Effect of Proteolytic Enzymes on Granuloma Formation. (22592)

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Recent studies have demonstrated the potent anti-inflammatory anti-edema properties of proteolytic enzymes(1-3). These studies have utilized protection against edema formation or erythema as a measure of the activity of the enzymes. Recently Ungar and Ginsburg reported a marked depression of granulation tissue surrounding surgically implanted gut in the livers of trypsin treated rats(4). Sherry and co-workers(5) also reported that they were able to prevent post-operative adhesions in dogs treated with plasminogen activators and attribute these results to activation of the blood fibrinolytic system. The purpose of this report is to present data demonstrating the effectiveness of the proteolytic enzymes, trypsin and chymotrypsin, as agents capable of modifying the tissue response to a foreign body *viz.* implanted cotton pellets.

Material and methods. Sprague-Dawley female rats weighing 95-110 g were used. The technic of Stucki and Meyer(6) was used for subcutaneous implantation of the weighed cotton pellets. The enzymes were subcutaneously administered as an aqueous solution daily, beginning immediately after pellet implantation and continued for 5 days. On the 6th day the animals were sacrificed, the pellets with the surrounding granulation tissue removed and dried at 105°C for 18 hours. The dried granuloma pellets were weighed, the pellet weight subtracted from the entire dry weight and the remainder recorded as granuloma weight. Control animals were injected with 0.5 ml 10% gelatin solution. One series of animals was treated with heat inactivated trypsin. *Pellet implantation* sites which had become infected were discarded. This situation occurred in several instances in the control animals, but was not observed in the enzyme treated rats. Korth and Bock(7) have similarly recorded a reduced incidence of infectivity in trypsin treated mice. The *enzymes* used were 2 x crystallized trypsin and chymotrypsin prepared by the method of Kunitz *et al.*(12).

TABLE I.

No. animals	Treatment	Dose, mg	Granuloma wt, mg	% inhibition
6	Controls		15.4 ± .93	
7	Trypsin	5	10.3 ± .98	33
7	Controls		13.7 ±1.80	
7	Chymotrypsin	2.5	6.8 ±1.37	51
14	Controls		8.8 ± .64	
14	Trypsin	2.5	6.0 ± .34	32
14	Chymotrypsin	2.5	6.0 ± .42	30
14	Controls		8.1 ±1.30	
14	Trypsin	1	5.5 ± .38	32
14	Chymotrypsin	1	7.2 ± .42	10
6	Controls		8.4 ± .83	
6	Trypsin (Heat inactivated)	5	9.0 ± .91	
	Hypophysectomized animals			
10	Control		8.5 ±1.31	
6	Trypsin	2.5	5.2 ± .58	39
6	Chymotrypsin	2.5	5.1 ± .67	40

Results. The data obtained are presented in Table I. The decrease in granuloma formation was consistently obtained in each of the 5 individual experiments. A quantitative effect could not be significantly demonstrated over a 2-fold increase in dose of enzyme. When the dose is reduced to 1 mg it is noted that although trypsin was effective in reducing the weight of the granuloma, chymotrypsin was ineffective. This difference may be due to the relative proteolytic activity of the 2 enzymes. Martin(8) has noted a correlation between effectiveness of the proteolytic enzyme as an inhibitor of egg white edema and proteolytic activity. Heat inactivated trypsin was ineffective in decreasing the size of granulomas.

Discussion. The effect of the proteolytic enzymes, trypsin and chymotrypsin, upon the granuloma induced by subcutaneous implantation of cotton pellets is another manifestation of the anti-inflammatory properties

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of these substances. The mode of action is not clear but presumably may be dissociated from an activation of the plasma fibrinolytic system, inasmuch as chymotrypsin, which is active in reducing the granuloma reaction, is ineffective as an activator of the plasma fibrinolysin system(9,10). Since the effect upon granuloma size occurs in hypophysectomized animals (Table I) this action cannot be ascribed to a stimulation of the pituitary-adrenal system with consequent hypersecretion of cortical steroids. This indicates that the enzymes probably exert their action either through their own proteolytic or depolymerase properties, or by the activation of an anti-inflammatory mechanism distinct from the profibrinolysin-fibrinolysin system. Martin(11) has suggested that these enzymes may be adsorbed or concentrated at the site of fibrin deposits and may exert their anti-inflammatory properties by depolymerization or digestion of these fibrin deposits.

During the course of these experiments it was also observed that the incision area of the trypsin and chymotrypsin treated animals healed with less granulation tissue than did the similar areas in the untreated control group.

Summary. The parenteral administration of trypsin and chymotrypsin to animals carrying subcutaneous implants of cotton pellets, significantly reduces the size of the granulomas formed around these pellets. Heat inactivated enzyme does not manifest this property.

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Effect of Antimetabolites on Growth of *Endamoeba histolytica*. II. Pantetheine and Coenzyme A Antagonist.* (22593)

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The identity of a number of growth factors for *Endamoeba histolytica* has recently been established by Nakamura and Baker (1,2). Purine and pyrimidine analogues were found to be potent inhibitors of the amebas by Nakamura and Jonsson(3). Aside from this report and that of Kun *et al.*(4), who studied the effect of metabolic inhibitors on carbon dioxide and hydrogen sulfide production by *E. histolytica*, no other information is available with regard to the effect of antimetabolites on the amebas. This situation arises from the fact that until recently very little was known regarding the specific nutritional requirements of this protozoan. Since coenzyme A has been identified as a growth factor for *E. histolytica*(2), it became desirable to confirm this observation using a coenzyme A antagonist.

Boxer *et al.*(5) reported that β-*d*-pantoyl-

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