CLXII. STRUCTURE AND ENZYME REACTIONS.

PART VII. THE SYSTEM GLYCOGEN-AMYLASE- LIVER TISSUE.

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THE researches described in the previous parts of this series [Przylecki *et al.*, 1927, 1928; Truszkowski, 1928], although performed upon models under conditions differing greatly from those obtaining within the living cell, yielded results possessing a certain biological importance, notably in the elucidation of the rôle of structure in the attainment of definite intracellular equilibrium.

It has been shown that the introduction of certain natural cell-constituents into the system amylase-glycogen leads to a retardation of the velocity of the reaction, due to the adsorption of either the ferment, the substrate, or both. We have, further, found that amylase becomes inactivated by adsorption upon a lipid mixture, whilst adsorption upon protein in no way impairs its activity. It appears, therefore, that the velocity of glycogenolysis in the presence of these cell-constituents depends not only upon that of the ferment.

One of the problems whose solution was sought in this series of researches was that of the domination within the cell of reactions of synthesis over those of hydrolysis. On the basis of the experiments described in Parts v and vi of this series it was concluded that, in view of the strong adsorption of glycogen upon cell-proteins, and of the feeble adsorption of glucose, the reaction $\text{glucose} \rightleftharpoons \text{polysaccharides}$ should be predominantly one of synthesis in a cellular environment. The conditions under which the experiments on which this conclusion was based are, however, not entirely comparable with those obtaining within the cell.

It was therefore necessary to study this problem under conditions closer to those actually found within the cell, not only in so far as concerns the nature of the adsorbent itself, but also in order to establish whether changes in the equilibrium point in favour of the reaction of synthesis of glycogen are due exclusively to adsorption of both constituents, or whether some further factors come into play, as indeed appeared to us to be probable.

In view of the high degree of adsorption of amylase found by us, and of the efficient action of such adsorbed amylase, the equilibrium point of the reaction

glucose $\rightleftharpoons$ glycogen, in the system glycogen-glucose-amylase-protein is, as we have shown, dependent upon the ratio of unadsorbed glycogen to glucose. The degree of glycogenolysis depends, therefore, upon the reversibility of the adsorption of glycogen.

The first question to be settled was whether glycogen in adsorption upon protein can be eluted by addition of water, and experiment showed that this was the case. In view of this, equilibrium in this system would depend upon the two equilibrium relations:

The elution of glycogen from protein would proceed until definite equilibrium had been attained in the system free glycogen-glucose, and this equilibrium again would depend upon the ratio adsorbed glycogen : free glycogen. Thus the greater the percentage of glycogen in adsorption, the sooner would equilibrium II supervene, and the quantity of adsorbed glycogen would be greater, the greater the ratio of free glycogen to glucose. In view of the above, we should expect to obtain minimum glycogenolysis in the presence of active amylase under the following conditions.

1. In order to bring the ratio of adsorbed to free glycogen to a maximum, very large quantities of protein together with small concentrations of glycogen should be taken; that is, however, inconsistent with biological conditions.

2. In order to attain an optimum ratio of free glycogen to glucose, solutions containing high concentrations of glucose and colloids should be taken. The latter point appeared to us to be of special importance.

The first question examined experimentally therefore was whether the deposition of glycogen in liver cells could be explained as being due exclusively, or to a large extent, to adsorption on the surfaces of the various hepatic colloids. This appeared to us to be *a priori* somewhat improbable, but, nevertheless adsorption isotherms of glycogen on liver pulp were constructed. The liver pulp used was left for a considerable period of time with antiseptics, which were then removed, and the pulp was thoroughly washed. The product contained no amylase or polysaccharides, and was also partly freed from lipoid substances. The reversibility of adsorption of glycogen on this pulp was next studied, and finally, having settled this point, the equilibrium point of the reaction glycogen $\rightleftharpoons$ glucose was determined in the system adsorbed amylase-glycogen-liver protein, using large quantities of the last-named substance.

EXPERIMENTAL.

I. Adsorption.

The degree of adsorption on liver pulp of glycogen from solutions of various concentrations was determined according to the methods described in Part III of this series [1928]. The liver pulp was prepared by adding to 1 kg. of comminuted, and, as far as possible, blood-free liver, 100 cc. of chloroform, 200 cc.

of glycerol and 1 litre of water. The whole was left for some months, during which time amylase and glycogen passed into solution, and the latter underwent hydrolysis to glucose. The contents of the flask were then poured through a sieve, on which the residue was washed in a strong current of water for 48 hours. The resulting product, which was free from amylase and polysaccharides, was allowed to drain, and was then used as the adsorbent. To 10, 20, or 30 g. portions of this pulp 30 cc. of 0.5–10 % glycogen solution were added, and the whole was shaken for 4 hours. The mixture was then either filtered, or centrifuged at low velocities in order to avoid concentration of glycogen in the lower layers. Glycogen was determined either in the filtrate or in the pulp, using the Pflüger-Bertrand method.

Table I. *Adsorption of glycogen on liver pulp.*

In every case 30 cc. of glycogen solution were used, and the pulp was rubbed through a sieve before weighing out.

Adsorption of glycogen on												
Glycogen solution used		10 g. pulp (analysis of pulp)		20 g. pulp (analysis of solution)						30 g. pulp (analysis of pulp)		
				Exp. 1			Exp. 2					
				Free glycogen in solution			Free glycogen in solution					
%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
0.5	150	53	79.5	96	144	0.018	82	123	0.088	—	—	—
1.0	300	52	156	94	282	0.054	85	255	0.179	99.0	297	0.099
2.0	600	46	276	66.6	400	0.60	79	474	0.416	—	—	—
3.0	900	43	387	68.8	619	0.87	70	630	0.891	93.0	837	0.21
5.0	1500	40	600	—	—	—	72	1080	1.39	—	—	—
7.5	2250	—	—	76.0	1710	1.62	—	—	—	—	—	—
10.0	3000	—	—	78.1	2343	1.97	74	2220	2.57	89.1	2673	1.08

The results, given in Table I, show that the pulp has an extraordinarily high adsorptive power for glycogen, and that, using appropriate concentrations of pulp, corresponding to those present in the liver, this adsorption may, even for 10 % glycogen solutions, amount to as much as 90 %. This fact appears to be of great interest, as it indicates that, within the cell, glycogen is concentrated on the protein surfaces. These results are, moreover, in conflict with certain histological evidence, whereby it would appear that glycogen is concentrated on lipid surfaces. We cannot yet say whether the inability to adsorb glycogen found by Truszkowski for certain lipoids applies to all lipoids, or whether the drops of lipid substances within the cell are coated with some protein, on the surface of which glycogen may be adsorbed. It is also possible that diminution of the surface of drops of lipoids due to several drops coalescing into one large one may lead to the irreversible precipitation of glycogen or starch adsorbed on these surfaces. This question will be considered in a later paper.

It follows from the results described in this paper that, where a cell system contains up to 10 % of glycogen, barely 0.8 % or even less can exist in solution, the remainder being adsorbed upon protein and possibly lipid surfaces.

II. *Reversibility of adsorption.*

Three points of biological significance were here to be elucidated. The first was to establish with what facility glycogen undergoes elution from protein as a result of dilution. The second was to determine the extent of adsorption of glycogen from solutions of various concentrations. Finally, the percentage of glycogen remaining in solution after the addition of different volumes of the same glycogen solution to equal quantities of adsorbent was measured.

The preliminary experiments were carried out on liver pulp or coagulated egg-white on which glycogen was adsorbed. Analysis of the adsorbent after washing in a stream of water for 24–48 hours showed that barely traces of glycogen remained. It hence follows that glycogen can be quantitatively eluted from adsorption on protein without the agency of elutive factors other than water. Further experiments were of a quantitative nature, the percentage elution at given dilutions being determined. The results are given in Table II.

Table II. *Elution by dilution.*

In every case 20 g. of liver pulp were taken.

In Exp. 4 30 cc. of 5 % glycogen were added to 20 g. of pulp, the whole was shaken for 4 hours, left for 3 hours, 270 cc. water were then added, the whole was shaken a further 6 hours and left 12 hours, after which glycogen was determined.

No. of Exp.	Glycogen g.	Water cc.	Glycogen %	Adsorbed glycogen		Glycogen in solution	
				g.	%	g.	%
1	0.150	30	0.5	0.123	82	0.027	0.088
2	1.500	30	5.0	1.170	78	0.330	1.09
3	1.500	300	0.5	0.688	46.5	0.812	0.27
4	1.500	300	0.5	0.741	49.4	0.759	0.25

III. *Definitive equilibrium.*

The following experiments have only a theoretical significance, their object being to establish whether in the presence of an adsorbent, definitive equilibrium supervenes before the hydrolysis of all the glycogen present in a system containing on the average 70–80 % of water.

These experiments were performed on solutions of glycogen, the adsorbent being coagulated egg-white, on which amylase had been adsorbed. 10 g. of egg-white with adsorbed amylase were taken, and 5 cc. of solutions or suspensions of 1 to 10 % glycogen. These systems were kept under aseptic conditions, without the introduction of antiseptics. In order to favour the reaction glucose $\rightarrow$ glycogen, glucose was added to 1–2 % of the reaction solution. The glycogen content of the whole system was determined after 1, 2 and 4 weeks. The results indicated that glycogenolysis proceeds practically to completion in these circumstances.

As to the cell system, the above experiments indicate that adsorption is able to inhibit glycogenolysis and to keep glycogen stabilised for an indefinitely long period only when glycogen is present exclusively in adsorption, or when

the reaction glycogen $\rightleftharpoons$ glucose is close to equilibrium, so that it is able to proceed in one direction or another when changes supervene in the concentration of one or the other substrate. Only in these circumstances can the synthesis of polysaccharides take place.

For the realisation of this synthesis not only the presence of an adsorbent which would remove large quantities of glycogen from solution is necessary, but, further, conditions should be such that the reaction glycogen $\rightleftharpoons$ glucose should be easily reversible. Such conditions cannot be brought about by the presence of an adsorbent. For this purpose, not only should the relative concentration of glycogen be diminished but also that of water. This matter will be considered more fully in a subsequent communication.

A further conclusion may be drawn from the above results. This is that our hypothesis is confirmed that the velocity of reaction is, in view of the traces of glycogen left in solution, determined in the first place by the equilibrium adsorbed glycogen $\rightleftharpoons$ free glycogen, even where the concentration of polysaccharide is high.

SUMMARY.

1. Liver tissue has an exceptionally high adsorptive action upon glycogen.
2. Adsorption of glycogen upon tissue and other proteins is of a reversible nature, elution taking place when the concentration of glycogen in solution falls.
3. The addition of protein to the system glycogen-amylase retards but does not inhibit the reaction of glycogenolysis.
4. Biologically, glycogenolysis is the resultant of the reactions: adsorbed glycogen $\rightleftharpoons$ free glycogen $\rightleftharpoons$ glucose.

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