Effect Upon Inflammation of Topical Treatment with Trypsin.* (20995)

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Several recent experimental studies deal with the effects of various enzyme-preparations upon inflamed tissue. Using the "granuloma-pouch" technic(1,2) as a test object, it has been noted that a fully developed granulomatous membrane is extraordinarily resistant both to trypsin(3) and to pepsin-containing fresh gastric juice(4). Consequently, the presence of inflamed tissue proved to afford an effective barrier against tissue-damage by these enzyme preparations. This could not have been solely a dilution effect with exudate accumulated in the pouch, since the contents of the latter had been evacuated prior to the introduction of the enzyme solutions. On the other hand, Seifter(5) reported that, if hyaluronidase is introduced into connective-tissue, simultaneously with a chemical irritant, (*e.g.*, paraphenylenediamine or allylisothiocyanate) the damage caused by the latter is actually aggravated, because the inflammatory response tends to spread over a greater area.

It has been our experience that the local protective value of irritated connective-tissue is essentially different during the acute stage, just after the eliciting injury is inflicted ("local alarm reaction") and during the more chronic stage, when the organization of a distinct granulomatous membrane has been completed ("local stage of resistance")(1). The experiments to be described in this paper have therefore been undertaken to compare the effect of trypsin upon normal, acutely inflamed and more chronically inflamed connective-tissue.

Materials and technics. Forty female Sprague-Dawley rats, weighing 130-150 g (average 138 g), were subdivided into 4 groups, as outlined in Table I. In all these animals, a symmetrical connective-tissue compartment was delimited by the insufflation of 25 ml of air, under the dorsal skin, between the shoulder blades. In Groups I, II, and III, this was immediately followed (without withdrawing the needle) by the injection into the cavity so produced, of 0.5 ml of a 1% croton-oil solution in corn oil. The details of this procedure, which leads to the formation of a typical granuloma-pouch, have been described elsewhere(2). In Group IV, air was insufflated in the same manner, but this was not followed by the injection of croton oil. Under these conditions, the connective-tissue lining the air-sac shows no inflammatory reaction and retains its essentially normal histologic structure. As a trypsin preparation, we used Armour's "Tryptar", in the form of a solution containing 5 mg/ml, freshly made up in Sorensen's buffer solution.† Two ml of this trypsin preparation was introduced into the airspace of each animal, in Groups II and IV immediately after the pouches were prepared, and in Group III 5 days later, when the croton oil had already transformed the lining into a well-organized granulomatous membrane.

TABLE I. Effect upon Inflammation Produced by Croton Oil of Topical Treatment with Trypsin.

Group	Treatment	Necrosis: Incidence (%)	Necrosis: Extent (mm^2)
I	Croton oil	0	0
II	Croton oil & trypsin simultaneously	100	75 ± 3.2
III	Croton oil & trypsin 5 days later	0	0
IV	Trypsin	20	5 ± .1

All experimental animals were killed (simultaneously with the controls of Group I), 48 hours after the trypsin injection. At that time, the entire dorsal skin was removed, flattened on a board, and the parchment-like dark-brown necrotic areas were measured planimetrically, as described in an earlier paper(2).

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† Buffered at pH 7.1 prepared by dissolving anhydrous dibasic sodium phosphate 6.6 mg/cc anhydrous monobasic potassium phosphate 2.7 mg/cc.

Results. As outlined in Table I, this, comparatively dilute, croton-oil solution caused no skin-necrosis in any of the controls (Group I), which were not treated with trypsin. On the other hand, the rats, in which croton oil and trypsin were administered simultaneously, all showed necrosis affecting considerable areas (75 mm^2 with a standard error of 3.2).

The rats in which the croton oil was administered 5 days before the trypsin (Group III), on the other hand, did not differ visibly from the controls, in that no skin-necrosis occurred in any of them. Under these conditions the trypsin also failed to induce any obvious change in the intensity of the inflammatory reaction itself. However, since this point was not relevant to the present study, we made no effort to appraise it exactly in quantitative terms. In any event more chronic experiments will have to be performed to assess the effect of the enzyme upon the later course of inflammation.

Among the animals receiving trypsin, without croton oil (Group IV), only 20% showed minute patches of necrosis (5 ± 0.1).

From these observations, it is evident that, in this experimental arrangement, trypsin given simultaneously with croton oil results in widespread tissue damage. This is presumably because here the irritant did not yet lead to the formation of a protective granulomatous barricade, and the damaging effect of the enzyme was merely superimposed upon that of the croton oil. It may be added that, in this group, the edematous and hemorrhagic inflammatory mass greatly exceeded the limits of the original air-sac, spreading towards the anterior surface of the chest. Apparently, the trypsin actually enhanced the dissemination of croton oil throughout the tissues. In view of the interesting observations of Innerfield and coworkers(6), who were able to produce antiphlogistic effects by intravenous and intramuscular trypsin treatment, this inverse effect is especially noteworthy. It calls attention to the fact that, at least under certain circumstances, trypsin can augment the tissue-damage produced by an irritant, conducive to an inflammatory response.

Conversely, after croton oil has already induced a well-formed granuloma, the tissue-damaging action of the enzyme is virtually abolished, and the adjacent tissues are protected. The mechanism of this protection is not yet known. Since, in the present experimental series, the trypsin was introduced on the fifth day, at a time when the pouch still contained only a few drops of exudate, it is unlikely that the overwhelmingly large dose of the concentrated trypsin solution (10 mg in 2 ml) could have been diluted beyond an effective concentration. This possibility is also rendered improbable by the experiments mentioned in the first paragraph of this paper, in which solutions of trypsin and pepsin had been introduced into fully developed granuloma-pouches, from which the exudate was completely removed by aspiration and yet, the phenomenon of protection remained evident. It is possible, on the other hand, that there occurs a considerable concentration of anti-enzyme factors in the granuloma-pouch, or in the few drops of exudate within the cavity, and such chemical inactivation might well be the basis of the protective effect here described. In man, approximately the same amount of trypsin is now given intravenously for the suppression of inflammatory tissue. Since here, activity is retained even after dilution with the entire blood-volume of the patient(6), such a concentration of antitryptic power in the granuloma-pouch is indeed rather improbable.

Finally, we had to consider the possibility that fatty acids, liberated from the oil, might have caused the inactivation of trypsin in the 5-day series, either because of their unsaturation or because of a lowering of the pH. To exclude this possibility and, at the same time, to show that a granuloma produced by an agent other than croton oil can also give protection, we performed a small additional experiment, in which carrageenin (kindly supplied by the Seaplant Chemical Corp.), was used as an irritant. The technics for the production of a granuloma-pouch and the experimental animals were similar to those employed in the main experimental series, except that here, in 10 rats, a granuloma-pouch was produced by the injection of 4 ml of a 1% aqueous solution of carrageenin, while 10 other rats acted as controls. On the fifth

day, the exudate which had accumulated in the carrageenin-treated animals was emptied and 4 ml of the above-mentioned trypsin solution was introduced into the air-sacs in both groups. With this doubled dose of trypsin, 80% of the controls showed necrosis of the skin above the pouch, while all the carrageenin-pretreated animals were perfectly protected. It may be concluded that an inflammatory granuloma, produced by an aqueous solution of an irritating polysaccharide, also gives protection against this enzyme.

Summary. Using the granuloma-pouch as an indicator, it has been shown that trypsin, given simultaneously with croton oil, causes particularly intense and widespread tissue-damage in the rat. On the other hand, if trypsin is introduced into the inflamed area 5 days after the croton oil, the granulomatous lining, formed under the influence of this chemical irritant, protects the adjacent tissues against otherwise toxic concentrations of this enzyme. The importance of the time-factor, in determining the effect of trypsin upon inflamed tissue, is emphasized.

1. Selye, H., In: *The Mechanism of Inflammation. An International Symposium.* Ed.: G. Jasmin and A. Robert, Acta Inc., Med. Pub., Montreal, 1953.
2. ———, *J.A.M.A.*, 1953, v152, 1207.
3. ———, *Exp. Med. and Surg.*, 1953, v11, 81.
4. ———, *Gastroenterology*, 1954, v26, 221.
5. Glassman, J. M., Blumenthal, A., Beckfield, W. J., and Seifter, J., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 323.
6. Innerfield, I., Angrist, A., and Schwarz, A., *J.A.M.A.*, 1953, v152, 597.

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A Possible Role of Iodinated Casein in Intestinal Assimilation of Vitamin B_{12}.* (20996)

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A widely used technic for increasing responses of animals to dietary vitamin B_{12} is the dietary inclusion of iodinated casein, as outlined by Betheil *et al.*(1), and others(2,3). It apparently is generally considered that the iodinated casein is effective because of its content of thyroxine and related compounds (4,5), which by increasing the rate of metabolism of a vit. B_{12}-deficient diet, makes animals thus conditioned more sensitive to dietary vit. B_{12}. That this may not be the whole explanation is indicated by the present results.

Experimental. Fifty day-old single comb White Leghorn cockerels were placed, after one day on corn meal, on a corn-soy diet† containing no animal products. An equal number was placed on this diet to which had been added 0.05% of iodinated casein.‡ Both diets contained 1.0% of formylsulfathiazole.§ Half of the birds under each dietary regimen were subjected to a 32-hour fast at 2 days, and again at 8 days of age, in an attempt to deplete yolk-sac reserves. Thus there were 4 experimental treatments in the depletion phase of the experiment: 1) basal, 2) basal plus fasting regimen, 3) basal plus iodinated casein, and 4) basal plus iodinated casein plus fasting regimen. At age 12 days, 5 birds, and again at age 15 days 4 birds were taken from each group for measurement of the experimental criteria. Each group then contained 16 residual birds. These were divided into halves. One half from each depletion treatment was placed on the same B_{12}-deficient basal diet‖

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† The same as described previously(6).

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