Pharmacists have successfully utilized this colorimetric test in their window displays of olive oil—one piece of apparatus showing a negative test on the brand of oil on display while another exhibited a positive test on some spurious brand. Since the heat may be removed a few minutes after the tubes are placed in the hydrocarbon oil, this apparatus lends itself well for window display purposes. The color is quite permanent. No change is perceptible in months.

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PEPSIN—METHODS USED FOR ITS QUANTITATIVE ESTIMATION—ITS PERMANENCE AND EFFECTIVENESS IN SOME OF ITS PREPARATIONS.*

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The oldest accurate and carefully recorded and carried out methods[1] for the quantitative estimation of pepsin, are those described by Bidde and Schmidt[3] in 1852. In the three-fourths of a century since, progress has been made in the quantitative estimation of the activity of this ferment, but it has been a progress in the art of manipulation and in the improvement of mechanical devices used rather than an advance in the understanding of the exact mechanism of the reactions involved in the process. Since the official or U. S. P. IX method for the quantitative estimation of pepsin has brought forth much criticism and many suggestions for its improvement, it was thought that time might well be spent in a study of the processes in an endeavor to improve the present method of assay. The older methods will be discussed more briefly and then a report of our success with them and also with the more recent ones suggested in the literature will be taken up.

At least two[1 & 2] excellent and comprehensive critical reviews of pepsin assay methods are to be found in the literature. Neither of these includes discussions of the more recent edestin methods (Fuld's and Brewster's), the recent modification of the electrolytic method[9] suggested by Northrup[20] or of the U. S. P. IX method and its development. Any method which requires more time for preparation and carrying out than the U. S. P. IX method does, with no advantage over it as to accuracy or reliability, need not be considered. This eliminates the Mett method which requires from twelve to twenty-four hours, Volhard's casein precipitation method which Geselschap[23] characterizes as "cumbersome and not particularly reliable," and the former U. S. P. method which required six hours for complete digestion. The Grützner and other colorimetric methods are not nearly as accurate as the U. S. P. IX method. With the Grützner carmine-fibrin method we have been able to detect only a 6 : 5 relationship between two solutions actually having a 2 : 1 relationship by the official test.

All the methods of the Jacoby, Solms, Fuld, Brewster "soluble protein" type

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* The authors wish to express sincere thanks to the many pepsin manufacturers who by their kind coöperation have aided in this investigation.

† Work done as holder of Stearns Fellowship for 1920–21, in partial fulfillment of the requirements for the Degree of Master of Science.

are open to the same difficulty, namely, that of obtaining a sharp end-point. Edestin offers the advantages over ricin of being non-poisonous and forming a clear solution in $\frac{N}{12}$ HCl. The pepsin assay method in which it is used is carried out somewhat differently by Brewster[21] than by Fuld.[14] The latter directs the preparation of four solutions: A, Normal 0.033 HCl; B, 0.1% solution of edestin in Normal 0.033 HCl; C, a standard pepsin solution; and D, 10% sodium chloride solution. Ten small test tubes containing from 0.1 to 1.0 Cc., inclusive, each of solution B and corresponding amounts of A to produce a constant amount of liquid are kept 30 minutes at room temperature and then 1 Cc. of the 10% NaCl solution added. If the strength of pepsin has been properly adjusted some of the tubes will be clear and some cloudy, the latter being due to the precipitate of unpeptonized edestin. It is, however, practically impossible to determine where turbidity ceases, even under the best of light conditions, and insofar as the minimum variation due to inability to discern cloudiness is 10%, it is quite evident that the method is far from being comparable to the U. S. P. method which permits of detection of a 3% discrepancy.

In the Brewster[21] modification a somewhat different procedure is used. Here the edestin is precipitated by a 10% sodium chloride solution and the agglutinated mass is digested by the pepsin—a process essentially different from the Fuld modification in that it may be classed, with the action of pepsin or coagulated egg albumen as in the U. S. P. IX method, as a typical peptonization process in which the dispersity of the precipitate is increased and which involves surface tension and adsorption phenomena, while in the Fuld modification we have a reaction taking place in solution, a peptonization.

We carried out the method as outlined by Brewster in his article using two different pepsins, but so diluted in solution that each liquid contained the equivalent of 1 Gm. of 1 : 3000 pepsin per 100 Cc. The bath was kept at 37.3° C. to 37.5° C. The results obtained were as follows:

TABLE I.

Tube Nos.		1	2	3	4	5	6
1% Edestin	Cc.	0.25	0.50	0.75	1.00	1.00	1.00
$\frac{N}{10}$ HCl	Cc.	0.75	0.50	0.25	0.00	0.00	0.00
10% NaCl	Cc.	1.00	1.00	1.00	1.00	1.00	1.00
1% Pepsin	Cc.	1.00	1.00	1.00	1.00	1.00	1.00
		TIME ELAPSED IN MINUTES.					
Pepsin No. 1		5.0	17	26	44	44	Undissolved
Pepsin No. 2		5.5	22	40	46	Undissolved	43

Thus in tubes No. I there is a difference of 10%, where there should be equality; in tubes No. 2 a difference of $\frac{22-17}{17}=\frac{5}{17}=29.4\%$, in tubes No. 3 a difference of $\frac{40-26}{26}=\frac{14}{26}=55\%$, and practically no difference in the 6 tubes (3 for each pepsin) containing equal quantities of all materials. It will be noticed, however, that 2 of these were totally useless for purposes of a comparison because the edestin had clumped so badly that it remained insoluble indefinitely. This clumping is not a very infrequent occurrence in our experience.

TABLE II—USING 0.1 CC. INCREMENTS AND DECREMENTS.

Tube Nos.	1	2	3	4	5	6	7	8	9	10
1% Edestin	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
$\frac{N}{10}$ HCl Cc.	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0
10% NaCl Cc.	1.0	throughout								
1% Pepsin Cc.	1.0	throughout								
	TIME ELAPSED IN MINUTES.									
Pepsin No. 1	3.5	6.0	6.5	8.5	12	15	20	28	43	45
Pepsin No. 2	5.0	10.0	9.0	8.0	23	23	35	44	44	63

From Table II it will be seen that the discrepancies referred to above are still larger than in Table I, since in either case solutions of identical peptic value were used throughout, and equal results should be expected. We find it very difficult to determine just when all the precipitate has dissolved and that the time required for solution varies not only with different operators, but with the same operator at different times. In tubes Nos. 8, 9 and 10 this discrepancy may amount to from 5 to 10 minutes. Our results lead us to believe that this method cannot be relied upon as an assay method for pepsin, even as a rapid comparison method using a pepsin standardized by the U. S. P. IX method as a control or standard of comparison, and of course not to replace the present official method.

Pepsin, like rennin, has the power of curdling fresh cows' milk. It will curdle milk with a rapidity proportional to the amount of pepsin present, but though it is possible to distinguish between pepsins related in proteolytic power in the ratio of 3 : 4 or sometimes even 4 : 5 by this method, it is not very reliable and not capable of greater delicacy.

We have also tried out the method recently outlined in detail by Northrup[20] and suggested by M. Oker-Blom[9] about twenty years ago. It is based on the fact that as pepsin and egg albumen interact in a solution the electrical conductivity of the latter changes in proportion to the amount of proteolysis taking place, that is, in such a determination the change in resistance divided by the time in minutes required to produce the change should give a factor directly proportional to the activity of the pepsin and amount of it used.

Our procedure was as follows (Bath adjusted to 37° ± 0.05°): The set-up did not include the Vreeland oscillator as suggested in Taylor and Acree's paper referred to by Northrup, but was the ordinary whetstone bridge set-up used in conductivity work, with microphone hummer and telephone. Into the conductivity cells were placed 25 Cc. portions of a 3% solution of egg albumen, and the liquid permitted to take the temperature of the bath. Then 2 Cc., 3 Cc. and 4 Cc. portions of a pepsin solution were added to the separate cells, and bridge readings taken with resistance adjusted to give middle of the bridge readings. The time of each reading was noted, and a table such as the following made out:

TABLE III.

Time.	Bridge reading.	Bridge reading difference.	Time difference.
3.34	.5015		
3.38	.5000	15	4
3.43	.4960	55	9
3.50	.4895	120	16
4.05	.4845	170	31

TABLE III (*continued*).

Time.	Bridge reading.	Bridge reading difference.	Time difference
4.15	.4805	210	41
4.24	.4790	225	50

$$\text{Factor} = \frac{225}{50} = 4.50$$

TABLE IV.

Time.	Bridge reading.	Bridge reading difference.	Time difference.
3.41	.5060		
3.46	.5050	10	5
3.55	.5000	60	14
4.02	.4965	95	21
4.11	.4935	125	30
4.19	.4900	160	38
4.29	.4875	185	48
4.46	.4835	225	65

$$\text{Factor} = \frac{225}{65} = 3.46$$

TABLE V.

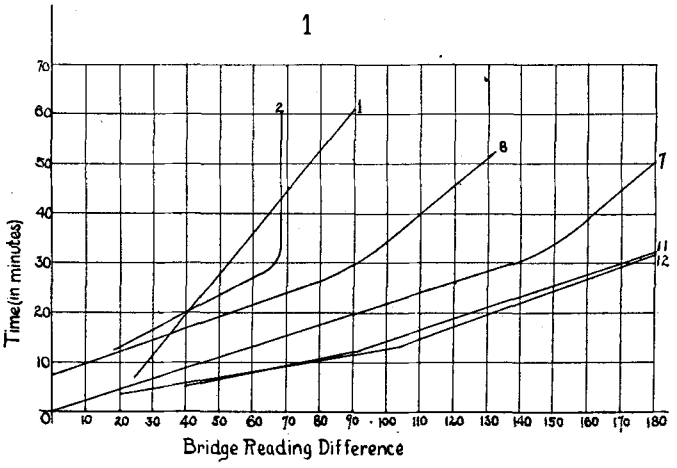
4.34	.5015		
4.40	.4985	30	6
4.43	.4960	55	9
4.48	.4930	85	14
4.54	.4900	115	20
5.25	.4800	215	51

$$\text{Factor} = \frac{215}{51} = 4.21$$

It will be noticed that the relationship of the factors obtained in Table III and Table IV is 3.46 : 4.50 or quite close to 3:4, the relationship of the amounts of pepsin used, but that the factor of Table 5 which should be about 2.25, because only 2 Cc. of pepsin solution was used, is 4.21. On two trials carried out identically where the relation should have approximated 1:1, we obtained a 4.37 : 4.41 relation, a very creditable result. An attempt to repeat this 1:1 relation in another test gave the relation 3.44 : 3.85, and in another case 2.95 : 3.43. Such results are typical of the many we obtained, so that while the method does sometimes give the true relationship existing it can by no means be relied upon. One feature of the method for which we have no practical use possibly at present, is the means it gives us of watching the rate of change taking place during the peptonization. By plotting the bridge reading difference against the time elapsed (column 3 against column 4 of the tables) we get a graphic representation of the progress of the reaction. (Chart I.) As Northrup points out the ideal curve is one approximating a straight line but we have not found this type of curve recurring with any great regularity. The accompanying graph shows some curves obtained.

Curves Nos. 1 and 2 were obtained from two identical samples, their factors are 1.47 and 1.11, respectively, and the curves are quite evidently not superimposable. Similarly 7 and 8 are from identical samples and although more concordant than

1 and 2 are also not superimposable. Curves 11 and 12 also are from identical samples and almost superimposable. Their factors are 4.4 and 4.7, respectively.



It was thought that by using a purified substitute in place of ordinary egg albumen considerable of the irregularity of the experiment might be eliminated, but the use of both edestin and purified egg albumen[29] showed no improvement over the use of ordinary egg albumen. It seems that the fundamental difficulty will not be eliminated until we thoroughly understand the "biologic" factor in the action of pepsin.

The official U. S. P. test seems to be a purely American method. We have not been able to find mention of it in the English or German literature. Its earliest forerunner seems to have been the "Manwaring" test introduced into the U. S. P. in 1890. The time of digestion was six hours and a 1:3000 pepsin was one which digested 3000 times its weight of freshly coagulated and disintegrated egg albumen in that time at 37° C. It was later discovered by Petit that the optimum temperature for pepsin digestion is about 50° C. and investigation of the constancy of egg albumen brought out that seemingly the albumen of eggs between 5 and 12 days old or more especially 5 to 7 days old[28] is less unconstant than that of eggs of other ages, so that these standards have found their way into the process.

In an effort to determine the variation in moisture content of the coagulated albumen of eggs of various ages with the intention of ascertaining the relation, if possible, between the ease of digestion and moisture content, we carried out about 200 moisture determinations (desiccated at 55–57° C.) on the albumen which was used in our assays, with using eggs from 1 day to 20 days old, and also storage eggs. Our results would indicate that the moisture content of the former is irregular and not at all constant, while that of storage eggs may often be a little less than that of the one to twenty day old eggs. On the other hand, the average storage egg albumen seems more easily digestible than that of a 5 to 12 day old egg, while that from eggs less than 5 days old often leaves noticeably large residues by the official method, that is, it is less easily digestible.

The especially designed, tapered, graduated sedimentation tube and the two and one-half hour time limit with 1 Cc. albumen residue are the later improvements of the method. Various suggestions have been made to standardize and make the method uniform, such as the official method of disintegrating the sieved albumen with a rubber-tipped glass rod, and more recently, the suggestion of

pounding a bottle containing the albumen a given number of times on a suitable pad. One excellent means of producing a uniformly disintegrated albumen mass is to pass the albumen through a No. 80 sieve after having first passed it through a No. 40 sieve, and to use a heavy (1 Cm.) glass rod with flattened end to produce disintegration. The resulting mixture (after the gradual addition of 0.3% HCl) contains the albumen more uniformly and highly dispersed and like an emulsion, than when it is run through a No. 40 sieve only. It is never lumpy and always uniform, although, of course, it requires additional labor. As far as we have been able to observe, the use of this modification does not produce either more rapid or more complete digestion although undoubtedly a much greater surface of albumen is subjected to the action of the pepsin. Evidently other more important factors than that of surface are involved. We find that passing the albumen through a No. 40 sieve is sufficient, but believe the flattened heavy glass rod superior to the rubber-tipped rod of the U. S. P. IX for disintegration. In order to obtain uniformity of results in such an assay method as this, it is of the greatest importance that a uniform technic be adhered to throughout.

The official method has been much criticized, usually for one or more of the following reasons:

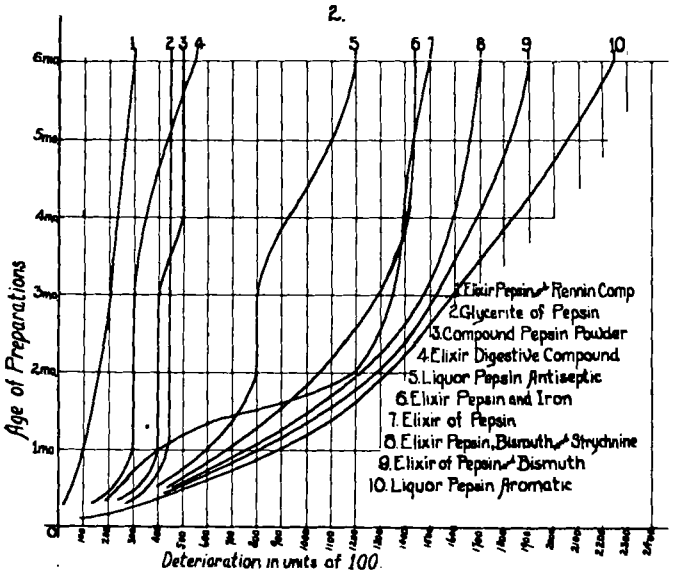
1. Results obtained by it are not always consistent and reliable.
2. To obtain eggs of the specified age is a decided objection and a handicap to the method.
3. The method is too long and cumbersome.

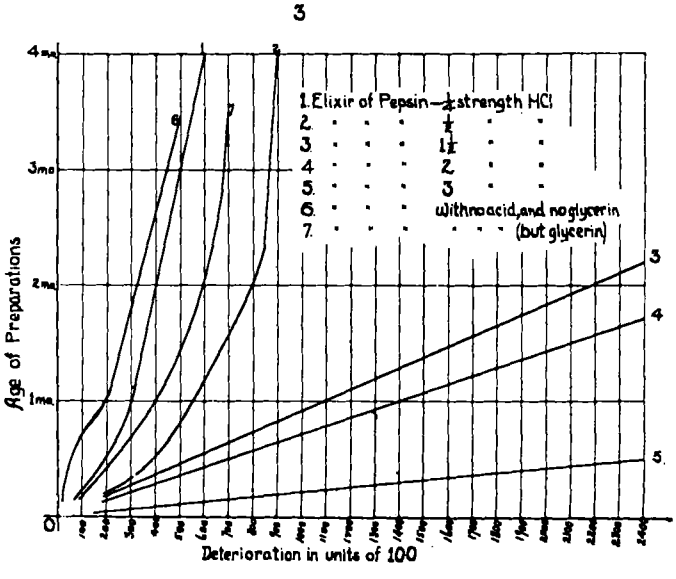
These criticisms will be discussed in this order.

We have found a method of procedure with the test which has given us reliable and consistent results, to be as follows: For pepsin in any form dissolve 0.1 Gm. in 150 Cc. $\frac{N}{12}$ HCl as the U. S. P. IX directs. Of this add 5.00 Cc. to the egg albumen prepared in the usual manner and suspended in $\frac{N}{12}$ HCl (it is assumed that the pepsin tests in the neighborhood of 1:3000, if not, preliminary tests covering the suspected range must first be run) and proceed in the usual manner (U. S. P. IX). Run six or more of such identical bottles side by side in the same bath. As a rule, after setting out in the sedimentation tubes, the amount of albumen residue varies anywhere from 0.5 to 2.0 or even 3.0 Cc. If six tubes are run discard those showing non-concordant residues, add up all the others and divide by their number. If this gives an average residue between 1.00 and 1.25 Cc. we would call the pepsin 1:3000. If the residue runs higher than 1.25 Cc. we would repeat with another six tubes at 1:2800 or 1:2900 as the amount of residue indicated, and if lower, at 1:3100, etc. A single determination showing a 1.00 Cc. residue really means nothing and two such but little more, and only if four or more tubes show 1.00 Cc. each of residue (a very unusual check) would we be willing to say a pepsin was of the value indicated by the strength run which gave these four checks. We believe it to be much more rapid and equally sure, to use the method of "Averages" outlined above. It has given excellent and consistent results.

A pepsin manufacturer called to our attention a comparative method which we have found to give good results and which obviates the necessity of continuous use of 5 to 12 day old eggs. It is first necessary to establish a "known" or standard pepsin of a convenient strength, let us say, 1:3000, by running a considerable

number of tests on it with fresh eggs by the official method. This is then used as a control when using albumen from ordinary storage eggs (as good a quality as is obtainable is used). Three to five control tubes containing the known 1: 3000 pepsin are placed at intervals throughout the bath and the average albumen residue left by them is used as a basis for calculating the value of the unknowns in the bath. Let us say the residue in the 1: 3000 standard averages 1.80 Cc. Then, if that of the unknowns averages the same or less they too are 1: 3000 or better, respectively. For example, in one test we ran two different known pepsins each close to 1: 3000, ten bottles each. The mean of the lowest seven residues was in one case 1.97 Cc. and in the other 1.83 Cc., a discrepancy of but 0.14 Cc., easily attributable to a slight difference in strength. This method, then, offers one means of solving the fresh egg difficulty, partially at least. We have also been working with commercial powdered egg albumen with the intention of preparing it for use in place of the albumen of storage eggs in this comparative method. If used in this manner it would not be necessary to standardize the commercial albumen in any way, nor would it matter if it varied in nature due to differences in source, because the method is purely comparative. We will have to await a later date for publication of more complete results on this work.





That the U. S. P. IX method is time-consuming and cumbersome there is no doubt. It has been suggested that the amount of albumen residue at the end of the test be increased and the time reduced from two and one-half hours to two or even one and one-half hours. There is a decided objection to this. The pepsin and albumen do not interact with the mathematical regularity and velocity of acid and alkali or oxidizing and reducing agents. Sometimes they react rapidly immediately after mixing, again it may take an hour or more before very vigorous action sets in. Hence the longer the time of the test the more chance for complete reaction and accurate results. Two and one-half hours seems to be a satisfactory length of time for good results. It has been our experience that two men, well versed in the technic of the method, without any mechanical device for turning bottles can easily run fifty to sixty bottles in an eight hour day. With a mechanical inverter they would have two and one-half hours for other work.

MEDICINAL PEPSIN PREPARATIONS.

The accompanying graphs Nos. 2 and 3 contain a list of some of the liquid and powder pepsin preparations which we have assayed at monthly intervals for several months with the total deterioration which they have shown. It will be noticed that all these preparations show more or less deterioration after being made up. We have also assayed a considerable number of similar preparations put on the market by various manufacturers, and find that in them, too, deterioration takes place. Our observation of these latter has only extended over four months, so that although they have shown somewhat less deterioration than the corresponding ones of our own, manufactured according to official formulas, they show no exception to the rule that the preparations of pepsin in general use for medicinal purposes do deteriorate considerably after manufacture.

Perusal of literature reveals a long list of substances which inhibit peptic activity. Aromatics and antiseptics of coal-tar origin are prominent among these. Corresponding to this our chart shows that Aromatic Solution of Pepsin N. F. IV, manufactured with enough 1:3000 pepsin to have assayed 1:3000 after manufacture, had dropped to 1:1000 after seven months, or a loss of 1:2000. The bismuth and iron (heavy metal) preparations show a similar deterioration. Elixir of Pepsin also contains aromatics, though in rather small quantities, and likewise the Antiseptic Solution of Pepsin. Elixir Digestive Compound N. F. III, the source of so much discussion in the past, shows comparatively little deterioration in peptic activity, although the same preparation lost seven-eighths of its diastasic value in three months. The other three preparations, Compound Pepsin Powder, N. F. III, Elixir Pepsin and Rennin Compound N. F. IV and Glycerite of Pepsin N. F. IV showed the least deterioration.

Just why pepsin in solution should gradually lose its activity, while in the dry forms it is comparatively permanent, it is quite impossible to answer definitely. E. Buchner,[25] toward the end of the last century, pointed out that cane sugar was an inhibitant, which may be an important factor here, for considerable sugar is present in almost all of these preparations. Pepsin in its dry forms, as scales, granulations, etc., we have found to be fairly permanent, losing very little activity over a period of years. Considerable difficulty is had by manufacturers of pepsin, however, because of the claims of the chemists that their pepsin is 100 to 200 or more

units below the labeled strength. This may, in the opinion of the authors, often be due to moisture taken up by the product when left in an unstoppered container. Pepsin is hygroscopic. We have found that the average samples of dry pepsin lose from 8 to 10% of their weight when kept in a desiccator over sulphuric acid for a month. This loss corresponds to a 1:300 loss in activity on a 1:3000 pepsin.

The question of proper acidity of pepsin preparations has always been a much-discussed one. It has been definitely established that pepsin cannot act in an alkaline medium. Schütz[26] has shown that free hydrochloric acid is not absolutely indispensable to peptic digestion and also that an energetic peptic digestion can take place with a noticeable hydrochloric acid deficit. We have it from such authorities as Hawk, Effront-Prescott, and Mathews that the strength of hydrochloric acid varies with: (1) The types of pepsin, that is, a certain concentration which may be optimum for pepsin from a hog's stomach is not so for that from a dog's or calf's stomach. (2) It varies with the protein acted upon, for example; 0.08 to 0.10 percent for fibrin and 0.25 percent for coagulated egg white (Hawk). Other figures are given by Effront.[27] (3) It varies with the degree of coagulation of the albumen.[27] Hence there cannot be such a thing as a *constant* optimum hydrogen ion concentration even for the same pepsin, also that the U. S. P. directions regarding the length of time for boiling the eggs is to be followed to the letter. In order to determine the keeping qualities of various preparations differing from the official Elixir of Pepsin in acidity and glycerin content only we made up those enumerated on Chart 3. beginning with an Elixir Pepsin containing only one-fourth of the quantity of hydrochloric acid of the official preparation. From our tabulated results, it would seem that this first preparation at the end of four months has more superior keeping qualities regarding its peptic activity than the official preparation containing four times as much acid, although we are not as yet able to say how rapid the deterioration will be from this point on.

In assaying any of these preparations a quantity of the preparation was taken which represented 0.1 Gm. of 1:3000 pepsin and sufficient $\frac{N}{12}$ hydrochloric acid was added to it to make 150 Cc., just as directed in the official method for pepsin in the dry form. 5 Cc. of this liquid should leave a 1 Cc. residue at the end of the process. Similarly, if run at 1:2900 we would use 5.17 Cc., at 1:2800 5.35 Cc., etc.

With the bismuth preparations a difficulty is encountered upon diluting 11.8 Cc. of either of the bismuth elixirs to 150 Cc. with $\frac{N}{12}$ hydrochloric acid in that a white precipitate of a basic bismuth compound is obtained. To assay the preparation one has the choice of three methods: (1) to shake it up and pipette off a portion, precipitate and all; (2) to filter and use the clear liquid only; (3) to just dissolve the precipitate by the cautious addition of 30% HCl. The first two methods give very irregular results, often hardly any albumen being dissolved at all; the last method is quite satisfactory though at times it seems that upon addition of the mixture to the albumen, when ready for digestion, the bismuth causes trouble again and we get a somewhat cloudy mixture, which seemingly, however, does not interfere with the test. Naturally an objection to this procedure presents itself in that, after the addition of concentrated HCl as suggested,

it would be expected that the resulting liquid would be far above the optimum in acidity for peptic digestion. In order to determine just how acid the resulting liquid was, that is, 11.8 Cc. Elixir Pepsin and Bismuth and 138.0 Cc. $\frac{N}{12}$ HCl plus a sufficient number of drops of concentrated HCl (usually 8 to 10 drops or 0.5 Cc. are sufficient), we titrated several such portions with $\frac{N}{12}$ KOH, using phenolphthalein as indicator. The mixture always required between 175 Cc. and 190 Cc. of $\frac{N}{12}$ KOH, while it should have required about 150 Cc. $\frac{N}{12}$ KOH, giving an acidity of about 0.35% HCl instead of about 0.30%. Now it must be remembered that only about 5 to 10 Cc. of this 0.35% HCl liquid are used in the assay and are added to about 35 Cc. of the $\frac{N}{12}$ HCl used to suspend the albumen, so that finally the pepsin in the digestion mixture is acting in a medium containing quite close to 0.30% HCl.

It also occurred to us to use distilled water instead of $\frac{N}{12}$ HCl to dilute the 11.8 Cc. of elixir to 150 Cc., for then no precipitate is obtained. Then a portion of this clear liquid is used. We have found that this procedure gives a larger number of cloudy mixtures when we get ready to set the digestion mixture out in the sedimentation tubes, and that the results are not as concordant as with the method suggested above where concentrated HCl is used. This is possibly due to a precipitation of the bismuth compound upon addition of the liquid to the digestion mixture and subsequent interference with the action of the pepsin by occlusion or some similar phenomena, or possibly to a too low acidity, because here the diluted pepsin preparation is very low indeed in acidity since water was used for dilution instead of $\frac{N}{12}$ HCl.

Besides the sample of Elixir Pepsin and Bismuth made up for assay over a period of months, we also made up two other separate samples, one of which, No. 1, was assayed on the seventh and eighth days after making; the other, No. 2, was assayed a sufficient number of times within the twenty-four hours immediately after it had been prepared to determine its strength. No. 1 (older sample) assayed 1: 2700 after eight days, and No. 2 assayed 1: 2800 within three hours after it was made up. As an example of the basis for our conclusions on figures obtained we submit the following Table VI.

TABLE VI.

Date.	No. of bottle.	Name of preparation.	Strength run.	Cc. of preparation used.	Albumen residue.	Remarks.
6/9/21	1	Elixir Pepsin and Bismuth	1:3000	5.00	1.70	clear
	2	of 6.2.21	1:2750	5.45	1.20	clear
	3		1:2500	6.00	0.80	clear
	4		1:2250	6.67	0.25	clear
6/10/21	2	same	1:2500	6.00	0.80	clear
	10		1:2500	6.00	0.70	clear
	11		1:2500	6.00	1.00	cloudy
	12		1:2500	6.00	0.60	clear
6/11/21	2	same	1:2700	5.55	0.95	clear
	7		1:2700	5.55	1.00	clear
	8		1:2700	5.55	0.95	clear
	9		1:2800	5.35	1.60	clear
	10		1:2800	5.35	1.70	clear

We should consider such results sufficient evidence for saying that this preparation on the last date given ran approximately 1:2700 and insofar as we were sure that the amount of scale pepsin used in its manufacture should have required it to assay about 1:3000 under these same conditions, we should say that a 10% loss in peptic value had occurred in the preparation in about one week's time.

Similarly the fresh preparation, No. 2 mentioned above, showed that the loss in peptic activity in this combination was 1/15 or about 6 $^{2}/_{3}$% immediately after making.

Finally, regarding our experience with the two pepsin-bismuth elixirs of the N. F. IV which were assayed over a period of seven months we found, as our chart No. 2 shows, that both preparations lost about 67% of their peptic value in seven months.

CONCLUSIONS AND SUMMARY.

1. The U. S. P. IX assay method for pepsin is both the most rapid and most reliable method at our command, and the edestin method (and its type using ricin, pea globulin and similar proteids) and conductivity methods are not reliable enough to supplant it.

2. The storage-egg modification of the official method is a reliable modification.

3. We have suggested a method for overcoming a difficulty in the assay of pepsin preparations containing bismuth.

4. From our results, showing deterioration in peptic value of manufactured pepsin preparations, we conclude that it would be to the manufacturers' advantage to strongly fortify such preparations with pepsin in considerable excess of the value as declared on the label.

5. That the moisture content of egg albumen is quite independent of the age of the egg and that ease of digestion of the albumen must be dependent on other factors.

6. A "standard" or "control" pepsin should be kept in containers (air-tight) of such a size that their contents will be completely dispensed before having taken up any considerable amount of atmospheric moisture.

7. We would suggest that the pepsin preparations containing bismuth, iron, aromatics, or antiseptics in the proportions found in the above official preparations be extemporaneously prepared only, and that it should not be expected that they retain their peptic activity as long as the other preparations.

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MODIFIED METHOD FOR DETERMINATION OF BISMUTH IN MAGMA BISMUTHI—BISMUTH MAGMA—MILK OF BISMUTH U. S. P. IX.

BY M. CRANE AND E. C. MERRILL.

Milk of Bismuth, U. S. P. IX, pages 260 and 261, calls for an assay requirement under the following method:

Assay—Evaporate to dryness about 10 Gm. of Bismuth Magma, accurately weighed, and ignite the residue to constant weight; the residue corresponds to not less than 5.6 percent nor more than 6.2 percent of the weight of Bismuth Magma taken.

This method of assay may be modified by use of Gooch method, by means of which speed of assay may be increased without affecting in any respect the accuracy of the determination. Such method is as follows:

Prepare a Gooch crucible in the regular manner, and place inside of a regular porcelain crucible, about size O. Dry and weigh both together. Pour about 20 Gm. of Magma into the Gooch and weigh again. Put the Gooch on a suction funnel and filter out the water. Then place Gooch on a pipe-stem triangle and heat up slowly. Finish at full heat of Bunsen. Cool in desiccator and weigh with regular crucible, which has been dried in the meantime.

The object of using the additional crucible is to hold the water that drains through the Gooch while weighing. This method cuts the time of assay to about one-third of the time required by the U. S. P. method and at the same time checks it.

Results given as follows:

Gooch method.		U. S. P. method.	
Gooch + Crucible + Magma	43.8408	Crucible + Magma	18.3955
Gooch + Crucible	23.7545	Crucible	8.3230
Weight taken	20.0863	Weight taken	10.0725
Gooch + Crucible + Bi_2O_3	24.9273	Crucible + Bi_2O_3	8.9090
Gooch + Crucible	23.7545	Crucible	8.3230
Bi_2B_3	1.1728	Bi_2O_3	0.5860
	5.85%		5.83%