HbCO is salted out. As can be seen (figure 1), small differences in salt concentration caused significant changes in HbCO solubility. Thus, the optical density varies with the molarity used. Red blood cell antigenic studies of Gallinaceous birds by Sanders and Novikoff (1962) demonstrated that a greater immunological affinity is indicated between *Colinus* and *Coturnix* than between *Colinus* and *Gallus* or *Coturnix* and *Gallus*. It is of interest to note (figure 1) the close similarities of the salting-out curves in *Colinus* and *Coturnix* hemoglobins. The crystals formed from the three hemoglobins were needle-like in appearance and varied as to size (Table 2). No intraspecific differences in needle formation were observed for *Gallus* and *Coturnix*. However, 3 *Colinus* hemoglobins failed to exhibit needle formation at 2.73 Molarity. Amorphous crystals were observed; however, the optical density readings were identical for all *Colinus* hemoglobins studied.

The final optical density readings were slightly less than the initial readings. This was probably due to some non-specific denaturation of labile hemoglobin.

CONCLUSIONS

1. Hemoglobin from adult *Gallus domesticus, Colinus virginianus,* and *Coturnix coturnix* were examined as to electrophoretic, solubility, and crystalline properties.

2. The three hemoglobins exhibited two electrophoretic fractions with identical mobilities.

3. Solubility and crystalline properties were characteristic for the particular species when examined with K_2HPO_4-KH_2PO_4 buffer maintained at pH 6.5.

4. Intraspecific diferences in needle formation and solubility were not observed in *Gallus* and *Coturnix* hemoglobin.

5. Intraspecific differences in needle formation were observed for *Colinus* hemoglobin at 2.73 M phosphate buffer.

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CELLULAR ENERGETICS IN LIVER AND KIDNEY HOMOGENATES OF THE CARP (*CYPRINUS CARPIO*)*

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ABSTRACT

Respiration rates of kidney and liver homogenates of *Cyprinus carpio* were measured using an oxygen electrode respirometer. The cellular factors regulating respiration in carp tissues proved to be the same as exist in warm-blooded animals; however, the respiratory enzyme concentrations were higher in carp kidney than in carp liver.

INTRODUCTION

Present knowledge of cellular energetics in vertebrates is mainly based on studies using tissues from homeothermic animals, most commonly the rat. Little information is available on metabolic regulatory mechanisms in tissues from cold-blooded animals. The need remains to determine the extent to which the cellular metabolic reactions demonstrated in mammalian tissues can be applied to explain metabolic regulatory mechanisms in poikilotherms, which differ from homeotherms in their oxygen dependence, metabolic requirements and temperature dependence (1, 2).

An approach which has proven useful in studies of tissue energetics is polarography, or oxygen electrode respirometry, since this method facilitates the rapid measurement of respiration rates in small samples of isolated tissue (3). The present study deals with the polarographic analysis of respiration rates in kidney and liver tissue from carp (*Cyprinus carpio*). Specifically, the investigation entails: (a) the measurement of endogenous respiration rates of carp kidney and liver tissue homogenates, (b) measurements of the effects of the metabolite, succinate, and of the effects of the two nucleotides, adenosinediphosphate (ADP) and adenosinetriphosphate (ATP) on the respiration rates of carp tissues, and (c) evaluation of these measurements in terms of the cellular mechanisms involved in the regulation of respiratory rates in carp kidney and liver tissue.

MATERIALS AND METHODS

Nine mature carp weighing about 1.6 ± 0.2 kg. served as the source of the tissue; of these, 6 were males and 3 females. Since there were no sex differences in the tissue respiration rates, the data obtained from these fish were combined. The carp were collected from the Benner Springs Hatchery, Bellefonte, Pennsylvania, and kept in an aerated laboratory tank for 1 to 4 days at a temperature of 20 ± 0.2° C., which corresponded to that of the collection stream. After stunning a carp with a sharp head blow, the entire liver and/or kidneys were removed and immediately placed into ice-cold homogenization medium, cut into small fragments, and homogenized with a Potter-Elvejhem homogenizer (4). The homogenization medium contained 250 mM (millimoles) sucrose and 0.10 mM EDTA (ethylenediamine tetraacetate), pH 7.4 ± 0.1. The reaction medium contained 50 mM sucrose, 40 mM KCl, 20 mM $MgCl_2$ and 20 mM potassium phosphate buffer, pH 7.3 (5).

The detailed procedures for preparing homogenates, calibrating the respirometer and making measurements of homogenate respiration are described in earlier reports (3, 6). The reagents were prepared in triple distilled water. Succinic acid (Calif. Biochem. Co.) was prepared as a 1 M solution, pH 7 ± 0.1; ATP (Sigma Chem. Co.) was prepared as a 0.1 M solution, pH 7 ± 0.1 and ADP (Sigma) as a 0.05 M solution, pH 7 ± 0.1. Small vials containing 1 ml of each of the above reagents were generally stored at -20° C. until used. The concentrations of ADP and ATP stock solutions were assayed spectrophotometrically at 259 mμ using an extinction coefficient of 15.4 mM^{-1} cm^{-1} (7).

The respirometer itself simply consists of a Clark oxygen electrode (Yellow Springs Instrument Co.) inserted in a 3.2 ml reaction vessel which is surrounded by a constant temperature water jacket (24° C.) (Fig. 1). The platinum cathode of the electrode was maintained at -0.6 volts relative to a silver anode; at this polarization voltage, oxygen is reduced as rapidly as it reaches the platinum tip, so that the electrode potential is dependent upon the oxygen concentration in the closed reaction vessel. This enables one to continuously monitor the rate of change in the oxygen concentration within the closed vessel, such as occurs when various substrates or reagents are added to a respiring homogenate sample. The physical theory of the oxygen electrode method is detailed in a recent review by Davies (8).

Briefly, the steps involved in making

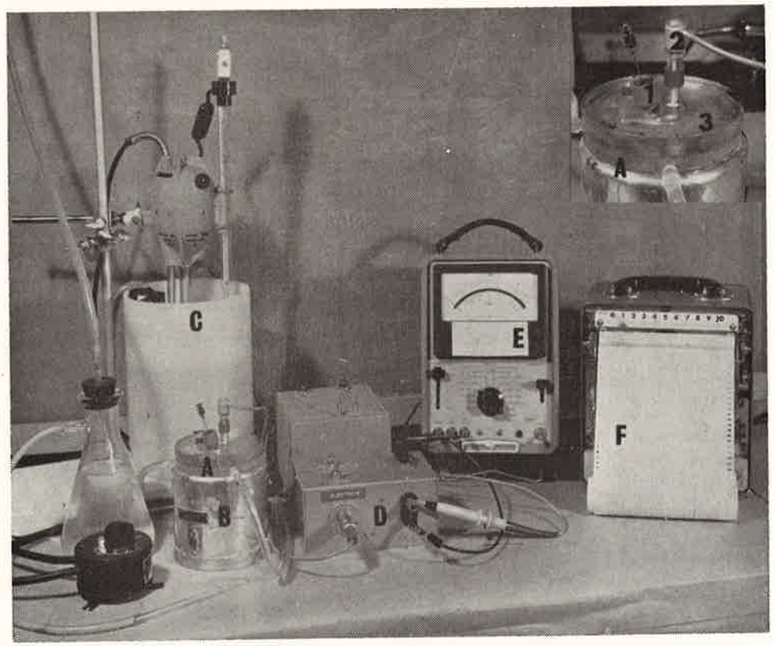

Fig. 1. Photograph of the equipment used for measuring liver homogenate and mitochondrial respiration. A. Electrode vessel (enlarged inset of reaction vessel. 1. Rubber bottle stopper and hypodermic needle. 2. Clark oxygen electrode. 3. Constant temperature water jacket with reaction vessel in center). B. Magnetic stirrer. C. Bronwill constant temperature circulator. D. Chassis containing battery and resistors. E. Hewlett-Packard D.C. precision volt meter with built-in amplifier. F. Varian recorder.

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measurements of tissue respiration can best be described by following a typical series of measurements obtained during one experimental run, such as is illustrated in Figure 3. After a homogenate is prepared, the respirometer vessel is filled with 3.20 ml of air-saturated reaction medium and measured volumes of homogenate, and subsequently of reagents, are injected into the system through a vaccine-bottle stopper. Before the homogenate is added, the initial tracing (reading the record from right to left) is a straight line since no oxygen utilization is occurring. The oxygen concentration of the air-saturated reaction medium was calculated as 240 μM O_2 (at an ambient pressure of 730 mm Hg at 24° C.) using data on solubility of gases in solution presented in the International Critical Tables. When a small volume of homogenate is introduced, the oxygen concentration immediately drops since the respiring homogenate had become anaerobic during storage in ice. After this initial drop, the slower decline in oxygen concentration reflects the endogenous respiration rate, since oxygen utilization by the homogenate depends upon the internal energy stores of the tissue sample. It is then possible to measure the effects of introducing an excess of an exogenous metabolite, such as succinate, or the effects of nucleotides on respiration. This is done by determining how much the rate of oxygen utilization has been increased relative to the endogenous rate. These respiration rates expressed in μM O_2/sec., are obtained from the slopes of the dotted lines in Figure 3. The nitrogen content of all the homogenates was determined by the micro-Kjeldahl

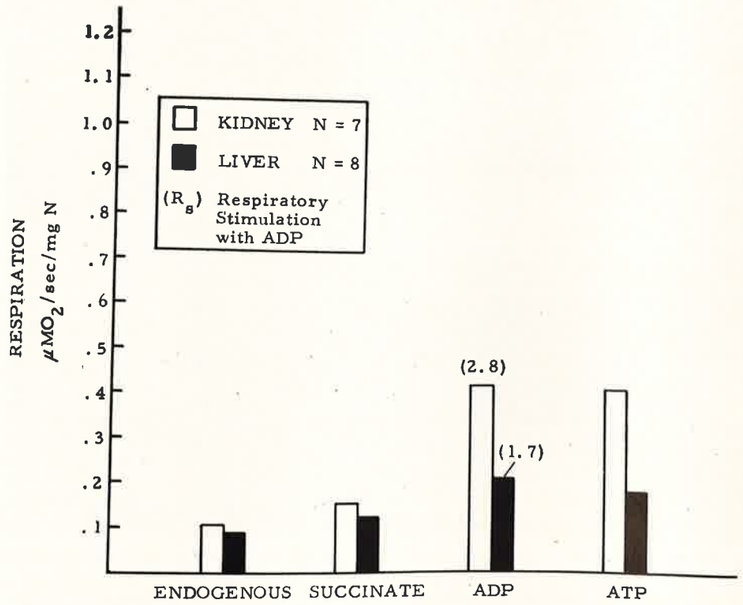

FIGURE 2. RESPIRATION OF LIVER AND KIDNEY HOMOGENATES OF THE CARP (Cyprinus carpio).

method (9) so that the average respiration rates could be expressed in μM O_2/sec/mg N.

The respiration data obtained are based on 15 tissue homogenates (8 liver, 7 kidney). Four types of measurements were made on each homogenate, with each respiratory rate measurement based on triplicate determinations. Thus, the average rates presented for endogenous respiration, succinate respiration, succinate-ADP respiration and succinate-ATP respiration of liver and kidney homogenates are based on a sum total of 180 respiratory rate determinations. The data were analyzed using Student's *t* test (10).

RESULTS

The respiration rate data are summarized for both liver and kidney homogenates in Figure 2 which shows the four types of measurements made along the ordinate. The endogenous rate is the average respiration rate in reaction medium containing no exogenous energy source. The succinate rate is the average oxygen utilization by the homogenate after the addition of an excess of succinic acid. The ADP and ATP rates reveal the extent of respiratory stimulation when an excess of either of these nucleotides is added to a homogenate containing excess succinate. The latter two rates will hence be referred to as succinate-ADP and succinate-ATP respiration rates, respectively. The R_s value is the ratio of succinate-ADP respiration to succinate respiration and is a useful index of the extent of respiratory stimulation caused by the addition of ADP. The standard errors of all the average values represented was approximately 10%.

The following conclusions can be drawn from the data shown in Figure 2.

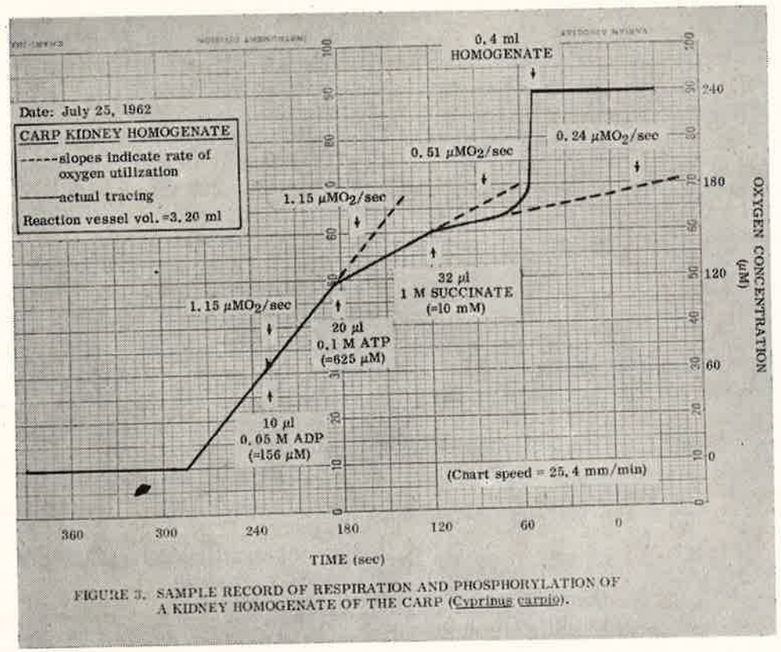

FIGURE 3. SAMPLE RECORD OF RESPIRATION AND PHOSPHORYLATION OF A KIDNEY HOMOGENATE OF THE CARP (Cyprinus carpio).

First, it is apparent that liver and kidney homogenates are comparable with respect to both the endogenous and the exogenous (succinate) rate of energy utilization. For example, the average endogenous respiration rates for kidney and liver homogenates were 0.10 ± .007 and 0.09 ± .01 μM O_2/sec., respectively. Similarly, the mean succinate respiration rate of the kidney (0.15 ± .02 μM O_2/sec.) was the same as that of the liver (0.12 ± .01 μM O_2/sec.).

A second conclusion is that kidney homogenates are more sensitive to ADP stimulation than are liver homogenates, e.g. the succinate-ADP rate of the kidney was 0.41 ± .04 and that of the liver was 0.20 ± .03 μM O_2/sec. ($P<.001$). This is also reflected in the R_s values which show that the succinate respiration rate of the kidney is enhanced 2.8 fold whereas that of the liver is increased only 1.7 fold following the addition of excess ADP.

A noteworthy finding was that ATP was as effective as ADP in stimulating the succinate respiration rate of both kidney and liver homogenates. For example, in Figure 3 it can be seen that the respiration rate following addition of ATP was identical to that obtained with ADP. This was a consistent finding irrespective of the sequence in which the two nucleotides were added. This could only occur in the presence of appreciable amounts of adenosinetriphosphatase (ATPase) in both liver and kidney homogenate preparations (see Discussion). Thus, one can conclude that carp liver and kidney homogenates both contain high concentrations of ATPase enzymes.

DISCUSSION

Since several types of measurements were made during the course of the present study, it is pertinent to briefly discuss the significance of each of these as it relates to the detailed regulation of cellular metabolism in carp tissue.

Endogenous respiration rates provide a basis for comparing general levels of metabolic activity in different tissues and are useful for comparing the effects of different substrates or nucleotides on respiration. The endogenous rate, however, does not enable one to ascertain the cellular basis for any differences in the respiration of various tissues since too many factors simultaneously control this rate. Some of the factors known to regulate endogenous respiration are: the type and concentration of substrate, endogenous nucleotides and ATPase enzymes (6). Thus the finding that the endogenous respiration rates were comparable in both liver and kidney homogenates demonstrates that both of these tissues have the same endogenous rates of energy utilization.

The fact that the addition of excess succinate stimulated endogenous respiration in carp liver and kidney tissue demonstrates the presence of the enzyme, succinic dehydrogenase, in both these tissues. This is in keeping with the recent reports of others that succinic dehydrogenase is present in carp tissue (11). Since it was also shown that the respiration rates in the two tissues were comparable, it can be concluded that the concentrations of succinic dehydrogenase in the liver and kidney were equivalent.

On the other hand, the succinate-ADP respiration rate provides one with an indirect measure of the functional respiratory units present in the tissue; that is, this measure reflects the concentration of respiring mitochondria or mitochondrial fragments (12). In this metabolic state, respiration proceeds at the maximal rate since all reactants known to be necessary are present in excess, and the respiratory rate is limited only by the

number of functional respiratory units (12). The finding that kidney homogenates had a higher succinate-ADP respiration rate relative to liver homogenates indicates that there is a higher rate of energy utilization and energy production in the kidney than in the liver per unit volume of tissue.

It has been demonstrated by other workers that the substrate-ATP respiration rate reflects the endogenous rate of ADP production (12). ATP *per se* has been shown to have no effect on the substrate respiration rate and acts indirectly when ATP is dephosphorylated to ADP (13). Since the extent of respiratory stimulation one obtains with ATP is directly dependent upon the amount of ADP formed from ATP (ATPase activity), the substrate-ATP respiration rate is useful as an indirect measure of ATPase. In the present study it was found that maximal respiratory stimulation was obtained with ATP in both kidney and liver homogenates. This could only mean that the rate of dephosphorylation of ATP to ADP was sufficiently high to result in the presence of an excess of ADP. In other words, both the liver and kidney contain high concentrations of ATPase enzymes.

In summary, the major conclusion that can be drawn from the present study is that tissues of the carp appear to have the same basic mechanisms of energy release at the cellular level as are known to exist in mammals. Thus it was evident from the finding that the substrate, succinate, is an energy yielding metabolite and that maximal respiration is obtained with ADP. The data on the effects of nucleotides extend the findings of Gumbmann (11), who compared the effects of various substrates on respiration of carp liver tissue and concluded that enzymes of the Krebs cycle are found in fish tissue. Our data are also in keeping with the concept that the same high energy compounds exist in all vertebrate animal tissues.

ACKNOWLEDGMENTS

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METHODS OF DIFFERENTIATING TREMATODES OF THE GENUS *NOTOCOTYLUS**

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ABSTRACT

From collections of snails in several different states it was found that one species of the genus *Notocotylus* develops in several species of the snail, *Stagnicola,* and another one in the snail, *Physa.* Cysts from *Stagnicola* infections readily developed in the ceca of birds and those from *Physa* infections in the large intestines of rodents.

INTRODUCTION

Yamaguti (1958) lists 29 different species of the genus *Notocotylus* from birds and five from mammals. A study of the drawings and descriptions of some of those species shows that some names are probably not valid. The reported methods of fixation and staining vary considerably. So few drawings were made of adults taken from feeding experiments that it seemed advisable to study adults recovered in this way as aids in substantiating species. This study includes data of previous feeding experiments as well as a goodly number of new feeding attempts from collections made in Minnesota, Michigan, Pennsylvania and Tennessee. An attempt is also made to reevaluate the most useful characters for the separation of species of the genus *Notocotylus.* A characterization of the family and the genus is presented first to give us points of reference.

The genus *Notocotylus* belongs to the family NOTOCOTYLIDAE and the sub-family NOTOCOTYLINAE. This family has the following diagnostic characteristics: small to medium-sized monostomes, body usually elongate, tapering anteriorly and rounded posteriorly. Ventral surface usually concave, and usually with longitudinal rows of glands or ridges. Cuticle spined ventrally. Oral sucker comparatively small and terminal: pharynx absent; esophagus short; intestinal ceca slender, often with short diverticula and terminating medial or posterior to testes. Testes symmetrical, close to posterior extremity. Cirrus pouch elongate, enclosing part or all of seminal vesicle, prostatic complex and long eversible ejaculatory duct; ovary median; usually inter-testicular; shell gland complex in front of ovary; uterus coiled transversely in intercecal field; eggs with polar filaments at each pole. Vitellaria generally follicular, lateral or dorsal to testes. Seminal receptacle absent. Genital pore median and usually directly posterior to intestinal bifurcation. Excretory pore dorsal, near posterior end of body; excretory vesicle with short stem and long arms which unite near anterior end of body. Parasitic in alimentary canal of birds and mammals.

The genus *Notocotylus* has these characteristics and more specifically three longitudinal rows of ventral glands; testes and vitellaria lateral to ceca, the testes varying in the number of lobations and the vitellaria, located anterior to the testes, varying in the number of follicles.

Genotype: *N. attenuatus* (Rudolphi 1809), syn. *N. triserialis* Diesing, 1839, in *Scolopax gallinago;* also in many other aquatic birds; cosmopolitan. Cercaria of *N. attenuatus* develops in *Planorbis rotundatus,* Joyeux (1922): cercaria matures in *Lymnea limosa,* encysted, develops in ducks in ten days, Mathias (1930); cercaria evolving in *Lynmea palustris* grows in ducks, L & U Szidat (1933).

In *N. stagnicolae,* a species similar to the genotype, Herber (1942) reported experimental infections in *Anas platyrhynchos, Gallus domesticus, Querquedula discors* and *Mergus merganser.* Eleven specimens of *N. stagnicolae* were taken from a natural infection in *Aegialitis semipalmata.* Cercaria of this form were found in *Stagnicola emarginata angulata* and *S. e. canadensis.* Wu (1953) made further studies of this monostome. He found the cercariae in *S. palustris* from Ottawa River.

In 1955, Herber reported studies on *N. urbanensis,* a species often confused with *N. stagnicolae.* In that article *Catatropis fimbriata* and *Paramonostomum echinum* were listed as cospecific with *N. urbanensis.*

In the latter two papers the following characters were listed as most helpful in distinguishing species of the *Notocotylinae*: (1) the number of ventral glands in each row, (2) the anterior extent of the vitellaria, (3) the lateral lobation of the testes, (4) the body and tail size of cercariae, and (5) the course of the excretory tubules of cercariae.

MATERIALS AND METHODS

Recent observations on studies of *N. stagnicolae* are based on 462 *S. caberata* snails collected at the source of the Mississippi River, Lake Itasca, Minnesota in 1961 and 81 *S. e. angulata* from Walloon Lake, Michigan in 1963. On *N. urbanensis* studies, 1004 *P. gyrina* from near Lake Itasca, 1642 *P. gyrina* from near Erwin, Tennessee, thousands from Carlisle and several hundred from the Cheboygan, Michigan area gave us the larval infections. Collections were brought to the laboratory in pond water containing sticks, stones or vegetation. When long distances were involved, ice was used to cool the jars containing the snails.

Isolation of snails in the laboratory followed standard methods. Local spring or lake water and four snails were added to stender dishes or wide mouthed bottles to a height of about an inch. Often lights were turned on to stimulate emergence of monostomes. At the end of 24 hours the dishes were examined with the aid of a dissecting microscope for evidence of cercarial emergence. The actual cyst can be seen or the discarded tails were evident. If infection was certain each of the four snails was isolated individually to locate the positive snail. If no cercariae or cysts were noted another 24 hours delay was allowed to give the laggards a chance to emerge.

Cercariae were studied in the living state, first without and later with the addition of neutral red, a vital dye. For permanent slide material, cercariae were fixed in hot 10% formalin and stained in Grenacher's Alum carmine.

For feeding experiment material, the infected snails were placed in lettuce-lined stender dishes partially filled with spring water. Sherman live traps were used to trap *Microtus* and *Peromyscus* to be used as experimental animals. The Highlands, North Carolina, area was ideal because there are no monostome infected snails in the area. Cysts become infective within a short time. If no feedings were planned the cysts were stored in the refrigerator. When laboratory rats were used, bits of lettuce containing cysts were packed into holes of cheese; for *Microtus* cysts were forced into slits of apple; chicks were forced fed.

Recently 23 bird and 18 mammal feeding experiments were attempted with *Physa* cysts. In all, previously reported, unreported and recent feedings, 147 mammals and 55 birds were used in *Physa* cyst research.

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Recently (1961-63) nine birds and two mammals were fed cysts from *Stagnicola* cercariae. In summary, 55 birds and 13 mammals were fed *Stagnicola* cysts.

After a predetermined interval following the feeding of cysts, the host's alimentary canal was cut into sections, slit and examined individually for flukes in petri dishes or enamel pans. Collected worms were examined for eversion of ventral glands, fixed and stained.

RESULTS

There was a 1% infection of notocotylid cercariae in *Physa* snails. Three of seven laboratory rats and five of eleven meadow mice were infected following cyst feedings from the cercariae mentioned. Only one of the 23 birds fed these cysts became infected and that was a doubtful positive. Including all *Physa* cyst feedings from the beginning of my experiments to date, 30 mammals out of 147 became infected, while only two birds out of 55 feedings were positive and these were immature forms. For the first time in my work a permanent slide was made of an experimentally produced adult. See Plate I. Nine other mature specimens were stained from recent experimental feedings.

In *Stagnicola* snails collected recently, slightly over 1% were infected with a notocotylid cercaria. This emerged later in the day than the one from *Physa* snails. Three of the nine birds used in *Stagnicola* cyst feedings developed adults. In the negative results several of the animals were fed very few cysts and some from a snail in the death throes. None of the mammals allowed the *N. stagnicolae* cysts to develop. Summarizing the total set of *Stagnicola* cyst experiments: 19 out of 26 birds became infected while only four mammals out of 11 showed any kind of development. Several of the latter animals were fed whole snails which makes the experiment a doubtful one. *Stagnicola caberata* is reported as a new host from the Lake Itasca area.

A very high incidence of a monostome infection was also noted in an amnicolid snail, *Fontigens*. It appears to be similar to *Quinqueserialis quinqueserialis* cercariae. So far no adults have been recovered from feeding experiments.

DISCUSSION

From the data presented it is evident that experimental birds can be readily infected with cysts from *Stagnicola* snails. Adults mature and shed eggs in eight days or more. Very few mature adults can be recovered from similar cysts fed to mammals. It takes longer to mature the same lot of cysts in a mammal.

Physa cysts fed to laboratory rats and meadow mice, under certain unexplained conditions, develop to maturity. Perhaps lowered vitality is conducive to infections since old and tumorus rats are more likely to become infected than young active animals. Pregnancy also seems to favor infection since one pregnant rat was fed cysts, gave birth to nine young, and after posting exhibited four adult worms. This would be an almost ideal laboratory experiment provided one knew all the conditions necessary for infection. Schell (1960) reported a *Q. quinqueserialis* infection in *Physa*. Normally this species is found in *Gyraulus*. This has not been reported from any other region but Idaho. It would be interesting to know how we could get our *Physa* to become infected with the *Quinqueserialis* monostome. In the areas mentioned all *Physa* cysts fed to mammals produced the notocotylid monostome.

Eggs shed by *N. stagnicolae* adults and placed with young *Physa* snails show no infections but if placed with young *Stagnicola* snails all became infected. Since *N. stagnicolae* does not develop to maturity in mammals as fast as in birds, it might be a good idea to keep such experimental animals at a higher temperature to see if that would speed up maturity or conversely to try to keep birds fed *Physa* cysts at a lower temperature.

It should be noted that the stagnicola form grows in at least four species of snail—*Stagnicola emarginata angulata, S. e. canadensis, S. caberata* (Minnesota), and *S. palustris*. This makes it appear as if it was less specific than *N. urbanensis*.

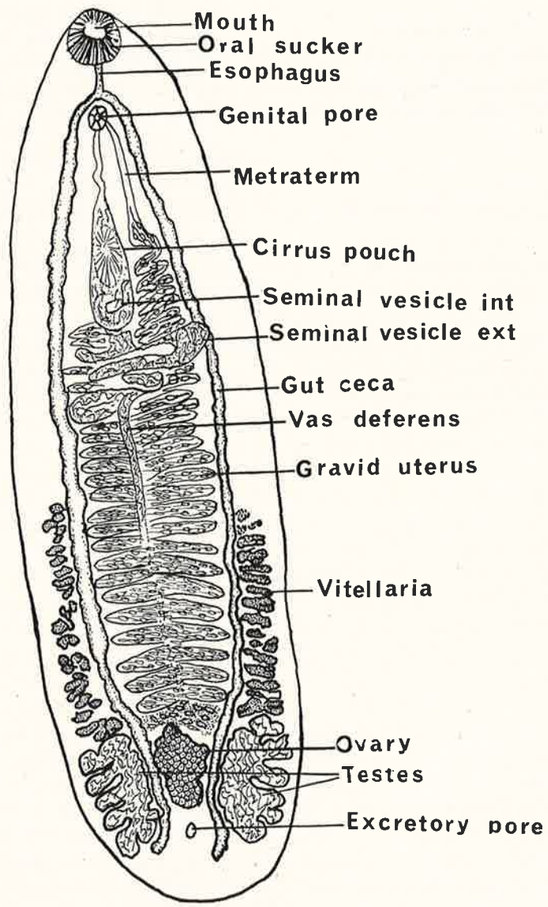

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PLATE I

Notocotylus urbanensis 24 da exp inf

Besides this evident specificity in both larval and adult forms the following characters are helpful in distinguishing these two species: (1) the anterior extent of the vitellaria, (2) the lateral and medial lobation of the testes, (3) the course of the excretory tubules in the cercariae, and (4) the time of emergence of the cercariae.

CONCLUSIONS

Stagnicola caberata is reported for the first time as a host for *N. stagnicolae*.

N. stagnicolae matures readily in birds in about eight days. Now at least four species of the genus *Stagnicola* are infected with the larval stage of this form.

N. urbanensis, under certain conditions, matures in both laboratory rats and *Microtus* in about 24 days. The larval stages are always found in *Physa* snails. A drawing is made from an experimentally produced adult. *N. urbanensis* is always found in the folds of the large intestine near the posterior end while *N. stagnicolae* always develops in the ceca of birds.

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AN INTESTINAL HELMINTH STUDY OF *CATOSTOMUS COMMERSONI* FROM THE BUSHKILL CREEK, NORTHAMPTON COUNTY, PENNSYLVANIA, WITH OBSERVATIONS ON SEASONAL DISTRIBUTION OF *TRIGANODISTOMUM* sp. (*TREMATODA*) AND *FESSISENTIS* sp. (*ACANTHOCEPHALA*)[1]

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ABSTRACT

Sixty-five (73.9%) of 88 *Catostomus commersoni* collected from the Bushkill Creek, Northampton County, Pennsylvania in 1963 were infected with one or more species of Caryophyllaeidae (Cestoda), *Triganodistomum* (Trematoda), and *Fessisentis* (Acanthocephala). The only species positively identified was *Triganodistomum attenuatum* Mueller and Van Cleave, 1932.

Specimens of *Triganodistomum* were abundant in the spring and sparse in the fall. *Fessisentis* sp., absent in the spring, was present in the fall. These findings suggest a seasonal distribution of species of *Triganoditomum* and *Fessisentis* from *C. commersoni* from the Bushkill Creek.

INTRODUCTION

White suckers, *Catostomus commersoni,* collected from the Bushkill Creek, a tributary of the Delaware River in Northampton County, Pennsylvania, during May, October, November, and December of 1963 were examined for intestinal helminths. Trematodes of the genus *Triganodistomum* Simer, 1929, an acanthocephalan of the genus *Fessisentis* Van Cleave, 1931 and monozoic cestodes of the family Caryophyllaeidae were found.

The purpose of this paper is to report the incidence of infection and seasonal distribution of the intestinal helminths found in *C. commersoni* in 1963.

MATERIALS AND METHODS

Suckers were collected with the aid of an electric shocking device. Two electrode grids attached to poles and operated by a 115 volt, 5.2 ampere generator driven by a gasoline motor, were placed in the water. The electric shock immobilized the suckers facilitating collection with hand nets.

Suckers were examined within a few hours after collection. Intestines were removed, cut into 6-inch sections and placed in 0.65% saline. Each section was opened longitudinally and examined under a dissecting scope. Parasites were removed, relaxed in cold tap water or saline, flattened with moderate cover slip pressure and fixed in hot A.F.A., or fixed in A.F.A. without flattening. Helminths stained in Gower's (1939) carmine, Harris' haematoxylin, and Grenacher's alcoholic borax-carmine were prepared as whole mounts according to procedures described in Meyer and Penner (1962).

RESULTS

The trematodes were identificed as members of the genus *Triganodistomum* from descriptions and figures in Mueller (1934), Mueller and Van Cleave (1932) and Van Cleave and Mueller (1934). The only species positively identified was *T. attenuatum* Mueller and Van Cleave, 1932. Some specimens were tentatively identified as morphological variants of *T. attenuatum* or as other *Triganodistomum* species. The acanthocephalans

1 This research was supported in part by a Research Grant from the Advanced Studies and Research Fund of Lafayette College.

were identified as a species of *Fessisentis* from Dr. Wilbur L. Bullock's Acanthocephala key published in Meyer and Penner (1962). The identification was confirmed by Dr. Bullock.

Sixty-five (73.9%) of 88 *Catostomus commersoni* collected from the Bushkill Creek in 1963 were infected with one or more species of Caryophyllaeidae, *Triganodistomum,* and *Fessisentis.* All species were from suckers collected in shallow, fast moving, slow moving, and deep water.

Twenty-two (91.7%) of 24 suckers collected in May were infected with a total of 1086 specimens of *Triganodistomum* sp. The maximum number of flukes found in one host was 190, and the minimum number was 2. The mean number of trematodes from the 22 infected hosts was 49.4. Five (7.8%) of 64 suckers collected in October, November, and December were infected with 6 flukes.

Fessisentis sp. was not found in May. Twenty-five (39.1%) of 64 suckers collected in October, November, and December were infected with 113 specimens of *Fessisentis* sp. The maximum number of acanthocephalans found in one host was 33 and the minimum number was 1. The mean number of acanthocephalans from the 25 infected hosts was 4.5.

Twenty-nine (33.0%) of 88 suckers collected in May, October, November, and December were infected with caryophyllideans. The number of cestodes per host was not recorded, but worm burdens were approximately the same in the spring and fall.

DISCUSSION

Species of Caryophyllaeidae, *Triganodistomum,* and *Fessisentis* were common intestinal helminths of *Catostomus commersoni* collected in the Bushkill Creek, Northampton County, Pennsylvania in 1963.

Reports of Caryophyllaeidae from *C. commersoni* are numerous and include those of Van Cleave and Mueller (1934) in New York, Hunter (1942) in Connecticut, Bangham and Venard (1946) in Ontario, Fischthal (1952) in Wisconsin, Sindermann (1953) in Massachusetts, Hoffman (1953) in North Dakota, Meyer (1954) in Maine, Meyer (1958) in Iowa, and Hugghins (1959) in South Dakota.

Species of *Triganodistomum* have been previously reported from *C. commersoni* in New York by Van Cleave and Mueller (1934), in Ontario by Bangham and Venard (1946), in Wisconsin by Fischthal (1952), in North Dakota by Hoffman (1953), in Iowa by Meyer (1958), and South Dakota by Hugghins (1959). *T. attenuatum* was the only species positively identified from *C. commersoni* from the Bushkill Creek. Van Cleave and Mueller (1934) found 2 of 36 *C. commersoni* in Oneida Lake, New York infected with a total of 6 speciments of *T. attenuatum.* Eleven of 53 *C. commersoni* examined in Ontario by Bangham and Venard (1946) were infected with *T. attenuatum,* and Fischthal (1952) found 9 of 89 *C. commersoni* from Wisconsin each infected with 1 to 10 specimens of *T. attenuatum.* Meyer (1958) recovered a total of 11 *T. attenuatum* from 3 *C. commersoni* from Trumball Lake, Iowa. *T. attenuatum* has also been reported from the longnose sucker, *C. catostomus* by Meyer (1954) in Maine, from the northern creek chub, *Semotilus a. atromaculatus* by Bangham and Venard (1946) in Ontario, and from the greater redhorse, *Moxostoma rubreques,* by Fischthal (1952) in Wisconsin. Haderlie (1950) described *T. polylobatum* from *C. occidentalis* in California, but Meyer (1958) reported that *T. polylobatum* was a possible synonymn of *T. attenuatum.*

There are no published reports of species of *Fessisentis* from *C. commersoni.* Two species of *Fessisentis* have been described, *F. fessus* from the freshwater drum, *Aplodinotus grunniens* in Mississippi by Van Cleave (1931), and *F. vancleavei* from the sunfish, *Lepomis gibbosus* in New Hampshire by Haley and Bullock (1953). Because specimens from *C. commersoni* differ from the description of *F. fessus* and *F. vancleavei* a specific identification was not made.

Specimens of *Triganodistomum* from suckers in the Bushkill Creek were abundant in the spring and sparse in the fall suggesting a seasonal distribution of this parasite. Data on seasonal variation are lacking since other workers have recovered species of *Triganodistomum* only during the summer months.

Fessisentis sp. was absent in the spring but present in the fall indicating a seasonal variation of this parasite. Van Cleave (1916) observed seasonal distribution of a fish acanthocephalan, *Neoechinorhynchus gracilisentis,* finding it present in the fall, winter, and early spring, but absent in the late spring and summer. Haley and Bullock (1953) examined 383 *Lepomis gibbosus* and found a total of 6 specimens of *F. vancleavei.* They did not state when their collections were made, but suggested that the low number of worms might be a result of seasonal variation. They also stated that *F. vancleavei* might be in an unnatural host. Because 39.1% of the suckers collected in the Bushkill Creek were infected with approximately 5 specimens per host it appears that *C. commersoni* is a natural definitive host of *Fessisentis* sp.

SUMMARY

1. Sixty-five (73.9%) of 88 white suckers, *Catostomus commersoni,* collected from the Bushkill Creek, a tributary of the Delaware River in Northampton County, Pennsylvania, during May, October, November, and December of 1963 were infected with one or more species of intestinal helminths.

2. Trematodes of the genus *Triganodistomum* Simer, 1929, an acanthocepalan of the genus *Fessisentis* Van Cleave, 1931, and monozoic cestodes of the family Caryophyllaeidae were found.

3. The only species positively identified was *Triganodistomum attenuatum* Mueller and Van Cleave, 1932.

4. Twenty-two (91.7%) of 24 suckers collected in May were infected with a total of 1,086 specimens of *Triganodistomum* sp.

5. Five (7.8%) of 64 suckers collected in October, November, and December were infected with a total of 6 specimens of *Triganodistomum* sp.

6. *Fessisentis* sp. was not found in May.

7. Twenty-five (39.1%) of 64 suckers collected in October, November, and December were infected with a total of 113 specimens of *Fessisentis* sp.

8. The results of the survey suggest a seasonal distribution of species of *Triganodistomum* and *Fessisentis* from *C. commersoni* collected from the Bushkill Creek.

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LABORATORY MAINTENANCE OF THE TURTLE BLOOD FLUKE, *SPIRORCHIS* sp. (*TREMATODA*)[1]

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ABSTRACT

Laboratory procedures used to maintain *Spirorchis* sp. (Trematoda) are described and illustrated. Miracidia hatched from spirorchiid eggs obtained from naturally infected *Chrysemys picta* lung tissue are used to infect a small planorbid snail, *Menetus dilatatus buchanensis* (Lea). Cercariae subsequently emerging from these snails are used to infect juvenile *Chrysemys picta*.

INTRODUCTION

Fried and Goodchild (1963) reported on the reproduction, growth, and survival of a planorbid snail, *Menetus dilatatus buchanensis* (Lea), an experimental host of *Spirorchis* sp. (Trematoda). Goodchild and Fried (1963) experimentally infected laboratory-reared *M. d. buchanensis* with miracidia from spirorchiid eggs obtained from naturally infected *Chrysemys picta bellei*. The purpose of this paper is to describe and illustrate procedures used to maintain the life cycle of this species of *Spirorchis* in the laboratory. Individuals of this species show considerable morphological variation depending upon their age and location in the final host. Identification of this species and taxonomic criteria no longer valid in distinguishing spirorchiid species will be published elsewhere.

METHODS AND DISCUSSION

The procedures used to maintain the snail phase of this life cycle have been described previously (Goodchild and Fried, 1963). In brief, they are as follows: *Menetus* snails are reared in enamel pans or finger bowls containing pond water and fed boiled lettuce. Spirorchiid eggs are obtained from the lungs of naturally infected *Chrysemys picta bellei* purchased from a commercial dealer in Minnesota (J. R. Schettle Frog Farm, Inc., Route 1, Stillwater). Lung tissue is teased and the eggs are prepared and embryonated essentially as described by Cable (1950) and Goodchild and Fried (1963). Individual snails are exposed overnight in shell vials with fully embryonated eggs or are placed in spot plates with miracidia (Figs. 2 and 4). Because miracidia are shortlived and usually hatch in the early morning hours it is technically difficult to expose large numbers of snails to miracidia in spot plates. Following exposure snails are maintained in 4-inch finger bowls. Sporocysts and cercariae are usually observable through transparent snails' shells 12 days later. Cercarial emission begins approximately 2 to 3 weeks after exposure and is temperature dependent. If snails are placed in a lightproof cabinet equipped with a timing switch operating a 15-watt fluorescent tube (Fig. 3), and exposed to an altered light-dark cycle, maximal cercarial emission occurs in the morning rather than evening (Goodchild and Fried, 1963).

Parasite-free juvenile painted turtles, *Chrysemys picta* are purchased from a commercial dealer in Wisconsin (The Lemberger Co., P. O. Box 482, Oshkosh) and maintained in 10-gallon aquaria containing tap water approximately 1 to 2 inches deep (Fig. 1).

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2 Based in large part on work done at the Department of Biological Sciences, Emory University, Atlanta, Ga.

Rocks placed in aquaria allow turtles to climb out of the water. Turtles are exposed to sunlight or artificial illumination and a temperature of 20 to 30 C is maintained. Once or twice a week turtles are fed cat food (Puss'n Boots Cat Food, The Quaker Oats Co., Chicago, Ill.) in separate aquaria to avoid fouling the water. Occasionaly the diet is supplemented with live guppies, *Gambusia,* mealworm larvae, and a variety of Wardley's (Long Island City, N. Y.) prepared turtle foods. Although the nutritional value of the various components of the diet have not been determined, uninfected and infected juvenilt *Chrysemys picta* have survived for over a year in the laboratory.

Turtles are exposed overnight to cercariae in 4-inch finger bowls or for a 1 to 2 hour period in exposure chambers. Cercariae are pipetted into finger bowls containing approximately 20 ml of water, and cercarial penetration is indicated when turtles forcefully open and close their mouths, or violently scratch their heads with their foreclaws. An exposure chamber consists of a 3/4 x 3/4 inch sewing thimble held in place with cotton in the bottom of a 2-inch petri dish (Fig. 5). A turtle is immobilized with modeling clay or with elastic bands so that its head is just above the opening of the thimble filled with 2 to 3 ml of water and a known number of cercariae. A turtle usually moves its head in and out of the water during the exposure period. This technique has the advantage of concentrating cercariae in the head region, and facilitates counting cercariae that have not penetrated. Turtles' carapaces are marked with finger nail polish, but since the polish occasionally rubs off, claws and digits are clipped according to a code.

Turtles are necropsied from approximately 2 to 12 months after exposure. Infections are determined by the presence of spirorchiid eggs in internal organs, or adult worms and eggs in the heart, pulmonary, systemic, mesenteric, and other arteries (Fig. 6).

Eggs obtained from laboratory infected juvenile turtles provide an excellent source of material to infect a new generation of laboratory-reared *M. d. buchanensis*.

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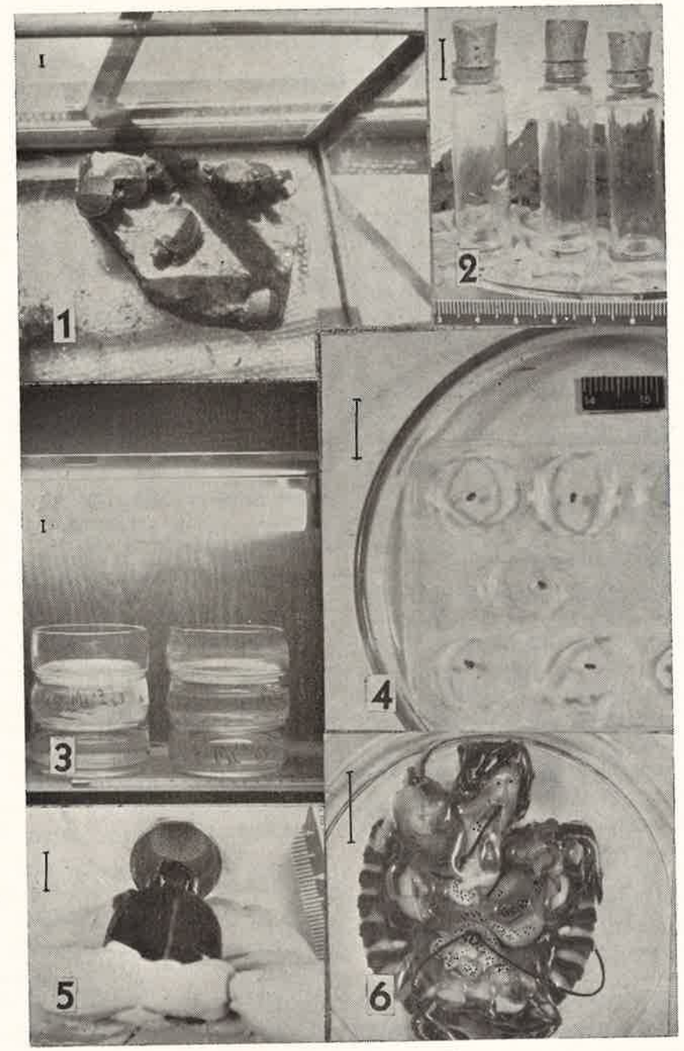

EXPLANATION OF FIGURES

The scale to the left of each figure represents approximately 1.0 cm.

Fig. 1. Juvenile *Chrysemys picta* maintained in an aquarium.
Fig. 2. Individual *Menetus d. buchanensis* exposed with spirorchiid eggs in shell vials.
Fig. 3. Infected *M. d. buchanensis* maintained in finger-bowls in lightproof cabinet.
Fig. 4. Individual *M. d. buchanensis* exposed to spirorchiid miracidia in a spot plate.
Fig. 5. Juvenile *Chrysemys picta* exposed to cercariae in an exposure chamber.
Fig. 6. Experimentally infected juvenile *Chrysemys picta*. The photo has been retouched with India ink to intensify the spirorchiid eggs in the tissues. The thread is looped around the mesenteric artery which contains an adult spirorchiid.

LARVAL DENSITIES AND ADULT BODY SIZE INTERACTIONS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

A highly inbred line of *Drosophila melanogaster* was produced at three larvae densities; 10, 30, and 50. The thorax length of all adults that emerged (55% survival) was measured. Significant differences were found among the three groups for the character measured. Correlations for males was 0.44; for females 0.84.

INTRODUCTION

The affect of variation in population densities in morphological characters in *Drosophila* have been studied by numerous workers, notably: Tantawy and Mallah (1961), Tantawy (1961). Parsons (1961) using ebony, wild type and heterozygous larvae grown at two levels of competition (25 and 100 larvae at 25°C) found that fly size decreased at the higher levels of competition. Moree and Krug (1961) concluded that larvae crowding produces a corresponding decrease in body weight. Further, the relationship between larvae density and adult body weight is non-linear. Come and Nash (1962) in a selection study for thorax size in *Drosophila* found a high correlation between size and adult densities, even at low density levels.

This study was undertaken to determine the extent of interaction between adult body size and larvae densities of fifty and below. At these levels it would appear that little competition for food would exist.

METHODS AND MATERIALS

The inbred stock used in this study was derived from a wild population of *Drosophila melanogaster* collected at Woodycrest, near State College, Pa. The line, designated A3V, had been inbred over 200 generations prior to this study, and is maintained at the Department of Zoology, Rutgers University.

Adult flies were produced in vials containing three levels of larval density; 10, 30, and 50. Five replicates were made at each density. Larvae were produced by placing adults from the A3V line in petri dishes with a thin layer of standard media. Two day old larvae were transferred to clean shell vials (25mm by 95mm) and allowed to develop. A number of methods for transferring larvae were tried; the method described gave about 50% survival.

Two day old larvae hatched in petri dishes were removed to the culture vials with a hooked *Drosophila* needle. The needle was moistened before each transfer and in most cases some food was transferred at the same time. In this manner it was possible to transfer from 3 to 5 larvae at a time.

The thorax length of all adults was measured with a special device designed specifically for use with *Drosophila* by the Drummond Scientific Company of Philadelphia. Details of the apparatus are furnished by Mitchell (1958). Thorax length was determined by the profile length between parallel lines at right angles to the body axis, at the head-thorax juncture, and at the posterior tip of the thorax. Measurements were taken of etherized flies lying on their side; measurements were taken to the nearest 0.005 mm.

Table 1 Mean thorax length for females and males with larval density controlled. Number below means is number of adults eclosing. A=10 larvae; B=30; C=50. Prime indicate males.

	(Replicates)					
	1	2	3	4	5	Totals
A	102.00 1	102.07 3	102.92 5	102.44 5	97.47 3	101.44 17
B	98.48 15	97.88 5	99.25 8	98.05 11	100.16 11	98.76 50
C	99.71 18	98.06 14	98.90 14	98.63 17	97.58 6	98.58 69
A′		85.88 5	91.95 2	90.60 1	89.48 4	89.48 12
B′	84.14 5	86.45 8	84.68 4	86.28 4	86.11 10	85.72 31
C′	87.42 10	85.53 12	85.85 18	83.44 21	84.02 6	85.25 67

RESULTS

The mean thorax length for all males and females eclosing is given in Table 1. A 55% survival of larvae was achieved using the method previously described. It may be safer then to interpret these results on a 5, 15, 25 larval density bases. At all densities less males eclosed than females. An analysis of variance for mean thorax length within each of the five replicates is given in Table 2. The results of the analysis favor the pooling of the information within each subgroup as no significant difference was found. A slight significant difference found among males in the C[1] replicate is ignored.

The mean thorax value for males and females declined with increasing larval density, the larger slope between the 10 and 30 larval densities. An analysis of variance of the pooled values for females and males is given in Tables 3 and 4, respectively. Highly significant differences are recorded in all cases, except the difference in mean female length at 30 and 50 densities. Here only a slightly significant difference was found.

The correlation between larvae density and thorax length is given in Table 5. A higher negative correlation was found for females than males: males—0.44, females—0.84.

Table 2 Analysis of variance for subgroups 1-5.

	df	ms	F
Among A	4	16.08	0.79 ns
Within A	12	20.37	
Among B	4	8.5	0.59 ns
Within B	45	14.46	
Among C	4	7.98	0.77 ns
Within C	64	10.32	
Among A′	3	21.13	2.65 ns
Within A′	8	8.35	
Among B′	4	5.96	0.678 ns
Within B′	26	8.77	
Among C′	4	32.9	3.66*
Within C′	62	9.00	

Table 3 Analysis of variance for females.

	df	ms	F
Among Groups	2	124.55	10.65***
Within Groups	133	11.70	
A vs. B	1	197.20	16.85***
B vs. C	1	65.90	5.63*

Table 4 Analysis of variance for males.

	df	ms	F
Among Groups	2	595.00	32.21***
Within Groups	107	18.21	
A vs. B	1	132.6	17.09***
B vs. C	1	139.0	16.49***

Table 5 Correlation between larvae density and thorax length.

					r
Males	Thorax Length (x)	89.48	85.72	85.25	—0.44
	Larval Density (y)	10	30	50	
Females	Thorax Length (x)	101.44	98.76	98.56	—0.84
	Larval Density (y)	10	30	50	

DISCUSSION

This study, undertaken to examine responses between larval density and body size in *Drosophila,* shows the necessity for careful control of laboratory conditions. Very low larval densities were effective in limiting body size; the actual competition in this study may have been as low as 5 larvae per vial. Also, at low densities, differences of 20 larvae per vial can produce a significant thorax length response. It is very probable that at higher densities and at extremely low densities (1-5 larvae per vial) variations in larval competition may not be as important a factor in determing body size. However, of particular concern here is that even small variations in larval density, between 10 and 30, could be effective in altering some quantitative morphological characters.

The affect of very small changes in larval density can be explained by the behavior of the female fly. The female lays most of her eggs along the perimeter of the vial; therefore, although per vial density may be small, any given larvae may find his immediate environment highly competitive. This same situation could have occurred in this study, as many larvae were transferred at one time, and located in "close" proximity to one another.

The results indicate that density effects on body size are linear between 5 and 50, and at low densities (1-5) and at extremely high densities (above 50) plateaus would be reached.

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EFFECTS OF VARIOUS ADRENAL STEROIDS ON WATER AND ELECTROLYTE SHIFTS IN THE ADRENALECTOMIZED FORCE-FED RAT

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ABSTRACT

The effects of eight 11-oxy-corticoids were tested for their effect on body weight, urinary sodium and potassium, and terminal serum sodium and potassium levels. Emphasis was placed on the effect of one steroid, Δ' u, with regards to its life-maintaining properties. It was found that these steroids have effects on mineral and water metabolism.

INTRODUCTION

The steroidal secretions of the adrenal cortex play important roles in regulating water, mineral, and carbohydrate utilization in vertebrate organisms. However, the actions of so called glucocorticoid steroids on water and electrolyte metabolism are not well understood. In the human, Soffer *et al.* (1), have shown that desoxycorticosterone acetate (DCA) decrease NaCl excretion in the normal individual and it is well known in Cushings syndrome that there is urinary loss of sodium and chloride. The work of Woodbury *et al.* (2) indicated a difference between the electrolyte response to ACTH when DCA was given concomitantly and when the animals had been pretreated with chronic doses of DCA. Selye (3) found that certain morphological effects of aldosterone were inhibited by cortisol when these two steroids were administered concurrently. Eversole and Romero (4), investigating the effects of several 11-oxy corticoids and their analogues on electrolyte metabolism, reported considerable differences between the steroids in the response of animals maintained on a forced feeding regimen. They found that while one steroid might have an effect of augmenting sodium and potassium excretion it may not change appreciably the serum levels of these electrolytes. More recently Uete and Venning (5) investigated the effects of cortisone, cortisol, and 9 α fluoro -16-OH-Δ' cortisol with respect to the action of DCA and aldosterone on electrolyte excretion. These workers demonstrated that one group of steroids antagonized the other with regards to natriuresis but both groups facilitated potassium excretion.

It was therefore felt that an investigation to ascertain the effects of various 11-oxy corticoids on sodium, potassium and water turn-over in the force-fed adrenalectomized animal might shed some light on the effects of certain glucocorticoids on salt and water metabolism. The force-fed adrenalectomized preparation was used in order to standardize consumption of water and salt and to avoid possible complication of interaction with endogenous corticosteroids. Cortisone, cortisol, their Δ^1 analogues (Prednisone and Prednisolone), corticosterone, 9 α fluorocortisol, and $\Delta^{1,4}$-pregnadiene -17, 20, 21-triol-3, 11-dione (Δ^1 U) were chosen as a representative series of steroids to be studied in attempts to shed some light on the effects of 11-oxy (glucocorticoid) steroids on water and electrolyte balance.

The investigtion was supported in part by grants from the National Institutes of Health to W. J. Eversole at the University of New Mexico, Albuquerque, New Mexico. The steroids used in this study were supplied by the Merck Institute for Therapeutic Research.

METHODS

Ten groups of Long-Evans male rats (approx. 230 g were used. One intact group and one adrenalectomized group served as controls and received no hormone while the others were adrenalectomized and treated with one of the following corticoids: cortisone, prednisone, cortisol, prednisolone, 9 α fluorocortisol, Δ^1U, corticosterone, or 11 dehydrocorticosterone. Daily urine volumes, daily excretion of Na and K, terminal blood plasma levels of Na and K, and body weight changes were criterea in evaluating hormonal effects. The rats were maintained in air conditioned quarters for at least ten days prior to commencement of each experiment. After this adaptation a tube feeding regimen was established, using a semiliquid diet (Ingle and Oberle, 6). The amount of food administered was gradually increased from 5 to 20 ml/day over a 6 day period. During the preliminary feeding Purina Chow and water were supplied *ad lib*. After 8 days on forced feeding the animals were bilaterally adrenalectomized and hormone treatment initiated. Each day for 9 days the rats were fed morning and evening and given 12 ml of water at noon. Each rat received 1 mg hormone/day: .4 mg in morning and evening, and .2 mg at noon. The steroids were dissolved in alcohol, suspended in Merck Aqueous Vehicle No. 1, and injected subcutaneously. Animals were housed in individual metabolism cages which separated urine and feces. Body weights and urine volumes were recorded daily. Urine collected on alternate days was used for Na and K assay by a flame photometer with an internal lithium standard. Terminally, blood collections were made from decapitated trunks, and clear plasma analyzed for Na and K. Standard error was calculated by use of the formula recommended by Fisher (7): $SE = \pm \sqrt{\frac{d^2}{N(N-1)}}$

RESULTS

Table 1 shows the values obtained for urine volume, urinary Na and K, and plasma Na and K. Adrenalectomy alone resulted in increased urinary output and 6 of the 8 steroids augmented significantly ($P<.05$) urine volume above the level found for the adrenalectomized animal; prednisone was most effective; corticosterone was ineffecitve in this respect while Δ^1U decreased the daily urine volume of the adrenalectomized animal to the level of the control. The total urinary Na was decreased with Δ^1U, 9 α fluorocortisol and corticosterone, slightly increased with cortisone, and not changed with the other steroids tested. The corticoids, excepting Δ^1U, increased urinary K levels above those found in intact or adrenalectomized controls. While Δ^1U had no effect on terminal plasma Na concentration the remaining steroids to varying degrees, elevated plasma Na concentration above the level of that found in adrenalectomized untreated animals. Of the compounds which lowered plasma potassium to levels below those characteristic of the adrenalectomized rat, 9 α fluorocortisol was most effective, even depressing the level below that found in intact controls. The steroids, excepting Δ^1U accentuated weight loss to a greater extent than adrenalectomy alone.

From Fig. 1 and Table 2 it can be seen that Δ^1U was the only steroid of those employed which gave a gain in body weight and recovery of the water from the level found in the adrenalectomized rat to near that found in the intact controls.

DISCUSSION

These experiments demonstrate that small daily dose of either cortisone, cortisol, their Δ^1 analogues, or 9 α fluorocortisol increased urine volume and potassium excretion in adrenalectomized force-fed rats. These steroidal effects are simi-

	Urine Vol. ml. day	Urinary Na m.Eq. day	Urinary K m.Eq. day	Plasma Na m.Eq. L.	Plasma K m.Eq. L.	Wt. Change Gms. day
Intact Control	9.2 ± 0.27 * (6)† (59)††	1.93 ± 0.05 (6) (28)	1.52 ± 0.04 (6) (28)	150 ± 1.62 (6) (6)	7.4 ± 0.43 (6) (6)	+2.3 ± 0.27 (6) (6)
Adx. Control	10.8 ± 0.31 (7) (56)	2.33 ± 0.08 (7) (25)	1.59 ± 0.06 (7) (24)	137 ± 4.3 (6) (6)	8.3 ± 0.67 (5) (5)	-0.7 ± 0.30 (7) (7)
Adx. Cortisone	13.5 ± 0.44 (5) (42)	2.44 ± 0.02 (5) (22)	1.89 ± 0.04 (5) (23)	149 ± 4.05 (4) (4)	8.7 ± 0.7 (4) (4)	-2.0 ± 0.27 (5) (5)
Adx. Hydrocortisone	13 ± 0.32 (6) (51)	2.34 ± 0.05 (6) (26)	1.98 ± 0.06 (6) (28)	150 ± 3.78 (6) (6)	8.2 ± 0.59 (6) (6)	-1.9 ± 0.28 (6) (6)
Adx. Prednisone	15.2 ± 0.36 (6) (56)	2.30 ± 0.05 (6) (27)	2.03 ± 0.02 (6) (27)	142 ± 0.24 (6) (6)	9.0 ± 1.03 (6) (6)	-3.2 ± 0.32 (6) (6)
Adx. Prednisolone	14.6 ± 0.36 (5) (46)	2.33 ± 0.05 (5) (23)	2.04 ± 0.04 (5) (23)	148 ± 0.13 (5) (5)	7.5 ± 0.55 (5) (5)	-2.7 ± 0.35 (5) (5)
Adx. 9∝Fluoro Hydrocortisone	14 ± 0.48 (6) (54)	2.09 ± 0.05 (6) (28)	1.94 ± 0.05 (6) (28)	145 ± 0.07 (5) (5)	5.5 ± 0.3 (6) (6)	-1.4 ± 0.23 (5) (5)
Adx. Δ^1 U	9.1 ± 0.36 (6) (50)	2.04 ± 0.10 (6) (25)	1.58 ± 0.05 (6) (23)	137 ± 3.0 (4) (4)	8.2 ± 0.20 (5) (5)	+0.4 ± 0.4 (6) (6)
Adx. Corticosterone	10.5 ± 0.53 (6) (54)	2.20 ± 0.13 (6) (30)	1.84 ± 0.04 (6) (30)	145 ± 4.16 (3) (3)	8.2 ± 0.15 (3) (3)	-1.5 ± 0.33 (6) (6)
Adx. 11 dehydro Corticosterone	12 ± 0.48 (7) (55)	2.25 ± 0.04 (5) (24)	1.90 ± 0.03 (5) (24)	142 ± 3.0 (5) (5)	7.8 ± 0.85 (5) (5)	-0.9 ± 0.26 (5) (5)

† Number animals
†† Number cases
* S.E.$= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

Table I. Effects of adrenal steroids on water and electrolyte balance in force-fed adrenalectomized rats (treatment 1 mg/day).

lar to those obtained in the intact force-fed animal (4). The effect on natriuresis in this study differs from that seen in the intact animal where it formerly was found that most of the steroids augmented Sodium excretion (4). In the present study, where the effects were studied in the adrenalectomized animal, there was little, if any, effect on sodium excretion. In other words these 11-oxy steroids were natriuretic in the intact animal but not in the adrenalectomized one. The reason for such a difference is not clear but it could be related to the probability

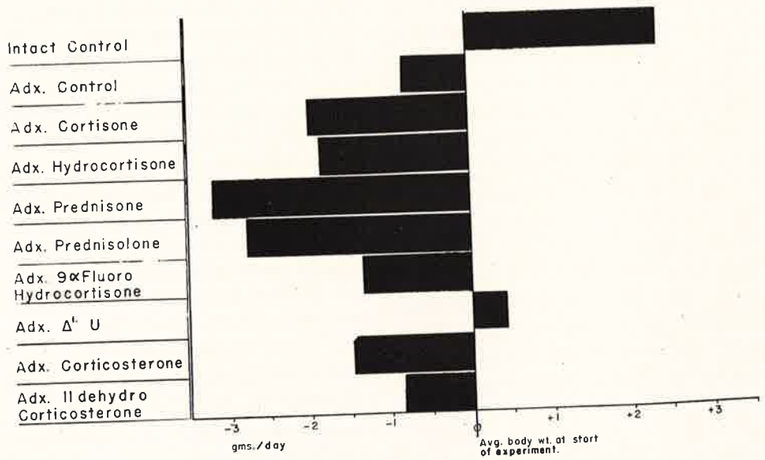

Fig. 1. Effects of adrenal steroids on body weight in force fed adrenalectomized rats (treatment 1 mg/day).

of an interaction of affects between the administered steroids and endogenous ones in the intact animal, whereas in the adrealectomized rat the administered steroid could exert its effect without interference from adrenocortical secretions. The finding by Uete and Venning (5) of antagonistic effects between mineralocortioids and glucocorticoids on sodium excretion but similar effects of these steroids on potassium excretion lend credence to the idea of complicated interrelationships between endogenous and exogenous steroids acting simultaneously on metabolic processes. A low plasma sodium characteristic of the adrenalectomized animal, was found in the force-fed adrenalectomized rats studied here and administration of the corticoids (excepting Δ^1U) elevated plasma sodium, cortisone and hydrocortisone being most effective. The steroids failed to lower the high plasma potassium level of the adrenalectomized rat while stimulating urinary loss of this element. Hormones effective in replacing adrenocortical function generally decrease urinary loss of sodium, elevate plasma sodium levels, increase urinary potassium excretion, and depress plasma potassium levels. Since the steroids tested here failed to depress plasma potassium and halt renal loss of sodium and water it is quite likely that they would have poor life-maintaining properties in adrenalectomized animals. This has been our experience, particularly with cortisone. Furthermore, it seems quite clear that the steroids used here, while generally are thought of as primarily active in carbohydrate metabolism (8, 9), have a marked but somewhat unpredictable influence on water and electrolyte metabolism.

Corticosterone and 11-dehydrocorticosterone considered to be hormones normally secreted by the adult rat (10), had little effect on renal excretion of water and electrolytes; this was formerly shown to be true for the intact animal (4). Reasons for such findings are not clear

	HOH	Na	K
Intact Control	57 %	76 %	72 %
Adx. Control	68 %	92 %	76 %
Adx. Cortisone	84 %	97 %	89 %
Adx. Hydrocortisone	81 %	93 %	94 %
Adx. Prednisone	94 %	91 %	96 %
Adx. Prednisolone	90 %	92 %	97 %
Adx. 9∝Fluoro Hydrocortisone	88 %	83 %	92 %
Adx. Δ^1 U	57 %	81 %	75 %
Adx. Corticosterone	66 %	87 %	87 %
Adx. 11 dehydro **Corticosterone**	**75 %**	**89 %**	**90 %**

Table II. Effects of adrenal steroids on the percent of administered water sodium and potassium recovered per day (treatment 1 mg/day).

but it may be that the rat metabolizes or inactivates these natural products more readily than other steroids, and hence requires larger doses to obtain similar effects.

Effects with Δ^1U were somewhat interesting in that in the adrenalectomized animal it was the only compound which promoted gain in body weight and depressed the urine volume to that characteristic of the intact animal. It did not appreciably affect plasma or urinary electrolytes. This compound then seems to have mild physiological properties favorable to survival in the adrenalectomized rats. At the termination of the experiment the rats treated with Δ^1U appeared healthier and were more active than the animals treated with any of the other steroids. Such results, lead us to tentatively suggest, that Δ^1U promotes anabolic effects more so than the other steroids used here. In confirmation of others (11), 9 α fluorocortisol increased urine volume and potassium, but depressed urinary sodium excretion. This steroid lowered the plasma potassium sufficiently to cause hyperexcitability and probably affected cardiac functions. It would be of interest to study its effects on cardiac activity.

All of the steroids tested, except Δ^1U either had no effect or augmented recovery of administered water, sodium, and potassium in adrenalectomized rats. Since Δ^1U was the only compound which depressed water recovery to normal levels and promoted gain in body weight it probably exerted beneficial homeostatic effects by acting somewhat selectively on the transfer water from one body compartment to another, or by promoting renal tubular reabsorbtion of water. Such assumptions are based mainly on the findings that plasma sodium and potassium were not appreciably affected by the steroid, and urinary sodium was only slightly depressed while urinary potassium remained unchanged. Effects somewhat similar have been reported in the adrenalectomized dog by Swingle *et al.* (12) after administration of 2 methyl-9 α fluorohydrocortisone.

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THE BIOLOGICAL AVAILABILITY OF RIBOFLAVIN SOLUBILIZED WITH SODIUM SALICYLATE

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ABSTRACT

The vitamin solubilized with sodium salicylate (25 g of sodium salicylate and 1.2 g of riboflavin per 100 ml of solution) was fully available when tested with *Lactobacillus casei* after being stored for three weeks at 37° C and nine weeks at 6° C and 23° C. The freshly solubilized vitamin was also found to be available when tested with chicks.

INTRODUCTION

The limited solubility of riboflavin in water has stimulated investigators to seek agents which increase the water solubility of the vitamin without interfering with its biological usefulness.

Miller (1946) and Sengupta and Gupta (1949) reported that sodium salicylate acts as a solubilizer for riboflavin.

The experiments reported in this paper were carried out to determine whether the sodium salicylate when used as a solubilizer interferes with the biological usefulness of the vitamin in solutions containing 25 g of sodium salicylate and 1.2 g of riboflavin per 100 ml of solution.

PROCEDURE

The sodium salicylate solubilized samples of riboflavin were compared with unsolubilized riboflavin by two biological procedures: microbiologically with *Lactobacillus casei* (Snell and Strong, 1939) and biologically with Kimberchik K-137 chicks as the test animals.

The procedure used in the microbiological assays is found in the ninth edition of the Difco Manual published by Difco Laboratories, Detroit, and is a slight modificaiton of that of Snell and Strong. The microbiological evaluations were based on the titration of the lactic acid produced after seventy-two hours of growth.

The procedure used in the chick test follows quite closely that used by Arnold, *et al.* (1952) in determining the availability of riboflavin solubilized with sodium 3-hydroxy-2-naphthoate. The day-old, male chicks were fed a riboflavin-deficient diet for one week prior to placing them on test. The tests were begun with twelve chicks in each group.

The riboflavin used in these studies was obtained from Nutritional Biochemicals Corporation and its activity was

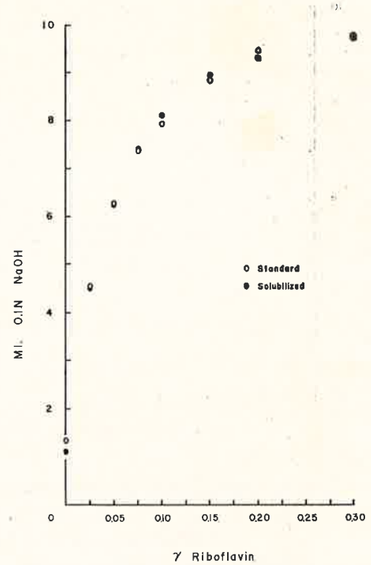

Fig. 1. Microbiological response to riboflavin freshly solubilized with sodium salicylate compared with that of unsolubilized riboflavin (standard).

found to be equivalent to that of U.S.P. riboflavin when tested with *Lactobacillus casei*. The riboflavin-deficient diet was obtained from the same source.

For the microbiological tests the solution was simply diluted to the appropriate concentrations before use. Tests were performed on the solution when freshly prepared and upon storage in ruby glass in a dark place for the periods of time and at the temperatures indicated under Results.

For the chick tests a suitable aliquot of the solution was dried on sucrose in a vacuum desiccator over calcium chloride prior to regrinding and incorporating into the diets. The solution and riboflavin powder were added to the diets at two levels of test to supply 116 ad 204 micrograms of riboflavin per 100 grams of diet.

RESULTS

The results of the microbiological tests on freshly prepared riboflavin-salicylate solution and on solutions stored at 37° C for three weeks and at 23° C for nine weeks are shown in Figures 1 to 3. The results from the unsolubilized and solubilized vitamin do not differ more than do the results of the duplicate tests made on either one. The results of the test of solution stored at 6° C for nine weeks agree equally well with the reference riboflavin.

Examination of the growth curves of the chicks given in Figure 4 and of the average weekly weight gains per 100 micrograms of riboflavin intake given in Table I indicates that the gains in weight of the chicks fed the two riboflavin sources were not appreciably different at each of the intake levels.

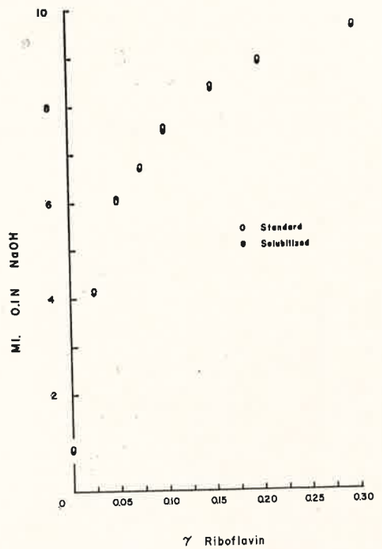

Fig. 2. Microbiological response to riboflavin solubilized with sodium salicylate and stored for three weeks at 37° C compared with that of unsolubilized riboflavin (standard).

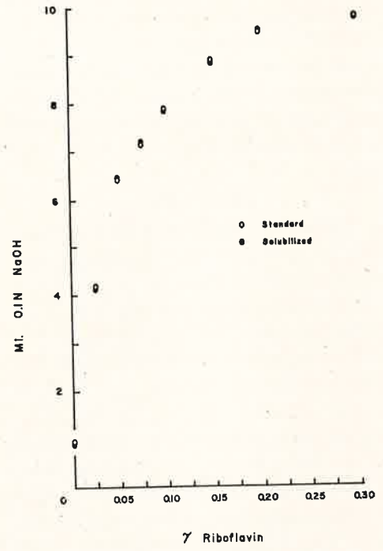

Fig. 3. Microbiological response to riboflavin solubilized with sodium salicylate and stored for nine weeks at 23° C compared with that of unsolubilized riboflavin (standard).

TABLE I

Growth response of one week riboflavin depleted chicks to each of two riboflavin preparations each fed at two levels of intake

Riboflavin source and dietary level	Number of chicks surviving test period	Total riboflavin intake per chick	Weights		Weekly gain per 100γ riboflavin intake
			Initial	4 weeks	
		γ	gm	gm	
Riboflavin powder, 116γ%	10	183.9	45.9	66.5	2.80
Riboflavin powder, 204γ%	10	386.5	46.4	89.7	2.80
Solubilized in sodium salicylate, 116γ%	11	144.8	46.2	62.7	2.85
Solubilized in sodium salicylate, 204γ%	10	335.6	43.6	77.5	2.52
None	3		41.7	43.6	

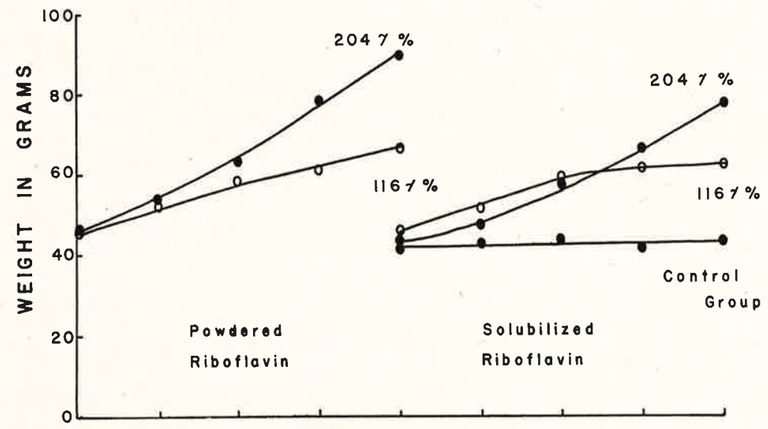

Fig. 4. Growth response of chicks fed a riboflavin-deficient diet supplemented with unsolubilized riboflavin or with riboflavin solubilized with sodium salicylate.

SUMMARY

The biological availability of riboflavin solubilized with sodium salicylate was determined with *Lactobacillus casei* and with chicks as the test organisms. The riboflavin in a solution containing 25 g of sodium salicylate and 1.2 g of riboflavin per 100 ml of solution remained fully available since the solubilizing agent had no determinable effect.

Tests showed that the solution was stable to storage for at least three weeks at 37° C and at least nine weeks at 6° C and 23° C.

ACKNOWLEDGMENTS

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RESEARCH APPROACH TO COLLEGE CHEMISTRY

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ABSTRACT

Since potential chemists are often lost because of students' disappointment with college freshman chemistry a laboratory method has been devised which stimulates interest and uncovers talents through student-chosen projects. Motivated by a desire to prepare chosen substances, the necessity for purifying them and proving he has them the student becomes skillful in his manipulations and acquires a working knowledge of basic chemistry superior to that obtained from "cook-book" laboratory experiments.

Any teacher of chemistry who has not, at some time, been dissatisfied with the results of his teaching has never fully realized the tremendous responsibility civilization has placed on his shoulders. Through our hands are passing the world's future chemists, and, unfortunately, through our hands are also slipping some *potential* chemists. These are the young people who loved chemistry—or thought they did—but, because of disappointment with their college freshman chemistry, became bored and soured on the science. We have all seen it happen, and many of us are trying to do something about it.

For a long time now teachers of freshman chemistry have been looking for something superior to the "cook-book" laboratory method. We are all aware that a student can fill every blank in the most modern and complete laboratory manual and still not know chemistry, but what can we do about it? Just adopting a newer, thicker laboratory manual is not the answer.

Several years ago I began to experiment with an entirely different type laboratory instruction, a "learning-by-doing" method which I call the *"research"* approach to college chemistry. What does the researcher have that keeps him plowing on in spite of vitriol, fire, and brimstone? Isn't it his self-chosen goal, his dream? When I allowed my students to attempt the materializing of their own dreams in the laboratory they became true researchers, young scientists who could plan and carry out intricate, difficult experiments, who could delve into volumes of reference material, endure failure after failure, and accept stained hands and acid-riddled clothing without losing the shine in their eyes. And, strange though it may seem, they not only had a wonderful time, but they learned chemistry, *really* learned it, far better than those who followed cut-and-dried conventional laboratory procedures. As one boy put it, "What I like about this method is, we learn *why*."

Of course the "research" that my freshmen do is not the frontier-moving research of the advanced scientists. They are not doing things that have never been done before—though at times they come close to it—but they are pushing their own frontiers of knowledge back while traveling their self-plotted courses. To *them* it is research, research with all its agonies and ecstasies.

First, let me say that I use a conventional laboratory manual—"cook-book" procedure and all—for the first half of freshman chemistry. Here, in Chemistry 101, the students learn the basic techniques and do the standard experiments which are essential for all beginning chemists. However, all along the way I keep pointing out the reason for this rigorous, difficult—and sometimes boring—training. They are told that this is

all preparation for a new exciting kind of chemistry where they will be given a great deal of choice in their experiments.

Near the end of Chemistry 101 each student is told to choose a metal which is to be the spring-board for his 102 "research." Any other type of base-material could be used, but I use the metals because the lectures at this time in the course usually deal extensively with the chemistry of the metals. Work with the chemistry of one metal may seem a narrow path for a freshman, but it soon develops that it is far from narrow. Before long the path begins to branch, and branch again, until it joins the chemistry of other metals and cuts deeply into the chemistry of the non-metals, thus connecting much of the whole, vast field of inorganic chemistry.

With the choice of a metal decided the student begins his reading. Starting with his own text book, and almost immediately adding more advanced works, he goes on to search the libraries and material from industries for information about his metal. As he reads he keeps looking for things *he* might be able to do: compounds to produce, processes to try, tests to make. At first he may feel completely bewildered, but eventually he spots some simple thing he can try, and once launched he soon realizes that almost every paragraph in a book such as MELLOR'S MODERN INORGANIC CHEMISTRY (1) contains a potential experiment.

The first day in the 102 laboratory the student is introduced to standard reference material and taught how to use it. He meets the Chemist's Handbook (2) (3), which soon become his trusted friends. He is also told more about other library facilities and how to use such encyclopedic works as Mellor's COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY (4).

There are certain other instructions the student must be given on this first day so he will know how to regulate his laboratory procedure. He is warned that never, absolutely *never,* is he to do an experiment without first clearing with me! This is an iron-clad rule and breaking it can lower a grade. He is instructed how to keep accurate, dated notes of every step he takes. Failure to do this reduces his experiments to "chemistry-set messsing around," and can result in failure for the course. He is also taught how to judge and limit quantities for safety and thrift. For a quick rule-of-thumb we say to use no more than two grams of a metal, and no more than five milliliters of anything. Special cases, of course, vary these quantities both ways.

On this first day, just as soon as the instructions are completed, the students whose projects and experiments have been approved are free to begin work. One by one they quietly get up from their tables and start to assemble apparatus and materials. This is a frightening time for me. As I watch those first hesitant steps I always ask myself, "Will it work? Will it really work this time?" But it always does. With the first two or three on their feet, it is not long before the others, too, timidly ask for permission to try something they have found in their reading.

Most students begin by attempting to make some simple compound, but even that has its problems, as it soon becomes evident that each metal presents a set of problems all its own. One student may find he can make a sulfate of his metal in one quick step, while another must go through a long, laborious procedure for his, and still another may find his metal forms no sulfate at all. There is no chance of trailing along in the footsteps of the "brain" of the class for everyone is working on a *different* metal. The "brain" has problems of his own. Within two or three weeks most have enough knowledge, skill, self-confidence, and daring to try any reaction they can find in their reading. I can actually see them grow as, day by day, they discover the reductions, the complexes, the different oxidation states, the individual, "personal" traits that characterize the elements themselves. And, no one is limited by a missing reagent. What he needs, he *makes!*

This "making what he needs" is one of several "secret" ingredients of the research approach which makes it such an effective way to learn chemistry. I keep only one or two sources of each metal on our supply shelf. By all sorts of chemical gymnastics the student has to change these into all the various substances he desires to make, and, in addition to making the compounds of his own metal, he is quite likely to find it necessary to make his other reagents. Complications build on top of complications, and each one teaches him a little more about chemistry.

Another "secret" ingredient is that nothing is ever thrown away just because "it didn't work." Students and teachers alike learn more from the failures than from the successes. If a reaction does not turn out as expected there is a reason for it that must be discovered, if possible. With the teacher as a consultant the student puts his hopeless-looking results through reaction after reaction in an effort to get it back into an identifiable form. Also, everything possible is reclaimed and purified for reuse. For instance, we rarely throw away a silver or mercury compound.

In every case the student must purify his product and prove he has what he says he has. This is accomplished by tracking down chemical and physical properties, bit by bit, from the Chemist's Handbooks (2) (3), Mellor's (1) (4), et cetera. It is here he learns about solubilities, crystallizations, and the delicate separations and washings of products.

There must be correct equations for every reaction. No lazy guesses will do. Sometimes it takes the whole department to find the accepted equation, or to write those we cannot find, but our student-teacher combination rarely gives up.

Although the student is on his own I am with him all the way, not in front of him, but beside him, ready to suggest a reference book, an improved set-up, a different technique, a safety measure, a possible solution to a knotty problem, and eternally, purification and proof.

When possible, purified samples of the final products from the various projects are mounted on uniform sheets and posted on a large bulletin board just outside the laboratory door. In addition to the sample with its name and formula these sheets give all the reactions which produced it and the reasons for their going to completion. Also the student must record how he separated and purified this substance from any others present. Little knots of students congregate in front of this display all during the day to exchange experiences and offer suggestions. In this way all students in all of the 102 classes share in the glory of each others successes and sympathize with their difficulties. They are proud of these samples because each bit of Saran-wrapped crystal or powder is the materialization of an idea, not just something they had to do to pass chemistry. Even the poor students take pride in their part of the display, for they know something of the "why" of each reaction.

It is difficult to describe what happens to both students and teacher as the

course gains momemtum. I can see each one pass from fumbling and doubts to poise and confidence. As interest becomes first warm, then hotter and hotter, "lab" ceases to be a smelly ordeal that must be endured for science credit, and becomes a time and place to work out ideas, a place where a bit of "goo" in an evaporating dish can become very precious because it is the tangible results of labor combined with a dream. Before long they are standing in line anxiously waiting for me to unlock the laboratory door at the beginning of the period. Soon they forget to watch the clock for their "break," and I have to drive them out at the end of the period. As for the teacher—I have more fun than anyone! At the end of each term I feel I have had another wonderful course in advanced inorganic chemistry.

It is impossible to tell adequately how this method functions. You would have to follow me around the laboratory for several weeks and actually see it in action to apreciate what is accomplished. Rarely do I say an experiment cannot be done. If someone insists on trying a project, even in the face of my doubts of his success, I usually give in. I have seen too many inspired young chemists do the impossible. Of course, I do not permit any reaction that could produce an extremely poisonous gas, such as arsine or cyanogen, and I am very careful about explosives. When allowing a reaction with which I am unfamiliar, one that could be dangerous in any way, we use only "pin-head" amounts and work up from that. I am constantly amazed at the ever-newness of the course taught in this way. Certainly many, many reactions are repeated term after term as each new class discovers them for themselves, but each term someone does something that has never been done in the class before.

A few examples will illustrate how the determination of the student to reach a chosen goal can carry him cross-grain in chemistry to learn many things. "Mr. Silver" read where a 25-75% silver-iron alloy could be made by the simultaneous aluminothermic reduction of the corresponding oxides of the two metals. This seemed simple enough until he discovered we had neither silver oxide nor iron oxide. Our source of silver was silver nitrate and our sources of iron were metallic iron and ferrous sulfate. He had recently reviewed nitrates and knew they would all decompose when heated, and all but the nitrates of the alkali metals would give the oxide. The silver oxide would be easy; but, would the ferrous sulfate decompose when heated? Some sulfates do, but they give the oxides? He could, of course, make iron nitrate and decompose that—provided nitric acid did not render iron passive as it does some metals. After studying the chemistry of iron and properties of sulfates for some time he chose the one-step method of heating the sulfate. (As a by-product of his reading he learned that the distillation of the hydrated ferrous sulfate was the first method for making sulfuric acid.) His reading done and his plans made, the next thing was to calculate the amount of hydrated ferrous sulfate needed. I told him to base all his calculations on three grams of ferric oxide. From this three grams of ferric oxide he figured both ways to determine the amounts of hydrated sulfate, silver nitrate, and aluminum needed. Some tedious figuring was necessary before he could even start his reactions, but he did not mind that, or the reading, or the careful weighing, because he knew why he was doing it. It was the vision of a little gray lump of metal that led him criss-cross through all sorts of by-ways of chemical knowledge.

And then there was little "Miss Barium" who had been so nervous and afraid of chemistry 101 that she had begged to drop the course, but in 102 she became so fascinated with the chemistry of barium that she was afraid of nothing. One day she asked for permission to try for metallic barium. When I asked her how she planned to do it, she rattled with confidence:

"By the electrolysis of barium chloride using mercury as a cathode. Then you distill off the mercury."

I doubted that she would get very far, but I told her she could try. In a few minutes she was back. "There isn't any barium chloride." Her eyes questioned me for a suggestion.

"I know," I laughed.

She grinned back at me. "I'll have to make it, won't I?"

"That's right," I said.

Soon she was back again. "It says here," she indicated a paragraph in "Mellor's", "that the chloride is made from barium oxide. But we don't have the oxide, only the peroxide . . ."

The look on my face answered that one. She spent half a period trying to adjust the heat so as to get the oxide without its reverting to the peroxide before she discovered that she could use the peroxide for making the chloride. She prepared her chloride, did a well-planned, successful electrolysis, and obtained her barium dissolved in the mercury cathode. During the distillation of the mercury she learned much about its properties and how to handle it safely. She did get her barium, a little round ball of metal left from the distillation, but it oxidized too rapidly to recover as a sample. Even though this project did not result in a sample that could be posted, it was far from a failure.

These are just two examples of the many experiments done by these students. Before the term ended all their activities had woven for them a net-work of chemical knowledge covering far more territory than that of the conventional laboratory manual.

This laboratory method is so unusual for the undergraduate that is raises a number of questions.

First, do the students actually cover the material and experience the techniques and disciplines presented by the accepted laboratory course? One doubting evaluator asked me to make a check-list to determine if this is so. I checked the material covered by my research method against that covered for chemistry 102 in three laboratory manuals which are used extensively in other colleges—Sorum's (5), Kanda and Burtt's (6), and Frantz and Malm's (7)—and I found it did not differ from these any more than the four differed from each other, except that most of my students go beyond the scope of these manuals, excellent though they are.

Second, with the students choosing their own activities how can I be sure that the experience of *each* will be broad enough? This is done by a little gentle steering. True, they are not dragged through the course by the force of my will, but neither are they left to flounder. The teacher is a partner in this "research program."

Third, does not this method require some kind of super-teacher, someone with a special gift of imagination, enthusiasm, and skill in the art of teaching? And, does it not require someone with a vast reservoir of chemical knowledge? With the first set of qualifications I agree. It does require such a person, but *any* chemistry course requires these in the teacher. As for the super-knowledge of chemistry, anyone who is qu
teach college chemistry by a
method is qualified to do it b

search approach. The teacher is not supposed to know all the answers; the best processes to use, the "whys" for all unpredicted results, the identities of surprising products. No one person could. When I am stumped, as I often am, I set the student to digging deeper into reference material, then, after all the evidence is in from experiment and reading, we draw our conclusions together. Some of the best learning situations we have ever had in my classes developed from my own ignorance. There is nothing that stimulates learning quite so much as beating the teacher to the answer.

Fourth, does this method prepare the student for advanced chemistry courses as well as the conventional method? Emphatically, yes! He is thoroughly at home in the laboratory. He can plan experiments, judge quantities, calculate amounts, devise methods, and improvise equipment; and the whole library is his text book. He has learned an independent way of study, the self-disciplined way of the researcher. He thinks as a scientist thinks; from vision, to plan, to procedure, to conclusion and proof. He is prepared to take the next step up with confidence and poise.

This is not a magic process that turns every student into a serious scientist. I am claiming only that I have found the results from this method far better than those from the "cook-book" laboratory manual method. I still have failures. Also I still have a few of the lazy, the vague, the flighty—and those who would be *bored* at a Cape Kennedy countdown. But even these usually get caught up in the excitement of some project before the course is finished, and surprise themselves by learning a little chemistry.

In evaluating this method, or any other, teachers must ask themselves the following questions. Which is more important: being able to answer the questions, by fair means or foul, in a laboratory manual, or being able to prepare a desired compound from almost any given source? Which is more important: dutifully performing a list of prescribed experiments in order to pass a course, or discovering latent talents and acquiring a deep love and appreciation for chemistry. Which is more important: learning *about* the laws of nature, or learning how to use these laws to bring about a desired result?

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SOME TEMPERATURE-VISCOSITY RELATIONSHIPS FOR CERTAIN ESTERS OF NAPHTHENIC ACID. IV. CHARACTERISTICS OF HIGHLY FRACTIONATED ETHYL NAPHTHENATE

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INTRODUCTION

It has been demonstrated that certain esters of naphthenic acid have unique temperature-viscosity relationships in comparison with dioctyl sebacate, whose temperature-viscosity relationships are well known (Brennan, 1961). More specifically, Appleton *et al.* (1962) have shown that the ethyl and n-propyl esters have superior temperature-viscosity relationships to the other naphthenic esters. (By "superior" is meant that the curves exhibit a small rate of change of viscosity with temperature. "Inferior" refers to those curves which show a marked or rapid increase of viscosity for some temperature change.) This publication revealed the heterogeneity of the low-boiling fraction of naphthenic acid and its esters by the use of gas liquid partition chromatography, as did the later work of Burti *et al.* (1963).

The present paper is an outline of further work using a more discrete cut of naphthenic acid and a finer fraction of its ethyl ester.

OBJECT AND PLAN OF INVESTIGATION

The object of this investigation was to obtain a relatively more homogeneous ethyl ester of naphthenic acid and to determine the temperature-viscosity relationships of this fractionated mixture. This was accomplished by fractionation techniques and improved methods of preparation. Gas liquid partition chromatography was utilized to demonstrate the degree of homogeneity involved. Also, infra-red spectra of the ester fractions were obtained for the purpose of identification and analysis.

METHODS

The initial phases of this investigation were concerned with the separation from the crude naphthenic acid of all components boiling below 250°C. The separation of this range of acid was accomplished at atmospheric pressure by means of a simple ground glass distillation apparatus. The distilled acid samples thus obtained were then fractionated at a reduced pressure of approximately 2 mm. Hg. The fractionating column employed in this operation was a 4-foot Podbielniak-type column, packed with 3/32 inch glass helices and rated at 14.7 theoretical plates (Weissberger, 1951). The fractionated acid is specified by the data listed in Table I.

The ethyl ester of the fractionated naphthenic acid was prepared by the method of Appleton *et al.* (1962) with one modification. It was observed that overheating of the acid chloride, in the process of removing excess thionyl chloride from the reflux mixture, resulted in polymerization of the acid chloride, as evidenced by a dark, tarry residue in the distillation vessel. Therefore, the major portion of the excess thionyl chloride was removed by the simple distillation method, but the last traces were driven off by a direct steam distillation from the two-phase system that exists following the esterification reaction and hydrolysis procedure.

The ethyl ester thus prepared was purified by successive washings with sodium bicarbonate and distilled water and was fractionated by means of the Podbielniak-type column described

Physical Character	Light Amber Liquid
Unsaponifiables	10% (maximum)
Acid Number	200-215
Iodine Number	10 maximum
Sulfur Test	negative
Low Temperature Stability	Stable at 40°F
Moisture (Karl Fisher)	0.3% (maximum)
Boiling Range (°C)	30-120°C (at 2.5 mm Hg.)

Table I: Specifications of Naphethenic Acid

above. The two fractions collected, designated as "A" and "B", respectively, were then washed and dried over anhydrous calcium chloride. The ester fractions are characterized by the data of table II.

Table II

	A	B
Acid Number	1.43	2.00
Saponification Number	191.1	188.1
Refractive Index	1.4479	1.4513
Boiling Range	56-69°C*	69-80°C*

* at 2mm Hg.

Table II: Characteristics of Ethyl Naphethenate Fractions Investigated

All viscosity measurements were made with a Brookfield Model LVF Synchrolectric viscometer, following the procedure of Appleton *et al.* (1962).

The gas chromatographic data presented herein were obtained with a Fisher-Gulf Model 300 Partitioner, fitted with a 7½-foot column packed with Johns Manville "Celite" and employing a liquid phase of Dow Corning silicone grease in the ratio of 1 gram of grease to 5 grams of "Celite."

RESULTS AND DISCUSSION

Infra-red Spectroscopy:

The infra-red spectrograms of the fractionated acid (Figure 1) and of the fractionated ester (Figure 2) were analyzed in order to obtain further structural information and characteristic data on these compounds. No functional groups other than those that would be anticipated for ordinary carboxylic acids and esters were found to be present. The spectra of the two ester fractions were found to be identical, the implication being that the esters differ only in length of side chains.

With regard to the acid spectrum, C-H stretching appears at 3.4 to 3.5 microns and C-H bending at 6.9 and 7.3 microns, as would be expected. The ester spectrum reveals similar bands at the above-indicated wavelengths. Absorption at 5.8 to 5.9 microns in both the ester and the acid is probably due to C=O stretching. The remaining bands in the region of 7 to 9 microns may seemingly be ascribed to C-O stretching or O-H bending.

Data for the above interpretation were obtained from Silverstein and Bassler's *Spectrometric Identification of Organic Compounds* (1963).

Gas Liquid Partition Chromatography:

The gas chromatograms illustrated in Figures 3 and 4 indicate the degree of homogeneity of the ester fractions investigated.

The "A" and the "B" fractions were each partially resolved into 4 peaks. These may be compared with the low-boiling ester fraction investigated by Burti *et al.* (1963), which was partially resolved into 8 components. These data would seem to illustrate the greater homogeneity of the finely fractionated sample utilized in the present investigation.

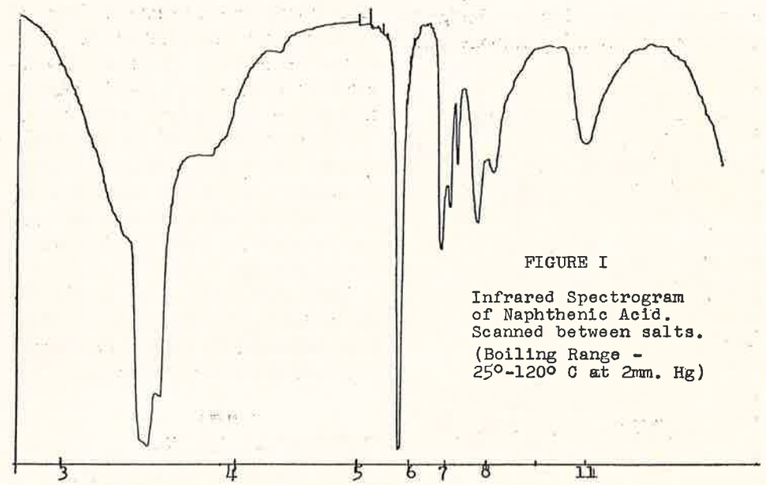

FIGURE I

Infrared Spectrogram of Naphthenic Acid. Scanned between salts. (Boiling Range - 25°-120° C at 2mm. Hg)

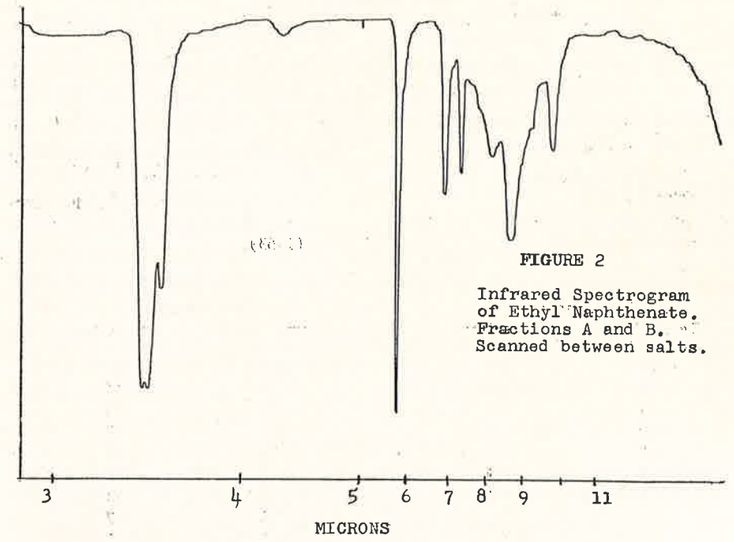

FIGURE 2

Infrared Spectrogram of Ethyl Naphthenate. Fractions A and B. Scanned between salts.

Fig. 1. Infrared Spectrgram of Naphthenic Acid.
Fig. 2. Infrared Spectrogram of Ethyl Naphthenate Fractions "A" and "B".

Temperature-Viscosity Data:

The temperature-viscosity data for the ester fractions investigated are listed in Table III and are illustrated in Figure 6. Temperature is in degrees Centigrade and viscosity is given in centipoise units.

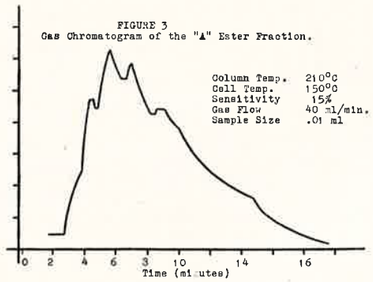

FIGURE 3
Gas Chromatogram of the "A" Ester Fraction.

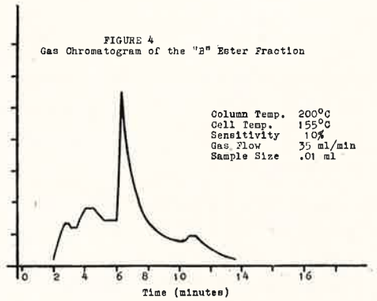

FIGURE 4
Gas Chromatogram of the "B" Ester Fraction

Fig. 3. Gas Chromatogram of the "A" Ester Fraction.

Fig. 4. Gas Chromatogram of the "B" Ester Fraction.

It is evident from the graphs of Figure 6 that the ethyl naphthenate fractions under present investigation are characterized by temperature-viscosity curves inferior with respect to rate of change of viscosity with temperature to those curves representing fractions investigated by Burti, *et al.* (1963). As the temperature approaches approximately -60°C., the viscosity of the finely fractionated ester begins to increase relatively rapidly. It may be noted that the curves of the fractionated ester are, however, superior to those of the control, dioctyl sebacate.

These data would seem to indicate that as the number of components of the ester decreases, its temperature-viscosity relationships become less favorable.

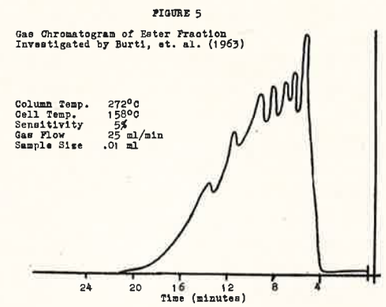

FIGURE 5
Gas Chromatogram of Ester Fraction Investigated by Burti, et. al. (1963)

Fig. 5. Gas Chromatogrgam of Ester Fraction Investigated by Burti, et al. **(1963).**

"A" Fraction		"B" Fraction	
Temp. (°C.)	Visc. (cps.)	Temp. (°C.)	Visc. (cps.)
—60	239	—60	244
—50	102	—53	144
—39	39	—42	90
—30.5	31	—37	68
—19	13	—28	49
— 7	8	—18	22
0	5	—13.5	13
		0	7
		+3	5

Table III. Temperature-Viscosity Data For Ethyl Ester Fractions

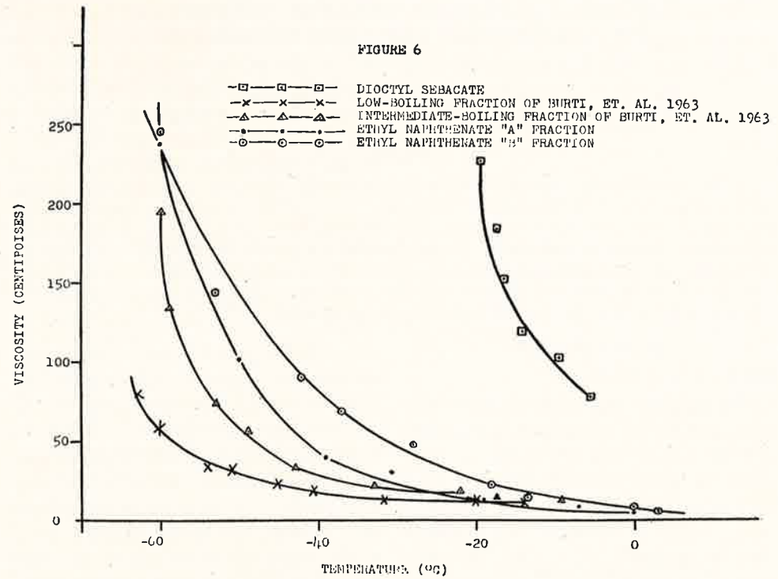

FIGURE 6

Fig. 6. Temperature-Viscosity Relationships of the "A" and "B" Ester Fractions, the Esters of Burti, et al. (1963), and of Dioctyl Sebacate.

SUMMARY AND CONCLUSIONS

1. The ethyl ester of naphthenic acid was prepared from a fractionated sample of the acid and the ester thus obtained was itself fractionally distilled through a 14.7 theoretical plate Podbielniak-type column.

2. The ester was divided into two fractions of boiling ranges 56 to 69°C. and 69 to 80°C., respectively.

3. Infra-red spectroscopy was utilized to further characterize the fractionated naphthenic acid and the ethyl naphthenate.

4. Determination of the low temperature-viscosity relationships of the finely fractionated ester indicates that relatively homogeneous ethyl naphthenate fractions exhibit temperature-viscosity relationships inferior to less discretely fractionated samples of the ester in that the rate of change of viscosity with temperature is greater in the more homogeneous samples.

ACKNOWLEDGMENT

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DYNAMIC ANALYSIS OF METALLIC DEFORMATION BY TRANSMISSION ELECTRON MICROSCOPY*

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ABSTRACT

This paper describes techniques which enable thin metal foils to be deformed directly, and immediately observed in the electron microscope. The advantages of these dynamic testing methods are discussed, and typical observations of deformation as a result of fatigue, impact, and contact stressing are presented. Some consideration is also given to the theoretical interpretation of the imaging of defects by transmission electron microscopy. Selected area electron diffraction techniques are also employed to investigate the mechanism of contact stress fracture, which is directly observed in the electron microscope.

INTRODUCTION

Historical Review

The electron microscope is essentially quite similar to an optical microscope. The basic difference lies in the fact that while the light beam is focused by glass lens reflection and refraction, the electron beam is focused by magnetic lens interactions. And, since the electron beam is composed of charged particles of finite mass, the electron beam also possesses energy or penetration power. This penetration ability thus allows the direct imaging of metallic crystals by transmission of a beam of electrons of sufficient energy through a foil, the thickness of which may vary from several hundred to several thousand angstrom units. Then too, since the electron beam has a very short wavelength, the resolving capability of the electron microscope is far in excess of the light microscope.

About 1948, Heidenreich (1) was successful in preparing a thin metal section from polycrystalline aluminum by electrolytically polishing a hole in the metal sheet. He found that the edges around the hole were transparent to the electron beam, and so the first transmission pictures of a metal crystal were obtained. It was later proven by Hirsch, et al (2) and Whelan and Hirsch (3), that metallic defects could also be observed and studied by the method of transmission-electron microscopy.

Essentially, two types of defects have been observed by electron transmission; namely, dislocations and stacking faults. The method of imaging these defects may be described, in the simplest form, as electron diffraction from the distorted area, or neighboring lattice points effected by the distortion.

The imaging of defects by electron diffraction is probably best described, both from a physical or analytical point of view, by the dynamical treatment developed originally by Howie and Whelan (4) through the application of the classical Schrodinger equation to the electron diffraction images observed. A working diagram for this concept is shown in Fig. 1. Here we imagine a thin metal

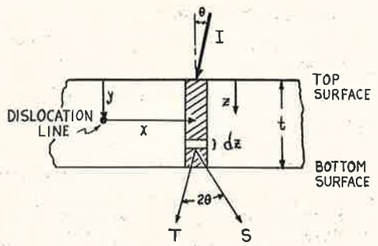

Fig. 1. Schematic representation of defected lattice section imaged by electron diffraction phenomenon.

* This research was supported in part by the Air Force Office of Scientific Research under Contract No. AF-AFOSR-165-63.

foil section which contains a defect (a dislocation in this particular instance). The basic equations descriptive of the transmitted (T) and diffracted (S) components of the incident electron beam (I) as a function of foil penetration are given by the following differential equations:

$$\frac{dS}{dz} = \pi i\left(\frac{1}{\xi_g} + \frac{i}{\xi_g'}\right)T \cdot e^{-2\pi i\left(s_g z + \bar{g}\cdot\bar{R}\right)} \quad (1)$$

$$\frac{dT}{dz} = \pi i\left(\frac{1}{\xi_g} + \frac{i}{\xi_g'}\right)S \cdot e^{2\pi i\left(s_g z + \bar{g}\cdot\bar{R}\right)} \quad (2)$$

where ξ_g and ξ_g' are constants which depend upon the atomic scattering factor (see any classical text on x-ray diffraction), $\bar{R}$ is the displacement of an atom in the shaded region of Fig. 1 caused by the dislocation present in the lattice, $\bar{g}$ is the reciprocal lattice vector, and s_g is the distance of the reciprocal lattice point (g) from the reflecting plane of the diffracted beam.

Actually, ξ_g and ξ_g' have the dimensions of length, and ξ_g is commonly referred to as the foil (image) extinction distance. This extinction phenomenon is similar to the familiar Newton rings observed optically on oil films and other striated layers. The extinction distance is then the distance between successive ring fringes or striae. ξ_g' is then simply a factor which accounts for the electrons absorbed by scattering and similar interactions within the foil as the beam traverses from the top to the bottom surface. It has been found experimentally that if

$$\xi_g/\xi_g' = (.075 \to .10)$$

then eqs. (1) and (2) are quite descriptive of the image contrast observed as a result of lattice imperfection.

As a simple example of what actually happens as the electron beam passes through a metal foil, we can consider the case where the dot product, $(\bar{g}.\bar{R})$ is zero; and where the absorption becomes quite small so that ξ_g' approaches infinity. Thus, eqs. (1) and (2) become

$$\frac{dS}{dz} = \frac{\pi i}{\xi_g} \cdot T e^{-2\pi i s_g z} \quad (3)$$

$$\frac{dT}{dz} = \frac{\pi i}{\xi_g} \cdot S e^{2\pi i s_g z} \quad (4)$$

Now if we differentiate eq. (3) with respect to the variable (z), we obtain

$$\frac{d^2S}{dz^2} = \frac{\pi i}{\xi_g} \cdot \frac{dT}{dz} e^{-2\pi i s_g z} + \frac{\pi i}{\xi_g} \cdot T(-2\pi i s_g) e^{-2\pi i s_g z} \quad (5)$$

and substituting eqs. (3) and (4) in eq. (5) there results

$$\frac{d^2S}{dz^2} = -\frac{\pi^2}{\xi_g} S - 2\pi i s_g \frac{dS}{dz} \quad (6)$$

which, written in operator form,

$$\left[D^2 + (2\pi i s_g)D + \left(\frac{\pi}{\xi_g}\right)^2\right]S = 0 \quad (7)$$

appears as a linear second-order differential equation with constant coefficients. The roots of the complementary solution are then

$$\rho_1 = -\pi i\left(s_g + \sqrt{s_g^2 + \frac{1}{\xi_g^2}}\right)$$

$$\rho_2 = -\pi i\left(s_g - \sqrt{s_g^2 + \frac{1}{\xi_g^2}}\right)$$

and if we let

$$\gamma = \sqrt{s_g^2 + \frac{1}{\xi_g^2}}$$

the complementary function becomes

$$S(z) = K_1 e^{-\pi i(s_g + \gamma)z} + K_2 e^{-\pi i(s_g - \gamma)z} \quad (8)$$

In evaluating the constants, K_1 and K_2, we can consider the boundary conditions associated with Fig. 1. That is, at the top surface of the foil we have

$$\{z = 0,\ T = 1,\ S = 0\}$$

since there is no diffraction at the top surface, and the transmitted wave at this point is essentially the incident wave.

Substituting these conditions into the preceding equations for T and S we find that

$$K_1 = -K_2 = -\left(\frac{1}{2\xi_g \gamma}\right)$$

Then substituting this solution into the complementary form of eq. (8), the diffraction amplitude (intensity) as a function of the foil penetration (z) is found to be

$$\left|S(z)\right|^2 = \frac{\sin^2(\pi\gamma)z}{\xi_g^2 \gamma^2} = \left(\frac{\pi}{\xi_g}\right)^2 \left[\frac{\sin(\pi\gamma)z}{(\pi\gamma)}\right]^2$$

It should be observed that eq. (9) represents a wave function, the amplitude of which oscillates with a sinusoidal periodicity. Simply stated, this then shows that the diffracted image will alternate in intensity from bright to dark as a function of the penetration dimension (z).

Similar contrast effects such as those predicted by eq. (9) are actually observed for stacking faults and dislocations, since each type of defect creates a distortion in the crystal lattice. In Fig. 2 is shown the basic concepts of the stacking faults encountered in metal foils, while Fig. 3 illustrates the complete dislocation consisting of an edge and screw component.

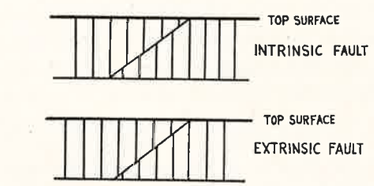

Fig. 2. Schematic representation of stacking faults in a crystal matrix.

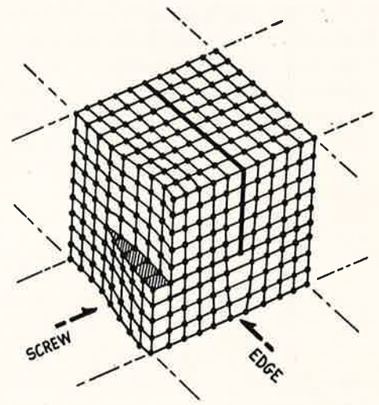

Fig. 3. Total dislocation in a crystal lattice composed of edge and screw components.

Both types of defects are created by a lattice disturbance, usually a deformation of some sort, or any mode of action upon the crystal which will create stresses sufficient to translate a matrix atom or groups of atoms with respect to the relatively undisturbed crystal matrix. Thus, following a brief review of the contemporary observations of defects by transmission electron microscope techniques, recent observations of defects created by various deformation modes acting directly on the thin foil specimens will be presented along with analyses and comparison of these results with this contemporary work dealing with bulk metal deformation.

Review of Literature and Statement of the Problem

Since the first examination of thin mental sections by Heidenreich (1) using the electron microscope, a new era of atomic physics has erupted. In less than ten years, the entire realm of dislocation theory in its basic form has been verified by the critical work of Hirsch, et al (2), Whelan and Hirsch (3), and countless

others. More recently, Hirsch, Partridge, and Segall (5), Whelan (6), and Howe, et al (7), have extended the basic knowledge and use of transmission electron microscope techniques to investigate such phenomena as simple tensile deformation in metals, fatigue of metals, and the interaction of dislocations during various simple deformation modes. These studies, for the most part, have been confined to the observation of deformation induced in the bulk metal specimens which have been reduced to foil thickness suitable for electron transmission (approx. 3000 A.U.) using the methods of Bollmann (8) and Tomlinson (9).

An obvious drawback in these techniques has been the inability to observe the succession of events which constitute or describe a mechanism of deformation. In this respect, the fatigue problem is of particular interest. At the time of writting of this paper (1964), the only reported attempts to actually observe the phenomenon of fatigue deformation directly by transmission electron microscopy have been those by Smith (10) and Murr and Wilkov (11). Preliminary investigations of other deformation phenomena by direct observation of the metal foil by transmission electron microscopy have also been performed by Murr (12) with some success.

There has developed a need, in the last few years, to investigate in detail the mechanisms of deformation in metals. This involves repeated observations of the same area, a feat only successfully accomplished for simple tensile stressing of thin foils directly within the electron microscope by Wilsdorf (13), Hirsch, et al (14), and very few others. Although the direct stressing of metal foils seems quite feasible at first glance, the accomplishment of such experimental work is impeded by the inability to produce good

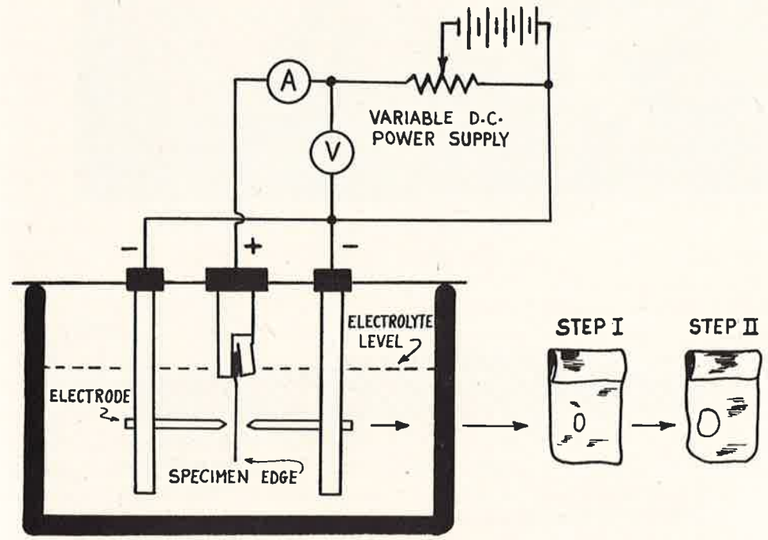

Fig. 4. Speciment preparation technique. The electrolyte in this process consists of 34% sulfuric acid, 42% phosphoric acid, 24% water. Electropolishing current is maintained at 9 amperes.

quality metal foils which are capable of distributing the applied stresses representatively throughout the structure; and the fact that deformations occurring in thin metal foils might be quite unrepresentative of the actual macroscopic deformations of particular interest to engineering and design personnel.

It is, therefore, the purpose of this paper to first show that simple deformation experiments involving such modes of deformation as impact, fatique, and contact stress, may be observed directly-dynamically by the direct deformation of these foils, and examination in the electron microscope immediately after or during deformation; and second, to show that there is strong evidence, in some cases, to suggest that thin foil deformation is representative of bulk metal behavior.

EXPERIMENTAL METHODS

The preparation of metal foil specimens was accomplished by electrolytically polishing type 304 rolled (.007 in.)-annealed stainless steel sheet. The method, basically a modification of the Bollmann (8) technique, is illustrated schematically in the diagram of Fig. 4. This technique consists in first polishing a hole in a ½ in wide strip of stainless steel sheet, and then to withdraw the electrodes and shift the specimen so that one edge of the hole and the nearest specimen edge polish towards one another. When the closest distance from edge to edge is approximately 1 mm, the areas all along these edges a distance sometimes of more than 100 microns, are electron transparent. Fig. 5 shows a typical test specimen prior to deformation testing. The schematic section in Fig. 5 also illustrates the foil thickness variation across the necked region produced by the electrolytic polishing.

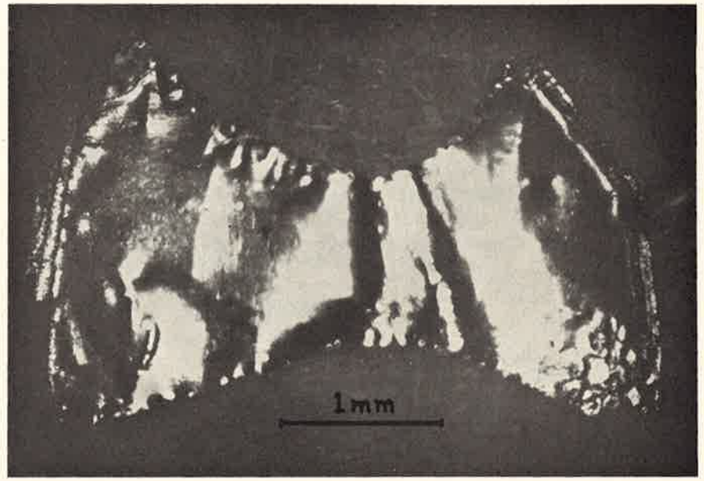

Fig. 5. Optical micrograph of a typical stainless steel test specimen. Transverse cross-section of necked section is shown in schematic form to illustrate specimen-edge thickness variations.

Fatigue experiments were performed on the Hitachi H.U. 11 electron microscope using specially designed cycling attachments previously described in the literature by Murr and Wilkov (11). In these experiments, the specimens were glued to the standard Hitachi type HD-1 tensile deformation specimen holder and fatigued at very low cycle rates (12.5 cpm). Repeated observations were then made of the same area at various intervals in order to detect structural changes representative of, or caused by the fatigue stressing. Observations in the Hitachi electron microscope were made at an operating voltage of 75 kilovolts. The strain amplitude was adjusted for approximately 20 microns.

Direct deformation of the thin foil stainless steel specimens was also performed using the modes illustrated in the diagrams of Fig. 6. Fig. 6(a) illustrates, essentially, an impact or high pressure type deformation; while Fig. 6(b) is designed to produce rolling stress deformation of the metal foil. It should be pointed out that each foil used in the deformation studies was carefully checked in the electron microscope before testing to be sure that the material was relatively free of imperfections.

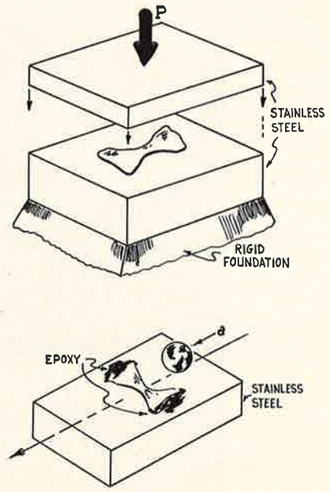

Fig. 6. Direct deformation of thin metal foils. (a) Impact-shock pressure testing technique, (b) Rolling contact stress technique.

Impact and compression tests of these thin foil specimens were performed by sandwiching the thin foils between two polished stainless steel blocks. The lower block was maintained rigid while the upper block was struck with a hammer. The deformation stresses cannot, in this case, be accurately measured, however; the stresses have been approximated to be of the order of several thousand bar during a time interval of application of about 10^{-4} second or less.

Observations of impact and contact stress tested foils were performed in the Jem 6A electron microscope operating at 100 kilovolts. Because of the stability and low specimen contamination rate of the Jem electron microscope, long and

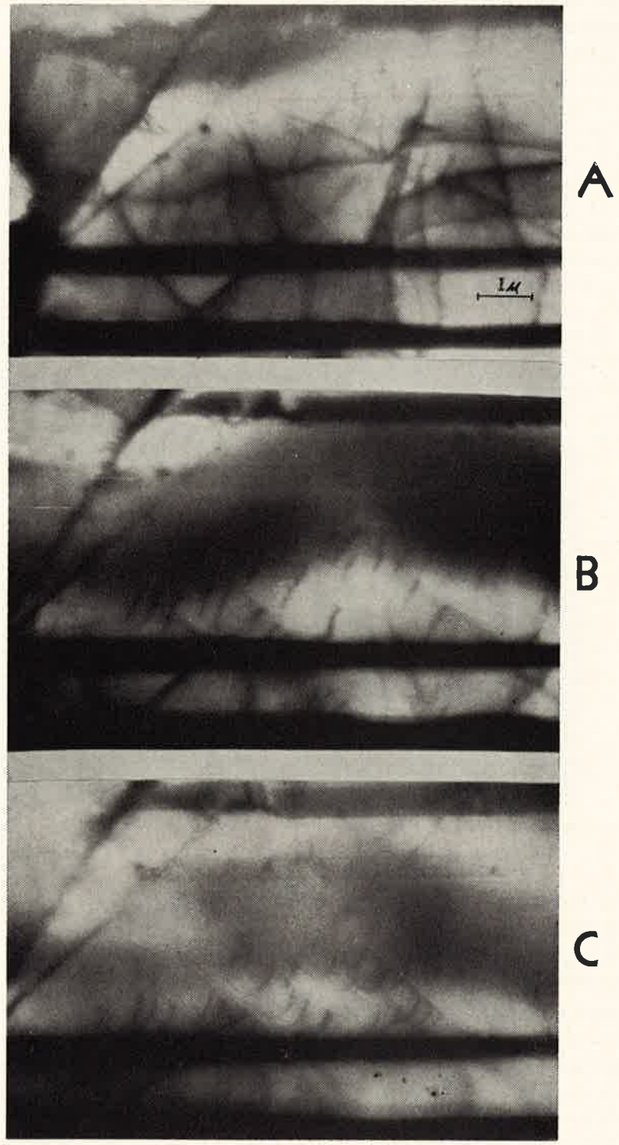

Fig. 7. Fatigue sequence in stainless steel showing (a) unstressed area (b) same area after 1400 cycles (c) same area after 2500 cycles.

detailed studies of all transparent foil areas could be accomplished with continued good resolution.

RESULTS AND DISCUSSION

The results of low cycle fatigue of 304 stainless foils in the Hitachi electron microscope were far from conclusive when considering an actual fatigue mechanism or characteristic dislocation phenomenon. Actually, at such low cycle rates it is difficult to determine whether or not a fatigue phenomenon is involved at all. Figure 7 illustrates a sequence showing dislocation motion after several thousand stress cycles. It is difficult to state, with absolute certainty, that the motion observed is representative of any fatigue activation; or whether the motion has resulted from residual unidirectional stresses.

Several features concerning dislocations generally can, however, be pointed out with reference to the sequence shown in Fig. 7. The fact that the dislocations have bowed during movement allows the direct calculation of the normal stress in the slip plane (111) by considering the situation shown in the diagrams of Fig. 8. With reference to Fig. 7, the projected radius of curvature (R′) of the dislocations after 2500 cycles of fatigue may be measured directly. Then since the (112) plane is parallel to the photographic surface in this case, the conditions expressed in Fig. 8 are satisfied by considering

$$R \cong \frac{R'}{\cos\theta} \qquad (10)$$

and since the normal stress is given by

$$\sigma_N = \left(\frac{G}{2R}\right)\bar{b} \qquad (11)$$

where G is the shear modulus, and $\bar{b}$ is the magnitude of the Burgers vector, then from eq. (10);

$$\sigma_N \cong \left(\frac{G\cos\theta}{2R'}\right)\bar{b} \qquad (12)$$

Now the angle, $\ominus$, between (112) and (111) is ~ 60°, therefore, eq. (12) may be written simply as

$$\sigma_N \cong \left(\frac{G}{4R'}\right)\bar{b} \qquad (13)$$

The direct measurement of R′ from Fig. 7 results in an average radius of 1000 A.U., and since G for 304 stainless steel is 12 x 10^3 psi (Marin and Sauer (15)), the normal stress becomes

$$\sigma_N \cong 3000\,\bar{b} \qquad (14)$$

where $\bar{b}$ may be any value less than or equal to 3.56 A.U. (the inter-atomic spacing for stainless steel). Thus, the maximum normal stress will be of the order of 10,500 psi or approximately 8 kg/mm². This latter value is approximately 40% smaller than calculations made by Menter and Pashley (16) for gold, and about 4 times greater than similar calculations made by Wilsdorf (13) for the maximum stress applied to the thin specimens to initiate dislocation motion. It will be observed that stresses of this order are sufficient to fatigue bulk stainless steel specimens. Thus, from a stress point of view, it might appear as

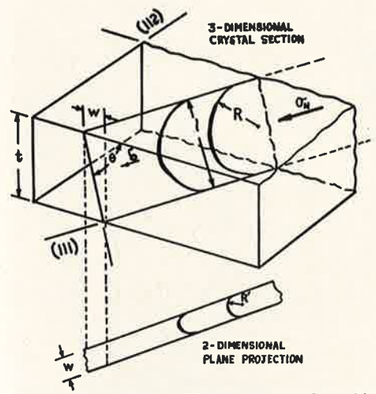

Fig. 8. Crystallographic geometry for thin foil thickness measurements and determination of normal stresses.

though the thin foils can be representative of bulk metal specimens in exhibiting the deformation phenomena desired.

Another simple, yet interesting property which may be directly determined from the electron micrographs of Fig. 7 is the foil thickness. By utilizing the geometrical characteristics shown in Fig. 8, it will be observed that

$$\tan\theta = \left(\frac{t}{w}\right) \qquad (15)$$

and since w is the direct projection of the slip plane (111) on the micrograph of Fig. 7, the thickness becomes

$$t = 1.73\, w \qquad (16)$$

where w is of the order of 1700 A.U. by direct measurement of the electron micrographs. Thus, the foil thickness from eq. (16) is approximately 3000 A.U. in the area under observation.

Figure 9 shows an electron micrograph typical of unstressed stainless steel foils prior to impact and compressive deformation tests. Dislocations observed eminating from the grain boundary may have been activated by stresses induced in handling of the foil prior to observation in the electron microscope. In all observations in thin foils prepared from the stainless steel sheet described previously, the surface is either a (110) or a (112) surface.

Following compressive stressing, many foil areas were dislocated as illustrated in Fig. 10. In this instance, dislocations have been generated by a grain boundary source, have move across the grain, and have piled-up against the opposite grain boundary. Such dislocation pile-ups are typical of unidirectional stressing, and have supposedly resulted from similar stress conditions in the grain section shown. These simple dislocation arrays are not necessarily typical of compressive stressing. Murr (12) has previously shown that large compressive stress

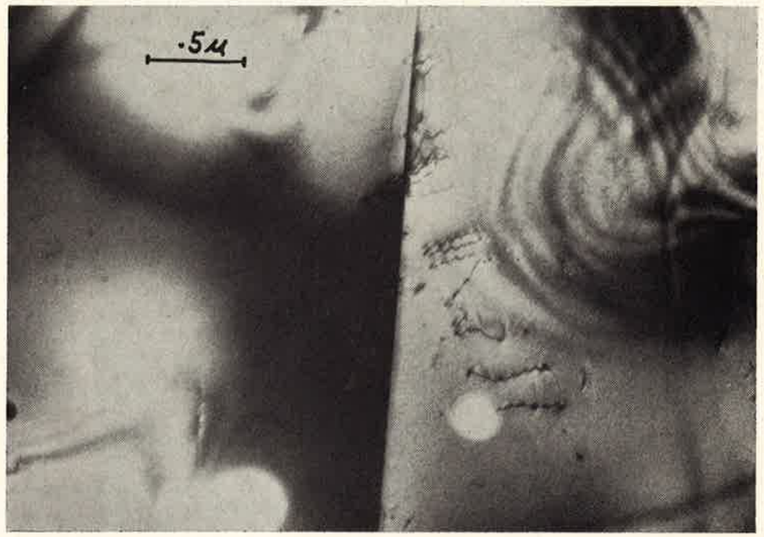

Fig. 9. Typical foil section electron micrograph of an unstressed stainless steel specimen showing dislocations eminating from a grain boundary.

deformation of thin foil specimens produces regular dislocation arrays and a quasi-stable cell structure; presumably resulting from some energy equilibrating process.

Dislocation arrays resembling energy-stable configurations are nearly always observed under impact and high-load compressive stressing. Figure 11 illustrates this phenomenon in two grain areas separated from each other by approximately 50 microns or more. Observations of this impacted foil specimen showed this effect to be extremely com-

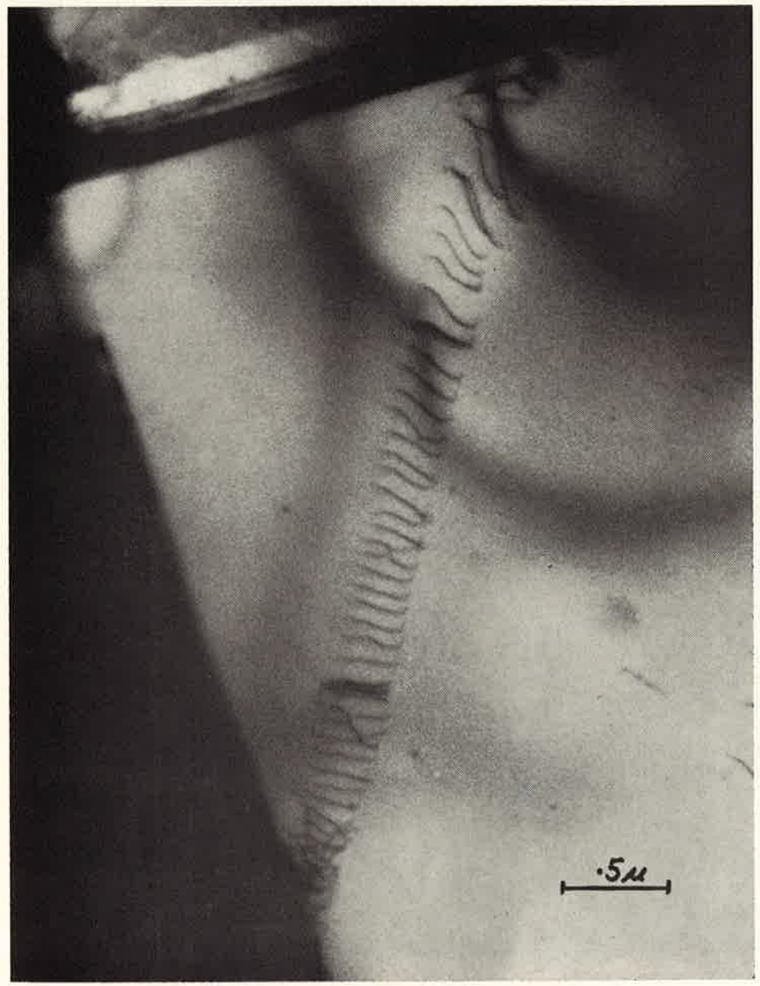

Fig. 10. Dislocation pile-up in compressive stressed stainless steel foil.

A

B

Fig. 11. Electron micrographs (a) and (b) showing typical dislocation structure in impact-shock tested thin foils.

mon. Another common example of impact stressing is shown in the electron micrograph of Fig. 12. Here is shown the random dislocation arrangement of the lattice caused by the large, complicated stress systems. Several stacking faults are shown, along with evidence of dislocations splitting into partials. Several areas indicative of dense dislocation cross-slip are also visible. The high re-

Fig. 12. Typical distortion in an impact tested foil in a low pressure grain section. Zig-zag contrast of dislocations is apparent as well as several well defined stacking faults. Note cross-slip in areas denoted by (cs), and splitting of dislocations into partials at p.

solution of this particular area in the electron microscope also shows clearly the zig-zag characteristics of the dislocations in the (111) slip-planes, and the contrast phenomena associated with stacking faults; all capable of explanation by properly solving the dynamic equations, (1) and (2).

It is interesting here to observe that the foil thickness in this area (Fig. 12) can be computed with great accuracy by observation of the stacking fault contrast. With reference to eq. (9) it will be observed that $\S_g$, the extinction distance, is the distance, from a maximum to a maximum in terms of the diffracted wave intensity. This quantity varies in different metals from a value of 200 A.U. to 600 A.U., and for stainless steel, the value is approximately 300 A.U. Therefore, the foil thickness is simply given by

$$t = N\S_g \qquad (17)$$

where N is simply the number of dark or white bands composing the stacking fault. In Fig. 12, N is observed to be approximately 10, and the thickness by eq. (17) is found to be about 3000 Angstrom units.

There is still more to be said about the dislocation structure observed in Fig. 11. In each example, a definite pattern has been formed by the dislocations. They appear to have originated in a twin boundary in similar (111) slip planes, and have oriented themselves in a linear fashion between adjacent slip planes. Most of the dislocations are straight, possibly indicative of the uniform-high stresses normal to the dislocations in the slip planes. Tangled and looped dislocations observed are probably the result of internal obstacles in the path of the moving dislocations.

From these observations it would appear that the impact or compressive

Fig. 13. Electron micrograph showing a compressed area in a stainless steel foil. Dislocations have been pinned at one surface, and have elongated in the slip planes under the action of the applied stress.

stress has the effect of producing a type of stable dislocation array as a result of their seeking a position in the lattice which constitutes the lowest energy assemblage in the stressed matrix. In similar high pressure tests on bulk nickel specimens, Nolder and Thomas (17) have observed the formation of dislocation cell structure resembling that produced when bulk metal specimens are

Fig. 14. Electron micrograph of a fracture trough in a stainless steel foil produced by rolling contact stresses. (rolling steel ball)

fatigued at high strain amplitudes as in the work of Feltner (18) and Grosskreutz (19). Since there has been no reported work on the direct fatigue of, or pressure deformation of thin metal foils at the time of writing this paper (1964), it is rather impossible to interpret these results in this respect. However, it may be possible that the stable dislocation arrays observed in this research are a form of the previously observed cell structure in the thin specimens prepared from the bulk deformed metal.

It should be pointed out that in the case of the impact and pressure studies presented, the results shown have been repeatedly observed in a number of experiments. The conclusion here is then that the results obtained by direct impact and high pressure deformation of the thin foil specimens are representative of the deformation phenomena observed.

One other interesting point not to be overlooked in the observation of pressure and impact deformation of these thin foil specimens is the phenomenon shown in the electron micrograph of Fig. 13. Here the pressure acting normal to the surfaces has caused one segment of the dislocation to be pinned, while the other segment is moved under the action of the resolved stresses acting normal in the slip planes. The result is an elongated dislocation. Stacking fault striae observed on a portion of many of these dislocations have resulted from the splitting of the dislocation into partials at one surface of the foil. Actually, the approximate depth of the partial may be computed by counting the number of fringes, and substituting this number for N in eq. (17). Such dislocation pinning and elongation has been previously observed by Murr (12) when thin stainless steel foils were subjected to very high compressive stresses.

Contact stresses produced by rolling a steel ball over a stainless steel foil as shown in Fig. 6 were sufficient, in several experiments, to cause a fracture trough to form in the path of the rolling surface in the thinner (edge) portions of the foils. Figure 14 shows a typical segment of a fracture trough created as described. The mode of fracture appears to be a translation slip and cross-slip phenomenon with a possibility of twin deformation as well. Twinning, however, does ot appear to be definitely indicated as shown by the superimposed electron diffraction pattern of Fig. 15.

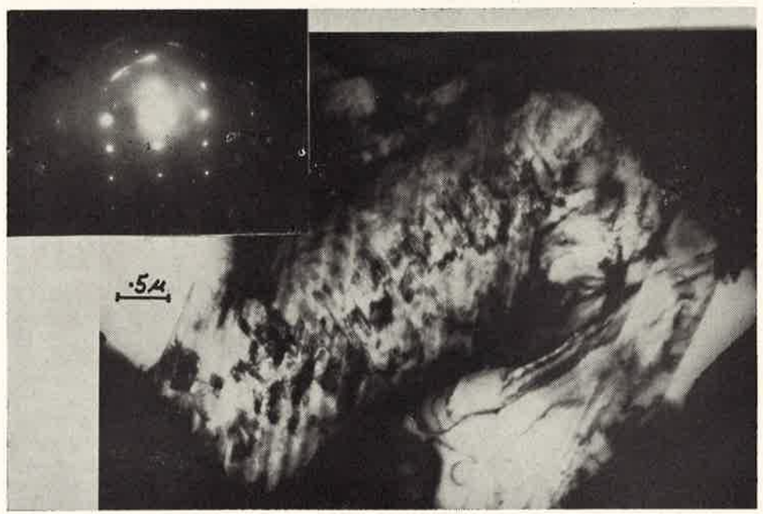

Fig. 15. Electron microgrgaph and superimposed selected area electron diffraction pattern of a typical fracture trough in a stainless steel foil.

Several examples of simple (111) faulting were observed in the thin areas near the fracture troughs in several foils investigated. Figure 16 shows a typical faulted area with a selected area electron diffraction pattern superimposed. The faulting shown in Fig. 16 is actually a double fault system, with the faulting occurring on adjacent (111) slip planes. The foil surface is a (112) surface, and

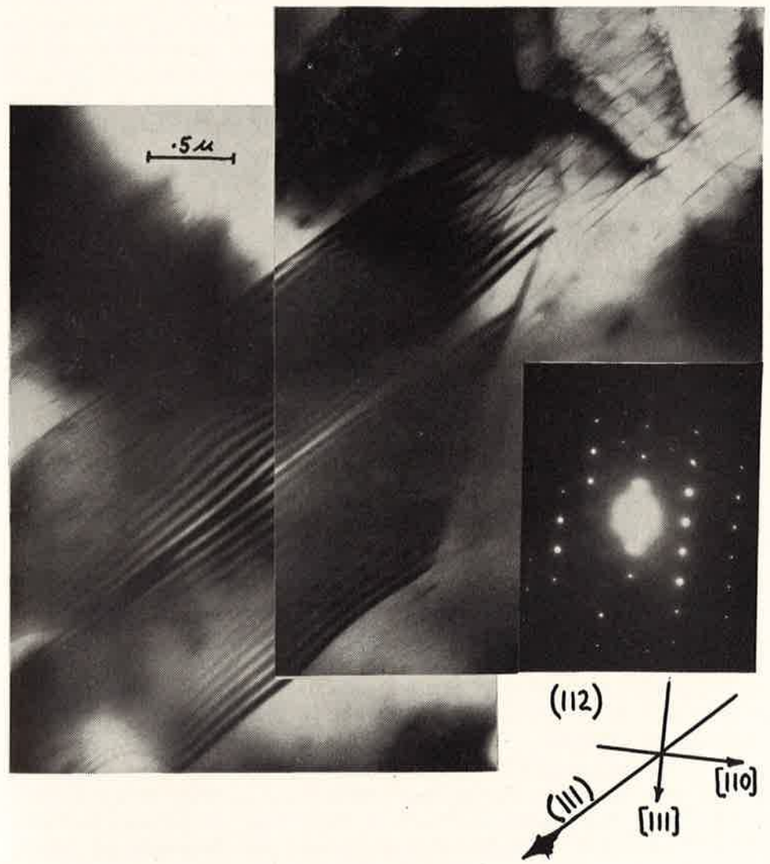

Fig. 16. Large stacking faults in a thin foil section several microns from a contact stress fracture trough. Note dislocation network at upper fault.

the slip planes are inclined approximately 19.5° to this surface. This geometry thus accounts for the very large extinction effects observed in the electron micrograph. A dislocation network of a sort is also visible at the upper extension of one fault in Fig. 16. The foil thickness here is approximately 3500 Angstrom units by application of eq. (17).

The apparent slip-type fracture observed in rolling stress experiments is interesting since similar fracture deformation has also been observed in nickel foils thinned from bulk specimens subjected to pressures of 350 to 700 kilobars (1 kilobar equals (986) atmospheres), by Nolder and Thomas (17). The deformation mode in the nickel was, however, definitely established as twinning.

Rolling contact stressing of thin foils is also of interest since the mode of deformation involves extremely high local compressive stresses at the base of the trough. Thus, as the steel ball is accelerated across a foil, the stress mode is somewhat analogous to high pressure shock phenomena.

CONCLUSIONS

Several interesting conclusions can be drawn from the results and techniques presented in this paper:

(1) Certainly the experimental advantage in the dynamic testing of thin metal foils directly has been clearly demonstrated in this paper. The methods introduced illustrate the potential for solving the problem of deformation physics in terms of a mode mechanism which can be directly observed.

(2) In the case of impact and compressive stress studies on thin stainless steel foils, the same results have been repeatedly observed. This reproducibility further attests to the validity and advantages of the direct thin foil testing and observation by transmission electron microscopy.

(3) The results of impact tested metal foils tend to show that stable dislocation arrays are formed, possibly as a result of some minimum energy phenomena.

(4) Thin foil deformation and fracture caused by rolling contact stresses have been observed to result from a slip, cross-slip mechanism which is similar to the twinning observed for shock loaded nickel in the bulk form.

(5) When considering all of the thin foil deformation phenomena it may be said that while the results do differ in some instances from those obtained after testing of bulk metal specimens, the basic characteristics from a mechanistic point of view are quite similar. Thus, if one also considers the proven reliability of thin foil testing (as in (2) above), it must be concluded that the results observed by the direct testing of metal foil specimens are closely associated with the stress motivated deformation mechanisms observed for each deformation mode.

(6) It must be pointed out, however, that while the experimental results have been representative of deformation resulting from simple impact, compressive, shock and contact stresses; tensile type deformation including the fatigue mode employed in the experimental work discussed in the text have not been successfully observed. Thus, acceptance of the previous conclusions with respect to fatigue observations must be reserved until further, more conclusive experimental evidence has been obtained.

(7) While testing and observation of bulk metal specimens may represent or present some distortions created by or as a direct result of the electro-thinning technique and the consequent inducement of foreign defect structures into the same through handling, the method introduced and successfully employed in this paper can, with some care and planning, give an exact account of the deformation phenomenon desired.

ACKNOWLEDGMENTS

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QUINONE TOXINS AND ALLIED SYNTHETICS IN CARCINOGENESIS

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INTRODUCTION

Tumorigenic properties have been most recently discovered in secretions of common flour beetles, *Tribolium* spp., and in metabolic products from the rice mold *Penicillium islandicum* (Ladisch, 1963; Davidson, 1963). These naturally occurring food contaminants are quinones. Reports scattered throughout the literature show that very similar synthetic quinones are carcinogenic. This fact has received very little attention. In view of the discovery of the quinoid food toxins, cancer caused by quinones obviously becomes of distinct interest. The present paper deals therefore with the occurrence of such agents, and with the significance of quinone carcinogenesis.

SCOPE OF QUINONE CARCINOGENESIS

The substances discussed in this report are organic aromatic compounds. Their most outstanding characteristic is the ease with which they are being oxidized and reduced. For example, oxidation transforms phenol to hydroquinone. The latter is readily oxidized to quinone. Sunlight reduces quinone to trihydroxybenzene. Analogous conversions are very common in the chemistry of living beings. The pharmacological properties of phenols resemble those of quinones; both give rise to hydroquinone: quinone systems during animal metabolism. Redox systems of this nature in the environment of man deserve consideration in relation to quinone carcinogenesis.

QUINOID CARCINOGENS AND PROMOTERS

Table I shows several carcinogenic and/or tumor-promoting chemicals of the class referred to. Bracketed numerals in the text refer to the compounds listed in the table.

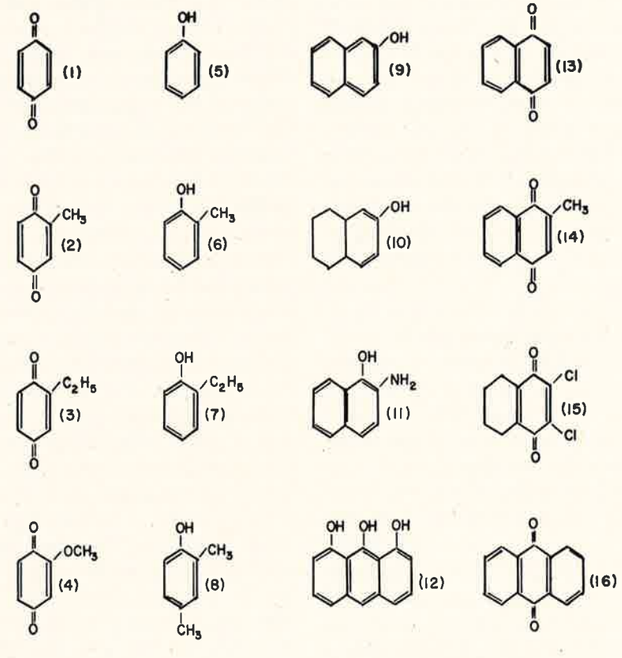
QUINONE (1), a coal-tar derivative used in the manufacture of dyes and for tanning hides, produces skin- and lung-cancers in mice (Takizawa, 1940). Hueper (1936, p. 580) has drawn attention to the systemic effects of this carcinogen.

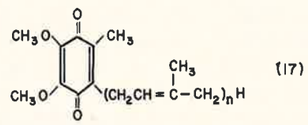
TOLUQUINONE (2) is a commercial product, obtained by the oxidation of toluidine. The latter causes bladder cancer in "aniline" workers. It provokes liver damage, also lympho- and myelosarcomatosis in mice (Dittmar, 1942).

GRAIN AND FLOUR BEETLES. Glandular secretions consisting of the quinones (2) and (3) have been identified in *Tribolium confusum, T. castaneum, T. destructor,* and *Latheticus oryzae* (Locontiand Roth, 1953). These beetles infest grains and flour; also cacao beans, nuts, powdered milk, and many stored foods (Ladisch, 1963). Insect secretion from *T. confusum* has provoked lymphomas and leukemias in rats (Ladisch, 1963).

ROACHES. The quinones (1), (2), and (3) are given off by the roach *Diploptera punctata* (Roth and Stay, 1958). *Periplaneta americana* and *Blatta orientalis,* frequent roaches in flour mills and in households, secrete a benzoquinone derivative (Brunet, 1955), which is chemically related to the hepatoma inducing safrole (Davidson, 1963).

ANILINE DYES AND ALLIED COMPOUNDS. "Butter-yellow," an extensively investigated liver carcinogen, is readily oxidized to benzoquinone (Kuhn and Beinert, 1943). Various





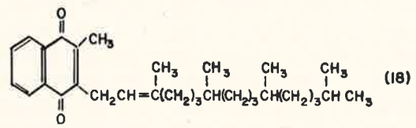


Table I.—Chemicals 1 through 16 provoke or promote cancer. Compounds 17 and 18 are innocuous. Consult text.

(1) 1,4-benzoquinone; (2) 2-methyl-1,4-benzoquinone; (3) 2-ethyl-benzoquinone; (4) 2-methoxy-1,4 benzoquinone; (5) phenol; (6) 2-methylphenol; (7) 2-ethylphenol; (8) 2,4-dimethylphenol; (9) beta-naphthol; (10) tetralol; (11) 2-amino-1-naphthol; (12) anthralin; (13) 1,4-naphthoquinone; (14) 2-methyl-1,4-naphthoquinone; (15) 2,3-dichloro-1,4-naphthoquinone; (16) 9,10-anthraquinone; (17) co-enzyme Q; (18) vitamin K_1.

other synthetic dyes may produce metabolic benzoquinone and/or substituted naphthoquinones. Because of the resultant possible cancer hazard, their use in food has been criticized (Bauer, 1950). Interestingly enough, Stoltzenberg and Stoltzenberg have proposed as early as 1922 that the *in vivo* transformation of Sudan III, Scarlet R, indole, skatole, aniline, benzidine, toluidine, and beta-naphthol produces carcinogenic quinones.

PHENOL (5) is a carcinogen for the mouse skin (Boutwell and Bosch, 1959). TOBACCO TARS contain many phenols, among them the compounds (6), (7), and (8). Such phenols initiate and/or promote cancers of the mouse skin (Boutwell and Bosch, 1959), and they are believed to be operative in human lung cancer (Smoking and Health, 1964).

BETANAPHTHOL (9), used in the manufacture of rubber antioxidants and dyes, is moderately active in promoting skin cancers in mice; TETRALOL, a solvent, is a potent promoter (Boutwell and Bosch, 1959). AMINO-NAPHTHOL (11), a metabolic product from the ingestion of the bladder carcinogen beta-naphtylamin (in dogs), is carcinogenic (Hueper, 1956). ANTHRALIN (12), which is employed in some medical ointments, produces malignant tumors in mice pretreated with a single dose of 125 micrograms of DMBA (Bock and Burns, 1963).

NAPHTHOQUINONE (13), the parent compound of polyhydroxy derivatives occurring in micro-organisms (Thomson, 1957), has been reported to be carcinogenic for mice (Takizawa, 1940). Tumor-promoting properties of this compound have also been seen in the mouse skin after DMBA initiation (Boutwell and Bosch, 1959), and after pretreatment with methylcholanthrene (Kline and Rush, 1944). MENADIONE (14), or synthetic vitamin K, a skin irritant and toxic agent for mice (Fieser and Fieser, 1944), promotes cancer in the mouse skin, and so does PHYGON (15), a seed disinfectant and fungicide (Boutwell and Bosch, 1959). The dye intermediate ANTHRAQUINONE (16), is carcinogenic for mice (Takizawa, 1941).

POLLUTED AIR. For every ton of rubbish burned, more than eight pounds of emission are phenols (Boutwell and Bosch, 1959). Quinoid compounds arise in the catalytic vapor-phase oxidation of benzene and naphthalene when oxidation is incomplete. In the oxidation of coal, polyquinones are formed from naphthalins (Yome et al., 1953). Phenol-quinone agents such as these are carcinogens and/or promoters (loc. cit.).

The benzoquinone derivative coenzyme Q (17), and the naphthoquinoid vitamin K_1 (18), have been added to table I as representatives of certain quinoid substances that occur naturally in the animal organism. These compounds have long-chain substituents. They are normally non-toxic.

FURTHER EVIDENCE FOR TUMORIGENIC ACTIVITY OF FLOUR BEETLE QUINONES

We have most recently studied the acute toxicity of the quinones secreted by insect *Tribolium confusum* and *T. castaneum* (Ladisch and Suter, 1964). Cba/ Jax mice, two to three months old, were used. Aqueous solutions of (a) the quinones isolated from the beetles, and (b) synthetic ethyl:methyl-p-benzoquinone (80:20), respectively, were injected under the skin of the dorsal area. The average lethal dose for both, the natural and the synthetic quinones, was found to be 70 mgs./kg. of body weight as computed from 24-hour mortality data.

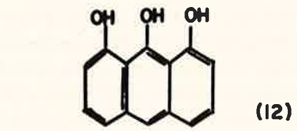
Forty-two animals survived a single dose each of 20 to 70 mgs. of these quinones per kg. of body weight. Within one year and two months following the injections, fourteen of the surviving mice died with large subcutaneous tumors, four additional tumor-bearing animals were sacrificed at the age of twelve months, and thirteen succumbed with no apparent lesions when they were 6 to 17 months old. The tumor quota was 60 percent. Cba/ Jax mice have been reared in this laboratory for many years. They do not develop more than an occasional tumor per hundred of these animals.

Of the four sacrificed animals, three had subcutaneous tumors. The fourth one had developed a cystic lesion of the kidney which was probably neoplastic. All the subcutaneous tumors revealed a similar microscopic appearance. There are well-formed, glandular structures in most areas. Occasional mitoses are noted. The nuclei are moderately irregular in size and shape. They show some irregularity in staining and polarity. Some of the better formed acini contain pinkish granular material. In two mice, the lesions were located in the ventral subcutaneous area (abdomen in nipple line). The third mouse had a tumor on the dorsal lateral neck (not in the nipple line). Skin adnexal gland origin appears most likely. The tumors are well encapsulated as adenomas but appear rapidly growing. At a later stage of development, adenocarcinomatous change would probably have occurred. Microscopic examination of the wall of the cystic lesion of the kidney reveals a lining of cuboidal cells resembling epithelial cells, many of which are partially or completely compressed to an atrophic basophylic membrane apparently due to the pressure within the cystic lesion. The wall of the cyst is made up of fibrous connective tissue in which occasional glands lined by similar columnar or cuboidal epithelium are noted, and there is infiltration of lymphocytes. In the outer portions of the wall, occasional fat cells are noted. Although this lesion could be developmental in type, its size and evidence of rapid growth favor a neoplastic origin, and because of epithelial and mesothelial elements, a cystadenofibroma appears to be a justifiable designation for this type of lesion.

CANCER-RELATED CHEMISTRY IN QUINOID TOXINS FROM STORED-FOOD MOLDS

Like flour beetles, stored-food fungi have become a problem since about 1900, when centralized processing of bulk foods was introduced. *Aspergillus* and *Penicillium* spp. are now predominant on stored grains.

Common stored-food fungi generate quinones in a great variety (Thomson, 1957). At least some of these quinones belong to the presently discussed class of carcinogenic chemicals (see table II). Anthralin (12) is a powerful promoter in mouse skin carcinogenesis, and anthraquinone (16) causes malignant tumors in mice (loc. cit.). The physcion anthrone (19), structurally very similar to either of these compounds, occurs in five species of the grain mold *Aspergillus*. A simple anthraquinone derivative, islandicin (20), is found together with the quinoid luteoskyrin (22) in the rice mold *Penicillium islandicum*. Luteoskyrin produces hepatomas, cholangiocarcinomas, and occasionally a sarcoma in rats and mice (Davidson, 1963). Di-anthrones are readily formed in biosynthesis from anthrones (Thomson, 1957). Thus fagopyrin (21) is related to compound (19). Fagopyrin occurs in buckwheat. The latter produces liver cancer (Kubo and Fujimoto, 1940).



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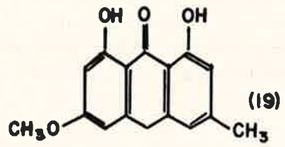

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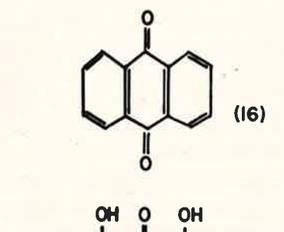

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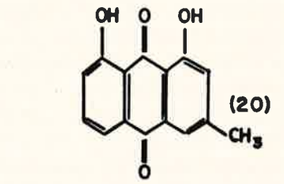

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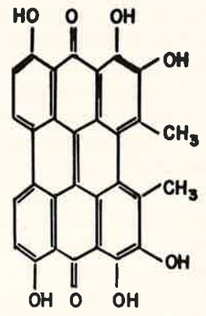

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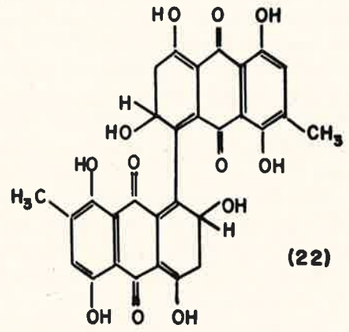

(22)

Table II—Consult Text.

DISCUSSION AND CONCLUSION

The data reveal that quinone chemistry—of great importance in the normal life processes—is likewise an outstanding phenomenon in carcinogenesis. One can only be impressed by the great number of environmental agents that share this distinct principle.

This paper has dealt with aromatic carcinogens. Bauer (1949, p. 249, 305) has stated with finality that, in the biochemical sense, most of the known carcinogenic chemicals are mere artifices from the world of synthetics. He believed, they cannot explain the genesis of "spontaneous" cancers, and they do not provide fundamental information on the actual cancer problem in man. This, he thought, is particularly true "bei den Azofarbstoffen, die ueberhaupt keine Beziehungen zu irgendwelchen Stoffen der lebenden Materie besitzen." The thesis has many proponents.

The present report, however, demonstrates that this approach to cancer is no longer tenable. One of the most familiar carcinogens, the azo-dye "butter-yellow," yields upon oxidation benzoquinone, i.e. a "synthetic" carcinogen. Common stored-food pests produce the same quinone biosynthetically. Therefore, the cancer-related chemistry of the azo-dye is directly applicable to that of the natural insect toxins. Also, in analogy to the well-recognized structural similarities between carcinogenic hydocarbons and steroids, benzoquinoid and napthoquinoid carcinogens are likewise structurally very similar to co-enzyme Q and the natural vitamin K.

The presently greatly improved concept of quinone carcinogenesis permits for the first time the unified consideration of two frequent and extensive potential cancer inciters, namely polluted air (cigarette smoke) and stored-food insect pests. This problem is obviously of considerable interest as it relates directly to allied chemical contaminants in the food we eat, and in the air we breathe.

ACKNOWLEDGMENT

I wish to thank Dr. Russel W. Weller of the Memorial Hospital of Chester County, who kindly furnished the description on the mouse tumors induced by insect quinones.

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INDEPENDENT JOINT SYSTEM SUPERIMPOSED ON METAMORPHIC FABRIC OF GLENARM SERIES NEAR COATESVILLE, PENNA.

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ABSTRACT

Orientations of 534 major joints were measured in the Mortonville area of Anderson, et al. (this volume) 5 miles SE of Coatesville, Penna. Steep joint sets are identical in six sub-areas of markedly different metomorphic fold pattern and are associated with the youngest S-surfaces or kink planes. The joints are apparently youthful and relatively independent structures superimposed on the older fabric of the region.

INTRODUCTION

Structural data of Anderson et. al. (this volume) provides background to contrast joint versus fold patterns in the Glenarm Series of the Mortonville area, near Coatesville, Penna. On the basis of this work the Mortonville area was subdivided into six regions (Fig. 1) of

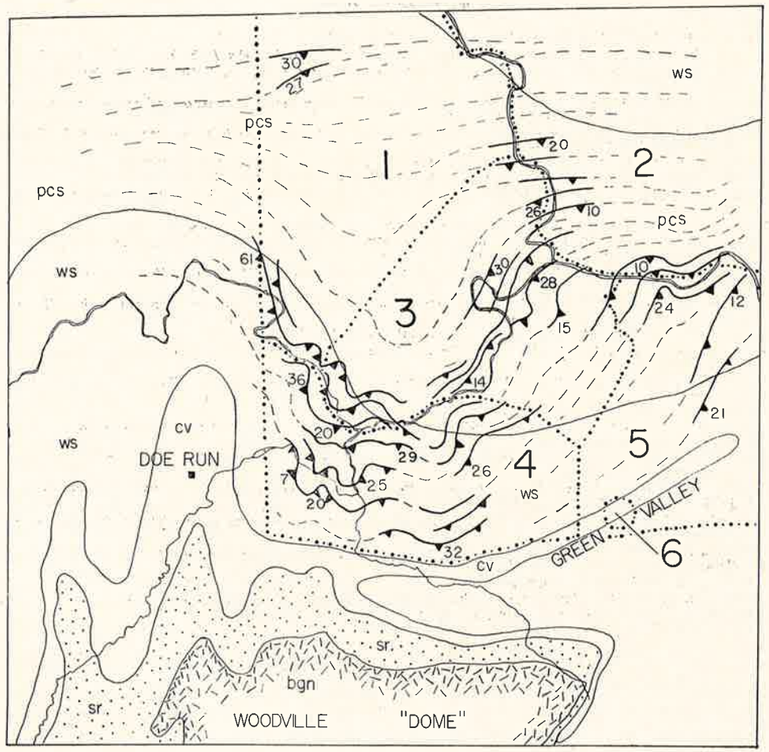

Fig. 1. Index map to Mortonville Area. Sub-areas 1-6 are indicated on basis of generalized S_1 orientations from Anderson, et al. (this volume).

differing fold dominance. Joint patterns in each sub-area were evaluated separately (fig. 2) for changes associated with the varying fold systems.

This study was part of a National Science Foundation undergraduate re-

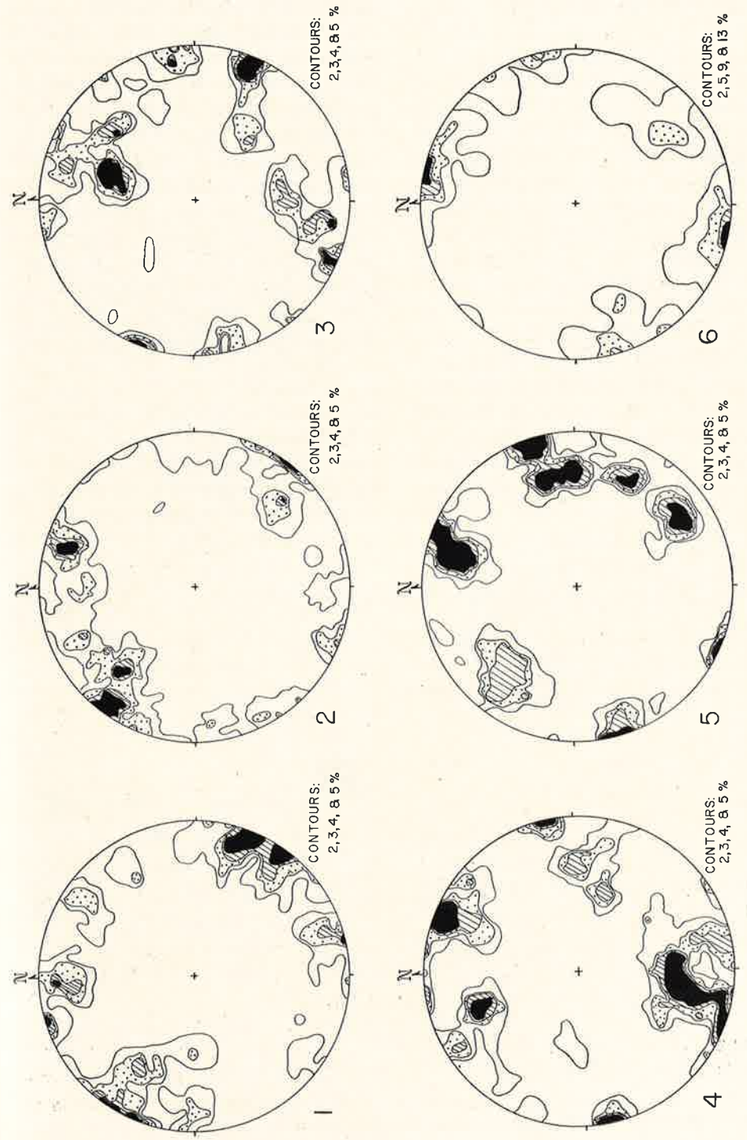

Fig. 2. Orientation of poles to major joint planes in sub-areas 1-6 (figure 1) of the Mortonville Area.

search training program to Franklin & Marshall College. David Drake and Thomas Anderson kindly provided the S_4 kink plane data of figure 3.

METHODS

Joint statistics used here are those of Billings (1954) for the lower hemisphere of equal area nets. Field technique differed from the customary by selection for measurement of only those large (plus 10 feet^2) joint surfaces which defined general shape of outcrops. One to four measurements were taken at each outcrop and any markedly irregular or arcuate surfaces were rejected.

With this technique an area of at least one square mile was needed to provide a meaningful population (about 100 measurements). The method automatically filters out lesser fractures and by increasing the area minimizes effects of selective joint enhancement by topography (Chapman, 1958).

RESULTS

Three steeply dipping major joint sets are strongly developed in the plots of figures 2 and 3A. Strikes of these sets are: N70W, N15W, and N35E. The set at N35E may be part of a pair, the other member striking N20E (Fig. 3A).

These joint sets maintain constant orientation despite radical changes in the older fabric of the rock as determined by Anderson, et. al. (op. cit.). Sub-areas 1, 3 and 4 (Fig. 1) are influenced by D_3 folding along NNW axes whereas the southern parts of sub-areas 4 and 5 are influenced by the ENE trending Green Valley anticline; sub-area 6 is in the marble core of this anticline. Nevertheless, all have the same joint pattern.

No simple relationship appears between jointing and the older fold systems as suggested by Ch'ih (1950, from Postel) or Goodwin (1961). This apparent independence is interpreted as the result of superposition of a younger joint pattern onto a metamorphic complex so thoroughly annealed as to be essentially isotropic to the younger stress system.

KINK PLANES AND JOINTS

Closely associated with joints of the Glenarm Series are 1 to 4 inch wide

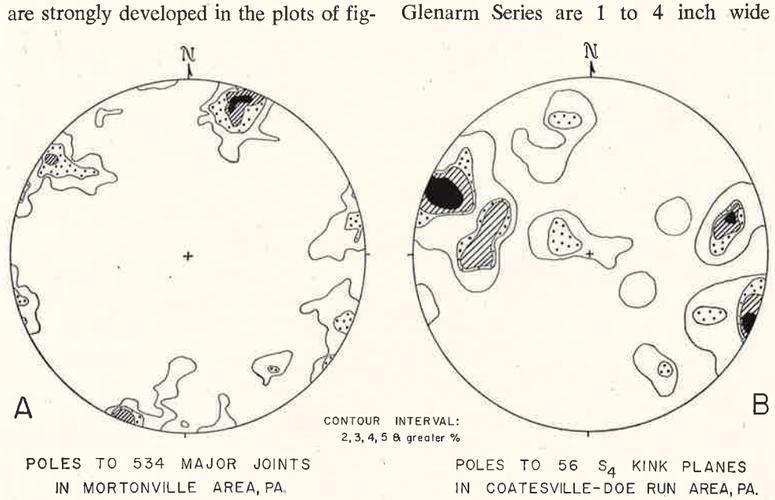

Fig. 3. Contrast of major joint orientations of the Mortonville Area with kink planes in the Coatesville-Doe Run Area. (Courtesy of Anderson, et al.)

planar zones of sharp kinking or bending of schistosity into chevron-like folds. Along strike these kink planes are transitional into small faults or into joints. Spacing of kink planes ranges from a few feet to several hundred feet. In the Mortonville area dips are steep and displacement sense may be normal or reverse. Near Coatesville sub-horizontal kink planes are more common and account for most of the flat dips of figure 3B.

These features have been described as S_4 planes by Anderson, et. al. (op. cit.) and by Freedman, et al. (in press). In the Lancaster area Wise (1960, p. 60) describes similar zones transitional into jointing but having strike-slip displacement.

Statistical parallelism of joint and kink planes (Fig. 3) plus observed transitions between the structures in outcrop proves some genetic relationship. At least some of the joints were approximately contemporaneous with this youngest S-surface in the region. The minor fault displacement on the kinks suggests a shear environment for the associated joints even though displacement on joint surfaces is not measureable on most outcrops.

The two major joint sets having associated kink planes show a conjugate relationship suggestive of paired shear planes (Fig. 3). Maximum compression would have been oriented along a NNE direction approximately perpendicular to the third joint set, the one without associated kink planes. Unfortunately, this simple explanation requires movement on the kink planes to be strike-slip rather than the observed dip slip. Clearly more complex mechanisms are needed but cannot be defined until a more exhaustive study of the kink and joint planes is completed in the region.

CONCLUSIONS

A genetic tie exists between some major joint sets and S_4 kink planes of Anderson, et al. (op. cit.). Both features are independent of the older metamorphic fabric and apparently superimposed on it. They seem to be the only major S-surface developed after the present dominant grain of the region was established. Continuation of this type of joint study around the Pennsylvania curve of the Appalachians may yield information on the nature of this youngest stress system and its relationship to mechanisms of Appalachian curvature.

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CROSS-BEDDING FORMED BY LATERAL ACCRETION IN THE CATSKILL FORMATION NEAR JIM THORPE, PENNA.

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ABSTRACT

This paper describes an occurrence of cross-beds whose dip azimuths are oriented perpendicular to the direction of sedimentary transport as judged from the orientation of associated rib and furrow structure and parting lineation. It is believed that the cross-beds formed on point bars by a process of lateral accretion.

INTRODUCTION

Cross-bedding, accordingg to Potter and Pettijohn (1963, p. 69), "is a structure confined to a single sedimentation unit (Otto, 1938, p. 575) consisting of internal bedding, called foreset bedding, inclined to the principal surface of accumulation." Cross-bedding almost always is assumed to have formed in response to currents acting parallel to the dip azimuth of the inclined beds. As such, statistical studies of the orientation of cros-bed dip azimuths are commonly employed to determine the direction of flow of paleocurrents.

This paper describes an occurrence of cross-beds whose dip azimuths are oriented perpendicular to the direction of sedimentary transport as judged from the orientation of other paleocurrent indicators closely associated with the cross-beds.

The cross-beds described occur in sandstones of the Catskill formation exposed along Highway 29 just south of the town of Jim Thorpe, Pennsylvania.

STRATIGRAPHIC CONSIDERATIONS

The Catskill formation, a molasse deposit of Upper Devonian age, at this locality consists of coarse- to fine-grained gray, pink, and red sandstones (the red sandstones are usually micaceous), conglomerates, micaceous red siltstones and mudstones, and red shales. According to Glaeser (1963, p. 57), the total thickness is 5,687 feet. Some disagreement exists in regard to stratigraphic subdivisions of the Catskill which can be recognized and delineated in this section. Willard (1939, Fig. 72 facing p. 276), for example, in his classic study of the Devonian of Pennsylvania, at this locality recognized the Damascus red shale, the Honesdale sandstone, and the Cherry Ridge red beds. Read (1953), who mapped part of the Catskill between Honesdale, Pennsylvania, and Jim Thorpe, accepted these divisions. Glaeser (1963) did not recognize the units used by Willard and simply divided the section into 15 informal members based on "lithologically distinct properties."

The several rock types described above clearly are cyclically interbedded. Some cycles are incomplete but all in general follow a similar sequence. Each cycle begins with a generally massive but in places cross-bedded coarse-to medium-grained sandstone or conglomerate or conglomeratic sandstone. Some basal units are gray or gray-green, others are pink or red. The base of this unit is always sharply defined and in places truncates on a small scale the bedding planes of the underlying unit. This contact must represent an ancient erosion surface. The massive sandstone or conglomerate *grades* upward into fine-grained slabby sandstones, usually micaceous and characterized by an abundance of parting lineation and rib and furrow structure. Cross-beds may or may not be present. The slabby sandstones in

complete cycles *grade* upward into red mudstones and shales which in their upper parts in places contain mud cracks. This completes the cycle. The top of each cyclic unit, complete or incomplete, is marked by the sharp basal contact with the next higher cycle.

Thus, each cycle is characterized by a kind of graded bedding on gross scale.

Within that part of the section designated as the Honesdale sandstone by Willard and Read, the cycles are incomplete —the red siltstones, mudstones and shales found in the subjacent and superjacent units are not present. These, or sediments of similar lithology, could have been present at the time of deposition—they may have been removed during the period of erosion which preceded deposition of the next higher cycle.

CROSS-BEDS AND OTHER DIRECTIONAL STRUCTURES WITHIN THE SANDSTONES

Directional sedimentary structures within the sandstones of the section include cross-beds, rib and furrow structure, and parting lineation.

Cross-beds are generally tangential but the planar variety also are present. The angle between cross-beds and normal beds is generally small, averaging between 12 and 20 degrees. Most cross-beds are in sets 6 inches to 18 inches thick defined by upper and lower surfaces which in outcrop appear to be parallel to normal bedding. In a few instances, the sets are inclined to normal bedding, truncating bedding planes in underlying units. The lower surfaces of such sets generally are convex downward; the upper surface is planar or concave upward.

In two sets, one of which is inclined, the surfaces of cross-beds were observed to contain easily recognized and well-defined parting lineation. In both sets, the parting lineation is oriented parallel to the strike of the cross-beds (after correction for tectonic change). A similar situation was observed in comparing the orientation of rib and furrow structure in normal beds immediately overlying a third cross-bed set with the orientation of the cross-beds. These relationships would indicate that the cross-beds in these sets formed in response to currents which were moving in a direction parallel to the strike of the cross-beds. Furthermore, the inclined set which contained the cross-beds herein described also was oriented parallel to current flow.

The surfaces of most cross-beds in this exposure are not marked with linear current features. Nevertheless, it is believed that many are oriented similarly to those described above with respect to paleocurrent direction. The orientation of 20 cross-bedding dip azimuths, measured and plotted on a circular histogram, indicated a bimodal distribution: 60% of the dip azimuths fell in the sector 30° to 60°; 25% of the dip azimuths fell in the sector 330° to 360°; the remainder were scattered in the northern half of the diagram. The mean of this distribution is 30°. The orientations of 20 linear features (axes of rib and furrow structure and parting lineation) also were measured and plotted on a circular histogram. For this purpose, the rib and furrow structures were assumed to be concave in the downstream direction. The azimuth of parting lineation was plotted in the hemisphere which would conform to that of the rib and furrow plot. The azimuths of all lineations measured fall betwen 264° and 310° (mean direction = 285°). Thus the paleocurrent direction as determined from measurements of linear markings was 285°—a direction roughly 90° from that which is suggested by the mean of the cross-bed dip azimuths.

The Catskill paleocurrent direction in this area as determined from measurements of parting lineation and rib and furrow structure compares well with that reported by Pelletier (1958) for "Upper Devonian" rocks of Maryland and Pennsylvania (based on 194 observations of 13 localities) and that reported by McGucken (1959) for the Honesdale sandstone (based on 189 readings at 16 localities between Jim Thorpe and Honesdale). Pelletier reported a mean paleocurrent direction of 304°; McGucken reported a mean paleocurrent direction of 308°.

CONCLUSIONS

Much of the cross-bedding in the Catskill formation at this locality was formed in response to currents flowing at right angles to the dip of the foreset beds. The presence of linear markings located on cross-bed surfaces and oriented parallel to the strike of the cross-beds, and the occurrence of the cross-beds in sets which are inclined to normal bedding would indicate that the cross-beds observed are not simply the flank or wing portion of troughs or festoons. It seems more likely that cross-beds of this type formed by a process of lateral accretion.

Lateral accretion as a process of cross-bed (or inclined bed) formation has been described for several depositional environments. Considering the various characteristics of the Catskill rocks, it seems likely that the cross-beds herein described represent successive positions of point bar surfaces in meandering braided streams. Deposition under these conditions has been described by Wright (1959).

Cross-bedding of this type is not common in the stratigraphic record and probably is not common in the sandstones of the Catskill formation at other localities.

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CYCLICITY OF THE CONOCOCHEAGUE FORMATION*

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ABSTRACT

The Conococheague Formation near Greencastle, Pennsylvania, was deposited cyclically. Each cycle is initiated by intraformation conglomerates-oolites-stromatolites which pass upward through interlaminated limestone-dolomite, into pure dolomites. Conochocheague equivalents east and west, over large distances, also exhibit cyclicity indicating uniform basin tectonics and sedimentation during Upper Cambrian time.

INTRODUCTION

In the Cumberland Valley the Conococheague Formation of Upper Cambrian Age outcrops principally along the western flank of South Mountain. The limestones and subordinate dolomites that constitute this unit have been little utilized to date and hence study of the formation has been neglected. During recent work conducted by the Pennsylvania Geological Survey, a section of the Conococheague was measured at Waynecastle Dairy, four miles east of Greencastle, Franklin County and a noteworthy cyclicity of the sediments was observed (Location I, Fig. 1).

Fig. 1. Index Map of Pennsylvania showing distribution of outcropping Upper Cambrian sediments (solid black pattern), various areas referred to in text (Roman numerals), and trend (not position) of the Upper Cambrian shoreline. The Waynecastle Dairy section is indicated by large arrow at I.

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PETROLOGY OF THE MEASURED SECTION

The uppermost 500 feet of exposed Conococheague was measured along State Highway 16 as previous workers Wilson (1951) and Long (1953) had done but the traverse was then offset 1600 feet northeast along strike because outcrops in the fields expose more detail. The section measured was 2100 feet thick; however, it is incomplete because the top of the formation is not exposed. Total thickness of Conococheague beds above the Big Spring Station Member is 1780 feet of which 43% is not exposed. The Big Spring Station Member is a limestone-orthoquartzite sequence at the base of the Conococheague Formation.

In the course of measuring this section it was apparent that several distinct lithologies, averaging two to five feet in thickness, are repeated in the Conococheague beds above the Big Spring Station Member. There are slight variations of these types but almost all the carbonates can be classified in the major categories. The lithologic types are described in the succeeding section and for reference are assigned numbers.

Rock type 1, intraformational limestone conglomerate. In this rock type are included both edgewise and flat-bedded conglomerates. This rock consists of pebbles of limestone, less frequently dolomite, discoidal in shape with rounded edges, usually ½ - 1 inch long

and clearly derived from the underlying or adjacent beds. The clasts are set in a micrite matrix admixed with varied amounts of calcarenitic and oolitic components. This unit is frequently disconformable on the underlying unit.

Rock type 2, complex of calcarenitic, algal, oolitic limestones. The calcarenitic limestone grains may be of bioclastic or of inorganic origin. They seldom range above coarse-sand size and are set in a micrite matrix. The few coquinoidal beds present are included here also.

The algal limestone is composed of stromatolites mostly of the cryptozoon form, both cabbage head and sheet varieties.

Also included in this category are oolitic limestones. The oolites, which show both concentric and radial structure, are well sorted attaining a maximum size of 1-2 mm. Tectonic processes have deformed the oolites to some degree. They are set in a micrite cement.

The calcarenite, algal, and oolitic limestone are all intimately associated. Seldom is there a bed composed entirely of one lithologic type; there is usually an admixture or intercalation of the rock types within this category.

Rock type 3, interbanded limestone and dolomite. This rock type consists of limestone interbanded with dolomite. The limestone bands which comprise 60%—70% of this rock type are usually ½″ - 1″ thick. Internally the bands may be finely laminated, structureless micritic, or, at times, finely clastic. The bands are wavy, imparting a ripple-marked aspect to some of the bands. The silty dolomite is in bands ¼″ thick and characteristically supports lichen growth. The contrast between the medium-gray limestone and the silty buff dolomite forms a dinstinctive banding. Occasionally there are intercalations up to 6 inches thick of rock types 1 and 2 within this sequence.

Rock type 4, interlaminated limestone and dolomites. This rock type consists of limestone interlaminated with dolomite. The limestone laminae which comprise usually about 60% of this rock type are 0.1 to 5 mm thick. The laminae are planar and parallel to bedding although gently undulose and cross-bedded laminae are also present. The limestone alternates with dolomite of similar thickness and fabric. The finely crystalline buff-colored dolomite is silty and weathers in relief relative to the medium-gray limestone laminae.

Rock type 5, dolomite. The thin dolomite beds weather characteristically to buff tones in contrast to most of the limestones which weather light to medium gray and are medium to light gray on fresh surfaces. The dolomite is uniformly finely crystalline throughout and harder than adjacent limestones. The dolomite beds are generally structureless with some internal laminations due to variations in crystal size.

Silt-size quartz and clay are more abundant in the dolomite than the limestone. Floating sand-size quartz grains occur in some of the dolomites, and several thin grains are intimately associated with the dolomite beds, "sandstone beds composed of medium to coarse grained, rounded, well sorted quartz."

The dolomite of this rock type and types 3 and 4, because of their texture and structure, are considered as either primary or penecontemporaneous dolomites.

CYCLICITY OF THE CONOCOCHEAGUE FORMATION

The five rock types described in the preceeding section occur repeatedly throughout the 1800 foot stratigraphic sequence. Types 1 and 2 are most abundant occurring more than 40 times. Types 3 and 4 are less abundant, and type 5 is least abundant occurring about

20 times. The relation to these rock types to each other at first was not apparent because no obvious pattern exists. After some study, however, it was concluded that the rock types are part of a simple depositional cycle (Fig. 2) and are sequential to each other. Forty-three cycles, of varying degrees of development, are exposed at the measured section.

The ideal cycle (Fig. 2) is initiated by a period of strong wave activity in which fragments of the limestone sea bottom are torn and broken forming a basal intraformational conglomerate (Rock type 1) that is frequently unconformable on the underlying stratum. Wave activity decreases and stromatalites, oolites, and calcarenites—(Rock type 2) are formed. In many places types 1 and 2 are so intimately associated that they could be classified as one rock type. With continued decreasing wave activity and probably increasing salinity of the waters, interbanded limestone and dolomites are precipitated succeeded by interlaminated limestone and dolomite. As the number of dolomite laminae increase they merge to ultimately culminate in a bed of pure dolomite. Some thin dolomites are developed in the banded sequence of type 3 also. The dolomite represents the culmination of the cycle, after a period of dolomite precipitation wave activity begins to increase and salinity to decrease, yielding an inversion of the depositional sequence. The ideal cycle is terminated whenever wave activity is of sufficient magnitude to form rock types 1 and 2.

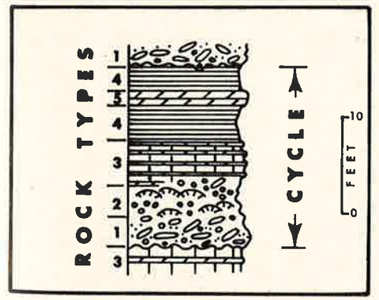

Fig. 2. Idealized sedimentary cycle and rock sequence in the Conococheague Formation near Greencastle, Pennsylvania.

This ideal cycle occurs in several places in the Concococheague at Waynecastle Dairy. However, there are numerous places where part of a cycle develops or where the cycle develops but one or more of the rock types is absent. Because of the geologic factors controlling the rock sequence it is to expected that development of the ideal cycle would be rare. Wave base action which exerts much control upon the formation of the high mechanical-energy rock types 1 and 2 would be affected by any shift in strand line or by periods of variable storm intensity. Geochemical conditions, in large part, control the formation of low mechanical-energy-sediments rock types 3, 4, and 5. Changes in such factors as temperature, pressure, ph, ionic concentration etc. could affect the type and amount of carbonate being precipitated. Thus the dependence of the rock sequence in the cycle upon both mechanical energy and chemical conditions, partly independent factors, explains the paucity of complete cycles in the section.

REGIONAL ANALYSIS

Regionally a cyclic pattern has been observed elsewhere in surface exposures of the Conococheague Formation and its equivalents. In central Pennsylvania near State College, Pelto (1942) and Krynine (1946) (Location II in Fig. 1) noted cyclicity of the dolomite beds comprising the Gatesburg Formation (Conococheague equivalent). The cycles, here, because of their partly arenaceous nature, are probably the best developed and most distinctive in the state. At Snake

Spring Valley, along the Pennsylvania Turnpike, Raymond Knowles (personal communication) has observed cycles similar to those recognized by Pelto (III in Fig. 2). In the Lancaster area Stose (1930, p. 33) remarked on the rhythmic occurrence of algal beds in the Conococheague and suggested seasonal growth as a control (IV in Fig. 2). The Richland and Millbach Formations of the Conococheague Group in Lebanon County are reported to be cyclical in character (Geyer, 1964) (V in Fig. 1). In easternmost Pennsylvania, in the Buckingham Valley area the Conococheague Formation according to Stose (in Bascom et al. 1931, p. 21) is marked by repetition of a tripartite limestone cycle (VI in Fig. 1). Thus, during the Upper Cambrian, carbonates accumulating in central and eastern Pennsylvania, a distance of at least 175 miles parallel to depositional strike, and 75 miles normal to depositional strike were subjected to conditions leading to cyclical deposits.

Unfortunately the paucity of good exposures, distance between outcrops, and lack of precise faunal markers for correlation cause difficulty in reconstructing the precise factors controlling the cyclicity of the Upper Cambrian carbonates. It appears that a single factor of regional extent controlls this widespread repetition and the simplest mechanism is an oscillatory onlap-offlap relation. Whether this is due to rhymthic crustal movements or eustatic sea level change is unknown. Rock types 1 and 2 would be deposited in shallow water during offlap and rock types 3, 4, and 5 would be deposited in deeper restricted waters during onlap. Both the fine varve-like alternation of limestone and dolomite and the coarser interbanding may be related to climatic changes superimposed on a major onlap period.

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PETROLOGY OF BASIC IGNEOUS INTRUSIVES IN THE MARTINSBURG FORMATION, LEBANON COUNTY, PENNSYLVANIA*

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ABSTRACT

Analyses of thin sections of rocks collected from traverses across two basic intrusives of uncertain age reveal that the major rock type is a quartz-bearing diabase. The following types of alteration, considerably more extensive than in many Triassic diabases, partially obscure the original textures and composition: amphibolitization, chloritization, sericitization, and serpentinization.

INTRODUCTION

South of Jonestown in Lebanon County, in an area of approximately twelve square miles, a group of altered quartz-bearing diabase plutons cut the Martinsburg Formation. Urry (1936), using the Helium method of age dating, obtained ages of 375 x $10^6 \pm 15$ and 355 x $10^6 \pm 15$ years for these intrusives and they are, therefore, considerably older than the Triassic diabase intrusives seen elsewhere in the region. The structure and areal distribution of the intrusives as well as volcanics occurring to the north (Fig. 1) have been described by Moseley (1954, 1956). Moseley concluded that the intrusives, sediments, and volcanics are cut by dip faults with offsets of a few hundred feet, and that the intrusives generally show a concordant relationship with the surrounding sediments. These sediments consist of red and green shales, and feldspathic sandstones and conglomerates, all belonging to the Martinsburg Formation. The purpose of this study has been to investigate the petrology of the intrusives, and to compare their extent of alteration to Triassic diabases.

Samples were collected at fifty foot intervals across the strike of two intrusives. The site of sample traverse "A" is a diabase quarry located west of Route 72, two miles south of Jonestown. The intrusive has a maximum thickness of 350 feet of which 250 feet of its interior are readily accessible to sampling. The quarry face is fractured and contains serpentinized slickensides, vein calcite and quartz. Sample traverse "B" was made across the strike of an intrusive having a maximum thickness of 400 feet located west of a north-south trending road about 500 yards south from Swope school. All samples collected from this site were taken from float boulders, some of which were covered by an orange limonitic stain. The depth of weathering of the boulders appears to be quite shallow. Samples collected from both traverses, whether from outcrop or float, are similar, both in hand specimen and thin section.

PETROGRAPHY—Megascopically, the diabase is a fine to medium grained, greenish gray phanerite having a color index of 45. Plagioclase feldspar is light green where alteration has been intense. Greenish black clinopyroxene is the dominate ferromagnesium mineral. Conspicuous phenocryst-like rounded plates, up to 4 mm in diameter, are serpentinized pyroxenes. Grains of pyrite and some ilmenite or ilmeno-magnetite are scattered throughout most specimens.

Under the microscope, thin sections from the intrusives exhibit sub-ophitic and diabasic textures. The average unaltered rock composition, taken from twelve modes appears in Table 1. The unaltered rock composition is arrived at

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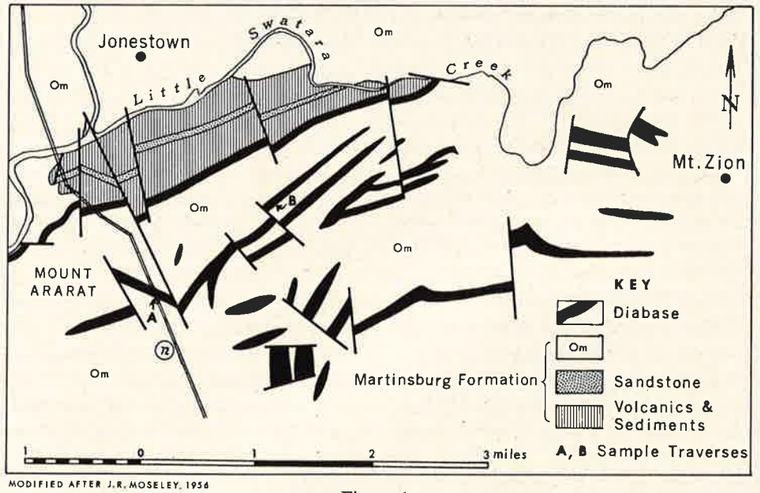

Figure 1.

by counting a particular secondary mineral as the primary mineral from which it was derived. Plagioclase often occurs in euhedral laths about 2.5 mm in length, and comprises from 34 to 47% of the unaltered rock composition. It is often dusted with iron oxide, or extensively altered to sericite, making the percent anorthite composition exceedingly difficult to determine. However, in many sections six or seven clear grains, with albite twinning and suitable orientation were available; these showed a range in anorthite content from 46 to 53%. No evidence of saussurite was found in any of the thin sections. Sericitized plagioclase of undetermined anorthite content is commonly associated with interstitial quartz. In weathered samples, serpentine forms along cleavage planes and fractures of plagioclase.

The predominant ferromagnesium minerals are pyroxenes; they constitute 38 to 52% of the unaltered rock composition. Two varieties of pyroxene are observed. The first is the clinopyroxene pigeonite, having a 2V of less than 20° and containing narrow augite exsolution lamellae. It comprises at least 60% of the total pyroxene content. Pigeonite occurs as elongate subhedral grains (sometimes crushed or bent, showing undulatory extinction) approximately 3 mm long and 0.5 mm wide, and as stubby prisms approximately 1.5 mm long and 1.3 mm wide. Serpentine, amphibole (uralite), and chlorite may form at the expense of pigeonite. Although some pigeonites are more extensively altered than others, as a whole, their degree of alteration is moderate. Pigeonite, because of its low Al content, alters more readily to serpentine than to chlorite; both may show relic herringbone twinning, exsolution phenomena, and resorbed pigeonite grains. Serpentine itself is sometimes partially replaced by magnesite. Pigeonite, which is partially altered to amphibole, is dotted with magnetite granules, and its relief is more pronounced. The second variety of pyroxene occurring in the diabase is pigeonite inverted to the orthopyroxene bronzite. Bronzite is found in one thin section collected along sample traverse "B". It occurs as large anhedral grains measuring 2.7 mm across, and as subhedral prisms 1.7 mm long and 1.2 mm wide. Some of these inverted pigeonites exhibit thick parallel augite lamellae approximately 0.03 mm in width, and resorbed rounded grains of pigeonite which have not inverted. This sample contains 24% pigeonite, and 15% bronzite (inverted pigeonite) which is crossed by fibrous serpentine veinlets. Much of the pyroxene which is now completely serpentinized is probably inverted pigeonite.

Quartz constitutes 7% of the samples taken from the two diabasic plutons. It is an interstitial mineral, and may also form myrmekitic and graphic intergrowths with plagioclase and with orthoclase feldspar. Quartz often contains euhedral crystals and long narrow prisms of apatite. Abnormal quartz, having a 2V of approximately 10°, has been observed.

Orthoclase feldspar comprises less than 2%, occurring as euhedral laths and as rods associated with plagioclase and quartz. Ilmenite altering to leucoxene is the dominant opaque mineral. Pyrite, magnetite, and in weathered samples, hematite, are other observed opaques. Primary hornblende associated with quartz and apatite, and appearing to alter to chlorite, is found in amounts less than 2%. Biotite occurs as a minor constituent in both intrusives.

Table 1.

Average Unaltered Rock Composition From 12 Modes (based on a 300 point count)

Mineral	*Percent*
plagioclase (An46-53)	39.75
pyroxene (clino and othro varieties)	43.58
quartz	7.56
opaques	3.17
orthoclase	2.80
amphibole (hornblende)	2.84
biotite	0.23
apatite	0.10
	100.03

CRYSTALLIZATION SEQUENCE OF PYROXENE—In the material studied, a high temperature magnesium pigeonite was the first mineral to crystallize out from a basaltic magma. The large grain size of these now inverted pigeonites and their thick augite lamellae indicate that this crystallization occurred before the magma invaded the country rock, in an environment of slow cooling. The magma then began the intrusion of the country rock, and with falling temperature magnesium pigeonite inverted to bronzite. The broad augite lamellae remain as a relic structure. The intruding magma was rich in volatiles and silica, which it may have obtained by contamination from the country rock. In the presence of volatiles, pigeonite (a more ferriferous variety) is more stable than orthopyroxene (Poldervarrt, 1947, p. 169), and the clinopyroxene once again begins to crystallize—this time showing very fine striations of augite. Biaxial quartz, and curved pigeonite grains showing crushing and undulatory extinction also suggest the possibility that crystallization began as the magma was being emplaced in the country rock.

ALTERATION OF TRIASSIC DIABASES—Extent of alteration in eight thin sections of ophitic Triassic diabase from Cornwall, Pennsylvania, ten miles southeast of Jonestown, is considerably less than the alteration of the sub-ophitic, quartz-bearing diabase from Jonestown. Secondary alteration of the Cornwall diabase is noticeable only where the diabase is associated with aplite, micropegmatite, or along vein contacts (personal communication, D. M. Lapham). Where this occurs, pyroxene is extensively uralitized, and plagioclase feldspar slightly sericitized. None of the plagioclase in these sections appeared to be turbid. Serpen-

tinized pyroxene psuedomorphs are also noticeably absent in the Cornwall samples. These differences in alteration between the Jonestown and Cornwall samples appear to be quite distinctive.

CONCLUSION

Altered quartz-bearing diabases occur in Lebanon County associated with sediments belonging to the Martinsburg Formation. Pyroxenes, crystallizing from a basaltic magma high in magnesium and low in iron and calcium show characteristics of both plutonic and hypabyssal environments of crystallization. Extensive low temperature alteration, after the crystallization of primary diabasic minerals, has made plagioclase turbid, and has also altered it to sericite; has altered pigeonite to chlorite, amphibole (uralite), and serpentine; and hornblende to chlorite. These diabases appear to be much more highly altered than younger Triassic diabases.

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CRETACEOUS TRANSATLANTIC MIGRANTS

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ABSTRACT

Among Cretaceous intvertebrates in the Bassler Perùvian collection at Lehigh University, some are identical to North African and European species. Assuming the distribution to have been westward, that of the mobile ammonites may be explained through migration. The benthonic species may reflect current-born larvae or eggs or continental drift.

INTRODUCTION

The Harvey Bassler collection of Peruvian fossils (Willard, 1961) at Lehigh University includes invertebrates of Carboniferous, Jurassic, Cretaceous and Tertiary ages. The Cretaceous invertebrates are the most abundant and most widely distributed. There are also vertebrate fossils (Willard, 1962) and plants.

FOREIGN RELATIONSHIPS

It is well established that many fossil vertebrates of South America are unique and confined to that continent. Isolation and radial evolution are the bases for such a development. Years ago, the eminent Argentine paleotologist, Florintino Ameghino, postulated that South America (particularly Argentina) had been a centre of vertebrate evoluution. From there the mammals, at least, spread world-wide. The hypothesis is of course no longer tenable. However, Peru furnishes typical South American vertebrate remains such as peccaries, toxodonts, ground sloths, glyptodonts and astracotheres, examples of which are illustrated in the Bassler collection. Today, the peccaries range as far north as Texas, and ground sloths once lived in the United States of America. Conversely, the mastodon worked its way south into Peru, as too did man, barring transpacific voyages.

The distribution of marine invertebrates is unlike that of the vertebrates, judging from the number and kinds which appear to be identical to South American and other continents. Some of the invertebrate faunas show a certain cosmopolitanism. The few Carboniferous fossils include productid brachiopods which suggest the Pennsylvanian of North America and the Upper Carboniferous of Europe. The most abundant Jurassic ammonite, *Arietites,* has a nearly worldwide distribution (Willard, 1963). The Cretaceous marine invertebrates include examples with broad geographic ranges.

The Cretaceous ammonites are a conspicuous illustration of wide dissemination. During the latter half of the nineteenth and into the twentieth century, many were described from Europe, Africa and North America. Some of these are identical to ones found in the Cretaceous of Peru. Benavides (1956) in his monograph on the Peruvian Cretaceous ammonites indicates the following as found in Peru and the Old World.

Protanisoceras blancheti Pictet and Campiche ..... Europe
Paraturrilites lewisensis (Spath) ..... England
Desmoceras latidorsatum (Michelin) ..... Europe
Desmophyllites gaudama (Forbes) ..... India
Dourvilleiceras monile (Sowerby) ..... Europe
Venezoliceras venezolanum (Steiler) ..... Portuguese E. Africa
Lyelloceras lyelli d'Orbigny ..... Europe

L. pseudolyelli Parona and Bonarelli	Italy
Mammites nodosoides afer Pervinquiere	N. Africa
Vascoceras cf. *silvanensi* Choffat (variety?)	Portugal
Texanites hourcqi Collignon	Madagascar
Barroisiceras (*Solgerites*) *brancoi* (Solger)	Camaroon, W. Africa
B. (*Forresteria*) *alluaudi* Boule	Madagascar
?*B. haberfellneri* (von Hauer)	Switzerland
Tissotia fourneli (Bayle)	N. Africa

In addition to the foregoing ammonites, Benavides lists one nautiloid, *Lissoniceras mermeti* (Coquand) originally described from North Africa (Coquand, 1862).

It is not particularly astonishing that presumably free-swimming or planktonic cephalopods should have a wide distribution, but when we turn to the pelecypods we again meet remarkably wide geographic spread of a number of species. Roemer (1852) names several pelecypods from the Cretaceous of Texas. His *Exogyra ponderosa, E. costata* and *E. arietina* are all reported from Peru by Steinmann (1929), and they are represented in the Bassler collection, as, too, are Roemer's *Vola texanus* and *Pecten texanus*. Three of Roemer's Texan echinoids, *Holoctypus planatus, Pseudodiadema texanum* and *Enallaster texanus* are known in Peru (Steinmann, *op. cit.* p. 129-130). The echinoid, *Hemiaster fourneli* Deshayes (first described from North Africa), or the variety *obliqua* of Brüggen, is recognized by Steinmann (*op. cit.* p. 172). All four are represented in the Bassler collection.

The suite of Cretaceous pelecypods which Coquand (1862) and others identified from Africa and Europe and which appear to be identical to ones in the Bassler collection is slightly longer than than for the cephalopods:

Arca maresi (Coquand)	N. Africa
Ostrea siphax Coquand	N. Africa
O. nicaisei Coquand	N. Africa
O. plicatuloides Coquand	N. Africa
O. tevesthensis Coquand	N. Africa
O. senaci Coquand	N. Africa
Exogyra (*Ostrea*) *fourneli* (Coquand)	N. Africa
E. (*O.*) *mermeti* (Coquand)	N. Africa
E. (*O.*) *minos* (Coquand)	N. Africa
E. (*O.*) *overwegi* (Coquand)	N. Africa
Pecten tenouklensis Coquand	N. Africa
P. aequicostatus Lamark	England
P. quinquecostatus (Sowerby)	England
Plicatula gurgitis Pictet and Roux	Switzerland
Plicatulopecten ferryi Coquand	N. Africa
Lima grenieri Coquand	N. Africa
Liopistha gigantea Sowerby	England
L. molli (Coquand)	N. Africa
Pholadomya elongata Muenst	Europe
P. nodulifera Muenst	Europe
Anisocardia hemitei Coquand	N. Africa
Venus desvauxi Coquand	N. Africa

Among the foregoing, *Ostrea nicaisei* and *Plicatulopecten ferryi* are the most abundant numerically and are identified from more places than any other Cretaceous invertebrates in the collection. Knechtel (1947) reports from Peru a few other pelecypods whose identification is attributed to European paleontologists. Their Old World affinities are not verified here. Few gastropods from the Peruvian Cretaceous appear to have been reported from beyond South America. Exceptions are *Aporrhais costae* Choffat from Portugal (Choffat, 1886-1902), *Fusus bleicheri* Thomas and Perón and *Fusus assailyi* Thomas and Perón from Switzerland and *Tylostoma cossoni* Thomas and Perón from North Africa.

Citation is due Gustav Steinmann for remarking on the faunal relationships between the Peruvian Cretaceous fossils and those of other regions in the Northern Hemisphere (*op. cit.*, p. 287-288). Although Steinmann cites no specific ties, he mentions similarities between Lower Cretaceous invertebrates of Peru and those of other South American countries, particularly Brazil, and with Mexico and the Mediterranean region. Among Upper Cretaceous fossils he recognizes similarities between Peruvian faunas and those of northern South America, Mexico, North Africa and southern Europe. Curiously, he does not allude to Roemer's (1852) discoveries in Texas, although he figures some of Roemer's fossils as present in Peru. Steinmann does not appear to have offered an explanation for the wide similarities, but his mention of northern Brazil suggests a possible step along a migration route between the Old World and the New.

The Tertiary invertebrates in the collection are of little significance beyond Peru. Few are Prepliocene. From the Pliocene sediments of the Amazon Basin, there are about a dozen pelecypods and perhaps two dozen gastropod species which are represented by countless individuals. Most of these molluscs are believed to have been fresh- or brackish-water animals, which may account for their relatively restricted distribution.

Like all half-known truths, the distribution of the Cretaceous marine pelecypods reported from Peru, Europe and Africa is enigmatic. Those few Peruvian species which coincide with North American kinds might have migrated along shallow coastal waters. If one assumes that the Old and New World continents were separated in Cretaceous time by an ancestral Atlantic Ocean, in which direction did the trans-oceanic species move, east to west or west to east? If from east to west, allowing the oceanic circulation to have been analogous to that of today, could larvae or eggs have been current-borne from the Old to the New World? Could some have survived the journey if given sufficient time for many trials and errors? An alternative assumption is that the eastern and western continents were contiguous in Late Cretaceous time and that the now separate faunas were in that time near neighbors which were subsequently parted during continental drift.

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EXPERIMENTS ON THE PRECIPITATION OF SALTS FROM SEA WATER

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ABSTRACT

Sea water was treated with ethyl alcohol to promote the precipitation of salts. At room temperature with a system closed to carbon dioxide the sequence of precipitation is gypsum-mirabilite-thenardite. A similar sequence is found with a system open to an atmosphere of carbon dioxide. Temperature effects are discussed.

INTRODUCTION

The origin of non-clastic sedimentary rocks has been a subject of conjecture, debate, and controversy for many years. Certainly some of the rocks formed are an accumulation of chemically precipitated crystals. Some such precipitates are undoubtedly altered by diagenetic processes. Before such rocks can be completely understood the nature of the material precipitated from saline water and the sequence of precipitation must be established. It is the purpose of this paper to present data from a study conducted to obtain information on the precipitates from sea water.

METHODS AND RESULTS

Some major problems of experiments concerned with precipitates from sea water are: first, and probably most important, the development of metastable crystals or the failure of stable crystals to form; second, the detection of phases that may form in small quantities; and third, the identification of crystals in the state in which they formed. This last problem is not simple because many of the hydrated crystals cannot be dried for oil immersion work or X-ray examination.

An experimental method was used that eliminated some of the above problems. The precipitation was carried out in a large seperatory funnel that initially contained one liter of sea water. The sea water was obtained from about 16 miles off the New Jersey coast, and it had a chlorinity of 32°/00. Ethyl alcohol was added to this sea water over a period of 25 days until the final density of the solution was 0.80_5. After addition of each 100 ml of alcohol, any precipitate that formed was carefully scraped from the sides of the seperatory funnel, and after it settled it was removed. The precipitate was collected on a 25 mm diameter millipore filter of 0.8 μ pore, and it was X-rayed while still on the filter and still moist. In this manner very small quantities of precipitates could easily be recovered and identified. Because precipitation was induced by the addition of alcohol, the salts did not cake or accumulate as a rim near the solution-air interface as they do in drying experiments.

In the first experiment the solution was closed to the air and therefore carbonate compounds would not be expected to form. Gypsum was the first mineral to precipitate. It formed at a solution density of 0.97_4 and it continued to form until the solution reached a density of 0.89_6. It was observed that the gypsum that formed early in the sequence was larger—better developed crystals than that which formed late. At solution densities from 0.89_6 to 0.88_0, mirabilite ($Na_2SO_4.10H_2O$) formed. This mineral appeared as very long needles ro threads under the mircoscope, but megascopically it appeared as a flocculent precipitate. At densities from 0.88_0 to 0.81_5 thenardite (Na_2SO_4) formed. No further precipitation occurred even through alcohol was added until the final solution had a density of 0.80_5. The temperature ranged from 21 to 25°C during the experiment.

The effect of alcohol on the pH of sea water was studied. It was found that there is a systematic increase in pH with the addition of alcohol. One part of sea water with thre parts of alcohol gave a pH of 8.4. Thus the pH conditions of the experiment were not greatly different from those expected in nature.

In another experiment the same method was used except that the solution was under an atmosphere of carbon dioxide. Under these conditions carbonate compounds could be expected; however none formed. In fact, the same minerals formed as in the first experiment; they formed in the same sequence; and they each formed over the same density range. Because of the atmosphere of carbon dioxide, the solution was acid and at a density of 0.86_1 the measured pH was 5.3. The temperature ranged from 23 to 25°C during the experiment.

Because of the salts that formed, and the absence of any magnesium salts, one must conclude that the residual solution is rich in magnesium and poor in calcium and sulfate ions. Thus it seems likely that magnesium carbonate compounds may form if the pH of the final solution could be increased and yet keep CO_2 as a component of the system.

The CO_2 content of the final solution is a function of the temperature of the solution and the partial pressure of CO_2 in the atmosphere over the solution. Because the solution is rich in alcohol, heating the solution would have a double-barreled effect. The solubility of gas in water decreases with increasing temperature; and further, the partial pressure of alcohol and water vapor in the atmosphere over the solution will increase with increasing temperature. Thus heating of the CO_2 bearing-magnesium rich solution from the second experiment could possibly give conditions under which magnesium carbonate compounds could form. The solution was heated to 77°C, within about a degree of the boiling point of ethyl alcohol, yet no precipitation occurred. This indicates either: 1) that the solution is still undersaturated with magnesium compounds even though it contains all the magnesium originally present in sea water; 2) that the magnesium is complexed with the alcohol or something in the sea water; or 3) that the temperature increase has a greater effect on the solubility than does the pH change brought about by the increased temperature. Why magnesium bearing compounds do not precipitate easily from sea water remains a frustrating problem.

In comparison to the pioneering work by Usiglio, more than a century ago, some major differences are found. Usiglio's sequence, listed by Steward (1963), is the following:

1. Fe_2O_3
2. $CaCO_3$
3. $CaSO_4.2H_2O$
4. NaCl, $MgSO_4$, $MgCl_2$
5. NaBr

The present study indicates the following partial sequences:

1. $CaSO_4.2H_2O$
2. $Na_2SO_4.10H_2O$
3. Na_2SO_4

Marked differences are immediately evident. Possibly ethyl alcohol affects the system in some manner other than simply abstracting water. The differences may be result of either or both studies giving metastable relations, and last, it is possible that products studied more than a century ago without the advantages of X-ray could be misidentified.

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PALEOECOLOGY OF TWO MARYLAND SECTIONS OF THE KEYSER LIMESTONE

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ABSTRACT

The lower third of the Keyser Limestone, of upper Cayugan age, was deposited during a cyclical change in turbulence, as indicated by changes in micrite content and clastic particle size. Coral and stromatoporoid populations predominated in the more turbulent phases of the cycle, with stromatoporoids most abundant during highest turbulence. Brachiopod and ostracode populations generally predominated during the less turbulent phases of the cycle.

INTRODUCTION

In the lowest 150 feet of Keyser Limestone, near Hancock, Maryland, the total faunas preserved in apparent life position change from a basal bryozoan—stromatoporoid zone, through about 80 feet of section in which brachiopods predominate, to a tabulate coral and stromatoporoid zone. These faunas preserved in apparent life position, remnants of ancient populations, varied presumably because of changing ecologic conditions rather than evolution. This study attemps to: (1) infer changes in ecologic variables during deposition of the lower part of the Keyser Limestone, and (2) relate these changes to variations in organism populations preserved in the rocks.

The outcrops studied occur in the Potomac River gap through Tonoloway Mountain, Maryland, opposite Great Cacapon, West Virginia; and in a quarry on the north side of US 40, ½ mile east of Licking Creek, which is approximately six miles east of Hancock, Maryland. The sections were remeasured on the basis of recurring zones of nodular and laminar bedding. Rock samples were taken from the middle of each bedding zone, and in the laboratory were analyzed for: insoluble residue, mineralogy by Alizarin Red S staining, micrite content and skeletal material plus other clastics by point count of polished surfaces. Mean diameter of pelmatozoan ossicles was also determined. Remains of organisms in apparent life position were counted on measured areas of polished surface to define the populations. Some results of field and laboratory study of the Tonoloway Mountain section appear in figure 1. The Licking Creek quarry section was found to be nearly identical in all aspects to the upper 60 feet of the Tonoloway Mountain section. Therefore, only the Tonoloway Mountain section is figured.

SETTING AND CONDITIONS

Sediments now exposed at the sections under study were deposited under water near the depocenter of a shallow trough which was parallel to the present mountains (Willard, et. al., 1939). Evidence for a possible Keyser shoreline in eastern Pennsylvania is the presence of redbeds of probable Cayugan age (Swartz and Swartz, 1941).

General ecologic conditions that are known to affect organism occurrence include: temperature, salinity, solar radiation, oxygen, and availability of plankton and nutrients (Hedgpeth et. al. 1957). Evidence indicates that these conditions changed little over the time of Keyser deposition. Salinity was probably normal for marine waters of the time, as brachiopods, corals, and echinoderms, indicators of marine water, are present through the section. Since extensive Recent shelf carbonates occur only in tropical or warm subtropical seas (Rodgers, 1957), it is believed that the extensive Cayugan carbonate deposits were laid down in a warm sea. Oxygen, plankton and nutrients were present in sufficient abundance to support the sessile epibenthic populations now preserved. Evidence for light penetration is weak, as spherical and irregular plate shaped fragments, possibly algal remains, are rare. If the fragments are of algal origin, they (and the other sediments) were probably deposited within the photic zone, as algal fragments do not transport readily (Chave, 1962). There is no evidence that could give a positive indication of changes in absolute or relative changes in depth of water. Since populations varied while there is no evidence for change in these conditions, it appears that these conditions had no effect on fluctuations of organic populations.

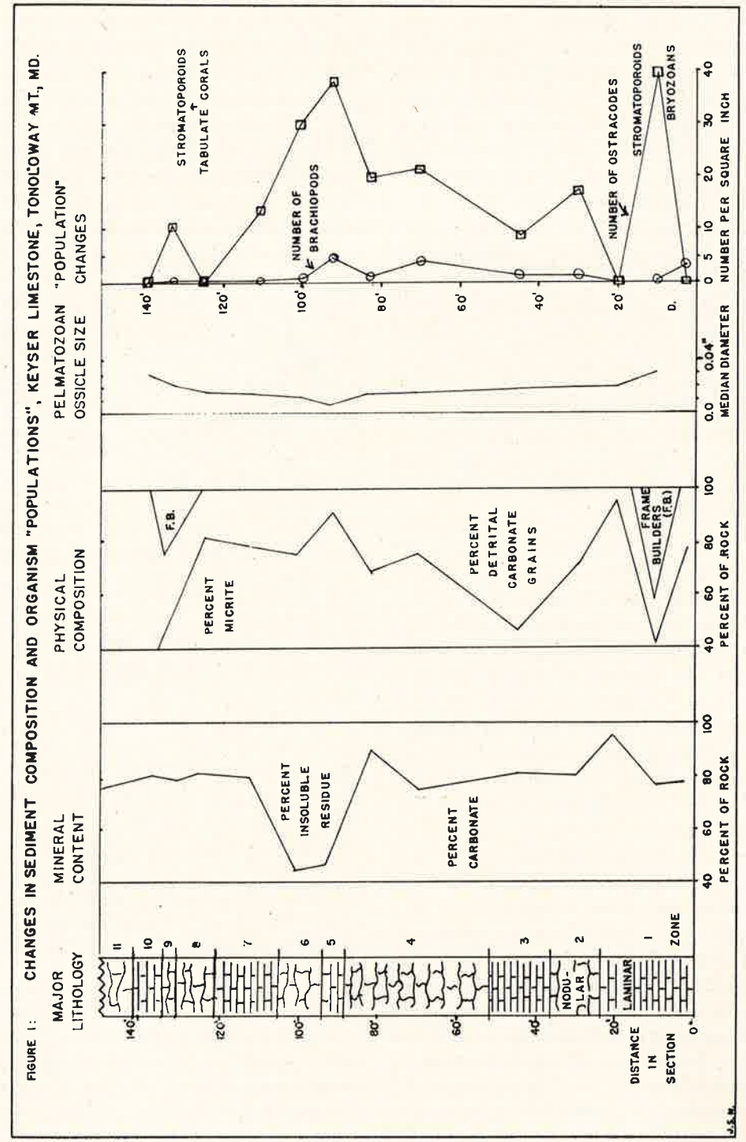
PROCESSES

Processes that could affect organic populations, as seen in the geologic record include: carbonate solution, biologic disturbance of sediments, water movement and turbulence, and clastic sedimentation. Since the faunal lists are comparable to those of other Cayugan rocks, it appears that there was no major removal of shell material by solution. If mottling or nodularity of sediments indicates biologic disturbance (Moore and Scruton, 1957), alternate nodular and laminar zones would indicate recurring episodes of biologic disturbance and non-disturbance (figure 1, major lithology). Micrite content and pelmatozoan ossicle size give a relative estimate of degree of water movement and turbulence. For example, high micrite content and low pelmatozoan ossicle median diameter signify quieter, less turbulent water. From figure 1, physical composition and pelmatozoan ossicle size columns, it can be seen that water movement and turbulence was greatest during deposition of the base of the section, and except for one reversal, gradually decreased to about two thirds of the way up section, whereupon it again increased. This change appears to represent one sedimentation cycle. Clastic dilution as measured by percent insoluble residue, also was greatest at the middle of the cycle. Dolomite content, not depicted in figure 1, also varied, with the curve of its changes varying conversely to the curve of pelmatozoan ossicle size. These three regular changes appear to define one sedimentary cycle. From published descriptions of the section under study, four more cycles of alternating coarse and fine grained limestone appear to be present in the Keyser (Stose and Swartz, 1912). Specific aspects of these cycles have been correlated into Pennsylvania (Reeside, 1918).

PALEOECOLOGIC RELATIONSHIPS

Results of population studies are presented in figure 1. Bryozoans (*Cyphotrypa*), stromatoporoid and ostracode populations are present near the base of the section, but quickly decrease upwards, and are followed by a brachiopod (*Rhynchospira*) and ostracode (*Leperditia* spp.) zone, in which populations gradually increase to a maximum two thirds of the way up the section, and then decline. Near the top of the section, tabulate corals appear, first branched forms (*Cladopora*), then massive forms (*Favosites*). These are then replaced upwards by stromatoporoids and ostracodes.

By comparing changes in populations to changes in inferred conditions and processes, it can be seen that population changes are related to changes in water movement and turbulence. Coral, bryozoan, and stromatoporoid populations developed in the more turbulent zones,



while brachiopod populations flourished in less turbulent water. Numbers of ostracodes fluctuated widely, but generally seem to vary as brachiopod populations. The transition from zones of branching tabulate corals through zones of massive tabulate corals to stromatoporoid zones in increasingly turbulent water was also noted by Lecompte (1960) in Devonian reefs of Belgium.

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MEGAPETROFABRIC OF THE COATESVILLE-DOE RUN AREA, PENNA.

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ABSTRACT

An older system of NE striking isoclinal folds related to the Peach Bottom synclinorium is disrupted by younger basement uplift of the Mine Ridge—Chester Valley zone and by N-S striking folds associated with the Woodville "dome." The relations are permissive of younger strike-slip faulting as suggested by McKinstry (1961) and support his interpretation of multiple deformation albeit in a different age sequence.

INTRODUCTION

The Coatesville-Doe Run area of the Pennsylvania Piedmont exposes the complex Peach Bottom synclinorium (Fig. 1) between basement uplifts of Mine Ridge on the north and a series of irregular basement masses on the south. The problematic Martic line along the Chester Valley on the south flank of Mine Ridge separates known Paleozoic rocks on the north from uncertain aged rocks of the Glenarm series on the south.

Surface distribution of these units is reasonably well known from the work of Bascom and Stose (1932) but the age and structural relationships have been intensely debated, most recently by McKinstry (1961) and Mackin (1962). Mackin interprets the irregular basement uplifts as erosional remnants of complex nappes whereas McKinstry interprets them as irregular gneiss-cored anticlines resulting from multiple folding. This paper is an attempt to test McKinstry's hypothesis by detailed work on the magapetrofabric.

The work was supported by the National Science Foundation undergraduate research training program of Franklin and Marshall College.

TERMINOLOGY

Terminology of structural elements in this paper partially follows that of Freedman, Wise and Bentley (1964) who applied it to the Susquehanna River Section, 25 miles to the west. The system is:

S_0 = bedding

S_1 = major schistosity of the region and axial planes of isoclinal folds (F_1) formed in the first deformation (D_1) of the area.

S_2 = a younger cleavage (poorly developed in this area and unrecognized in the Susquehanna area)

D_3 = a basement related deformation younger than S_2 but older than S_4. Folds are discernible in map patterns but have no detectable S-surface counterparts in outcrop (probably correlates in part with D_2 of Freedman, et. al.)

S_4 = kink bands transitional into joints or small faults

OLDER FOLD SYSTEM (F_1 and S_1)

Equal area plots of figure 2 represent orientations of the dominant schistosity (S_1) along a line of stations across the region. All are lower hemisphere plots of lineations or poles to planes collected within quartermile radii of the areas indicated on figure 1. Although low angle southeasterly dips dominate the S_1 pattern of figure 2, younger deformations have caused highly variable strikes in the south and sharp increases in dip in the north.

A very tight isoclinal fold system (F_1) with S_1 as its axial plane appears wherever bedding is well enough preserved.

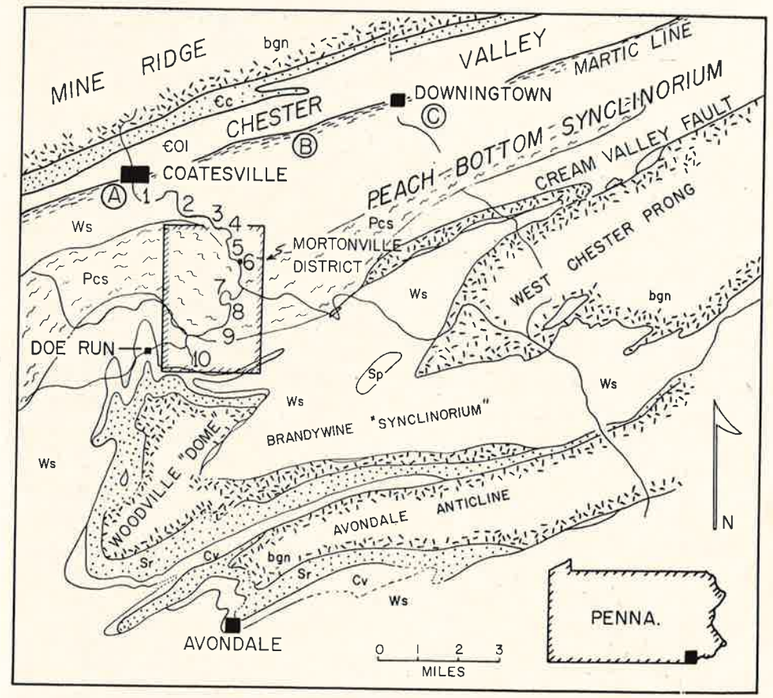

Fig. 1. Index Map of the Coatesville-Doe Run Area. Locations 1-10 refer to figure 2 and A-C refer to figure 5, Pcs: Peters Creek schist; Ws: Wissahickon schist; Cv: Cockeysville marble; Sr: Setters quartzite: bgn: Baltimore gneiss; Cc: Chickies quartzite; CO1: Cambro-Ordovician limestones; Sp: serpentine.

The F_1 fold axes (Fig. 3) strike N to NE at sharp angles to the dominant grain of the area as defined by basement uplifts. Although basement may have been involved to some extent with the F_1 deformation, the bulk of basement disturbance is a younger feature (D_3) disrupting older F_1 axes and S-surfaces.

The rotation sense of 132 F_1 fold axes from the south limb of the Peach Bottom synclinorium is presented in fig. 3. A single movement sense of deeper rocks forcing northwestward with respect to shallower rocks dominates the plot. The movement picture is in accord with the squeezing of material up and out of the center of a very tight synclinorium. The exceptions to this picture (20%) are minor reversals associated with subsidiary digetations scattered through the outcrop area.

The rotations indicate that the south limb of the Peach Bottom synclinorium acted as single movement domain during F_1 development. An inadequate number of fold rotations were collected on the north half of the synclinorium to test for reversal on that limb. Farther south in Sportman's Quarry at Avondale, 11 rotation determinations indicate a reversal with respect to the pattern of figure 3. This method of delimiting movement

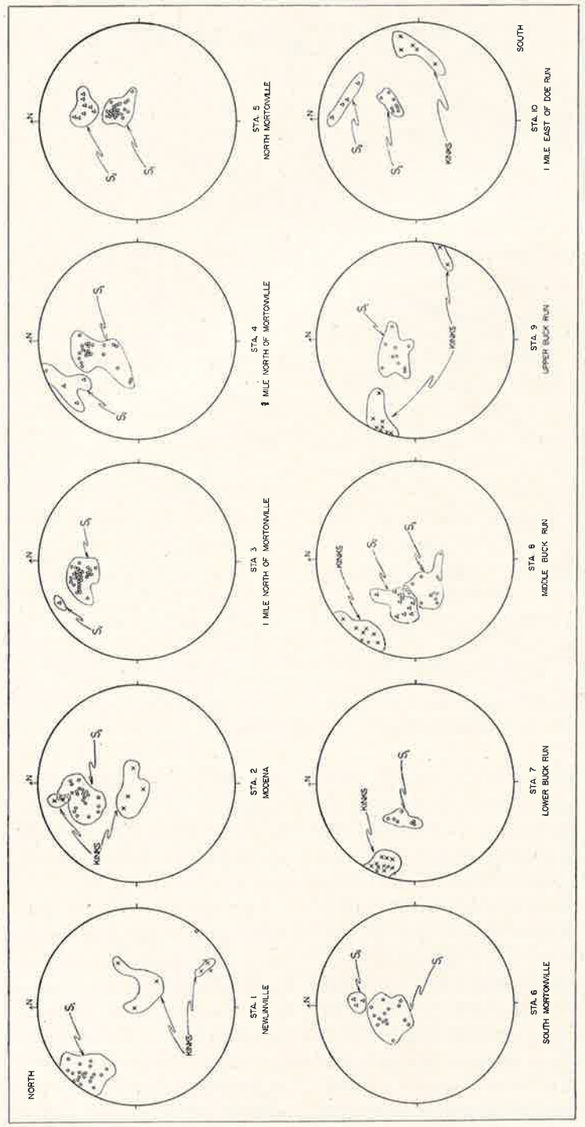

Fig. 2. Equal area plots of poles to S-surfaces in the Coatesville-Doe Run Area. Locations indicated on figure 1.

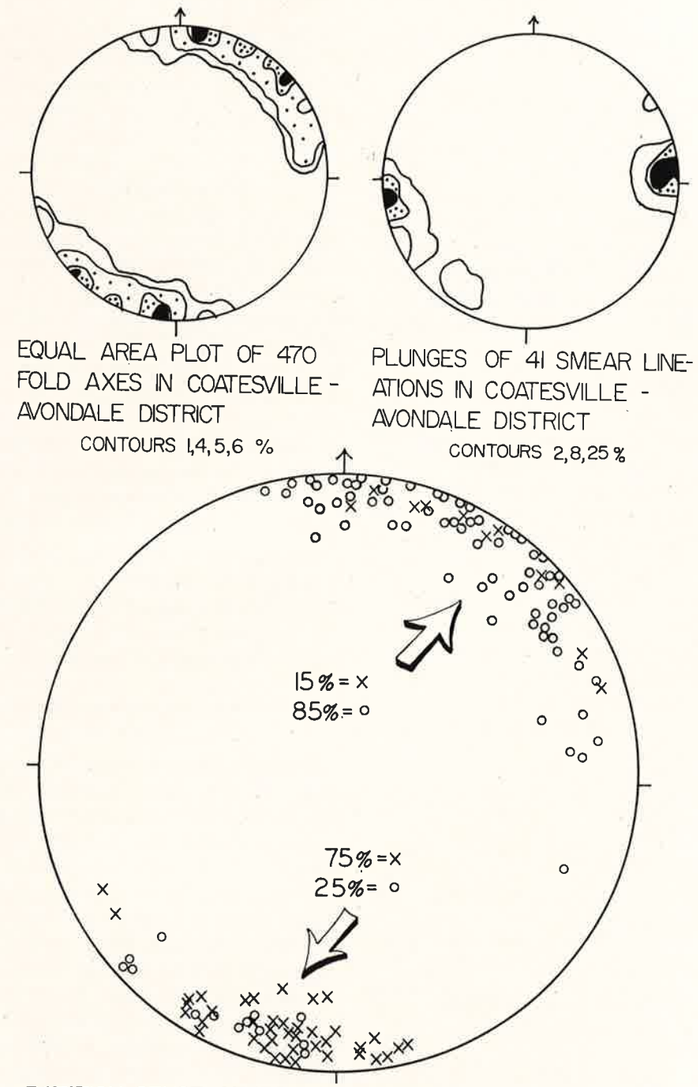

Fig. 3. Lineation orientations in the Coatesville-Doe Run-Avondale Area. Rotation sense of lower figure are plotted as viewed down the plunge.

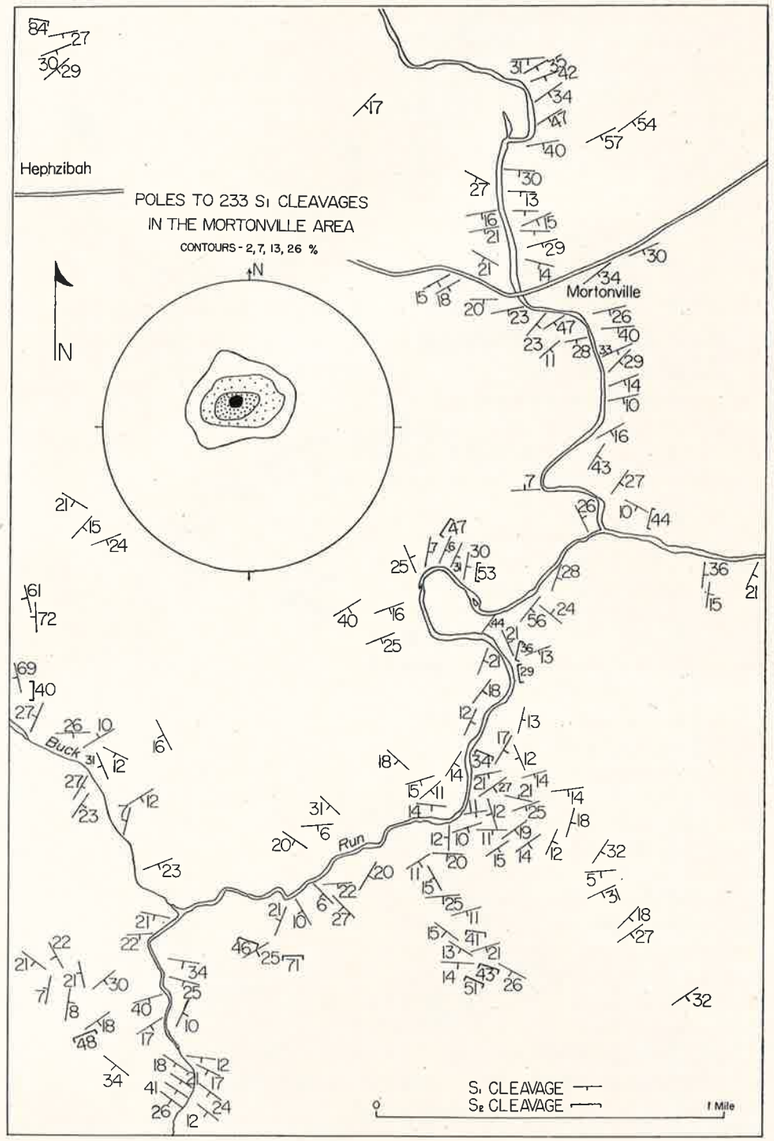

Fig. 4. Orientation of S_1 and S_2 planes in the Mortonville Area (location on figure 1). Generalized orientations on index map of Newell and Wise (this volume).

domains and fold limbs without the help of stratigraphic units seems promising to decipher more of the F_1 system in adjoining areas.

S_2 PATTERN

A poorly developed fracture or strain-slip cleavage (S_2) with little or no associated folding occurs sporadically through the area. Its planar character transecting S_1 in rocks with intense F_1 folding indicates an age younger than D_1. S_2 generally dips southward at 20°-40° (Fig. 2) parallel in strike but of steeper dip than S_1. The detailed mapping in the Mortonville area (Fig. 4) shows this parallelism most clearly where both S_1 and S_2 maintain a constant relative orientation despite marked changes in strike associated with a younger (D_3) folding.

S_2 in this area seems a different structure, older and more weakly developed, than the strong, basement-related structures designated S_2 by Freedman, et. al. (op. cit.) along the Susquehanna River.

YOUNGER BASEMENT-RELATED DISTURBANCES (D_3)

Irregular basement uplifts and/or distorted S-surfaces mark one or more younger disturbances (D_3) in which the present dominant N60-80E grain of the region was established. The Chester Valley represents erosion along weaker carbonates sharply tilted by basement uplift of Mine Ridge with respect to Glenarm rocks in the Peach Bottom synclinorium. S_1 planes along this zone are more sharply tilted (Fig. 2), more irregular, rippled, phyllitic, and shattered than in most of the area. The upturned S_1 planes have variable strike along the zone (Fig. 5) but, like the F_1 axes and Peach Bottom synclinorium, strike more northeasterly than the N75E crosscutting structures of Mine Ridge and the Chester Valley. Strike-slip movement is possible along the zone but was undetected in this study.

The Martic contact lies within the Chester Valley disturbed zone but that contact, whatever its nature, is older than the basement uplift of Mine Ridge as shown by relationships to the west (Cloos and Heitenan, 1941; Freedman, et. al, 1964). Accordingly the Chester Valley zone is emphasized here to avoid confusion with the older but locally coincident Martic line.

Two NNW trending folds (Fig. 1) in the Doe Run area project northward from the basement mass of the Woodville "dome." Northward in the Mortonville area these folds warp both S_1 and S_2 in identical fashion (Fig. 4) and twist the core of the Peach Bottom synclinorium into a sigmoidal shape as mapped by Bascom and Stose (1932).

These folds, disrupting the uniform movement domain of S_1 (Fig. 3) and the S_2 patterns, must be younger features, despite their aproximate parallelism with F_1 axes, the basis on which McKinstry interpreted Doe Run folds as part of the

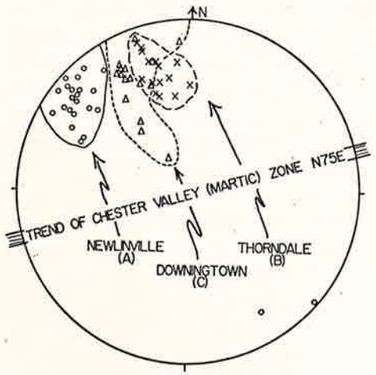

Fig. 5. Equal area plot of 53 poles to S_1 cleavage on the south edge of Chester Valley between Coatesville and Downingtown. (Locations A, B, C refer to fig, 1, index map.)

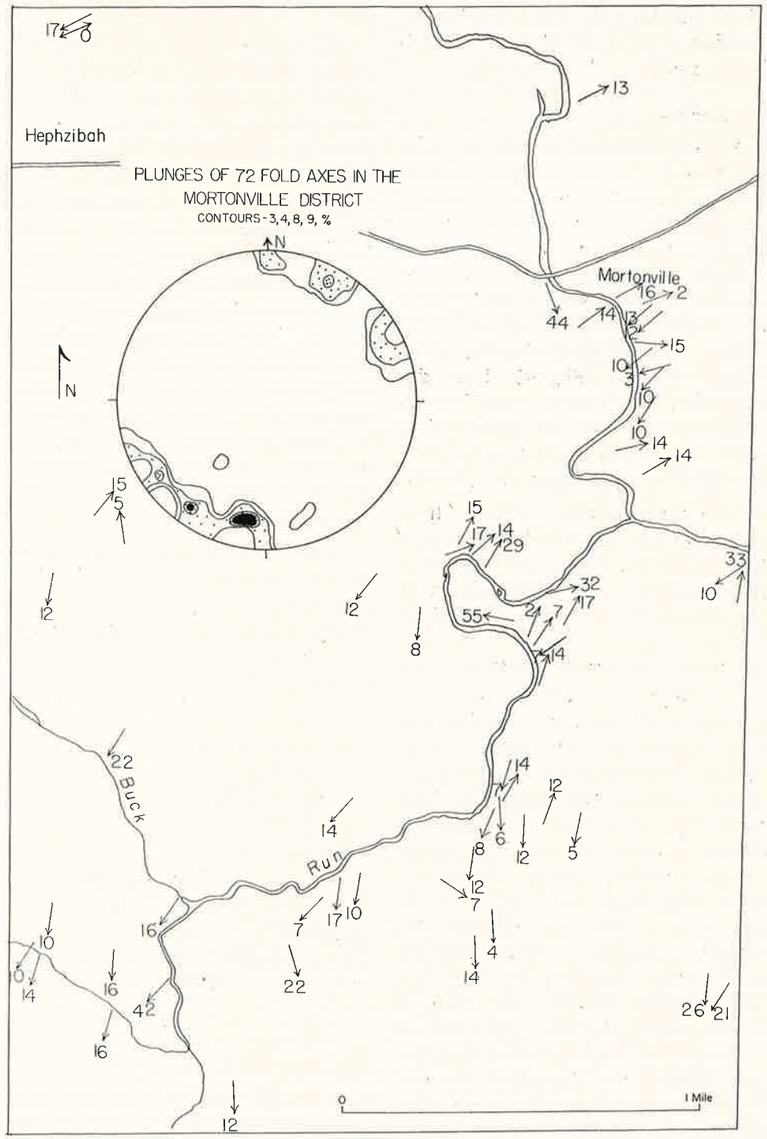

Fig. 6. Orientation of F_1 fold axes in the Mortonville Area (location on figure 1).

F_1 system. Post-S_2 age and association with basement in the Woodville "dome" suggests some genetic tie with the Mine Ridge basement uplift. Accordingly all these younger basement related elements have been grouped as D_3 disturbance in spite of diverging fold axes and uncertainty whether several sub-phases of deformation are involved. S-surfaces associated with this deformation were not recognized in the area studied.

One explanation of the unique location and orientation of the Doe Run folds utilizes McKinstry's (1961) suggestion of right-lateral shearing along the Cream Valley fault zone (Fig. 1). Westward dissipation of right lateral basement movements into the covering Glenarm series would result in the observed localization and sense of shortening of the Doe Run folds as well as the proper sense of rotation for the sigmoidal twist of the Peach Bottom Synclinorium core. Thoughout the area, but mostly in the Setters and Cockeysville formations near Avondale, are strongly developed late-stage grooves and slickenside-like smears showing little relationship to the local fold pattern. This orientation (Fig. 3) is in perfect parallelism with these proposed strike-slip movements. In addition McKinstry (p. 564) notes right-lateral shearing on young S-surfaces east of the present area of study.

S_4 Pattern of Kink Planes

Thoughout the area are 1"-4" wide zones of kinked schistosity. They are steeply dipping in the south part of the area but relatively flat in the north. The zones are unaffected by all the older folds discussed above and hence post-date them. Transitional into jointing and small faults they are the youngest readily discernible structures in the area. Apparently the rocks had just enough mobility to flex locally at the time S_4 formed. Orientation and relationship to jointing are discussed in the companion paper by Newell and Wise.

CONCLUSIONS

The Coatesville-Doe Run Area has undergone multiple deformation but present data is insufficient to distinguish between McKinstry's interpretation of complex multiple uplift versus Mackin's complex nappe theory for the Woodville "dome." The oldest deformation involved development of major schistosity and isoclinal folding along NNE trending axes. At that time the south limb of the Peach Bottom synclinorium was acting as a single movement domain squeezing NW with respect to its cover. A second deformation had little effect on the area other than development of a weak strain-slip cleavage (S_2).

A major (D_3) deformation broke the area into basement blocks and established the present dominant N60-80E grain of the region with little regard to the older F_1 trends. Part of the D_3 involved basement uplift of Mine Ridge and upturning of the Chester Valley zone. Presently available evidence on D_3 structures is permissive of (but not conclusively for) McKinstry's right lateral displacement of basement masses. This interpretation warrants further investigation in view of the discovery of C. L. Drake, et al (1963) of major oceanic faulting of the east coast of North America. That offset projects toward this area and may well be related to the changing tectonic trends of the Appalachians.

A final deformation superimposed a system of S_4 kink planes on the area subsequent to the establishment of the new tectonic trends.

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DISTRIBUTION OF THE AGED IN PENNSYLVANIA

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ABSTRACT

In 1960, one of every 10 persons in Pennsylvania was 65 years of age or older. This study examines the distribution of this group within the state by size and class of community and by minor civil divison. Gross variations in the distributional patterns reflect the major recent migratory readjustments of the population.

INTRODUCTION

The relatively rapid and continued increase in the proportion of the total population which is 65 years of age or older has fostered a growing literature on the distribution, characteristics, and problems of this group. However, few research efforts have made intensive use of the age data available in published census reports for minor civil divisions, i.e., townships and boroughs. This study examines the distribution of the aged in Pennsylvania using minor civil division data to determine whether generalizations about the distribution of the aged in other areas are true for Pennsylvania, and whether detailed mapping of this distribution can add to, or require a modification of, existing generalizations.

THE AGED IN PENNSYLVANIA AND THE NATION

In 1960, one of every 10 persons in Pennsylvania had reached or passed his 65th birthday. The relative number of aged in Pennsylvania was thus somewhat greater than in the nation as a whole (9.2 per cent) and higher than in 38 of the other 49 states. Furthermore, the relative increase of the aged in Pennsylvania between 1950 and 1960 far outstripped the growth of the total population. During the last census decade, the number of oldsters in the Pennsylvania population increased by 27.3 per cent, whereas the total population of the state grew by only 7.8 per cent. While a similarly disproportionate increase of the aged was characteristic of the United States population, the degree of disparity between the growth of; the total and the aged population was considerably greater for Pennsylvania than for the nation[1]. As a result, the rank of Pennsylvania among the states according to the proportion of their population over 65 years rose from 25th to 18th between 1950 and 1960.

Primarily responsible for the increase of the aged in the United States has been a general decrease in fertility levels during the last part of the 19th and the early 20th centuries, although a portion of the increase can be credited to increased longevity[2]. The almost universal fertility and mortality declines in the United States, however, mean that these variables are of limited utility in explaining large differences among the states and other political units in the relative number of the aged. Most commonly, local differences in the distribution of the older population are a function of the age selectivity in the migratory movements which affect these localities. Age selective migration has been particularly important in shaping the distribution of the aged in Pennsylvania, from which an estimated 460,000 persons were lost by net migration during the 1950 decade[3]. The majority of these migrants were relatively young, having a median age of 26.7 years[4]. The loss of these younger age groups obviously increased considerably the proportion of the aged in Pennsylvania's population.

RURAL-URBAN DISTRIBUTION OF PENNSYLVANIA'S AGED

In the United States generally, the proportion of the population 65 years and over is highest in the rural farm areas, intermediate in the cities, and is lowest in the rural non-farm areas. Pennsylvania departs from this pattern, although very slightly, in that relatively more of the urban population is in the older ages than is either of the rural classes of residence. However, the proportion of the aged in the total population of the non-metropolitan counties, hence chiefly rural, is greater than in the metropolitan counties. This apparent reversal is due largely to the inclusion of large numbers of rural non-farm suburbanites in the metropolitan counties. The differences between the distribution of the aged population in Pennsylvania and the nation by community class is illustrated in Table 1. In Pennsylvania, while categories of urban have somewhat higher proportions than do corresponding categories in the nation, the greatest departure from the national norm occurs in the larger cities (over 10,000) outside the metropolitan areas. The causes for this discrepancy are unknown, although out-migration of young people from the cities of this class which are located in the depressed coal regions may be a major contributory element.

In the rural areas, the greatest departure from the national norm is the smaller relative numbers of the aged in the small rural towns and hamlets. However, in Pennsylvania, as in the nation, there is a tendency for the proportion of the aged in the population to increase as the size of the hamlet decreases. In the smallest boroughs of Pennsylvania, those having fewer than 500 people, nearly 14 per cent of the total population is 65 years or over[5].

The analysis of the distribution of the aged population in Pennsylvania communities when extended to differences in sex ratios adds a new and complex dimension. Sex ratios among the urban aged in Pennsylvania are somewhat higher than in the nation in all classes of communities except the smaller urban places outside the cetropolitan counties (Table 1). The greatest departure from the national average is in the urban fringe. Within the United States as a whole, the sex ratio of the aged in all urban fringe areas is 76.7 males per 100 females. The comparable ratio for Pennsylvania is 78.5, indicating a somewhat greater pull of the urban fringe for males over 65 than is true in the nation. Greater discrepancies in sex ratios among the aged occur in the rural areas. The rural aged population in the nation has a sex ratio of 100.1; thus, the aged population is almost equally divided between males and females. Since the females considerably outnumber the males among the aged group, such a ratio is indicative of a sizeable female out-migration from the rural to the urban areas. The sex ratio for the rural aged in Pennsylvania, on the other hand, falls to 97.4. Thus, unlike the nation, the rural population of Pennsylvania is dominated numerically by females. The greater representation of older females in rural Pennsylvania could be produced by either a greater rural to urban out-migration rate for aged males or lower mobility rates for older rural females than is true within the nation.

TABLE I

AGED POPULATION: PENNSYLVANIA AND THE UNITED STATES
by Community Class

	UNITED STATES		PENNSYLVANIA	
	% over 65	Sex Ratio	% over 65	Sex Ratio
Total	9.2	82.8	10.0	81.8
Urban	9.2	76.2	10.3	76.7
Central Cities	10.0	76.5	11.0	76.6
Urban Fringe	7.3	76.7	8.5	78.5
Other Urban				
10,000	9.8	73.8	12.0	73.5
2,500-10,000	11.0	76.8	11.8	75.9
Rural	9.3	100.1	9.2	97.4
1,000-2,500	12.2	80.5	10.9	83.4
Other Rural	8.9	104.3	8.9	100.6

REGIONAL DISTRIBUTION OF AGED IN PENNSYLVANIA

The migration currents experienced by Pennsylvania during recent years would seem to account very largely for the gross patterns of the distribution of the aged within Pennsylvania. Areas with relatively high concentrations of aged are located in the northeastern quarter of the state, the rural north-central counties, the tier of townships northward from northern Indiana county to the New York state border, and scattered townships in the bituminous producing areas in western Pennsylvania. Within this latter area, particularly strong concentrations of aged are located in southwestern Fayette County, in Greene County, and in western Washington County. Among these counties, while there are several exceptions, the share of the total population composed of the aged is generally highest in the most rural areas, intermediate in the anthracite region, and lowest in the bituminous producing counties. Although there is a lack of direct correspondence between the relative size of the aged group and the degree of population loss by out-migration between 1950 and 1960, it is in these counties that the heaviest out-migration relative to total population has occurred. The five main counties of the anthracite region combined, for example, lost an estimated 177,000 people (equivalent to 17.3% of their 1950 population) by net migration durin gthe 1950 decade[6]. Even heavier migration losses relative to total population were experienced by Greene (23.2%) and Fayette (21.4%) counties.

But while the general areas of out-migration correspond closely with those having relatively large concentrations of older persons, the detailed distributional patterns of the aged cannot be so easily explained. A striking example is offered by Pike County. Pike County has the highest median age of any county in Pennsylvania, the highest proportion of persons 65 years and over, and is an area designated for redevelopment under the Area Redevelopment Act of May 1, 1961, because of low rural incomes[7]. All of these conditions would lead one to expect a net outflow of young people and a consequent increase in median age. Yet, Pike County registered a gain by net migration between 1950 and 1960. Unless Pike County has experienced an unusual population development in the past, the most likely explanation is that the in-migration included a substantial portion of aged retiring to this resort area.

Other areas in which out-migration of younger people cannot be employed to explain the distribution of the aged population occur in the anthracite region[8].

Between 1950 and 1960 every minor civil division within the Northern Anthracite Field underlain by coal experienced an indicated net out-migration. Many of the minor civil divisions immediately peripheral to the area underlain by coal, however, gained net migrants during this period. Yet, the distribution of aged persons shows little association with these migration patterns. Indeed, north and west of Scranton several of the divisions which apparently gained population by migration during the 1950 decade have the highest proportions of aged of any of the minor civil divisions of the anthracite region. The explanation of this apparent anomaly may again be in-migration of older people. It could well be that people who have accumulated capital in the anthracite area during their productive years have built retirement homes outside of but adjacent to the area of their previous residence. Equally baffling are boroughs wtihin the anthracite region which lost heavily by out-migration (and presumably lost younger persons) during the 1950's but which have relatively low percentages of aged persons in their populations. Nor can satisfactory explanations be offered at this stage of investigation for the belt of low proportions of aged people northward from Clearfield County, a belt which divides the north-central region of high aged concentrations into two segments. One can only conclude at this point that the migration patterns of the aged in rural Pennsylvania are highly varied, and hope that subsequent study can provide solutions.

The areas having the lowest proportions of aged in their populations are concentrated in the southeastern and south-central parts of the state, the area surrounding Pittsburgh, and, to a lesser degree, along the extreme western borders of the state. In these regions, net migration during the 1950 decade added rather large numbers to the population. Since these migrants were concentrated in the younger ages the older component of the population experienced a corresponding decrease. Older people are conspicuously infrequent in the population of lower Bucks County, into which came nearly all of the 117,000 net migrants added to that county's population between 1950 and 1960. Falls Township, which grew from 3,540 persons in 1950 to 29,082 persons in 1960, had the lowest proportion of aged of any minor civil division in the state (2.2%). Northern Bucks County, in contrast, in which the population was rather stationary or declined during the 1950's, had an aged component higher than the average for the state. While lower Bucks County is conspicuous because of the low proportion of aged in its population, similar areas are found in the other counties surrounding Philadelphia as well as peripheral to most of the larger cities of the state.

The major metropolitan areas of Pennsylvania exhibit highly varied distributional patterns of their aged populations. In every central city in the state, with the single exception of Chester, the proportion of the aged among the total population is higher than the average for the state. Among these large cities the highest concentration of the aged is found in Reading in which 14.4 per cent of the total population was 65 years or older. While the central cities which experienced the greatest population losses between 1950 and 1960 are cities which have the highest proportion of persons over 65, the association is far from perfect. Away from the central cities into the suburbs, the aged component of the population decreases, although the decrease is far from regular. It would appear as though the degree to which the aged are represented in the population of the suburban minor civil divisions is directly related to the age of the suburb. Such a speculation is certainly in keeping with findings of studies in other areas[9]. Residents of suburbs developed in earlier years, unless replaced by subsequent in-migrants, will age as the suburbs age. It is interesting to note that around nearly every major city in Pennsylvania, the suburban areas nearest the city, and presumably the longer-developed areas, commonly have higher proportions of aged than do those located at greater distances from the city.

In non-metropolitan Pennsylvania, and often intermingled with areas having high proportions of older people, are numerous townships and boroughs having relatively small numbers of aged in their populations. Some of these areas can be readily explained as in the case of State College Borough and the adjacent townships where the large numbers of students decrease the proportional representation of the aged to less than 5 per cent of the total population. Other areas remain puzzles which can be solved only by detailed studies.

Throughout the state, but especially in the rural areas, a conspicuous feature of the distribution of the older population is the degree to which the boroughs exceed the townships which surround them in the percentage of the population 65 years of age or over. In the non-metropolitan counties the average borough has about 3.5 per cent more of its population in the older ages than does the township in which it is situated, a distribution which reflects the tendency of the rural town to function as a retirement center for the surrounding population.

CONCLUSION

This study represents the first phase of what is to become an intensive examination of the distribution and characteristics of the aged population of Pennsylvania. Already, in this stage, the utilization of minor civil division age data has revealed highly varied and complex patterns of distribution and migration of Pennsylvania's older population, patterns which remain obscured when analysis is limited to county totals of aged persons. Subsequent research will examine the sex ratios of the aged for the minor civil divisions in hopes that the patterns revealed will aid in the explanation of distribution of the state's older people and in the unraveling of the complex threads of their migrations.

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5. Census data on age are provided for all incorporated boroughs in Pennsylvania. The degree to which the age structure of the small boroughs is reflective of all hamlets of similar size is unknown.
6. Calculated from the Pennsylvania Statistical Abstract. 1961. Pa. Dept. Internal Affairs. p. 11.
7. Redevelopment Areas, April 20, 1962 (map). Area Redevelop. Admin., U. S. Dept. Com.
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9. *c.f.* Sheldon, H. D. 1958. The Older Population of the United States. Wiley. pp. 34-37.

SHIFTS IN TRANSPORTATION ROUTES: THE RHODE ISLAND EXAMPLE

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ABSTRACT

The relocation of a major transportation line has such far-reaching consequences on the places newly contacted or by-passed that it is worthwhile examining the causes and nature of such relocations. An examination of route shifts in a particular area sheds light on the kinds of factors that are important as well as on the general processes involved.

When a major road or railroad changes its course, there are important consequences both for the places newly contacted and for those no longer so served. Lost or gained are fast and frequent access to large urban markets, possibilities for tapping supplies of raw materials and finished goods passing along the route, and opportunities to serve travelers. Places newly on a major transportation line tend to enjoy increased employment and population whereas places no longer so situated commonly lose employment and population.

Relocations of a major route have such far-reaching consequences that the *causes* of such shifts are well worth considering. The present paper considers first the general nature of route changes, and then the particular historical developments in one area as illustrative of the kinds of factors important in other places as well.

The course of a road is controlled primarily by its destinations, i.e., by its two ends, and secondarily by intermediate points such as mountain passes, river crossings, swamps, and the like. If changes occur in either the terminal or intermediate control points, then the route may shift. Let us forget the case of a change in a terminal point (obviously the route *must* then change) and focus our attention on the route whose termini remain constant but which, nevertheless, experiences shifts in at least some parts of its course. That shifts do occur in such routes there is abundant historical evidence. In the great majority of such cases, the change makes the route more nearly straight, as for example, the early elimination of twistings around rocks and fallen trees or, in a later stage of development, the complete relocation of a portion of the route. This suggests that the normal development is a progressive straightening of a route more direct may be considered an exception to the rule, and that any shift in a route which does *not* make the route.

Why should a straightening of routes be so typical? Primarily, it would appear, because men everywhere seem to desire the least expenditure of effort necessary, and the straightest route is the shortest and quickest at least in theory, and commonly in reality as well. Man's desire not to expend any more effort than necessary would also seem to be responsible for the exceptional cases where a shift takes place which does not make the route straighter. In such cases, however, there is almost inevitably some particular condition so that in fact the shift does result in a saving of time or effort, such as the elimination of a steep grade, or of the delay at a ferry crossing, or the avoidance of congested urban traffic.

The speed at which a route becomes straight depends on a number of factors: topography, technologic know-how, financial ability, and, perhaps, the degree of social organization. Thus a new form of transportation such as the railroad, or a new road surface such as macadam, or a transportation company uniting several formerly independent links into one through system may be the necessary and sufficient cause of a shift in a route.

A case history can best illustrate these ideas, and Rhode Island serves nicely (see map). Situated on the coast of southern New England, part-way between New York and Boston, Rhode Island found itself from earliest times on an increasingly busy thoroughfare. Between the two major cities, four routes developed. One was by water eastward from New York along the coast past Rhode Island to Cape Cod and then north to Boston. A variant of this route was even more popular because it avoided the dangerous, exposed passage around Cape Cod; it traversed Narragansett Bay as far inland as possible, thus passing through Rhode Island. From the head of Narragansett Bay, at Providence, travelers then went by land the last 50 or so miles to Boston. From Boston there developed three land routes which converged in Connecticut: the Upper, Middle, and Lower Post Roads as they were known in colonial times. The Upper and Middle Post Roads ran from Boston westward and southwestward to the Connecticut River, then southerly to the coast. They did not touch Rhode Island and need not concern us here other than that they offered alternative routes to the Lower Post Road. This latter route led from Boston to the head of Narragansett Bay, and then followed the western shore of Narragansett Bay to Long Island Sound whose coast it then followed to New York. The Lower Post Road thus essentially traversed Rhode Island diagonally. Let us look further at this route and the variants of it which developed.

Rhode Island is so small, its greatest diagonal dimension measuring only about 58 miles, that one might not expect the main route across it to have shifted much. It did, however, repeatedly. Eight land routes have been of prime importance at one time or another, and some of them experienced recurring periods of favor. The shift from, and return to, the several routes illustrates nicely the effects of topography, technologic skills, social organization, and other factors.

The earliest important land route through Rhode Island followed the coast. Travel by land was commonly preferred to travel in the small, unwieldy sailboats of the time, especially when most people did not know how to swim. Of the several Indian trails across the land, the one along the coast was preferred for two main reasons: the route lay near sea level much of the way instead of going up hill and down as did trails further inland, and, perhaps, of greater importance then, it was along the coast that most of the early settlers were located. A traveler quite naturally took a route through as many settlements as possible in order to minimize his chances of getting lost and to increase his opportunities for obtaining food and shelter. In Rhode Island the coastal route was known in the 17th century as Pequot Path because it led to the Pequot Indian country along the Thames River in eastern Connecticut. After 1703 when the road was officially laid out it was known as Post Road, a name still used. Along this road passed Madame Knight in 1704 on her way to New York, and her diary, one of our earliest colonial travel records, vividly portrays the problems and dangers then of getting lost, hiring guides, crossing hazardous fords and ferries, horses going lame, and dirty hostels with inedible food.

During the early decades of the 1700's, settlement spread inland from the coasts of the Bay and the Sound into western Rhode Island and eastern Connecticut. Then a shortcut became feasible. A road, laid out in Rhode Island in 1714, led

southwestward from Providence through Plainfield, a town in eastern Connecticut, to Norwich at the head of the Thames estuary. Plainfield Road crossed more hilly country in Rhode Island than did Post Road, but it was more direct and had the additional great advantage of avoiding a dangerous crossing of the Thames near its mouth. Throughout the 18th century, Plainfield Road became increasingly popular, and during Revolutionary times it was used by George Washington and by the French army which disembarked in Rhode Island.

So popular was this route that toward the end of the century, when the turnpike fever which had swept England began to be felt in this country too, it was Plainfield Road toward which attention was directed first. But in 1805 when Plainfield Pike was completed, much of it lay north of old Plainfield Road; the towns through whose southern parts old Plainfield Road passed refused to allow any turnpike except one more centrally located within them, an example of the influence of political boundaries on the course of a road. The resulting Plainfield Pike crossed more hilly country than did old Plainfield Road, but the turnpike surface was smoother and better maintained and so travel was faster on it. For some two decades in the early 1800's, Plainfield Pike was the preferred route across Rhode Island.

Meanwhile, other developments were taking place to the west in Connecticut and New York which were to have far reaching effects on travel in Rhode Island. Steam-power had been found to be applicable to water transportation, and steam boats began scheduled runs between New York and points up the Connecticut coast: first to New Haven, next to the Connecticut River towns, and then to New London at the mouth of the Thames. Steamboats offered a ride which was not only faster than that by stagecoach but also much more comfortable. When steamboat service reached New London, turnpike connections with Providence were commenced. Before the turnpike was finished, however, Connecticut had granted permission for steamboats to use its southeasternmost town, Stonington, and so the turnpike through Rhode Island ran to Stonington although it was known by the name of its original destination, New London.

Opened in 1820, the New London turnpike was listed in the tourist guides of the time, but was actually important for less than eight years. Steamboat service to Providence began in 1822 and grew rapidly in popularity; in 1828 the New London turnpike company sold its equipment.

Steamboats became faster and larger, and traffic between Boston and New York increased tremendously, especially passenger travel. Until about the end of the century, the water route was by far the most popular way through Rhode Island.

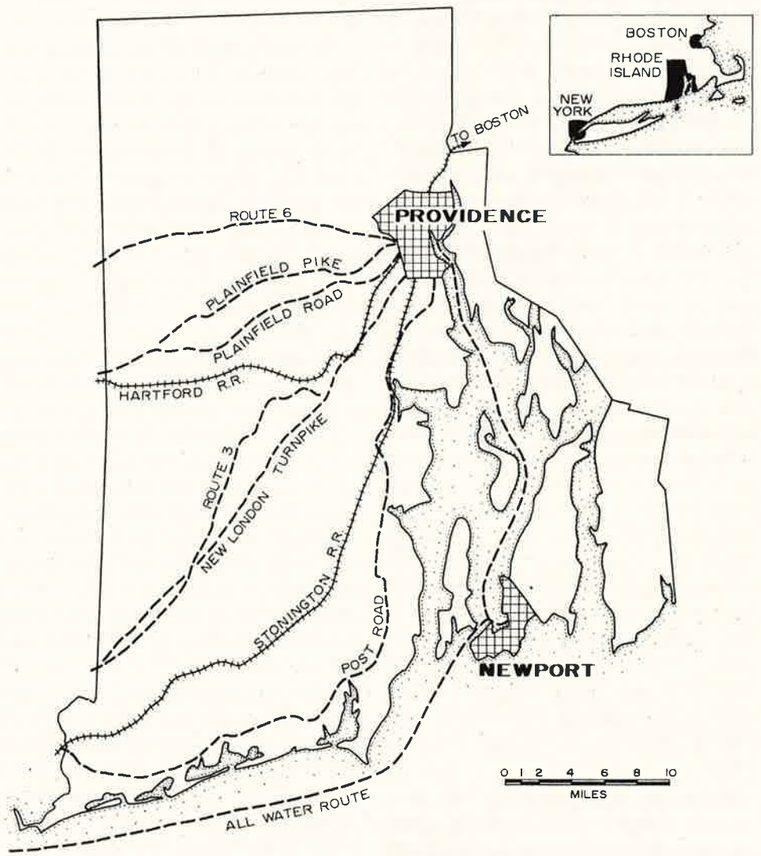
Still, there were other alternatives that developed. The conspicuously large and rapidly increasing amount of travel between Boston and New York made further improvements in transportation feasible, and one of the first three railroads built from Boston ran to the head of Narragansett Bay, replacing the so-called boat coaches. So successful was this Boston and Providence Railroad that an extension was opened two years later, in 1837, to Stonington where it connected with the steamboats to New York. Travel from Providence to Stonington by rail was much faster than by boat and also was more comfortable than the choppy passage around Point Judith at the southwesternmost corner of Narragansett Bay. The railroad, compared to the old Post Road, followed a somewhat shorter, more inland route constructed across several swamps which the Post Road had avoided.

Throughout the rest of the 19th century, Rhode Island, along with the rest of New England, was caught up in a railroad fever, but no serious competitor to the water route developed until near the 1890's. A variant of the water route started at Fall River, Massachusetts, on the eastern shore of Narragansett Bay. The Fall River steamboat line ran for some 90 years, until 1937. In 1854 a railroad was opened from Providence to Hartford, where other rail connections could be made for New York. This route was the only land route which was not, basically, a variant of the Lower Post Road along the coast of Connecticut. There were, however, no through trains from Boston to New York, because several competing railroad companies owned the various links. Thus the great advantage of the railroad over the steamboat, speed, was never adequately realized. Still, many people preferred an all-land trip and took this route between Providence and New York. The trains could never match the so-called floating palaces, however, in comfort and elegance.

Meanwhile in Connecticut, links in a coastal railroad were gradually completed by several companies. The Connecticut River was bridged near its mouth in 1870, and the Thames in 1889, so that finally a no-ferry rail line existed along the shore. No through New York to Boston trains used this route, however, until the several links all came under the control of one company, in 1893. Thereupon the almost gradeless shore line route rapidly supplanted the interior routes with their sharper curves and heavy grades. It also eventually supplanted the much slower steamboats. Thus did the degree of business organization affect the location of the most traveled route across Rhode Island.

The final chapter of the shifting main route concerns the modern highways. The automobile had one great advantage over the railroad, convenience. A person determined his own timetable. Roads at the turn of the century were in very bad repair, as they had been ever since the railroads destroyed the turnpike companies. As first bicyclists and then motorists demanded better roads, improvements were made: first in the cities, and then progressively farther out. From Providence the first lengthy improved road ran southward to the various beaches and resorts, to which there was the greatest flow of traffic. Motorists driving to New York quite naturally took the best road, and so the old coastal route across Rhode Island, the Post Road, came back into favor. As the most used road, it was continually improved, whereupon it drew still more traffic. When numbered highways were established nationally, old Post Road became part of U. S. 1.

Other modern highways also were built, but they did not begin to rival the coastal route until after the Second World War. Then an increased demand for a shorter, faster way across the State, avoiding the heavy resort traffic, resulted in the construction of a modern highway generally along the course of the old New London turnpike but swinging away from it to avoid some steep grades. As sections of this super-highway, Route 3, have been opened, more and more through traffic has used it. In the past half dozen years, Connecticut has built a new toll road from its New York border eastward, essentially parallel to the old coastal or Lower Post Road. Hoping to attract Boston traffic, the Connecticut Turnpike leaves the coast near New



Successively Important Providence-New York Routes

London and swings inland past Norwich toward the northeastern corner of the State. Its route thus passes within a couple of miles of the Rhode Island boundary and less than 30 miles west of Providence. Accordingly, Route 6, the highway running most directly west from Providence to the Connecticut Turnpike, has recently become the fastest way across Rhode Island from Providence to New York.

In conclusion, it is evident from what has been stated that even such a small area as Rhode Island reveals much about the nature of routes and their changes. The particular course which a main road takes may be influenced not only by topography and technologic inventions but also by such factors as political boundaries and social organization. Distant as well as local developments may be significant. Underlying all shifts is the desire for the fastest, easiest route possible.

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AREAL DIFFERENCES IN THE IMPACT OF THE DECLINE IN MINING ON THE ANTHRACITE REGION*

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ABSTRACT

This study is concerned with areal differences in the impact of the 1950-1960 decline in coal mining on the 55 towns of the anthracite region which have a population of 2,500 or more. Its purpose is fourfold: (1) to document the development of such areal variations during the decade; (2) to demonstrate, cartographically, the marked degree of spatial correlation between such variations; (3) to advance an hypothesis as to possible causes for such variations and their spatial correlations; and (4) to speculate on some implications of these conditions insofar as they may be related to attempts at revitalizing the anthracite area.

INTRODUCTION

The economic and demographic characteristics of the Pennsylvania anthracite region (Fig. 1) have been undergoing marked alterations since the beginning of secular decline in the coal mining industry of the area about 1930. The changes have been most traumatic in intensity since 1950, when mining employment began to experience drastic percentile decline (Fig. 2).

Literally volumes have been written in recent years about the declining anthracite industry of the state, and the effects of a shrinking mineral base on the people and economy of the anthracite region as a whole. However, to the authors' knowledge, no comprehensive study has thus far been made of *areal* differences in the impact of the contracting industry on its dependent communities. It is the purpose of this paper, therefore: (1) to document the development of such areal variations during the decade, 1950-1960; (2) to demonstrate, cartographically, the marked degree of spatial correlation between such variations; (3) to advance an hypothesis as to possible causes for such variations and their spatial correlations; and (4) to speculate on some implications of these conditions insofar as they may be related to attempts at revitalizing the anthracite area.

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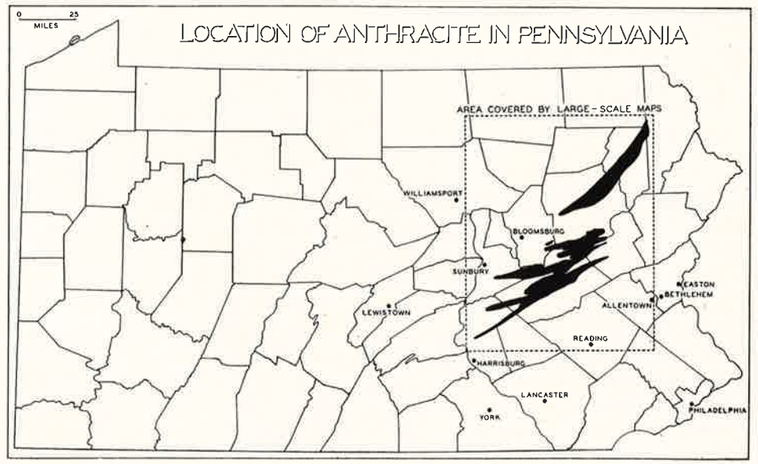

Fig. 1. Location of Anthracite in Pennsylvania.

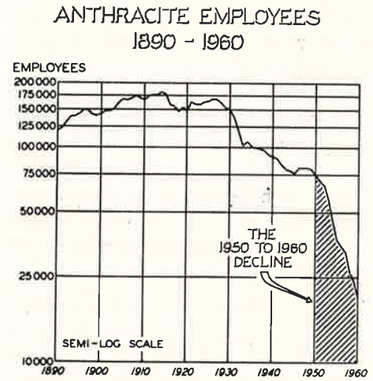

Fig. 2. Anthracite Employees, 1890-1960. Note use of semi-log scale.

It is assumed throughout the paper that changes in the economy and demography of the region since 1950 are primarily the result of the drastic decline in its coal mining industry. On the basis of previous studies that have been made of the region as a whole, the assumption is undoubtedly in large measure correct.

SOURCE AND LIMITATIONS OF DATA

Most of the facts and conclusions contained in this paper are based upon United States census data[1] concerning the 55 towns in the anthracite region having a 1960 population of 2,500 or more (Fig. 3). Thirty-three of these are located in the Northern Field and include eight towns with populations of over 10,000, namely, Carbondale, Dunmore, Scranton, Pittston, Kingston, Wilkes-Barre, Plymouth, and Nanticoke. Four of the 55 towns are situated in the Eastern Middle Field and one, Hazleton, has a population in excess of 10,000. Nine of the 55 towns are in the Western Middle Field, and three (Shenandoah, Mt. Carmel, and Shamokin) exceed 10,000. The remaining nine of the 55 towns are found in the Southern Field, with two (Tamaqua and Pottsville) having over 10,000 population.

It is evident from Figure 3 that, except for the southwestern portion of the Southern Field, most parts of the anthracite region are relatively highly urbanized. The essentially urban character of much of the area is confirmed by a study of population data concerning its towns and minor civil divisions; these data indicate that some four-fifths (79.1 per cent) of the approximate total of 719,000 people reside in towns of 2,500 or more population.

The highly urbanized character of the anthracite region is emphasized here because of the vital significance of this point to all that follows. For the analysis of spatial variations in the effect of the contracting mining industry on the region is dependent solely upon census data pertaining to the population of its urban centers, and such an analysis is valid only to the degree that people of the region reside in towns.

TYPES OF DATA

Based upon a subjective evaluation of the relative merits of various indicators of economic and demographic change, and subject to the constraint of availability from census sources, it was decided that 11 measures of change would be employed to ascertain intra-regional differences in the impact of the decline in mining on the character of the 55 anthracite towns. Four of the indicators relate to the employment situation, and include: percentage decline in total labor force; and percentile point increase in total unemployment, male unemploy-

ment, and female component of total employed. Three additional indicators are concerned with the occupational structure, and include: percentile point increase in manufacturing employment, and services and trade[2] employment; and percentile point decrease in mining employment. The remaining four indicators deal with the age structure of the 55 anthracite towns, and include: percentile point increase in the 0 to 14, 45 to 64, and 65 and older age groups; and percentile point decrease in the 15 to 44 year age group.

Obviously, data relating to additional or alternative measures of economic and demographic change are available from census sources. However, use of added indices probably would produce redundant results, and certainly would expand this study beyond permissible limits of available space; and substitution of other

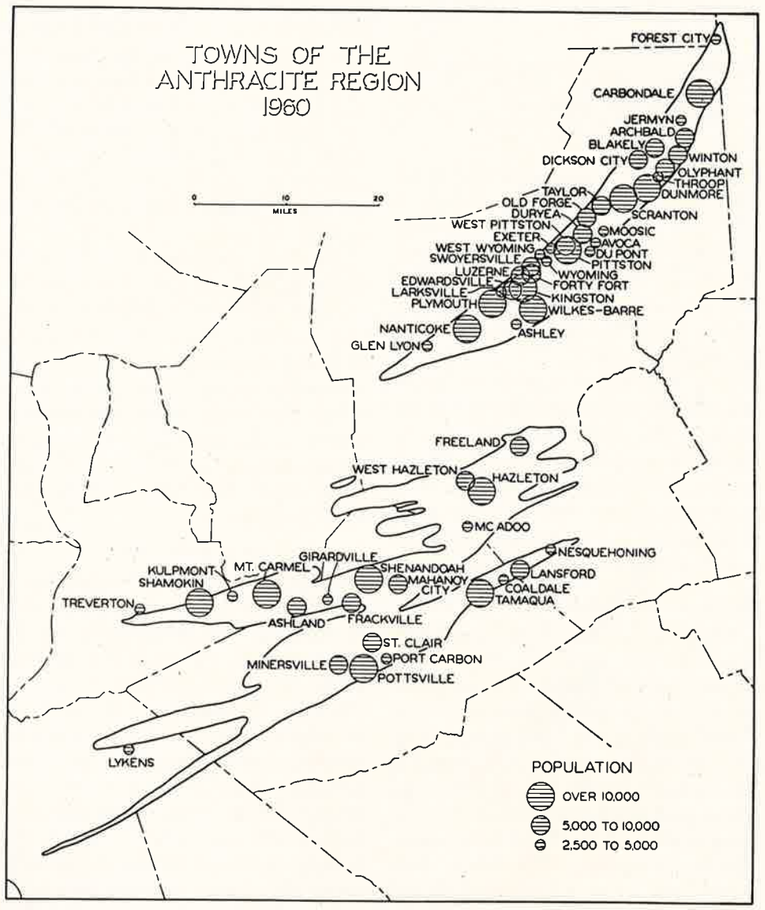

Fig. 3. Towns of the Anthracite Region, 1960.

indices to replace those selected could be validated only on the same subjective basis employed in the original choice.

MANIPULATION OF DATA

The following procedures are employed to manipulate raw census data into appropriate form for utilization in this paper. These procedures apply to all 11 indices of change listed above except the first, which is evolved by self-evident means. The over-all unemployment situation is selected to illustrate the process.

First, the percentage[3] of unemployed in the 1950[4] total civilian[5] labor force[6] is calculated for each of the 55 anthracite towns. Next, equivalent urban unemployment percentages are determined for 1960, and the difference between 1950 and 1960 percentages for each town (either plus or minus) is plotted on a map (Fig. 4). These figures, which

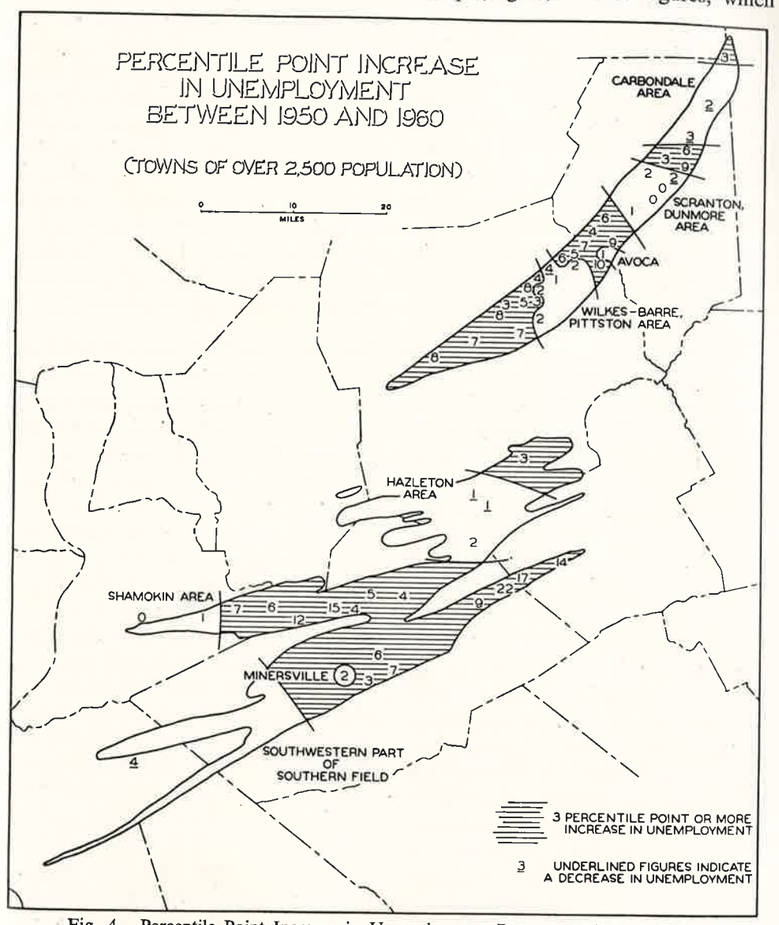

Fig. 4. Percentile Point Increase in Unemployment Between 1950 and 1960.

represent percentile point (not percentage) changes in unemployment between 1950 and 1960, are seen on the map to range from an increase of 22 percentile points to a decrease of 4.

Then the average percentage of unemployment among the combined populations of all 55 anthracite towns in 1960 is calculated (10 per cent), the equivalent 1950 percentage is determined (7 per cent), and the latter is subtracted from the former to yield an average decennial percentile point increase in unemployment for the entire anthracite region (3 percentile points). On the map (Fig. 4), shading is placed on those portions of the anthracite region having towns with percentile point values of increase in unemployment equal to or higher than the regional average, thereby indicating graphically the above-average impact of the decline in mining on the unemployment situation in those areas. Remaining portions of the anthracite region, which are characterized by towns having values below the regional average, are left unshaded and are named, in most instances the names being those of the largest city or cities within their limits (Fig. 4). The unemployment situation in such unhaded areas, of course, is least aggravated by the decline in coal mining between 1950 and 1960, or, indeed, conditions actually might have improved over the period.

The positions of boundary lines between ruled and unruled areas on the map are, in some cases, rigidly determined by towns in close proximity to one another having sharply differing values; but, in other instances, the boundary lines are more or less arbitrarily positioned by judgment between widely spaced towns belonging in opposite categories[7]. In the latter situation, when equivalent boundary lines between towns must be constructed on more than one map, care is taken to duplicate the position and configuration of such lines on all of the maps involved.

As a final step in the data manipulation process, the ruled areas on the overall unemployment map (Fig. 4) are transferred to an appropriate summary map (in this case, Fig. 5) dealing with all phases of the unemployment situation under analysis, and are identified by symbol in the legend of that map.

Applying the above procedures, or their equivalent, to each of the remaining 10 previously selected indices, one is in a position to accomplish two of the objectives of this paper, namely: to document the development of areal variations in the impact of the declining anthracite industry on its 55 dependent communities during the 1950-1960 period; and to demonstrate, cartographically, the presence of a marked degree of spatial correlation between such variations.

IMPACT OF THE DECLINE IN MINING ON THE EMPLOYMENT SITUATION

As is well known, the employment situation in the anthracite region has deteriorated drastically in recent years. The area experienced an average 13 per cent decline in its labor force between 1950 and 1960, as many workers moved elsewhere in search of jobs. The percentage of unemployment among the region's remaining labor force increased from 7 to 10 per cent during the decennium, and male unemployment advanced from over 8 per cent to more than 12 per cent. As mines closed or reduced output, and laid off more and more male employees, the female component of the region's work force grew from an already high 31 per cent in 1950 to an even more extreme figure of 39 per cent by 1960.

Analysis of 1950 and 1960 employment data relative to each of the 55 towns of the anthracite region reveals, however,

that the effects of the dying mining industry were not everywhere equally severe. Decennial change in the size of the labor force varied all the way from a decrease of 39 per cent in one town to an increase of 31 per cent in another. Change in total unemployment, as was pointed out earlier (Fig. 4), ranged from an increase of 22 percentile points in one town to a decrease of 4 in another; and, for male unemployment, the equivalent figures were an increase of 38 percentile points and a decrease of 6. The female component of the laboring force grew by 22 percentile points in one town, but by a mere 1 percentile point in another. It is apparent from these figures that individual anthracite towns have been reacting in widely divergent fashion to the recent traumatic contraction of the

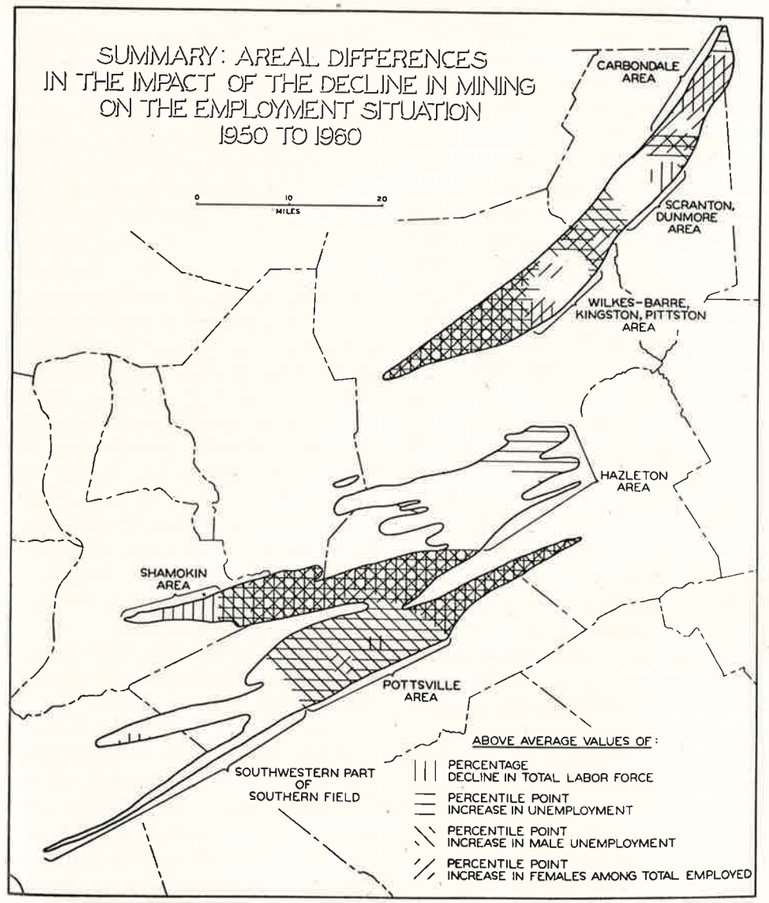

Fig. 5. Summary: Areal Differences in the Impact of the Decline in Mining on the Employment Situation, 1950 to 1960.

region's once dominant coal mining industry.

The map in Figure 5 summarizes these areal divergencies in employment trends, and indicates the spatial correlations that exist between such areal variations. As is indicated in the legend on the map, towns in the vertically ruled areas experienced greater decennial percentage declines in size of total labor force than the 13 per cent average for the anthracite region as a whole. Towns in horizontally ruled areas had an increase in total unemployment that exceeded the 3 percentile point regional average; and towns in areas ruled diagonally from upper left to lower right were characterized by a larger increase in male unemployment than the 4 percentile point regional average. Finally, towns in areas ruled diagonally from upper right to lower left realized an increase in the female component of their laboring force that was larger than the 8 percentile point average for the entire region.

It is evident from Figure 5 that the employment situation in parts of the anthracite region has undergone relatively little change between 1950 and 1960. Scranton, Dunmore, Wilkes-Barre, Kingston, Pittston, Hazleton, and some of their small neighboring towns, as well as the southwestern part of the Southern Field, all have escaped the more traumatic employment conditions that have shaken the very economic foundations of less fortunate parts of the anthracite region. Several other areas have experienced above-average impact in only one or two of the four aspects of employment that are mapped; these include the Shamokin area, in which there was an above-average decline only in size of labor force; the greater Pottsville area, which in most parts had an above-average increase only in total and male unemployment; and the Carbondale area, which nowhere was characterized by above-average values in more than two of the adverse aspects of employment. The towns of the remaining, unnamed, portions of the anthracite region, in contrast, were simultaneously severely affected by above-average values of most or all of the four employment factors mapped.

IMPACT OF THE DECLINE IN MINING ON THE OCCUPATIONAL STRUCTURE

Equally as drastic changes have occurred in the anthracite region's occupational structure as have characterized the employment situation during the 1950-1960 decade. The mining industry, which accounted for more than 20 per cent of the area's total working force in 1950, hired less than 5 per cent of all workers by 1960 and thus had dwindled to insignificance. On the other hand, manufacturing expanded in importance as a regional employer, its share increasing during the decade from 27 to 36 per cent of the laboring force. Decennial employment in services and trade also increased, from approximately 48 to 52 per cent. Other occupational categories —agriculture, construction, fishing, and forestry—have remained insignificant, none of them accounting for as much as 4 per cent of the total employed in either 1950 or 1960, and none changing in significance over the decade by more than a fraction of a per cent.

Analysis of 1950 and 1960 occupational data for each of the 55 towns in the anthracite region, moreover, reveals the same extremes in values from locality to locality that characterized the employment data previously described. Decline in mining employment between 1950 and 1960, for instance, ranged from a maximum of 54 percentile points in one town to a minimum of 4 in another. Similarly, the decennial change in manufacturing employment varied from a 36

percentile point increase to an 11 point decrease; and, for services and trade employment, from a 31 percentile point increase to a 17 point decrease. Obviously, the recent occupational transformation of the 55 towns in the anthracite region has been anything but uniform.

The map in Figure 6 summarizes areal differences in the impact of the decline in mining on the occupational structure of the anthracite region between 1950 and 1960. Towns in vertically ruled areas enjoyed a greater decennial increase in manufacturing employment than the 9 percentile point average for the region as a whole; and towns in horizontally ruled areas were characterized by greater growth in services and trade employment than the 4 percentile point average. On the other hand, urban centers in the di-

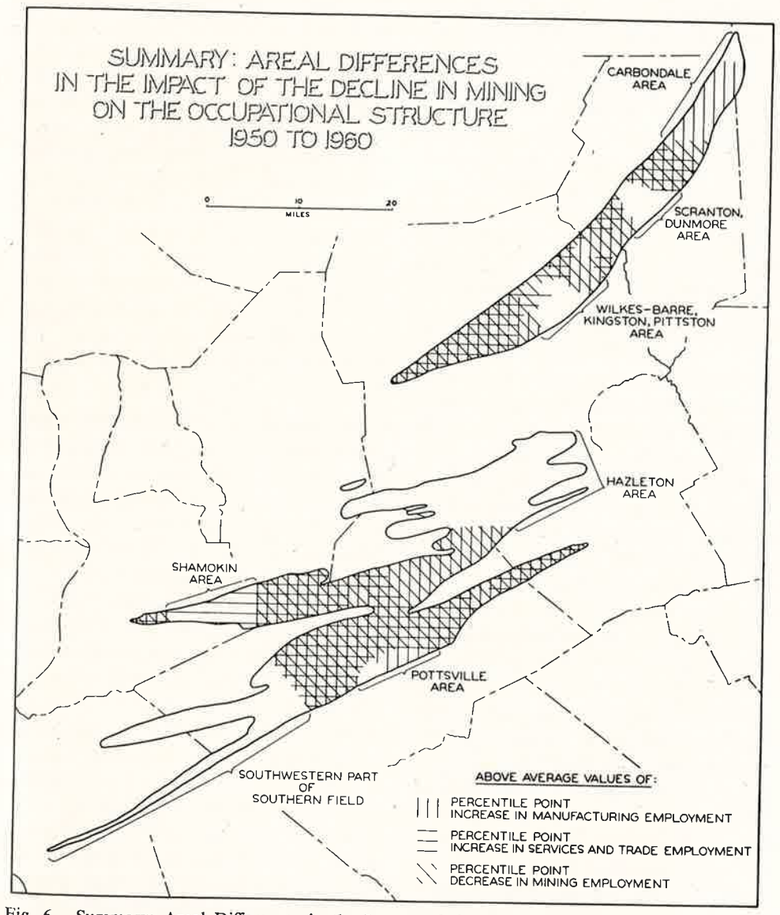

Fig. 6. Summary: Areal Differences in the Impact of the Decline in Mining on the Occupational Structure, 1950 to 1960.

agonally ruled areas experienced declines in mining employment exceeding the 16 percentile point mean for the region. In combination, the patterns of superposed lines on Figure 6 indicate graphically those portions of the anthracite region whose occupational framework, between 1950 and 1960, has been literally wrenched out of all semblance to its former configuration; as well as other segments of the region in which there has been considerably less occupational dislocation. Among the latter class of areas are those in the vicinity of Carbondale, Scranton, Dunmore, Wilkes-Barre, Kingston, Pittston, Hazleton, Shamokin, and Pottsville, as well as the southwestern part of the Southern Field. Note that the patterns of occupational changes represented on Figure 6 are strikingly similar to those relating to the employment situation that are shown on Figure 5.

IMPACT OF THE DECLINE IN MINING ON AGE COMPOSITION

The movement of people away from the depressed anthracite region during the 1950-1960 period, referred to earlier in this paper, has been a selective rather than an indiscriminate process. Younger workers, having fewer personal and employment reasons for remaining in the region than older workers and retirees, and with more opportunity to find jobs elsewhere, tended to form, together with their children, a disproportionately large percentage of the out-migrants. The residual population of the anthracite region, consequently, has been increasingly characterized by an age mix differing from that of Pennsylvania as a whole. For example, people in the anthracite region aged 65 years and older increased from approximately 8 to 12 per cent of the total population between 1950 and 1960, while the corresponding change for the state was only from 9 to 10 per cent. Likewise, during the decennium, people in the 45 to 64 year age bracket increased from approximately 22 to 26 per cent of the total population of the anthracite region; whereas, in the state as a whole, the equivalent population group remained essentially static in percentage. Moreover, people of age 15 to 44 declined from approximately 46 to 37 per cent in the anthracite region, while the percentage for the state dropped only from 45 to 39. Lastly, the 0 to 14 year age group in the anthracite region merely increased from approximately 23 to 25 per cent, whereas the same age group in the state expanded from 25 to 29 per cent. These variations in percentages, while seemingly of small proportions, actually represent significant modifications in the age mix of the anthracite region which, if continued into the future for an appreciable length of time, would result in major losses of the more vigorous and promising components of the population and hence would render more difficult the economic rehabilitation of the depressed region.

Examination of 1950 and 1960 population data for each of the 55 towns of the anthracite region reveals that the effects of out-migration on age composition were not everywhere the same. Decennial increase in the 65 year and older age group, for instance, varied all the way from 10.3 percentile points in one town to 1.0 in another. Change in the 45 to 64 year age group ranged from an increase of 14.7 percentile points in one locality, to a decrease of 0.3 elsewhere. Similar extremes can be found in other age brackets: 15 to 44 years—a maximum decrease of 16.7 percentile points to a minimum of 5.1; 0 to 14 years—an increase of 5.7 percentile points versus a decrease of 8.3. As in the case of previously examined indices of change in the anthracite region, it is obvious that the impact of the decline in

mining also has not been identical on the age composition of individual towns in the area.

The map in Figure 7 summarizes changes in age structure that have occurred during the 1950-1960 decade in the anthracite region. As shown in the legend, towns in vertically ruled areas experienced an increase in the 0 to 14 year age group that was less than the 1.7 percentile point average for the region as a whole. Towns in horizontally ruled areas were characterized by a decrease in the 15 to 44 year age group exceeding the 9.2 percentile point regional average; and those in areas ruled diagonally from upper left to lower right had an increase in the 45 to 64 year age group in excess of the 3.8 percentile point regional average. Finally, localities in areas ruled

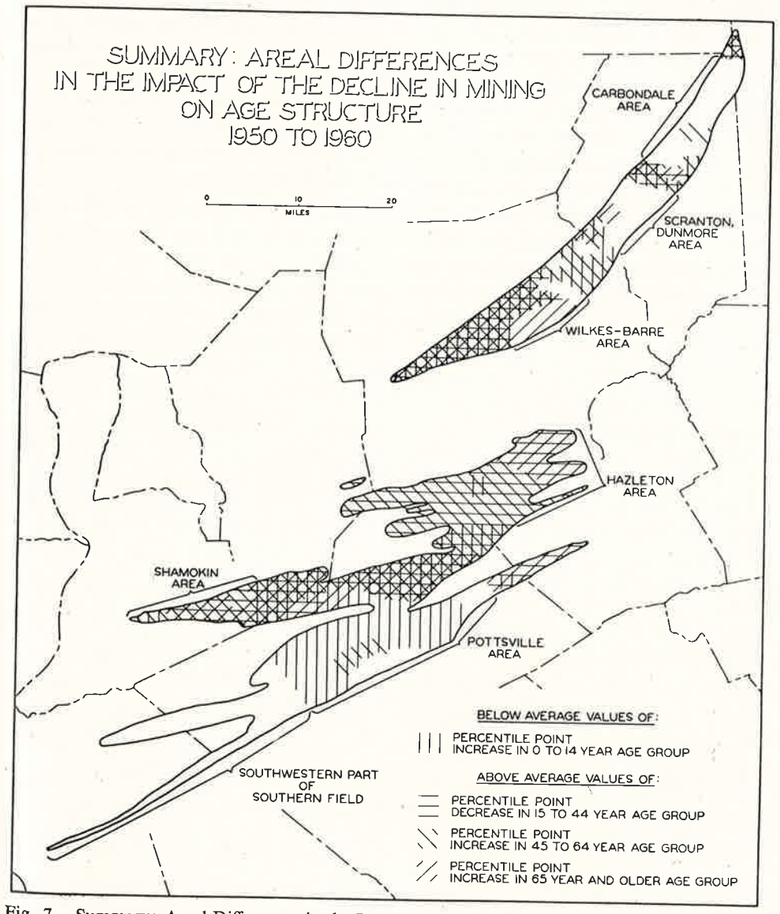

Fig. 7. Summary: Areal Differences in the Impact of the Decline in Mining on Age Structure. 1950 to 1960.

diagonally from upper right to lower left recorded an increase in the 65 year and older age group surpassing the 3.7 percentile point average for the region.

The patterns formed by the superposed lines on Figure 7 reveal widespread local divergencies in the effects on age composition of the declining mining industry. In the more darkly shaded portions of the map, major demographic modifications have been in progress since 1950. In more lightly shaded and unpatterned areas, changes in the relative importance of the various age components of the population were minimal. This latter group of areas includes many whose regional designations on the map are, by now, familiar to the reader: Carbondale, Scranton, Dunmore, Wilkes-Barre, Pottsville, and the southwestern part of the Southern Field. Note, however, the relative severity of population modifications in the Hazleton and Shamokin areas.

CAUSES OF AREAL VARIATIONS IN IMPACT

Causes for areal differences in the impact of the decline in mining on the 55 towns of the anthracite region during the 1950-1960 period are undoubtedly manifold and complex, and any attempt at complete identification and verification of such causes might well prove to be a hopeless task. Yet, the fact that approximately identical patterns are present on all three maps summarizing spatial variations in selected economic and demographic changes (Figs. 5, 6, and 7) leads one to suspect that only a limited number of causal factors play a really dominant role in determining areas of major and minor impact.

Recurrence of the names of such major urban centers as Carbondale, Scranton, Wilkes-Barre, Hazleton, and Pottsville among the areal designations used on the summary maps to identify sections least affected by change, suggests that perhaps

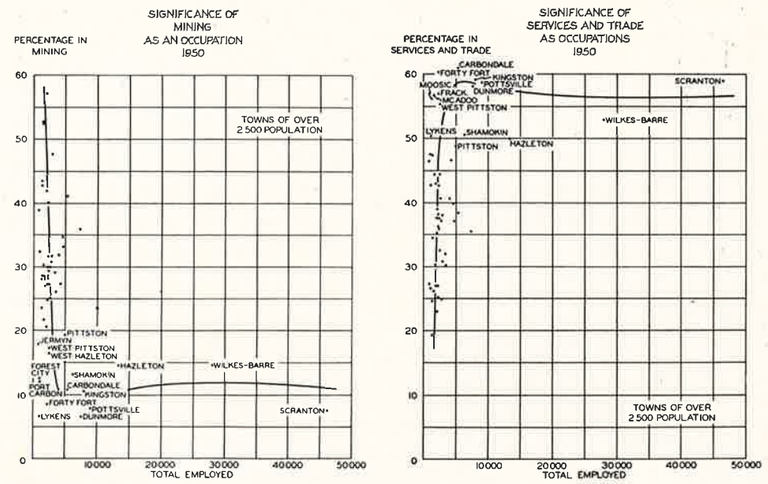

Fig. 8. Significance of Mining, and of Services and Trade, as Occupations, 1950. The term, total employed, represents the number of individuals, 14 years of age and older, engaged in all types of gainful occupations.

it is the larger cities which are most lightly touched by the convulsions now racking the region, whereas the smaller towns are not so fortunate. But this supposition is disproven when one recalls that, of the 14 anthracite towns with populations of 10,000 or more in 1960 mentioned at the beginning of this paper, five of them—Mt. Carmel, Nanticoke, Plymouth, Shenandoah, and Tamaqua—are invariably located in areas of maximum impact on all three summary maps (compare Fig. 3 with Figs. 5, 6, and 7). Conversely, a number of small towns are to be found in areas of intermediate or minimal impact on some or all of the summary maps. Examples of such small towns include: Lykens and Port Carbon in the Southern Field; Trevorton and Frackville in the Western Middle Field; McAdoo, West Hazleton, and Freeland in the Eastern Middle Field; and Forty

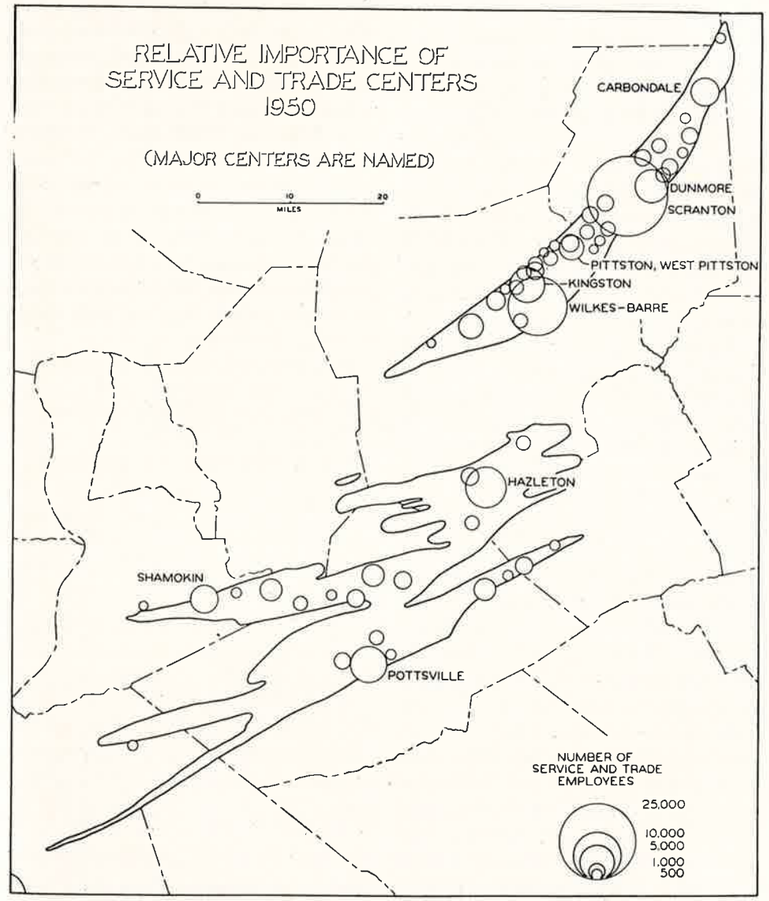

Fig. 9. Relative Importance of Service and Trade Centers, 1950.

Fort, West Pittston, Moosic, Jermyn, and Forest City in the Northern Field (compare Fig. 3 with Figs. 5, 6, and 7).

A further possible explanation comes to mind: if size of population is not significant in determining the relative degree of modification in the economic and demographic structure of the 55 anthracite towns, then perhaps differences between towns in the occupational composition of their working force may play a major causal role. To test this hypothesis, two graphs are presented, one representing the 1950 percentile significance of mining as an occupation, and the other the 1950 percentile significance of services and trade as occupations, for all 55 towns (Fig. 8). In theory, towns with limited percentages of their laboring force engaged in mining in 1950 should have been least affected economically and demographically by subsequent major decline in the coal industry; and, conversely, towns concentrating most heavily on service and trade functions in 1950 should have benefited to the greatest extent from the inherent stability of such occupations even in the face of sharp contraction in a basic industry. Examination of the graphs in Figure 8 confirms the validity of these assumptions. All nine towns whose names are used to designate areas of limited impact on one or more of the three summary maps—Scranton, Wilkes-Barre, Hazleton, Shamokin, Carbondale, Kingston, Pottsville, Dunmore, and Pittston—are seen to be characterized in 1950 by minimum dependence on mining and maximum reliance on services and trade as occupations. On the contrary, other towns in the 10,000 and above population category—Mt. Carmel, Nanticoke, Plymouth, Shenandoah, and Tamaqua—are not so favored. In addition, every small town on the graphs in Figure 8 having less than 20 per cent of total employed in mining, and/or more than 50 per cent in services and trade, during 1950, is located in an area of minimum impact on at least one of the three summary maps. These small towns are named on the graphs, and most of the names correspond to those listed above in the text.

As a check on the significance of the service and trade functions of each of the 55 anthracite towns in 1950, figures were compiled on the actual number of employees in such occupations for each town. The map in Figure 9 represents the results, and demonstrates graphically the importance of Carbondale, Dunmore, Scranton, Pittston (including West Pittston) Kingston, Wilkes-Barre, Hazleton, Shamokin, and Pottsville as major regional service and trade centers. Conversely, the relative insignificance of Plymouth, Nanticoke, Shenandoah, Mt. Carmel, and Tamaqua in these occupational categories, despite their large total populations, is evident from the map (compare Fig. 9 with Fig. 3).

In an effort to determine whether or

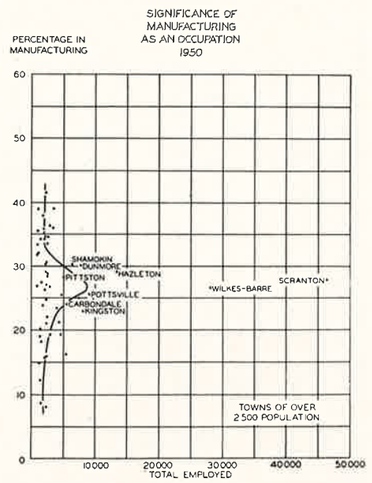

Fig. 10. Significance of Manufacturing as an Occupation, 1950.

not the percentage of manufacturing employment in 1950 also was of importance in differentiating between high and low impact towns, a graph (Fig. 10) of this factor was constructed. It is apparent from the graph that the nine large towns of minimum impact, which are named on the graph, were characterized by neither appreciably above, nor below, average percentages of manufacturing employment.

One can only assume, on the basis of the above facts, that differences in the impact of the decline in mining on the towns of the anthracite region between 1950 and 1960 are at least in major part a function of the degree of initial dependence of each town on service and trade occupations, and an inverse function of the initial dependence on mining. The size of a town's population, and the degree of dependence of a town's working force on manufacturing, seem to have no major significance in determining its reaction to the sharp regional decline in mining. One must also presume, in view of the evidence presented, that neither an especially strong community spirit, nor intense promotional drives, nor other human endeavors to mitigate the effects of a contracting mining industry have thus far been able to appreciably modify the reaction patterns established by the occupational factor.

CONCLUSION

In conclusion, it appears to the authors that this study can be justified on two grounds. One is purely academic, i.e., information concerning the variety of reactions of individual towns in a well defined economic area to drastic decline in a basic regional industry is desirable simply for its contribution to the fund of human knowledge. The second basis for justification is more immediately practical in character. For it is only through such studies that federal- and state-financed area redevelopers can assess the extent and types of damage to individual communities in an economically deteriorating region; can evaluate the relative merits of available corrective measures that might be prescribed for each of these communities; and can, with wisdom and judgment, apply such remedial procedures with greatest precision to precisely those segments of the region most severely affected.

In the opinion of the authors, the current federal area redevelopment program does not employ a sufficiently fine screen to separate areas requiring assistance from others that are presently self-sufficient. Most redevelopment areas, and areas eligible for redeveolpment assistance, in the United States today consist of large labor market areas or counties,[8] both of which criteria obviously are not appropriate for the anthracite region where requirements for aid differ so greatly from locality to locality within the same county or labor market area. Some anthracite towns demand immediate and concentrated attention; others close by have been relatively successful in maintaining, or even improving, their economic status. Such matters as this must surely plague the conscientious area redeveloper, once the facts are laid before him, for only the unsophisticated or unscrupulous official would prescribe one set of economic nostrums for the entire anthracite region.

The findings of this study raise an interesting question. Which of the 55 anthracite towns have received to date the major share of state and federal redevelopment assistance, and which the least? Have such towns as Scranton, Wilkes-Barre, and Hazleton, together with some of their nearby smaller neighbors, obtained most of the aid? If so, the possibility of redirection of funds

might well be considered, for this investigation has shown that such places have been least hurt by the decline of mining in the region. Or have some other, very badly ailing, towns which are situated in areas of maximum impact been the chief recipients of aid? The answer is unknown to the authors, but the subject would form a most suitable topic for subsequent study.

REFERENCES AND NOTES

1. *Census of Population: 1950*, Vol. II, Part 38, Pennsylvania; *U. S. Census of Population: 1960*, Final Reports PC(1)-40A, -40B, and -40C, Pennsylvania. Bur. of Census, U. S. Dept. Com. Many of the census tabulations employed in this paper are based on a total count of the relevant populations, but some are derived from 20 or 25 per cent samplings.
2. The term services and trade, as used in this paper, includes such gainful activities as transportation, communication, utility and sanitary service, wholesale and retail trade, finance, repair, personal service, entertainment, hospital service, education, religion, professional service, and public administration. It does not include agriculture, forestry, fishing, mining, construction, and manufacturing.
3. Percentage figures used in this paper in all instances are rounded off to the nearest whole per cent, except in the case of certain figures relating to age groups.
4. The years 1950 and 1960, as used throughout the study, refer specifically to the April 1 dates on which the censuses were taken.
5. The data employed in this paper relate only to the civilian component of the relevant populations. Individuals in the armed services, however, represent only a small fraction of one per cent of the anthracite region's total population.
6. The labor force, as defined in this study, comprises individuals 14 years of age and older who are engaged in gainful employment or are seeking such employment.
7. In the case of the southwestern part of the Southern Field, there is little available information because of the absence of towns having populations of 2,500 or more. Hence, conditions in this area may be misrepresented on some or all of the maps in this study.
8. See *Summary List of Redevelopment Areas and Eligible Areas, Public Works Acceleration Act*, Area Designation Status Report No. 9, Area Redevelopment Admin., U. S. Dept. Com., July 1, 1963.

THE ASHLAND, PENNSYLVANIA, TOURIST FACILITY: A GEOGRAPHIC CASE STUDY*

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ABSTRACT

Data are virtually non-existent concerning operational details of a geographical character for specific individual private tourist facilities in the United States. Despite this fact, a body of generalized theory is being developed concerning the tourist industry of the country, and major Federal and state plans for expansion of tourist facilities are being formulated—all without benefit of detailed knowledge concerning present day operational units of the industry. This paper: (1) presents a case study of certain geographic facts concerning one such unit—that at Ashland, Pennsylvania; (2) proposes tentative hypotheses concerning some of these facts; and (3) pleads the case for similar studies to be made at other tourist sites in the country.

INTRODUCTION

The Ashland, Pennsylvania, tourist facility, located in the east-central part of the state at a distance of approximately 85 miles northwest of Philadelphia and 55 miles southwest of Scranton (Fig. 1), is an assemblage of anthracite-mining memorabilia consisting of a small-scale rehabilitated underground mining operation, a steam-powered narrow gauge outdoor mine railroad, an abandoned strip mining trench, and various related miscellany. It is operated as a tourist attraction by a private non-profit corporation under the leadership of local business and professional men who donate their services. This project, through the kind cooperation of its directors, has been under study by the authors since its inception in September, 1962.

An earlier article,[1] based on data relating to operations of the Ashland facility during the terminal portion of the 1962 tourist season: (1) comprehensively describes its site characteristics and facilities, (2) estimates in broad outline some aspects of its initial impact on the depressed economy of the local area, and (3) analyzes in detail the number of visitors and the sources from which they originate.

The present paper, which utilizes much more comprehensive data for the full tourist season of 1963, continues the previous analysis of number of tourists and their major source area, and, in addition, investigates several aspects of the Ashland project that had not been considered previously because of lack of information. Among these are studies of: (1) routes of approach to the facility used by visiting groups; (2) status of the attraction as a destination for visitors, i.e., whether terminal or intermediate; (3) average dollar expenditures, by facility users, at business establishments in and near Ashland; (4) types of local businesses patronized by the tourists and the extent of their patronage; and (5) dominant factors inducing visits to the tourist site. In each case, geographical associations and variations in the patterns that evolve are noted, and, when possible, hypotheses are proposed to explain such variations.

There is ample justification for such a study. Despite the fact that tourism is a multi-billion dollar industry in the United States[2] in which almost all Americans participate, and that recreation and tourism are reported to be the first, second, or third industry in terms of finan-

* Contribution No. 63-76 from the College of Mineral Industries, The Pennsylvania State University. This investigation is part of a program of research sponsored by the Mineral Conservation Section of the University.

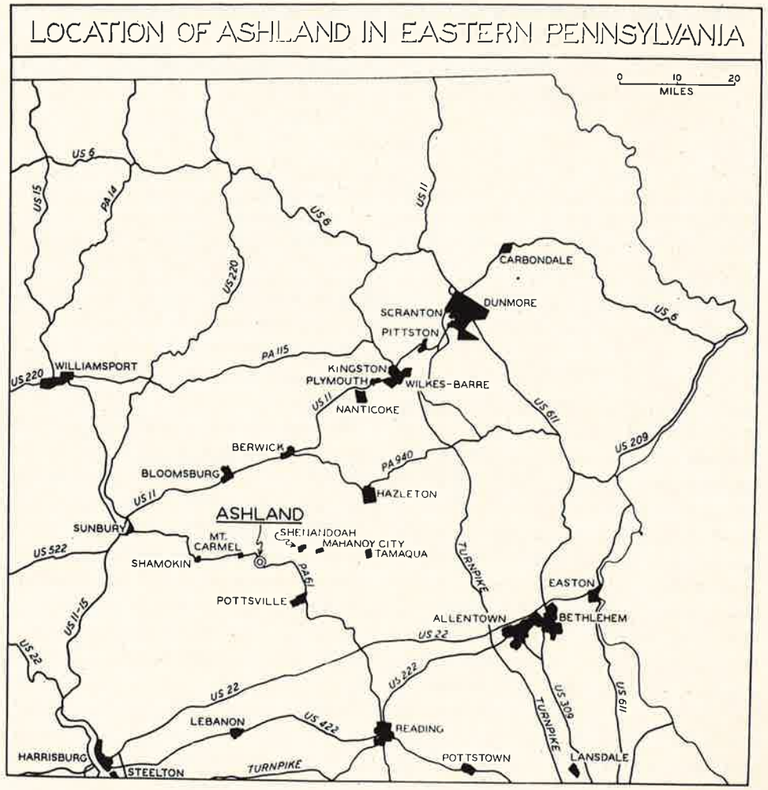

Fig. 1. Location of Ashland in eastern Pennsylvania. Only selected major highways and towns of over 10,000 population are shown, except for Ashland.

cial returns in approximately half of the states,[3] detailed published data are virtually non-existent for specific individual private tourist facilities.[4] Instead, only the most generalized estimates are available concerning the number of visitors, their seasonal concentration, their sources, their avenues of travel, their impact on the local economy, the kinds of publicity that attract them, and similar relevant data. Moreover, no attempt has thus far been made to demonstrate, and if possible to suggest explanations for, patterns of geographical association and variation that may exist with regard to each of these topics. Hence, this case study of the Ashland tourist facility may be considered somewhat in the light of a pioneer attempt in the field of the geography of individual recreational sites. To the extent that it is successful in achieving meaningful goals, it is hoped that it will serve as a stimulus for a host of similar research studies of other individual tourist attractions of both private and public ownership. The need for such studies is pressing, for a body of generalized theory concerning the tourist

and recreation industry, based largely on intuition, is even now in process of formulation, despite the lack of information concerning many of the most elemental aspects of the industry. Only through detailed research studies of individual operating units can a body of basic factual knowledge and specific explanatory hypotheses be developed that will make it possible to verify, modify, or refute presently propounded generalizations and to evolve additional principles.

NUMBER OF VISITORS

The number of individuals visiting the Ashland tourist facility during the 1963 season is unknown, for the grounds are unfenced and entrance is open to all without charge. Tickets purchased for rides on the railroad number 32,995, and for admission to the underground mine 16,724, but there is undoubtedly almost complete overlap between these figures so that total attendance would not be the sum of the two. It is known that some visitors to the site do not purchase tickets for either attraction, and that others, especially children, buy multiple rides on the railroad during a single visit, but the extent of such conditions is believed to be limited. In the absence of more specific data, therefore, it may be asssumed that the total number of visitors to the operation during 1963 approximates 33,000.

A guest registry, voluntarily signed by many visitors, is maintained at the central office and contains the names and addresses of 11,051 individuals for 1963, or approximately one-third of the total estimated to have visited the site. Analysis of the registry shows 903 names and addressses for May, 1,599 for June, 2,787 for July, 3,396 for August, 1,379 for September, 949 for October, and 38 for November, thus conforming to the August attendance peak that is believed to characterize almost all summer-oriented outdoor tourist attractions. Further examination of the registry indicates a concentration of attendance during the week-end days of those months in which the facility was in operation on a daily, rather than on a week-end only, basis: 72 per cent on Saturdays and Sundays in June, 38 in July, 45 in August, 71 in September, and 87 in October. To be noted, however, is the lesser degree of significance of week-end utilization during the traditional family vacation months of July and August. Of the two week-end days, Sunday proves to be by far the more popular, accounting for 65 per cent of week-end attendance during the full May through November season, and 36 per cent of total attendance for that period. Thus, the Ashland facility conforms to the common concept of the tourist industry as a whole in that it is not only strongly seasonally peaked during the year, but also daily peaked during the week.

All of these facts, of course, have critical implications with regard to returns on capital investment and stability of employment conditions at the Ashland tourist facility,[5] just as they are presumed to pose problems at most other outdoor recreational attractions, for grounds, equipment, and personnel must be adequate to handle temporary large surges in demand whereas at other times they remain under-utilized. Development of a multipurpose tourist facility at the site, with emphasis on winter-oriented attractions, would assist in the achievement of more nearly year-round operations, but thus far no consideration to this possibility has been given by the directors of the infant Ashland operation because of lack of time and limitations of capital. Smoothing out of week-end peaks in attendance is probably impossible, given present weekly work and school schedules of potential visitors.

MAIN SOURCE OF VISITORS

One of the most intriguing aspects of the present study of the Ashland facility, to the authors, is the opportunity it provides to evaluate their previously published facts and conclusions concerning the main source area for visitors to the project. Their 1962 paper on the subject,[1] referred to above, delimits such a source area, and arrives at a tentative hypothesis concerning the cause for deviations in the location and configuration of the actual main source are from that to be expected on the basis of theoretical principles stated in the literature.

Unfortunately, the information available at that time concerned only the first two months of limited operations for a newborn attraction that had come into being near the end of a tourist season. The representativeness of such data might be open to question on the grounds that there had been insufficient time to adequately publicize the attraction. Thus, the main source area for visitors derived from the data might be correspondingly warped in location and configuration, and the hypothesis employed to explain its aberrations would be invalid.

But, by the end of the 1963 tourist season, the Ashland project was a full-fledged operation that had been in existence for over a year and had been rather intensively publicized over a wide area for a protracted period of time. Its present main source area for visitors, therefore, should more nearly reflect the true tributary territory from which it can normally expect to derive major patronage. If the 1963 main source area duplicates, or closely approximates, that for 1962, the validity of the previously stated explanation for deviation from the expected norm is strengthened. On the other hand, if the 1963 data should result in considerable revision in the location and configuration of the main source area, the earlier explanatory hypothesis would be suspect.

The map in Figure 2 records, on a town-by-town basis, the sources of all 1963 registered guests at the Ashland operation coming from eastern Pennsylvania and states or parts of states to the immediate north, east, and south; Figure 3 is a continuation of the above map and shows, at a much small scale, similar data for a more extensive area in northeastern United States. No really important contributors of tourists lie outside the territory covered by these two maps. The main source of visitors, as determined by inspection, is outlined by a dashed line and labeled. The area thus enclosed consists of two parts: (1) east-central and southeastern Pennsylvania, and (2) the almost continuous line of large cities and associated suburbia extending from Washington, D. C. northeastward to and slightly beyond New York City. Reference to the authors' previous paper on the Ashland facility discloses a virtually identical delimitation of the 1962 main source area, the two lines differing only in the considerably greater convolutionary detail of the 1963 boundary. Obviously, 1962 data on sources of tourists are truly representative of conditions, and the previously advanced explanatory hypothesis is not invalidated by more recent information.

The eccentric shape and off-center location of the main visitor source area for the Ashland project conflict with two basic economic principles, proposed in the literature, concerning the sources of visitors to a recreational facility. The first of these states, in essence, that the number of visitors to a tourist site from two or more towns with the same populations will be inversely proportional to the costs involved in traveling to the site, the costs being expressible in terms of dollars, time, or travel distance.[6] In other words, the farther away from a tourist attraction that a potential source town is located, the fewer the visitors it will contribute to that attraction. The second principle states that the number of visitors to a tourist site from two or more towns located at equal distances from that site will be directly proportional to the populations residing within those towns.[6] Both of these principles appear highly plausible, but, to test their applicability in the case of the Ashland tourist facility, the map in Figure 4 is presented. This map shows: (1) the location and approximate population (in part inferred by the physical size of the larger

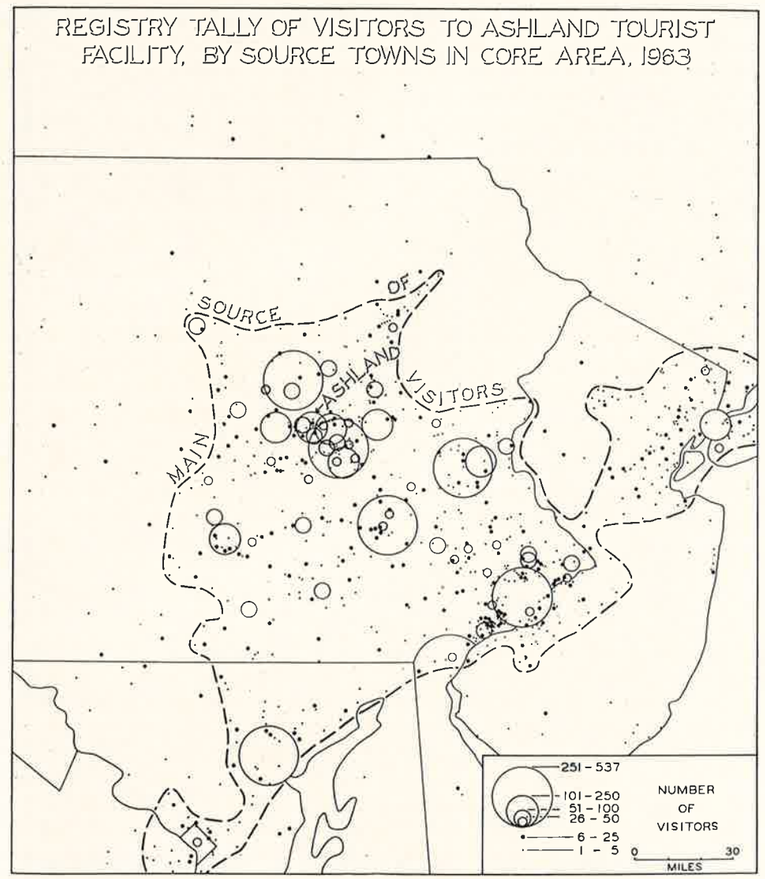

Fig. 2. Registry tally of visitors to Ashland tourist facility, by source towns in core area, 1963. Data are based on a random sampling of tourists during the seven-months period, May through November. The heavy dashed lines bound the main source area for tourists. Names of some of the more important tourist source towns in Pennsylvania may be identified by comparing this map with Figure 1.

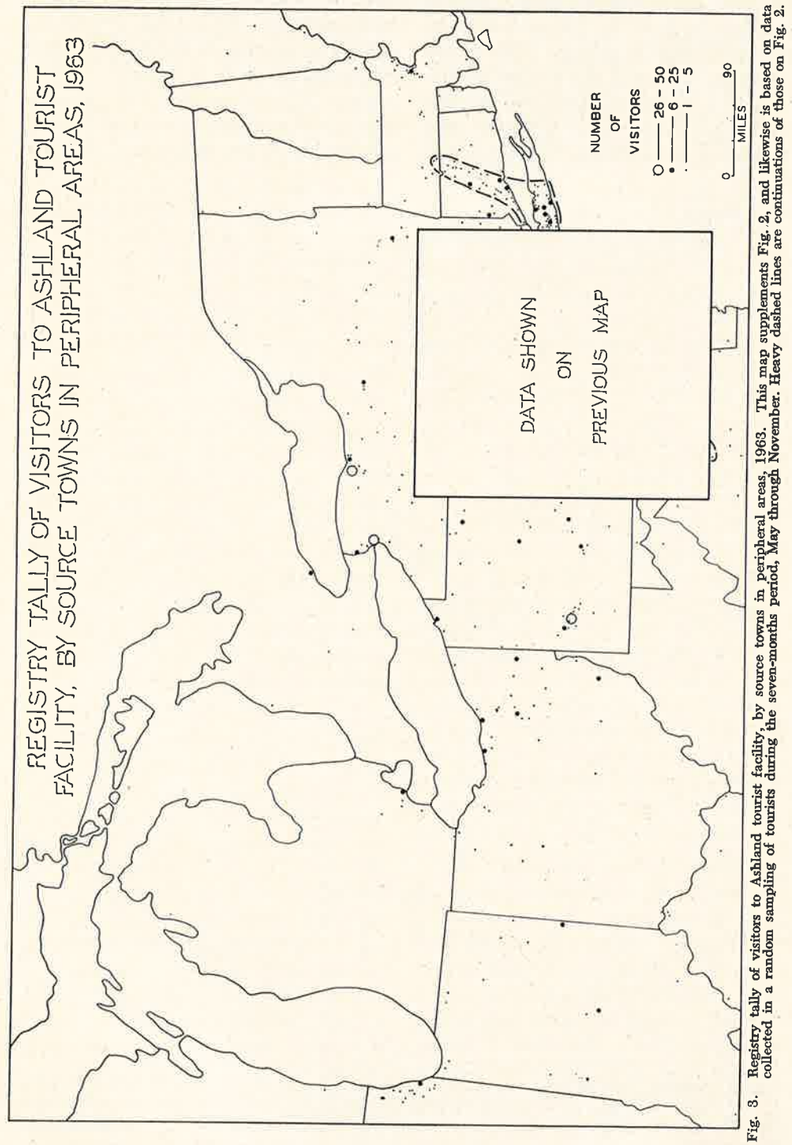

Fig. 3. Registry tally of visitors to Ashland tourist facility, by source towns in peripheral areas, 1963. This map supplements Fig. 2, and likewise is based on data collected in a random sampling of tourists during the seven-months period, May through November. Heavy dashed lines are continuations of those on Fig. 2.

towns as mapped) of all settlements in central and eastern Pennsylvania; (2) airline distances of these towns from Ashland, by means of 20-mile interval concentric circles; and (3) a generalized boundary of the main tourist source area for the Ashland operation. Visual comparison of the data on this map with those showing the number of visitors from source towns in the equivalent area in Figure 2, demonstrates almost innumerable specific instances of the inapplicability of both of the above so-called basic principles to this particular situation. Obviously, some other factor, or factors, are so dominantly in control that the effects of relative distance and relative population are largely nullified. One such factor has been suggested in the earlier study by the authors.

SAMPLING STUDY

All of the materials that follow are based upon a sampling study utilizing information obtained from a rather lengthy questionnaire that was completed by some of the visitors to the Ashland tourist facility. The forms were prominently displayed at the central office each day throughout the 1963 season, so that results would be representative of the entire period; the number of questionnaires completed ranged from none per day during slack periods to more than a dozen on busier days. No attempt was made by operating personnel to coerce selected persons into filling out the form; the action was strictly voluntary on the part of individual tourists. Questionnaires were completed by young and old, men and women, individuals traveling

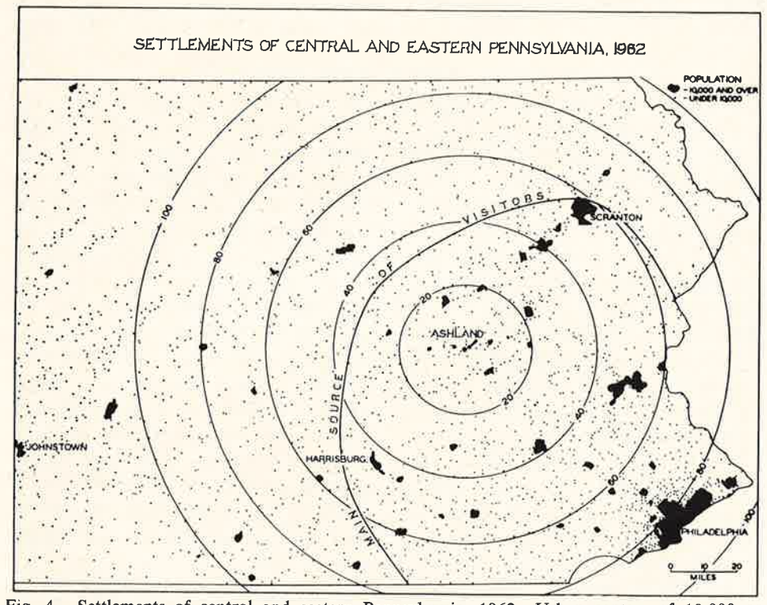

Fig. 4. Settlements of central and eastern Pennsylvania, 1962. Urban centers of 10,000 or more population are represented by large symbols; smaller settlements are indicated by dots. Concentric circles are centered on Ashland and spaced at 20-mile intervals. The heavy irregularly-curved line indicates the generalized boundary of the main source area of visitors to the Ashland tourist facility.

alone and those representing both small and large groups. The returns, therefore, may be considered to reflect the results obtainable from random selection.

A total of 352 questionnaires were wholly or partially completed by visitors. Of these, 14 are not used in this study because information on the home towns of the respondents is omitted; and an additional 47 are discarded because the home towns are so widely scattered and distant[7] as to prevent development of valid geographical generalizations.

The answers on the remaining 291 completed questionnaires, representing the eastern Pennsylvania area and portions of states immediately to the north, east, and south, form the basis for the series of sampling study maps and related analyses that follow. It should be noted that all questions are not answered on every form, and hence the degree of completeness of the various maps differs somewhat. The 291 forms represent 2,112 individuals, or slightly more than six per cent of the total estimated 33,000 visitors to the Ashland facility in 1963. The greatest number of questionnaires come from residents of Philadelphia (59). Other towns and selected regions with four or more completed forms include: greater New York City (27), the Middle and Southern Anthracite Region (21), greater Baltimore (20), Allentown-Bethlehem (16), greater Reading (14), greater Pottsville (11), the Northern Anthracite Region (8), greater Aberdeen, Md. (6), greater Easton (5), greater Harrisburg (5), greater District of Columbia (4), and greater York (4). There also are smaller numbers of questionnaires for several score additional towns. On the whole, the distributional pattern of questionnaires completed by residents of eastern Pennsylvania and vicinity conforms in essential details to the pattern formed by the much larger number of registered visitors from that area (Fig. 2). Such correlation strengthens confidence in the representativeness of the questionnaires.

ROUTES OF APPROACH

Two state highways pass through the town of Ashland, Pa. 61 and Pa. 45, and all visitors to the town's tourist attraction must approach it from west or east via one of these routes. A query on the questionnaire concerns the highway that was followed by the respondent and his party. Answers to the question are mapped in Figure 5, which shows the location of the two highways, and, by symbol, the town of origin for each visiting group together with the route it utilized and the direction it traveled. On the basis of these data, generalized boundaries between the several highway tributary territories are plotted on the map, and appropriate designations are applied to each tributary area.

It is evident from Figure 5 that most Ashland tourist visitors tend to follow typical business type direct-line routes to their destination, rather than selecting circuitous approaches that might perhaps be considered more appropriate for holiday-oriented journeys. Indeed, the areas mapped as tributary to each of the various highway segments, with the one minor exception of the area due north of Ashland, could just as well have been derived from data relating to the movements of trucks and other commercial vehicles traveling to Ashland from the tourists' home towns. Of course, individual exceptions to this generalization are apparent, but on the whole, Americans, even when on vacation, remain "people with a purpose."

A second generalization can be derived from data on Figure 5, namely, most visitors to the Ashland facility stop there on the out-bound portion of their journey rather than on the return trip to their homes. This is evidenced by the preponderance of solid black symbols, indicating approach from the east, to the east of an imaginary north-south line extending through Ashland; and the contrasting dominance of outline symbols, indicating approach from the west, to the west of the line. Apparently visitors to Ashland either select that town as the final destination on a short trip, or else schedule it as an early stop on the outgoing portion of a longer trip. Relatively few groups (only those represented by outline symbols to the east of the imaginary north-south line, and others represented by solid black symbols to the west of that line) choose to stop at the Ashland site during the return journey from a more distant destination, due to the fact, one might surmise, that either little or no vacation time or funds remain available at that late stage of the trip, or that the urge to reach their nearby homes will not permit further delay.

Still a third generalization is possible, namely, that visitors to the Ashland faci-

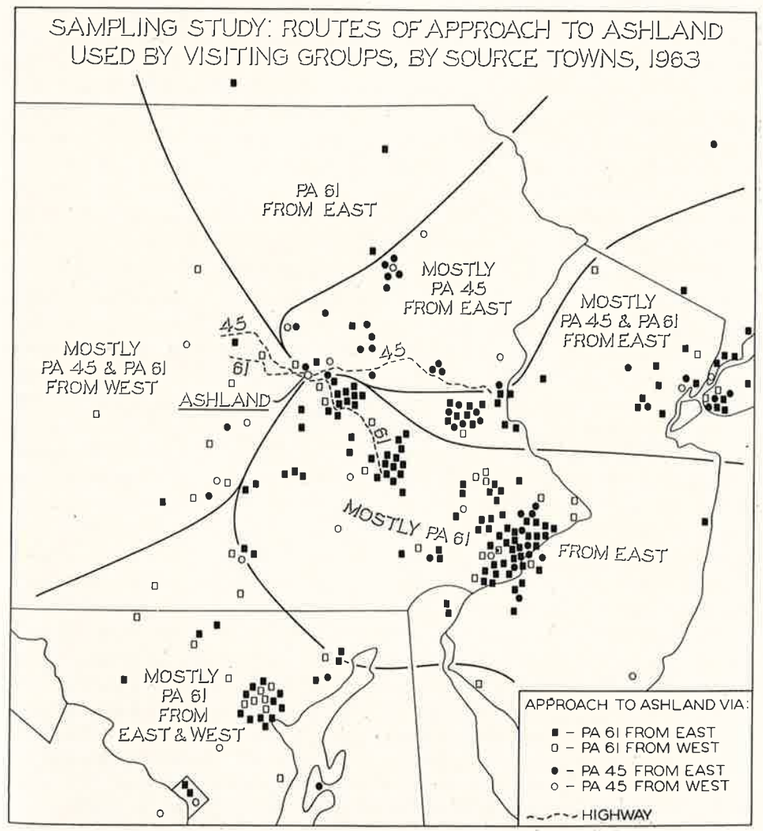

Fig. 5. Sampling study: routes of approach to Ashland used by visiting groups, by source towns, 1963.

lity tend to select the more important and prominently mapped road when offered alternative routes of approximately equal directness, even though pleasure-bent rather than on business. Pa. 61 is a relatively heavily traveled highway, and is commonly shown by means of a wide red line on road maps of the state; Pa. 45 is less intensively used, and is typically mapped as a narrower black line. In tributary areas plotted on Figure 5 lying equally direct via either road, the choice of routes by the tourist is most commonly Pa. 61.

STATUS AS A DESTINATION

One of the inquiries on the questionnaire concerns the status of the Ashland tourist attraction as a destination, namely, is it the terminal goal of a trip or merely one stop on a longer journey. Replies to the question are given on the

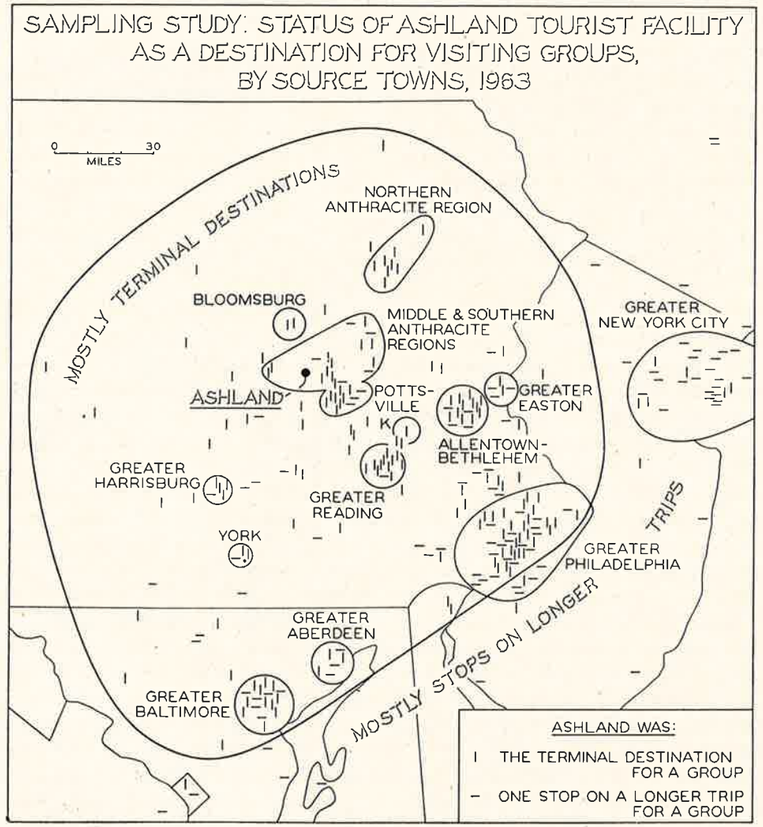

Fig. 6. Sampling study: status of Ashland tourist facility as a destination for visiting groups, by source towns, 1963. Towns and selected regions contributing 20 or more visitors are circled and named. The heavy irregularly-curved line encloses the area in which terminal destinations predominate. The letter, K, represents Kutztown.

map in Figure 6, with an appropriate due east and due west of Ashland, where access to that town is approximately symbol being plotted at the approximate site of origin for each visiting group. Towns[8] contributing 20 or more visitors are circled and named on the map.[9]

Analysis of the data indicates that, within roughly a radius of 100 miles of Ashland, most visitors to that town's tourist attraction select it as their ultimate objective, and, presumably, make the round trip from home within one day. Groups originating at greater distances, however, are most likely to visit the Ashland site only incidentally while heading for another destination farther away. Such a pattern of geographical differentiation conforms to the commonly accepted principle that visitors to a recreational area who wish to confine their trip to a single day must be located within 100 miles of it in order to have adequate time for enjoyment of the facility.[10]

The heavy boundary line on Figure 6 which separates the inner, or terminal destination, zone from an outer zone, however, is not a 100-mile radius circle centered on Ashland, but instead is an irregular closed curve lying at distances ranging from 65 to 120 miles from that town. Presumably, if data upon which this visually determined line are correct,[11] many visitors from Baltimore are willing to travel considerably longer distances for a one-day visit to the Ashland attraction than are other tourists whose homes lie to the west, north, and east of the town. Reasons for irregularities in this zonal boundary line have not been investigated by the authors. One might expect that, in some cases (greater New York City, for instance), highway congestion is a factor, but the traffic element certainly is not involved in such relatively uncongested areas as central Pennsylvania and south-central New York. Another possible cause might be the very attractive and prominent publicity given the Ashland facility by a large Baltimore newspaper,[12] undoubtedly the most elaborate news media story to date, which may have provided added incentive for Maryland residents to travel longer than normal distances to visit the site during a single day. Certainly, the analysis of this problem would involve an instructive study in the various factors motivating tourist movements.

EXPENDITURES BY TOURISTS

Among the various items of information requested in the questionnaire is an estimate of the amount of money spent, or to be spent, by each group at business establishments in and near Ashland during its visit. To simplify the procedure for the respondent, a series of bracketed sums ($0.00 to $1.00; $1.00 to $5.00; $5.00 to $10.00; etc.) is listed on the form so that it is only necessary to check the most appropriate amount.

In tabulating the results, the mean figure for the bracket checked is selected as the amount spent by a party, the assumption being made that differences in expenditures by all parties checking a particular bracket will average out to something approaching the mean for that bracket. Division of the appropriate mean by the number of individuals in a group yields the average dollar expenditure per person for that group. When a town[8] is represented by more than one questionnaire, expenditures of all of the groups involved are totaled, and divided by the sum of the individuals composing the groups to arrive at the average expenditure per tourist from the town as a whole. The resulting data, on a town-by-town basis, are plotted on Figure 7. Towns with total expenditures of $20.00 or more are emphasized by being circled and ruled;[13] the names of most of these

are given on the preceding map (Fig. 6). Finally, expenditures by all groups are totaled, the sum is divided by the number of individuals in these groups, and the average expenditure per tourist for the entire area is determined, namely, $1.35. This figure is employed to plot a line on Figure 7 enclosing an area—"the low expenditure zone"—within which almost all town figures are below the regional average. Beyond the line, most figures exceed $1.35.

Analysis of the data mapped on Figure 7 reveals a number of interesting facts as well as a problem. First, there is no relationship between the size of a town's population and average expenditure per tourist. Large towns (generally those

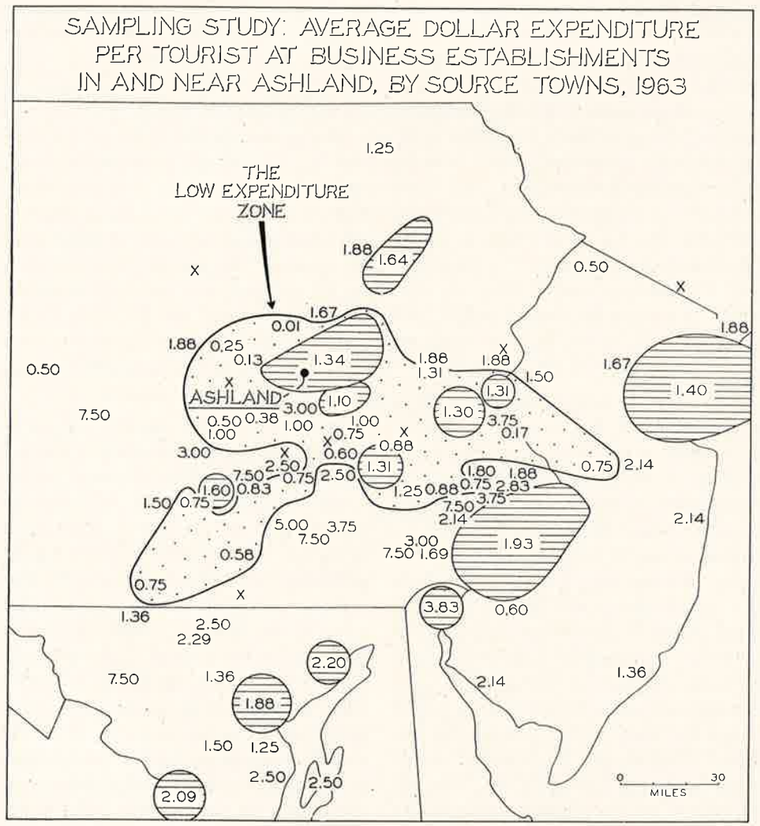

Fig. 7. Sampling study: average dollar expenditure per tourist at business establishments in and near Ashland, by source towns, 1963. Towns and selected regions spending a total of $20.00 or more are circled and ruled, except Lansdale, Pa., which cannot be so indicated on the map because of crowding; the names of many of these are indicated on the previous map. The "low expenditure zone" includes most towns with an average expenditure per tourist of less than $1.35, the mean regional figure. The letter, x, indicates no data for a town.

ruled) and small both are characterized by a considerable range of expenditures. However, the extent of the range is far greater for small towns, due probably to the fact that many of them are represented by single questionnaires which quite often reflect extremes of spending among individual groups. Second, while there is no regularly progressive increase in individual spending outward from Ashland, there certainly is present the expected inner core of towns with below average per capita expenditures. Third, this inner "low expenditure zone" lacks the relatively symmetrical shape that theoretically is supposed to characterize such an area. Instead, long fingers extend outward from its main body toward the southeast and southwest, to or beyond the state border. The reason for such extensions is unknown to the authors. The fingers are not aligned along particular routes of transportation; nor do they conform in position to the economically depressed regions of eastern Pennsylvania; nor do they encompass, exclusively, either large or small towns. They present a most intriguing problem in the geography of tourism that thus far has not been solved.

BUSINESS PATRONAGE

A further item included on the questionnaire involves the use of specific types of business establishments in or near Ashland by visiting groups. A check list of the four presumably most commonly patronized facilities (gasoline station, restaurant, retail store, and hotel or motel) is provided on the form, together with a blank space for naming other types of establishments not individually specified.[14]

Tabulation of returns from the forms is here limited to those representing the 14 tourist source towns[8] providing total expenditures of $20.00 or more each (Fig. 8). Such a limitation is dictated by the cartographic impossibility of mapping the complex results of the tabulation for all source towns on a small-scale map. However, returns from unmapped source towns, in a majority of cases, do not differ significantly from those given for the nearest mapped town.

The percentage figures plotted on Figure 8, for each of the 14 towns, are derived by determining the total number of individuals represented by all questionnaires from a town, and dividing this figure into successive sums of individuals represented only by those forms on which each of the specific business establishments is checked. On many forms, of course, more than one type of establishment is designated, so that the sum of all percentages for most towns exceeds 100. Using greater New York City as an example of the process followed, the questionnaires for the town represent a total of 223 individuals, of which 25 (11 per cent) made purchases at gasoline stations located in or near Ashland according to the forms, 41 (18 per cent) dined at Ashland's restaurants, 11 (5 per cent) visited the town's retail stores, none (0 per cent) stopped at a local motel or hotel, and 177 (79 per cent) patronized no business establishment whatsoever in or near Ashland.

When the data plotted on Figure 8 are analyzed and classified geographically, some striking patterns evolve. Visitors from the two anthracite areas, for example, indicate strong predilection toward the use of dining facilities in Ashland, and lesser degrees of patronage for other types of business facilities. These visitor sources are designated on the map as the "restaurant group." In contrast, visitors from Pottsville and greater Reading to the southeast of Ashland, as well as from greater New York City to the east, apparently have an aversion to us-

ing any business establishments in and near Ashland, for the great majority report no use whatsoever of local facilities. These sources are labeled the "penurious group." The remaining nine source towns, located mostly to the south and southeast of Ashland, give a predominance of their business to gasoline stations, and are so named.

Factors underlying such geographical differentiation in the employment of Ashland's business facilities by tourists have not been investigated by the authors. However, on the basis of personal knowledge concerning conditions existing in the area as well as general observations concerning tourists, a number of hypotheses can be advanced. The preference for Ashland's alimentary facilities evidenced by tourists originating in the anthracite region can perhaps be explained in large part by the general absence of quality dining establishments in most towns of the coal region, together with the existence in Ashland of a hotel whose cuisine is of some regional renown. Elsewhere in eastern Pennsylvania and adjoining states there are many facilities of equivalent or superior quality, and hence visitors from these areas are less likely to be attracted by what Ashland has to offer. The status of Ashland's gasoline stations as chief beneficiaries of the tourist trade from most towns, of course, scarcely merits comment. The almost universal need for their products and services by a motoring public that has traveled scores of miles to reach its destination virtually assures such businesses a major role. The presence of the "penurious group" of tourist source towns shown in Figure 8, however, defies explanation by the authors. They can offer no reasons to account for its existence or location. Again, therefore, a neat little problem in recreational geography presents itself for investigation.

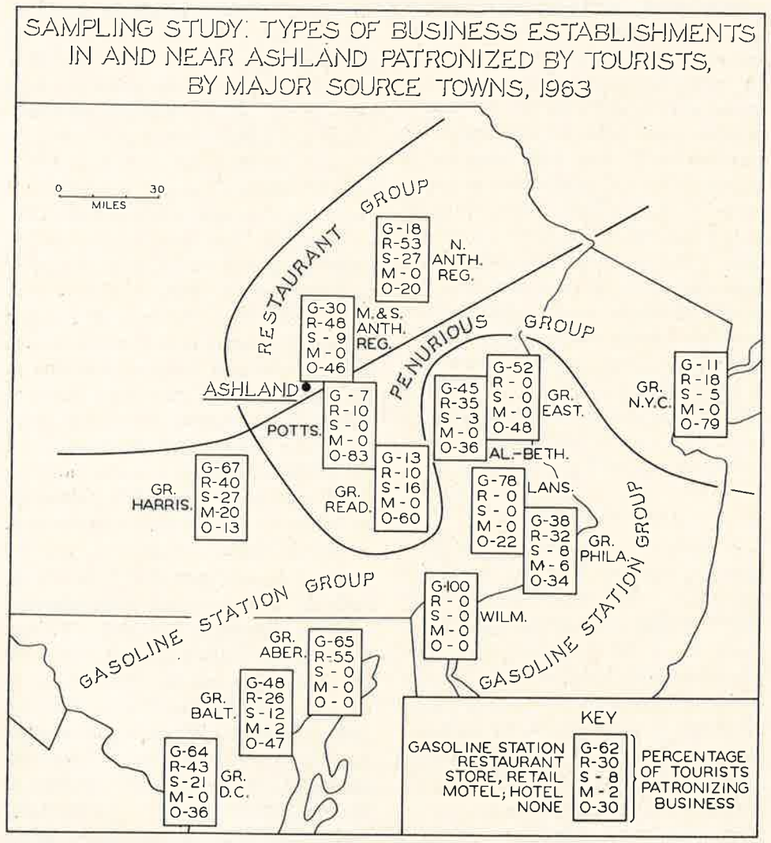

Fig. 8. Sampling study: types of business establishments in and near Ashland patronized by tourists, by major source towns, 1963. See text for full explanation of percentage figures. Includes data only for towns and selected regions spending a total of $20.00 or more at business establishments.

PUBLICITY

The last of the questionnaire items used in this study deals with types of publicity that induced each party to visit the Ashland tourist attraction. Items listed in the form for checking by respondents include: (1) a brochure that was distributed both by the operators of the facility (approximately 100,000 copies) and by the Pennsylvania Travel Promotion Bureau (approximately 200,000 copies); (2) automobile bumper signs (13,000 distributed); (3) news stories in newspapers, ranging in importance from local weeklies to metropolitan dailies (circulated over a wide area in northeastern United States, and probably elsewhere); (4) newspaper advertisements (on a small scale in a limited number of large city papers); (5) news stories carried over radio (on many nearby stations, and probably elsewhere); (6) news stories over television (on a very limited number of nearby stations, but including several lengthy presentations); (7) letters to friends from Ashland residents (probably in considerable numbers because of the intense local pride in the facility); (8) conversations with friends by Ashland residents (probably in considerable numbers); (9) roadside signs (only a few local signs in use during 1963); and (10) postage meter advertisements (employed by certain local businesses on their out-going mail). A number of other means of advertising were initiated as the 1963 season progressed, but these were not introduced into the questionnaire because of the undesirability of modifying its contents during the survey period. The impact of these latter types of publicity, therefore, is not assessed, but their omission does not affect seriously the evaluation of the main promotional devices surveyed in the questionnaire.

Returns relative to the matter of publicity are tabulated and manipulated in exactly the same manner as that described above in connection with business establishments, except that publicity items applicable to less than 10 per cent of the tourists from a source town[8] are not included. This restriction is employed to simplify the cartographic presentation of the data on Figure 9. Note on this map that neither news stories on television nor postage meter advertisements are mentioned by as much as 10 per cent of the visitors coming from any of the 14 towns mapped.

Figure 9 reveals major geographical differentiation in the kinds of promotion that induce tourists to visit the Ashland facility. In the surrounding Middle and Southern Anthracite Region and the associated town of Pottsville, as might be expected, visitors most commonly learn of the attraction and decide to visit it on

the basis of conversations with friends. Surprisingly, however, word-of-mouth publicity is also the most potent factor in enticing Philadelphians to the facility, presumably because of the fact that many earlier residents of the general Ashland area who now reside in greater Philadelphia maintain close personal ties with their relatives and friends back home. Newspaper stories about the Ashland operation are, understandably, the most influential means of attracting visitors from most of the more distant source towns; but brochures, for reasons unknown to the authors, are the dominant factor in southeastern Pennsylvania and parts of adjacent states. Also to be noted on Figure 9 is the wide diversity in types of publicity operative in many of the towns in east-central and southeastern Pennsyl-

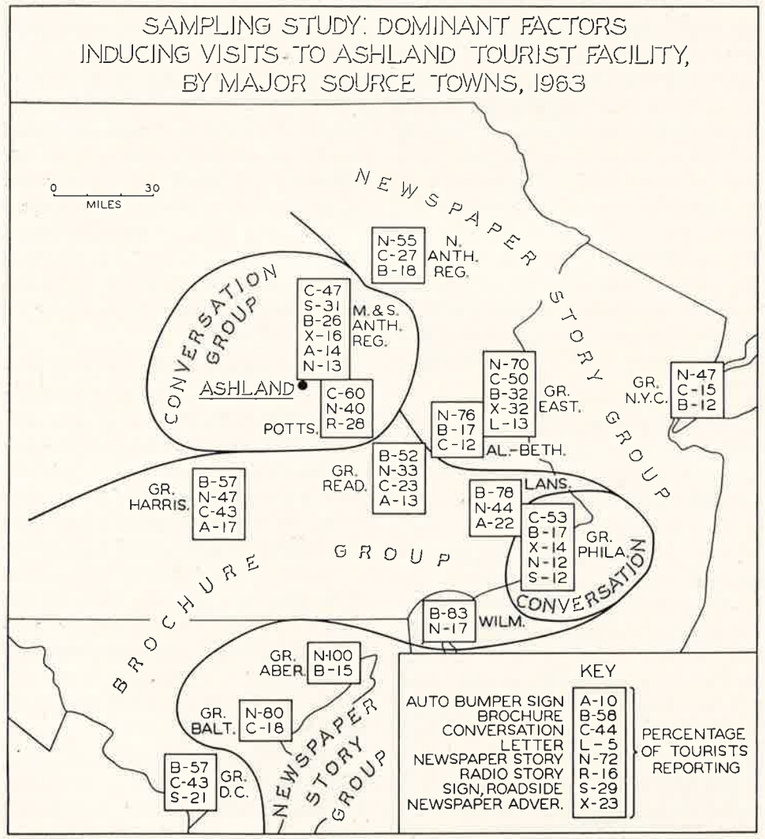

Fig. 9. Sampling study: dominant factors inducing visits to Ashland tourist facility, by major source towns, 1963. See text for full explanation of percentage figures. Iincludes data only for towns and selected regions spending a total of $20.00 or more at business establishments. Includes only factors rated at 10 per cent or higher for individual towns.

vania, as compared with the more limited number of types that are effective elsewhere. For example, six types are influential in the Middle and Southern Anthracite Region; five in greater Philadelphia; and four each in greater Harrisburg, greater Reading, and greater Easton. In contrast, only two types persuade significant percentages of visitors to come from greater Baltimore, greater Aberdeen, and Wilmington; and only three types induce tourists to make the trip from greater District of Columbia, greater New York City, and the Northern Anthracite Region.

CONCLUSIONS

This study has raised far more questions than it has answered. Certainly, no pretense is made by the authors that it is comprehensive in coverage or definitive in treatment. Its primary contributions have been to demonstrate the exceedingly complex nature of the effects of one relatively small tourist attraction, to indicate a few of the many as yet unsolved problems associated with an understanding of such effects, and to plead, by example, with workers in the field of tourism to produce a multitude of similar or improved studies of individual operating units before embarking on the momentous task of developing a set of basic theoretical principles applicable to the industry as a whole. For theory that does not rest on an ample foundation of facts is little better than guesswork, and facts concerning almost all facets of the tourist industry are virtually non-existent.

Despite its primarily academic focus, there is much of practical value in this study both for the operators of the Ashland facility and for administrators in governmental agencies at the state and Federal levels that are charged with promoting and financing tourism. If its contents are carefully studied, they can provide a basis for the more successful conduct of the specific operation under consideration, and can serve as a guide for assessing and beneficially modifying the effects of future tourist attractions to be established elsewhere in the nation.

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4. In referring to the authors' earlier study of the Ashland tourist facility, Mr. A. H. Underhill, Assistant Director of the Bureau of Outdoor Recreation, U. S. Department of the Interior, states: "We believe your study will have widespread applicability in agencies responsible for the promotion of tourism . . Few such follow-up studies have been made in the field of tourism and recreation." Personal letter dated Feb. 5, 1964. See, also, Clawson, M., Land And Water For Recreation, 1963, Rand McNally, p. 96: "Outdoor recreation is provided by a large number and by many kinds of private groups; unfortunately, their number and variety is matched by the lack of information about them."
5. The Ashland facility gives year around employment to four men and one woman. During the summer season it also provides daily work for three months, and week-end work for another three months, for an additional four women. It further furnishes hourly work on busy days to eight or ten other persons, both male and female, although only one or two of the extras are working at any one time.
6. Clawson, M. *op. cit.* p. 47.
7. There are a number of forms from respondents whose home towns are in western Pennsylvania, upstate New York, Michigan, Massachusetts, Illinois, Ohio, Connecticut, and Indiana; and one form each from Arizona, Florida, Nebraska, Oregon, Rhode Island, and Wisconsin.
8. The term, town, as used in this and following sections of the study, refers also to the Northern Anthracite Region, and the Middle and Southern Anthracite Region.
9. The number of visitors contributed by each such town or area is a follows: Bloomsburg (401), greater Philadelphia (360), greater New York City (223), Middle and Southern Anthracite Region (105), greater Baltimore (93), Pottsville (72), greater Reading (69), Allentown-Bethlehem (66), Northern Anthracite Region (45), Kutztown (42), greater Easton (31), York (31), greater Harrisburg (30), and greater Aberdeen (20).
10. Clawson, M. *op. cit.* pp. 15, 24.

11. In the construction of this line, replies from a number of questionnaires are used which could not be mapped on Figure 6. These forms represent several towns in central Pennsylvania located immediately west of the map border, and one town situated just north of the Pennsylvania-New York border which is unmappable because of interference from the title of the map. In all these cases, respondents designate Ashland as one stop on a longer trip.

12. Baltimore Sun, Magazine Sect. Aug. 4, 1963.

13. Total expenditures by visitors from each of these towns or areas are as follows: greater Philadelphia ($674), greater New York City ($256), greater Baltimore ($160), Middle and Southern Anthracite Region ($133), greater Reading ($85), Allentown-Bethlehem ($82), Pottsville ($77), Northern Anthracite Region ($74), greater Harrisburg ($48), greater Aberdeen ($44), Lansdale ($26), greater District of Columbia ($23), Wilmington ($23), and greater Easton ($21).

14. Almost no other types of establishments are named by respondents other than the four specifically designated on the questionnaire. Whether this indicates a wise selection of specific establishments for the questionnaire, or unwillingness on the part of the respondents to take the time to write the names of other types of businesses, is unknown.

TRENDS IN THE COAL MINING INDUSTRY OF THE UPPER SUSQUEHANNA BASIN (WEST BRANCH), 1925-1962

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INTRODUCTION

The development of coal mining was one of the first economic activities in the Upper Susquehanna Basin (West Branch). As early as 1785 a tract of land was purchased along the Susquehanna River for the purpose of mining coal which was to be shipped to the eastern seaboard market. Records indicate that the first coal was shipped eastward using the Susquehanna River in 1804. Coal mining thus has been an important economic activity since the early nineteenth century.

It is the purpose of this study to investigate modern trends in this mature coal mining economy and to analyze the importance of coal mining in the industrial structure of the Upper Susquehanna Basin (West Branch). This study is confined to the four counties of Clearfield, Centre, Clinton, and Lycoming.

COAL RESOURCES OF THE REGION

In the Upper Susquehanna Basin (West Branch) the coal is located primarily in the Allegheny group of the Carboniferous period. The Allegheny formation is not evenly distributed over the area. The greater portion of Clearfield County is underlain by coal bearing rocks belonging to this group. In Centre, Clinton, and Lycoming counties its distribution is much more limited. In Centre County there are two principal coal areas; the first lies southeast of Moshannon Creek in Rush Township and the second lies in Snow Shoe and Burnside townships, extending north and east from Snow Shoe. In Clinton County the coal areas are small and confined to the highest elevations of three basins which cross in a general northeast-southwest direction in the western portion of the county. The coal bearing areas of Lycoming County are confined to the Little Pine Creek Basin in Pine and McHenry Townships and to McIntyre and McNett townships. Only the highest points in these townships contain coal.

There are eight coal beds in the region. Of these the Lower Kittanning and Lower Freeport are most important. The Brookville, Upper and Middle Kittanning, and Upper Freeport are mined locally. The Clarion and Mercer coals are thin and unimportant.

The Lower Kittanning is a persistent coal seam in the region. The bed is usually 2 to 3 feet in thickness but may reach 6 feet in thickness in two or three benches. In places the bed is rather badly split with partings which range from one-half to 10 inches in thickness. The Lower Kittanning coal ranges from 20 to 24 per cent volatile matter, 59 to 67 per cent fixed carbon, 6 to 16 per cent ash, and 0.6 to 3 per cent sulphur. It is a soft, friable, non-coking coal with stick and block structure.

The Lower Freeport is less regular than some of the other coal beds in the region, but it is one of the most intensively mined seams, particularly in Clearfield County. In large areas it has a thickness of 3 to 5 feet, and locally may attain a thickness of 9 feet. In places the coal is found in two benches which may be as much as 55 feet apart. The coal usually has one parting in it. The roof is commonly shale but may be bony coal. This coal has the same percentage of fixed carbon and volatile matter as the Lower Kittanning coals of the region,

but usually has a higher ash and sulphur content.

The Middle Kittanning coal is not mined extensively in the region. Its average thickness is only about 30 inches, but has the advantage of being a solid bed without partings. The Upper Kittanning is one of the most irregular of the principal beds of the Allegheny group. It is mined only when it attains a good workable thickness of from 2 to 4 feet. The Upper Freeport is a thin but fairly persistent bed in the coal region. Its thickness is commonly less than 3 feet, but locally may attain 5 feet or more.

The original coal reserve of the Upper Susquehanna Basin was 4,670,865,000 tons of which 3,992,000,000 tons were found in Clearfield County and 514,485,000 tons in Centre County. Cumulative production of the region between 1800 and 1962 totaled about 598,272,000 tons, of which about 480,000,000 tons were produced in Clearfield County. If 50 per cent of the coal is lost in mining, the coal produced and lost in the mining process totals approximately 1,196,000,000 tons. Thus within the area the remaining reserves total about 3,474,865,000 tons. Of this amount 3,050,000,000 tons are located in Clearfield County and about 350,000,000 tons in Centre County.

THE COAL MINING INDUSTRY

Distribution of coal mining. Within the Upper Susquehanna Basin (West Branch) coal is mined in each of the four counties (Fig. 1). However, Clearfield dominates with 80 to 85 per cent of the total output. Centre County is second in importance with 10 to 15 per cent of total production. Clinton County has from 5 to 8 per cent and Lycoming from 1 to 2 per cent of the regional production.

Underground and strip production trends. Since 1925 two distinct coal production trends can be distinguished in the Upper Susquehanna Basin (Fig. 2). The period from 1925 to 1940 was dominated by underground production. Since 1940 strip mining has become increasingly important, surpassing underground production in 1945.

Underground production was highest in 1925 when 6,563,696 tons were produced. The economic recession of the early 1930's caused coal production to decline. From 1930 to 1945 underground output remained fairly stable. Since 1945 there has been a steady decline with underground production decreasing from 3,810,000 to 1,233,414 tons in 1962. This was but 16 per cent of the total coal output in the region in 1962.

Strip mine production became important in the Upper Susquehanna Basin during World War II when demands for coal soared. In the post-war period the output of the strip mines has continued to rise from 3,892,000 tons in 1945 to a peak of 6,685,653 tons in 1962. The proportion of coal mined by stripping operations rose from 51 per cent in 1945 to 84 per cent in 1962. As a result of the rise in strip production the total annual output of coal since 1945 has been remarkably stable varying from 7,532,563 to 7,834,437 tons.

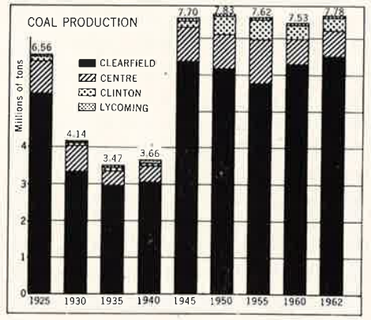

Fig. 1. Coal production by counties.

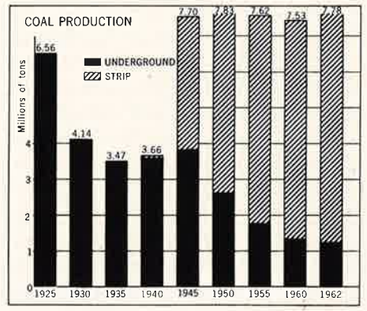

Fig. 2. Coal production by type of mine.

Employment. Total employment in the coal mining industry has steadily declined from a high in 1925 of 11,130 employees to a low of 2,858 in 1960 (Fig. 3). In underground mining the employment decline was from 11,130 in 1925 to a low of 842 in 1962. The employment in strip mining has been rather stable varying from 1,700 to 2,300 employees since 1945. The proportion of workers in underground mines to total mines has declined from 100 per cent in 1925 to 28 per cent in 1962. In contrast, the number of strip miners increased from 39 per cent of the total in 1952 to 72 per cent of the labor force in the coal mining industry in 1962. Although coal production has remained stable since 1945, the number of miners has declined about 50 per cent. This illustrates the greater productivity per miner in the region.

Average number of days worked. There is a significant difference between the average number of days worked annually by the underground and strip miners (Figs. 4 and 5). The number of days worked annually by the underground worker rose from 153 in 1925 to a peak of 265 in 1945 during World War II. Since then the underground miner has worked between 180 and 211 days per year. The strip miner normally works 20 to 40 more days per year than the underground miner. The average number of days worked per year by the strip miner has varied from 200 to 236 since 1945.

Within the region there are some variations in the number of days worked per year. The underground miner in Clear-

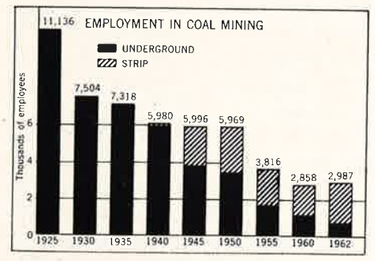

Fig. 3. Employment in coal mining by type of mine.

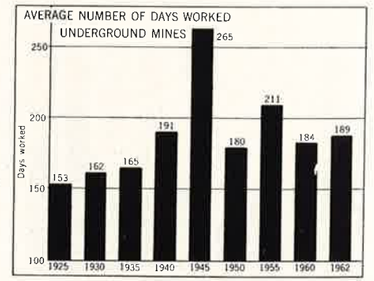

Fig. 4. Average number of days worked per miner in underground mines.

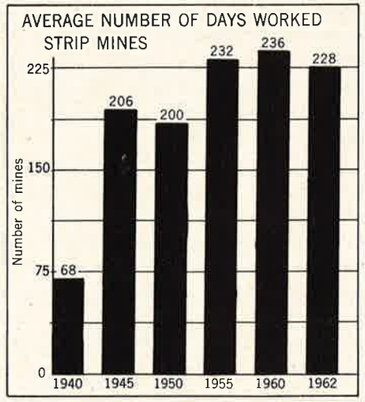

Fig. 5. Average number of days worked per miner in strip mines.

field County has a greater number of work days than in Centre, Lycoming, or Clinton counties. For example, in 1962 the underground miner in Clearfield County averaged 193 days work while in Centre the number was 163, Lycoming 126, and Clinton only 86 days. In the coal basins of Centre, Lycoming, and Clinton counties coal mining is frequently a part-time operation engaged in by men during the slack work period of the year.

Number and employment in mines. The number of underground mines has varied from a high of 231 in 1925 to a low of 85 in 1955 (Fig. 6). The number of mines in operation from year to

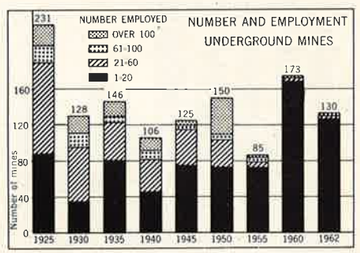

Fig. 6. Number and employment in underground mines.

year fluctuates considerably. For example, in 1960 there were 173 underground mines in operation, but in 1962 there were but 130. This illustrates the ease of opening an underground mine, and also the possible short duration of its existence.

Between 1925 and 1962 there has been a major change in the size of the labor force in underground mines. In 1925 of a total of 231 mines 85 had 1 to 20 employees. This was 27 per cent of the total underground mines. By 1962, of 130 mines 125 had an employment of 1 to 20 miners, or 96 per cent of the mines. In 1925, 24 of the mines had an employment of over 100 workers, or about 10 per cent of the total. In 1962 there were no mines with 100 or more employees and only one mine with an employment between 61 and 100 workers. The largest underground mine in the region employed 880 miners in 1935.

Today the underground mine is normally developed by a small number of workers who open a mine and operate it for a short period. As a consequence the traditional coal mining community of the nineteenth and early twentieth centuries that developed at a mine with a large employment is not developing today. Of even greater consequence the decline of the large coal mine has stranded the one economy coal towns. It is a rare instance when a new economic activity has replaced the declining coal economy of these towns.

The number of strip mines has steadily increased from 105 in 1945 to a peak of 144 in 1962 (Fig. 7). There have also been changes in the size of the labor force in strip mines but not of the magnitude of the underground mines. The number of strip mines with a labor force of over 50 has always been small. The proportion of mines with employment of

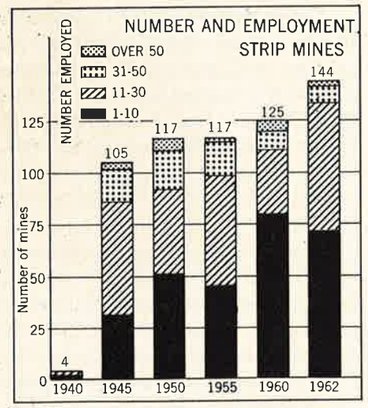

Fig. 7. Number and employment in strip mines.

31 to 50 has gradually declined from a maximum of 18 in 1950 to 8 in 1962. At the same time the proportion of mines with 1 to 10 employees has increased. In 1962 one-half of the 144 mines had 1 to 10 employees while in 1950, 32 of the 105 mines had 1 to 10 workers, or only 30 per cent of the total. As the strip mining equipment becomes larger and more efficient the size of the labor force is reduced.

Average employment per mine. The average employment in the underground mine has steadily declined from a high of 67 in 1930 to 6 workers in 1960 and 1962 (Fig. 8). In 1925 there were essentially no variations of the average employment per mine within the region in the underground mines. By 1962 regional variations were apparent. In Clearfield County the average employment per underground mine was 7. It was, however, only 4 in Centre County, 2 in Clinton County, and 1 in Lycoming County.

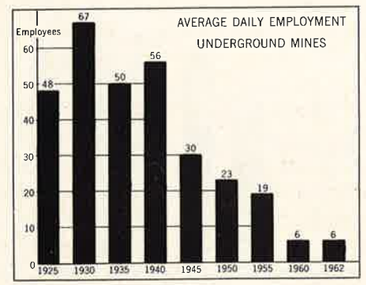

Fig. 8. Average daily employment in underground mines.

Fig. 9. Average employment in strip mines.

The average employment in strip mines has also declined from 20 in 1945 to 15 in 1962 (Fig. 9). Regional variations also exist in strip mining employment. In 1962 Clearfield and Centre counties each had an average employment in strip mines of 15 workers, while Clinton County had 10 and only 7 miners were in the average strip mine in Lycoming County. On the margins of the coal field in Clinton and Lycoming counties the magnitude of the operation is smaller than in Clearfield and Centre counties.

Average tons per man per day. The average tons mined per man per day has risen in both underground and strip mines (Figs. 10 and 11). In the underground mine the output per man per day has risen from an average of 2.87 tons in 1935 to 7.73 tons in 1962. In the strip mine the productivity has risen from an average of 9.02 tons per man per day in 1945 to a high of 15.38 tons

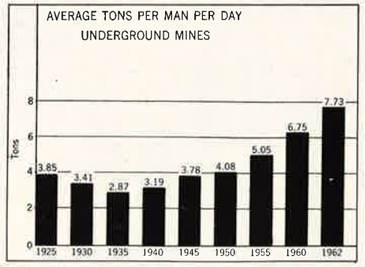

Fig. 10. Average tons per man per day in underground mines.

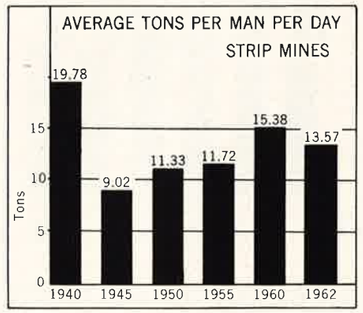

Fig. 11. Average tons per man per day in strip mines.

in 1960. In 1962 there was a slight decline to 13.57 tons per man per day in strip mines.

There is a large variation in the output per man per day in both underground and strip mines. In 1962 the productivity in the underground mines varied from a low of 0.95 tons per man per day in one mine in Clinton County to a high of 19.31 tons in one mine of Clearfield County. The large mines were the most productive. As a consequence the small mines were usually producing below the regional average.

1. The Pennsylvania Deparment of Mines and Mineral Industries has devised a formula for estimating acres in strip pits and acres in strip pits plus spoil banks. The formula for acres in strip pits is: $\frac{\text{tons}}{5{,}000}$ = acres in strip pits. Acres in strip pits plus spoil banks is derivedd by multiplying the acres in the strip pits by 1.5.

There is also considerable variation in the productivity of the strip mine. In 1962 the productivity varied from a low of 2.19 tons per man per day to 61.4 tons. The variation in strip mine productivity is primarily due to the size of the shovels used in the mining operation, the ease of mining the coal, and the thickness of the seams.

Acres in strip pits and spoil banks. Between 1945 and 1962 it is estimated that 20,132 acres were converted to strip pits, and the acreage of strip pits and spoil banks combined was 30,199.[1] In 1962 the acreage affected by strip pits and spoil banks totaled 1,998 (Fig. 12).

Within the region, southern and central Clearfield County has been most affected by strip mining (Fig. 12). Nearly 20 per cent of this area is in strip pits and spoil banks. Only a small percentage of the land has been reclaimed by leveling and planting in trees. Acid water from the abandoned strip pits has adversely affected the streams. Fishing has been destroyed and the polluted waters discourage certain types of industry from entering the region. An effective conservation policy is needed to restore the strip mined areas. With a minimum of

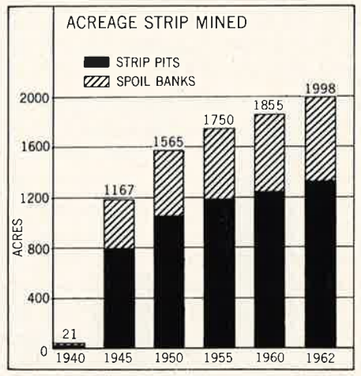

Fig. 12. Acreage in strip pits and spoil banks.

effort many of the strip pits could be converted into small lakes which could be utilized for fishing, boating, and swimming. The fees collected from strip mining should provide the necessary capital to restore the forests of this region so that a revitalized forest industry could develop in the future.

Transportation of coal. The coal from the Upper Susquehanna Basin has always been primarily marketed outside the area. The market within the region has been limited due to its relatively small market for coal to be used for domestic purposes, the limited industrial development in the region, and the lack of a coking industry. Although coal was first shipped from the region by barges on the principal water routes, a network of railroads was developed at an early date in Clearfield County. In the early 1860's a branch of the Pennsylvania Railroad entered Clearfield County from the south at Osceola Mills and extended to Philipsburg, Clearfield, and Curwensville with mining branches to Houtzdale and Philipsburg. Another branch line extended into Clearfield County from Bellwood to Coalport and Irvona. At about the same time the Buffalo, Rochester and Pittsburgh Railroad entered the region and within a few years had routes covering most of the southern part of Clearfield County. The railroad line along the West Branch of the Susquehanna was built in the early twentieth century.

The coal districts of Centre County are served by two railroads. The Pennsylvania Railroad serves the Philipsburg district and a branch line enters the Snow Shoe area. The New York Central Railroad extends through the mining districts of Moshannon and Snow Shoe. In Clinton County the coal districts are served by the Pennsylvania and New York Central railroads. The railroads of Lycoming County do not serve the coal regions directly.

Until the early 1930's well over 90 per cent of the coal was marketed by railroad transportation (Figs. 13 and 14). Since then the importance of railroads has declined with the introduction of truck transportation. In 1962 about 20 per cent of the coal mined from underground and 16 per cent of the coal from strip mines was marketed by trucks.

There are some significant differences in marketing practices among the counties. The marketing of coal by railroads from either underground or strip production remains most important in Clear-

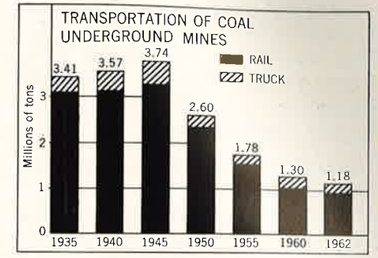

Fig. 13. Transportation of coal from underground mines.

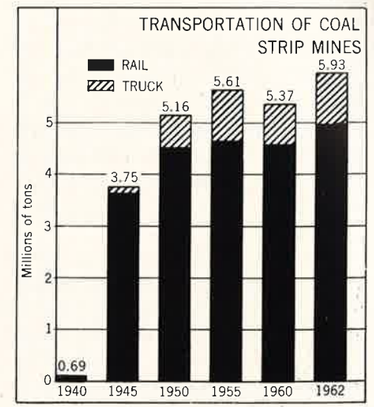

Fig. 14. Transportation of coal from strip mines.

field County. In 1962, 87 per cent of the coal from underground mines and 82 per cent from strip mines in Clearfield County were marketed by railroads. In contrast, in Centre County only 60 per cent of the strip mined coal and none of the underground coal production was marketed by rail. In Clinton County 93 per cent of the strip mined coal was marketed by rail, but none of the underground coal production. All coal, whether by underground or surface mining, was marketed by truck in Lycoming County in 1962.

A number of observations may be made about the transportation of coal. Railroads still play a dominant role in transporting coal from the region. Their importance within the region depends on their accessibility to the coal mines. Truck transportation will undoubtedly continue to increase in importance as the railroad system declines.

ECONOMIC IMPORTANCE OF THE COAL MINING INDUSTRY

Within the Upper Susquehanna Basin (West Branch) the coal mining industry is most important in the economic structure of Clearfield County. The economy of Clearfield County was long dominated by the primary economic activities of agriculture, mining, and lumbering. As recently as 1930 mining provided the greatest source of employment with 27 per cent of the total, followed by trade and services 22 per cent, manufacturing 19 per cent, transportation and communications 12 per cent, and agriculture 11 per cent (Fig. 15).

Since 1930 the importance of mining as a source of employment has declined greatly and in 1960 provided only 10 per cent of the employment in Clearfield County. Employment in agriculture has also declined as well as employment in transportation and communications. In contrast employment in trade and services is now most important with 41 per cent of total employment, followed by manufacturing with 30 per cent of the total. Total employment in Clearfield County declined slightly between 1930 and 1960 from 26,972 employees to 25,470.

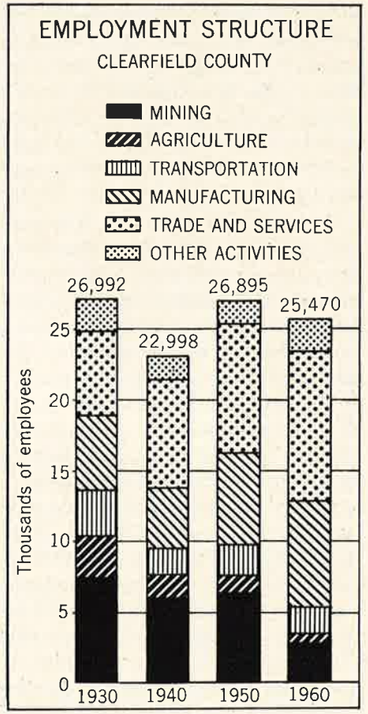

Fig. 15. Employment structure of Clearfield County.

Although employment in mining has declined, the coal mining industry remains important to the economy of the Upper Susquehanna Basin (West Branch). The annual value of coal between 1945 and 1962 varied from $25,646,000 to $33,841,000 (Fig. 16). Of the annual value of coal produced, between 77 and 85 per cent came from Clearfield County.

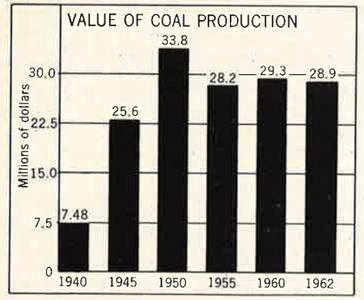

Fig. 16. Value of coal production.

Within Clearfield County the annual payroll of all employees in bituminous coal mining totaled $9,387,000 in 1958. During the same year the capital expenditures totaled $3,327,000; cost of supplies were valued at $12,955,000, and the value added in the mining process totaled $17,411,000. Nevertheless the mining industry has a lower economic impact on the economy of Clearfield County than manufacturing. In 1958 manufacturing had an annual payroll for all employees of $22,027,000 and the value added in manufacturing totaled $40,144,000. However, capital expenditures in manufacturing totaled only $1,895,000.

CONCLUSION

The coal mining industry of the Upper Susquehanna Basin (West Branch) remains one of the most productive in the state. While coal production within the state declined from 135,266,612 tons in 1925 to 65,648,015 in 1962, a decrease of 69,618,597 tons, coal production in the four counties of the Upper Susquehanna Basin rose from 6,563,696 to 7,784,353 tons, an increase of 1,220,657 tons. Because coal reserves in the region are large and efficiency in mining has increased productivity, the production of coal will continue to be important for the foreseeable future.

Although production has been maintained the coal region has been plagued with economic problems in recent years. As a response to increased mechanization and growth of surface strip mining, the importance of the coal mining industry as a generator of employment has drastically declined. Unemployment of 10 to 11 per cent of the labor force in Clearfield County places this county near the top in unemployment in Pennsylvania. The availability of fuel, a dense network of railroads, and an excess supply of skilled, mechanical labor have not overcome the handicaps of lack of most industrial raw materials, long distances to major markets, and high-cost labor in attracting new manufacturing plants to the area in sufficient numbers to stabilize employment. As a result of economic problems Clearfield County is experiencing one of the highest rates of out-migration of population in Pennsylvania. In conclusion, the coal mining industry of the Upper Susquehanna Basin which once was a dynamic force in the growth of the regional economy has lost its effectiveness in maintaining the economy of the coal region.

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Phyllis C. Martin
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