A STUDY OF THE CYTOLOGY OF THE REPTILIAN PANCREAS WITH SPECIAL REFERENCE TO THE ORDER TESTUDINATA.

BY

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INTRODUCTION

The pancreas in most vertebrate forms consists of an exocrine part, which elaborates certain digestive juices: and an endocrine portion whose internal secretions play such an important role in the control of the intermediate carbohydrate metabolism. The cells of these regions are distinctly different, although, frequently they may be interchangeable as far as their origin is concerned.

Little seems to recorded in reference to cytological studies of the pancreas in reptiles. "Pischinger (1895) was the first to identify the islets of Langerhans in the pancreas of the reptiles. His work was closely followed by several reports (Giannelli and Giacomini, 1896; Laquesse, 1898; and Diamare, 1899) which substantiated and extended the observations to many additional forms."[1] The workers, at that time, did not, however, show any particular interest in the acinar tissue as they took for granted the fact that it did not show any unusual characteristics of interest and of importance. Subsequently, with the discovery of the importance of the islets of Langerhans in respect to carbohydrate metabolism, later and more recent investigations have been concentrated on mammalian forms. Therefore, a more thorough and complete study of the reptilian pancreas has not been made. The most recent work on the reptilian pancreas is that of Thomas, who has investigated the cytology of the pancreas of the snakes. Kendall

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[1] Thurlo B. Thomas, "The Pancreas of Snakes." The Anatomical Record, Vol. 82, No. 3, P. 327, March,1942.

observes that the pancreas of the lizards does not show well defined islet groups, but he fails to give any reference for his statement.[2] The author has been unable to locate any other reference to the pancreas of the lizard. Our present knowledge of the reptilian pancreas, therefore, rests upon scattered observations in the literature and on detailed study on the snakes.

The present investigation was initiated to contribute to our general knowledge of the reptilian pancreas by making a study of the "normal" or non-secretory cytology of the pancreas of the order Testudinata, so that it may form the basis for subsequent comparative and experimental studies. As Thomas has pointed out the mammalian pancreas is poorly adapted for experimental studies because of the variability of the cell population, and he has suggested that the reptilian pancreas might prove more satisfactory for research purposes.[3] With this fact in mind, it was planned to give consideration to the possibility of utilizing turtles in laboratory investigations of the pancreas on the basis of the results obtained from this current work.

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[2] James I. Kendall, The Microscopic Anatomy of the Vertebrates (Philadelphia: Lea and Febiger, 1940), P. 196.

[3] Thomas, Op. Cit. PP. 327-328.

MATERIALS

The data presented in this paper is based on the study of representatives of the order Testudinata. From one to three individuals of five different genera were acquired either by field collection or by purchase. All animals were received alive and retained for the same period of time in terraria under suitable environmental conditions. The following specimens, which are classified according to Stejneger and Barbour, were used in this survey:[4]

Class Reptilia

Order Testudinata — Common Names

Sternotherus odoratus (Latreille)... The musk turtle

Chrysemys picta (Schneider)..... The painted terrapin

Pseudemys elegans (Wied)..... The cumberland "

Terrapene carolina carolina (Linné). The box turtle

Chelydra serpentina (Linné)..... The snapping turtle

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[4] Leonard Stejneger and Thomas Barbour, A Checklist of North American Amphibians and Reptiles (Cambridge: Harvard University Press, 1923), 2nd edition, PP. 127, 129, 130, 134, 135.

METHODS

In order to obtain tissue from the animals in as uniform a cytological state as possible, the turtles were not fed prior to the removal of their pancreases. Ignorance of the previous feedings and diet habit tend to confuse and complicate the picture by yielding inexplainable divergences. It was felt that such a procedure would eliminate most of the unknown factors and tend to equalize the secretory state and behavior of the pancreatic cells. This would also produce a cytological picture that could be used for comparative observations. As reptiles normally go long periods without food of any sort, feeding only occasionally in either the natural or captive state; it was believed that such a procedure would not lead to starvation effects which might occur in higher forms. In removing the pancreas from the turtle further efforts were made to excise it in a state as "normal" as possible free from any effects of anesthesia or degeneration resulting from any prolonged exposure in the operative technique. Therefore, the pancreas was removed by vivisection made possible when the carapace was first displaced. The pancreas was transected into numerous small pieces and immediately dropped into waiting containers filled with Zenker's standard fixation fluid.[5] After subsequent washing and dehydration the material was embedded, sectioned at 8u, and mounted on slides.

To enhance the cellular differentiation, sections were

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[5] Michael F. Guyer, Animal Micrology (Chicago: The University of Chicago Press, October, 1943), pp. 8 and 232.

stained with Heid enhein's azocarmine modification of Mallory's triple connective tissue stain.[6] The use of this stain gives the following color reactions; the nuclei and their inclusions, especially chromatin, stains a dark red. The cytoplasm appears pink, while the zymogen granules become deep red. This is the picture for the staining reaction of the acinar cells. The adjacent collagen and reticular connective tissue assumes a bright blue color. This stain, furthermore, has special affinities for the islet cells of Langerhans, whereby the granules of the alpha cells are stained a ruby red, the beta cells orange, and the delta cells bright blue or violet.[7] The cytoplasm of the islet cells remains relatively unstained. According to Bloom, this is the best stain for the acinar cells.[8] Most workers in cytology consider that these reactions are very satisfactory because of their specificity and the fact that the colors do not fade readily. However, there may be some color variations due chiefly to the use of acidic fixatives and subsequent treatment with dehydration alcohols. In these it is not the stain which is effected but the texture and granular composition of the various cells.[9]

Approximately four hundred slides of sectioned material

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[6] B. B. Bensley and S. H. Bensley, Handbook of Histological and Cytological Technique (Chicago: The University of Chicago Press, 1941), P. 75.

[7] J. Louis Bremer and Harold L. Weatherford, A Textbook of Histology (Philadelphia: The Blakiston Co., October, 1944), P. 393.

[8] William Bloom, "A New Type of Granular Cell in the Islets of Langerhans in Man." The Anatomical Record, Vol. 47, PP. 363-372, 1931.

[9] S. H. Bensley, "Solubility Studies of the Secretion Granules of the Guinea Pig." The Anatomical Record, Vol. 72, No. 2, P. 135, October, 1938.

were prepared representing samples of all of the specimens. From a preliminary examination of these, thirty six selected slides were used in the detailed study. All of these slides were examined using an oil immersion lens. Furthermore, from these slides desirable photomicrographs were made to demonstrate some of the grosser cytological aspects. Such photomicrographs in addition provided the means of measuring cells and their nuclei by the use of a calibrated scale, which was photographed at the same magnification as the prepared material (Plate VII, Figure 11). The photomicrographs were taken through a 15x ocular and 20x objective lens system, giving a magnification of 300x and through a 15x ocular and 45x objective lens system resulting in a magnification of 675x. The final prints were enlarged to double these magnifications. To increase the details and improve the quality of contrast of the photomicrographs, standard red and green filters were used.

OBSERVATIONS

GENERAL TOPOGRAPHY

The pancreas of the turtles is a relatively compact and ribbon-shaped organ ranging in color from flesh white to pinkish yellow. It is intimately associated with the duodenal portion of the intestine and held in the hepatoduodenal ligament. The pancreas is covered by a serose of thin connective tissue derived directly from the parietal peritoneum which does not, however, form a definite fibrous capsule.[10] Approximately the first two thirds of the pancreas lies in contact with the anteriodorsal surface of the intestine just distad of the pylorous of the stomach. Its latter third flexes so that it is directed posteriorly and comes to lie along the laterodorsal face of the concurrently curved small intestine. Since various genera and species were considered it is not possible to give average figures of the respective length and width of the turtle pancreas. However, individual measurements can be computed mathematically to show a fairly constant ratio. It was observed that the length of the pancreas is approximately six times as great as the width. The pancreatic duct opens near the bile duct into the dorsal surface of the duodenum as previously reported by Noble and Noble.[11] The pancreas is firmly and intimately

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10 Alexander A. Maximow and William Bloom, A Textbook of Histology (Philadelphia and London: W. B. Saunders and Company, February, 1943), 4th edition, P. 446.

11 Glenn A. Noble and Elmer R. Noble, A Brief Anatomy of the Turtle (California: The Stanford University Press, March, 1940), PP. 5-6.

adherent to the intestine and only the single pancreatic duct mentioned above persists in the adult condition. Unlike the pancreas of the snakes as observed by Thomas, the pancreas of the turtles bears no relationship to the spleen either in proximity or association.[12] The topography of the pancreas may be readily observed in Plate I, Figure 1, and Plate II, Figure 2, which were photographed in situ.

MICROSCOPIC ANATOMY

THE EXOCRINE TISSUE

The pancreas is a compound acinous gland bound together by relatively loose connective tissue through which run blood vessels, nerves, lymphatics, and excretory ducts.[13] (Plate IV, Figure 5 and 6.) The internal structure of the pancreas shows the relative distribution of the acinar cell groups, and the collecting ducts, with their numerous branching collecting tubules. The latter anastomose throughout the exocrine tissue and are easily discernible. It was observed that the connective tissue which accompanies the ducts and tubules is continued around and distributed among the secretory acini. Further, the connective tissue has the tendency to insulate clusters of acini into characteristic lobes. In most instances the acinar groups have lumina that are occluded by the close arrangement of the exocrine cells. Some few cases, comparatively speaking, nevertheless show a small lumen which may be and usually is

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12 Thomas, Op. Cit. P. 329.

13 Maximow and Bloom, Op. Cit. P. 447.

empty. In no instance do the lumina appear to contain mucous or any cellular extrusions or debris. (Plate III, Figure 4). Plugging of the lumen with centro-acinar cells as occurs in the mammals was not observed in any of our preparations.

The exocrine cells of the acini are typically and coarsely granulated. On the average they are more or less pyramidal measuring 12u across their lesser diameter and 25u through their greater diameter. The granules are dispersed in uniform fashion rather evenly throughout the entire cytoplasm of each cell. It is observed, however, that there is a slightly greater concentration of granules between the nuclei and the supernuclear portion of the cell opposite the lumen of the acinus. These may be considered as zymogen granules which are probably in an inactive state. (Plate V, Figure 8). Cowdry states that when there is a retention of the secretion, then the cytoplasm becomes filled with them.[14]

The nuclei of the secretory cells, which are similar both in type and appearance to those of the tubular epithelial cells, are round or broadly spherical in shape and lie in or near the basal portion of each cell. On the average they measure approximately 11u across their greatest diameter. In the peripheral region of the nucleus just inside the nuclear membrane there is an abundant concentration of deeply staining chromatin granules which appear to form a meshwork which tends to form radial extensions toward the nucleolus. Occasionally some of the nuclei show such a heavy concentration of chromatin that it gives them

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14 E. V. Cowdry, A Textbook of Histology (Philadelphia: Lea and Febiger, 1938), 2nd edition, P. 317.

the character of a massive or compact nucleus as viewed from the surface or on a tangent. In those nuclei which possess the more characteristic vesicular structure the chromatin assumes a radial pattern suggestive of spoke-like connections between the periphery and the central portion of the nucleus. In the rete formation of this chromatin material the inner termini of these strands coalesce over the surface of the nucleoli. This distribution of the chromatin granules is similar to that observed in the nuclei of pancreatic cells of other vertebrate forms and was also shown by Thomas as occurring in the nuclei of the cells of the pancreas of the snakes, (Plate I, Figure 5).[15] As these chromatin granules stain a deep red with azocarmine they are to be considered as acidophilic structures. (Plate IV, Figure 6 and Plate V, Figure 7).

The nucleoli of the vesicular nuclei are eccentric in position and of two major types, massive and vesicular in character, with a series of variable gradations from one to the other. Like the parent nuclei, they stain dark red with azocarmine and are, therefore, acidophilic in character. As in the rat, a central granule has been observed by the author quite frequently. This granule, the significance of which, if any, is undetermined, it may be nothing more than an aggregation of chromatin. Whether the presence of these granules depends on the reticular state of the inclusions or not, has been undetermined. (Plate V, Figure 7 and Plate VI, Figure 10).

The cytoplasm of the acinar cells contains granules, which

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[15] Thomas, Op. Cit. P. 343.

are quite uniform in distribution, as noted above. On the basis of the staining reaction which they give, red with azocarmine, they may be considered as zymogen granules. They extend into the basal portion of the cells unhampered by the presence of the nucleus in that area. While it may be observed that some cells appear to have more granules than others, in general the distribution appears to be uniform throughout all of the acinar cells. No cells were observed to be devoid of granules or to have markedly fewer granules than average. To a certain extent, it appears as if the number of granules present tend to influence their own distribution within the cell. There is no tendency toward clumping or undue aggregation of granules which might suggest a degenerating condition. (Plate III, Figure 4 and Plate V, Figure 8). Excessive accumulation of these indicates a rather latent or nonactive state of secretion.[16] There is, in addition, no evidence of granules having been extruded into adjacent lumina of the acini or tubules. Thomas observed that some of the acinar cells of the pancreas of snakes showed a tendency toward vacuolization in the cytoplasm which he interpreted as indicative of a degenerating condition of the pancreatic cells after extrusion or collapse of the zymogen granules.[17] No similar configuration appears in the acinar cells of the tissue used in this study. Regardless of the disposition of the zymogen granules there is no means by which we may interpret their metabolic state upon the position or character of the nucleus,

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16 Cowdry, Op. Cit. PP. 322-325

17 Thomas, Op. Cit. P. 331.

since the latter appears to remain in a constant position. Thus, the variability of the zymogen granules may be nothing more than the normal appearance encountered in cells differing only in the state of their activity.

The acinar cells of the turtle pancreas do not show signs of degeneration or collapse nor do the nuclei show any loss of nuclear contents or destruction of nuclear membranes. Occasionally such elements are involved in the formation of a "Nebenkern" or a paranuclear structure. Further, there is not any breakdown of the reticular structure or granular inclusions which preclude the use of azocarmine. There is no evidence to indicate that any cells or cell contents have been extruded into the lumina at any time. Also, it was not observed that there is any indication that the acinar groups or individual cells are compressed or distorted by adjacent cellular structures, thereby causing the elimination of the former. It seems reasonable to conclude, therefore, that the structures described are of a definite and constant nature rather than artifacts resulting from fixation.

In the choice of materials used for this investigation, care was taken to insure adequate representation of the head, body, and tail portions of the pancreas. It seems fair to state, therefore, that the cellular picture of the acini of the pancreas of the turtle presents a more or less universally similar cytological picture within the various genera examined.

The epithelial lining of the tubules is made up of cells having approximately the same height and general cytological appearance as those of the acini. It may seem that the cells

of the acinar tubules, however, are spaced somewhat apart by the invaginations of the basement membrane. It should also be mentioned that the cellular elements making up the epithelial lining of the tubules contain granules similar in position, distribution, and staining reaction; but fewer in number, to those of the acinar tissue. Since the nuclear structure of the epithelial cells of the tubules is essentially the same as that of the acinar cells, it does not seem desirable to repeat that information at this point. The complex histological differentiation of the tubules characteristic of the mammalian pancreas is not observed in the turtle pancreas. Thus, the tubules present the appearance of expanding at the ends to form acini made up of similar cells which are, however, pyramidal in shape with their distal ends compressed around the lumen of each acinus. (Plate III, Figure 3 and Plate V, Figure 8). Whenever acini are formed they do not tend to expand nor to enclose some of the most terminal duct cells. Examination of several figures will show that not all tubules end in a typical acinus, as some show simple blunt endings instead of typical acini. In these, the cells are very similar to those of the epithelium of the tubules suggesting either an incompleted acinus or a variable type. (Plate VI, Figure 9). It is at these points that Thomas reports that evidence for endocrine cell groups is observed in the pancreas of the snake.[18]

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[18] Ibid, P. 333.

THE ENDOCRINE TISSUE

In his survey of the pancreas of the order Ophidia, Thomas reported that islet tissue is present as a massive unit or several smaller islet groups in that portion of the pancreas which is enjoined by the spleen. These islets are located more or less closely to the splenic fimbriae which enter into the pancreas of the snakes in that region. Other islets have been described by Thomas and Laquesse as being scattered throughout the anterior half of the pancreas in contact with the epithelial ducts.[19] The latter were considered by them to be temporary islets of acinar origin which eventually return to their former state. However, during their transient establishment they migrate away from their point of origin and are bounded by connective tissue. Kendall shows photographic evidence of the appearance of islet tissue so isolated amid the acini.[20] Careful examination was made to determine the presence of islet tissue in the pancreas of the turtles. In each and every section of prepared material, regardless of the specimen involved, there was no evidence that islet tissue was present either in isolated clusters or individual islet cells. In mammals, islet tissue is frequently associated with the acini or the pancreatic tubules or ducts; however, at these foci in the turtle pancreas there was no indication that such islet tissue was present. It was impossible to locate even transitory remains or vestiges of islet tissue.

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[19] Ibid, P. 333.

[20] James I. Kendall, The Microscopic Anatomy of the Vertebrates (Philadelphia: Lea and Febiger, 1940), P. 196.

As has been previously noted, Thomas reported the occurence of islet tissue at the end of an uncompleted acini in the pancreas of the snakes. The presence of these endocrine cell groups was observed chiefly between the basement membrane and the acinus or its duct.[21] In this study it has not been possible to observe any configuration in the turtle pancreas in these regions that would lend itself to interpretation as endocrine tissue. In all instances the acini and their tubules were directly supported by a basement membrane allowing no intrusions of other cells. At points of contact between these elements the gradation of cells was too uniform to allow the possibility that the endocrine cells might arise either from the acini or the tubular epithelium. In sharks the islet tissue retains its primitive relationship to the duct system. In these instances, the specialized endocrine cells form an irregular row on the outer surface of the duct epithelium.[22]

Since the islets in the snake seem more permanent in those areas where the spleen makes contact with the pancreas, it is obvious that such a condition could not exist in the turtles because the spleen is completely unattached and distinctly removed from the pancreas in the latter. Further, no trace of islet cells was found in relation to the vascular system, which is another well known focus for islet tissue in the vertebrates. It is, therefore, necessary to conclude that no cellular evidence exists for the presence of islet tissue in the pancreas of the turtles.

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[21] Thomas, Op. Cit. P. 552.

[22] Thurlo B. Thomas, "Islet Tissue in the Pancreas of Elasmobranchs." The Anatomical Record, Vol. 76, PP. 1-18, 1940.

DISCUSSION

THE ACINAR CELLS

This study of the pancreas of the order Testudinata has brought out certain features that are of considerable interest from a point of view of comparative morphology, as well, as others bearing on the endocrine behavior of the organ. In its general morphology, the turtle pancreas is, as would be expected, similar to that of all the other vertebrates in that it arises as a diverticulum from the intestine. This diverticulum forms the excretory or pancreatic duct which divides into the interlobular ducts that in turn subdivide to form the intralobular tubules; whose small branches form the secretory ducts, each of which ends in an acinus or a simple blunt tubule. The entire gland is supported by a very thin covering of connective tissue which does not form a definite capsule. Elements of this connective tissue sheath continue into the gland and ramify so as to form the basement membranes and sustentative tissues about the ducts and their end pieces. Therefore, it may be said definitely that the gross anatomy of the gland follows the general picture that is recognized for all other vertebrate animals. It differs somewhat from the mammalian pancreas in the degree to which internal lobules can be recognized. Unlike the pancreas of the snake, mentioned previously, the turtle pancreas bears no intimate relationship to the spleen or any other visceral organ except the duodenum. Furthermore, the turtle pancreas may be considered as a compact structure never to be confused with the diffused composition of the pancreas in the teleost

fishes. As observed by MacLeod, the teleost pancreas occurs as narrow and loosely held bands in the mesentery of the intercaecal spaces and frequently microscopic examination is required to ascertain its presence.[23] In the birds the pancreas is supported in the mesoduodenum and occupies the space created by the duodenal loop being, thus, somewhat removed from the pylorus. Externally the turtle pancreas is smooth and does not possess any auxiliary modifications: for example, a pancreatic bladder which has been seen and described in the cat by Boyden.[24] It is of interest to consider the contrasting relationship of the turtles and snakes because of the fact they are members of the same class. Differences between their pancreases serve to lend additional weight to the generally accepted opinion of comparative morphologists; that the class Reptilia, while being one of the most homogenous in major characteristics, is one of the most heterogenous as regards specific systems and organs.

Microscopically the general picture of the gland is similar to that of all other vertebrates in having a tubulo-alveolar structure; but here again, sufficient differences are apparent so that it is justifiable to consider the demonstrated cytology as peculiar to the Testudinata.

In mammals, the various portions of the tubular system may be readily recognized by differences in the cytology of the cells.[25]

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[23] J. J. R. MacLeod, "The Source of Insulin." The Journal of Metabolic Research, Vol. 2, P. 15, 1922.

[24] Edward Boyden, "The Problem of the Pancreatic Bladder." The American Journal of Anatomy, Vol. 36, No. 1, PP. 151-183, September, 1925.

[25] Bremer and Weatherford, Op. Cit. P. 391.

One of the most striking features of the turtle pancreas is the similarity of the cells which make up the tubules and acini. As has been noted, in the observations of this study, all of the cells in the turtle pancreas are similar in height and alike in cytological and nuclear structure with the exception of the fact that the tubular cells have a fewer number of cytoplasmic granules. In fact, the entire cytological structure of the pancreas of the turtles presents an unusually uniform appearance. Only in the Aves do we find a similar condition existing. In this group Batt has reported for the domestic hen that cytologically the epithelium of the tubules is morphologically the same as that of the acinar cell groups.[26] In observing the relationship of these same structures in the snakes it has been observed by Thomas that the duct and secretory cells are of the same height and both rest on the basement membrane; but as the exocrine cells differentiate and increase in size they appear to compress the most terminal duct cells into the lumen so that the latter resemble the centro-acinar cells of mammals.[27]

As regards the general cytology of the acinar cells of the turtle certain aspects should be discussed. In interpreting these observations it may be informative to compare them to the cytology of the mammalian pancreas and that of the other forms. Little, if anything, would be gained by extending this

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[26] H. E. Batt, "The Normal Histology of the Endocrine Glands of Gallus Domesticus." Report of the Ontario Veterinary College, Ontario, Canada, PP. 1-12, 1934.

[27] Thurlo B. Thomas, "The Pancreas of Snakes." The Anatomical Record, Vol. 82, No. 3, P. 330, March, 1942.

comparison further, since too little in the way of definite information is available in regard to the cytology of many of the lower forms.

Comparison of the sections of the turtle pancreas with the section of the guinea pig pancreas show that cytologically the exocrine portion of both organs is comparable. However, it was observed by the author that the zymogen granules of the pancreas of the guinea pig were comparatively more flocculent and less discernible. As the cytology of all of the turtle pancreases is identical, it is logical to assume that all were, therefore, in the same metabolic state. Since the granules are well defined and present in relatively large numbers, it appears safe to assume that the attempts to obtain the pancreas in a state of rest or non-zymogenic activity, by allowing the annimals to go without food before sacrificing them was successful. Since it is not stated by Thomas just what conditions prevailed prior to obtaining the pancreases of the snakes, he used, it is questionable how valid any comparison with our data will be. However, the granules in the pancreas of the snakes, in general, may be said to be similar to those of the turtles. In his observations, it was noted that more granules were present in the distal portion of the cell than were observed in the same region of the acinar cells of the turtle pancreas. As is well known, this difference might be due solely to the variable state of secretory activity in the pancreatic tissue investigated by him.

As this study was solely an attempt to determine the "normal" or resting cytology of the turtle pancreas efforts were not

made to demonstrate mitochondria or the gogli apparatus. Observations by the author indicate that the cytoplasm of the acinar cells seems relatively uniform and homogenous in texture in response to the staining technique employed. For example, mammalian acinar cells stained with Janus Green B show noticeable mitochondria in the basal region of the cell to which they impart a characteristic striated appearance. The mitochondria frequently extend into the supernuclear region of the cell in which are found the zymogen granules. Cowdry states that the topographic arrangement and subsequent absence of the mitochondria may be due to some partial role they play as antecedents of the zymogen granules.[28] Working with the toad, Key observed that the number, size, and arrangement of mitochondria were independent of the formation and function of the zymogen granules.[29] The same considerations can be made in regard to the golgi apparatus and its role with respect to the origin of zymogen granules and their content and distribution in the acinar cells. However, since the origin and source of the zymogen granules has, at one time or another, been attributed to every cell inclusion, in whole or in part, and because this matter is of experimental nature not considered here, no further comment need be made about it. In toto, the presence of the many zymogen granules in the basal region of the cells suggests that the digestive secretion is at a very low or latent level. Normally, in active secretion, most of the

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[28] Cowdry, Op. Cit. PP. 317-318.

[29] J. Albert Key, "On the Relation of Mitochrondria to Zymogen Granules." The Anatomical Record, Vol. 10, PP. 215-216, 1916

zymogen granules would be confined in the distal part of the cells and, therefore, it seems that this excessive formation of secretion granules at the expense of the homogenous basal cytoplasm indicated a resting condition between periods of active secretion.[30] It was felt that any extended study on the turtle along these lines would merely contribute structural configurations without offering any experimental data to support conclusions drawn from such configurations and their relationships in the acinar cells.

All of the nuclei observed in this study had a characteristic reticulated structure inside of the nuclear membrane. The significance of this formation probably should be considered as a quality of acinar cell nuclei since it is identical with observations reported for the cat, rat, snake and other forms. All acinar nuclei observed in different forms show the same reticular arrangement of chromatin with radial projections from their point of origin, in a dense peripheral region of nucleoplasm, directed toward the nucleoli. In shape, the nucleus of the turtles is more spherical than the nuclei of the guinea pig and other mammalian forms but more or less identical with those of the snakes. There is no visual evidence that the nuclei in their from and position influence the behavior of the zymogen granules within the cell.

The eccentric nucleoli of the observed material are larger in proportion to the body of the cell than those of the guinea pig and rat, and appear to be greater than those of the snake:

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[30] Maximow and Bloom, Loc. Cit.

but otherwise seem identical as regards their massive and vesicular character. It was observed in certain sections of the guinea pig pancreas that individual cells had smaller nucleoli or that frequently they were absent. In some cases in the rat, it was observed that the nucleoli contained a heavy cortical concentration of acidophilic material and a large eccentric granule or close aggregation of similar material. Saguchi reports in the frog the presence of several nucleoli in many pancreatic cells of which, however, one appears to be predominent nucleolus.[31] Such variable elements were not observed in the turtle acinar cells.

Obviously, from a study of the resting pancreas it is not possible to make definite conclusionsin regard to the exact secretory or functional behavior of the acinar cells. However, certain observations were made in this study that do have bearing on the problems of secretion. It has already been stated that the source and origin of the zymogen granules cannot be told with accuracy. Probably every cell constituent plays some part in their formation and subsequent function, especially those elements which are most frequently associated with cell activity, i.e., the mitochondria and golgi apparatus. The most valid statement that can be made here is the simple fact that the zymogen granules encountered were spread over and throughout the entire cytoplasm of the cell. This fact offers proof that despite periods of long duration between feedings the turtle pancreas is fully prepared to engage in active digestive secretion

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[31] . Saguchi, "Studies on the Glandular Cells of the Frog's Pancreas." The American Journal of Anatomy, Vol. 26, No. 3, P. 350, January, 1920.

at very short notice. Furthermore, the size of the lumina varies with the state of the functional condition of the gland; thus, it is small at rest, but during active secretion becomes distended with secreted matter.[32] It has been noted previously that the lumina were more or less compressed and always empty, therefore, suggesting a normal non-functional picture.

THE ISLET CELLS

This study has been noteworthy in that it has been impossible to demonstrate tissue which could be termed islet tissue in the pancreas proper. Such a finding deserves considerable comment when we consider that insulin is one of the most fundamental hormones in that it regulates carbohydrate metabolism in the animal body. Even though an animal may not utilize foodstuffs that are preponderantly carbohydrate in its diet, insulin is supposedly necessary for the proper utilization by the liver of the carbohydrate material which is associated with any foodstuff. Further, the normal functioning of the liver as an organ of food storage, the metabolism of muscular contraction, and many other functions of the organisms seem to depend upon the presence of insulin.[33] In the case of elasmobranchs and teleost fishes MacLeod mentions the problem of animals on a non-carbohydrate diet. Furthermore, the pancreas of these forms does not have islet tissues for the manufacture of insulin. Special extrapancreatic structures produce the insulin

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[32] Maximow and Bloom, Op. Cit. PP. 447-449.

[33] Eugene Opie, "Cytology of the Pancreas." Special Cytology (New York: Paul B. Hoeber, 1932), Vol. 1, PP. 350-402.

which he suggests may be important in the regulation of fats and oils in addition to the glycogen that the liver may store.[34] Another idea is expressed by Saguchi, working with Rana temporaria, that islet cells appear and secrete insulin only when there is any need for these, hence, they are non-existant or very indistinguishable in the pancreas.[35] With debatable and controversial reports such as these and others it seems desirable to offer some explanation for the absence of recognizable islet tissue in the turtle pancreas.

The typical picture in one's mind is that of the organ as it exists in the mammals where the islet tissue is relatively common. For example, Bensley observed that the number of islets in the pancreas of the guinea pig ranged from 13,318 to 56,722.[36] According to the findings of Clark the pancreas of man has between 120,323 to 1,760,000 individual islets. Overholser noted between 1,494 to 14,579 in the white rat.[37] Such results would lead one to believe that islet tissue was comparatively prevalent and even abundant in the vertebrate pancreas. When we consider the rest of the vertebrate animals, however, it is observed that a wide variation actually is found to exist, first, in the number of islets present and, secondly, in the

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34 MacLeod, Op. Cit. P. 151.

35 S. Saguchi, "Cytological Studies of Langerhans's Islets, with Special Reference to the Problem of their Relation to the Pancreatic Acinus Tissue." The American Journal of Anatomy, Vol. 28, No. 1, PP. 1-57, November, 1920.

36 R. R. Bensley, "Structure and Relationships of the Islets of Langerhans." The Harvey Lectures, Vol. 10, P. 250, 1915.

37 Cowdry, Op. Cit. P. 325.

actual presence of the islet tissue itself.

MacLeod reports the islet tissue of the elasmobranchs is in intimate contact with the duct epithelium especially in the dorsal lobe of the pancreas. In his observations he further observed, as had Rennie before him, that the endocrine tissue was absent in the pancreas of the teleost fishes but was collected in a separate organ to which the name "Principal Islets" has been applied.[38] This comparison may be continued to point out that in most teleost fishes the pancreas is present only as diffuse masses of tissue that are not collected together to form a definite organ. Jackson supplements this observation further by reporting that often this diffuse pancreas is microscopic in quality and when examined shows no evidence of any transitional cells.[39] On the other hand we find that in the elasmobranch fishes the endocrine tissue is well defined and is observed in the pancreas to be associated with the duct system or in mammalian-like islands. In these foci their distribution and relations to the rest of the gland are of primitive nature.[40] Noguchi's report on toads shows the endocrine tissue to be distributed frequently in one cell units scattered throughout the gland in close association with the acini.[41]

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[38] MacLeod, Op. Cit. P. 154.

[39] Slator Jackson, "The Islands of Langerhans in Elasmobranchs and Teleost Fishes." The Journal of Metabolic Research, Vol. 2, PP. 141-148, 1922.

[40] Thurlo B. Thomas, "Islet Tissue in the Pancreas of Elasmobranchs." The Anatomical Record, Vol. 76, No. 1, PP. 1-18, January, 1940.

[41] Noguchi, Op. Cit. PP. 53-54.

The lizard pancreas is noteworthy in that the islet tissue is not conspicuous.[42] The islet tissue in the snake pancreas, on the other hand, appears to be well defined because it is chiefly localized in the anterior end of the pancreas which is in contract with the spleen. Other so called "Secondary Islets" are scattered through the exocrine tissue and are believed to be temporary islets which are transitional, eventually returning to their original state as acinar cells.[43] The only available study on the pancreas of the birds is a report by Bett in which it was observed in the domestic hen that the islet tissue was slight in amount and appeared as closely packed cords of cells. Since this study was performed through the use of routine hematoxylin and eosin staining further information was not observed in regard to the type of cells present, the islets, however, were well defined.[44] Essentially, therefore, the pancreas is a highly variable organ in the vertebrates and islet tissue is by no means a universal characteristic of this gland.

From an evolutionary point of view, the pancreas actually develops from three outgrowths; one dorsal connecting to the duodenum by the duct of Santorini, and two ventral which unite to connect through the duct of Wirsung or the true pancreatic duct.[45] In the mammals, these three parts of the pancreas unite

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[42] Kendall, Loc. Cit.

[43] Thurlo B. Thomas, "The Pancreas of Snakes." The Anatomical Record, Vol. 82, No. 3, P. 332, March, 1942.

[44] Bett, Loc. Cit.

[45] L. E. Adams, An Introduction to the Vertebrates (New York: John Wiley and Sons, Inc., 1934), PP. 264-265.

to connect with the duodenum by a single duct, the duct of Wirsung. It is the general opinion of the comparative morphologists that the dorsal portion of the pancreas represents the primitive and true pancreas which was concerned primarily with the endocrine secretion in that it contained the islet tissue. In the course of evolutionary time, the enzymatic tissue was added to this and the ventral outgrowths from the alimentary tract were developed. In support of this theory, we have the fact that the pancreas of the elasmobranchs represents only the dorsal portion of the gland, connecting to the duodenum by the duct of Santorini, often misnamed, the pancreatic duct because of the misconception that it is homologous with the pancreatic duct of the higher forms.[46]

In the teleost fishes we find that the ventral portions of the pancreas begin formation as diffuse acinar elements. The latter, in the more advanced forms, unite into a more or less fairly well defined pancreas that lacks islet tissue and is connected by the duct of Wirsung to the intestine. The turtles, which are placed next to the fishes in our present day evolutionary classification scheme, have a well defined pancreas connected by a single duct, the duct of Wirsung, to the intestine.[47] As there is no embryological study available on the pancreas of the turtle, it is impossible on the basis of this report to determine whether the dorsal portion of the

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[46] Herbert Eugene Walter, Biology of the Vertebrates (New York: The MacMillan Company, 1939), 2nd edition, P. 327.

[47] A. S. Romer, Man and the Vertebrates (Chicago: The University of Chicago Press, 1937), 2nd edition, PP. 385-390.

pancreas is associated with the definitive pancreas.

On the basis of the above discussion, it is not illogical nor unexpected to find that islet tissue was not observed in the turtle pancreas. From a strictly evolutionary point of view it might be assumed that the pancreas of the turtles was intermediate between the typical teleost condition and that observed in the higher vertebrates. Therefore, it might well be postulated that the islet tissue of the turtles was located elsewhere, either in the mesenteries near the duodenum or adjacent to some other vascular center. The author is quite certain that an additional structure such as the "Principal Islets" of the teleosts is not to be found in the turtles.

It should also be pointed out that many of the islets present in the pancreas of the snakes appear to be transitory, it is quite possible that the endocrine tissue of the turtles, if present, might have the same character, and thus was not observed in this study. We have some evidence available that insulin may be produced in the absence of islet tissue in the pancreas, in that insulin was able to be extracted from the enzymatic tissue of teleost fishes which contain no islets. The amount of insulin so extracted was approximately one-tenth of that extracted from the "Principal Islets" of the same individuals.[48] Jackson observes that insulin can be recovered from the pancreas of the teleost fishes; but the yield depends upon the freshness of the gland and the method of extraction.[49] Both of the above

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[48] S. Vincent, F.C. Dodds, and J. Dickens, "The Pancreas of the Teleost Fishes and Source of Insulin." The Lancet, Vol. 2, pp. 115-116, 1924.

[49] Jackson, Op. Cit. P. 145.

mentioned reports agree that the islets present in the "Principal islets" are homologous with corresponding structures in the other vertebrates, and each suggests that the absence of these in the zymogenous tissue makes it possible that the latter may also function in the production of insulin by some other emergency internal secretion.

Lastly, some consideration must be given to the possibility that the previous treatment of the turtles resulted in the obliteration of the insular tissue. If this is true, then the metabolism of the islet tissue of the turtle is quite different from the higher forms, where there is no evidence that starvation results in the destruction of the islet tissue. It is well known, however, that starvation induces atrophy in the acinar groups. Since the acinar groups of the material used in this study did not show any effects of prolonged starvation it seems logical to conclude that the procedure is not responsible for the absence of endocrine tissue. It appears, therefore, that we must conclude that the pancreas of the turtles, either does not contain insular tissue in common with the teleost fishes, or that the endocrine tissue is transitory in nature, arising as needed from the acinar cells and, consequently, was not capable of demonstration in this study.

In regard to the order Testudinata it should be noted that this investigation has included representatives of four out of the seven generally recognized families of this group. Therefore, it is probable that the cytological features reported herein are constant for all members of the order.

SUMMARY AND CONCLUSIONS

This investigation which represents the first study that has been made of the pancreas of the turtles, was initiated to determine; first, the general cytology of the gland and secondly, the extent to which the organ approached the cytology of other vertebrate groups. Since so many investigations in the past have been unnecessarily complicated by the fact that the pancreas has been obtained in various unknown stages of secretory activity, the animals used in this study were kept without food. It was assumed that this treatment previous to sacrificing them would make it possible to observe the glandular tissue in a "normal" or resting state. For this reason no attempt was made to investigate either mitochondria or golgi, as the morphology of these two cellular organoids is of little value unless correlated with experimental data.

Material from five species of turtles, representing four of the seven families of the order Testudinata, were utilized in this study. Therefore, it seems reasonable to assume that these results will apply equally to all members of the group.

The major conclusions established by t is investigat ion may be tabulated as follows:

1 The gross morphology of the pancreas of the turtle is similar to that of most vertebrates. It is a flattened rectangular organ bound to the duodenum just caudad of the pylorus by the hepatoduodenal ligament. The color is relatively constant, ranging from flesh white to a pale pink.

2 The cells of the lining epithelium of the tubules appear quite similar cytologically to the acinar cells proper. The

tubules and either in typical acini, or in atypical acini or they may simply terminate bluntly.

3 The acini are composed of 8 to 12 cells, pyramidal in shape, with eccentric nuclei which are close to or in the basal region of the cell. The cells measure 12u in width and 20u in length. All of the acinar cells were similar; in that, no transforming stages were observed.

4 The acinar cell nuclei are spherical in shape, measuring approximately 11u in diameter, and having a characteristic distribution of chromatin material, which forms a reticular meshwork with a well defined peripheral border adjacent to the nuclear membrane.

5 Nucleoli are eccentric and show variations from typically massive ones to typical vesicular characteristics.

6 The zymogen granules are prominent, well defined, and equally distributed throughout the cytoplasm of the acinar cells.

7 The cytology of the epithelial cells of the tubules is identical with that described above for the acinar cells with the exception of the fact that they show fewer zymogen granules which are also present in these cells.

8 Lumina of the acini and tubules are relatively closed; but whether closed or partially open they do not show cellular or nuclear extrusions and no mucous is evident. Centro-acinar cells are not present.

9 Typical islets of Langerhans were not present. Endocrine tissue was not observed in individual cell units or in clusters either in connection with the acini and ducts, or in the connective tissue elements. Several possible explanations

for their apparent absence has already been discussed above.

10 On the basis of this study, it is concluded that cytologically the pancreas of the turtle is quite specific in character. It is believed that this study will serve as a useful basis for any desirable experimental work utilizing the turtle pancreas. However, the author does not believe that this vertebrate form is very satisfactory as a typical laboratory experimental animal.

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ILLUSTRATIONS

PLATE I

Figure 1.

This is a photograph of Pseudemys elegans taken of the live animal with the carapace removed. The viscera, principally, the liver are deflected in order to expose the pancreas. Directly in the center of the picture is to be seen the duodenum of the intestinal tract. Immediately in contact with its anterior surface and just to the right of the stomach a major portion of the pancreas is visible. Note that the stomach is quite deflated and that the pancreas begins at the pyloric end of the stomach. The pancreas is firmly held to the duodenum by mesentery which is not readily seen. Magnification x3.

PLATE I

Figure 1.

PLATE II

Figure 2.

This photograph of Terrapene carolina carolina in vivo also has the carapace removed. It is again possible to see here the position ofthe pancreas in situ. Note that the pancreas occupies the anterior wall of the duodenum just distad of the pyloris. Approximately, one third of the pancreas is visible; the remainder is concealed on the dorsal surface of the intestine as it flexes and becomes directed caudad. The viscera are relatively well exposed showing a very much reduced and empty stomach. Most of the gasto-hepatoduodenal ligament has been destroyed; but fragments of it can still be seen attached to the pancreas and adjoining intestine. The liver had to be deflected completely causing the ruptured condition of this mesentery. Magnification x3.

PLATE II

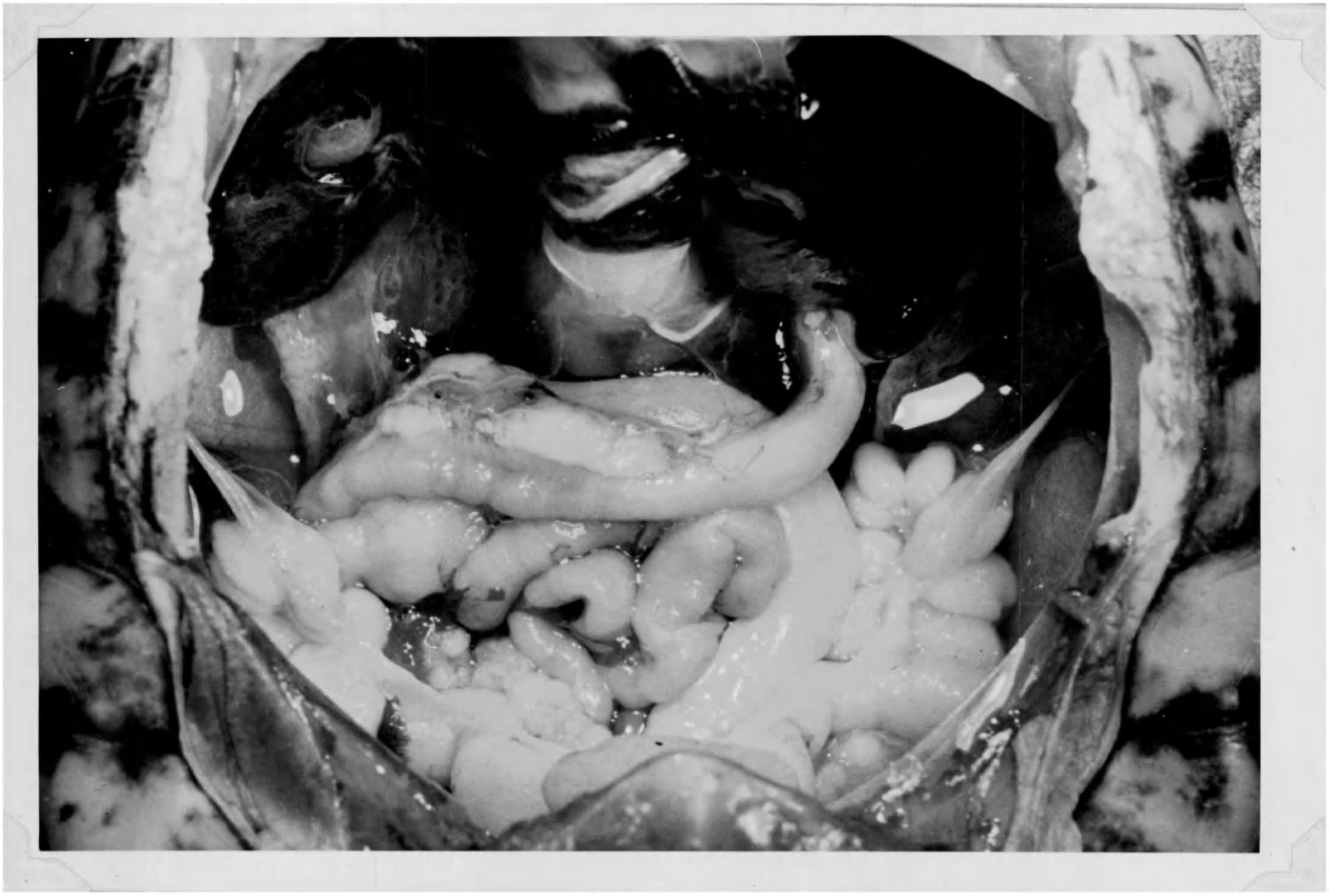

Figure 2.

PLATE III

Figure 3.

A portion of a prepared slide containing pancreatic material from Terrapene carolina carolina. Note the cellular arrangement of the acinar cells. Due to the angle of cutting several excellent cross sections are visible, i.e., observe the one in the lower left hand corner. In the center of the photomicrograph is an excellent longitudinal section of an acinus and its tubule. Magnification x600.

Figure 4.

Similar to the specimen in figure three, Terrapene carolina carolina; but enlarged twice. The enlarged condition makes it possible to see more clearly the texture of the acinar cells. Note the relatively uniform distribution of zymogen granules in the various acini. Because the chief attention was directed to focusing upon the cell bounderies, the nuclei appear extremely massive. Observe the two clear and open lumina in the acini at the top of the figure. Magnification x1200.

PLATE III

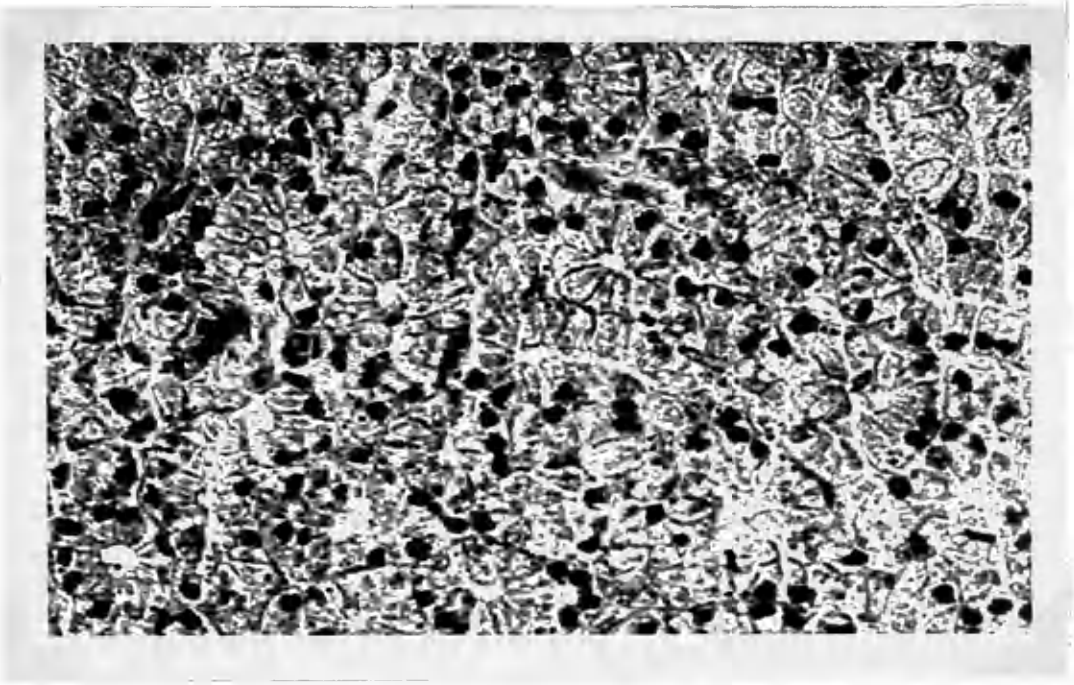

Figure 3.

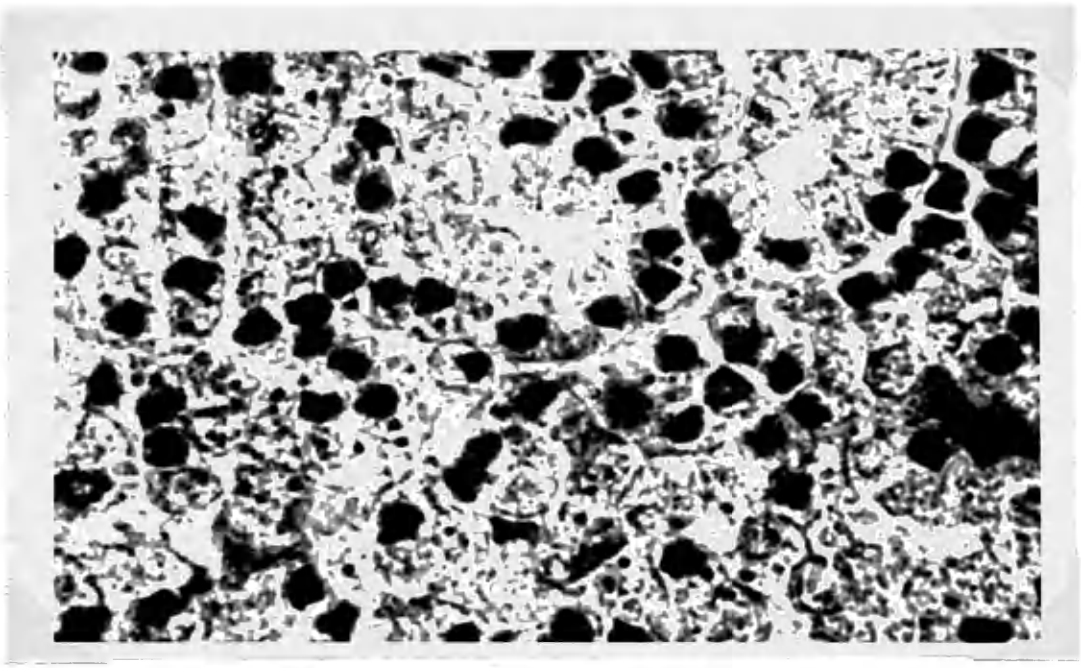

Figure 4.

PLATE IV

Figure 5.

Observe in this section of the pancreas of Chrysemys picta the general distribution and arrangement of the acini and tubules. Note the characteristic nucleoli in their relation to the nuclei. Magnification x600.

Figure 6.

As above, this is a photomicrograph of pancreas from Chrysemys picta. In this specimen it is relatively easy to see portions of the pancreatic duct. Note the position of the nuclei in the basal region of the cells in which many of them are extremely close to the basement membrane. Also notice the vesicular nature of the nuclei and the concentration of chromatin lining the periphery of the nuclear membrane. Most of the nuclei show the characteristic eccentric nucleoli. Obviously all of the nuclei are not in focus. Magnification x1350.

PLATE IV

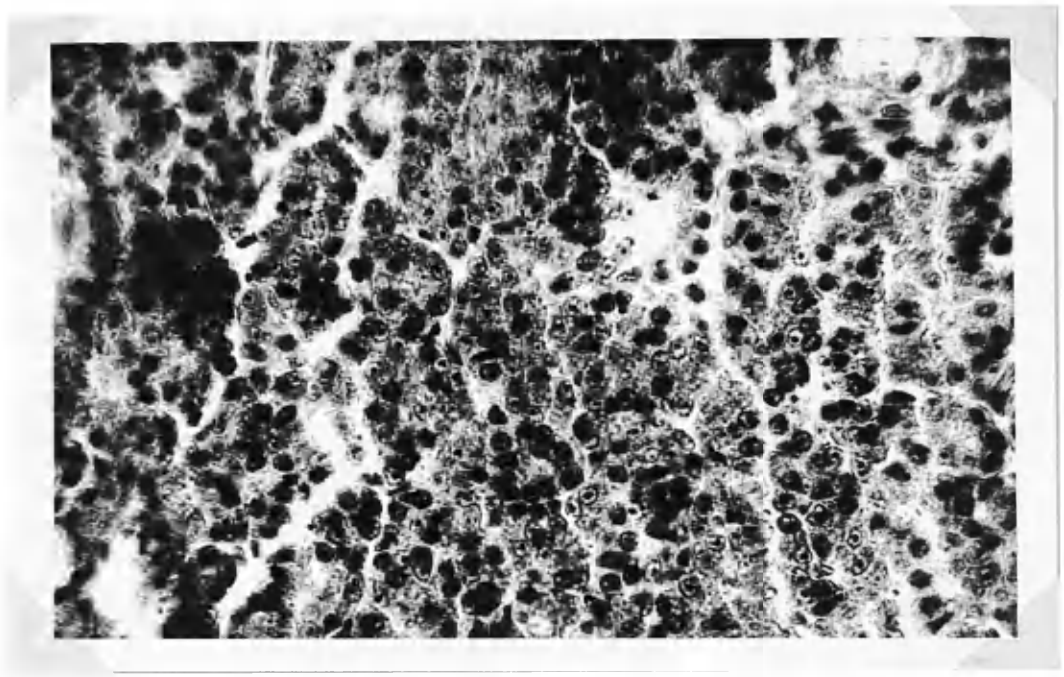

Figure 5.

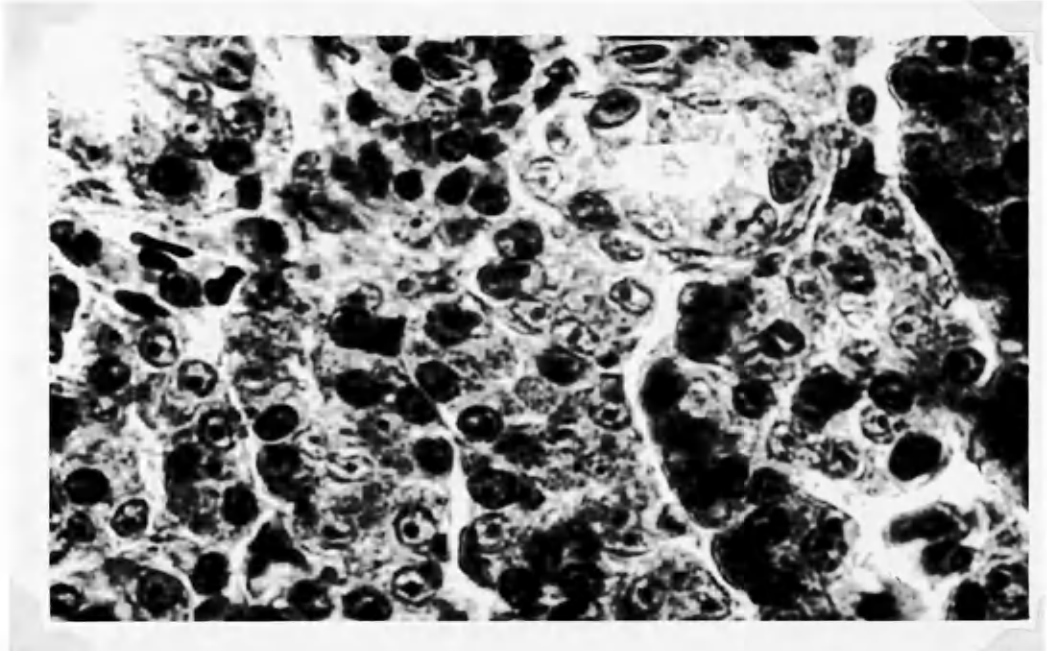

Figure 6.

PLATE V

Figure 7.

This specimen was photographed to show very clearly the position of the nucleus within an acinar cell. Furthermore, examination shows the reticular nature of the nuclei and clearly demonstrates the presence of a heavily stained eccentric nucleolus. Chromatin strands which radiate toward the nucleoli are not readily seen because of the difficulty in focusing. Note in the lower left hand corner the extremely large acinar cells. Immediately to the right of this group there are seen several segments of pancreatic ducts. Magnification x1250.

Figure 8.

This material taken from Pseudemys elegans was photographed through a red filter in order to accentuate the cytoplasmic granules. It can be seen at a glance that the distribution of zymogen granules is extremely uniform. The nuclei of pancreatic acini are demonstrated here more accurately because of the filter effect. This section demonstrates very clearly the investment of the acini by the connective tissue formed into a basement membrane. Magnification x1250.

PLATE V

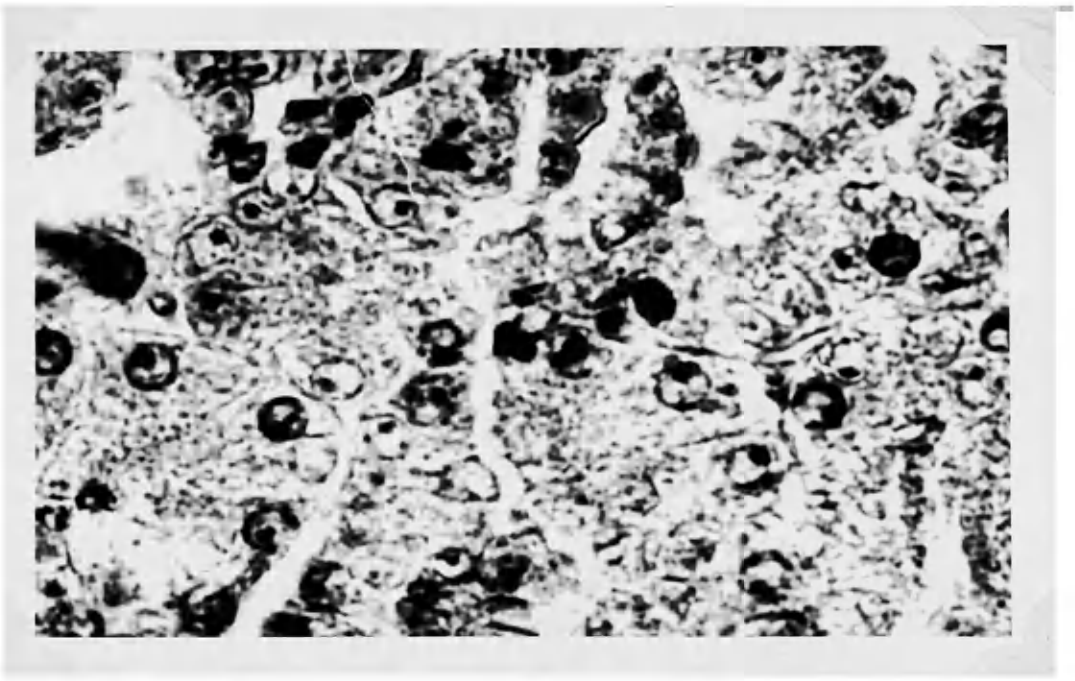

Figure 7.

Figure 8.

PLATE VI

Figure 9.

In this section taken from Terrapene carolina carolina are seen numerous pancreatic ducts and several secretory tubules. It may be seen that the epithelial cells are of approximately the same size as the acinar cells; although the former are more frequently rectangular in outline. As in most cases, the nuclei are again seen to occupy a position deep in the basal portion of the cells. Even at this lesser magnification the nucleoli are evident within the chromatin reticulum of the nuclei. Magnification x600.

Figure 10.

This photomicrograph of Pseudemys elegans was taken also using a red filter to intensify the cellular composition as brought out by the stain. Again, in this picture the zymogen granules are shown to be well distributed throughout the cytoplasm. Notice the basement membrane bounding the acini. As in previous demonstrations the nuclei show characteristic concentrations of chromatin material lying within the periphery. In addition the nucleoli are all shown to be relatively massive but this is not quite correct. Many of the nucleoli have a reticular structure which is not demonstrated in the photograph due to the difficulty of focusing sharply before the camera. Magnification x1250.

PLATE VI

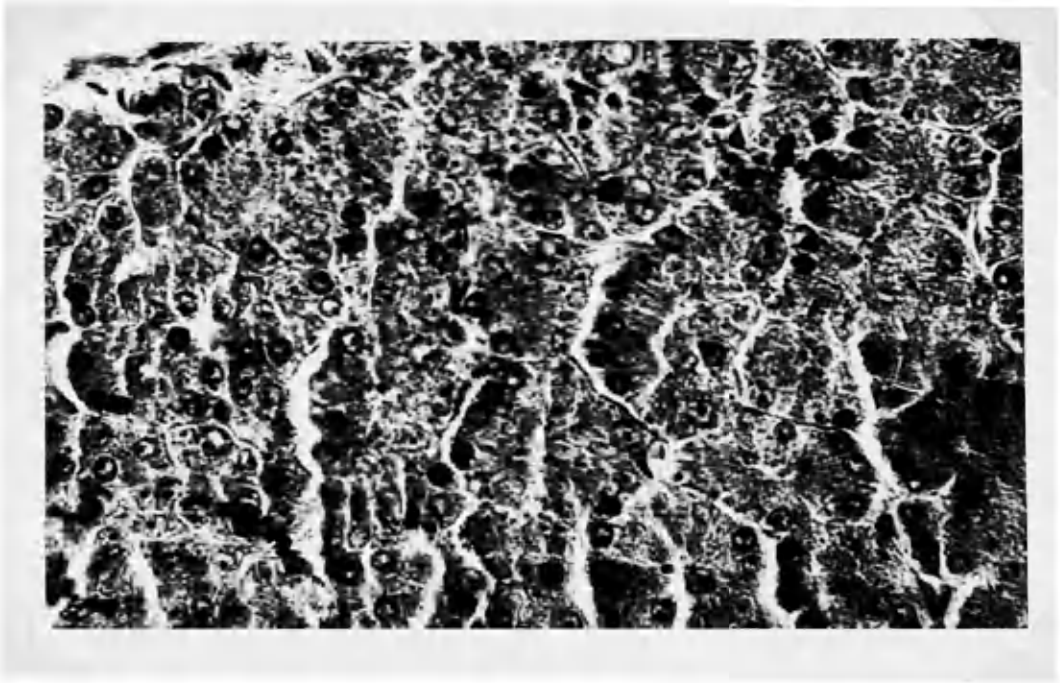

Figure 9.

Figure 10.

PLATE VII

Figure 11.

This figure is a photomicrograph of a precision scale. It is a portion of a calibrated scale from a slide made by Carl Zeiss of Jena. The entire scale represented 1 mm. divided into units of one hundredths. Therefore, in the accompanying figure each division is 0.01 mm. Being photographed under the same conditions as the entered figures, it was possible to use this scale in measuring the diameters of the pancreatic cells and their nuclei.

PLATE VII

Figure 11.