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The hydrolysis of collagen by trypsin.

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The generally accepted statement that collagen is not hydrolyzed by trypsin unless previously swollen in acid or alkali, shrunk in hot water, or treated with pepsin, rests entirely on qualitative work by Kühne and Ewald[1] in 1887 and 1890.

Using finely sifted hide powder as a source of collagen, we found that it was readily digested by trypsin in concentrations of the protease exceeding 10 mg. per liter. The hide powder was treated in 10 c.c. conical graduated centrifuge tubes with buffer solutions at various hydrogen ion concentrations, centrifuged, and the volume of powder measured. The buffer was then replaced by a trypsin solution made up in a buffer of known P_H, and digestion carried on, with continuous shaking, at 40.00°. By suitable variation of the buffer we found that the tryptic hydrolysis was not affected by pretreatment of the collagen at different P_H's between 1.1 and 8.9 and that the optimum reaction for the hydrolysis was at P_H 5.9. The time-hydrolysis curve for trypsin-collagen is of the same nature as with trypsin and proteins in general; since here the substrate is insoluble though hydrated, tryptic action appears to take place at the surface of the substrate particles, whether these be coarsely or colloidally dispersed. The degree of hydrolysis increases with increasing concentration of trypsin and decreasing size of hide powder particles. Complete hydrolysis was not reached in four periods of 20 minutes each, the experiment being then stopped owing to increasing hydrolysis in the control (hide powder and buffer solution without trypsin).

The shape of the hydrolysis-time curve suggests that the reaction is very slightly reversible. Experiments on pelt, using concentrations of trypsin up to 0.4 per cent., showed measurable

[1] *Verh. d. Naturhist. Med. Ver. in Heidelberg,* (N.F.) 1887, i, 451. *ibid.,* (N. F.), 1887, i, 451; *Z. f. Biol.,* (N. F. 8), 1890, xxvi i.

hydrolysis of the collagen, although the organized skin structure inhibited diffusion of the enzyme and greatly decreased the speed of the reaction.

Specimens of collagen tanned with quinone, gallotannic acid, copper sulfate and formaldehyde were all hydrolyzed by trypsin while chrome tanned collagen was not.

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The specific soluble substance of pneumococcus.

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In 1917 Dochez and Avery[1] showed that there was contained in filtrates from pneumococcus cultures and in the body fluids of experimentally infected animals and of patients suffering from pneumonia, a soluble substance which reacts specifically in anti-pneumococcus serum of the homologous type. This substance, which was found to be thermostable, precipitable by alcohol or acetone, non-dialyzable, and not digested by trypsin, is now being subjected to a more intensive chemical study.

Eight-day, autolyzed cultures of Type II Pneumococcus in phosphate broth were concentrated to 1/15 volume and precipitated with 1.2 volumes of alcohol. The precipitate, centrifuged at high speed, yields a compact middle layer containing the specific soluble substance. By repeated fractionation with alcohol or acetone, first in neutral, then in dilute acetic acid solution, followed by repeated fractional precipitation with ammonium sulfate and final dialysis, about 1 gm. of a highly purified preparation was obtained for each 75 liters of culture used.

In its present state of purity the specific soluble substance is amorphous and yields a viscous solution in water. A 1 per cent. solution gives no biuret test, yields no precipitate with phospho-

[1] Dochez and Avery, *J. Exp. Med.*, 1917, xxvi, 477.