solution. Digestion is performed for 20 minutes at 37° C, at the end of which time the pepsin is inactivated by the addition of 2 cc of .4 N NaOH and incubation for 30 minutes at 37°C. Thereafter, 4 cc of the inactive mixture is pipetted into a colorimeter tube and 20 cc of 6% sulfosalicylic acid is added. After 15 minutes the optical density is read in the photoelectric colorimeter with a No. 420 filter.

Since an aliquot of ⅓ is taken for the albumin determination, the mg albumin digested as obtained from Fig. 4 are multiplied by 3 to obtain the actual milligrams of pepsin digested.

When the mg albumin digested is plotted against the concentration of crystalline pepsin on semilogarithmic paper, a straight line is obtained. The range of this test is from 5 to 2000 γ. The antilogs of 0 to 3.5 would cover this range without distortion so that the antilog of the mg albumin digested unmultiplied by 3 is used to convert to pepsin equivalents.

It will be noted that the range of this procedure is much smaller than that utilizing egg albumen. Hence it may be occasionally necessary to use a greater initial dilution than is indicated. Although its range is smaller its sensitivity is correspondingly greater.

The digestion pH for this test has been found to be 1.75 as determined by the glass electrode.

To assay juice of extremely low peptic power it is more accurate if half concentrated albumin is used for the substrate.

*Summary and Conclusion.* The disadvantages and inaccuracies of many tests for pepsin have been discussed. A method for determining total pepsin which does not embody these disadvantages is described. Egg albumen is used as substrate and a photoelectric colorimeter used as a densimeter is employed to determine the amount of albumen solution digested. An alternative method of greater sensitivity is also described utilizing purified bovine and human serum albumin. Little technical skill is required and the execution of the tests is exceedingly rapid.

Thanks are expressed to Dr. William H. Fishman for his aid in the preparation of this manuscript.

## 15568

## Active Pepsin Fraction, Resting Peptic Activity, and Percentage of Pepsin Inhibition in Gastric Juice.*

HARRY H. LEVEEN. (Introduced by Dallas B. Phemister.)

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Total pepsin is only an index of the secretory activity of the pepsinogenic cells and gives no indication of the digestive action *in vivo* as modified by inhibitory substances and pH. Because of the importance of pepsin in experimental ulcer formation[1,2] one should know the quantitive effects of both inhibitory substance and pH.

Total pepsin can be assayed only after high dilution of gastric juice, whereupon it is completely freed from inhibitory peptones. If gastric juice were assayed in its undiluted state, only that portion of pepsin uncombined with inhibitor (active pepsin fraction) would be measured.

If digestion is carried out at different pH's, a correction chart for varying digestion pH's can be constructed. By this means, the actual digestive power of the juice can be obtained after ascertaining the pH of the gastric juice.

The digestive action of juice corrected for pH and inhibitor substances has been designated resting peptic activity, and is a measure

* Done on a grant from the Otho S. A. Sprague Memorial Institute.

1 Schiffrin, M. J., and Warren, A. A., *Am. J. Dig. Dis.*, 1942, **9**, 205.

2 Driver, R. L., Chappell, R. H., and Carmichael, E. B., *Am. J. Dig. Dis.*, 1945, **12**, 166.

of the digestive power which the intestinal mucosa is resisting.

*Preparation of Albumen Substrate.* One hundred grams of dried egg albumen is dissolved in 300 cc of distilled water with frequent agitation over a period of hours. Merthiolate is added as a preservative. The insoluble material is cleared by filtering for 24 hours or by centrifugation.

*Technic for Performance of Test.* Three cc of albumen solution, ½ cc of .25 N HCl and 1½ cc of gastric juice, in order indicated, are pipetted into a test tube. After shaking, the mixture is incubated for 30 minutes at 37°C. Pepsin is inactivated at the end of the digestion period by the addition of 1 cc of N/2 NaOH, and, after shaking, incubation for 30 minutes. Three cc of the mixture is then diluted to 50 cc with N/150 NaOH. Five cc of this dilution is pipetted into an Evelyn colorimeter tube and 19 cc of 6% sulfosalicylic acid is added. After 15 minutes, the density is determined in the Evelyn photoelectric colorimeter. Because only 5 mg of the original 100 mg of albumen are taken for determination, the density curve previously obtained may be used. The mg albumen digested are obtained by subtracting from 5 mg, the possible total. Since an aliquot of 1/20 was taken for determination the result is multiplied by 20.

*pH of Digestion Mixture.* It is well nigh impossible to obtain adequate buffering by the usual methods; therefore, the pH of the digestion mixture is taken in each determination with a glass electrode. The mixture for pH determination is prepared as for digestion with the exception that boiled gastric juice is used (active digestion disturbs the pH readings).

*Effect of pH upon Digestion.* If digestion is performed at different pH's, a curve is obtained relating pH to the percentage of digestion at pH 1.75 which is chosen as 100% (Fig. 1). By means of this graph all results are corrected to pH 1.75 which is the digestion pH of the test for total pepsin.

*Effect of Low Dilution on Digestion and Interpolation to Undiluted State.* If the square root of the milligrams albumen digested per cc of gastric juice (after correction to pH 1.75) is plotted against dilution, a

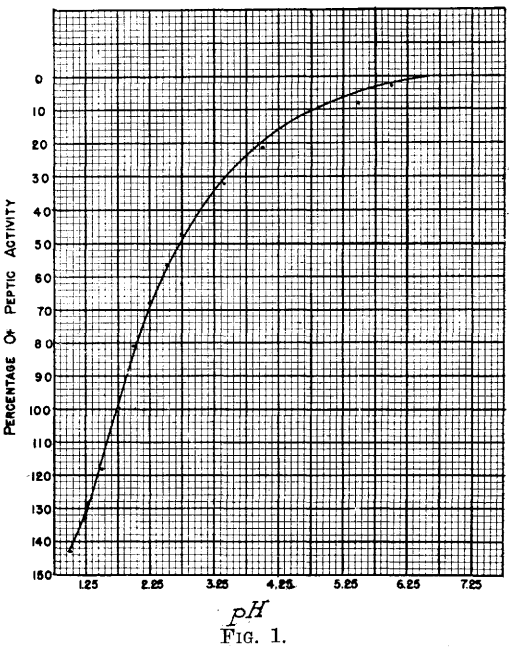


FIG. 1.

The percentage of egg albumen digestion as compared to pH 1.75 (which has been taken as 100%) is represented on the ordinate. The pH of the digestion mixture is represented on the abscissa.

linear relationship is obtained (Fig. 2). This relationship holds up to a dilution of 1:5 in resting juices 8 to 12 hours old, but only up to 1:4 or 1:4.5 in the fresh juice of active secretion. It becomes feasible, therefore, to extrapolate to the point of no dilution without the introduction of a sizable error.

The relationship presented is such that an increase of one in dilution increases the square root of the mg albumen digested by one. To extrapolate to point of no dilution from 3.33 dilution, subtract 2.33.

*Calculation of Peptic Power.* The actual calculation becomes as follows: mg albumen digested as obtained from the chart is multiplied by 20 and corrected to pH 1.75 using Fig. 1 and then divided by 1.5 (number of cc used for test). From square root of the result, 2.33 is subtracted. This final figure squared represents the number of mg albumen digested per cc of undiluted gastric juice at standard digestion pH of 1.75. To obtain the "power" of the juice at its own pH, the latter figure is adjusted to that pH by use of Fig. 1.

*Conversion to Pepsin Units.* The results

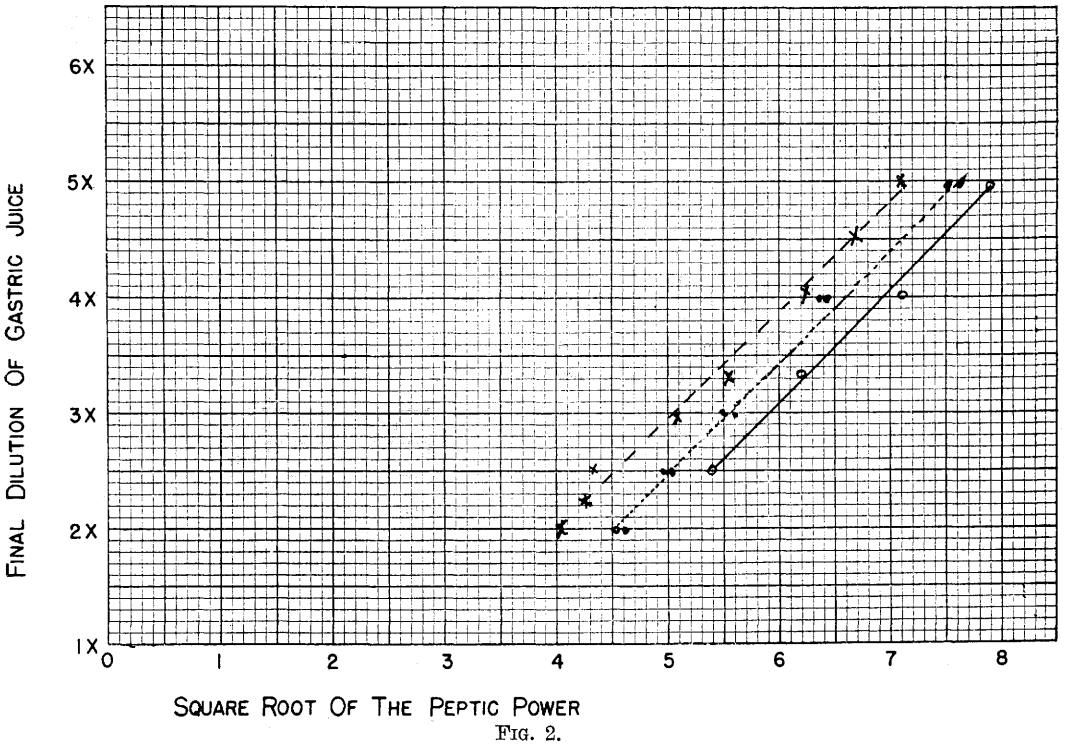


FIG. 2.

The square root of the milligrams of egg albumen digested per cc of gastric juice is plotted against dilution. A linear relationship is observed only in low dilutions (*i.e.*, not over 1 to 5). Interpolation to zero is done by simply extending the straight line to 1x which represents the undiluted state. The relationship is such that on all 3 different specimens plotted, an increase in dilution of 1 increases the square root of the milligrams albumen digested by one.

may be expressed in pepsin units so adjusted that the tests for total pepsin and active pepsin fraction are comparable. One can, thereby, determine that percentage of the total pepsin which is normally inhibited.

Determinations made with crystalline pepsin in both tests show that 2.5 times the enzyme concentration in the test using concentrated substrate produce 20 times the amount of digestion (Fig. 3). Hence, if the mg albumen digested in the concentrated test is divided by 20 and the antilogarithm of this result divided by 2.5, pepsin units for the active fraction and resting peptic activity are obtained which are comparable with the test for total pepsin.

It should be understood that conversion of the resting peptic power to pepsin units is an artificial comparison and that really the action of pH is on the substrate and not on the enzyme. It represents a comparable activity which would be obtained if the enzyme concentration were reduced and the pH remained constant.

*Percentage of Pepsin Inhibited.* Subtracting the active fraction from the total pepsin content (both being in identical pepsin units) yields that fraction of pepsin which is inactive. This latter figure divided by the total pepsin concentration and multiplied by 100 yields the percentage of pepsin inhibited.

*Sources of Error.* The sources of error are the same as in the test for total pepsin, with the exception that dissolved mucin in alkaline gastric juice can produce more major differences in nephelometric readings. This necessitates an occasional blank determination which is performed by pipetting .05 cc of gastric juice into 5 cc of distilled water and adding 19 cc of sulfosalicylic acid.

The sulfosalicylic acid must always be added to the albumen solution (not vice versa), and preferably at the same relative rate; otherwise large errors in the nephelo-

metric readings may occur.

*Alternative Method Using Crystalline Protein.* Crystalline bovine or human albumin are used the same as described in the test for total pepsin.

Two cc of a 70 mg/cc solution of albumin is mixed with 1 cc of .15 N HCl and 2 cc of gastric juice. Incubation is performed for 20 minutes at 37°C after which inactivation is accomplished by the addition of 3 cc of .4 N NaOH and incubation for 30 minutes. Two cc is then diluted to 50 cc with N/150 NaOH. To 5 cc of the diluent are added 19 cc of sulfosalicylic acid. Since the range of this test is small, the digestion pH should be taken in advance and adjusted by altering the normality of the acid added to the digestion mixture so as to be in the range of pH 3.5. If the digestion is too great or too small, the digestion pH may be adjusted so as to bring the juice into the range of the test. Correction for pH is made according to Fig. 4.

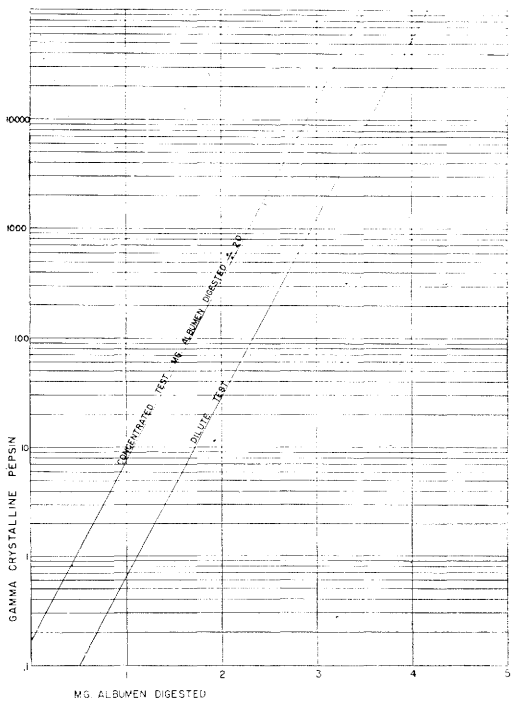


FIG. 3.

The relationship of the test using dilute albumen to that using concentrated albumen is shown. The plots are on semilogarithmic paper. Two and one-half times the crystalline pepsin concentration produces 20 times the amount of digestion in the test using concentrated albumen. The milligrams albumen digested in this latter test have been divided by 20.

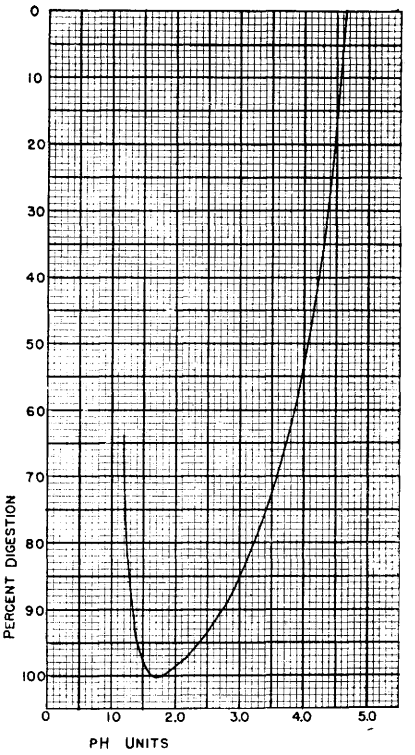


FIG. 4.

Shows relationship of pH of digestion mixture to percentage of digestion of human serum albumen at pH 1.75 which is represented as 100%.

In extrapolating to the undiluted state, the milligrams albumen digested, after pH correction, is divided by 2 (number of cc of gastric juice) and the square root taken. From this figure 1.03 is subtracted. This difference is squared and multiplied by 40, since an aliquot of 1/40 was taken for albumen determination. The result yields the milligrams albumen digested per cc of undiluted gastric juice. If the results are to be expressed in pepsin units, the multiplication by 40 is omitted.

If no correction is made for the aliquot taken for determination in the concentrated test, 13.5 times the amount of crystalline enzyme in the concentrated test digested an amount identical in the dilute test. Therefore, the antilog of the mg albumin digested (after correction for dilution and pH and unmultiplied by 40) is divided by 13.5. Pepsin units thus obtained are of the same magnitude as in the dilute test so that the per-

centage of pepsin inhibition may be obtained. Resting peptic power cannot be determined with the alternative method.

*Summary and Conclusions.* Simple procedures have been described whereby the amount of active pepsin, the percentage of pepsin inhibition and the resting peptic power can be obtained when used in combination with the test for total pepsin. The test employs a nephelometric method to determine the diminution of an albumen substrate after digestion.

15569 P

## An Electro-Magnetic Sphygmograph* of New and Simple Design.

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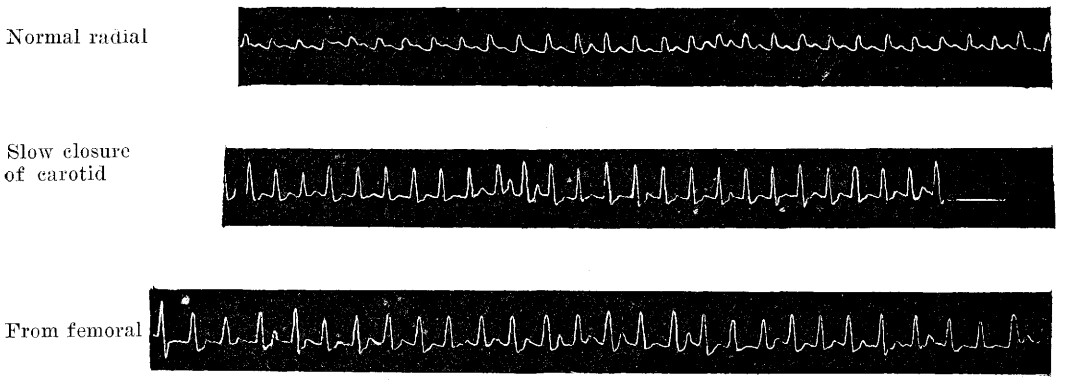
While there are several more or less satisfactory mechanical devices adapted for the transcription of pulse pressure waves from superficial arteries (carotid,† radial,‡ digital§), excellent results are not always easily attained. The adaptation of the electro-magnetic principle here described, while simple, seems to present a sturdy as well as a foolproof device. The chief assemblage includes a 200 ohm, single Button Model W carbon granule microphone¶ of excellent sensitivity which, by the adaptation of a short rod fixed upon its diaphragm, is held or strapped over a suitable throbbing artery serving in place of a dispatching tambour. It is to be noted here that its function is the reception *not* of sounds but of the pulse pressure waves as such, and it acts as a variable resistance to the passage of an electric current. Thus the resistance is caused to vary inversely with the differential pressure throbs of the artery. At the recording end of the electrical circuit in this assemblage, a permanent cylindrical alnico magnet, 3/8″ in diameter and 5″ long is fixed in an upright position, supported upon an adjustable iron stand. About the magnet is placed an ordinary 6″ test tube with bottom removed, upon which is wound about 200 turns of 28 gauge cotton covered magnet wire of approximately 1.7 ohm total resistance; each end of




* Acknowledgment is here made to Dr. R. E. Vollrath of the Physics Department for his interest and helpful suggestions.

† Usual assemblage of two Tambours in series.

‡ The Dudgeon Sphygmograph.

§ See A Simplified Digital Sphygmograph, Baldwin, F. M., *Science*, N. S. **69**, No. 1792, May, 1929.

¶ Manufactured by Universal Microphone Co., Inglewood, Calif.