STUDIES OF THE ISLANDS OF LANGERHANS AFTER CONTINUOUS INTRAVENOUS INJECTION OF DEXTROSE [1, 2]

C. ARTHUR WOERNER
Department of Anatomy, The University of Chicago

THREE TEXT FIGURES AND THREE PLATES (ELEVEN FIGURES)

INTRODUCTION

These experiments were undertaken to determine the effect of continued elevation of the blood sugar on the islands of Langerhans in the intact pancreas. Homans ('15) and Allen ('22) working independently, studied the islands of Langerhans after the removal of a sufficient amount of the pancreas in dogs and cats to produce a continuous glycosuria on a diet of bread and meat. They both found hydropic degeneration in the cells of the islands, particularly the beta cells: this was taken as an indication that continuous hyperglycemia would produce the hydropic degeneration by overwork and fatigue of the beta cells, and, thereby, establish a true diabetes. If this were the most important factor it would seem that continuous injection of large amounts of dextrose might produce similar changes in the intact pancreas.

Woodyatt ('15), who first designed an apparatus for continuous intravenous injection, injected into dogs large quantities of dextrose. He found that quantities greater than about 0.9 gm. per kilogram per hour would produce glycosuria

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[2] This work was done under the direction of Dr. Sylvia H. Bensley. Submitted in partial fulfillment of the requirements of The University of Chicago for the degree of Doctor of Philosophy.

within 30 minutes; that the amount of dextrose necessary to produce glycosuria was approximately twice the amount consumed in producing the total heat lost under the conditions of his experiments; that there was a marked increase in metabolic rate, but that this increase could account for but a small part of the total amount of dextrose injected; and that there seemed to be a rather definite maximum above which the total metabolic rate could not be increased by the injection of larger quantities of dextrose. Jacobs and Colwell ('36) studied blood chemistry during the continuous injection of as much as 4.25 gm. of dextrose per kilogram per hour, injected as long as 6 days.

Since in the experiments of Woodyatt ('15) and Jacobs and Colwell ('36) the continuous injection of dextrose was maintained for no longer than 6 days with no resulting diabetes, and no cytological studies were made of the islands of Langerhans, it seemed worthwhile to study the pancreas by the cytological methods developed by Bensley ('11), after the prolonged injection of various amounts of dextrose.

MATERIALS AND METHODS

Forty apparently healthy male guinea pigs weighing between 360 and 840 gm. were injected continuously with a 30% solution of dextrose to which had been added 0.75% of sodium chloride. As controls, two animals were injected with 2 cc. of 0.75% solution of sodium chloride per hour, one for 7 days and the other for 14 days.

Under ether anaesthesia, with aseptic technique, an incision was made in the neck of the animal and a small rubber tube, as described by Jacobs ('33), was put into the external jugular vein through a large hypodermic needle so that about 2 cm. of the tube were in the vein. The other end of the tube was brought out through the incision and the incision was closed. The small rubber tube was connected through a special glass cannula (fig. 2) (attached to the neck of the animal) with a larger rubber tube that went to a 50 cc. syringe held in an apparatus driven by a synchronous motor so that exactly

2, 3 or 4 cc. of the solution were injected during each hour, that was, between 0.71 and 2.45 gm. of dextrose per kilogram of body weight per hour.

The continuous injection apparatus consisted of a device into which from one to three 50 cc. syringes were clamped and arranged so that the plungers of the syringes were pushed by means of a synchronous motor, which assured a constant rate (fig. 1). The syringes, the tubing, the bottle containing the solution, and the glass cannula were wrapped in a towel and autoclaved. The apparatus was so designed that the syringes and the connecting tubing would not have to be taken apart in order to connect them with the apparatus.

In order to make the conditions of the experiment as nearly normal as possible, the animals were kept in cages rather than tied down to a board. It was found necessary to make the cages narrow enough so that the animals could not turn around, about twice the length of the animals and just high enough so that the animals could stand, move forward and backward comfortably and yet could not climb up on the side of the cage and turn over. In the top of the cage was a slit through which the tubing passed (fig. 3). (If there is enough room in the cage the animals will invariably turn around and twist the tubing so that the injection will be stopped, and if they can reach it, they will chew the tubing and make it leak.)

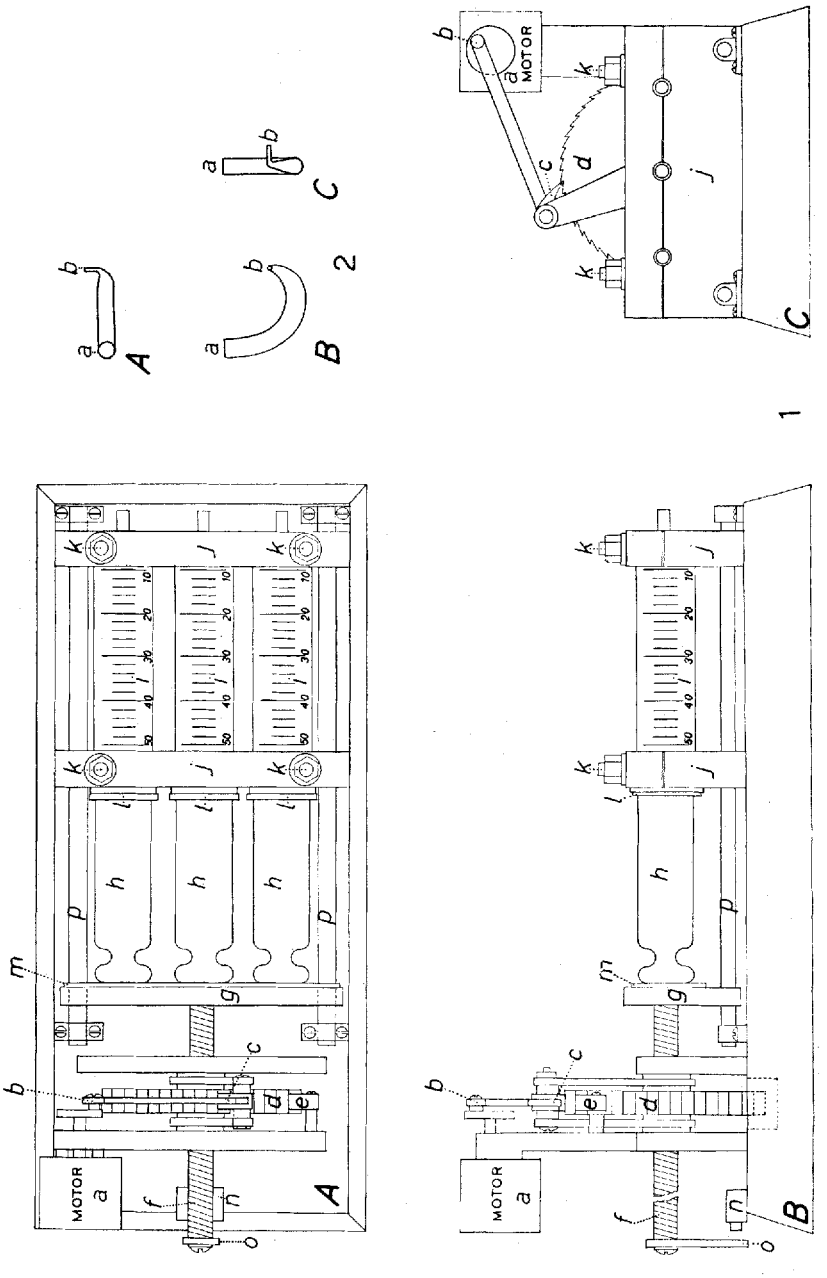
At the end of each experiment the tissue was fixed in formalin-Zenker (for adequate preservation of secretion granules), Bensley's AOB, and Regaud (for adequate preservation of mitochondria). Pieces of the livers were fixed in formalin-alcohol for glycogen studies. In a few of these experiments the animals were perfused with Janus green by Bensley's method, fixed with ammonium molybdate, dehydrated in absolute alcohol, cleared with benzol, and mounted in balsam. In dehydrating and clearing these tissues it was found necessary to cut the pancreas into three or four pieces as this considerably facilitated the dehydration and clearing. After clearing, the benzol was poured off and balsam poured in and the vessel containing the tissue was placed under a

bell jar that was evacuated. This facilitated the evaporation of the benzol so that when the tissue was mounted between glass plates the balsam was more easily hardened. These preparations were then bound with lantern slide tape. If adequate illumination is available, these can be projected and the islands of Langerhans show up very well (figs. 11, 12, 13 and 14). The tissue fixed in formalin-Zenker was stained with neutral gentian and the tissue fixed in Bensley's AOB was stained with aniline acid fuchsin and methyl green (Bensley, '11).

In one experiment about 80% of the pancreas was removed 7 days before injection was started. At the end of 7 days of injection the animal was perfused with Janus green. A piece of the remnant of the pancreas was fixed in formalin-Zenker. The larger part of the pancreas was fixed with molybdate and made into a whole mount. The tissue fixed in formalin-Zenker was embedded in paraffin and sections of it were stained with neutral gentian.

Fig. 1 The continuous injection apparatus. *A*, viewed from above; *B*, viewed from the side; *C*, viewed from the delivering end. *a*, synchronous motor that drives the apparatus at a constant rate. The shaft of the motor rotates at 2 r.p.m. *b*, eccentric on the shaft of the motor. The position of the eccentric can be changed so that the rate of injection can be changed. *c*, dog, that is activated by the eccentric (b). *d*, ratchet gear activated by the dog (c). *e*, a second dog that keeps the ratchet gear (d) from reversing. *f*, screw mounted in the center of the ratchet gear (d). *g*, bar driven by screw (f) when the ratchet gear (*d*) is rotated. *h*, plunger of 50 cc. syringe driven by the bar (g). *i*, 50 cc. syringe. *j*, split wooden blocks that hold the 50 cc. syringes in place. *k*, bolts that hold the blocks (j) together. *l*, rubber washers that protect the syringes from breakage. *m*, thin rubber pad that protects the plungers of the syringes. *n*, safety switch. *o*, metal strip placed on the end of the screw (f) to contact the safety switch (n). The length of the screw is just enough so that the switch will turn off the motor and prevent breaking the syringes should one fail to reset the apparatus. *p*, guide rods that keep the bar (g) aligned so that the plungers of the syringes are driven together.

Fig. 2 The special glass cannula. *A*, viewed from above; *B*, viewed from the side; *C*, viewed from the front. *a*, the larger end of the cannula that is connected to the rubber tubing leading from the continuous injection apparatus. *b*, the smaller end of the cannula that has been drawn small enough to pass into the special rubber tubing, after the special rubber tubing has been put into the external jugular vein of the animal. The cannula has been bent in three dimensions so that it fits closely to the neck of the animal.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In several of these experiments samples of blood were taken from the heart at different periods and the amount of reducing sugar determined by the Somgyi modification of

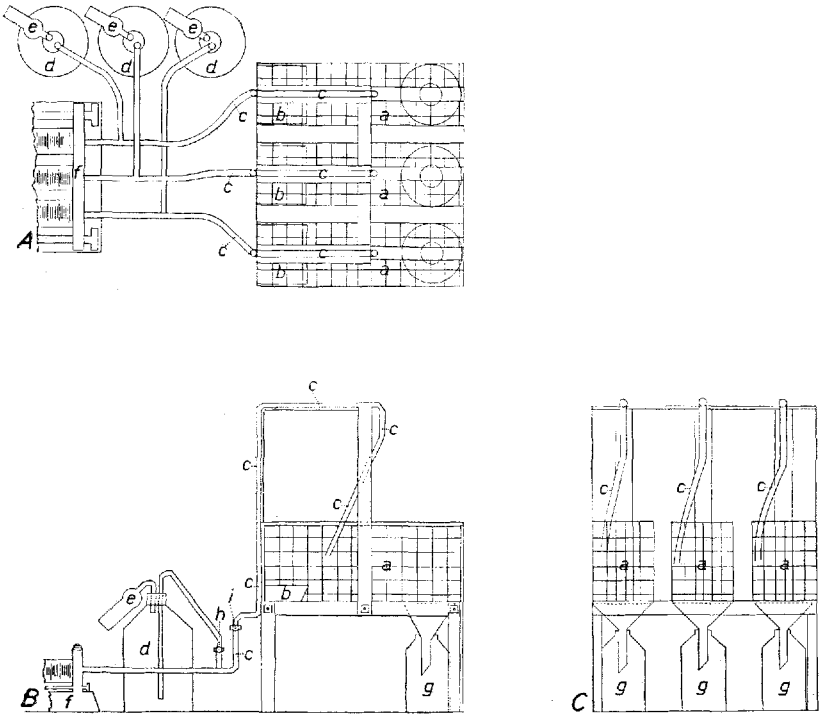

Fig. 3 The cages. *A*, viewed from above; *B*, viewed from side; *C*, viewed from end. *a*, cage in which guinea pig is confined. *b*, metal tray in which food is placed. *c*, glass and rubber tubing leading from injection apparatus to the animal. *d*, bottle containing 30% dextrose solution to which has been added 0.75% of sodium chloride. *e*, calcium chloride tube filled with cotton to prevent contamination of solution. *f*, the continuous injection apparatus. *g*, bottle in which the urine is collected for analysis. *h*, clasp on tube leading to bottle when machine is injecting. *i*, clasp on tube leading to animal that is closed when syringes are being refilled from the bottle (d).

the Shaffer-Hartman method as described by Peters and van Slyke ('32). Samples of the urine were tested by the same method. These determinations are summarized in the following table.

Summary of data on experimental animals discussed

NUMBER OF DAYS	NUMBER OF ANIMAL	FIRST WEIGHT (GRAMS)	INJECTION (GRAMS/KILO PER HOUR)	NUTRITION	BLOOD SUGAR (MG./100 CC.)
1	4	430	1.39	Fair	–
4	25	500	1.20	Fair	–
4	28	550	1.00	Fair	–
5	11	450	1.33	Fair	–
5	14	360	1.66	Fair	–
6	9	780	0.76	Fair	6th day— 270
6	12	440	1.37	Poor	–
7	17	600	1.00	Fair	–
16	19	840	0.71	Fair	Before starting injection— 109 16th day— 147 One hour later on 16th day after injection stopped— 107
28	20	780	0.76		Before starting injection— 87 48 hours— 140 10th day— 147 11th day— 126 Injection stopped: 20 min.— 113 60 min.— 105
10	29	600	2.00	Fair	–
12	24	440	2.25	Fair	Before starting injection— 107
7	26	480	1.00	Fair	
7[1]	27	520	1.00	Fair	

[1] One week after removal of 80% of the pancreas.

DESCRIPTION OF MATERIALS

Of the forty animals which were injected the following have been chosen for cytological description because of adequate fixation and staining and because they appear to be characteristic of the stages which they represent.

Animal no. 4, injected 1 day. The islands are of moderate size and stain palely. The beta cells are small, closely packed and contain few granules. The alpha cells are not numerous. There is no vacuolation in the acinar cells (figs. 5 and 5a).

Animal no. 28, injected 4 days. There is apparently a large amount of island tissue. The beta cells are small and well

filled with large discrete granules. The alpha cells are not numerous. A few of them stain poorly and are somewhat vacuolated. The acinar cells are poor in granules but not vacuolated.

There are a number of small islands consisting of a few beta cells, and individual beta cells are found within some acini. That these cells are true beta cells and not degenerating cells contain Mankowski granules may be determined by the size and staining of the granules. These may be compared in the same section, for a few Mankowski cells do occur. The granules in these intra-acinar beta cells are small, in some cases near the limit of visibility, while the Mankowski granules are larger, about halfway in size between the zymogen and beta granules. The beta granules stain a pale blue, whereas the color of the Mankowski granules approaches the gentian of the zymogen granules.

Occasionally a single cell containing both beta granules and zymogen granules may be found within an acinous (figs. 6 and 6 a). That these two types of granules occur within the same cell was determined by the examination of adjacent serial sections. They were studied in cells in which both types of granules are in focus in the same plane as the nucleus. These cells usually line the terminal portion of the lumen of the acinous, containing 1) less chromophile material than the adjacent acinar cells, and 2) granules that are identified as zymogen granules by their large size, intense stain, and position next to the lumen. The mitochondria are shorter than those of the typical acinar cells, but not as rod-like as those in the typical beta cells. These single cells are usually adjacent to a dilated capillary and contain specifically stained beta granules in the base of the cell.

Another animal (no. 25) injected for the same period, died some time before the pancreas was removed. Although there is some evidence of postmortem change, several mitoses occur in beta cells in many of the larger islands. The alpha cells are almost uniformly fragmented.

Animal no. 11, injected 5 days. There is apparently a large amount of island tissue. Occasional cells, identified as intercalated duct cells by their size, shape and position, contain specifically staining beta granules. In some of the acini near small islands individual cells with beta granules are found among cells containing zymogen granules (figs. 7 and 7 a). These cells were identified by the same characteristics which are exhibited by newly formed beta cells in the pancreas of the preceding animal, no. 28. That these cells are not a part of larger islands inserted between the acini was determined again by examining adjacent serial sections.

The larger islands stain darkly but some of them appear patchy due to the presence of alpha cells and large and small beta cells poor in granules. Most of the beta cells contain large discrete granules grouped at the poles of the cells.

The acinar cells are poor in granules and exhibit a moderate amount of vacuolation of the cytoplasm. The osmic acid preparations suggest that these vacuoles represent swollen and fatty mitochondria.

In another animal (no. 14) injected for the same period of time, the findings are essentially the same.

Animal no. 9, injected 6 days. The beta cells are of two types, one large and elongated with a polar distribution of the granules, and another small and polygonal with a variable granule content.

The alpha cells are not numerous and stain paler than normally. The mitochondria in the acinar cells are moderately swollen.

Another animal (no. 12) injected with almost twice as much dextrose for the same period of time exhibited essentially the same picture except that the changes in the alpha and acinar cells were more marked.

Animal no. 17, injected 7 days. The islands stain darkly but have a punched-out appearance. The light areas consist of alpha cells and other cells which have many of the characteristics of beta cells that have lost their granules, but are very difficult to differentiate from alpha cells. A few of

these pale staining cells exhibit hydropic degeneration (figs. 8 and 8 a). Many of the small beta cells are rich in large discrete granules. In the acinar cells the mitochondria are swollen and fatty (figs. 9 and 9 a).

Animal no. 20, injected 28 days. The islands stain darkly but are patchy due to the presence of many beta cells that are large, poor in granules and resemble alpha cells. The alpha cells are not numerous and appear pale except for a dark area close to the nucleus which stains with the nuclear dye (figs. 10 and 10 a). There is no vacuolation in the acinar cells and the mitochondria are normal.

Animal no. 29, injected 10 days with 4 cc. per hour. The beta cells are uniformly filled with large granules. The alpha cells are not numerous but stain normally. The mitochondria in the acinar cells are normal.

Animal no. 24, injected 12 days with 3 cc. per hour. The islands stain dark but are patchy due to alpha and beta cells which are poor in granules. Beginning hydropic degeneration is present in some of the alpha cells and in duct cells associated with the islands. The mitochondria in the acinar cells are moderately swollen.

Animal no. 26, injected 7 days and perfused with Janus green. The number of islands does not appear to be much more than that found in some normal animals (fig. 13). However, it should be noted: 1) that the larger islands are larger than in the normal 2) that the staining is more diffuse in many of the larger islands and 3) that there is considerable peripheral island formation in relation to acini, the so-called 'acinar type' of island. This would suggest that merely counting the number of islands is not adequate to demonstrate an increase in island tissue. But the perfusion method does give us a reliable technique not only for determining the total number of islands in the pancreas but also for visualizing changes in size and character of the islands throughout the pancreas, since it agrees with the histological findings.

Sections of a piece of this tissue fixed (after perfusion) in formalin-Zenker confirmed histologically the findings from perfusion.

Animal no. 27, injected 7 days, after partial pancreatectomy; perfused with Janus green. The preparations of the remaining piece of pancreas show an unusually large amount of island tissue (fig. 14). In sections of a piece of this tissue fixed (after perfusion) in formalin-Zenker there is considerable evidence of new island formation. There are many very small islands of the 'acinar type' and several mitoses occur in the islands near large ducts.

DISCUSSION

In these experiments the quantity of solution injected during 24 hours was about 10% of the body weight of the animal. The volume was thus restricted lest excessive amounts of fluid cause the animal to become markedly abnormal. In some of the experimental animals and in two control animals injected with physiological salt solution there developed a moderate amount of edema. The smallest amount of dextrose injected was 0.71 gm. per kilogram per hour in an animal that was injected for 16 days. The largest amount that was injected without increasing the volume of fluid was 1.66 gm. per kilogram per hour. Another animal that was injected with twice the volume of fluid received 2 gm. per kilogram per hour. The amount of dextrose injected may be estimated as approximately 150 to 500 mg. of sugar per 100 cc. of blood per minute. The increase in blood sugar was approximately only 50 mg. per 100 cc., which is very similar to that which was found in the experiments of Jacobs and Colwell ('36). In only one of the animals of this series was any appreciable amount of sugar excreted in the urine.

The animals receiving dextrose ate poorly, but that this loss of appetite was not due to the operative procedure was indicated by the fact that other animals, injected similarly with physiological saline or with insulin, ate very well. There was a loss of weight amounting to 20 to 30% of the original weight of the animal.

The absence of extensive 1) hyperglycemia, 2) glycosuria, or 3) storage of glycogen in the liver or muscles studied, but a marked loss of body weight seems to indicate a rapid oxidation of the sugar and an increase in basal metabolism, with the utilization of the animal's own fat and tissue protein. Woodyatt ('15–'16) in his experiments demonstrated such a marked increase in metabolic rate following dextrose injection.

From the sections of these tissues it was found that during the first 24 to 48 hours there is a marked decrease in the number of granules in the beta cells. From 4 to 5 days there is evidence of an increase in island tissue.

That this increase is due in part to growth in preexisting islands is evidenced by mitoses in beta cells in the larger islands, as observed in the tissue from the 4-day animal, no. 25, and from the 7-day animal, no. 26. The development of beta cells from centro-acinar cells and intercalated duct cells has also been observed in the tissue from the 5-day animal. Moreover, there is evidence in the tissue from the 4-day animal (no. 28) that even acinar cells may directly transform into island cells. Here are found acini among whose cells a single cell occurs which shows the properties of both acinar and beta cells. That is, a cell in the position of an acinar cell, containing a small amount of chromophile material at the base and zymogen granules toward the lumen, all characteristics of a differentiated acinar cell, at the same time is adjacent to a dilated capillary and contains specifically staining beta granules in the base of the cell toward the capillary. The mitochondria, instead of being the long filamentous type characteristic of the active acinar cell are shorter, approaching the small rod-like type, characteristic of the beta cell, but not fragmented or swollen as they are in the Mankowski cell.

Such unquestionable evidence of the direct transformation of acinar cells into beta cells has not been presented before. Many investigators have claimed to have observed such transitions. But when the criteria for these assertions are examined they are found to be inadequate.

Bensley ('15, p. 272), after reviewing the specific characteristics of the acinar and beta cells, pointed out:

Considering these properties of the cell it is obvious that to establish a transition between them we must find cells which partake of the properties of both, ... We cannot accept the presence of undifferentiated cells *per se* as evidence of transition but must insist on the intermediate phases which demonstrate progress and its direction.

Examining on the basis of these criteria the assertions of those authors who describe transition, we find that they fall into three classes: first, those who state that, as the acinous cell loses its zymogen and chromidial substances, it becomes more like an islet cell; second, those who have lightly and without due consideration interpreted the A cells as transitions because they frequently occur on the surface of the acinus; and third, those who perceived the logical necessities of the case and attempted to prove a real transition by showing the aquisition by the acinous cells of positive islet characters. The first class requires no discussion because the statements rest upon a negative interpretation of the islet cell. The second group has been sufficiently answered by the demonstration by Lane and others that the A cell was not an intermediate but a specialized cell with characters peculiar to itself. The third group, consisting of Mankowski and Laguesse, requires a more extended discussion.

Bensley goes on to show that the granular cells which Mankowski described as transitional cells and which are now called by his name are in reality degenerating forms of acinar cells whose mitochondria swell, become spherical and finally disappear in the cytoplasm, and whose zymogen granules disappear. He points out that the granules in these cells may be differentiated from the beta granules by their larger size, their intense staining and by their resistance to fixatives in which the beta granules are lost. The transition cells which Laguesse described in the normal pancreas Bensley was unable to confirm and suggested that these might be optical illusions of partially superimposed cells rather than actual independent intermediate cells.

Bensley considered that after ligation of the pancreatic duct in the guinea pig, the dedifferentiated acinous cells and cells of the ducts participated in equal measure in the regeneration of island tissue. He did not observe a direct transformation

of fully differentiated acinous cells into island cells, but he did not assert that this could not occur.

The demonstration, therefore, of positive characteristics, of position and cell constituents, of both acinar and beta cells within the same cell, by Bensley's cytological methods, would seem to fulfill his requirements for adequate evidence of the direct transformation of differentiated acinar cells into beta cells. This demonstration of a direct transformation of one specialized type of cell into another specialized type of cell is one more argument against the theory of irreversibility of differentiation.

Toward the end of the first week, compensatory and degenerative changes may occur within the same pancreas. At this period the beta cells generally contain many granules and these are larger than the normal granules of the beta cells. There are, however, a number of unusually large and a few small beta cells that contain very few granules. These beta cells, in the absence of a specific granule content, are difficult to distinguish from alpha cells which are also deficient in granules. Here the differentiation must be based largely on nuclear detail and this is not always reliable. There are a few cells, usually alpha cells, that show hydropic degeneration. All of the alpha cells stain paler that the normal tissue. In the acinar cells the mitochondria are swollen and some of the mitochondria contain large fat droplets.

Later, after 2, 3, or 4 weeks, the tissue appears more like the normal except that there may be many beta cells poor in granules, some containing unusually large granules, and the alpha cells are pale with an area staining dark with methyl green, close to the nucleus. The acinar tissue approaches the normal.

Like the experiments of Homans and Allen, these results indicate that increase in the blood sugar does affect the beta cells and may cause hydropic degeneration in them. But in contrast to their results are the compensatory increase in island tissue, the absence of complete hydropic degeneration of the beta cells and also the occurrence of degenerative changes in the alpha cells and acinar cells.

A possible explanation for these differences may lie in the fact that Homans and Allen removed the factor of safety by surgical reduction of the pancreas to a point where the excess of sugar constituted such a strain on the remaining beta cells that they degenerated and the sugar was thereafter not available for body metabolism but was largely excreted in the urine, due perhaps to insufficient insulin production. In the intact pancreas, on the other hand, the excess sugar is met with an increase in island tissue and perhaps insulin production, is rapidly oxidized so that the blood sugar level is rarely above that of a feeding animal and therefore constitutes but a moderate strain on the beta cells, but results in an increase in metabolic rate and upset in the fat and protein metabolism, which may be responsible for the later degenerative changes in the other types of cells. These changes, however, may be due to incomplete nutrition and vitamin deficiency.

It may be that larger amounts of sugar injected for short periods of time, or moderate amounts for longer periods would cause more extensive hydropic degeneration of the beta cells in the intact pancreas. This is at present a subject of further experimentation.

The staining methods used in this research are not adapted for the study of the delta cells discovered by Bloom and therefore no data have been accumulated as to their response to these experimental procedures.

SUMMARY

Continuous intravenous injection of moderate amounts of dextrose into guinea pigs for as long as 28 days indicate that:

1. There is a great amount of individual variation in the reaction of guinea pigs to continuous sugar in the blood.
2. It produces anorexia.
3. It produces no extensive hyperglycemia, glycosuria, or glycogen storage, but a marked loss in body weight, which is interpreted as an increase in basal metabolism.
4. At first the beta granules are exhausted in the islands.
5. Subsequently there may be an extensive increase in island tissue.

6. The number of beta cells may be increased: a) By mitoses of pre-existing beta cells; b) by transformation of duct and centro-acinar cells; c) by direct transformation of acinar cells.

7. Following the initial period of exhaustion followed by increase in island tissue there may be a period when degenerative changes occur.

8. The degenerative changes may involve both alpha and beta cells in the form of hydropic degeneration, and also the acinar cells in the form of swollen and fatty mitochondria.

9. In animals that survive the injection for longer than 2, 3 or 4 weeks both island and acinar cells approach the normal condition, although in the islands there may be many beta cells devoid of granules and alpha cells which stain poorly save for a dark staining cap near the nucleus.

10. No evidences of an established diabetes occur in animals injected with this amount of sugar even after 28 days.

CONCLUSION

When a moderate increase in the blood sugar level is maintained by continuous intravenous injection of dextrose, a diabetic condition does not result in the guinea pig. Although the beta cells in the islands of Langerhans appear to be affected by continuous injection of sugar, in many cases there is an intense compensatory increase in the island tissue. Beta cells may arise by mitoses from pre-existing beta cells, by transformation of duct and centro-acinar cells and even by direct transformation of acinar cells. With this increase in island tissue and insulin secretion, there appears to be an increase in metabolic rate resulting in considerable loss of body weight. Hydropic degenerative changes do occur in the alpha cells and duct cells as well as in the beta cells.

I wish to acknowledge my indebtedness to Dr. Sylvia H. Bensley, Dr. N. L. Hoerr and Dr. R. R. Bensley for their generous advice and encouragement during the course of this work, and to Mr. R. D. Bensley for the photomicrographs.

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PLATE 1

EXPLANATION OF FIGURES

The optical system used for figures 4 to 10 consisted of a Zeiss apochromatic oil immersion lens H.I. × 35, 0.85 aperture and a Leitz × 8 ocular with a bellows length which gave an initial magnification of × 400 (except where otherwise indicated). A subsequent enlargement of two diameters was made by enlargement of the negative.

4 and 4 a Four micra section of normal guinea pig pancreas, fixed in formalin-Zenker, stained in neutral gentian. Cells in an island of Langerhans. × 800. *a*, α cells. *b*, β cells with many granules. *bn*, nucleus of β cell containing few granules.

5 and 5 a Four micra section of pancreas of guinea pig no. 4 injected for 1 day with dextrose, fixed in formalin-Zenker, stained in neutral gentian. Island cells. × 800. *b*, β cell with vacuoles. *bn*, nuclei of closely packed small β cells devoid of granules.

6 and 6 a Four micra section of pancreas of guinea pig no. 28 injected for 4 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. Acinar cells. × 950. *ac*, acinar cell containing zymogen granules near the lumen and small β granules at the base of the cell.

7 and 7 a Four micra section of pancreas of guinea pig no. 11 injected for 5 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. A peripheral island in relation to acini. × 800. *ac*, acinar cell. *acs*, group of acinar cells. *b*, β cell rich in granules; *b'*, β cell containing small granules.

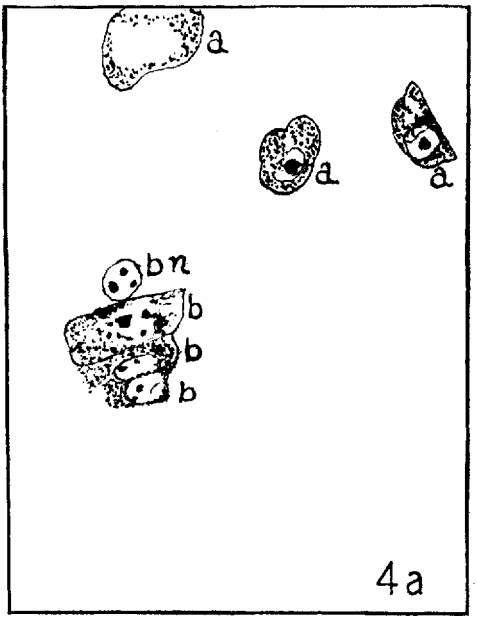
PLATE 2

EXPLANATION OF FIGURES

8 and 8 a Four micra section of pancreas of guinea pig no. 17 injected for 7 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. The edge of an island. × 800. *ab*, β cell lacking granules. *gb*, β cell rich in large discrete granules. *hd*, cell showing hydropic degeneration.

9 and 9 a Four micra section of pancreas of guinea pig no. 17, fixed in Bensley's AOB, stained in aniline acid fuchsin and methyl green. The edge of an island. × 800. *a*, pale staining α cell. *bn*, nucleus of β cell. *sm*, swollen mitochondria in acinar cells.

10 and 10 a Four micra section of pancreas of guinea pig no. 20 injected for 28 days, fixed in formalin-Zenker, stained in aniline acid fuchsin and methyl green. Island cells. × 800. *as*, group of pale-staining α cells with dark-staining area near the nucleus. (Compare these with figs. 6 and 8 a.) *nab*, nucleus of β cell poor in granules. *ngb.s*, nuclei of β cells rich in large discrete granules.



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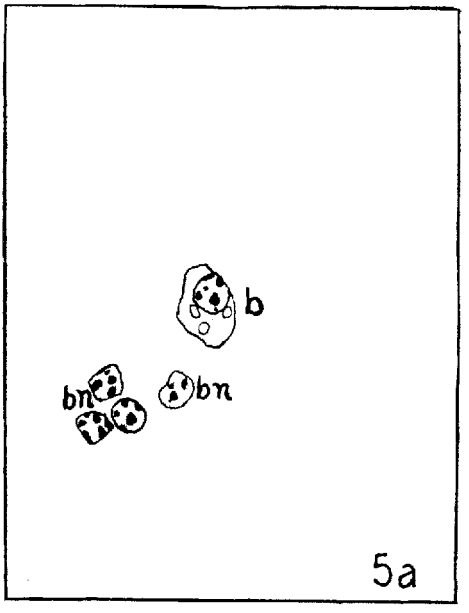

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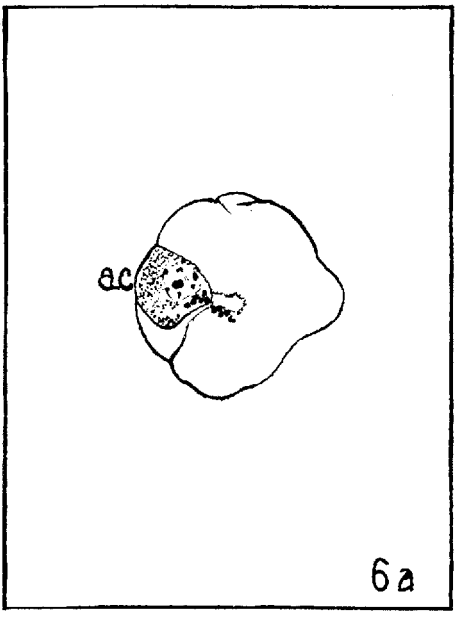

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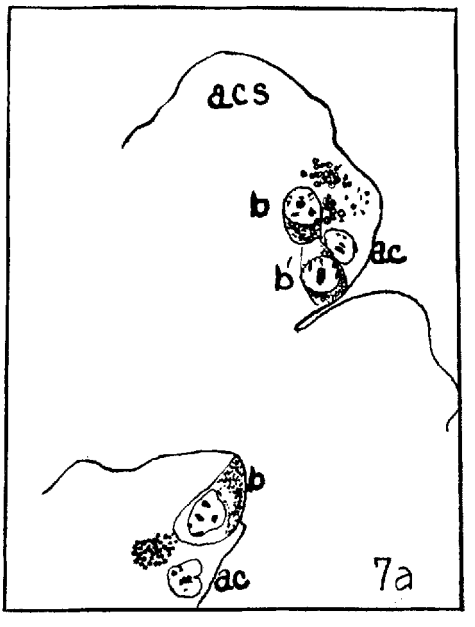

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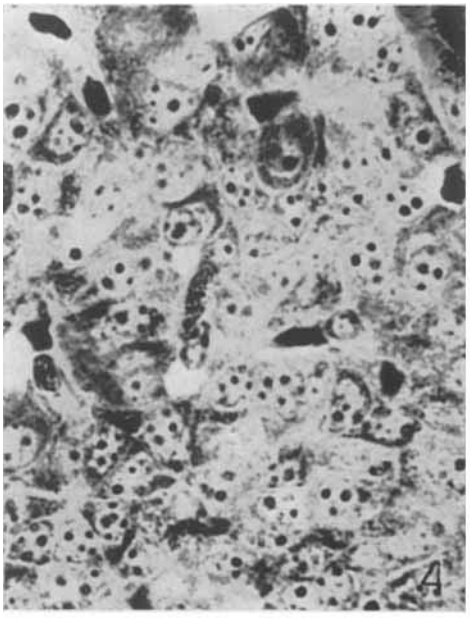
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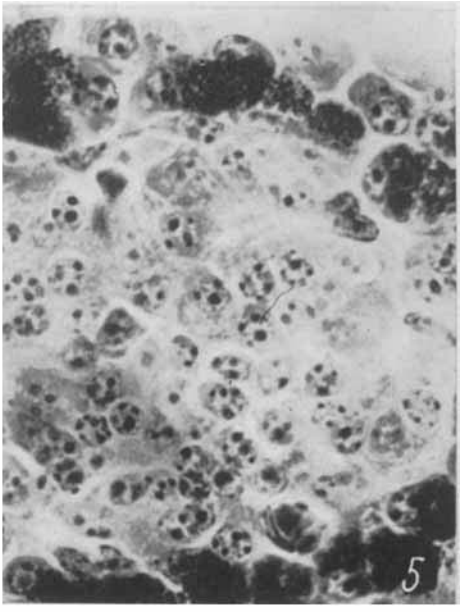
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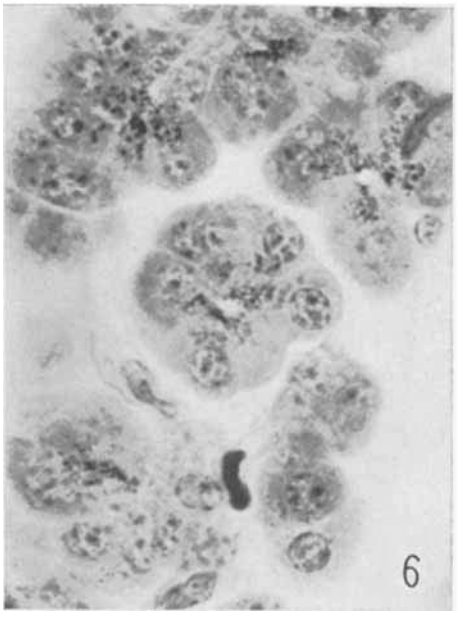
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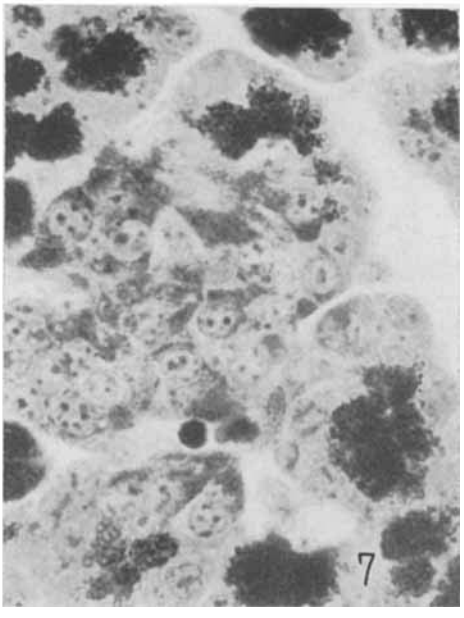
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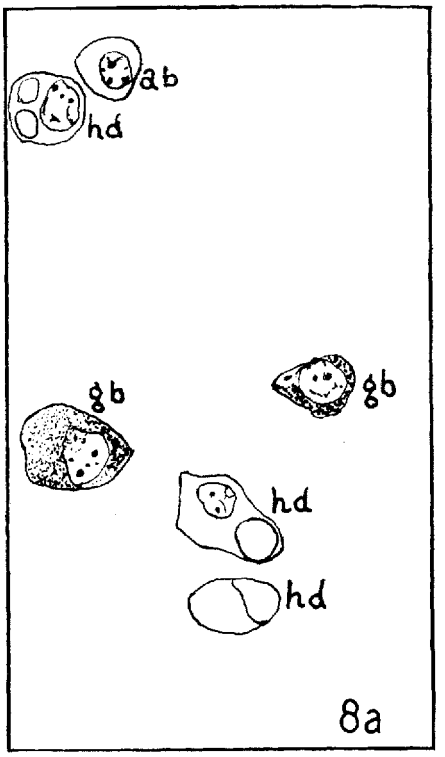
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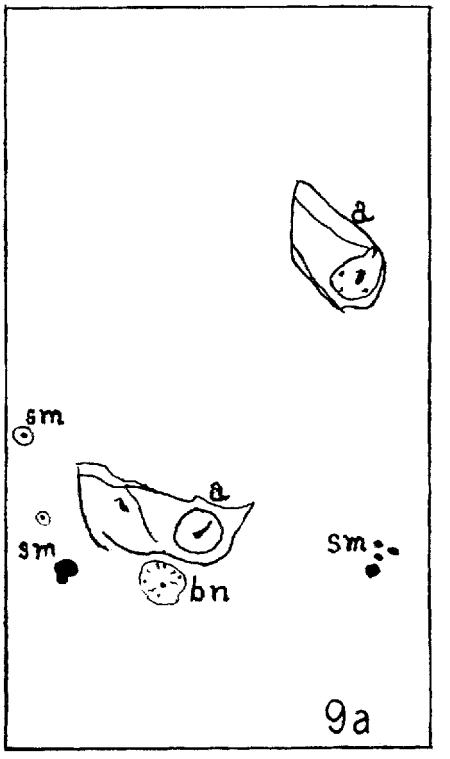
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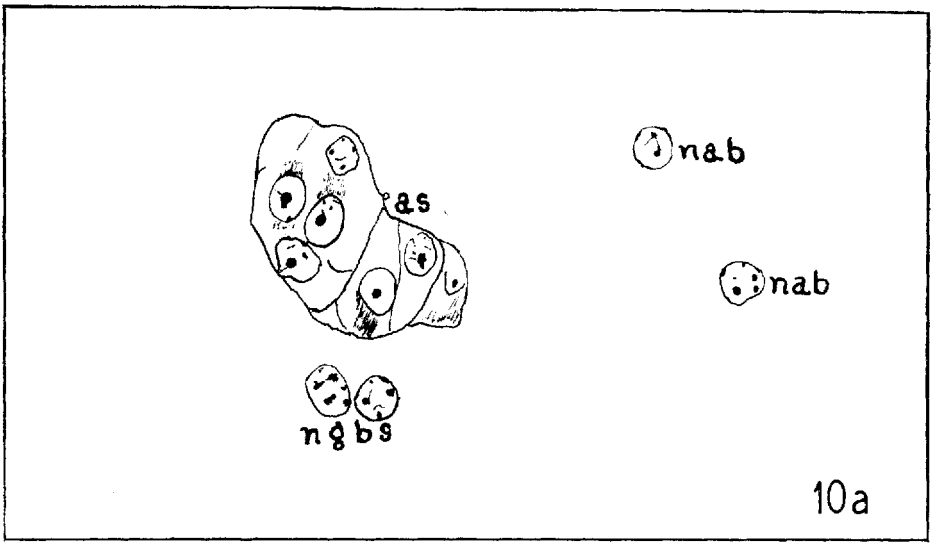
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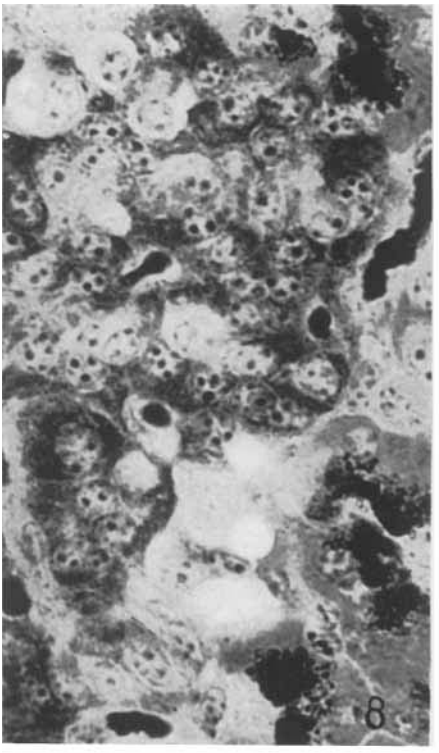
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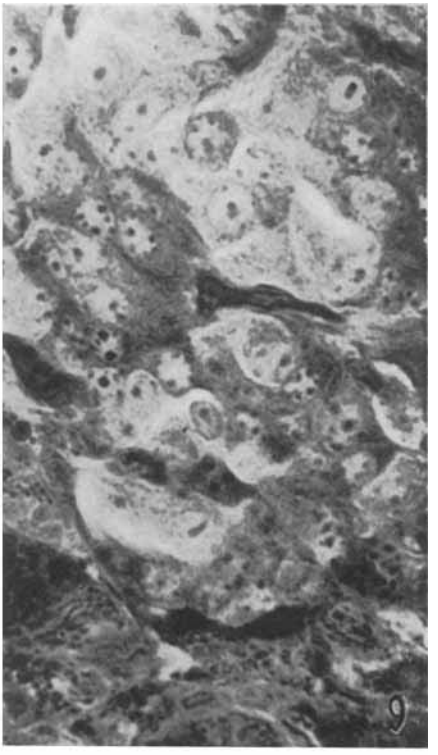
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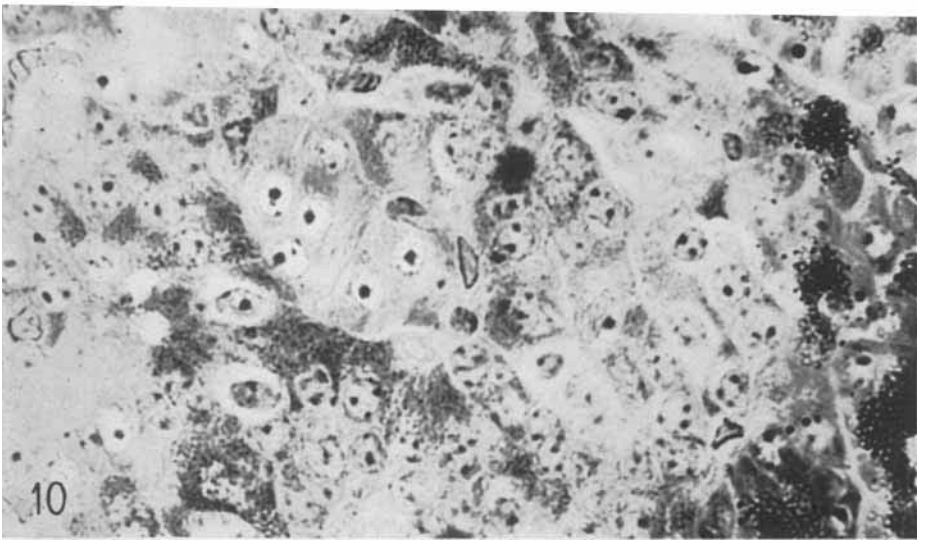

10

PLATE 3

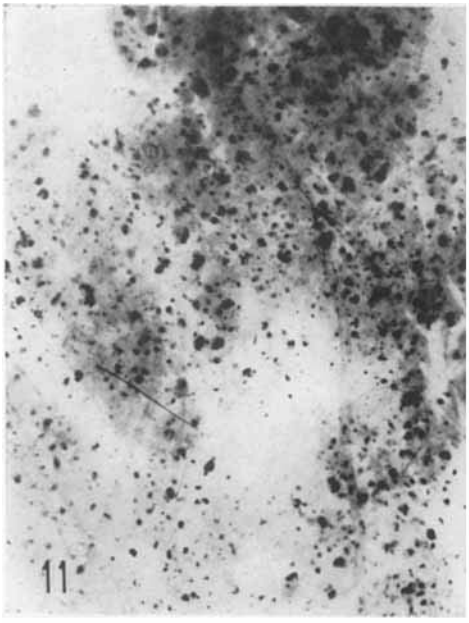
EXPLANATION OF FIGURES

The optical system used for figures 11 to 14 consisted of a Zeiss 32 mm. planar lens with bellows length to give a magnification of × 10.75.

11 and 12 From a whole mount of the pancreas of a normal animal perfused with Janus green. × 10.15.

13 From a whole mount of the pancreas of guinea pig no. 26, injected for 7 days with dextrose and perfused with Janus green. × 10.75. Note the increase in the large islands and the increase in the acinar type of island.

14 From a whole mount of the residual pancreas of guinea pig no. 27, injected for 7 days with dextrose after partial pancreatectomy, and perfused with Janus green. × 10.75. Note the acinar type of islands.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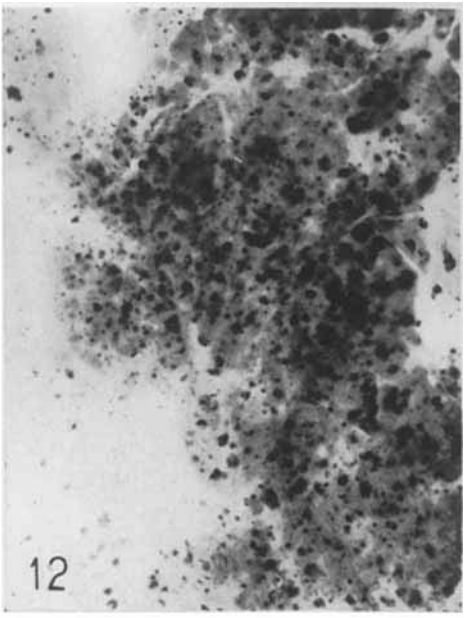
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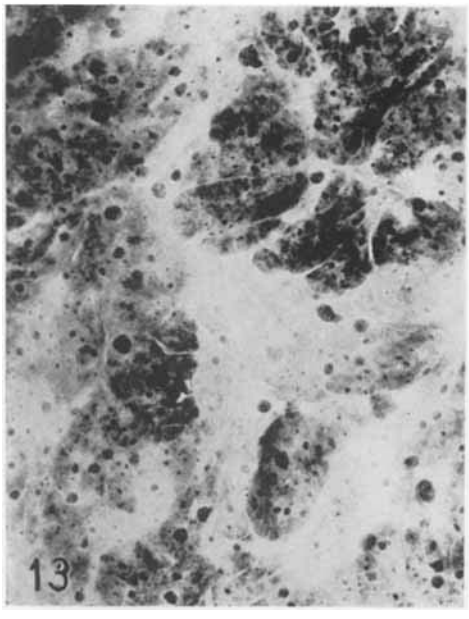
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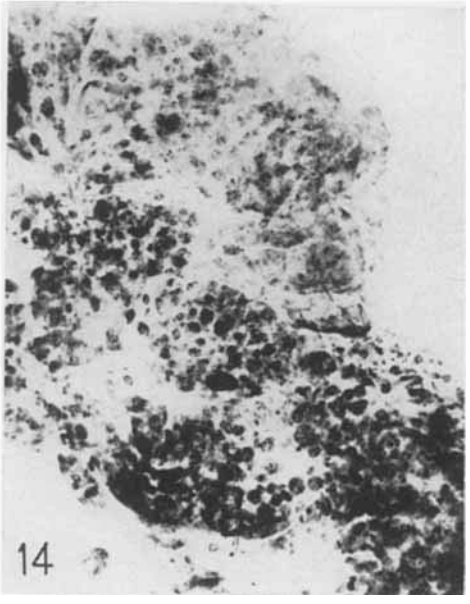
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