CLIV. THE RELATION OF LACTIC ACID AND ALANINE TO GLYCOGEN FORMATION.

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THE intermediary stages in the transformation of alanine into glucose are not clearly understood. The amino-acid may either undergo hydrolytic deamination yielding lactic acid, or be converted into pyruvic acid by oxidative deamination. Evidence that it is finally converted into sugar and the quantitative relationship which exists have been furnished by making use of the glycosuric organism. Thus Ringer and Lusk [1910] gave 20 g. of *dl*-alanine to a phlorrhizinised dog and observed its complete elimination as 20 g. of urinary glucose. Mandel and Lusk [1906] showed that *d*-lactic acid was completely converted into glucose in the diabetic organism and that 70 % of the *dl*-lactic acid could be so transformed. Ringer [1913] showed that pyruvic acid is convertible into glucose when administered to the glycosuric organism.

An alternative method of demonstrating the conversion of amino-acid into glucose, and one which can be shown in a normal organism, seems to be the measurement of liver- and muscle-glycogen following administration of the amino-acid. Neuberg and Langstein [1903] gave 20–30 g. of *dl*-alanine to starving rabbits and found from 1 to 2 g. of glycogen in the liver. Muscle-glycogen was not measured. They recovered from the urine of their animals an amount of lactic acid corresponding to 2 g. of zinc lactate. Regarding the formation of glycogen from lactic acid itself contradictory results have been obtained. Barrenscheen [1914] perfused the isolated livers of rabbits and dogs with sodium lactate and was unable to demonstrate glycogen formation. Abramson, Eggleton and Eggleton [1927], using dogs anaesthetised with ether and amytal, were unable to demonstrate glycogen synthesis in the liver following intravenous injection of sodium *dl*-lactate, although the injection of glucose under the same conditions yielded glycogen in the liver. Cori and Cori [1929] found that sodium lactate, when fed by mouth or injected subcutaneously, led to glycogen deposition in the liver.

Efforts to demonstrate an increase in liver-glycogen in normal animals are complicated by such important considerations as the obtaining of reliable controls and the use of operative procedures designed to prevent excessive excitation. Adult rabbits are not a good medium for work of this kind on account of the marked irregularity in the glycogen content of the tissues even when every precaution is taken to have the animals fed and otherwise treated alike. Young rabbits on the other hand are capable of furnishing reliable controls, especially when of the same litter, because of the uniformity of their liver- and muscle-glycogen content.

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EXPERIMENTAL.

Young rabbits of the same litter were used. Blood-sugar was determined by MacLean's method, blood from the carotid artery being taken. Glycogen was determined in liver and muscle in the following manner. The feet and head of the animal were held and the carotid artery on one side severed with a sharp razor. From the blood thus obtained appropriate amounts for the various estimations were immediately taken. The animal was killed by a sharp blow on the back of the head, extended and the abdomen rapidly opened. The liver was quickly removed, the gall bladder detached, the posterior lobe cut off, weighed and dropped into boiling 60 % potassium hydroxide (1 cc. to 1 g. tissue). This process, from the time of killing the animal until the addition to the potassium hydroxide, did not occupy more than 90 seconds. Meanwhile the posterior extremities were skinned and the anterior tibial and gastrocnemii muscles removed, weighed and dropped into boiling potassium hydroxide. The remainder of the liver was weighed and discarded. The tissue was allowed to hydrolyse in the potash for 3 hours, then 3 cc. of glacial acetic acid were added and the mixture filtered. Of the filtrate an aliquot part was taken into a hard glass centrifuge-tube and precipitated with absolute alcohol. After standing one-half hour the tube was centrifuged, the supernatant fluid drained off, 2 cc. of 2.2 % hydrochloric acid added, and the glycogen hydrolysed to glucose in a boiling water-bath. After cooling and neutralising with KOH the solution was made up to a known volume and filtered through a starch-free filter-paper. The glucose was estimated by the method of von Issekutz and von Both [1927] which depends on the reduction of potassium ferricyanide, and the quantities were so adjusted that amounts of glucose only up to 15 mg. could be estimated. This technique is particularly suitable for accurate glycogen determinations.

Blood- and urine-lactic acid estimations were made by the method of Friedemann, Cotonio and Shaffer. Blood- and urine-amino-acid estimations were made by the method of Folin.

Exp. 1. Ten young rabbits were taken. They were deprived of food for 24 hours before experiment. Five were used as controls and the other five were each given an intravenous injection of 2 g. of sodium *dl*-lactate. The animals were killed 3 hours after injection. The control animal and the corresponding injected animal are from the same litter in each case.

No.	Weight (g.)	Blood-sugar (mg./100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Blood- lactic acid (mg./100 cc.)
			Liver	Muscle		
<i>Controls :</i>						
1	1000	90	0.481	0.371	143	64
2	1100	58	0.513	0.584	163	44
3	850	45	0.292	0.110	63	33
4	900	45	0.643	0.592	195	44
5	850	51	0.726	0.362	163	24
<i>Injected animals</i> (2 g. sodium <i>dl</i> -lactate intravenously):						
6	1100	67	1.635	0.314	579	83
7	1100	79	1.512	0.450	553	96
8	1000	62	1.594	0.148	503	93
9	930	168	2.056	0.461	744	66
10	850	330	1.850	0.371	445	67

These results show an average increase in liver-glycogen of 400 %. There is no significant change in the glycogen content of muscle, nor in the blood-

sugar values. The average blood-lactic acid value rose from 41.8 mg./100 cc. in the control animals to 81 mg./100 cc. in the injected animals. The amounts of lactic acid in the urines of Nos. 7, 9 and 10 were measured and found to be 0.39 g., 0.113 g. and 0.196 g. This shows a variable excretion of from 5 to 20 % of the injected sodium lactate as lactic acid in the urine.

Exp. 2. Four animals from the same litter were taken and two of them given 2 g. of sodium *dl*-lactate intraperitoneally each. Before experiment, Nos. 1 and 3 were deprived of food for 12 hours, Nos. 2 and 4 for 24 hours, in order to show whether a greatly depleted liver-glycogen was a *sine quâ non* for glycogen formation.

No.	Weight (g.)	Blood-sugar (mg./100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Blood- lactic acid (mg./100 cc.)
			Liver	Muscle		
<i>Controls:</i>						
1	1300	85	4.990	0.612	2065	53
2	1300	81	0.533	0.409	208	50
<i>Injected animals</i> (2 g. sodium <i>dl</i> -lactate intraperitoneally):						
3	1300	95	10.43	0.604	5704	117
4	1200	171	2.284	0.268	768	110

It will be seen that the results obtained by intraperitoneal injection parallel those resulting from intravenous injection. Also that an initial level of 5 % glycogen in the liver does not preclude a rise to 10 % following lactate administration.

In view of the rapidity with which the young rabbit forms liver-glycogen from lactic acid it is reasonable to expect a similar increase in liver-glycogen from alanine, if lactic acid be considered an intermediary product in the transformation of alanine into glucose.

Exp. 3. Three animals from the same litter were deprived of food for 24 hours before experiment. Two of the animals were injected intravenously with 2 g. of *dl*-alanine made up to 10 cc. with isotonic saline. Killed 3 hours after injection.

No.	Weight (g.)	Blood-sugar (mg./100 cc.)	Glycogen (%)		Blood- lactic acid (mg./100 cc.)
			Liver	Muscle	
<i>Control:</i>					
1	550	75	0.583	0.242	40
<i>Injected animals</i> (2 g. <i>dl</i> -alanine intravenously):					
2	525	100	0.517	0.228	37
3	550	75	0.570	0.178	44

It will be observed that there is no significant change in liver-glycogen or in muscle-glycogen. Nor is there a rise in blood-lactic acid as was observed when lactic acid was injected.

An experiment was then carried out to see what was happening to the injected *dl*-alanine. With this end in view the amino-acid and urea contents of both the blood and the urine were estimated.

Exp. 4. Five young rabbits from the same litter were taken and deprived of food for 24 hours before experiment. One animal was used as a control and into each of the others were injected 2 g. of *dl*-alanine. The animals were killed 1½, 3, 4½ and 6 hours after injection. The control animal received 10 cc. of isotonic saline intravenously. Before being injected the urinary bladders of the animals were emptied, and afterwards the animals were placed over receptacles and the urine in these and from the bladder was collected at the end of the experiment.

Weight (g.)	Killed (hours after injec- tion)	Glycogen (%)		Blood- amino-acid (mg./ 100 cc.)	Urine- amino-acid (total amount mg.)	Urine- urea (total amount mg.)	Urine- lactic acid (total amount mg.)
		Liver	Muscle				
<i>Control:</i>							
1150	3	1.67	0.262	9.39	5.48	115	0.594
<i>Injected animals (2 g. dl-alanine intravenously):</i>							
1100	1½	1.47	0.266	24.7	84.2	50	6.9
975	3	1.62	0.490	21.8	80.4	54	5.23
1300	4½	1.75	0.230	15.0	106.2	105	16.6
1150	6	1.75	0.315	13.4	98.3	192	9.76

The values obtained for blood-urea and blood-lactic acid were without significance. The remarkable constancy of the liver-glycogen values will be at once apparent. There is no significant change in muscle-glycogen. On an average less than 5 % of the injected amino-acid was excreted as such in the urine. The excretion of urea does not show the increase that might have been expected. Although there is a slight increase in lactic acid excretion, the absolute values are very small.

Having in mind the possibility that enough time had not been allowed to show glycogen deposition, the following experiment was devised.

Exp. 5. Two young rabbits from the same litter were deprived of food for 24 hours before experiment. The control animal was given three 10 cc. injections of isotonic saline at intervals of 2 hours. The other animal was given three injections containing 1 g. of *dl*-alanine in each injection, also at 2-hourly intervals. Both were killed 24 hours after the first injection. Urine was collected as in *Exp. 4*.

Weight (g.)	Glycogen (%)		Blood- amino-acid (mg./ 100 cc.)	Urine- amino-acid (total amount mg.)	Urine-urea (total amount mg.)	Urine- lactic acid (total amount mg.)
	Liver	Muscle				
<i>Control:</i>						
1050	0.282	0.136	9.3	5.25	3488	0.09
<i>dl-Alanine-injected animal (3 g.):</i>						
1025	0.303	0.240	8.1	155.2	3183	0.09

The same negative results as in *Exp. 4* were obtained again. Of 3000 mg. of *dl*-alanine injected only 155 were excreted in the urine. The lactic acid excretion in each case is an equal but negligible quantity.

Finally, to investigate the problem under conditions as nearly normal as possible the alanine was given orally.

Exp. 6. Four young rabbits from the same litter were deprived of food for 24 hours before experiment. Two were used as controls and the other two each received 4 g. of *dl*-alanine by stomach tube. The animals were killed at the end of 6 hours.

No.	Weight (g.)	Glycogen (%)		Blood- amino-acid (mg./ 100 cc.)	Urine- amino-acid (total amount mg.)	Urine- urea (total amount mg.)	Urine- lactic acid (total amount mg.)
		Liver	Muscle				
<i>Controls:</i>							
1	850	0.505	0.174	10.9	3.5	812	3.6
2	625	0.494	0.165	8.9	—	—	—
<i>dl-Alanine-administered animals (2 g. orally):</i>							
3	875	0.390	0.228	22.6	25.0	580	Nil
4	500	0.445	0.173	43.5	—	—	—

The urine secreted by Nos. 2 and 4 was insufficient for quantitative investigation. No lactic acid could be recovered from the urine of No. 3. The excretion of urea over short periods varies so much in different animals, that not much reliance can be placed on comparative figures. The foregoing experiment demonstrates the inability of the animals to lay down liver-glycogen from orally administered *dl*-alanine.

DISCUSSION.

The ease and speed with which lactic acid is transformed into liver-glycogen in the young rabbit is clearly demonstrated. But when *dl*-alanine, an amino-acid closely allied structurally to lactic acid, the immediate deamination product of which may indeed be lactic acid, is injected no increase in liver-glycogen is obtained. To explain the discrepancy between these results and the above-mentioned findings of Neuberg and Langstein it is necessary to review the work of the latter in the light of present day knowledge. In their work no mention is made of the length of starvation, the age of the animals, the time interval between the giving of the amino-acid and killing of the animal, nor what methods of control, if any, were used. Without careful attention to these very important considerations an increase in liver-glycogen may be apparent but unreal. Moreover these investigators did not estimate the muscle-glycogen. If there had been much excitation of the animals during the experiment a decrease in muscle-glycogen might have been observed; and the consequent liberation of lactic acid in the muscles could have been responsible for formation of glycogen in the liver and the substantial excretion of lactic acid which they found in the urine of their animals, amounting to about 10 % of the weight of alanine given. This is approximately the lactic acid excretion obtained in my work after injecting sodium lactate itself. When, in my experiments, alanine was given quite negligible amounts of lactic acid were obtained. It is possible that inability to demonstrate appreciable quantities of lactic acid in the urine under these conditions may indicate that lactic acid is not an intermediate product in the breakdown of alanine to glucose.

Since only up to 5 % of the injected alanine can be recovered as such in the urine, and since urea excretion figures show little evidence of any substantial deamination, it is obvious that the excess of amino-acid is taken up by the tissues.

In such conditions as diabetes and phlorrhizin poisoning the processes of gluconeogenesis are proceeding at a rate much greater than is normally called for, and the deamination of protein degradation products is so rapid that administered amino-acid is at once transformed into glucose, and thence under suitable conditions to glycogen. In the normal animal, however, the process of utilisation of amino-acid is comparatively slow and the tissues therefore take up the excess administered, and the rate of its removal from the tissues is determined by the necessities of calorie requirements.

SUMMARY.

1. The ease with which the young rabbit forms liver-glycogen from injected sodium *dl*-lactate is demonstrated.
2. Similar injections of *dl*-alanine cause no increase in liver-glycogen and this result serves to emphasise the great difference between the metabolism of amino-acids in diabetic or phlorrhizinised animals and normal animals. The most

probable interpretation of this result is that in the normal animal the tissues take up the amino-acid rapidly and release it so slowly that no appreciable deposition of glycogen can be detected.

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