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ORIGINAL ARTICLES

THE INTERNAL SECRETION OF THE PANCREAS*

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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.[1] From the passage in this article, which gives a résumé of degenerative changes in the acini 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[2] 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 and fatal cachexia after this operation. The fact was thus established that the pancreas was responsible for this form of diabetes. In 1884, Arnozan and Vaillard[3] 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

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round-celled infiltration. On the fourteenth day the parenchyma was mostly replaced by fibrous tissue. Sscobolew[4] 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[5] believed that the islets were modified acinous cells. Laguesse,[6] 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[7] and Sscobolew[8] independently furnished the first clinical foundation for the belief that the islets were involved in pancreatic diabetes.

W. G. MacCallum,[9] 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,[10] in 1912, repeated and corroborated MacCallum's findings and, by the use of Lane's[11] method of staining, proved that the atrophic tissue contained healthy islets.

Kamimura[12] 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.[13] 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,[14] 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,[15] using eviscerated animals were unable to confirm the above results. Patterson and Starling[16] 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[17] prepared an alkaline extract of pancreatic tissue and after injection of this solution, secured a reduction in sugar excreted in a diabetic animal. Kleiner[18] 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[19] 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.[20]

METHODS

The first chart is a record of an animal depancreatized by the Hédon method. The details of this operation are given in Hédon's article.[20] The remaining records are of animals (females) completely depancreatized at the initial operation. 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 experiment. 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[21] modification of the Lewis-Benedict method. The results of this method were corroborated by the Schaffer-Hartman[22] 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

Chart 1 contains the record of a 6.5 kilogram dog (410). This experiment is not conclusive but is interesting to us at least, since we administered the first dose of extract of degenerated pancreas to this animal. On July 11, the pancreas, with the exception of the processus uncinatus, was removed. The processus was allowed to remain until July 18. In the interval between the operations there was no hyperglycemia or glucosuria. The curves on subsequent days show the effect produced by the removal of the pedicle. It will be noted that as the experiment progresses the percentage of blood sugar did not rise to the level usually attained in completely depancreatized animals, and also that there was a marked decrease in the daily amounts of nitrogen and sugar excreted and the volume of urine voided. The animal continued to lose weight and seemed to be entering the cachexial condition characteristic of depancreatized animals which had become infected.

The chart for July 27 shows the effect produced on the percentage of

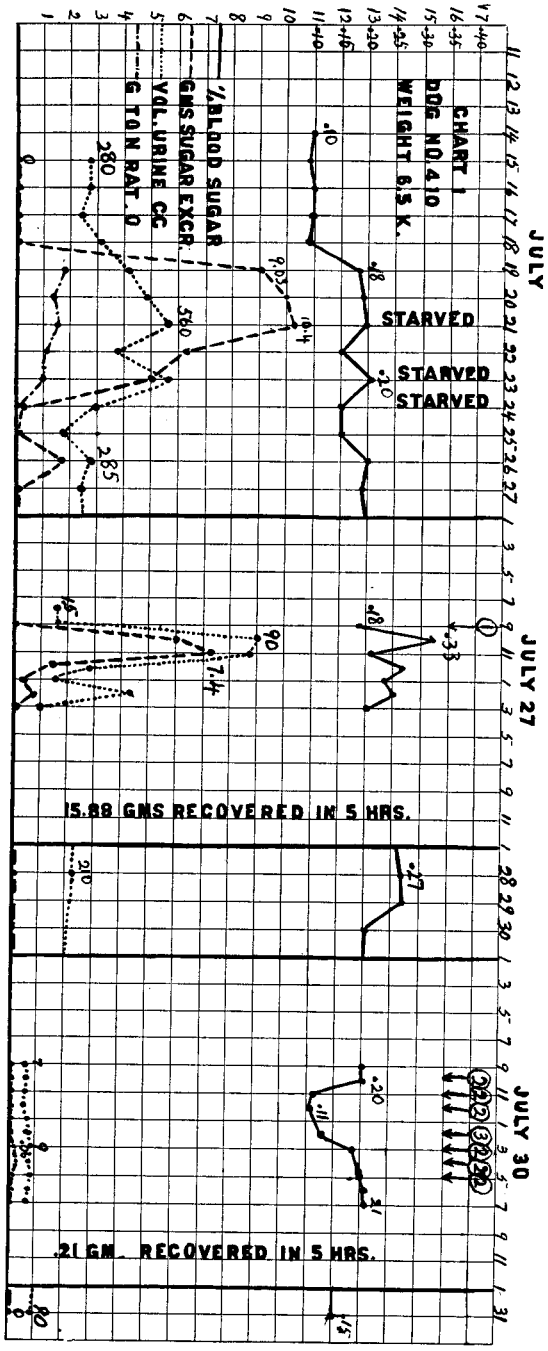

Chart 1.—(1) 25 gms. glucose in 250 c.c. water by stomach tube. (2) Degenerated gland extract, 5 c.c. dose intravenously. (3) .20 gms. glucose in 200 c.c. water by stomach tube. Dog died July 31st—cachexia.

blood sugar and on the sugar excretion by the oral administration of twenty-five grams of dextrose in two hundred and fifty c.c. of water.

At 10 A.M. July 30, the percentage of blood sugar was 0.20. Four c.c. of extract of degenerated pancreas were injected intravenously. At 11 A.M. the blood sugar had fallen to 0.12 per cent. The injections of extract are shown

in the chart. At 12 A.M. twenty grams of sugar in two hundred c.c. of water were given by stomach tube. The chart records the effect.

The obvious criticism of this experiment is that the animal was moribund when the effect of the extract was tried. The interesting features, which gave us great encouragement are (1) the extract caused a sudden fall in the blood sugar and (2) that in the presence of the extract the animal excreted .21 grams of a 20 gm. injection in a period of five hours following the injection, in contrast to an excretion of 15.88 grams of a 25 gm. injection in the same interval, when no extract was administered.

Chart 2 is the record of dog 92, weight 11.9 kg. A complete pancreatectomy was performed on this animal at three P.M. August 11. The first injection of extract was given six hours after the operation and subsequently an injection every four hours. This extract was freshly prepared from a ten Kg. dog whose pancreatic ducts had been ligated for ten weeks. One hundred and twenty-five c.c. of extract were prepared from the gland residue but this supply was exhausted by two P.M., August 13, after which other extracts were used. Blood samples were always taken before the injections of extract.

On August 12, the blood sugar curve shows that neither five nor eight c.c. of this extract every four hours were sufficient to counterbalance the upward trend of the percentage of sugar of the blood. At 10 P. M. the dose was increased to twelve c.c. and a marked fall is noted. The chart at 10 A. M. August 14 records the reduction of the percentage of sugar in the blood below its normal level, as a result of extract from another degenerated gland. (The exceptionally higher values for the volume of urine and the urinary nitrogen for August 15 and 16 may be due to the adulteration of the urine with vomit.) On August 15 at 10 A. M. the chart shows the effect produced by ten c.c. of the same gland extract made 0.1 per cent acid with HCl. This extract made 0.1 per cent alkaline with NaOH causes a slight reduction (August 15, 8 P. M.) The effect may be due to the alkali.

The extract administered at 10 A. M. August 16 was neutral and made from the same degenerated gland.

On August 16 and 17 effects of extracts from normal glands were tested. A normal pancreas from a ten kg. animal was divided into three equal parts. One third was extracted with neutral saline, the second portion with 0.1 per cent HCl and the third with 0.1 per cent NaOH. On August 17 at four P. M. the neutral whole gland extract was administered. A marked fall in blood sugar resulted. The acid and alkaline extracts were injected at 12 P. M. August 17, and 7 A. M. August 18. The last two injections were perhaps not given a fair opportunity to develop their effects. We do not take colorimeter readings by artificial light and therefore did not have an accurate knowledge of the height of blood sugar at these times.

The conclusion from this experiment is that freshly prepared neutral or acid extracts of the whole pancreas do have a reducing effect on blood sugar, thus confirming Kleiner. It may be stated here that repeated injections of whole gland extracts cause marked thrombosis of the veins where the injections are made and a noticeable interference with kidney function. It is obvious from the chart that the whole gland extract is much weaker than that from the degenerated gland.

On August 20, we attempted to exhaust the pancreas of a nineteen kg. dog by continued injections of secretin and repeated stimulation of the vagus nerve below the diaphragm. We obtained eighty-five c.c. of pancreatic juice and considered the gland exhausted. It was swiftly removed and immediately chilled. The marked effect of injection of this material is shown on the chart at 7 P. M. August 20. On August 21 we incubated ten c.c. of the extract and five c.c. of pancreatic juice for two hours at

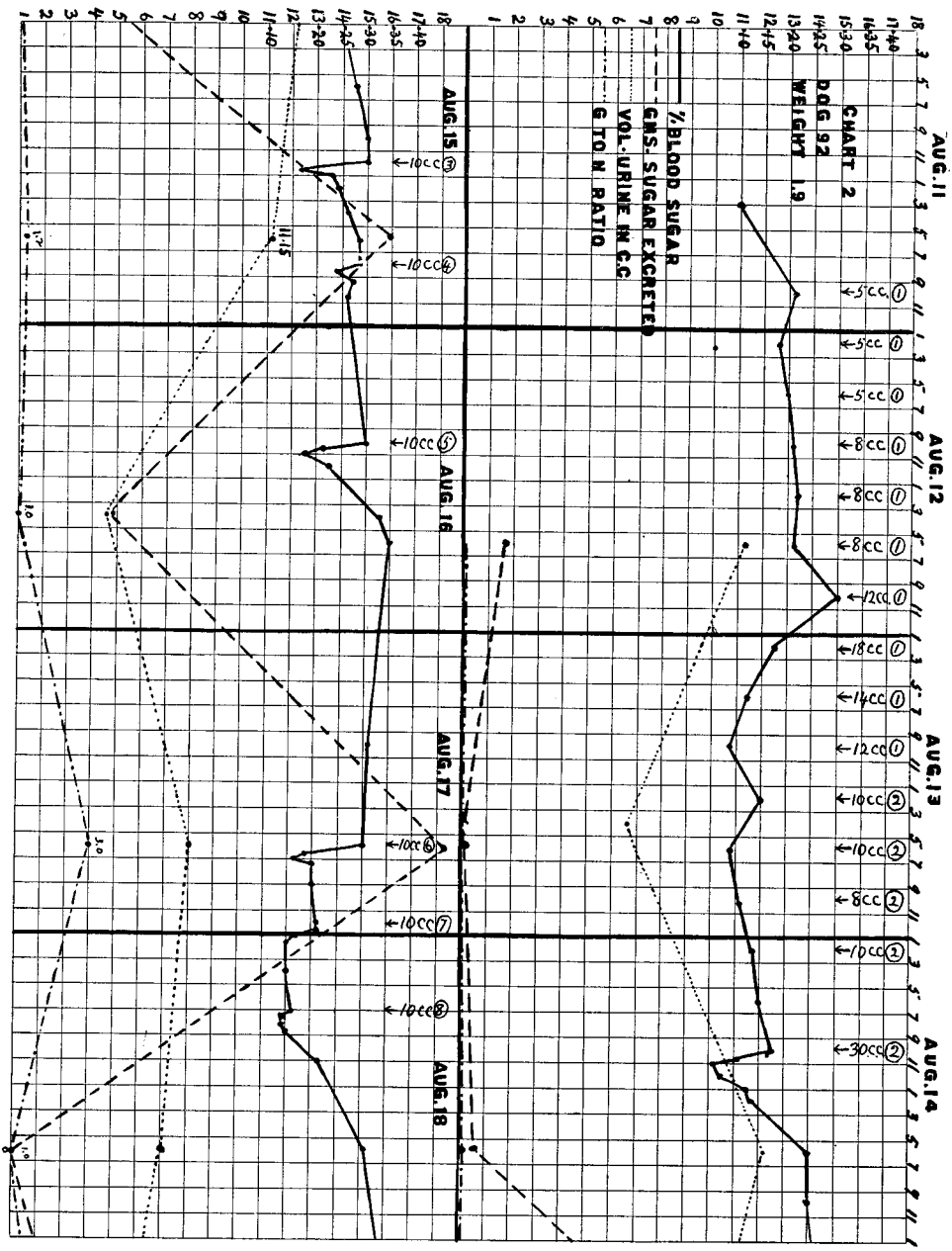

Chart 2.—(1) Degenerated pancreas, dog 394. (2) Degenerated pancreas, dog 390. (3) Degenerated pancreas, + .1% HCl. (4) Degenerated pancreas + 0.10% NaOH. (5) Degenerated pancreas, + .1% HCl. (6) Whole gland extract, fresh, cold. (1) Whole gland extract, + .1% HCl. (8) Whole gland extract + .1% NaOH. (9) Exhausted gland extract. (10) 10 c.c. exhausted gland extract + 5 c.c. pancreatic juice incubated 2 hours. (11) 10 c.c. exhausted gland extract (– pancreatic juice) incubated 2 hours. (12) Whole gland, cat. Dog died August 30.

body temperature in alkaline solution. This solution was injected at 6 P. M. August 21. The curve shows the very slight effect produced. As a control on the above, ten c.c. of extract and five c.c. of saline were incubated under similar conditions for two hours. The chart at 10 P. M. August 21 records the marked effect of the injection of this second solution. On August 22 at 6 P. M. eight c.c. of extract from the normal

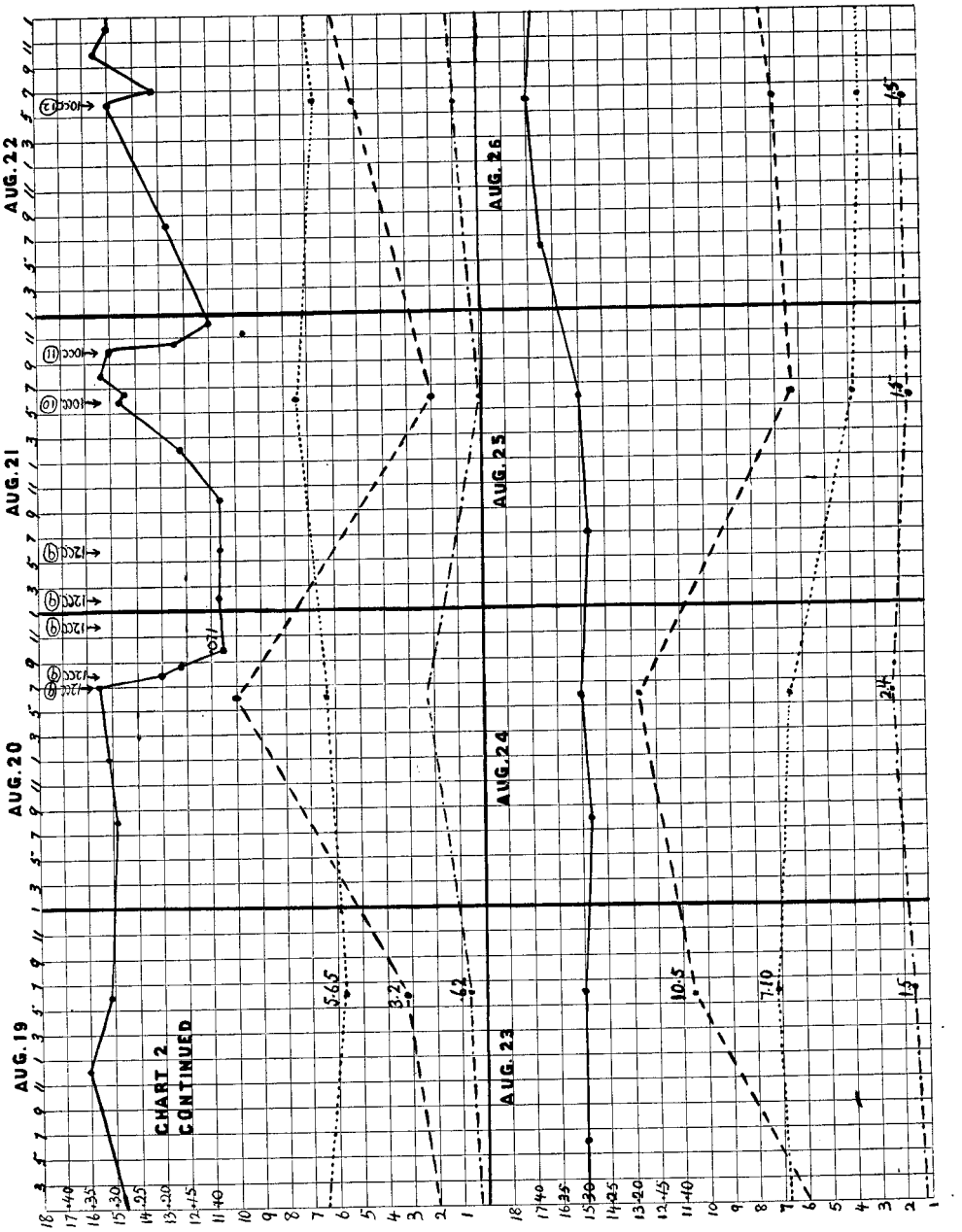

Chart 2.—Continued

pancreas of a cat were injected. We obtained a marked anaphylactic-like reaction. The curve shows the effect upon the blood sugar.

No further injections were given to this animal after August 22. The dog died on August 30, nineteen days after the operation. The autopsy showed consolidation and necrosis of a large area in lower lobe of right lung, infection in right pleural sac. The operation wound was well healed. There was no sign of pancreatic tissue. The abdomen was not infected.

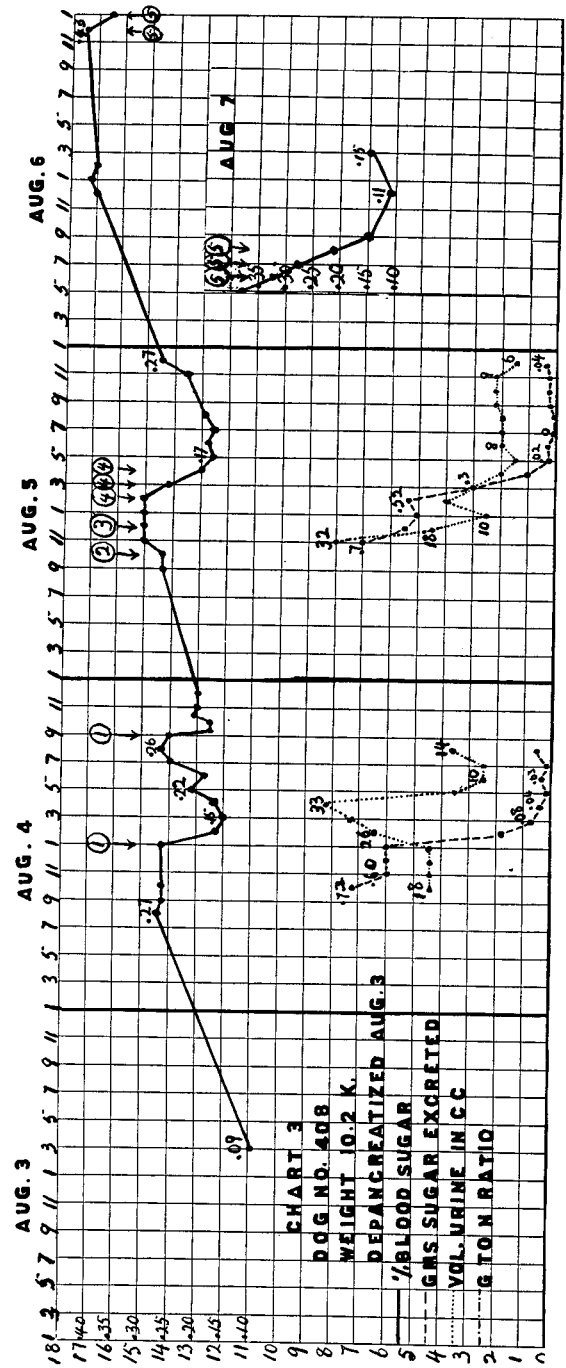

Chart 3.—(1) 5 c.c. 4 day old extract of degenerated pancreas. (2) 5 c.c. extract of liver. (3) 5 c.c. extract of spleen. (4) 5 c.c. extract of degenerated pancreas. Dog died August 7—general peritonitis.

Chart 3 is the record of dog 408. The weight of this animal was 9 kg. The details of the experiment will be given rather fully.

The normal blood sugar of dog 408 was .090 per cent. Eighteen hours after pancreatectomy the percentage of sugar in the blood was .27. Twenty-two hours after

the operation, 1 P. M. August 4, the blood sugar was .26 per cent. During the twenty-two hours 3.10 grams of sugar were excreted. The volume of urine was 494 c.c. At one P. M. we administered five c.c. of extract of degenerated pancreas which had been prepared four days previously and kept in cold storage. At two P. M. the blood sugar was .16 per cent. At three P. M. the percentage of sugar in the blood had fallen to .15. From one to three P. M. .19 grams of sugar were excreted in a volume of twenty-

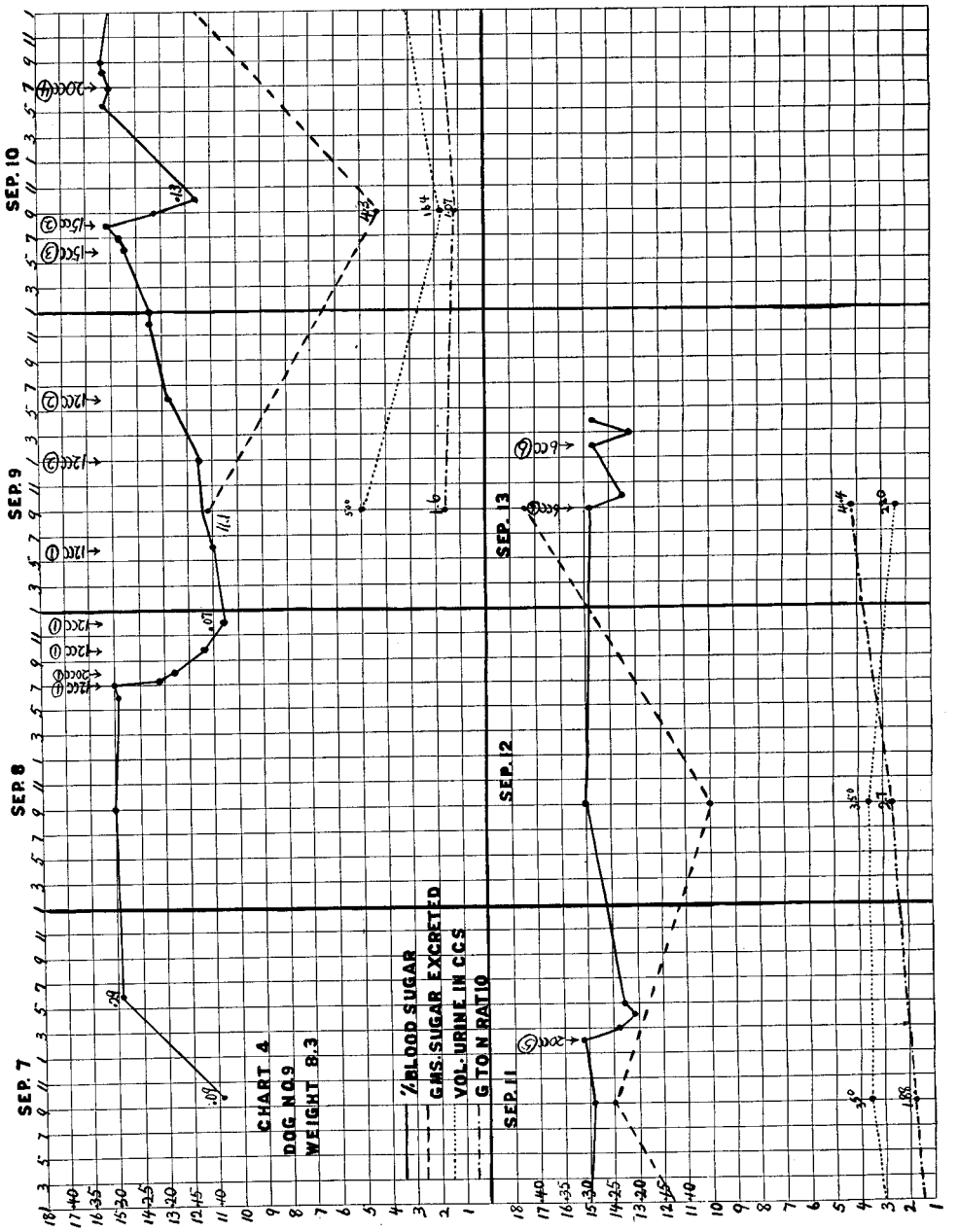

Chart 4.—(1) Exhausted gland extract 1% HCl. (2) Exhausted gland extract neutralized. (3) Exhausted gland extract neutralized per rectum. (4) 20 c.c. exhausted gland extract and alkali + 10 c.c. pancreatic juice incubated 3 hours at 37°C. then neutralized. (5) 20 c.c. exhausted gland extract incubated 3 hours at 37° C., 0.1% HCl. (6) Exhausted gland of cat, 0.2% HCl.

six c.c. of urine. Three and forty-three hundredths grams of urinary nitrogen were excreted in the twenty-four hours following the operation. The G. to N. ratio for this period was 1.4:1. From 3 P. M. to 7 P. M. the percentage of sugar in the blood shows a gradual rise from .15 to .25 per cent. This latter level was maintained until 9 P. M. The chart shows a slight rise in sugar excretion following the rise of blood sugar. At 9 P. M. five c.c. of extract which had been exposed to room temperature for one hour was injected intravenously. The blood sugar was reduced to a value of .18 per cent. The chart shows a gradual ascent from this value to .27 per cent. At 10 P. M. the percentage of sugar in the blood was .27. At this hour five c.c. of extract of liver, prepared in precisely the same manner as the pancreatic extract, were administered intravenously. One hour later the blood sugar was .30 per cent. This level was maintained during the following three hours. It was unaffected by an injection of 5 c.c. of extract of spleen. The chart shows the rise in volume of urine and amount of sugar excreted. At 2 P. M. (b. s. .3 per cent), five c.c. of an extract of degenerated pancreas were injected. A sharp fall in the blood sugar resulted. At 3 P. M. and again at 4 P. M. a similar dose of extract was given. The chart records the lasting effect. The 2 P. M. level of .30 per cent was regained twelve hours after the first injection. The hourly excretion of sugar ran approximately parallel with the percentage of sugar in the blood. Between 1 P. M. and 2 P. M. .52 grams were excreted. Less than .02 grams were excreted between 7 P. M. and 8 P. M. The highest glucose to nitrogen ratio observed in this experiment was a 3:1 value for the 22-hour interval between 2 P. M. the 6th of August and noon the following day. At 12 noon August 6 the percentage sugar in the blood was .40 per cent. Five c.c. of boiled extract of degenerated pancreas was injected intravenously at this stage and caused no reduction of blood sugar. At twelve midnight August 6 five c.c. of extract of degenerated pancreas which had been prepared 48 hrs. previously were administered. The blood sugar fell from .43 per cent at 12 P. M. to .37 per cent at 1 A. M. Five c.c. doses were given at 1, 2 and 3 A. M. and a twenty-five c.c. dose at 4 A. M. The chart shows the reduction in blood sugar to a normal level and the beginning of an upward trend five hours after the last injection of extract. The animal died at 12 A. M. August 7.

A brief description of the clinical condition of the animal at various stages of the experiment is necessary for the correct interpretation of the above results. The animal made a good postoperative recovery and was able to retain water and meat after the second day following the operation. On the morning of August 5 we noticed that the condition of the animal was much worse. It appeared excessively tired, did not eat, and vomited after drinking water and also after extract of spleen given intravenously. At 5 P. M. August 5 the animal appeared considerably improved. It retained water and ate meat. On August 6 at 10 P. M. the abdominal wound was moist with exudate, and the animal was not so active as on the preceding day. No marked variation from this condition was observed until 4 A. M. when 25 c.c. of extract were administered. After this injection the animal had a marked reaction and appeared to be dying. It was revived slightly by intravenous and intraperitoneal injections of warm saline. Considerable improvement was noted at 7 A. M., dog was able to stand. The improvement was short-lived. The dog died at 12 A. M. August 7. The postmortem showed a widespread abdominal infection. There was no sign of pancreatic tissue.

The entire degenerated pancreas from one 8 kg. dog and approximately one-half the degenerated gland from a 6 kg. dog was the substrate of the extract used in this experiment.

Chart 4, Dog 9, gives additional evidence on several important points which have been referred to previously. 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 chart records the effect of this and sub-

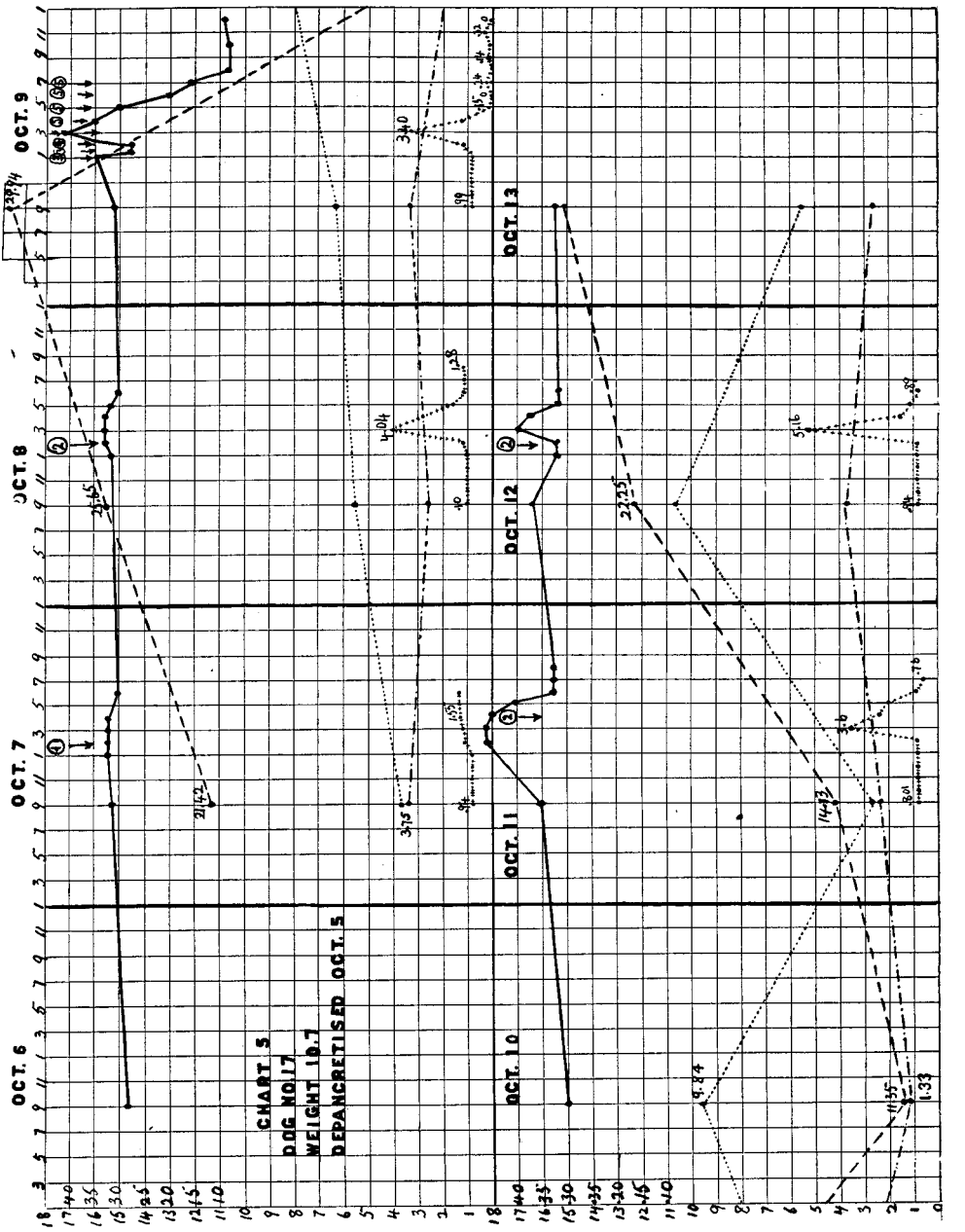

Chart 5.—(1) 100 c.c. saline. (2) 10 gms. sugar + 100 c.c. water. (3) 10 c.c. extract A. (4) 10 gms. sugar, 80 c.c. water, 20 c.c. extract A. (5) 20 c.c. extract B. *Note*: Extract A was made from uncinate process. Extract B from tail portion of pancreas.

sequent injections of the same material. At 6 A.M., September 10, 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 was very marked. Twenty c.c. of exhausted gland extract, made 1 per cent alkaline with NaOH, were

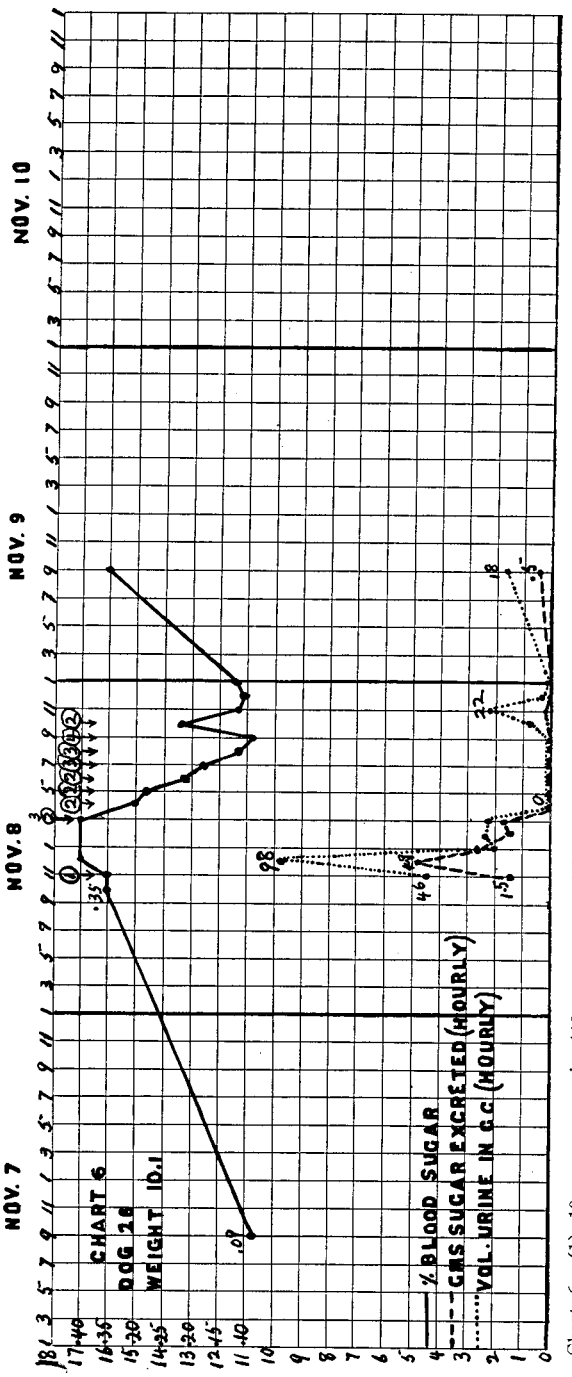

Chart 6.—(1) 10 gms. sugar in 100 c.c. water. (2) 15 c.c. extract degenerated pancreas (4 weeks after ligation of ducts). (3) 6 c.c. extract degenerated pancreas (7 weeks after ligation of ducts). (4) 10 gms. sugar in 100 c.c. water. Dog died November 10—duodenal ulcer.

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. The curve shows the drop in blood sugar. On September 13 at 9 A.M. and

2 P.M. the effect of extracts from the partially exhausted gland of a cat is shown. 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.

Chart 5 is the graphic record of an experiment on a 10 Kg. dog in which we have attempted to prove that the reduction of the percentage sugar in the blood is not a dilution phenomenon. Our plan of campaign was to inject at a given hour on the first day (2 P.M., October 7), 100 c.c. of isotonic saline (*1*). On the second day at the same hour the animal received 100 c.c. of 10 per cent sugar solution (*2*). Extract (*3*) (10 and 15 c.c. doses) was given one hour, and a second dose thirty minutes before the corresponding hour on the third day and 70 c.c. of distilled water containing 10 gms. of sugar were injected on the hour (*4*). Extract in 20 c.c. doses was injected at 3, 4, 5, 6 and 7 P.M. (*5*). To interpret correctly the curve for this day, a brief description of extracts (*3*) and (*5*) is necessary. The pancreas from which these extracts were made was not completely degenerated. The pancreatic ducts of the animal had been tied six weeks. Extract (*3*) was made from the processus uncinatus. Extract (*5*) was made from the remainder of the gland. The extract from the uncinate process was much weaker than the latter. In the four hours following the first injection of sugar we recovered 9.94 grams in the urine. In the corresponding period on the following day after the injection of sugar plus extract 4.49 grams were recovered. Had we used the more powerful extract first, the reducing action might have been even more strikingly demonstrated.

We were surprised that we did not secure a raised blood sugar one hour after the first injection of sugar. The rise after the second injection was very marked. We gave the animal one day's rest and repeated the consecutive injections with the same results as above. The interpretation of the results of the first of the later injections is complicated by an unexplainably high percentage of sugar present before the injection. This phenomenon cannot be wholly explained by the rate of output of sugar since on the fourth injection, the first hour excretion was the maximum of the series and we did obtain a definite rise in blood sugar. An injection of 1 gram of sugar per kg. given to a normal dog showed a pronounced rise in percentage sugar of the blood after a 15-minute interval. This hyperglycemia rapidly subsided and at the end of an hour the blood sugar had regained its normal level; 2.29 grams of sugar were excreted.

Hemoglobin estimations were made (1) one hour after the first sugar injection, (2) just before the first injection of extract and (3) at 12 P.M., Oct. 9. The blood sugars at these times were .33 per cent, .35 per cent and .09 per cent respectively. The hemoglobin was identical at the second and third determinations; a slightly lower value was obtained at the first determination.

Chart 6 is the record of a short, but very interesting experiment which again demonstrates the remarkable effect of the extract of degenerated pan-

creas upon the power of a diabetic animal to retain sugar. On Nov. 8 at 11 A.M. (b.s. 35 per cent), 10 gm. of sugar were injected intravenously. The curve shows the rise in blood sugar. 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. At 9 P.M. (b.s. .09 per cent), 10 gm. of sugar were injected. The curve shows the effect on blood sugar and sugar excretion. The effect of partially degenerated gland extract, 5 weeks after ligation of the ducts, upon the kidneys is shown here. This extract may produce a raised threshold to sugar or a condition of anuria, as in this experiment. Hemoglobin estimations before and after administration of extract were identical. 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.

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.

CONCLUSIONS

The results of the experimental work reported in this article may be summarized as follows:

Intravenous injections of extract from dog's pancreas, removed from seven to ten weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine.

Rectal injections are not effective.

The extent and duration of the reduction varies directly with the amount of extract injected.

Pancreatic juice destroys the active principle of the extract.

That the reducing action is not a dilution phenomenon is indicated by the

following facts (1) hemoglobin estimations before and after administration of extract are identical; (2) injections of large quantities of saline do not effect the blood sugar; (3) similar quantities of extracts of other tissues do not cause a reduction of blood sugar.

Extract made 0.1 per cent acid is effectual in lowering the blood sugar.

The presence of extract enables a diabetic animal to retain a much greater percentage of injected sugar than it would otherwise.

Extract prepared in neutral saline and kept in cold storage retains its potency for at least seven days.

Boiled extract has no effect on the reduction of blood sugar.

We wish to express our gratitude to Professor Macleod for helpful suggestions and laboratory facilities and to Professor V. E. Henderson for his interest and support.

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