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GLYCOGEN AND ADIPOSE TISSUE

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It has been shown by Hoffmann & Wertheimer [1927] and by Wertheimer [1928] that rats or dogs which are placed after fasting on a diet rich in carbohydrates transiently store a polysaccharide in their adipose tissue. The polysaccharide is precipitable by the method of Pflüger and is presumably glycogen. Recent investigations [Loew & Krćema, 1929; Scoz, 1932*a*; Schoenen, 1932; Wetzel & Held, 1936; Hausberger & Gujot, 1937; Hausberger & Neuenschwander, 1939] have confirmed and added to this finding.

The experiments described in the present paper complete and extend the earlier investigations and deal more particularly with (1) the identification of the polysaccharide deposited in adipose tissue as glycogen, (2) the particular factors which induce the storage of glycogen in fatty tissue, and (3) the ultimate fate of adipose tissue glycogen in vivo.

Methods

Experiments on an extensive scale could only be carried out with rats. These were of the laboratory breed and were fed until the beginning of the experiment on the ordinary oat, bran and vegetable diet. Dogs deposit relatively greater quantities of glycogen in adipose tissue, but are comparatively difficult to maintain under standardized conditions. Pflüger's micro-method for the determination of glycogen was found to be applicable to adipose-tissue glycogen. Sugar was determined after hydrolysis according to the method of Folin-Wu. The results are presented as g. glucose/100 g. fresh tissue. Total carbohydrate content was determined in fatty tissue by the method of West, Scharles & Petersen [1929]. Phosphate was determined by the method of Lohmann & Jendrassik [1928].

For purposes of comparison, series of experiments were necessary to define the conditions under which glycogen storage in adipose tissue was maximal. It was found that in general young animals gave the best results. Prolonged, partial starvation was more effective than a brief but complete fast. Fasted, sexually mature females may contain much more fat than males. Standard experiments were therefore conducted as follows: young male rats weighing 95–120 g. were maintained for 7–10 days on a diet which induced a loss of body weight of about 20%. The rats lost about 2–3 g. daily. Greater weight losses were undesirable. For some time subsequently the rats were allowed to consume as much food as they desired of the following composition: carbohydrate, 70% (45% starch and 25% cane sugar); protein (casein), 20%, fat, 10%; mineral salt mixture, 4 g.; dry yeast, 2 g.; cod-liver oil, 25 drops. Glycogen was estimated in groin, in testicle and perirenal adipose tissue.

All three types of fatty tissue were quantitatively removed and carefully mixed on an ice-cooled plate. Weight was determined by a hand balance. In a few cases separate analyses of each of the stated types was undertaken, but showed no definite and reproducible differences between them. Interscapulary fat, the so-called brown, fat body, was separately investigated.

Results

Identification of the polysaccharide in adipose tissue

The adipose-tissue polysaccharide could be readily and completely hydrolysed to a fermentable monosaccharide by $N/1$ H_2SO_4. The iodine stain of the purified polysaccharide was identical with that given by similarly purified liver glycogen. A sample of a material purified by Kerr's method [1938] gave the following analysis: Optical rotation determined in 0·87% solution in $\frac{1}{2}$ dcm. tube: $[\alpha]_D^{20} + 201$; on hydrolysis 0·0213 g. gave 0·020 g. sugar; nitrogen and P_2O_5 absent. A sample purified according to Somogyi's method [1934] gave the following values: $[\alpha]_D^{20} + 196$; 0·0696 g. on hydrolysis with $D/1$ H_2SO_4 gave 0·0697 g. glucose by the method of Somogyi. In view of these data the polysaccharide deposited in adipose tissue of fasted animals placed on a carbohydrate-rich recovery diet is glycogen.

To determine whether glycogen in adipose tissue was easily soluble (lyo-glycogen), or was closely bound to the tissue (desmo-glycogen), the method of Willstätter & Rohdewald [1934] was used. The following results are typical: a dog was maintained for a week on a starvation diet and was then given a carbohydrate-rich ration during 2 days. Analysis

gave: total fat-tissue glycogen, 1·06%; desmoglycogen, 0·615%; lyoglycogen, 0·32%. It is therefore clear that the greater part of the glycogen in fat tissue is in a closely bound form.

Glucose was only demonstrable in the fatty tissue in traces, if at all. Its presence was questionable, even after incubation under conditions which favour glucose formation. In a series of experiments parallel determinations were made of glycogen and of total carbohydrate by the method of West *et al.* [1929]. Typical data are presented in Table 1.

TABLE 1. Glycogen and total carbohydrate content of adipose tissue

Glycogen %	Total carbohydrate %	Remarks
0·76	0·85	Tissue left for 3–5 min. on ice before analysis
0·96	1·06	
0·17	0·21	
0·32	0·35	
0·35	0·41	
0·19	0·26	
0·59	0·61	Tissue immersed in hot KOH solution for glycogen determination immediately after death
0·42	0·42	
0·60	0·66	

The difference between the glycogen and total carbohydrate level is very small only if the analysis is carried out immediately after death. It may therefore be assumed that much and possibly all the carbohydrate, other than glycogen, is formed after removal of the adipose tissue from the parent organism.

Storage of adipose tissue on ice for 15 min. caused a 12–20% decrease in glycogen content and a corresponding increase in the quantity of unknown carbohydrate. The nature of the latter product will be considered separately. In any case, the greater part of the carbohydrate material deposited in fat tissue during recovery feeding is clearly glycogen.

Occurrence of glycogen in adipose tissue

Scoz [1932*b*] claims that glycogen and glucose are normal constituents of fatty tissue. He emphasizes in particular that glycogen in this tissue is also increased when the metabolism of the organism is changed over from the state of equilibrium to one of hunger and mentions the possibility that adipose tissue fat may be convertible into carbohydrate. Adler-Mönnich & Tiberi [1937] claim furthermore that sugar formation from fat can be obtained with adipose tissue in vitro. Experiments concerning these questions are dealt with in Table 2.

The term 'traces' is used in the table to indicate a glycogen content of less than 0·03%. The experiments showed that when rats are either fasted, maintained continuously on a carbohydrate-rich diet, transferred

TABLE 2. The influence of diet on the glycogen and total carbohydrate content of adipose tissue

Series	No. of exps.	Diet	Glycogen	Total carbohydrate
1	10	Continuous basic carbohydrate ration 8 days	Traces	Traces
2	6	As in (1), then fasted for 3 days	,,	,,
3	6	As in (1), then maintained on a starvation diet until loss of body weight was 20%		
4	6	Thyroxine continuously administered to a total dose of 4 mg. in 10 days. Phloridzin-hunger. Dinitrophenol in a dose of 30 mg. per 100 g. body weight	,,	,,
5	5	Continuously maintained on a ration composed of 70% casein, 20% carbohydrate and 10% fat or meat	,,	,,
6	5	Maintained for 6 days on a ration as in (5), then 2 days on ration as in (1)	,,	,,

from such a diet to a starvation ration, or changed from a protein-rich to carbohydrate-rich diet, neither glycogen, nor any other polysaccharide is formed in the adipose tissue in appreciable and definitely demonstrable quantities. Even when intensive formation of carbohydrate is necessary for the organism, e.g. as in thyroxine, phloridzin, or dinitrophenol poisoning, no increase of carbohydrate in the fatty tissue was demonstrable.

It may be stated in the light of all the experiments now available that glycogen deposition only occurs in the adipose tissue of rats which show an increase in body weight, when placed on their normal diet after a prolonged fast.

Time relationship between recovery feeding and deposition of glycogen in adipose tissue

Under standard conditions, as described above (loss of 20% body weight in fast followed by recovery on 70% carbohydrate diet), glycogen deposition in adipose tissue takes the time course shown in Table 3. These results suggest that the glycogen formed during recovery feeding after starvation has a specific and limited role to play, and that this role is limited to a restricted time, after which glycogen is removed from the adipose tissue through the establishment of a new state of equilibrium.

The loss in weight which is necessary for the deposition of adipose-tissue glycogen need not be brought about simply by hunger. Glycogen deposition will also occur on addition of 8% dry yeast to the ration, after a 20% loss in weight has been brought about by absence of vitamin B

TABLE 3. Glycogen percentage in adipose tissue and liver after recovery diet

Duration of recovery diet ...	0	1–2 hr.	5 hr.	1 day	2 days	3 days	4 days	5 days	6–8 days
No. of exps.	8	8	7	28	10	7	7	8	8
Glycogen in adipose tissue	Tr.	0·05	0·24	0·60	1·05	0·48	0·03	0·18	Tr.
Liver glycogen	—	3·0	5·2	6·8	3·2	3·5	3·7	2·7	2·8

Glycogen percentage in interscapular fat

Duration	0	1–2 hr.	5 hr.	1 day	2 days	3 days	4 days	5 days	6–8 days
No. of exps.	6	4	11	26	15	6	5	—	8
Glycogen in interscapular fat	Tr.	0·37	1·02	1·48	0·90	0·56	0·22	—	Tr.

Glycogen percentage in adipose tissue after vitamin B-free diet

Duration	0	5 hr.	1 day	2 days	3 days	4 days	5 days
No. of exps.	8	2	4	3	5	3	2
Glycogen in adipose tissue	Tr.	Tr.	Tr.–0·2	0·45	0·54	0·19	Tr.
Liver glycogen	0·82	—	—	4·4	4·0	2·35	—

complex (Table 3). Glycogen deposition in this case is delayed. Possibly during the first day after yeast is added a mechanism, which was paralysed by the absence of vitamin B, may be set in motion within the adipose tissue [McHenry & Gavin, 1938].

The influence of fat-soluble vitamins A and D on glycogen and fat deposition in adipose tissue was also studied. Young rats of 80–100 g. were maintained on a vitamin A and D-free ration until growth disturbances were definitely evident. The animals were then given a vitamin A and D-free starvation diet, and so maintained until they had lost about 15% of their weight. They were then transferred to a carbohydrate-rich vitamin A and D-free ration. In five experiments it was uniformly observed that a normal and adequate deposition of glycogen and fat in adipose tissue had occurred. The amount of glycogen deposited after 6 hr. was 0·7% in the adipose tissue and 2·2% in the interscapulary fat.

Deposition of glycogen after starvation is far more marked and occurs more rapidly in the brown fat body than in other fatty tissue (Table 3). In this respect it is comparable to liver, with the difference, however, that brown fat tissue is already empty of glycogen within 4–5 days.

It was noted that the glycogen levels attained in adipose tissue are subject to seasonal fluctuations. In winter lower levels were noted than in summer (June to November), and deposition of glycogen only began 5 hr. after feeding, though otherwise the time course of deposition was unchanged. At the commencement of summer the glycogen values of adipose tissue were particularly high. In ten experiments in May the

average glycogen deposition after 1 day recovery feeding was 1·18% for adipose tissue, and 4·2% for interscapulary fat, most of the values for the latter being between 4 and 6%.

Influence of diet on glycogen deposition in adipose tissue

Experiments with different diets are set out in Table 4. On a predominantly protein ration given after fasting, glycogen deposition in adipose tissue does not occur. Three separate high-protein rations gave

TABLE 4. Influence of diet on glycogen deposition in adipose tissue

Diet	No. of exps.		Adipose tissue glycogen %		Liver glycogen %		Remarks
	1 day	2 days	1 day	2 days	1 day	2 days	
70% carbohydrate 20% casein 20% fat	10	6	0·58	1·00	6·8	3·2	
40% carbohydrate 40% casein 20% fat	5	5	0·20	0·17	3·3	1·2	
40% carbohydrate 40% fat 20% casein	5	5	0·09	0·10	1·2	1·65	
70% casein 20% carbohydrate 10% fat	4	7	0·02	0·05	2·85	1·9	After 3, 4, 8 days only traces of adipose-tissue glycogen
85% casein 15% carbohydrate	4	4	Tr.	0·034	—	—	
Meat	2	2	Tr.	Tr.	—	—	
85% carbohydrate 15% fat	5	5	0·30	0·215	7·9	4·1	
1·75–3·2 g. glucose by stomach tube	6 (6–8 hr.)		Tr.		2·6		

this result: (1) 70% casein, 20% carbohydrate and 10% fat; (2) 85% casein, 10% carbohydrate and 5% fat; (3) meat only. It was further found that on a ration containing protein and carbohydrate in equal proportions (45% protein, 45% carbohydrate and 10% fat), glycogen deposition in adipose tissue is much reduced. The same result was obtained with a ration containing carbohydrate and fat in equal proportions (40% fat, 40% carbohydrate and 20% casein). However, in this latter case it was probable that the deposition of glycogen was being inhibited by the deposition in the adipose tissue of fat from the ration itself. With a ration rich in carbohydrate, poor in fat and lacking in protein (85% carbohydrates, 15% fat), glycogen deposition in the adipose tissue was considerably less than on the standard diet.

Large quantities of glucose (1·75–3·2 g.) administered by stomach tube after fasting, though absorbed rapidly, induced no deposition of

glycogen in ordinary fatty tissue within 6–8 hr. and only a slight glycogen deposition (0·5%) in the interscapulary fatty tissue. Corresponding control experiments using 0·9% NaCl solution instead of glucose did not influence deposition of glycogen in adipose tissue.

In vitro experiments

An attempt was made to obtain a deposition of glycogen in adipose tissue from fasted animals in vitro. Various conditions were tried. Unless otherwise specified, the experiments were carried out in a water bath at 37° C. under continuous passage of oxygen. The suspension medium was phosphate-Ringer, as indicated by Krebs. In parallel experiments tests for the appearance of glucose in the suspension medium were carried out. Synthesis of glycogen or production of sugar could not, however, be demonstrated in vitro. A finely minced suspension of 0·4 g. adipose tissue from a fasted rat in 2 c.c. phosphate-Ringer solution was used for each test in the following series:

(1) Adipose tissue and 10 mg. glucose, incubated for ½ and for 4 hr. with or without hexokinase (as in experiments on synthesis of muscle glycogen by Willstätter & Rohdewald [1940]) and with or without addition of either monoiodoacetate 1 : 1500, or KCN 1 : 2500 and 1 : 5000.

(2) Adipose tissue and either *M*/50 pyruvate, lactate, or butyrate, incubated for 4 hr.

(3) Adipose tissue and 10 mg. Cori ester, incubated for 15 min., 2 hr. or 20 hr., with, or without addition of *M*/200 NaF (as in the experiments of Ostern, Herbert & Holmes [1939]).

An attempt was then made to obtain deposition of glycogen in fatty tissue by perfusion experiments, in which 0·5% glucose-phosphate-Ringer solution was added to blood in the proportion of 2 : 1. In cats perfusion was effected via the aorta and in rats via the cava. The perfusion fluid was saturated with oxygen. Glycogen deposition could not, however, be demonstrated.

Deposition of fat in adipose tissue during recovery feeding on a carbohydrate-rich diet after fasting

Rats, maintained under the standard conditions already described, were fasted, and after various intervals of recovery feeding, killed and examined. The groin, testicle and perirenal adipose tissue was carefully collected and weighed. The results are summarized in Table 5.

These results, together with those in deposition of glycogen, indicate that a correlation probably exists between the deposition of fat and

TABLE 5. Deposition of fat in adipose tissue. Results expressed as g./100 g. body wt.

No. of exps.	Nutrition	Wt. of fat g.
34	Fast	1·02
32	Standard ration: 1 day	1·33
23	2 days	1·65
7	3 days	1·86
14	4 days	2·52
19	5–10 days	2·75
7	without fast	2·90
7	70% casein 2 days	0·94
6	70% casein 4 days	1·50
6	70% casein 9 days	1·88
9	40% casein 2 days	1·25
10	40% fat 6–10 days	2·70

glycogen in adipose tissue. Deposition of fat is definitely demonstrable 1 day after recovery feeding, continues on subsequent days side by side with glycogen deposition, attains a maximum on the 4th day, when the main deposition of glycogen is already at an end and is almost negligible in subsequent periods when glycogen deposition is both small and uncertain. The disappearance of adipose glycogen may thus be said to mark the re-establishment of a fat equilibrium in the organism. It is remarkable, indeed, that within 4 days, and after a considerable loss of body weight, the fat depots of rats on a standard diet should be fully replenished and that, moreover, the completion of this process should coincide with that at which deposition of glycogen in the adipose tissue ceases. When rats were maintained on a protein-rich ration, the course and character of fat deposition were markedly different. Fat deposition was delayed and the amount was smaller than under standard conditions. With the high fat ration the amount of adipose fat deposited after 6–10 days equalled that found on the standard ration. In this case deposition in adipose tissue of fat from the ration itself may be assumed to have taken place.

Glycogen and fat deposition in the adipose tissues of herbivora

The course of deposition in guinea-pigs and rabbits is very different from that found in rats, dogs, and cats (unpublished experiments). Experiments with the herbivores followed the exact procedure described above for rats. The results are given in Table 6. The low total values, irregular nature and considerable extension of the period of glycogen deposition in guinea-pigs are striking. The maximum values were attained on the average after 5 days; zero value was only attained after 2 weeks of recovery diet. The comparatively uniform liver and muscle glycogen values throughout the period of recovery feeding averaged 7·2 and 0·84%

TABLE 6. Glycogen deposition in the adipose tissue of guinea-pigs and rabbits

(*a*) Guinea-pigs.

Recovery diet days	Gain in body wt. g.	Glycogen %			Exps. with negative glycogen deposition	Exps. with significant increase of fat in adipose tissue	No. of exps.
		Groin fat	Abdominal fat	Inter-scapular fat			
1	36	0·12	—	0·16	1	—	5
2	40	0·10	0·10	0·22	1	—	5
3	52	0·23	0·08	0·07	1	—	4
4	54	0·10	0·11	0·07	2	—	5
5	56	0·30	0·37	0·30	—	1	5
6	70	0·15	0·23	0·14	—	3	5
7–8	79	0·16	0·15	0·24	—	5	5
9–11	89	0·18	—	0·15	—	4	4
12–13	97	0·11	—	0·08	2	5	5
14	110	Tr.	—	Tr.	3	3	3
(*b*) Rabbits.							
1	168	0·16	—	0·23	0	—	5
2	172	Tr.	—	0·13	3	—	5
3	184	0·05	—	0·052	2	—	4
4	184	0·05	—	0·03	2	—	4
5	247	0·042	—	0·06	2	—	4
6–10	266	Tr.	—	Tr.	6	—	6

respectively. Fat deposition in guinea-pigs is much less regular and in the first days so small as to be insignificant. Appreciable fat increments in adipose tissue were only recorded after a week of recovery feeding.

In rabbits glycogen deposition is smaller and even less regular. Glycogen deposition was uniformly recorded here only in the first day of recovery feeding. Fat deposition was so uncertain as to render any quantitative expression of the results impossible.

Stimulation of adipose tissue

It has been shown already [Wertheimer, 1926, 1927] that scission of suitable nervous connexions renders popliteal fat relatively immune to exploitation during phloridzin starvation. Hausberger [1935] was able to cut the nerve supply of the interscapular fat body of the mouse on one side only, and showed that an increase of glycogen and in its wake an accumulation of fat already became noticeable in the denervated half of the interscapular fat body 10 hr. after denervation. The fat content of the denervated side exceeded that of the control side also during starvation. Beznák & Hasch [1937] have made similar observations.

In view of the technical difficulty of isolating and stimulating the nerve supply of the fat body, experiments on the influence of a direct, purely mechanical type of stimulus, viz. massage, seemed desirable. Groin fat tissue was chosen for these experiments. One side of the groin was massaged, while the second served as a control. Each massage

period lasted 2 min. and this was repeated at equal time intervals four times daily. The massage was of the usual type.

The first question to which an answer was sought was whether massage could influence deposition of glycogen on the adipose tissue. Normally, approximately equal quantities of adipose glycogen are found on opposite sides of the groin. At the very most a difference of 10% may occur. The usual experimental procedure was employed. Fasting was instituted to obtain a loss of 20% body weight and was followed by a period of recovery feeding during which massage was performed on one side of the groin only. The animals were then killed and the groin fat and groin muscle tissue of both sides isolated (over ice). The results of the experiments are presented in Table 7, and show that muscle glycogen remains the same on both sides, whereas two massage treatments only—applied 6 hr. before death—suffice to lower the adipose glycogen level on the massaged side.

TABLE 7. Effect of massage on adipose-tissue glycogen during recovery diet

Body wt. g.	Groin-fat glycogen %		Glycogen in abdominal fat %	Glycogen in muscle %		Recovery diet days	Massage, side	Duration days	Rest, days after massage
	Left	Right		Left	Right				
101	0·19	0·60	—	0·61	0·60	1	L.	1	—
105	0·27	0·68	—	0·67	0·77	1	L.	1	—
84	Tr.	0·165	0·134	0·39	0·44	1	L.	1	—
106	0·03	0·116	0·133	—	—	2	L.	1	—
98	0·44	0·65	0·98	0·44	0·44	1	L.	Only twice	—
261	Tr.	0·126	0·20	0·31	0·32	2	L.	1	—
115	0·08	0·28	0·23	0·37	0·44	2	L.	2	—
105	0·214	0	0·266	0·38	0·38	1	R.	1	—
250	0·09	Tr.	0·15	0·40	0·45	1	R.	1	—
103	0·54	0·22	0·61	0·40	0·42	2	R.	2	—
107	0·24	0·32	0·47	0·80	0·80	3·5	L.	2	1·5
145	0·29	0·25	0·22	0·54	0·50	4·5	L.	2	2·5
210	0·167	0·157	0·22	0·50	0·50	1	—	1 electrical stimulation	
95	0·42	0·43	0·53	0·44	0·50	1	—	1 electrical stimulation	
117	0·255	0·244	—	—	—	1	—	1 direct electrical stimulation of fat tissue	

One-sided electrical stimulation by induction shock applied along the full length of the adipose tissue at repeated intervals failed to affect the glycogen level.

More prolonged treatment by massage was next tried to see if the deposition of fat was affected. The same experimental procedure was followed, except that the massage periods were spread over 3–8 days, since fat deposition only attains maximum level several days after the beginning of recovery feeding. Where massage was not applied, the adipose

TABLE 8. Effect of massage on fat deposition under various conditions

Body wt. g.	Extracted fat: Left g.	Extracted fat: Right g.	Difference %	Duration of massage days	Side
(*a*) During a normal recovery diet.					
110	0·911	0·763	16	6	R.
116	0·941	0·699	26	8	R.
109	0·755	0·781	3	5	L.
105	0·490	0·628	22	4	L.
102	0·384	0·434	11·5	4	L.
100	0·338	0·449	24	3	L.
120	0·507	0·643	21	6	L.
(*b*) During continuous maintenance on a normal diet.					
121	0·372	0·383	3	5	L.
110	0·672	0·724	7	5	L.
112	0·614	0·561	8·6	7	R.
115	0·496	0·603	16·6	8	L.
130	0·760	0·899	18	9	L.
120	1·037	0·808	22	8	R.
(*c*) During a fat-rich recovery diet.					
128	0·694	0·792	12	6	L.
109	0·710	0·811	12	6	L.
110	0·998	0·745	15	7	R.
93	0·533	0·610	12·6	4	L.
100	0·487	0·585	16·7	4	L.

fat content of both groin sides was practically equal. The results of some typical experiments are presented in Table 8.

The figures show that the fat content of the massaged groin is uniformly smaller than that of the unmassaged. The difference in fat content is already noticeable 3 days after beginning the application of massage; the effect of the duration of massage on the fat content is not clear from these experiments however, as individual differences between the animals were too considerable for any decision in this respect. The fat levels of the massaged and unmassaged groin show differences of 11–37% (eighteen experiments) and a mean difference of 20·7% (in one experiment a difference of only 3% was found). Massage carried out during the period of weight loss (starvation, or starvation plus phloridzin) failed to effect a considerable change in the rate at which the adipose tissue was depleted.

When the groin of rats which have not been starved and which are maintained on a normal diet is massaged (twelve experiments), the fat content of the massaged groin falls below that of the unmassaged side, but does so rather slowly (Table 8). After 7 days' massage the lesser fat content of the massaged groin is clearly discernible. The maximum difference between massaged and unmassaged groin was found after 8–10 days when the average decrease was 23%.

If in recovery experiments as described above, the usual carbohydrate-rich diet is replaced by a fat-rich but carbohydrate-poor diet (40% fat, 40% carbohydrate, 20% protein), and massage is applied during recovery feeding lasting 4–7 days, the differences noted are smaller than with a carbohydrate-rich diet—13·6% on the average, as against 20·7% in the carbohydrate diet experiments (Table 8). A similar relationship was established in experiments on the effect of prolonged massage treatment (8–10 days) which was not preceded by starvation. On the fat-rich diet the difference produced by prolonged massage was found to be 11%, as against 23% on a carbohydrate-rich diet.

Mechanical stimulation by massage thus inhibits both glycogen and fat deposition in adipose tissue under the conditions described. The inhibitory effect on fat deposition is evident even when the rats are not starved but are continuously maintained on a normal diet, though in this case massage must be prolonged to be effective. Since the inhibitory effect of massage is generally found to be smaller on a fat-rich rather than a carbohydrate-rich recovery diet, it seems probable that massage inhibits especially neogenesis of fat from carbohydrate in adipose tissue. The same experiments also demonstrate the extreme reactivity of adipose tissue metabolism to relatively feeble stimuli, e.g. massage applied for 2 min. four times daily. If the effect is mechanical and due to the pressing out of fat from the adipose tissue or to the facilitation of fat absorption by the vascular system, the extension of the effect to the process of adipose tissue depletion in starvation is to be expected. In fact, however, massage was found to be without effect on the latter. Moreover, if the effect of massage was merely mechanical, massage should be at least equally effective in experiments where the normal diet is given throughout, as in recovery experiments.

Influence of toxins on the deposition of glycogen and fat in adipose tissue

Experiments were undertaken to elucidate the effect on adipose glycogen deposition of poisons which are known to affect carbohydrate metabolism. For the first experiments bacterial endotoxins whose influence on carbohydrate metabolism has been the subject of systematic study [Delafield, 1932; Olitzki, Leibowitz & Berman, 1937] were chosen. A dead, washed preparation of *Salmonella typhi murium* was used.[1] The dose was of sufficient strength to cause an appreciable decrease in the glycogen content of the liver and was separately assayed therefore for each preparation.

[1] We are indebted for this preparation to Dr L. Olitzki and to Mr P. Koch.

In the first experiments, the effective dose (1 mg./100 g. body weight) was injected twice, 24 and 4 hr. before the animals were killed. In later experiments only a single injection, 24 hr. before the determination, was given. The following results were obtained:

No. of exps.	Body weight of rats g.	Liver glycogen %	Adipose tissue glycogen %	Inter-scapular fat glycogen %	Blood sugar mg. %	Remark
3	98	1·42	Traces	0·15	89	Two injections of 1 mg. toxin
6	115	4·4	Traces	0·83	120	Single injection of 1 mg. toxin

The bacterial toxin prevented deposition of glycogen in typical adipose tissue, inhibited deposition of glycogen in interscapulary fatty tissue and in liver, and failed to influence the glycogen content of muscle. The food intake by the poisoned animals was lower than normal, though not to an extent which might affect the amount of glycogen deposited. Control experiments with normal animals which received similarly restricted quantities of food confirm this view. The effect of the toxins on adipose-tissue, glycogen was further investigated as follows: After a day of recovery feeding, groin fat from one side was removed and analysed. At the same time an effective dose of toxin was injected. The fat of the remaining groin side was sampled in experiment (1) after 1 hr., in experiment (2) after 4 hr. The following glycogen values were recorded: in experiment (1) at the beginning 0·19%, after 1 hr. 0·15%; in experiment (2) at the beginning, 0·27%, after 4 hr., 0.

By similar methods information as to the influence of toxin on fat deposition was obtained. In these experiments the rats received injections of effective toxin in daily doses for 4–5 days during recovery feeding. Unpoisoned rats contained 2·5 g. fat per 100 g. body weight after this time. The toxin-treated rats yielded the following values:

No. of exps.	Adipose tissue, g./100 g. body weight	Liver glycogen %	Muscle glycogen %
7	1·33	1·7	0·40

In other experiments the effect of administration of strychnine on the glycogen content of adipose tissue was examined. The procedure for these tests was as follows: After 1 day of recovery feeding, as in the standard experiments, groin fat was removed from one side under light ether narcosis. The operated animals were then given cramp-inducing doses of strychnine (0·12 mg. strychnine sulphate per 100 g. body weight) and

killed after ½–1 hr. This treatment exerted no measurable effect on the adipose tissue glycogen though it caused a marked fall in the glycogen content of muscle. The following results are typical:

No. of exps.	Adipose-tissue glycogen % Before strychnine	Adipose-tissue glycogen % After strychnine	Liver glycogen %	Muscle glycogen %
6	0·70	0·72	6·8	0·09

The influence of endocrine glands on the deposition of glycogen and fat in adipose tissue

If deposition of glycogen and the accompanying deposition of fat in adipose tissue are not passive processes of storage but co-ordinated activities of the adipose organ, their subjection to hormonal regulation may be expected.

(*a*) *Thyroidin.* The experimental animals received a daily portion of 10 mg. thyroidin for 4–5 days as a supplement to their ordinary diet. After this time the food given was so far reduced as to effect a 20% loss in body weight within 6 days. Recovery feeding with a supplementary portion of 10 mg. thyroidin per day followed. In all, the rats were subject to the action of thyroidin over a period of 10–16 days. The effectiveness of the thyroidin treatment was not only evidenced by the decrease in the amount of liver glycogen but was also reflected in the results of gas exchange tests which revealed an increase in the rate of oxygen consumption of 40–50%.

Table 9 and Figs. 1*a* and 1*b* show that the time curves of glycogen deposition in normal and thyreotoxic rats are markedly different. The curve in thyreotoxicosis is much steeper, its high peak is attained within the first day which also sees the beginning of the descent. The figures for liver glycogen in thyreotoxicosis are almost negligible. It seems probable that here too a disturbance in deposition accompanies the enhanced rate of utilization. In adipose tissue, glycogen storage is not inhibited but is actually favoured, though concurrently combustion or other means of utilization of glycogen are stimulated.

The enhanced utilization of glycogen in the adipose tissue of thyreotoxic animals may be demonstrated by the following procedure: Fasting until 20% loss of body weight occurs, followed by 24 hr. standard diet, then sampling of groin fat from one side, and after 1 hr. sampling from the second side, both operations being carried out under amytal narcosis. In normal animals the glycogen content after 1 hr. is practically unchanged, but in thyreotoxic animals the glycogen content drops from

TABLE 9. Glycogen and fat deposition in adipose tissue in thyreotoxic and thyroidectomized rats on recovery diet

Duration of recovery diet	No. of exps.	Glycogen in adipose tissue %	Glycogen in inter-scapular fat %	Wt. of fatty tissue g.	Glycogen in liver %	Glycogen in muscle %
Thyreotoxic rats.						
0	6	0	0	0·63	Tr.	—
1·5 hr.	2	0·08	0·06	—	Tr.	0·36
5 hr.	5	0·89	1·43	—	0·61	0·58
1 day	18	0·78	0·72	1·16	0·34	0·48
2 days	7	0·41	0·40	1·41	0·64	0·64
3 days	4	Tr.	Tr.	—	Tr.	0·42
4 days	4	0·07	0·03	1·30	Tr.	0·62
6 days	4	Tr.	Tr.	1·26	Tr.	0·5
Thyroidectomized rats.						
5 hr.	2	0	—	—	—	—
1 day	3	0·20	0·88	—	5·6	—
2 days	3	0·42	0·40	—	5·2	—
4 days	2	0·05	0·28	—	5·0	—
8 days	3	Tr.	0·10	—	3·4	—

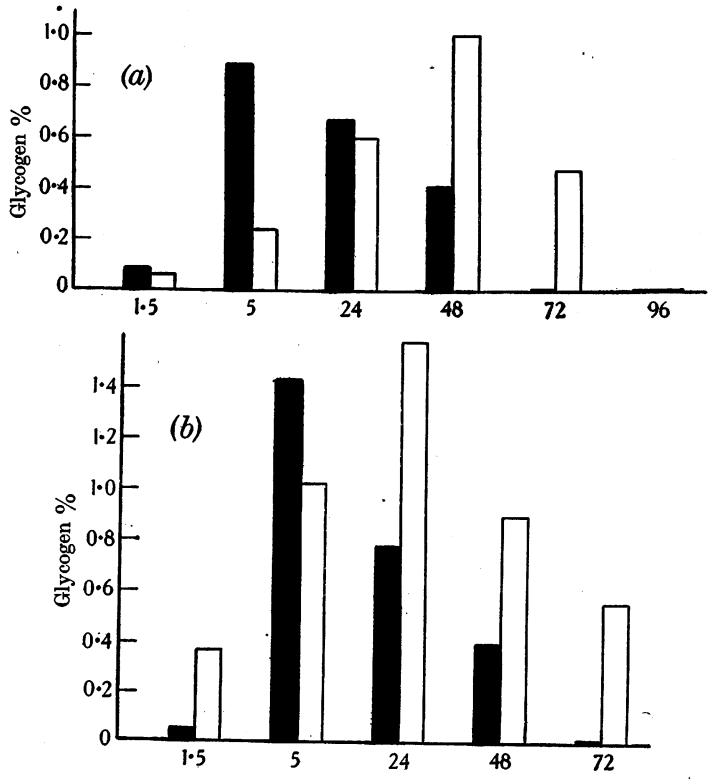

Fig. 1. *a*, glycogen in adipose tissue; *b*, glycogen in interscapular fat. Ordinates: glycogen%. Abscissae: duration of recovery diet in hours. ■ Thyroidin-treated animals, □ controls.

0·72 to 0·49 g. %. As may be seen from Table 10, an appreciable drop in the glycogen content also occurs in the other adipose tissues.

1:2:4-Dinitrophenol administered under corresponding conditions exerts a qualitatively similar but quantitatively feebler effect on adipose tissue. Fat and glycogen deposition accompany one another. When glycogen is at peak level an appreciable increase in fat content is evident, but with the disappearance of glycogen the deposition of fat ceases.

(*b*) *Thyroidectomy.* Thyroidectomized rats deposited less glycogen fat than did normal rats. In no case was obesity observed in these animals (Table 9).

(*c*) *Insulin.* The method of groin-fat analysis was used. It was found in such experiments that doses of insulin which sufficed to depress the blood-sugar concentration by 50% failed to induce an increase in the concentration of adipose-tissue glycogen (Table 10). In addition, experiments using small doses of insulin which affected the blood-sugar concentration only slightly were carried out but no effect of insulin was found. Also, no effect of insulin on adipose glycogen was observed during fasting.

TABLE 10. Effect of various endocrine substances and dinitrophenol on the glycogen content of adipose tissue

			Glycogen %						
			Groin fat						
Remarks	Body wt. g.	No. of exps.	Before lb.	After lb.	Abdominal fat	Inter-scapular fat	Liver	Muscle	Blood sugar mg. %
Controls	109	6	0·71	0·75	0·70	4·0	6·2	0·72	105
0·03–0·1 mg. adrenaline/100 g.	103	10	0·67	0·65	0·82	3·1	6·6	0·42	240
Insulin	106	5	0·71	0·64	0·81	3·7	4·6	—	50·2
Thyroidin 10 days 10 mg. daily	108	7	0·72	0·49	0·36	0·46	0·68	0·41	126
Dinitrophenol 3 mg./100 g.	109	8	0·82	0·60	0·92	3·3	2·1	0·28	231

(*d*) *Adrenaline.* Fat was taken from one groin after 1 day of recovery feeding and then adrenaline was injected. Fat was taken from the remaining groin 1 hr. later. Under these conditions no effect of adrenaline on the glycogen content of adipose tissue was discernible. Severe hyperglycaemia was produced. Muscle glycogen was diminished (Table 10).

(*e*) *Adrenalectomy.* It was observed that adrenalectomized rats which died several weeks after operation and which had not suffered considerable losses of body weight contained only minimal quantities of adipose tissue, such as are only rarely found in normal rats even after most severe weight loss (30–40%). Thyreotoxic rats suffer comparable fat depletion only

exceptionally and after fasting. For the analysis pooled samples of groin, testicle, and perirenal fat of rats weighing 140–200 g. were used. Whereas the adipose tissue in adrenalectomized rats after loss in body weight from 0 to 20% weighed only 0, 10–0, 40 g., the weight of adipose tissue in normal rats under essentially similar conditions was 2–5 g. (eight experiments).

The experiments on the deposition of glycogen and fat in adipose tissue of adrenalectomized animals suggest a possible explanation of the above phenomenon.

Rats weighing 130–170 g. were adrenalectomized by dorsal approach under amytal-ether narcosis. On a diet rich in bread and with Rubin-Kriek salt solution to drink, the operated animals generally recovered rapidly. They were subsequently placed on restricted diet until they had dropped approximately 20% in body weight and were afterwards transferred, as in the preceding experiments, to a carbohydrate-high recovery diet for varying lengths of time. The food intake and consequent weight increase of the adrenalectomized animals was 30–40% below normal. Except as specified the technique of these experiments was the same as that followed previously.

TABLE 11. Glycogen and fat deposition in adipose tissue of male adrenalectomized rats on recovery diet

Duration of recovery diet	No. of exps.	Weight of fat tissue g.	Glycogen %				Blood sugar mg. %
			Adipose tissue	Interscapular fat	Liver	Muscle	
0	6	1·1	0	0	Tr.	0·16	64
5 hr.	3	—	0	0	Tr.	0·19	90
1 day	7	1·0	0·06	0·10	2·1	0·23	100
2 days	7	1·06	Tr.	Tr.	1·96	0·28	96
3 days	4	1·8	Tr.	0·036	0·80	0·23	85
4–5 days	Adren. 3	1·13	0	0·4	1·7	0·28	—
	Controls 3	4·43	0	0·15	2·8	0·44	—
6 days	Adren. 4	1·2	0	0·11	1·5	0·31	91
	Controls 2	5·0	0·11	0·22	4·2	0·63	—

In over forty experiments involving different times of recovery feeding, typical adipose tissue was found to contain at the most only traces of glycogen. Interscapulary fat, after extended time intervals, was seen to contain slight quantities of glycogen but considerably less than was contained in the brown fat of control animals after similar time intervals. Deposition of glycogen in liver and muscle is diminished under similar treatment, but remains considerably higher than during hunger. The blood-sugar concentration also remains high (Table 11).

It may be noted that all animals used in the above experiments were in good condition. It was arguable that their inability to deposit glycogen

was conditioned by a lowered food intake and consequent weight loss. Experiments show, however, that this is not the case. Normal rats, and vitamin A- and B-deficient rats showed normal glycogen deposition at subnormal food intake rates. On the other hand, adrenalectomized rats have been known to show normal rise in weight but no accompanying deposition of adipose-tissue glycogen.

When the food intake was restricted during recovery feeding so as to limit weight gain to 6 instead of the normal 14 g. in the first day, the following glycogen values were recorded:

No. of exps.	Adipose-tissue glycogen %	Liver glycogen %	Weight of rats g.
6	0·35	6·8	102

After short recovery times (8 hr.) normal amounts of adipose glycogen were deposited by rats on a restricted recovery diet. Adrenalectomized rats only rarely took as little food or experienced such low rates of gain in weight as were established by restriction of the ration. It appears, therefore, that the adrenal cortex is indispensable to glycogen formation in adipose tissue.

In a recent comprehensive paper, Long, Katzin & Fry [1940] state that glycogen deposition in liver and muscle by adrenalectomized rats is normal if the animals are given daily injections of a suitable dose of NaCl and $NaCO_3$. In a separate series of experiments, therefore, rats which had lost 20% of their weight were transferred for 2 days to a recovery diet, during which time they also received salt injections as prescribed by Long *et al.* Such rats showed very satisfactory and nearly normal increase in weight. Their general condition was good. In view of the fact that the liver and muscle glycogen content of fasted adrenalectomized rats is generally abnormally low the figures found after recovery feeding may be regarded as evidence of vigorous deposition. The glycogen content of the adipose tissues under the same conditions nevertheless remained near zero level; a value of 0·22% was only found on a single occasion. In interscapulary fat a slight amount of glycogen deposition was noted, but this too was abnormally low. Values recorded in these experiments were as follows:

No. of exps.	Weight g.	Weight gain (average) g.	Liver glycogen %	Muscle glycogen %	Adipose-tissue glycogen %	Interscapular fat glycogen %	Blood sugar mg. %
5	139	14·5	2·74	0·293	0·051	0·128	125

In fourteen experiments with adrenalectomized rats which were given various quantities (up to 1 mg./day) of desoxycorticosterone acetate (doca organon) for varying time periods, glycogen synthesis in adipose tissue was noted only on two occasions, the levels then obtained being 0·43 and 0·30%. Under similar conditions low amounts or only traces of glycogen occurred in interscapulary fat. The averages noted show that apart from a small improvement in the glycogen values of liver, desoxycorticosterone is without effect on glycogen deposition.

Using a fresh extract of cattle adrenals prepared according to Cartland & Kuizenga [1936], it was possible to obtain some deposition of glycogen in adipose tissue in the majority of investigated cases (Table 12). The effect on liver glycogen was more marked. The extract in these experiments was given twice daily in a dosage corresponding to 5 g. adrenal gland for 2–3 days before the determination.

TABLE 12. Effect of adrenal extract on the deposition of glycogen

No. of exps.	Weight g.	Liver glycogen %	Muscle glycogen %	Adipose-tissue glycogen %	Interscapular fat glycogen %	Remarks
14	133	3·2	0·32	0·05	0·13	Treatment with desoxycorticosterone
5	140	6·2	0·41	0·31	1·2	Treatment with fresh adrenal extract

It may be noted in connexion with the above observations that semi-adrenalectomized rats behave, as regards glycogen deposition, like normal rats.

Similarly uniform results were also obtained in experiments on the deposition of fat in the adipose tissues of male adrenalectomized rats (Table 11). The occurrence in this case of a slight fat deposition after 3 days' recovery feeding is exceptional. On the fourth and fifth day when fat deposition by normal rats reaches a turning point no deposition at all was observed. The figures for normal animals maintained under similar conditions show the difference most clearly. After extended recovery periods, moreover, an actual loss of adipose-tissue fat by adrenalectomized males was observed. The losses noted after extended recovery times were such as are observed in these animals before death.

Results obtained with adrenalectomized female rats must be considered separately (Table 13). In these animals glycogen was not deposited in the adipose tissues but the deposition of fat in the first days of recovery feeding was frequently considerable though always subnormal. It was

TABLE 13. Glycogen and fat deposition in adipose tissue in female adrenalectomized rats on recovery diet

	Duration of recovery diet	No. of exps.	Wt. of fat tissue g.	Glycogen %				Blood sugar mg. %
				Adipose tissue	Inter-scapular fat	Liver	Muscle	
Adren.	0	6	3·0 (1, 2–5, 4)	0	0	Tr.	0·15	—
Adren.	1 day	3	1·2	0·05	0·14	4·9	0·4	111
Controls		3	1·4	1·18	2·1	7·7	0·46	—
Adren.	2 days	2	2·5	Tr.	0·06	2·35	0·28	108
Controls		2	3·3	0·465	0·233	2·35	0·40	—
Adren.	3 days	1	4·25	Tr.	0·02	1·73	0·29	—
Controls		1	5·23	0·95	2·57	4·70	0·89	—
Adren.	4 days	5	2·7	0	0·11	1·34	0·38	97
Controls		5	3·1	0·16	0·12	3·2	0·48	105
Adren.	6 days	5	1·5	0	0·06	1·35	0·30	102
Controls		5	3·8	0·06	0·06	2·9	0·36	—

found, however, that no new fat formation had occurred here. In medium-sized female rats, as has already been noted, considerable fat reserves (1, 2–5, 4 g.) are retained in the body even after 20% weight loss. The reason for this peculiarity has not been cleared up. The fact itself, however, provides a possible explanation for the findings obtained during the first days of recovery feeding. If the observation time is extended to say 4–6 days the quantity of fat does not continue to rise as in normal rats but rapidly and progressively drops.

It follows from the experiments reported above that the absence of adrenal glands renders fat and glycogen deposition in the adipose tissues impossible.

It should be noted that because of the desirability of greater resistance to the operation animals used for adrenalectomy were of larger size than those normally used in the standard tests. Since the larger animals should have contained more fat than the smaller ones used in the standard test, the use of the larger-sized animals does not therefore vitiate the significance of comparisons with normal-sized rats. In the principal experiments, moreover, some control groups of larger sized rats were examined as may be seen in Tables 12 and 13.

It is further noteworthy that thyroidectomy or castration does not change the effect of subsequent adrenalectomy on the carbohydrate metabolism of adipose tissue.

(*f*) *Castration.* Castration of either males or females failed to influence glycogen deposition in adipose tissue after various periods of recovery feeding. Obesity of castrated rats was not observed (six experiments).

Discussion

The presence of glycogen in adipose tissue during recovery feeding on ordinary diet after fasting raises questions regarding the manner of its deposition. Since peripheral fatty tissue has been regarded as a tissue of minimal activity, consideration must be given first to the possibility that the deposition of glycogen in this tissue is a passive process. Glycogen must be assumed, on this view, to reach the adipose tissue via the blood stream, though it should be noted that well-preserved adipose-tissue preparations contain glycogen within the cells but not in the intercellular spaces. As glycogen cannot diffuse through the cell membrane it is necessary to conclude that it is formed within the cell. The view that glycogen is formed from tissue fat itself is utterly lacking experimental support. There remains the possibility that during recovery feeding, after fasting, glycogen is synthesized within the adipose cell itself from the sugar with which it is supplied. This latter seems to be the only remaining explanation possible; nevertheless, attempts to demonstrate synthesis of glycogen from sugar or sugar breakdown products after starvation by adipose tissue in vitro have so far failed. As against this it should be borne in mind that under corresponding conditions it is also difficult to demonstrate synthesis of glycogen even by liver.

The second question to arise concerns the fate of the glycogen of adipose tissue. A rapid disappearance of this glycogen occurs if the recovery diet is withheld. If recovery feeding on a standard diet is continued the typical time curve of glycogen deposition is obtained: a progressive increase in glycogen values to the end of the second day followed by a decrease which is complete on the fourth day. Two principal lines of explanation present themselves.

(1) Excess carbohydrate is deposited as glycogen in the empty adipose tissue and is later drawn into the main circle of carbohydrate metabolism. (2) Adipose glycogen may be the first step towards the formation of fat from carbohydrates in the adipose tissue cell itself. The latter view is supported by the following: (*a*) As far as is known all deposition of fat is regularly preceded by deposition of glycogen in adipose tissue. Such has been demonstrated in the deposition of fat during recovery feeding after fasting. A similar precedence has also been observed in the deposition of fat in the embryo [Hausberger & Gujot, 1937], and the same relationship has been shown to hold when the deposition of fat follows denervation of adipose tissue. (*b*) The parallel course of the time curves of fat and glycogen deposition in rats con-

stitutes strong evidence for the interdependence of these processes. The deposition of fat in adipose tissue of rats approaches a maximum after 4 days' recovery feeding when glycogen disappears from the adipose tissue. (*c*) Deposition of glycogen in the adipose tissue of guinea-pigs was of another order, smaller in magnitude and prolonged for a period of roughly a fortnight; the period of fat deposition in comparison to that found in rats was also prolonged. (*d*) Treatments which decrease or prevent deposition of glycogen have been shown to effect that of fat in a similar manner. Such, for instance, is the effect of high-protein feeding. This inhibits deposition of glycogen in the fatty tissue and, if the percentage of protein is sufficient, inhibits the deposition of glycogen completely whilst markedly inhibiting that of fat. Massage diminishes the deposition of both glycogen and fat. A similar influence is exerted by certain bacterial toxins. In thyreotoxicosis and during the first stage of recovery feeding a considerable deposition of both glycogen and fat occurs; glycogen deposition then diminishes very rapidly and at the same time fat deposition comes to an end. The best example of the parallel nature of fat and glycogen deposition is perhaps presented by adrenalectomized rats. These fail to deposit glycogen in adipose tissue during recovery feeding but are also unable to deposit fat. The adipose tissue therefore grows ever leaner until at death only traces of fat can be found, even though the loss of body weight may not be considerable.

It is therefore very probable that rat adipose tissue is able at times to effect the conversion of glycogen into fat. It is not to be inferred therefrom that such conversion is impossible to other tissues, particularly to liver. It is also self-evident that in addition to the form of fat deposition which has here been described other forms exist, of which the longest known is that in which fat is deposited directly from the fat of the diet. On a high-fat diet (40%) for instance, the deposition of glycogen in adipose tissue is very slight but that of fat is abundant. Also on a high-protein diet, fat deposition does not occur via glycogen.

The prevalent opinion as to the nature of adipose tissue ascribes to the latter the purely passive role of a fat-receiving depot. The amount of fat stored in the depot is supposed to be dependent on the nutrient content of the blood. The genesis of fat from carbohydrates is regarded as an exclusive function of liver tissue. The replenishment of the fat depot is supposed to take place by an infiltration of fat molecules through the cell walls as through a sieve [cf. for instance Maximow, 1927]. Rosenfeld, however, as long ago as 1902, expressed the opinion that fat might be formed from carbohydrates within adipose tissue itself. At that time

experimental support for this opinion could not be cited. The demonstration above that adipose tissue is capable of synthesizing glycogen during the deposition of fat, the finding that this tissue is able to break down glycogen, and the discovery of nervous and hormonal regulatory systems within the fatty tissue itself point clearly to the conclusion that adipose tissue fills more than a merely passive role and constitutes in fact a regulated organ with a definite role to play in the metabolism of carbohydrates and of fat. It is of interest to note, therefore, that a new conception of the mode of formation and role of adipose tissue has also been developed recently in a purely anatomical approach [Wassermann, 1929; Hausberger, 1937; Wells, 1940].

It is evident that a relationship exists between the experiments described above and the problem of obesity.

A further point remains to be discussed. The glycogen in adipose tissue has been shown to be subject to the influence of different factors which are specific up to a certain point for this glycogen. Adrenaline, for instance, which at first affects muscle glycogen and then liver glycogen, has no effect at all on adipose-tissue glycogen. Strychnine again, which causes a severe diminution of muscle glycogen, is without effect on adipose-tissue glycogen. Certain bacterial endotoxins, which are without effect on muscle glycogen, exert a marked effect on adipose-tissue glycogen but a relatively weak effect on liver glycogen. Thyroxin causes a sharp diminution of liver glycogen and a relatively small diminution of muscle glycogen, but may even induce a primary accumulation of the glycogen in adipose tissue. Adrenalectomy under favourable conditions need cause only a slight drop in the glycogen contents of liver and muscle, but almost always renders accumulation of glycogen in adipose tissue impossible. The specific regulation of cardiac glycogen has been dealt with in an earlier paper [Stein & Wertheimer, 1940].

Summary

When rats which have been starved are placed on a diet rich in carbohydrates, glycogen occurs in their adipose tissue in the first days of recovery. The amount may reach 1%. This adipose-tissue glycogen resembles liver glycogen in all physical and chemical properties so far investigated.

Glycogen regularly occurs in adipose tissue only under defined conditions, i.e. only when prolonged starvation is succeeded by a diet rich in carbohydrates. Carbohydrates other than glycogen are present in adipose tissue under the same conditions in only minimal quantities, if at all.

The deposition of glycogen in adipose tissue depends on the nature of the recovery diet. A diet of 70% carbohydrate, 20% protein and 10% fat is roughly optimal. If the dietary percentage of protein or fat is increased at the expense of carbohydrate, or if the amount of protein is decreased and an excess of carbohydrate is given, the amount of glycogen deposited is lessened.

In the brown interscapulary fat, deposition of glycogen is particularly rapid and large, and comparable in rate and amount with its deposition in liver.

The deposition of fat in adipose tissue is already apparent 1 day after the beginning of recovery feeding and approaches a maximum after 4 days, i.e. when glycogen disappears from the adipose tissue. After this time glycogen occurs in the adipose tissue only irregularly and in very slight amounts, if at all, and deposition of fat in the same tissue is minimal. Deposition of glycogen and fat in the adipose tissues of guinea-pigs and rabbits is less regular, smaller in order of magnitude and shows a different time curve to that in rats.

Brief but repeated massage of groin adipose tissue during recovery feeding leads to a lessened deposition of glycogen and fat on the massaged side.

Bacterial endotoxins of the *Salmonella* group prevent deposition of glycogen and markedly inhibit deposition of fat in adipose tissue during recovery feeding. Muscle glycogen is not affected under similar conditions, and liver glycogen is only slightly diminished.

Cramp-inducing doses of strychnine which cause a marked diminution of muscle glycogen do not affect the glycogen content of adipose tissue.

Deposition of adipose-tissue glycogen in thyreotoxic rats during recovery feeding is enhanced, takes place earlier and diminishes sooner than in normal animals.

Adipose tissue of thyreotoxic rats consumes glycogen more rapidly than that of normal rats. Deposition of fat in adipose tissue of thyreotoxic rats is marked in the early stages of recovery feeding when glycogen is deposited, but ceases at a low level when the glycogen values approach zero.

Insulin and adrenaline do not influence glycogen deposition in adipose tissue.

Adrenalectomized male rats at death contain only minimal amounts of peripheral fat irrespective of weight loss. After partial fasting followed by recovery feeding on high carbohydrate diet the deposition of glycogen and fat in adipose tissue is generally negligible.

The differences between glycogen deposition in liver and adipose tissue were particularly large when the adrenalectomized rats received injections of NaCl and $NaHCO_3$. The deposition of glycogen in adipose tissue was induced by administration of fresh cattle adrenal extract but not of desoxycorticosterone acetate (doca).

It is concluded (1) that adipose tissue can synthesize glycogen, (2) that the glycogen metabolism of adipose tissue is specifically regulated, (3) that adipose tissue can probably effect the conversion of carbohydrate into fat, and (4) that adipose tissue thus appears to play a more active part in carbohydrate-fat metabolism than has hitherto been assumed.

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