**CXXXVI. STRUCTURE AND ENZYME
REACTIONS. PARTS I AND II.
THE SYSTEMS UREA-UREASE-CHARCOAL AND
POLYSACCHARIDE-AMYLASE-CHARCOAL.**

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THE question of the applicability of the law of mass action to reactions in the living organism has long been considered as one of the most important problems in the dynamics of life phenomena.

The chief difficulty in the solution of this problem is that these reactions take place in a heterogeneous medium, in which, apart from purely chemical reactions, we encounter processes of diffusion, and the whole question is further complicated by the fact that most of the reacting substances are in the colloidal state.

For this reason, all except ionic reactions and those connected with the last stages of disintegration or the first stages of synthesis, are reactions involving all those factors connected with reactions of hydrophile colloids in heterogeneous media, *i.e.* reactions of adsorption, and in general, all surface phenomena, to which the law of mass action is, at least in the state of present day knowledge, inapplicable.

In this paper we shall consider the questions of the applicability of the law of mass action to enzyme reactions. In general, in so far as the kinetics of enzyme reactions in homogeneous systems are concerned, if we can, in general, consider any enzyme system to be homogeneous, these conform to the law of mass action, as has been pointed out by Michaelis and Menten [1913] and Josephson [1925].

It is a question whether the same holds good for enzyme reactions *in vivo*, and whether reversible enzyme reactions may here be interpreted on the basis of the concentration of the substrates. Here we encounter a whole series of incomprehensible facts, apparently irreconcilable with the law of mass action, a classical example of this being the different behaviour of amylase *in vitro* and *in vivo*.

Some factor must therefore exist in the structure of the cell which alters the course of reactions and their final equilibrium point. This factor need not necessarily be connected either with the nervous or the endocrine system, but may be connected with the actual structure of the liver cell. A whole series of examples could be cited, showing that this phenomenon is not limited to amylase, but may also be encountered among the most varied reactions of enzymic hydrolysis, involving colloidal constituents, such as proteins, carbohydrates and nucleic acids.

Apart from the question of the direction of the reaction and its final equilibrium point, it is a point of considerable interest that the velocity of enzyme reactions depends to a large extent upon the structural conditions of the environment in which the enzyme acts. In general, enzymic and vital processes may be classified into those in which the velocity of reaction is accelerated by the destruction of structure, and those in which it is retarded, most hydrolytic ferments belonging to the former class.

Hofmeister [1901], on the basis of Bütschli's theory of the alveolar structure of protoplasm, considers that reactions take place in the more liquid portions of the cell. Thus ferments, to which the colloidal parts of the partitions are impermeable, cannot liberate themselves from the latter, in contrast with those crystalloids, taking part in the reactions of the cell, which are able freely to circulate.

Hofmeister did not analyse more closely the possible causes of the binding of the enzymes. These may be extremely heterogeneous. Hedin [1915] was one of the first to draw attention to the phenomenon of adsorption as a possible factor in the explanation of variability of the velocity of enzyme reactions *in vivo*.

Lesser [1920] showed that the retardation of the decomposition of glycogen may take place, not only *in vivo*, but may also be reproduced experimentally by the addition of some adsorbent to the solution. Lesser interpreted his results in the following way. Only that portion of ferment which is in solution is active, the adsorbed portion being inactive. Substances which lower surface tension increase the active concentration of ferment by elution of the latter. This effect is similar to the hypoglycaemia produced by alcohol and other narcotics *in vivo*.

This phenomenon has been the subject of numerous researches, based upon the assumption that adsorbed enzymes are inactive. Certain facts tending to discredit this assumption may, however, be found, such as Meyerhof's [1914] discovery that saccharase, adsorbed on colloidal ferric hydroxide, retains its enzymic activity. Admitting therefore, that adsorption need not necessarily deprive enzymes of their activity, the interpretation of Lesser's experiments becomes exceedingly difficult.

The possibility may arise that, if enzymes lose their activity not through adsorption, but in accordance with Hofmeister's [1901] conception, at least in certain cases, changes in the velocity of enzyme reaction in heterogeneous

systems may be due to the separation of the ferment from its substrate. In that case, we should have to admit that this retarding effect would arise when both the enzyme and its substrate are adsorbed, and this appears to be the case for the system amylase-glycogen.

Certain unpublished experiments carried out by one of us [cf. Przylecki, 1922], in 1918–1920, also support this interpretation. In these experiments we found that, during the months in which Lesser found almost complete absence of glycogenolytic action in the frog's liver, *i.e.* from November to January, the liver synthesises glycogen quite efficiently from glucose. Thus frogs placed in 7 % glucose at 15° exhibited a considerable increment of glycogen, the content of the latter in the liver increasing on the average by 40 % over a period of two weeks. Almost identical results were obtained in experiments in which the glycogen content of the liver was determined at the beginning of the experiment, using in this case summer frogs at the same temperature (see Table I).

Table I. *Glycogen content of lobes of the livers of frogs kept 10 days in a 7 % solution of glucose at 15°.*

Month	No. of experiments	Content in the analysed lobe at beginning of experiment in % live weight		Same after ten days	
		Average	Deviations	Average	Deviations
December	5	5.5	4.8–6.0	7.8	7.1–8.3
June–July	5	4.8	3.9–5.9	6.8	5.3–7.9

If therefore, Lesser's hypothesis were correct, it could be reconciled with our findings only on the assumption that the synthesis of glycogen depends upon a different enzyme from amylase, since any other interpretation would necessarily involve accepting the view that a free ferment acts as efficiently as when adsorbed. The difference in the velocity of hydrolysis of glycogen between summer and winter livers would not, in that case, be due to differences in the amount of amylase present, but to the distance separating the enzyme from its substract. Should this be so, it would be clear that the enzyme acts equally efficiently for the synthesis as for the hydrolysis of glycogen, and further, the reason for the existence of seasonal differences in the velocity of the latter process would be explained, since the enzyme and substrate may be more or less separated from one another, according to the season.

In order to decide which of the above views is correct, *i.e.* to prove that the velocity of enzyme reactions is affected, not by adsorption of the enzyme, but by spatial separation of the latter from the substrate, a whole series of experiments would be necessary.

The experimental part of this research has as its object the verification of the following postulates upon which the above reasoning is based.

1. That adsorbed enzymes retain their activity; *i.e.* that the velocity of enzyme reactions, in systems where the enzyme is strongly adsorbed on to

some adsorbent, and the substrate slightly or not at all, is equal to or only slightly less than in the absence of the adsorbent.

2. That, in systems containing an adsorbent, the velocity of enzyme reactions depends upon the concentration of unadsorbed substrate.

Experiments conducted upon the system urease-charcoal-urea confirmed the first postulate, showing that the velocity of decomposition of urea is independent of the presence of an adsorbent, in spite of the fact that 90 % of the urease is adsorbed. Urea causes scarcely any elution of the ferment and the addition of alcohol has little influence upon the velocity of decomposition of urea.

We may hence conclude that in those conditions where an adsorbed enzyme suffers no loss of catalytic power, and the substrate is not adsorbed, the change from a homogeneous to a heterogeneous system, due to the introduction of the adsorbent, does not influence the direction of the reaction, its final equilibrium point, or the velocity of enzymic reaction.

We shall next proceed to examine those systems in which both the enzyme and the substrate undergo adsorption. Such a case is afforded by the systems amylase-dextrin or glycogen-charcoal. Here we find that dextrin and glycogen are strongly adsorbed by charcoal, that the addition of such substances as butyl and propyl alcohols, ether and chloroform greatly diminishes the degree of the adsorption, that amylase is very strongly adsorbed by charcoal and that the addition of alcohols, chloroform or ether, in such quantities as diminish the degree of adsorption of dextrin or glycogen by 50 % does not produce this effect upon amylase. Since the completion of these experiments we have found a confirmation of this fact in a paper published by Unna [1926].

Experiments carried out with the object of comparing the velocity of enzymic hydrolysis in the presence or absence of charcoal show that this velocity increases inversely as the quantity of added adsorbent. In this way, the quantity of glycogen hydrolysed by amylase in the presence of charcoal may be reduced to 5 % of that which would normally be decomposed.

Alcohols added to such systems in such quantities as cause no elution of adsorbed amylase, but only of glycogen or dextrin, cause a commensurate increase in the velocity of hydrolysis of these polysaccharides.

The above results show that, in those cases examined, the cause of differences in the velocity of enzyme reactions lies in adsorption, not of the enzyme, but of the substrate. It would therefore appear that adsorbed enzymes act quite efficiently, and in order to show that this is the case we have been able, making use of the fact that the adsorbed ferment does not undergo elution on treatment with water or glycogen solution, to prove that amylase completely adsorbed on charcoal is capable of hydrolysing glycogen or dextrin.

The next point which we wished to establish was that an adsorbed ferment is as active as when not adsorbed. This would also show that differences in reaction velocity depend, not upon adsorption of ferment, but of substrate. That such is the case was shown by the fact that the velocity of hydrolysis

of glycogen or dextrin in the presence of charcoal depends upon the concentration of unadsorbed substrate, and that where the same quantity of ferment was taken and the concentration of free substrate was the same, the presence of charcoal made no difference to the velocity.

From the results obtained for the system amylase-polysaccharide-charcoal may be drawn the following conclusions. Firstly, adsorbed or free amylase possesses the same activity. Secondly, differences in the velocity of hydrolysis of polysaccharides in the presence or absence of charcoal are due solely to the above described spatial separation of the enzyme from its substrate. This would mean that, whether the enzyme combines chemically with the substrate or whether, as Bayliss [1924, 1925] assumes, an adsorption compound is formed, an adsorbed enzyme is only exceptionally able to enter into contact with an adsorbed substrate. Our figures show that, under these conditions, combination of enzyme with substrate is very limited, if the solution is not stirred, and even with stirring only a minimal combination takes place between the adsorbed enzyme and polysaccharide.

A further extremely important conclusion may be drawn from our experiments, namely, that the law of mass action applies also to enzyme reactions in heterogeneous systems. Up to the present, the equation used to express this law had included the following factors: quantity of enzyme, of substrate and of water. If absolute values be applied in this equation, it is obvious that fundamental differences will exist between ferment systems in the presence or absence of an adsorbent. On the other hand, this need not be the case if not the absolute quantities of the above three components be taken into consideration, but only that part of them which is free to take part in the reaction. In this case, we have shown that the whole of the enzyme present is reactive, whilst, in those experiments in which the whole of the enzyme is adsorbed only the free unadsorbed portion of the substrate is at liberty to enter into chemical or adsorptive combination with it. Hence only this portion takes part in the reaction, and should be exclusively considered in the equation. Introducing this correction, we find that the law of mass action is here followed, and that the velocity of reaction between the whole of the enzyme present and the unadsorbed part of the substrate is independent of the heterogeneity of the system.

Conditions are somewhat more complicated if part of the enzyme is unadsorbed. In this case the possibility would arise that this unadsorbed portion would be free to combine with adsorbed substrate. If as above, we assume that only the unadsorbed fraction of the substrate is free to enter into reaction, and accept this as the active concentration, higher values would be obtained than the theoretical.

Thus we see that an examination of the systems urease-urea-charcoal and amylase-polysaccharide-charcoal yields concurrent results, fully in conformity with our theoretical reasoning. The spatial separation of an enzyme from its substrate by the adsorption of both is a factor inhibitive of enzyme action in heterogeneous systems.

It would be of interest to consider firstly, how general is this phenomenon of the influence of structure, and secondly, whether from the above experiments any other conclusions as to influence of structure on enzyme reactions might be drawn. As to the former problem, Meyerhof and Willstätter and his colleagues [1922, 1925] have shown that adsorbed ferments may act quite normally, without any difference in the degree of their activity.

Hedin's work, however, shows that this fact cannot be generalised, since he found that trypsin and rennin lose their activity on adsorption. We would in this connection emphasise that Willstätter, Waldschmidt-Leitz and Kraut found extremely different degrees of adsorption for the pro- and co-enzymes of trypsin, and it may be that their separation and fixation on the adsorbent leads to the inhibition of their action.

This different behaviour of various enzymes, or even of the same enzyme, using various adsorbents, is probably due to differences in the place of attachment of the enzyme to the adsorbent. Thus it is conceivable that in one case, the adsorbent may be bound to a grouping belonging to the accessory non-characteristic part of the enzyme, whilst in a different case, a group indispensable to the action of the ferment is bound.

Such phenomena as changes in velocity of reaction may, within the living organism, depend not exclusively upon sorption in the most general sense of the word, but may equally well be due to chemical combinations, leading to the local fixation of the enzyme or substrate. In this case, glycogen may enter into some loose combination with some other organic colloid.

Certain analogous phenomena may be produced by mechanical occlusion and precipitation during the passage of some colloid from sol to gel, as exemplified in certain of Henri's [1906] experiments. An enzyme reaction which takes place in a solution of gelatin is inhibited by the presence of gelatin gel.

A certain rôle may also be played here by the degree and condition of saturation of the substrate. Thus, owing to changes in the state of solution of difficultly soluble substances, which readily tend to reprecipitate, their active concentration may vary in spite of the fact that their total concentration remains constant. A number of further possibilities may also exist. Thus certain factors may exist which lead to elution of the enzyme but not of the substrate. Such cases have not so far been noted in the literature of the subject, but are none the less possible. Their effect would be to augment velocity of reaction, since the unadsorbed enzyme would be enabled to react with the substrate.

If we accept the above reservations, and substitute for adsorptive influences all factors tending spatially to separate the enzyme from its substrate, many of the characteristics peculiar to the phenomena of the living cell would be explicable.

A further important conclusion follows from this conception of spatial separation as an inhibitive factor in enzyme reaction. Accepting that in homogeneous systems the total concentrations of enzyme and substrate enter

into the equation expressing the equilibrium constant, whilst in heterogeneous systems, in which the enzyme is almost completely adsorbed, only the concentration of unadsorbed substrate enters, then, for reversibly acting enzymes, the final equilibrium point must be different in these two systems, wherever the synthesised body and its disintegration products are differentially adsorbed.

Let us imagine a reversible reaction in a homogeneous system, where, at the equilibrium point the concentration of synthesised product to disintegration products is as 40 : 60. If the former body is more strongly adsorbed than are the latter, then the presence of an adsorbent would shift the equilibrium point in the direction of synthesis, since in the equilibrium equation would figure concentrations of unadsorbed synthesised product and disintegration products. Since the latter are, in the hypothetical case here considered, less strongly adsorbed than are the former, the proportion of the synthesised product in solution would from the commencement be less than that required for equilibrium, and would remain so throughout the experiment. Equilibrium would supervene when considerably more than 40 % of synthesised substance is present; this increment would be larger, the greater the difference in the degree of adsorption of the substrates. Thus a much greater amount of the body should be synthesised in a heterogeneous than in a homogeneous system, and, on the contrary, a much smaller proportion of the synthesised substance should suffer decomposition under the same conditions. Experiments are now in progress with the object of verifying this hypothesis; these are not yet completed, but we consider that they will in all probability confirm our views.

Should this be the case, this would to a certain extent explain why in intracellular conditions the equilibrium point of enzymic hydrolyses lies on the side of synthesis. In such cases as in the synthesis of polysaccharides or proteins, the end-products of reaction are strongly adsorbed, whilst their progenitors or decomposition products are considerably less so.

EXPERIMENTAL.

Solutions of urea and of urease, or of both of these substances together, were added to charcoal, equal proportions of the two former substances taken separately without charcoal, and the amounts of urea converted in both cases were compared. The solutions were filtered either at the beginning of the experiments or after certain intervals of time. The results shown (Tables II to V) are derived from experiments in which both series were carried out simultaneously, using charcoal taken from the same stock. The urease taken was urease powder, purchased from Squibb, a fresh solution being prepared for each series of determinations. In this way, the substances used in each series, both as regards enzyme and adsorbent, were as far as possible uniform.

Urea-ammonia was determined by Schlösing's or Folin's method at a point at which not more than 15 % of the urea present was decomposed. The quantity of urea adsorbed on the charcoal was taken as the difference between that added and that found in the filtrate.

Table II. *Percentage adsorption of urea measured in 2 cc. of solution.*

Amount charcoal added g.	No. of experiments	% urea	% of original urea adsorbed	
			Average	Deviations
0.2	4	0.2	2.4	2.2-2.8
0.4	4	0.4	4.6	3.9-4.8

Table III. *Mg. urea decomposed in the filtrate. Average of five experiments.*

Urease without charcoal	Urease with charcoal	Urease with charcoal and butyl alcohol	Urease with charcoal and propyl alcohol
20.0	0.1	0.1	0.3
19.2	0.3	0.2	0.2

Table IV. *Mg. of urea decomposed.*

Urea, charcoal and urease left in contact without filtration		Urea, charcoal and urease with filtration		Urease and urea		Urease and charcoal filtered and urea added	
0.2 % urea	0.4 % urea	0.2 % urea	0.4 % urea	0.2 % urea	0.4 % urea	0.2 % urea	0.4 % urea
11.8	19.9	3.5	4.8	11.9	20.2	0.0	1.5
11.6	19.8	3.3	5.1	11.5	19.9	0.9	1.0

Table V. *Mg. of urea decomposed.*

			0.1 g. urease, 0.5 g. charcoal and 20 cc. 0.2 % urea					Urease + 0.2 % urea				
Time Series (hours)			I	II	III	IV	V	I	II	III	IV	V
α	2	No alcohol	5.3	5.5	6.6	5.4	2.7	5.1	5.0	—	5.6	2.8
		Propyl alcohol	—	—	—	—	—	5.1	5.1	—	—	2.8
		Butyl alcohol	—	—	—	—	—	5.0	5.3	—	—	2.7
	4	No alcohol	10.4	10.8	13.1	10.5	5.5	10.2	10.2	13.5	10.7	5.7
		Propyl alcohol	—	—	14.9	11.0	5.7	14.0	10.4	13.5	10.9	5.6
		Butyl alcohol	—	—	15.1	10.8	5.4	—	10.1	13.5	10.7	5.4
β	2	No alcohol	5.2	5.6	6.1	5.7	2.8	5.1	5.2	—	5.8	2.4
		Propyl alcohol	5.3	5.7	—	5.9	2.8	5.2	5.3	—	—	2.3
		Butyl alcohol	5.3	5.4	—	5.9	2.9	5.1	5.3	—	—	2.4
	4	No alcohol	10.5	10.4	12.1	10.7	5.7	10.1	—	11.8	10.8	5.0
		Propyl alcohol	10.7	—	13.9	11.0	5.8	10.2	—	11.8	11.0	5.0
		Butyl alcohol	10.6	—	14.1	11.0	5.8	10.2	—	11.5	10.9	4.9
			0.1 g. urease, 0.5 g. charcoal and 20 cc. 0.4 % urea					Urease + 0.4 % urea				
α	2	No alcohol	10.6	11.4	—	11.7	—	10.2	10.5	—	11.1	5.9
		Propyl alcohol	11.0	11.5	—	11.7	—	10.3	11.2	—	11.3	5.8
		Butyl alcohol	10.8	11.4	—	11.9	—	10.6	—	—	11.1	5.8
	4	No alcohol	21.1	22.7	—	21.2	—	21.0	20.3	—	21.1	11.9
		Propyl alcohol	21.4	23.0	—	21.4	—	20.3	—	—	21.4	12.0
		Butyl alcohol	21.5	22.8	—	21.4	—	22.5	—	—	21.0	12.0
β	2	No alcohol	10.4	—	—	11.6	—	10.6	11.0	—	11.4	5.6
		Propyl alcohol	10.7	—	—	11.8	—	—	—	—	11.2	—
		Butyl alcohol	10.7	—	—	11.7	—	—	—	—	11.6	—
	4	No alcohol	20.7	22.0	—	21.4	11.2	20.9	21.6	—	22.1	11.3
		Propyl alcohol	—	22.1	—	22.0	—	21.0	22.5	—	22.1	11.3
		Butyl alcohol	—	22.0	—	21.8	—	20.8	21.8	—	22.3	11.2

The second type of experiments was carried out on amylase and dextrin or glycogen. The enzyme used was purified amylase, purchased from Merck;

in some cases the ferment contained in frog's blood was taken, proceeding according to Lesser [1924]. Here too, determinations were made at such intervals that up to 15 % of the polysaccharides taken had been decomposed. The degree of hydrolysis of dextrin was estimated by Bertrand's method. The amount of polysaccharides adsorbed by charcoal was determined by the hydrolysis of the filtrate.

A. *The system urease-urea-charcoal.*

1. *Adsorption of urea.* The values shown in Table II indicate that the specimen of charcoal used adsorbs urea very feebly.

2. *Adsorption of urease.* 0.5 g. of charcoal was added to solutions containing 0.1 g. of urease, the temperature of the solutions being 20°. After 15–30 minutes, the solutions were filtered, the residue was washed with two 5 cc. portions of water, and 0.2 g. of urea added to the filtrate, so that the latter is a 0.4 % solution of urea. Simultaneously an equal quantity of urea was added to a second, also filtered, solution of urease, without previous charcoal treatment. The results obtained, given in Table III, show that in these experiments 85–95 % of the enzyme present undergoes adsorption by charcoal.

The adsorption of urease in the presence of butyl and propyl alcohols was next studied. The results obtained show that neither of these alcohols is able either to cause elution of adsorbed urease, or to diminish the degree of adsorption of the latter when added simultaneously with it (Table III).

3. *Activity of adsorbed urease.* The degree of adsorption of urease was found to be unaffected by the subsequent addition of urea. This fact is of great importance for demonstrating that adsorbed urease retains its enzymic properties, and that the conditions under which free urease in a homogeneous system and adsorbed urease in a heterogeneous system are examined are strictly comparable.

1 cc. of 0.2 % urease solution was added to 10 cc. water containing 0.2–0.5 g. of charcoal. After 30–60 minutes, half of the solution was filtered and urea solution added to both portions, the second portion being also filtered after from 5 to 10 minutes. The amount of unadsorbed urease was estimated by determining the amount of urea decomposed in the first filtrate within two hours. Since the concentration of urea was the same in all cases, differences in the final concentration could arise only from different urease contents. The values given in Table IV show a certain difference between those experiments in which urease was added before and after filtration. These discrepancies are probably largely due to the urea being in contact with the enzyme before filtration. A further cause of these differences lies, however, in the slight elution of urease by urea, as is shown by those experiments in which the quantity of ammonia formed in two hours by filtered urease solutions to which urea is added, is compared with the differences between the amount of ammonia formed in two hours after filtration of a similar solution containing urea, and that present immediately after filtration.

Having thus shown the degree of adsorption of urease by charcoal to be extremely high, and that urea has scarcely any elutive effect, it became possible to investigate the question of the efficiency of adsorbed urease for the reaction of the decomposition of urea.

The velocities of decomposition of urea in the system urease-urea-charcoal, with and without shaking, were compared, and found to be identical. Hence, it would follow that the efficiency of action of urease is independent of its distribution throughout the solution.

B. *The system polysaccharide-amylase-charcoal.*

1. *Adsorption of polysaccharide.* 0.2 or 0.4 g. of charcoal was added to 20 cc. of a solution of dextrin or glycogen, which was filtered after a lapse of 30 minutes. Table VI shows that, in those cases where 0.2 g. of charcoal was added, on the average 50.5 % of the dextrin was adsorbed, whilst with 0.4 g. of charcoal the degree of adsorption was considerably greater, on the average 72.8 %. Glycogen is adsorbed to a slightly greater extent, the average adsorption in a solution containing 0.4 g. of charcoal being 74 %.

The adsorption of the above substrates is therefore quite considerable, as only about 20 % of the given polysaccharide can exist in solution. Using smaller concentrations of substrate, even greater differences between the total content of the latter and its concentration in solution were obtained.

Table VI. *Mg. reducing sugars found after hydrolysis.*

Compound	No. of experiments	In absence of charcoal	0.2 g. charcoal	0.4 g. charcoal
Dextrin	7	Average 13.5 Deviations 13.1-14.0	Average 6.7 Deviations 6.3-7.2	Average 3.7 Deviations 3.3-4.6
Glycogen	4	Average 14.1 Deviations 14.1-14.2	—	Average 3.4 Deviations 3.2-3.8

Table VII. *Mg. reducing sugars found after hydrolysis. Averages.*

Compound	No. of experiments	Without charcoal	With charcoal		With charcoal and alcohols added		Alcohol added
			0.2 g.	0.4 g.	0.2 g.	0.4 g.	
Dextrin	12	12.2	6.9	3.2	8.5	4.7	Butyl; propyl
Glycogen	4	14.2	—	3.6	—	4.5-4.9	Butyl; propyl

Table VIII. *Mg. reducing sugars found after hydrolysis. Average figures for six experiments.*

Without charcoal	0.4 g. charcoal added	0.4 g. charcoal and various other substances	Other substances added
12.8	3.3	7.0	Amyl alcohol
12.0	2.9	7.6	Chloroform
12.6	3.4	8.2	Ether

In the presence of bodies strongly adsorbed by charcoal, such as propyl or butyl alcohol, considerable elution of glycogen or dextrin took place (see

Table VII). The behaviour of systems containing glycogen is analogous, as may be seen from the results of two experiments conducted on this system, and probably all polysaccharides behave in this way.

The action of amyl alcohol, of ether and of chloroform is, as we see from Table VIII, similar to that of the above alcohols. Thus it appears that more strongly adsorbed substances, such as narcotics, lead to the elution of adsorbed polysaccharides. By making use of this important fact we can change at will the concentration of dissolved polysaccharide without affecting the total amount present.

In all probability, the above observation is of considerable significance in physiological reactions. A second perhaps equally important conclusion which may be drawn from this observation is that the degree of elution of polysaccharides by alcohols is proportional to the degree of adsorption of the former.

2. *Adsorption of amylase by charcoal.* The enzyme-adsorbent system was filtered, a solution of dextrin added to the filtrate, and the whole left at 37° in a thermostat for 7–8 hours. For experiments in aqueous solution, 10 cc. of 1 % amylase purchased from Merck and 0.5 g. of charcoal were taken. After filtration, 10 cc. of 1 % dextrin were added to the filtrates (Table IX). Over 90 % of the amylase present undergoes adsorption, and is present in suspension as an adsorption compound.

From Table X we see that the degree of adsorption of amylase is unaffected by the addition of such powerful elutive agents as butyl and propyl alcohols. Similar results were obtained using diastase from frog's blood. Here a slightly different experimental procedure was adopted. Instead of filtration, the solutions were centrifuged. Almost identical results were obtained with those mentioned above for Merck's amylase, *i.e.* strong adsorption, without elution by alcohols. The only difference is in the slightly lower degree of adsorption of frog amylase.

Table IX. *Mg. dextrin decomposed by filtered amylase solutions.*

Average figures for eight experiments.

Without charcoal		With 0.5 g. of charcoal	
Average	Deviations	Average	Deviations
72.1	69.7–74.2	6.8	6.6–7.1

Table X. *Mg. of dextrin decomposed by filtered solutions of amylase.*

Average figures and deviations for six experiments.

1	2	3	4	5	6	7	8
Without charcoal	0.5 g. charcoal	Charcoal and propyl alcohol	Charcoal and butyl alcohol	Propyl alcohol	Butyl alcohol	Charcoal filtered and propyl alcohol added to filtrate	Charcoal filtered and butyl alcohol added to filtrate
70.6	6.8	6.7	6.7	69.6	69.2	6.6	6.7
68.7–73.0	6.7–7.1	6.6–6.9	6.6–6.9	67.8–71.8	67.8–72.3	6.5–6.8	6.5–6.8

Differences in the velocity of hydrolysis of dextrin.

We have shown that the enzymic hydrolysis of dextrin proceeds in every case more rapidly in a homogeneous than in a heterogeneous system. The retardation of reaction increased with the amount of adsorbent added, until finally only 5 % of the hydrolysis taking place in a homogeneous system was observed.

Two factors might lead to this retardation: the enzyme might be inactivated by adsorption or the enzyme might be spatially separated from its substrate by the fixation of both on the adsorbent. Since we have evidence that adsorbed enzymes may still be active, it remained to establish which factor or factors enter into the present case. It appeared that in order to elucidate this point, certain properties of the enzyme in question would have to be considered. With this end in view, three types of experiments were undertaken. These had as their object to establish, firstly, that the addition of alcohols in such quantities as cause elution of substrate but not of ferment accelerates enzymic hydrolysis; secondly, that completely adsorbed amylase can catalyse the decomposition of dextrin; and thirdly, that the velocity of enzymic hydrolysis is proportional to the concentration of unadsorbed dextrin in solution, and is equal to that of the same concentration of dextrin in the presence of the same quantity of ferment in a homogeneous system.

In our experiments upon the influence of alcohol, the concentrations of substrate, ferment and charcoal were the same as before. Four determinations were made of hydrolysis in the systems charcoal-amylase-dextrin and amylase-dextrin, with and without the addition of butyl and propyl alcohols in such concentration as to cause no elution of the ferment.

Table XI. *Mg. of polysaccharide decomposed. Average figures.*

Substrate	No. of experiments	Time hours	1 % amylase-charcoal-polysaccharide				Amylase-polysaccharide
			Without alcohol	Butyl alcohol	Propyl alcohol	Charcoal g.	
Dextrin	3	6	32.1	47.8	46.1	0.2	55.5
	3	6	16.1	28.1	26.4	0.4	55.2
	3	12	63.9	79.0	76.6	0.2	102.7
	3	12	24.1	36.3	37.2	0.4	103.4
Glycogen	2	6	10.8	16.7	17.1	0.4	46.9
	3	6	26.7	31.2	33.0	0.2	44.7
	3	12	19.9	32.0	31.8	0.4	80.2

The results shown in Table XI indicate that the addition of alcohols considerably accelerates the velocity of hydrolysis of polysaccharides. This effect can be due only to an increase in the concentration of unadsorbed dextrin. It hence follows that, in the presence of substances which adsorb both enzyme and substrate, the addition of alcohols or other narcotics leads to an augmentation of the velocity of reaction due to elution of the substrate, but not of the ferment.

It remains to be proved, however, whether the adsorbed ferment has any hydrolytic action upon dextrin. Thus, in the above case, the possibility may

exist that, both in the presence or absence of alcohols, only the unadsorbed portion of the enzyme has any action, differences in velocity of hydrolysis being due exclusively to elution of substrate. We see, however, no *a priori* reason for believing that adsorption should inactivate an enzyme, unless, of course, attachment to the adsorbent binds some group indispensable to the action of the ferment.

In order, however, to establish that amylase remains active after adsorption, the following experiments were conducted. 1 g. of charcoal was added to 10 cc. of 0.5 % amylase, and the mixture shaken mechanically at 25° for two hours, after which it was centrifuged and the supernatant liquid completely poured off. The residue was then washed five times with water at 25°, glycogen solution was added, and the whole left for from 2 to 4 hours at 37°. We know from Unna's experiments that under these conditions adsorbed amylase is not eluted by glycogen, so that any hydrolytic action observed must be due solely to the adsorbed ferment. Table XII shows that the hydrolytic action of such enzyme-adsorbent preparations upon glycogen is quite considerable.

Table XII. *Non-elution of amylase by polysaccharides.*

Substrate	No. of experiments	Mg. reducing sugars found after contact of from 2-8 hours of adsorbed ferment with substrate		Mg. reducing sugars produced in solution after centrifuging and decanting
		Average	Deviations	
Dextrin	6	21.8	10.6-31.5	0
Glycogen	4	26.3	11.5-33.2	0

That longer contact of adsorbed amylase with glycogen solution does not lead to elution of ferment is shown by the following type of experiment.

The concentration of reducing sugars found after a 4-8 hours' contact of glycogen solution with the charcoal-amylase preparation is determined, and the mixture centrifuged. A portion of the clear supernatant liquid exhibits no increment in the amount of reducing sugars present after a further lapse of 6-8 hours (cf. Table XII).

We may hence consider the activity of adsorbed amylase to be conclusively established, as also its non-elution by glycogen solutions.

The above result is, however, only a proof that adsorbed amylase possesses enzymic activity; whether this activity is as great as that of the free ferment remains to be proved.

Thus the retardation of hydrolytic action in systems of the type amylase-charcoal-polysaccharide may be ascribed either to depression of enzyme action due to adsorption, or to diminution in the concentration of free substrate owing to its fixation on the adsorbent. With a view to the elucidation of this question, the following experiments were carried out.

Charcoal and dextrin solution were added to a solution of amylase at 0°, and after 20 minutes the mixture was filtered, and the dextrin content of the filtrate determined. Two further portions were then prepared, one of

which was identical with the above, whilst the other from which charcoal was absent contained an identical concentration of amylase but a concentration of dextrin equal to that found in the filtrate in the preliminary experiment. Both portions were placed in a thermostat and their reducing sugar content was determined after from 2 to 8 hours.

Concordant results were obtained from a number of experiments in which the velocity of hydrolysis was equal in both cases (cf. Table XIII). It hence follows that the efficiency of adsorbed amylase is quantitatively equal to that of the free enzyme.

Differences in the velocity of hydrolysis of polysaccharides by amylase, caused by the addition of charcoal to the system, can therefore be ascribed solely to the spatial separation of the enzyme from its substrate by this fixation on the adsorbent.

Table XIII.

No. of experiments	Amylase-charcoal-dextrin		Amylase-dextrin	
	Average % dextrin unadsorbed	Average glucose found mg.	Average % dextrin	Average glucose found mg.
4	0.71	31.2	0.71	32.0
4	0.53	18.8	0.53	18.9
4	0.29	6.2	0.29	6.1
4	0.12	3.7	0.12	3.6

As to the question of the applicability of the law of mass action, it follows that the velocity of enzyme reaction is, within certain limits, independent of the absolute concentration of the substrate, but is proportional to that of the unadsorbed substrate. For this reason the velocity of reaction diminishes proportionately to the amount of adsorbent added (Table XIII).

SUMMARY.

1. The effect of heterogeneity of the medium upon the course of enzymic reactions is considered in relation to reactions in the living organism and is studied experimentally upon models consisting of enzyme, adsorbent and substrate.

2. In the case of the system urease-charcoal-urea, urease is shown to be almost quantitatively adsorbed, whilst urea is adsorbed only to a very small extent. The addition of propyl or butyl alcohol produces no measurable elution of the ferment.

3. In such a system, in which the ferment, but not the substrate, is adsorbed, the velocity of enzymic reaction is practically the same as in the absence of an adsorbent, and is unaffected by the addition of narcotics.

4. In the case of the system amylase-charcoal-glycogen or -dextrin, the ferment is, like urease, practically quantitatively adsorbed, whilst the percentage adsorption of dextrin or glycogen amounts to from 50 to 75 %, depending upon the quantity of charcoal added.

5. The addition of propyl or butyl alcohol, or of other narcotics, leads to an elution of about 50 % of the substrate; at the same time no measurable elution of ferment takes place. The percentage elution of polysaccharide by alcohols is greater, the greater the percentage adsorption.

6. In this system, in which both enzyme and substrate undergo adsorption, the velocity of enzymic hydrolysis is considerably lower than in the absence of an adsorbent.

7. This diminution in reaction velocity is explained as being due to the spatial separation of enzyme and substrate by fixation on different parts of the adsorbent surface.

8. The addition of narcotics to this system accelerates the reaction by increasing the concentration of unadsorbed substrate, which alone is able to enter into reaction.

9. The law of mass action is followed in enzyme reactions in a heterogeneous medium, with the reservation that the effective concentration of the substrate be taken as that part of the latter which is in solution.

10. The non-degradation of glycogen by frog's liver during the winter months is explained on the basis of the above findings, amylase and glycogen being supposed to be fixed on different parts of the structural surface of the liver cell.

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