A MODIFIED METHOD FOR THE CLINICAL ESTIMATION OF PEPSIN *

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In the chemical examination of stomach contents attention has lately been directed anew to the estimation of the enzyme content of the gastric secretion, and a number of relatively simple methods of analysis have been proposed. Among these are the methods of Volhard,[1] Fuld and Levison,[2] Gross,[3] and Jacoby and Solms.[4] The Jacoby-Solms procedure appears to offer the greatest advantages, and has received favorable consideration for clinical purposes in this country from Goodman[5] and Einhorn,[6] and abroad from Witte[7] and others. The method involves the preparation of an imperfect solution of commercial "ricin" (prepared from the castor bean). This fluid is rendered more turbid by addition of a small amount of acid. Diluted gastric contents are added in varying quantity to a series of test-tubes containing the same volume of the turbid ricin solution; and after the total volumes of fluid are made alike in all the tubes they are allowed to stand in an incubator for three hours.[8] At the end of this time one examines the tubes to ascertain with what dilution the cloudy solution has cleared up, i. e., in which tube the suspended protein has just been completely dissolved by the enzyme present. The pepsin content of 1 c.c. of gastric juice which, in a dilution of 1 to 100, is just capable of carrying out the reaction as outlined, is expressed as 100 pepsin units. On this basis the enzyme content of variously diluted gastric contents can be calculated.

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* From the Sheffield Laboratory of Physiological Chemistry, Yale University.

1. Volhard: München, med. Wchnschr., 1903, L, 2129.
2. Fuld and Levison: Biochem. Ztschr., 1907, vi, 473.
3. Gross: Berl. klin. Wchnschr., 1908, xlv, 643.
4. Solms: Ztschr. f. klin. Med., 1907, lxiv, 159.
5. Goodman: Am. Jour. Med. Sc., 1908, cxxxvi, 734.
6. Einhorn: Berl. klin. Wchnschr., 1908, xlv, 1567; New York Med. Rec., 1908, lxxiv, 351.
7. Witte: Berl. klin. Wchnschr., 1907, xliv, 1338.
8. Einhorn (Berl. klin. Wchnschr., 1908, xlv, 1567; New York Med. Rec., 1908, lxxiv, 1338) uses a glass vacuum bottle instead of a thermostat for heating the tests. The vacuum bottle contains twelve graduated pepsin tubes showing marks at 2 c.c., 3 c.c., and 3.5 c.c. By this means he dispenses with pipettes. The whole apparatus is filled with water having a temperature of 50 or 60 C., well corked, and digestion allowed to continue for thirty minutes.

In principle the process really consists in obtaining a saline extract of the crude "ricin," containing sufficient globulin to yield an incipient protein precipitation on addition of very small quantities of acid. This behavior is in no way specifically characteristic of the proteins of the castor bean (*Ricinus*). The precipitation of most seed proteins by acid depends largely on the presence of inorganic salts in their solutions.[9] The selection of the so-called ricin for the pepsin test is an unfortunate one. The commercial product is not always readily available, although Jacoby has suggested a source of supply in Berlin. The purchasable preparations are crude mixtures of varying and inconstant make-up, containing considerable insoluble denatured protein (probably proteans). But the most serious objection lies in the extreme toxicity of castor bean products. This is shown in the studies of Osborne, Mendel, and Harris[10] with the purified protein ricin isolated by them from *Ricinus* seeds. Fractions of a milligram of pure ricin will kill a rabbit, and the necessity for care in handling such a product has not been properly emphasized.

Furthermore, true ricin is an albumin, as the researches mentioned have shown; and proteins of the globulin type are better adapted for the preparation of cloudy solutions. Indeed, the Jacoby-Solms reaction with castor bean proteins should properly receive some designation other than the "ricin test."

A globulin preparation well adapted for the purpose of the Jacoby test can be made cheaply from the ordinary garden pea, *Pisum sativum,* as follows: The finely ground peas, freed as much as possible from the outer coating, are repeatedly extracted with large quantities of 10 per cent. sodium chlorid solution, the extracts combined, strained through fine bolting-cloth, and allowed to stand over night in large cylinders to deposit insoluble matter. The supernatant fluid is siphoned off and saturated with ammonium sulphate. The precipitate of albumins and globulins is filtered off, suspended in a little water, and dialyzed in running water for three days, until the salt has been removed, and the albumins have been dissolved. The globulins are filtered off and washed two or three times with water to remove the last trace of albumins. To purify further, the precipitate is extracted with 10 per cent. sodium chlorid solution, and filtered until perfectly clear. The resulting solution is exactly neutralized to litmus paper by the cautious addition of dilute sodium hydroxid, and again dialyzed in running water for three

9. Cf. Osborne: The Vegetable Proteins, 1909.

10. Osborne, Mendel, and Harris: Am. Jour. Physiol., 1905, xiv, 259. Cf. also B. W. McFarland: Yale Med. Jour., 1910, xvi, 379.

days to remove the salts completely. The precipitated globulins are then filtered off, and dried on a water-bath at 40 C. During the complete process of separation the proteins should be preserved with a mixture of alcoholic thymol and toluene. The globulins so prepared dissolve practically completely in 10 per cent. sodium chlorid solution, and after slight acidification with hydrochloric acid yield a turbid solution, which does not settle out on standing.

Since in the Jacoby-Solms method the stomach contents are not neutralized, the estimations are not made under like conditions of acidity. It was thought advisable, therefore, to modify the method in this respect also, and to make all digestions in a solution whose total acidity is 0.2 per cent. of hydrochloric acid. By this means one determines not the digestive power of the gastric fluid, but the relative amount of pepsin present. The necessity of knowing whether or not the gastric juice in question is normal in respect to acid before diluting is also avoided.

METHOD

The complete method as modified follows: 0.25 gm. globulin of the pea, prepared as described above, is dissolved in 100 c.c. of 10 per cent. sodium chlorid solution (by warming slightly if necessary) and filtered.[11] Portions of the clear filtrate of 1 c.c. each are introduced into a series of eleven small test-tubes about 1 cm. in diameter. To each tube is added 1 c.c. of 0.6 per cent. hydrochloric acid, and about five minutes are allowed for the development of the turbidity. A measured volume of the stomach contents is then exactly neutralized to litmus paper with dilute alkali. If a precipitate of acid protein forms, this is filtered off, and the clear neutral solution is diluted a known number of times (usually five) with distilled water, allowance being made for the dilution of neutralization. A portion of the diluted juice is boiled and filtered, and amounts decreasing by 0.1 c.c. added to the tubes of turbid protein: to the first, 1.0 c.c.; to the second, 0.9 c.c.; to the third, 0.8 c.c., and so on to the eleventh, to which none is added. The unboiled juice is then rapidly added in increasing amounts as follows: to the first, none; to the second, 0.1 c.c.; to the third, 0.2 c.c.; to the fourth, 0.3 c.c.; to the fifth, 0.4 c.c.; to the

11. Such a solution will keep perfectly well for two months if covered with a thin layer of toluene. At the end of that time, acidification produces no more turbidity than when freshly prepared solutions are used. This avoids the objection raised against the Jacoby-Solms method by Farr and Goodman (THE ARCHIVES INT. MED., 1908, i, 648). These authors, while in general considering the method favorably, point out that solutions of commercial ricin which have been prepared for some time are rendered so much more turbid by the addition of acid that fresh solutions must always be employed.

sixth, 0.5 c.c.; to the seventh, 0.6 c.c.; to the eighth, 0.7 c.c.; to the ninth, 0.8 c.c.; to the tenth, 0.9 c.c., and to the eleventh, 1.0 c.c. Each of the tubes thus has a total volume of 3.0 c.c., and a total acidity of 0.2 per cent. of hydrochloric acid, as shown in the following scheme:

Tubes.	1	2	3	4	5	6	7	8	9	10	11
0.25 % globulin solution, c.c.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.6 % HCl, c.c.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Boiled gastric juice, c.c.	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0
Unboiled gastric juice, c.c.	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Total volume, c.c.	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total acidity, % HCl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

The measurements of the solutions may be easily and accurately made with a pipette of 1 c.c. capacity, graduated to 0.01 c.c. The tubes are well shaken and allowed to stand in a thermostat or water-bath for fifteen minutes at a temperature of 50 to 52 C. Exactly the same end-point is obtained by keeping the tubes at a temperature of 35 or 36 C. for one hour. At the end of the digestion time that tube in the series is selected which contains the least amount of gastric juice and which exhibits no turbidity. The peptic activity is calculated on the basis of the amount of gastric juice used in this tube. The enzyme content is expressed by the number of cubic centimeters of the 0.25 per cent. globulin solution that would be digested by 1 cubic centimeter of the undiluted gastric juice under examination, if the activity were exerted for a period of one hour at 35 or 36 C., or for fifteen minutes at 50 or 52 C. For example, if 0.5 c.c. of a gastric juice diluted five times clears up one cubic centimeter of the 0.25 per cent. globulin solution in fifteen minutes at the given temperature, the activity of the solution would be expressed:

$$\text{Peptic activity} = (1 \div 0.5) \times 5 = 10.$$

For clinical purposes it suffices to use the scale of pepsin units here proposed, which gives figures about one-tenth of those expressed on the Jacoby-Solms scale. Lately it has been found that six tubes ordinarily afford a range of trial which suffices for all diagnostic purposes.

SCHEME OF SIX TUBES

Tubes.	1	2	3	4	5	6
0.25 % globulin solution, c.c.	1.0	1.0	1.0	1.0	1.0	1.0
0.6 % HCl, c.c.	1.0	1.0	1.0	1.0	1.0	1.0
Boiled gastric juice, c.c.	1.0	0.9	0.7	0.5	0.2	0
Unboiled gastric juice, c.c.	0	0.1	0.3	0.5	0.8	1.0
Total volume, c.c.	3.0	3.0	3.0	3.0	3.0	3.0
Total acidity, % HCl	0.2	0.2	0.2	0.2	0.2	0.2

In the above method the conditions are constant in every trial, in respect to acidity, volume, protein content, and temperature. One determines the proteolytic activity of the gastric filtrate independently of the variations in acidity. Thus an additional variable of the original Jacoby-Solms procedure is eliminated. Goodman[5] concludes from a limited number of observations that uniform acidity makes no difference in the final result. Using the method here proposed, I am unable to accept these conclusions. Several series of tests showed that, while variations within the normal range of acidity produced no difference in the final results, large decreases, such as occur pathologically, have a marked influence on the rapidity with which the turbid solutions are cleared. The results of two typical series of tests, one made with artificially prepared gastric juice, and the other with normal human gastric juice, are shown in Table 1.

TABLE 1.—TESTS WITH HUMAN AND ARTIFICIAL GASTRIC JUICE

——Artificial Gastric Juice——		——Human Gastric Juice——	
Acidity.	Pepsin Content.	Acidity.	Pepsin Content.
0.4 % HCl	..	0.4 % HCl	10
0.2 % HCl	14	0.2 % HCl	10
0.1 % HCl	14	0.1 % HCl	10
0.05 % HCl	18	0.05 % HCl	15

It will be seen that the enzyme content is expressed by much larger numbers when the total acidity is 0.05 per cent. of hydrochloric acid than when it is 0.2 per cent. This is not due to greater activity of the pepsin under such conditions, but to the decreased turbidity of the globulin solution, resulting from the decreased acidity. Less enzymolysis is necessary for the clearing of the solution, since the cloudiness is so much less intense. When the acidity is further decreased, the loss in peptic activity far overbalances the loss in turbidity, with the result that much smaller figures are obtained.

In the Jacoby-Solms method the total acidity in the tubes is frequently as low as 0.05 per cent. of hydrochloric acid. In many pathological conditions, such as pernicious anemia, gastric carcinoma, gastric diseases of children, etc., the acidity is practically or absolutely *nil.*[12] If the unmodified ricin method is employed in such cases, the acidity in the tests will be only that resulting from the addition of the 0.5 c.c. of tenth-normal hydrochloric acid, used to render the protein solutions turbid. Errors in analysis will then probably occur. Indeed, Witte[7] found, using

12. See Willcox (Quart. Jour. Med., 1909, iii, 93-106), for a review of the literature on acidity of the gastric juice in various diseases.

the Jacoby procedure, that the enzyme activity was much reduced by neutralizing the samples of stomach contents before making the determinations. Nirenstein and Schiff[13] and Roth,[14] in studying the composition of the gastric secretion under various conditions with the Mett method, also recognized the importance of the hydrochloric acid content on the enzymatic activity. They state that for comparable results all estimations must be made under like conditions of acidity.

TABLE 2.—RESULTS OF ANALYSIS MADE BY PROPOSED MODIFICATION

Sample.	Diagnosis.	Peptic Activity.	Remarks.
1	Normal.	10	
2	Normal.	10	
3	Normal.	10	
4	Normal.	9	
5	Normal.	11	
6	Practically normal.	10	
7	Practically normal.	10	
8	Practically normal.	10	
9	Practically normal.	10	
10	Constipation.	9	Acidity very low.
11	Hypoacidity.	10	
12	Nervous hypoacidity.	10	
13	Nervous hypoacidity.	10	
14	Hyperacidity.	12	
15	Gastritis.	10	No free hydrochloric acid.
16	Neurasthenia.	10	Very low acidity.
17	Neurasthenia.	10	No free hydrochloric acid.
18	Gastric ulcer.	12	Very high acidity.
19	Ulcer of duodenum.	10	High acidity.
20	Gall-stones.	3	
21	*Gastric carcinoma.	2	
22	Gastric carcinoma.	1	Young man, 26 years old.
23	*Gastric carcinoma.	Trace.	No free HCl. Rennin-negative.
24	Gastric carcinoma.	Trace.	
25	*Carcinoma of intestine.	3	Young woman, 24 years old. No free HCl.
26	Carcinoma of esophagus.	2	
27	*Cancer of the rectum.	10	
28	*Cancer of the breast.	2	Total acidity, 42. No free HCl.

* Diagnosis verified by operation.

13. Nirenstein and Schiff: Arch. f. Verdauungskr., 1902, viii, 559.
14. Roth: Ztschr. f. klin. Med., 1900, xxxix, 1.

In the tabular scheme (Table 2) the results of analysis made by the proposed modification are summarized. The gastric contents were withdrawn after an ordinary Ewald test meal.[15]

It will be noted that the "normal" peptic activity on the scale here proposed is about 10, corresponding with the figure 100 on the scale of Jacoby and Solms. No attempt has been made to select cases illustrative of the incidence of peptic activity in various diseased states. The characteristic low figures in carcinoma are typically shown.

The possible presence of an antienzyme should be taken into consideration in making an examination of the gastric secretion. Oguro[16] has shown that blood-serum contains an antipepsin, which may be demonstrated by heating the neutralized gastric juice with the serum at body temperature. It is advisable, therefore, before estimating the proteolytic activity, to make a test for blood in stomach contents containing little or no free hydrochloric acid.

I wish to express my gratitude to Prof. Lafayette B. Mendel, at whose suggestion and under whose guidance the study was made, for much helpful advice.

15. Most of the specimens were furnished by Dr. L. M. Gompertz, instructor in gastroenteric diseases, who made the clinical diagnosis in each case.

16. Oguro: Biochem. Ztschr., 1909, xxii, 266-277.