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A Method for the Determination of Enterokinase.

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Any method of assay for enterokinase is indirect, and consists in a determination of the amount of trypsin which the enterokinase can produce from a given amount of trypsinogen. The amount of trypsin is determined by means of its digestive action on some suitable protein substrate. The methods used by Waldschmidt-Leitz,[1] and Linderstrom-Lang and Steenberg[2] consist in a determination of that amount of kinase which will just give maximal activity to a given quantity of trypsinogen or only one-half of the maximal activity in a given period of time, usually 30 minutes.

Such methods of assay involve all of the variables and possible errors of a trypsin assay plus a great number involved with the activation process. A brief study has shown that many gross errors are easily incurred during activation. Activation was found to occur in the presence of the alkaline protein substrate so that any method of partial activation before addition of the protein substrate is complicated by the additional amount of activation which occurs subsequently. The amount of kinase which will impart maximal activity to a given quantity of trypsinogen is not a clean-cut quantity. As one adds increasing amounts of enterokinase to a constant quantity of trypsinogen a maximum value is almost never reached. It is reached very gradually so that a small error at any point in the determination would be magnified in the final estimation.

In assaying enzymes it is customary to have present an excess of substrate. Assuming the action of enterokinase to be that of an enzyme and since activation occurs during digestion and at a slower rate than at the neutral pH usually used for activation, it seemed logical to attempt to assay by using an excess of trypsinogen with no activation with the added enterokinase, other than that occurring simultaneously with the digestion. Such a system is obviously complex but no more so than when activation is carried out as a separate step. The results, however, show very simple relationships and the assay of a sample of enterokinase is easily accomplished in less time than that of a sample of trypsinogen since the period of activation is omitted.

[1] Waldschmidt-Leitz, E., *Z. Physiol. Chem.*, 1924, **132**, 181.

[2] Linderstrom-Lang, K., and Steenberg, E. M., *Compt. rend. trav. Lab. Carlsberg*, 1929, **17**, 1.

The materials used for the enzyme solutions were dry trypsin-free trypsinogen powders and acetone defatted duodenal mucosa. The protein substrate was 6% casein in 0.4% sodium carbonate. The amount of digestion was determined by observing the change in refractive index of isoelectric filtrates of the substrate at once and after 4 hours digestion at 37°C.

It has been found that below a change of R.I. of 5 scale divisions on a Bausch and Lomb immersion refractometer, the change is almost directly proportional to the concentration of the trypsin. By using trypsinogen equivalent to 4 times the amount of trypsin which will cause a refractive index change of 5, and adding variable amounts of enterokinase, it was found when the total resulting change in refractive index is less than 5 scale divisions, that this change is directly proportional to the concentration of enterokinase added.

$$\Delta \text{ R.I.} = k \times \text{mg. enterokinase.}$$

Also, if both trypsinogen and kinase are varied the product of the two, times a constant, gives the change of refractive index:

$$\Delta \text{ R.I.} = k \times \text{mg. trypsinogen} \times \text{mg. enterokinase.}$$

For practical work, however, it is simpler to plot a curve using a constant amount of trypsinogen and variable amounts of a known enterokinase preparation and to read subsequent values from this curve. This method is not free from errors, but is rapid and gives values more easily reproduced than other proposed methods.

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Action of Morphine on the Intestine in Peritonitis.

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For many years the administration of morphine in peritonitis has been advocated by many surgical authorities. It has been advocated not only for the relief of pain and distress, but for the purpose of putting the intestine at rest in order to restrict or prevent the spreading of the infection that intestinal motility might produce. The well established observation that morphine tends to produce constipation was interpreted to mean that morphine did stop intestinal motility.