STUDIES ON THE PHYSIOLOGY OF SECRETIN

III. FURTHER STUDIES ON THE EFFECTS OF SECRETIN ON THE BLOOD SUGAR

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There is not a unanimity of opinions concerning the effects of secretin on the blood sugar. Some authors have described hyperglycemia after the injection of secretin in rabbits, dogs, and man; others have described hyperglycemia followed by hypoglycemia; while another group have observed only hypoglycemia. Mellanby (1928) described a new method of purification of secretin yielding a product which he says possesses great secretagogue activity and no effects on either blood pressure or blood sugar of rabbits or cats. However, Zunz and La Barre (1929; this paper contains a complete bibliography) using a product prepared in Mellanby’s laboratory found on injecting large enough doses in dogs to obtain rapid secretion of the pancreas, that there also was a lowering of the blood pressure and sugar. Still and Shipner (1929) reported that highly purified secretin did not possess hypoglycemic properties in normal or diabetic dogs. They used both barbitalized and unanesthetized animals.

One of us (E. U. S., 1929) described a method of preparing a crude secretin which was a powerful secretagogue but possessed no vaso-dilatin. It consisted of precipitating an acid extract of the fresh duodenum with solid NaCl, subsequent solution of the precipitate formed in acid and reprecipitation by trichloracetic acid. This second precipitate was dried by mixing with absolute alcohol and acetone and the addition of ether.

Series I. In this series of experiments we have studied the effects of this crude secretin on the blood sugar. We used chloralosed dogs (80 mgm. in saline per kilo intravenously) having the necessary cannulae in place for the collection of pancreatic juice, samples of blood or injections. A sample of blood was taken, then 1 to 2 mgm. of crude secretin per kilo was injected by vein. Subsequent samples of blood were collected at 15 or 30 minute intervals for 2 hours. Blood sugar determinations were made on Folin and Wu filtrates by the method of Hagedorn and Jensen. Figure 1 shows the results of four experiments of this kind. In these experiments there seemed to be a relationship between the dosage of crude secretin and the change in blood sugar. In experiment I of this group the effects were minimal. The
dog had respiratory difficulties until artificial respiration was resorted to. It is believed the poor results are due to that complication. It is demonstrated by these experiments that crude secretin may be prepared which is a powerful secretagogue, produces hypoglycemia but is not in the least hypotensive. The mechanism of the hypoglycemia was then studied.

**Series II.** If crude secretin is extracted several times with acidified 90 per cent alcohol the secretin (fraction A) will pass into the alcohol leaving behind a residue (fraction B). Fraction A, secretin, on treating with brucine, pyridine and methyl alcohol extraction as described in the first paper of this series yields a white and very active secretagogue which is not in the least hypotensive nor produces hypoglycemia, even in dosages of 3 to 5 mgm. per kilo. Figure 2 shows 4 such experiments using dosages which gave, as the legend shows, enormous secretions of pancreatic juice, with no hypoglycemia. On examining fraction B (residue) it was found to possess very little secretagogue activity and not to be hypotensive but to greatly depress the blood sugar. Figure 3 shows the results of two experiments. The blood sugar curve after crude secretin or the residue are quite similar.

**Series III.** Since Mellanby suggested the hypoglycemic properties of some secretin preparations may be due to included insulin, the point was investigated. Taking advantage of the fact that insulin is inactivated by pepsin-HCl, that method was used to see if insulin was present in crude

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Fig. 1. The effect of intravenous injection of crude secretin into normal dogs under chloralose.

1, 18 kilo male dog, 1 mgm. crude secretin per kilo, gave 4.2 cc. pancreatic juice in 20 minutes; 2, 9 kilo female dog, 2 mgm. crude secretin per kilo, did not measure secretion; 3, 11 kilo male dog, 1 mgm. crude secretin per kilo, 2.7 cc. pancreatic juice in 20 minutes; 4, 8 kilo female dog, 2 mgm. crude secretin per kilo, 2.5 cc. pancreatic juice in 20 minutes.

Fig. 2. The effects of intravenous injection of highly purified secretin on the blood sugar. 1, 10 kilo male dog, 0.04 mgm. pure secretin per kilo, 4 cc. juice in 20 minutes; 2, 10 kilo male dog, 0.09 mgm. pure secretin per kilo, 5.1 cc. juice in 20 minutes; 3, 9 kilo male dog, 0.23 mgm. pure secretin per kilo, 7 cc. juice in 20 minutes; 4, 9 kilo male dog, 0.22 mgm. pure secretin per kilo, 8.2 cc. juice in 20 minutes.

Fig. 3. The effect of intravenous injection of secretin residue on the blood sugar. 1, 11 kilo female dog, 1½ mgm. residue per kilo, 1 cc. juice in 90 minutes; 2, 11 kilo male dog, 3 mgm. residue per kilo, 0.8 cc. juice in 90 minutes.

Fig. 4. Effects of intravenous injection of inactivated crude secretin on blood sugar. 1, 21 kilo male dog, 0.9 mgm. crude secretin per kilo inactivated by pepsin per kilo, only 0.4 cc. juice; 2, 9 kilo male dog, 1 mgm. crude secretin per kilo inactivated by pepsin per kilo, only 0.6 cc. juice.

Fig. 5. Pepsin and insulin controls for experiments in figure 4. 1 and 2, effect of intravenous injection of 10 units of insulin inactivated by pepsin or the blood sugar. 3 and 4, effects of intravenous injection of 5 mgm. of inactivated pepsin on the blood sugar.

Fig. 6. The effects of intravenous injection of crude secretin in totally depancreatized dogs.
Figs. 1 to 6
secretin. Large doses of crude secretin were incubated for 10 minutes with pepsin-HCl, neutralized, heated to inactivate the pepsin, and injected. The secretagogue activity was destroyed but the hypoglycemia characteristic of the crude secretin was observed as shown in figure 4. Control experiments were made using heat inactivated pepsin alone or insulin inactivated by pepsin. Figure 5 shows these experiments. These experiments show that the hypoglycemia which follows the intravenous injection of insufficiently purified secretin is not due to included insulin but to some other substance which is not heat labile and is not inactivated by pepsin-HCl as is insulin.

Series IV. It would be of interest to know if crude secretin produces hypoglycemia by increasing insulin secretion or by some other mechanism. This point was studied by two groups of experiments.

1. Dogs rendered totally diabetic some days before were injected intravenously with 2 mgm. of crude secretin per kilo and the blood sugar studied. Figure 6 shows the results obtained from two such experiments. This is in agreement with the reported findings of Takacs (1927-1928).

2. The question still remained as to whether or not all of the effects were due to the preparation per se, for diabetic animals are known to be especially sensitive to substances affecting the carbohydrate metabolism and because Zunz and La Barre (1929) showed by transfusion experiments that secretin increased the output of insulin which largely was responsible for the hypoglycemia. Using their technique the question was investigated. The pancreatic vein of a large dog A was anastomosed with the jugular vein of small dog B. A clamp was placed between the dogs, samples of blood taken and 2 to 4 mgm. of crude secretin injected intravenously into dog A. Three minutes later the clamp was removed permitting transfusion to take place from the pancreas of dog A into dog B. The transfusion continued for 15 minutes, then the anastomosis was severed. Samples of blood were taken each 30 minutes for 3 or 4 hours. The blood sugar of dog A (donor, being physiologically depancreatized) was lowered by 10 to 20 mgm. per cent. In the normal dog the decrease would have been 30 to 60 mgm. per cent. The blood sugar of dog B (receiving the pancreatic blood from dog A) decreased by 20 to 30 mgm. per cent. In some experiments dogs were used with adrenals intact, and in others with the adrenals removed.

These results agree, qualitatively, with those reported by Zunz and La Barre. The greater hypoglycemia observed by them indicates that their secretin was more impure with respect to freedom from physiologically active substances than the crude secretin used in these experiments.

It seems then that the mechanism of the hypoglycemia is due to at least two known factors: 1. The crude secretin per se diminishes the blood sugar as shown by the effects on the diabetic dogs. 2. The crude secretin augments the secretion of insulin, as shown by the transfusion experiments.
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CONCLUSIONS

1. Crude secretin may be prepared which is not hypotensive, possesses great potency as a pancreatic secretagogue and contains varying amounts of hypoglycemic substances.

2. Crude secretin may be fractioned into one part which is only secretagogue and another which will lower the blood sugar.

3. The hypoglycemic fraction from crude secretin is not hypotensive, is not insulin, and produces hypoglycemia by at least two known factors—1, stimulation of secretin of insulin (most important); and 2, effects of the preparation per se.

4. Purified secretin has no effect on the blood sugar.

BIBLIOGRAPHY

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