

## ASSAY OF THE N. F. PEPSIN PREPARATIONS.\*

R. K. SNYDER, LOUIS GOLDBERG AND E. N. GATHERCOAL.

Elixir of Pepsin N. F. V and N. F. VI, Elixir of Pepsin and Rennin N. F. V and N. F. VI, Compound Elixir of Pepsin N. F. VI and Glycerite of Pepsin N. F. VI were made up and each preparation was divided into three parts and placed in Pyrex bottles for storage. One bottle of each was kept on an open shelf, one in a dark cupboard and one in a refrigerator.

The proteolytic activity of each at the end of a year was determined. The average results are as follows:

Elixir of Pepsin N. F. VI,	175 per cent
Elixir of Pepsin N. F. V,	60 per cent
Elixir of Pepsin and Rennin N. F. VI,	162 per cent
Elixir of Pepsin and Rennin N. F. V,	140 per cent
Compound Elixir of Pepsin N. F. VI,	175 per cent
Glycerite of Pepsin N. F. VI,	112 per cent

It should be remembered when inspecting the table that all of the preparations except the glycerite were made up to the N. F. formulas, that is, 200 per cent strength, and are up to the official standards as long as they are above 100 per cent strength. The glycerite was made up to 114 per cent strength and is up to standard as long as it is above 100 per cent strength.

As would be expected, the preparations kept in the refrigerator held their activity the best, those in the dark cupboard next and those on the shelf the least. However, the differences between the three are very small and do not warrant the extra care in storage.

It will be observed from the table that the N. F. VI preparations maintained a higher level of proteolytic activity than the N. F. V. This is particularly noticeable in the Elixir of Pepsin. This is due to the fact that the N. F. VI product has a higher hydrogen-ion concentration (slightly over  $p_H$  4) than the N. F. V (about  $p_H$  2.5), and is buffered. The difference in proteolytic activity due to this change in  $p_H$  is of a much larger order than that due to storage conditions and indicates that the proper  $p_H$  of the solution is of vital importance.

In connection with these assays of the N. F. pepsin preparations, certain refinements in the official assay process (see N. F. VI page 119) were tried out and seemed to be real improvements. These refinements may be briefly mentioned as follows:

(a) The amount of dilution of the Elixir was changed from "5 cc. of Elixir to 145 cc. of this dilute acid" to "3.33 cc. of Elixir to make 100 cc. with this dilute acid." It was found that a more accurate dilution could be prepared by using a pipette carefully calibrated to deliver 3.33 cc. and a 100-cc. volumetric flask.

(b) The solution of Reference Pepsin is likewise prepared in volumetric glassware to insure greater accuracy.

(c) A sufficient quantity of the egg albumin is prepared at one time for the

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\* National Formulary Research Project F 3 in progress in the N. F. Laboratory, Chicago, Illinois.

assays of the day and kept in a well-stoppered container. The coagulated albumin passed through the sieve is well mixed, so that a uniformity is attained even if there be a variation in the albumin of different eggs.

(d) The granulated albumin is reduced in a mortar with some of the dilute acid to a uniform suspension and then quantitatively transferred to the digestion bottle. This insures a more uniform final precipitate and apparently does not influence the amount of albumin digested.

(e) The period of "standing" to permit complete and uniform precipitation is increased from "30 minutes" to "over night." This may appear as a very marked increase, yet it has been found that the "reading" of the precipitates is much improved and apparently no "errors" are developed by the increased time of standing.

(f) Finally, the whole set-up of six standards graduated in steps of 0.5 cc. of the dilution of standard pepsin (equivalent to less than 0.0003 Gm. of pepsin per "step") and the two tubes of "unknown" of different strengths, permits a close comparison of the precipitates in the tubes of the "unknown" with that in the tubes of the standard. If the reading of the "unknown" should not be exactly equivalent to that of one of the standards, but falls in between two of them, the value may be interpolated.

The detailed assay process as used in this work is as follows:

Mix 35 cc. of normal hydrochloric acid with 385 cc. of distilled water. Dilute exactly 3.33 cc. of Elixir of Pepsin to 100 cc. with this dilute acid in a 100-cc. volumetric flask. Dissolve 0.1458 Gm. of Reference Pepsin (see U. S. Pharmacopœia XI, page 486) in 250 cc. of this acid. Immerse a sufficient number of eggs in boiling water for 15 minutes to provide sufficient coagulated albumin for the assays of the day. Remove the eggs and when they have sufficiently cooled to handle, remove the shell, pellicle and all of the yolk, and at once rub the albumin through a clean, dry, hair or brass, No. 40 sieve, rejecting the first portion that passes through the sieve and keeping the remainder in a stoppered container. Place a 10-Gm. portion of the granulated egg albumin in a porcelain mortar with 10 cc. of the dilute acid and rub until the albumin is completely suspended. Quantitatively transfer this suspension to a wide-mouthed bottle of about 100 cc. capacity using 20 cc. of the dilute acid to rinse the mortar. Repeat this procedure until a sufficient number of bottles have been prepared. Place them in a water-bath warmed to 52° C. To one bottle add exactly 3 cc. of acidulated solution of Elixir of Pepsin and 7 cc. of the dilute acid. To a second bottle add exactly 5 cc. of the acidulated solution of Elixir of Pepsin and 5 cc. of the dilute acid. Prepare a set of standards in a similar manner to the above using exactly 4.0 cc., 4.5 cc., 5.0 cc., 5.5 cc., 6.0 cc. and 6.5 cc. of the acidulated solution of Reference Pepsin, and in each case a sufficient amount of the dilute acid to bring the total liquid to a volume of 40 cc. At once cork the bottles securely with smooth cork stoppers, invert them three times, and place in a bath adjusted to maintain a temperature at 52° C. Keep the bottles at this temperature for two and one-half hours, agitating the contents equally every 10 minutes by inverting the bottles once, making sure that no particles of albumin adhere to the stopper or walls of the bottle. Remove the bottles from the bath; pour the contents of each into conically shaped measuring vessels of suitable capacity having a diameter not exceeding 1 cm. at the bottom, and graduated from the tip to the 1.0-cc. mark in 0.05-cc. divisions and from the 1.0-cc. to the 5.0-cc. mark in 0.1-cc. divisions. All of the measuring vessels must have the same internal taper. Transfer the undigested egg albumin which adheres to the sides of the bottles to the respective measuring vessels with the aid of small portions (about 15 cc. at a time) of distilled water until the total amounts measure 50 cc. each. Stir the mixtures well, and allow to stand over night. Keep the measuring vessels in a perfectly vertical position during the sedimentation time. Compare the tubes containing the Elixir of Pepsin with the tubes containing the Standard Reference Pepsin and observe which tubes have approximately the same amount of undigested egg albumin. Calculate the percentage of active pepsin in the Elixir of Pepsin. If neither the 3-cc. nor the 4-cc. samples of the acidulated solution of Elixir of Pepsin produce amounts of undigested egg albumin which fall within the range of the set of

standards, it is then necessary to use either less or more of Elixir of Pepsin as the case may be until the amount of albumin falls within the range of the standards.

The following is a sample of the calculations used:

If it is found that 3 cc. of the acidulated solution of Elixir of Pepsin is equal to 5 cc. of the acidulated solution of Reference Pepsin, the calculations would be as follows:

One cc. of the acidulated solution of Reference Pepsin contains 0.000583 Gm. of Pepsin, therefore, the amount of Pepsin in the 3 cc. is equal to  $0.000583 \times 5$  or 0.002915 Gm. Therefore, the amount of pepsin in 100 cc. of an Elixir of Pepsin of this strength would contain 1000 times as much pepsin or 2.915 Gm. Dividing this amount of pepsin by 1.75 Gm. (the standard required in the Elixir of Pepsin) gives the percentage as used in the above table of figures.

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## THROUGH THE APOTHECARY TO THE MEDICAL INTERN.

BY ROLAND T. LAKEY.\*

The evidence of our changing professional attitudes and viewpoints is embodied in the records of our state association's annual convention programs. A comparison of the papers and discussions of 10, 20, 30, 40 or 50 years ago will reveal a gradual waning of interest in the professional side of retail pharmacy and a corresponding increase of interest in the commercial development of the drug store. It is not my intention to add to the often repeated expression of regret that this has taken place, but rather to point out that we still have only one ground upon which our extremely diversified experiences can make contacts for the purpose of professional improvement, namely, our common interest in the arts and sciences of pharmacy. Due to the failure of our state associations in maintaining this interest, we must seek other agencies for promoting a professional consciousness. The AMERICAN PHARMACEUTICAL ASSOCIATION, of course, provides for this nationally, but has been unsuccessful thus far in enlisting sufficient numbers to make much of an impression locally. It is true that the branch programs are mainly educational and professional in character, but they are not systematically arranged and do not consistently provide for attaining a definite objective. The mixed character of the membership is another reason why they cannot confine their programs to professional subjects. There is in each metropolitan center, however, a number of registered pharmacists whose interests are wholly professional; I refer to the hospital and professional pharmacy apothecaries. With this thought in mind last May, the writer sent invitations to all of the hospitals, professional pharmacies and other institutions conducting an exclusive compounding and dispensing service in the Detroit district, to meet at the Receiving Hospital for the purpose of organizing a Section of Apothecaries of the Detroit Branch of the A. PH. A. Twenty-seven attended the meeting. These men and women welcomed the suggestion of an organization of their own and proceeded to elect officers. R. L. McCabe, proprietor of a professional pharmacy, was chosen as *President*. James Early, chief pharmacist of the Detroit Receiving Hospital, was elected *Secretary* and Helen Linsky, chief pharmacist, St. Joseph Hospital, *Treasurer*. It is estimated that there are about 100 eligible for membership in the metropolitan area and it is expected that practically all will be enlisted when the fall season starts.

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\* Section on Education and Legislation, A. PH. A., Dallas meeting, 1936.