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TOXÆMIA AND CARBOHYDRATE METABOLISM.

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In an earlier paper, one of us [Corkill, 1930], following the observations of Goldblatt [1929], discussed the meaning of the glycogen deposition seen in the liver of the young rabbit, but in no other kind of animal yet tested, in response to an injection of insulin, or of a small adjusted dose of adrenaline alone, or of insulin and adrenaline together. The suggestion was put forward that the production of this effect by injection of either hormone alone requires the action, not only of the one injected, but also of the other, secreted in response to the commencing change of blood-sugar level; and additional, more direct evidence for this conception will be given in a following paper by Cope and Corkill. If adrenaline was thus involved in any case, it was natural to think of the phenomenon as involving part of the carbohydrate cycle described by Cori, muscle glycogen → lactic acid → liver glycogen.

In a later paper Corkill [1932] showed that early diphtheritic toxæmia caused a changed response of the young rabbit to insulin, including failure of the normal storage of liver glycogen. It was shown that adrenaline, with or without insulin, similarly failed to cause, under such conditions, any deposition of glycogen in the liver. Since glucose, injected or given by the mouth, caused the usual glycogen storage at this stage of the toxæmia, the defect was presumably at some link of the cycle above mentioned. The experiments here to be described have accordingly been planned with the object of determining whether the toxæmia produces any departure from the normal in,

(1) the formation of lactic acid from muscle glycogen; or

(2) the formation of liver glycogen from lactic acid.

For the former, we have studied the process *in vitro*, using the methods of Meyerhof [1926] for measuring the enzymatic formation of lactic acid by muscle extracts from glycogen or hexose diphosphate (H.D.P.). In

the presence of these substrates it is possible to obtain practically uniform results for any particular type of muscle extract, so that it enables comparison to be made of the normal extracts with those prepared from the muscles of toxæmic animals. For the study of glycogen formation from lactate we have made experiments on the living animals, following the recent observations of Hodgson [1933], who, in extension of the Cori's observations, has found that the intravenous or intraperitoneal injection of Na *dl*-lactate into young fasting rabbits leads to a deposition of liver glycogen. We have compared the effects of such injections of lactate on liver glycogen in normal and toxæmic young rabbits.

Methods.

Young rabbits from 8 to 9 weeks old were used and in all instances a preliminary fast of 24 hours was observed. This precaution was necessary for studies on liver glycogen, though not for the enzyme studies; for the sake of uniformity, however, all animals were similarly treated. Liver glycogen was estimated by Evans' method [1931] with the exception that the glucose after hydrolysis was determined by a modified Bertrand method. The muscle enzyme studies were carried out according to Meyerhof's manometric technique [1926]. The rabbits were killed by a blow on the head followed by bleeding from the cut vessels of the neck. As soon as possible samples from both hindlimbs were taken and placed in a covered glass beaker surrounded by a freezing mixture. When partially frozen the muscle was removed and passed through a mincer. Extracts were made by grinding up in a mortar containing ice-cold 0·9 p.c. KCl in the proportion of 4 c.c. KCl to 3 g. muscle. After standing for $\frac{3}{4}$ hour on ice with occasional stirring, the extracts were centrifuged and filtered through muslin. Extract mixtures were then made by adding $NaHCO_3$ to give a final concentration of 0·5 p.c. To those extracts intended for use with glycogen, phosphate was added in addition to bicarbonate. The extract mixtures were saturated with a nitrogen +5 p.c. CO_2 mixture and placed on ice until ready for use. The glycogen substrate consisted of a 1·14 p.c. glycogen solution in 0·9 p.c. KCl. The H.D.P. was prepared from the acid barium salt of fructose-di-phosphate, which is fairly soluble in 0·9 p.c. KCl. The barium was precipitated with the calculated amount of K_2SO_4, and the solution was then neutralized to litmus with NaOH and the volume made up as desired. The actual solution used was 1·14 p.c.

For the experiments with sodium lactate a 20 p.c. solution of Na *dl*-lactate was prepared. The requisite amount of solution was slowly in-

jected into the ear vein and the animals sacrificed 3 hours later. They were stunned and bled, and the liver was then quickly removed and worked up for glycogen.

Results.

(1) *The enzymatic formation of lactic acid, from glycogen and hexose diphosphate, by normal and toxæmic muscle extracts.*

The results of eight experiments (four normal and four toxæmic animals) were regularly in the same direction. The experimental procedure can best be indicated by the following illustrative record.

Exp. A normal young rabbit fasting for 24 hours was killed by stunning and bleeding and extract mixtures were prepared from the hind-limb muscles. The final mixtures were as follows:

(1) 10 c.c. muscle extract + 2 c.c. 5 p.c. $NaHCO_3$ + 1·8 c.c. phosphate solution.

(2) 10 c.c. muscle extract + 2 c.c. 5 p.c. $NaHCO_3$.

Both extract mixtures were saturated with the nitrogen + 5 p.c. CO_2 mixture and placed in stoppered flasks on ice until ready for use. The filling of the Warburg vessels followed the usual technique. The volume of fluid in the vessel was in all cases 1·7 c.c. (*Vf*) and the temperature at which we worked was 28° C. The contents of the different Warburg vessels were so arranged that we could correct for changes in pressure due to mixing of the extracts with KCl and also, in the case of H.D.P., for any CO_2 evolved by the reaction of this substance and the $NaHCO_3$ in the muscle extract. We have not made any allowance for CO_2 retention by the extract mixtures. This was not considered necessary, since we had actually determined the so-called retention factor of Meyerhof, and had found it to be of small proportions under the actual conditions of our experiments.

The arrangement of the Warburg vessels, in this and other experiments of a similar nature, was as follows:

No. 1	Thermocontrol, vessel contained	1·7 c.c. 0·9 p.c. KCl
No. 2	Vessel: muscle extract with phosphate	1 c.c.
	Bulb: 0·9 p.c. KCl	0·7 c.c.
No. 3	Vessel: extract without phosphate	1 c.c.
	Bulb: 0·9 p.c. KCl	0·7 c.c.
No. 4	Vessel: $NaHCO_3$ 1 c.c. ($NaHCO_3$ 1 c.c. + 5 c.c. 0·9 p.c. KCl)	
	Bulb: H.D.P. 0·7 c.c.	
No. 5	Vessel: extract with phosphate	1 c.c.
	Bulb: 0·3 c.c. KCl + 0·4 c.c. glycogen solution (1·14 p.c.)	0·7 c.c.
No. 6	Vessel: extract without phosphate	1 c.c.
	Bulb: H.D.P. solution	0·7 c.c.

Vessels Nos. 2 and 3 served as the controls for changes of pressure due to mixing of extracts with KCl without the complication of substrate, while No. 4 enabled corrections to be made for CO_2 evolved from the reaction of the H.D.P. substrate and $NaHCO_3$ in the extract mixture. The final calculations were expressed as c.mm. CO_2 evolved and mg. lactic acid formed.

TABLE I. Lactic acid formation in rabbit's muscle extract from glycogen and hexose-di-phosphate. Manometric method.

CO_2 in c.mm. evolved at N.T.P. Glycogen 4 mg.; H.D.P. 7 mg.

Extract ...	Muscle with phosphate	Muscle without phosphate	$NaHCO_3$	Muscle with phosphate	Muscle without phosphate
Substrate ...	Nil	Nil	H.D.P.	Glycogen	H.D.P.
Time (min.)					
10	0	0	86	180	217
30	0	0	88	267	275
50	0	0	88	313	288
90	1·5	1·5	88	329	288
110	3	4	88	349	—

Lactic acid formed: from glycogen in 110 min. 1·40 mg.
from H.D.P. in 50 min. 1·16 mg.

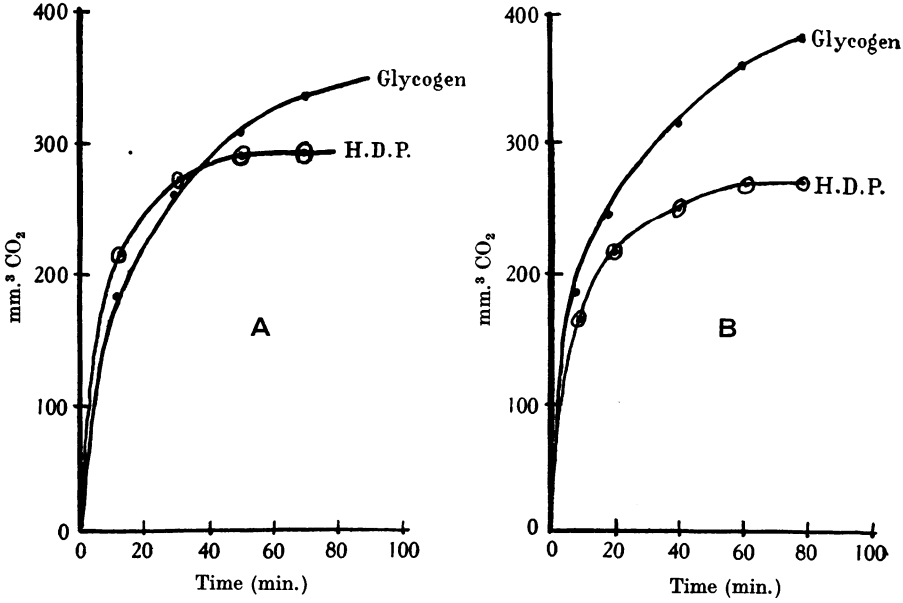

Fig. 1. A. Normal young rabbit. Lactic acid formation, from glycogen 90 min. 1·38 mg.; from H.D.P. 50 min. 1·16 mg. B. Toxæmic young rabbit. Lactic acid formation, from glycogen 80 min. 1·52 mg.; from H.D.P. 80 min. 1·08 mg.

In Table I the method of recording results is shown. Since the readings in manometers Nos. 2 and 3 usually indicated an absorption of gas, the first readings, calculated as c.mm. CO_2, have been expressed as zero, and the readings in the other manometers corrected on this basis.

In the experiments on toxæmic animals the method followed was precisely similar to that described in the preceding one, except that the animal used had been injected with diphtheria toxin 24 hours before the experiment. The diphtheria toxin was supplied by Dr Hartley and its M.L.D. (guinea-pig) was 1/50 c.c. It was found that 4 M.L.D. would usually kill a young rabbit within 4 days, and in the present series of experiments we have used from 3·5 to 4 M.L.D.

For the purpose of comparing the results of such an experiment with the previous one we have presented the results of both as curves (see Fig. 1). Several other experiments on similar lines were carried out and it was apparent that the capacity of muscle extracts, to form lactic acid, did not significantly differ in normal and toxæmic animals.

(2) *Liver glycogen formation from sodium lactate in normal and toxæmic young rabbits.*

Recently Hodgson [1933] has extended Cori's investigations in rats to young rabbits and has shown that intravenous or intraperitoneal injections of Na *dl*-lactate cause a marked storage of liver glycogen. An average increase of 400 p.c. was observed, without any significant changes in muscle glycogen or blood-sugar values. It would also appear from Hodgson's observations that an initial high value for liver glycogen does not preclude a further rise following lactate. Thus, one animal starved for half the usual period had a liver glycogen content of 4 p.c., whilst another injected with lactate showed a value of 10 p.c. These observations are of particular interest in relation to the problem at present under consideration. The capacity of the normal young rabbit to store injected lactate as liver glycogen provides a means of examining the final stage in the Cori cycle already referred to.

In the preliminary experiments we first attempted to obtain standard conditions for demonstrating glycogen storage from Na *dl*-lactate in normal animals.

Exp. Seven young rabbits were taken and kept without food for 24 hours. Three served as normal fasting controls, whilst the remainder were injected with lactate as shown in Table II. The injected animals were killed 3 hours after the lactate administration.

TABLE II.

Controls				Injected with Na *dl*-lactate				
No.	Wt. g.	Blood sugar p.c.	Liver glycogen p.c.	No.	Wt. g.	Blood sugar p.c.	Liver glycogen p.c.	Dose of lactate g.
1	395	0·13	0·68	4	390	0·095	1·26	1·3
2	710	0·105	0·54	5	382	0·114	1·50	1·3
3	700	0·098	0·58	6	704	0·13	1·11	2
				7	703	0·111	1·13	2
		Average	0·60				1·25	

It is clear that the lactate has caused a definite deposition of liver glycogen, and in our next experiments we investigated the same phenomenon in toxæmic animals. In Table III the combined results from two litters are shown. In all, ten young rabbits were used. Two were used as normal fasting controls, two as toxæmic fasting controls, whilst the remainder were treated as shown in the table. The toxæmic animals received 3·5 M.L.D. of diphtheria toxin 24 hours before the actual experiment.

TABLE III.

No.	Wt. g.	Blood sugar p.c.	Liver glycogen p.c.	Remarks
1	763	0·108	0·10	Normal fasting control
2	663	0·095	0·57	" " "
3	702	0·088	0·22	Toxæmic fasting control
4	774	0·103	0·34	" " "
5	707	0·098	1·56	Toxæmic rabbit injected with 8 c.c. 25 p.c. glucose (intra-peritoneal). Killed 3 hours later.
6	642	0·113	1·80	Normal rabbit, 2 g. Na *dl*-lactate intravenously.
7	761	0·104	1·58	Normal rabbit, 2 g. Na *dl*-lactate.
8	638	0·098	0·35	Toxæmic rabbit, 2 g. Na *dl*-lactate.
9	666	0·114	0·10	" " " "
10	750	0·095	0·30	" " " "

The above experiment brings out a clear difference between the normal and toxæmic animals. Rabbit No. 5 clearly indicates that the degree of toxæmia did not prevent the liver from forming glycogen from glucose. The normal animals Nos. 6 and 7 injected with Na *dl*-lactate show a definite deposition of liver glycogen; but this is entirely absent in the toxæmic animals Nos. 8, 9 and 10, which were injected with the same amount of lactate. It would therefore appear that the toxæmic animal cannot form liver glycogen from lactate. This fact has been confirmed with complete regularity in two experiments similar to that shown above.

Discussion.

The failure of adrenaline to cause a deposition of liver glycogen in young toxæmic rabbits appears to be directly related to the fact that these animals cannot synthesize glycogen from lactate. Since the toxæmia has not prevented orally administered glucose from forming glycogen, it would further appear that glycogen synthesis, from lactate and from glucose, proceeds by two different mechanisms.

Since the toxæmic muscle forms lactic acid normally from glycogen and hexose diphosphate, the broken link in the cycle is that concerned with the formation of liver glycogen from this lactate. This will also be the missing link in the chain of events leading to the deposition of glycogen in the normal young rabbit in response to the injection of insulin, if the suggestion is correct, that this action involves also a reactive output of adrenaline. More direct evidence on this point, however, can only be obtained by studying the effect of insulin on young rabbits deprived of the suprarenal medulla. Such evidence is given in the following paper by Cope and Corkill.

Our experiments give no direct indication of the mechanism by which the diphtheria toxin so early deprives the liver of the power of making glycogen from lactate. According to Britton and Silvette [1934] absence from the system of the hormone of the suprarenal cortex is accompanied by depression of the power of the liver to make glycogen from either glucose or lactate. That does not correspond to the condition seen in early toxæmia, in which formation from lactate has apparently vanished, at a stage when that from glucose appears still to be normal. The recognizable effects of diphtheria toxin, at a later stage, on the suprarenal cortex make it desirable, however, to keep in mind the possibility that the early defect of formation of glycogen from lactate may represent an early and selective injury of the suprarenal cortex, rather than a direct effect on the liver.

Summary.

1. The effect of toxæmia on the factors concerned in the storage of liver glycogen has been further studied in young rabbits.

2. While the formation of lactic acid from muscle glycogen appears to proceed in a normal manner, the toxæmic liver is unable to form glycogen from lactate. The failure of adrenaline to cause glycogen storage in the livers of toxæmic young rabbits appears thus to be explained.

3. The bearing of these facts on the actions of insulin and adrenaline is discussed.

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