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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

IV. The effect of some anæsthetics.

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It has been shown [Evans, Tsai and Young, 1931] that the liver glycogen of the cat decreases under both ether and amytal anæsthesia. Since it would be desirable, if possible, to find an anæsthetic which would disturb the liver glycogen and blood sugar as little as possible, it was decided to carry out an investigation of the effect of some anæsthetics on the liver glycogen level in cats.

Methods.

The determination of liver glycogen was carried out in a manner similar to that described in a previous paper [Evans, Tsai and Young, 1931] with the following modifications:

After neutralization of the potash hydrolysate the glycogen was precipitated by adding alcohol up to 70 p.c.: the centrifuged precipitate, dissolved in 1 c.c. of water, was reprecipitated by adding 3 c.c. of alcohol, again centrifuged and hydrolysed. For the analysis of an aliquot portion of the neutralized hydrolysate we used the modified Shaffer-Hartmann method. Prof. Shaffer has kindly furnished us (through Prof. Lovatt Evans) with the unpublished details of the modifications of the original procedure, and with his permission we herewith include these, as we find the method as now modified to be entirely satisfactory and to give theoretical results with pure glucose.

Modified Shaffer-Hartmann reagent.

Na_2CO_3	25 g. per litre
$NaHCO_3$	20 „ „ „
Rochelle Salt	25 „ „ „
$CuSO_4$	7·5 „ „ „
KIO_3	100 to 300 c.c. of a solution 0·1N as to I_2.

(We add 0·801 g. of KIO_3 per litre.)

5 c.c. of the neutralized glycogen hydrolysate are added to 5 c.c. of the above reagent and the mixture heated, in test-tubes (25 × 200 mm.) covered with glass bulbs, for 15 min. in a rapidly boiling water bath. After cooling in water to each is added 2 c.c. of a solution

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containing 2·5 p.c. of potassium iodide and 2·5 p.c. of potassium oxalate, and this is followed by 5 c.c. of $N H_2SO_4$. The tubes are kept covered by glass bulbs and, after shaking and standing for 5 to 10 min. to ensure complete solution and reoxidation of cuprous oxide and iodide, the mixture is titrated with 0·005 N thiosulphate. For calculating the results a curve may be constructed from the following data, supplied by Prof. Shaffer.

TABLE I.

Mg. glucose in 5 c.c.	Titration difference c.c. of 0·005 thiosulphate
2·00	18·20
1·75	15·95
1·00	9·00
0·50	4·48
0·10	0·85
0·05	0·42

Effect of sampling on glycogen content of lobes of the liver.

In accordance with the results described in the previous paper [Evans, Tsai and Young, 1931] the glycogen contents of different lobes of the liver have been considered to be comparable.

In order to determine whether the glycogen content of a lobe was significantly altered by the taking of samples, a number of simultaneous determinations were made on lobes, some of which had previously been sampled, and some of which had not, with the following results.

TABLE II. Glycogen content of used and unused liver lobes.

Exp.	Used lobe p.c.	Unused lobe p.c.
206	0·78	0·44
206	0·17	0·13
206	1·33	1·43
208	1·26	1·02
208	1·16	1·06
222	0·47	0·19
223	0·31	0·22
227	3·22	3·29
230	2·45	2·27
232	0·12	0·05
238	0·40	0·31
239	1·82	1·95
	Average 1·124	Average 1·030

Standard deviation = 0·087. Standard deviation = 0·094.

Unexpectedly it seems that the average of the used lobes is 0·094 p.c. greater than that of the unused lobes, *i.e.* nearly a 10 p.c. relative error.

Calculation of the standard deviation of the average for used and unused lobes suggests that this difference may or may not be significant, but whatever conclusion is drawn, it is certain that the glycogen content of used lobes is not lower, on the average, than that of unused lobes.

In a few cases one or more lobes appeared dark and bruised in consequence of handling or other injury. The glycogen content of such lobes was lower than that of the lobe which appeared quite normal, and histological examination showed that in the bruised sample there was stasis and congestion in the vessels and shrinkage and cytoplasmic cloudiness in the cells.

Blood sugar has been determined throughout by the method of Hagedorn and Jensen [1923]. Although Somogyi [1926] and others have shown that this method includes in the result what Benedict [1931] has termed "saccharoids," yet since we were interested principally in the relative blood glucose changes, we considered the method to be sufficient for our purpose.

At the beginning of each experiment we took a sample of normal blood from the ear for sugar determination. It was usually found possible to obtain such a sample without the slightest struggle while the cat was sitting peacefully on the table. In the few cases where struggling occurred the blood sugar level was always considerably above the average, and those results were not included in the average figures.

All the cats used in this investigation were fasted for 44–48 hours before the experiment.

RESULTS.

Normal blood sugar of cats.

During the course of investigations in this laboratory blood samples from 89 unanæsthetized cats have been taken. The average blood sugar level of these animals was found to be 94·5 mg. p.c. (min. 62, max. 122).

In Table III are given the averages over various periods.

TABLE III. Average blood sugar for cats fasted 48 hours (Hagedorn and Jensen method).

Period	No. of cats	Blood sugar (mg. p.c.)		
		Max.	Min.	Average
11. xi–10. xii. 31	22	120	79	98·8
11. xii. 31–26. i. 32	29	118	70	95·7
27. i–9. ii. 32	15	122	87	97·0
10. ii–25. iii. 32	23	121	62	86·9

During the period 9. ii–4. iii. 32, which included the coldest weather during the time of these experiments, the average blood sugar for 12 cats was 81·7 mg. p.c. The houses in which the animals are kept before experimentation are maintained thermostatically at a temperature of 60° F. The average duration of stay in the animal house is 4 days.

Whether the variation of average blood sugar for different periods shown in Table III is fortuitous, seasonal or dependent on temperature is, however, impossible to determine.

Effect of chloroform.

The induction of chloroform anæsthesia was effected by the use of an open mask, and continued after insertion of a tracheal T-cannula, by connecting this with a Woulffe's bottle containing chloroform, access of air being regulated by a screw clip on the side tube. In Fig. 1 are shown the average curves for liver glycogen and blood sugar obtained from three experiments. The liver in each case initially contained about 2 p.c. of glycogen, and the average curve has been extrapolated to 0 min.

It has been shown [Evans, Tsai and Young, 1931] that etherization for 50 min. reduces the liver glycogen to about 50 p.c. of its original level in cats. After 50 min. the liver glycogen content tended to rise slightly.

As was to be expected from its known toxic action on the liver, chloroform has a considerable effect on the glycogen content, reducing it to about one-quarter of the initial amount in 50 min. The blood sugar was also higher than that found under ether anæsthesia.

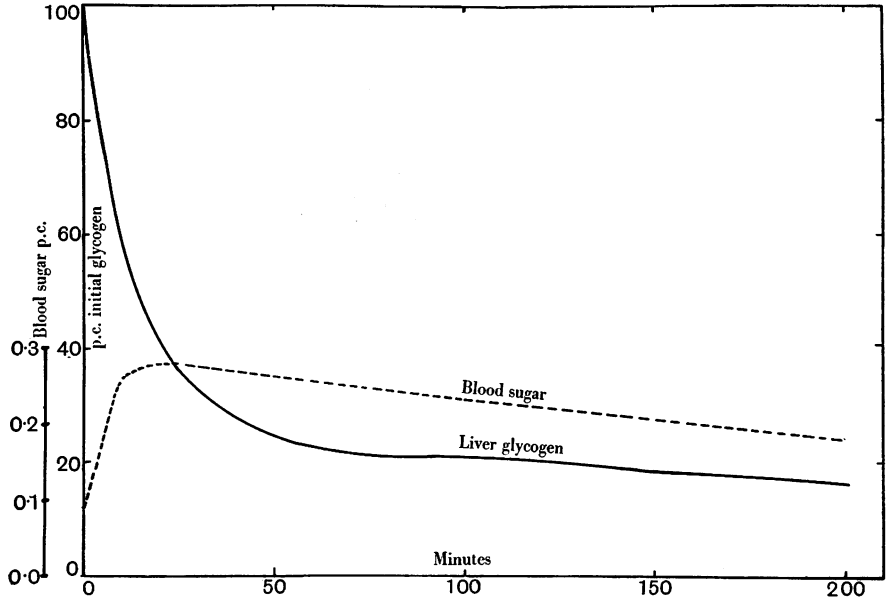

Fig. 1. Effect of chloroform anæsthesia on blood sugar and liver glycogen. Mean curves for three experiments. Chloroform begun at 0 min.

Effect of amytal.

Amytal (isoamyl ethyl barbituric acid) was one of the first anæsthetics shown to have little effect on the blood sugar level [Page, 1923; Edwards and Page, 1924], but in a previous paper [Evans, Tsai and Young, 1931] from this laboratory it was stated that amytal anæsthesia causes a considerable loss of liver glycogen in cats. This has been confirmed in the present investigation and the average blood sugar and liver glycogen curves for four experiments are given in Fig. 2.

We have found 100 mg. per kg. a generally satisfactory dose for intramuscular or intraperitoneal injection, but greater amounts (up to 160 mg. per kg.) are required subcutaneously.

Dann and Chambers [1932] have recently noted that the dose of amytal we used for cats (70–160 mg. per kg.) was considerably in excess of that required for dogs in their experiments (60 mg. per kg.), and consider that for dogs, at any rate, amytal anæsthesia is suitable for the study of carbohydrate metabolism.

We have invariably found with cats that if less than 70 mg. per kg. was injected intramuscularly satisfactory anæsthesia did not result even after 2 hours, at which time a further injection of 30 mg. per kg. produced surgical anæsthesia in 30 min. Our amytal was supplied by British Drug Houses, Ltd., and for injection was completely dissolved in 5–6 c.c. of dilute NaOH by warming to 50° C., the excess alkali being neutralized by dilute acetic acid, until the solution was faintly opalescent, one drop of alkali then being added to ensure complete solution.

Zerfas *et al.* [1928] find the intravenous anæsthetic dose for dogs to be 40–50 mg. per kg., whilst in man it is 20–25 mg. per kg., and they stress the fact that the *p*H of a 10 p.c. solution of the sodium salt of amytal in distilled water is 9·5–9·8, and claim that this *p*H gives a maximum anæsthetic effect in dogs with a minimum degree of toxicity. Lowering of the alkalinity of a 10 p.c. solution to *p*H 9·2–9·3, which causes some cloudiness, causes a striking loss in anæsthetic power, and a definite increase in toxicity.

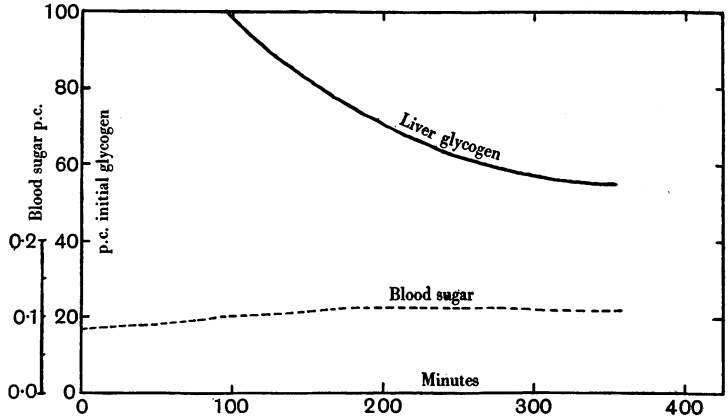

Fig. 2. Effect of amytal anæsthesia on liver glycogen and blood sugar. Mean of four experiments. Surgical anæsthesia about 100 min. after injection.

Page and Coryllos [1926] state that whereas 50 mg. per kg. intravenously is the anæsthetic dose for dogs, 15–20 mg. per kg. more is required for intraperitoneal injection. Fitch and Tatum [1932] find that the minimum lethal dose intraperitoneally for rabbits is 90 mg. per kg.

We are informed by Dr L. E. Bayliss (private communication) that he finds the amytal available in this laboratory to be less potent than that used by him at Harvard University (presumably Lilly's), of which he found 100 mg. per kg. to produce surgical anæsthesia in cats in 15 min. The amytal he used at Harvard was incompletely soluble in alkali, and after filtration was injected in alkaline solution, without neutralization.

We cannot explain this difference in potency of the amytal from these two sources.

Effect of dial.

Diallyl barbituric acid or dial (Society of Chemical Industry in Basle) was found to be effective in doses of 0·12 g. per kg. when dissolved in the same way as with amytal, surgical anæsthesia being induced in about an hour. The effect of dial in lowering liver glycogen and raising blood sugar

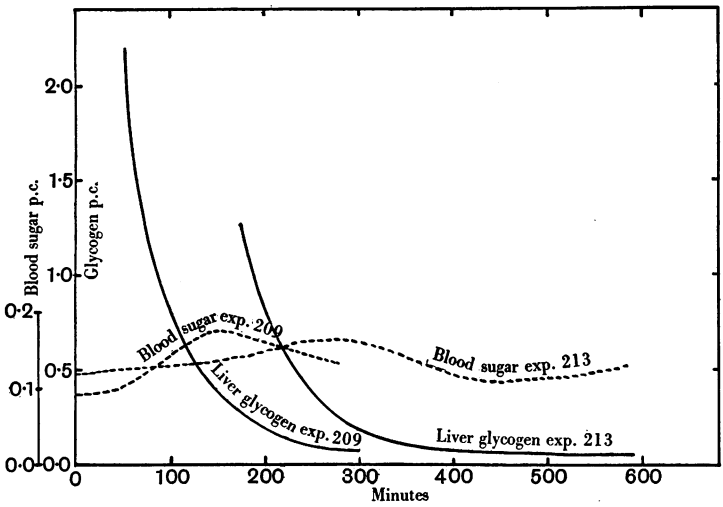

Fig. 3. The effect of dial anæsthesia on liver glycogen and blood sugar.

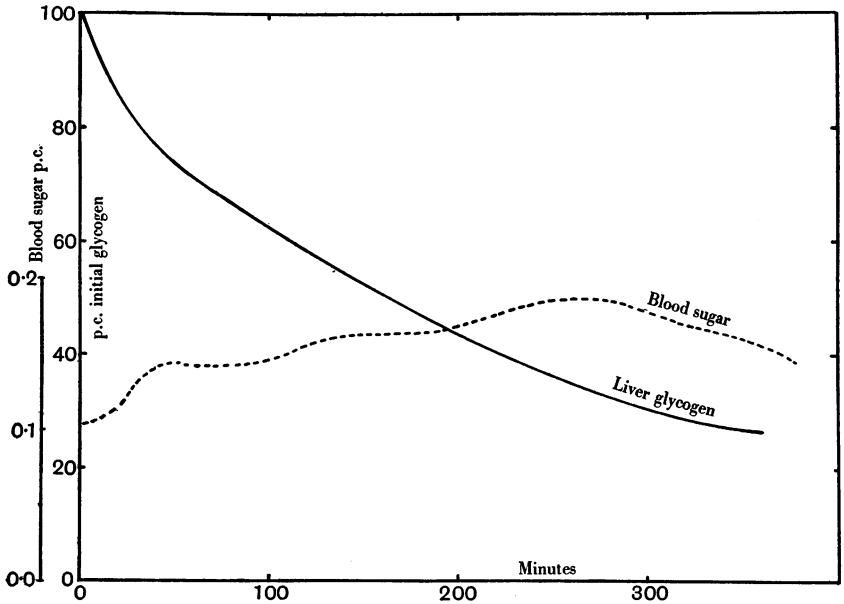

Fig. 4. Effect of luminal anæsthesia on liver glycogen and blood sugar. Mean of three experiments. Curve extrapolated to 100 p.c. liver glycogen at 0 min.

was found to be considerably greater than that of amytal, and the results are given in Fig. 3.

In spite of the fact that anæsthesia was very deep, the fall of liver glycogen content was extremely rapid, although the blood sugar was not raised above 175 mg.

Effect of luminal.

Sodium luminal (the sodium salt of phenyl ethyl barbituric acid (Bayer Products, Ltd.) in doses of 120–150 mg. per kg. in aqueous solution intraperitoneally was found to produce surgical anæsthesia in 20 min. In Fig. 4 are given the average liver glycogen and blood sugar curves for

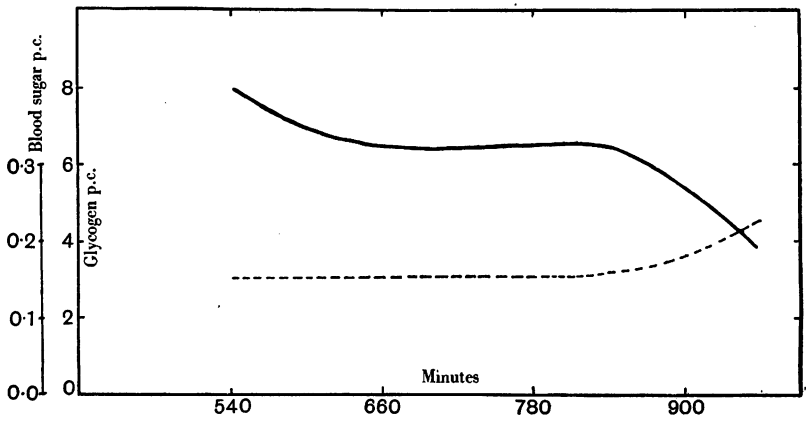

Fig. 5. Effect of luminal anæsthesia on liver glycogen and blood sugar after 9 hours of anæsthesia without liver sampling.

three experiments in which the initial liver sample was taken immediately after induction.

Arnell [1928] has stated that sodium luminal is without action on the sympathetic and the "parasympathetic" nervous systems. If this is so then it might be expected that if reflex effects were avoided this anæsthetic would have little effect on the glycogen stored in the liver.

This, however, was found not to be the case. The blood sugar progressively rose for 270 min. after which it fell, whilst the liver glycogen continuously diminished.

Figs. 5 and 6 give the results of two experiments in which the animal was allowed to remain anæsthetized during a long preliminary period before a liver sample was taken for analysis. By this means it was hoped that the liver glycogen would come to a steady state in which glycogenolysis was equal to glyconeogenesis.

During this preliminary period the temperature of the animal was carefully maintained at 37·5–38·5° C. on a hot plate, and water administered by a stomach tube if necessary. In one case blood sugar samples

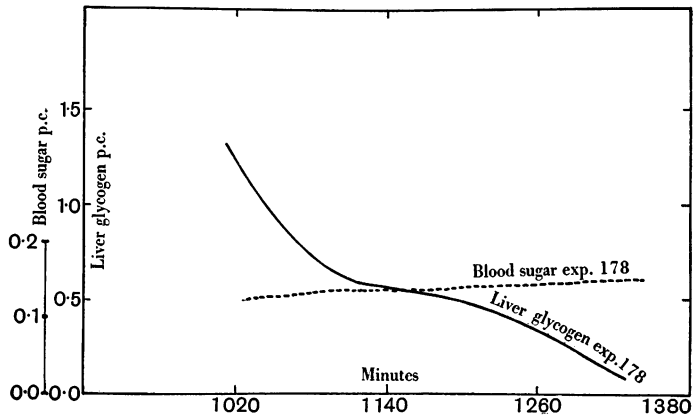

Fig. 6. Effect of luminal anæsthesia on liver glycogen and blood sugar, after 1000 min. of anæsthesia without liver sampling.

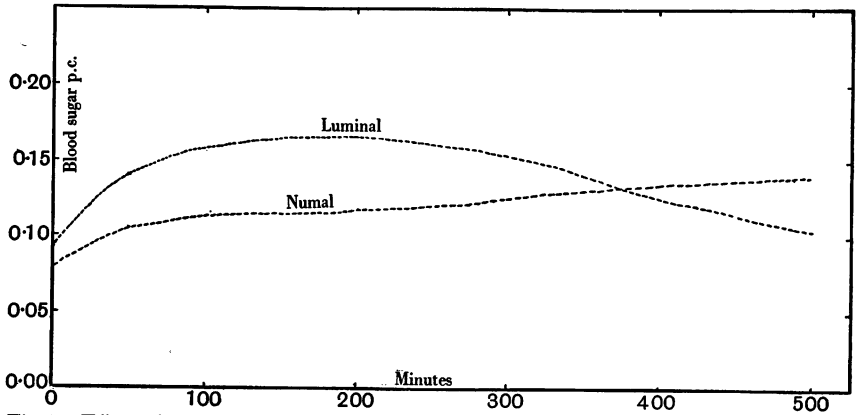

Fig. 7. Effect of luminal and numal anæsthesias on blood sugar. No liver samples taken. (Numal curve average of two experiments.)

were taken from the ear vein during this time, the curve being given in Fig. 7.

In the case of Fig. 5 the initial liver sample, which contained 8 p.c. glycogen, was taken after 9 hours of anæsthesia, and during the further period of 180 min., from 660 until 840 min., the liver glycogen and blood sugar showed little change.

In the experiment shown in Fig. 6, however, in which the initial liver sample was taken 17 hours after induction of anæsthesia, and contained 1·4 p.c. of glycogen, the liver glycogen content did not remain steady for any appreciable period, although from 1100 until 1220 min. the fall was very small.

Effect of numal.

Numal-Roche (the diallyl isopropryl barbiturate of diethylamine) is supplied by the makers in a 10 p.c. solution. Hoet and Ernould [1930] have found that this anæsthetic does not affect the blood sugar in rabbits,

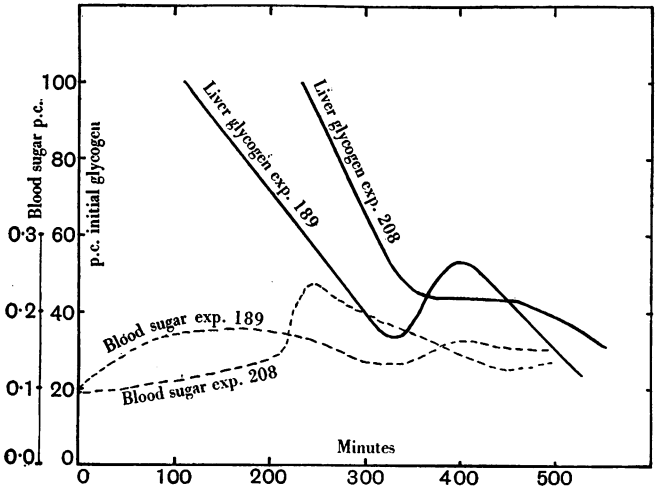

Fig. 8. Effect of numal anæsthesia on blood sugar and liver glycogen. Dose 0·7 c.c. per kg. intraperitoneally. Glycogen content of first sample in both cases approximately 3 p.c.

but Clark [1931] found that during surgical anæsthesia by numal in rabbits, and probably in cats, the hyperglycæmia following the intravenous injection of glucose is prolonged.

We have found (Fig. 7) that in an experiment in which blood samples were taken at intervals from the ear veins of two cats before and during induction of anæsthesia with numal, the average blood sugar steadily rose from 80 to 150 mg. in 500 min.

We have found the intraperitoneal injection of 0·5–0·8 c.c. per kg. of numal to be effective in producing surgical anæsthesia in about 100 min. in cats.

In Fig. 8 are given the results of experiments in which the liver samplings extended from 100 to 500 min. after the injection of numal,

whilst in Fig. 9 are given the results of two experiments in which a preliminary period of about 10 hours of anæsthesia preceded the liver sampling. In another experiment (Fig. 10) the first liver sample was

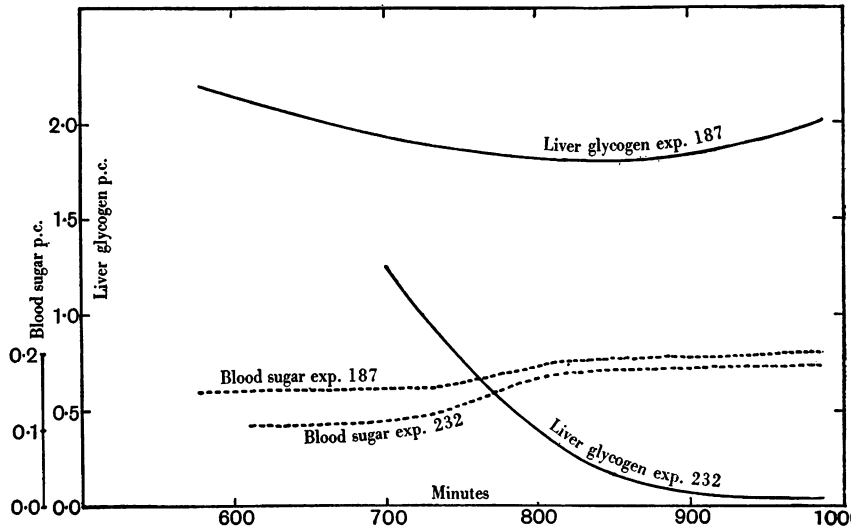

Fig. 9. The effect of numal anæsthesia on blood sugar and liver glycogen: 600–700 min. of anæsthesia previous to liver sampling.

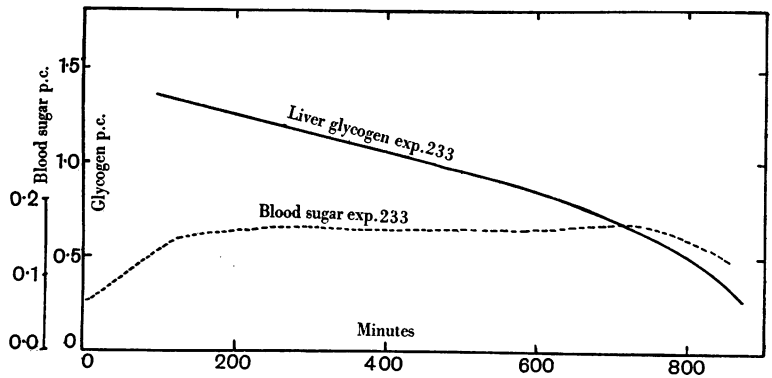

Fig. 10. Effect of numal anæsthesia on blood sugar and liver glycogen. First liver sample taken immediately after induction, the second 10 hours later.

taken immediately after anæsthesia had become satisfactory, the next being taken 10 hours after.

It seems from these results that the effect of numal anæsthesia on liver glycogen is very variable, though in all cases except one there was a

decided fall in the liver glycogen content, but it is difficult to explain the discrepancy between the glycogen curves shown in Fig. 9, as these two experiments were performed under similar conditions.

Effect of pernocton.

Pernocton is the sodium salt of sec. butyl bromallyl barbituric acid and is supplied by its makers (J. D. Riedel, Berlin) in 10 p.c. solution. The dose recommended is 1 c.c. per 12½–15 kg. body weight, but we found 0·5 c.c. injected intraperitoneally did not anæsthetize a cat weighing 2 kg. in 2 hours, after which time a further injection of 1 c.c. produced

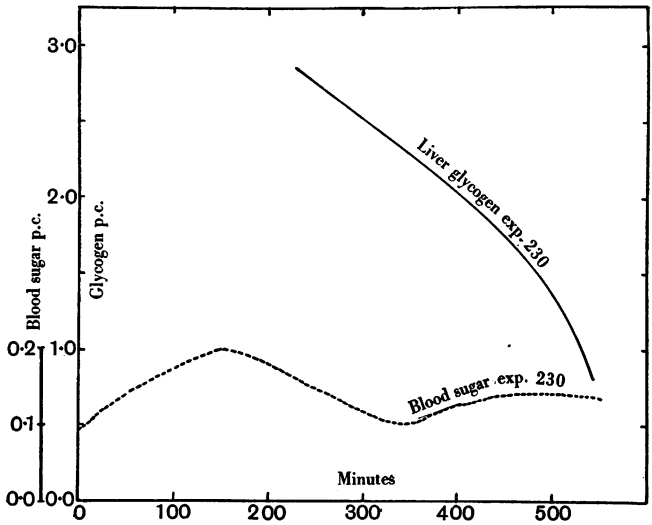

Fig. 11. The effect of pernocton anæsthesia on liver glycogen and blood sugar.

surgical anæsthesia after 1 hour. The effect on liver glycogen and blood sugar is shown in Fig. 11.

According to Dimitrijević [1930], in dogs the margin between surgical anæsthesia and death is narrow, and small rises of blood sugar occur under pernocton anæsthesia, though Matakas [1931] finds blood sugar and lactate are unaffected.

Effect of chloralose.

Vincent and Thompson [1928] found that chloralose raised the blood-pressure of cats and suggested that the secretion of adrenaline had been stimulated, but Tournade and Hermann [1928] could find no evidence for this theory.

We have found, in confirmation of Griffith [1923], that chloralose affects the blood sugar level very little, the theory of increased adrenaline

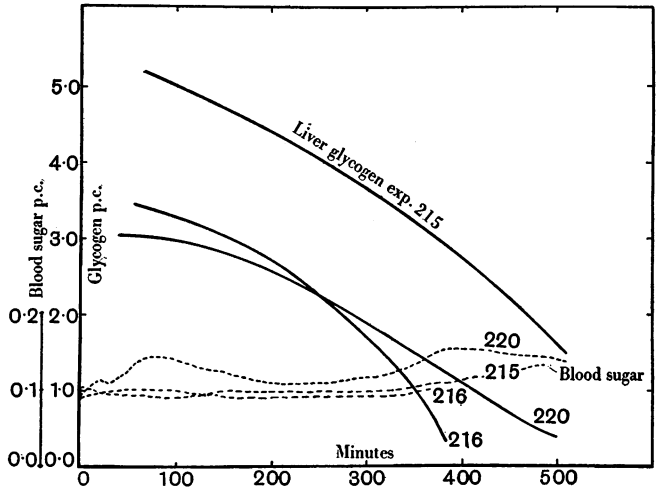

Fig. 12. Effect of chloralose anæsthesia on blood sugar and liver glycogen.

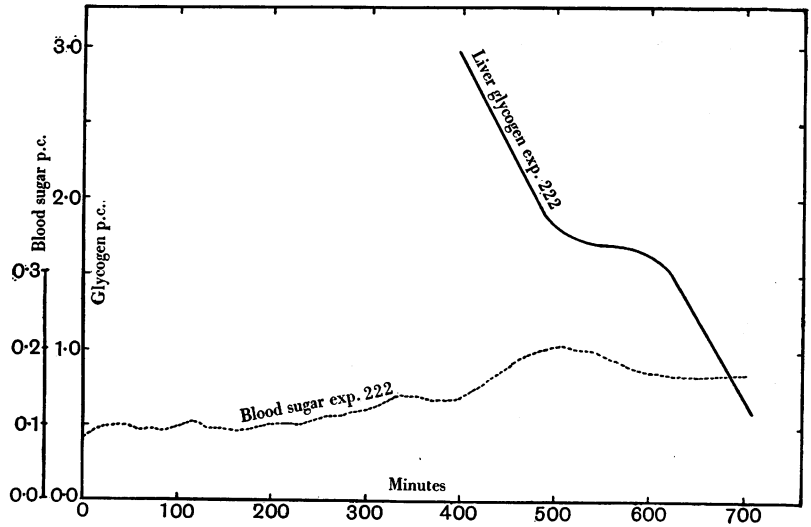

Fig. 13. Effect of chloralose anæsthesia on blood sugar and liver glycogen: 6½ hours of anæsthesia previous to liver sampling.

stimulation being thus not substantiated. Nevertheless, under the anæsthesia produced by subcutaneous injection of 0·1 g. per kg. body weight,

it was found, as it was by Bodo and Neuwirth [1931] in dogs, that the liver of cats rapidly lost glycogen (Fig. 12).

In Fig. 13 is given the result of one experiment in which a preliminary period of blood samples under chloralose anæsthesia preceded the taking of liver samples. In this cat the fall of glycogen was rapid at first followed by a period (500–620 min.) during which there was little fall, and again succeeded by another rapid fall. On the whole, however, the glycogen disappeared from the liver more quickly than in the short period experiments with chloralose.

In view of the very small change in blood sugar of cats anæsthetized with chloralose, the increasing rate of disappearance of liver glycogen with increasing time is rather surprising.

Magenta [1927] finds that chloralose causes a rise of blood sugar in the first 15 min., this being followed by a fall for 30–60 min. We found that the effect on the blood sugar was rather a slight fall at first with a slight rise after 5 hours. This eliminates the possibility that chloralose exerts its narcotic effect by the liberation of small quantities of either chloral or chloroform, as both of these anæsthetics produce a considerable hyperglycæmia [Steinmetzer and Swoboda, 1928].

Nembutal.

It has been suggested that nembutal (E. H. Spicer and Co.) has less effect on the liver than other anæsthetics used clinically, and we have carried out one experiment with this. As with other anæsthetics the liver glycogen fell continuously while the blood sugar rose somewhat.

Discussion.

Of the various anæsthetics we have tried, the blood sugar was least affected by chloralose, and, of the barbituric acid derivatives, least by amytal.

It is obvious that no anæsthetic that we have tried leaves the carbohydrate metabolism of the body undisturbed, under the conditions of our experiment, which involved the taking of successive samples of the liver. It would in fact appear very unlikely that any method of anæsthetization would eliminate the reflex glycogenolysis consequent upon operative procedures, without exerting some direct toxic action on the liver instead.

It seems, on the evidence of extrapolation, which is only approximate in view of the long period between induction and commencement of sampling, that the fall of liver glycogen, which is probably continuous from induction, is accelerated by the process of sampling.

It was also clear from experiments in which blood sugar determinations were made during a preliminary period of anæsthesia that the blood level was raised somewhat as soon as sampling of the liver commenced.

The rise in blood lactate and blood glucose, and the fall in alkali reserve [Fuss, 1931] under ether anæsthesia, together with the fall in liver glycogen, can in part be attributed to asphyxia, to a direct action of the anæsthetic on the liver, and to the liberation of adrenaline from the suprarenals [Macleod, 1926; Evans, Tsai and Young, *loc. cit.*]. Chloroform would appear to have a still more potent action of the same nature.

Brown and Garry [quoted by Clark, 1931] find that chloralose has less effect on the autonomic nervous system than any other anæsthetic they have tried. In experiments with cats under chloralose, Clark [1931] denervated the liver and tied off the suprarenals: he then found that there was a steady fall in blood sugar which was accelerated for a short time by the cutting of the right vagus. If his interpretation of this, *i.e.* that the vagus carries inhibitory fibres to the islets of Langerhans, be accepted, it seems possible from our experiments that chloralose may decrease the action of these inhibitory fibres, thus resulting in a liberation of insulin.

In those experiments in which the animal was kept under luminal or chloralose anæsthesia for a considerable period before the first liver sample was taken, there was, as shown by the curves (Figs. 5 and 13), a period during which the fall of liver glycogen was negligible.

During this period glycogenolysis was apparently either suppressed completely, or else was equal to the glycogen formation from protein fat or lactate: formation of glycogen from glucose is ruled out, in the case of luminal at least, by consideration of the blood sugar curves.

With numal anæsthesia in two cases, Fig. 8, Exp. 189, and Fig. 9, Exp. 187, the liver glycogen actually began to rise after the preliminary fall, the rise in each case being accompanied by a slight rise in blood sugar. It is clearly evident that glycogen formation could not have occurred from blood glucose, as this was rising, so that it must have taken place from some other source, possibly by glyconeogenesis, although evidence considered later tends to the view that amytal anæsthesia inhibits glyconeogenesis.

In most cases it is clearly impossible to account for the disappearance of liver glycogen by an increase in blood sugar. This is most clearly

seen in the action of chloralose, as the following detailed protocol shows:

Cat. ♀. 2·10 kg. Fasted 44 hours. Time		Blood glucose p.c.	Liver glycogen p.c. (mean of two)
11.05	Blood sample from ear	0·101	—
11.06	Chloralose, 0·12 g./kg. intraperitoneally	—	—
11.27	Blood sample	0·098	—
11.58	Liver samples	—	3·48
11.59	Blood sample	0·104	—
3.07	Blood sample	0·097	—
3.14	Liver samples	—	2·21
5.00	Blood sample	0·111	—
5.01	Liver samples	—	0·88
5.50	Blood sample	0·113	—
5.52	Liver samples	—	0·19

Assuming that the blood sugar is in equilibrium with a volume of blood *plus* tissue fluids together equivalent to one-third of the body weight, the cat used in this experiment had 700 c.c. of fluid available for diffusion. At the beginning of the experiment the liver weighed 47 g., so that a fall of liver glycogen content from 3·48 p.c. at 11.58 a.m. to 2·21 p.c. at 3.14 p.m. should cause a rise of blood sugar of 0·085 p.c., *i.e.* glucose was being removed at the rate of 0·026 p.c. per hour (= 0·18 g. per hour).

In three experiments in which muscle and liver glycogens were determined at 60 and 350 min. after chloralose injection it was found that on the average the fall of liver glycogen during the period 100–350 min. was 0·65 p.c., whilst the muscle glycogen fell by 0·04 p.c.

The livers were weighed in each case, and the average loss of liver glycogen was calculated to be 0·46 g. Assuming that the musculature of the animal was equal in weight to one-half of the total weight of the animal the fall in muscle glycogen accounted for the disappearance of 0·93 g. of glycogen. During this period the blood sugar rose by an average of 20 mg. p.c., whilst the blood lactate fell by an average of 5 mg. p.c.

If the assumption is made that the volume of fluid in equilibrium with the blood sugar and lactate is equal to one-third of the body weight [Evans, Tsai and Young, 1931], then the rise of blood sugar and fall of lactate can together account for the disappearance of 0·17 g. of glycogen from the liver and muscles.

Thus of the 1·39 g. of glycogen which disappears from the glycogen stores of the body, only 0·17 g. can be accounted for in the blood, leaving a balance of 1·22 g. A similar result is obtained for experiments under amytal; presumably the 1·22 g. of glucose disappearing in 4 hours can be

more than accounted for by combustion in our experiments [Best, Dale, Hoet and Marks, 1926].

The question that remains, however, is, why do the glycogen stores of the anæsthetized cat suffer this depletion during a period that leaves the glycogen of an unanæsthetized cat materially unaffected?

The basal metabolic rate of amytalized animals differs little from the normal [Lee, 1928; Deuel, Chambers and Milhorat, 1926], and similar results would be expected for other anæsthetics, so that increased metabolism would not account for this difference.

The most reasonable assumption would seem to be that in the normal unanæsthetized animal the liver is continuously manufacturing glycogen, and that this "secretion" is inhibited by anæsthesia.

That amytal inhibits experimental hyperglycæmia has been confirmed by Olmsted and Giragossintz [1931] in the case of hyperglycæmia due to morphia and to asphyxia, and by Donhoffer and Macleod [1932] in the case of that due to asphyxia; Olmsted and Giragossintz [1931] suggest that amytal anæsthesia tends to inhibit glycogenolysis.

Donhoffer and Macleod [1932] have suggested that in the fasting rabbit decerebrated through the pons and with little liver glycogen, hyperglycæmia is due to stimulation of the glyconeogenic process in the liver by way of the parasympathetic nerves, and that this glyconeogenesis is inhibited by administration of atropine with section of both vagus nerves, and by amytal.

In view of recent evidence suggesting that amytal inhibits the parasympathetic [Weiss, 1929; Leib and Mulinos, 1929; Shafer, Underwood and Gaynor, 1930; Garry, 1930; Olmsted and Giragossintz, 1930; Donhoffer and Macleod, 1932] it would seem possible that the fall of liver glycogen under amytal anæsthesia is due to inhibition of the parasympathetic, and consequently of glyconeogenesis. Whether such a statement is true for other anæsthetics, in particular, for chloralose, cannot be decided on the available evidence.

However, as all anæsthetics that we have tried cause a decrease of liver glycogen under the conditions of our experiments, we must draw the conclusion that such conditions are unsuitable for the sudy of carbohydrate metabolism of the normal unanæsthetized animal.

Summary.

1. The effect of anæsthesia by chloroform, amytal, luminal, dial, numal, pernocton, chloralose on blood sugar and liver glycogen of the cat has been investigated.

2. In all the experiments in which liver samples were removed at intervals for analysis, it was found that the liver glycogen content fell throughout, or at some time.

3. Of the anæsthetics tried, on the average there was least fall of glycogen with amytal, whilst chloralose had least effect on the blood sugar.

4. In experiments in which the animal was anæsthetized some hours before the first liver sample was taken, the liver glycogen curve showed a "plateau," for the duration of which the fall of liver glycogen was small.

5. This can be considered to be evidence of the formation of glycogen in the liver, from non-carbohydrate sources.

6. It is concluded that experiments carried out under the above anæsthetics involving the taking of liver samples from experimental animals are unsatisfactory.

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