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GLYCOGEN IN ADIPOSE TISSUE

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When rats are fed on a carbohydrate-rich diet after prolonged starvation, appearance of glycogen within adipose tissue is an early indication that deposition of fat is impending. Other findings also suggest that glycogen is converted in the adipose tissue into fat (Tuerkischer & Wertheimer, 1942; Mirski, 1942). On this view it is expected that glycogen will precede fat deposition in adipose tissue whenever newly synthesized fat, derived from carbohydrate, is stored. The present paper describes the continuation of experiments on the relation between glycogen and fat within adipose tissue.

I. *Insulin treatment.* Clinicians have long assumed that insulin favours deposition of fat. The effect is generally believed to be an indirect one due to a stimulation of the appetite. Drury (1940) and MacKay, Callaway & Barnes (1940) showed that insulin increased appetite, body weight, and fat storage in rats and rabbits. Is such fat deposition under insulin treatment preceded by deposition of glycogen within the adipose tissue? Is the insulin effect direct, or only secondary to increase in appetite for carbohydrate? As early as in 1927 Hoffmann & Wertheimer demonstrated that insulin sometimes induces glycogen deposition in the adipose tissue of dogs. Insulin was found to be without effect on glycogen deposition in the adipose tissue of rats fed a carbohydrate-rich diet after being starved (Tuerkischer & Wertheimer, 1942).

II. *Alternate starving and feeding.* MacKay & Drury (1941) have shown that, when rats are fed on a diet consisting almost entirely of carbohydrates and starved on alternate days, so that their average body weight and food intake become constant, the food carbohydrate comes to be stored largely in the form of fat. Is fat deposition in this case also associated with the appearance of glycogen in adipose tissue?

III. *Carbohydrate excess.* Is glycogen deposited in the adipose tissue after excessive intake of carbohydrates?

Methods

The rats used were of laboratory stock. As a rule, young male animals weighing 80–110 g. were selected. They were maintained on a standard diet consisting of wheat and vegetables or on a carbohydrate-rich synthetic diet (70% carbohydrate, 20% casein, 10% fat with the usual

supplements of minerals and vitamins). In order to make sure that the rats were showing a regular increase in weight, they were weighed daily for about a week before their use in an experiment. The room temperature was not allowed to fall below 20–21° C. and large fluctuations were carefully avoided. Glycogen deposition in adipose tissue following insulin administration was easier to demonstrate in summer than in winter. In winter experiments a higher insulin dose was found to be necessary. Protamine-Zinc-Insulin (PZIns.) was more effective than ordinary insulin.

The following conditions proved suitable: the rats were given a first dose of PZIns. in the morning. After 3–4 hr. a second injection was given. Six to eight hours after the first insulin injection the rats were killed.

The adipose tissue was treated in the manner described by Tuerkischer & Wertheimer (1942). Groin, testicle and perinephric fat were pooled and weighed and are referred to as 'mixed fat'. Fat from mesentery and brown interscapular fat were weighed and treated separately (Hook & Barron, 1941). For glycogen determination, samples were taken as follows: 'mixed' fat 0·5 g., mesentery fat 0·2 g., interscapular fat 0·1–0·2 g. Chemical procedures employed have been already described (Tuerkischer & Wertheimer, 1942). Glycogen values are given as g. glucose per 100 g. fresh tissue.

The experiments in series 1, 2 and 3 were carried out in summer, those in series 4 to 7 in winter and spring at a room temperature of 22 to 23° C.

1. In this series of 11 experiments the rats were given 25 % glucose solution to drink during the 24 hr. period before insulin injection. During the time of insulin action normal diet and tap water were allowed. The dose of insulin was $2 \times 0{\cdot}3$ units PZIns. Included in this series were 4 experiments comparing the reaction of young and old rats. The old rats had an average weight of 180 g.

2. In 10 experiments the rats received 3·5 c.c. 25 % glucose by stomach tube just before the first insulin injection. Otherwise the experimental conditions were as in series 1.

3. In 19 experiments the rats were given 25 % glucose solution to drink during the experimental period. The other experimental conditions were unchanged.

4. In 11 experiments the conditions were as in series 3 but the dose of insulin was 2×3 units PZIns.

5. In 19 experiments the rats received only tap water to drink and the dose of PZIns. was 2×1 units.

6. In 13 experiments the conditions were as in series 5 but the rats were on three different diets: (*a*) 70 % casein, 20 % carbohydrate, 10 % fat with the usual supplements; (*b*) 50 % fat, 25 % carbohydrate, 25 % casein; (*c*) 70 % casein, 30 % fat.

7. In 5 experiments the conditions were as in series 6 (*a*) but the insulin dose of $2 \times 0{\cdot}3$ units PZIns. was given after a 20 hr. fast. The rats were killed 5 hr. later.

Results

I. *Influence of insulin on glycogen deposition in adipose tissue*

Table 1 gives a typical result for series 1 to 6 (*a*) inclusive. In 6 (*b*), 6 (*c*) and in 7 glycogen was found only in the interscapular fat. When insulin administration (2–6 units PZIns. daily in winter experiments) was prolonged for from 2 to 9 days, and normal diet was allowed, the occurrence of glycogen in adipose tissue became a constant feature. This was observed in 36 experiments. In similar experiments with rats maintained on the 70 % casein diet, glycogen deposition in the adipose tissue of the same order was observed only on the first and second days of the experiment.

The rise and fall of the glycogen level in adipose tissue was investigated in 275 experiments. Glycogen deposition appears in the interscapular fat as

TABLE 1. Glycogen deposition in adipose tissue after administration of protamine-zinc-insulin

	Intake of glucose sol. (c.c.)	Glycogen, g./100 g., in			Blood sugar mg./100 c.c.
		Interscap. fat	Mesent. fat	Mixed fat	
Insulin	23	0·30	0·21	0·11	71
Control	27	0·03	Traces	Traces	112

early as about ¾ hr. after administration of 1–3 units PZIns. The curve of the glycogen deposition rises with the fall of the blood sugar, reaches its peak at about 10–14 hr., and falls to zero again after 16–20 hr. with the return of the blood sugar to normal. Glycogen deposition in the 'mixed' and in the mesentery fat begins only at 4 hr. after insulin administration, and follows in its rise and fall the curve of the glycogen deposition in the interscapular fat. The glycogen values in the 'mixed' and the mesentery fat were much smaller than in the interscapular fat. With normal insulin the glycogen curves returned to zero sooner than with PZIns.

All time-curves on glycogen deposition were carried out in summer experiments.

Control tests were carried out at different times on 45 untreated rats. The values obtained in the controls for 'mixed' fat and for mesentery fat were uniformly nil; for interscapular fat a single value of 0·05% and one of 0·2% were recorded. The findings, with data published in our earlier paper (Tuerkischer & Wertheimer, 1942), support the conclusion that, in normal conditions, glycogen very rarely occurs in adipose tissue.

Do carbohydrates other than glycogen appear in adipose tissue following insulin injection? Both glycogen and total carbohydrate were determined in adipose tissues following insulin injection. The method of West, Scharles & Peterson (1929) was employed. Analyses were carried out on 'mixed' fat both before and after glycogen begins to appear as the result of insulin injection. In all cases the carbohydrate was present as glycogen only.

Glycogen in adipose tissues of herbivores after insulin treatment. In rabbits and guinea-pigs maintained on an ordinary diet (bran, oats, vegetables), insulin treatments (3–12 units PZIns. per kg.) failed to induce glycogen deposition in the adipose tissues. MacKay *et al.* (1940) have emphasized that 'the rabbit is not a very good animal for the type of experiment to gain much weight with insulin administration. The reason for this is the low energy value of its food when calories per unit volume are considered'. According to Long & Bischoff (1930) insulin treatment does not induce increase of body weight in rabbits.

If, however, rabbits are given a diet rich in concentrated carbohydrates, a very small deposition of glycogen can be observed in the adipose tissue. Following insulin administration the amount of glycogen in the adipose tissue is doubled.

The following procedure was found most suitable: small thin rabbits weighing about 1 kg. were given 30–40 c.c. 25% glucose solution by stomach tube and 10 units PZIns. per kg. body weight in the morning; 3 hr. later the insulin injection was repeated. After 5–7 hr. the animals were killed.

In fat animals the glycogen values were lower. In two cases no definite difference between the experimental animal and the control could be established. The results of the analyses are summarized in Table 2.

TABLE 2. Mean values for adipose tissue glycogen in rabbits treated with protamine-zinc-insulin

	No. of expts.	Glycogen, g./100 g., in				Blood sugar mg./100 c.c.
		Interscap. fat	Mesent. fat	Mixed fat	Liver	
Insulin	14	0·12±0·010	0·09±0·017	0·10±0·007	7·0	97
Controls	15	0·06±0·007	0·05±0·009	0·05±0·006	7·3	128

II. *The effect of alternate starving and feeding*

Rats were subjected to the MacKay & Drury regimen of alternate starving and feeding. Animals which failed to maintain their weight were eliminated from the tested group. Experiments were performed in winter. Table 3 gives the results.

TABLE 3. Glycogen in adipose tissues of rats maintained on a McKay-Drury regimen and killed 6 hr. after recovery feeding

Mean values for 3 animals in each case

No. of fast days	Glycogen, g./100 g., in		
	Interscap. fat	Mesentery fat	Mixed fat
1	0·52±0·16	0·10±0·02	0·08±0·03
2	2·20±0·59	0·21±0·09	0·25±0·07
3	2·64±0·35	0·35±0·07	0·23±0·10
4	2·48±0·56	0·52±0·09	0·33±0·11
5	2·70±0·35	0·55±0·18	0·56±0·12
6/7	1·12±0·24	0·52±0·17	0·17±0·05

In order to time the glycogen deposition, groups of rats which had completed their fourth fast day on the MacKay & Drury regimen were killed at different times after feeding. The results of an experiment carried out in winter are presented in Table 4.

TABLE 4. Glycogen in adipose tissues of rats killed at different times after recovery feeding in the MacKay-Drury test

Mean values for 3 animals in each case

Time after recovery feeding	Glycogen, g./100 g., in		
	Interscap. fat	Mesentery fat	Mixed fat
30 min.	0·14±0·04	0	0
1 hr.	0·70±0·16	0	0
2 hr.	0·96±0·08	0	0
4 hr.	1·90±0·12	0·23±0·08	0·08±0·01
6 hr.	2·15±0·38	0·52±0·15	0·22±0·10
1 day	0·61±0·11	0·26±0·08	0·37±0·17
2 days	0·40±0·05	0·21±0·06	0·23±0·05
3 days	0·50±0·28	0·08±0·03	0·08±0·02
4 days	0	0	0

In summer, higher values were obtained. In five summer experiments, after 4 days fast and in the sixth hour after feeding, glycogen values (g./100 g.) were found as follows: 'mixed' fat 0·58, mesentery fat 0·82, interscapular fat 4·3.

If animals in the MacKay-Drury test after 4 days fast are fed and then again starved, a rapid disappearance of glycogen from the adipose tissues occurs. Following 6 hr. of fast after 6 hr. of feeding, glycogen values (g./100 g.) were obtained as follows: 'mixed' fat 0·15, mesentery fat 0·06, interscapular fat 0. If the fast was prolonged to 12 hr. all the values were zero. There were three rats in each experiment. Experiments showing the effect of different duration of fast and of feeding on glycogen in adipose tissue are assembled in Table 5.

TABLE 5. Glycogen deposition in adipose tissue after different fast and feeding periods
Mean values for 3 animals in each case

Fast period hr.	Hours after end of starvation	Glycogen, g./100 g., in		
		Interscap. fat	Mesentery fat	Mixed fat
24	6	0·53±0·10	0·10±0·04	0·08±0·03
	24	0·39±0·08	0·08±0·04	0·16±0·03
	32	0·09±0·05	0·08±0·03	0
10	6	0·40±0·04	0·22±0·05	0·08±0·03
	24	0	0	0
6	6 and 24	0	0	0

In all the experiments the fast periods were arranged so that they occurred at night. The findings in experiments according to MacKay & Drury (1941), as well as those with prolonged hunger (Tuerkischer & Wertheimer, 1942), support the conclusion that the quantity and duration of the glycogen deposition in the adipose tissues following feeding vary positively with the length of the preceding hunger period, i.e. with the amount of the fat loss and the corresponding increase in fat-storage capacity.

In the MacKay-Drury test, as well as in the earlier experiments of Tuerkischer & Wertheimer (1942), fat of adrenalectomized rats in good condition was found to be practically free from glycogen. Adipose tissue glycogen deposition in rats kept on a B_1-free diet is diminished.

III. *Carbohydrate excess*

A brief period of glycogen deposition in the adipose tissue can be distinguished in rats maintained on a diet excessive in carbohydrates and which are not starved at all. Carbohydrate saturation is most easily effected by giving rats, on an ordinary diet, 25% glucose solution, rather than water, to drink. The clearest results were obtained in experiments of 6–8 hr. duration. The majority of the experiments were performed in winter. The results are shown in Table 6.

Table 6. Mean values for glycogen in adipose tissue after excess intake of carbohydrate

No. of expts.	Intake of sugar sol. c.c.	Glycogen, g./100 g., in Interscap. fat	Mesentery fat	Mixed fat	Blood sugar mg./100 c.c.
15	15	0·24±0·05	0·15±0·03	0·17±0·04	119

After excess intake of carbohydrate had been continued for 12–24 hr. glycogen was rarely detected in adipose tissue. In summer the amount of glycogen deposition in the adipose tissues following excess intake of carbohydrates was smaller.

Discussion

Tuerkischer & Wertheimer (1942) suggested that glycogen *always* accumulates in adipose tissue whenever synthesis of fat from carbohydrate occurs. The simplest example of this is found in chronic underfeeding followed by recovery-feeding with carbohydrate. Further examples have been described in the present communication. MacKay & Drury (1941) found that animals, which are alternately starved and fed, store carbohydrate temporarily as fat. This storage too is associated with deposition of glycogen in adipose tissues. A fleeting deposition of glycogen in adipose tissue can be induced even without preceding fasting if a large excess of carbohydrates is fed.

MacKay, Barnes & Carne (1941) showed that rats fed on a carbohydrate-rich concentrated diet, and treated with Protamine-Zinc-Insulin, react with marked increase in appetite, in body weight and in fat deposition. In this condition of fat synthesis from carbohydrate, glycogen also appears within the adipose tissues. The findings seem to be best explained by the assumption that glycogen is an intermediary in fat synthesis from carbohydrate in adipose tissues.

A further question to be considered is whether the effect of insulin on the fatty tissue is direct or indirect. The marked increase in appetite which insulin produces might suggest that insulin acts merely by increasing carbohydrate intake. This conclusion is at variance, however, with the following: 1. The effect of insulin is frequently demonstrable in the interscapular fat within about 45 min. of insulin injection, i.e. before any effect on appetite has become apparent and certainly before any such effect could become of importance. 2. MacKay *et al.* (1941) have shown that insulin fails to induce increased appetite and food intake in rats maintained on a protein-rich diet; nevertheless, glycogen is deposited in the adipose tissues in these conditions. It occurs, moreover, in the interscapular fat also in rats starved for 24 hr. after protein rich feeding. 3. In adrenalectomized animals, insulin fails to increase the carbohydrate appetite. Nevertheless, glycogen deposition in the adipose tissue of adrenalectomized rats, following insulin injection, is especially marked. It is proposed to discuss this result further in a succeeding communication.

It may be concluded therefore that the influence of insulin is primary and direct on glycogen synthesis by the adipose tissue. The reactivity in this respect is a new proof of the active role of the adipose tissue in the carbohydrate-fat exchange. On quite different grounds Longenecker (1941) writes: 'The recognition that the fat depots are centres of continuous metabolic activity represents a fundamental change in the conception of a tissue which previously had been considered inert.'

A third question to be considered is whether the present findings have any special bearing on the role of insulin in carbohydrate balance. Glycogen disappears from the adipose tissue simultaneously with the disappearance of the insulin effect on the blood sugar level and reappears only following a renewed insulin stimulus. It has also been shown that prolonged insulin action induces an increase in the fat deposits. Experiments *in vitro* by Mirski (1942) give strong support to the view that glycogen within the adipose tissue is converted into oxygen-poor substances, probably fatty acids. We assume that the amount of glycogen found in the adipose tissue is merely the resultant of simultaneously proceeding processes of both synthesis and breakdown. The conversion of carbohydrate into fat, a uniform accompaniment of a carbohydrate-rich diet, has not yet been given sufficient consideration. Pauls & Drury (1942) concluded as follows: 'Most ingested carbohydrate is normally stored as fat, and it is considered the most likely possibility that this is a fate of the bulk of the sugar stored under the influence of insulin.'

Schur, Loew & Krcma (1934), even before this, pointed out the role of insulin as a hormone regulator of nutrient storage. The few experiments carried out, however, did not prove this conclusion.

Summary

1. In suitable conditions, insulin induces synthesis of glycogen in the adipose tissues of the rat. The synthesis is particularly rapid and marked in the brown interscapular fat. Glycogen disappears from fatty tissues simultaneously with the disappearance of the insulin effect on the blood sugar level. Synthesis of glycogen in adipose tissues can also be demonstrated in the rabbit, but is less marked.

2. It is shown that insulin directly affects synthesis of glycogen in adipose tissue and does not act through an effect on carbohydrate food intake.

3. It is considered that synthesis of glycogen in adipose tissues, under the influence of insulin, is a primary step in the transformation of carbohydrate into fat.

4. In animals which are alternately starved and fed (MacKay & Drury, 1941), deposition of fat has been found to be preceded by deposition of glycogen in the adipose tissues.

5. In animals first starved and then fed, the amount and the duration of glycogen deposition in the fat tissues depend on the length of the fast period.

6. In rats, which have not been starved, administration of an excess of carbohydrate frequently induces brief deposition of glycogen in fatty tissues.

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