A STUDY IN EXPERIMENTAL DIABETES

THE EFFECT OF INTRAVENOUS INJECTION OF PANCREATIC
PERFUSATES ON THE D/N RATIO FOLLOWING
PANCREATECTOMY *

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The relation of the pancreas to diabetes mellitus was proved in 1889 when Von Mering and Minkowski discovered that fatal diabetes always followed complete pancreatectomy. Two principal hypotheses have been advanced to explain how the pancreas is linked with carbohydrate metabolism, viz., the detoxication and the internal secretion theories. In the former the pancreas is supposed to remove a toxic substance from the blood stream, the presence of which interferes with the oxidation of carbohydrates by the tissues; in the latter the pancreas is said to contribute something to the blood stream which acts as a necessary link in the process of carbohydrate assimilation. Attempts to establish either of these theories have met with little success although the preponderance of evidence to date seems to favor the theory of an internal secretion. Notwithstanding a vast amount of experimentation the exact role of the pancreas in carbohydrate metabolism remains unknown.

The parabiosis experiments by Forschbach,¹ the transplantation and cross circulation experiments by Hedon,² pancreas feeding experiments and many others have yielded results either negative or else contributory alike to the detoxication and the internal secretion theories.

The blood transfusion experiments by Carlson and Ginsberg;³ and by Drennan; the study of experimental diabetes in pregnant dogs by Carlson⁴ and the experiments by Clark⁵ in which the surviving mammalian heart and pancreas were perfused in circuit, all support the theory of an internal secretion. These conclusions are also supported by the in vitro studies carried out by Cohnheim⁶ and Levene.⁷

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1. Forschbach: Arch. f. exper. Path. u. Pharm. **9**:131, 1908.
2. Hedon: Arch. intern. di. Physiol. **13**:4, p. 255, 1913.
3. Carlson and Ginsberg: Am. J. Physiol. **36**:280, 1915.
4. Carlson: Am. J. Physiol. **28**:391, 1911.
5. Clark, A. H.: J. Exper. Med. **24**:621, 1916.
6. Cohnheim: Ztschr. f. Physiol. Chem. **39**:., 1903.
7. Levene, P. A., and Meyer, G. M.: J. Biol. Chem., 1911.

Of these methods of attack, perfusion experiments seem most promising, for if it can be shown that the pancreas contributes something to a physiologically inert solution passing through its vessels by which utilization of sugar by the tissues is augmented, the theory of an internal secretion would be made tenable. Besides the perfusion experiments of Clark already mentioned, only two references to the direct perfusion of the pancreas could be found. Hustin perfused the pancreas in a study of its external secretion and de Meyer found that by adding a solution previously perfused through a pancreas to one subsequently perfused through a liver there was an increase in liver glycogen.

In the experiments reported herewith an attempt has been made to determine the effect of pancreatic perfusates upon dextrose utilization in depancreatized dogs.

Fig. 1.—1. Woodyatt pump. 2. Mercury manometer for measuring the pressure of the perfusate. 3. Air cushion. 4. Perfusion chamber. 5. Supporting rod to which the pancreas is fastened. 6. Cannula for the pancreaticoduodenal artery. 7. Water bath maintained at 42 C. 8. Water bottle for saturating air with moisture. 9. Bath for maintaining second water bottle at 75 C. In this way the air entering the chamber was heated to from 30 to 35 C. 10. Cotton filter for air entering apparatus. 11. Artificial lung composed of glass beads. 12. Small manometer for registering negative pressure in perfusion chamber. 13. Receiving bottle containing 150 c.c. of perfusion medium. 14. Vessel containing warm water used to warm the perfusate to body temperature previous to injection. 15. Attachment for vacuum pump. 16. Woodyatt pump by which perfusate was injected. 17. Cannula for the saphenous vein. The black tubing carries air.

Method of Procedure.—The D/N ratio was taken as a criterion for the carbohydrate consuming power of the animal. Dogs were depancreatized and the D/N ratios were determined daily. When these figures became rather constant for each animal, it was the plan to inject intravenously a quantity of pure dextrose dissolved in Tyrode's solution. Subsequent determinations of the D/N ratio

should show the amount of dextrose retained after such injections. It was then proposed to perfuse the pancreas of a normal animal with Tyrode's solution containing dextrose, and to inject this perfusate intravenously, at the same rate, into an experimentally diabetic animal, with the expectation that the subsequent D/N ratios would show some change in dextrose utilization.

Females under morphin-ether anesthesia were used for removal of the pancreas. The technic of pancreatectomy was developed so that the complete extirpation of the gland could be assured. Asepsis was given particular attention. The postoperative recovery of the animals was satisfactory and they survived from five days to five weeks.

Fig. 2.—Showing the effect on the sugar and nitrogen excretions of repeated injections of pancreatic perfusates. Black line indicates dextrose. Dotted line indicates nitrogen. Figures represent time of injection of perfusate.

Immediately following pancreatectomy the animals were placed in clean metabolism cages and the urine was collected every twenty-four hours. The dogs were kept on a constant daily diet of 480 gm. raw lean meat. Water was given freely. The urine was collected at the same time every day, alcoholic thymol being used as a preservative. The volume was measured and the sugar and nitrogen determinations were made. The total nitrogen was determined by the Kjeldahl-

Gunning procedure. The Munson-Walker-Bertrand method was used for the determination of the urine sugars.

The perfusion medium used in all experiments was Tyrode's solution modified so that it contained no dextrose. The formula was as follows: Sodium chlorid, 0.7 per cent.; potassium chlorid, 0.02 per cent.; magnesium chlorid, 0.02 per cent.; calcium chlorid, 0.02 per cent.; sodium bicarbonate, 0.01 per cent., and monobasic sodium phosphate, 0.005 per cent. Fresh solutions were always used.

The pancreas was perfused as follows: The peritoneal cavity of a dog under morphin-ether anesthesia was opened aseptically by a right rectus incision so as to expose the pancreas. The superior pancreatico-duodenal artery and vein were isolated and ligated close to the origin of the former. The vessels were then cannulated so as to send the perfusion medium through the pancreas in the direction of the blood flow. To render the subsequent steps of the experiment practically bloodless, the thorax was rapidly opened, the aorta clamped

Fig. 3.—Showing effect of the intravenous injection of 1 c.c. of the freshly prepared perfusate on the blood pressure of an etherized animal.

above the diaphragm and the chest cavity quickly closed. The apparatus for maintaining the circulation through the gland was designed so that it could be sterilized in an autoclave. It consisted of a motor driven syringe (Woodyatt transfusion pump) connected through a manometer to the cannula in the pancreatico-duodenal artery. The solution as it came from the pancreatico-duodenal vein poured over glass beads through which a current of moist air was being drawn. The perfusate then flowed into a closed bottle from which it was drawn by the syringe and again sent through the gland. During the procedure the dog was kept under light anesthesia. The perfusate was maintained at 37 C. and a pressure of 120 mm. of mercury. The gland at first became mottled and later white in color as the perfusate continued to pass through its vessels. After fifteen or

twenty minutes moisture was noticed, due to the escape of the perfusate from the surface of the pancreas, and in this way a few cubic centimeters of the solution were lost. By the addition of small quantities of Tyrode's solution at intervals to compensate for this loss on the surface of the gland, a continuous flow of Tyrode's solution through the vessels could be maintained indefinitely.

One hundred and fifty cubic centimeters of Tyrode's solution containing 10 gm. dextrose was allowed to pass through the gland for one hour. At the end of this period a small sample was withdrawn for analysis and the remaining portion injected by means of a Wood-yatt apparatus at the rate of 10 c.c. per minute, directly into the saphenous vein of a diabetic animal. Local anesthesia was used to cannulate the vein. The concentration of dextrose in the perfusate was determined in the sample withdrawn for that purpose, and the

Fig. 4.—Showing effect of the intravenous injection of 1 c.c. of the perfusate allowed to stand for forty hours at room temperature on the blood pressure of an etherized animal.

quantity of sugar injected into the diabetic animal calculated. It was the plan that dogs so treated should show a fairly constant D/N ratio for a period of at least three days preceding the injection. Also the quantity of sugar excreted in the urine following injections of known quantities of pure dextrose was to be determined before injection with the perfusate containing dextrose. It was thought that the pancreatic factor found present by Clark in his perfusates might enable the diabetic animal to utilize all or a portion of the injected dextrose.

Such experiments were attempted on a series of about fifteen depancreatized dogs. Necropsy on these animals revealed complete

removal of the pancreas in almost every instance. Death in a large percentage of cases was caused by pneumonia. Table 1 shows that Dog 8 was injected with 3.27 gm. pure dextrose seventeen days following pancreatectomy. For some unknown reason the daily sugar excretion, preceding the day the animal was injected, rose from 10.54 gm. to 20.35 gm. The following day only 15.15 gm. sugar were excreted in spite of the injection of 3.27 gm. dextrose. In no instance did we feel justified in drawing conclusions as to dextrose utilization from such fluctuating figures.

Having found this procedure useless in the solution of the problem, it was decided to attempt to influence the onset and course of experimental diabetes by the intravenous injection of pancreatic perfusates alone. Such an experiment, if successful, might also throw some light on the hypothetical internal secretion of the pancreas.

TABLE 1.—EXPERIMENTAL RESULTS FROM OBSERVATION OF DOG 8, RECEIVING 480 GM. RAW MEAT PER DAY. PLENTY OF WATER

Date*	Time	Amount of Urine	Sugar, per Cent.	Sugar Total	Total Nitrogen	D/N
April 3	10:00 a.m.	350	4.70	16.15		
April 4	10:00 a.m.	470	2.30	11.28	6.063	1.86
April 5	10:00 a.m.	490	2.40	11.76	11.270	1.05
April 6	10:00 a.m.	527	2.00	10.54	12.648	0.83
April 7	10:00 a.m.	260	Contaminated urine			
April 8 †	10:00 a.m.	370	5.50	20.35	9.250	2.20
April 9	10:00 a.m.	505	3.00	15.15	9.595	1.56
April 10	10:00 a.m.	265	4.80	12.72	7.950	1.60
April 11	10:00 a.m.	200	4.80	9.60	6.000	1.60
April 12	10:00 a.m.	185	4.00	7.79	7.326	1.06

* Complete pancreatectomy March 22.

† Intravenous injection at 3:00 p.m. of 3.27 gm. pure dextrose dissolved in 100 c.c. of Tyrode's solution. Fifteen minutes was required for the injection. The saphenous vein was cannulated under local anesthesia.

Clarke's work as previously quoted, indicates the existence of such secretion in pancreatic perfusates.

The first line of experiments were conducted as follows: Proceeding on the theory that the animal would most likely respond to treatment while the cells were in a normal physiologic state rather than after they became altered by an abnormal carbohydrate metabolism, intravenous injections of pancreatic perfusates were made immediately following pancreatectomy. In this way the onset of experimental diabetes might be delayed.

The following technic was used in preparing these perfusates. The abdomen was opened by an upper right rectus incision. The splenic end of the pancreas was first liberated as far as the pylorus. The duodenal end was then cut from the mesentery up to the point of its attachment to the bowel wall. The superior pancreatico-duodenal artery was isolated and ligated close to its origin. The thorax was opened and the aorta clamped immediately above the diaphragm so

as to make the subsequent steps practically bloodless. The gland with the attached piece of duodenum, 8 cm. in length, was quickly extirpated and washed in a warm, glucose-free, Tyrode's solution. The pancreatico-duodenal artery was then cannulated and the gland suspended by passing the supporting rod of the perfusion chamber through the lumen of the attached bowel. The splenic end of the pancreas was then tied by a thread to a loop in the supporting rod, and the gland placed in the perfusion chamber. The cannula was connected and the perfusion started. This procedure was carried out in from five to seven minutes. A pressure not to exceed 120 mm. of mercury was maintained and about 15 c.c. fluid was passed through the gland per minute. The perfusate was maintained at 37 C.

Fig. 5.—Curve showing dextrose and nitrogen excretion for a period of sixty-two hours following the injection of 175 c.c. pancreatic perfusate. The perfusate was prepared by allowing the solution to flow through the gland once. It was injected immediately into the animal following pancreatectomy. Solid lines indicates dextrose. Broken line indicates nitrogen.

For these experiments a special effort was made to devise a perfusion apparatus which would approximate, as nearly as possible, physiologic conditions. A diagram of this is shown and explained in Figure 1. Following each experiment the apparatus was carefully washed and filled with 75 per cent. alcohol. Immediately preceding each experiment the alcohol was removed and repeated washings of Tyrode's solution were pumped through the system. One hundred

and fifty cubic centimeters of fresh, glucose-free Tyrode's solution were then placed in the receiving bottle.

Twenty-seven dogs were used in this series of experiments. None of the animals were fed after operation. The perfusate was allowed to pass through the vessels of the pancreas for one hour. Small, white areas appeared on the surface of the gland almost immediately after the perfusion fluid had entered its vessels. These regions gradually enlarged as the perfusion proceeded until the body and head of the pancreas became white. Aside from this whiteness, due to the washing out of the blood, the tissue remained entirely normal in gross

Fig. 6.—Curve showing dextrose and nitrogen excretion for a period of seventy-six hours following injection of 100 c.c. pancreatic perfusate, prepared as described below. Solid line indicates dextrose. Dotted line indicates nitrogen. The perfusate was prepared by passing once through a pancreas 100 c.c. of defibrinated blood drawn from a diabetic dog. It was injected intravenously into a second animal immediately following pancreatectomy.

appearance. Peristaltic waves were often observed traveling along the attached bowel, and these persisted throughout the time of perfusion. While the gland was being perfused, a pancreatectomy was completed. The perfusate was then injected, intravenously, into the depancreatized dog by the Woodyatt apparatus at the rate of 10 c.c. per minute. This operation was done aseptically while the dog was still under ether

anesthesia. Following this the animal was at once placed in a metabolism cage and the urine collected as soon as possible after being voided. The time of the appearance of glycosuria was noted and quantitative sugar and nitrogen determinations were made from this point until the death of the animal.

Fig. 7.—Curve showing the dextrose and nitrogen excretion for a period of ninety-two hours following injection with 150 c.c. pancreatic perfusate. The perfusate was allowed to circulate through the gland for one hour and injected immediately following the completion of pancreatectomy. Solid line indicates dextrose. Dotted line indicates nitrogen.

It was found that the course of diabetes following complete removal of the pancreas was fairly constant. Sugar invariably appeared in the urine from six to eighteen hours after complete pancreatectomy and reached its maximum (from 5 to 8 per cent.) in from twenty-four to forty-eight hours. Aside from the glycosuria, the usual diabetic syndrome was noted, viz.: excessive thirst, polyphagia, polyuria, failure of wounds to heal, rapid loss of weight, progressive weakness, and death from complications or extreme inanition.⁸

8. Drennan, F. M.: *Am. J. Physiol.* **28**:396, 1911.

Dogs injected immediately following pancreatectomy with 150 c.c. of perfusate prepared as described, showed no delay in the onset of glycosuria. There were, however, two exceptions in which sugar did not appear in the urine of animals so injected until forty and fifty-eight hours, respectively, following complete removal of the pancreas. Glycosuria, on appearing, followed its usual course until a few days later when the dogs died of pneumonia. Necropsy on these animals revealed the duodenum to be smooth and free from bits of pancreas visible to the naked eye. Both dogs were in early pregnancy, fetuses measuring from 1 to 1.5 cm. being found.

Fig. 8.—Showing glucose and nitrogen output per twenty-four hours on a constant protein diet. Solid line indicates dextrose. Dotted line indicates nitrogen. Arrow indicates intravenous injection of 3.27 gm. pure dextrose.

Glycosuria developed within from six to eighteen hours following pancreatectomy in the remaining ten dogs of this series. As is noticed in Table 2, subsequent injections after glycosuria had developed produced no consistent change in the D/N ratio. Figure 2 shows that the urine sugar did not reach its maximum until 158 hours following pancreatectomy. During this period the dog was injected three times with pancreatic perfusates. The result is typical of those obtained in this series. The delayed sugar excretion is probably apparent rather than real, since it is always followed by a compensatory rise lasting through a period of several hours. The total quantity of glucose excreted for the given period remains unchanged.

This interpretation seems justified by examination of the tracing (Fig. 3) which shows the action of these perfusates on the blood pressure of an etherized animal. Their marked vasodilator effect indicates that they are in this respect similar to tissue extracts. It is known that a fall in blood pressure resulting from the injection of tissue extracts into experimentally diabetic animals is usually accompanied by a decrease in glycosuria followed by a compensatory rise.

TABLE 2.—EXPERIMENTAL RESULTS FROM OBSERVATION OF DOG 20*

Date	Time	Amount of Urine	Sugar, per Cent.	Sugar, Gm.	Total Sugar	Nitrogen, Gm.	Total Nitrogen	D/N
July 6-7 †	9:00 a.m.	175	2.61	4.568	4.568	5.0225	5.0225	0.91
July 7-8 ‡	8:45 a.m.	170	4.29	7.293		4.875		
	12:55 p.m.	30	2.83	0.849	8.142	0.843	5.718	1.42
July 8-9	8:50 a.m.	75	3.31	2.483	2.483	2.160	2.160	1.15
July 9-10	10:45 p.m.	118	4.64	5.475		7.434		
	6:00 p.m.	50	2.74	1.370	6.845	3.150	10.584	0.65
July 10-11	No urine							
July 11-12	8:35 a.m.	65	4.42	2.873		2.152		
	11:00 a.m.	105	4.33	4.547		3.476		
	7:45 p.m.	85	4.42	3.757		2.814		
	9:00 p.m.	50	6.72	3.360	14.537	1.655	10.097	1.44
July 12-13	7:30 a.m.	80	4.80	3.84	3.84	2.176	2.176	1.77
July 13-14	8:00 a.m.	65	1.69	1.099	1.099	1.768	1.768	0.62
July 14-15	12:00 n.	215	2.96	6.364	6.364	5.354	3.354	1.89
July 15-16	5:00 p.m.	180	1.60	2.880	2.880	4.446	4.446	0.64
July 16-17 §	8:00 a.m.	85	0.70	0.595	0.595	1.394	1.394	0.43
July 17-18	10:00 a.m.	70	1.91	1.337	1.337	1.890	1.890	0.71

* Pancreatectomy complete 12:45 p.m. July 5, 1919. Injected immediately following pancreatectomy with 150 c.c. pancreatic perfusate prepared as described.

† Second injection 12:30 p.m. July 6.

‡ Third injection 12:45 p.m. July 7.

§ Fourth injection 9:00 p.m. July 16.

TABLE 3.—EXPERIMENTAL RESULTS FROM OBSERVATION OF DOG 26*

Date	Time	Amount of Urine	Sugar, per Cent.	Sugar, Gm.	Total Sugar	Total Nitrogen	D/N
July 17	10:00 p.m.	450	1.30	5.896	5.896	4.158	1.42
July 18	7:10 a.m.	395	4.33	17.104			
July 18	8:35 p.m.	185	6.40	11.846	28.944	10.759	2.68
July 19	8:45 a.m.	340	3.67	12.478			
July 19	11:00 a.m.	45	4.60	2.070			
July 19	4:00 p.m.	50	4.51	2.255	18.224	6.777	2.69
July 19	1:30 p.m.	35	4.06	1.421			

* Operated on 4 p.m. July 17; injected with 175 c.c. pancreatic perfusate directly as it came from the gland.

The marked depressor action of the perfusates may be a factor in explaining their apparent influence on glycosuria (E. G. Kirk).⁹ Figure 4 shows an increase in this property if the perfusate is allowed to stand thirty hours, probably a result of autolysis caused by pancreatic digestive enzymes present in the solution. However, throughout the experiments no rise in temperature or visible signs of toxemia or depression were noted.

9. Kirk, E. G.: Arch. Int. Med. **15**:39 (Jan.) 1915.

It was suggested that the apparent negative results were due to a decomposition of the internal secretion contained in the perfusate, and that decomposition rather than concentration resulted from an hour's perfusion of the gland. The findings of Clark as to the stability of the secretion made this theory probable. Consequently, the solution

TABLE 4.—EXPERIMENTAL RESULTS FROM OBSERVATION OF DOG 30 *

Date	Time	Amount of Urine	Sugar, per Cent.	Sugar, Gm.	Total Sugar	Total Nitrogen	D/N
July 23	9:30 a.m.	90	4.87	4.183	4.183	3.384	1.26
July 24	7:30 a.m.	120	6.17	8.124			
July 24	9:15 p.m.	115	4.64	5.336	13.460	9.823	1.37
July 25	9:30 a.m.	80	6.68	5.344			
July 25	8:15 p.m.	40	2.61	1.044	6.388	4.392	1.45

* Pancreatectomy on Dog 30 was completed at 3:45 p.m., July 22, 1919, and the animal immediately injected with a perfusate prepared by passing defibrinated blood of a diabetic dog through the normal gland once.

TABLE 5.—EXPERIMENTAL RESULTS FROM OBSERVATION OF DOG 22 *

Date	Time	Amount of Urine	Sugar, per Cent.	Sugar, Gm.	Total Sugar	Nitrogen, Gm.	Total Nitrogen	D/N
July 11-12	7:00 a.m.	80	2.70	2.100		2.296		
	8:35 a.m.	98	4.47	4.380		3.900		
	7:45 p.m.	255	3.27	8.338	14.818	10.276	16.472	0.89
July 12-13	7:30 a.m.	210	3.27	6.867		4.767		
	1:00 p.m.	35	3.89	1.362		0.795		
	3:30 p.m.	55	2.09	1.150		1.249		
	6:55 p.m.	50	1.52	0.760	10.139	1.135	7.946	1.28
July 13-14	8:30 a.m.	20	6.04	1.206		0.67		
	12:30 p.m.	20	7.88	1.576	2.784	0.67	1.340	2.07

* Pancreatectomy on Dog 22 was complete at 3:30 p.m., July 10, 1919. The animal was injected immediately following pancreatectomy with 150 c.c. pancreatic perfusate as described.

TABLE 6.—DOG 2 WAS FED 480 GM. RAW, LEAN MEAT PER DAY, AND RECEIVED PLENTY OF WATER *

Date	Time	Amount of Urine	Sugar, per Cent.	Total Sugar	Total Nitrogen	D/N
April 26	10:00 a.m.	645	2.60	16.77	12.90	1.30
April 27	10:00 a.m.	427	Spilled by accident			
April 28	10:00 a.m.	350	3.00	9.11	5.950	1.54
April 29	10:00 a.m.	227	2.30	5.22	4.994	1.05
April 30	10:00 a.m.	270	3.10	8.37	5.670	1.48
May 1	10:00 a.m.	70	2.92	2.04	1.680	1.22
May 2	10:00 a.m.	240	3.20	7.68	5.280	1.45

* Complete pancreatectomy April 16.

was passed once through the gland and injected immediately into the depancreatized animal. The same technic and precautions were observed as previously described.

Figure 5 shows the typical curve from such an experiment. Glycosuria appeared six hours following pancreatectomy and rose to its maximum within thirty hours. As will be seen in Table 3, the

D/N ratios rose from 1.42 to 2.69 during the sixty hour period immediately following pancreatectomy.

A third series of experiments was then attempted in which defibrinated blood from a depancreatized animal was used instead of Tyrode's solution. It was hoped that this would be a better medium for collecting the internal secretion from the gland. Defibrinated blood from depancreatized animals had been previously shown by Drennan to have no effect on the D/N ratio in experimental diabetes.

Under light anesthesia, blood was drawn aseptically from the jugular vein of an animal depancreatized three days previously. The animal was diabetic, having a glycosuria of 3.36 per cent and a hyperglycemia of 1.22 mg. dextrose per c.c. of blood. The Munson-Walker method for blood sugar determination was used. One hundred cubic centimeters of this blood, after being defibrinated, was passed through a normal pancreas and then injected into an animal immediately following pancreatectomy.

Figure 6 shows the dextrose nitrogen curves for this experiment. There was apparently no effect on the onset of glycosuria. Table 4 shows a uniform rise of the D/N from 1.26 to 1.45 during a period of seventy-five hours following injection.

CONCLUSIONS

1. As compared with the figures reported in the literature, especially by Lusk, the D/N ratios are uniformly low.

2. The percentage of sugar in the urine and the total sugar excretion per day show wide variations both on a constant diet and during starvation.

3. Although the total nitrogen excretion in some animals was relatively constant, others under identical conditions showed marked variations.

4. All factors within our control, such as diet, water, etc., did not maintain a D/N ratio sufficiently constant to be of use in carrying out the first series of experiments.

5. Perfusates prepared by passing glucose-free Tyrode's solution for one hour through the blood vessels of a normal pancreas did not delay the onset of diabetes.

6. Two exceptions to the above conclusion are recorded. It seems unlikely, in view of the work of A. J. Carlson and co-workers on the Control of Diabetes in Pregnancy, that the fetuses described in these animals could have altered the course of diabetes. Since we were unable subsequently to confirm these results, they are merely of suggestive value.

7. Maximum elevation of glycosuria is delayed by injection of pancreatic perfusates.

8. The influence on glycosuria is probably apparent and not real, since it is followed by a compensatory rise. This effect may be explained by the action of depressor substances shown to be present in the perfusates.

9. Solutions passed once through the gland when injected intravenously showed no effect either on the onset or course of experimental diabetes.

10. Defibrinated blood drawn from pancreatectomized animals and passed once through the normal pancreas had no effect on experimental diabetes.

11. The internal secretion of the pancreas, if it can be obtained by the perfusion experiments described, may be too labile or in too low concentration to influence the sugar metabolism of the diabetic animal.

We wish to record our thanks to Dr. F. C. Koch for his many valuable suggestions and his untiring interest in our work.