LII. THE EFFECT OF INSULIN ON THE GLYCOGEN IN THE TISSUES OF NORMAL ANIMALS.

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THE immediate and most striking effect of insulin when injected into normal animals, is the fall of blood sugar.

Experiments made by one of us in collaboration with Laidlaw, Boock and Trevan [1923] suggest that this disappearance of sugar is not due simply to an increased combustion of carbohydrate, since the output of carbon dioxide and the oxygen consumption of normal animals under the influence of insulin both fall continuously and rapidly to a very low level, while the respiratory quotient, after a short, transient rise also falls. Kellaway and Hughes [1923] studying the normal human subject, which behaves in a similar way, consider that the glucose disappears, not because it is burnt, but rather because it is converted into some complex substance.

The recent suggestion of Dale [1923] that the action of insulin is to shift the incidence of combustion on to the carbohydrate and to inhibit or at any rate spare the normal catabolism of fats and proteins must be borne in mind and experiments to test this hypothesis are being performed.

The evidence, as far as it goes, being rather in favour of the view that the vanished glucose has not left the body as carbon dioxide and water, it was anticipated that possibly the effect of insulin might be to favour the laying down of glycogen by the liver, as is apparently the case in diabetic (depancreatized) dogs [Banting and colleagues, 1922], and that consequently the glucose of the blood might be found as glycogen in that organ. But on putting the matter to the test, it was found that not only is the sugar not stored in this manner, but the livers of normal animals which have received sufficient insulin to bring them to the verge of hypoglycaemic convulsions contain very little glycogen. It appears, in fact, that as soon as the blood sugar falls to a certain level the liver mobilises its glycogen and pours glucose into the blood stream, in an effort to maintain an adequate concentration of sugar there.

The possibility of a transference of glycogen from the liver to the skeletal muscles and a temporary storage of glycogen in them was considered and

investigated. Again, as in the case of the liver, a disappearance and not an accumulation of glycogen was observed. The experiments on this question seem to indicate that the glycogen of the muscles remains more or less unaffected until that of the liver is greatly depleted, and that then the muscle glycogen is called upon to provide glucose for the circulating blood.

The heart muscle, as might be expected, appears to retain its glycogen to the last. It may be mentioned that the fat content of the livers of mice which had been given large doses of insulin was compared with that of the livers of normal mice. No difference either in the quantity of fat or its degree of unsaturation, as indicated by iodine values, was detected. Apparently, then, the effect of insulin is not to cause a conversion of carbohydrate into fat. Further experimental work on the fate of the sugar in normal animals under the influence of insulin is being carried out.

EXPERIMENTAL.

The glycogen content of the livers of normal mice and of mice after insulin.

Three normal mice were killed, the livers rapidly removed and worked up separately by Pflüger's method. The sugar in the hydrolysed fluid was estimated by Shaffer and Hartmann's micro-method [1920].

Weight of mice in g.	Weight of livers in g.	% glycogen in liver
24	1.79	2.02
21	1.47	2.36
18	1.22 (cyst in liver)	0.89

Three mice were each given 1/400 mg. of "insulin picrate" [Dudley 1923] subcutaneously, and were killed immediately on the appearance of convulsions, which happened within an hour of the injection.

Weight of mice in g.	Weight of livers in g.	% glycogen in liver
23	1.17	0.075
21	1.15	
20	1.12	

As preliminary qualitative experiments had shown the disappearance of glycogen from the livers of such mice the three livers were combined and worked up together.

It will be seen that the average glycogen content of these livers is less than one-tenth of the lowest of the three control animals. It should be noted that there was a large cyst in the liver of the third control animal; this may account for the glycogen content of its liver being so much below that of the other two controls, whose livers were quite normal.

This experiment has been performed several times and the result is always the same. A quantitative determination of the glycogen is not necessary to convince one of the main fact of the disappearance of glycogen under the

influence of insulin. On precipitation of the glycogen with alcohol it is at once strikingly apparent that the glycogen of the insulin-treated livers is much less than that of the normals.

Glycogen in the liver and muscles of the normal and insulin-treated rabbit.

Two rabbits, which had been well fed on carrots and beetroot for a couple of days previous to the experiment were used in this experiment. One of the rabbits, weighing 2.3 kilo. received an injection of 10 mg. (10 2-kilo. rabbit units) "insulin hydrochloride" [Dudley, 1923]. It developed hypoglycaemic convulsions six hours after the injection. It was then killed. The other rabbit, weighing 2.8 kilo., which had been kept in a cage without food from the time of the injection of the first rabbit, was killed at the same time. The livers, hearts, and about 50 g. of thigh muscle, were removed from the animals promptly and worked up for glycogen according to Pflüger's method.

The following results were obtained:

	Normal rabbit % glycogen	Insulin rabbit % glycogen
Liver	5.53	1.86
Heart	0.26	0.54
Skeletal muscle	0.57	0.0

It will be noted that the glycogen content of the insulin rabbit's liver is much less than that of the normal, and there was so little glycogen in the insulin rabbit's muscle that it could not be estimated.

The glycogen content of the normal rabbit's heart was only about half that of the insulin rabbit's, but no special significance is attached to this result, since it is known that such differences do occur in normal animals [Abderhalden, 1914], and another determination on a normal rabbit's heart gave 0.57 % glycogen.

The fat content of the livers of normal mice and of mice after insulin.

Thirteen normal mice with an average weight of 20.7 g., the extremes being 25 g. and 18 g., were killed by first stunning and then bleeding by cutting the vessels of the throat. The livers, bulked together, weighed 14.91 g. The tissue was hydrolysed in 60 % KOH on a boiling water-bath, a stream of hydrogen being passed through the flask to prevent oxidation of the unsaturated fatty acids. The hydrolysed liquid was strongly acidified with HCl and the fatty acids were shaken out with ether. The ethereal solution was thoroughly washed with water and then evaporated to dryness *in vacuo*, a brownish crystalline mass being obtained. This was dissolved in 50 cc. of absolute alcohol.

Thirteen mice with an average weight of 21.5 g., the extremes being 24 g. and 19 g., were injected subcutaneously with insulin hydrochloride (1/1000 mg. per gram of mouse) and placed in a room warmed to 28°. In three-quarters of an hour most of the mice were dead and the rest moribund. Their livers were

collected and weighed 18.95 g. The fatty acid was extracted by the same technique as in the case of the normal mice livers and the resultant fatty acid was dissolved in 50 cc. of absolute alcohol.

10 cc. of each of the solutions of fatty acid were titrated with alcoholic $N/10$ KOH using phenolphthalein as indicator and the amount of KOH required was practically identical in each case; namely, 5.4 cc. for the control and 5.32 cc. for the insulin experiment.

The remaining 40 cc. of alcoholic solution were in each case evaporated to dryness and the residues were taken up in chloroform.

Iodine values were determined by Hübl's method. There was no significant difference in the volumes of $\text{Na}_2\text{S}_2\text{O}_3$ required to titrate the excess iodine.

A comparison of the weights of the liver taken in these experiments is not justifiable since the insulin livers, which weighed considerably more than the normal livers, were obviously gorged with blood. The total weight of the normal mice was 269.1 g. and that of the insulin mice was 279.5 g. It is therefore a fair assumption that the total weight of liver tissue taken in the two experiments was the same within, say, 5 %. Therefore, as the amounts of fatty acid obtained were practically identical in both cases, the experiment shows satisfactorily that the sugar which disappears under the action of insulin is not converted into fat in the liver.

We are greatly indebted to Dr H. H. Dale for valuable help and advice during the course of these experiments.

Since this paper was written we have learned that Professor Babkin, working independently at University College, has been studying the same problem, and that his results are in agreement with ours.

SUMMARY.

1. The sugar which disappears from the blood of normal animals under the influence of insulin is neither converted into nor stored as glycogen either in the liver or in the skeletal muscles.
2. The glycogen of both liver and skeletal muscle disappears almost completely in animals which are given a dose of insulin sufficient to cause hypoglycaemic convulsions.
3. There is no evidence of a conversion of carbohydrate into fat in normal animals under the influence of insulin.

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