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Enzymatic Breakdown of Glycogen in Liver Extracts.

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It has been shown in previous papers[1] that dialyzed liver extracts contain an enzyme which forms glucose-1-phosphoric acid (1-ester) from glycogen and inorganic phosphate and that this enzyme is activated by adenylic acid. When 1-ester is added to liver extract, inorganic phosphate is split off due to the presence of a phosphatase. It is shown in this paper that the combined action of these two enzymes converts glycogen to glucose, a reaction which has hitherto been ascribed exclusively to a diastatic enzyme. A typical experiment is recorded in Table I. The liver of a fasted rabbit was cooled, ground in a mortar and extracted twice with ice-cold distilled water. The extract was dialyzed for 4 hours in thin collodion sacs against running tap water of 10°; it was then centrifuged at high speed for 10 minutes and used at once. Additions were made to the extract as shown in Table I, and analyses were performed before and after incubation. The glycogen, after digestion in 30% NaOH, was precipitated from boiling alcohol, centrifuged, redissolved in water and again precipitated. The fermentable sugar was determined in $HgSO_4$-$BaCO_3$ filtrates, inorganic phosphate in trichloracetic acid filtrates. The formation of hexosemonophosphate was calculated from the amount of inorganic P which was esterified during incubation.

Table I shows that addition of phosphate to the reaction mixture increases very markedly the disappearance of glycogen and that addition of adenylic acid causes a further increase. In the latter case the rate of disappearance of glycogen corresponds to 1.4 g per 100 g liver per hour which would be sufficiently rapid to meet physiological needs of blood sugar formation in that organ. Phlorhizin, which is known to inhibit the disruptive phosphorylation of glycogen in muscle hash or extract,[2] also has an inhibitory effect in liver extracts.

By means of acid and alkaline fermentation (Somogyi[3]) it was

1 Cori, G. T., Colowick, S. P., and Cori, C. F., *J. Biol. Chem.*, 1938, **123**, 375; 1938, **124**, 543.

2 Lundsgaard, E., *Biochem. Z.*, 1933, **264**, 209; Ostern, P., Guthke, J. A., and Terszakowec, *Z. physiol. Chem.*, 1936, **243**, 9.

3 Somogyi, M., *J. Biol. Chem.*, 1937, **119**, 741.

TABLE I.
Enzymatic Breakdown of Glycogen in Dialyzed Liver Extract.
10 cc of reaction mixture corresponds to 5 g of liver. Incubation period 1 hr, temperature 37°.

Composition of reaction mixture	Changes during incubation (mg per 10 cc mixture)			
	Glycogen	Hexosemono-phosphate (as hexose)	Fermentable sugar	% glycogen accounted for
1. 1% glycogen .004 M $MgSO_4$	—12		+ 6	50
2. 1% glycogen .004 M $MgSO_4$ .02 M phosphate pH 7.2	—39	(+0.3)	+30	77
3. 1% glycogen .004 M $MgSO_4$ .02 M phosphate .001 M adenylic a.	—70	+19	+48	96
4. Same as 3. .005 M phlorhizin	—40			

ascertained that the fermentable sugar formed was mostly glucose. The glucose formation was highest in the experiment with adenylic acid in which case there was also an accumulation of hexosemonophosphate, its rate of formation being apparently greater than its rate of breakdown to glucose and inorganic phosphate. It may be mentioned that in muscle extract such a breakdown of hexosemonophosphate does not occur, because phosphatases are absent.

The effects of addition of phosphate, adenylic acid and phlorhizin are characteristic for the phosphorylating enzyme system. It is difficult to prepare an active diastase from liver and when the organ is first washed free of blood, hardly any diastase can be extracted (Davenport[4]). Furthermore, the diastatic enzyme causes a stepwise breakdown of glycogen, so that after short periods of incubation, such as were used in the experiment in Table I, dextrines predominate. Only after much longer periods of incubation do larger amounts of maltose and glucose make their appearance in the reaction mixture.[5]

It is concluded that there is present in dialyzed liver extracts an enzyme system which after addition of phosphate buffer and adenylic acid is capable of rapid glucose formation from glycogen. It seems possible that the blood sugar of mammals is produced by this enzyme system rather than by a diastase. In this connection it is suggestive that when liver slices are shaken in oxygenated phosphate-Ringer's solution, addition of phlorhizin exerts an inhibitory effect on the breakdown of glycogen.

[4] Davenport, H. A., *J. Biol. Chem.*, 1926, **70**, 625.
[5] Somogyi, M., *J. Biol. Chem.*, 1938, **124**, 179.