THE EFFECT OF VARIOUS ACIDS ON THE DIGESTION OF PROTEINS BY PEP SIN.

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I.

The widespread occurrence of antagonistic salt action on living tissues raises the question whether the underlying cause of the phenomenon might not be found in the action of the salts on the activity of the enzymes. A similar effect has been described by Falk in the case of lipase. As Loeb has shown, it is also possible to demonstrate antagonistic salt action on the physical properties of a protein; i.e., gelatin. It seemed important therefore to determine whether or not such an effect was to be found in the influence of various acids on the digestion of proteins by pepsin.

The relative action of the various acids on the pepsin digestion of proteins has already been the subject of many investigations. The literature on the subject is confused and contradictory, however, due largely to the fact that in the early work the effect of the hydrogen ion concentration was not taken into account and that the acids were usually compared in equimolecular or even equipercentage strengths. Attempts were made by Berg and Gies to rule out this disturbing factor by using solutions containing equal calculated amounts of hydrogen ion. They took no account, however, of the "buffer" action of the weaker acids and of the proteins so that their

4 Pfleiderer, R., Arch. ges. Physiol., 1897, lxvi, 605. A review of the early literature is given in this article.
solutions probably did not have the same hydrogen ion concentration. Sörensen has shown that the activity of pepsin depends very largely on the hydrogen ion concentration, and at the same time has developed accurate methods for the determination of the latter. In view of his work it is obvious that the comparison of the action of the different acids must be made in such a way as to keep this factor constant. Failure by the earlier workers to do this probably accounts in large measure for the great differences in the observed efficiency of the various acids.

More recently Michaelis and Mendelsohn have shown that the optimum acidity for the digestion of edestin by pepsin is the same for hydrochloric, nitric, tartaric, and oxalic acids. Ringer states that the optimum reaction depends on the protein used and coincides with the maximum hydration of the protein as measured by the viscosity. A somewhat similar theory was proposed by Pfleiderer who attempted to show a relation between the rate of digestion of fibrin and the amount of swelling in various acids.

In all these investigations the amount of digestion was followed by determining (1) the amount of solution of an insoluble substrate, (2) the amount of precipitable protein left in solution, or (3) the rate of liberation of carmine from carmine fibrin. As has been pointed out by various authors, there is considerable doubt as to whether any of these methods actually follows the chemical changes in the structure of the protein during hydrolysis.

The recent improvements in the technique of the determination of amino nitrogen by the Van Slyke method make it possible to follow the increase in the number of free amino groups. From our knowledge of the changes involved in the hydrolysis of proteins it would seem that this increase probably follows accurately the amount of hydrolysis. The changes are small, however, and even with the greatest care it is difficult to get strictly accurate figures.

In the present work the rate of pepsin digestion of gelatin, egg albumin, edestin, blood albumin, and casein in the presence of hydrochloric, nitric, acetic, sucruic, oxalic, phosphoric, and citric acids has been followed by this method. The determinations were made at two ranges of hydrogen ion concentration, pH 1.0 to 1.5 and pH 2.5 to 3.5.

A summary of the results obtained with edestin at a reaction of pH 2.6 is given in Table I. The results obtained with the other proteins were practically identical with these and therefore will not be given here. The experiments show that the rate of hydrolysis of all the proteins studied is identical for all the acids (except acetic) within the rather wide range of error of the method used (about 5 per cent). With gelatin acetic acid also behaves quantitatively like the other acids even in concentrations as high as 25 volumes per cent. With the other proteins the rate of hydrolysis in the presence of acetic acid is slower than with HCl, HNO₃, H₂SO₄, oxalic, citric, or phosphoric acids. The effect therefore is evidently on the protein.
and not on the enzyme. These experiments also show that the physical properties of the solution, such as viscosity, have little or no effect on the rate of digestion since Loeb has shown that there is a striking difference in the viscosity of gelatin in sulfuric as contrasted with hydrochloric acid solution. This is still more strikingly shown in the experiments with edestin, which is practically insoluble in sulfuric acid and yet digests under these conditions at the same rate as when dissolved in hydrochloric acid. The simplest explanation of these results would seem to be that the rate of digestion of the protein is determined by the amount of acid protein salt formed. As Loeb has shown, the physical properties of a gelatin solution are also functions of this same quantity.

II. EXPERIMENTAL.

Method of Keeping Samples.—Samples were withdrawn from the bottles containing the digestion mixtures at intervals of 4 and 24 hours and placed in iced bottles containing three drops of saturated ferric chloride solution. They were kept at 2-4°C. until analyzed. No change could be noted in the amino nitrogen content in 24 hours under these conditions.

Temperature.—Some of the experiments were conducted at 37° ± 0.1, and some at 35° ± 0.1.

Analysis.—The technique was the same as described by Van Slyke except that 10 cc. of solution were analyzed in a large size apparatus and the gas was measured in a small (3 cc.) burette. This was necessary owing to the small amount of amino nitrogen present. The protein solution was allowed to stand 15 minutes in contact with the nitrous acid and then shaken rapidly for 5 minutes. The reaction was complete under these conditions.

Pepsin Solution A.—30 gm. of Fairchild's pepsin were dissolved in 500 cc. of water and allowed to digest at 37° for 24 hours. The solution was dialyzed and filtered, and made up to 3 liters. It contained 0.1 cc. of amino nitrogen per 10 cc. This quantity did not further increase digestion in 24 hours and therefore does not enter into the figures obtained for the increase of amino nitrogen in the solutions.
It was found that 20 cc. of this solution added to 100 cc. of the protein solution caused the hydrolysis to be about one-third complete in 4 hours and two-thirds complete in 24 hours. This concentration was therefore used.

_Edestin Solution A._—25 gm. of crystalline edestin were dissolved in 300 cc. of dilute NaOH and precipitated by the addition of dilute HCL. The reaction was adjusted to the isoelectric point of edestin and the solution then dialyzed for a week against tap water and 2 days against distilled water. It was then diluted to 500 cc. A fine suspension was obtained which could be accurately pipetted. The conductivity was about that of an m/1,500 KCL solution, showing that only traces of electrolytes were present. The other protein solutions were purified in the same way by dialysis at the isoelectric point (Loeb).²

_Adjustment and Measurement of the Reaction._

The required amount of protein solution was pipetted into a 100 cc. volumetric flask and a drop of indicator added (methyl orange or thymol blue, depending on the reaction desired). Hydrochloric acid was then added until the approximate reaction desired was reached. The solutions containing the other acids were prepared in the same way by adding the acid to the protein solution until the color matched exactly that of the flask containing the hydrochloric acid solution. In this way solutions of the same pH could be easily prepared.

Control experiments showed that the indicator had no effect on either the rate of digestion or the analysis. The absolute reaction of the mixtures could not be measured colorimetrically owing to the "protein error." A sample of the solution was removed shortly after adding the pepsin therefore and the pH determined by the e.m.f. method. It was found as stated by Sørensen that the change in reaction during the digestion was insignificant.
SUMMARY.

1. At equal hydrogen ion concentration the rate of pepsin digestion of gelatin, egg albumin, blood albumin, casein, and edestin is the same in solutions of hydrochloric, nitric, sulfuric, oxalic, citric, and phosphoric acids. Acetic acid diminishes the rate of digestion of all the proteins except gelatin.

2. There is no evidence of antagonistic salt action in the effect of acids on the pepsin digestion of proteins.

3. The state of aggregation of the protein, i.e. whether in solution or not, and the viscosity of the solution have no marked influence on the rate of digestion of the protein.

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