THE COMPARATIVE HISTOLOGY OF THE VERTEBRATE PANCREAS: A STUDY OF CERTAIN NORMAL AND EXPERIMENTAL CONDITIONS RELATED TO THE PANCREAS

A Thesis

Presented to

the Faculty of Graduate Studies and Research

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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1955

The author wishes to
express her appreciation to
her advisor, Dr. J. A. McLeod,
for his helpful suggestions
and the use of his private library.

ABSTRACT

THE COMPARATIVE HISTOLOGY OF THE VERTEBRATE PANCREAS: A STUDY OF CERTAIN NORMAL AND EXPERIMENTAL CONDITIONS RELATED TO THE PANCREAS

Sharon Gladys Campbell

All the animal species studied were members of the subphylum Vertebrata. Except for the primative fishes, the pancreas is located in a similar position in all species, closely associated with the stomach, a loop of the duodenum nearby and the spleen.

The pancreatic organs of the different species studied were found to vary in the following respects:

(a) the amount and distribution of the connective tissue present, giving rise to the variations in the gross appearances of the organs;

(b) the sizes of comparable cells, presenting a slightly different morphological picture in each species;

(c) the distribution and size of the islets of Langerhans.

While the first two features are species characteristic, the distribution and size of the islets may vary within the species.

Although the variations noted did exist, the cells of the pancreas in the different species were found to stain in a similar fashion. The basic histological pattern of the gland was comparable in the types studied.

Two groups of stimuli were applied to the experimental rats. The first group were acute stimuli which consisted of either starvation alone or starvation followed by the feeding of a restricted diet for two or three days. The second group

were chronic stimuli which consisted of either starvation or the continuous feeding of a restricted diet for five or more days.

The reactions to the acute stimuli were cytological and noted as follows:

a. vacuolation of the cytoplasm

b. increase in the number and size of the secretory granules

c. slight increase in the size of the glandular cells.

Degenerative changes appeared as a result of the chronic stimulation and were noted as follows:

a. swelling of the glandular components, especially noticeable in the beta cells with long periods of glucose feeding

b. disintegration of the connective tissue

c. infiltration of both the exocrine and endocrine portions of the pancreatic gland.

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CHAPTER I

THE PROBLEM

The early stages of research on pancreatic tissue was concerned only with the digestive portion of the organ. In fact, an endocrine function was not suspected until 1869 when Langerhans observed the islets of tissue distinct from the acini. When it was later proven that the pancreas was a mixed gland, interest was revived in the organ. Today, using cyto-chemical methods, more detailed observations of the secretory cells are possible, limited only by the resources and ingenuity of the investigator. The purpose of this present research was to study some of the aspects of the histology of the pancreas under normal and special dietary conditions, comparing the pancreas of the vertebrate classes.

CHAPTER II

REVIEW OF THE LITERATURE

HISTORY OF THE PANCREAS

The first description of the pancreas was recorded by Aristotle around 350 B.C. During the Dark Ages, previous research seems to have been ignored. Not until 1641 was the pancreas and its ducts noticed by Hofman but he did not publish his results until 1648. By this time Wirsung had found, and published, his results on the pancreas. Further dissections of the pancreas revealed more details and a digestive function was assumed. Only in 1869 did Paul Langerhans observe the islets of Langerhans, later named by Laguesse in 1893, who guessed their function to be endocrine. As far back as 1500 B.C. the Egyptians had known diabetes; the Greeks named it. In 1776 Dobson demonstrated the sugar in the urine, noticing its connection with diabetes. De Mayer named the still hypothetical endocrine product, insulin, which regulates blood sugar concentration, in 1909. In 1921 Banting and Best prepared the first pancreatic extract which consistently alleviated diabetes in pancreatomized animals.

EMBRYOLOGY OF THE PANCREAS

The pancreas arises from two diverticula of the duodenum just anterior to the hepatic diverticulum. There are usually two or three ducts which secondarily unite to give one or two ducts. The ventral ducts are termed Wirsung's ducts, the dorsal ducts are those of Sanatorini. These duct components fuse and the ventral duct becomes the main duct. However, in some forms the ducts unite with the bile duct or, as in the lamprey, are lost altogether. Most of the pancreas is produced by the dorsal outgrowth which gives rise to the body, tail and part of the head. The duct of this primorduim becomes the future Sanatorini duct, which is the accessory duct in man. Only a small portion of the organ grows from the ventral duct, which is destined to become the main or Wirsung's duct. These primordial ducts grow out into their normal, adult positions, and form a network of anastomosing tubules lined by a single layer of cells which then develop into typical acini and islets. Figure I illustrates the development of the pancreas.

The arterial supply of the pancreas arises from the celiac and superior mesenteric arteries. Veins accompany the arteries throughout the gland and lead the blood either directly into the portal vein or indirectly through the splenic vein. The exact

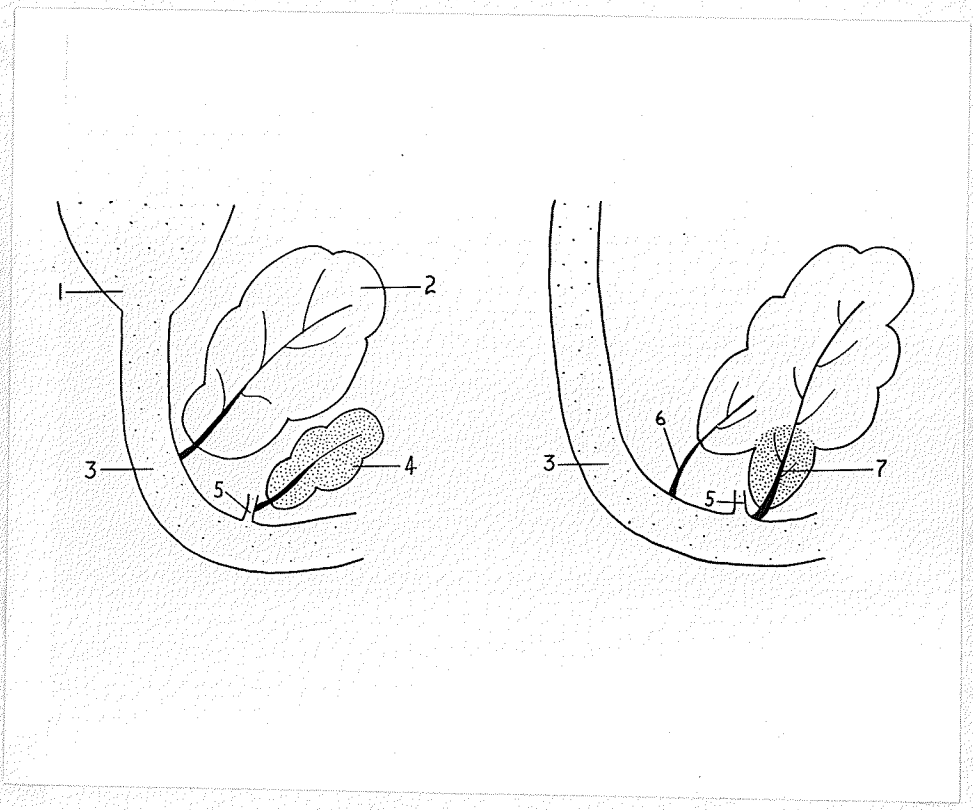

FIGURE I

Development of the pancreas
The diagram on the left represents development in a very young embryo; the right diagram is a later stage

1. stomach
2. dorsal pancreas
3. duodenum
4. ventral pancreas
5. bile duct
6. Sanatorini's duct
7. Wirsung's duct

(after Neal and Rand)

lymphatic supply of the gland is not yet known. The nerve supply consists largely of unmyelinated fibers from the celiac plexus. These fibers accompany the arteries into the gland and end about the acini in fine terminals. Some myelinated fibers also enter the pancreas but their function is not fully understood (61,62,63).

DISTRIBUTION OF THE PANCREAS IN THE VERTEBRATE KINGDOM

The pancreas is strictly a vertebrate characteristic as far as is known. No comparable structure has been found in the invertebrates nor indeed in the lower chordates. In the cyclostomes, the pancreatic tissue is buried in the liver and walls of the small intestine. Most authors believe that the vertebrates, beginning with the elasmobranchs, possess both dorsal and ventral pancreases. One author disputes this belief, stating that sharks develop only the dorsal pancreas (68). There is great variety in form, position and size of the pancreas throughout the vertebrate kingdom. Occasionally a pancreatic bladder occurs in cats which acts as a storage organ for the pancreatic digestive juices, as the gall bladder stores bile from the liver (13,14).

FUNCTIONS OF PANCREATIC TISSUE

The pancreas functions to produce:

1. pancreatic juice, a mixture of various digestive enzymes and 2, insulin, a hormone which regulates the blood sugar level. Possibly

further endocrine functions will be attached to this organ (22,48). Since no duct appears with the pancreatic tissue of the cyclostomes, it is often assumed that the pancreas is primarily endocrine rather than exocrine in function. Another fact which seems to support this idea is that while islet tissue is invariably present in all true vertebrates, pancreatic enzymatic secreting cells may be absent (68). Figure 2 illustrates the relationship of the acinar to the insular portion of the pancreas.

EXOCRINE PHYSIOLOGY OF THE PANCREAS

The earlier physiologists were chiefly interested in the digestive functions of the pancreas. During the latter half of the nineteenth century many attempts were made to study the effects of pancreatomizing animals, but most of them failed due mainly to poor surgical technique. Since the time of Pavlov shortly after 1900 surgery involving the digestive system has been very successful.

During fasting periods there is probably only slight secretion, from the pancreatic duct, corresponding with the occasional spontaneous activity of the intestines. The reflex response closely follows the intake of food, due to vagus stimulation which results in the production of glairy, concentrated secretion rich in enzymes. Stimulation of splanchnic nerves results in slight secretion. Bayliss and Starling

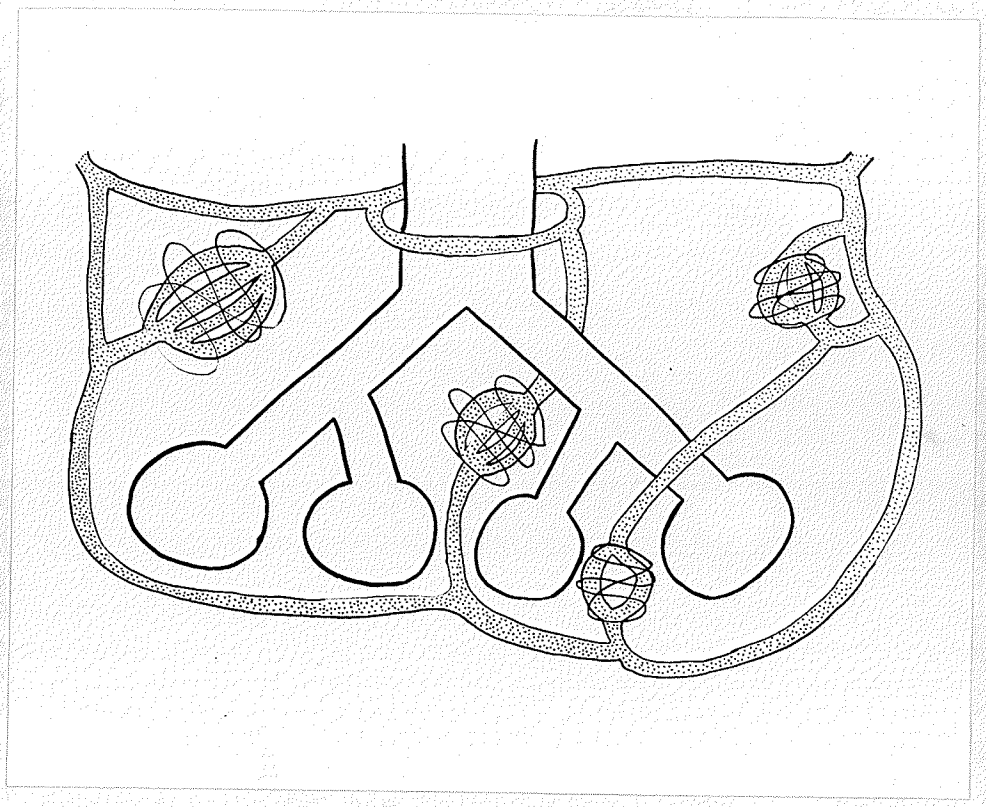

FIGURE 2

Relationship of the exocrine and endocrine portions of the pancreas. The cords of islet cells (stippled), join at the islets of Langerhans which are surrounded by a meshwork of blood vessels. The acini are not physiologically connected with the endocrine portion.
(after Selye)

(21) found that a secretion of pancreatic juice is produced even if acid is introduced into a loop of the small intestine entirely freed from any possible nervous connections with the rest of the body. The hormone involved is secretin. It is present in the mucous membrane of the intestines. Immediately after the acid chyme enters the duodenum, a certain amount of secretin is liberated by the acid, is carried by the blood stream to the cells of the pancreas and excites there the secretion of strongly alkaline pancreatic juice. As soon as sufficient pancreatic juice has been secreted to nearly neutralize the chyme, the formation of secretin halts. Actually two hormones are involved in the process, the two usually being grouped as secretin. However, secretin causes only a secretion of water and salts while pancreozymin is responsible for the discharge of the digestive enzymes.

Pancreatic juice is a clear or slightly opalescent fluid which is alkaline, pH 7-8, from the presence of sodium bicarbonate. Proteins present include the enzymes and serum proteins. The juice tends to become defficient in enzymes, but not in alkali, as secretion proceeds. The pancreatic tissue contains the same enzymes as its juice, and in the same relative amounts. Trypsin is a mixture of several proteolytic enzymes: amylase or amylopsin is an amylolytic enzyme: lipase converts neutral fat to glycerol and fatty acids.

ENDOCRINE PHYSIOLOGY OF THE PANCREAS

The endocrine glands are specialized tissues which secrete hormones into the circulation to influence other cells, tissues or organs of the same organism or the organism as a whole. They are highly vascular and subject to control by the central nervous system, other endocrine glands, certain metabolites or a combination of these. There seems to have been an evolution of the pancreatic endocrine tissue from areas within the central nervous system possessing a few secretory cells to a condition where the cells are specialized for producing only hormones apart from any concentration of nerve cells (56). Simard has illustrated various phases of this proposed evolution by his neuro-insular complexes (62).

In 1889 Von Mering and Minkowski depancreatomized dogs to study their digestive processes. By accident they discovered diabetes mellitus of the dogs. Since then, it has been found that the islets produce insulin which regulates the storage and utilization of carbohydrates. Possibly lipocaic, with the function of preventing fatty livers, is produced by the islets (67). Glucagon, a substance antagonistic to insulin, has been reported to be produced by the alpha cells (22).

TRANSFORMATIONS OF CELL TYPES

Many authors claim that the acinar and insular tissue, once

formed, are permanent (7,22,27,31). Others contradict this belief (28,35,59,61,62). Minkowski described a cell in 1902 which he interpreted as a transitional type between acinar and islet tissue. Gomori since has suggested that this cell was due to a pathological condition. He states that alpha cells develop from the duct epithelium; gamma cells are probably aged alpha cells and the origin of beta cells is unknown. The smallest islets consist chiefly of gamma cells with a few beta cells. Larger islets show an increase of beta cells with alpha cells in the center. Some authors believe that this fact supports the idea of gamma cells producing alpha cells (7,12). Selye points out that although one type of islet cell may be transformed into another, when beta cells are destroyed by alloxan they are not replaced. Some have demonstrated cell types which seem to be exocrine and endocrine in function (4,59,62). The latest experiments on this subject were done in 1954 and seem to prove that cell types once formed are fixed (22).

RECENT EXPERIMENTS RELATED TO THIS STUDY

Many types of surgical experiments have been performed involving hypophysectomy and ligations of the pancreas (34,45,60,61,63). More extensive work has been done using substances toxic to certain cells, such as alloxan which selectively destroys the beta cells of the islets of Langerhans (24,26,36,37,38,39,40,41). Hormones and growth factors

have been and still are the subject of much debate. A few facts seem to have been confirmed however. The beta granules are believed to represent stored insulin (8,22,44,48,52). A substance produced by the alpha cells, glucagon, acts antagonistically to insulin. This study has demonstrated that alpha cells may be present in the mucosa of the stomach and intestines (11,15,22,33,64). On the other hand, it has been postulated that the alpha granules store an insulin containing compound (4).

CHAPTER III

MATERIALS AND METHODS

ANIMALS STUDIED

All the animal species studied were members of the subphylum Vertebrata. Except for the primative fishes, the pancreas is located in a similar position in all species, closely associated with the stomach, a loop of the duodenum nearby and the spleen.

Class Pisces: the dogfish shark, Squalus acanthus and the pike, Esox lucius; all specimens were adults

Class Amphibia: the frog, Rana pipiens; various ages

Class Reptilia: the snapping turtle, Chelydra serpentina; one 40 pound adult

Class Aves: the black-bellied plover, Squatorola squatorola; the greater yellow legs, Totanus melanoleucus; an immature herring gull, Larus argentatus; the lesser scaup, Marila affinis; the mallard, Anas platyrhnchos; the common tern, Sterna hirundo; the domestic chicken, Gallus domesticus

Class Mammalia: the guinea pig, Cavia cobaya; the mouse, Mus domesticus; the rabbit, Oryctolagus cuniculus; the golden hamster, Mesocricetus auratus; the rat, Rattus norvegicus albinus; the cat,

Felis catus; the dog, Canis familiaris. The white rats were of the Sprague-Dawley strain and the hooded rats of the McGill strain.

MICROTECHNIQUE METHODS USED

Fixatives used were 10% formalin, Bouin's picro-formal and Zenker's fluid. Guyer (30) was used for the formulae of the solutions.

Three methods of embedding blocks were tested; a chloroform-anilin method, a xylol technique and a cedarwood oil procedure. Guyer contains the latter method.

CHLOROFORM - ANILIN METHOD

1. The tissue was fixed as desired.
2. The tissue was washed as necessary.
3. Two changes of 95% alcohol during 24 - 48 hours were used.
4. Two changes of anilin during 24 - 48 hours were used.
5. Three changes of chloroform during 1 hour were used.
6. A 50:50 mixture of chloroform and wax in the oven for 30 minutes was used.
7. Wax, in the oven for 3 - 5 hours, was used.
8. The tissues were embedded.

XYLOL METHOD

1. The tissue was fixed as desired.
2. The tissue was washed as necessary.
3. The tissues were run through a graded series of alcohols, each for one hour, ending with two changes of absolute alcohol for one half hour each.
4. The tissues were left in xylol for 12 hours.
5. The tissues were put in fresh xylol for 1 hour.
6. The tissues were left in wax in the oven for 5 hours.
7. The tissues were embedded.

Cutting was done with a Bausch and Lomb rotary microtome, the sections being cut at 4 or 6 microns of thickness.

A variety of stains were employed. Different haematoxylin staining methods were tried (25,30,42) but the author's choice was that formula provided by Dr. J. M. Isa of the Provincial Veterinary Laboratory:

1. 1 gram of haematoxylin was dissolved in 10cc. of 95% ethyl alcohol.
2. 20 grams of potassium or ammonium alum were dissolved in 100cc. of distilled water.
3. The two solutions were mixed and quickly brought to a boil until the solution was purple.
4. 0.5 grams of mercuric oxide were added and the mixture cooled

rapidly in cold, running water.

5. 100 cc. of glycerine were added.

6. 4 cc. of glacial acetic acid were added.

As a counter stain, 0.5% eosin was used in both 70% alcoholic and aqueous solutions.

Several special staining techniques were tried (6,27,32,51). The Bencosme method was used as a general cytological and differential stain. It consisted of the following steps; the formulae were taken from the original paper.

1. The sections were immersed in water.
2. The sections were treated with a 1% iodine alcohol solution for 10 - 30 minutes.
3. The sections were rinsed in water.
4. Treatment with a 5% sodium hyposulphite solution for 1 minute followed.
5. The sections were washed in tap water for 5 - 15 minutes.
6. The sections were rinsed with distilled water.
7. The sections were treated with a 5% iron - alum solution for 5 minutes or longer at 52°- 56°C.
8. Careful rinsing with distilled water followed.
9. Regaud's haematoxylin stain was applied for a minimum of 5 minutes at 52°- 56°C.
10. A 95% alcohol rinse was used until no more colour came out of

the section.

11. This step was controlled under the microscope: the tissue was differentiated at 52°- 56°C using a picric acid alcohol solution until the chromatin stood out sharply.

12. Washing in running water for 20 minutes followed.

13. The tissues were stained for at least one hour with a ponceau fuchsin mixture.

14. A brief rinsing in 1% aqueous acetic acid followed.

15. The tissues were differentiated in a 5% phosphomolybdic acid solution until the gamma cells were unstained or very pale, approximately 2 hours.

16. A distilled water rinse was used.

17. Anilin blue was applied for 10 - 20 minutes.

18. The sections were rinsed briefly with distilled water.

19. The anilin blue was differentiated with 1% aqueous phosphomolybdic acid for 10 - 45 seconds.

20. The sections were placed in 1% acetic acid for about 5 minutes or until all the colours and structures were clearly defined.

21. The sections were dehydrated with absolute alcohol.

22. The sections were mounted in permount or Canada balsam.

In addition, to determine certain normal characteristics, the pancreatic organs of twenty normal rats were subjected to the following techniques. The Feulgen stain was applied to sections to determine the

desoxyribose-nucleic acid content and location. Here the tissue was treated with Schiff's reagent after acid hydrolysis according to the method of Lillie. From each of the twenty rats, five sections were chosen and counts made of the islets present in an area of 5mm. by 5mm. measured grossly and marked on the slide with ink. The counting was done using a microscope with a mechanical stage, as a differential count of a blood smear is made.

DIETS TESTED IN THE EXPERIMENTS

A normal diet consisted of "Victor fox cubes" supplemented by wheat germ oil, mixtures of various cereals and milk. The protein diets tested were gelatin (Difco), egg albumen (Merck) and isoelectric casein (Difco). "Pure cane" lump sugar was the carbohydrate used, while Maple Leaf Tendersweet pure lard constituted the fat ration.

EXPERIMENTS PERFORMED

The rat was employed in the experimental studies. Both the albino and hooded strains were used, but unless otherwise stated, the albino rat was employed. A young rat was considered by the experimenter to be up to six weeks for the male, or nine weeks for the female. The mature rat was seven to eight weeks for the male and ten to eleven weeks for the female. An adult rat was fullgrown at nine to twelve months.

In every experiment, water was given freely.

Experiment 1: complete starvation; winter temperature (60°F average, often below 50°F), 10 albino rats; summer temperature (65°F or higher), 10 albino and 10 hooded rats.

Experiment 2: starvation 3 days, fed normally, 1 killed each day after the commencement of normal feeding; 5 albino and 5 hooded rats.

Experiment 3: protein fed up to death; gelatin 2 rats, egg albumen 5 rats, casein 7 rats.

Experiment 4: sugar fed up to death; 5 rats.

Experiment 5: lard fed up to death; 5 rats.

Experiment 6: starvation one day, followed by two days of casein feeding; 5 rats.

Experiment 7: starvation one day followed by two days of sugar feeding; 5 rats.

Experiment 8: starvation one day followed by two days of lard feeding; 5 rats.

Experiment 9: two days of starvation followed by casein feeding. One rat was killed each day after feeding was begun for five days.

Experiment 10: two days of starvation followed by sugar feeding. One rat was killed each day after feeding was begun for five days.

Experiment 11: two days of starvation followed by lard feeding. One rat was killed each day after feeding was begun for five days.

Experiment 12: starvation for two days, followed by casein feeding for four days and then normal feeding. One rat was killed each day after resuming normal feeding for five days.

Experiment 13: starvation for two days, followed by sugar feeding for four days and then normal feeding. One rat was killed each day after resuming normal feeding for five days.

Experiment 14: starvation for two days, followed by lard feeding for four days and then normal feeding. One rat was killed each day after resuming normal feeding for five days.

CHAPTER IV

RESULTS OF THE EXPERIMENTS

EXPERIMENT I

TABLE I

RATS ON STARVATION DIETS

strain of rat	number of rats	age	sex	temperature of environment	average length of life
albino	10	adult	random	65°F.	4.2 days
albino	4	adult	random	60°F.	4.1 days
albino	3	young	random	46°F.	3.1 days
albino	3	adult	random	46°F.	3.1 days
albino	10	adult	random	70°F.	7.9 days

The average life span of a starving hooded rat was longer than that of an albino rat. Although the hooded rats survived for and average of 7.9 days, their endurance did not match that of Nerenberg's rats which lasted 12 days (53). His rats however had a mortality rate of about 50 per cent, considerably higher than in this experiment.

Histologically, very large apical zones appeared in the acinar

cells. These zones increased in size, becoming filled with zymogen granules and containing prominent mitochondria, especially after four days. The beta cells gradually lost their granules as time progressed.

EXPERIMENT 2

Evidence of fasting was present on the first day of normal feeding. Beginning with the second day, the gland began to return to normal but a transient vacuolar condition appeared in a few cells. By the fifth day, the gland was normal.

EXPERIMENT 3

TABLE II

RATS ON PROTEIN DIETS

Protein	Number of Rats	Age	Sex	Average Life	Results
Gelatin	2	adult	random	3	normal tissue
Egg albumen	5	adult	random	5.2	normal tissue
Casein	7	adult	random	6	degenerative changes on the sixth day

The degenerative changes in the pancreatic gland were characterized by a general loss of structure, as shown in figure 17. Apparently the animals did not live long enough on the gelatin or egg albumen diet to produce changes.

EXPERIMENT 4

The average life span of the rats in this experiment was 11.2 days with one rat lasting 16 days. The acini remained quite normal, contrary to previous reports (16). By the fourth day, the islets had increased in size and become permeated with engorged blood vessels. Nerenberg has completed various studies related to this experiment (52,53,54). He reports on the secretion of insulin by monosaccharides and disaccharides given orally. Degenerative changes were beginning to show in some cases.

EXPERIMENT 5

The average life of these rats fed until death with lard was 15 days. Lard was found to be effective in producing degranulation of the beta cells, possibly because the rats die rapidly if fasted completely (5). The rats in this experiment showed a remarkable change in the colour of their hair and skin. They became an intense yellow colour after three days of eating lard, and very emaciated. The

acini showed signs of overstimulation. It has been found that by using the sodium salt of beta hydroxybutyric acid that islet size may be increased with 27 days of feeding but the islets then degenerate (49).

EXPERIMENTS 6, 7, 8

The changes in these experiments were similar to those of experiment 2 in the early stages. The changes noticed were cytological, the glandular components being slightly enlarged due to an abundance of secretory granules. The second day of feeding produced a condition closer to normalcy.

EXPERIMENTS 9, 10, 11

The result of these experiments paralleled those of experiment 2 for the first two days of feeding. From the third day onward, the animals gradually recovered although the casein fed animals recovered very quickly. This fact seems to be explained by the results of experiment 3, where the pancreatic cells were slower to alter to an abnormal condtion and therefore did not have to change a great deal to be normal again. In both experiment 10 and 11, the islets seem to have been damaged more as they did not recover until the fourth day of feeding.

EXPERIMENTS 12, 13, 14

Again the results showed a recovery pattern similar to that of experiment 2 although the casein fed animals recovered more quickly. The feeding of normal food apparently brought the animals of the three experiments to an equal condition.

CHAPTER V

OBSERVATIONS

REVIEW OF THE METHODS USED

Formalin was found to be the most satisfactory fixative for this work as it was easy to handle when collecting specimens outside the laboratory and the specimens could be left in it for a considerable time without appreciable damage. The chloroform-anilin embedding process was preferred by the author. For a routine haematoxylin stain, that of Dr. Isa seemed most dependable. It does not overstain the sections while the acidic property increases its nuclear staining power. Aqueous eosin gave better results than the alcoholic variety.

Of the special stains tried, the Bencosme method previously listed gave the best results. The alpha cells were identified as a brown - purple colour in the seemingly homogenous cytoplasm, the individual granules not being visible at 4 microns. Bencosme states that the granules in sections of 2.5 microns thickness do show up as individuals. The mitochondria did not stand out in the alpha cells but the Golgi apparatus was situated near the nucleus. Beta cell granules stained salmon pink. Here the mitochondria stained an intense red - brown, although they could not always be distinguished from the secretory granules. The Golgi network was more obvious than in the alpha cells. The delta cells had a clear blue cytoplasm with

a few small, red mitochondria in evidence. In the case of each cell type, certain cyanophilic or blue staining granules were present. The ductular cells and materials within stained a pale blue colour with red mitochondria. Occasionally cells were either unstained or overstained. Acinar cells were lilac at their basal regions and possessed red mitochondria and purple zymogen granules. The nuclei stained black with red nucleoli often standing out.

In using the special staining procedures, certain changes were made from the original methods mentioned. For example, it was found that the routine acid haematoxylin was as satisfactory as the Regaud's haematoxylin. The ponceau fuchsin techniques of Sergeyeva (61) or Lillie (42) seemed to stain equally as well as the one listed. Minor variations of timing the staining procedures had to be incorporated.

The Feulgen stain technique is a specific test for desoxyribose-nucleic acid. Results found were the same as those outlined by de Robertis (20), who suggests the use of absorption rays to demonstrate the nucleic acids. Since both types of nucleic acid show up equally with absorption rays, the Feulgen stain should be used to demonstrate the desoxyribose-nucleic acid, then the rest of the nucleic acid would be ribosenucleic acid. It was found that the Feulgen reaction demonstrates large granular shaped portions of the nucleus, leaving unstained the nucleoli. Additional information

on this topic is being obtained by the use of the labelled antibody technique (43).

REACTIONS OF THE RATS TO EXPERIMENTAL CONDITIONS

It has been stated that the amount of food eaten by an animal is partly determined by its caloric value (2). The feeding reactions of the rats support this statement for by weight, they did consume more carbohydrate and protein than fat. Wet foods were definitely preferred to dry. If food was denied for more than five or six days, the rats would not recover if given food. They die of starvation (1). Accompanying this fact is the observation made by Nerenberg (52,53,54) that there is a high mortality rate in rats starving one or two days. The experimenter has found that the temperature influences greatly the life of a starving rat even though water is given freely. In winter, when the animal room averaged 60°F with the temperature occasionally falling to 50°F, the rats lasted only a few days, whereas in the warmer temperature they lived ten or more days. Young rats did not live as long as the adult animals, nor female as long as males. As was expected, the rats deprived of food became greatly emaciated with enlarged spleens. Extensive studies have been made on normal and obese mice which demonstrated the differences in the gross appearance of the pancreas (10). It was found that the female rat has a heavier pancreas than the male of the same body weight.

DISCUSSION OF THE SECRETORY CYCLE AND HISTOLOGY OF THE PANCREAS

Histologically and functionally the pancreas has two completely separate groups of cells, although both are situated in the same gland. The phases of the secretory cycle will be discussed, followed by the features of the acinar and insular portions of the pancreas.

The secretory cycle of the pancreatic cells followed a routine pattern. Characterizing the fasting stage was the apical zone packed with secretory granules. This condition is illustrated in figures 7, 8 and 9, which show the apical zones of the acinar cells progressively filled up with secretory granules as the experiment progressed. Many mitochondria were present around the nucleus and there was a basophilic substance in the cytoplasm. This material was chromidial in nature, composed chiefly of nucleoproteins. The Golgi apparatus us present. It has been stated that during this phase, exocrine secretion is inhibited (16). The following stage was the stimulatory phase where secretion begins with the excretion of the secretory granules. Vacuolation of the cytoplasm was noted, the mitochondria were abundant as noted by others (65), the Golgi increased and the chromidial substance absent. This phase occured after feeding. With overstimulation by constant feeding of a certain diet, such as sugar or lard, the condtion was exaggerated and the blood vessels were enlarged as well as great vacuolation being present. When secretion halted, the gland entered the recovery stage where the mitochondria almost disappeared completely, the Golgi

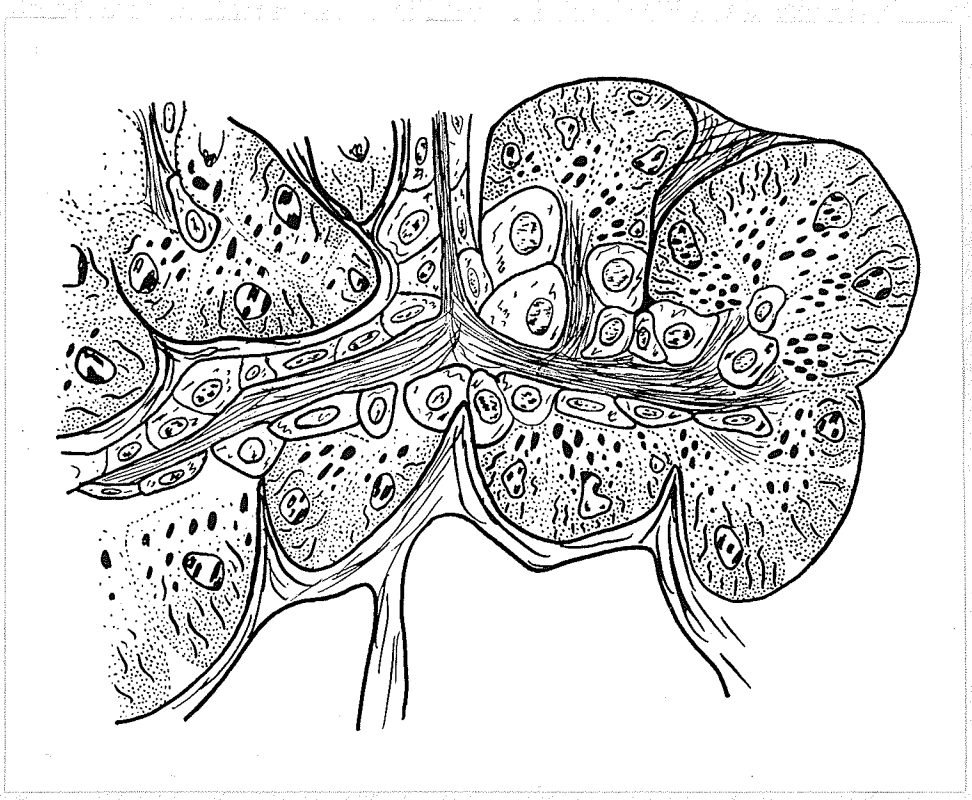

FIGURE 3

Section of pancreatic acini:
showing the cellular relationships and cytology of the individual cells. The nucleus is surrounded by rod shaped mitochondria and the protein rich cytoplasm. The apical zone of the acinar cells is occupied by zymogen granules. A secretory duct, lined by ductular epithelium joins the acini.
(after Bensley and Nixon)

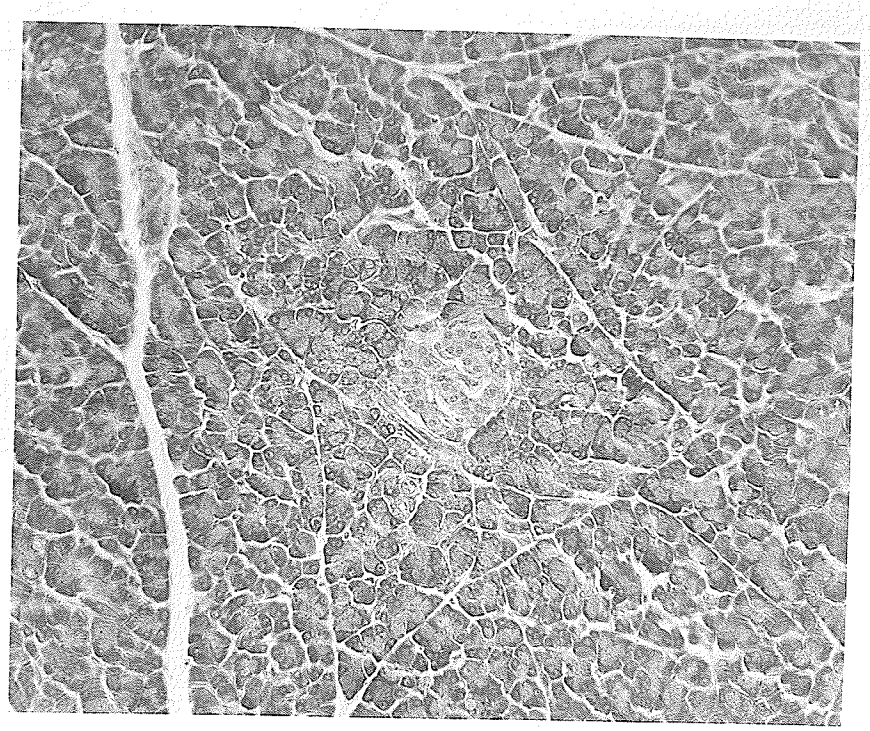

FIGURE 4

Section of a normal rat pancreas;
showing exocrine and endocrine portions X200

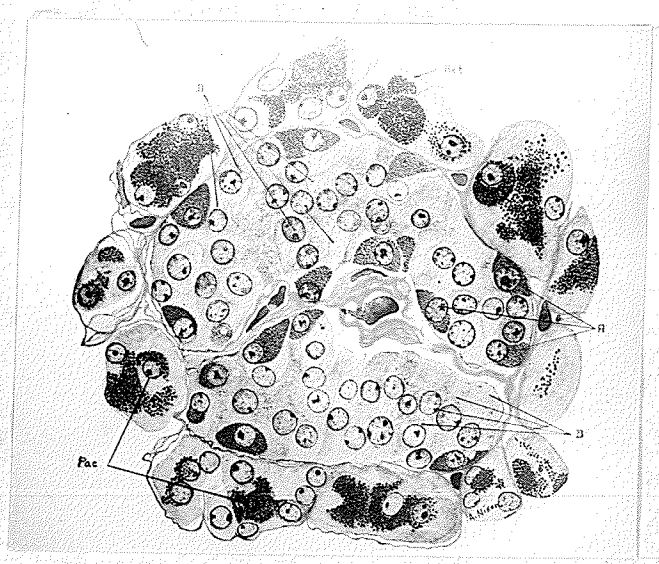

FIGURE 5

Section of mammalian pancreas:
cell types A, B and D of the islet of Langerhans;
Ret, reticular fibers; Pac, pancreatic acini
(from Maximow and Bloom) X960

apparatus became prominent and the chromidial substance was again obvious. As well as these general phases of secretion, many histological features were observed.

The pancreatic acinus consisted of a single row of pyramidal epithelial cells resting on a basement membrane and converging towards a central lumen. These acini were arranged to form a tubular acinus gland. The size of the lumen varied with the functional condition of the organ. Between the acinar cells were fine secretory capillaries, which are reported to be connected with the central lumen (43). The base of the cell was occupied by the nucleus and by elongated mitochondria. The apical region was occupied by refractile granules with a high protein concentration. The relationship between the basophile homogenous zone at the base and the amount of secretory granules depends on the stage of production of the digestive juices (61). The nucleus was spherical, containing much chromatin and one or two prominent oxyphile nucleoli, and showing desoxyribosenucleic acid with the Feulgen reaction. Among the zymogen granules in the apical zone there is a reticular Golgi apparatus (46,47). Figure 4 and 6 illustrate the pancreatic acini.

It is now believed that the zymogen granules of the pancreatic acini are composed of the enzymes found in the pancreatic digestive juices. These enzymes are probably in the form of proenzymes. One investigator (20), noted the following results. When the content of the granules of the acinar cells is determined quantitatively at various times after stimulation with pilocarpine, and at the same time the concentration

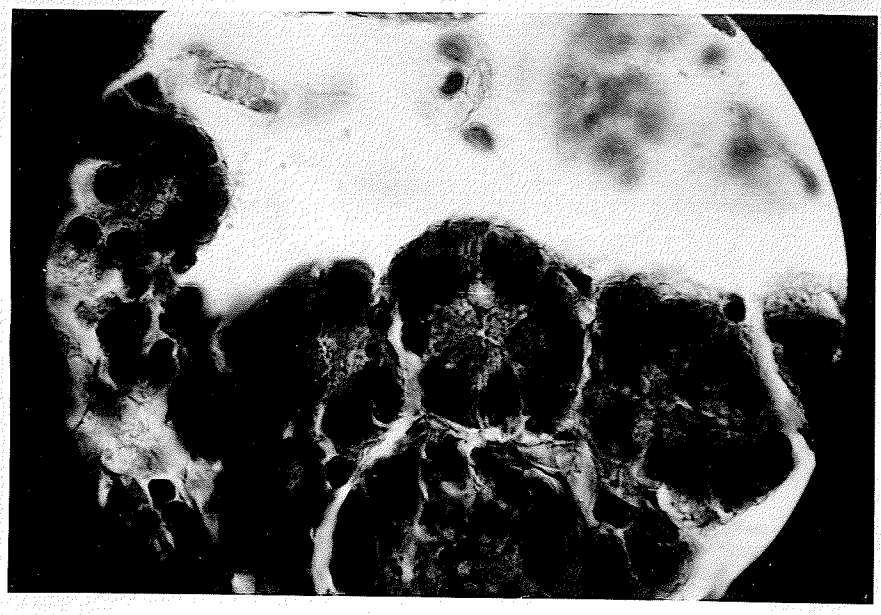

FIGURE 6

Mammalian pancreatic acini: resting stage. X1250

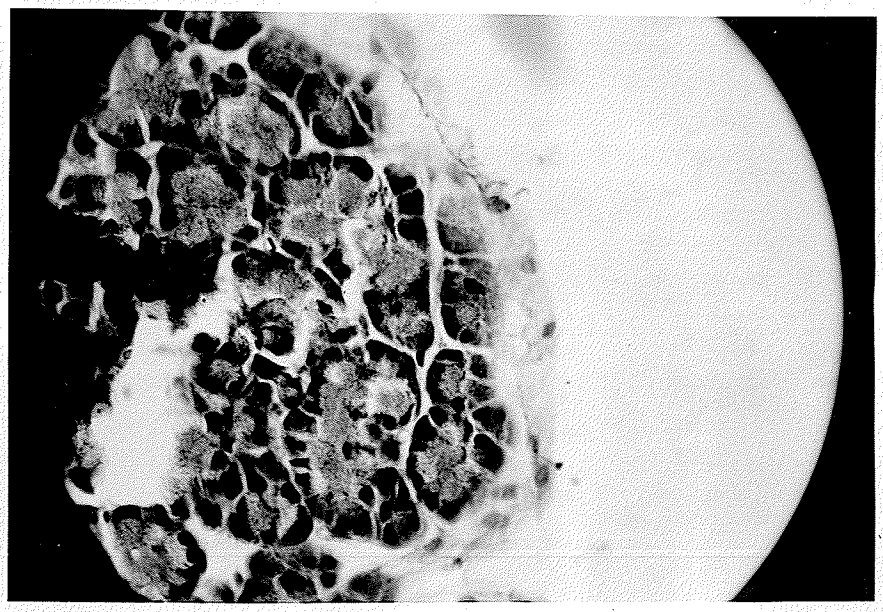

FIGURE 7

Mammalian pancreatic acini:
apical zone enlarged with secretory
granules after three days of starvation. X600

of carboxy-polypetidose is analyzed, a close correlation exists between the two.

In a single gland the cells are generally found in various phases of secretory activity. To compensate for this natural condition, stimuli, such as pilocarpine, may be applied to rapidly modify all the glandular cells, directing their activity to one stage. In studying such a secretory cycle fixation by the freezing - drying technique presents great advantages as cellular processes are stopped instantly and products are preserved unchanged. Bourne believes that certain facts about enzyme action, such as whether or not they are altered in the experimental techniques, must be fully understood before the newer methods are completely acceptable (11).

The islets of Langerhans comprise the secreting cells of the endocrine pancreas. They have no connection with the excretory ducts from which they originate. Their size varied from single cells to masses of several hundred. It has been estimated that in the pancreas of the guinea pig there may be as many as 25,000 islets (68). Trautman believes that one islet exists for every [illegible] to [illegible] square millimeters of pancreatic tissue, with islets varying from 0.04 to 0.2 millimeters in diameter in mammals (66).

The investigator noticed the following results on a study of 100 counts of normal rat pancreatic sections cut at six microns of thickness. The range of the diameter of the islets was 0.06 millimeters to 0.25 millimeters. Since these were random samples, an attempt was made to

classify the type of islet. A diameter of 0.06 millimeters or less was considered small, 0.07 to 0.17 millimeters medium, and 0.18 millimeters or more large. The average count for a section of 25 square millimeters in area was four small, two medium and one large islets, or one islet for every 3.6 square millimeters of tissue.

The endocrine gland unit was an encapsulated network of cords of cells with small capillaries between. Special staining procedures demonstrate three, as seen in this study, and possibly four types of islet cells (9,43). Bowie describes a fourth type of cell which he suggests may possibly be related to the gamma cell (12). One author has even outlined five cell types (57).

The alpha or A cell were usually seen occupying a position near the center of the islet. The relative number of alpha cells varied, the small islets having no alpha cells and the larger ones many. These cells were the most densely granular of the islet cells. The nucleus was located at the base of the cell. The beta or B cells were usually closely associated with the capsule, even along the trabeculae which grow inward into the gland. They were pyramidal in shape, often having an elongated process reaching to a nearby capillary. Usually the beta cells were fairly evenly distributed throughout the gland but were sometimes in groups in the large glands. Their size was larger than the other islet types and their granules were evenly distributed throughout the cytoplasm. The nucleus was spherical and has a distinct

central nucleolus. The gamma or D cells (often called delta) were located at the periphery of the islets. They were mixed in with the other cell types but their boundaries were not distinct. They were relatively large and more numerous than the other cell types, their granules being large and scarce. The central nucleus was of variable shape with one or two small nucleoli. The islets occurred in the interstitial tissue or among the acini. The islet cell types are illustrated in figure 5. In addition to these general features common to all the species studied, there were many minor variations which will be discussed in the following paragraphs.

COMPARISON OF THE VERTEBRATE PANCREATIC ORGANS

The foremost difference noted in the various pancreatic glands studied was the amount of connective tissue present. On gross appearance, the fish pancreas was a very compact, elongated, oval - shaped gland. The pancreatic organs of the frog and turtle were similar but somewhat more loosely organized. In the bird, the pancreas was definitely less firm in consistency with the macro lobules being distinct while the mammalian pancreas did not exhibit the distinct shape of the primitive forms. These higher forms had a gland in which the lobules were visible with the naked eye and held together in such a manner that although the gland was attached to the spleen and duodenum, the connection was very loose and the lobules arranged in amongst the intestinal loops, the spleen and the stomach. This condition was different than in the fish and

and frog where the attachment to the intestine and stomach was firm and the organ had a definite location in a small area, held there by the tight binding action of the glandular capsule.

On histological examination the variation in the connective tissues was evident immediately. The lower forms had little connective tissue present in a definite capsule while in the mammalian type the parenchymal cells were permeated with trabeculae of connective tissue. Occasionally large areas of fatty connective tissue were seen in the fish pancreas, as shown in figure 10. The variation of shape in acinar cells can be seen in figures 11 and 12, although the cell shapes of the mallard, turtle and rat were very similar as shown in figures 8,13,14,15 and 17, except for minor differences (7,19,62). The islet cell types were found to be similar in all species. One author reports two alpha cell types and two beta cell types in the fish (17). Other investigators suggest different types of insular cells, (3), but the experimenter was unable to demonstrate these unusual cell types.

PANCREATIC REGENERATION AND DEGENERATION

Pancreatic acini have great regenerative power. Grauer (29), reduced the pancreas of a rabbit to a system of branching ducts. After twenty five days the exocrine pancreas was almost normal. Pancreatic tissue has been transplanted to various parts of the animal body quite successfully (23). New islets sprout throughout life from duct tissue (10). Disappearance of diabetes, presumably due to recovery of islet

cells, has been reported in rats (41). The investigator found in the experiments that regenerative changes, such as regranulation and disappearance of vacuolation, occurred in cases where loss of cellular structure was not yet evident.

Degeneration may be caused by toxic substances or malfunction (55). Hypophysectomy leads to atrophy of the acinar tissue (48), as do certain fats (49). Diabetes results in a degranulation of the beta cells, while not affecting the islet as a whole (28), as does the injection of insulin (45). Deprivation of the abdominal sympathetic nerve supply results in a progressive sclerosis of the gland. Cavity formation throughout the lobules of the pancreas occurs with age and the islets may atrophy in later years (1). Degenerative changes occurred in the experiments performed. They followed a pattern of loss of cellular structure with the intercellular connective tissue disintegrating, as illustrated in figure 17. This condition occurred after overstimulation of the gland by one type of food or from starvation.

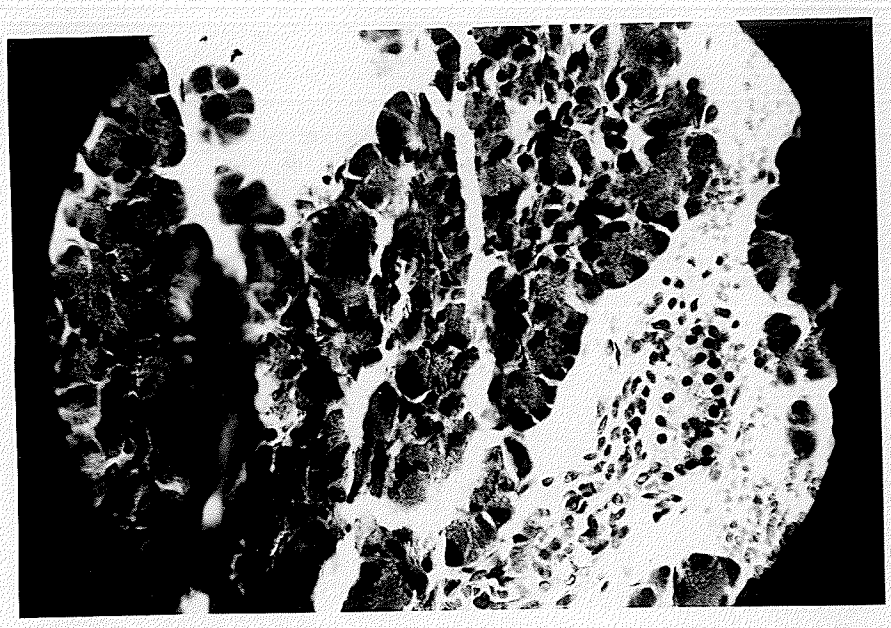

FIGURE 8

Mammalian pancreatic acini:
a later stage of figure 7, after four days of starvation. X600

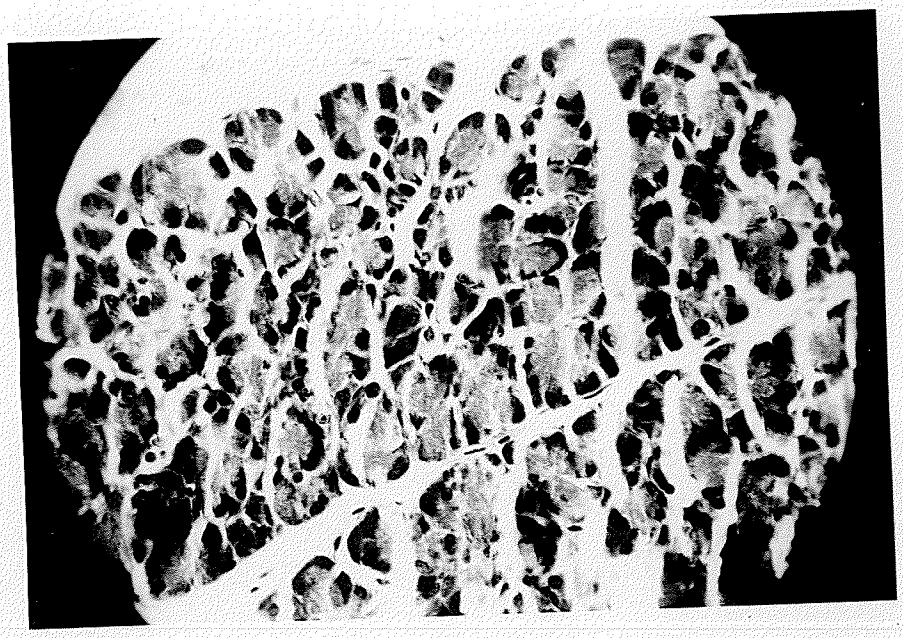

FIGURE 9

Mammalian pancreatic acini:
a later stage in the sequence of figures 7 and 8,
after five days of starvation. X600

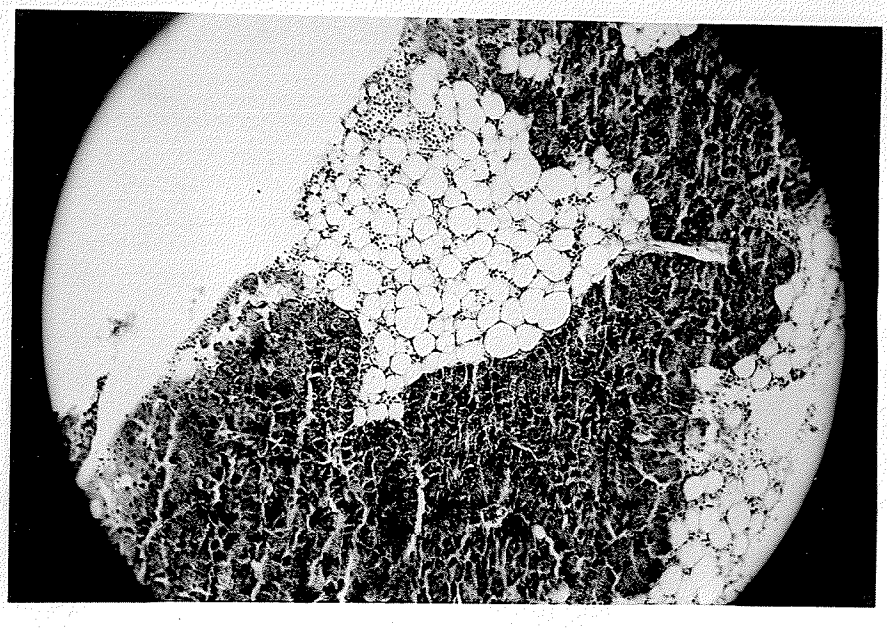

FIGURE 10

Section of fish pancreas
showing fatty connective tissue. X600

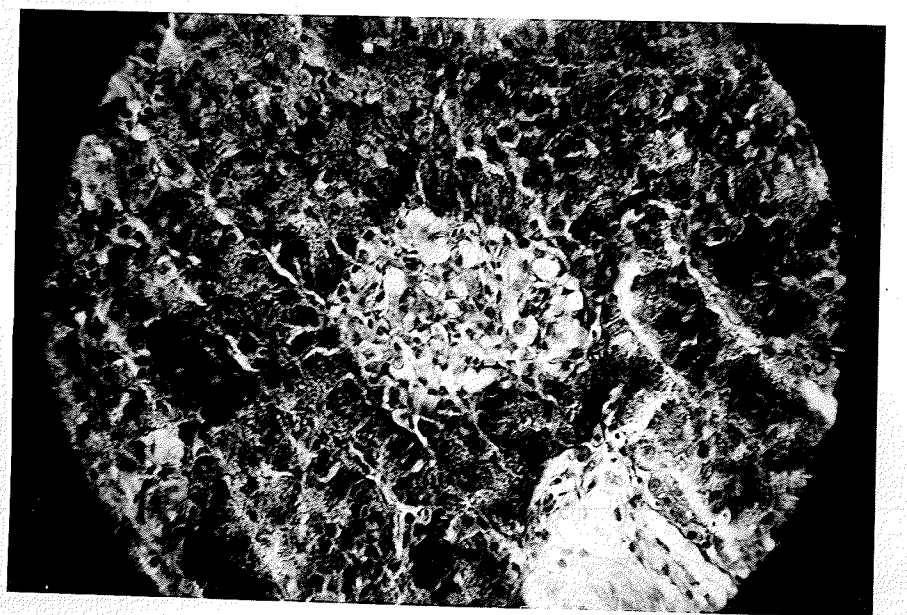

FIGURE 11

Fish pancreas showing an islet of Langerhans. X600

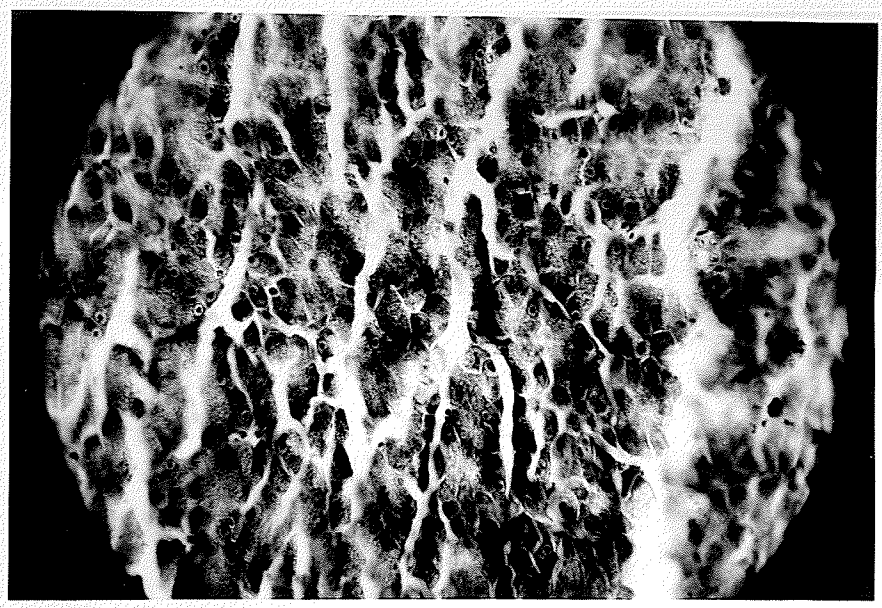

FIGURE 12

Acinar portion of fish pancreas,
illustrating the variability in cell shape. X600

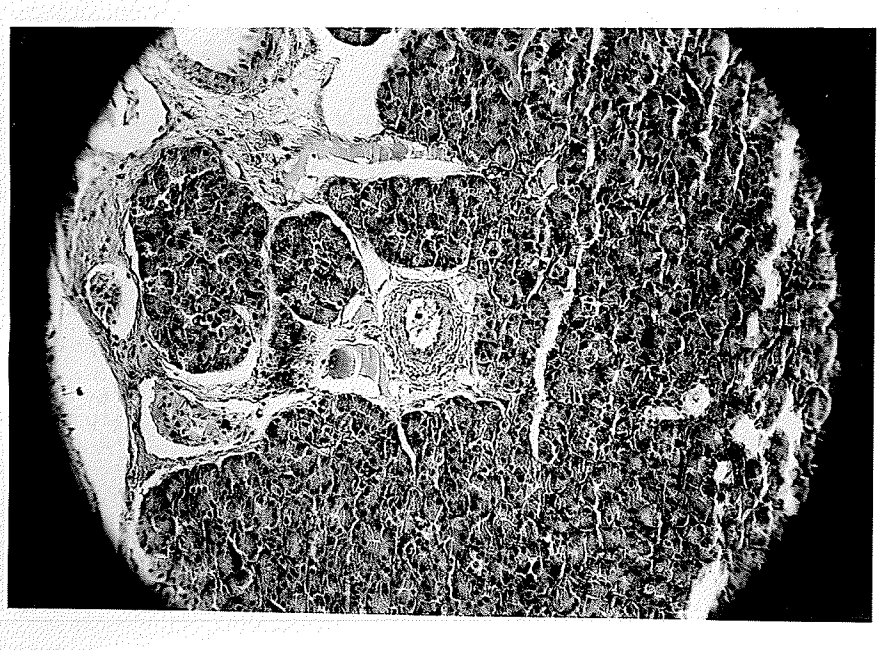

FIGURE 13

Section of turtle pancreas. X200

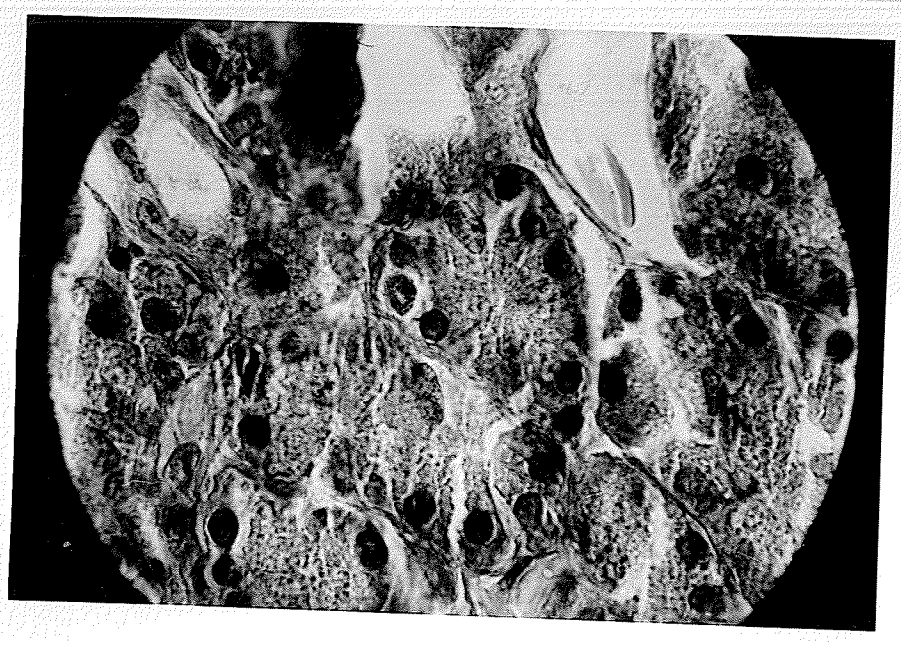

FIGURE 14

Turtle pancreas:
note the similarity to mammalian pancreatic tissue. X1250

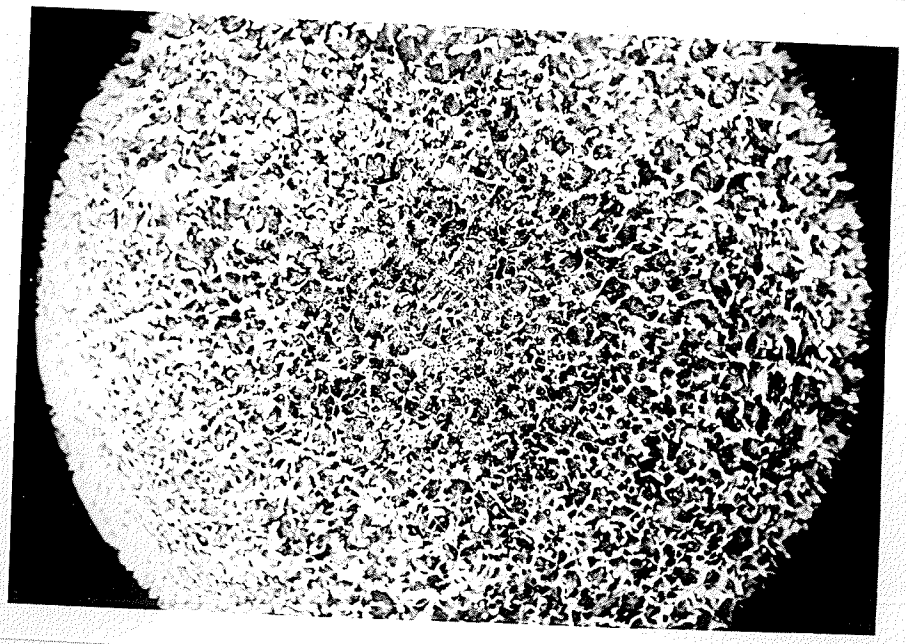

FIGURE 15

Pancreas of a mallard duck. X200

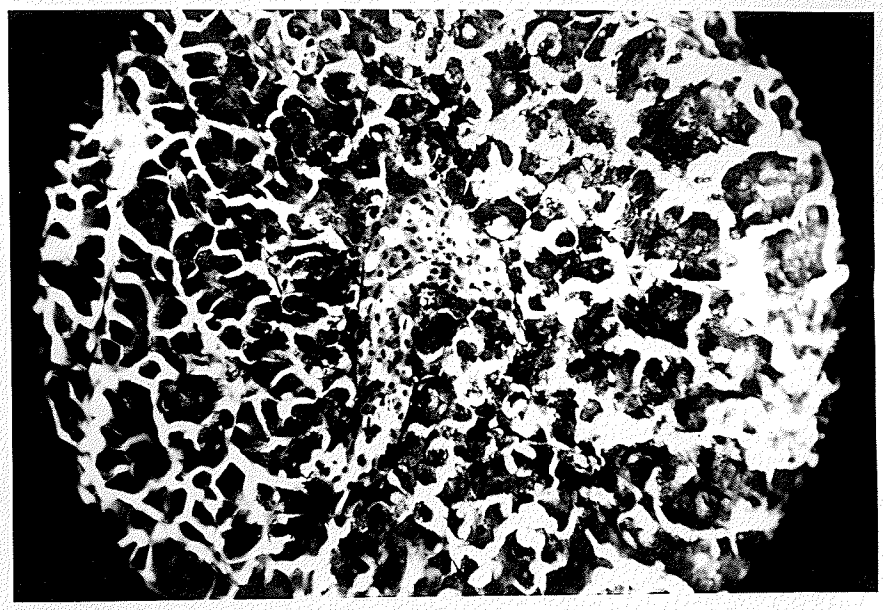

FIGURE 16

Pancreas of a mallard duck:
large islet in the center of the picture. X600

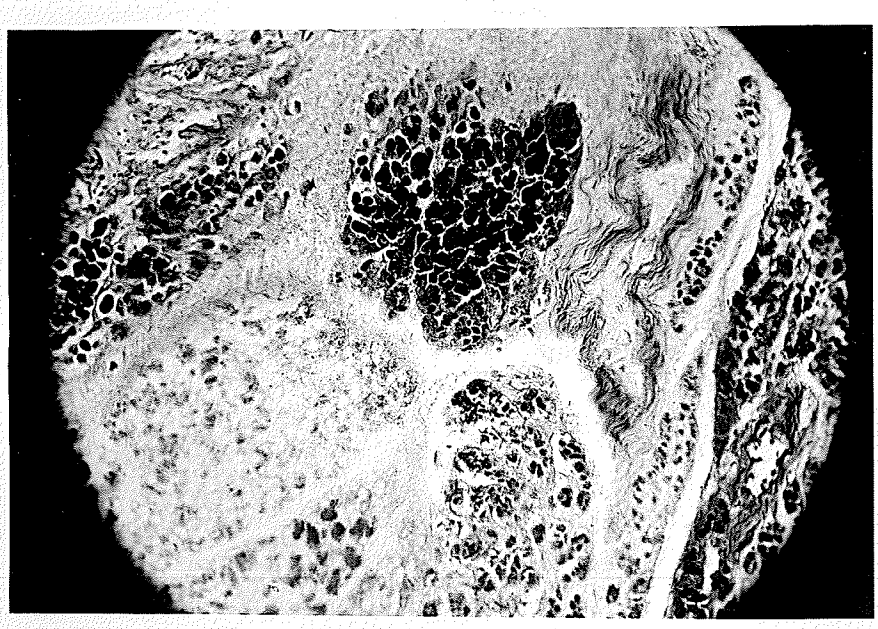

FIGURE 17

Pancreas in the degenerative phase.
Certain areas of the gland are losing their cellular structure and the connective tissue fibers are becoming separated; some areas are still functional. X200

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. A comparative investigation of the pancreas was made using representatives of each vertebrate class. The gross appearance, histological relationships and cytological features of the different pancreatic organs were described and illustrated.

2. An experimental investigation of the secretory cycle of the pancreas was carried out. Rats were starved, fed carbohydrate, protein and fat diets to observe any cyclic changes which might occur in the cellular components of the pancreas.

3. The pancreatic organs of the different species studied were found to vary in the following respects:
   (a) the amount and distribution of the connective tissue present, giving rise to the variations in the gross appearances of the organs;
   (b) the sizes of comparable cells, presenting a slightly different morphological picture in each species;
   (c) the distribution and size of the islets of Langerhans.

   While the first two features are species characteristic, the distribution and size of the islets may vary within the species.

4. Although the variations noted did exist, the cells of the pancreas in the different species were found to stain in a similar fashion. The basic histological pattern of the gland was comparable in the types studied.

5. Two groups of stimuli were applied to the experimental rats. The first group were acute stimuli which consisted of either starvation alone or starvation followed by the feeding of a restricted diet for two or three days. The second group were chronic stimuli which consisted of either starvation or the continuous feeding of a restricted diet for five or more days.

The reactions to the acute stimuli were cytological and noted as follows:

a. vacuolation of the cytoplasm
b. increase in the number and size of the secretory granules
c. slight increase in the size of the glandular cells.

Degenerative changes appeared as a result of the chronic stimulation and were noted as follows:

a. swelling of the glandular components, especially noticeable in the beta cells with long periods of glucose feeding
b. disintegration of the connective tissue
c. infiltration of both the exocrine and endocrine portions of the pancreatic gland.

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