The Internal Secretion of the Pancreas.

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Diabetes mellitus has been known to man since the early days of the Roman Empire. However, it was not correlated with functional defect of the pancreas until 1889. Von Mering and Minkowski, by means of improved surgical techniques, successfully removed the pancreas from several dogs. These experimental animals soon developed symptoms remarkably similar to those characteristic of human diabetes and died within three or four weeks. Parallel chemical studies revealed the striking finding that although sugar was absent in the urine of control or sham operated animals, it was abundantly present in the urine of experimentally altered animals. From these findings it was soon realized that diabetes is essentially a defect of carbohydrate metabolism. Investigators

were quick to attempt the preparation of pancreas extracts that might ultimately be used for the specific treatment of the disease. These attempts were uniformly unsuccessful. The principal difficulty lay in the apparent destruction of the hormone by powerful proteolytic enzymes produced in the exocrine portions of the pancreas.

Reprinted in the following paper are the detailed methods employed by Banting and his collaborators which ultimately led to the preparation of extracts sufficiently potent to relieve the symptoms of expeximentally produced diabetes. Crucial to the successful demonstration of the hormone, and its partial isolation in the form of active extracts, was the application of an improved surgical technique leading to degeneration of the exocrine portion of the pancreas but without effect upon the islet tissue responsible for the synthesis of insulin. In recognition of these accomplishments Banting and his former teacher, Professor Macleod, were awarded the Nobel Prize in 1923.

Five years after Banting's revolutionary findings were published, Abel and his associates reported the isolation of crystalline insulin. Various analytical studies since that time indicate that insulin is a protein molecule, though one of the smallest. In 1954, the Cambridge University biochemist Frederick Sanger and his colleagues, after a decade of intensive research, succeeded in describing its molecular structure. Insulin thus became the first protein for which a structural formula could be written.

THE HYPOTHESIS underlying this series of experiments was first formulated by one of us in November, 1920, while reading an article dealing with the relation of the isles of Langerhans to diabetes. From the passage in this article, which gives a résumé of degenerative changes in the acini [sac-like chambers] of the pancreas following ligation of the ducts, the idea presented itself that since the acinous, but not the islet tissue, degenerates after this operation, advantage might be taken of this fact to prepare an active extract of islet tissue. The subsidiary hypothesis was that trypsinogen or its derivatives was antagonistic to the internal secretion of the gland. The failures of other investigators in this much-worked field were thus accounted for.

The feasibility of the hypothesis having been recognized by Professor J. J. R. Macleod, work was begun, under his direction, in May, 1921, in the Physiological Laboratory of the University of Toronto.

In this paper no attempt is made to give a complete review of the literature. A short résumé, however, of some of the outstanding articles which tend to attribute to the isles of Langerhans the control of carbohydrate metabolism, is submitted.

In 1889 Mering and Minkowski found that total pancreatectomy in dogs resulted in severe and fatal diabetes. Following this, many different observers experimented with animals of various species and found in all types examined, a glycosuria [sugar in the urine] and fatal cachexia [poor physical condition] after this operation. The fact was thus established that the pancreas was responsible for this form of diabetes. In 1884, Arnozan and Vaillard had ligated the pancreatic ducts in rabbits and found that within twenty-four hours the ducts become dilated; the epithelial cells begin

to desquamate; and that there are protoplasmic changes in the acinous cells. On the seventh day there is a beginning of round-celled infiltration. On the fourteenth day the parenchyma was mostly replaced by fibrous tissue. Sscobolew in 1902 noted in addition to the above, that there was a gradual atrophy and sclerosis of the pancreas with no glucosuria. However, in the later stages, from thirty to one hundred and twenty days after ligation of the ducts, he found involvement of the islets and accompanying glucosuria.

Lewaschew believed that the islets were modified acinous cells. Laguesse, an anatomist, first suggested that the islets might be the organ of pancreatic internal secretion. He showed that there were comparatively more islets in the fetus and the newborn than in the adult animal. Opie and Sscobolew independently furnished the first clinical foundation for the belief that the islets were involved in pancreatic diabetes.

W. G. MacCallum, in 1909, ligated the ducts draining the tail third of the pancreas. After seven months he excised the remaining two-thirds. This was followed by a mild glucosuria. Three weeks later he removed the degenerated tail third. This second operation resulted in an extreme and fatal glucosuria. Kirkbridge, in 1912, repeated and corroborated MacCallum's findings and, by the use of Lane's method of staining, proved that the atrophic tissue contained healthy islets.

Kamimura in 1917, working on rabbits, traced the degenerative changes in the parenchymatous tissue of the pancreas after ligation of the ducts, and found that the islets remained normal and that the animal did not develop glucosuria as long as the islets were left intact.

The first attempt to utilize the pancreas in defects of carbohydrate metabolism was made by Minkowski. This worker tried the effect of pancreatic feeding, with no beneficial results. Up to the present time only useless or even harmful effects have been obtained from repeated attempts to use this method.

Knowlton and Starling, in 1912, published experiments which showed a marked decrease in the power of using sugar of a diabetic heart perfused outside the body, as compared with a normal heart under similar conditions. Macleod and Pearce, using eviscerated animals, were unable to confirm the above results. Patterson and Starling subsequently pointed out that a serious error was involved in the early experiments due to (1) excess glycogen present in diabetic hearts, and (2) to the irregular disappearance of glucose from the lungs.

Murlin prepared an alkaline extract of pancreatic tissue and after injection of this solution, secured a reduction in sugar excreted in a diabetic animal. Kleiner has pointed out that the reduction secured by Murlin might be due to the alkali *per se*. Kleiner himself has shown that "unfiltered-water extracts of fresh pancreas diluted with .90 per cent NaCl when administered slowly usually resulted in a marked decrease in blood sugar." There was no compensating increase in urine sugar, but rather a decrease, which Kleiner suggests may be partly due to a temporary toxic renal effect. Hemoglobin estimations made during the experiment showed that the reduction in blood sugar was not a dilution phenomenon. Paulesco has recently demonstrated the reducing effect of whole gland extract upon the amounts of sugar, urea and acetone bodies in the blood and urine of diabetic animals. He states that injections into peripheral veins produce no effect and his experiments show that second injections do not produce such marked effect as the first.

From the work of the above-mentioned observers we may conclude: (1) that the secretion produced by the acinous cells of the pancreas are in no way connected with carbohydrate utilization;

(2) that all injections of whole gland extract have been futile as a therapeutic measure in defects of carbohydrate utilization; (3) that the islands of Langerhans are essential in the control of carbohydrate metabolism. According to Macleod there are two possible mechanisms by which the islets might accomplish this control: (1) the blood might be modified while passing through the islet tissue, i.e., the islands might be detoxicating stations and (2) the islets might produce an internal secretion.

We submit the following experiments which we believe give convincing evidence that it is this latter mechanism which is in operation.

In the ten-week interval which we considered necessary for complete degeneration of the acinous tissue, we secured records of dogs depancreatized by the Hédon method.

METHODS

The procedure is as follows: under general anesthesia an upper right rectus incision is made through the abdominal wall. The duodenum is delivered through the abdominal wound, and the pancreas traced to the tail portion. The mesentery beyond is cut between clamp and ligature. Vessels from spleen are then isolated, ligated and divided. Little dissection is then required until the duodenum is reached. The superior pancreatico-duodenal vessels are located and great care is exercised to avoid damaging them. The pancreas is stripped from the duodenum by dry dissection. The vessels to the uncinate process are ligated and divided, and the process freed from its mesenteric attachments. The larger duct of the pancreas is then ligated close to its entry into the duodenum and the pancreas is removed. Special care must be exercised to preserve the splenic vessels. The superior pancreatico-duodenal vessels must be left intact. Failing this, duodenal ulcer is a frequent development. If this procedure is carried out the whole gland with the exception of the portion in contact with the duodenum is covered with mesentery. The abdominal wound is closed layer by layer with catgut. A collodion dressing is used. The urethral orifice is exposed by a midline incision of the perineum and the edges of the wound drawn together to facilitate healing.

We have found that animals between eight and sixteen months old are the most suitable for this operation. At this age the pancreas is not so firmly fixed as it becomes later.

We first ligated, under general anesthesia, the pancreatic ducts in a number of dogs. (Blood sugar estimations on these animals were recorded from time to time. We have no record of a hyperglycemia).

The extract was prepared as follows: The dog was given a lethal dose of chloroform. The degenerated pancreas was swiftly removed and sliced into a chilled mortar containing Ringer's solution. The mortar was placed in freezing mixture and the contents partially frozen. The half frozen gland was then completely macerated. The solution was filtered through paper and the filtrate, having been raised to body temperature, was injected intravenously.

We have never found it necessary to cut down on a vein under general or local anesthetic. The skin surface above the vein is shaved and the needle inserted into the vein which is dilated by compression. The dogs make very little resistance to this procedure and after the first few punctures lie quietly during the operation. Sugar injections (100 c.c. of fluid) as well as the numerous administrations of extract were conducted by this method.

We performed several experiments with the object of exhausting the zymogen granules of the pancreas. Prolonged secretin injections and vagus stimulation below the diaphragm were practiced. Fortune favored us in the first experi-

ment. In subsequent attempts we were never able to exhaust the gland sufficiently to obtain an extract free from the disturbing effects of some constituent of pancreatic juice.

The blood sugar estimations were made by the Myers-Bailey modification of the Lewis-Benedict method. The results of this method were corroborated by the Schaffer-Hartman method at high and low percentages of blood sugar. The former method gave results which were consistently slightly higher (.01 per cent) than those obtained by the Schaffer-Hartman method. We find the average normal blood sugar, from observations on thirty normal dogs, to be .090 per cent.

Hemoglobin estimations were made by the carbon-monoxide saturation method, using the du Boscq colorimeter.

RESULTS

[*The authors here present many detailed protocols, of which we reprint only the following excerpts.*]

At 6 P.M., September 8, we administered ten c.c. of extract of degenerated pancreas *per rectum*. There was no reduction in blood sugar at 7 P.M. when we gave 12 c.c. of extract of exhausted gland intravenously. [The blood sugar rapidly fell from .30 per cent to .21 per cent; subsequent injections of the same material produced a further drop in blood sugar to .07 per cent.] At 6 A.M., September 10, [the blood sugar having returned to .27 per cent] we administered 15 c.c. of extract of exhausted gland per rectum. There was no effect. At 8 A.M., September 10, fifteen c.c. of extract of exhausted gland were injected intravenously. The drop in blood sugar [to .13 per cent] was very marked. Twenty c.c. of exhausted gland extract, made 1 per cent alkaline with NaOH, were incubated three hours at body temperature with 10 c.c. of active pancreatic juice. This solution was neutralized and injected intravenously at 7 P.M. September 10. No reduction in blood sugar resulted, At 2 P.M. September 11, 20 c.c. of acid extract incubated for three hours at 37.5° F. were injected. [There followed a] drop in blood sugar. On September 13 at 9 A.M. and 2 P.M. extracts from the partially exhausted gland of a cat [were administered.] This extract produces a pronounced general reaction.

We observe that extracts prepared from these more or less exhausted glands, while retaining to some extent the reducing effect upon blood and urine sugar, produce many symptoms of toxicity which are absent after injections of extracts from completely degenerated glands. . . .

A short, but very interesting experiment again demonstrates the remarkable effect of the extract of degenerated pancreas upon the power of a diabetic animal to retain sugar. On Nov. 8 at 11 A.M. (blood sugar .35 per cent), 10 gm. of sugar were injected intravenously. [In one hour the blood sugar rose to .40 per cent.] In the four hours following the injection, 10.88 gm. of sugar were excreted. From 3 to 9 P.M. 78 c.c. of dilute extract were injected in 13 c.c. doses. [The blood sugar fell to .09 per cent.] At 9 P.M. (b.s. .09 per cent), 10 gm. of sugar were injected. [At 10 P.M. the blood sugar had risen to .22 per cent.] Hemoglobin estimations before and after administration of extract were identical.[1] Duodenal ulcer was the cause of the early termination of the experiment.

A more detailed description of the histologic sections obtained during our experiments will be included in a subsequent communication. Suffice it here to note that the pancreatic tissue removed after seven to ten weeks' degeneration shows an abundance of healthy islets, and a complete replacement of the acini with fibrous tissue.

[1] Evidence that the fall in blood sugar is not a dilution phenomenon.—*Editors.*

In the course of our experiments we have administered over seventy-five doses of extract from degenerated pancreatic tissue to ten different diabetic animals. Since the extract has always produced a reduction of the percentage sugar of the blood and of the sugar excreted in the urine, we feel justified in stating that this extract contains the internal secretion of the pancreas. Some of our more recent experiments, which are not yet completed, give, in addition to still more conclusive evidence regarding the sugar retaining power of diabetic animals treated with extract, some interesting facts regarding the chemical nature of the active principle of the internal secretion. These results, together with a study of the respiratory exchange in diabetic animals before and after administration of extract, will be reported in a subsequent communication.

We have always observed a distinct improvement in the clinical condition of diabetic dogs after administration of extract of degenerated pancreas, but it is very obvious that the results of our experimental work, as reported in this paper, do not at present justify the therapeutic administration of degenerated gland extracts to cases of diabetes mellitus in the clinic.[2]

[2] The history of insulin has spectacularly exceeded these cautious expectations. It is estimated that 90 billion units of insulin are used annually in the treatment of America's half million or more diabetics. The life expectancy of diabetics has increased remarkably since pre-insulin days, particularly for younger patients. Before 1913 a thirty-year old diabetic could expect to die in four years; today he can expect to live to sixty. In the pre-insulin era, diabetic children were doomed to die in a year or two; today they can live to middle-age or beyond and their death rate is only 0.3% above that of the general population.—*Editors.*