The first point in question in this method is whether or not the iodine solution should be shaken after the addition of the digested starch paste. It will be found that on carrying this method out as the end-point is approached the addition of the required amount of digested starch paste to the iodine solution will give a blue to violet color, and this color will remain dispersed through the solution. If the solution is now inverted several times to thoroughly mix, the blue color will totally disappear. With high strength samples of pancreatin this is particularly noticeable and whether or not the iodine solution is shaken has a considerable influence on the assay results.

I would recommend that in the next revision of the U. S. P. the method be so modified as to state definitely that the iodine solution be not shaken after the addition of the starch paste. In this way the point at which the starch totally disappears could be more accurately determined.

The second point of indefiniteness in the method is the temperature of the iodine solution. It has been observed that the color reaction between starch and dilute solutions of iodine is very sensitive to temperature; the lower the temperature the deeper is the color. It is possible to obtain a divergence in results on this point, which is shown by carrying out the following experiment:

Two tubes of iodine solution are prepared ready for the test; one is adjusted to a temperature of 20° C.; the other is brought to a temperature of 30° C. To these two tubes the required amounts of a starch paste digested by a U. S. P. pancreatin are added. A blue or violet color will be produced with the iodine solution at 20° C. while the one at 30° C. will usually show no color or possibly a very faint coloration. The color reaction can be brought out in the latter tube by cooling it down; the lower the temperature the deeper the color becomes. This shows that if approximately identical temperatures of the iodine solution were not observed some divergence in results would be obtained.

To overcome this possible source of inaccuracy in the test, it would be recommended that in the next revision the temperature of the iodine solution used in the test be definitely stated. A temperature of 23° C. has been found convenient in carrying out this method.

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THE ASSAY OF PANCREATIN.*

BY F. E. WILLSON.

Numerous methods have been suggested for the determination of the tryptic activity of pancreatin. The majority of these are impractical as routine laboratory methods either because of the indefiniteness of the end-point or the time required for carrying them out. Practically all such determinations are based upon the digestive action of trypsin on some weakly alkaline substrate, such as casein or egg albumin. Casein solutions have been favored because of the fact that the undigested casein remaining, after the digestion has been carried on for a definite length of time, can be easily thrown out of solution by the addition of a mixture of

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

alcohol and acetic acid. However, the end-point in such determinations is very difficult to determine and the method is sensitive to conditions under which the test is run, thereby giving divergent results in the hands of different operators.

This is true both of methods in which the amount of digestion produced by a standard sample and of those which use the total disappearance of substrate as the end-point as a means of standardization. The latter has the added disadvantage that the last portions of the substrate are very slow in digesting, perhaps due to the decomposition products of digestion having an inhibiting action. Determinations based upon the peptonization of milk by trypsin as a means of standardization also give widely varying results, probably partly due to the condition of the milk.

In the search for a more accurate method Sorenson[1] suggested that his formol titration for amino acids be applied. This method is based upon the breaking down of the polypeptide linkages of a casein solution into carboxyl and amino acid groups by the trypsin. The amount of amino groups formed by the hydrolysis of the casein solution cannot be determined directly because of the amphoteric properties of the amino acid groups. This is overcome by the addition of a solution of formaldehyde which converts the amino groups into methyleneimino groups which allows the free carboxyl groups to be titrated by a standardized alkali solution.

This method was further improved upon by Smith[2] for use as a laboratory method for determination for the tryptic activity of pancreatin. However, it was found that some changes could be made in his method that would make the method more accurate and rapid. Following is an outline of the method as now followed:

SOLUTIONS REQUIRED.

A. A 4% casein solution (Hammerstein's casein as supplied by Merck) adjusted to neutrality by the addition of *N*/1 sodium hydroxide, phenolphthalein being used as an outside indicator.

B. A 40% formaldehyde solution to which 1 cc. of a 0.5% phenolphthalein solution in 50% alcohol has been added. This solution is brought to neutrality with *N*/1 sodium hydroxide.

C. A 1% unfiltered pancreatin solution in distilled water.

METHOD.

The casein solution and a supply of recently boiled distilled water is heated to 55° C. Then 24 cc. of the distilled water is added to 25 cc. of the casein solution and placed in a water-bath at 55° C. At the same time 25 cc. of the distilled water is added to 25 cc. of the casein solution in another flask or tube and also heated to 55° C. This is to be used as a blank. Add 1 cc. of the pancreatin solution to the first casein and water mixture, mix, and allow to digest for exactly 20 minutes at 55° C. At the end of this time 20 cc. are withdrawn and placed in a 50-cc. Nessler tube and 10 cc. of the formaldehyde solution (Solution B) added, mixed well and then titrated with *N*/10 sodium hydroxide to bring the solution to the same pink color as a previously titrated blank.

While the digestion is proceeding the blank test can be titrated by withdrawing 20 cc. of the solution, to which no pancreatin has been added, treating with 10 cc. of Solution B and titrating with *N*/10 sodium hydroxide, noting the number of cc. required.

To compute the strength of the pancreatin the number of cc. of *N*/10 sodium hydroxide used in the blank is subtracted from the number of cc. used in titrating the digested sample. Multiply the number of cc. thus obtained by 100 and the result will be the percentage strength of the tryptic activity.

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[1] S. P. Sorenson, "Études enzymatiques," *Comp. rend. du Carlsberg*, 7 (1907).

[2] A. R. Smith, "The Measurement of Relative Tryptic Activity," *Pharm. J.*, 35 (1912), 137.

This method of determination was arrived at after numerous samples had been subjected to the test, which had been previously assayed and reassayed, according to the U. S. P. method. At first $N/5$ sodium hydroxide was used in the final titration. It was found that pancreatin samples assaying U. S. P. or 100% invariably required 0.5 cc. of $N/5$ alkali after the blank had been subtracted. Therefore, it was decided to accept 0.5 cc. of $N/5$ NaOH as representing 100% tryptic activity. This standard was found to apply equally well in higher strength pancreatin samples. For instance, a sample assaying 300% by the U. S. P. method required 1.5 cc. of $N/5$ NaOH. In order to increase the accuracy of the method $N/10$ NaOH was used and results checked against those obtained with $N/5$ NaOH. It was found that the accuracy was increased and it had the added advantage that the strength can be determined directly by multiplication of the cc. by 100.

The actual test requires little discussion as it is quite simple in manipulation. Some difficulty might be found when carrying this test out for the first time in the preparation of the casein solution. Smith's method is followed rather closely in this respect. To prepare a liter of solution, 40 Gm. of casein is added to 900 cc. of recently boiled distilled water to which approximately 25 cc. of $N/1$ sodium hydroxide has been added. The casein is brought into solution by stirring or mixing from time to time. If at the end of an hour or two all the casein is not dissolved a few more cc. of $N/1$ alkali are added so as to accomplish this. After all the casein has been dissolved, it is usually necessary to add a few more cc. of $N/1$ NaOH to bring the solution to neutrality or a very faint pink color, using phenolphthalein as an outside indicator. Then 5 cc. of chloroform are added as a preservative and the volume made up to one liter. The casein solution is now ready for use and will keep for several months, providing it is saturated with chloroform.

A number of laboratories using the method as outlined ($N/5$ alkali was used in the titration) were able to obtain fairly concordant results. The results varied no more than the results obtained using the U. S. P. test and the method had the disadvantage of being unfamiliar to practically all the investigators. It was suggested that possibly these divergencies were due partly to the adjustment of the casein solution and some evidence was brought out to substantiate this claim. In order to find out whether or not this were true, it was decided to carry out the test using casein solutions of varying hydrogen-ion concentrations. In order to do this a large volume of casein solution was prepared using only enough alkali to completely bring the casein into solution. This solution was divided into portions and each portion brought to different hydrogen-ion concentrations with alkali. The Hellige hydrogen-ion comparator was used for determination of the p_H values of the various solutions.

Four samples of powdered pancreatin were selected for study of which the tryptic activity had been checked and re-checked by the U. S. P. casein method. According to the U. S. P. test the samples gave the following assays:

1. 80%; 2. 125%; 3. 350%; 4. 325%.

The outlined method was then applied, using the casein solutions of varying hydrogen-ion concentrations.

In the following table the results obtained are tabulated. The p_H values and the percentage strengths obtained on assay are recorded.

	p_H* 6.9.	p_H* 7.5.	p_H† 7.9.	p_H† 8.4.	p_H† 8.9.	p_H† 9.1.	p_H† 9.4.
Sample 1	78%	85%	79%	68%	66%	59%	52%
Sample 2	121%	124%	123%	113%	103%	79%	76%
Sample 3	362%	363%	350%	335%	332%	307%	290%
Sample 4	350%	353%	334%	326%	328%	299%	271%

* Casein solution p_H value determined using brom-thymol blue color disc in comparator.

† Casein solution p_H value determined using thymol blue color disc (alkaline range) in comparator.

The results obtained indicate clearly that the hydrogen-ion concentration of the casein solution must be taken into consideration in carrying out this method in order to insure consistent results. Data obtained indicate that a preference is shown for casein solutions with a p_H value of 7.5. However, pancreatin samples assayed using casein solutions with p_H values of 6.9 and 7.9 show little variance from the results obtained with the former casein solution mentioned. We would, therefore, suggest that the casein solution for use in this method be adjusted to a p_H value not less than 7.0 and not more than 8.0.

The advantages of this method over the present U. S. P. method are:

(*a*) A definite end-point is obtained.

(*b*) It is much shorter, providing a supply of the preserved casein solution is kept on hand.

(*c*) More consistent results can be obtained, especially with the higher strength samples of pancreatin.

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ACCELERATED PRODUCTION OF SPECIFIC URINARY PIGMENTS BY DRUG ADMINISTRATION.*

I. EFFECT OF PHENYLDIMETHYLPYRAZOLON ON UROBILIN FORMATION.

BY FREDERICK G. GERMUTH.

INTRODUCTION.

The relation of chemical constitution to physiological activity in synthetic therapeutic agents has long been a subject of fascinating interest. Aside from purely theoretical aspects, the action of chemical agents upon the animal organism, and upon mankind in particular, is avowedly of the greatest importance. A knowledge of the relationship obtaining is of fundamental significance and importance—not alone to those contemplating the exploration of this vast field, but is certainly of no less import to the practitioner whose duty it is to prescribe the employment of this particular class of medicinal substances. It is believed, therefore, that the paper here presented—the first of a contemplated series dealing with urinary pigment formation engendered by the administration of specific organic medicinals—will be of interest alike to the members of the medical and pharmaceutical professions as well as to the chemist and others engaged in scientific work.

In a previous research,[1] the author has furnished data describing the pro-

* Contribution from the Division of Research, Bureau of Standards, City of Baltimore.

[1] Germuth, *American J. Pharm.*, 99 (1927), No. 11.