various salts tried, NH_4Cl and $CaCl_2$ had the greatest accelerating effect on the rate of protein cleavage. The proteolytic enzymes present in wheat flour caused a more rapid hydrolysis of the proteins when desiccated egg albumen was present, but not when casein was used.

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[CONTRIBUTION FROM THE ORGANIC LABORATORY, COLUMBIA UNIVERSITY AND THE HARRIMAN RESEARCH LABORATORY No. 263.]

ADSORPTION OF INVERTASE.

BY J. M. NELSON AND EDWARD G. GRIFFIN.

Received March 11, 1916.

Hedin and his collaborators, Jahnson-Blohm, and Eriksson,[1] found that the presence of certain substances like serum, egg albumin, saponin, cholestrin, and in some cases the substrate itself, lessened the inhibition of the activity of enzymes like rennet, trypsin and invertase brought about by the presence of solid powders (charcoal), or substances soluble in water as colloids (serum and egg albumin). They considered this inhibition as due to the adsorption and removal of the enzyme from the sphere of action by the charcoal or serum. When, however, certain substances capable of being adsorbed by the inhibitor are present or added to the reaction mixture, they can replace part or all of the enzyme in the enzyme-adsorbent, and the liberated enzyme becoming active again, decreases the amount of inhibition.

It has been shown in a previous paper[2] that, although charcoal does adsorb invertase (and possibly serum and egg albumin do likewise), the apparent inhibiting effect is not due to this, but to a change in the hydrogen ion concentration of the reaction mixture produced by the charcoal, etc. In the light of these results, the explanation given by Hedin for the decrease in inhibition is untenable in the case of invertase at least, since the activity is independent of whether the invertase is adsorbed or not. This is further substantiated by the results indicated below, where the addition of a second substance such as saponin, serum or egg albumin to the enzyme-inhibitor mixture does affect the amount of invertase adsorbed by an inhibitor like charcoal or aluminium hydroxide. In no case is there any noticeable change in the activity as long as the hydrogen ion concentration is kept constant by means of suitable buffers.

Since very little experimental data were given in the previous paper concerning the activity of invertase while adsorbed by charcoal and gelatinous aluminium hydroxide, and since the results have an important bearing on the chemistry of enzyme action in general, as can be seen from the statements based on Hedin's conclusions, occurring in many text-

[1] *Z. Physiol. Chem.*, **72**, 324 (1911); **82**, 175, 178 (1912).

[2] THIS JOURNAL, **38**, 722 (1916).

books,[1] it was deemed advisable to include in this paper a description of the following experiments:

Solutions A, B, C and D contained 100 cc. of a 10% cane sugar solution, 10 cc. of an invertase solution, and 20 cc. of a buffer solution which would give the desired hydrogen ion concentration, and 0.3 g. of finely powdered animal charcoal.

In the case of E, 0.6 g. of the charcoal, 40 cc. of buffer and 20 cc. of invertase solution were allowed to stand for one hour after first being thoroughly mixed, then filtered and 30 cc. of the filtrate added to the 100 cc. of the cane sugar solution.

Solution F was made up similarly to A except that no charcoal was used, and served as a control.

B, C and D were filtered after the inversion had progressed for certain lengths of time, and the amount of inversion in the filtrate compared with that of the unfiltered solution A, as well as with that of the control F.

Solutions G, H and I and J were similar to A, B, C and D, except that instead of the charcoal and buffer, 20 cc. of a suspension of gelatinous aluminium hydroxide, equivalent to 0.12 g. of aluminium oxide, were used.

TABLE I.

Solution.	p_H^+.	Change in degrees of rotation after 18 hours.	24 hours.	42 hours.	48 hours.
A	5.3	4.15	5.40	8.21	8.70
B	5.3	4.02[2]	4.34	4.95	5.20
C	5.3	..	5.36[2]	5.86	6.04
D	5.3	..	..	8.17[2]	8.28
E	5.3	0.40	0.58	1.03	1.20
F	5.3	4.14	5.42	8.27	8.77
G	4.95	4.74	6.08	8.87	9.49
H	4.95	4.82[2]	4.82	4.85	4.87
I	4.95	..	6.07[2]	6.09	6.10
J	4.95	..	..	8.95[2]	8.97
K	4.95	0.01	0.02	0.05	0.06
L	5.0	4.80	6.13	8.99	9.63
		13 hours.	38 hours.	64 hours.	88 hours.
M	7.5	0.22	0.48	0.56	..
N	7.5	0.23[2]	0.22	0.25	..
O	7.5	..	0.45[2]	0.44	0.48
P	7.5	..	..	0.52[2]	0.52
Q	7.5	0.01	0.01	0.01	..
R	7.5	0.24	0.47	0.60	..

[1] "General Chemistry of the Enzymes," Euler, Wiley & Sons, **1912**, p. 77, 81 and 82. "The Nature of Enzyme Action," Bayliss, 2nd ed., p. 105. "Die Fermente und ihre Wirkungen," Oppenheimer, Leipzig, **1913**, p. 79. "Physikalische chemie der Zelle und Gewebe," Höber, 4th ed., p. 738, and others.

[2] Indicates the time when the adsorbent was removed by filtration.

Solution K corresponded to E, except that 30 cc. of a filtrate from a mixture of 40 cc. aluminium hydroxide and 20 cc. of invertase solution was used in place of the filtrate described under E.

Solution L served as a control for G, H, I and J and differed from them in that it contained 20 cc. of a buffer solution (sodium citrate and hydrochloric acid), instead of the aluminium hydroxide.

Solutions M, N, O, P, Q and R only differed from solutions G, H, I, J, K and L in having a lower hydrogen ion concentration, $p_H^+ = 7.5$ instead of 4.9. The change in hydrogen ion concentration was brought about by adding 10 cc. of 0.1 *M* hydrochloric acid to 150 cc. of the aluminium hydroxide suspension used for Solutions G, H, I and J.

On comparing the values from A, G and M with those from the respective controls, F, L and R, containing no adsorbent E, it is apparent that the presence of the latter is without effect on the rate of inversion. The values for G and M show that the same holds for solutions containing different hydrogen ion concentrations. Values for B, C, D, H, I, J, N, O and P show that the enzyme is practically removed (especially when aluminium hydroxide was used), by filtering off the adsorbent, since the filtrates show no appreciable additional change in rotation.

Since the charcoal and aluminium hydroxide carrying the invertase settled to the bottom of the bottle in which the inversion was taking place, it became evident that it made no difference whether the enzyme was uniformly distributed throughout the solution or not. This behavior agrees with the opinion held by many that enzymes are colloids, and that the action between the invertase and cane sugar solution depends on the contact of the cane sugar solution with the insoluble enzyme, either in suspension or adsorbed to the charcoal or aluminium hydroxide. The simplest way to account for the uniformity in activity when the enzyme occurs in the bottom of the bottle in an adsorbed state as when uniformly suspended throughout the entire system, is that the inversion depends on an interaction between two distinct phases, where the amount of surface of contact between the enzyme phase and cane sugar phase remains constant. Why the extent of the enzyme phase exposed to the cane sugar phase should appear to be the same in the adsorbed condition as when it is uniformly distributed throughout the solution, is at present hard to say. If the idea of the amount of surface contact between the two phases is correct, then the surface area of the invertase particles must be constant under the same hydrogen ion concentrations, since so many workers have found a definite ratio to exist between the amount of invertase used and the rate of inversion. This also brings up the question whether the concentration of the hydrogen ion is the same on the surface of the charcoal as it is in the solution. Another question which suggests itself is: Is the size of the enzyme particles dependent on the hydrogen ion

concentration of the cane sugar solution in which the inversion is taking place? To the latter question Bayliss[1] has ventured the answer that it does. In this communication Bayliss also points out an argument for the view that enzymes are of colloidal nature, since there are cases where the enzymes have been insoluble in the solution of the substrate and still were active. This is in agreement with the results described above.

The following series of experiments were undertaken to study whether substances like saponin and egg albumin might have any influence on the activity of invertase in the presence of an adsorbent such as charcoal or egg albumin, as claimed by Hedin and his co-workers.

For this purpose the following solutions were prepared: Solutions I and II consisted of 70 cc. of water, 1 g. of finely powdered animal charcoal, 20 cc. of a buffer solution, and 10 cc. of invertase. Solution III. This solution was similar to I and II, except it contained 45 cc. of water instead of 70.

One gram of saponin was added to II, and 25 cc. of a 20% egg albumin solution (in which $p_H^+ = 5.1$), to III. Each of these solutions, II and III, were then divided into three separate portions and marked IA, IB, IC, IIA, IIB, IIC, IIIA, IIIB, and IIIC. All of the B mixtures were filtered and 20 cc. of the filtrate added to 80 cc. of a 10% cane sugar solution. All of the A mixtures were centrifuged instead of filtered, in order to show that the process of filtering had no influence on the activity of the invertase, and 20 cc. of the supernatant liquid added to 80 cc. of 10% cane sugar solution. In the case of all the C mixtures, 20 cc. of the original was added directly to the 80 cc. of 10% cane sugar solution.

TABLE II.

Solutions.	IA.	IB.	IC.	IIA.	IIB.	IIC.	IIIA.	IIIB.	IIIC.
p_H^+	6.6	6.6	6.7	6.4	6.4	6.5	6.0	6.0	6.1
Change in rotation after									
20 hours	0.02°	0.01°	0.75°	0.86°	0.88°	0.82°	1.22°	1.20°	1.16°
After 48 hours	0.01°	0.00°	1.59°	1.77°	1.82°	1.67°	3.15°	3.13°	3.00°

The values obtained from solutions IA and IB, when compared with those from IC, show that on filtering or centrifuging off the charcoal, when no saponin or egg albumin was present, the invertase was also removed. The values from IIA, IIB, IIIA and IIIB show that the presence of both the saponin and egg albumin prevented the charcoal from removing the invertase, and in this respect the results agree with the claims of Hedin and Jahnson-Blohm. A comparison of the values from IIA and IIB with those from IIC, and the values from IIIA and IIIB with those from IIIC, shows that, even when the saponin prevents the adsorption of the invertase by the charcoal, no effect is noticeable on the activity. This behavior of the saponin and egg albumin indicates that they act as

[1] *Science*, 42, 513 (1915).

protective colloids towards the invertase, and according to the present theories concerning protective colloids, the invertase is therefore adsorbed to the saponin and egg albumin as well, which is contrary to the views of Hedin and co-workers. Therefore it seems that the invertase adsorbed to colloids like saponin and egg albumin behaves in a manner similar to that adsorbed to charcoal, and in each case the activity is not affected. It furthermore indicates that the adsorption of invertase to charcoal is influenced by these protective colloids in a way similar to the coagulation of metal sols, etc. If this is so, and if activity is dependent on the amount of surface of the enzyme particles, we might then have an additional property to aid us in the study of the relationship existing between the adsorbent and the substance adsorbed in the adsorption combination.

Since all preparations of invertase whose composition have been examined always contained a polysaccharide and probably a protein, both of which substances would very likely act as protective colloids, the possibility suggests itself, that the true invertase principle might be something adsorbed to this material. For this reason, some gelatinous aluminium hydroxide, free from nitrogen, was added to an invertase solution whose nitrogen content was known. The aluminium hydroxide was subsequently filtered off, and the amount of nitrogen in the filtrate again determined, as well as the activity of the aluminium hydroxide, containing the adsorbed invertase, and the amount of nitrogen it carried with it. It was thought that it might be possible that only the active principle might be adsorbed to the aluminium hydroxide, and if it were nitrogen free, this fact would be revealed. The aluminium hydroxide however did carry with it some nitrogenous material and therefore no definite conclusions could be drawn.

Jahnson-Blohm[1] claimed that the addition of saponin or egg albumin as the second colloid to a mixture of rennet or trypsin and their respective substrates containing serum or egg albumin, also affected the activity of the enzymes just as when charcoal was used as the adsorbent.

In order to see whether any effect of this kind occurred when an invertase cane sugar solution, containing serum or egg albumin, was treated with saponin or egg albumin as the second colloid, the following experiments were undertaken. The results obtained again show that in the case of invertase, there is no such effect on the activity produced by this addition of a second colloid.

The solutions used in these experiments were:

A. Ten cc. of invertase solution, 5 cc. of a 25% serum solution, in which the serum had been neutralized previously with 0.1 molar hydrochloric acid to $p_H^+ = 6.8$, were mixed and allowed to stand for some time, and then added to 5 cc. of water and 50 cc. of a 20% cane sugar solution.

[1] *Loc. cit.*

B and C differed from A in having 5 cc. of 1% saponin solution and 5 cc. of a 20% egg albumin solution, respectively, in place of the 5 cc. of water in A. D, E and F were controls for A, B, and C, respectively, and contained 10 cc. of buffer solution in place of the 5 cc. of serum and 5 cc. of water in A, in order to give them the same hydrogen ion concentration.

TABLE III.

Solutions.	A.	D.	B.	E.	C.	F.
p_H^+	7.1	7.1	7.0	7.0	6.4	6.4
Change in rotation after 18 hours	2.02°	2.04°	2.12°	2.15°	2.77°	2.75°

In these solutions serum was used as the first colloid or adsorbent. The value obtained from A, where the second colloid was absent, agrees with its control, D, containing no serum. The values of B and C, which contained both the first colloid or adsorbent (serum), and the second colloid (saponin and egg albumin, respectively), also are the same as those of the controls E and F, which contain no saponin, egg albumin nor serum. It is therefore evident that the presence of one or more colloids does not affect the activity of invertase.

Similar results were obtained when the order in which these three substances, serum, egg albumin and saponin were mixed, was interchanged. Thus when invertase and egg albumin were permitted to stand for some time and then saponin or serum added, no difference in the activity of the enzyme was noticed.

Eriksson[1] found that the inhibition became less as the length of time before filtering increased when the cane sugar solution was added to a mixture of charcoal and invertase in water, which had stood for some time to allow the charcoal to adsorb the enzyme, and then filtering off the charcoal after different lengths of time after the substrate had been added. This he considered due to the cane sugar gradually liberating the invertase adsorbed to the charcoal and again becoming active.

Explained in the light of the results indicated in this and previous papers,[2] the filtering off of the charcoal would carry with it some of the invertase, and the longer this invertase adsorbed to the charcoal was permitted to be in contact with the sugar solution, the greater would be the amount of inversion taking place, and therefore there is no indication whatever that the cane sugar liberates the invertase from the charcoal-invertase combination.

For a description of the methods employed in making the above measurements, etc., see Experimental Part in previous paper.[3]

[1] *Loc. cit.*

[2] THIS JOURNAL, 38, 722 (1916).

[3] *Ibid.*, 38, 722 (1916).

Summary.

1. It has been shown, in a new way, that invertase is colloidal in nature, and the reaction between the enzyme and cane sugar solution depends on the contact of two phases.

2. The activity of invertase (the product obtained from yeast and called invertase), is not affected whether or not the enzyme is adsorbed to a solid like charcoal, or to a colloid like saponin, serum, or egg albumin, distributed uniformly throughout the solution of the substrate.

3. Displacing the adsorbed invertase by a second colloid is without effect on the activity, contrary to the views held by many.

4. Invertase can be removed from an aqueous solution by adsorption to a solid, and again brought into solution by a second colloid suspended uniformly throughout the solution.

5. Eriksson's proof that cane sugar can liberate invertase adsorbed to charcoal is not valid.

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ON THE REACTION OF THE PANCREAS AND OTHER ORGANS.

[SECOND PAPER.]

BY J. H. LONG AND F. FENGER.

Received March 27, 1916.

In a recent paper[1] we showed that the "press juice" obtained from the pancreas of the hog, sheep and beef by centrifugal separation, is characteristically acid, the degree of acidity being nearly constant. This unexpected result suggested the importance of further experimentation, as it has usually been assumed that the reaction of the fluids of the body is not far from that of the serum. But this assumption leaves out of consideration the fact that the external secretion of the pancreas is rather markedly alkaline. A compensating acidity should then be expected somewhere, and most naturally within the organ.

In our view the reaction might be due, in part at least, to the presence of acid phosphates in the juice, or possibly to acid organic compounds of phosphoric acid, since the acid is abundantly present. In the discussion following the presentation of the paper at the Seattle meeting, Sept. 1, 1915, the suggestion was made by several colleagues that the acidity might be due to ferment action through the lipase present. This and other points had already been considered by us, but in view of the importance of the phenomenon we have thought it desirable to present further evidence bearing on the question, this evidence being in the form of data collected since the first paper was published.

[1] THIS JOURNAL, 37, 2213 (1915).