CIII. STRUCTURE AND ENZYME REACTIONS. PARTS V AND VI.

THE SYSTEMS GLUCOSE-ENZYME AND ESTER-CATALYST.

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IN the introduction to Part I of this series [Przylecki *et al.* 1927] it was shown that the introduction of an adsorbent into certain enzyme-substrate systems diminishes the velocity of reaction in those cases where both enzyme and substrate undergo adsorption. As was shown in Part III [Przylecki and Niedzwiecka, 1928] retardation of reaction may also be observed in systems where the presence of an adsorbent leads to a reduction in the number of collisions taking place in unit time between enzyme and substrate particles. Similarly, it might be conceived that insufficient stirring or agitation of the suspension of adsorbent on which either the enzyme or the substrate is adsorbed could reduce the velocity of reaction. As was shown in Part I, this phenomenon is not of general occurrence and depends upon the velocity of reaction and rate of diffusion for each particular case; where the latter factor is negligible in comparison to the former, no retardation should be observed.

The previous papers of this series deal with practically irreversible reactions, whose velocity is proportional to the concentrations of enzyme and substrate, whilst the products of reaction are adsorbed to a considerably less extent than the substrate. The latter factor, while of no great importance in the case of irreversible reactions, may become of importance in those cases where only the products of reaction are adsorbed. In such cases, the presence of an adsorbent may lead to an acceleration in the velocity of the later stages of an enzyme reaction, since the velocity constant may be expressed

$$K = \frac{[\text{enzyme-reaction products}]}{[\text{enzyme}] [\text{reaction products}]}$$

Thus a part of the products of reaction is separated by adsorption from chemical combination with the enzyme, leaving the latter free to combine with the substrate.

An adsorbent might therefore be chosen such that it not only does not retard, but even accelerates, irreversible reactions, and this acceleration will be greater the greater the value of K , *i.e.* the greater is the concentration of the products of degradation and the smaller that of the initial substance.

In Part I the possibility was raised of the acceleration of reversible reactions and of the shifting of the equilibrium point under the influence of an added adsorbent. This might occur in those cases where either the products of synthesis or of degradation are differentially adsorbed. The following conclusion, based on the law of mass action, might hence be drawn, only those cases being considered in which the adsorption of the substrates is reversible, and in which the adsorbent does not enter into chemical reaction with the substrates.

The kinetics of enzyme reactions would here depend upon two factors—the shifting of the point of equilibrium and the degree of adsorption. Where the degrees of adsorption of substrates and products are similar, both when the products of synthesis or of degradation are present in excess, the velocity of reaction diminishes, as a result of the removal by adsorption of the substrates from the sphere of action of the enzyme. If, however, the products of synthesis are adsorbed to a greater extent than the substrates the reaction will depend kinetically upon its relation at any given moment to the definitive equilibrium point, *i.e.* upon the quotient

$$K = \frac{[\text{products of degradation}]}{[\text{products of synthesis}]}.$$

Where the products of synthesis are present in excess, so that the reaction is proceeding in the direction of degradation, the addition of an adsorbent should retard the reaction, as in the case of the system amylase-charcoal-polysaccharide (Part II). Should, however, the reaction of synthesis be dominant several possibilities might arise.

If the substrates of the reaction of synthesis do not undergo adsorption, whilst at the same time the adsorbed enzyme retains its activity, the reaction should be accelerated almost from the commencement, since the products of synthesis are being continually removed by adsorption from the reaction mixture. The difference between reaction in a homogeneous and a heterogeneous system will be greater the closer the former system is to its equilibrium point, and the quantity of synthesised product present at any one moment in the former system after the attainment of equilibrium will be smaller than in the latter. In such a case, the value of K for the system as a whole diminishes with increasing adsorption of the synthesised products, although in the disperse phase alone it will not have undergone any change, except that the concentrations of all the constituents will be smaller.

In those systems, however, in which both the substrates and synthesised bodies undergo adsorption, but the latter to a greater extent than the former, conditions are more involved. Whilst, in the above case, the velocity of the reaction of synthesis is almost from the beginning greater in the presence than in the absence of an adsorbent, in the present case two stages of the reaction may be distinguished. In the first stage, the concentration of substrates in solution is, as a result of their adsorption, smaller than in a homogeneous system, whilst the adsorption of products of synthesis is too small noticeably

to affect the velocity of reaction. As a result, the addition of an adsorbent to a system at this stage may retard the reaction of synthesis. The second stage supervenes when the reaction is approaching equilibrium; here adsorption of the substrates is insignificant, but that of the synthesised products is so large as to cause acceleration of the reaction. Graphically, we would here obtain two curves—one rising steeply but soon becoming flatter, and the second at first less steep, but retaining its initial slope longer, so that eventually it cuts the former curve and rises above it. This reasoning applies, of course, only to reactions for which the velocity of reaction is a function of the concentration of the substrates. In a system in which the latter are present in excess, or are very feebly adsorbed, the adsorption of a part of them need have no perceptible effect upon the velocity of reaction in its initial stages. The kinetics of such a reaction would be unaffected by the addition of an adsorbent.

In the case of certain biological syntheses, the application of the above illustrations may be exceedingly involved. Thus, for such heterogeneous reactions as the synthesis of glycogen where the eventual product of synthesis undergoes adsorption, and the products of the various stages of synthesis are located in different, spatially separated parts of the cell, the velocity of reaction would be diminished by the presence of an adsorbent. This, however, need not apply to a reaction of synthesis catalysed by a number of enzymes, in which the whole process takes place in one part of the cell, whilst the intermediate products are relatively not adsorbed to any great extent. Whether the synthesis of polysaccharides takes place under such conditions it is not yet possible to decide, as our knowledge of this process is insufficient. The biological synthesis of fats is, however, in all probability of this type. Proteins are possibly synthesised in a system of the first type, in which the reaction proceeds in several stages, spatially separated from each other. Little consideration has, however, up to the present been given to this problem.

A third general possibility is of the adsorption of the products of degradation being greater than that of the substrates. In such cases, in which the synthesised body does not undergo adsorption and is present in excess, the addition of an adsorbent should accelerate the reaction of degradation almost from the beginning, whilst, where the product of synthesis undergoes adsorption, parallel conditions obtain to those found in certain systems of the second type, *i.e.* the velocity of degradation would be retarded in its initial and accelerated in its final stages. In systems of this type, the velocity of synthesis should be diminished by the addition of an adsorbent, which would lower the concentration of substrates.

Thus the addition of an adsorbent to systems of the type enzyme-products of synthesis-products of degradation, in which the enzyme acts practically reversibly, may cause the following changes.

1. *In the equilibrium point.* K remains unaltered in the disperse phase, but in the system as a whole, including those of its constituents present in the

adsorbed condition, it is either changed in favour of the reaction of synthesis, should the products of the latter be more strongly adsorbed than the substrates, or in favour of the reaction of degradation, should the contrary be the case.

2. *In the velocity of reaction.* This may remain unaltered, be retarded or be accelerated.

The influence of the adsorbent on these two factors, K and velocity of reaction, has been expressed mathematically by Sym, as follows.

If a be the concentration of the reacting substance in the absence of an adsorbent, then after the addition of the latter the concentrations in solution and on the adsorbent will be together $a_1 + \frac{a}{m} a_1^{1/n}$, whilst at some time during the course of the reaction, they will be

$$a_1 - x + \alpha/m (a_1 - x)^{1/n} + x_1 + \beta/m \cdot x'^{1/p},$$

and the quantity of substance y which has entered into reaction would be given by the equations

$$y = x' + \beta/m x'^{1/p} = x + \alpha/m [a_1'^{1/n} - (a_1 - x)^{1/n}].$$

The velocities of reaction and the equilibrium constants would then be as follows, for systems in which adsorption is reversible and mutually independent for each constituent.

1. In the absence of an adsorbent,

$$\frac{dy}{dt} = k_1 (a - y) - k_2 y, \text{ and } K_1 = \frac{a - y}{y_1}.$$

2. Adsorption of the reacting substance,

$$\frac{dy}{dt} = k_1 (a_1 - x) - k_2 y, \text{ and } K_1 = \frac{a - x}{y}.$$

$$K_2 = \frac{(a_1 - x_1) + \alpha/m (a_1 - x_1)^{1/n}}{y_1}.$$

3. Adsorption of product of synthesis,

$$\frac{dy}{dt} = k_1 (a - y) - k_2 x', \text{ and } K_1 = \frac{a - y_1}{x_1},$$

$$K_3 = \frac{a - y_1}{x_1' + \beta/m \cdot x_1'^{1/p}}.$$

4. Adsorption of both substances,

$$\frac{dy}{dt} = k_1 (a_1 - x) - k_2 x', \text{ and } K_1 = \frac{a_1 - x_1}{x_1'},$$

$$K_4 = \frac{a_1 - x_1 + \alpha/m (a_1 - x_1)^{1/n}}{x_1' + \beta/m \cdot x_1'^{1/p}}.$$

In view of the great number of enzymic reactions which take place in the living organism, often reversible, and whose substrates, products or enzymes are adsorbed by substances found within the cell, the above theoretical reasoning calls for experimental verification. A number of researches with this end in view are now being conducted at this laboratory; in this paper the results obtained for two systems will be described. These are the systems

enzyme-glucose-charcoal and hydrochloric acid-aliphatic acid-alcohol and ester-charcoal.

In the first system we have the reaction:

The reaction attains a definite equilibrium point the value of which, in systems without charcoal, depends upon the initial concentration of glucose. This system can be used for the demonstration of the postulate that structure may influence reaction by changing the value of K and so augmenting synthesis, since the hexobiose formed is considerably more adsorbed than glucose, in solutions containing over 20 % of the latter and from 1 to 2 % of maltose.

The reaction, acid + alcohol $\rightleftharpoons$ ester + water, proceeds under the catalytic influence of hydrochloric acid in a medium containing excess of alcohol. The addition to this system of preparations of charcoal, which adsorbs the organic acid to a considerable extent, but not the ester, brings about the acceleration of the reaction of hydrolysis.

THE SYSTEMS CHARCOAL-ENZYME-GLUCOSE AND ENZYME-GLUCOSE.

The work of Hill [1898], Emmerling [1901], Fischer and Armstrong [1902] and Bourquelot *et al.* [1913] has established that if an appropriate enzyme be added to a concentrated glucose solution, a reaction of synthesis of disaccharide proceeds to a certain equilibrium point.

This system has been selected as suitable for the experimental establishment of the hypothesis that the equilibrium point of reversible reactions may be shifted in favour of synthesis by the addition of a substance on which the products of synthesis are adsorbed to a greater extent than are the substrates.

A fundamental condition for this is the almost quantitative adsorption of the enzyme. Whilst from theoretical considerations it follows that the ratio of glucose to maltose in solution would be changed in favour of the former, a consideration of adsorbed glucose and maltose should indicate augmented synthesis of the latter.

A number of preliminary experiments had first to be undertaken:

(1) with a view to establishing that glucose and maltose are adsorbed by charcoal in concentrations approaching those which are obtained in the enzymic synthesis of maltose;

(2) for the analysis of these two sugars in adsorption on charcoal;

(3) in order to carry out a reaction lasting several months under sterile conditions, without the use of antiseptics, which would affect the relative adsorption of the sugars, and diminish their absolute adsorption;

(4) in order to elaborate a method for the quantitative determination of maltose in the presence of a large excess of glucose.

The researches of Bourquelot, of Emmerling and of Hill were of great assistance in the execution of our research. From the papers of these authors were taken the concentration of glucose used, the time of reaction, and the selection of the enzyme, as well as the method for the estimation of small quantities of disaccharide in the presence of a large excess of glucose.

A. *The equilibrium of the system enzyme-glucose.*

Our first experiments had as their objects the establishment of the necessary technique for working over long periods of time without the use of disinfectants; further, the achievement of conditions relatively close to those of final equilibrium in the absence of charcoal, and the determination of the degrees of adsorption and of elution, together with the conditions under which the latter takes place.

(a) *Sterilisation.* The elutive effect of ordinary antiseptics militated against their use in these experiments, and the following technique was therefore used for the maintenance of aseptic conditions. The glucose solutions, with or without charcoal, were sterilised by heating in an autoclave for 30 minutes in 250 cc. Erlenmeyer flasks stoppered with cotton-wool. The enzyme solutions were sterilised either by passage through a Chamberland filter or by the addition of powerful antiseptics such as ethyl alcohol, which were then removed under diminished pressure at 20°. The sterilised enzyme solutions were in some cases measured out from sterilised graduated cylinders and then added to the sterilised glucose solutions. In others, the flask containing the glucose solution was tightly stoppered with cotton-wool, through which passed two tubes, one connected to a filter pump and the other to a graduated vessel containing sterilised enzyme solution. The diminution in volume of the contents of the latter vessel represented the volume of enzyme solution added to the glucose solution. In many cases, as an additional precaution against infection, methyl alcohol was added. This is a fairly strong antiseptic, whilst at the same time its elutive power is inconsiderable.

The above measures enabled us to maintain sterile conditions for over 6–7 months. Before each determination, the solutions were tested for sterility, and only those from which micro-organisms were absent were taken for analysis.

(b) *Choice of enzyme.* Emmerling [1901] showed that isomaltose and dextrin are produced in concentrated solutions of glucose in the presence of yeast maltase, whilst Bourquelot *et al.* [1913] used several enzymes such as emulsin for the synthesis of disaccharides. In this research, yeast maltase prepared according to Emmerling was used.

(c) *Determination of synthesised disaccharide.* The method described by Emmerling was applied, with slight modifications. Glucose was completely decomposed by fermentation, and the remaining disaccharide hydrolysed by hydrochloric acid, the glucose thus formed being then determined by Bertrand's method. The fermentation was carried out by the agency of *Saccharomyces marxianus*, *S. exiguus* or *Willia anomala*.

(d) *Establishment of equilibrium.* Two points had here to be proved; firstly that the velocity of synthesis is almost unaffected by the addition of charcoal at the initial stages of the reaction, and secondly that the high degree of adsorption of maltose on charcoal shifts the equilibrium point of the system as a whole in the direction of synthesis. For the establishment of these facts absolute equilibrium was not essential, conditions close to this state being sufficient. Since solutions from which samples had been taken for analysis could not be used further, and as the entire contents of a flask had to be used for the determination of the degree of elution, the attainment of conditions close to equilibrium had to be judged by the determination in different flasks of the ratio of glucose to maltose at fortnightly intervals beginning a month after the commencement of the experiment. In this way, it was found that in the absence of charcoal initially 20 % glucose solutions contained at equilibrium 1.48 % of disaccharide (see Table I).

Table I. *Synthesis of hexobiose.*

Initial concentration of glucose (%)	Hexobiose found after 5 months (%)
20	1.29, 1.58, 1.71, 1.34
	Mean 1.48

B. *Adsorption of glucose and disaccharide on charcoal.*

Having determined equilibrium conditions in the absence of charcoal the system enzyme-20 % glucose could now be studied in its presence.

In order to obtain measurable differences in the equilibrium point of the reaction it was necessary to create conditions such that the quantity of adsorbed disaccharide was as great as practicable. This was done by introducing large quantities of adsorbent, the components of the systems examined being present in the following quantities: 5 g. of charcoal, 50–100 cc. of a solution containing 10–20 % of glucose and 1–2 % of maltose. The charcoal preparations used were either of local manufacture or Merck's "Carbo medicinalis." The mixtures were placed in a mechanical shaker for 4–6 hours, after which they were left in a thermostat at 37° for 12 hours, and then filtered. The results obtained are given in Tables II, III and IV. The values found show that barely 7.2 % of glucose is adsorbed on local charcoal from a 16.6 % solution, and 8.1 % from a 9.0 % solution. Maltose is adsorbed far more intensely, to the extent of 63.5 % from a 0.86 % solution, and 43.2 % from a 1.4 % solution. Considerably higher degrees of adsorption were observed for Carbo medicinalis, being 12 % from a 20 % solution of glucose and 97 % from a 2 % solution of maltose.

From the point of view of our experiments, it was of great importance to establish the degrees of adsorption of glucose and maltose in systems containing originally only the former sugar, to which maltose solution is added later. Such conditions would correspond to those obtaining during the enzymic synthesis of hexobiose in the presence of an adsorbent.

Table II. *Adsorption and elution of maltose and glucose.*

	Without charcoal	With charcoal			
		Before elution		After elution	
		In solution	% adsorption	In solution	% adsorption
Glucose solution	9.0	8.29	8.1	9.0	0
Maltose solution	0.86	0.31	63.5	0.70	18.6

Table III. *Adsorption of glucose and maltose separately and together.*

Percentage concentrations.

Solution	Without charcoal	With charcoal	% adsorption
Glucose	16.6	15.4	7.2
Maltose	1.4	—	43.2
Maltose 1.4 %, with glucose 16.6 %	—	—	33.7

90 cc. of 22 % glucose were added to 5 g. of charcoal, the whole was shaken 3 hours and left overnight. 10 cc. of 2 % maltose solution were then added drop by drop and with constant shaking, an interval of one hour being allowed after the addition of each 2 cc. The results obtained are given in Tables III and IV.

Table IV. *Adsorption and elution of glucose and maltose together and separately.*

Percentage concentrations.

Solution	Without charcoal	With charcoal			
		Before elution		After elution	
		% in. solution	% adsorption	% in. solution	% adsorption
Glucose	20.11	17.7	12.0	19.3	4.0
Maltose	1.97	0.058	97.0	0.97	50.0
Maltose and glucose together		0.674	65.8	1.50	24

These values are based upon analyses and calculations of the concentrations of solutions of maltose with glucose with and without charcoal. Maltose was determined after the fermentative destruction of glucose by hydrolysis in the presence of hydrochloric acid, the glucose thus formed being then determined by Bertrand's method. The amount of adsorption of glucose was determined polarimetrically and was calculated from the difference between the optical rotations with and without adsorbent of the solution of maltose and glucose less that of the maltose alone. Comparative values are given only for the adsorption of maltose, which alone concerns us in the study of equilibrium conditions.

The adsorption of maltose in the presence of 16.6 % glucose on charcoal preparation No. 1 (Table III) is considerably smaller than in its absence. Thus adsorption is here diminished by 22.2 %, from 43.2 to 33.7 %. The

second charcoal preparation which normally adsorbs 97 % of maltose from solution exhibits an adsorption of maltose of only 66 % in the presence of 20 % glucose, a diminution of 31 %.

A number of experiments, the detailed results of which will not here be given, showed that the diminution of adsorption of maltose caused by the addition of glucose depends upon the concentrations of these sugars. Thus the greater the concentration of maltose used the more closely do the adsorptions of glucose and maltose separately approach each other, and the same is the case when the concentration of glucose is lowered. On the other hand, the difference in the degree of adsorption of maltose in the presence of glucose is intensified as the concentration of the former sugar rises, and this difference becomes even more accentuated in more concentrated solutions of glucose. These considerations possess a certain biological significance. Thus, changes in the enzymic equilibrium of this reaction will, up to a certain point, be the more marked the greater the relative concentration of glucose, and the augmented yield of biose due to the presence of an adsorbent will vary inversely as the concentration of maltose in equilibrium in its absence. These conclusions follow from the very similar degrees of adsorption of these two sugars. The attainment of the differential adsorption of glucose and maltose thus depends upon their concentrations being chosen so as to fall in different regions of the adsorption isotherms.

The above would be of considerably less importance in those cases in which the degree of adsorption of the synthesised body differs greatly from that of its progenitors, as in the case of glycogen and glucose, where glucose undergoes only negligible adsorption upon protein whilst that of glycogen is very considerable. In this case, the presence of an adsorbent may accelerate synthesis and change the equilibrium constant even with large concentrations of glycogen and small concentrations of glucose.

C. *Elution of the sugars.*

Changes in the equilibrium point of this enzymic reaction are apparent only if the adsorbed biose be taken into consideration. Thus, for the determination of K it was necessary to find the sum of glucose and maltose adsorbed and in solution. This was done by elution of adsorbed sugars by means of amyl alcohol, which had been shown by Przylecki and Niedzwiecka [1928] to act as a powerful elutive agent of uric acid from charcoal. The procedure was as follows. Solutions of glucose and maltose separately or together were added to charcoal in the same order and applying the same precautions as described previously. Amyl alcohol was then added to the mixture, which was then mechanically shaken for 3–6 hours, left tightly stoppered for 12 hours, and again shaken for 3–6 hours. The mixture was then filtered into separating funnels, and the aqueous layer of the filtrate taken for analysis. The optical rotation of the solution of sugars, either mixed or separate, was now measured,

and the same analytical procedure applied as before to the determination of the concentrations of the individual sugars.

The determination of maltose in the presence of excess glucose and of amyl alcohol was with certain modifications effected according to Emmerling. In order to establish that the presence of amyl alcohol did not in any way influence the reaction of fermentation, the following experiments were carried out. Yeast maltase was added to the above filtrates, containing glucose and maltose separately or together. The mixtures were then heated, to drive off amyl alcohol and to destroy the hexobiose-synthesising enzyme, and the excess glucose was destroyed by fermentation.

The results obtained showed that no reducing bodies remained after the fermentation of glucose, and that when maltose was present the content of this substance did not diminish under these conditions. Emmerling's method is therefore applicable to this analysis.

The results given in Tables II and IV show that for the sugars taken separately glucose undergoes almost quantitative elution, whilst maltose is only partially eluted, 18.6 to 50 % of the total quantity remaining in adsorption. Where a mixture of maltose and glucose is taken, a far smaller quantity of the former remains adsorbed after the addition of amyl alcohol, the percentage adsorption falling from 65 to 24 %.

D. *Comparison of enzymic equilibrium in the presence and in the absence of charcoal.*

The technique employed in these experiments was similar to that used in the preliminary investigations. 50–100 cc. of 20 % glucose solution containing yeast maltase was added to 5 g. of charcoal, and analyses were made of the sugars after 1–2 weeks and 2–6 months. The relative concentrations of glucose and hexobiose were determined separately for the solution and for the whole system after elution with amyl alcohol. Glucose was determined either polarimetrically or by Bertrand's method as the difference between the total amount of glucose found after acid hydrolysis of the whole mixture and that produced by hydrolysis of the biose alone, which, as before, was isolated by fermentation of glucose.

Table V. *Synthesis of hexobiose in the presence of 5 g. of charcoal to 50–100 cc. of 20 % glucose.*

No. of determinations	Time	Volume of solution cc.	Without charcoal	With charcoal		Hexobiose after elution
				In solution	Reversibly adsorbed	
5	3–7 days	50	0.250	—	—	0.270
4	"	100	0.304	—	—	0.310
7	2 months	50	1.40	1.34	0.688	2.03
8	5 "	—	1.67	1.61	0.81	2.42

The results given in Table V afford a satisfactory experimental confirmation of the postulates put forward in the theoretical part of this paper. During

the first stages of the reaction, lasting 3-7 days, the presence of charcoal has no appreciable effect upon the velocity of the reaction of synthesis. Neither in the solution alone, nor in the whole system can any great difference be seen in the yield of synthesised hexobiose. If anything, it would appear that the amount of hexobiose synthesised in the presence of charcoal is smaller than in its absence, although this difference is so small as hardly to exceed the limits of experimental error.

This phenomenon appears to be due to the fact that at this stage the reaction is still far from its end-point, and its velocity should not therefore be greatly affected by the adsorption of the small quantities of hexobiose present. Similarly the removal by adsorption of glucose from solution, leading to a diminution in its concentration of from 18.6 to 20 % is unable greatly to diminish the velocity of the reaction of synthesis. As at this stage the very small quantities of hexobiose produced render its determination difficult, only its total amount in solution and adsorption was determined.

The results obtained after a lapse of 2-5 months are entirely different. Here very considerable differences exist between the amounts of hexobiose synthesised in the presence or absence of charcoal. Whilst in the latter case 1.40 % of biose is found after 2 months and 1.67 % after 5 months, in the presence of charcoal we find 2.03 and 2.42 % after 2 and 5 months respectively. This represents in the absence of an adsorbent a yield of 6.0 and 8.35 % respectively on the glucose used, whilst in the presence of charcoal the yields are 10.1 and 12.1 % respectively. The actual difference is undoubtedly even greater, since the uneluted hexobiose has not here been taken into consideration.

No attempt has been made to express the above differences in terms of K , since we have no certainty that definitive equilibrium has been attained—indeed, in those systems examined after 2 months there can be no doubt that equilibrium had not been reached, and for this reason the differences are expressed only as percentages. Thus, after 2 months the presence of charcoal increased the yield of hexobiose by 45 % and after 5 months by 44.1 %.

As to the solutions alone, the concentrations of biose are substantially the same whether an adsorbent is present or not. The cause of augmented synthesis lies solely in the charcoal, on which 0.688 % of the biose was adsorbed after 2 months, and 0.81 % after 5 months, corresponding respectively to 34.4 and 33.8 % of the total quantity synthesised.

INFLUENCE OF ADSORBENT UPON REACTIONS OF ESTERIFICATION.

The effect produced by the addition of adsorbent charcoal (Merck's) to systems containing alcohols, aliphatic acids and their esters, and hydrochloric acid as catalyst, was investigated.

The acid content was determined by titration with barium hydroxide solution, using phenolphthalein as indicator, and esters were determined by

hydrolysis with excess sodium hydroxide solution and back-titration with standard acid.

The procedure generally applied was as follows. Two flasks containing an aqueous solution of alcohol (solution III) of known concentration were taken, and a known quantity of aliphatic acid or ester was added to one flask (solution I). To the other a known quantity of hydrochloric acid was added (solution II); the concentration of alcohol in both flasks was kept equal.

After these solutions had been warmed to the reaction temperature of 37° the concentrations of their constituents was determined. Equal volumes of solutions I and II were then mixed, and the resulting solution IV analysed. The concentration of the constituents of solution IV amounted in every case to half the sum of those of solutions I and II separately. The time at which solutions I and II were mixed was noted, and represents the time of commencement of reaction.

To a portion of solution IV adsorbent charcoal was added immediately after mixing, and, after violent shaking over a short period, the degree of adsorption was determined in this mixture, VII. The degree of adsorption of the individual constituents of the latter mixture was determined by adding adsorbent charcoal to solutions prepared by mixing equal volumes of solutions I and III (solution V) or II and III (solution VI) and analysing after shaking. The sum of the quantity of compounds adsorbed from solutions V and VI was always close to that adsorbed from solution VII.

The concentrations of the substrates of solutions IV and VII were determined at various intervals of time up to the attainment of equilibrium, after which the degree of adsorption of the substrates and reaction products was determined.

10 cc. of solution was in every case taken for titration.

RESULTS.

I. *The system glycerol-butyric acid-charcoal-catalyst.*

Applying the same numeration as above, the following solutions were prepared:

- I. 400 cc. of glycerol (sp. gr. 1.233) + 350 cc. water + 50 cc. butyric acid.
- II. 400 cc. " " + 388 cc. " + 12 cc. conc. HCl.
- III. 400 cc. " " + 400 cc. "

The concentration of acid in solution I was equivalent to 336.9 cc. and in II to 88.6 cc. of 0.05 *N* barium hydroxide, half of the sum of which amounts to 212.7 cc., whereas that of solution IV was 212.3 cc. The concentrations of solutions V and VI were respectively 168.2 and 44.4 cc., after adsorption 137.5 and 44.4 cc. respectively, a total of 181.9 cc.

The concentration of hydrochloric acid in VI is thus unaffected by the addition of 2 g. of adsorbent per 100 cc. of solution. It was noticed incidentally that the filtrate from VI after the addition of charcoal gave a preci-

pitate of barium sulphate on the addition of barium hydroxide. Solution VII contained 182.2 cc. of 0.05 *N* acid, or only 0.3 cc. more than solutions V and VI together.

Table VI. cc. of 0.05 *N* butyric acid or ester.

Date ...	15. xii		16. xii		18. xii		$K = \frac{[\text{acid}]}{[\text{ester}]}$		
	6. xii Acid	7. xii Acid	Acid	Ester	Acid	Ester			
Without adsorbent	167.9	133.3	107.3	60.6	106.5	61.4	106.2	61.7	1.72
With adsorbent } in solution	137.8	112.0	90.2	53.6	89.6	54.1	89.6	54.1	1.66
adsorbent } in adsorption	30.1	26.1	21.0	2.8	21.3	2.9	21.3	2.9	—
Total content	167.9	138.1	111.2	56.4	110.9	57.0	110.9	57.0	1.97

The results are given in Table VI. The quantity of adsorbed butyric acid was determined by direct titration of the residue left after filtering 200 cc. of solution VII, and deducting from the value obtained the quantity of acid contained in the solution absorbed by the filter-paper and between the grains of charcoal. Filtration of solution VII before the addition of charcoal showed that the filter-paper retains 5.6 cc. of 0.05 *N* acid, and it may be taken that the second item amounts to about 4 cc. of solution or about 36.0 cc. of 0.05 *N* acid. Since the filter-paper with residue after the filtration of 200 cc. of solution contains the equivalent of 469.0 cc. of 0.05 *N* acid, and the correction is altogether 41.6 cc., the figure of $427.4/20 = 21.3$ cc. 0.05 *N* butyric acid is arrived at for the adsorption of this acid upon charcoal. The amount of adsorption of the ester is arrived at by subtracting from the difference between the acid + ester content with and without charcoal, namely

$$167.9 - 143.7 \text{ cc.} = 24.2 \text{ cc. } 0.05 \text{ } N \text{ acid + ester,}$$

representing adsorption of butyric acid and of its glyceride, the above value of 21.3 cc. found for acid alone, leaving a value of 2.9 cc. of 0.05 *N* ester adsorbed. Equilibrium in the heterogeneous system is thus represented by $K = 1.97$, as against a mean value of 1.69 for the dispersing medium alone. The introduction of an adsorbent into this system increases, therefore, the equilibrium constant, which is changed in favour of hydrolysis.

II. The hydrolysis of ethyl acetate.

The procedure adopted for this system was identical with that of the preceding, except that the substrates were ethyl acetate dissolved in 44.4 % alcohol. The quantity of charcoal used here was 3 g. per 100 cc. of solution, and as before, 10 cc. of solution were in all cases taken for titration, using 0.0397 *N* barium hydroxide. Using the same numeration of solutions as previously, solutions IV and VII contained initially 93.5 and 82.5 cc. of 0.0397 *N* ester, and 7.9 and 7.5 cc. of 0.0397 *N* hydrochloric acid respectively. Equilibrium was attained in solution IV in 13 days, the initial and final conditions being illustrated in Table VII.

Table VII. *cc. of 0.0397 N acetic acid or ester.*

	Initial content of		Final content of		$K = \frac{[\text{acid}]}{[\text{ester}]}$
	Acid	Ester	Acid	Ester	
Without adsorbent	0	93.5	47.5	46.0	1.03
With adsorbent { in solution	0	82.5	43.2	41.3	1.02
in adsorption	0	11.0	1.7	7.5	—
Total content	0	93.5	44.9	48.8	0.92

The degree of adsorption of the compounds present in the equilibrium mixture is derived from the values found for each constituent separately at concentrations close to those found at equilibrium. Thus 7.4 cc. of 0.0397 *N* ethyl acetate were adsorbed from a solution containing 40.9 cc. Assuming that a certain proportionality exists between the amounts in adsorption and in solution for small changes in concentration, it is here taken that 7.5 cc. are adsorbed from a solution containing 41.3 cc. of 0.0397 *N* ester. Similarly for acetic acid 2.3 cc. were adsorbed from a solution containing 57.9 cc., whence 1.7 cc. would be adsorbed from the equilibrium mixture containing 43.7 cc. of 0.0397 *N* acetic acid. These calculated values are in satisfactory agreement with experimental values, as is shown by a comparison between the sum of adsorbed and unadsorbed acid and ester found in solution VII, and the value for the amount of ester present at the beginning of the reaction, namely the equivalent of 93.7 and 93.5 cc. of 0.0397 *N* barium hydroxide respectively.

In this case the degrees of adsorption of the acid and ester are different from those existing for the first reaction studied, and the equilibrium constant is therefore lowered by the addition of an adsorbent, *i.e.* the reaction of hydrolysis does not proceed as far in the presence as in the absence of an adsorbent. At the same time it will be seen that the equilibrium constant remains unaffected in the dispersing medium.

III. *The synthesis of ethyl acetate.*

This system consisted of 12.4 % ethyl alcohol containing acetic acid, with hydrochloric acid as catalyst; 5 g. of charcoal per 100 cc. of solution were added. The results, expressed in terms of cc. of 0.0397 *N* barium hydroxide are given in Table VIII.

Table VIII. *cc. of 0.0397 N ethyl acetate or acetic acid.*

Date ...	2. iv HCl	2. iv Acid	3. iv Acid	4. iv Acid	5. iv Acid	6. iv Acid	16. iv Acid	18. iv		$K = \frac{[\text{acid}]}{[\text{ester}]}$
								Acid	Ester	
Without adsorbent	6.7	56.0	49.4	48.4	48.2	48.0	47.8	47.8	8.2	5.8
With { in solution	5.0	46.5	43.6	41.3	38.8	36.2	34.7	34.0	6.1	5.6
adsorbent / in adsorption	—	9.5	9.0	8.4	7.9	7.6	7.4	7.4	9.2	—
Total content		56.0	52.6	49.7	46.7	43.8	42.1	41.4	15.3	2.7

In this case a very considerable enhancement of synthesis has taken place as a result of the addition of charcoal. This is due entirely to adsorption, since the equilibrium attained in the dispersing phase ($K = 5.6$) is little

different from that of the homogeneous system, for which K is 5.8. In the system as a whole, however, including that part of its constituents which is in adsorption, 7.1 cc. more of ester have been synthesised, representing an increase in yield of over 86 %, with a consequent diminution of K to 2.7. Curves illustrating this system are given in Fig. 1. It will be seen that synthesis, although at first more rapid in the absence of an adsorbent, soon slows down, and that the first portion of Curve II, representing reaction in the presence of charcoal, is practically a straight line. This would be due to

Fig. 1. Curves expressing concentration of acid in the absence (I) and presence (II) of adsorbent.

the practical absence of ester in the first stages of the reaction, which would only commence to exhibit retardation when the charcoal surface is under the given conditions close to saturation with ethyl acetate. The smaller initial velocity of reaction in the presence of charcoal is probably due to adsorption of acetic acid, as a result of which its active concentration is diminished.

The above three cases fully confirm the theoretical reasoning put forward in the first part of this paper and afford excellent examples of the mechanism of reversible reactions in the presence of adsorbents.

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