Polarization Optical Properties of the Pancreatic Acinar Cell of the Mouse

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(Received for publication, October 28, 1957)

ABSTRACT

The birefringence of fresh and fixed mouse pancreatic acinar tissue was studied, utilizing whole mounts of pancreas from which the mesentery had been removed. Fresh pancreas in Tyrode's solution demonstrated positive birefringence with respect to the radial axis (the axis radiating from the nucleus as spokes from a wheel). Formol fixation reversed the sign of birefringence to negative with respect to the radius. The magnitude of birefringence increased with longer fixation. Neutral formal also reversed the sign to radially negative, but the magnitude did not increase with longer fixation. Tissue fixed in 2 per cent osmium tetroxide or potassium permanganate demonstrated strongly negative birefringence with respect to the radius. The cytoplasm of tissue fixed in acetone, though finely granular, still possessed radially positive birefringence. Birefringent areas could be seen in tissue fixed in potassium dichromate, but the sign of birefringence could not be determined. Ethyl alcohol, chromic acid, picric acid, and mercuric chloride all produced a brilliant cytoplasm in which no birefringence could be demonstrated. Freezing markedly decreased the radially positive birefringence of fresh tissue.

Fresh tissue placed in increasing concentrations of glycerol demonstrated increasing radially positive birefringence. When formol-fixed tissue was placed in glycerol, the radially negative birefringence decreased. Osmium tetroxide-fixed tissue in 50 per cent glycerol was isotropic. The granularity of ethyl alcohol-fixed tissue disappeared in 50 per cent glycerol, and radially positive birefringence was evident. Frozen tissue showed increasing radially positive birefringence in increasing concentrations of glycerol.

The results are discussed in relation to theories of fixation.

Prior to the application of electron microscopy to biological investigations, submicroscopic structure was deduced from studies on tissue with the polarizing microscope and x-ray diffraction camera. Observations on fixed tissues with the electron microscope have confirmed in large part the suggestions of structure based on earlier work. The presence of parallel membranes in myelin was deduced from studies of the birefringence (23, 36, 38) and x-ray diffraction patterns (17, 40). The radially positive birefringence of myelin has been pictured classically as the difference between the form birefringence of concentric laminated protein sheets and the intrinsic birefringence of lipide molecules oriented perpendicularly to the protein (21). In myelin the intrinsic birefringence is greater than the form birefringence. Similarly x-ray diffraction data are consistent with a lipide-protein membrane complex (17, 40). Schmidt (37) suggested a similar type of membrane structure in the retinal rods on the basis of birefringence studies. Electron microscopic studies of myelin (16, 19) and of retinal rods (14, 41, 42) have demonstrated membrane structures much as predicted from earlier studies. Furthermore, studies on myelogenesis have demonstrated that myelin is

* This work was completed during the tenure of a Student Summer Research Fellowship from Washington University, School of Medicine, St. Louis, Missouri.

J. BIOPHYSIC. AND BIOCHEM. CYTOLO. 1958, Vol. 4, No. 2
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A study of changes in birefringence occurring with fixation have been studied by many technics. Strange-ways and Canti (46), using the dark field microscope, followed changes of cell structure in single cells during the process of fixation. Similar studies with the phase microscope were performed by Crawford and Barer (12) and by Buchsbaum (7). During the latter half of the 19th century model systems were studied by Hardy (24), Mann (29), and Bütschli (8). The behavior of protein solutions in the presence of fixatives provided knowledge of the changes in protein states accompanying fixation. Despite many thorough studies such as those above, the mechanisms of fixation are still poorly understood.

A study of changes in birefringence occurring with fixation can yield information relevant to the changes in submicroscopic structure induced by fixation. The mouse pancreas is a particularly favorable organ for such a study. Much is known about the structure of pancreatic acinar cells from electron microscope studies (13, 34, 44, 45, 49). Since the mouse pancreas lies within the mesentery of the small intestine, thin sheets of pancreatic tissue can be dissected free from mesentery, thus making whole mounts of fresh tissue available for study.

**Materials and Methods**

The pancreases of mature white mice, which had been killed by a quick blow on the head, were placed in Tyrode’s solution at room temperature. The mesenteric investments were pulled away with jeweler’s forceps under a dissecting microscope, in order to reduce extraneous birefringence. A small (1 mm. x 1 mm.) lobule was detached at its base from the mass of tissue and transferred to a slide. Excess Tyrode’s solution was removed and replaced by immersion media. The coverslip was lowered gently onto the preparation, and the edges were rimmed with vaseline. The coverslip was lowered gently onto the preparation, and the edges were rimmed with vaseline.

Two microscopes were used during the course of the investigation, a Spencer research model polarizing microscope, and the special polarizing microscope built by Dr. S. Inoué (26). Retardations were measured according to the field-match method of Bear and Schmitt (3). Duplicate readings were taken in two quadrants, and the differences were taken for the 2δ term in the equation (3):

\[ m = m_e \sin 2\delta_e \]

in which \( m \) is the unknown retardation, \( m_e \) is the retardation of the rotating compensator, \( \delta_e \) is the number of degrees from extinction when the compensator is rotated to produce equal intensity of specimen with respect to isotropic background. Accurate measurements of cell thickness were not feasible in whole mounts of the above type; hence, magnitudes of birefringence were not calculated. Values for retardations cited below are the medians of several determinations made on the same preparation. All values for the determinations of retardations are given in angstrom units.

In order to have a systematic reference nomenclature for the discussion of the various areas of a single pancreatic acinar cell, the following definitions are used throughout this paper. The base of a cell is the portion that is immediately beneath the mesentery and is free from granules. The apex of a cell is the portion that contains zymogen granules and presumably connects with the ductal system. The margins of a cell are those areas that are in contact with adjacent cells.

The sign of birefringence is conventionally designated as positive when the slow axis of transmission of light is parallel to a reference axis. Similarly, the sign of birefringence is negative when the slow axis of transmission is perpendicular to a reference axis. To determine the sign of birefringence in pancreatic acinar tissue, both a first order (5) red plate and a rotating mica compensator (5 wave length) were used. When the compensator was rotated so as to darken the base of an acinar cell, the margins became bright. Rotating the compensator in the reverse direction produced opposite results. Thus, the pancreatic acinar cell demonstrates non-uniform extinction, as does the myelin sheath of nerve. Since the axis of myelin is regarded as radial, a similar convention was adopted for pancreatic tissue. In this investigation the reference axis for pancreatic acinar tissue was considered to radiate from the nucleus as spokes from a wheel. Measured birefringence is thought to represent the algebraic sum of the intrinsic (radially positive) birefringence and form (radially negative) birefringence of concentric laminated lipide-protein lamellae.

Fixatives which were chosen for study are listed below. For uniformity in the discussion, formol solutions are expressed as percentage of the commercial...
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solutions; thus, 10 per cent formol is a 1:10 dilution of the commercial 40 per cent solution of formaldehyde. 10 per cent neutral formol is formol that has been neutralized for a period of time over calcium carbonate.

10 per cent formol
10 per cent neutral formol
2 per cent osmium tetroxide
2 per cent potassium permanganate
10 per cent acetic acid
3 per cent potassium dichromate
95 per cent ethyl alcohol
Saturated aqueous picric acid
2 per cent chromic acid
Saturated aqueous mercuric chloride

The technic of demonstrating birefringence photographically was the following: The point of extinction of the rotating compensator was determined. The $\lambda_0$ wave length compensator was set at plus 8° and minus 8°, respectively, for each of two pictures. All pictures were taken on 35 mm. film and enlarged photographically to a final magnification of approximately 2,300.

OBSERVATIONS

Observations on Fresh Tissue.—Birefringence was easily demonstrated in most of the cells from preparations of fresh pancreas in Tyrode's solution. To examine the birefringence of single cells, the margins of the preparation were inspected for acini which projected beyond the tissue mass where areas of single cell thickness were located. A single cell was anisotropic at the base and at the margins; the nuclear area was isotropic. Birefringence could not be demonstrated in the apex of the cell, where the brilliance of the highly refractile zymogen granules masked any birefringence that might have been present. The sign of birefringence was positive with respect to the radius in all preparations of fresh pancreas examined. The median retardation of fresh tissue was 110.4 A (Figs. 1 and 2).

Observations on Fixed Tissue.—When placed in 10 per cent formol, the sign of the birefringence of pancreatic acinar tissue became negative with respect to the radius. When fixed in 10 per cent formol, the magnitude of the negative birefringence increased with time. After 2 hours fixation in 10 per cent formol, the median retardation was -96.3 A, and after 24 hours was -257.1 A. Ten per cent formol neutralized over CaCO$_3$ differed from 10 per cent formol in that no change in the magnitude of the birefringence occurred with longer fixation. The median retardation after 2 hours' fixation in 10 per cent neutral formol was -80.7 A, and after 24 hours -77.6 A (See Text-fig. 1). The appearance of the tissue was similar, regardless of the type of formol used. The cytoplasm became finely granular, and the nucleus became more prominent. The apical granules remained unchanged. Mitochondria, which were difficult to distinguish in fresh tissue, were easily identified after formol fixation (Figs. 4 and 5).

Upon contact with 2 per cent osmium tetroxide, the tissue began to darken at the edges of the preparation and the birefringence immediately became negative with respect to the radius. The magnitude of the birefringence was larger with osmium fixation than any other fixative examined with a median retardation of -413.6 A. The appearance of the cells changed only slightly. The cytoplasm remained homogeneous, and the mitochondria blackened more than the surrounding cytoplasm (Fig. 7).

Tissue fixed in 2 per cent potassium permanganate rapidly blackened. The sign of the birefringence was radially negative, but accurate measurement of the magnitude was impossible, since the tissue appeared darker than the background at all compensation settings.

When placed in 10 per cent acetic acid, cytoplasm of pancreatic acinar cells appeared finely granular and the nuclei were prominent. Radially positive birefringence was clearly evident with a median retardation of +215.1 A (Fig. 10).

Tissue fixed in 3 per cent potassium dichromate exhibited a coarsely granular cytoplasm with little discernible cell structure. The apical granules were not prominent, and cell margins were indistinct. Scattered areas of birefringence were demonstrable with rotation of the compensator, but no systematic organization of anisotropic areas could be discerned. Thus, the sign of birefringence was not determined (Fig. 9).

A group of fixatives classified by Baker (1) as "protein precipitants" (ethyl alcohol, chromic acid, picric acid, and mercuric chloride) produced identical results under the polarizing microscope. The entire tissue became brilliant, and no cytoplasmic detail could be seen. No polarization optical evidence of preferential organization of cytoplasmic components was evident after treatment of pancreatic cells with fixatives of this type (Fig. 11).

When pancreatic tissue was frozen on the mounting block of a freezing microtome and
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Text-Fig. 1. Values represented on the chart are individual determinations of the magnitude of birefringence under the stated conditions. The arrow points towards the median value. Tyr, Tyrode's solution, Gl, glycerol, Form, 10 per cent formol, N Form, 10 per cent neutral formol.

Thawed in Tyrode's solution, the tissue appeared translucent under the dissecting microscope. The birefringence was markedly altered with a median retardation of +48.3 Å. The sign of the birefringence remained radially positive. In all preparations of fresh tissue that had been frozen, the nuclei appeared swollen and prominent and the cytoplasmic details and cell borders could not be distinguished (Fig. 3).

Observations with Glycerol as an Imbibition Media.—Three concentrations of glycerol (10 per cent, 50 per cent, and 100 per cent) were studied in this investigation. The refractive indices of the three solutions were determined by means of an Abbé type refractometer to be:

- 10 per cent glycerol: 1.354
- 50 per cent glycerol: 1.403
- 100 per cent glycerol: 1.473

The birefringence of fresh tissue which had been placed in glycerol was positive with respect to the radius for all concentrations of glycerol studied. The magnitude of the retardation increased with increasing concentrations of glycerol from +110.4 Å for fresh tissue in Tyrode's solution to +142.0 Å in 10 per cent glycerol, +218.5 Å in 50 per cent glycerol, and +372.1 Å in 100 per cent glycerol. Pancreatic tissue gradually swelled in all concentrations of glycerol, and the motion of this swelling blurred photographs of these preparations. Apart from the increased magnitude of birefringence, the appearance of the cells was similar to fresh tissue in Tyrode's solutions for all concentrations of glycerol (Text-fig. 1).

Formol-fixed tissue placed in glycerol of varying concentrations demonstrated marked changes in birefringence. The sign of birefringence of 10 per cent neutral formol-fixed pancreas was negative in 10 per cent glycerol, but the sign reverted to positive in 50 per cent and 100 per cent glycerol. The respective median retardations were −60.2 Å in 10 per cent glycerol, −165.7 Å in 50 per cent glycerol, −277.1 Å in 100 per cent glycerol. When 10 per cent formol-fixed material was placed in glycerol, the sign of birefringence remained negative for all concentrations of glycerol. The magni-
tude of the negative birefringence decreased with increasing concentrations of glycerol from \(-90.1\) Å in 10 per cent glycerol to \(-74.4\) Å in 50 per cent glycerol, and to \(-45.0\) Å in 100 per cent glycerol (See Text-fig. 1). Thus glycerol in all concentrations increased the positive birefringence or decreased the negative birefringence with respect to the radius. The appearance of neutral formol-fixed pancreas in 50 per cent glycerol was comparable to fresh tissue in 50 per cent glycerol (Fig. 6).

Osmium-fixed material placed in 50 per cent glycerol became isotropic. The cytoplasm appeared amber and translucent. The apical granules were indistinct (Fig. 8).

The appearance of tissue fixed in ethyl alcohol and placed in 50 per cent glycerol was similar to that of fresh pancreas in Tyrode's solution. The brilliance of the alcohol-fixed tissue disappeared, and radially positive birefringence was evident. Similar results were obtained with tissue fixed in the other "protein precipitants" (Fig. 12).

Tissue which had been frozen and then thawed in 10 per cent glycerol showed little change from that already described for frozen tissue. When 50 per cent glycerol was used as a mounting medium, birefringent areas became visible. The sign of birefringence of these areas was positive with respect to the radius. The peculiarity of these preparations was the distribution of birefringence in needle-shaped masses arranged circumferentially around the nucleus. The tissue gradually swelled, and all pictures of the preparations were badly blurred.

When neutral formol-fixed tissue was frozen and then examined with the polarizing microscope, no change from the original preparation could be detected. The birefringence remained radially negative. When formol-fixed frozen tissue was placed in 50 per cent glycerol, no observable change resulted. The birefringence remained radially negative.

**DISCUSSION**

Radially positive birefringence in the pancreatic acinar cell is predicted on the basis of current concepts of pancreatic morphology and membrane structure. The pancreatic acinar cell as seen by electron microscopy is packed with parallel membranes arranged concentrically around the nucleus (13, 34, 43, 44, 49). These concentric membranes are known as the endoplasmic reticulum (31), or the ergastoplasmic sacs (49). In other tissues where parallel membrane systems exist, such as myelin and the retinal rods, lamellar birefringence has been found (35, 36). The membrane structure of such lamellar systems is classically pictured as protein sheets with perpendicularly oriented lipides (6, 21, 37). If the membrane structure of the ergastoplasmic sacs is similar to the membrane structure of myelin, the pancreatic acinar cell should show positive birefringence with respect to the radius, if the radially positive intrinsic birefringence of oriented lipides predominates over the radially negative birefringence. Thus the radially positive birefringence of fresh pancreatic acinar tissue is a finding consistent with the lipide-protein model of membrane structure. The present investigation, however, does not prove that such a model is necessarily correct. Though the ergastoplasm of exocrine pancreas may be significantly different from nerve myelin in respect to membrane structure, a gross similarity to the lipide-protein model of myelin is presently most plausible.

Structural changes accompanying formol fixation have been carefully studied by Crawford and Barer (12), using phase microscopy on individual cells before and after fixation. They noted a fine granularity in the cytoplasm, and a similar change was noted with the polarizing microscope in the present study. The reversal of the sign of birefringence after formol fixation can be interpreted as the result of (1) an increase of the form birefringence of the protein lamellae due to increasing the refractive index of the protein or due to a more perfect orientation of protein molecules, or (2) a decrease of the intrinsic birefringence of the lipide molecules by disorienting or removing lipide molecules. An increase in the refractive index of the protein lamellae is unlikely. French and Edsall (20) discuss the small increase in mass of various proteins after reacting with formol, and any change in density or refractive index would similarly be small. A more perfect orientation of protein molecules cannot be ruled out at this time. Baker (2) has presented evidence that phospholipides go into solution when tissue is fixed in formol, and he states that calcium-formol prevents the leaching of lipides into solution. Cain (9) has also found the calcium ion necessary to prevent the phospholipides from going into solution. During the present study, formol which had not been neutralized over CaCO$_3$ produced more negative birefringence with longer fixation. Thus, a continued leaching of the
lipides could explain the observed results; and neutral formol, which contains calcium ion, prevented lipides from going into solution. Actual leaching of lipides was not demonstrated in these experiments.

The group of fixatives classified by Baker (1) as the "protein precipitants" were found to produce identical results under the polarizing microscope. Lassek (27) has also found more material precipitated from liver emulsion with these fixatives than with other fixatives. Thus Baker's classification is valid from a functional standpoint. Final evaluation of the classification proposed by Baker must await elucidation as to the nature of the cytoplasmic component precipitated under these conditions.

Birefringence was demonstrable after fixation with potassium dichromate. Potassium dichromate is not a protein precipitant according to Baker's classification (1), although chromic acid is. Casselman (10) has also studied fixation by chromates, and he reports that fixation with potassium dichromate is superior to fixation with chromic acid. From the studies on birefringence given above, a similar distinction between potassium dichromate and chromic acid is obtained.

Osmium tetroxide provides excellent preservation of cytoplasmic structure as seen by the light microscope (29). Buchsbaum (7), in studying the changes in single cells with the phase microscope, found that fixatives containing OsO₄ produced less alteration in cytoplasmic structure than other fixatives. The qualities of OsO₄ fixation for electron microscopy are well known (30, 32). In 1936, W. J. Schmidt (36) demonstrated that osmium fixation altered the birefringence of myelin. When frog sciatic nerve was fixed in OsO₄, the sign of birefringence of the myelin reversed from axially negative to axially positive. A similar reversal of sign occurred when pancreatic tissue was fixed in osmium. Finean (18) has also observed a change in the x-ray diffraction pattern of myelin that has been fixed in OsO₄. Thus osmium, though known to preserve cytoplasmic detail, alters physical properties of cells dependent upon submicroscopic structure.

Wiener (50) has derived equations representing the birefringence of isotropic lamellar plates when one component becomes absorbing. In his paper he chose representative values for the refractive indices, absorption coefficients, and partial volumes of the two components and calculated examples of changes in birefringence in the presence of absorption. One example given by Wiener is a positively birefringent lamellar system which, when one lamella became absorbing, demonstrated a reversal of sign to negative. The reversal of sign of birefringence of pancreas accompanying osmium fixation might be explained on a similar basis. To behave as an absorbing body according to the Wiener equations, osmium must be partitioned between the components of the lamellar system and be deposited selectively in one component. Finean (18) has reached a similar conclusion regarding the fixation of myelin by osmium. The existence of a well ordered x-ray diffraction pattern of myelin after fixation by OsO₄ according to Finean, "...indicates a deposition (of osmium) at specific points which repeat in a regular fashion in the radial direction." (18). The nature of the component which selectively partitions the osmium in the lamellar system cannot be determined from the Wiener equations (50) or from the x-ray diffraction data (18).

The process of freezing markedly alters the birefringence of fresh pancreas. Elkes and Finean (15) have also demonstrated alterations in the submicroscopic structure of myelin that has been frozen. Though their work was based on x-ray diffraction data, a similar result is seen with the polarizing microscope. Lipoproteins are known to be especially susceptible to denaturation by freezing (33). Since membranes are thought to be composed of lipides and proteins in some form of a complex (6, 17, 21, 37), freezing might be expected to alter the structure of this complex.

Many studies of tissue birefringence have involved the use of glycerol as a mounting medium (23, 25, 39). Glycerol is also a compound of biological interest, and its use in preserving the viability of frozen cells is well known (45). The effect of glycerol on birefringence was first described by Göthlin (23), who found a reversal of the sign of birefringence in some invertebrate nerves when placed in glycerol. The reversal of sign was called the "metatropic reaction." Bear and Schmitt (4) localized this change in the sign of birefringence of lobster nerve to the nerve sheath. Since prior extraction of the nerve with lipide solvents prevented the metatropic reaction, both investigators concluded that the reaction was due to a decrease in the form birefringence of the protein lamellae. The decrease in the form birefringence was thought to be due to a change in the refractive index of the surrounding media (4, 11).

To demonstrate more conclusively the mecha-
nism by which glycerol alters birefringence, a mathematical treatment of the problem was attempted. Thornburg (48) has recently derived an equation for lamellar birefringence of three components. The form birefringence equation as given by Thornburg is:

\[ BR = \frac{-1}{n_3 + n_o} \left[ \frac{d_1 d_2 n_1^2 (n_2^2 - n_0^2)}{n_1 n_2^2 + d_2 n_1^2 n_2^2 + d_3 n_1^2 n_2^2} + \frac{d_2 d_3 n_2^2 (n_3^2 - n_0^2)}{n_2 n_3^2 + d_3 n_2^2 n_3^2 + d_1 n_2^2 n_3^2} \right] \]

in which \( BR \) = form birefringence, \( d \) = partial volume, and \( n \) = refractive index, subscripts 1, 2, and 3 refer to the first, second, and third components, \( n_3 \) is the refractive index parallel to the optical axis, and \( n_o \) is the refractive index perpendicular to the optical axis. Using the above equation and choosing values for the \( n \)'s and \( d \)'s, one can derive the value of the form birefringence which will be significant in this discussion, if the system is in fact a three component system as described by the equation. Considering glycerol to replace the water component without changing the other two components, the variation in the form birefringence with different concentrations of glycerol can be calculated. The following values were chosen for such a calculation, with the first component water, the second lipide, and the third protein:

\[ \begin{align*}
  d_1 &= 0.9, \quad d_2 = d_3 = 0.05 \\
  n_1 &= 1.33, 1.35, 1.40, \text{ and } 1.47 \text{ (refractive indices} \text{ of the three concentrations of glycerol used} \\
  &\text{substituted for the water in successive trials)} \\
  n_2 &= 1.4 \text{ (approximate refractive index of lipide)} \\
  n_3 &= 1.5 \text{ (approximate refractive index of protein)} \\
  n_o &= n_o = 1.35
\end{align*} \]

These values were chosen to represent approximates to biologically accurate values. Small changes in \( n \) and \( d \) values alter the resultant \( BR \) only slightly. Calculations were done using \( d_1 = 0.6, \ d_2 = 0.2, \ d_3 = 0.2, \) and \( n_2 = 1.52, \) and the calculated \( BR \) was altered only slightly. Thus, though these values may not be correct, the changes in \( BR \) that occur by alterations in \( n_1 \) will none the less be in the same direction as to those illustrated. The magnitudes of the form term with varying concentrations of glycerol were calculated from the above equation substituting the appropriate values to be:

\[ \begin{align*}
  \text{Water} & \quad \ldots \quad 2.1 \times 10^{-4} \\
  10 \text{ per cent glycerol} & \quad \ldots \quad 1.6 \times 10^{-4} \\
  50 \text{ per cent glycerol} & \quad \ldots \quad 6.6 \times 10^{-4} \\
  100 \text{ per cent glycerol} & \quad \ldots \quad 2.7 \times 10^{-4}
\end{align*} \]

Thus, the Thornburg equation predicts a decrease in the form birefringence for all concentrations of glycerol studied. The changes in the birefringence of fresh tissue, tissue that has been frozen, and tissue that has been fixed in formol, osmium, and alcohol can be explained as a decrease in the form birefringence when placed in glycerol. The observed increase in the radially positive birefringence is thus due to a decrease in the radially negative form birefringence. Thus the “metatropic reaction” is explained qualitatively on the basis of a change in the refractive index of one component of a three component lamellar system.

Hillarp and Olivecrona (25) have studied the birefringence of formol-fixed, frozen sections of pancreas. Such a preparation was isotropic until placed in glycerol of a concentration greater than 30 per cent. At this concentration, negative birefringence with respect to the base-apex axis appeared. The present investigation has demonstrated that formol fixation, the process of freezing, and glycerol as a mounting media change the birefringence of pancreatic tissue. Deductions based on such material are valid only if the changes in the tissue accompanying each of the processes have been evaluated.

Sjöstrand (43) in a preliminary note describes a radial axis to pancreatic acinar cell birefringence, but he claims the sign to be negative. Since no descriptive paper has appeared regarding his methods and results, any evaluation of his results are impossible at this time.

CONCLUSIONS

1. The radially positive birefringence of pancreatic acinar tissue is consistent with current concepts of pancreatic morphology and membrane structure. The observed birefringence is compatible with a lipide-protein membrane model.

2. Formol fixation decreased the intrinsic birefringence of oriented lipides, while formol neutralized over CaCO3 prevents the leaching of oriented lipides into solution.

3. Osmium tetroxide is deposited on regularly repeating units within the cytoplasm of the cell during fixation. The reversal of the sign of the birefringence occurring with osmium fixation is predicted from the Wiener equations for isotropic lamellar plates with an absorbing body.

4. Glycerol consistently decreases the form birefringence of the protein lamellae. The decrease in form birefringence, known in the past as the “metatropic reaction” is predicted from the
Thornburg equation for lamellar birefringence of three components.

5. Freezing and fixation alter the submicroscopic organization of membrane systems. Conclusions based on the birefringence of tissue that has been fixed or frozen require careful interpretation.

The author wishes to thank Dr. H. Stanley Bennett and the Department of Anatomy of the University of Washington, School of Medicine, for use of laboratory facilities during the course of this investigation, and to thank Dr. Wayne Thornburg for helpful suggestions and encouragement.

References

24. Hardy, W. B., J. Physiol., 1899, 24, 158.
EXPLANATION OF PLATES

All figures are paired photographs of the same preparation, taken at plus and minus 8° from extinction with the rotating compensator. Arrows point to identical areas of the two pictures. Birefringence is present when a given area is bright in one photograph and dark in the other. All magnifications are approximately 2,300.
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Fig. 1. Fresh pancreas in Tyrode's solution. The area illustrated is one cell thick at the margins. The base (B) and the margins (M) are alternately dark and light in the two pictures, illustrating the radial axis of birefringence. The apex (A) contains zymogen granules and birefringence cannot be demonstrated.

Fig. 2. Fresh pancreas in Tyrode's solution. The area of the base of the cells is birefringent. The margins of the cells in this preparation are not evident. The base (B) illustrates the birefringent area which contains no zymogen granules.

Fig. 3. Fresh, frozen tissue in Tyrode's solution. The nuclei (N) are large and prominent, and cellular details are obscure. The birefringence has been markedly altered as compared to Fig. 1.
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Fig. 4. 10 per cent formalin fixation for 24 hours. The birefringence is more prominent than seen in fresh tissue. The alternation in brightness of base (B) and margins (M) of the cell is well illustrated.

Fig. 5. 10 per cent neutral formalin fixation for 24 hours. The amount of birefringence is less than in Fig. 4. Cellular details are obscure, and a faint granularity is prominent. The base and margins of individual cells cannot be identified.

Fig. 6. 10 per cent neutral formalin fixation for 24 hours placed in 50 per cent glycerol. The cytoplasm appears homogeneous, and the cytoplasmic granularity seen in Figs. 4 and 5 is absent. The alternating birefringence of base (B) and margin (M) is well illustrated.
(Munger: Polarization optical properties)
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Fig. 7. 2 per cent osmium tetroxide fixation. The apposed margins (M) of two adjacent cells demonstrate birefringence opposite to that of the base (B).

Fig. 8. 2 per cent osmium tetroxide fixation placed in 50 per cent glycerol. The cytoplasm appears homogeneous, and no birefringence is demonstrable.

Fig. 9. 3 per cent potassium dichromate fixation. Randomly arranged anisotropic areas can be seen, but no systematic organization of these areas is evident.
Fig. 10. 10 per cent acetic acid fixation. This photograph demonstrates a single cell that has become detached from the mass of tissue during fixation and manipulation. The nucleus (N) is in the center of the cell. The radial axis of birefringence is demonstrated by the opposite sides of the cell showing identical birefringence.

Fig. 11. 95 per cent ethyl alcohol fixation: The appearance is similar to that of the other protein precipitants. The brilliance of the tissue precludes any observation of birefringence that might be present.

Fig. 12. 95 per cent ethyl alcohol fixation placed in 50 per cent glycerol. The brilliance demonstrated in Fig. 11 is absent, and scattered areas of birefringence can be seen. The magnitude of birefringence is very small.
(Munger: Polarization optical properties)