25. THE CONVERSION OF LAEVULOSE AND FATTY ACID INTO GLUCOSE

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It has often been suggested that laevulose is converted into glucose in the animal body and a good deal of evidence has accumulated to support this view. Most of the evidence is indirect, but Cori & Shine [1936] claimed, by incubating liver slices with Ringer's solution, to have demonstrated the direct conversion of laevulose into glucose: they gave no experimental details. Goda [1937; 1938] showed that laevulose disappeared when incubated with liver or kidney slices suspended in Ringer's solution. In an attempt to prove more conclusively the direct conversion of laevulose into glucose, tissue slice experiments have been carried out and a complete carbohydrate balance sheet drawn up.

The possibility of glucose formation from laevulose was first shown by Reilly *et al.* [1898] who used phlorhizinized dogs. Mann & Magath [1922] found that laevulose was effective in hepatectomized dogs only if given some time before hypoglycaemic convulsions developed and suggested that glucose formation must be slow. Later, Bollman & Mann [1931] showed that such dogs could be kept alive for many hours without glucose if laevulose was given in sufficiently large amounts before the blood sugar was greatly lowered. It has been shown by Noble & Macleod [1924], Herring *et al.* [1924] and Corley [1929] that laevulose is not nearly so effective an antidote for preventing shock following the injection of insulin as glucose, unless it is given very soon after the insulin. Immediate protection would, it is presumed, have been afforded in all cases if laevulose could effectively replace glucose without being first converted into glucose or glycogen.

That the liver is the main site of the conversion is indicated by the work of Isaac [1920], Bodansky [1923], Lampson & Wing [1926], Corley [1929] and Mann & Magath [1922]. It has, however, been suggested that other tissues may have the ability to convert laevulose into glucose. Bollman & Mann [1931] showed that whilst laevulose was effective in preventing hypoglycaemic convulsions in hepatectomized dogs if given early enough, it was ineffective in eviscerated hepatectomized dogs, from which it was concluded that the intestines are a second site for the transformation. This is supported by Verzář & McDougal [1936] and Laszt [quoted by Verzář & McDougal, 1936] who consider that laevulose is converted into glucose in the intestine. These results have been contraverted by Steinberg [1927], Oppel [1929], Burget *et al.* [1932] and by Griffith & Waters [1936] who found that laevulose is utilized and can prolong life in dogs deprived of their liver and viscera. Goda [1938] showed that laevulose disappeared and glucose concentration was increased when laevulose was incubated with kidney slices. Steinberg [1927] showed that the isolated surviving skeletal muscle was able to utilize laevulose directly as efficiently as it could utilize glucose. This result supports McGuican [1908] and is confirmed by Griesbach [1929], Bornstein & Völker [1929] and Okamura [1938]. Griffith & Waters [1936]

also suggest that laevulose is utilized by the muscles and brain because it prolongs the life of eviscerated dogs. On the other hand, Maclean & Smedley [1912] and Stewart & Gaddie [1934] were unable to demonstrate any utilization of laevulose by the heart muscle of the dog, rabbit or frog, whilst a similar negative result was obtained by Ashford [1933] for brain tissue.

EXPERIMENTAL

The R.Q., O_2 uptake and lactic acid formation of freshly sliced tissue were determined by the method of Dickens & Šimer [1931], 100 mg. moist tissue slices (dried between filter papers) and 1.5 ml. of physiological salt solution [Krebs & Henseleit, 1932] to which laevulose was added, being used for each experiment. Total sugar and laevulose were determined before and after respiration by the method of Hagedorn & Jensen and a modification of that of Herbert [1938] respectively, whilst total carbohydrate was determined by the method of Ochoa [1930]. The respiration time in every case was 2 hr. The carbohydrate oxidized was calculated from the R.Q. and the O_2 uptake. Liver, kidney, brain, skeletal and cardiac muscle, spleen, and intestinal muscle and mucosa of the rat were the tissues examined. A typical balance sheet for liver slices is shown in Table 1.

Table 1. *Balance sheet for liver slices*

O_2 uptake	426 μ l.	
R.Q.	0.78	
CO_2 equivalent of lactic acid formed	146 μ l.	
	Before (mg.)	After (mg.)
Laevulose	2.33	1.26
Total sugar of medium (as glucose)	3.47	3.92
Total carbohydrate of tissue (as glucose)	1.25	0.45
Glucose converted into lactic acid	—	0.59
Carbohydrate oxidized (as glucose)	—	0.12
Total carbohydrate accounted for	4.72	5.08
Laevulose disappeared		1.05 mg.

Table 2 shows the results obtained with the various tissue slices. In the first experiments, in which laevulose was the only sugar present in the medium, a big leakage of carbohydrate from the tissue into the medium occurred. This was common to all experiments in which the medium did not contain glucose and so in the subsequent experiments glucose as well as laevulose was present in the medium.

These experiments clearly show the direct conversion of laevulose into glucose by the liver and the kidney, since the amount of laevulose which disappeared is greater than the sum of the carbohydrate oxidized and that converted into lactic acid. All the remaining tissues examined failed to bring about the conversion, the amounts of laevulose which disappeared being negligible. This is especially interesting in the case of the intestine, since it contraverts the conclusions, from indirect evidence, of Bollman & Mann [1931], Verzář & McDougal [1936] and Laszt [quoted by Verzář & McDougal, 1936]. That the conversion is an aerobic reaction is shown by Exp. 14, in which liver slices were incubated with the usual laevulose-containing medium in an atmosphere of N_2 without the disappearance of any laevulose. It may be mentioned here that many attempts have been made, without success, to prepare an active liver brei which would bring about the conversion of laevulose into glucose (or glycogen). No stress can be laid on the R.Q. in the experiments with muscle and mucosa, since the

Table 2

Table 2

Exp.	O ₂ uptake μl.	R.Q.	μl. CO ₂ equiv. of lactic acid formed	Laevulose mg.	Total sugar carbohydrate (medium) mg.	Total carbohydrate (tissue) mg.	Glucose converted into lactic acid mg.	Carbohydrate oxidized mg.	Total carbohydrate accounted for mg.	Laevulose disappeared mg.
Liver	6	0.87	85	B 2.53	4.35	0.63	—	—	4.98	—
				A 1.28	4.25	0.85	0.34	0.24	5.68	1.25
				(8 other exps. gave closely similar results)						
				Kidney						
13	740	0.81	68	B 2.08	3.42	0.68	—	—	4.10	—
				A 1.14	2.80	0.55	0.28	0.31	3.94	0.94
				(8 other exps. gave closely similar results)						
				Liver in an atmosphere of N ₂						
14	—	—	109	B 3.02	3.78	0.40	—	—	4.18	—
				A 2.92	3.78	0.45	0.44	Nil	4.67	0.10
				(1 other exp. gave a closely similar result)						
				Brain						
16	236	1.09	28	B 2.74	3.55	0.20	—	—	3.75	—
				A 2.69	3.20	Nil	0.11	0.29	3.60	0.05
				(2 other exps. gave closely similar results)						
				Skeletal muscle						
20	44	(1.56)	Nil	B 2.75	3.95	0.62	—	—	4.57	—
				A 2.75	4.10	0.67	Nil	0.05	4.82	Nil
				(1 other exp. gave a closely similar result)						
				Duodenum (muscle and mucosa)						
22	157	1.07	125	B 2.53	3.37	0.80	—	—	4.17	—
				A 2.42	3.05	0.50	0.50	0.19	4.26	0.11
				(1 other exp. gave a closely similar result)						
				Duodenum (mucosa)						
25	60	(0.42)	100	B 3.30	3.92	0.23	—	—	4.15	—
				A 3.25	4.08	0.27	0.40	Nil	4.75	0.05
				(2 other exps. gave closely similar results)						
				Cardiac muscle						
27	20	(1.55)	4	B 3.00	3.90	0.30	—	—	4.20	—
				A 2.86	3.80	0.25	0.01	0.02	4.08	0.14
				(1 other exp. gave a closely similar result)						
				Spleen						
29	366	0.80	89	B 3.14	3.47	0.25	—	—	3.72	—
				A 3.06	2.98	0.25	0.36	0.15	3.74	0.08
				(1 other exp. gave a closely similar result)						
				B. Before respiration. A. After respiration.						

B. Before respiration. A. After respiration.

respiratory exchange was too small for accurate measurement. Had conversion of laevulose into glucose occurred at the same rate, proportional to the O_2 usage, as in liver, it would have been detectable by the direct estimations of laevulose, but nothing can be said as to the production of 'new' carbohydrate in these experiments.

It will be noticed that in all the experiments with liver slices, including those under anaerobic conditions, there was an apparent gain in carbohydrate, a phenomenon which was found with no other tissue except duodenal mucosa. Such an increase in total carbohydrate when liver slices were incubated with Ringer's solution had been previously reported by Gemmill & Holmes [1935], who because of the low R.Q. obtained with liver slices from butter-fed rats, attributed the increase to a conversion of fat into carbohydrate. This was criticized by Cori & Shine [1935] who pointed out that the increase could be attributed to the glycerol and phosphoric ester fraction of the fat. Bach & Holmes [1937] showed that the formation of carbohydrate under their experimental conditions was partially inhibited by insulin and that this fraction appeared to be derived from amino-acids. The precursor of the remaining carbohydrate formed they again suggested was fat.

That the increase in carbohydrate was not due to fortuitous errors of analysis is shown further by Exps. 30-35 (Table 3) which represent simultaneous experiments with liver and kidney slices. In every case liver slices gave an increase in carbohydrate, whilst kidney slices gave an almost perfect balance.

Exps. 30-35 show that the gain in carbohydrate was greatest with the livers from rats which had been on a high carbohydrate diet, and was practically non-existent with the livers from starved rats.

There are several possible precursors of carbohydrate in such a system as that used in liver slice experiments, e.g. protein and protein derivatives, the glycerol fractions of the fats and phospholipins, whilst the formation of reducing trioses from hexoses would produce an apparent gain in carbohydrate.

Since, in liver especially, some of the 'lactic acid' may actually be acetoacetic and β -hydroxybutyric acids, the figure for glucose converted into lactic acid is probably somewhat high, and so consequently is that for total carbohydrate accounted for at the end of the experiment. Even if the whole of the 'lactic acid'

Table 4

Exp. 38. Estimation of urea before and after the incubation of 100 mg. moist liver slices with 1.5 ml. medium at 37°.

mg. urea N			Glucose equivalent of increase (G/N = 6.25)
Initial	Final	Increase	
0.008	0.012	0.004	0.025
0.004	0.004	Nil	Nil
0.012	0.012	Nil	Nil
0.012	0.028	0.016	0.100

Exp. 39. Estimation of non-protein N. Conditions as in Exp. 38.

mg. non-protein N			Glucose equivalent of increase (G/N = 6.25)
Initial	Final	Increase	
0.140	0.135	Nil	Nil
0.267	0.275	0.008	0.05
0.180	0.200	0.020	0.13

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were derived from non-carbohydrate sources, there would still, however, be an apparent gain in carbohydrate.

Only minute increases in urea or non-protein nitrogen could be detected after incubation (Exps. 38 & 39, Table 4), and these could only account for a small fraction of the observed gain in carbohydrate even assuming an optimum conversion of protein into carbohydrate, i.e. a G/N ratio of 6.25.

If hexoses are broken down to reducing trioses, i.e. triosephosphates, or if phosphoric esters are the actual substances which appear as 'carbohydrate' and so account for the apparent increase, there might be some disturbance of the inorganic P/organic P ratio and there should be some increase in the organic acid-soluble phosphate. It would be peculiar if such changes in phosphate were confined to liver tissue when other tissues are also metabolizing carbohydrate. Experiments show very little disturbance of this ratio and very little, if any, increase in organic phosphate.

Table 5

Exp. 40. Acid-soluble P estimations before and after the incubation of 100 mg. moist liver slices with 1.5 ml. medium at 37°. Rat on high carbohydrate diet.

	Initial		Final	
	Inorganic P mg.	Organic P mg.	Inorganic P mg.	Organic P mg.
(a)	0.0965	0.0265	0.0937	0.0400
(b)	0.094	0.047	0.097	0.044

In one of these experiments there is no evidence of any increase at all in the reducing phosphoric acid esters; in the other, the increase found in the organic acid-soluble phosphate would, if it were all present as newly formed reducing substance (i.e. triosephosphate), account for 0.09 mg. 'glucose' in the calculated balance sheet. If it were triosephosphate formed from a non-reducing precursor (e.g. glycerol or glycerophosphoric acid) this is then the maximum amount of apparent carbohydrate which could be accounted for; if it were formed from a reducing or potentially reducing precursor (glucose or glycogen) only half this would be a gain in 'carbohydrate'. In either case the amount is much less than the apparent gains in carbohydrate actually found. It appears then that only a small part of the 'carbohydrate' which appears during the incubation of liver slices can be derived from protein and amino-acids or by an increase in reducing power due to formation of reducing phosphoric esters. It remains to consider the possibility of carbohydrate formation from fat.

In studying the changes in the fat during incubation, the fat was extracted from the tissue and medium by a mixture of alcohol and ether (3 : 1). Eventually the fatty acids were weighed after a treatment involving saponification, acidification with H_2SO_4 to liberate the fatty acids and their final extraction with light petroleum (B.P. 40–60°). The experiments reported in Table 6, show that in the case of liver slices, an increase in carbohydrate corresponds to a decrease in fatty acids whilst the kidney slices gave neither the apparent gain in carbohydrate nor any decrease in fatty acids. That the increase in carbohydrate cannot be attributed to the glycerol fraction of the fat is clearly shown by these experiments. Assuming the glycerol fraction of a fat to be approximately one-tenth of the molecular weight, then a loss 0.87 mg. of fat (as was found in Exp. 41) would correspond to only 0.09 mg. of carbohydrate derived from glycerol, whereas the actual gain was 0.52 mg.

Table 6

Exp.		Laevulose mg.	Total sugar (medium) mg.	Total carbohydrate (tissue) mg.	Glucose converted into lactic acid mg.	Carbo- hydrate oxidized mg.	Total carbohydrate accounted for mg.	Laevulose disappeared mg.	Increase in carbohydrate mg.	Total fatty acids mg.	Decrease in fatty acid mg.
Liver. Medium containing laevulose and glucose											
41	B	3.25	4.12	0.28	—	—	4.40	—	—	5.68	—
	A	1.65	3.76	0.52	0.34	0.33	4.95	1.60	0.55	4.81	0.87
(1 other exp. gave a closely similar result)											
Liver. Medium containing glucose only											
45	B	—	4.60	1.22	—	—	5.82	—	—	4.34	—
	A	—	5.65	0.75	0.36	0.10	6.86	—	1.04	3.62	0.72
(2 other exps. gave closely similar results)											
Kidney. Medium containing glucose only											
47	B	—	3.00	0.35	—	—	3.35	—	—	3.31	—
	A	—	2.65	0.35	0.09	0.41	3.50	—	0.15	3.40	-0.09
(1 other exp. gave a closely similar result)											
Liver. Medium containing glucose only. Atmosphere of N ₂											
49	B	—	3.40	0.77	—	—	4.17	—	—	2.75	—
	A	—	3.68	0.45	0.36	—	4.49	—	0.32	1.77	0.98
(2 other exps. gave closely similar results)											

DISCUSSION

Even if one adds together the amounts of carbohydrate possibly obtainable from protein and amino-acids, increase in phosphoric ester reducing substances, the glycerol of fats and the factor introduced by the formation of acetoacetic and β -hydroxybutyric acids making the total carbohydrate accounted for too high, there is still a very considerable amount of carbohydrate unaccounted for. The quantity involved is such that only the fatty acids would seem capable of being regarded as the source. Incidentally it may be remarked that the increase in carbohydrates with a corresponding decrease in fatty acids is not a property peculiar to experiments in which the medium contains laevulose, but is also found when the medium contains only glucose (Exp. 45).

It is evident that, of the factors considered, only the disappearing fatty acid is quantitatively sufficient to account for the apparent new formation of carbohydrate in liver slices. The further fact that in other tissue slices, in which no carbohydrate formation is detected, there is no loss of fatty acid, considerably strengthens the conclusion that fatty acids are converted into carbohydrate by liver.

There are, however, difficulties in accepting this conclusion without reserve. Thus the observed O_2 consumption is too low to allow the production of the observed carbohydrate from fatty acid together with the oxidation of the remainder of the fat which disappears. It is true that since the formation of carbohydrate and the loss of fatty acids seemed to occur in liver slices anaerobically (Exp. 49, Table 6) one may invoke an unknown hydrogen acceptor and imagine it to be functioning also in the presence of O_2 . But this is not entirely satisfactory, and without further proof of the existence of such a hydrogen acceptor there remains a suspicion that some experimental error may have been overlooked.

It is important to remember that the carbohydrate balance sheets were calculated, at a time when the new formation of carbohydrate and the disappearance of fatty acid were unsuspected, on the assumption that the respiratory exchange represented complete oxidation. If fatty acid or anything else is converted into carbohydrate this assumption is unjustified and the calculations are wrong. They cannot be corrected, for, apart from the question of the anaerobic changes which may be occurring, recalculation would involve the assumption of the fatty acid conversion which the figures are designed to prove (or disprove). In any case, such a calculation leads to impossible results, which is not surprising in view of the apparent anaerobic changes in the carbohydrate and fat contents of the system. This error in the calculations does not affect the observation that carbohydrate (or at any rate, reducing substance) is formed in liver slices, for the increase in reducing power (calculated as glucose), plus the lactic acid formed is usually greater than the calculated carbohydrate loss by oxidation. In some experiments the actual reducing power of the system is definitely increased. Anaerobically, there is, of course, no loss of carbohydrate by oxidation, and the increase in reducing power is again real.

The apparent gain in carbohydrate has been observed by so many workers that it may be accepted, but since the gain is one of reducing power only, there is no complete proof that it is really due to formation of hexoses or polysaccharides. The exclusion of phosphoric ester production, does not amount to proof. Similarly the loss of fatty acid seems real and careful checking of the gravimetric analyses by a titrimetric procedure confirms the results and shows the disappearance of fatty acid to be due to complete removal of fatty acid molecules and not to a

shortening of the carbon chain. Incidentally it is interesting to note that if the whole of the 'glucose converted into lactic acid' could be attributed to the keto-acids, it may account for the loss in fatty acids, since they are in the ratio of about 2.5 to 1, as are their molecular weights. The unexpected suggestion of an anaerobic conversion of fatty acid into carbohydrate with its corollary of a non-oxygen hydrogen acceptor is, however, disturbing, and indicates that much more work (which war conditions prevent) is required.

SUMMARY

1. It is shown that the liver and kidney, but not the brain, spleen, cardiac muscle, skeletal muscle or duodenum, are capable of converting laevulose into glucose or glycogen. The conversion occurs only in the presence of oxygen and of intact liver cells.

2. All experiments with liver and duodenal mucosa showed an apparent gain in carbohydrate, whilst the other tissues did not.

3. The increase in carbohydrate was greatest with the tissues of rats which had received a high carbohydrate diet and least with those from starved rats.

4. The increase in carbohydrate corresponded to a decrease in fatty acid, but though the evidence is suggestive it is not regarded as proved that the fatty acid is converted into carbohydrate.

5. It is suggested that some hydrogen acceptor other than oxygen may, in these experiments, have been concerned in a disappearance of fatty acids and production of carbohydrate.

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