XLI. THE FORMATION OF CARBOHYDRATE FROM FAT IN THE LIVER OF THE RAT.

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THERE have been many attempts to test whether or not fat can be transformed into carbohydrate in the animal body. The majority of the experiments have been negative, and the few which have given positive results have met with criticism. Burn and Marks [1926] reported an increase in glycogen in a perfused liver taken from a cat or dog after a high fat diet. At the same time they observed an increase in the sugar of the perfusion fluid. Gregg [1933] repeated these experiments and found, in the majority of his experiments, a decrease of liver glycogen. He explained the results of Burn and Marks and similar results obtained by earlier investigators on the grounds that feeding fat to dogs and cats does not completely rid the liver of glycogen. Also, he found that the distribution of the glycogen in the liver was so variable that a sample from one lobe did not give any information concerning the carbohydrate content of the whole liver.

Apart from perfusion experiments, evidence of this transformation is provided by the increase of glycogen found to occur in the livers of intact animals fed on fat diets. Takao [1926], Burn and Ling [1929] and Magnüsson [1929] have reported high glycogen values under this condition. For example, Burn and Ling found values as high as 5·0 % after 96 hours of fat feeding. In contrast to these results are those of Bodey *et al.* [1927], Gregg [1931] and Greisheimer [1931]. These observers failed to find such high percentages of glycogen as the workers quoted above, although in the majority of their experiments they did find a small but definite increase. Gregg [1931] claimed that this rise was due to the glycerol content of the fat. In support of this view, he found that when a rat was given the soaps of butter fat, there was no increase in the glycogen content over that shown by his series of fasting rats. As Gregg reported only one experiment of this nature, his conclusion is only suggestive.

Another type of evidence has been obtained from a study of the D/N ratio in depancreatised dogs. Soskin [1929] fed fat and lecithin to diabetic dogs. He found in 3 out of 15 experiments an increase in sugar in the urine. He concluded from this evidence that gluconeogenesis from fatty acids was proven. Page and Young [1932] were unable to obtain any increase in urinary sugar following an intravenous injection of lecithin into phlorhidzinised dogs. These experiments do not contradict those of Soskin, as it is now well recognised that the metabolism of a diabetic animal and a phlorhidzinised one are not the same; it is very difficult to transfer the results from one type of experiment to the other. The three positive experiments of Soskin are the most direct evidence that we have had, up to the present time, for transformation of fat into carbohydrate in the animal body.

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The respiratory quotient of man and animals on fat diets has given suggestive evidence for this transformation. This subject has been studied recently by Hawley *et al.* [1933]. They found many respiratory quotients of less than 0·70 for men on a diet of cream. They concluded that these low quotients could be explained by the supposition that the process of desaturation of fats occurred more rapidly than that of oxidation, although they did not offer any experimental proof in support of this suggestion. Gregg [1931] in the same laboratory, found that rats on a diet of fat had an average R.Q. of 0·72.

The difficulty of interpreting the results from respiratory exchange, in man and animal, is that the process of conversion may take place in one organ, but the metabolism of the rest of the body may obscure the lowered respiratory quotient of that single organ. Therefore, it would be more significant to determine the respiratory quotient of individual tissues. Meyerhof and Lohmann [1926] obtained the respiratory quotient in serum of liver slices taken from fasting rats. They obtained quotients of 0·48, 0·76 and 0·67 for this preparation. Dickens and Šimer [1931] reported quotients of less than 0·70 for liver slices from fasting rats whether the experiments were made with phosphate or bicarbonate-buffered Ringer's solution. However, when serum was used for the determinations, an average value of 0·71 was obtained. Neither of these observers claimed a transformation of fat into carbohydrate from these low quotients. Needham observed figures as low as 0·35 for the respiratory quotient of the developing yolk sac. He was unable to detect sufficient transformation of fat into carbohydrate to explain these low quotients and concluded that they were due to some unknown retention of carbon and oxygen for synthetic purposes.

A fourth type of experimentation has been a study of the output of sugar in the urine following the injection of adrenaline into a depancreatised dog. Chaikoff and Weber [1928] obtained a definite increase of sugar by this method. They claimed that the excess sugar came from fatty acids. However, it is now well known, from the work of Cori and Cori [1928], that adrenaline mobilises muscle glycogen. Chambers *et al.* [1932] and others have demonstrated that depancreatised dogs have considerable amounts of carbohydrate in their muscles and other tissues. Therefore, the results of Chaikoff and Weber can be explained by the liberation of carbohydrate from the tissues under the action of adrenaline.

Since the majority of workers have observed a small but definite increase in the glycogen content of the liver of rats fed on fat, we thought that this problem might be solved for this particular tissue by two types of experimentation; a determination of the respiratory quotient of the fatty livers and an estimation of any change in the carbohydrate content of slices shaken in bicarbonate-Ringer for several hours.

Methods.

Feeding of rats. The normal rats were fed on the usual laboratory diet. The rats on a fat diet were given butter, the essential salts and water. The amount of butter eaten each day varied from 3 to 7 g. After the 4th day of fat feeding, the rats were generally in poor physical condition. For this reason, the majority of experiments were made on animals that had been on butter diets for 2 or 3 days only. The rats were killed by a blow on the head. Portions of the liver were removed as quickly as possible for the glycogen determinations. The remainder of the liver was sliced for the manometric or other experiments.

Manometric methods. The method developed by Dixon and Keilin [1933] was used for the determinations of the respiratory quotient and the O_2 consumption.

Two major difficulties complicated the determination of the respiratory quotient and Q_{O_2} of the fatty livers. In the first place, it was noticed that fat separated from the tissue and collected on the sides of the vessel. Owing to this separation of fat, it was impossible to obtain the exact weight of the tissue used in each vessel. In our later experiments, this difficulty was overcome by weighing several slices of tissue before and after drying, in order to obtain the ratio for that particular liver. The slices for the manometric method were cut with a razor blade slightly moistened with Ringer's solution and were placed on a watch-glass in a covered dish which had moistened filter-paper on the sides and top until enough slices had been collected for the experiment. The slices were weighed and transferred to the vessels as quickly as possible. This gave an accurate measure of the wet weight of the tissue, and having obtained the ratio of the wet to the dry weight for that liver, the dry weight was calculated for the tissue used in the vessel of the manometer. When the calculated value of dry tissue was compared with the observed value of the tissue taken from the vessel, washed in water and dried in an oven, the calculated value was always much higher than the observed. The difference was due to the amount of substance lost by the usual procedure for obtaining the dry weight of the tissue. Since we determined the true ratio for the wet and dry tissues only in our later work, we are unable to give Q_{O_2} values for the earlier experiments.

The second major difficulty was the formation of acetoacetic acid. If aceto acetic acid is formed by the slices from a fatty liver, oxygen will be absorbed, but no corresponding amount of CO_2 will be liberated. We determined the amount of acetoacetic acid formed, using the manometric method, and we found that considerable quantities of this acid were produced in 2 hours by the fatty liver slices. A manometric method was devised by Ostern [1933] for the determination of oxaloacetic acid and it was later used by Quastel and Wheatley [1933] for their study of the production of acetoacetic acid by the liver. Krebs (unpublished experiments) has recently improved this method by using aniline citrate in place of the aniline hydrochloride. We have adopted this modification in our work. The initial value of this substance in the liver was practically zero. Liver slices from the liver of rats on normal diet produced very little acetoacetic acid. Unfortunately our observations on acetoacetic acid formation were made before we obtained a definite ratio of wet to dry tissue in each experiment. Therefore, we are unable to give absolute values of the acetoacetic formation per mg. dry tissue per hour. We can say, however, that there is a definite formation in the livers of fat-fed rats, and that this formation would affect the respiratory quotient. In the manometric method, aniline citrate was used to liberate carbon dioxide from the acetoacetic acid. This fact suggested that hydrochloric acid used in the Dixon-Keilin method might also liberate carbon dioxide in a similar way. Several experiments were made to study this reaction, using separately aniline citrate and HCl on pure solutions of acetoacetic acid. The results of one experiment are given in Fig. 1. The carbon dioxide was quantitatively liberated by HCl, as well as by the aniline citrate, but the time courses of the two processes were very different. These experiments were made at 37°. There was a delay of 15 minutes on account of the preliminary shaking to ensure thermal equilibrium. During this time, small amounts of acetoacetic acid may be broken down, especially in the presence of the citric acid, which would account for the recovery of only 80 % of the theoretical yield. The same results were obtained when similar experiments were made on normal tissue to which acetoacetic acid had been added. Thus, the routine procedure of Dixon and Keilin may be used for determining the respiratory quotient of the fatty livers provided that a sufficiently

long period be allowed for the breakdown of the acetoacetic acid. This period was, in our experiments, generally about 5 hours after spilling over the hydrochloric acid.

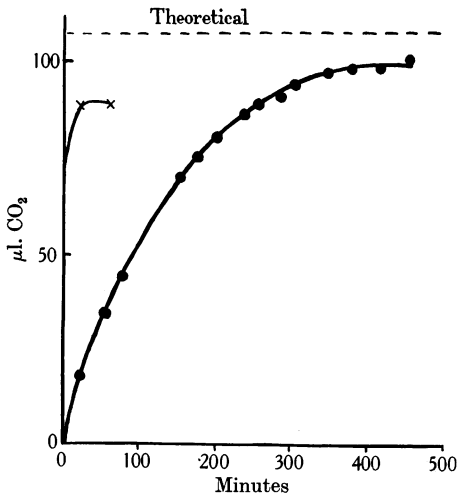

Fig. 1. Liberation of CO_2 from acetoacetic acid after adding aniline citrate and HCl.
×——× Aniline-citric acid, 80 % recovery. •——• HCl, 94 % recovery.

The remainder of the procedure for the manometric experiments was identical with the original description of Dixon and Keilin. We observed, however, that the readings, after the KOH had been admitted to the vessels, did not become constant for several hours. Elliot and Schroeder [1934] have observed similar changes, lasting for two or more hours.

Chemical methods. The Hubbard [1921, 1] method was used for the determination of total acetone bodies in the urine and the method described by Good *et al.* [1933] was used for the glycogen estimations. The glycogen estimations were made in duplicate. Only one acetone determination was made, as the amount of urine collected from a rat rarely exceeded 10 ml. for the 24-hour sample. The total fermentable carbohydrate in liver slices was determined by the method described by Cori and Cori [1933] for muscle. Estimations of β-hydroxybutyric acid in the liver slices were made by grinding the tissue in ice-cold water and carrying out the procedure described by Hubbard [1921, 2] for determining this substance in blood.

For the determination of the total fermentable carbohydrate and the β-hydroxybutyric acid in the tissue slices the following procedure was used. The tissue was cut in the same manner as for the manometric method. Alternate slices were taken for each series in order to ensure a good sampling of the liver tissue. The tissue was weighed and was placed in a small flask containing 3 ml. of bicarbonate-Ringer solution. Generally about 1·00 g. of slices was used for each determination. The solution had previously been saturated with 5 % CO_2 in oxygen. The small flask was closed with a rubber stopper through which passed two tubes, so that 5 % CO_2 in oxygen could be passed through the vessel while it was shaken in the water-bath. The gas mixture was passed through the vessels for 10 minutes. The stop-cocks were then closed and they were shaken for an additional 5 minutes before the contents of one vessel were taken for the initial value.

Results.

Respiratory exchange: The results obtained on normal and fatty liver slices are given in Tables I and II and in Fig. 2. The respiratory quotients of liver slices taken from rats on a normal diet were between 0·61 and 0·87 with an average of

Table I. *Respiratory metabolism of liver tissue taken from rats on a normal diet.*

Date 1934	Weight of rat g.	Sex	Ratio of wet to dry weight of liver	Duration of exp. hours	R.Q.	Q_{O_2}	Liver glycogen %
July 4	233	M	—	1·35	0·83	—	3·09
					0·86	—	—
5	253	M	—	1·55	0·87	—	4·25
							4·45
9	398	M	—	1·38	0·82	—	4·48
					0·84	—	4·78
23	273	M	—	1·17	0·84	—	4·60
							5·07
August 2	—	M	—	1·57	0·63	—	1·56
					0·61	—	1·52
3	87	M	—	1·30	0·76	—	0·09
							0·09
7	90	M	—	1·37	0·75	—	—
					0·80	—	—
8	—	M	—	1·3	0·80	—	—
					0·76	—	—
31	276	M	—	1·5	0·72	—	3·12
					0·74	—	2·64
September 10	156	F	—	1·6	0·73	—	—
October 16	207	M	—	3·0	0·80	−7·02*	—
17	234	M	3·4	3·0	0·74	−5·46	—
18	272	M	—	3·0	0·75	−5·80*	—
22	213	M	3·5	2·0	0·83	−6·50	—
					0·83	−8·34	—

* Ratio of wet to dry tissue assumed to be 3·5.

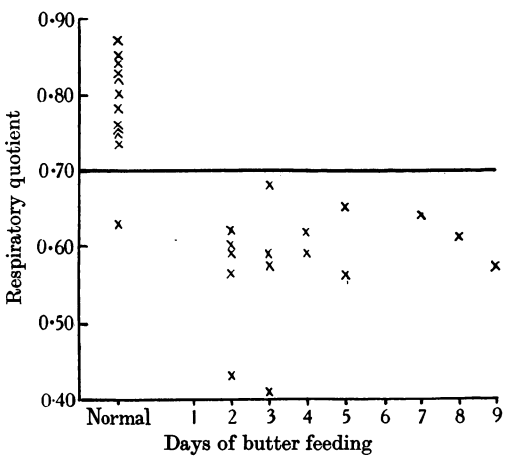

Fig. 2. Respiratory quotients of normal and fatty livers.

0·79, although only one determination, that of August 2nd, gave a value less than 0·70. Therefore, the majority of the respiration quotients for normal tissue fell within the normal limits of tissue oxidation. The range of these determinations is shown in graphic form in Fig. 2. When, on the other hand, determinations were made with the liver slices taken from fatty livers, the respiratory quotient was always below the normal level for fat oxidation, the lowest value observed was 0·41 and the highest 0·68, giving an average of 0·58. These results are given in Table II and in Fig. 2. This series of determinations on fatty livers was made

Table II. *Respiratory metabolism of liver tissue taken from rats on a fat diet.*

Date 1934	Weight of rat g.	Sex	Days of feeding butter	Ratio of wet to dry weight of liver	Wet weight of tissue g.	Duration of exp. hours	R.Q.	Q_{O_2}	Liver glycogen %
September									
11	165	M	2	—	—	2·0	0·60	—	0·03
									0·03
12	147	M	3	—	—	1·75	0·41	—	1·27
									1·36
13	194	M	4	—	—	2·0	0·59	—	0·65
							0·59	—	0·52
14	178	M	5	—	—	2·03	0·60	—	0·10
							0·72	—	0·09
17	123	—	8	—	—	1·75	0·62	—	0·43
18	135	M	9	—	—	2·00	0·55	—	2·31
							0·58	—	2·50
October									
24	123	M	2	2·6	0·300	2·0	0·42	−3·18	0·39
							0·43	−3·70	0·42
25	101	M	3	2·5	0·300	2·0	0·58	−6·33	0·68
							0·59	−5·06	0·65
26	126	—	4	2·3	0·300	2·0	0·62	−3·90	0·57
									0·57
27	90	—	5	3·1	0·300	2·0	0·56	−5·00	0·56
									0·54
29	96	M	7	3·1	0·300	2·0	0·68	−1·86	0·47
							0·60	−3·17	0·57
31	181	M	2	3·0	0·300	2·0	0·62	−6·47	0·00
									0·00
November									
1	161	M	3	2·8	0·300	2·0	0·68	−3·54	0·49
							0·67	−4·00	0·43
5	172	M	2	3·0	0·300	2·0	0·57	−5·88	0·87
							0·60	−5·49	0·61
7	190	M	2	2·8	0·300	2·0	0·58	−3·90	0·00
							0·56	−4·65	0·00
							0·54	−5·00	—
8	205	M	3	3·3	0·300	2·0	0·55	−6·20	1·52
							0·56	−4·13	1·57
							0·62	−7·48	—

after we had overcome the difficulties of obtaining accurate weights of the wet and dry tissue and was made in such a way as to eliminate the error involved in the fixation of oxygen in acetoacetic acid. The Q_{O_2} values, therefore, can be given for this series. They show some variation, not only from experiment to experiment but also in the same experiment. For example, in the experiment of November 8th, where the respiratory quotients of those determinations on the same liver were 0·55, 0·56 and 0·62, the Q_{O_2} values were 6·20, 4·13 and 7·48. However, closer agreement was obtained in other experiments.

A previous series of respiratory quotient determinations was made before we realised the necessity of waiting 4 or 5 hours to ensure the complete breakdown of acetoacetic acid. They fall within a somewhat lower range than those reported in Fig. 2, although the difference is not very great. This is certainly accounted for by the fact that, in this series, there was a retention of O_2 by reason of the formation of acetoacetic acid.

It is interesting to note that the ratio of wet to dry weights was different for the normal and fatty tissues. The ratio for normal tissue averaged 3·5, while that for the fatty tissue averaged 2·9.

In order to determine whether the formation of β-hydroxybutyric acid had any effect on the lowering of the respiratory quotient, two determinations of this substance were made. The results are given in Table IV. They show that the

Table III. *β-Hydroxybutyric acid in fatty liver slices.*

Date	Days of feeding butter	Duration of exp. hours	β-Hydroxybutyric acid initial %	Increase mg. per g. of tissue	O_2 equivalent per 0·3 g. tissue per 2 hours μl.
November 19	2	3	0·0041	0·198	4·3
20	3	3	0·0214	0·37	8·0

formation of 0·37 mg. β-hydroxybutyric acid per g. tissue would bind only 8 μl. O_2 per 0·3 g. of wet tissue per 2 hours. This is a negligible amount compared with the total uptake of over 1000 μl. observed in these experiments.

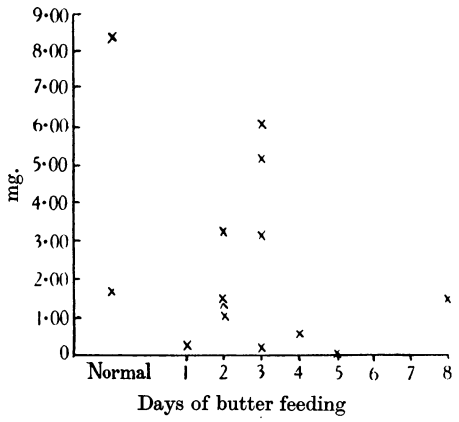

Fig. 3. Increases in total carbohydrate per g. of tissue.

Total fermentable carbohydrate. These results are given in Table IV and in Fig. 3. In order to see if comparable results could be obtained for the total fermentable carbohydrate in two series of liver slices, cut from the same liver, determinations were made on two such batches, shaken in the water-bath for the same length of time. The results of these determinations show close agreement both for the initial and final values (Table IV), illustrating that this method is adequate for determining changes in the carbohydrate content of the tissues. After these preliminary experiments, determinations were made in which one series was removed at the end of 15 minutes in the water-bath for the initial value and the other series at the end of 3 hours. Two determinations on normal

Table IV. *Total carbohydrate in normal and fatty liver slices.*

Date 1934	Sex	Weight of rat g.	Days of feeding butter	Duration of exp. hours	Total carbohydrate: Initial: Fermentable %	Total carbohydrate: Initial: Non-fermentable %	Total carbohydrate: Final: Fermentable %	Total carbohydrate: Final: Non-fermentable %	Increase in total fermentable carbohydrate mg. per g. tissue	Initial lactic acid mg. per g. tissue
September										
28	—	—	Normal	0	8·03	0·31	—	—	—	—
					8·12	0·33	—	—	—	—
October										
2	—	—	Normal	0	4·68	0·145	—	—	—	—
					4·47	0·24	—	—	—	—
3	—	—	Normal	2·0	—	—	3·92	0·26	—	—
							4·02	0·22	—	—
4	—	—	Normal	2·0	—	—	3·83	0·16	—	—
							3·78	0·06	—	—
5	—	—	Normal	2·0	2·48	0·09	2·64	0·20	1·60	—
6	M	150	Normal	2·0	8·72	0·29	9·55	0·00	8·30	—
8	F	95	1	3·0	0·427	0·00	0·454	0·00	0·27	—
9	F	87	2	3·0	0·457	0·00	0·585	0·00	1·28	—
10	F	94	3	3·16	1·03	0·054	1·34	0·053	3·10	—
11	F	77	4	3·0	1·19	0·00	1·24	0·00	0·50	—
12	F	89	5	3·0	3·42	0·00	3·41	0·00	−0·10	—
15	F	72	8	3·0	1·51	0·09	1·64	0·06	1·30	—
November										
10	M	261	2	3·0	0·574	0·00	0·890	0·00	3·16	—
11	M	141	3	3·06	3·12	0·08	3·63	0·00	5·10	—
12	—	234	2	3·0	1·66	0·10	1·80	0·08	1·40	—
13	M	214	3	3·5	2·53	0·16	3·13	0·05	6·00	0·64
14	—	300	2	3·0	1·38	0·00	1·48	0·00	1·00	1·14
15	—	314	3	3·0	1·38	0·00	1·39	0·00	0·10	1·00

tissue gave an increase in carbohydrate during this period of shaking. These results are in contrast to those of Takane [1926]. Takane found a decrease in total carbohydrate in normal tissue and only observed an increase in the presence of added lactate. We determined the lactic acid content of the tissue at the beginning of three experiments (Table IV). The maximum initial lactic acid content was 1·14 mg. per g. tissue. If all the lactic acid were converted into fermentable sugar, it would account for a rise of the same amount. We did not determine the content of lactic acid at the end of our experiments. If lactic acid had disappeared, there would be no evidence that it had passed into carbohydrate and thus no correction could be applied to the carbohydrate increase. We then turned our attention to the livers of fat-fed animals. Again, an increase in carbohydrate was observed. The most marked increase was obtained on November 13th, where the increment was 6 mg. per g. tissue. In this experiment, the initial lactic acid value was only 0·64 mg. per g. tissue. Therefore the conversion of lactic acid into carbohydrate could account for only a small proportion of the total increase. In a few of our experiments the carbohydrate increase could be explained by assuming that all the initial lactic acid was converted into fermentable carbohydrate, as in the experiment of November 14th. The majority of our experiments gave increases greater than 1 mg. per g. wet tissue, therefore the increment of carbohydrate must have come from some other source. Taken in conjunction with the low respiratory quotients, we regard this as most important evidence for the formation of carbohydrate from fat.

Glycogen. The changes in the liver glycogen of the rats on a butter diet are given in Fig. 4. These results are stated in terms of g. glucose per 100 g. wet tissue. The values of the glycogen content of livers from rats on a normal diet varied between 1·52 % and 5·25 % with one exception. This value was very low,

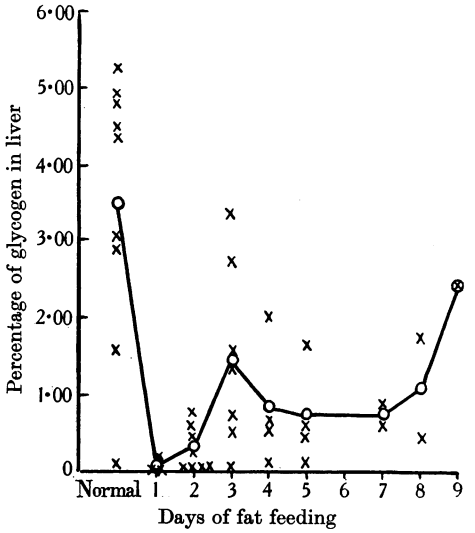

Fig. 4. Variation of liver glycogen on normal and fat diets. The circle gives the average value in each case.

0·10 %. On the diet of butter, the liver glycogen practically disappears on the 1st day and then gradually returns, reaching an average value of about 1 %. A high value was obtained for the liver glycogen of a rat fed for 9 days on butter. The averages for our 7, 8 and 9-day experiments are not as conclusive as for the 2nd, 3rd and 4th days, owing to the smaller number of experiments made on the longer periods of fat feeding. Our results show an increase of glycogen under

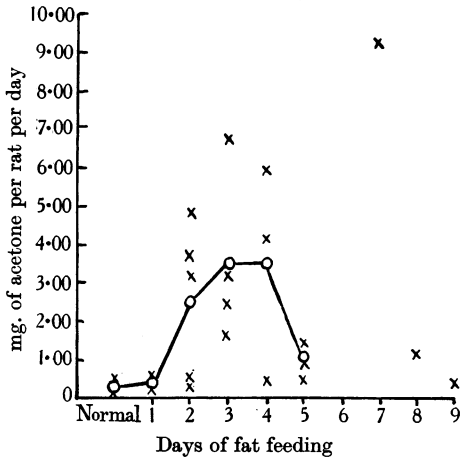

Fig. 5. Total acetone bodies excreted per rat per 24 hours.

these conditions. In one experiment, we kept a rat on butter diet for 7 days and then on the normal diet for 2 days. This rat gave an exceptionally high value for liver glycogen, 9·5 %.

Total acetone bodies. The results are given in Fig. 5 in terms of mg. acetone per rat excreted in the urine during the 24 hours before the animal was killed. There is a slight correlation between the appearance of glycogen in the liver and the disappearance of the total acetone bodies from the urine. The results in both series are so scattered that it is difficult to draw any conclusions from the averages. In general the acetone bodies showed an initial rise, starting on the 2nd day, reaching a peak on the 3rd and 4th days and then falling again, although the one experiment on the 7th day gave a very high value. Wigglesworth [1924] has observed similar changes in the acetone bodies of rats on a diet of butter.

An attempt was made to determine the changes in the fat content and the iodine number of liver slices in order to see if any increase in desaturation or if any decrease in fat content could be detected during a 3-hour period of metabolism. The results were so variable for two series of slices shaken for the same period of time that this procedure was discontinued.

Discussion.

The experiments described in this paper offer good evidence in favour of the hypothesis that fat can be converted into carbohydrate in the liver of the rat. In view, however, of the sharp controversy which the question has raised in the past, we are anxious to define as precisely as possible exactly what we believe our experiments to have proved. We have shown that, on a fat diet but not on a normal one, the respiratory quotient of rat liver slices *in vitro* is well below 0·7. Therefore there is a causal connection between fat feeding and low respiratory quotients. Further, we have shown that carbohydrate can be synthesised by liver slices *in vitro*. The matter may, perhaps, be put into more general terms, as follows. The respiratory quotient in our experiments is a function of the fat diet: carbohydrate synthesis also takes place under these conditions. Since we have eliminated by direct experiment many of the fallacies which surround the determination of the respiratory quotient, we feel that there is strong presumptive evidence that the low quotients do, in fact, indicate carbohydrate synthesis; this presumption is greatly strengthened when we find such a synthesis actually occurring. We should feel that neither finding, taken by itself, would be very conclusive; on the other hand, both taken together, represent a strong case.

One or two further points merit attention. In the livers of two rats on a normal diet, we found carbohydrate synthesis, and in one case this synthesis was larger than any found in the livers of the fat-fed animals. If gluconeogenesis were a process occurring only in animals fed on an entirely abnormal diet of pure fat, it would probably be of little importance. It has, on the contrary, been assumed by many authors, without experimental proof, to occur in normal animals. The R.Q. of the normal liver slices may be assumed to have been in the neighbourhood of 0·79, the average figure for normal livers, and one which does not indicate the formation of carbohydrate from fat. We have, in fact, no evidence to show that the carbohydrate was not formed in these cases from protein. Supposing, however, that it was formed, in whole or in part, from fat, it is quite possible that in a carbohydrate-rich liver the process would result in a lowering of the R.Q. from unity to a level which was still well above that of fat oxidation. On the other hand, in carbohydrate-poor livers, such as are those on the 2nd and

3rd days of a fat diet, the liver is probably oxidising fat, and carbohydrate synthesis would lower the R.Q., starting from a base line, not of 1·0, but of 0·7. We have calculated, in fact, that the synthesis of 3·0 mg. carbohydrate per g. liver slices would lower the R.Q. from 0·79 to 0·63 in our respiratory experiments. Although some of our experiments showed a synthesis of carbohydrate of less than 3·0 mg. per g. tissue, in four experiments the carbohydrate synthesis was greater than 3·0 mg. Similarly, in a number of experiments, the R.Q. was less than 0·63. Our average value was in fact 0·58.

The increase in liver glycogen in the rats fed on butter occupies an intermediate position between the results of those observers who have reported large increases and those who have found very small changes. The averages for our determinations for each day show a decided increase on the 3rd day in contrast to the absence of glycogen on the 1st and 2nd days. Without our other data, it might be assumed that this increase is due to the glycerol fraction of the fat, an assumption made by Gregg [1931] to explain similar results. With the lowered respiratory quotient and the determination of an actual increase in carbohydrate in liver slices the more probable explanation for this rise is that part of the carbohydrate is coming from fat.

SUMMARY.

1. The respiratory quotient of slices of liver from a rat fed on a normal diet averages 0·79, while that from a rat fed on butter averages 0·58.

2. The carbohydrate content of liver slices taken from a liver of a rat fed on butter shows a definite increase after 3 hours' shaking in bicarbonate-Ringer medium at 37°.

3. The glycogen content of the liver of rats fed on butter falls to practically zero on the 1st day of this diet. It gradually rises reaching a level of almost 1·00 % on the 4th and 5th days of butter feeding.

4. The acetone bodies in the urine of rats increase, reach a maximum on the 3rd or 4th day of butter feeding and then decrease.

5. The lowering of the respiratory quotient and the increase in carbohydrate of the liver slices indicate that conversion of fat into carbohydrate takes place in the liver of a rat fed on butter. The same conversion may take place in a normal liver.

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