A PHOTOELECTRIC METHOD FOR THE DETERMINATION OF PEPTIC ACTIVITY IN GASTRIC JUICE

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The need for a rapid, accurate method for the determination of peptic activity of gastric juice from clinical cases prompted the development of the method here reported. Procedures dependent upon chemical analysis, such as that of Anson and Mirsky,1 are too time-consuming, while the widely used clinical method in which Mett's tubes are employed requires incubation for 24 hours and as a rule is not sufficiently accurate.

In principle, the method depends upon the use of a substrate consisting of a standardized, homogenized suspension of coagulated egg white. When acted upon by pepsin, the turbidity decreases with time. It is assumed that the amount of protein digested in unit time is proportional to this decrease in turbidity, and since turbidity can be measured by means of a photoelectric colorimeter, the peptic activity of the system can thus easily be determined. Since peptic hydrolysis of protein follows a monomolecular course, and since within limits its velocity constant is a function of the concentration of pepsin, it is convenient to express peptic activity as a velocity constant.

EXPERIMENTAL

Substrate—A fresh hen's egg is boiled for 10 minutes and the coagulated white is washed free of membrane and yolk and mashed in a mortar. With 5 ml. of water added per gm., the mash is passed through a homogenizer2 several times. (With instruments of the Elvehjem type 2 drops of caprylic alcohol per 100 ml. may be needed to prevent foaming.) The volume is doubled with distilled water, homogenization is repeated, and the product is centrifuged at 1500 to 1800 r.p.m. for 15 minutes. After the removal of any surface film the supernatant homogenate is analyzed for total nitrogen and diluted to contain 0.5 mg. per ml. (which will usually mean approximately doubling the volume). Merthiolate is added to give a concentration of 1:10,000. Substrates thus prepared and stored below 10° have given reproducible results for 2 months.

2 An inexpensive, satisfactory instrument is carried by the Fisher Scientific Company, Pittsburgh, under the name of "laboratory homogenizer."
**Standard Turbidity Curve**—The relation of turbidimetric readings to protein concentration in the standard substrate is obtained as follows: With Filter 42 (blue-violet) for the Klett-Summerson instrument, a reading is taken of a 1:1 dilution of substrate in distilled water. With this as 100 per cent, the readings of eight to ten increasing dilutions are plotted against their respective percentage concentrations and a smooth curve is drawn through the points thus obtained. On the logarithmic scale of the Klett-Summerson apparatus the curve is nearly rectilinear, as shown in Fig. 1. If the same instrument is used, subsequent batches of substrate may be adjusted to give the same 100 per cent reading as the first, thus eliminating further nitrogen determinations. The original curve will also be found to apply.

**Buffer Solution**—This consists of 0.2 \text{n} hydrochloric acid and 0.2 \text{n} (or 0.067 \text{m}) sodium citrate in the proportion 4:1. When diluted with substrate and distilled water to the concentration used in the digestion mixture, the pH is approximately 1.6. Sodium phosphate and hydrochloric acid in suitable proportions can as well be used.

**Gastric Juice**—The specimens are centrifuged at about 1500 R.P.M. for a minimum of 5 minutes and the relatively homogeneous middle layer is used for analysis. Because of the appearance of particles of food following the use of most test meals and variations in gastric emptying time, it is suggested that fractional drainage following insulin or histamine stimulation be employed. One of the authors (B. C. R.) has obtained satisfactory
results using 1 unit of insulin per 10 pounds of body weight. Specimens are collected every 10 minutes for 50 minutes. The insulin is given intravenously and the test is concluded with the introduction through the stomach tube of about 100 ml. of a 50 per cent solution of Karo syrup, which has been found to counteract successfully any symptoms of hypoglycemia.

Specimens of gastric juice have been found to retain their activity without appreciable change for 12 hours at room temperature or for 48 hours if refrigerated immediately.

**Procedure**

A constant temperature water bath is set at 30° ± 0.1°. For preparation of the blank 1 ml. of the specimen is diluted with 4 ml. of the buffer and 5 ml. of distilled water and read in the colorimeter. Since the turbidity of gastric samples is often a function of pH, the buffer is added to reproduce the pH of the digestion mixture.

Two acid-cleaned tubes are set up, a colorimeter Tube A and a test-tube of similar size, Tube B. In Tube A are placed 5 ml. of the substrate and in Tube B are placed exactly 1 ml. of the centrifuged specimen and 4 ml. of the buffer. Both tubes are set in the water bath. When temperature equilibrium is reached (a minimum of 5 minutes), the contents of Tube B are poured into Tube A, the mixture into Tube B, and back to Tube A which is replaced in the water bath immediately. The time is taken at the beginning of the mixing, which should occupy 8 to 10 seconds. At 30 seconds the tube is placed in the colorimeter, read at exactly 1 minute, and replaced in the bath. It is again removed at 5½ minutes and read at exactly 6 minutes. If in the case of a very weak specimen the concentration of undigested substrate is reduced by less than 10 per cent, the tube is replaced in the bath and the final reading is taken at some multiple of 5 minutes after the first, such as 30 minutes. Time is measured to within 2 seconds, the outsides of the tubes being dried carefully before mixing and before each reading.

**Calculations**

Since under the conditions of the assay protein hydrolysis follows a monomolecular course, the equation expressing the velocity of the hydrolysis is \( \frac{dC}{dt} = kC \). \( C \) is the relative concentration of protein at the time \( t \) and \( k \) is the velocity constant, indicating the proportion of protein hydrolyzed per minute. On integration between two time limits \( t_1 \) and \( t_2 \) the velocity equation becomes \( k = \frac{1}{(t_2 - t_1)} \times 2.3 \log \frac{C_1}{C_2} \).

\(^2\) Generously supplied by Eli Lilly and Company.
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The two readings, after subtraction of the blank, are converted from the concentration curve to percentages of undigested substrate. If $C_1$ and $C_6$ are the values of these percentages at 1 and 6 minutes respectively, then the velocity constant is by substitution $k = 2.3/5 \log C_1/C_6 = 0.46 \log C_1/C_6$. For the longer time intervals used with weak specimens, the result is divided by the multiple of 5 minutes between readings.

DISCUSSION

That the monomolecular velocity equation is followed under the conditions of the assay is shown in Fig. 2. The concentration of protein in the standard substrate in the presence of 0.2 per cent of Merck's granular pepsin was determined every 30 seconds. The plot of the values of $2.3 \log C_0/C_t$ against time is rectilinear after the 1st minute. The slope of the line is $k$. The reasons for the non-linearity of the 1st minute of the curve are not known. It is possible that the substrate is not completely homogeneous and the rate of hydrolysis of the smallest particles may be governed by factors not operating in the remainder of the substrate. The mechanical process of mixing is also a probable factor, since the length of this initially rapid period is not materially altered by extreme dilution of the enzyme, and is varied widely by variations in the time and method of mixing. In

4 A nomogram converting the values of the two readings directly into $k$ values has been found convenient and may be obtained upon request from the authors.
practice, therefore, the initial concentration is measured at 1 minute. Furthermore, as might be anticipated, the curve falls off when observations are carried beyond 70 per cent of complete digestion. The determinations are therefore reliable only if completed below this limit, which has been the case with all human gastric samples thus far encountered. Fig. 2 also shows that within the limits defined above two readings will give sufficient accuracy for most purposes.

With a constant amount of enzyme the velocity constant of the reaction was found to increase rapidly with increasing dilution of the substrate. This was to be expected, since the substrate became relatively more saturated with the enzyme. The concentration specified in the method was selected independently of this fact in order to utilize the most accurate range of the colorimeter. Within this range, however, closely reproducible results have been obtained with variations up to 5 per cent in the substrate concentration.

The effect of inhibiting substances may be minimized by working with dilute solutions of the enzyme—indeed consistent determinations were made in the range of 1 to 10 \( \gamma \) of one commercial preparation of crystalline pepsin with a 24 hour observation period. However, the desirability of a rapid method led to the selection of a concentration of gastric juice such that the determination can usually be completed in 6 minutes. The variability under these circumstances remains within 3 per cent. The reproducibility with crystalline pepsin is illustrated by Table I.

It was found that a variation of about 0.3° produced a maximum variation of about 4 per cent in the velocity constants at approximately 30°. This

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**Table I**

Degree of Reproducibility Possible in Determinations on Same Solution of Crystalline Pepsin

<table>
<thead>
<tr>
<th>Determination No.</th>
<th>Velocity constant ( k )</th>
<th>Per cent deviation from mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0842</td>
<td>1.32</td>
</tr>
<tr>
<td>2</td>
<td>0.0824</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>0.0828</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>0.0824</td>
<td>0.84</td>
</tr>
<tr>
<td>5</td>
<td>0.0838</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean..............</td>
<td>0.0831</td>
<td>0</td>
</tr>
</tbody>
</table>

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\(^6\) Plaut Research Laboratories, Lehn and Fink Products Company, Bloomfield, New Jersey.
temperature was selected because it was found to lie in the range most conveniently controlled during the manipulations.

Variations in $k$ due to pH changes from 1.4 to 1.8 were found to be less than 5 per cent. However, a buffer is used, because, since the protein substrate is insoluble, it does not itself function as a buffer.

The concentration of an enzyme is usually expressed in terms of some arbitrary unit, for it is rarely possible to determine the concentration in terms of weight. Moreover, the conditions of the assay must also be definitely specified. We have selected the velocity constant of the monomolecular hydrolysis of a standard protein homogenate determined under strictly defined conditions as a convenient unit of peptic activity because it has certain advantages: (1) The velocity constant appears in the equation expressing the measured hydrolysis and hence requires no further definition. (2) It has physicochemical meaning, since it gives the proportion of the protein being hydrolyzed at any instant. (3) It is a function of the concentration of the enzyme. This is demonstrated in Fig. 3, where $k$ is plotted against increasing concentrations of pepsin. We found, however, that $k$ is not a rectilinear function of the concentration of crystalline pepsin. The almost exact coincidence of the two curves in this figure, although the dialyzable impurities have been removed in one preparation, seems to eliminate these impurities as an important factor in this connection. Our
results differ from those of Anson and Mirsky,¹ who by their method found a rectilinear relation between pepsin concentration and peptic activity. This non-linearity, however, is no real handicap to the use of the method for clinical purposes, since \( k \) still serves even at high levels as a sufficiently accurate measure of peptic activity.

**Table II**

**Gastric Analysis 30 Minutes after Intravenous Insulin Stimulation**

The ten patients were selected at random from the hospital wards, with no evidence of digestive disease. Acidity is measured by the glass electrode method.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>Color</th>
<th>Diagnosis</th>
<th>Gastric analysis</th>
<th>( \text{pH} )</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.</td>
<td>20</td>
<td>White</td>
<td>Orchiopexy</td>
<td>2.77</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>41</td>
<td>Negro</td>
<td>Inguinal hernia</td>
<td>2.30</td>
<td>0.114</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>35</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.14</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>35</td>
<td>&quot;</td>
<td>Knee injury</td>
<td>2.01</td>
<td>0.251</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>46</td>
<td>&quot;</td>
<td>Hemorrhoids</td>
<td>6.08</td>
<td>0.130</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>56</td>
<td>&quot;</td>
<td>Neurasthenia</td>
<td>1.89</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F.</td>
<td>52</td>
<td>Negro</td>
<td>Carcinoma of vulva</td>
<td>7.14</td>
<td>0.288</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>37</td>
<td>&quot;</td>
<td>Myoma uteri, preoperative</td>
<td>1.55</td>
<td>0.325</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>57</td>
<td>&quot;</td>
<td>&quot; postoperative</td>
<td>2.09</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>39</td>
<td>White</td>
<td>Finger infection</td>
<td>2.13</td>
<td>0.225</td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

**Fractional Gastric Analysis after Intravenous Insulin Stimulation**

The subject was a 43 year-old white male with a healed gastric ulcer, receiving no medication. The flow of juice ceased after 30 minutes.

<table>
<thead>
<tr>
<th>Specimen time</th>
<th>Volume</th>
<th>( \text{pH} )</th>
<th>Pepsin ( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>min. after insulin</td>
<td>ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>13</td>
<td>7.40</td>
<td>0.005</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>6.49</td>
<td>0.048</td>
</tr>
<tr>
<td>20</td>
<td>13</td>
<td>5.96</td>
<td>0.027</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

However, determinations made on the highly active juice from Pavlov pouches⁶ showed so rapid a decrease in turbidity that it was necessary to use 1 ml. of a 1:4 dilution of the specimen. Under these circumstances the \( k \) values varied from 0.15 to 0.30. Extrapolation of the curve in Fig. 3

⁶Kindly supplied by Dr. M. H. F. Friedman, Jefferson Medical College, Philadelphia.
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shows that these results cannot simply be multiplied by the factor of the dilution to be comparable with the $k$ values obtained from human specimens. This is a distinct limitation of the method, although if the same dilution is used in all specimens from a given source the results will be comparable for that source.

No attempt has been made to relate $k$ to units of measurement obtained by other methods. If such a relationship is desired, it could easily be obtained by comparison of an activity-concentration curve such as that of Fig. 3 with a similar activity-concentration curve obtained by any other method.

In the presence of regurgitated bile sufficient to give marked coloration, the substrate has occasionally been observed to flocculate during the digestion, making it necessary to repeat, or even discard the determination. The other most frequently met sources of error, in the order of their apparent importance, are variations in the time and method of the manipulations—particularly the mixing, cleanliness of the glassware, and errors in reading the colorimeter due to imperfect timing.

Tables II and III show the results of single analyses 30 minutes after insulin stimulation and one fractional analysis after insulin stimulation. These values are given merely as an orientation of the values that may be expected, and do not imply a level of normality. Further study on the clinical application of the method is being made by one of the authors (B. C. R.).

SUMMARY

A method for the estimation of peptic activity is described which combines rapidity with accuracy, and is particularly adapted to clinical studies.

Enzyme activity is measured photoelectrically as the decrease in turbidity of a standardized, homogenized suspension of coagulated egg white under specified conditions. Accurate measurement is possible down to a level equivalent to 1 $\gamma$ of crystalline pepsin.

Protein hydrolysis so measured follows a monomolecular course and hence the enzyme activity is expressed as a velocity constant.

The velocity constant is a curvilinear function of pepsin concentration and serves throughout the range encountered clinically as a convenient measure of the peptic activity of gastric samples.

Representative clinical results are given.
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