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DIET AND THE INSULIN CONTENT OF PANCREAS[1]

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It is well known that fasting, or feeding diets rich in fat and poor in carbohydrate, leads to a change in the metabolism of sugar as judged by (1) the glucosuria following glucose administration, (2) the diabetic type of sugar tolerance curve when glucose is given, and (3) the absence of the normal rise in respiratory quotient after glucose administration. There is evidence that the administration of insulin at least partially restores the normal metabolism of carbohydrate. An excellent review of the relevant literature has recently been published by W. H. Chambers [1938].

We have reported in a preliminary communication [Haist, Ridout & Best, 1939] that a very definite change in the insulin content of pancreas may be produced by alterations in diet. In order to investigate this subject it is necessary to have available (1) an experimental animal which will ingest the diets provided and one from which all the pancreatic tissue can be removed without undue difficulty; (2) an extraction procedure which consistently gives optimal yields of insulin from pancreatic tissue; and (3) a method of testing which gives accurate results when relatively small amounts of insulin are available. The first requirements are satisfied when the Wistar rat is used as the test animal. A suitable method for the extraction of insulin is that outlined by Jephcott [1932] and by Scott & Fisher [1938*a*]. Satisfactory assays of the insulin content of the extracts are obtained by the mouse method of testing.

The results of these investigations which will now be described demonstrate, among other points, that a very definite decrease in the insulin content of pancreas is brought about by fasting or by the ingestion of diets rich in fat.

[1] The material in this and in subsequent papers on this subject will be incorporated in a thesis to be presented by one of us (R. E. H.) in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Toronto.

Methods

In most of the experiments male rats weighing from 200 to 300 g. were used. They were from 100 to 200 days old. In any one experiment the rats were from the same age group and the initial weights of each group of ten animals were equal. The rats were kept in individual cages. The diet for each animal was weighed and the uneaten residue was recovered and weighed daily. Unless otherwise stated, the food was removed 14 hr. before pancreatectomy. Each test solution was made from the pancreatic tissue of ten animals. The rats were usually anaesthetized by the intraperitoneal injection of a solution of "sodium amytal". All the pancreatic tissue was carefully dissected from the anaesthetized animals and added to the extraction fluid immediately after its removal. The extraction fluid was made by mixing 750 c.c. absolute alcohol, 250 c.c. distilled water and 15 c.c. conc. HCl. Approximately 5–6 c.c. of this solution per gram of pancreas were used. The actual procedure for the preparation of the insulin-containing extract was as follows. The container with the required amount of extraction fluid was weighed before and after the addition of the pancreatic tissue. After the second weighing, the pancreas was thoroughly minced with scissors. The mixture was shaken at frequent intervals, allowed to stand overnight in the refrigerator, and was then filtered through cheese cloth. The solid material was pressed until nearly dry and re-extracted with the same volume of the acid solution. The mixture was allowed to stand for 2 hr. and was again filtered through cheese cloth. The two filtrates were combined, made just alkaline to litmus by addition of ammonium hydroxide and the total volume of the extract was measured. After filtering through Whatman no. 1 filter paper, five 9 c.c. aliquots were placed in 50 c.c. centrifuge thimbles. Fifteen c.c. of absolute alcohol and 25 c.c. of ether were then added to each thimble. These were placed in the refrigerator overnight. The next day the mixtures were centrifuged, the supernatant fluid discarded and the tubes drained. The precipitate in each tube was dissolved in isotonic saline (*p*H 2·5) and the solutions were combined and made up to a definite volume.

The potency of these solutions was estimated by the mouse method of assay. The procedure which we have followed was essentially the same as that described by Trevan & Boock [1926] and by Trevan [1927]. This test depends upon the relative number of mice convulsing when the standard and unknown solutions are administered under comparable conditions. The ratio of the potencies of the two solutions is obtained by

reference to a standard curve. The characteristic dose-response curve for our mouse colony was obtained by injecting some 6000 mice with various dilutions of the standard solution. From 200 to 300 mice were used in assaying the potency of each extract. The animals were given dextrose as soon as convulsions appeared and those which recovered were used again after one week. For the most part, the insulin content of the pancreas is expressed in terms of units per group of ten rats and also as units per kg. initial body weight of rats. Since the amount of pancreatic fat is so variable, it was thought that any expression based on pancreatic weight would be less reliable. However, where portions of a homogeneous mixture of pancreatic tissue are compared, as in the test of the method, results are expressed as units of insulin per gram of pancreatic tissue.

Assay of pancreatic tissue

It will be appreciated that the method of preparation of insulin used in these experiments provides us with a relatively crude material. Nevertheless, it is perfectly adequate for determinations of activity and further purification would probably involve a loss of potency. Jephcott [1932] has shown that crude insulin added to minced pancreatic tissue can be recovered quantitatively by the extraction procedure we have used. This has been confirmed repeatedly by D. A. Scott in the Connaught Laboratories and by E. T. Waters in the Department of Physiology. It was decided that the best way to test our methods was to secure a homogeneous preparation of pancreatic tissue and conduct extractions and assays on weighed portions of this material. Two such preparations were used. Each was obtained by removing the pancreatic tissue from fifty rats. The first group consisted of normal animals, fasted overnight, while the second had been maintained for 7 days on a diet consisting only of beef fat, agar and vitamins A, B_1 and D. As each pancreas was removed it was dropped into liquid air and, when fifty had been collected, the tissue was ground to a very fine powder in a cooled mortar. The powder was thoroughly mixed and five aliquots were taken for extraction and assay. The results of these experiments, which are collected in Table I, demonstrate the consistency of the findings under these conditions. No further comment is required here except that it may be stated that *the variations on which we have placed significance are far greater than any which could be expected to result from the errors inherent in the method of extraction and testing.*

In practically all cases the solutions were tested on mice within a week after preparation. They were kept in the refrigerator during this

interval. Tests have shown, moreover, that under these conditions the change in potency of the solution is barely appreciable when 2 weeks are allowed to elapse between extraction and test.

TABLE I. Test of the method

Control diet			Fat diet, 7 days		
Aliquot no.	Weight of aliquot of pancreatic tissue g.	Units of insulin per g. of pancreas	Aliquot no.	Weight of aliquot of pancreatic tissue g.	Units of insulin per g. of pancreas
1	14·6	2·08	1	11·8	1·25
2	12·3	1·83	2	10·2	1·12
3	14·6	2·20	3	11·7	1·22
4	14·0	2·21	4	10·8	1·24
5	13·5	2·18	5	12·0	1·20
	Average	2·10			1·21

Effect of anaesthesia

In order to determine the effect of certain anaesthetics upon the insulin content of pancreas, three groups of animals were studied. One group was anaesthetized with urethane, one with "sodium amytal" and the third was stunned. In each group half the rats were fasted overnight, and the other half were fasted for 7 days. The length of time under anaesthesia was the same for the urethane and "sodium amytal" groups. In the stunned animals, the pancreas was removed immediately. The results in Table II show that no significant difference exists between

TABLE II. Effect of anaesthesia on the insulin content of pancreas

		No. of rats	Units of insulin per group of 10 rats	Units of insulin per kg. of initial body weight
Urethane	Control	10	31·4	8·4
	Fasted 7 days	10	16·6	4·4
Sodium amytal	Control	10	28·8	7·7
	Fasted 7 days	10	15·2	4·1
Stunned	Control	10	31·4	8·4
	Fasted 7 days	10	14·5	3·9

the insulin contents of the pancreatic tissue of the three groups of animals. Blood sugars were determined upon the groups receiving urethane and "sodium amytal" by the Shaffer-Somogyi method [1933]. While the sugar content of the blood was definitely higher in the group which received urethane, this apparently produced no effect upon the insulin content of the pancreatic tissue. In most of the subsequent experiments "sodium amytal" was used as the anaesthetic.

Effect of fasting

The effect of fasting on the insulin content of pancreas is shown in Table III. The total number of fasted rats was 130, and 110 control animals were used. All the animals had previously received a well-balanced diet. The average loss in weight of the fasted animals was 23%

TABLE III. Effect of fasting on the insulin content of pancreas

No. of rats	Units of insulin per group of 10 rats: Fasted* 7 days	Units of insulin per group of 10 rats: Control	Units of insulin per kg. of initial body weight: Fasted* 7 days	Units of insulin per kg. of initial body weight: Control
20	11·1	20·0	3·9	6·9
20	11·0	19·0	3·8	6·6
20	13·5	28·4	4·0	7·9
20	16·6	28·8	5·0	8·1
10	16·2	—	4·9	—
20	11·9	20·5	3·3	5·8
20	16·0	23·9	4·5	6·8
10	17·1	—	4·8	—
20	16·6	31·4	4·4	8·4
20	15·2	28·8	4·1	7·7
20	11·1	29·7	3·0	8·1
20	12·2	29·7	3·3	8·1
20	14·5	31·4	3·9	8·4
Average	14·1	26·5	4·1	7·5

* Average loss in weight 23%.

of the initial value. Since there was considerable variation in the initial weights of the different groups, it is best to compare the values for the starved and control animals in each individual experiment. It is evident from these figures that fasting produces a definite decrease in the insulin content of the pancreas.

Effect of feeding fat or sugar

The results of an experiment designed to study this point are collected in Table IV. In the first experiment one group of fifty rats ate the balanced diet and a similar group consumed the fat diet *ad libitum*. The definite fall in the insulin content of the pancreas of animals receiving a diet rich in fat is evident. In the second experiment a paired feeding test was conducted on two groups of thirty rats each. The value for the sugar-fed animals, 18·8 units of insulin per group of ten rats, is below the average value for normal animals, but the insulin content of fat-fed animals receiving the same caloric intake as those which received sugar is much lower than that of the sugar-fed group. A very important point emerges from a consideration of the latter results. The weight loss in the

TABLE IV. Effect on the insulin content of pancreas of diets containing only fat or sugar*

No. of rats	Duration of exp. days	Loss in weight %	Diet	Units of insulin per group of 10 rats	Units of insulin per kg. of initial body weight
50	7	3	Balanced†	28·9	8·8
50	7	13	Fat†	13·7	4·2
30	14	17	Sugar‡	18·8	6·5
30	14	20	Fat‡	10·9	3·8

* Fat or sugar actually constituted 90% of the material given. Agar, salt mixture, and vitamins A, B_1 and D made up the remainder.

† Rats ate *ad lib.*

‡ These two groups had the same caloric intake.

group which received sugar only was 17% of the initial value, while that of the group receiving the same caloric intake in the form of fat was 20% of the initial value. The initial weights of the two groups were the same at the start of the experiment. *These figures show that weight loss alone is not the factor which is responsible for the change in the insulin content of the pancreatic tissue.*

It may be remarked here that when animals are placed on diets composed only of sugar, protein, or fat, the insulin content of pancreas falls in all cases. However, the caloric intake in these groups is not normal. The animals which are provided with protein eat so little that undernutrition plays a large part in the results obtained. Even when this is involved, results of some preliminary experiments indicate quite definitely that the fall in insulin content is not as great in the group receiving protein as in the group where fat only is ingested. For example, in one series the control value was 31 units of insulin per group of ten animals, while the group which received sugar only gave a value of 22 units. In the protein-fed animals 18 units were present in the pancreatic tissue of ten rats, whereas those animals which received fat showed approximately 9 units per group.

In the first experiment described in Table IV the animals on the diet rich in fat ate as much as they desired. The weight loss in this group was 13% of the initial value after 7 days. It will be noticed that the decrease in the insulin content was as great as in the group which had been starved for the same period (Table III) although the loss of weight was not nearly so extensive.

In the second experiment described in Table IV the animals receiving sugar and those ingesting fat were fed in pairs so that each group had an equivalent caloric intake. The sugar-fed group ingested 11·6 g. per day

which provided 41·8 cal. The group receiving the fat diet ate 4·8 g. per day which was equivalent to 38·9 cal. The loss of weight in the two groups is comparable but, as noted above, the insulin content of the pancreas of the two groups is quite different. These results, in addition to showing the fall in insulin content which occurs when diets rich in fat are used, demonstrate that carbohydrate tends to prevent the decrease.

Effect of various diets in fasted animals

In this experiment all the animals were starved for 7 days. They were then divided into various groups and placed on different diets. Three groups received sugar only, three fat only, and three were given a

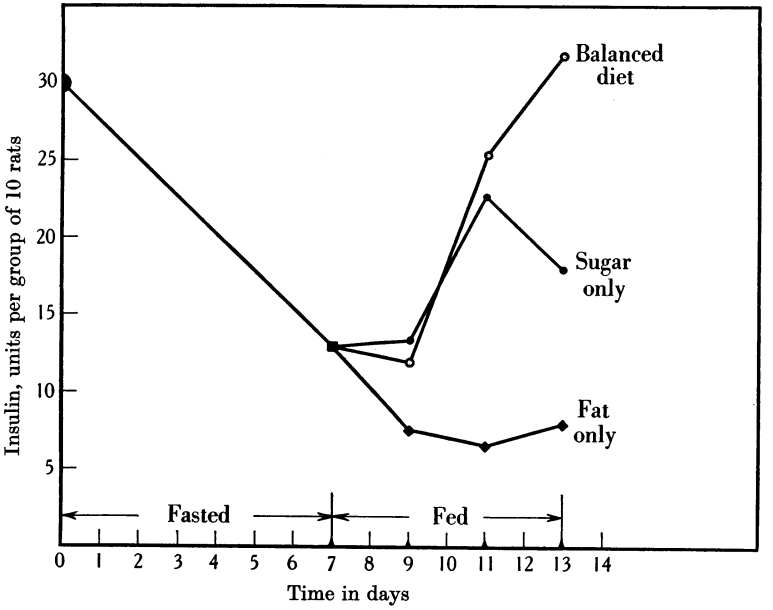

Fig. 1. Diet and the insulin content of pancreas.

balanced diet. All the rations provided approximately the same caloric intake and this was based on the food consumption of the carbohydrate-fed group. Groups were killed on the 2nd, 4th and 6th days. The insulin content of the pancreas was determined and the results are shown in Fig. 1. It will be quite evident that feeding fat alone does not lead to a restoration of the insulin content. On the contrary, there appears to be a significant further drop. Sugar alone leads to a return towards the normal value, while the adequate diet results in a complete restoration

of the normal value within 6 days. At the end of 2 days, however, there was no indication of recovery. The results of this experiment furnish further evidence that loss of body weight may not be an important factor in the decrease in the insulin content of pancreatic tissue. Several sets of figures which illustrate this point are given in Fig. 2.

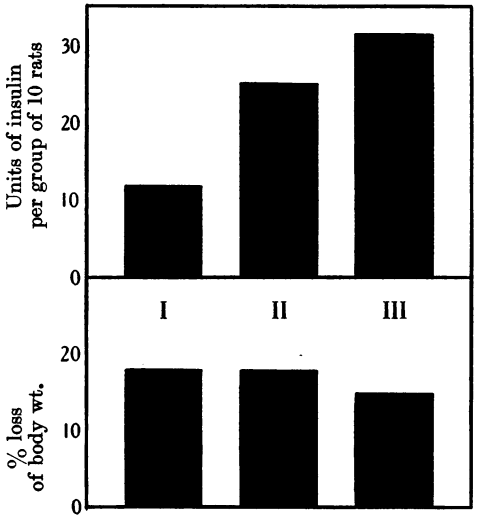

Fig. 2. Following a fast of 7 days, a balanced diet was fed for: I, 2 days; II, 4 days; III, 6 days.

Effect of diets moderately rich in carbohydrate and fat

In order to determine whether or not a diet *moderately* rich in fat would exert an effect on the insulin content of pancreas over longer periods of time, a diet containing 28% carbohydrate, 15% protein, and

TABLE V. Effect of moderately high fat and carbohydrate diets on the insulin content of pancreas

No. of rats	Diet	Duration of exp. months	Average weight per group of 10 rats: Initial g.	Average weight per group of 10 rats: Final g.	Units of insulin per group of 10 rats
30	Control	2	1043	2345	26·7
30	Fat	2	1033	2373	20·1
60	Control	6	955	2661	29·9
59	Fat	6	959	2618	23·3
30	Control	3	737	2525	25·3
30	Carbohydrate	3	655	2246	23·9
18	Control	7	691	3337	25·0
67	Carbohydrate	7	613	2347	24·9

46% fat by weight was provided. The diet high in carbohydrate contained 69% carbohydrate, 15% protein, and 5% fat. The control diet consisted of 50% carbohydrate, 18% protein, and 16% fat. These diets contained a salt mixture, yeast, and cod-liver oil. The results of these experiments are summarized in Table V.

While there appears to be a decrease in the insulin content when the animals are fed the diet moderately rich in fat, the findings do not suggest that extensive depletion of insulin could be rapidly produced by diets of this type.

Vitamin B_1 deficiency

While we are contemplating a thorough investigation of the effects of deficiency of the various vitamins, our results thus far are confined entirely to a study of the lack of vitamin B_1. The diet was made up of beef muscle powder 10%, beef fat 20%, sucrose 62·7%, salt mixture 5%, agar 2%, choline chloride 0·3%, and vitamins A and D in the form of a cod-liver oil concentrate. One group received 16 μg. of crystalline vitamin B_1 for every 10 g. of diet. The rats in the first group ate freely while those in the second group were given the same amount of food as the animals which received the diet deficient in vitamin B_1. During the first 2 weeks 16·5 μg. of vitamin B_1 per rat per day were ingested, in the third week 10·9 μg. and in the fourth week 7·5 μg. were ingested. The results of the insulin determinations are given in Table VI and show

TABLE VI. Vitamin B_1 deficiency and the insulin content of pancreas

No. of rats	Diet	Units of insulin per group of 10 rats	Units of insulin per kg. of initial body weight
29	B_1 deficient	12·0	9·4
29	B_1 added	13·4	10·5

that, while in both the control and the test group there is a very definite decrease in the insulin content as compared with normal animals, the addition of vitamin B_1 had no effect. Presumably the decrease was due, in large part, to deficient caloric intake. These results are being extended.

Liver fat and the insulin content of pancreas

In some of the experiments in which diets rich in fat were fed, the rats developed fatty livers. It was therefore considered advisable to determine whether or not fatty changes in the liver might be associated with a change in the insulin content of pancreas. We had the opportunity to conduct tests on the pancreatic tissue from (1) rats poisoned with carbon tetrachloride, (2) rats poisoned with carbon tetrachloride and given

choline, and (3) control rats receiving the same diet as those in the first group and a similar caloric intake. The results in Fig. 3 show clearly that the insulin content of the pancreas is not affected under these conditions when extensive changes in liver fat have occurred. Further details of these experiments are given in a paper by Barrett, Best, MacLean & Ridout [1939].

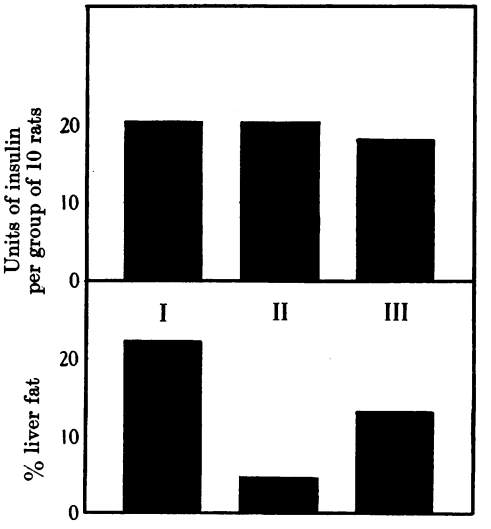

Fig. 3. I, animals poisoned with carbon tetrachloride; II, animals poisoned with carbon tetrachloride but receiving choline; III, control animals receiving same caloric intake as group I.

General discussion

It is obvious that the altered carbohydrate metabolism observed in starvation or as a result of feeding diets rich in fat might be produced by factors affecting the production of sugar, the rate of utilization, or both. Factors affecting the production of sugar in the liver or the rate of utilization in the tissues might act directly on these structures, or might exert their effects through one or more of the endocrine glands. At present there are very few facts available which enable one to decide which of these mechanisms are in operation. Our results show conclusively that in rats one definite finding is present under these conditions, namely, that there is a decrease in the insulin content of pancreatic tissue.

In many of the experiments reported the loss of body weight was considerable. In all the experiments, however, the change in insulin content of the pancreas was definitely greater than that which might be expected merely from loss of weight if one makes the unjustifiable

assumption that the insulin-producing tissue shares equally with the other tissues in this loss. The exact weight of the pancreatic tissue in rats is difficult to determine since the amount of fat closely associated with the pancreas may vary. *In the experiments where food was administered after fasting, the weight loss still remained extensive even in those animals whose pancreas showed a recovery of the normal insulin content* (Fig. 2).

The insulin content of the pancreas represents a balance between the rates of production and liberation of the hormone. A lowered insulin content such as we have observed after fasting or fat feeding may therefore be due to either diminished production or increased liberation or both.

Although no work has yet been done in our laboratory on the glucose tolerance of starved rats, or of rats fed on a diet rich in fat, it would appear from the results of other investigators on other species that the impaired carbohydrate tolerance occurs at a time when, in the light of our experiments, a decrease in the insulin content of the pancreas would be expected. As stated previously, the normal carbohydrate metabolism is partially restored by the administration of insulin [Cori & Cori, 1926, 1927; Dann & Chambers, 1930; Ellis, 1931]. Since the diminished carbohydrate tolerance in starvation and after feeding fat may be explained, in part at least, on the basis of decreased liberation of insulin, it would seem logical to assume that the low insulin content of pancreas which we have observed is caused by decreased formation rather than by increased liberation of insulin.

While no general relationship between the insulin content of the pancreas and the rate of insulin liberation has been established, there are experimental results which indicate that the liberation is related to the insulin content under certain conditions. It has been shown, for example, that in the permanent diabetes produced in dogs by the administration of anterior pituitary extracts, the insulin content of the pancreas is reduced to extremely low values [Campbell & Best, 1938]. Furthermore, in many diabetic patients the results obtained by Scott & Fisher [1938*b*] show very clearly that the insulin content of the pancreas is reduced well below the normal value. In these cases in which the insulin content of the pancreas is low the organism suffers from severe diabetes and is favourably affected by small doses of insulin. Under these circumstances it is reasonable to suppose that the low insulin content leads to a reduced rate of liberation. There is certainly no evidence at present that there is an increased rate of liberation and that the effect of this is overbalanced

by opposing factors. On the other hand, in those clinical cases suffering from liberation of excessive amounts of insulin, i.e. hyperinsulinism, it has been established that the tumours of islet tissue may contain abnormally large amounts of the hormone. For example, in one of the recent cases reported by Campbell, Graham & Robinson [1939] in which D. A. Scott estimated the insulin content of the tumour, the concentration of insulin was 8 units per gram. The patient suffered from a very definite hyperinsulinism which was alleviated by removal of the tumour.

We have merely cited evidence which suggests that under certain conditions there is a relationship between the insulin content of pancreas and the rate of insulin liberation. It is quite possible that under other conditions this relationship may not hold or may be completely obscured by compensatory physiological adjustments. We are postponing a full discussion of the significance of our findings until further studies on the insulin content of pancreas have been made. It may be mentioned here, however, that research along this line will certainly throw further light on such problems as the mechanism of the effect of the undernutrition treatment of diabetes [Allen, Stillman & Fitz, 1919], the role of dietary substances in the aetiology of diabetes [Himsworth, 1935], the influence of obesity on the diabetic state [Newburgh, Conn, Johnston & Conn, 1938], and the use of diets very rich in fat in the treatment of hyperinsulinism.

Summary and conclusions

1. Fasting or the feeding of diets rich in fat produces a very definite decrease in the insulin content of the pancreas.

2. While an abnormally low caloric intake may be one of the factors producing this result in the animals given the diet rich in fat, it is not the only one, since those receiving a certain caloric intake as fat show a much greater decrease in insulin content than those provided with the same caloric intake in the form of carbohydrate.

3. The insulin content of the pancreas, depleted by fasting, is restored within 6 days to a normal value by feeding a well-balanced diet. Carbohydrate alone effects a partial restoration but fat produces no rise in insulin content.

4. The results of preliminary studies on the effect of (1) short periods of anaesthesia and (2) vitamin B_1 deficiency do not suggest that these conditions affect the insulin content of the pancreas in a specific manner.

5. The possible relationship between the low insulin content of the pancreas and the altered carbohydrate metabolism of animals which have been fasted or fed diets rich in fat is discussed.

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