LVI. STUDIES ON THE METABOLISM OF ANIMALS ON A CARBOHYDRATE-FREE DIET.

II. VARIATIONS IN THE SENSITIVITY TOWARDS INSULIN OF DIFFERENT SPECIES OF ANIMALS ON CARBOHYDRATE-FREE DIETS.

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IN each set of experiments described in our previous communication [Hynd and Rotter, 1930], a number of animals were tested for their response to insulin. It was thus possible to compare the effects produced by an injection of the hormone not only with regard to the previous dietetic treatment of the animal, but also in relation to the existing blood-sugar level, the muscle-glycogen content, and the distribution of glycogen and fat in the liver.

All the experiments were conducted in a room the temperature of which was maintained at 20–22°, for although the symptoms develop less rapidly at this temperature than at higher temperatures such as 30–37°, as first shown by Krogh, our conditions are more normal and hence less likely to bring other factors into operation. The same sample of insulin was used throughout the experiments recorded in this paper, and the injections were made subcutaneously. The dose employed for the several species varied slightly, the amount used being that found necessary to produce in control animals the train of symptoms accompanied by what are usually referred to as “hypoglycaemic convulsions.”

Experiments with mice.

For the experiments with mice the insulin was diluted 1 in 10 with 0.05 % hydrochloric acid, 7 minims of this diluted insulin being the dose for a mouse of 20 g. body weight. As the diets employed have already been described [Hynd and Rotter, 1930], it is only necessary to indicate the number of animals employed in the several experiments. On carbohydrate-free diets 41 mice were tested for their response to insulin, namely 15 on cheese, 14 on butter-fat, and 12 on caseinogen, and these tests were controlled by 23 animals fed on a normal diet.

With one exception all the mice on the control diet showed the characteristic insulin symptoms between 1 and 1½ hours after the injection, and re-

quired to be resuscitated by the administration of glucose or of maltose. The cheese-fed mice, on the other hand, were without exception resistant to the same dose of insulin, and, even with twice the dose, the only effect of the injection of insulin was to render the animals somewhat quiet and drowsy. All returned to normal in 2–3 hours without treatment of any kind. To elicit severe symptoms including convulsions, the dose required to be increased 3–4 times. Cheese-fed mice, which had been proved resistant to insulin, showed a normal response after 12 days' subsequent feeding on oats, marked lowering of the blood-sugar (0.062) occurring in 30 minutes after the insulin injection and the convulsive level (0.054) being reached in 65 minutes.

Similar results were obtained with mice reared on caseinogen until 7 weeks old, although one animal died 30 minutes after being injected. Of the remaining 11 mice used, 7 showed no symptoms except drowsiness, and the other 4, in addition to being drowsy, showed slight sprawling of the limbs. This, however, did not develop further and the animals were normal in the course of 3 hours. Mice rendered resistant to insulin by a diet of caseinogen remained so, though supplied for 5 days with as much (a) glucose, or (b) fructose, as they would take in addition to the usual amount of caseinogen. These experiments were not continued further, as the animals refused after a time to eat the pure sugars.

Of the 14 fat-fed mice, 6 showed no symptoms except drowsiness, 6 others became slightly sprawling for a time, and only 2 showed severe symptoms requiring glucose injection to bring about recovery.

Evidently either for maintaining the growth and health of the animals, or for producing resistance to the effects of insulin, a carbohydrate-free diet, consisting exclusively of either fat or protein, is not so satisfactory as one in which both protein and fat are present. Nevertheless, on all the carbohydrate-free diets employed, mice showed a marked difference in their sensitivity towards insulin from animals on a normal diet. After 2 weeks' feeding on a carbohydrate-free diet, resistance to insulin is well established, and the mice were still resistant when the cheese diet had been continued for $2\frac{1}{2}$ months.

Experiments with rats.

From the experience gained in feeding carbohydrate-free diets to mice, only two diets were attempted with rats, namely a carbohydrate-rich diet of bread and milk, and a carbohydrate-free diet of cheese. Twenty-two animals on each of these diets have been tested with insulin, the dose being proportionately less than that given to mice, namely 10 minims of 1 in 5 insulin per 100 g. of body weight. As we found that the rate at which hypoglycaemic symptoms developed in a normal rat seemed to depend, in part at least, on the strength of the insulin solution injected, we have used invariably for the experiments with rats 1 part of insulin diluted with 4 parts of 0.05 % HCl. Also as piebald rats are somewhat variable in their response to insulin, all the experiments here reported were carried out on albinos.

Under the dosage indicated above, rats on the cheese diet showed no notable symptoms except drowsiness, while the control rats without exception had severe convulsions after about 2 hours, and required to be relieved by the injection of glucose:

The following is typical of the different behaviour of the two sets of rats.

	Rat, ♂, 161 g. Diet of bread and milk	Rat, ♂, 160 g. Diet of cheese for 14 days
9.43 a.m.	Injected with 16 minims of 1:5 insulin	Injected with 16 minims of 1:5 insulin
11.0 "	Not quite normal: climbs wire cage on being disturbed	Not quite normal: remains quiet
11.5 "	Irritable; no sprawling	Legs sprawling; feels rigid when handled; no fall of body temperature; not irritable
11.15 "	Symptoms developing	Improving; looks drowsy; otherwise almost normal
11.45 "	Sprawling limbs; cold and limp	Quiet and drowsy; otherwise normal
11.51 "	Severe convulsion	Drowsy
11.57 "	Severe convulsions	Drowsy
12.1 p.m.	Severe convulsion	Drowsy
12.5 "	Another convulsion	No further symptoms developed
12.10 "	Injected 1 cc. 20 % glucose solution	Taking food (cheese moistened with milk)

In the experiments with rats, it has been noticed that almost invariably, previous to the stage when symptoms develop in the carbohydrate-fed animals, the cheese-fed rats appear somewhat worse than the controls. They are not so irritable, but much less active, and show slight sprawling of the hind limbs. However, they do not become cold and when handled appear rigid rather than limp. This effect lasts only a very short time, and the animals then improve without further symptoms developing. If the dose of insulin has been too large, or the carbohydrate-free diet has not been continued long enough, mild insulin symptoms may develop, but if so, they appear later than in the control rats, as noted by Bainbridge [1925].

As we have already shown that the livers of cheese-fed rats have a maximum fat content after a fortnight's feeding, experiments were carried out to determine (1) whether the resistance to insulin is also at a maximum at the same time, and (2) when resistance first develops. The results obtained were inconclusive. Partial resistance to insulin could be detected after 4 days on the cheese diet, as after this period of feeding the symptoms were delayed in development and less severe than those of the control animals. After 8-10 days, resistance was well established, and was still well marked after 1 month. This is in agreement with the observations of Abderhalden and Wertheimer [1924, 1], and of Bainbridge [1925]. The former workers noted resistance in rats after 10 days' feeding, while the latter employed periods of 2-6 weeks' feeding. In our experiments, all animals after 14 days' feeding on cheese were resistant to insulin. It was also found that, after the withdrawal of food for 24 hours, rats which had been fed on cheese for a fortnight were still resistant to the effects of insulin.

Experiments with rabbits.

Only one experiment was carried out on rabbits, in which we confirmed the observation made by several workers (Page [1923], Blatherwick *et al.* [1924], Abderhalden and Wertheimer [1924, 3], Pénaud and Simonnet [1925], Geiger and Kropf [1929]), that rabbits on green food are less resistant to insulin than animals fed on oats. The object of our experiment, however, was not merely to confirm this behaviour of rabbits, but rather to determine whether any departure from the normal could be detected in the respective reducing power of the blood-plasma and corpuscles of such animals. As is evident from the results tabulated below, no significant differences were obtained on the two types of diet.

Diet	Whole blood-sugar %	Plasma- sugar %	Corpuscle- sugar %	Plasma volume	:	Corpuscle volume
Greens	0.113	0.144	0.047	76.66	:	23.34
Oats	0.134	0.147	0.060	77.71	:	22.29

Experiments with kittens.

As we had found previously that kittens were more responsive than rabbits to the same dose of insulin, it seemed desirable to extend our observations to this species. The carbohydrate diet was again bread and milk, while the carbohydrate-free diets employed were (a) white fish, and (b) cream, the former consisting largely of protein, the latter having a high fat content, and both suited to the type of animal under investigation. Four sets of experiments have been carried out. In each set the kittens employed were of the same litter and were 6 weeks old at the commencement of the experiment. After 3-4 weeks on the diets indicated above, they were injected subcutaneously with 20 minims of insulin per kg. of body weight.

The kittens on all three diets responded to the injection and displayed the characteristic symptoms, including leg-weakness, mewing, salivation and convulsions [Olmsted and Logan, 1923]. Relief followed the administration of glucose, but there were frequent relapses. Hence a smaller dose of insulin, namely 10 minims per kg., was tried, but with similar results. Typical symptoms ensued in all cases, and the only difference noted was the length of time required for the symptoms to develop. The time of onset of the first convulsion in each case is summarised in the following table, from which it is seen that the animals on the carbohydrate-free diets were always much earlier in showing symptoms than the carbohydrate-fed animals. In fact, on two occasions the kittens fed on bread and milk did not have convulsions.

Diet	Period which elapsed before first convulsion (minutes)			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Fish	60	75	75	75
Cream	80	80	90	—
Bread	180	(No convulsion)	(No convulsion)	105
Minims of insulin	20	10	10	10

DISCUSSION.

On a carbohydrate-free diet rats and mice, while showing no significant change in muscle-glycogen when compared with animals on a carbohydrate-rich diet, tend to have a high blood-sugar, an increased liver-fat content, and a diminished liver-glycogen [Hynd and Rotter, 1930]. Can any of these differences be correlated with the resistance to insulin that such animals develop when maintained on a carbohydrate-free diet?

In the table which follows are summarised the blood-sugar levels of mice on different diets and their conditions at varying periods after the injection of insulin. On the control diet of bread and milk, the blood-sugar is seen to fall rapidly and the convulsive level is soon reached. On the carbohydrate-free diets, on the other hand, though the actual range through which the blood-sugar is lowered by insulin is approximately the same, the change takes

Experiments with mice.

Diet ... Time after insulin injection (mins.)	Without being fasted						Fasted 18 hours			
	Bread		Cheese		Fat		Bread		Cheese	
	Blood- sugar %	Con- dition of animal	Blood- sugar %	Con- dition of animal	Blood- sugar %	Con- dition of animal	Blood- sugar %	Con- dition of animal	Blood- sugar %	Con- dition of animal
0	0.124	Normal	0.157	Normal	0.191	Normal	0.174	Normal	0.171	Normal
30-40	0.05	Very irritable	0.087	„	0.076	„	0.087	Slightly affected	0.073	„
50-60	0.039	Convulsion	—	„	0.069	„	0.056	Convulsion	—	„
70-80	0.038	„	0.060	„	—	„	—	—	—	—
90	0.039	„	—	„	0.049	„	—	—	—	—
150	—	—	0.057	„	0.056	„	—	—	0.064	Slightly sprawling
240	—	—	0.131	„	—	„	—	—	0.064	Normal
Max. fall	0.086		0.100		0.142		0.118		0.107	

place much more slowly and the animals remain more or less normal. Further, as animals which have fasted 12-24 hours have their blood-sugars at the same level as controls, but are still resistant to insulin, it is not the initial high blood-sugar level but rather the diminution in the rate at which the fall in blood-sugar is produced that is concerned with the resistance to insulin. This is supported by the experiments with rats on carbohydrate-free diets carried out by Abderhalden and Wertheimer [1924, 2], who have reported that such animals after the injection of insulin display a less rapid fall in blood-sugar. Tiitso [1925] also found that the blood-sugar falls less rapidly in starved rabbits than in those fed with abundant carbohydrate.

Since the blood-sugar level represents the balance between two sets of opposing processes, it is necessary to inquire if we can discover any factor responsible for the diminished rate at which the blood-sugar falls in non-carbohydrate-fed rodents, since it appears that there is some connection between the diminution in rate of fall of blood-sugar and the response to insulin.

The difference in the effects of insulin on well-fed as compared with starved animals was considered by McCormick, Macleod, Noble and O'Brien [1923] to depend primarily upon the amount of liver-glycogen. Lawrence [1926], also, in emphasising the importance of the glycogen content, stated that when the glycogen stores were empty insulin was much more effective in its hypoglycaemic action than at any other time. Accordingly, as we have found that animals on a carbohydrate-free diet show a reduced liver-glycogen content, one would predict that such animals should respond more readily to an injection of insulin. Experiment, however, shows just the opposite result. Non-carbohydrate-fed rodents withstand insulin much better than those receiving abundant carbohydrate, both when judged by the rate of fall of the blood-sugar and the incidence of convulsions. The amount of glycogen stored in the liver, therefore, is not, in all circumstances, a reliable index of the sensitivity of an animal towards insulin. In other words, there is some factor more important than the available amount of stored glycogen.

Bainbridge [1925] believed resistance to be due to excess of fat rather than to absence of carbohydrate from the diet, and sought to establish a connection between resistance to insulin and the fat content of the diet. Our results do not support this contention. The livers of rats and of mice on a cheese diet show a high liver-fat content, which reaches a maximum at about that period when maximum resistance to insulin develops, but as the excess of liver-fat is extremely mobile and disappears after 24 hours' starvation, lack of response to insulin is not connected with the excessive amount of fat, for starved animals are still resistant to insulin. Moreover, we have succeeded in producing marked resistance by feeding mice on a purely protein diet, namely on caseinogen, while several workers have reported on the resistance of rabbits fed on diets containing little or no fat. Again, it is well recognised that animals on a high carbohydrate diet after a time become distinctly fatty. Yet such fattened animals do not show resistance to insulin. On the other hand, the absence of carbohydrate from the diet was considered by Abderhalden and Wertheimer [1924, 1, 2] to be a factor of importance in establishing resistance to insulin. Some support for this opinion is found if one accepts Laufberger's [1924] view that the essence of insulin action is to inhibit gluconeogenesis, for when no preformed carbohydrate is supplied, the formation of carbohydrate from other substances must of necessity become accentuated, and consequently a diminished insulin effect would result in animals on a carbohydrate-free diet. However, the investigations of Grevenstuk, de Jongh and Laqueur [1925] indicated that the resistance was not due to any specific type of foodstuff, but depended on the relative proportions of carbohydrate, protein and fat.

Generally speaking our observations on the resistance of rodents to insulin are in agreement with those recorded by other workers: and it is quite clear that neither the stores of glycogen nor of fat are responsible for the resistance to insulin. But a further difficulty arises when the results of the experiments

with kittens are considered, because in this type of animal neither a diet consisting entirely of protein, nor one containing a large amount of fat, brought about resistance to insulin. In fact, our experiments showed that kittens on a carbohydrate-rich diet were somewhat less responsive to insulin than animals on carbohydrate-free diets. However, the carbohydrate-fed kittens could not be described as resistant to insulin, as, though in two cases no convulsions occurred, the animals were definitely affected. Had a smaller dose of insulin been given, resistance might have been claimed, but the chief point for the present is that kittens differ from mice and rats in that feeding a carbohydrate-free diet does not render them resistant to the action of insulin.

Eadie [1930] has recently reported a difference in the action of adrenaline in the cat and the rat, and Corkill [1930] found the young rabbit to differ from several other species with regard to the increase of liver-glycogen after insulin. Funk [1922], also, has pointed out that dietary changes have often an influence on the toxicity of certain drugs. Consequently we have here a parallel to our results with regard to the action of insulin. In other words, there is a distinct species difference not only between kittens, which may be taken as representative of carnivora, and rodents, but also amongst the rodents themselves.

Now it is well known that while the alkali reserve of a rabbit is easily changed, it is exceedingly difficult to alter that of a dog or a man, and hence probably also that of a cat [McClendon, 1919]. Feeding on oats was found by Page [1923] and also by Abderhalden and Wertheimer [1924, 3] to lower the alkali reserve of a rabbit, and a non-carbohydrate diet would be capable of a similar effect in the case of a mouse or a rat [Abderhalden and Wertheimer, 1925], but not in a dog, cat or man [Michalowsky, 1930]. We thus arrive at the reasonable conclusion that an animal when maintained on a diet which is capable of bringing about a lowering of its alkali reserve will tend to show resistance to the action of insulin. There is thus an element of truth in the views of both Abderhalden and Wertheimer [1924, 1, 2] and of Bainbridge [1925], already referred to, as either absence of carbohydrate or excess of fat inevitably leads to a diet tending to reduce the alkali reserve. Though certainly not the only factor, the acid-base equilibrium of the body is thus a fundamentally important one in the mechanism of insulin action, in that it probably regulates the effect not only of the internal secretion of the pancreas, but also the effects of the other ductless glands, such as the suprarenals, thyroid and pituitary, which are known to be concerned in carbohydrate metabolism. In a forthcoming paper further experiments will be described in support of this view.

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