XII. ON THE OPTIMAL REACTION FOR PEPSIN.

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That the action of most ferments depends upon the hydrogen ion concentration is now a well-known fact. Sørensen [1909] was the first to study the optimal hydrogen ion concentration for pepsin. He made acid albumin solution from white of egg and, after digestion of it with pepsin, determined the increase of soluble nitrogen left after precipitation by tin chloride or tannic acid. Thus he found that in short durations of digestion ($\frac{1}{2}$ or 1 hour) the optimal hydrogen ion concentration was about $[H^+] = 2.3 \times 10^{-2}$, in longer durations (12 hours or longer) nearly 6.0×10^{-2} , gradually moving to the acid side. Michaelis and Davidsohn [1910] used caseinogen as substrate and found the optimal concentration $[H^+] = 1.5 \times 10^{-2}$, with only short duration of digestion (10–15 minutes). Michaelis and Mendelssohn [1914] found, using edestin as substrate, that the optimal point of this ferment is, on the average, 4.0×10^{-2} ; concentrations between 2.0×10^{-2} and 8.0×10^{-2} being usually nearly the same in effect. They found further that the optimal hydrogen ion concentration does not depend upon the kind of acid nor on the presence of salts; in the latter case, it moves slightly to the less acid side.

Sørensen made all his experiments of the same chlorine concentration. In my experiments, the NaCl concentration was kept constant. The details are as follows:

(1) 1 % acid albumin solution as substrate. About 3.3 % neutral protein solution was first made. This was obtained by mixing 600 cc. white of egg, 1146 cc. water and 54 cc. N/10 HCl. After shaking for two hours, this was put in the cold store until next day, when it was filtered. Then 1600 cc.

of this neutral protein solution were mixed with 2640 cc. water and 800 cc. N/1 HCl and the mixture put in an incubator (38°), and after 24 hours 30 cc. 5N HCl were added: 4 hours later 50 cc. 5N HCl were added and the mixture then kept another 24 hours at 38°.

(2) *Pepsin solution.* 10 g. scale preparation of pepsin was dissolved in 1000 cc. of 0.01N HCl; 10 cc. of this solution contained 1.6 mg. nitrogen.

For each experiment, as the table shows, 360 cc. acid albumin solution, 40 cc. of various mixtures of sodium hydrate and salt solution were mixed, and after warming to the temperature required there were added 100 cc. pepsin solution. The various solutions contained always the same salt concentration (about 0.16N NaCl). The digestion was carried out in an incubator at 38° C.

Precipitation with tin chloride or tannic acid solution was carried out in the manner of Sørensen. The hydrogen ion concentration of each test was electrometrically measured. It was found that the change of this

concentration during the course of digestion was quite small and could be neglected.

Controls were made by heating the pepsin in a water bath to 100° for one hour, so that the ferment was thoroughly destroyed. From these experiments it was found that the nitrogen not precipitated by tannic acid or tin chloride solution in the control was constant during the whole digestion. 30 cc. of control solutions contained at the beginning as well as at the end of digestion 0.98 mg. nitrogen not precipitated by tannic acid, and 1.68 mg. not precipitated by tin chloride, so that these values were subtracted from the values obtained in the experiments with the active enzyme.

The results are shown in the accompanying table and curves.

From the table it is to be seen that the increase in the amount of nitrogen not precipitable with tin chloride is greatest in Exp. 5, $[H'] = 1.7 \times 10^{-2}$, then follow Exps. 4, $[H'] = 4.3 \times 10^{-2}$, and 3, $[H'] = 5.8 \times 10^{-2}$, showing no considerable difference between them. The increase of that not precipitable by tannic acid is most obvious in Exp. 4, then come Exps. 3 and 5. Therefore the optimum seems to be at about Exp. 4, or between Exps. 4 and 5.

CONCLUSION.

The optimal point for the action of pepsin is about $[H'] = 4 \times 10^{-2}$. Between $[H'] = 5.8 \times 10^{-2}$ and 1.7×10^{-2} there is no considerable difference in the rate of hydrolysis.

REFERENCES.

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