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INSULIN AND THE STORAGE OF LIVER GLYCOGEN IN ANÆSTHETIZED CATS

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The effect of insulin on glycogen storage in the liver has been studied by many workers. Cori [1931] and Macleod [1934] have given an adequate review of the relevant literature. Recently, Cope and Corkill [1934], in a paper which appeared while the experiments in this paper were in progress, showed that the increase in liver glycogen when insulin was given to young fasting rabbits involved the cooperation of adrenaline, secreted in response to insulin hypoglycæmia. Our results for anæsthetized cats, however, appear to justify the presentation of a different point of view.

Methods

It was shown by Reid [1936] that cats under chloralose were suitable subjects for showing deposition of glycogen in the liver from certain precursors. In this investigation cats, fasted for 48 hours, were used throughout and were subjected to the same treatment before use, *e.g.* method of anæsthetization, blood, liver and urine sampling and analyses, method of infusion into a vein, etc., as described in the above paper, but blood glucose was determined by the Shaffer-Hartman method. The duration of the initial, infusion and post-infusion periods was about 2 hours each, unless stated to be otherwise.

Results

Insulin and liver glycogen in normal cats

Insulin was given to nine cats by slow infusion into a vein for 2 hours in amount varying from 0·07 to 0·28 unit per kg. per hour. The results are shown in Table I.

TABLE I. Behaviour of liver glycogen under insulin in normal cats. (1), (2), (3) = samples at end of initial, infusion and post-infusion periods respectively.

Cat	Insulin unit per kg. per hour	Blood glucose mg. per 100 c.c. (1)	(2)	(3)	Liver glycogen p.c. (1)	(2)	(3)
191	0·07	72	24	—	1·54	1·08	—
190	0·09	100	40	55	0·18	0·30	0·07
184	0·10	176	58	90	1·57	1·88	0·60
177	0·11	119	22	24	1·88	2·06	1·03
189	0·11	102	43	66	2·00	1·75	0·17
193	0·13	90	20	49	2·82	2·37	0·62
168	0·13	153	82	100	0·42	0·62	0·21
209	0·23	80	22	26	0·30	0·37	0·05
215	0·26	123	27	22	2·06	1·98	1·40
216	0·28	82	38	41	2·03	2·07	0·68
191*	0·05	72	16	—	1·54	0·54	—

* Infusion period of 3·75 hours.

Two factors apparently affect the behaviour of liver glycogen during the 2-hour period: (1) the size of the dose of insulin, (2) the initial blood glucose concentration. With the larger doses or with an initial blood glucose of over 100 mg. per 100 c.c., liver glycogen remained stationary or increased slightly; with smaller doses (sufficient also to produce hypoglycæmia) and with an initial normal blood glucose, liver glycogen decreased. Controls, however, showed a fall in liver glycogen of about the same order. The blood lactic acid remained throughout within normal limits, viz. 5–10 mg. per 100 c.c.

In other experiments in which the infusion of insulin lasted for more than 2 hours, liver glycogen had fallen definitely at the end of the infusion period.

Although the severe hypoglycæmia induced in nearly all experiments lasted for several hours during the infusion and post-infusion periods, it was not accompanied by convulsions and did not result in any obvious deterioration of the condition of the animals. Occasionally towards the end of an infusion period fibrillary twitchings were seen in the skeletal muscles and were taken as a sign for discontinuing insulin.

In order to confirm that a raised initial blood glucose was a factor in determining the behaviour of liver glycogen during the infusion of insulin, a small amount of glucose, 0·2 g. per kg., was injected by a syringe into a vein so that the infusion of insulin was begun 15 min. later in some and 2 hours later in other experiments, while the blood glucose was still above the normal fasting level. In this way it was confirmed that a raised blood glucose was a factor in determining a rise in liver glycogen

under insulin, but apparently the additional insulin was not more effective in causing glycogen storage in these animals.

27. vi. 35. Short protocol of cat No. 200: wt. 3·1 kg.

Hour	
09.35	Chloralose 0·24 g.
12.15	Liver glycogen 0·73 p.c.
12.40	Glucose 0·2 g. per kg.
13.00	Blood glucose 182 mg. per 100 c.c.
14.45	Blood glucose 146 mg. per 100 c.c.; liver glycogen 0·93 p.c.
15.00–17.10	Insulin given 0·2 unit per kg.
17.15	Blood glucose 65 mg. per 100 c.c.; liver glycogen 1·14 p.c.

In a third series of experiments on normal cats, an attempt was made by infusing glucose and insulin together to increase the rate of glycogen storage above the maximum found for anæsthetized cats given glucose only [Reid, 1936]. Although these experiments were not successful in demonstrating an increased rate of deposition, since it was likely that the maximal rate of increase had already been attained in the experiments with glucose alone, they showed that glycogen storage was not improved under chloralose by giving additional insulin.

Insulin and liver glycogen in cats adrenalectomized or deprived of their adrenal medullas

Two groups of cats were studied: (1) a group of five cats in which the adrenals were tied off and removed after anæsthetization with chloralose, (2) a group of four cats in which the adrenal medullas were destroyed by thermo-cautery by two-stage operation with intervals of at least 2 weeks between operations and before use.

In the former group it was not always possible to avoid a rise in blood glucose, but the period before the infusion of insulin was never less than 3 hours. The animals of the latter group were not used unless their weight records showed that they were thriving. Their glands were examined later by serial sections to confirm the absence of functioning medullary tissue.

From Table II it is evident that the behaviour of the liver glycogen was on the whole similar to that in the series of intact animals in Table I. The blood lactic acid again remained within normal limits in all experiments.

In order to raise the blood glucose, as was done for normal cats, glucose, 0·2 g. per kg. and followed later by insulin, was given to four cats, adrenalectomized or with their medullas destroyed at previous operations. These experiments merely confirmed the findings of similar procedures in normal cats.

TABLE II. Behaviour of liver glycogen under insulin in cats deprived of adrenal glands or medullas. (1), (2), (3) = samples at end of initial, infusion and post-infusion periods respectively.

Cat	Insulin unit per kg. per hour	Blood glucose mg. per 100 c.c. (1)	(2)	(3)	Liver glycogen p.c. (1)	(2)	(3)	Nature of preparation
185	0·05	172	66	44	1·67	1·57	0·60	A.A.
205	0·07	101	65	103	1·69	1·42	0·43	M.M.
186	0·10	142	52	—	0·28	0·47	—	A.A.
206	0·11	108	38	—	1·35	1·29	—	M.M.
181	0·15	102	16	—	0·75	0·85	—	A.A.
180	0·16	88	28	14	1·05	1·32	0·69	A.A.
208	0·18	104	50	—	0·22	0·28	—	M.M.
182	0·19	162	92	90	0·91	1·10	0·81	A.A.
207	0·28	88	27	19	0·11	0·14	0·12	M.M.

A.A. = adrenalectomized; M.M. = adrenal medullas destroyed.

The behaviour of liver glycogen and blood glucose in the post-infusion period after insulin

Shortly after the infusion of insulin was stopped, the glycogen of the liver fell quickly in both intact and adrenalectomized cats. The rate of fall lay between 0·5 and 1·0 p.c. per hour for the former and 0·3 and 0·7 p.c. per hour for the latter group, provided the store of glycogen was sufficient to allow this. The blood glucose rose slowly in normal cats by about 5–10 mg. per 100 c.c. per hour, but the blood glucose showed usually no tendency to rise in the other group despite the decrease in glycogen.

Preliminary discussion

From the results detailed in Tables I and II, it may rightly be claimed that additional insulin, given in 2 hours to fasting cats which were normal or deprived of their adrenal medullas, had little or no effect on the storage of glycogen in the liver. If the period of infusion was prolonged to nearly 4 hours, as was done in some experiments, *e.g.* cat No. 191, Table I, liver glycogen decreased. The similarity of the behaviour of glycogen in both groups of cats, normal or deprived of their adrenal medullas, is not surprising in view of the quiet anæsthesia obtained which, in addition, would largely inhibit the usual reactions of the non-anæsthetized animal to hypoglycæmia.

The rapid fall in liver glycogen after insulin was stopped can depend, in part only, on adrenaline, and suggests that "secretion" of glucose by the liver is slowed during insulinæmia. It does not appear to be suppressed entirely, since it occurs during the infusion of a small dose of insulin which was adequate, nevertheless, to produce hypoglycæmia.

The above suggestion must be reviewed in the light of subsequent experiments dealing with the influence of insulin on the relation between carbohydrate and protein metabolism.

Insulin and the interrelation of carbohydrate and protein metabolism

It is well known that carbohydrate is a sparer of the body proteins. This holds good for normal cats under chloralose [Reid, 1936], and, as shown in Table III, for cats deprived of their adrenal medullas. To determine if glucose acted as a protein sparer in the absence of the pancreas, several cats were depancreatized shortly after the induction of chloralose anæsthesia. This operation could be carried out rapidly and without raising the blood glucose. After an interval of 3 hours, glucose, 2·0 g. per kg., was injected slowly into a vein during 2 hours, and the effect on the excretion of sulphate noted during the infusion and post-infusion periods. Control experiments were also done.

TABLE III. The relation of glucose to the excretion of sulphate in normal and depancreatized cats. (1), (2), (3) = initial, infusion and post-infusion periods respectively.

	Excretion of sulphate mg. per hour			
Cat	(1)	(2)	(3)	Nature of experiment
135	2·47	2·26	2·23	Normal: control
195	1·37	1·25	1·44	Depancreatized: control
141	2·20	1·45	1·82	Normal: glucose
164	1·91	2·08	1·93	Depancreatized: glucose
165	1·16	1·10	1·14	Depancreatized: glucose
194	1·48	0·80	1·05	Normal: glucose + insulin
166	1·35	0·70	0·62	Depancreatized: glucose + insulin
206	2·30	1·52	—	Adrenalectomized: glucose
208	1·33	0·70	1·20	Adrenalectomized: glucose + insulin

It has already been shown that variations in the rate of excretion of sulphate in the fasting cats will indicate approximately variations in the catabolism of protein [see Reid, 1935 and 1936]. By comparing the results in Table III from a depancreatized control and normal depancreatized animals given glucose, it is clear that the organism failed to use glucose as a sparer of the body proteins soon after pancreatectomy. In support of this finding of the early disturbance of metabolism after pancreatectomy, Hédon and Giraud [1920] had shown that subsequent removal of the transplanted uncinate process of the pancreas after excision of the rest of the gland was soon followed by a rise in blood glucose.

The behaviour of the excretion of sulphate during the infusion of insulin appeared to be related to the initial blood glucose concentration (Table IV); (1) if the blood glucose was above 100 mg. per 100 c.c., the excretion of sulphate decreased and therefore the metabolism of protein

TABLE IV. Behaviour of blood glucose, liver glycogen and excretion of sulphate during and after a slow infusion of insulin lasting 2 hours. Group A = averages from four cats with an initial blood glucose of from 123 to 176 mg. per 100 c.c. Group B = averages from six cats with an initial blood glucose of from 72 to 100 mg. per 100 c.c. (1), (2), (3) = samples at end of initial, infusion and post-infusion periods respectively.

	Blood glucose mg. per 100 c.c.			Liver glycogen p.c.			Excretion of sulphate mg. per hour		
Group	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
A	158	60	62	1·55	1·62	0·55	1·86	1·51	1·33
B	88	32	38	1·32	1·27	0·42	1·68	2·13	1·99

decreased at the expense of the circulating glucose; (2) if the blood glucose was below 100 mg. per 100 c.c., the excretion of sulphate was increased. Presumably, the period of decreased excretion would be short and masked by the increased excretion of sulphate when the blood glucose fell to 20–40 mg. per 100 c.c. Evidently during the maintenance of hypoglycæmia by insulin in anæsthetized cats increased oxidation of protein occurred. After the infusion of insulin was stopped, glycogenolysis in the liver was rapid and the excretion of sulphate decreased as a rule. If, however, the store of glycogen was initially low, this decrease was not observed during our post-infusion period.

Glycogen storage in the livers of depancreatized cats

Some of the depancreatized cats mentioned in the previous section were tested for glycogen storage when glucose was given slowly into a vein. It was established that glycogen storage definitely occurred, but the number of experiments did not justify a comparison with normal cats. The following protocol illustrates the occurrence of glycogen storage in a depancreatized cat when glucose was given and also that glucose did not cause the usual fall in the excretion of sulphate as seen in normal cats. Presumably, the tissues must have contained little or no insulin at the beginning of the infusion of glucose.

22. ii. 35 Short protocol of cat No. 165: wt. 1·7 kg.; depancreatized and given glucose.

Hour

09.45 Chloralose 0·14 g.

10.00–10.20 Pancreatectomy.

13.00 Blood glucose 97 mg. per 100 c.c.; liver glycogen 1·38 p.c.; sulphate 2·08 mg. per hour.

13.15–15.35 Glucose 2 g. per kg.

15.40 Blood glucose 580 mg. per 100 c.c.; liver glycogen 2·24 p.c.; sulphate 2·10 mg. per hour.

18.00 Blood glucose 250 mg. per 100 c.c.; liver glycogen 2·29 p.c.; sulphate 2·10 mg. per hour.

Discussion

If glucose is given to a normal animal or one deprived of its adrenal medullas (Table III), insulin from the pancreas is necessary in order to replace partly the protein metabolism of fasting by the metabolism of carbohydrate. There still remains, however, to try to explain the relation of insulin to glycogen storage in the liver. It is clear from the results detailed in this paper and those of other workers that additional insulin, given by slow infusion to a normal anæsthetized animal in sufficient amount to produce hypoglycæmia in the course of 2 hours, does not increase or decrease very significantly the store of glycogen in the liver. In our experiments some of the complications, *e.g.* convulsions, introduced by the effects of hypoglycæmia on the nervous system of the non-anæsthetized animal, are avoided.

Shortly after the infusion of insulin was stopped, glycogenolysis in the liver was rapid (Tables I and II). Moreover, it is clear from Table III, cat No. 166, depancreatized and given insulin + glucose, that sufficient insulin remained in the tissues in the post-infusion period to ensure the protein-sparing action of carbohydrate. The apparent inhibition of glycogenolysis in the liver during the period of infusion may not be a true inhibition. It may simply mean that the liver cells under the influence of additional insulin will use glucose preferentially for their energy requirements and glycogen precursors, not required for this purpose, would tend to maintain the store of liver glycogen in our insulin-treated animals under chloralose anæsthesia, during which depletion of the glycogen store occurs at the rate of about 0·1–0·2 p.c. per hour in normal controls.

Cruickshank [1914] and Fisher and Lackey [1925] have reported a low glycogen content in the livers of fasted and fed dogs, depancreatized a few days previously. It is well known also that insulin restores glycogen storage in the livers of depancreatized animals. This finding is understandable in terms of the restoration by insulin of normal metabolism whereby carbohydrate can again be used to spare the metabolism of protein. It should be noted, however, that within a few hours after pancreatectomy (protocol of cat No. 165) liver glycogen increased when glucose was given at a time when apparently insufficient insulin remained to ensure the protein-sparing action of glucose. Previously Evans, Tsai and Young [1931] found that regeneration of liver glycogen occurred in the decapitate acute depancreatized cat but not in the decapitate chronic depancreatized animal.

No examination has been made of the effect on the metabolism of fat by the different experiments dealt with in this paper, but this problem requires different methods of examination.

Summary

The relation of additional insulin to the storage of glycogen in the liver has been investigated in fasting anæsthetized cats which were normal or deprived of their adrenal medullas.

With regard to the above, the anæsthetized animal deprived of its adrenal medulla behaved in a manner similar to the intact anæsthetized animal.

In agreement with the work of others, it has been shown that the deposition of liver glycogen in the intact animal given glucose is not further increased by giving additional insulin.

Within 5 hours after pancreatectomy, glucose ceased to act as a protein-sparer, but glycogen storage can occur during infusion of glucose. The power to spare protein is restored by insulin.

The application of the preceding finding to the storage of liver glycogen has been shortly discussed.

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