XXIV. STRUCTURE AND ENZYME REACTIONS.

VIII. THE PHYSICAL STATE OF THE SORBENT.

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INTRODUCTION.

PREVIOUS research on the influence of structure on enzyme reactions has shown that the sorbent may influence the course of such reactions in two ways: (1) by inactivation of the adsorbed enzyme, and (2) by the spatial separation of the substrate from the enzyme, in those cases in which both undergo adsorption [cf. Przyłeczki, 1929].

If we represent the total quantity of enzyme present in a given heterogeneous system by E , that of the adsorbed and free enzyme by E_1 and E_2 respectively, of active and inactivated enzyme by aE and iE respectively, whilst total, adsorbed, and free substrate are represented by S , s_1 and s_2 , then the quantity of enzyme-substrate compound formed will be some function of E and S in the absence of a sorbent, and of $(E_1, S) + (E_2, S)$ in its presence. In those cases in which $S = s_2$, the velocity of reaction in the presence of a sorbent will depend upon the degree of activity of E_1 and E_2 . Where $aE_1 = aE_2$, it was considered that the velocity of reaction in the presence of a sorbent not affecting the substrate would be the same as in a homogeneous system, whilst where $E_1 = iE_1$ and $E_2 = aE_2$, it would depend solely upon E_2S .

The effect of spatial separation of the enzyme from the substrate is most marked in those cases in which $aE_1 = aE_2$.

Where both enzyme and substrate are adsorbed, the velocity of reaction is the sum of the velocities of the individual reactions taking place between adsorbed and unadsorbed enzyme and substrate in the various phases of the system. The term E_1s_1 is in macroheterogeneous systems negligible, as a result of the difficulty of contact between E_1 and s_1 . This view has been confirmed by experiments in which the sorbent was present as a suspension (charcoal) or as a colloidal gel (ovalbumin), whilst experiments carried out conjointly with Dr J. Niedzwiecka [1928] on the influence of ovalbumin sols have indicated that the physical state of the sorbent influences the reaction between E_1 and s_1 .

The subject of this paper is the detailed examination of the influence exerted on the velocity of enzymic reactions by changes in the physical state of the sorbent. This problem is extremely interesting in view of the fact that

within the cell, according to the recent researches of Spek [1928], the colloids present, which may act as adsorbents, are in a state of flux.

On the whole, biologically important sorbents may be divided into two classes—dispersoid colloids, and those present at liquid-liquid interfaces, such as between drops of fats and plasma. Changes in the former class may consist in changes in the degree of dispersion, which are often associated with passage from sol to gel, or the reverse, and may result in diminution of the adsorbent surface.

The surface tension at the boundaries colloid-solvent often changes with the degree of dispersion of the colloid. As the colloidal micelles increase in size, the concentration of physically adsorbed substances increases. In particular, abrupt changes in systems in which membrane formation takes place, or in which transient surface tension changes occur, may lead to changes in the above relations E_1/E and s_1/S .

Thus, for example, if starch solution be added to a protein solution in which a foam has been formed by agitation, a considerable portion of the starch will undergo adsorption on the protein membrane-water interfaces. At the moment that the foam is broken, and the protein membranes consequently disappear, the adsorbed starch should return into solution. In our experiments this did not, however, take place. The adsorbed starch apparently undergoes some physical alteration, as a result of which, after the resolution of the foam, it separates as a precipitate which settles at the bottom of the vessel; this precipitation is absent from systems which have not been shaken.

The formation of this precipitate leads to changes in the velocity of reaction. The system contains finally s_1 , s_2 and s_0 (precipitate), an equilibrium

probably existing, and s_0 does not to any considerable extent enter into combination with E_1 or E_2 . The velocity of reaction will now consist of the following terms:

$$f_1(E_2s_2) + f_2(E_1s_1) + f_3(E_1s_2) + f_4(E_2s_1) + f_5(E_1s_0) + f_6(E_2s_0).$$

The reaction is thus retarded to an extent commensurate with the magnitude of s_0 , and relatively independent of E_1/E .

The existence of such phenomena will be demonstrated on the system shaken, ovalbumin solution-starch solution, in which kinetic experiments show that at least 85 % of s_0 does not enter into the reaction (see p. 186).

The passage of monons (primary particles) into polyons, as well as increase in the size of monons, may lead to the occlusion of originally adsorbed micelles of enzyme or substrate, the quantity of micelles thus occluded being proportional to the ratio original quantity of monons : final quantity of polyons. This phenomenon may be expressed as follows: $E_1 \rightarrow E_1^a + E_1^b$, and

$s_1 \rightarrow s_1^\alpha + s_1^\beta$, the terms designated by α and β denoting respectively free and occluded enzyme or substrate. In those systems in which only S is occluded, the velocity of reaction is represented by the sum of various functions of the following terms: $E_1 s_1^\alpha + E_1 s_1^\beta + E_1 s_2 + E_2 s_1^\alpha + E_2 s_1^\beta + E_2 s_2$. The values of the various terms containing s_1^β depend upon the distance between monons of the same micelle as well as on the dimensions of the particles of the substrate and the extent to which they are adsorbed. The effect of occlusion of the substrate upon the velocity of reaction is as a rule greater than that of occlusion of the enzyme, in view of the generally greater size of the particles of the latter, which renders their entry within the polyon difficult. An example of this is given by the system amylase-occluded glycogen-caseinogen, in which the hydrolytic action of the enzyme is completely inhibited.

If s_1^β is excluded from the reaction, the equilibrium point of reversible reactions will be altered, whilst in the case of irreversible reactions these do not practically proceed to completion, as in the above-cited cases of occluded glycogen and amylase. The occluded substrate can take part in the reaction only as a result of a change in the state of dispersion of the sorbent. Occlusion of the enzyme, however, leads in the above case only to a retardation in the velocity of reaction, but not to its complete inhibition. We may similarly conceive that the formation of membranes may be associated with the incarceration of enzyme or substrate particles in separate cells, and with consequent retardation or partial inhibition of reaction.

It is of importance that substances which normally powerfully elute substrates or enzymes from a given sorbent are unable to do so in those cases in which occlusion has taken place, and this appears to us to be possibly a very potent factor in the regulation of enzymic equilibria *in vivo*.

The occlusion and consequent inactivation of enzymes or substrates depends, apart from the distance separating the monons of a given polyon, upon factors influencing the degree of dispersion of the sorbent. Further, substances provoking elution of the adsorbate before aggregation of the sorbent to a large extent prevent subsequent occlusion.

An example of retardation of reaction due to occlusion of the enzyme alone is given by the system ovalbumin-occluded saccharase-chloroform (W. Giedroyć, unpublished work). In this case, as a result of the selective elutive effect of chloroform upon the substrate alone, the ovalbumin, on coagulation, contains only the ferment.

Diminution of the degree of dispersion of the sorbent is associated with diminution of the Brownian motion of its particles. Since, as has been shown in a previous part of this series, practically no reaction can, as a result of their spatial separation, take place between enzyme and substrate particles adsorbed on one and the same micelle, enzyme and substrate are free to interact only by the collision of different micelles on which they are adsorbed, and the possibility of such collisions is greater the greater the degree of dispersion of the sorbent, and consequently, the greater the Brownian motion of its

micelles. This was illustrated in Part II of this series, by the system ovalbumin-amylose-glycogen.

Cessation of Brownian motion may be accompanied by the arrangement in some definite order of the monomers combining to form polyions, whereby structure formation commences; this may still further retard enzyme reactions, in two ways:

(a) even stirring or shaking cannot facilitate contact of enzyme adsorbed on one micelle with substrate adsorbed on another;

(b) quite apart from sorption, the velocities of diffusion of enzyme and substrate are diminished.

In systems containing two liquid phases, such as oil-water, changes in the oil-water interface may take place with the greatest facility. This interface possesses surface energy, which is diminished by the presence of starch or glycogen, which thereby undergo adsorption on the oil surfaces. Increase in the dispersion of the oil will therefore lead to an increase in the proportion of adsorbed glycogen, with a corresponding diminution in its concentration in solution. The reverse does not, however, apply. Coagulation of the drops of oil with a consequent decrease in free surface leads not to a corresponding increase in concentration of dissolved polysaccharide, but to the precipitation of the latter.

The above considerations show that retardation of reaction may be due not only to inactivation of adsorbed enzyme and to spatial separation of simultaneously adsorbed enzyme and substrate; a number of other possibilities may exist in which retardation is due to the physical state of the substrate, whilst the enzyme may remain active and unadsorbed. Thus both the precipitation of the substrate and its occlusion may reduce the velocity of reaction, as well as affect its equilibrium point. These are particular cases of the effect of the state of the substrate on enzyme reactions; this problem will further be considered in the subsequent papers of this series.

EXPERIMENTAL.

All experiments were comparative. Sørensen's phosphate buffers were used to ensure uniform p_H , which was 6.2 in the systems containing oil or protein and 6.6 in those with gelatin. Adsorption was measured at room temperature immediately after shaking, whilst precipitation was examined 24 hrs. after commencement of shaking. The kinetics of all systems was examined either in a thermostat at $37^\circ \pm 0.5^\circ$, or at $25^\circ \pm 1^\circ$. In all cases protein was removed before determination of glucose.

I. THE SYSTEM OIL-STARCH-AMYLASE.

Adsorption of starch. 30 cc. of 0.5 and 1 % solutions of starch are shaken for 2 hrs. with, in some cases, 5 cc. of olive oil, in 100 cc. Erlenmeyer flasks, the contents of which are then filtered; 10 cc. of the filtrate are hydrolysed at 100° for 4 hrs. with 1.5 cc. of concentrated hydrochloric acid, and reducing sugars

determined by Bertrand's method. The results, given in Table I, show that whilst in the absence of oil, 10 cc. of 1 and 0.5 % starch solutions yield respectively 92.5 and 52 mg. of sugar, in its presence only 68.2 and 34.2 mg. are obtained. It follows that 26.3 % of the starch present in 1 %, and 34.2 % of that in 0.5 % solutions have undergone concentration on the oil surfaces; increasing the time of shaking from 2 to 4 hrs. does not appear to affect the values obtained. At the same time, the emulsion is stabilised by the starch, in the absence of which separation of the phases takes place in 30–60 mins., whilst in its presence the emulsion lasts over 24 hrs. As the globules of oil coalesce, the greater part of the adsorbed starch is precipitated, a certain amount remaining, however, in adsorption. The addition of propyl alcohol to 1.4 % or of butyl alcohol to 0.71 % leads to a considerable diminution in the degree of adsorption of starch, which now amounts to 11.8 and 14.7 % respectively (see Table I).

Table I. *Adsorption and elution of starch on oil.*

Mg. glucose in 10 cc. of filtrate, using 30 cc. of 0.5 and 1 % starch, and 5 g. of oil.
Each result is the mean of four determinations.

		Oil							
		No alcohol				With propyl alcohol		With butyl alcohol	
Starch %	No oil	2 hrs. shaking	% sorption	4 hrs. shaking	% sorption	% sorption		% sorption	
0.5	52.0	34.2	34.2	34.3	34.0	45.9	11.8	44.4	14.7
	(51.0–53.0)	(32.6–36.2)		(32.4–36.5)		(41.4–47.1)		(43.3–47.8)	
1.0	92.5	68.2	26.2	—	—	—	—	—	—
	(91.5–93.5)	(66.1–70.4)							

Adsorption of amylase. Parallel experiments to the above were carried out using 0.5 % amylase (Merck's) in 1 % sodium chloride solutions in place of starch. To 20 cc. of the filtrates after shaking, 20 cc. portions of 1 % starch solution were added and the flasks left at 37° for 4 hrs. after which reducing sugars were determined as before. The results, given in Table II, show that in the presence of oil 20 cc. of filtrate produced 67.5 mg. of sugar, and in its absence 71 mg., a difference of 4.9 %. It follows that amylase is not to any great extent concentrated on the oil-water interface.

Table II. *Sorption of amylase on oil.*

Mg. glucose in 40 cc. solution, containing 20 cc. of 1 % starch, after 4 hrs. at 37°.

No. of expts.	Without oil	With oil	% sorption
4	71.0	67.5	4.9
	(70.0–72.5)	(65.5–69.3)	

Kinetics of the system oil-water-amylase.

(a) *Emulsion.* A number of flasks containing 30 cc. of 0.5 % starch solution and 10 cc. of 1.5 % amylase solution were shaken for 5 hrs. at 25°, and the reducing sugar content of the filtrates compared with that of a similarly

treated series of flasks to which 5 cc. of olive oil had been added. The results, given in Table III, show that in the first case 47.9 mg. of sugar were found in 10 cc. of the filtrate, whilst in the presence of oil 45.4 mg. were found, a difference of 5.2 %.

Table III. *Influence of oil on kinetics of hydrolysis of starch by amylase.*

No. of exps.	Mg. glucose in 20 cc. of filtrate, using 0.5 % starch solution.					
	No oil		Oil			
	With shaking	Without shaking	Amylase added before shaking	% inhi- bition	Amylase added after shaking	% inhi- bition
4	47.9	48.0	45.4	5.2	37.8	20.1
	(47.2-48.5)	(47.0-49.1)	(45.2-46.6)		(37.6-38.9)	

This inhibition may be due either to simultaneous adsorption of substrate and enzyme or to the inactivation of the latter. In the first case, substitution in the equation $ES = E_1s_1 + E_1s_2 + E_2s_1 + E_2s_2$ gives

$$0.05 \times 0.34 + 0.05 \times 0.66 + 0.95 \times 0.34 + 0.95 \times 0.66 = 1.7 \% ES,$$

i.e. the maximal retardation of reaction due to simultaneous adsorption is 1.7 %; in reality the value obtained would be even lower in view of the fact that the system is shaken, whereby the possibility of contact occurring between substrate and enzyme particles in adsorption on different globules of oil is increased. The observed inhibition of reaction must therefore be due to inactivation of adsorbed enzyme. It is in this connection of interest that Truszkowski [1928] found that amylase adsorbed on lipid surfaces is thereby inactivated, and, as this is the first case published of inactivation due to physical adsorption, it is our intention further to investigate this phenomenon.

(b) *The precipitate.* The above experiments were repeated, with the difference that amylase was added 24 hrs. later, when a considerable portion of the starch had been precipitated. The results, given in Table III, show that in the absence of oil 47.3 mg. of sugar were formed, and in its presence 37.8 mg., a difference of 20.2 %. This retardation is due preponderantly to precipitation and consequent exclusion from reaction of starch as s_0 . Since about 30 % of the starch is, in emulsions, present at the interface, it follows that only 67.7 % of the adsorbed substrate undergoes precipitation.

II. THE SYSTEM OVALBUMIN-AMYLASE-STARCH.

Adsorption and elution of starch. To one series of flasks, containing 30 cc. of 0.5 % starch, 5 cc. of 8 % Merck's ovalbumin solution are added, and to another 5 cc. of water; all flasks are then shaken for 2 hrs., their contents filtered, and the reducing sugar content of the filtrates determined after hydrolysis with hydrochloric acid, as before. The results, given in Table IV, show that, in the presence of ovalbumin, 62.7 % less starch is present in the filtrates than in its absence. Substantially the same results are obtained if the flasks are shaken 4 hrs. instead of 2. This phenomenon appears to be due to the adsorption of starch on the protein membranes present in the foam formed by shaking, and these membranes are retained by the filter-

paper. Starch adsorbed on dissolved protein passes together with the latter through the filter-paper, so that determinations made on the filtrates apply to both free starch and to that adsorbed on the colloidal sol.

The addition of alcohols in the same concentrations as those used with the corresponding system containing olive oil similarly diminishes the amount of adsorption. Thus, as is shown in Table IV, the proportion of adsorbed starch is diminished by 67.5 % by propyl alcohol and 27.0 % by butyl alcohol.

If the system is left for 24 hrs. after shaking, a precipitate of starch forms, as in the presence of oil, and the amount of this precipitate is considerably smaller in the presence of alcohols. No precipitate forms if the system is not shaken.

Table IV. *Sorption and elution of starch in shaken ovalbumin solutions.*

Mg. glucose after hydrolysis of filtrate of the system 30 cc. 0.5 % starch + 5 cc. 4 % ovalbumin.

No. of exps.	Ovalbumin							
	No ovalbumin	Shaken 2 hrs.				Shaken 4 hrs.		
		No alcohol	% sorption	Propyl alcohol	% sorption	Butyl alcohol	% sorption	No alcohol
4	45.2 (44.9-45.5)	14.7 (14.4-16.0)	67.5	37.3 (35.1-38.6)	17.5	33.0 (31.9-35.2)	27.0	12.5 (12.2-13.5)
								72.2

Adsorption of amylase. To one series of flasks containing 20 cc. of 1 % amylase 5 cc. of 10 % ovalbumin solution are added, and to another series 5 cc. of water. All are then shaken for 2 hrs., filtered, and to 10 cc. of each filtrate 20 cc. of 0.5 % starch solution are added, and the amylase allowed to act for 4 hrs. at 37°. The results given in Table V show that about 29.6 % of the amylase present is adsorbed on the protein membranes.

Table V. *Sorption of amylase in shaken ovalbumin sols.*

Mg. glucose 4 hrs. after addition of 10 cc. of filtrate from 20 cc. of 1 % amylase solution and 5 cc. of water or of 10 % ovalbumin to 20 cc. of 0.5 % starch solution.

No. of exps.	Without ovalbumin	With ovalbumin	% sorption
4	42.2 (40.8-43.4)	29.7 (27.0-32.4)	29.6

Kinetics.

(a) *In constantly shaken systems.* Assuming that on the average 67 % of the starch and 29.6 % of the amylase are in adsorption on the protein membranes present in the foam, then, even neglecting possible retardation of reaction due to simultaneous adsorption of enzyme and substrate on the particles of the sol, $E_1s_1 = 19.8\%$ *ES*, i.e. the retardation of reaction should amount to about 20 %. A comparison of the reducing sugar contents of the filtrates of flasks shaken for 2 hrs. at 25°, and containing 30 cc. of 0.5 % starch solution and 10 cc. of 1 % amylase solution, with the addition of 5 cc. of 10 % ovalbumin solution or of water, indicates (see Table VI) that hydrolysis

is barely 3.5 % less in the presence of ovalbumin. It follows that retardation due to the term E_1s_1 is only 17.5 % of the theoretical maximum. In this case, therefore, the presence of a sorbent of both enzyme and substrate does not involve the absence of reaction between E_1 and s_1 .

(b) *After cessation of shaking.* The preceding experiment was repeated, with the difference that shaking took place at 5°, after which the flasks were left for 2 hrs. at 25°, when the reducing sugar content of the filtrates was determined. These modifications should not lead to any great changes in the ratios E_1/E and s_1/S , yet, as is shown in Table VI, the percentage inhibition is now 12.7 %, as compared with 3.5 % in the preceding experiment, i.e. the reaction between E_1 and s_1 is inhibited to the extent of 60.3 %. In no case was precipitation of starch observed. The retardation of reaction should therefore be ascribed to the absence of agitation, which in the previous experiment facilitated contact of enzyme and substrate particles adsorbed on different micelles.

Table VI. *Influence of ovalbumin on kinetics of hydrolysis of starch.*

Mg. glucose in 20 cc. of filtrates of systems containing 30 cc. of 0.5 % starch, 10 cc. of 1 % amylase, and 5 cc. of water or of 10 % ovalbumin solution.

No. of exps.	No ovalbumin	Ovalbumin					
		Shaken 2 hrs.	% inhi- bition	Shaken 2 hrs. at 5°, then left 2 hrs. at 25°	% inhi- bition	Shaken 2 hrs., then left 24 hrs., amylase added, and left 2 hrs. at 25°	% inhi- bition
4	43.3 (42.8-43.6)	41.8 (41.6-42.1)	3.5	37.8 (35.7-38.3)	12.7	21.6 (20.4-22.1)	50.0

(c) *24 hrs. after cessation of shaking.* Exp. a is repeated with the difference that amylase is added 24 hrs. after cessation of shaking, i.e. after the formation of a precipitate of starch. We see from Table VI that the reaction is now inhibited to an extent of 50-54 %. Since 55-60 % of the starch has been precipitated, 10 % of this must have entered into reaction.

Table VII. *Kinetics of enzymic hydrolysis in the presence of ovalbumin at different degrees of coagulation.*

Duration of heating of ovalbumin secs.	Mg. glucose	% inhibition
0	39.0 (37.9-40.1)	0
15	37.8 (35.6-39.2)	3.1
60	34.0 (32.8-35.0)	12.9
90	32.2 (30.7-33.1)	17.4
300	30.9 (30.1-32.0)	20.7

(d) *The same system containing ovalbumin in various stages of coagulation.* A number of flasks containing 20 cc. of 1 % ovalbumin solution were heated with constant shaking for 0, 15, 30, 60 and 90 secs., and 5 mins. in a water-bath at 100°. The flasks were then cooled and to each 15 cc. of 1 % starch solution

and 10 cc. of 1 % amylase solution were added. The flasks were then shaken for 2 hrs. and left at 25° for 3 hrs., after which the volume of solution was in all cases made up to 50 cc., and the reducing sugar content determined. The results, given in Table VII, indicate that inhibition of reaction between E_1 and s_1 rises from 0 in the presence of unheated protein to 100 % where the latter had been heated 5 mins.

III. THE SYSTEM CASEINOGEN-GLYCOGEN-AMYLASE.

10 cc. of 2 % glycogen were added to 100 cc. of milk, the whole was shaken for 2 hrs., after which hydrochloric or acetic acid was added to p_H 3.0–4.2, and the solution left for 6 hrs. for precipitation of caseinogen, after which N sodium hydroxide solution was added to p_H 5.0–5.2. A second series of flasks was submitted to the same operations, with the difference that the 10 cc. of glycogen solution were added after alkalisation. All flasks were now shaken for 2 hrs., after which 25 cc. of 1 % malt amylase solution were added, and the flasks left at 25°. After 7, 30 and 90 days glycogen was determined in the whole solution, in the filtrate and in the residues.

The results, given in Table VIII, show that not even a trace of glycogen was present in any of the filtrates, and glycogen was also absent from the residues of those flasks to which it had been added after precipitation of caseinogen, as a result of the reaction $s_1 \rightarrow s_2$, and of the interaction of s_1 with E_2 and of s_2 with E .

Table VIII. *Inhibition of glycogenolysis due to occlusion.*

Each result is the mean of four experiments.

Mg. glycogen found in the systems

Duration of exp. days	Curdled milk to which glycogen and amylase were added			Glycogen and amylase added to milk which is then curdled								
	No alcohol		2 % Butyl alcohol ppt.	Without alcohol			Alcohol added after curdling of milk			Alcohol added before curdling of milk		
	Whole system	Ppt.		Whole system	Ppt.	Filtrate	Whole system	Ppt.	Filtrate	Whole system	Ppt.	Filtrate
0	200.0	176.8	69.4	202.1	179.6	23.0	201.7	174.0	23.2	201.0	84.6	118.2
7	39.7	36.1	23.8	143.5	141.0	3.0	140.9	138.7	0	70.7	63.7	0.7
30	0	0	0	138.1	137.4	0	137.0	136.8	0	62.2	61.2	0
60	0	0	0	137.2	137.1	0	136.8	136.3	0	62.4	60.2	0

In those flasks to which glycogen had been added before precipitation, on the other hand, it was present in the residue in all cases, and to an equal extent after 90 days as after 30 days.

In one experiment, for example, 89.4 % of the glycogen present was adsorbed on the caseinogen. After the action of amylase, 68 % of the glycogen remained unhydrolysed, whence it follows that 76.3 % of the adsorbed glycogen was protected from hydrolysis by occlusion.

Action of alcohols on the above system. Repetition of the above experiments, with the addition of propyl alcohol to 2 % before addition of amylase, shows

that where glycogen is added after precipitation of caseinogen considerable elution takes place, whilst this is almost entirely absent from those systems to which glycogen had been added before precipitation, and similarly, the velocity of hydrolysis of glycogen is accelerated in the former case by the addition of propyl alcohol but not at all in the latter case.

Entirely different results are obtained if the propyl alcohol be added at the same time as the glycogen. In this case, the proportion of occluded glycogen is greatly diminished, so that after 60 days we find 62.4 mg. per 100 cc. instead of 137.1 mg. as in the preceding experiment.

IV. THE SYSTEM GELATIN-AMYLASE-STARCH.

To a number of flasks containing 10 cc. of 0, 0.9, 2.1, and 3.9 % gelatin, 10 cc. of 0.6 % starch and 10 cc. of 0.9 % amylase were added, and the flasks left for 5 hrs. at 37° and at 25°, after which reducing sugar was determined. The results, given in Table IX, show that 0.3 % gelatin retards reaction by 9.3 % at 37° and by 9.9 % at 24°, 0.7 % gelatin by 17.5 % at 37° and by 21.2 % at 24°, whilst 1.3 % gelatin produces a retardation of 41 % at 37° and of 56.7 % at 24°. The inhibitive action of gelatin thus increases with concentration and with temperature, the relative effect of the latter factor being greater at higher than at lower concentrations of gelatin. The percentage inhibition due to a temperature difference of 13° is 6.5 % with 0.3 % gelatin, 21 % with 0.7 % gelatin, and 38 % with 1.3 % gelatin.

Table IX. *Kinetics of enzymic hydrolysis of starch in presence of gelatin.*

No. of exps.	Conc. of gelatin %	Mg. glucose in 20 cc. of solution after 2 hrs.				Percentage excess of inhibition at 25° over that at 37°
		at 37°	% inhibition	at 25°	% inhibition	
4	0	37.8 (37.6-38.0)	0	28.3 (28.0-28.7)	0	—
4	0.3	34.3 (34.0-34.6)	9.3	25.5 (25.2-25.8)	9.9	6.5
4	0.7	31.2 (30.9-31.5)	17.5	22.3 (22.0-22.6)	21.2	21.5
4	1.3	22.3 (22.0-22.6)	41.0	12.3 (12.1-12.7)	56.7	38.0

SUMMARY.

1. An examination of the system oil-starch-amylase yielded the following results.

Starch undergoes adsorption at the water-oil interface to an extent of 26.3 % from 1 %, and 34.2 % from 0.5 % solutions, in the presence of 5 g. of oil.

The presence of 1.4 % propyl alcohol, or of 0.71 % butyl alcohol, reduces adsorption to about one-half.

About 68 % of the adsorbed starch is deposited as a precipitate from emulsions of oil in starch solution on the resolution of the emulsion.

The adsorption of amylase on oil is very small, not exceeding 5 % under the experimental conditions.

The adsorbed amylase appears thereby to be inactivated and the precipitated starch does not react with amylase.

2. An examination of the system soluble ovalbumin-starch-amylase leads to the following conclusions.

Starch undergoes adsorption to about 63 % on the protein membranes present in the foam formed when ovalbumin solutions are shaken.

The addition of alcohols as in (1) leads to elution of starch.

A portion of the adsorbed starch is deposited as a precipitate on the resolution of the foam.

Amylase is adsorbed on the protein membranes to an extent of about 30 % under the experimental conditions.

In systems containing ovalbumin, starch, and amylase, the velocity of hydrolysis is only 3.5 % less than in the absence of protein if the system is constantly shaken; without shaking, retardation amounts to 12.7 %, whilst when amylase is added 24 hrs. after shaking it amounts to 52 %.

The degree of inhibition due to ovalbumin is proportional to the extent to which it is coagulated.

3. The system caseinogen-glycogen-amylase has been studied, with the following results.

When caseinogen is coagulated in the presence of glycogen, the latter is occluded within the precipitate, and is thereby protected from the action of amylase in the medium, only 24 % of the adsorbed glycogen being hydrolysed.

The occluded glycogen is not eluted by alcohols.

4. A study of the system gelatin-starch-amylase shows that the inhibitive effect on the hydrolysis of starch due to the presence of gelatin increases with concentration of the latter, and is greater at 25° than at 37°.

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