THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE INACTIVATION OF PEPSIN SOLUTIONS.

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One of the many factors which must be taken into consideration in any experiments with enzymes is the possible inactivation of the enzyme during the course of the reaction. This factor in the case of pepsin has been suggested by Sörensen[1] as the cause of the displacement of the optimum acidity for the digestion of protein to the acid side during the course of the digestion. He considers that the enzyme is more rapidly destroyed by the weak than by the strong acid. Arrhenius,[2] on the other hand, considers that the decrease in the rate of digestion on the acid side of the optimum hydrogen ion concentration for digestion is due to the more rapid destruction of the enzyme by the strong acid. If this explanation is correct the optimum phenomenon loses much of its significance and becomes a secondary characteristic of enzyme activity comparable to the optimum temperature. The possibility also arises that the peculiar falling off of the rate of digestion during the course of the reaction, at any hydrogen ion concentration, is also due to the destruction of the enzyme.

Several investigations[3] have been made on the stability of pepsin in acid solutions from various points of view but the results are not at all concordant. Much of this variation in results is probably due to the failure to realize the importance of the hydrogen ion concentration rather than the total acid concentration.

[1] Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 1909, viii, 162. Sörensen's experiments were made at 52°. They are therefore not strictly comparable with the present results.

[2] Arrhenius, S., Quantitative laws in biological chemistry, London, 1915, 44.

[3] Biernacki, E., *Z. Biol.*, 1891, xxviii, 49. Grober, J. A., *Arch. Exp. Path. u. Pharmacol.*, 1904, li, 103. Liebmann, P., and Johannesen, L., *Ugesk. Læger*, 1911, lxxiii, 902. Ramsay, C. F., *J. Am. Pharm. Assn.*, 1917, vi, 1047.

In the experiments considered in this paper the effect of the following variables on the inactivation of pepsin in solution has been studied: (1) the hydrogen ion concentration; (2) the anion of the acid; and (3) the purity of the enzyme solution.

The results of the experiments are given in Tables I and II and in Figs. 1 and 2. The figures in the tables are the relative amounts of active enzyme present in the solution after 24 or 48 hours. The total active enzyme present at the beginning of the experiment is taken as 10 units. The time required to cause a constant change

TABLE I.

Influence of the Purity of the Enzyme Solution on the Destruction of Pepsin at Various Hydrogen Ion Concentrations.

pH	Relative amount of pepsin per cc. of solution after 48 hrs. at 38°C.		
	0.25 per cent active pepsin.	2.5 per cent weak pepsin.	1.5 per cent weak pepsin in 3 per cent egg albumin solution.
6.2		1.0	
5.9			4.6
5.5	2.0	7.4	7.2
5.1	10.0		
4.7		8.4	8.2
4.0	10.0		7.9
3.6	9.0	6.8	7.2
2.0		6.3	7.0
1.2	8.0	6.0	6.8
0.6	7.8		

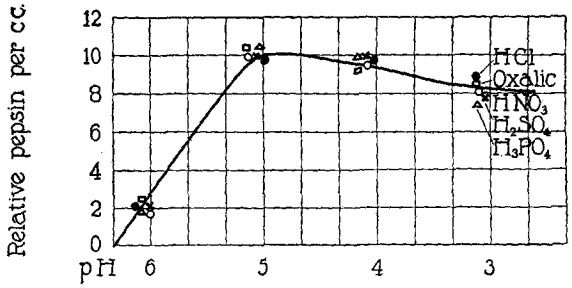

FIG. 1. Relative amount of active pepsin at different hydrogen ion concentrations with various acids after 24 hours at 38°C.

in the conductivity of an egg albumin solution under constant conditions is considered as inversely proportional to the amount of active enzyme present and was used as a measure of the enzyme concentration. The experiments with different enzyme solutions were not done at the same time and are not strictly comparable. The experiments with the various acids, however, are comparable.

It will be seen that in all the experiments the enzyme is most stable at a pH of about 5.0, irrespective of the anion of the acid and of the purity of the solution. Increasing the alkalinity of the solution causes a very great increase in the destruction of the enzyme. There is some indication that the impure solutions are inactivated more slowly under these conditions than the purer ones.

TABLE II.

Influence of Various Acids on the Destruction of Pepsin at Various Hydrogen Ion Concentrations.

pH	Relative amount of pepsin per cc. of solution containing the acids noted below after 24 hrs. at 38°C.				
	HNO_3	H_2SO_4	H_3PO_4	Oxalic.	HCl
6.0–6.2	1.7	2.1	1.8	2.5	2.2
5.0–5.2	10.0	10.0	10.3	10.0	9.8
4.0–4.2	9.5	10.0	10.0	9.2	9.6
3.0–3.2	8.0	7.9	7.6	8.5	8.8

Increasing the acidity of the solution above pH 5.0 causes a very slow increase in the amount of pepsin destroyed, and the quantity inactivated is not influenced either by the purity of the solution or by the anion of the acid. It would seem necessary to conclude from the marked asymmetry of the curve for the destruction of the enzyme, as plotted against the hydrogen ion concentration, that the process of inactivation of the enzyme on the acid side of pH 5.0 differs from the process of inactivation on the alkaline side of pH 5.0.

Fig. 2 shows that the amount of pepsin remaining in solution after 24 hours at 38°C. is about the same throughout the range of acidity in which the enzyme is active. The rate of destruction of the enzyme therefore differs very little at a pH of 1.0 and a pH of 3.0. As is well known, the activity of the enzyme varies greatly within this range.

This is shown by Curve D, Fig. 2, which is taken from Sörensen's paper and which represents the rate of digestion of egg albumin by pepsin at various hydrogen ion concentrations. If the decline in the rate of digestion on the acid side of pH 2.0 was due to the increased destruction of the pepsin by the acid in greater concentration than this, the same drop should be noticed in Curves A, B, and C as in Curve D, since these curves represent the actual amount of destruction of the enzyme at the acid concentration in question. Fig. 2 shows that this is not the case. But little more enzyme was destroyed at a

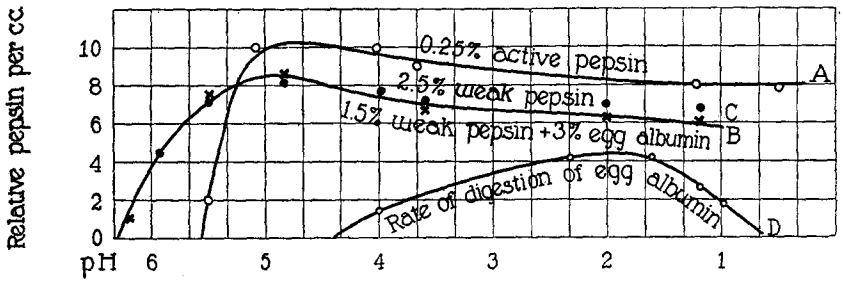

FIG. 2. Relative amount of active pepsin in various solutions at different hydrogen ion concentrations after 24 hours at 38°C.

pH of 1.0 than at a pH of 2.0 or 3.0. The rate of destruction is in any case much too slow to account for the rapid drop in the rate of the digestion curve. This drop is noticeable in the first few minutes of the reaction, while, as the figures show, only 10 to 20 per cent of the enzyme is destroyed in 48 hours at this hydrogen ion concentration.

The fact that the action of the acid on the enzyme is nearly the same across the whole range of hydrogen ion concentration in which the enzyme is active may be considered as indirect evidence that the optimum phenomenon is connected with changes in the substrate rather than in the enzyme. It is apparent from the figures that the enzyme is most stable at a pH of about 5.0; *i.e.*, the same as that for the isoelectric point of many proteins. There is no evidence, however, that pepsin is isoelectric at this point. A series of migration experiments made by the writer confirmed those of Michaelis and Davidsohn[4] (except that the enzyme was never found to migrate to both

[4] Michaelis, L., and Davidsohn, H., *Biochem. Z.*, 1910, xxviii, 1.

poles at the same pH) and gave a change in the direction of migration at about pH 3.0. There is no relation between this point and either the resistance of the enzyme to acid or the rate of its action on proteins. It is probable that this is not the isoelectric point of pepsin itself but that of a compound formed between pepsin and some other substance in the solution, since Peckelharing and Ringer[5] found that very pure pepsin solutions showed no isoelectric point.

No evidence was found that the inactivation of the enzyme was reversible under the conditions of these experiments although many experiments were made with this point in view.[6]

The results show that digestion experiments with pepsin cannot be carried out at 38° for longer than 24 hours without being complicated by the fact that the enzyme concentration is lower at the end of the experiment than at the beginning. They also show that in experiments on the decomposition temperature it is necessary to consider the reaction of the medium.

The general effect of the hydrogen ion concentration on the stability of the enzyme resembles that described by Falk[7] for lipase, and by Frankel[8] for papain. In the case of papain, however, the influence of the reaction is reversed; *i.e.*, papain is more sensitive to acid than to alkali.

Experimental Procedure.

Pepsin Preparations Used.—Active: Fairchild's pepsin U. S. P. 1:19,500. Weak: Pepsin U. S. P. 1:3,000.

Hydrogen Ion Determinations.—All determinations were made by the E. M. F. method.

Determination of the Relative Amount of Pepsin in Solution.

The enzyme solution was made up as shown in the tables and placed in a water bath at 38 ± 0.1°C. 5 cc. of the solution were pipetted out for analysis and 5 cc. of an acid solution added of such strength as to make the final acid concentration in each case equal

[5] Peckelharing, C. A., and Ringer, W. E., *Z. physiol. Chem.*, 1911, lxxv, 282.
[6] Tichomirow, N. P., *Z. physiol. Chem.*, 1908, lv, 107.
[7] Falk, K. G., *J. Biol. Chem.*, 1917, xxxi, 97.
[8] Frankel, E. M., *J. Biol. Chem.*, 1917, xxxi, 201.

to that of the solution containing the highest amount of acid. 1 cc. of this diluted solution was then added to a standard egg albumin solution and the time necessary to cause a 10 per cent change in the conductivity of the latter determined as described in a previous paper.[9] The relative concentration of active pepsin was about the same at the beginning of each experiment. This quantity was taken as 10 in each case. Under the conditions of these experiments neither the products of the digestion of the egg albumin nor the inactivated pepsin interferes with the determination; *i.e.*, the reciprocal of the time to cause a given change is directly proportional to the total quantity of active pepsin present.

SUMMARY.

1. Pepsin in solution at 38°C. is most stable at a hydrogen ion concentration of about 10^{-5} (pH 5.0).

2. Increasing the hydrogen ion concentration above pH 5.0 causes a slow increase in the rate of destruction of pepsin.

3. Decreasing the hydrogen ion concentration below pH 5.0 causes a very rapid increase in the rate of destruction of the enzyme.

4. Neither the purity of the enzyme solution nor the anion of the acid used has any marked effect on the rate of destruction or on the zone of hydrogen ion concentration in which the enzyme is most stable.

5. The existence of an optimum range of hydrogen ion concentration for the digestion of proteins by pepsin cannot be explained by the destruction of the enzyme by either too weak or too strong acid.

[9] Northrop, J. H., *J. Gen. Physiol.*, 1919–20, ii, 113.