STUDIES ON THE FUNCTION OF THE ALPHA CELLS
OF THE PANCREAS

DOGS WITH A REDUCTION IN THE NUMBER OF ALPHA CELLS

SERGIO A. BENCOSME, M.D., PH.D., S. MARIZ, M.D., AND J. FREI, M.D.

Department of Pathology, Queen's University, Kingston, Ontario, Canada

The relation of glucagon to the alpha cells of the pancreas, and the function of this substance and of these cells, were critically reviewed in a recent paper by Korp and LeCompte. Evidence observed in this laboratory indicates that the alpha cell is the site of origin for pancreatic glucagon. In the course of a systematic study of the function of alpha cells, some morphologic and biochemical changes were observed in dogs that were deprived of alpha cells. This paper deals with our observations on dogs with a beta:alpha cell ratio that was greater than normal, after the absolute number of their alpha cells was reduced by means of a selective partial pancreatectomy.

MATERIAL AND METHODS

Fifteen healthy mongrel dogs of both sexes were divided into 3 groups:

Group 1. Six dogs were used as controls for the morphologic studies.

Group 2. Under anesthesia with Nembutal, 5 dogs were operated upon as follows: as close as possible to the origin of the main pancreatic duct, the pancreas was severed between silk ligatures, without damaging the pancreaticoduodenal vessels or the main duct. A complete transverse section was removed from the portion adjacent to the body of the pancreas, for the purpose of histologic examination (biopsy A). After this, the rest of the pancreas (the body and the tail) was removed in the usual manner, thereby leaving the uncinate process (which is normally devoid of alpha cells) undisturbed in its natural anatomic position, together with a small amount of pancreas that contained alpha cells (Fig. 1).

Group 3. Under anesthesia with Nembutal, and using 4 dogs and 1 puppy, the main pancreatic duct and its branch entering the uncinate process were completely stripped of pancreas as far as possible toward the uncinate process, varying from 3 to 6 cm. according to the size of the dog. The pancreatic duct entering the body of the pancreas was stripped of pancreatic tissue and cut between silk ligatures; the rest of the body and the tail of the organ was then removed by means of standard procedures. Two pieces of tissue (labeled A) were removed for biopsy of the free edges of the uncinate process (Fig. 6). Postoperatively, dogs were treated with 60,000 I.U. of penicillin intramuscularly for 4

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Dr. Bencosme was Associate Professor of Pathology; his present address is Department of Pathology, University of California Medical Center, Los Angeles 24, California. Dr. Mariz and Dr. Frei are Research Fellows.

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GLUCOSE TOLERANCE

W 46
WT 36 lbs

SURGICAL PROCEDURE

UNCINATUS

BLOOD GLUCOSE (mg%)

GLUCOSE CONCENTRATION OF PANCREAS

V 69
WT 28 lbs

DUODENUM

BLOOD GLUCOSE (mg%)
days. It was hoped that this operation, in contrast with that performed on animals of Group 2, would remove all, or almost all, of the portion of the pancreas that contained alpha cell, but, at the same time, provide for the external secretion of the uncinate process being emptied into the intestine.

Animals were kept in individual metabolic cages until the completion of the experiment. They had free access to water, drank approximately $\frac{1}{2}$ pint of homogenized milk daily, and ate raw tripe. Blood was collected from a vein in the leg, and the level of glucose was determined by means of a micro-modification of the Folin-Wu method. Glucose in 24-hr. specimens of urine was determined by means of the quantitative Benedict method. Intravenous glucose tolerance tests were performed on fasted, anesthetized dogs as follows: 0.3 Gm. of glucose per kg. of body weight was injected in a 20 per cent solution. Duplicate samples of blood were collected prior to, and at 5, 10, 15, 20, 25, 30, 60, and 90 min. after the injection of glucose.

In order to provide some idea of the general metabolic state of these animals, the body weight and fasting level of blood sugar on the day of the glucose tolerance tests were plotted on the individual protocols.

For the purpose of making a quantitative estimate of the content of glucagon in the pancreatic tissue, extracts prepared according to the method of Best and his associates were tested with the technic described by Staub and Behrens. The details of these procedures as applied in this laboratory have been previously published. Pancreatic extracts were tested in duplicate whenever sufficient material was available.

At the completion of the experiment, dogs were killed by an over-dose of Nembutal at a specified time, as indicated in the individual protocols. Immediately after death, all of the remaining portion of the pancreas was removed and a tracing of each of the specimens was made. The pancreases removed post-mortem from 2 dogs (V 69 and V 39) were used only for morphologic observations, and they were sectioned sagittally, as indicated in Figures 1 and 2, in order to facilitate the histologic examination of the entire organ with a minimal amount of sectioning. The pancreas removed postmortem from each of the other dogs was divided, in order to provide tissue for histologic examination and extraction of glucagon from the region containing alpha cells, and also the portion that was devoid of them (see protocols in Figures 1 to 9).

From all of the animals, the following organs were examined histologically: the pituitary, thyroid, and parathyroid glands, the abdominal aorta, the liver, the adrenal glands and gonads, the duodenum, and the kidneys. Careful search was made at the site of operation for any fragment of pancreas that might have been left in the animal and regenerated, and suspected tissue was removed and examined histologically.

Tissues were fixed in Zenker-formol solution and in 10 per cent formalin, and then processed according to methods previously described; paraffin sections were routinely cut at 2.5 $\mu$ and stained with Masson's hemalum-phloxin and saffron method. Sections of pancreas and pituitary gland were also stained by means of the aldehyde-fuchsin technic, and with a modification of Gomori's chrome-alum
Fig. 3-4
hematoxylin and Masson's trichrome.\textsuperscript{2, 3} Glycogen was studied in all sections of the pancreas, the liver, and the kidneys, by means of the periodic acid-Schiff (PAS) reaction, controlled by digestion with diastase.\textsuperscript{19}

RESULTS

Group 1. The morphology of the pancreas of the control dogs was identical with that previously described.\textsuperscript{5-8} Glycogen was also found in the cells of the ducts, as reported in a previous paper.\textsuperscript{7}

Group 2. No significant change was found in the weight, fasting levels of blood sugar, glucose tolerance, or volume of urine from these partially pancreatetomized dogs, nor was glucose ever detected in their urine.

Alpha cells were observed in biopsy A of all the dogs. No morphologic changes were found in the alpha cells, nor in any of the other elements of the pancreas removed at autopsy. The extent to which these cells were distributed in the pancreas are indicated for each of the dogs in the individual tracings of the pancreas (see protocols in Figures 1 to 9). The amount of glycogen in ductal cells in sections B and C was not greater than that observed in biopsy A.

The results of testing the pancreatic extract reveal a close correlation between the presence of alpha cells and hyperglycemic activity (see protocols in Figures 1 to 9). No determinations were possible on the extract of V 65 B because it was accidentally lost.

The normal vacuolization observed in the renal epithelium of structures that were regarded as collecting tubes was absent in dogs that had survived surgery for more than 30 days. In the other dogs, such vacuolization was greatly diminished in one (17 days), moderately in another (20 days), and slightly in a third animal (18 days). No abnormalities were observed in the other tissues examined.

Group 3. One of the 4 operated dogs died of pneumonia on the fifth day after operation. Another dog, T 81, became diabetic 3 days after surgery, and this condition remained until the end of the experiment. The lack of glycosuria observed in this animal between the eighth and fourteenth days is related to an unintentional, daily injection of 5 units of insulin (P.Z.I.) from the sixth to the eleventh days after the operation. No alterations were observed in the glucose tolerance of W 46, and little, if any, in W 32. The glucose tolerance of T 81 was abnormal only once (Fig. 8). The fasting level of blood sugar on the days of the tests for glucose tolerance were within normal limits for all of the dogs except T 81, in which progressive hyperglycemia developed. Immediately after the operation, a transient polyuria and polydipsia developed in W 32 and T 81. These symptoms disappeared between the tenth and twelfth postoperative days.

At the time of autopsy, the pancreases of W 32 and W 46 were well preserved, whereas that of T 81 was mostly sclerosed. The number, distribution, and general cytology of the alpha cell was not affected by the operation (see protocols in Figures 1 to 9). The pancreas of T 81 manifested conspicuous amounts of glycogen in beta cells and ductal cells, although well-granulated beta cells were also occasionally observed.
As anticipated, all of the pancreatic extracts from Group 3 elicited some hyperglycemic activity. Because of advanced sclerosis, no glucagon was extracted from the pancreases of T 81 and W 44.

The kidneys of W 32 and W 46 manifested a complete disappearance of the vacuolization of the epithelium in the collecting tubules. In T 81, however, these cells were no different from those observed in the controls. Moderate amounts of glycogen were present in the tubular epithelium of T 81.

As a result of technical difficulties, the puppy (i.e., W 44) could not be studied as completely as the larger animals. The recorded data are included in this paper because the animal was a puppy in which the alpha cells were considerably reduced. This animal did not gain weight. Lecithin was administered in order to prevent the development of a fatty liver as a result of occlusion of the pancreatic duct. Although some bouts of glycosuria were observed, the levels of blood sugar a week after the operation and at the time of autopsy were within normal limits. Moreover, 7 days after operation, the glucose tolerance was also normal. Post-mortem examination revealed that the pancreas was selerosed as a result of occlusion of the duct. The regional distribution of alpha and beta cells was similar to that observed in the mature dogs, and there was no indication of damage to the alpha or beta cells. The other organs were not remarkable. The epithelium of the convoluted tubules of the kidneys manifested a great reduction in the number of vacuoles.

Specific alterations of the other organs examined were not observed in any of the animals.

**DISCUSSION**

Both methods of partial pancreatectomy resulted in a great reduction in the absolute number of alpha cells in the dogs; such reduction was greater in the animals of Group 3. The method used for this group led more readily to complications such as diabetes and occlusion of the pancreatic duct. Despite the great reduction in the numbers of alpha cells, no alteration of the carbohydrate metabolism was detected, judging by the results of the laboratory tests, unless complicating factors occurred, such as in T 81 and W 44. Although our conclusions are based on a small number of animals, they are also supported by similar results observed in dogs that were deprived of the portion of the pancreas that contains alpha cells.9

The lack of proliferation, new formation, or other morphologic alteration of the alpha cells in the operated dogs is remarkable in view of the drastic reduction in the absolute number of this type of islet cells. Although it is possible that the alpha cells were not provided with the proper conditions for regeneration, it seems more likely that the numbers of alpha cells left in the operated dogs of Groups 2 and 3 were sufficient for the needs of their bodies. Indeed, if this were not the situation, one would expect evidence of stress in the remaining alpha cells, such as degranulation, hydropic degeneration, necrosis, and, finally, disappearance of the alpha cells. One can not exclude the possibility that alpha cells are associated chiefly with growth, in which case, unless growing animals are
studied, one may never produce the syndrome caused by a deficiency of alpha cells. From the literature, there is some suggestion that alpha cells may be related to the process of growth. The histologic findings in the puppy's pancreas do not suggest that the alpha cells are more active during growth than in mature animals, although more work must be done with growing dogs prior to excluding such a possibility. The fact that no alpha cells were observed in the uncinate process of the pancreases of the operated dogs is in agreement with our recent observations that alpha cells also failed to develop in the uncinate process of dogs from which all of the pancreas that contained alpha cells was removed.

Because of the small number of animals in which polyuria was observed, we should not draw conclusions with regard to its significance. It seems advisable, however, to study the water metabolism and the histology of the kidneys when studying the function of the alpha cells. In this respect, it is of special interest to mention the paper of Staub and his associates, who found that glucagon considerably increases the excretions of chloride, sodium, phosphate, and potassium.

In conclusion, on the basis of the findings described in this paper, no positive suggestion should be made with regard to the function of alpha cells beyond confirming (once more) that glucagon seems to be produced by alpha cells. The experimental observations summarized herein do not exclude the possibility that alpha cells participate in the metabolism of carbohydrate, but the observations also do not provide evidence that supports such a possibility.

SUMMARY

This paper deals with a description of 2 methods that are suitable for reducing the number of alpha cells in the pancreas of a dog to an extremely low level, by means of a partial pancreatectomy. Dogs do not necessarily become diabetic as a result of the procedures. The external secretion of the portion of pancreas left in the body drains into the duodenum, following its natural course. Unless complications occurred, no changes were noted in the glucose tolerance of such animals postoperatively. The alpha cells of the operated dogs did not manifest morphologic changes that were suggestive of stress, even though the animals lived for as long as 60 days with an exceedingly small number of alpha cells.

Once again, there is evidence that suggests the alpha cell of the pancreas as the site of origin of glucagon. Although it is possible that water metabolism and renal function may in some manner be related to the function of alpha cells, we presently have, on the basis of the experiment described in this paper, no positive suggestion with regard to the physiologic role of alpha cells in the pancreas.

SUMMARIO IN INTERLINGUA

Iste articulo describe 2 methodos pro reducir le numero del cellulas alpha in le pancreas de canes a bassissime nivellos per medio de pancreatectomia partial. Le canes non deveni necessarimente diabetic como resultato del manovra. Le secretion externe del portion del pancreas que remaneva in le corpore disbuccava a in le duodeno secundo su curso natural. In le absence de complicaciones special, nulle alterationes esseva notate in le animales in lor tolerantia postoperatori pro
Each of the illustrations represents a summary of data pertinent to 1 animal in the series of 9, and may be interpreted according to the following key:

**Daily observations** (lower half of each figure):
- ▽ = Time when pancreatectomy was performed.
- □ = Sugar in urine per 24 hr. (measured only for animals T 81 and W 32).
- □ = Intake of water per 24 hr. (present only in animals T 81 and W 44).

**Glucose tolerance** (upper left of each figure): The graphs indicate the results of the tests performed prior to operation (— — — —) and after the operation (— — — —). The number at the end of each curve indicates the numerical order of that test, prior to or after the operation, as the type of line indicates.

**Surgical procedure** (upper right of each figure): The type of line in the sketch indicates pancreatic tissue removed at the time of operation (— — — —) or during the postmortem examination (— — — —). The location of blocks removed for histologic section and whether or not alpha cells were observed in the sections are also indicated.

**Assay of glucagon in extracts of pancreas**: The amount of glucagon was indicated by the changes in the level of blood sugar in cats that were treated with the extract from 1 Gm. of the B portion of the pancreas and 1 Gm. of the C portion. The sites of removal and whether or not these B and C portions of pancreas contained alpha cells are indicated in the upper right of each figure, under the heading "Surgical Procedure."

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**glucosa. Le cellulas alpha del canes operate non manifestava alterationes morphologic que poteva indicar un efecto de stress, ben que le animales superviveva usque a 60 dies con excessivamente basse numeros de cellulas alpha.

Le observationes corroborra le notion que le cellulas alpha del pancreas es le
sito de origine de glucagon. Ben que il es possibile que le metabolismo de aqua e
le function renal es relationate in un maniera o un altere al function del cellulitas
alpha, al tempore presente e super le base del experimentos hic descripte nos
possede nulle indicationes positive relativo al rolo physiologic del cellulitas alpha
in le pancreas.

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