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FURTHER OBSERVATIONS ON THE EFFECTS OF SOME COMPONENT OF CRUDE LECITHINE ON DEPANCREATIZED ANIMALS.

By C. H. BEST and J. M. HERSHEY.

(*Department of Physiology, University of Toronto.*)

In two previous communications from this laboratory [Hershey, 1930; Hershey and Soskin, 1931] the remarkable effects of crude egg yolk lecithine upon the symptoms and signs exhibited by depancreatized dogs under certain conditions have been discussed. Since the depancreatized animals have been kept from 3 to 11 months before the typical condition which we believe is largely attributable to failure of liver function developed, the progress of the research has necessarily been very slow. During the past three years, however, we have had the opportunity of confirming and extending the experimental results previously reported.

Methods.

The general procedures used in studying the diabetic animals have been the same as those discussed in previous communications. These animals need a great deal of care and attention, and the results of the chemical determinations on samples of blood or urine would be of little value if they were not considered with reference to the history and general condition of each animal at the time the determinations were made. To save space, a detailed report of these matters will not be included here. After the complete removal of the pancreas each animal was placed upon a diet of 300 g. of lean beef muscle and 100 g. of sucrose daily. This was the diet received by all the animals mentioned in Table I during the experimental periods which will be under discussion. The entire ration was in all cases completely consumed. The dose of insulin, which was administered subcutaneously twice a day, was adjusted in each animal after pancreatectomy to permit a slight or moderate glucosuria. The insulin dosage was usually constant throughout each experiment, and the ex-

ceptions to this noted below are considered separately. Blood sugar, urinary sugar and urinary nitrogen were determined by the Shaffer-Hartmann, Bertrand and Kjeldahl methods respectively.

Experimental results.

The actual figures which it is necessary to report are presented in Table I. The results which these figures help to illustrate may be discussed under several headings.

Table I.

Dog No.	Dates	Materials added to lean meat and sugar diet	Daily average of sugar excretion (g.)	Daily average of nitrogen excretion (g.)
10	(Depancreatized October, 1929)			
	1–4. xi. 29		4·5	—
	9. xi.–2. xii. 29	Lecithine	15·2	—
	3–6. xii. 29		12·4	5·6
	7–19. xii. 29		5·5	5·3
	8–22. ii. 30	Lecithine	2·4	6·2
	Dog killed Feb. 25, Liver fat 2·5 p.c., iodine No. 125.			
11	(Depancreatized May, 1930)			
	20. i–12. ii. 31	Vitamins A, B, C, D	1·2 (High 1·9)	8·1
	17. ii–8. iii. 31	Lecithine	5·0 (High 13·4)	6·4
15	(Depancreatized March, 1929)			
	14–20. xii. 29		3·1	5·3
	31. xii. 29–4. i. 30		2·2	4·5
	7–20. i. 30	Lecithine	17·0	5·6
	11–21. ii. 30		4·3	5·6
	23–30. xi. 30	Suet	1·1	—
	30. i–3. ii. 31	Suet and lecithine	4·6	—
16	(Depancreatized February, 1930)			
	14. xi–1. xii. 30		14·5	—
	30. xii. 30–8. i. 31	Vitamins and suet	4·3	—
	16–24. i. 31	Lecithine and suet	10·5	—
	27–31. i. 31	Suet	1·3	—
	7–21. ii. 31	Lecithine and suet	7·0	—
	1–25. iii. 31	Suet	1·0	—
17	(Depancreatized December, 1930)			
	31. i.–12. ii. 31		4·6	5·9
	13–23. ii. 31	Lecithine	9·2	—
	24. ii.–21. iii. 31	Lecithine and suet	15·4	6·4

The effects of lecithine on the general condition of the animals. The most interesting aspect of the effect of lecithine is probably the relief of a condition which in many instances borders upon a moribund state. It requires considerable experience to recognize this condition in its early stages, since the animals may only exhibit slight weakness and, in some

cases, jaundice. Liver function tests have not as yet been conducted. Later the weakness may be very marked, but it is not expedient to permit the development of this stage of the condition if it is desired to demonstrate the recovery of the animals when lecithine is provided. The falling off of the sugar excretion is the most valuable sign. Within a few days after lecithine is given the animals usually appear definitely stronger and brighter. These effects were observed in all the animals referred to in Table I. Several of the depancreatized animals which we have studied in this investigation have lived for very long periods after complete pancreatectomy. The longest record is that of dog No. 15, which lived for more than $2\frac{1}{2}$ years after the removal of its pancreas. On numerous occasions its condition was critical, but the addition of lecithine to the diet restored it apparently to normal health. The terminal illness was not associated with any signs of failure of liver function.

The effect on sugar excretion. It usually happens that the sugar excretion of the depancreatized animal after the first week or 10 days following the operation reaches a fairly constant level, which may be maintained with minor fluctuations for several months. During this time the animal is usually in very good health. When the characteristic condition which is associated with fatty infiltration and degeneration of the liver appears, the animals become weaker, bile pigments may appear in the urine, and the sugar excretion may be greatly diminished. It is sometimes necessary to decrease the dose of insulin to avoid fatal hypoglycæmia from a dose which previously permitted glucosuria. We have not included any figures in Table I for periods in which the insulin dosage had to be decreased. In all the animals referred to in this table there is convincing evidence that some component of the crude egg yolk lecithine added to their diet produces a very definite increase in the sugar excretion. This increase in sugar excretion is not accompanied by any very definite change in urinary nitrogen. Although we do not intend to discuss at length the bearing of these results on the vexed question of sugar production from fat, it is very difficult to account for the production of the great excess of sugar excreted in some cases during the period of lecithine feeding by any other mechanism. For example, in the experiment on dog No. 15, the average daily sugar excretion before the administration of lecithine was not over 3 g., while during the lecithine period of 13 days the average excretion was 17 g. It is very difficult to believe that the carbohydrate reserves have provided this excess sugar. From experiments on other depancreatized animals we have reason to believe that the liver and muscle glycogen are quite as high or higher during the

lecithine period as during the time when the sugar excretion is lower and the animals are suffering from other symptoms which are probably also attributable to disturbed liver function. The figures for nitrogen excretion indicate that little or none of this excess sugar is derived from protein. The question arises as to whether or not 10 g. of lecithine, which was the amount administered each day to these animals, could be the source from which the extra sugar is formed. If lecithine could be converted into dextrose in the animal body it is obvious that a large part of the excess sugar might be provided by the amount of phospholipine available. While further studies, in which a different plan of attack will be necessary, are required to answer these questions, the findings mentioned in the next section may furnish a clue. Unless the state of the carbohydrate reserves of the individual animal is accurately known it is obvious that experiments of the type reported here cannot provide unassailable evidence for sugar production from fat.

Failure of lecithine to increase sugar excretion. On only two occasions during the last three years have we encountered conditions in which lecithine failed to increase the sugar excretion of animals which were in a condition that previous experience led us to expect would be suitable for this demonstration. One case is referred to in the experiment on dog No. 10. The first time lecithine was administered to this animal a very definite increase in sugar excretion resulted. Later, however (February 8–22), 10 g. of lecithine daily failed to raise the sugar excretion. The animal was in good condition, and was sacrificed on February 25. The liver contained 2·5 p.c. fatty acid, which had an iodine number of 125. These values, and the general appearance of the liver, are typical of the lecithine livers. It is possible that the sugar excretion was not increased because there had been insufficient opportunity, previous to the second administration of lecithine, for the production of those changes which are responsible for the accumulation of fat in the liver.

The effects of vitamins. Fairly satisfactory evidence has been obtained in this series of experiments that the effect of lecithine is not due to the presence of vitamins. Some experiments, indicating that vitamins A and C are not present in the preparations of lecithine we have used, have been reported previously. In the experiments on dog No. 11 adequate amounts of vitamins A, B, C and D in concentrated form were provided during a period when the animal continued to excrete very little sugar. A definite rise in sugar excretion occurred subsequently, however, when lecithine was provided. The vitamins were also administered to dog No. 16 without any trace of the lecithine effect being observed.

Acceleration of the onset of the characteristic condition by fat feeding. In the experimental results secured from the observations made on dogs 15, 16 and 17, convincing evidence is provided that the onset of the condition characterized by lowered sugar excretion can be accelerated by the feeding of fat. Beef suet, approximately 10 g. daily, was usually effective within a few weeks in producing the desired condition. Great care must be exercised, however, in feeding this material, as some dogs are unable to tolerate more than very small amounts at first. Some refuse to eat sufficient fat to produce the required effect. The results of one experiment also suggest that when beef suet and lecithine are administered together, the sugar excretion is increased to a greater extent than when lecithine alone is given (dog No. 17). Further work is, of course, necessary to establish the significance of this result. The amounts of lecithine and suet provided are of course kept constant throughout any one experiment. These experimental results do furnish evidence, however, that a condition which appears to be very similar to that observed after prolonged periods in depancreatized animals kept on a sugar and lean meat diet, can be produced much more quickly by the addition of fairly saturated fats to the diet.

The effect of fat feeding in diabetic animals. The very definite decrease in sugar excretion produced by adding large amounts of saturated fats to the diet is well illustrated in the experiments on dogs 15 and 16 (Table I). In certain experiments where fat was fed the fasting blood sugar level became so low and the sugar excretion diminished to such an extent that the dose of insulin had to be decreased. None of these results is included in the table. The diet of certain of these animals was changed slightly and we were able to show that fat feeding may affect a completely depancreatized animal in such a way that it is able to tolerate a diet of fairly high caloric value without exhibiting glucosuria or hyperglycæmia when no insulin is administered for fairly long periods. One result of this kind is summarized in the following protocol.

Dog No. 11, weight 4·0 kg. This depancreatized animal had been excreting varying amounts of sugar on a daily diet of 300 g. of lean meat and 80 g. of sucrose (insulin 10 units). The excretion was approximately 1 g. per day. When fat was added to the diet the sugar output was definitely less and dangerous levels of hypoglycæmia were sometimes observed. On June 4, the diet was changed to the following: 300 g. lean meat, 20 g. beef dripping (iodine number about 40). No insulin was given. For the next 3 weeks the sugar excreted was less than $\frac{1}{2}$ g. a day. On many days only a trace of sugar was present. From June 25 to July 17, lecithine was added to the lean meat and fat diet, but in this instance (the second that we have observed) no increased sugar excretion was produced. That is, for 6 weeks the animal received no insulin. Fasting blood sugars determined on several occasions during

the 6 weeks showed values within the range found in normal dogs. During all but the last few days of this 6 weeks' period, when the animal suddenly became weak, it was apparently in excellent condition. No trace of pancreas was found at autopsy.

We suggest that the explanation of these results is that the fat feeding produces changes in the liver which inhibit gluconeogenesis in this organ to such an extent that the blood sugar is only slightly raised above the normal level, and very little sugar appears in the urine. These animals are apt to die very suddenly, however, and the livers are then found to be very friable and to contain large amounts of saturated fatty acids. A possible application of these results to treatment of diabetics in clinics where large amounts of saturated fats are permitted is obvious. It is possible to improve the "chemistry" of the diabetic organism without improving the clinical condition. It is remarkable, however, that depancreatized dogs without insulin may appear perfectly bright and normal for a month or longer, or without hyperglycæmia and glucosuria before they succumb to the effects of the liver damage. Since a series of depancreatized animals are at present under observation in an attempt to gain more information on these points, further discussion of this aspect of the subject can be reserved for a subsequent communication.

Histological studies. Specimens of the livers from animals which developed the characteristic condition without fat feeding, or livers from animals which have been fed large amounts of saturated fat, may show fatty degeneration as well as infiltration. The livers of the animals which had received lecithine are free from detectable fatty degeneration. A detailed account of these changes and those encountered in the normal animals referred to in the following papers will be given when the histological work is completed.

Summary.

The alleviation of a critical condition, characterized by signs of failure of liver function, by the addition of lecithine to the diet of depancreatized animals has been repeatedly demonstrated. These results confirm previous observations from this laboratory. Very satisfactory evidence has been obtained that the sugar excretion of those animals which developed the characteristic condition is greatly increased by the administration of lecithine. The source of this extra sugar has been briefly discussed. The action of the lecithine is not due to the presence of vitamins A, B, C or D. A condition similar to that which occurs spontaneously in animals kept on a lean meat and sugar diet can be produced

in a relatively short time by the addition of fairly saturated fats to the diet. The results of the studies on normal animals bear so directly on the questions raised by this one that a detailed discussion of the action of lecithine in diabetic animals will be reserved until certain experiments suggested by the recent findings have been completed.

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