

THE EFFECTS OF PROTEOLYTIC ENZYMES ON THE ISOLATED RABBIT INTESTINE *

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Since enzymes have relatively specific substrate requirements, by treating a biologically active substance with a specific enzyme the chemical nature of that substance can often be determined. Thus the disappearance of some kind of activity after enzymic action would be considered as an evidence of the presence of a certain vulnerable structure in a substance. This kind of study is especially useful in determining the chemical nature of substances which have not yet been purified. For instance, the fact that the posterior pituitary hormones lose their activities after tryptic digestion suggests that they are proteins or polypeptides.

In recent years, as crystalline preparations of various proteolytic enzymes have become available and their specific activities have been demonstrated, they have been used in differentiating proteins. Thus Wagtendonk (1948) observed that paramycin lost its capacity to kill *Paramecium aurelia* after being treated with desoxyribonuclease, pepsin, or chymotrypsin, but not after tryptic digestion. He concluded that a nucleoprotein was essential for the activity of paramycin, and that basic amino-acids such as arginine and lysine were probably not necessary constituents of it.

Investigations of this sort have been carried out mainly on non-living substances; little attempt has been made to apply the same principle to living tissues. The following experiments represent such an effort.

METHOD

A section of rabbit's ileum (the animal having been deprived of food for 24 hours) was suspended in Tyrode solution at 38° C. The volume of the bath was 100 c.c. A frontal writing point was used to record the movement.

Crystalline enzymes obtained from Armour & Co. were used in all experiments. The trypsin and chymotrypsin contained "not more than 50 per cent MgSO_4 ." The weights stated in this paper refer to the preparations as supplied. The weights of acetylcholine chloride and of barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) refer to those of the salts. The proper amounts of various agents, contained in 1 c.c. of Tyrode solution, were pipetted into the bath. They were washed out after about one minute unless otherwise stated.

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RESULTS

The effects of chymotrypsin.—Ten mg. of chymotrypsin (Fig. 1, C) caused an initial depression followed by an increase in the intestinal movement. The former is secondary to the Mg content which alone produced similar depressions (Fig. 4, F), and the latter is probably a result of liberated histamine or related substances (Rocha e Silva, 1943). Twenty minutes later the bath was washed out and more chymotrypsin was added (F). Thirty minutes later the bath was washed out thrice. The spontaneous movement as well as the stimulating actions of acetylcholine and barium, as can be clearly seen, were greatly diminished. In other experiments in which larger doses of chymotrypsin were added, the spontaneous movement disappeared and the stimulating actions of acetylcholine and barium were completely abolished.

Pepsin.—Repeated additions of pepsin (Fig. 2, C and D) had no obvious effect on either the spontaneous movement or the actions of acetylcholine or barium. This was to be expected, as the optimum pH for pepsin is about 2, whereas the pH of Tyrode solution is about 8.

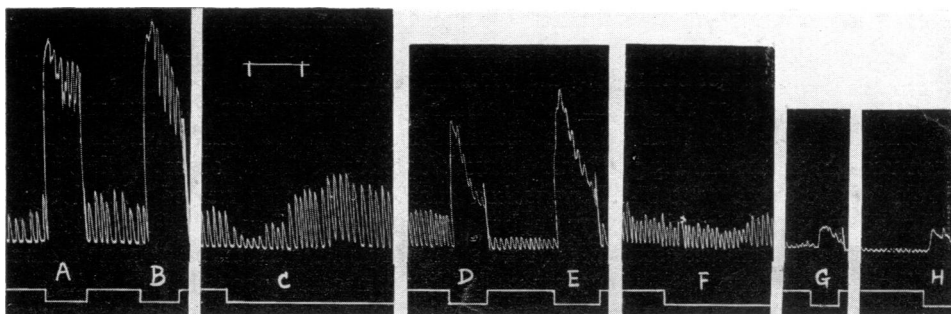


FIG. 1.—Isolated rabbit ileum in 100 c.c. bath. A,D,G—5 μ g. acetylcholine. B,E,H—20 mg. barium chloride. C—Chymotrypsin (10 mg.) left in bath for 20 minutes. F—Chymotrypsin (10 mg.) left in bath for 30 minutes.

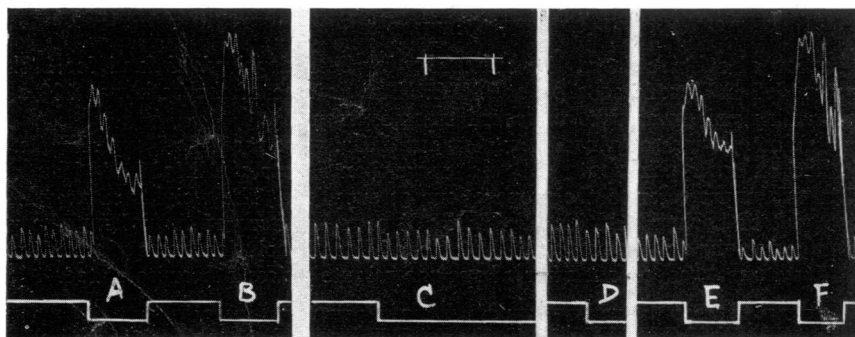


FIG. 2.—Same as Fig. 1. A,E—5 μ g. acetylcholine. B,F—25 mg. barium chloride. C,D—Pepsin (10 mg.) left in bath for 30 minutes each.

Trypsin.—The effects of trypsin on the intestinal movement, as shown in Fig. 3 at C (5 mg.) and E (10 mg.), were similar to those of chymotrypsin, an initial depression resulting from its Mg content and a delayed stimulation being probably due to released histamine and related substances (Ramirez de Arellano, Lawton, and Dragstedt, 1946 ; Rocha e Silva, 1940, 1943). Trypsin, however, differed from chymotrypsin in that it diminished greatly the spontaneous movement and the acetylcholine action without affecting the barium action. In some experiments in which 10 to 20 mg. trypsin was added to the bath the spontaneous contraction disappeared and the stimulating action of acetylcholine was completely abolished, while the action of barium was only slightly affected.

Trypsin, inactivated by heating at 60° C. for 1 hour, exerted no effect upon either the spontaneous movement or the intestinal responses to acetylcholine or barium (Fig. 4). The slight depression which occurred immediately after the addition of the enzyme is due to its Mg content, as shown at F in the tracing.

DISCUSSION

The foregoing experiments appear to indicate that chymotrypsin exerts its effects on the intestine by attacking the contractile substance of the smooth muscle. Trypsin, on the other hand, does not seem to affect the contractile substance, since the smooth muscle retains its contractility to barium after trypsin digestion. Thus the abolition of the action of acetylcholine by trypsin tends to indicate the destruction of a receptive substance which is necessary for its action but not for that of

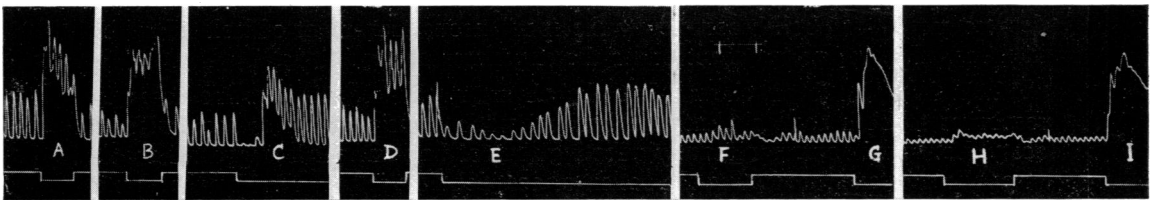


FIG. 3.—Same as Fig. 1. A,G,I—20 mg. barium chloride. B,D,F,H—2 μ g. acetylcholine. C—Trypsin (5 mg.) left in bath for 20 minutes. E—Trypsin (10 mg.) left in bath for 30 minutes.

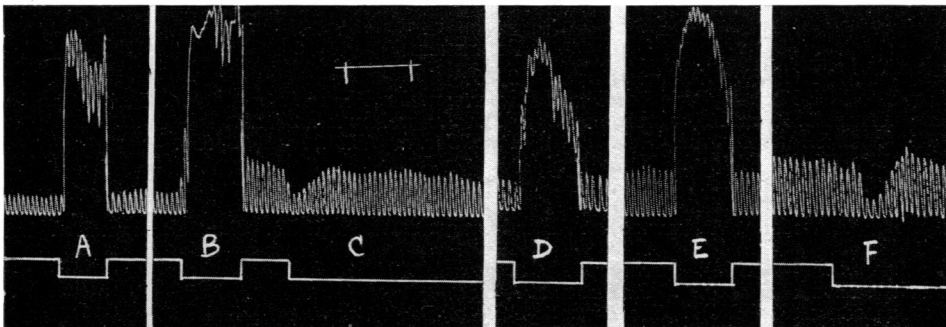


FIG. 4.—Same as Fig. 1. A,D—2 μ g. acetylcholine. B,E—20 mg. barium chloride. C—Inactivated trypsin (5 mg.) left in bath for 20 minutes. After washing out, 10 mg. inactivated trypsin was added and left in bath for 30 minutes (not shown in the figure). F—2.5 mg. magnesium sulphate.

barium ; the existence of such a receptive substance has already been suggested by other investigators (Clark, 1937 ; Ing, 1949 ; Langley, 1905 ; Pfeiffer, 1948 ; Welsh, 1948).

If the presence of an acetylcholine receptive substance can be taken as proved, the results described in this paper may be considered as evidence that it is protein in nature. It may be of interest to note in this connection that Welsh (1948) and Welsh and Taub (1951) have suggested acetylcholine receptive substances consist of a family of proteins (enzymes) and that acetylcholine acts as a coenzyme.

Since there appears to be little doubt that the contractile substance is also a protein and is not protected in some way by layers of non-protein structures (as evidenced by its vulnerability to chymotrypsin), its insusceptibility to trypsin is probably a result of the absence at its exposed surface of side groups characteristic for arginine and lysine residues, the presence of which in the substrate has been shown to be essential for the action of trypsin (Bergmann, 1942).

Although proteolytic enzymes similar to trypsin have been found in plasma, the normal functions of the receptive substances are not affected in the intact animal as in the experiments described. This may be because (1) the enzymes are in inactive forms in the plasma under normal conditions (Christensen and MacLeod, 1945 ; Clifton and Downie, 1950 ; Schwitz, 1937) or (2) the constituents of the body are in a dynamic state, destruction and construction being carried out continuously (Schoenheimer, 1946).

SUMMARY

1. It has been shown that both chymotrypsin and trypsin at suitable doses depress greatly or abolish completely the spontaneous movement of isolated rabbit intestine. Pepsin had no effect under the experimental conditions.

2. It has also been shown that the chymotrypsin-treated intestine loses its responses to both acetylcholine and barium, while trypsin abolishes the action of acetylcholine without significantly affecting that of barium. It is suggested that a receptive substance mediating the action of acetylcholine is destroyed by the tryptic digestion.

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