glycerin alone can produce reactions which sucrose does not produce. The use of the alkali bismuth tartrates in aqueous solution is accompanied by marked local reactions including pain, local swelling and lump formation at the site of injection. These reactions are due in each case to the alkali bismuth tartrate and are in no way related to the nature of the aqueous media in which they are dissolved. Therefore, it would appear that in spite of their low toxicity and ready absorption aqueous solutions of the alkali bismuth tartrates are far from satisfactory therapeutic agents in syphilis; the severe local reactions which they produce on intramuscular injection in animals are almost certain to be paralleled in man. It does not appear to be possible to materially reduce these local reactions by modifying the aqueous media in which the compound is dissolved.

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A PHYSICO-CHEMICAL METHOD OF MEASURING THE ACTIVITY OF PEPSIN. PART I—A PRELIMINARY STUDY.*†

BY EDWIN R. THEIS.

The usual procedure in measuring the activity of pepsin, has been to allow the enzyme to act in acid medium upon the substrate (coagulated egg albumin) at a more or less constant temperature for several hours and then measuring the amount of digestion by one of several methods. While this method is being used universally, it lacks many of the real essentials of being completely quantitative in nature and the personal error is in many cases rather large.

The writer, in studying the nature of pepsin and the kinetics of enzyme action in detail, came upon a physico-chemical method that may be of more than passing interest. It has been shown[1] in former work that when any protein is placed in water, a net contraction in volume of the entire system results, in other words the protein hydrates. As hydration proceeds, the net contraction of the system continues until an equilibrium condition is attained. This contraction of the system is the result of an internal compression of the absorbed liquid within the protein. It has been further shown[2] that if an enzyme, such as pepsin, is added to the liquid medium, hydration proceeds as before, but a temporary equilibrium results at an earlier period, at which time hydration ceases and dehydration

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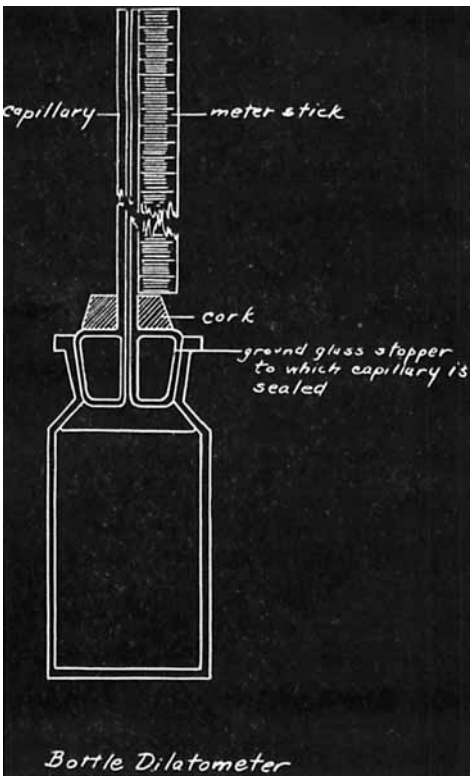
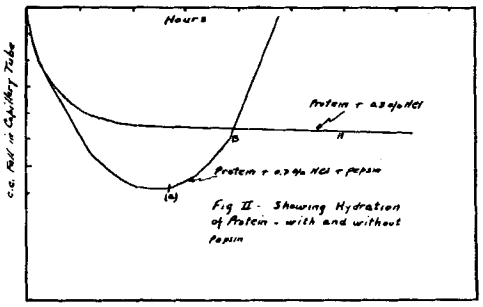
[1] *Ind. Eng. Chem.*, 21 (1929), 377; 22 (1930), 64.

[2] *Ibid.*, 22 (1930), 64.

occurs (evidenced by a net expansion of the system). This condition can be more easily explained by the use of Fig. 1. This figure shows several curves. Curve A is that of a protein placed in 0.3 per cent hydrochloric acid solution and the net volume decrease quantitatively measured over a period of time. It is seen from this curve that after a short time an equilibrium condition is soon reached. Curve B shows the type of curve obtained when pepsin is added to the above system. It is seen that only a temporary equilibrium condition results and in a short time the curve shows an upward trend—indicating a dehydration of the system. This





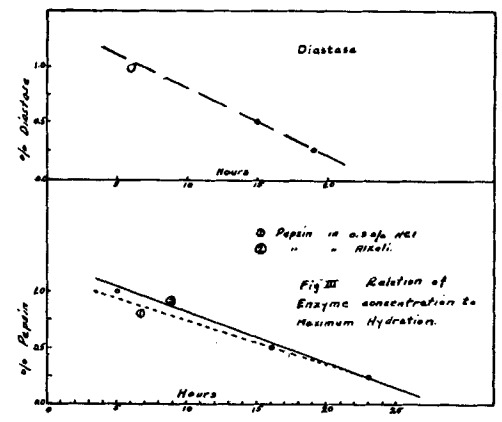


Fig. 1.

upward trend of the curve is caused by the peptic activity of the enzyme upon the protein—changing its potential hydration capacity. The early part of the curve represents the mean value of the hydrating and dehydrating forces. It can readily be seen from the curve that after a certain interval, the peptic digestion of the protein completely changes the hydration capacity and these changes are clearly indicated by the upward trend of the curve.

EXPERIMENTAL.

In developing this preliminary study, an improved dilatometer was used. This piece of apparatus is shown in Fig. 2. As protein material, cured calf skin

(cut into small cubes of 1 cm. edge) was used. Twenty grams of this material was placed in the dilatometer bottle, brought to a constant temperature of 37.5° C., 0.3 per cent hydrochloric acid solution added (where pepsin was used the enzyme was added to the acid solution), the glass stopper containing the capillary tube inserted into the bottle and the whole apparatus placed in a constant temperature bath, maintained at 37.5° C. Readings of the decrease in volume in the capillary tube were made until such a time as the system changed and an increase in the volume of the system occurred. The time for this change was noted for each pepsin concentration.

DISCUSSION.

Figure 3 shows in graphical form the results obtained by the method outlined. If the per cent of enzyme used is plotted against the time of maximum hydration (just at the point where dehydration begins as shown at "a" in Fig. 2) of the protein, we obtain the curves shown in Fig. 3. It is readily seen from the figures that a straight line function results, making it possible to determine just what percentage of pepsin (in this case 1:6000) was used.

These curves can be interpolated for any strength of pepsin. Suppose the sample is thought to be 1:12,000 pepsin, by making a 0.25 per cent solution of this pepsin and placing it together with the protein in a dilatometer, all that it is necessary to do is to determine the time of maximum hydration, by the method described previously. Suppose that we find that the time of maximum hydration is 17 hours—from Fig. 3, we see that this period of hydration corresponds to a 0.45 per cent solution (on the 1:6000 pepsin curve). By calculation

$$\frac{0.45}{0.25} \times 6000 = 10{,}800 \text{ pepsin,}$$

and our sample instead of being a 1:12,000 material is really a 1:10,800 sample.

As a general rule, the amount of hydration is increased by the addition of pepsin. This will be shown in Part 2 of these studies, which will be given at a later date. Work is now underway to obtain similar data, as outlined in this preliminary paper, using standard gelatin rather than animal skin as the protein material. The use of gelatin or some related material will make for a much more simple and more far-reaching standard physico-chemical method.

CONCLUSION.

A physico-chemical method for determining the activity of peptic enzymes is given in a preliminary way. This method is based upon the ability of proteins to hydrate. The amount of hydration is measured by the amount of net decrease in volume of the entire system. It is shown that the ability to hydrate is affected by the addition of such enzymes as pepsin. The effect upon hydration is measured and the amount of peptic digestion is gaged from the plotted graphs. The method offers interesting possibilities for future work upon more standard protein material, such as gelatin and casein, and this work will be reported upon.