REFERENCES

(1) Levine, D. D., *Hygeia*, 14 (1936), 728.

(2) Osborne, E. D., and Hitchcock, B. S., *J. Am. Med. Assoc.*, 97 (1931), 453.

(3) Goodman, H., "Treatment of Common Skin Diseases," Medical Lay Press (1932).

(4) Shaffer, L. W., and Cary, W. H., Jr., *J. Mich. State Med. Soc.*, 32 (1933), 648.

(5) Osborne, E. D., Putman, E. D., and Rickloff, R. J., *N. Y. State J. Med.*, 33 (1933), 1270.

(6) Circular, "Control of Ringworm in Gymnasia," *Bull. Indiana State Board of Health* (Jan. 10, 1938).

(7) Gershenfeld, L., "Bacteriology and Sanitary Science," Lea & Febiger, Philadelphia (1929).

A Method for the Determination of Peptic Activity*

By C. J. Klemme† and Lee Worrell‡

INTRODUCTION

This study is the result of an attempt to find in the literature a relatively short, convenient, accurate method of assay for preparations containing pepsin, which could be used for routine assays in a study of the stability of such preparations.

Although numerous assay methods for pepsin have been proposed, original references to many of which may be found in publications by Sherman and Neun (1), Vahlteich and Glover (2), Northrop (3), Greenberg (4), Jenkins and Hoshall (5) and Waksman and Davison (6), none have proved entirely satisfactory. The variability of the substrate and correlation between the actual measurement of the amount of digestion and the concentration of enzyme causing the digestion have been outstanding difficulties involved.

EXPERIMENTAL

Preliminary experiments, in which several of the above-mentioned methods were tested, indicated that the method of Jenkins and Hoshall (5) offered the most convenient means of determining the amount of digestion. It was necessary to make several important changes, however, before accurate results could be obtained.

Method.—Using a solution 10 cc. of which contain 0.05 Gm. of reference pepsin, determine the K value of the lot of casein to be used in the assays by the method described below. The reference pepsin is preferably a sample of the pepsin used in the preparations to be assayed; if this is not available, the "Reference Pepsin" of the U. S. P. XI may be used.

To about 200 cc. of 0.0800N hydrochloric acid contained in a one-liter Erlenmeyer flask, add 17.2 Gm. of casein (according to Hammarsten). Shake the flask until the casein is thoroughly moistened and evenly dispersed. Add enough 0.0800N hydrochloric acid to make the total volume of acid exactly 400 cc. Stopper the flask loosely and place in a bath of boiling water for exactly 30 minutes.[1] Remove and quickly cool (under the tap) to room temperature. The product should contain no undissolved particles of casein. Pipette 70 cc. of this substrate into a 125-cc. Erlenmeyer flask. Stopper the flask loosely and place it in a constant temperature bath, previously regulated to maintain a temperature of 55° C., in such a position that the neck of the flask is above the water in the bath. After the flask has remained in the bath exactly 10 minutes, and without removing it from the bath, add by means of a pipette 10 cc. of a dilution of the preparation to be assayed and mix by gently shaking. The preparation is diluted with distilled water so that 10 cc. of the dilution contain 0.02 Gm. to 0.08 Gm. of pepsin. Preliminary trial assays may be necessary in order to determine the proper dilution. Allow the flask, loosely stoppered, to remain in the bath exactly 30 minutes after the sample is added. Remove from the bath and immediately add exactly 20 cc. of a solution of sodium sulfate (20 Gm. of the anhydrous salt in 100 cc. of distilled water), mix well and cool under the tap to 25° C. Filter through hardened filter paper. Titrate a 25-cc. aliquot portion of the clear filtrate with $N/10$ sodium hydroxide, using phenolphthalein as the indicator. Run a blank, using 10 cc. of distilled water instead of the pepsin solution, in exactly the same manner. At the end-point of the blank titration, add 2.50 cc. of the dilution used in the determination, and complete the titration. The difference in acidity between the determination and blank, expressed as cc. of $N/10$ acid, is an index of the relative proteolytic activity and is designated in the calculations as X.

Calculations: To determine the K value of the casein, substitute the value obtained for X in the reference determination into the equation

$$K = X/2.24$$

To determine the amount of active pepsin in the preparation assayed, substitute the value obtained for X in the assay into the equation

$$\sqrt{E} = X/K$$

* An abstract of a thesis submitted to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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[1] In high altitudes where the boiling point of water is appreciably below 100° C., the time required for solution may be longer. This time would have to be determined experimentally.

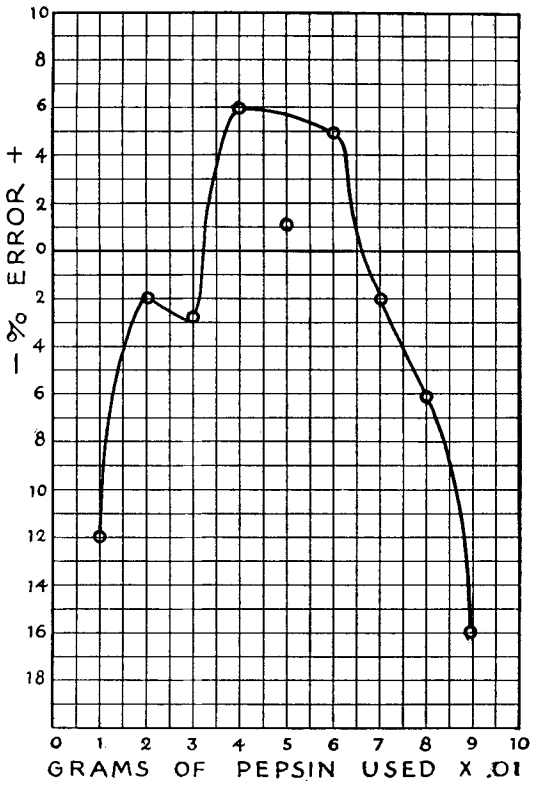

Fig. 1.—Graph Showing the Amount Digested in Relation to Pepsin Concentration.

Solve for E, which is the relative concentration of pepsin. Divide E by 100 to obtain the fraction of a Gm. of pepsin in the 10 cc. of the dilution used. Multiply by the appropriate factor, according to the dilution used, to obtain the concentration of pepsin in the preparation assayed.

The entire method of preparation of the substrate has been changed to eliminate errors found to be due to variations between the individual substrates of the older method. Experiment showed that it is necessary to heat the mixture at least 30 minutes before a homogenous substrate is produced. Longer periods of heating increase slightly the amount of digestion subsequently obtained, but the increases are not sufficient to compensate for the extra time involved. It was found that the use of $0.0800N$ hydrochloric acid provides a substrate of the optimum p_H for peptic digestion under the conditions of the method. Substrates prepared in the manner described give reproducible results as long as the same lot of casein is used; when different lots of casein are compared, slight variations occur in the results. Hence it is necessary to standardize each lot of casein used against a reference pepsin.

Experiments to Determine Optimum Digestion Temperature and Time.—The results of experiments to determine the optimum time and temperature for digestion indicate that digestion for 30 minutes at 55° C. is most suitable.

The preliminary experiments showed that within a certain range of concentrations of pepsin the Schütz Law is valid, *i. e.*, the amount of digestion is proportional to the square root of the amount of enzyme used. It was necessary to determine the exact range of pepsin concentrations in which this proportionality held true. The results tabulated below indicate that the calculated results check the known amounts of pepsin used within 6 per cent, when the range of pepsin concentration is from 0.02 Gm. to 0.08 Gm. This is more clearly shown in the accompanying graph. The abnormal value obtained at 0.05 Gm. is probably due to the end-point error.

Table I.—Results of Digestion Experiments

Casein Lot 79080. $K = 2.10$

Gm. Pepsin Used	Diff. in Acidity Cc. 0.1N HCl	Gm. Pepsin Found	Per Cent Error
0.0900	5.78	0.0756	−16.0
0.0800	5.75	0.0751	− 6.1
0.0700	5.50	0.0686	− 2.0
0.0600	5.27	0.0630	+ 5.0
0.0500	4.73	0.0506	+ 1.2
0.0400	4.32	0.0424	+ 6.0
0.0300	3.59	0.0292	− 2.7
0.0200	2.94	0.0196	− 2.0
0.0100	1.98	0.0088	−12.0

Twelve different samples of pepsin and thirty-eight different samples of liquid preparations containing pepsin have been assayed by this method. Six assays were run on each of the liquid preparations, three each on two different dilutions and four assays were run on each sample of pepsin. In every case checking results were obtained. Most of these samples were purchased from retail drug stores.

CONCLUSIONS

1. A convenient method of assay for peptic activity, especially suited for stability studies, has been devised using the method of Jenkins and Hoshall (5) as a starting point.

2. An investigation of the peptic activity of liquid pepsin preparations purchased from various retail stores indicates that the majority of liquid pepsin preparations so purchased are above the N. F. VI standard for peptic activity, but none of them have activity equivalent to the amount of pepsin added at the time of preparation.

REFERENCES

(1) Sherman, H. C., and Neun, D. E., *J. Am. Chem. Soc.*, 38 (1916), 2199.

(2) Vahlteich, H. W., and Glover, C. C., JOUR. A. PH. A., 10 (1921), 595.

(3) Northrop, J. H., *J. Gen. Physiol.*, 16 (1932), 41.

(4) Greenberg, H. L., JOUR. A. PH. A., 20 (1931), 1032.

(5) Jenkins, G. L., and Hoshall, E. M., *Ibid.*, 22 (1933), 625.

(6) Waksman and Davison, "Enzymes" (1926), Williams and Wilkins Co.

A Study of the Assay of Ginger*

By Robert Tzucker† and C. B. Jordan‡

INTRODUCTION

Ginger has been known and used in medicine for a great many years. Although the use of ginger in medicine has fallen off to a great extent, it is still widely employed as a spice and in the preparation of various beverages.

The method of assay for ginger has never been a satisfactory one. The establishment of an accurate assay method is difficult due to the resinous consistency of the active constituents. The Ninth Revision of the United States Pharmacopœia was the first revision to include a standard for ginger. In this revision, the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined. The Tenth Revision determined the water-soluble extract and the non-volatile ether-soluble extract. The Eleventh Revision determines the amount of ether-soluble extract, assuming, and rightly so, that the active or pungent principle is ether-soluble.

The method of the Eleventh Revision consists of extracting a portion of the drug with ether and weighing the residue after volatilization of the ether. Water-bath temperature is used to volatilize the ether. The objections to this method of assay are:

1. Individuals vary in their olfactory sensitivity to ether. Some operators may be unable to detect ether odor even though ether still remains in the extract; this, of course, will introduce an error in weight.

2. The active volatile principles may be partially but not entirely removed by heating at water-bath temperature, and therefore, the residue may not be a measure of all of the volatile constituents. Since the amount of volatile material coming off may vary, the weight of the residue will vary with different samples.

This study was carried out in an attempt to find a more satisfactory assay method.

HISTORICAL REVIEW OF THE METHODS OF ANALYSIS FOR GINGER

A method for the standardization of ginger was not introduced until the Ninth Revision of the United States Pharmacopœia. In that Revision the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined.

Garnett and Grier (1909) used a shake-out method to determine the amount of gingerol, which has been shown to be the active principle. The method consisted of extracting the drug with ether, volatilizing the ether and boiling the residue with repeated portions of petroleum spirit. The petroleum ether is shaken out with successive portions of sixty per cent alcohol, leaving the volatile oil, fatty oil and much coloring matter in the petroleum spirit. The alcoholic solution is then washed with further portions of petroleum spirit to remove the last traces of fat. The alcohol is recovered or volatilized and the residual liquid shaken out with three successive portions of ether. The ether is then volatilized and the gingerol is dried to constant weight and weighed. Garnett and Grier (1907) claim that capsicum is a common adulterant of ginger. They give a method for determining the presence of capsicum. It consists of digesting on a water-bath about ten cc. of the extract with a small quantity of caustic alkali for fifteen minutes. The solvent is volatilized and the residue is acidified slightly with hydrochloric acid. It is then shaken out with a small portion of ether and the ethereal solution is tasted. In the case of pure ginger, the pungency will be found to have entirely disappeared, while if capsicum be present, the pungent, biting taste is at once recognized. One part of capsicum in one hundred parts of ginger may be detected in this manner.

Ginger extracts also give a specific color reaction. This test is carried out as follows:

Dilute 10 cc. of the extract to 30 cc.; evaporate to 20 cc. and extract this solution with 20 cc. of ether. Allow the ether to evaporate spontaneously in an evaporating dish and add 5 cc. of seventy-five per cent sulfuric acid and 5 mg. of vanillin to the residue. Allow this mixture to stand for about fifteen minutes and then add an equal volume of water. An azure-blue color develops in the presence of ginger extracts.

* An abstract of a thesis submitted to faculty of Purdue University in partial fulfilment of the requirements for the Master of Science degree, June 1938. The thesis contains a bibliography of fifty references.

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