CRITICAL REMARKS ON THE U. S. P. PANCREATIN ASSAY.*

BY. F. E. WILLSON.

The U. S. P. X included under Pancreatin an assay method for the determination of the tryptic activity.

This method is based upon the total disappearance of the substrate, casein, by the digestive action of the trypsin. To meet the U. S. P. requirements the directions state that at the end of the digestion period, upon the addition of three drops of a mixture of alcohol and acetic acid, no precipitate is produced. In other words, there should be no casein present—which can be thrown out of solution.

For routine analytical purposes a series of tubes are prepared to which varying amounts of the pancreatin solution are added. In this way the strength of the sample may be found without running several consecutive tests, if the sample is either above or below U. S. P. strength. The tube in the series next to the one in which precipitation first appears should, therefore, represent the tryptic strength of the sample under test.

In reading over the U. S. P. assay method it would seem that the end-point described would be quite definite. However, in actual practice this is not the case. There is a gradation in the appearance of the tubes from the perfectly clear to hazy, then to distinctly precipitated. The tube in which precipitation first takes place is very difficult to judge because of this gradual change. The tube in the series immediately after the clear ones, though hazy or even slightly cloudy in appearance, can hardly be recorded as precipitation. Because of this indefinite appearance of precipitation at the end of the test different observers will choose different tubes as the point at which precipitation takes place. This was proven to be the case when samples of pancreatin were submitted to a number of different laboratories for assay. The results obtained were far from concordant, especially in the case of samples two or three times the U. S. P. strength. It was attempted to trace these divergencies to the casein supply, but practically as divergent results were obtained using samples of the same lot of casein.

The suggestion has been made that a U. S. P. sample be chosen as a standard and unknown strength pancreatins be assayed, using this standard sample for comparison. Two series of tubes would, therefore, be compared and from the one representing 100% with the standard sample, the strength of the unknown would be determined. However, this seems as unsatisfactory as the present U. S. P. method, since to match appearances of two slightly cloudy or cloudy tubes is practically as difficult and as liable to variance in the hands of different operators as when the end-point is determined in the present U. S. P. method.

It would, therefore, seem desirable that in the next revision of the Pharmacopœia that either a totally new method be adopted or such modifications in the present method be made that more consistent results may be obtained.

The assay method for the determination of the amylolytic activity of pancreatin in the present Pharmacopœia is for the most part quite satisfactory. There are, however, at least two points which seem worthy of consideration.

The method depends upon the absence of blue, red or violet color when a portion of the digested starch paste is added to dilute iodine solution.

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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.*

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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

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