CCXXX. FORMATION AND BREAKDOWN OF GLYCOGEN IN THE LIVER

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(Received 25 September 1939)

UNTIL a few years ago it was generally accepted that glycogen breakdown in animal tissues is initiated by an enzymic hydrolysis, giving glucose, or as some authors thought, a special "reactive form of hexose". Then Parnas & Baranowski [1936] discovered a new reaction of glycogen. They found that autolysed rabbit muscle extract forms from glycogen and phosphate a difficultly hydrolysable hexosemonophosphate which Ostern *et al.* [1936] showed to be the Embden equilibrium ester. This reaction, called by Parnas "phosphorolysis" of glycogen, differs from hydrolysis of glycogen in that phosphoric acid replaces water as the disruptive agent:

Hydrolysis of glycogen: $(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$.

Phosphorolysis of glycogen: $(C_6H_{10}O_5)_n + nH_3PO_4 = nC_6H_{11}O_6 \cdot PO_3H_2$.

The only other carbohydrate which reacts similarly in muscle extract is starch. Simple sugars and disaccharides are not phosphorylated. The extent of phosphorylation depends on the concentrations of glycogen and phosphate; with excess of phosphate present, all the glycogen is converted into hexosemonophosphate. With excess of glycogen, its breakdown is limited by the amount of phosphate available [Ostern *et al.* 1937].

Phosphorolysis seems to be the only method of breakdown of glycogen in muscle, and is the first step of the glycolytic cycle. A most important contribution to our knowledge of the mechanism of phosphorolysis was made by Cori *et al.* [1937], who showed that the hexosemonophosphate initially formed from glycogen is a new ester, glucose 1-phosphate (Cori ester), which is then converted into glucose 6-phosphate by the action of a second enzyme. Cori *et al.* [1938] further showed that this enzyme system also occurs in heart, brain, liver and yeast.

Liver extracts, dialysed for a short time, convert glycogen into Cori ester which is partly dephosphorylated to glucose. Since Davenport [1926] found that after removal of blood by perfusion little amylase could be found in liver, Cori & Cori [1938] suggested that glucose was formed in the liver by phosphorolysis rather than by the action of an amylase.

Later the presence of the phosphorylating enzyme in yeast was further confirmed by Schäffner [1939] and by Kiessling [1939], and Hanes [1939] has found in extracts of pea meal an enzyme which converts starch into Cori ester in the presence of inorganic phosphate.

Kiessling [1939] also showed that the reaction

is reversible. He isolated a protein fraction ("Protein C") from yeast which formed glycogen from Cori ester, and Cori ester from glycogen and phosphate, the same equilibrium being reached in both cases. An analogous reaction has very recently been described by Cori *et al.* [1939, 3] in muscle extracts from which

the enzyme converting the Cori ester into the Embden ester had been removed. From added Cori ester such extracts form glycogen which only differs from ordinary muscle glycogen in giving a blue colour with iodine.

In a preliminary note [Ostern & Holmes, 1939] we have reported a synthesis of glycogen from Cori ester in liver brei, and other experiments on the phosphorylase of glycogen in liver. In the present paper we shall extend these findings and describe further experiments on the breakdown and synthesis of glycogen in the liver.

The material used has been mainly a concentrated (1/1) brei prepared from the livers of starved rabbits by mincing in a Latapie mincer. We found it very difficult to prepare liver extracts that would actively phosphorylate glycogen and such extracts were always very unstable. The use of a brei has the great advantage that the concentration relationships of the different enzymes are not disturbed, as they are in extracts, and the conditions more nearly approximate to those of the intact tissue.

If glycogen is added to such a brei, it is rapidly converted into glucose and the rate of breakdown is accelerated by phosphate; there is, however, no esterification of inorganic phosphate and no formation of phosphoric esters.

If, however, NaF is added in concentrations as low as $M/200$, although added glycogen is broken down at the same rate, no glucose is formed. Instead, a rapid esterification of inorganic phosphate occurs exactly equivalent to the amount of glycogen disappearing; i.e. one mol. P to one glucose unit of glycogen. The inorganic phosphate which has disappeared all appears as a difficultly hydrolysable phosphoric ester which was identified as hexose 6-phosphate (the Embden equilibrium ester).

If in the presence of NaF more glycogen is added than phosphate, glycogen breakdown goes on until all the inorganic phosphate has been esterified and then ceases, and the excess glycogen remains unaffected. In the absence of NaF, however, glycogen breakdown proceeds at a steady rate for periods up to 2 hr. at least, even with very low concentrations of phosphate.

The only probable explanation of these experiments is the following one. The first step in the breakdown of glycogen in the liver is phosphorylase, by which it is combined with phosphate to form Cori ester; this reaction is not affected by NaF. In normal brei, the Cori ester is hydrolysed as soon as it is formed, to glucose and phosphate. The net result of these two reactions is that glycogen is quantitatively converted into glucose.

As we shall show later, reaction (2) is considerably faster than reaction (1); this explains why no esterification of inorganic phosphate is found during the breakdown of glycogen in normal brei.

It will be seen that in the above scheme there is a circulation of phosphate between inorganic and organic combination; thus small amounts of phosphate can bring about the breakdown of large amounts of glycogen, playing the part of a "coenzyme" in the conversion of glycogen into glucose in the liver.

In the presence of NaF, on the other hand, the dephosphorylation of Cori ester to glucose is inhibited and under these conditions the Cori ester is converted, not into glucose, but into Embden ester; therefore, in presence of NaF, glycogen is quantitatively converted into hexose 6-phosphate, as it is in normal muscle extract.

By this means, if there is more glycogen present than phosphate the inorganic phosphate will ultimately all be esterified and so no more glycogen can be phosphorylated. This explains the experimental observations mentioned above.

In confirmation of this hypothesis we find that Cori ester added to normal liver brei is very rapidly dephosphorylated; in the presence of NaF, however, the dephosphorylation to glucose is inhibited and little inorganic phosphate liberated; instead, the Cori ester is mainly converted into the Embden ester. This explains why, in presence of NaF, glycogen is converted not into glucose but into Embden ester.

Embden ester is not the only product formed, however, when Cori ester is added to NaF-poisoned liver brei. There is also a synthesis of glycogen, or at least of a polysaccharide indistinguishable from glycogen by all the chemical and physical tests applied. After 15 min. about 25% of the added Cori ester is converted into glycogen, and the rest into Embden ester. This synthesis of glycogen from Cori ester is a reversal of the process of phosphorolysis of glycogen, and is accompanied by a liberation of inorganic phosphate; thus in liver, as well as in muscle and in yeast, the reaction

is shown to be reversible.

Thus there are three ways in which the Cori ester may be metabolised in the liver: (a) dephosphorylation to glucose, (b) conversion into Embden ester, (c) synthesis to glycogen. Of these (a) is the most rapid and if Cori ester is added to liver brei it is almost all converted into glucose. This reaction, however, unlike (b) and (c), is inhibited by NaF, and if NaF is present only (b) and (c) can take place. In the absence of NaF, no synthesis of glycogen from Cori ester takes place, since (a) being much faster occurs almost exclusively.

Not only Cori ester is dephosphorylated to glucose by liver tissue; the Embden ester is also dephosphorylated, and since Embden ester accumulates from glycogen in NaF-poisoned liver brei, this dephosphorylation is also presumably inhibited by NaF.

We may therefore draw up the following schemes, comparing the breakdown of glycogen in liver and in muscle:

EXPERIMENTAL

Methods

Preparation of liver brei. Rabbits starved 24–48 hr. were killed by a blow on the neck, and bled at the throat as thoroughly as possible; the livers were then rapidly removed, the gall-bladder excised, and adherent blood washed off.

No further precautions were taken to render the livers free from blood. They were then minced in a Latapie mincer, and weighed amounts of the minced tissue ground in a mortar with an equal volume of fluid containing the necessary substances. The brei was allowed to stand at room temperature, samples being taken at intervals.

Cori ester. The Cori ester used was the di-potassium salt. Some of it was prepared from muscle by the method of Kiessling [1938]; most of it was prepared from pea meal extracts by the method of Hanes [1939]. We wish to express our thanks to Dr Hanes for his great kindness in providing us with large amounts of the pea enzyme, and affording invaluable assistance in the preparation of the Cori ester.

Methods of estimation. Phosphate was estimated in trichloroacetic acid filtrates by the method of Fiske & Subbarow [1925]. Glycogen was estimated in most cases by the method of Good *et al.* [1933]. In some cases it was estimated by precipitation from trichloroacetic acid filtrates by the addition of three volumes of alcohol, and subsequent hydrolysis. Glucose was estimated by the method of Hagedorn & Jensen [1923] in trichloroacetic acid filtrates after neutralization.

(1) *Phosphorylation of glycogen and formation of hexosemonophosphate and glucose*

Table I. *Phosphorylation of glycogen in liver brei, and effect of NaF*

Rabbit starved 24 hr. Dilution of brei 1/1. Room temperature. All figures in mg. per 10 ml. brei.

Exp.	Additions	Time (min.)	Inorganic P	Difficultly hydrolysable P*
1	0.4% glycogen (40 mg.) <i>M</i> /45 phosphate† (6.7 mg. P)	3	8.25	5.0
		30	7.70	5.0
		60	8.40	4.6
		120	9.10	4.2
2	Do. + <i>M</i> /200 NaF	3	7.35	5.85
		30	3.10	8.55
		60	1.90	9.45
		120	1.65	9.55
3	None	0	1.67	5.25

* Calculated from $P_{\text{total}} - P_{30 \text{ min.}}$

† In this and all other experiments, unless stated to the contrary, Sørensen's phosphate buffer, pH 7.2, was used.

If glycogen and phosphate are added to liver brei in the presence of *M*/200 NaF there is a rapid disappearance of inorganic P, and a parallel formation of "difficultly-hydrolysable" organic P (i.e. organic P not hydrolysable in 30 min. by *N* HCl at 100°). As we shall show later, this difficultly-hydrolysable ester, formed by the phosphorolysis of glycogen by inorganic P, is hexosemonophosphate.

In the absence of NaF, however, there is no esterification of inorganic P, and no accumulation of difficultly-hydrolysable organic P—in fact a small decrease, due to hydrolysis of the phosphoric esters of the liver tissue.

In the experiment shown in Fig. 1, the conditions were exactly the same as in Table I, but the disappearance of glycogen was measured, as well as that of inorganic P.

Glycogen breaks down at almost exactly the same rate in the presence or absence of NaF, but in absence of NaF almost no inorganic P is esterified, while in presence of NaF an amount of inorganic P is taken up almost exactly equal to the glycogen broken down.

This clearly shows that NaF has no effect on the phosphorylation of glycogen, but only affects the dephosphorylation of the phosphoric ester formed.

These, and all the following experiments, were done on rabbit liver brei. In a few experiments (not shown) rat and pigeon livers were used; the results were exactly the same as those obtained with rabbit liver.

Fig. 1. Breakdown of glycogen and esterification of inorganic phosphate in liver brei with low concentrations of glycogen and phosphate. Rabbit starved 48 hr. Dilution of brei 1/1. Room temperature. Additions: 0.4% glycogen, $M/37.5$ phosphate, $M/200$ NaF.

Fig. 2. Breakdown of glycogen and esterification of inorganic phosphate in liver brei with high concentrations of glycogen and phosphate. Rabbit starved 24 hr. Dilution of brei 1/1. Room temperature. Additions: 1.0% glycogen, $M/15$ phosphate, $M/200$ NaF.

Although in the above experiments no P is esterified in the absence of NaF, a direct demonstration of the phosphorylation of glycogen in unpoisoned liver is possible if high concentrations of phosphate are used. In the experiment shown in Fig. 2 concentrations of glycogen and phosphate are $2\frac{1}{2}$ times as great as in Fig. 1, and here there is a definite esterification of P even without NaF. The P esterified, however, is only equivalent to about $\frac{1}{3}$ of the glycogen broken down, while in the presence of NaF the P esterified is almost exactly equivalent to the glycogen disappearing.

As we shall show later, the reason for the uptake of P in presence of NaF is that the NaF inhibits the dephosphorylation of the Cori ester formed by the phosphorylation of glycogen; it is probable that high concentrations of phosphate have the same effect to a lesser degree, since it is a well-known fact that phosphatases are inhibited by inorganic phosphate [e.g. Martland & Robison, 1927].

Table II. *Effect of phosphate concentration on glycogen breakdown in liver brei*

Rabbit starved 24 hr. Dilution of brei 1/1. Room temperature. All figures in mg. per 10 ml. brei.

Exp.	Additions	Time (min.)	Glycogen broken down†	Inorganic P esterified†
1	1.5 % glycogen*	30	25	0.13
		60	43	0.07
2	Do. + <i>M</i> /45 phosphate	30	35	1.2
		60	50	1.8
3	Do. + <i>M</i> /15 phosphate	30	39	3.4
		60	67	4.8

* In this sample, where no phosphate was added, the initial inorganic P content of the liver tissue was 0.326 mg. per g., i.e. the brei diluted 1/1 contained *ca.* *M*/200 phosphate.

† Amount present after *x* min. subtracted from amount initially present.

In Table II it is shown that addition of phosphate increases the rate of glycogen breakdown in liver brei. The stronger the added phosphate, the faster the glycogen breaks down.

Confirming the results shown in Fig. 2, there is a definite esterification of phosphate (in absence of NaF) in the experiment with *M*/15 phosphate, but a much smaller esterification with *M*/45 phosphate. Even in the former case, the P esterified is much less than the glycogen broken down.

The fact that glycogen breakdown is accelerated by phosphate confirms the theory that phosphorylation is the main mode of glycogen breakdown in the liver.

This is further confirmed by the experiments shown in Table III and Fig. 3, where the breakdown of glycogen and the esterification of phosphate were simultaneously measured, with and without fluoride, the amount of glycogen added being three times higher than the amount of phosphate needed to esterify it. In the experiments with and without NaF, the rates of glycogen breakdown run parallel for the first 30 min.; at this time, when about 1/3 of the added glycogen has vanished, nearly all the inorganic P has disappeared in the experiment with NaF, though in the experiment without NaF none has disappeared. At this point, in the experiment with NaF, where little inorganic phosphate remains, the rate of glycogen breakdown changes abruptly and thereafter proceeds at only 1/5 of its former velocity, while in the experiment without NaF glycogen breaks down at the same rate as before.

This is explained as follows. In absence of NaF, glycogen is phosphorylated, with uptake of inorganic P to form Cori ester. This is then dephosphorylated to glucose, and the esterified phosphate is set free, to be used for the phosphorylation of more glycogen. Thus, even if the phosphate concentration is very low, glycogen can still break down (cf. Table II, exp. 1). In the presence of NaF, however, the Cori ester formed is not dephosphorylated, so the esterified P is not set free again, and, when it has all gone, no more glycogen can be phosphorylated.

Table III. *Glycogen breakdown in liver brei—glycogen concentration three times higher than the equivalent amount of phosphate present*

Rabbit starved 24 hr. Dilution of tissue 4/5. Room temperature. All figures in mg. per 4 g. liver.

Exp.	Additions	Time min.	Glycogen present	Inorganic P present
1	<i>M</i> /67.5 phosphate	3	0	6.07
		30	0	6.67
		90	0	6.36
2	<i>M</i> /67.5 phosphate + 1.1% glycogen	3	88.3	5.85
		30	68.5	6.07
		90	34.9	6.75
3	Like (2) + <i>M</i> /200 NaF	3	88.3	5.21
		30	75.5	1.47
		90	70.5	1.35

Fig. 3. Glycogen breakdown in liver brei: glycogen concentration three times higher than the equivalent amount of phosphate present. For details see Table III.

The slow breakdown of glycogen that then occurs is probably due, in part at least, to amylase activity. In the period from 30 to 90 min. in the experiment with NaF 5 mg. of glycogen are broken down; in the experiment without NaF 37.6 mg. Therefore only 13.3% of the observed glycogen breakdown in the liver under these conditions can be due to amylase activity; the rest is due to the phosphorylating mechanism.

(It will be observed that in the experiment with NaF, though the inorganic P sinks to a very low level, it does not entirely disappear. It is not improbable that the inorganic P apparently found arises from the decomposition of labile phosphoric esters on standing in the acid molybdate reagent, since no attempt was made in this work to measure the true inorganic P as distinct from the "directly-estimable" P.)

In Table IV are shown the effects of various poisons on the rate of glycogen disappearance and uptake of inorganic P in liver brei. With NaF, as in previous experiments, glycogen breaks down at an undiminished rate, and a nearly equivalent amount of inorganic P is esterified. With iodoacetate, however, no

Table IV. *Effect of various poisons on the phosphorylation of glycogen in liver brei*

Mixed liver tissue of two rabbits, starved 24 hr. Dilution of brei 1/1. Incubated at room temperature. All figures in mg./10 ml. brei. All experiments contained *M*/7.5 phosphate.

Exp.	Additions	Time (min.)	Inorganic P esterified	Glycogen broken down
1	<i>M</i> /200 NaF	45	3.5	—
		120	2.0	—
2	2% glycogen + <i>M</i> /200 NaF	45	11.0	77
		120	17.7	140
3	2% glycogen + <i>M</i> /400 iodoacetate	45	2.9	45
		120	4.9	124
4	2% glycogen + <i>M</i> /100 phloridzin	45	1.0	26
		120	0.7	54

more phosphate is taken up than in the control without glycogen, though the breakdown of glycogen is little affected. (The small inhibition observed—11% after 1 hr.—may be attributed to the general toxic effect of iodoacetate in the concentration used.) This shows that oxido-reductive processes are not concerned in the phosphorylation of glycogen.

With phloridzin also, esterification of phosphate is no larger than in the control, but here the breakdown of glycogen is greatly inhibited—nearly 70%. This is of the same order as the inhibition of phosphorolysis by phloridzin in muscle extract or brei, and strongly supports the view that glycogen breakdown in liver is mainly brought about by phosphorylation.

On the same sample of liver used in this experiment, attempts were made to demonstrate a phosphorylation of glucose and fructose. The results (not shown) were completely negative; no phosphorylation could be detected, aerobically or anaerobically, even in presence of NaF.

This of course does not prove that glucose and fructose cannot under any circumstances be phosphorylated in the liver. It does show, however, that under conditions where glycogen is rapidly phosphorylated glucose and fructose are not, and therefore neither glucose nor fructose can be intermediates in the breakdown of glycogen; as in muscle, it is the glycogen molecule itself that is phosphorylated.

Table V. *Phosphorylation of glycogen and formation of hexosemonophosphate under aerobic and anaerobic conditions*

Same sample of liver brei as in Table IV. Dilution of brei 1/1. Diluted brei shaken in Krebs bottles in a thermostat at 37°, in atmospheres of pure N₂ or O₂, for 35 min. Hexosemonophosphate isolated as described in text. All figures in mg./10 ml. brei.

Exp.	Additions	Gas	Inorganic P esterified	Hexose-monophosphate formed as mg. P
1	<i>M</i> /7.5 phosphate + <i>M</i> /200 NaF	O ₂	0	Trace
2	Do.	N ₂	— 2.8	Trace
3	Do. + 2% glycogen	O ₂	17.1	10.1
4	Do. + 2% glycogen	N ₂	11.6	8.16

In the experiments shown in Table V, the phosphorylation of glycogen under aerobic and anaerobic conditions in the presence of NaF was measured, and the phosphoric ester which accumulates as a result of this phosphorylation was isolated in the following way.

After deproteinization with trichloroacetic acid, the filtrate was made

alkaline to phenolphthalein with strong NaOH, and excess 50 % Ba acetate added. After standing, the precipitate was centrifuged off and rejected. The supernatant fluid was treated with 2 vol. of alcohol and kept in the refrigerator overnight. The precipitate was centrifuged off, washed with alcohol, dissolved in water, the small insoluble residue centrifuged off and the supernatant again precipitated with 2 vol. EtOH after adding a few drops of $N/10$ Ba(OH)₂. The precipitated Ba salt was washed with alcohol and ether and dried in a desiccator. The salts isolated from expts. 3 and 4, Table V, contained respectively 7 and 7.2 % of P (of which no more than a trace was inorganic P), and had ratios of Reduction (Hagedorn-Jensen) of 3.67 and 3.87. The theoretical values for pure Embden ester are 7.86 % P, and $\frac{\text{Organic P}}{\text{Reduction}} = 3.88$ % . The values for the isolated salts are thus a little low, but it is very difficult to obtain the Ba salts completely dry. From the analytical figures, the method of isolation and the hydrolysis curves in N HCl, which agreed with that of the Embden ester, there can be little doubt that the Ba salts isolated were those of the Embden hexosemonophosphate.

In the aerobic experiment 60 % of the inorganic P esterified was isolated as hexosemonophosphate; in the anaerobic experiment 70 %. Allowing for the losses in isolation, which are considerable (20–25 %), it seems probable that, in NaF-poisoned brei, the glycogen phosphorylated is quantitatively converted into Embden ester, at any rate under anaerobic conditions.

The rate of phosphorylation of glycogen is faster in O₂ than in N₂. We suggest that this is because hexosemonophosphate accumulates anaerobically, while it is removed aerobically by direct oxidation [cf. Warburg & Christian, 1937; Lipmann, 1936; Dickens, 1936; 1938]. This would account for the lower percentage yield and slightly lower purity of the hexosemonophosphate isolated in the aerobic experiment.

The speed of phosphorolysis was very great in this experiment. At 37° and with a glycogen concentration of 2 %, 5 g. liver can bring about the disappearance of 17.1 mg. P in 35 min., corresponding to the phosphorylation of 3.06 g. glycogen per 100 g. liver per hr. At room temperature the velocity is less than half of this. It also depends on the glycogen concentration, and falls to a value of less than 1 g. per 100 g. liver per hr. if the glycogen concentration is less than 0.5 %, and, as shown in Table II, it also depends on the phosphate concentration. In addition there is considerable variation from one liver to another.

In another experiment glycogen breakdown was measured directly, on the same liver, at 18 and at 37°, using 1.5 % glycogen and $M/15$ phosphate. (For the experiment at 37°, the brei was shaken in a Krebs bottle in a thermostat in an atmosphere of N₂.) The rate of glycogen breakdown at 18° was 1.04 g. per 100 g. liver per hr.—rather less than usually found. The rate at 37° was very high indeed—4.06 g. per 100 g. liver per hr.

The experiments shown in Table VI illustrate the “sugar” formation from glycogen in presence and absence of NaF. In both cases there is a rapid increase in reducing value; in the brei containing NaF, however, this only reaches 2/3 of the value reached in the “plain” sample. In the sample with NaF we find, as usual, a rapid esterification of inorganic P, while none occurs without NaF.

If we assume that in the experiment with NaF all the P esterified has been converted into the Embden ester, the calculated reduction values for the 30, 60 and 150 min. periods (obtained by multiplying the amounts of phosphate esterified by 3.94; i.e. the ratio $\frac{\text{Reduction}}{\text{P}}$ of the Embden ester) are 23.6, 25.6 and 26.4. The observed values, corrected for the control, are 24.1, 26.4 and 26.6.

Table VI. *Breakdown of glycogen and glucose formation in liver brei in presence and absence of NaF*

Rabbit starved 24 hr. Dilution of brei 1/1. Room temperature. Reduction determined in trichloroacetic acid filtrates (after neutralizing) by method of Hagedorn & Jensen. All figures in mg./10 ml. brei.

Exp.	Additions	Time (min.)	Inorganic P esterified	Increase in reduction as mg. glucose
1	None	30	-0.04	1.6
		60	-1.0	1.8
		150	-1.6	2.7
2	0.5% glycogen M/45 phosphate	30	-0.05	38.7
		60	-0.45	40.5
		150	-1.85	43.2
3	0.5% glycogen M/45 phosphate M/200 NaF	30	6.0	25.7
		60	6.5	28.2
		150	6.7	29.2

Recovery of added glucose

5 g. liver + 5 ml. H ₂ O	Glucose found, 29.3 mg.
5 g. liver + 5 ml. 1% glucose (50 mg.)	Glucose found, 78.4 mg.

Recovery = 98.4%.

If we assume that in the experiment without NaF the same breakdown of glycogen has occurred, but that the product is glucose instead of Embden ester, the amount of glucose formed in exp. 2 can be calculated from the amount of P esterified in exp. 3. The calculated values for the 30, 60 and 150 min. periods are 34.8, 37.8 and 38.9; the observed values (corrected for the control exp.) are 37.1, 38.9 and 40.5.

We conclude that, with or without NaF, the same amounts of glycogen undergo phosphorylation to Cori ester; without NaF the Cori ester is all hydrolysed to glucose, while with NaF this reaction is inhibited and it is all converted into Embden ester. The good agreement between observed and calculated reduction values supports this theory.

Glucose added to the brei was quantitatively recovered (98.4%) and, up to 150 min., did not disappear. This shows (a) that the technique used was reliable, and (b) that glucose is not metabolized by the liver to any significant extent under these conditions, which are virtually anaerobic.

(2) *Dephosphorylation of Cori ester and glycogen synthesis*

Cori ester added to liver brei is very rapidly dephosphorylated, as shown in Fig. 4. The rate depends on the concentration of Cori ester; with high concentrations it is very high indeed. The initial velocity in curve 1, Fig. 4, corresponds to a hydrolysis of 3.9 g. Cori ester (expressed as glucose formed) per 100 g. liver per hr. at room temperature.

It has already been suggested that the dephosphorylation of Cori ester is inhibited to some extent by high concentrations of phosphate. Table VII shows that glucose also causes partial inhibition. This is similar to the hydrolysis of glycerophosphate by bone phosphatase, which is inhibited by both glycerol and phosphate [Martland & Robison, 1927].

Table VIII shows that the dephosphorylation of the Cori ester is inhibited by NaF. This affords direct proof of our theory that, in normal liver brei, glycogen

Fig. 4. Dephosphorylation of Cori ester in liver brei. Rabbit starved 48 hr. Dilution of brei 1/1. Room temperature. Curve 1: Cori ester, 2.0 mg. P per ml.; Curve 2: Cori ester, 1.0 mg. P per ml.; Curve 3: Cori ester, 0.33 mg. P per ml.; Curve 4: Cori ester, 0.13 mg. P per ml.; Curve 5: no additions.

Table VII. *Effect of glucose on dephosphorylation of Cori ester on liver brei*

Rabbit starved 24 hr. Dilution of brei 1/1. Room temperature. All figures in mg./10 ml. brei.

Exp.	Additions	Time min.	Inorganic P formed
1	None	15	0.30
		30	0.37
2	Cori ester (12.5 mg. P)	15	5.77
		30	7.72
3	Do. + 2% glucose	15	3.83
		30	5.45

Table VIII. *Effect of NaF on dephosphorylation of Cori ester in liver brei*

Rabbit starved 36 hr. Dilution of brei 1/1. Room temperature. Glycogen measured in initial and 15 min. periods only. All figures in mg./10 ml. brei.

Exp.	Additions	Time min.	Inorganic P formed	Glycogen formed
1	None	15	0.4	0
		30	0.65	—
		60	1.3	—
2	Cori ester (5.5 mg. P)	15	3.15	0
		30	4.00	—
		60	4.55	—
3	Cori ester (5.5 mg. P) + M/200 NaF	15	0.57	7.6
		30	-0.85	—
		60	-0.85	—

is phosphorylated to Cori ester, which is then hydrolysed to glucose and phosphate so fast that there is no observable uptake of phosphate and very little formation of Embden ester; with NaF present the Cori ester cannot be dephosphorylated to glucose, and is therefore converted into Embden ester. The result

is that phosphate is taken up and Embden ester formed in amounts equivalent to the glycogen broken down and there is no formation of glucose.

When glycogen is phosphorylated, the Cori ester produced is converted as fast as it is formed into either Embden ester or glucose, and never accumulates to any extent. (We have never observed any considerable accumulation of easily hydrolysable P during the phosphorylation of glycogen, with or without NaF.) But when Cori ester itself is added in high concentration to NaF-poisoned brei in the absence of glycogen and phosphate, a new phenomenon makes its appearance—the synthesis of glycogen. This does not happen without NaF, for then the Cori ester is converted into glucose so rapidly that there is no time for any glycogen to be formed. But when this pathway of escape is blocked by NaF, Cori ester remains in fairly high concentration in the liver for a relatively long time, and during this time some of it is converted into glycogen.

In this experiment, in the presence of NaF, about 25% of the added Cori ester was converted into glycogen in the first 15 min.; without NaF no glycogen was formed.

Table IX. *Synthesis of glycogen from Cori ester in NaF-poisoned liver brei*

Rabbit starved 36 hr. Dilution of brei 1/1. Room temperature. All figures in mg./10 ml. brei.

Exp.	Additions	Time min.	Inorganic P formed	Glycogen formed
1	<i>M</i> /200 NaF	3	0	0
		15	-0.15	0
		30	-0.30	0
		60	-0.25	0
2	Cori ester (15 mg. P) + <i>M</i> /200 NaF	3	1.75	4.7
		15	3.45	10.0
		30	3.05	4.8
		60	2.75	0.5

Fig. 5. Synthesis of glycogen from Cori ester in NaF-poisoned liver brei. For details see Table IX.

In Table IX and Fig. 5 the synthesis of glycogen from Cori ester was followed over time intervals from 3 to 60 min. In the presence of NaF glycogen is rapidly synthesized during the first 15 min. and then disappears again, falling almost to zero in 1 hr.¹ This is because, of the two reactions Cori ester can undergo in NaF-poisoned brei,

- (1) Cori ester $\rightleftharpoons$ glycogen + phosphate,
- (2) Cori ester $\rightarrow$ Embden ester,

only the first is reversible. The equilibrium between Cori ester and glycogen is therefore continually disturbed by the irreversible transformation of Cori ester

¹ A similar effect was observed in muscle by Cori *et al.* [1939, 3].

into Embden ester, which ultimately goes to completion. Without NaF, or without Cori ester, no glycogen is formed at any stage; in the former case, the Cori ester is all converted into glucose.

In the experiment with NaF there is a liberation of phosphate parallel to the formation of glycogen, which disappears again as the glycogen disappears. This inorganic phosphate does not come from the dephosphorylation of Cori ester to glucose, but from its synthesis to glycogen and phosphate.

The properties of the glycogen synthesized from Cori ester (isolated by alcohol precipitation from trichloroacetic acid filtrates, and purified by reprecipitation from aqueous solution) are as follows:

(1) It is a white amorphous powder, forming a strongly opalescent solution in water.

(2) The aqueous solution gives with I_2 a red-brown colour of the same shade and intensity as a solution of authentic glycogen of the same strength.

(3) The substance is non-reducing, but liberates the theoretical amount of glucose after 2 hr. hydrolysis at 100° in 0.6 *N* HCl. It is not affected by 30 % KOH.

(4) A solution containing 0.98 mg. per ml. gave a rotation in a 2 dm. tube of 0.37° . $[\alpha]_D + 188^\circ$.

The substance is therefore indistinguishable from glycogen.

Table X. *Formation of Embden ester and glycogen from Cori ester in NaF-poisoned liver brei*

Rabbit starved 36 hr. Dilution of brei 1/1. Room temperature. Incubation time 15 min. in both experiments. All figures in mg./10 ml. brei.

Exp.	Additions	Embden ester found (as mg. P)	Glycogen found
1	M/200 NaF	1.42	0
2	Cori ester (18.75 mg. P) + M/200 NaF	14.06	12.8

In Table X Cori ester was added to NaF-poisoned liver brei, and Embden ester and glycogen were isolated as previously described. In the control without Cori ester no glycogen and almost no Embden ester were found. With Cori ester, however, there was a large and rapid formation of both these substances. The Ba salt isolated had a ratio of $\frac{\text{Reduction}}{\text{Organic P}}$ of 3.85, and its hydrolysis curve in *N* HCl agreed with that of the Embden ester, so there is no doubt of its identity.

Allowing for the small loss in isolation of the Embden ester, and the rather larger loss incurred in the purification of the glycogen, it will be seen that in the 15 min. of the experiment Cori ester is converted into Embden ester and glycogen in the approximate ratio of 3/1.

In the experiments shown in Table XI, the enzyme dephosphorylating Cori ester was investigated in rather more detail. The possibility was considered that not only an enzyme, but also a coenzyme might be involved; for example, phosphate might be transferred from Cori ester to adenylic acid, forming glucose and adenylypyrophosphate, the latter then being dephosphorylated. The addition of adenylic acid to the brei, however, caused no increase in the rate of dephosphorylation of Cori ester.

It was also found that Embden ester is dephosphorylated by liver brei at about the same rate as Cori ester; here again the addition of adenylic acid caused no increase in the rate of dephosphorylation.

Table XI. *Dephosphorylation of Cori ester and Embden ester in liver brei and the effect of adenylic acid*

Rabbit starved 48 hr. Dilution of brei 1/1. Room temperature. All figures in mg./5 g. liver.

Exp.	Additions	Time min.	Inorganic P
1	None	2	1.60
		15	1.72
		30	1.85
		60	2.12
2	Adenylic acid* (1.79 mg. P)	2	1.82
		15	1.72
		30	1.96
		60	2.28
3	Cori ester (4.5 mg. P)	2	2.72
		15	4.43
		30	4.88
		60	5.10
4	Cori ester (4.5 mg. P) + adenylic acid (1.79 mg. P)	2	2.76
		15	4.43
		30	5.10
		60	5.57
5	Embden ester (5 mg. P)	2	2.00
		15	4.90
		30	5.20
		60	5.55
6	Embden ester (5 mg. P) + adenylic acid (1.79 mg. P)	2	1.93
		15	4.43
		30	5.00
		60	5.55

* Muscle adenylic acid.

Table XII. *Dephosphorylation of Cori ester in liver extracts, and the effect of adenylic acid*

Liver of starved rabbit extracted with one part distilled water. Autolysed 5 hr. at room temperature and dialysed 12 hr. against distilled water at 5°. Each experiment contained 3 ml. extract made up to 5 ml. with additions. All figures calculated for whole sample. Extracts incubated at room temperature.

Exp.	Additions	Time min.	Inorganic P formed mg.
1	None	15	0.03
		30	0.07
		60	0.15
2	Adenylic acid (0.88 mg. P)	15	0.08
		30	0.13
		60	0.36
3	Cori ester (1.5 mg. P)	15	0.72
		30	1.11
		60	1.53
4	Cori ester (1.5 mg. P) + adenylic acid (0.88 mg. P)	15	0.86
		30	1.36
		60	1.73

The above experiments do not entirely exclude the possibility that adenylic acid may be concerned in the dephosphorylation of Cori ester, since it might be that there was already enough adenylic acid in the liver to "saturate" the enzyme. Experiments were therefore made (Table XII) on the effect of adenylic acid on the dephosphorylation of Cori ester in liver extracts autolysed 5 hr. to destroy

adenylic deaminase and dialysed to remove adenylic acid. Here again adenylic acid does not accelerate the dephosphorylation, which seems to show that adenylic acid does not act as a phosphate-carrier in this reaction.

Table XIII. *Dephosphorylation of Cori ester in liver extracts. Effects of dialysis, Mg and NaF*

Liver of 48 hr.-starved rabbit extracted with 1 vol. distilled water at 0°. Dialysed for 14 hr. against running distilled water at 5°. In each exp. 2 ml. extract made up to 4 ml. with additions. Incubated at room temperature. Figures in mg. calculated for whole sample.

Exp.	Additions	Time min.	Inorganic P formed
1	None	30	0.04
		60	0.10
2	Cori ester (4.24 mg. P)	30	2.02
		60	3.40
3	Do.	30	1.94
		60	3.62
4	Do. + MgCl ₂ (4 mg. Mg)	30	1.84
		60	3.33
5	Do. + M/200 NaF	30	0.80
		60	1.24

Exps. 1, 2, 4 and 5. Dialysed extract. Exp. 3. Undialysed extract.

Table XIII shows further experiments on liver extracts. The velocity of dephosphorylation of Cori ester is the same in dialysed and undialysed extracts. This seems to show that no coenzyme is required; however, we should not like to commit ourselves on this point, since it is possible that a coenzyme is involved which is difficult to remove by dialysis. (The difficulty of completely removing cozymase from muscle extracts, even by prolonged dialysis, is well known.)

Mg, however, is fairly easily removed by dialysis, and since dialysis causes no inhibition, and addition of Mg to the dialysed extract causes no acceleration, we may conclude that the enzyme dephosphorylating the Cori ester, unlike some phosphatases, does not require Mg.

Table XIV. *Dephosphorylation of hexosediphosphate by liver brei*

Rabbit starved 24 hr. Dilution of liver brei 1/1. Room temperature. All figures in mg./10 ml. brei.

Exp.	Additions	Time min.	Inorganic P formed
1	None	15	0.17
		30	0.21
		60	0.87
2	Hexosediphosphate (10.5 mg. P)	15	0.54
		30	0.86
		60	1.46
3	Do. + M/200 NaF	15	0.04
		30	0.04
		60	0.17

The specificity of the enzyme hydrolysing the Cori ester has not been thoroughly investigated. Tables XI and XII show that Cori ester and Embden ester are hydrolysed at about the same rate, while adenylic acid is only very slowly dephosphorylated. Table XIV shows that hexosediphosphate is only very slowly hydrolysed.

As far as is known, therefore, the enzyme only hydrolyses the 1- and 6-hexose-monophosphates.

(3) *Synthesis of glycogen from glucose in liver slices*

In none of the experiments we have carried out on liver brei have we succeeded in demonstrating either the synthesis of glycogen from glucose or fructose, or even the phosphorylation of these sugars. In the living animal, on the other hand, both these substances, and many others, give rise to liver glycogen, and it has been possible to demonstrate glycogen formation *in vitro* by liver slices in their presence [Cross & Holmes, 1937]. Experiments with liver slices have therefore been undertaken in connexion with the present work.

The experiments of Cross & Holmes were all made by shaking the liver slices in a NaHCO_3 -buffered medium containing sugar in an atmosphere of $\text{O}_2\text{-CO}_2$. Since glycogen is formed in liver from glucose 1-phosphate, it seemed likely that glycogen synthesis in the slices should go forward, and might be increased, in a phosphate buffer, though of course it was possible that no phosphate would penetrate the cells. Since the synthesis of glycogen from Cori ester in brei can only be demonstrated in the presence of NaF, it seemed necessary to test the effect of NaF on liver slices. Ca is precipitated by phosphate buffer of the strength and pH used in the experiments with brei, so that it was omitted from the Ringer solution used for the liver slices shaken in phosphate buffer; the fluid used contained Na, K and Mg in the proportions used by Krebs [1933], and various concentrations of phosphate. The pH was 7.2. The glycogen determinations were carried out by adding the required amount of 80% KOH to the tissue while it was still being shaken in the thermostat as previously described [Cross & Holmes, 1937]. We investigated the synthesis of glycogen both after long and short periods of incubation; we also tested the effect of aerobic and anaerobic conditions on the synthesis, and the effect of insulin.

Our findings may be summarized as follows. Liver slices synthesize glycogen from glucose in Krebs Ringer solution containing Ca and buffered with CO_2 and NaHCO_3 . The synthesis only occurs under aerobic conditions; no synthesis, or even a slight decrease in glycogen is observed anaerobically. The synthesis is not increased, but slightly decreased by insulin. It is relatively slow, the highest values being obtained after $2\frac{1}{2}$ hr., the longest time for which experiments were continued. If the fluid is buffered by phosphate instead of NaHCO_3 , the synthesis is very small. This is partly due to the absence of Ca, for if the phosphate concentration is lowered to $M/60$, so that Ca can be added to the Ringer solution, the synthesis is somewhat increased, and approaches the value found in NaHCO_3 -buffered but Ca-free Ringer solution. If Ca is added to the NaHCO_3 -Ringer solution in various concentrations, the maximum synthesis is observed when the concentration of Ca is 10 mg./100 ml.; i.e. approximately that found in blood. NaF completely inhibits the synthesis, whether the Ringer solution be buffered with phosphate or NaHCO_3 .

Similar experiments were done with brei, but no synthesis of glycogen from glucose was ever observed with or without NaF. No accumulation of organic phosphate was observed in the liver slices in any of the experimental conditions investigated either with or without NaF.

These findings are illustrated by the experiments shown in Table XV.

Group A illustrates the synthesis of glycogen in NaHCO_3 -Ringer solution containing glucose. There is no synthesis of glycogen in the absence of glucose.

Group B shows that synthesis of glycogen does not occur anaerobically.

Group C shows that glycogen synthesis is very small in a NaHCO_3 buffer without Ca.

Table XV

Rabbits starved 24 hr. 0.5 g. liver slices used for each experiment, shaken in 3 ml. Ringer solution containing 1% glucose, and otherwise of composition as indicated in col. 6. Ca of usual concentration (10 mg./100 ml.) unless otherwise indicated.

	Glycogen, mg./g. fresh tissue			Time of incubation min.	Gas phase	Composition of fluid
	Initial	Final	Increase			
A	1.36	5.22	3.86	60	O ₂ /CO ₂	NaHCO ₃ /CO ₂ + Ca
	0.74	4.00	3.26	120	"	"
	0.74	4.35	3.61	150	"	"
	0.74	0.53	-0.21	120	"	No glucose
	0.74	0.47	-0.27	150	"	No glucose
	0.15	3.80	3.65	160	"	"
B	1.31	4.93	3.62	120	O ₂ /CO ₂	NaHCO ₃ /CO ₂ + Ca
	1.31	4.95	3.64	120	"	"
	0.72	0.60	-0.08	120	N ₂ /CO ₂	"
	0.60	0.59	-0.01	120	"	"
C	0.31	0.41	0.10	15	O ₂ /CO ₂	NaHCO ₃ /CO ₂ . No Ca
	0.31	0.45	0.14	30	"	"
	0.31	0.36	0.05	60	"	"
	0.31	0.87	0.56	90	"	"
	0.16	0.80	0.64	60	"	"
	0.16	1.10	0.94	120	"	"
	0.15	0.88	0.73	160	"	"
D	0.55	0.35	-0.20	60	O ₂	Phosphate, M/20. No Ca
	0.77	0.32	-0.45	60	"	"
	0.16	0.20	0.04	60	"	Phosphate, M/30. No Ca
	0.16	0.68	0.52	120	"	"
	0.15	0.29	0.14	160	"	Phosphate, M/60. No Ca
	0.15	1.94	1.79	160	"	Phosphate, M/60 + Ca
E	0.36	3.08	2.72	120	O ₂ /CO ₂	NaHCO ₃ /CO ₂ . No Ca
	0.36	7.31	6.95	120	"	NaHCO ₃ /CO ₂ . Ca 5 mg./100 ml.
	0.36	8.76	8.40	120	"	NaHCO ₃ /CO ₂ . Ca 10 mg./100 ml.
	0.36	8.51	8.15	120	"	NaHCO ₃ /CO ₂ . Ca 15 mg./100 ml.
	0.15	0.12	-0.03	160	"	NaHCO ₃ /CO ₂ . No Ca + M/200 NaF
F	0.29	6.16	5.87	120	O ₂ /CO ₂	NaHCO ₃ /CO ₂ . Ca
	0.29	5.33	5.04	120	"	NaHCO ₃ /CO ₂ . Ca + 1 mg. insulin
	0.52	4.27	3.75	120	"	NaHCO ₃ /CO ₂ . Ca
	0.52	3.13	2.61	120	"	NaHCO ₃ /CO ₂ . Ca + 1 mg. insulin

Group D shows that glycogen synthesis is small or absent in a phosphate buffer without Ca, but that appreciable synthesis occurs if the phosphate concentration is decreased and Ca added.

Group E shows the influence of varying concentrations of Ca, and includes an experiment showing the inhibition of glycogen synthesis by NaF.

Group F shows the effect of insulin.

The interpretation of these experiments is difficult. Liver brei and muscle extract will form glycogen from Cori ester, but apparently neither phosphorylate glucose nor form glycogen from it under the conditions which we and other workers have used. On the other hand it is certain that in the living animal both liver and muscle form glycogen from glucose, and we have repeatedly demonstrated that isolated liver slices do so. To say that the special activities of the intact cell are necessary for the process may be true but it is not helpful. It is possible that phosphate fails to penetrate the cell, and it will, if present in any considerable concentration, precipitate Ca at the cell surface and in the fluid. If, in the absence of Ca, glucose cannot penetrate the cell, the removal of Ca may account for the absence of synthesis in phosphate-Ringer solution. Synthesis can

go on in weak phosphate if Ca is added, as it does in a "Ca-free" NaHCO_3 -Ringer solution, but in such a case "Ca-free" is only a relative term, since some Ca must be added with the tissue. The effect of Ca recalls the work of Minot *et al.* [1934], who observed the marked curative effect of Ca on animals whose livers had been damaged by chloroform, carbon tetrachloride and guanidine.

Since NaF inhibits the synthesis in liver slices, it is difficult to obtain any evidence as to whether the glucose is phosphorylated or not, since even in brei the formation of phosphate esters from glycogen can be demonstrated only with difficulty unless NaF is added to prevent subsequent dephosphorylation.

DISCUSSION

We consider that the evidence given lends ample support to the scheme of glycogen breakdown put forward in the introduction, and that phosphorylation is the main path of glycogen metabolism in the liver. We should like to draw particular attention to the high velocities of the reactions involved. 100 g. rabbit liver can phosphorylate 4 g. glycogen per hr. at 37° anaerobically. If we assume that the rate in human liver is about the same, then the average human liver (i.e. of about 1.5 kg.) will phosphorylate about 60 g. glycogen per hr. The rate of dephosphorylation of the Cori ester to glucose is even faster. In the intact liver the velocities must be greater still, since our conditions must have been far from optimum. The velocities of the reactions glycogen $\rightarrow$ Cori ester $\rightarrow$ glucose are therefore more than fast enough to maintain the blood sugar level, even in exercise.

One point in our scheme needs amplification. We have shown that both Cori ester and Embden ester are dephosphorylated in the liver at about the same rates, and also that Cori ester can be converted into Embden ester. During the breakdown of glycogen in normal liver, is the Cori ester itself dephosphorylated, or is it first converted into Embden ester and the Embden ester dephosphorylated? We should not like to be too dogmatic on this point, but it seems probable that the Cori ester formed from glycogen is dephosphorylated immediately, without going to Embden ester. The final result of course would be the same in either case; the glycogen is ultimately all converted into glucose.

If the Cori ester before dephosphorylation were converted into the Embden ester, which is an equilibrium mixture of glucose and fructose 6-phosphates, one would expect that dephosphorylation of the latter would give rise to a mixture of glucose and fructose; however, we could never detect the formation of any fructose during the breakdown of glycogen in normal liver brei. This argument is of course not completely conclusive, since it may be that only the glucose component of the Embden ester is dephosphorylated.

In our experiments, in normal brei, glycogen is entirely converted into glucose, while in NaF-poisoned brei, it all forms Embden ester. Probably both these routes are physiological, and the Embden ester serves as a substrate for the metabolism of the liver itself.

The metabolism of Embden ester in liver and in muscle appears to be different. In muscle, it reacts with adenylypyrophosphate, forming hexosediphosphate and adenylic acid [Ostern *et al.* 1936], but in NaF-poisoned liver brei glycogen is converted almost quantitatively into hexosemonophosphate, which does not seem to react further, at least, under anaerobic conditions. This is doubtless due to the fact that liver contains very little adenylypyrophosphate, and as is well known forms little lactic acid. It seems probable that the Embden ester is not converted into lactic acid to any great extent, but is mainly directly oxidized as it is in yeast and in brain [Lipmann, 1936; Dickens, 1936; 1938; Warburg & Christian, 1937].

From this comparison of the metabolism of glycogen in muscle and in liver we venture to draw some conclusions as to the well-known difference in the effect of adrenaline on these tissues. In liver adrenaline accelerates the conversion of glycogen into glucose; in muscle the formation of lactic acid. Both effects can be attributed to the acceleration of phosphorolysis; they differ in subsequent reactions only. In liver, Cori ester is hydrolysed to glucose; in muscle it is converted into lactic acid, by way of Embden ester and hexosediphosphate. If the action of adrenaline is to accelerate phosphorolysis of glycogen, this would also explain the results of Cori & Cori [1931], who found that injection of adrenaline caused an increase in the hexosemonophosphate content, as well as a decrease in the glycogen content, of intact muscles *in situ*.

It must be pointed out, however, that Cori *et al.* [1939, 1] and Gill & Lehmann [1939] failed to find any effect of adrenaline on phosphorylase activity in muscle extracts, a result we can confirm in liver brei. If adrenaline stimulates phosphorolysis of glycogen, its effect must therefore be an indirect one, possibly depending on the integrity of the living cell.

We have dealt for the most part with the breakdown of glycogen to glucose; there remains to be considered the reverse process. Glycogen is synthesized from glucose in the intact animal, the perfused liver and in liver slices. We have directly shown the synthesis of glycogen from Cori ester in liver brei, and there is no doubt that the enzymic conversion of glycogen into Cori ester is reversible. It therefore seems highly probable that in the synthesis of glycogen from glucose, the glucose is first converted into Cori ester. The mechanism of this process is as yet entirely unknown. We are inclined to doubt that it is a simple reversal of the dephosphorylation of the Cori ester to glucose, for the following reasons: (1) In liver slices, glycogen is only synthesized from glucose aerobically. This suggests that the phosphorylation of glucose is coupled with the energy of some oxidative process, as in yeast, for if it were a simple reversal of the dephosphorylation of Cori ester, it should occur equally readily aerobically and anaerobically. (2) Although it has been stated that the action of phosphatases is reversible, and Martland & Robison [1927] have claimed that glucosemonophosphate and glycerophosphate are synthesized by the action of bone phosphatase on glucose and glycerol respectively in the presence of phosphate, the evidence for this is hardly satisfactory, on account of the small extent of the synthesis, the slowness with which it proceeded, and the enormous concentrations used. In Martland & Robison's experiments, for example, using 50 % glycerol and $M/2$ phosphate, only 5.4 % synthesis occurred after 14 days; using 60 % glucose and $M/8$ phosphate, they obtained a 5.3 % synthesis after 21 days. It must be concluded that phosphatases are reversible to a very small extent, or not at all. In any event, we are not altogether certain that the dephosphorylation of the Cori ester is brought about by a simple phosphatase of the ordinary type. (3) The fact that, in muscle, glucose can be synthesized to glycogen but Cori ester cannot be hydrolysed to glucose, shows that the two processes are not necessarily interdependent.

In yeast, Lutwak-Mann & Mann [1935] have shown that glucose is phosphorylated by adenylypyrophosphate. This is probably not the mechanism in liver, which contains very little adenylypyrophosphate. Finally, the possibility that glucose is phosphorylated first to Embden ester, which is then converted into Cori ester and then into glycogen, can be excluded, since we agree with all other workers in finding that the reaction Cori ester $\rightarrow$ Embden ester is not reversible, and have never observed any synthesis of glycogen from Embden ester.

SUMMARY

1. The breakdown of glycogen has been studied in rabbit liver brei.
2. The main pathway of glycogen breakdown in the liver is through phosphorylation with inorganic phosphate, the product being glucose 1-phosphate (Cori ester), which is then dephosphorylated to glucose. Only a small part (not more than 15 %) of the glycogen breakdown in liver is due to amylase activity.
3. Reasons are given for the supposition that the above scheme represents the mechanism by which liver glycogen is converted into blood sugar *in vivo*.
4. In the presence of NaF, the dephosphorylation of the Cori ester is inhibited, and under these conditions, the Cori ester formed by the phosphorylation of glycogen is all converted into Embden ester.
5. When Cori ester is added to NaF-poisoned liver brei, part of it is converted into Embden ester, and the rest to glycogen. Thus the reaction $\text{glycogen} + \text{phosphate} \rightleftharpoons \text{Cori ester}$ has been shown to be reversible, and it is suggested that the reverse reaction is an intermediate step in the synthesis of liver glycogen from glucose.
6. Neither glucose nor fructose is phosphorylated or converted into glycogen in liver brei. Glycogen is, however, synthesized from glucose in liver slices. This synthesis only occurs aerobically. It is strongly inhibited by NaF, and slightly inhibited by insulin. It does not occur unless Ca is present in the Ringer solution in which the liver slices are suspended.

APPENDIX

Since this work was completed, a paper has appeared by Cori *et al.* [1939, 2] who have arrived at substantially the same conclusions as ourselves. These authors were successful in preparing active liver extracts, and by adsorption on alumina Cy, elution and ammonium sulphate precipitation, were able to separate the enzyme phosphorylating glycogen from the enzymes converting Cori ester into glucose and Embden ester. By this elegant method they were able to show, in the clearest possible way, that the reaction $\text{glycogen} + \text{phosphate} \rightleftharpoons \text{Cori ester}$ is a true reversible equilibrium, about 82 % of the added Cori ester being converted into glycogen when equilibrium is attained.

This paper incidentally explains the great difficulty we experienced in preparing actively phosphorylating liver extracts; apparently, fasted rabbits, on which all our experiments were made, give much less active liver extracts than well-fed animals.

The results of Cori *et al.* differ from our own in one respect; they state, as the result of one experiment, that NaF has no effect on the formation of glucose or hexosemonophosphate from glycogen. We have performed a great number of experiments with NaF, and have never failed to observe a marked effect. This discrepancy may be due to the fact that the above authors used concentrations of NaF 40 times greater than those used in our experiments. The possibility also remains that the dephosphorylation of Cori ester in the intact liver, and in brei, may be brought about by a more complicated mechanism than the (apparently) simple phosphatase present in liver extracts.

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