612.352.1:547.457.3

GLYCOGEN STORAGE AND LÆVULOSE TOLERANCE.

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(*Received December* 12, 1933.)

Carbohydrate tolerance tests, especially when done with lævulose, are thought to give some indication of liver function [Isaac, 1920; Isaac and Siegel, 1928; von Bergmann, 1932; Stroebe, 1932; Kimball, 1932, etc.]. Hofmeister [1890] was the first to point out that dogs who had been starved for a long time developed glycosuria after the ingestion of carbohydrates (hunger-diabetes). Barrenscheen [1914] showed by transfusion methods that less glycogen than normal was formed in a liver if it were from a starved animal and contained little glycogen. Bang [1913] demonstrated a high and prolonged blood-sugar curve following glucose ingestion in rabbits after a period of starvation. Staub [1922] found a similar type of blood-sugar curve after fasting in man. In view of the previous work of Barrenscheen, this type of blood-sugar curve was taken to mean that the liver cell could not form glycogen when it contained little itself [Lesser, 1920; Staub, 1922; Lichtwitz, 1930]. Isaac [1920] showed that after ingestion of lævulose the blood sugar in normal man rises only slightly, whereas with a damaged liver there is a considerable rise.

The small normal rise of blood sugar in man after ingestion of lævulose was believed to be due to unchanged lævulose [Isaac, 1920]. The micro-method of estimating lævulose introduced by van Crefeld [1927] showed, however, that it is always mainly due to glucose [van Crefeld and Ladenius, 1928; and others]. In the case of experimental damage to the liver (chloroform, hydrazine sulphate) and disease in man the larger rise is partly due to unchanged lævulose but much more to glucose, in some cases indeed to it alone; Corley, 1929; Heinecke and Peters, 1930; Steinitz, 1932; and others]. The unchanged lævulose when found is in all probability due to the failure of such a liver to convert lævulose fully into glucose, a conversion which was shown by Mann and Magath [1921] to be a function of the normal liver. The increase in

glucose was assumed, on the basis of Barrenscheen's experiments, to be due to the inability of a liver containing too little glycogen to make any more. Vogt [1932], under Stroebe, held the definite view that "only a liver rich in glycogen can deal adequately with large quantities of carbohydrate. A raised and prolonged blood-sugar curve after lævulose justifies the conclusion that the liver contains little glycogen." Buettner and Neuhaus [1931], Stroebe [1932], Steinitz [1932] and others consider this type of blood-sugar curve as evidence of the inability of the damaged liver to form or store glycogen. There is no experimental evidence to justify these opinions.

The following paper, therefore, deals with the problem whether a lack of glycogen in the liver cells impairs the power of forming glycogen after ingestion of lævulose and whether the blood-sugar curve is determined by the readiness with which glycogen can be formed or stored in the liver.

Methods.

Groups of male rats were submitted to different periods of starvation. The blood sugar and the average resting glycogen content of the liver and muscles were determined, and then blood sugar, liver and muscle glycogen estimated at intervals after the administration of a standard dose of lævulose.

Each series consisted of nine or ten animals of about 150 g. body weight which were kept under the same conditions two weeks before the experiment. Some (3 to 5) were then killed, while the rest received the sugar and were killed one at the end of each hour up to the sixth hour after it. The standard meal consisted of about 500 mg. of lævulose which was dissolved in 1 c.c. of water and introduced into the stomach by a fully filled 1 c.c. syringe fixed to the end of a stomach tube. At the end of the experiment the syringe was again fully filled with the lævulose solution employed in the experiment, then emptied into a 200 c.c. flask, made up with water and the exact amount of lævulose estimated in an aliquot part by Hagedorn-Jensen's method. Immediately after the death of the rat the liver and the skinned left leg were frozen in liquid air, and the glycogen content estimated by Pflüger's method. The muscular tissue remained almost perfectly intact by removing the leg from the pelvis. Blood was obtained from the heart with filter paper or by bleeding into a dish containing oxalate crystals. Estimation of blood sugar was made by Hagedorn-Jensen's method. The amount of lævulose absorbed was calculated by subtraction of the sugar still found in the alimentary tract after death from the total amount administered.

In order to determine the lævulose content of the gut, the whole alimentary tract was removed between ligatures and cut in pieces. The fluid was expressed from the segments which were then washed and all the fluid so obtained was filtered through glass wool. The sugar content of an aliquot part of the filtrate was then determined. No appreciable sugar content was found by this method in the empty alimentary tract.

Results.

Lævulose absorption from the gut.

The amount of sugar absorbed has been stated by Cori [1925, 1926] to depend very much on the length of the previous fasting period. He found that 77 mg. of lævulose were absorbed per hour per 100 g. rat with a previous fasting period of 48 hours, whereas with only 24 hours more than 100 mg. were absorbed. In the present experiments (Table I) no marked difference in absorption was found in rats starving for 12, 24, 48 or 96 hours. It was, however, considerably faster in winter, average 200 mg. in the first hour, than it was in the summer, when it averaged 131 mg. The average for all rats examined was found to be 174 mg. in the first hour, 142 mg. in the second and 125 mg. in the third. Absorption was complete at the end of the third hour. The values of blood sugar were not affected by the rate of absorption.

Table I. Lævulose absorption from the alimentary tract.

	Rate of absorption (in mg. per 100 g. rat)		
	1st hour	2nd hour	3rd hour
Rats starving 12 hours	180	148	128
„ „ 24 „	156	130	—
„ „ 48 „	149	135	115
„ „ 96 „	164	142	123
Rats killed during winter	202	157	127
„ „ „ summer	131	119	114
Average of all rats	174	142	125

Glycogen formation in the wall of the intestine.

If glycogen were formed in the intestinal wall during the absorption, the subsequent breakdown of this glycogen might contribute to the raising of the blood sugar. Accordingly estimations of glycogen in the intestinal wall were made; the amounts found were negligible and showed little or no change during and after lævulose absorption.

The blood sugar.

In this and the following sections the results are best set out by grouping the experiments thus.

Group I consisted of well-fed rats without a long previous fasting period. They were fed with 1 g. lævulose per 150 g. body weight on the eve of the experiment (12 hours before it) and then kept starving. In this group 52 rats were employed, 25 of them being killed at different intervals after the lævulose meal. In these latter the average increase of blood sugar was 7 mg. per 100 c.c.; in 68 p.c. of them not more than 10, in 32 p.c. between 10 and 50 mg. per 100 c.c.

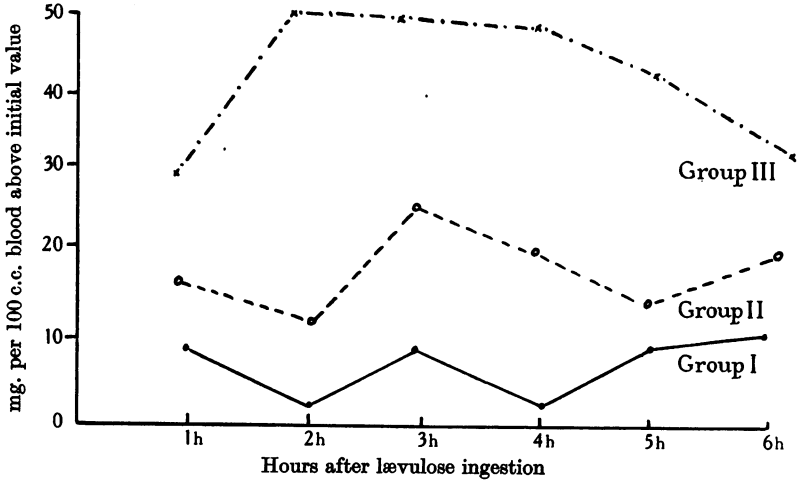

Fig. 1. Blood-sugar increase after lævulose meal. Figures given in mg. per 100 c.c. above initial level. Group I: well-fed rats. Group II: short fasting period. Group III: long fasting period.

Group II, 70 rats starving 12–48 hours, of which 47 were examined after getting lævulose. In these the average rise of blood sugar was 18 mg. per 100 c.c.; in 19·2 p.c. less than 10, in 78·7 p.c. between 10 and 50, and in 2·1 p.c. more than 50 mg. per 100 c.c.

Group III, 46 rats fasting for 96 hours, of which 28 were examined after lævulose. In these the average rise of blood sugar was 41 mg. per 100 c.c., in 3·6 p.c. of them less than 10, in 71·4 p.c. between 10 and 50, and in 25 p.c. above 50 mg. per 100 c.c.

The blood-sugar curves of groups I and II (Fig. 1) show a moderate increase during the course of the experiment, whereas the curve of group III corresponds to a "high and prolonged blood-sugar curve"

[Vogt, 1932]. If the statements referred to above were correct groups I and II ought to have shown a good initial glycogen content in the liver and good formation and storage after lævulose, whereas a lack of liver glycogen in the resting rats and a deficiency of glycogen formation and storage should have been found in the third group.

The glycogen formation in the liver.

Group I. This consisted, as already mentioned, of rats which were fed on the eve of the experiment with lævulose and then kept starving. This procedure was adopted because when animals were killed without any starvation period or after a fasting period of four hours the glycogen in the liver varied very widely. By the method adopted livers were obtained which showed a good resting glycogen content. There was a considerable difference in the glycogen content of different series which may well be accounted for by difference in the season and in external circumstances. In any one series, however, the resting liver glycogen content was found to be of approximately the same value (Table II).

TABLE II. Resting glycogen content in the livers of rats.
Figures given in g. per 100 g. liver.

Group I, well-fed rats. Group II, short fasting period. Group III, long fasting period.

Group I		Group II	Group III
Series S	4·90	0·14	0·26
	4·40	0·12	0·18
	5·79	0·25	0·36
Series J	2·41	0·32	0·16
	2·68	0·42	0·39
	2·60	0·07	0·52
	2·19	0·07	0·34
Series W	6·16	0·08	0·54
	5·86	0·06	0·54
	7·03	0·07	0·88
Series V	3·19	0·15	0·08
	3·64	0·04	0·15
	3·60	0·08	0·21
		0·06	0·53
		0·13	0·39
		0·14	0·12
		0·12	1·18
		0·08	0·13
		0·15	
		0·23	
		0·10	
		0·15	
		0·19	
		0·12	
		0·16	
	Average	0·14	0·39

After the standard dose of lævulose the average content of glycogen found in the liver (Table III) decreased in the course of the experiment. The most marked decrease was observed in series with the higher initial glycogen values, above 5 p.c. With an initial glycogen value below that figure there was a slight increase in the second and third hour, but a marked decrease occurred in the later hours of the experiment. As a control experiment water only was given. This was followed by a rapid and progressive decrease in liver glycogen. After the end of absorption (fourth hour of experiment) the glycogen in the liver was in every series actually less than at the beginning of the experiment.

TABLE III. Glycogen formation in the liver after 0·5 g. lævulose.
Figures given in g. per 100 g. liver above resting value.
+ indicates increase, − indicates decrease.
Group I, well-fed rats. Group II, short fasting period. Group III, long fasting period.

	Group I		Group II		Group III	
Time	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats
1st hour	−0·64	3	+0·80	7	+0·43	5
2nd hour	−0·64	4	+2·05	9	+2·29	5
3rd hour	−0·40	3	+2·27	7	+3·05	5
4th hour	−1·57	3	+2·57	6	+3·74	4
5th hour	−1·71	3	+2·04	5	+4·01	5
6th hour	—	—	+1·89	5	+2·97	4

Group II. The liver glycogen after a short fasting period was found to be practically constant, usually below 0·2 and never exceeding 0·5 p.c. After the dose of lævulose the average amount of glycogen deposited in the liver was found to be 0·80 after 1 hour and 2·05 p.c. after 2 hours. The glycogen formation was generally smaller than the average in those rats in which absorption from the gut was low. After 4 hours 25 p.c. of the sugar absorbed was recovered as glycogen in the liver. The average absolute glycogen content after that period was 2·57 p.c. which is only slightly less than the absolute average figure of the first group (2·84) in spite of the considerable difference in the resting glycogen of these two groups.

Group III. The glycogen values after prolonged fasting were found to be practically constant and definitely higher than those in group II (Table II). A strong positive acetone reaction was present in the urine at the beginning of the experiment.

After the dose of lævulose the rate of glycogen formation in the liver during the first hour was poor, the average found being 0·46 p.c. in contrast with 0·80 in group II. At the end of the second hour it was the same as in group II (Table III); at the end of the fourth hour it was

3·74 p.c., which corresponds to 33 p.c. of the sugar amount absorbed, both these figures being higher than those of groups I and II.

The results given so far show that the amount of glycogen already in the liver does not determine the capacity of the liver to build up glycogen, as was suggested by Barrenscheen's experiments. The rats of group II, although they had but little glycogen in the liver, showed good glycogen formation, whereas the rats of group III with a somewhat higher resting glycogen value formed definitely less in the first hour. The store of glycogen built up after the end of the absorption period (fourth hour) was in both groups II and III better than in group I with a high initial glycogen value. Furthermore, the results do not confirm the explanation of the blood-sugar curve after lævulose hitherto given. According to the blood-sugar curves, groups I and II should have had a good store of glycogen in the liver to start with, and after lævulose have increased it considerably, whereas the reverse should have held for group III. It is, however, evident that group II had the smallest amount of glycogen in the liver before the dose of lævulose and group I the smallest amount after it; the best glycogen formation was found in rats of group III. When the increase of blood sugar is compared with the glycogen deposition in the liver (Table IV) it is seen that a high rise of blood sugar does not correspond to a small glycogen formation in the liver nor a small one with a large. The blood-sugar curve after lævulose has in these experiments no relation to the liver glycogen.

TABLE IV. Increase in liver glycogen in relation to the blood-sugar rise. Increase in liver glycogen in p.c. of lævulose absorbed.

Increase in blood sugar mg. per 100 c.c.	1st hour		2nd hour		3rd hour		4th hour		5th hour		6th hour	
	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats
0–10	9	9	22	4	18	2	5	4	7	3	10	3
10–50	12	9	27	10	21	10	27	7	30	7	22	6
Above 50	13	1	31	2	42	1	27	2	24	1	—	—

The glycogen formation in the muscles.

Group I. The initial glycogen content of the muscles was approximately the same for all rats of this group of well-fed animals in spite of the considerable differences in the amount of liver glycogen in the different series (Table V). The average glycogen formation after lævulose was considerable; a comparatively large amount of glycogen was already deposited in the first hour after lævulose which was in all probability due to the breakdown of liver glycogen (Table VI).

Group II. The resting glycogen in the muscles of this group was low, as it was in the liver. After lævulose ingestion a good glycogen formation was found which increased every hour until the maximum was reached in the fourth hour of the experiment when absorption was finished. No appreciable difference is found in the average rate for the whole experiment between groups I and II (Tables V and VI).

TABLE V. Resting glycogen content in the muscles of rats.
Figure given in g. per 100 g. muscle weight.

Group I, well-fed rats. Group II, short fasting period. Group III, long fasting period.

	Group I	Group II	Group III
	0·31	0·16	0·20
	0·31	0·17	0·24
	0·36	0·14	0·24
	0·32	0·14	0·36
	0·38	0·15	0·38
	0·31	0·20	0·19
	0·22	0·18	0·20
	0·40	0·15	0·32
	0·26	0·28	0·21
	0·31	0·22	0·27
	0·27	0·15	0·26
	0·41	0·19	0·12
	0·26	0·22	0·23
	0·26	0·18	
	0·32	0·16	
	0·24		
	0·28		
Average	0·31	0·18	0·25

TABLE VI. Glycogen formation in the muscle after lævulose.
Figures given in g. per 100 g. muscle above resting value.
+ indicates increase, − indicates decrease.

Group I, well-fed rats. Group II, short fasting period. Group III, long fasting period.

	Group I		Group II		Group III	
Time	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats
1st hour	+0·12	3	+0·06	6	−0·01	5
2nd hour	+0·06	5	+0·08	8	+0·06	5
3rd hour	+0·13	5	+0·11	4	+0·09	5
4th hour	+0·13	5	+0·18	6	+0·07	4
5th hour	+0·13	3	+0·08	3	+0·05	4
6th hour	+0·12	2	+0·12	5	−0·02	4
Average	+0·12	23	+0·10	32	+0·04	27

Group III. The average initial glycogen content in this group held, as in the case of the liver, an intermediate position between the figures for groups I and II. No glycogen formation occurred during the first hour; the maximum was found after 3 hours and was about half as much as the maximum in group II. At each hour the figures are lower than

those for either group I or II, the average for the whole experiment being only one-third (Tables V and VI).

It must be noted that the increase of muscle glycogen is small like the initial glycogen content. The variations observed in the resting glycogen content in the liver, especially if this is low, do not affect the results after lævulose because comparatively large quantities of glycogen are newly formed. The same variations, when they occur as they do, in the resting muscle glycogen are much more liable to affect the result owing to the comparatively small amount of glycogen formed. The number of animals, however, from which these averages are calculated is such that the figures certainly indicate a diminished power of building up glycogen in the muscles of rats that have fasted long (group III).

There is a definite relationship between the formation of muscle glycogen and the corresponding increases in blood sugar (Table VII). Rats showing a marked increase in blood sugar (above 50 mg. per 100 c.c.) show little if any formation of glycogen in the muscle; the amount formed is largest when the blood sugar rises least. The capacity of the muscle to build up glycogen seems, therefore, to have a definite relation to the blood-sugar curve. The blood sugar after lævulose is high when the muscles cannot form glycogen.

TABLE VII. Increase in muscle glycogen in relation to the blood-sugar rise after 0·5 g. lævulose meal.

Figures given in g. per 100 g. muscle weight above resting value and in mg. per 100 c.c. blood. Where the – sign appears there is no increase but a diminution.

Increase in blood sugar	1st hour		2nd hour		3rd hour		4th hour		5th hour		6th hour	
	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats	Glycogen	No. of rats
0–10	0·12	5	0·10	5	0·12	4	0·15	5	0·17	2	0·16	3
10–50	0·03	9	0·06	8	0·11	10	0·11	6	0·09	6	0·03	7
Above 50	–0·08	1	0·01	3	0·02	1	0·03	2	0·05	2	—	—

The effect of thyroxine.

The following experiment points to a similar conclusion. Abderhalden and Wertheimer [1930] showed that after thyroxine the livers of rats are unable to form glycogen. A series of seven rats were, therefore, injected daily with 0·5 mg. thyroxine (Schering) for 7 days. The animals were then kept fasting 12 hours before giving the lævulose. The liver glycogen of the fasting rats was very low, 0·06 p.c. Following lævulose ingestion there was hardly any increase, the maximum after 3 hours being 0·16 p.c. The muscle, however, the initial glycogen content of

which was also very low (0·09 p.c.), had risen after 3 hours to a maximum, 0·25 p.c., an increase similar to that observed in rats of group II. There was no marked rise in the blood-sugar level. This result is very much against the old explanation that the failure of the liver to form glycogen accounts for the rise of blood sugar after lævulose, while on the contrary the relation between glycogen formation in the muscles and blood sugar is the same as that in the experiments recorded above. With larger doses of thyroxine the blood sugar tends to rise considerably after lævulose [Kugelmann, 1930] and the glycogen formation in the muscles of rats becomes poor [Abderhalden and Wertheimer, 1930].

Summary.

1. It has been commonly held that (*a*) the power of the liver to form glycogen is impaired when its store of glycogen is low; and that (*b*) an excessive rise of blood sugar after taking lævulose is due to the inability of the liver to form and store glycogen. The experimental basis of these opinions has been critically examined.

2. The power of the liver to form glycogen has been shown not to be impaired when its store of glycogen is low.

3. An excessive rise of blood sugar after lævulose has been found to be associated neither with a low glycogen store nor with inability of the liver to form and hold glycogen.

4. On the other hand such a rise of blood sugar after lævulose was associated with little or no glycogen formation in the muscles.

5. The results suggest that inability, not of the liver, but of the muscles to build up glycogen is at least one factor of importance in an excessive rise of blood sugar after lævulose. The clinical observation that such a blood-sugar curve is given in disease of the liver is not disputed, nor is it incompatible with the above results if the anabolism of glycogen in the muscles is influenced by the metabolic changes which carbohydrates undergo in the liver.

I wish to express my thanks to Prof. von Bergmann for many kindnesses shown to me while working at the Charité Krankenhaus, Berlin, and for facilitating this investigation in every way. I am also extremely indebted to Dr A. F. Hurst for his encouragement and advice which has enabled me to correlate my researches and present them in this form; and to Mr Spurrell and Dr S. L. Simpson for very helpful criticism.

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