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# CHOLINE AND LIVER FAT IN DIABETIC DOGS.

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In the first completely depancreatized dog which was observed for a prolonged period (70 days) in this department [Banting and Best, 1922], all the symptoms of diabetes were apparently alleviated by insulin. Later, however, several animals were kept in good condition for much longer periods of time. In these animals [Allan, Bowie, Macleod and Robinson, 1924] signs of failure of hepatic function, which autopsy findings suggested were attributable to fatty degeneration of this organ, appeared at varying intervals after pancreatectomy, but the condition did not develop when minced pancreas was added to the lean beef and sucrose diet. In a series of recent publications [Hershey and Soskin, 1931; Best and Hershey, 1932; Best, Hershey and Huntsman, 1932; Best and Huntsman, 1932] results have been presented which support, among others, the following conclusions: (1) that crude lecithine will prevent or alleviate the extensive fatty changes found in the livers of these diabetic dogs, (2) that purified lecithine will prevent the deposition of fat in the livers of normal rats when these animals are ingesting large amounts of fat, (3) that the amount of choline which may be derived from an adequate dose of lecithine will also prevent the fatty changes in the livers of the rats. These results raise numerous questions, one of the most interesting of which is whether choline is, like lecithine, effective in preventing or alleviating the fatty degeneration which may develop in the livers of diabetic dogs. That this question can be answered in the affirmative is the result of an investigation which has been carried out on the ten members of our diabetic colony during the last twelve months.

## Methods.

Very little space need be given to the description of methods. Some of these have been described in previous publications. The diabetic dogs were very carefully treated after pancreatectomy and were always in

excellent condition before the experiment was started. Each animal was then placed on a diet of approximately 300 g. lean beef, 100 g. sucrose daily with small amounts of "Bemax," orange juice and cod-liver oil. The insulin was administered 15 min. before each of the two meals and the dose was adjusted to permit a moderate glucosuria. Urinary volume and sugar excretion were determined daily and various other estimations on urine and on blood were made at frequent intervals. Fatty acids of blood were determined by the Stoddard and Drury procedure [1929]. Total fatty acids of the liver were estimated by the Leathes and Raper modification of the Liebermann's saponification method and the iodine numbers were determined by the Rosenmund-Kuhnhenn technique [1923]. As will be discussed later it has been found necessary to remove samples of liver repeatedly from the same animal. Ether anæsthesia was used, and in the earlier experiments a wedge-shaped bit of liver was quickly removed from the most easily available lobe. Bleeding was stopped by oversewing. Later, however, with the help in some experiments of Dr J. Markowitz, a small lobe of the liver was removed after ligation of the pedicle. This latter procedure is much more satisfactory, and even the animals with fatty livers recover very rapidly after the operation. Saline or glucose solution was given intravenously after the operation and the animals were kept quiet for 24 hours by the appropriate use of morphine.

Choline hydrochloride was obtained from the British Drug Houses. The dosage is expressed in terms of the base.

## Experimental results and discussion.

The first animal whose history will be outlined is dog "H," which was depancreatized January 12, 1932. The daily sugar excretion and the amount of insulin and choline received are given in Fig. 1. The sugar excretion of these diabetic dogs is usually much higher during the first week or two after pancreatectomy than it later becomes with the same dose of insulin. This is due, in part, to decrease in the carbohydrate reserves. In this animal the glucosuria gradually diminished until the third week after the operation when signs of insulin overdosage were exhibited, and the dose had to be diminished to avoid fatal hypoglycæmia. During the last few days of February a trace of bile appeared in the urine and the animal was unable to retain food. On the assumption that these signs were associated with fatty changes in the liver choline was administered (March 1) and the condition of the dog gradually improved.

There was considerable delay, however, in the appearance of increased sugar excretion. This may have been partly due to inadequate amounts of choline. Later, however, the sugar excretion reached a high level and the animal was apparently in excellent condition. This experiment was then

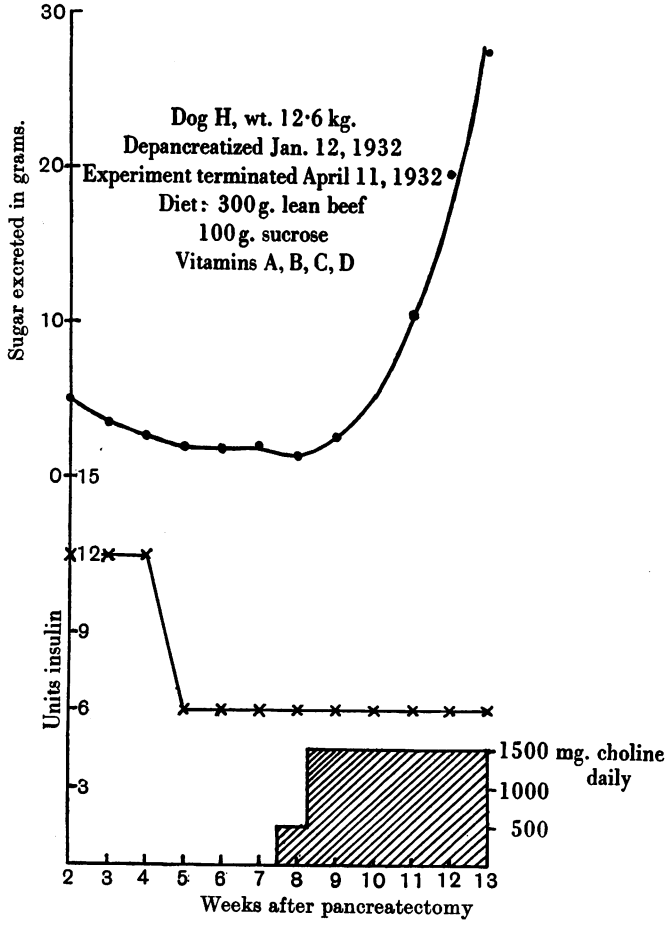


Fig. 1.

terminated and the total fatty acids of the liver were found to be 3·74 p.c. Based on our previous results we believed that this animal had a fatty liver before choline was given and that the accumulation of liver fat interfered with sugar production and possibly with other hepatic functions. If this assumption was correct, the results of this experiment furnish conclusive evidence for the effect of choline on liver fat. In later

experiments analysis of liver samples eliminated the necessity of relying on indirect evidence.

The second case to be considered is that of dog "S," Table I. This animal demonstrated a rather rapid decrease in sugar excretion, and 3 months and 5 days after pancreatectomy the level became so low that 1·5 g. of choline were added to the diet. A week later this amount was increased to 2·25 g., but there was no increase in sugar excretion. The

TABLE I. Dog "S," wt. 16 kg. Depancreatized June 8, 1932.

| Weeks after pancreatectomy | Insulin units | Sugar excretion daily (g.) | Fat daily (g.) | Choline daily (g.) | Condition |
|---|---|---|---|---|---|
| 4 | 12 | 56·7 | — | — | — |
| 5 | 12 | 41·2 | — | — | — |
| 6 | 12 | 28·9 | — | — | — |
| 7 | 12 | 29·4 | — | — | — |
| 8 | 12 | 19·0 | — | — | — |
| 9 | 12 | 7·8 | — | — | — |
| 10 | 12 | 3·8 | — | — | — |
| 11 | 12 | 5·2 | — | — | — |
| 12 | 12 | 0·7 | — | — | — |
| 13 | 12 | 0·4 | — | 1·5 | Frequent hypoglycæmic reactions in late evening |
| 14 | 8 | 0·5 | — | 2·25 | — |
| 15 | 5 | 1·0 | 10 | 2·25 | — |
| 16 | 5 | 0·8 | 20 | 2·25 | Animal thin but bright and very active |
| 17 | 5 | 0·7 | 20 | 2·25 | — |
| 18 | 5 | 1·7 | 20 | 2·25 | — |
| 19 | 5 | 1·0 | 20 | 2·25 | Bright and active |

animal was, however, perfectly well and very active. The insulin dose was decreased from 8 to 5 units daily. Fifteen weeks after pancreatectomy 10 g. and the next week 20 g. beef fat daily were added to the diet. The results of previous experiments indicated that very fatty livers and extensive interference with hepatic function were quickly produced by this amount of fat in animals receiving no lecithine or choline. This animal apparently tolerated the fat very well for 26 days. The experiment was then terminated. A complete autopsy revealed no obvious abnormalities. The value for the liver fatty acids (average of three determinations) was exceptionally low, 1·40 p.c. The iodine number was 115. In this experiment choline appeared to prevent the deposition of liver fat which would otherwise have been produced. But although the liver fat had apparently been reduced even below the normal values, there was no evidence of increased sugar production, a result for which previous experience with lecithine had partly prepared us. It is possible, and even probable when considered in the light of the results on other animals, that this would

have been observed if the experiment had been prolonged, but we can no longer associate, as intimately as previously seemed justified, the elimination of excess liver fat and increased production of sugar.

To illustrate the effects of larger amounts of fat, brief reference may be made to dog "L." This animal followed the usual course for the first month after pancreatectomy with the exception that the sugar excretion remained at a considerably higher level than in other dogs under similar conditions. After approximately 5 weeks fat was added to the diet (20 g. daily). The sugar excretion diminished somewhat and the amount of fat was doubled. On this diet, 300 g. of lean beef, 100 g. of sucrose, 40 g. of fat and 14 units of insulin, the dog became very ill and died. Choline, 1·7 g. a day, was administered for the last 2 days of its life. The fatty acids of the liver were 12 p.c., iodine number 66. If choline had not been given the fatty acid content might have been greater. None of the dogs has been able to tolerate a daily dose of 40 g. of fat without lecithine or choline, and we are convinced that even 20 g. daily cannot be administered for more than a very short time without deleterious results if a source of choline is not also provided. The histories of several other animals support this conclusion and higher fatty acid values have been observed in the livers in some cases—26 p.c. (iodine number 71) in dog "B," for example, whose history is similar to that of dog "L." There is great individual variation in the extent of the fatty changes in the livers of different animals of approximately the same size which have received the same amount of fat. There are no data available which permit us to decide whether or not large doses of choline will enable a diabetic animal to tolerate indefinitely more than 20 g. of added fat per day.

This brings us to a consideration of dog "Sa" whose history provides very convincing evidence for the efficacy of choline. This animal, which is still alive and in good condition, was depancreatized on May 26, 1932. For 4 months the dog was maintained on the usual diet and there was a gradual falling off in sugar excretion. On September 21, 20 g. of fat were added daily to the diet, but it was found necessary to reduce this to 10 g. to avoid vomiting. From October 3, 1·5 g. of choline were given and 2 weeks later (October 18) it was found possible to raise the fat to 20 g. a day without causing gastro-intestinal disturbances. The animal was in very good condition from that date until December 6, when a lobe of liver was removed for determination of fatty acids. The average value was found to be 16·5 p.c. with an iodine number of 66. As soon as the animal recovered from the operation the choline was stopped but the fat was continued. Although the animal made a rapid recovery from the operation, the high

fat diet without choline soon began to produce signs of liver failure and by January 4, the animal was in an extremely poor condition and was hardly able to stand. The condition of the animal at this time was in striking contrast to that displayed 1 month previously. The second specimen of liver was secured on January 4 and was found to contain 20·6 p.c. fatty acid, iodine number 57. Choline was then added to the diet in

TABLE II. Dog "Sa," Wt. 15 kg. Depancreatized, May 17, 1932.

| Weeks after pancreatectomy | Insulin units | Sugar excretion daily g. | Fat daily g. | Choline daily g. | Liver fat p.c. | Blood fat mg. per 100 p.c. | Condition |
|---|---|---|---|---|---|---|---|
| 16 | 16 | 3·9 | — | — | — | 284 | Excellent |
| 17 | 16 | 7·9 | 20 | — | — | 256 | Fat caused vomiting |
| 18 | 16 | 1·7 | 10 | — | — | — | — |
| 19 | 16 | 1·1 | 10 | 1·5 | — | — | — |
| 20 | 16 | 0·8 | 10 | 1·5 | — | — | — |
| 21 | 16 | 0·6 | 15 | 1·5 | — | — | — |
| 22 | 16 | 0·3 | 20 | 1·5 | — | — | — |
| 23 | 16 | 0·5 | 20 | 1·5 | — | — | — |
| 24 | 16 | 0·7 | 20 | 1·5 | — | — | — |
| 25 | 16 | 0·8 | 20 | 1·5 | — | — | — |
| 26 | 16 | 1·1 | 20 | 1·5 | — | — | — |
| 27 | 16 | 0·7 | 20 | 1·5 | — | — | — |
| 28 | 16 | 0·5 | 20 | 1·5 | — | — | — |
| 29 | 16 | 7·4 | — | 1·5 | 16·5 I.N. 65 | — | Rapid recovery from operation |
| 30 | 16 | 1·2 | 20 | — | — | — | — |
| 31 | 16 | 0·6 | 20 | — | — | 95 | — |
| 32 | 16 | 0·8 | 20 | — | — | 136 | — |
| 33 | 16 | 3·1 | — | — | 20·6 I.N. 57 | 158 | Vomiting, very weak, condition serious |
| 34 | 12 | 0·6 | 20 | 2·5 | — | — | — |
| 35 | 12 | 0·7 | 20 | 2·5 | — | — | Very weak after operation, saline intravenously |
| 36 | 12 | 0·4 | 20 | 2·5 | — | — | — |
| 37 | 16 | 0·6 | 20 | 2·5 | — | — | Gradual improvement |
| 38 | 16 | 0·6 | 20 | 2·5 | — | — | — |
| 39 | 16 | 0·8 | 20 | 2·5 | — | — | — |
| 40 | 16 | 0·5 | 20 | 2·5 | — | — | — |
| 41 | 16 | 0·6 | 20 | 2·5 | 6·7 I.N. 77 | 373 | Condition excellent |

slightly larger doses than during the first period of administration and, as our supply of choline was getting low, approximately 1 g. of betaine was also fed daily. The condition of the animal rapidly improved in spite of the 20 g. of fat consumed each day. There was, however, no increase in sugar excretion. On March 5 another lobe of liver was secured and was found to contain 6·7 p.c. fatty acid, iodine number 77. The animal made an uneventful recovery from the fourth operation and appears to be in excellent health. To recapitulate, the history of this dog shows that when the diet, including 20 g. of fat, and insulin dosage were

maintained constant, a small amount of choline kept the liver fat at approximately 16·5 p.c. When choline was withheld the animal became seriously ill and the liver fat rose to 20·6 p.c. When the dose of choline was subsequently increased the fatty acids of the liver fell to 6·7 p.c. and the animal was restored to excellent health.

TABLE III. Dog "J," Wt. 17·5 kg. Depancreatized April 15, 1932.

| Days after pancreatectomy | Insulin units | Choline g. | Sugar excretion daily (g.) | Added fat in diet g. | Wt. kg. | Condition |
|---|---|---|---|---|---|---|
| 30 | 8 | 0 | 1·2 | — | 15·3 | Good |
| 30–50 | 8 | 0 | 1·2 | — | 15·4 | ,, |
| 50–70 | 8 | 1·27 | 5·3 | — | 16·0 | ,, |
| 70–90 | 8 | 1·5 | 4·6 | — | 15·8 | ,, |
| 90–110 | 8 | 1·5 | 3·2 | — | 15·1 | ,, |
| 110–130 | 8 | 1·5 | 1·6 | — | 15·0 | ,, |
| 130–150 | 8 | 1·5 | 4·4 | 20 | 14·8 | ,, |
| 150–170 | 8 | 1·5 | 3·9 | 20 | 14·8 | ,, |
| 170–190 | 8 | 1·5 | 2·0 | 20 | 15·1 | ,, |
| 190–210 | 8 | 1·5 | 4·5 | 20 | 15·4 | ,, |
| 210–230 | 8 | 1·5 | 4·8 | 20 | 15·5 | ,, |
| 230–250 | 8 | 1·5 | 6·8 | 20 | 15·7 | ,, |
| 250–270 | 8 | 1·5 | 20·6 | 20 | 15·4 | ,, |
| 270–290 | 12 | 1·5 | 26·2 | 20 | 15·4 | Excellent |
| 290–310 | 14 | 1·5 | 25·9 | 20 | 16·0 | ,, |
| 310–330 | 14 | 1·5 | 20·8 | 20 | 15·8 | ,, |

Only one other animal's history will be reviewed. In this dog (Table III) choline administration was begun about 7 weeks after pancreatectomy, although no symptoms of hepatic insufficiency were observed. The animal remained in excellent condition. About 11 weeks later 20 g. of beef fat were added daily to the diet. The daily dose of 1·5 g. of choline has apparently been sufficient to protect the animal against this amount of fat, because she has remained in excellent condition on it for 200 days, at the time of writing. When choline was first provided the increase in sugar excretion was very slight. When the fat was also added to the diet there was perhaps a slight increase of sugar output. This would probably not have been observed if choline had not been available. Four months later, the sugar excretion had risen to such a high level that it was considered wise to increase the dose of insulin. The condition of the animal was excellent. The insulin was raised gradually from 8 to 14 units per diem and the glucosuria was still rather high. On the basis of experience with other animals we believe that this dog without choline would succumb rapidly to the effects of accumulation of fat in the liver. The animal has now been under observation for over a year and is still in good condition. It has already shown that a diabetic dog receiving choline can be maintained in excellent condition on a diet

containing much fat for a longer period than it has been possible to keep similar animals on a very low fat diet without choline. The choline content of the lean beef may be of interest in this connection but the amount has not as yet been accurately determined. The total fatty acid fraction of the daily ration of 300 g. is approximately 4·5 g., iodine number 75.

The beneficial effect of the administration of choline to these diabetic animals has been shown to result in a diminished risk of accumulation of fat in the liver, as shown by direct analysis. Up to the present time data obtained by indirect methods do not enable us to form more than a rough idea of the condition of the liver cells in these animals. The estimations of the fat in the blood that we have made are interesting in that they show that there may be little fat in the blood with a very fatty liver, but this subject need not be discussed here because an attempt to correlate the results of blood and liver-fat determinations with those of various liver function tests and with the histological appearance of the tissue in these diabetic dogs is being made.

The nature of the experiments, some of the results of which are reported here, makes it imperative that data on many points not in the direct line of the main investigation should be collected. Much that is new has been learnt about the general care and treatment of diabetic dogs. Pathological changes in kidneys, arteries and eyes have been observed in some animals, but these have not yet received the attention which they merit. A report on the results of a histological study of the livers of dogs "H," "S," "Sa," "B," "L," and others is in the press.

## Summary.

The condition in diabetic dogs which is associated with accumulation of liver fat and degeneration of liver cells can be prevented or cured by providing choline in the diet. This conclusion is supported not only by the results of analysis of liver tissue secured from animals which had been restored to health by choline, but samples of liver have been obtained from the same animal during the stage of fatty infiltration and degeneration and later after the amount of liver fat had been reduced by choline. A diabetic dog receiving choline and a considerable amount of fat in its diet has been maintained in excellent condition for a longer period than it was previously found possible to keep these animals, even when they got very little fat, without providing a source of choline. The fatty acid content of the liver may be lowered by choline without an accompanying increase in sugar

excretion, though such an increase may be observed in some animals. These results are briefly discussed, and one direction which further work will take is indicated.

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