# A COMPARISON OF DIFFERENT METHODS OF DETERMINING THE PROTEOLYTIC ACTIVITY OF GASTRIC JUICE

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Both in health and in disease, the principal indications of gastric secretion are the amount of the juice secreted per hour, its acidity, and its enzymatic activity.

There is no difficulty in measuring the acidity, but despite a number of investigations spread over nearly two hundred years, the problem of determining proteolytic activity cannot yet be considered to be satisfactorily solved.

We will not describe the many methods which are now only of historical interest [13, 20], but will deal with those which are widely used at the present time both in the USSR and abroad.

In 1889, S. G. Mett [4] working in I. P. Pavlov's Laboratory used a method based on macroscopical determination of loss of solid substrate (fibrin, ricin, and denatured egg-white) and worked out in detail a means of determining the proteolytic activity of gastric juice. Because of its simplicity it was possible to examine a large number of hourly samples of gastric juice, and being more accurate than other methods, it became widely used in laboratories both here and abroad. However, there are several shortcomings. A long period of incubation is required, it is difficult to obtain tubes of precisely equal diameter, to fill them with protein, and to supply a stable substrate for digestion; it is not easy to ensure optimal conditions for diffusion of the enzyme into the substrate, and there is some inaccuracy in the visual determination of the length of the digested column of protein. According to several authors [2, 5, 6, 19], for these reasons it is not suitable for accurate quantitative work. We have,therefore,sought other simpler methods.

A method which at one time was quite widely used was based on the formation of a clot due to the action of the enzyme on homogenized milk or restored from the powdered form [7, 8]. However, although they were used chemically, such methods were not sufficiently precise, on account of the variability of any one kind of substrate.

A method which is in quite common use at the present time is that of Gross. The quantitative determination of pepsin by this method is made in terms of the nephelometric effect (the appearance of cloudiness in a test tube after the addition of sodium acetate). According to many investigators [5, 19], Gross's method is not sufficiently precise (according to results which have been communicated to us, in T. J. Shlygin's Laboratory Gross's method was changed so as to increase its accuracy). V. S. Kozlovskii [3] compared the method with that of determining proteolytic activity in terms of the accumulation of the non-protein nitrogen, and found that when Gross's method was used, the variation in the results was 43.7%. Thus, although the method is more precise than that of S. G. Mett[2], it is capable of indicating only very great abnormalities in enzymatic content.

Recently, methods based on the determination of breakdown products, and in particular of tyrosine have been widely used. One such method was proposed in 1932 by Anson and Mirsky [11, 12], and consisted of determining proteolytic activity from the amount of tyrosine formed in the breakdown products of hemoglobin. Hemoglobin was used as the substrate, because it could be produced in large amounts and preserved without change for a long time; also it was rapidly digested, and the amount digested by a standard pepsin solution was fairly constant. Thus, with this method, the conditions of digestion were optimal. Pepsin activity was determined from the amount of tyrosine groups formed in a given time in the action of protease on the substrate. Tyrosine reacts with the added phenol reagent of Folin and Ciocalteu [1, 15] to form a blue color whose intensity depends on the amount of tyrosine present,

which can therefore be estimated by means of a photoelectric colorimeter. The pepsin activity is expressed in pepsin units as proposed by Anson and Mirsky [11, 12], and it may easily be transformed into milli-equivalents of tyrosine. After Anson and Mirsky published their report, many modifications were described [9, 10, 14, 16, 17]. In the later methods, either a different substrate was used for peptic digestion, or the technique of determination was modified.

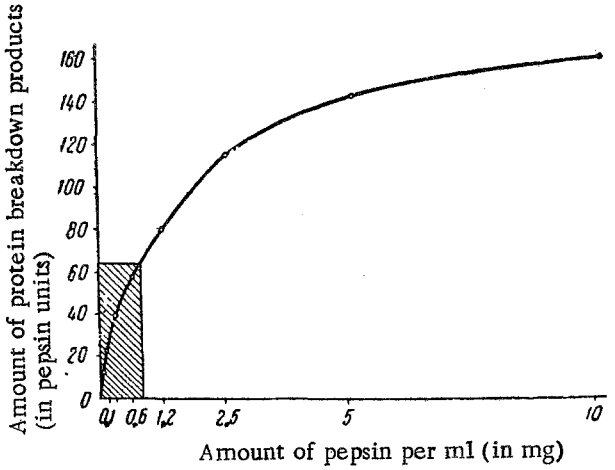


Fig. 1. Relationship between enzyme concentration and the amount of breakdown products. Time of incubation 15 min. Shaded portion represents the region of enzymatic activity of 1 ml of gastric juice.

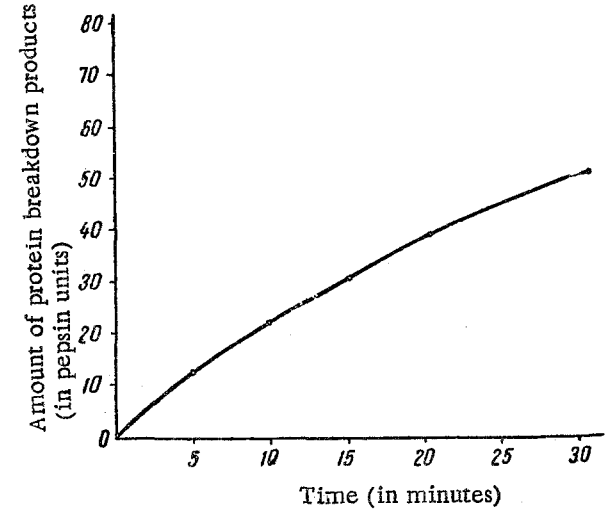


Fig. 2. Relationship between the time of action of the enzyme and the amount of protein breakdown products formed.

In our studies we have modified Hunt's method [17] of determining the proteolytic activity of gastric juice, as we consider it one of the simplest and most convenient. As substrate, Hunt used either plasma or human serum which was not suitable for intravenous application, and dissolved it in hydrochloric acid at a pH of 2.1, to make a solution of 5.6 - 2.4%. Serum and plasma are freely soluble in water. The gastric juice to be studied was mixed with an equal volume of hydrochloric acid at pH 2.1. Test tubes were arranged in pairs, one of which contained 5 ml of a 5.6% solution of serum (substrate) and the other 10 ml of 0.35 N trichloracetic acid, and were then placed in a water bath at 35°. A test tube containing gastric juice to be estimated was also placed in the water bath. After 20 minutes, when the contents of the tube had reached the temperature of the bath, to both test tubes (one containing substrate, and the other trichloracetic acid) were added 1 ml of gastric juice, the contents were mixed by shaking, and then left to stand in the water bath. The time at which the gastric juice was added was noted. In the tube containing the substrate, peptic digestion began after the gastric juice was added. After precisely 15 minutes, digestion was arrested by pouring 10 ml of trichloracetic acid from the second tube into the first. The liquids were poured back and forth between the tubes two or three times to ensure thorough mixing, so as to bring the digestion to an end as quickly as possible. The tubes were then left to stand in the water bath for four minutes. The contents of the tubes were then filtered through a fine filter paper. Fifteen to thirty minutes after filtering, 2 ml of filtrate were placed in a 50 ml flask containing 20 ml of 0.25 N NaOH, and vigorously mixed by rotating the flask, then 1 ml of the phenol reagent diluted 3 times with distilled water immediately before use was added. A blue color appeared and was estimated with a photoelectric colorimeter between the 15th and 40th min. Readings were taken with a red filter. Because blood serum and the gastric juice may themselves contain tyrosine, its amount must be determined. Therefore, for each determination, a control was performed. To a test tube were added 5 ml of serum, 10 ml of trichloracetic acid, and 1 ml of gastric juice; they were mixed, placed for 4 minutes in a water bath, and the whole of the remaining procedure carried out, starting with filtration, and proceeding as before. The figures for the control were substracted from those of the experiment. Pepsin activity was expressed in Hunt's pepsin units.

A gastric juice of 50 pepsin units will be one which gives a coloration equal to that produced by 2 ml of a freshly prepared 5% phenol solution to which has been added 20 ml of a 0.25 N NaOH solution and 1 ml of Folin-Ciocalteu reagent diluted 3 times. By calculations from the readings obtained by placing this standard solution in

the photoelectric colorimeter, a curve may be obtained which enables the apparatus to be calibrated in pepsin units. Curves may also be constructed where necessary to show the relationship of the pepsin units to mg of tyrosine or of crystalline pepsin [18].

In our experiments, we introduced the following simplifications of Hunt's method, which in no way reduced the accuracy of the results. We used as substrate native serum (preserved in chloroform) of large-horned cattle; it is produced in large quantities for bacteriological media by the A. I. Mikoyan Moscow Meat Plant. The serum was freeze-dried and a 2.4% solution prepared daily by dissolving weighed amounts in a 0.06 N hydrochloric acid. The measurements were made on undiluted gastric juice. Instead of 2 ml of filtrate, we used 5 ml. Corresponding changes were made in the amount and concentration of the reagents [17], and 10 ml of a 4% solution of trichloracetic acid, and 10 ml of 0.5 N NaOH solution were used; (to obtain the standard color, 10 ml of 0.5 N NaOH solution were also taken). We carried out the reaction in test tubes instead of flasks.

Comparison of Methods of Determining the Proteolytic Activity of Gastric Juice

| Statistical quantity | Anson-Mirsky method (in Anson-Mirsky pepsin units) | Hunt's method (in Hunt's pepsin units) |
|---|---|---|
| Variability of the results (M ± σ) . . . . | 2.69 ± 0.139 | 24.3 ± 3.1 |
| Mean error (m) . . . . | ± 0.019 | ± 0.52 |
| Number of experiments (n) . . . . . | 54 | 36 |
| Percentage error (P) . | 0.7 | 2.14 |

We have not yet sufficient figures to describe the activity of gastric juices secreted in response to different food stimuli, but we can give some examples. Thus, in two dogs, one with a Pavlov and the other with an Heidenhain pouch, in response to a blood substitute, the gastric juice secreted had a strength of 12 and 20 pepsin units respectively, and to meat the values were 30 and 44, while in response to sham feeding of a dog with a Basov fistula, the strength was 46 pepsin units.

To investigate the relationship between pepsin concentration and the amount of breakdown products of the protein substrate, we carried out experiments with gastric juice and solutions of crystalline pepsin in which the concentration of the enzyme was up to 20 times greater than that of canine gastric juice (Fig. 1). The samples were agitated, and incubated in a Warburg's apparatus. The results showed that within certain limits there was a direct relationship between the two quantities, which completely justified working with the concentrations of pepsin usually encountered in canine gastric juice. We also found such a relationship to obtain between the time of action of the enzyme and the amount of breakdown products formed (Fig. 2), as did also Hunt [17] in studying human gastric juice. In cases when the sample of gastric juice is small, if it contains sufficient pepsin, satisfactory results may be obtained by diluting it 2 or 4 times with 0.06 N hydrochloric acid. Our results confirm that the amount of protein breakdown products is constant for a given dilution of pepsin and a given time of digestion [17].

We compared our modification of Hunt's method with that of Anson-Mirsky. We used a solution of crystalline pepsin in 0.06 N hydrochloric acid containing 0.1 mg of pepsin per ml. The results were treated statistically, and are shown in the table.

The results confirm the high accuracy of our method, which we recommend. Its comparative simplicity and the fact that it is possible to examine several portions of gastric juice simultaneously makes the method suitable for scientific research work and for clinical purposes.

## SUMMARY

Certain methods used for determining the proteolytic activity of the gastric juice are characterized. Modification of the colorimetric Hunt's method is described. Precision of this and of Anson-Mirsky's methods is compared; the results of investigation were treated statistically. Average figures of the gastric juice activity, obtained in experiments on dogs, are presented. Precision of the modified Hunt's method recommends it both for scientific investigations and in clinical purposes.

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