15689

Changes in Concentration of Enzymes in Pancreatic Juice After Giving Insulin.*

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Babkin[1] has recently reviewed the literature dealing with the effects of hypoglycemia and hyperglycemia on the external secretory function of the pancreas, hence only a brief summary of the conclusions to be drawn from previous experimental work will be given here. In cross-circulated dogs hypoglycemia of the head diminishes and hyperglycemia augments the output of fluid and enzymes from the pancreas (La Barre and Destrée[2-4]). In rabbits hypoglycemia decreases the output of enzymes from the pancreas through a central action and hyperglycemia increases the output through a peripheral action (Baxter,[5] Hebb[6]). Also in rabbits the normal "trophic" effect (increase in enzyme output) of vagus stimulation is reversed by hypoglycemia (Hebb[6]). Experiments on human subjects have given quite different results from those obtained on anesthetized animals. Insulin hypoglycemia stimulates the flow of pancreatic juice in man (Okada[7]) but not if gastric juice is excluded from the intestines (Frisk and Welin[8]). The same is true of fistula dogs (Scott *et al.*[9]) When given with secretin, insulin increases the output of pancreatic enzymes in man (Lagerlof and Welin[10]).

Apparently no information is available regarding the effect of insulin-induced hypoglycemia on the output of pancreatic enzymes in unanesthetized experimental animals. This report is intended as a contribution to the study of that problem.

Methods. The experiments were performed on 3 dogs having tubulated gastric and duodenal fistulas with the duodenal fistula opposite the main pancreatic duct. Pancreatic juice was collected through a temporary glass cannula inserted into the duct via the duodenal fistula.[9,11] The stomach was drained with the aid of suction through a double drainage tube passed through the gastric fistula all the way to the pylorus. The drainage tube was made up of a rather large soft rubber outer tube within which was a much smaller inner tube. Both tubes had numerous perforations near the pyloric end. The inner tube was connected through a trap to a water-operated vacuum pump.

Because insulin alone does not stimulate pancreatic secretion in the dog,[9] secretin was given by continuous intravenous injection throughout each experiment by means of a motor-driven pump. The rate of secretin injection was adjusted to give an estimated 3 to 4 cc of pancreatic juice in 10 minutes but these limits were frequently exceeded in practice.

* We are indebted to Dr. M. H. F. Friedman for making the determinations of tryptic activity and to Dr. Friedman and to Wyeth, Inc., for adequate supplies of secretin. We are also indebted to Prof. Abraham Cantarow in whose laboratory the blood sugar determinations were made.

1 Babkin, B. P., *Secretory Mechanism of the Digestive Glands*, Hoeber, New York, 1942.

2 †La Barre, J., and Destrée, P., *C. R. Soc. Biol. Paris*, 1928, **98**, 1237; *Ibid.*, 1928, **98**, 1240; *Ibid.*, 1928, **99**, 1056; *Ibid.*, 1928, **99**, 1874; *Ibid.*, 1929, **101**, 147; *Ibid.*, 1935, **110**, 1177.

3 † La Barre, J., *C. R. Soc. Biol. Paris*, 1928, **99**, 1053; *Am. J. Physiol.*, 1930, **94**, 117.

4 † Destrée, P., *C. R. Soc. Biol. Paris*, 1930, **104**, 1038.

† Cited by Babkin.[1]

5 † Baxter, S. G., *Quart. J. Exp. Physiol.*, 1932, **21**, 355.

6 †Hebb, C. O., *Quart. J. Exp. Physiol.*, 1937, **26**, 339.

7 †Okada, S., *Nagoya J. med Sci.*, 1933, **7**, 91.

8 † Frisk, A. R., and Welin, G., *Acta med. scand.*, 1937, **91**, 170.

9 Scott, V. Brown, Collingnon, U. J., Bugel, H. J., and Johnson, G. C., *Am. J. Physiol.*, 1941, **134**, 208.

10 †Lagerlof, H., and Welin, G., *Acta med. scand.*, 1937, **91**, 397.

11 Hart, Wm. M., and Thomas, J. E., *Gastroenterology*, 1945, **4**, 409.

The dogs were fed once daily and were used for experiments before feeding, when the stomach was generally empty. If any food residues remained they were removed through the fistula before starting the experiment.

Pancreatic juice was collected continuously and divided into separate samples, each collected during a 10-minute interval. After a control period during which at least 5 10-minute samples were collected with secretin alone, insulin was given intravenously in doses ranging from 7.5 to 20 units (total dose). The experiment was then continued for one hour or longer without further modification. Blood samples were collected from a leg vein at intervals throughout each experiment. Blood sugars were determined by the Benedict method.[12]

The volume and specific gravity of each 10-minute sample of pancreatic juice were measured. Specific gravity was determined by weighing the juice in a 1 ml specific gravity bottle at room temperature. The control samples and the samples collected after giving insulin were then poured into separate containers and the proteolytic activity determined on each group of pooled samples. The first sample collected and any additional samples obtained while adjusting the rate of secretin injection were excluded from the control pool.

Results. Sixteen reasonably satisfactory experiments were performed. Four experiments were discarded because of technical difficulties, the most common being the appearance of excessive amounts of acid in the duodenal contents. Only those experiments were discarded in which, in our judgment, the technical failures materially influenced the results.

Blood Sugars. The range of control blood sugar concentrations was approximately the same in the 3 dogs used. The highest control value was 95 mg per 100 ml and the lowest 51 mg. Only 3 were above 70 mg and 3 were below 60 mg. The doses of insulin were sufficient to lower the blood sugar to between 30 and 40 mg in most instances. In 2 experiments, both in the same animal (2-46) the blood sugar fell to below 30 mg. Restlessness, dilation of the pupils, increase in pulse rate and, occasionally, drowsiness were observed in association with hypoglycemia. Blood sugar values are recorded along with other data in Table I.

Changes in Specific Gravity of Pancreatic Juice. Specific gravity was used as an index of the nitrogen content of the pancreatic juice with which it has a linear relation.[13] The nitrogen, in turn, is believed to vary with the enzyme content.[14] After giving insulin the specific gravity invariably increased. The increase was generally evident in the first 10-minute sample collected after giving insulin and reached a maximum within 30 to 40 minutes. The subsequent decline was gradual and seldom reached the control level during the one to 2 hours that the observations were continued. Detailed data are given in Table I and a typical experiment is illustrated in Fig. 1. The significance of the changes in specific gravity will be more evident if the estimated values for average total nitrogen before and after giving insulin are compared. Increases of well over 100% of the control value for total nitrogen are common among the average values after insulin. The average values, however, fail to reveal the full effect of insulin since they include data on samples collected before the response was fully developed. In this situation the maximum values are more significant.

Changes in Tryptic Activity. Tryptic activity is expressed in Table I in terms of the velocity constant, k, as determined by the rate of decrease in turbidity of a colloidal suspension of coagulated egg white.[15,16] It is evident that in the 10 experiments in which enzyme determinations were made the tryptic activity increased with increasing specific gravity in all but 2. In one of these

[12] Benedict, S. G., *J. Biol. Chem.*, 1920, **83**, 165; 1928, **76**, 457.

[13] Crider, J. O., and Thomas, J. E., *Am. J. Physiol.*, 1944, **141**, 730.

[14] Babkin, B. P., and Tichomirov, N. P., *Z. f. physiol. Chem.*, 1909, **62**, 468.

[15] Friedman, M. H. F., in press.

[16] Riggs, B. C., and Stadie, W. C., *J. Biol. Chem.*, 1943, **150**, 463.

TABLE I.
Effect of Insulin on Pancreatic Secretion Induced by Secretin.

		Control period					After giving insulin (first hour)						
Dog No.	Date 1946	Avg vol. of 10 min. samples, cc	Specific gravity, avg	Avg total N (estimated) mg/cc	Trypsin, "k"	Blood sugar, mg/100 cc	Dose of insulin, units	Avg vol. of 6 10-min. samples, cc	Specific gravity Max.	Specific gravity Avg	Avg total N (estimated) mg/cc	Trypsin, "k"	Min. blood sugar, mg/100 cc
1-44	5/22	4.5	1.0108	1.96	0.143	77.5	20	3.2	1.0156	1.0133	3.44	0.170*	35
"	31	4.4	1.0107	1.90		63.7	15	4.3†	1.0167	1.0144	4.10		31
"	6/ 4	3.9	1.0119	2.61		69	15	5.3	1.0177	1.0158	4.93		33
"	17	3.9	1.0114	2.32	0.155	68.5	15‡	3.7	1.0154	1.0138	3.75	0.101§	50
"	7/30	2.8	1.0118	2.55		95.5	15	2.3	1.0184	1.0160	5.05		49
1-46	6/ 3	4.8	1.0118	2.55		51.5	20	5.1‖	1.0156	1.0141	3.93		31
"	7	3.5	1.0109	2.02	0.250	68	10	4.5	1.0145	1.0134	3.51	0.425	36.5
"	19	5.1	1.0109	2.02	0.110	66	10	3.7	1.0132	1.0121	2.73	0.105	40
"	24	3.4	1.0092	1.01	0.05	69	7.5	6.3	1.0113	1.0102	1.66	0.09	51
"	7/ 1	3.4¶	1.0098	1.36		58	15	4.0	1.0135	1.0119	2.61		34
2-46	6/ 5	2.8	1.0104	1.72	0.141	86	20	3.8	1.0192	1.0156	4.81	0.450	22.5
"	10	4.9	1.0112	2.20	0.260	66	10	3.6	1.0152	1.0126	3.03	0.310	34.5
"	18	4.2	1.0100	1.48	0.120	52	10	2.6	1.0159	1.0125	2.97	0.19**	28
"	21	3.0	1.0096	1.25	0.075	60.5	15††	4.5	1.0116	1.0105	1.78	0.120	55
"	"						5‡‡	5.4	1.0147	1.0131	3.33	0.260	30
"	7/ 8	2.3	1.0111	2.14		60.5	15	2.0	1.0178	1.0159	4.99		36
"	25	4.4	1.0099§§	1.43	0.115§§	61.5	7.5	5.4	1.0115	1.0101	1.54	0.170	31

* 2 samples. Avg sp. g. 1.0114.
† Traces of acid in duodenum.
‡ Injected subcutaneously.
§ See text.
‖ 2 samples omitted because of change in rate of secretin injection.
¶ Acid in duodenum at start, none later.
** 5 samples. Avg sp. g. 1.0138.
†† Injected subcutaneously.
‡‡ Second dose of insulin; given intravenously.
§§ Includes 4 samples excluded in estimating volume because of difference in rate of secretin injection.

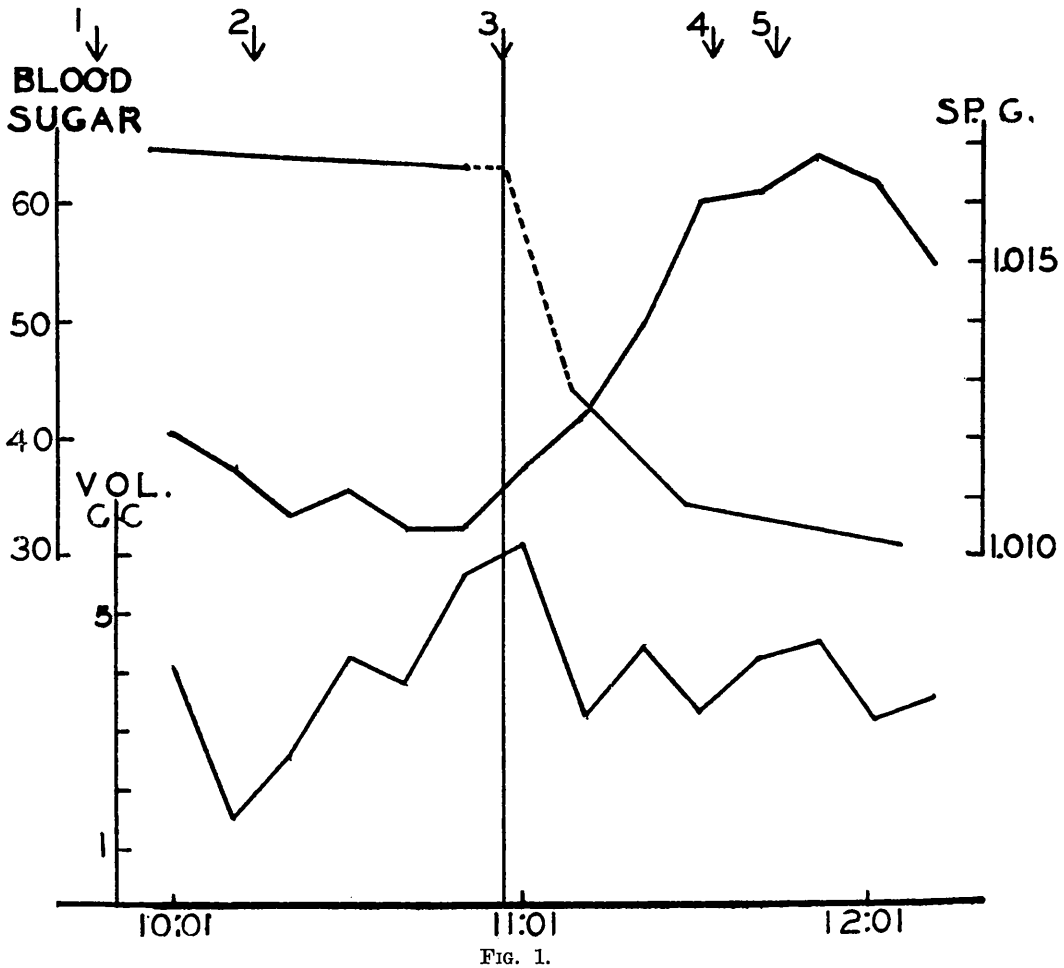

FIG. 1.

Curves showing, in order from above downward, blood sugar, specific gravity of pancreatic juice, and volume of juice in a typical experiment with insulin on Dog 1-44, May 31, 1946. Meaning of arrows:

1. Secretin injection started.
2. Rate of secretin injection increased from 0.6 mg to 1.0 mg in 10 minutes.
3. Fifteen units of insulin (Mulford) given by vein.
4-5. Traces of acid in duodenal contents.

(note "d" in Table I), the experiment was on a dog (1-44) in which discrepancies between tryptic activity, and total nitrogen as determined by the Kjeldahl method, were common. Such discrepancies were rare or did not occur in a considerable number of other dogs that we have studied and we believe this dog to be unique in this respect. In the other instance (Dog 1-46, 6/19/46) the difference is small and probably within the range of experimental error.

Effects on the Volume of Pancreatic Juice. In 8 of the experiments summarized in Table I the average volume of the 10-minute samples of pancreatic juice secreted during the first hour after insulin was greater than during the control period. In 5 it was less and in 3 there was practically no change (less than 0.5 cc). The differences in either direction seldom exceed the normal range of variation and are clearly not significant. However, in a few experiments there was a definite decrease in volume after insulin which, when it occurred, became more evident toward the end of the experiment.

Discussion. According to the available

evidence the changes in visceral function following administration of insulin are caused by stimulation of the vagus centers through the influence of hypoglycemia. Our experiments provide no additional evidence for or against that view. The occurrence of restlessness and tachycardia in dogs, convulsions in other animals, and of sweating and convulsions in the human indicates that the stimulation is general and is not confined to the vagus or the parasympathetic centers. The increased output of epinephrin which tends to counteract the fall in blood sugar may be taken as a specific indication of stimulation of thoracolumbar sympathetic mechanisms.

In such a situation the effect on the pancreas, which receives both excitatory and inhibitory innervation, could hardly be predicted. It would doubtless represent a summation of antagonistic influences and would probably be readily modified by experimental conditions such as anesthesia and operative trauma. It is not surprising, therefore, that our results in unanesthetized dogs are quite opposite to those previously reported on anesthetized, operated animals. Since they agree with the results obtained in human experiments they probably represent the normal reaction of the pancreatic secretory mechanism to the complex situation resulting from acute hypoglycemia.

The inhibitory effects of hypoglycemia were manifest in some of our experiments only as a late decrease in volume of pancreatic juice secreted in response to a constant stimulus (secretin). Babkin[1] has suggested that these effects may be due to inability of the pancreas to function normally without an abundant supply of carbohydrate for its metabolic needs. In our experiments the volume, when low, could be promptly restored to normal by slightly increasing the rate of secretin injection. The specific gravity of the juice remained high in these circumstances. Clearly the pancreas was still capable of normal function when adequately stimulated. The decreased response to secretin in these experiments could be attributed to vascular changes or to preponderance of the inhibitory innervation.

Summary and Conclusions. Specific gravity and tryptic activity of dog's pancreatic juice secreted in response to continuous injection of secretin are increased following administration of insulin. The average volume secreted during the first hour after giving insulin is not significantly affected. Inhibitory effects of hypoglycemia described as occurring in anesthetized, operated animals are not readily demonstrated in unanesthetized dogs.

15690

Some Effects of Desoxycorticosterone Acetate on Mice Irradiated with X-rays.*

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Total body irradiation with X-rays produces fatty changes in the livers of irradiated animals.[1] We have recently demonstrated that the administration of desoxycorticosterone acetate protects the livers of mice irradiated with various lethal doses of X-rays against these fatty liver changes.[2] It was noted in addition that there was a decrease in the mortality rates produced by the previously used X-ray doses. It is the purpose of this paper to analyze these phenomena

* Aided by grants from the John and Mary R. Markle Foundation, New York, and the Schering Corporation, Bloomfield, N.J.

1 Ellinger, F., *Radiology*, 1945, **44**, 241.

2 Ellinger, F., *Science*, 1946, **104**, 502.