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Proteolytic Enzyme in the Latex from the Fig Tree (Ficus Glabrata). The pH of Optimal Activity.*

BENJAMIN H. ROBBINS. (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

For many years the sap from various species of the fig tree (genus Ficus) has been used in Central and South America as a vermifuge.[1, 2] It has been shown to have a fair action against Ascaris and *Trichuris trichiura.*[3] We[4] found that the active anthelmintic principle was a proteolytic enzyme that was active over a pH range of 4 to 9 and that it would hydrolyze coagulated egg albumen, gelatin and casein. From the hydrolysis of casein, crystals of tyrosine and 'leucine balls' separate out. Dilute solutions of the sap (0.5 to 1%) will digest the body walls and organs of live Ascaris.

The present paper gives the results of studies on the influence of different hydrogen-ion concentrations upon the proteolytic activity of the enzyme as determined by its action on gelatin.

Preparation of the dry enzyme mixture. The crude sap, a thick creamy mixture, contains about 25% solids, a part of which can be separated by centrifuging (this part is inactive); the remaining or active material is precipitated by 3 parts of acetone and is separated by decanting and centrifuging. The active part is redissolved in water and again precipitated by acetone and dried over $CaCl_2$ in a vacuum desiccator. One hundred cc. of the sap yields about 10-12 gm. of the dried powder to which we have given the name Ficin, from the generic name of the trees from which the active sap is obtained.

For determining quantitatively the enzymatic action on gelatin the formol titration was used. To 100 cc. of a 2% gelatin solution is added the sample of enzyme under study. A formol titration is carried out immediately on a 20 cc. aliquot. The mixture is then incubated at 35° for 24 hours and a second titration made. The difference between the 2 determinations is due to the hydrolysis and is proportional to the activity of the enzyme. Toluene was used as a

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1 Paez, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 217.

2 Mouat-Briggs, C. E. F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1914, **8**, 216.

3 Caldwell, F. C., and Caldwell, E. L., *Am. J. Trop. Med.*, 1929, **9**, 471.

4 Robbins, B. H., *J. Biol. Chem.*, 1930, **87**, 251.

preservative. The results are reported in milligrams of amino nitrogen set free by the action of the enzyme upon 100 cc. of 2% gelatin in 24 hours at 35°C.

Seven samples of a solution containing 40 mg. each of ficin were adjusted to pH values of 3 to 9 by the addition of hydrochloric acid or sodium hydroxide. These mixtures were then placed in an incubator at 35° for 3 hours. At the end of this time they were neutralized and added to flasks containing gelatin and diluted to 100 cc. (final gelatin concentration 2%). Formol titration was immediately carried out and again after 24 hours incubation at 35°C. The results of 2 such series are given in Fig. 1. The mixtures in series 2 were incubated 24 hours longer and again titrated and the data shown in curve 3 of Fig. 1.

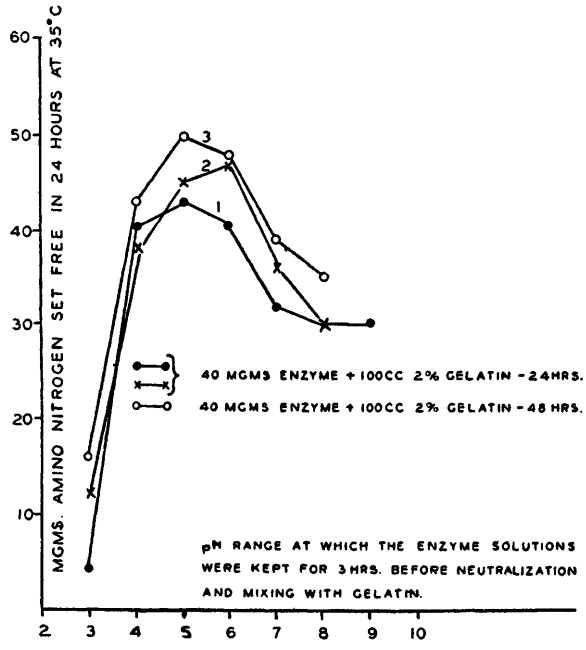

FIG. 1.

Hydrolysis of gelatin by samples of enzyme, obtained from the sap from the fig tree *F. glabrata*, which had been kept at varying pH values from 3 to 9 for 3 hours before neutralization and mixing with the gelatin for digestion.

Seven samples of gelatin (80 cc. of a 2.5% solution) and 7 samples of enzyme (20 cc. each containing 40 mg. of ficin) were adjusted to pHs of 3 to 9 and then mixed. Formol titrations were made immediately upon 20 cc. aliquots and again at the end of 24 hours incubation at 35°. The results of this series and one using 80 mg. of ficin are given in Fig. 2.

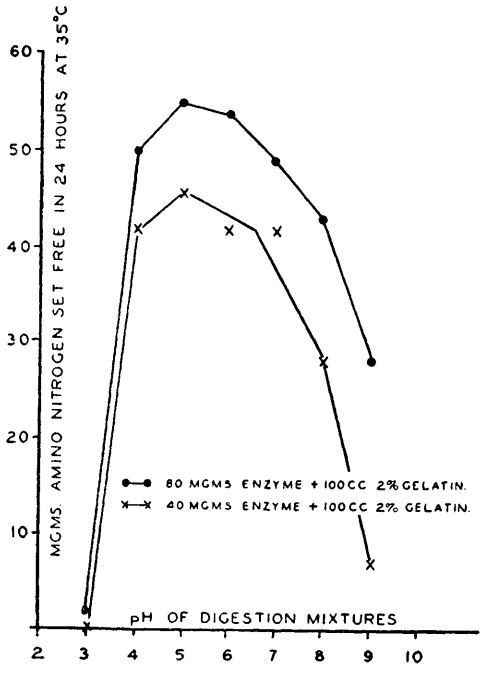

FIG. 2.

Hydrolysis of gelatin mixtures, of pH values from 3 to 9, by the proteolytic enzyme in the sap from the fig tree *F. glabrata.*

It can be seen from the Figs. 1 and 2 that the pH of optimal activity for ficin is 5. That a pH of 3 destroys the enzyme action whereas a pH of 8 is inhibitory, is shown by comparing the curves of Figs. 1 and 2 and also by data obtained when the digestion mixtures used in curve 1 in Fig. 2 were neutralized and incubated for 24 hours longer. There was no increase in the free amino nitrogen in the mixture originally incubated at a pH of 3 whereas in the mixture originally at pH of 8 showed an increase of 13 mg.

Summary. The optimum hydrogen-ion concentration for the ficin-gelatin proteolysis is pH 5.