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FORMATION OF GLYCOGEN IN THE LIVER OF ANÆSTHETIZED CATS WITH NOTES ON SPECIFIC DYNAMIC ACTION

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In the study of glycogenesis in the liver, the use of anæsthetics has been condemned by many workers because the glycogen of the liver decreased during anæsthesia. In spite of this drawback non-volatile anæsthetics may have a limited range of usefulness. The present investigation deals with the behaviour of glycogen in the livers of cats under chloralose when some possible sources of glycogen, carbohydrate and non-carbohydrate, are given slowly into a vein.

Methods

(1) *Experimental*

Cats, fasted for 48 hours, were anæsthetized by giving an intraperitoneal injection of 0·08 g. chloralose per kg. body weight. The induction period should be quiet and free from muscular movements. After anæsthetization it is important to maintain the rectal temperature of the cats at about 38° C. [Reid, 1935].

The behaviour of liver glycogen was studied during and after the administration of the following substances: glucose, lactic acid (boiled previously to hydrolyse anhydride), glycerol, propionic acid, alanine, amino-acetic acid, glutamic acid and aspartic acid. Solutions of these were filtered before use and injected slowly into the femoral vein by the infusion apparatus of Burn and Dale [1924].

The periods before, during and after infusion are referred to as the initial, infusion and post-infusion periods respectively.

Carotid blood and liver samples (by the method of Evans, Tsai and Young [1931 *a*]) were taken at the end of each of these periods.

A glass cannula with rubber tubing and clip was tied through the proximal part of the urethra into the bladder, which was emptied and washed out with 0·9 p.c. NaCl about 1·5 hours after anæsthetization. Subsequently samples of urine were obtained after the blood samples and kept for analysis.

Blood-pressure readings were taken occasionally during each experiment.

The liver was weighed at the end, and, in some experiments, the heart was excised and its glycogen content determined.

(2) *Chemical*

The following determinations were made as far as possible in duplicate:

(1) Blood: glucose—Hagedorn and Jensen [1923]; lactic acid—Friedmann, Cotonio and Shaffer [1927]; amino-N—Folin [1922].

(2) Urine: glucose—Benedict [1911]; lactic acid—as for blood; inorganic sulphate—Fiske's [1921] modification of the method of Rosenheim and Drummond [1914]; urea—urease tablets (B.D.H. Ltd.).

(3) Liver: glycogen—Evans, Tsai and Young [1931 *a*]; water content—drying at 100° C. to constant weight.

As the variations in water content were small, it was not considered necessary to allow for this in the calculation of the percentage of glycogen.

Results

(1) *Glucose and lactic acid*

Twenty p.c. glucose was given during a 2-hour infusion period so that 2·0 g. per kg. were given in four and 1·5 g. per kg. in two experiments. Lactic acid, neutralized with NaOH to phenol red, was given in doses of 1·0–1·5 g. per kg. in six experiments, but the infusion period lasted 5 hours, as it was inadvisable to give lactic acid as rapidly as glucose. The results, showing the yield of liver glycogen, are set out below (Table I). Increases in glycogen were recorded in each experiment, while controls showed continuous decreases of about 0·1 p.c. per hour.

Table I. Infusion of glucose or lactic acid into a vein: averages from six experiments in each group.

Retained per kg. body wt.	Liver glycogen p.c.			Liver wt.
Hour...	Initial	2·5	5·0	g.
Glucose 1·32 g.	0·98	1·51	1·85	58
Lactic acid 1·18 g.	0·98	1·83	1·39	60
Controls	1·12	0·82	0·60	57

(2) *Glycerol*

Glycerol, 1·5 g. per kg., was given as a 10 p.c. solution in three experiments during an infusion lasting 2 hours. Continuous increases in liver glycogen were recorded in each experiment. Blood glucose increased by 0·06–0·09 p.c. in two experiments but not in the third, which showed the largest rise in liver glycogen. Blood lactic acid rose by nearly 0·01 p.c. at the end of the experiment. This might be due to formation of lactic acid from glyceric aldehyde.

13. xi. 34. Short protocol of cat No. 136: wt. 2·6 kg.; given glycerol 1·5 g. per kg. intravenously.

Hour	
09.30	Chloralose 0·2 g.
11.30	Blood glucose 88 mg. per 100 c.c.; liver glycogen 2·38 p.c.
12.03–14.15	Glycerol 3·9 g.
11.05–12.55	Sulphate excretion 1·81 mg. per hour.
14.20	Blood glucose 83 mg. per 100 c.c.; liver glycogen 2·85 p.c.
14.55	Sulphate excretion 2·06 mg. per hour.
16.00	Blood glucose 93 mg. per 100 c.c.; liver glycogen 2·98 p.c.; blood pressure 110 mg. Hg.
16.03	Sulphate excretion 2·22 mg. per hour.

(3) *Propionic acid*

From a few experiments in which propionic acid, neutralized, was given slowly into a vein, it was concluded that conversion of propionic acid to glycogen in the liver was not sufficiently rapid to be shown unequivocally in our anæsthetized cats. In one experiment, cat No. 196, for example, the glycogen of the liver fell slowly from 2·65 to 2·48 p.c. in 5 hours during the infusion of propionic acid.

(4) *Amino acids*

Alanine, amino-acetic acid, glutamic acid and aspartic acid, 1·5–2·0 g. per kg., were given in 10 p.c. solution. As the last two are not very soluble, NH_4OH or NaOH was added until complete solution was obtained.

Alanine.

In ten experiments with alanine, four showed continuous increases in liver glycogen, four a fall followed by a significant rise, one a stationary level and one a continuous decrease. The increases were substantial, and, as controls showed continuous decreases, they are taken as significant.

Blood glucose rose by about 0·05 p.c. and blood lactic acid, which had risen to between 0·03 and 0·04 p.c. at the end of the infusion, fell in

3 hours nearly to the initial level. The large increase in the blood lactic acid may indicate the conversion of alanine to lactic acid and other substances, *e.g.* pyruvic acid, which are estimated as lactic acid. In the experiments with amino-acetic acid the increase in the blood lactic acid was small and of doubtful significance. Since its specific dynamic action is of the same order as that of alanine [Rapport and Beard, 1927], it is unlikely that the increased lactic acid of the blood with alanine is derived from the muscles.

About 5 p.c. only of the alanine was excreted in the urine from the commencement to 3 hours after the infusion period.

3. x. 34. Short protocol of cat No. 120: wt. 2·8 kg.; given alanine 2 g. per kg. intravenously.

Hour	
09.30	Chloralose 0·21 g.
12.00	Blood sample: glucose 96 mg. per 100 c.c.; amino-N 12 mg. per 100 c.c.; lactic acid 16 mg. per 100 c.c.; liver glycogen 1·21 p.c.
12.20–15.20	Alanine 5·6 g.
10.45–12.45	Sulphate excretion 1·68 mg. per hour.
15.30	Sulphate excretion 2·18 mg. per hour.
15.30	Blood sample: glucose 134 mg. per 100 c.c.; amino-N 26 mg. per 100 c.c.; lactic acid 38 mg. per 100 c.c.; liver glycogen 1·35 p.c.
16.50	Blood pressure 115 mm. Hg.
17.45	Sulphate excretion 2·22 mg. per hour.
18.00	Blood sample: glucose 147 mg. per 100 c.c.; amino-N 19 mg. per 100 c.c.; lactic acid 15 mg. per 100 c.c.; liver glycogen 1·75 p.c.

Amino-acetic acid.

In seventeen out of nineteen experiments with amino-acetic acid, liver glycogen decreased rapidly during the infusion period, but increased very slowly during the post-infusion period in fourteen of these. Continuous increases were recorded in two experiments only.

2. x. 34. Short protocol of cat No. 131: wt. 2·3 kg.; given amino-acetic acid 1·5 g. per kg.

Hour	
09.30	Chloralose 0·18 g.
13.00	Blood sample: glucose 82 mg. per 100 c.c.; amino-N 16 mg. per 100 c.c.; lactic acid 11 mg. per 100 c.c.; liver glycogen 1·14 p.c.
13.12–15.55	Amino-acetic acid 3·45 g.
10.40–13.20	Sulphate excretion 1·20 mg. per hour.
16.00	Blood sample: glucose 121 mg. per 100 c.c.; amino-N 30 mg. per 100 c.c.; lactic acid 18 mg. per 100 c.c.; liver glycogen 0·30 p.c.
16.20	Sulphate excretion 1·59 mg. per hour.
18.20	Blood sample: glucose 109 mg. per 100 c.c.; amino-N 18 mg. per 100 c.c.; lactic acid 11 mg. per 100 c.c.; liver glycogen 0·46 p.c.; blood pressure 85 mm. Hg.
18.25	Sulphate excretion 1·36 mg. per hour.

Blood glucose rose during the infusion when glycogenolysis was rapid, but fell after the infusion was stopped. The behaviour of the amino-N of the blood and the urine was on the whole similar to that found for alanine.

When amino-acetic acid was given by slow infusion into the upper jejunum, the results confirmed those obtained by the intravenous route.

Glutamic acid and aspartic acid.

On the whole, liver glycogen decreased more rapidly when the NH_3 or Na salts of these amino acids were given than in controls with one exception. Evidently these amino acids are not readily available in the course of a few hours for conversion to glycogen in the liver of anæsthetized cats.

The blood glucose increased in all experiments and the lactic acid with aspartic acid but not with glutamic acid. In contrast to alanine and amino-acetic acid, the amino-N of the blood decreased only slowly after the infusion was stopped.

Discussion

Glucose and formation of glycogen in the liver

Some workers maintain that failure to show that the hepatic inflowing blood contained more glucose than the outflowing during deposition of glycogen, when glucose was given, is a point against the direct formation of liver glycogen from glucose. In recent investigations [Evans, Tsai and Young, 1931*b*; Tsai and Yi, 1934*b*] the source of regenerated liver glycogen after decapitation, etc., in the cat was not glucose, but the further conclusion of Tsai and Yi [1934 *a*] that the liver did not form glycogen from glucose is based on their failure to show a higher percentage of glucose in the inflowing hepatic blood. When our anæsthetized cats were given large amounts of glucose, the largest average increase in liver glycogen was 0·44 p.c. per hour. If the hourly circulation through the liver weighing 60–75 g. is about 4 litres [see Tsai and Yi, 1934 *b*], the difference in the glucose content of nearly simultaneous samples of the inflowing and outflowing blood of the liver during glycogen deposition of this order must be small, and might be lessened or reversed by the addition of glucose to the outflowing blood from glycogen formed from other sources or by accidental manipulation of the liver. In a recent paper which appeared while we were investigating the glucose content of blood going to and coming from the liver, Tsai and Yi [1935] claim that, during absorption of glucose from the cat's intestine and the concomitant deposition of glycogen in the liver, slightly more glucose passed

into than out of the liver. Their observation cannot be taken as conclusive. The small difference might merely show utilization of glucose by the liver for purposes other than the formation of glycogen.

Indirect evidence from the present investigation is suggestive. From the nitrogen excreted in the urine during and after the infusion of glucose, the glucose which could be derived from protein metabolism was calculated and found to be less than the glycogen as glucose laid down in the liver, notwithstanding the raised N excretion during the diuresis due to the infusion.

It is also of interest to note from other experimental records that in cats under chloralose the highest average increase in liver glycogen per hour was 0·44 p.c. when glucose, a protein sparer, was given and 0·47 p.c. for lactic acid, which is not a direct protein sparer.

The behaviour of liver glycogen in relation to specific dynamic action and protein sparing action

Evidence as to the substances used in this investigation which acted as protein sparers is obtained by reference to Table II, which shows the excretion of urea-N and sulphate during the experimental period. In

TABLE II. Excretion of urea-N and sulphate. Chloralose at 0 hour; A = initial period (1·5–3·5 hours); B = infusion period; C = post-infusion period.

Experiments (No.)	Urea-N mg. per hour			Sulphate mg. per hour		
	A	B	C	A	B	C
Controls (3)	20	48	32	1·70	1·50	1·42
*Controls (2)	42	39	38	1·57	1·40	1·42
Glucose (5)	25	48	20	1·87	1·26	1·27
Lactic acid (2)	39	80	55	1·33	1·30	1·29
Glycerol (3)	48	62	37	2·04	2·42	1·93
Alanine (10)	29	80	88	1·68	1·80	1·97
Amino-acetic acid (19)	26	70	68	1·70	2·13	1·82
Glutamic acid (5)	26	19	16	1·65	1·14	1·47
Aspartic acid (3)	30	25	22	2·09	1·13	1·84
Ammonia	52	49	60	2·07	1·67	2·55

* No infusion of NaCl given.

controls not given an infusion the excretion of urea decreased slightly under chloralose, but increased in controls receiving an infusion of NaCl. On the other hand, the rate of excretion of sulphate was not unduly disturbed in these two series of controls but decreased 10 p.c. approximately subsequent to the initial periods. Accordingly, the rate of excretion of the latter has been taken as indicating the rate of protein metabolism when different substances were given, provided these were sulphur-free. The propriety of doing so is afforded further support by

the behaviour of the excretion of sulphate which increased when the body temperature of fasting anæsthetized cats rose above and decreased when it fell below 38° C. [Reid, 1935].

From Table II it is seen that glucose spared protein but neither lactic acid nor glycerol did so directly. Nevertheless, formation of glycogen in the liver occurred from all of these substances.

With regard to the specific dynamic action of the amino acids tried, the complete data of the individual experiments showed that the possession of a considerable carbohydrate store, as represented for example by a glycogen content in the liver of 2–4 p.c., prevented a rise in the excretion of sulphate. On the other hand, if the glycogen store was low, the excretion of sulphate was significantly increased by the administration of both alanine and amino-acetic acid. Further, deposition of glycogen in the liver was readily shown after alanine, but not from amino-acetic acid. On these grounds, therefore, an accurate comparison between the intensity of their specific dynamic effects was not possible from the data of the excretion of sulphate.

Ringer and Lusk [1910] claimed that both amino-acetic acid and alanine were completely converted into glucose in the phlorrhizinized dog, and Rapport and Beard [1927] showed that their specific dynamic action per molecule was of the same order. In our experiments formation of glycogen occurred when alanine was given, but during the administration of amino-acetic acid a rapid fall in liver glycogen occurred. Such a result appears surprising in view of the metabolic paths suggested by Ringer and Lusk. Cori and Cori [1935] wrote that "it would appear that only such amino acids as contain or can break up into odd-numbered carbon chains can yield glucose and that amino-acetic acid seems to be an exception to this rule". It is of interest to observe, however, that Ringer and Lusk [1910], confirmed by Milhorat and Deuel [1926–7], have shown that acetic acid cannot be transformed into glucose in the phlorrhizinized dog.

Glutamic and aspartic acids, given as the NH_4 or Na salts, diminished the excretion of urea N and of sulphate (Table II). In regard to this finding it may be noted that Abderhalden [1912], showed that ammonium salts, when given with non-protein foodstuffs, greatly reduced protein metabolism without establishing nitrogenous equilibrium. In the post-infusion period the excretion of sulphate rose to or above the original rate, but the excretion of urea continued low especially when NH_4OH was used. The slow infusion of a 2 p.c. solution of NH_4OH resulted in decreased excretion of sulphate and little change in the urea

excretion during the experimental period, but the implications of this and the preceding findings require further elucidation.

Notwithstanding these effects on the excretion of nitrogen by the administration of the salts of glutamic or aspartic acid, formation of glycogen in the liver was not shown in our fasted anæsthetized animals.

Summary

1. The usefulness of a non-volatile anæsthetic, chloralose, in the study of glycogenesis in the liver of the cat has been examined, and it has been shown that formation of glycogen in the liver from certain substances, if not unaffected by chloralose anæsthesia, is not thereby prevented.

2. Formation of glycogen in the liver occurred when glucose, lactic acid, glycerol or alanine was slowly infused into a vein; propionic, glutamic and aspartic acids did not increase the glycogen content of the liver during our experimental periods in anæsthetized cats, while amino-acetic acid infusion caused a decided fall, much greater than that shown by controls.

3. No correlation has been found between the ability of an amino acid to increase metabolism and its power to cause the deposition of glycogen in the liver.

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