ON THE RELATIVE VALUE OF DIFFERENT PEPSIN TESTS.

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The gastric juice, secreted by the peptic glands of the stomach, contains two important principles, viz., hydrochloric acid and a ferment called pepsin.

By the combined action of these two principles it performs its physiological function, which consists in digesting albuminoid matters, *i. e.*, it disintegrates, dissolves and converts them into soluble, non-coagulable products, known as peptones.

Wasmann first isolated pepsin in 1839, but it must be said that his process, as well as those which have since been proposed for the preparation of this article, do not furnish perfectly pure pepsin. The product obtained is always more or less mixed with extractive matter, mineral matter and peptone, sometimes in considerable quantity, which our present knowledge of the art has not enabled us to completely remove. Perfectly pure pepsin is, therefore, not known, but we are nevertheless enabled to prepare products of very high digestive power.

Many methods have been proposed for testing pepsin, but none of them are perfectly satisfactory.

Thus, Bidder and Schmidt place a known weight of small cubes of coagulated white of egg in contact with a liquid containing a known weight of pepsin, dissolved in hydrochloric acid of 0.2 per cent. strength, and heat the mixture for about five hours at 45° C. At the end of this time the non-dissolved albumen is washed and weighed. The loss in weight of this albumen indicates the digestive power of the pepsin.

This method of assay has the inconvenience of taking into account only the amount of albumen dissolved, without paying attention to the amount of albumen really converted into peptone. The same applies also to the two following methods.

Grünhagen allows fibrin to swell in hydrochloric acid of 0.2 per cent. strength, and places the thick jelly thus obtained in a funnel,

closed at one end with a little glass wool, and exposes the whole in a drying oven to a heat of 45° C. After all excess of water has drained off, a certain number of drops of a solution, containing the pepsin under examination, is allowed to trickle upon the jelly contained in the funnel. In about two minutes the liquid will begin to drop from the funnel, and the rate of dropping in a given time is said to be proportional to the strength or activity of the pepsin.

P. Grützner determines the value of pepsins by allowing flocks of fibrin, stained with carmine, to digest under similar conditions, and then estimating colorimetrically, at the end of a certain time, the intensity of the coloration of the liquid separated by decantation from the non-dissolved fibrin, by comparing it with a certain number of standard solutions of carmine. It is very easy to stain fibrin evenly with carmine, and, therefore, the intensity of the coloration of the solution is proportional to the amount of fibrin dissolved by the pepsin.

The methods I propose to discuss in this paper are three, viz. : the U. S. P. test, the Manwaring test and the Kremel test.

According to the experiments of numerous investigators, the peptic digestion of albuminoids depends upon several conditions.

1. The temperature.

The pepsin of fish acts energetically at 20° C., but the pepsin of mammals requires a higher temperature, and it has been found that peptonization is most active between 35° C.–50° C. Above this, digestion runs much slower and ceases totally towards 70–80° C.

2. The quantity of pepsin.

There being no such thing as absolutely pure pepsin, it has been impossible to determine, with accuracy, the amount of albumen which can be converted into peptone by a given quantity of the ferment. We know only that the amount is very large, provided that from time to time a little acid and water is added in order to maintain a certain degree of dilution.

The quantity of albuminoid which can be digested in a given time increases rapidly with the quantity of pepsin employed till it reaches a maximum, and then decreases slowly. The quantity

of peptone finally obtained increases with the proportion of pepsin.

3. The quantity of water.

As the products of digestion accumulate, the rate of peptonization gradually decreases. The addition of a fresh quantity of acidulated water causes the peptic action to recommence until it has reached a certain limit, beyond which the reaction ceases entirely.

4. The nature and quantity of the acid used.

A large number of acids may take the place of hydrochloric acid in peptic digestions, but none of them are as efficient as the latter. A. Mayer found that with the use of hydrochloric acid, complete peptonization occurred in from 3 to 5 hours; with nitric acid in about 5 hours, with oxalic acid in 13 hours, and with sulphuric acid in 19 hours.

According to Brücke, peptonization is already very active in a medium containing only 0.8 pts. of hydrochloric acid per 1,000, and attains its maximum, with a concentration of 1 pt. of acid in 1,000 of water. A too large proportion of acid hinders peptonization, 7 pts. of acid per 1,000 of water being sufficient to make the action very slow. Mayer thinks that the most favorable proportion of acid is 2 pts. per 1,000 water, or 0.2 per cent.

5. The time of action.

6. The variety and character of the albumen.

One of the most largely used tests in this country is the U. S. P. test, which reads as follows:

"One pt. of saccharated pepsin dissolved in 500 pts. of water, acidulated with 7.5 pts. of hydrochloric acid, should digest at least 50 pts. of hard boiled egg albumen, in 5 or 6 hours, at 100–104° F. (37.5–40° C.)"

The above test seems simple, but, in reality, it is unreliable and misleading, as no two persons using the same pepsin can obtain the same or even approximate results; it is, therefore, not surprising that we meet with such a diversity of conclusions.

The weak points in the above test are the following:

1. The test is based upon the amount of albumen which can be dissolved in a given time (including peptone and intermediary products), but does not take into consideration the

amount of peptone actually formed, and this I claim to be of the greatest importance.

2. It directs that a given pepsin shall digest at least 50 pts. of coagulated albumen. Now, in order to determine how much albumen has actually been dissolved, it is necessary to use an excess of albumen, and then weigh what remains undissolved. The test in question does not specify how much albumen shall be used, but leaves it entirely to the option of the experimenter. I consider this to be a weak point, as it makes quite a difference whether only a small or large quantity of albumen is used.

3. It is difficult to see how accurate results are to be obtained by weighing the amount of undissolved albumen remaining after a digestion, because it is impossible to find two samples of coagulated albumen, which contain exactly the same quantity of moisture; and besides this, the quantity of moisture is very liable to vary during the weighing, owing to the loss of moisture by evaporation.

4. It is not stated how long the eggs shall be boiled! This is a very important matter, as digestion differs greatly according to whether the eggs are boiled for a short or a longer time.

5. No provision is made for the size of the pieces of coagulated albumen. This, also, is very important, as it has been found that the greater the surface of the albumen exposed to the peptic ferment, the greater will be the amount of albumen digested.

6. This test applies only to saccharated pepsins, and no provision is made for other brands of pepsin.

It will, therefore, be seen that the U. S. P. pepsin test is absolutely unreliable and misleading.

Lately my attention has been called to a new pepsin test, which I will designate by its author's name, the "Manwaring test." In this test Manwaring has tried to avoid as much as possible the bad points of the U. S. P. test; but in doing this he has stumbled against other sources of error which I will try to make clear further on.

The test can best be described in the words of its author:

"The design of the following mode of testing the dissolving power of pepsin is to conform as nearly as possible to the U. S. P. test, which, contemplating the testing of the saccharated form,

makes no provision for the proportion of acidulated water to be used with a pure pepsin.

"On the basis that 1 part of a pure pepsin is capable of dissolving 1,000 times its weight of coagulated egg albumen in 6 hours, a saccharated pepsin made with a pure pepsin of U. S. P. strength would contain 5 per cent. of pure pepsin; therefore if 1 grain of a U. S. P. *saccharated* pepsin is to be tested in the presence of 500 grains of acidulated water, then 1 grain of a pure pepsin should be tested in the presence of 10,000 grains acidulated water, to equal the same proportion of water and acid used for the *actual* quantity of pure pepsin contained in a U. S. P. saccharated pepsin when tested according to the U. S. P."

In order to render the weighing of small quantities of pure pepsin as easy as possible to the pharmacist, Manwaring recommends that it should be saccharated, and for this purpose he gives the following recipe:

R. Saccharated pepsin, consisting of:

Pure pepsin ..... 1 grm.
Milk sugar ..... 19 "

To make the test take of the above saccharated pepsin 0.3 grm. (= 0.015 grm. pure pepsin).

Coagulated egg albumen ..... 22.5 grms.
Acidulated water consisting of:
Distilled water 100 c.c.
Hydrochloric acid U. S. P. 1.25 } ..... 154 c.c.

The eggs are to be boiled for 15 minutes and the whites pressed (by means of a spatula) through a (preferably flat) 30 mesh sieve. For the sake of uniformity, the egg whites should be cut into small pieces and thoroughly mixed before being passed through the sieve.

The mixture should be maintained at 100–105° F. for six hours, and agitated thoroughly about every half-hour.

At the end of six hours the temperature of the bath should be quickly run up above 145° F. to destroy the pepsin, then the bath with contained bottles allowed to remain undisturbed over night, that the undissolved albumen may settle.

If the test bottle has been kept securely corked during the test, or if by previously weighing bottle and contents and afterwards

making up with water any loss from evaporation, the quantity of albumen dissolved may be easily determined as follows :

From the settled contents of the test bottle pipette off 10 c.c. and evaporate to dryness—until weight is constant—in a watch glass. From this dry residue figure as follows (1 pt. of pepton or intermediate products representing 1 pt. of original albumen):

Suppose 10 c.c. of the liquid = 0.2 grm. dry residue ; $\frac{7}{1.4}$ times its weight = the quantity of water contained in the 10 c.c. that was derived from the albumen dissolved ; 10 c.c. of liquid less 1.4 c.c. of water leaves 8.6 c.c. water taken from the 154 c.c. of acidulated water in making the test.

1.6 grms. or 8 times 0.2 grm. dry residue = the quantity of albumen in its natural state as originally used, that has been dissolved in the 10 c.c. of liquid evaporated to dryness.

Therefore, if 8.6 c.c. acidulated water holds 1.6 grm. egg-albumen,
then 154 c.c. " " " 28.5 " "

Then, as 0.015 grm. pepsin dissolved 28.5 grms. coagulated egg-albumen, 1 pt. would dissolve 1900 times its weight.

The use of the multiplier 7 and 8 is based on the fact that egg-albumen averages 12½%, or $\frac{1}{8}$ dry.

As will be seen, this test is quite a departure from the U. S. P. test, and in some respects is an improvement upon the latter, but I object to it on several points, viz.:

1. It makes no provision for other than concentrated, or, as Manwaring calls them, *pure* pepsins, while in reality the number of these is small compared to the saccharated pepsins.

A person desiring to assay a saccharated pepsin by this test, would be at an utter loss to know how much pepsin should be weighed out, or how much acidulated water should be employed, unless he knew that the pepsin under examination was of U. S. P. strength.

This cannot be ascertained without submitting the sample to a complete chemical analysis, and hence a great deal of trouble and erroneous results are apt to ensue.

I have encountered this very trouble myself, as may be seen from the following figures :

A sample of pepsin, purchased as a pure pepsin (pepsin C in the tables), but which in reality was a saccharated pepsin, gave by this

method 420, *i. e.*, it is supposed to dissolve 420 times its weight of coagulated albumen.

Not knowing the strength of this saccharated article in pepsin, I was at a loss to know how much should be used in the test, and therefore decided to look upon it as a concentrated pepsin. In consequence of this decision on my part the pepsin was saccharated according to M.'s directions, and 0.3 grm. of this resaccharated pepsin used in the test, with the above result. The result obtained undoubtedly does the pepsin injustice, for it is probable that if I had not resaccharated the original article, and consequently diluted it to a much greater extent, that a much higher test would have been recarded.

The same applies also to the following pepsins, which were in the saccharated condition when bought and were resaccharated by me before testing.

Brand of Pepsin.	Grs. Albumen dissolved in six hours.
Pepsin A	550.6
" B	516.6
" C	420.
" D	398.6

2. We are further instructed to raise the temperature of the mixture after six hours' digestion up to and above 145° F. to destroy the peptic principle, and then the bath, with contained bottles, is allowed to rest over night. It has been shown by numerous experiments that the peptic principle is not killed at this temperature (145° F. = 62.2° C.), but that on the contrary digestion may and does continue up to 80° C., though of course much more slowly than at a lower temperature. If, therefore, a bottle containing a quantity of undissolved albumen be subjected to a temperature of 62.2° C., and that only for a comparatively short time (or during the time that the water bath is cooling), it is manifest that the peptic principle would not be injured, and that consequently digestion will progress all night. We should thus obtain a much too high and an erroneous result.

3. The next step in the process is to pipette off 10 c. c. from the settled contents of the test bottle, and evaporate to constant weight. Although I have found that as a rule the undigested albumen remaining in the test bottle settles pretty well over night,

yet there is always a little left suspended in the liquid, which, if pipetted off, as it must be, would add to the weight of the dry residue obtained from the 10 c. c. pipetted off, and hence would be a new source of error.

4. We are next directed to evaporate the 10 c. c. to dryness, and in doing this the residue chars or carbonizes, owing to the free hydrochloric acid contained in the water, so that in reality we weigh a certain amount of carbonaceous matter, instead of peptone, etc. Does this represent the amount of coagulated albumen dissolved by the pepsin? It may, but I strongly doubt it.

5. Assuming that the dry residue had not charred, what would we then be weighing? We would be weighing a mixture consisting of undigested albumen, partially digested albumen, and fully digested albumen, or peptone, together with all the intermediary products which always are to be met with in peptic digestions. Does this represent the efficiency of the pepsin, or is it not more likely that the amount of true peptone formed is an indication of the strength of the pepsin? The latter, in my opinion, is the more plausible explanation, especially if it be remembered that only the peptone is assimilable in the human system, while the intermediary products are not. In short, if we let the weight of all the products formed in a peptic digestion represent the efficiency of a pepsin, we would be obtaining a result considerably above what it ought to be.

6. As far as the calculations involved in this test are concerned, I would say that they are very ingenious and eminently fitted to make a pepsin test as high as possible. I will not dispute the accuracy of the multipliers 7 and 8, but do not believe that their accuracy is infallible in every test.

As this test was particularly gotten up to determine the digestive power of concentrated pepsins, I therefore append tests made by myself upon two such pepsins.

Pepsin E.—One grain of this pepsin was found to digest 819.2 grains coagulated egg albumen in six hours.

Pepsin F.—One grain of this article was found to digest 784 grains of coagulated egg albumen in six hours.

I have found by personal experience that the accumulation of peptone during peptic digestions hinders and finally stops the

action of the pepsin upon the albuminoid matter entirely. If now the liquid be diluted, digestion will recommence again and proceed until the pepsin has become inert. (I do not believe in the theory that one grain of pepsin can go on digesting to infinity.)

As Manwaring lays particular stress upon this question of dilution, I think his test is a decided improvement over the U. S. P. test.

The next good point in his test lies in the fact that he does not attempt to weigh the undigested albumen, as is done in the U. S. P. test, and thereby does away with a great source of error; but instead of this he figures the amount of albumen (?) digested upon a dry basis, and then tries to convert this dry basis by calculation into albumen on the wet basis. In doing this errors are apt to occur, as I have already pointed out, but I do not think that they are errors of such magnitude as are apt to be obtained with the U. S. P. test.

Finally, I wish to say a few words about a test which I consider to be the only approach to an accurate method of testing pepsin that I know of. I do not claim that this test is absolutely accurate either, as slight errors are apt to occur, which, however, do not materially injure the final result. I refer to the Kremel test, which was published some time since in the Druggists' Circular.

In devising this test Kremel has made a radical departure from the usual methods, and bases his test upon the fact that under the conditions in which artificial peptic digestions take place, pepsin alone has the property of converting albuminoid matter into peptone, and that, therefore, from an analytical as well as from a physiological standpoint, the only correct method is to take the quantity of peptone produced as a gauge of the action of the pepsin; or in other words, the test is made to resemble as nearly as possible the conditions existing in the natural process.

Without going into any further detail, the test is made as follows:

One grm. of egg albumen (soluble) dried at 40° C. and pulverized, and 0.1 grm. of the pepsin to be tested, are placed into a 100 c.c. flask, and dissolved in 50 c.c. of 0.2 per cent. hydrochloric acid. The solution is heated to 38–40° C. for three hours, and then exactly neutralized with sodium carbonate; it is then

heated on a water bath to 90° C., and cooled after coagulation has taken place. The flask is then filled to the mark with distilled water, and 50 c. c. are filtered off and evaporated to dryness in a platinum dish on a water bath.

The residue is dissolved in hot distilled water, filtered through a moist filter into a platinum dish, and the filter carefully washed. The solution is again evaporated to dryness and weighed. The peptone is then incinerated with ammonium carbonate, and the weight of the ash deducted leaves the weight of the pure peptone, or the representative of the digestive power of the pepsin.

The good qualities of the above test are the following :

1. Simplicity.

2. No guesswork, troublesome calculations or the use of questionable factors.

3. No weighing of albumen dissolved in hydrochloric acid, undigested albumen and intermediary products along with the peptone. This is all obviated by the use of soluble egg albumen, coagulation and filtration or removal of the undigested portion as detailed above.

4. The ease with which it is possible to duplicate and still obtain concordant results.

On the other hand, the objections to this process are the following :

1. The great difficulty of procuring absolutely pure soluble dried egg albumen. This source of error, however, in my opinion, is very slight, because in each test a large excess of albumen is always used, and consequently the pepsin always has enough albumen to act upon. Besides this it must be remembered that only the peptone formed is weighed, and not the amount of undigested albumen, as is the case with the U. S. P. test.

2. It may be objected to this test that the results obtained are expressed by the weight of peptone formed and not by the weight of albumen dissolved, and consequently the figures, being based upon dry peptone, will be much lower than when the result is expressed as so much moist or coagulated albumen. If this, however, be objected to, it is comparatively easy to obtain higher figures by a simple calculation. Assuming that the amount of dry peptone obtained is equivalent to so much dry albumen, then by

multiplying the weight of the latter by 8 (Manwaring's multiplier) we would obtain the equivalent in coagulated or moist albumen. I do not think it necessary or advisable to follow this course, as it involves the use of a multiplier which, as already pointed out, is questionable.

3. It takes a little longer to make a test by this process, but if accuracy is thereby gained the process is to be preferred.

To further illustrate the test, I append the following results obtained with commercial pepsins:

	Peptone formed from 0.1 grm. pepsin in 3 hours.
Pepsin G	0.5844
" E	0.4972
" B	0.4722
" F, crystal	0.4682
" C (saccharated)	0.4676
" H	0.4598
" A (saccharated?)	0.4370
" A (saccharated)	0.4246
" D plain, soluble	0.3470
" D pure, scales	0.3250
" D pure, another sample	0.3146
" I (saccharated)	0.2780
" J French	0.1848
" K (saccharated)	0.1738

These tests were all made with the same quantity of pepsin, whether the latter was saccharated or not, and I think are a fair indication of the relative values of the different pepsins.

It may be objected that this test does not do a concentrated pepsin full justice, on the ground that the latter would form a much greater proportion of peptone and thus retard if not completely arrest any further action of the pepsin upon the albuminoid matter.

In order to test this question, I saccharated samples of E, F and H respectively, according to Manwaring's directions, which is equivalent to diluting with mere acidulated water, and submitted them to the same conditions as before and obtained the following results:

	Peptone formed from 0.1 grm. pepsin in 3 hours.
Pepsin E	0.2620
" F	0.1240
" H	0.1250

It will be observed that in these tests the figures are considerably lower than in the former ones; but it must be remembered that the pepsins with which the tests were made were twenty times weaker, or rather more diluted, than in the previous tests, and notwithstanding this the peptone formed is proportionally larger than before. This would clearly show that dilution is beneficial in the case of concentrated pepsins, as it corrects the retarding action of peptone. As the dilution in these last tests was twenty times greater than in the previous ones, we ought, by multiplying each of the above results by twenty, to obtain the amount of peptone which would be formed by using the pepsins in their concentrated forms, viz.:

	Peptone that should be formed from 0.1 grm. concentrated pepsin in 3 hours.
Pepsin E	5.240
" F	2.480
" H	2.500

The above figures are not, however, obtained as has already been shown, and therefore the calculation is erroneous.

As all the results obtained by strictly following Kremel's directions are comparable among themselves, I do not see how the process can well be improved upon.

The mere fact that increased dilution increases the yield of peptone is not, in my opinion, sufficient reason for condemning the process. As the conditions prevailing in the stomach of a full grown man do not differ materially as to dilution from day to day, it is safe to say that pepsins of varying strength administered to such a person will only perform a certain amount of work and no more, and that, consequently, the results obtained by this test more closely resemble the conditions prevailing inside the stomach than any other.

In conclusion, it will be seen that all the tests mentioned in this paper are subject to faults and imperfections, some having more than others; and, therefore, all we can do under the present unsatisfactory state of affairs is to select the one which is least objectionable, and this, in my opinion, is the Kremel test.