XLVIII. STUDIES IN CARBOHYDRATE METABOLISM.

V. EFFECT OF ADMINISTRATION OF DEXTROSE AND OF DIHYDROXYACETONE UPON THE GLYCOGEN CONTENT OF MUSCLE IN DEPANCREATISED CATS.

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INTRODUCTION.

It has been shown by Burn and Dale [1924] and by Lambie [1926] that administration of insulin to decerebrated and eviscerated cats results in a more rapid disappearance of dextrose from the blood stream than when no insulin is given. It has further been shown by Best, Hoet and Marks [1926] and by Best, Dale, Hoet and Marks [1926] that a large proportion of the sugar which thus disappears is laid down as glycogen in the muscles.

Much discussion has centred around the question as to whether the essential action of insulin is to convert dextrose into some intermediary substance such as dihydroxyacetone. It therefore seemed desirable to carry out experiments with a view to ascertaining whether dihydroxyacetone causes the deposition of glycogen more readily than does dextrose. Mostowski [1911] showed that the administration of dihydroxyacetone to chickens results in the deposition of glycogen in the liver, whilst in more recent years Isaac and Adler [1924] have claimed that dihydroxyacetone, when injected intraperitoneally into mice and rats, effects more rapid deposition of glycogen in the liver than does dextrose. Cori and Cori [1928] have studied the distribution of glycogen in the livers and muscles of rats fed on these sugars, and found that at the end of 4 hours the rats fed on dextrose deposited 18 % of the sugar absorbed as glycogen in the liver and 25 % as muscle-glycogen, whereas in the case of dihydroxyacetone 21 % was laid down as liver-glycogen and only 15 % as muscle-glycogen. When insulin was administered the distribution of glycogen as between liver and muscle was 6 % and 36 % for dextrose and 2 % and 33 % for dihydroxyacetone, respectively. In both cases dihydroxyacetone failed

to lay down glycogen in the muscles as readily as dextrose but in the case of the liver the percentages were approximately the same. As Cori and Cori point out, these figures cannot be taken as representing the ease of formation of glycogen, since the fraction deposited in the muscles would, other things being equal, be decreased either by increased oxidation or by increased deposition in the liver. No conclusion can therefore be drawn from these experiments as to the relative ease with which the two sugars form muscle-glycogen.

Campbell and Markowitz [1927, 1, 2] have brought forward evidence which seems to prove that in the hepatectomised animal the administration of dihydroxyacetone is unable to cause recovery from the symptoms of hypoglycaemia and that the triose is quite unattacked by the organism under these conditions. These results suggest that dihydroxyacetone is converted in the liver either into dextrose or into some active form of the triose. It is known that dihydroxyacetone can, under certain conditions, be converted by the liver into dextrose, but, whilst this would account for certain resemblances in the physiological behaviour of the two sugars, the differences which they exhibit could not be satisfactorily explained on this hypothesis alone. It therefore seemed possible that experiments on animals in which the liver was intact might afford evidence that an active substance was formed from dihydroxyacetone. The following experiments were therefore carried out upon decerebrated and depancreatized cats in which the liver remained intact.

EXPERIMENTAL.

Cats were employed in all experiments. After brief etherisation the brain was destroyed with a probe through a trephine opening in the skull, and artificial respiration was carried out by means of a cannula inserted into the trachea. Cannulae were also placed in the left jugular vein and the left carotid artery. After withdrawing a sample of blood for analysis (Blood 1 in table), the abdomen was opened and the pancreas was removed. In one experiment only (No. 19 in table) was the animal eviscerated and the liver and kidneys tied off, thus excluding them from the general circulation. After pancreatectomy or evisceration, as the case may be, a second sample of blood (Blood 2 in table) was taken and then transfusion of the sugar under examination was begun. The sugar (dextrose or dihydroxyacetone) was dissolved in saline in the requisite concentration and, by means of the apparatus previously described [Lambie, 1926], was run in at a constant rate through the cannula in the jugular vein. Immediately after the perfusion had begun a ligature was placed round the right femoral artery and the muscles of the right lower limb were removed, cut into small pieces with scissors, weighed *en masse*, and then dropped into boiling 60 % potassium hydroxide solution. After the transfusion had continued for a given time, a third sample of blood (Blood 3 in table) was withdrawn and the left hind limb was removed and added to potassium hydroxide solution after cutting and weighing. The

glycogen content of the muscles was then determined by means of the following method.

Estimation of glycogen in muscle.

The weighed muscle (about 100 g.) was dropped into a conical flask containing hot aqueous 60 % potassium hydroxide (100 cc.) as described above and the mixture heated in a boiling water-bath for about 6 hours when the solid material had been practically completely decomposed and a reddish brown solution remained. On cooling, this solution was poured into a standard 250 cc. flask and diluted with water to the mark. After thorough mixing 5 cc. of the solution were pipetted into a 25 cc. centrifuge tube. Absolute alcohol (20 cc.) was then added and the mixture allowed to stand for 20 hours when the glycogen was completely precipitated. The precipitated glycogen was centrifuged off and washed twice with 66 % alcohol, then with absolute alcohol and finally with ether. It was then dissolved in 2 % hydrochloric acid (10 cc.) and heated on the boiling water-bath for 4 hours. The acid solution was washed into a 25 cc. standard flask and diluted to the mark. The glucose present in a convenient volume of the solution was then estimated by the Hagedorn-Jensen method [1923] and the percentage of muscle-glycogen calculated from this result.

In certain experiments curare was employed in order to paralyse the motor nerve endings of the muscle and eliminate any muscular twitching which might alter the glycogen content of the muscles. A solution, in saline, of the residue formed by evaporation to dryness of an alcoholic extract of the bark of *Strychnos toxifera*, 1 cc. of which had in control experiments been found to paralyse completely the motor nerve endings of the muscles of the lower limb of the cat, was injected in the requisite dose immediately after decerebration had been performed and artificial respiration begun.

As it was advisable to avoid as far as possible stimulation of the pancreas, ergotamine tartrate (5 mg.) was injected 2 hours before the beginning of the experiment with a view to paralysing the terminations of the splanchnic nerves in the liver and thus limiting the initial hyperglycaemia occasioned by the destruction of the brain and the ether anaesthesia, until the pancreas could be removed. It was found, however, that the ergotamine, by paralysing the sympathetic endings in the heart, often caused excessive slowing of that organ and the resulting depression of the circulation was frequently so great that circulatory failure followed the administration of the anaesthetic. When this threatened, adrenaline, 0.2 cc. of 1 in 1000 solution, was injected intravenously in order to save the animal. The results are summarised in Table I.

The total reducing power of the blood, as given in the table, in terms of glucose, was determined by the method of Hagedorn and Jensen [1923], whilst the method of Campbell [1926] was used for the determination of dihydroxyacetone. It was found by the Hagedorn and Jensen method that 1 g. of dihydroxyacetone is equivalent to 1.48 g. of glucose by this method.

DISCUSSION.

The single experiment in which the liver was excluded from the circulation and the animal perfused with dihydroxyacetone showed a slight fall in muscle-glycogen in spite of the fact that the blood at the end of the experiment contained 0.1 % of dihydroxyacetone. This seems to indicate, in agreement

Table I.

No. of exp.	Wt. of cat g.	Sugar perfused	Concn. of sugar in perfusion fluid %	Rate of perfusion per hour g.	Time of perfusion mins.	Total wt. of sugar perfused g.	Blood-sugar (total reducing power) mg. per 100 cc.			Glycogen in muscle mg. per 100 g.			Remarks
							1	2	3	Before	After	Difference	
1	2600	Dextrose	40.6	2.02	90	3.04	276	369	1560	234	298	+ 64	Pancreas removed. Liver intact
2	—	"	40.0	2.0	130	4.30	222	276	318	253	201	- 52	
3	2300	"	34.5	1.72	117	3.44	—	—	—	476	170	-306	
4	—	"	30.0	1.5	175	4.50	84	106	588	359	238	-121	Ergotamine. Pancreas removed. Liver intact
5	—	"	30.0	1.5	100	2.50	282	438	—	307	455	+148	
6	—	"	30.0	1.5	135	3.37	254	326	580	574	562	- 12	Ergotamine. Adrenaline. Pancreas removed. Liver intact
7	—	"	60.0	6.25	106	11.04	144	198	2220	279	206	- 73	
8	—	"	30.0	1.5	130	3.25	—	104	456	658	687	+ 29	
9	2300	Dihydroxyacetone	30.0	1.5	120	3.00	258	326	870	402	407	+ 5	Pancreas removed. Liver intact
10	3200	"	50.0	2.5	70	2.91	—	318	1560	194	242	+ 48	
11	2250	"	50.0	2.5	90	3.75	140	158	(0.42 % Di.) 1140	592	421	-171	
12	2270	"	35.4	1.77	90	2.66	222	343	(0.1 % Di.) 1020	274	288	+ 14	
13	—	"	40.0	2.0	80	2.66	258	404	(0.173 % Di.) 1230	149	206	+ 57	
14	—	"	40.0	2.0	110	3.66	154	220	1710	381	372	- 9	Curare. Pancreas removed. Liver intact
15	2100	"	31.5	1.58	120	3.15	—	—	—	274	223	- 51	
16	2400	"	37.4	1.87	90	2.80	—	—	—	499	577	+ 78	
17	—	"	30.0	1.5	80	2.0	230	426	1008	559	743	+184	Ergotamine. Adrenaline. Pancreas removed. Liver intact
18	—	"	60.0	6.25	90	9.37	272	282	3180	989	564	-425	
19	3450	"	53.0	2.69	90	4.03	—	254	(0.1 % Di.) 1140	252	232	- 20	Complete evisceration. Liver tied off

The calculated Blood 3 glucose values in experiments Nos. 10, 11, 12 and 19 are 938, 992, 764 and 992 mg. per 100 cc. respectively.

with the conclusion of Campbell and Markowitz [1927, 1, 2], that glycogen is not produced readily from dihydroxyacetone in the absence of the liver, but further experiments are necessary to settle this point.

It will be noted that in certain experiments ergotamine or curare was administered to the animal. As explained above, this was done with the aim of preventing glycogenolysis in the liver or muscles respectively but there was no evidence that the curarised animals gave more constant results than the untreated ones, whilst the animals which had been treated with ergotamine frequently suffered from circulatory failure and usually died if not given adrenaline. Moreover the ergotamine was frequently ineffective in paralysing the splanchnics completely.

It is evident from the figures in the preceding table that no significant difference is observable under the above conditions in the power of dextrose and of dihydroxyacetone to cause deposition of glycogen in the muscles. In the eight experiments with dextrose the mean decrease in muscle-glycogen from the beginning to the end of the perfusion period was 0.040 ± 0.051 %, whilst when dihydroxyacetone was used the decrease was 0.027 ± 0.054 %. It is obvious, however, that in individual experiments a change in the glycogen is quite considerable and the question arises as to whether this can be correlated with any of the other factors which vary from experiment to experiment.

Inspection shows that with the exception of experiment No. 18, a marked fall in glycogen is usually associated with a lower initial blood-sugar, whilst a marked rise in glycogen is usually observed with a high initial blood-sugar. The figures (including Exp. 18) were therefore examined statistically, taking the 13 experiments in which the initial blood-sugar had been determined, in order to find out whether any correlation existed between the level of initial blood-sugar and the change in the muscle-glycogen. The value of the partial correlation coefficient between initial blood-sugar level and change in muscle-glycogen for a constant initial glycogen content was found to be -0.30 ± 0.15 . This correlation coefficient would have been considerably greater but for experiment No. 18. It may be noted that in this experiment an unusually high initial muscle-glycogen existed and the large fall in glycogen content may be related to this factor. Because of the comparatively small number of animals (13), the probable error of the correlation coefficient is high and too much emphasis must not be laid on the result, but the above experiments suggest that the level of the initial blood-sugar in such a perfusion probably plays a rôle in determining the alteration in glycogen. This may be due to the stimulation of the pancreas, in the short time that elapses before it is excised, so that it secretes insulin and in this way brings about the deposition of glycogen.

Whilst these experiments do not prove conclusively that dihydroxyacetone cannot cause deposition of glycogen in the muscles more readily than dextrose, they show that there is no striking tendency towards such glycogen deposition and to this extent are in accord with the observations of Cori and Cori which have been mentioned above. In some experiments, the amount of dihydroxyacetone administered was certainly large enough to effect a definite rise in muscle-glycogen provided the triose readily polymerised to form the polysaccharide. In experiment No. 18, in which 9 g. of dihydroxyacetone were administered, a fall of glycogen occurred of 0.425 %, which is by far the greatest fall observed in all experiments, and further, the four experiments in which a fall took place with dihydroxyacetone are those in which the animal received the largest amounts of the triose, and the animal which received the smallest amount of dihydroxyacetone showed the biggest rise. If the correlation coefficient between the amount of triose administered and the change in glycogen be calculated it is found to be 0.95 ± 0.02 , which indicates a strong relation between fall in glycogen content and the amount of triose given. As mentioned above, too much emphasis cannot be laid upon the exact figure, because of the comparatively small number of animals used. When the experiments in which dextrose was administered are examined in a similar way, the correlation coefficient is found to be 0.56 ± 0.15 . This also indicates the important effect which a large amount of dextrose has in causing the muscle-glycogen to fall. We do not at present propose to discuss the various theories which may be advanced to explain this remarkable result. Since the amount of carbohydrate injected has this effect upon the

disappearance of glycogen from muscle, it is obvious that no conclusion can be drawn, from experiments of this type, regarding the comparative ease with which glycogen can be laid down in muscle.

SUMMARY.

1. When dihydroxyacetone is administered intravenously to decerebrated cats from which the pancreas has been removed, the change in muscle-glycogen depends largely upon the amount of triose administered. With small quantities an increase tends to occur, with large quantities a decrease.

2. When dextrose is administered under similar conditions, results of an analogous type are obtained, although, in this case, the relation between the amount of glucose administered and the change in glycogen may not be so great.

3. The level of the initial blood-sugar, before pancreatectomy, appears to have some effect upon the change in muscle-glycogen, high initial blood-sugars tending to cause an increase. This is probably due to stimulation of insulin secretion by the pancreas.

4. From the experiments, no evidence could be obtained that muscle-glycogen was more readily formed from dihydroxyacetone than from dextrose in presence of the liver.

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