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THE REDUCED SENSITIVITY TO INSULIN OF RATS AND MICE FED ON A CARBOHYDRATE-FREE, EXCESS-FAT DIET.

By H. W. BAINBRIDGE.

(*From the Wellcome Physiological Research Laboratories.*)

The purpose of these experiments was to discover whether the sensitivity of animals to insulin could be altered by increasing the proportion of fat in the diet.

Method. In the first series of experiments on rats the animals used were divided into two groups. One group was fed on the diet of Halliburton and Drummond (called the complete diet), the other group on an exactly similar diet except that the starch was replaced by butter (the excess-fat carbohydrate-free diet) the two diets being of approximately equivalent caloric value (Table I).

Table I. Diets.

	Excess-carbohydrate fat-free grms.	Complete grms.	Excess-fat carbohydrate-free grms.
Casein	18	18	18
Butter	—	20	40·7
Starch	91	47	—
Salt mixture	5	5	5
Marmite	5	5	5

The rats were kept in cages—two or three in each cage—provided with water and as much food as they could eat. At the end of a period, which varied from two to six weeks in the different experiments, the rats, which were starved for about 20 hours before injection, were placed in a room at a temperature of approximately 33° C. and injected subcutaneously with insulin. The dose given in all the experiments on rats was four clinical units per kilogram rat. Three clinical units per kgm. brings the blood sugar of 60 p.c. of rabbits down to ·045 p.c. at room temperature. Rats are evidently normally less sensitive to insulin than rabbits. A room temperature of 33° C. was used because it has been found in these laboratories (Trevan and Boock, private communication) that rats are more susceptible to insulin, and show less range of variability at the higher temperature. Krogh (using mice) was the first

to notice that the action of insulin was much affected by the temperature at which the animals were kept. They were, therefore, placed in a room at this temperature a few minutes before injection and kept there afterwards for a definite period, usually two hours.

Results. Table II is a detailed record of two experiments made on growing rats of about 100 grms. weight which had previously been fed on ordinary laboratory diet (bread, milk and green food), and were transferred to the experimental diet six weeks before the date of these experiments. The symptoms, characteristic of insulin, were severe unless stated to be otherwise. Convulsive movements appear to be less usual in rats than extreme prostration.

The animals were kept in sets of three or four in separate cages and their body weights and sex noted. These details are omitted in the accounts of the experiments, since they were not associated with any difference in result.

TABLE II.

Diet	Rat	Time after injection at which symptoms became severe
Complete diet	1	45 mins. Dead in 1 hr.
	2	Convulsions 35 mins. Glucose
	3	35 mins. Dead in 1 hr.
	4	None
	5	35 mins. Dead in 1 hr.
	6	55 mins. Dead in 1 hr.
	7	2 hrs. Recovered without glucose
	8	2 hrs. Glucose
	9	1 hr. 25 mins. Convulsions 1 hr. 35 mins.
Carbohydrate-free excess-fat diet	10	1 hr. 40 mins. Dead 1 hr. 55 min.
	11	1 hr. 40 mins. Recovered in cold 1 hr. 55 mins.
	12	1 hr. Recovered in cold 1 hr. 20 mins.

Six other rats on the carbohydrate-free excess-fat diet were similarly treated and insulin injection caused no symptoms.

In most of these cases one or more injections of insulin had been made during this period of six weeks without producing any obvious symptoms, the dose given (about a quarter of that finally adopted) being apparently too small to be effective. When rats 1–3 and 10–12 were tested the temperature was allowed to rise to 37° C., and this may possibly account for the fact that it was the only case in which all the rats on the excess-fat carbohydrate-free diet showed symptoms. Even here the onset of the symptoms was delayed in the fat-dieted rats.

The fact that rats fed on an excess-fat diet are less susceptible to the action of insulin than those on the complete diet is confirmed by further experiments, a few of which are summarised in Table III. These rats were given the special diet as soon as they were old enough to be taken from their mother, and were kept on it for a period varying from

2–6 weeks before injection. No previous injection of insulin had been given.

TABLE III.

Diet	Rats	Duration of Exp.	Symptoms
Complete	19–21	1 hr.	All in 35 mins., one died later.
	22–24*	2 hrs.	2 in 1 hr. 20 mins., 1 in 30 mins.
	25–27	3 to 4 hrs.	2 in 1 hr. 20 mins., 1 in 20 mins. All died later
	28 and 29	3 to 4 hrs.	Both in 1 hr. 40 mins. Both died later
Excess-fat carbohydrate-free diet	30–32	1 hr.	None
	33–35*	2 hrs.	One slight symptoms, left in warm room, no worse in 2 hrs.
	36–38	3 to 4 hrs.	None
	39 and 40	3 to 4 hrs.	One showed symptoms 2 hrs. Convulsions 2 hrs. 45 mins.

* These rats received 0·1 c.c. cod liver oil each per day for one week before injection.

In the cases in which the rats on the carbohydrate-free excess-fat diet developed symptoms, these symptoms were delayed and were less severe than those of the rats on the complete diet. Thus rat 22 on the complete diet exhibited convulsive symptoms half an hour after injection. These symptoms became so severe after twenty more minutes in the incubator, that even though it was removed to room temperature and given 5 c.c. of 50 p.c. glucose, it was dead half an hour later. The other two rats in this experiment (rats 23 and 24) showed severe symptoms 1 hr. and 20 mins. after injection and were dead ten minutes later. The effect of an equal dose of insulin on rats fed on an excess-fat diet is very different. Only one of the rats (rat 33) injected at the same time showed symptoms which developed 1 hr. 40 mins. after injection. They were much less severe than those of the complete diet rats and had not increased in severity at the end of the experiment twenty minutes later. This rat was removed to room temperature and given 4 c.c. of 50 p.c. glucose—it was able to walk about immediately and was apparently perfectly well next day.

It is well known that animals vary widely in their response to insulin and these results might be due to the fact that the rats on the excess-fat diet chanced to be among the extremely refractory group. To exclude this the following experiment was made:

(*a*) Seven rats after being fed on the complete diet for 13 days were given insulin. Two of these developed severe symptoms in 1 hr. 5 mins.; three in 1 hr. 30 mins. to 2 hrs. and one had no symptoms. They were then fed on excess-fat carbohydrate-free diet for 13 days and on giving insulin, six developed no symptoms and one slight only.

(*b*) Six rats were fed on excess-fat carbohydrate-free diet for 13

days; insulin caused no symptoms in any of these; after feeding for 13 days on the complete diet, four developed severe symptoms in 1 hr. 40 mins. to 2 hours, one had less severe symptoms and only one had none.

In addition to this series of experiments (on 128 rats) a second series was made with different insulin, the strength of which was possibly rather lower than that used in the first series. The dose of insulin and the conditions were the same as in the first series. The rats used were placed on the diet about six weeks after birth, their weight being about 40 grms. They were kept on this diet for a period varying from three to ten weeks before injection, the average time being under six weeks. The results of the two series are given in Table IV.

TABLE IV.

Diet	No. of rats injected	Dead	With symptoms	Percentage
Complete	118	24	59	70·3
Excess-fat	131	2	18	15·3

TABLE V.

Cod liver oil, excess-carbohydrate. No fat	19	—	14	73·6
Complete	22	—	14	63·6
Excess-fat, no carbohydrate	22	—	3	13·6

In a few experiments rats were fed on a diet containing no butter. The calorie value of the diet was maintained by an increased proportion of starch (see Table I). The necessary amount of vitamin A was supplied by cod liver oil—0·3 c.c. of which was given daily by pipette to each rat on the no-fat diet. The results are summarised in Table V.

Experiments on mice. Similar experiments were made with mice instead of rats. The mice were kept under suitable conditions in large wooden boxes and were fed on the diets for about a week before injection. The diets used for mice were the same as those used for the rats except that cod liver oil was added to each diet. The amount added in Series 4 (Table VI) was equivalent to over 5·0 mg. per rat and it was assumed that this would be sufficient for an equal weight of mouse. The mice

TABLE VI. Mice. Series 3.

Dose in clinical units per 20 grm. mouse	Excess-fat diet			Complete diet			No fat excess-carbohydrate		
	No. injected	No. symptoms	Percentage	No. injected	No. symptoms	Percentage	No. injected	No. symptoms	Percentage
·01	20	3	15	20	1	5	20	2	10
·013	27	1	3·7	37	9	24·3	42	19	42·2
·02	40	7	17·5	40	22	55	36	23	63·9
				Series 4.					
·013	51	7	13·7	60	38	63·3	62	47	75·8

in Series 3 (Table VI) received ten times this amount in their diet with the exception of mice on the complete diet from which the cod liver oil was unfortunately omitted. The mice ate almost all the food provided for them during the twenty-four hours. Table VI shows the results.

All these results seem to show clearly that a diet from which carbohydrate is completely absent and which contains in its place an increased amount of butter-fat, produces in rats and mice a decreased sensitivity to insulin. Whether the further deduction can be made, that the reduced sensitivity is proportional to the amount of butter-fat present is less certain. Though the difference in the percentage number of symptoms on the complete and excess-carbohydrate no-fat diets is probably a significant figure, this cannot be held to be definitely established. It is assumed that the important factor is the presence of increased amounts of fat and not the absence of carbohydrate.

A few rats were put on a carbohydrate-free diet in which the butter-fat was replaced by heated lard (21 grms.). These rats were old stock rats weighing from 150 to 250 grms. at the beginning of the experiment. They were injected with the usual dose of insulin a month later. Table VII gives the results compared with those shown by similar rats on the complete and excess-butter-fat diet. All the rats were injected at the same time. One rat only on the excess-butter-fat diet showed more than very slight symptoms and those developed by the rats fed on the diet containing the lard were almost equally slight.

TABLE VII.

Diet	No. of rats injected	Dead	Other symptoms
Complete	10	2	8
Excess-butter-fat	10	none	3; 2 very slight
Excess (lard) fat	9	none	4; all slight

Blood sugars. The blood sugar was estimated in a small number of cases. Miss Boock kindly bled the rats for me by cardiac puncture. The average blood sugar of 10 normal rats was found to be 0·13 p.c. That of dieted rats one or two hours after injection is shown in the following table (Table VIII).

TABLE VIII. Blood sugars of rats 1–2 hours after injection with insulin.

Diet	No. of rats	With symptoms	Average blood sugar values	Lowest value	Highest value
Complete	11	10	·0696	·055	·082
Excess-fat	13	4*	·0808	·054	·112

* Only slight symptoms in each case.

There are various other factors which might influence the results.

Condition of animals. The rats on all diets appeared to be strong and healthy throughout the experimental period. The gain in weight of rats on the excess-fat diet was at least equal to that of rats on the complete diet. The gain in weight in four weeks of 71 rats on the complete diet was 104 p.c., that of 67 rats on the excess-fat diet was 135 p.c.

The mice on the excess-fat diet were in bad condition after two or three days and got progressively worse. Mice on the other two diets remained well and healthy (Table IX).

TABLE IX. Relative mortality of mice on diets for 6 days.

Diet	No. of mice	Deaths in 6 days
Carbohydrate only (No fat)	100	3
Complete	100	6
Excess-fat (No carbohydrate)	100	32
Excess-fat (No carbohydrate) Phosphate water	50	2

During the six days the 100 mice were on the excess-fat diet, there were thirty-two deaths. Eighteen of these deaths occurred overnight among seventy of these mice, which were being starved for injection. 50 of these 52 mice alive next day were given ·013 clinical unit of insulin and only seven of the 50 showed any symptoms, whereas the corresponding figures, after the same dose of insulin, for complete and carbohydrate diet mice on the same day were 27 out of 39 injected and 31 out of 40 respectively. The mice on the excess-fat diet had no doubt developed a considerable degree of acidosis, and looked ill, with rough greasy coats and many were in a state of coma before death. A 5 p.c. solution of Na_2HPO_4 in tap water had been substituted for the ordinary drinking water in the case of other fifty mice on the excess-fat diet with the result that the mortality before injection was only 4 p.c.; their response to insulin was exactly the same as that of the excess-fat mice which developed acidosis.

Action of cod liver oil. The different susceptibility to insulin of rats and mice fed on diets differing in their contents of butter-fat does not appear to be due to a deficiency of vitamin fat—soluble A in the diet containing the smaller amount of butter, as the addition of an amount of cod liver oil more than sufficient to supply an adequate amount of this vitamin has no influence on the results. Table X shows the results of insulin injection on dieted rats with and without cod liver oil, and they are confirmed by the experiments on mice already referred to.

TABLE X. Rats fed on diets with and without cod liver oil.

Diet	Plus 0·4 c.c. cod liver oil daily			Diet without cod liver oil		
	No. injected	With symptoms	Percentage	No. injected	With symptoms	Percentage
Complete	12	9	75	12	10	85
Carbohydrate-free	26	11	42	23	11	47

Importance of the thyroid. Dr Burn suggested that the resistance of rats on an excess-fat diet might be due to the action of fat on the thyroid gland. Burn(2) has shown that thyroidectomised rabbits are more than normally sensitive to insulin, but recover their normal resistance when fed with extracts of that gland. E. and M. Mellanby found that puppies living in confinement show marked hyperplasia of the thyroid when fed on butter-fat, the weight of the gland increasing five-fold or even more. The thyroid glands of a number of these experimental rats were therefore dissected out and weighed as soon as possible after death. They were also examined histologically. The average weight of 18 rats on a complete diet was ·0135 grm. (standard deviation of mean = 0·000566), that of 21 rats on excess-fat diet was ·0163 (standard deviation of mean = 0·00071), a difference just not large enough to be significant. No histological changes were obvious.

Results. The results show that a diet from which carbohydrate is absent and which contains excess-fat reduces the sensitivity to insulin of the animals tested to such an extent that a dose which would cause 70 p.c. of those on the complete diet to convulse causes symptoms in under 20 p.c. of the cases on the excess-fat diet; no explanation has been satisfactorily demonstrated. The variation in sensitivity is apparently not due to variations in the amount of vitamin present and it has not been proved that it is due to variations in the activity of the thyroid gland.

SUMMARY.

1. Rats and mice were fed on a diet containing protein, salts and vitamins, from which carbohydrate was omitted, the necessary calorie value being maintained by an increased amount of fat. Such animals developed a high degree of resistance to insulin.

2. Certain experiments make it appear probable that an increased amount of carbohydrate and complete absence of fat in the diet render the animals still more sensitive to insulin than they would be on a complete diet, but this cannot be considered to be definitely proved.

3. The variations in sensitivity are not due to deficiencies in the amount of vitamin A present.

4. The evidence as to the importance of the thyroid gland is inconclusive.

REFERENCES.

(1) Halliburton and Drummond. This Journ. 51. p. 235. 1917.
(2) Burn and Marks. Ibid. 59. 1924; Proc. Physiol. Soc. p. viii.
(3) E. and M. Mellanby. Ibid. 55. 1921; Proc. Physiol. Soc. p. vii.

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