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THE EFFECT OF TRYPSIN ON LOCALIZED INFLAMMATION IN THE LIVER *

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ABSTRACT

1) The effect of trypsin on the organization of surgical gut in the liver of rats was investigated.
2) Intravenously administered trypsin caused marked depression of granulation tissue surrounding implanted surgical gut as well as marked fibrinolysis.
3) These effects did not occur when trypsin in oil was injected intramuscularly.

The antiphlogistic effect of trypsin has been reported both in clinical studies (Innerfield et al. 1953; Innerfield 1954, 1954a) and in experimentally induced edema (Martin et al. 1953, 1954; Beiler et al. 1955; Cohen et al. 1955; Adamkiewicz et al. 1955). The present report describes the effect of parenteral trypsin on the tissue response to implanted surgical gut in the liver.

MATERIALS AND METHODS

Male albino rats bred at the Hebrew University weighing 200—300 g were maintained on Purina Laboratory chow, and tap water *ad libitum*. Details of the technique used and its critical evaluation have already been reported (Ungar and Neuman 1952, Ungar and Feldman 1953).

During laparatomy under ether anesthesia, plain surgical gut 3/0 ("Ethicon brand") was introduced into the main lobe of the liver using a straight needle.

The following preparations of trypsin were used:

1) Trypsin twice crystallized containing 50% $MgSO_4$ (General Biochemical Inc.).
2) Trypsin 1 : 250 (Difco Laboratories).

These preparations were administered intravenously via a tail vein. All solutions were made up in phosphate saline buffer M/15 *p*H 7.4 and were freshly prepared daily.

3) Trypsin in oil (Parenzyme) obtained through the courtesy of the National Drug Company, Philadelphia. This was injected intramuscularly.

Determination of proteolytic activity

To mixtures containing 0.2 ml rat plasma and various concentrations of trypsin, thrombin (Upjohn Co.) was added to a final concentration of 5 units per ml of mixture

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and the time taken for complete lysis of the clot was determined. Thus the dosage of a single injection was based on the minimal amount of trypsin needed for complete lysis of the normal rat plasma clot in 15 minutes at 37°. Blood volume was taken to be 10% of body weight.

The animals were examined on the fourth day following implantation of gut. Controls without trypsin were included in each experimental group. Slices of liver containing the gut were fixed in Zenker's acetic acid solution. Paraffin sections were stained with hematoxylin-eosin and by Laidlaw's silver impregnation counterstained with Van Gieson. For some animals Weigert's fibrin stain was used.

RESULTS

Untreated controls

The surgical gut was surrounded by abundant neutrophiles mingled with remnants of necrotic liver cells. Around this area was a ring of granulation tissue 250—300 μ in thickness. The tissue contained moderate numbers of argyrophilic fibres, capillaries, young fibroplasts and histiocytes and was clearly defined against the adjacent liver tissue (Figure 1). These findings were identical with those reported previously (Ungar and Neuman 1952, Ungar and Feldman 1953). There was no mortality in this group and clot lysis was not observed.

Twice crystallized trypsin

Of eight rats receiving trypsin, five survived to the end of the experiment, four days following the implantation of gut. Four to seven injections were given, starting 6—10 hours after the operation and continuing for 4 days. The total dose injected was 64—210 mg/kg body weight.

Blood coagulation time was considerably prolonged in all treated animals and ranged between 40 and 50 minutes as compared with 5 minutes in the controls. Marked fibrinolysis occurred in all animals, lysis of the clot being completed within 2—5 hours.

Histological findings

The surgical gut was surrounded by neutrophile leukocytes which were present in diminished numbers in rats which had received more than 130 mg/kg of trypsin, as compared with those treated with the smaller dose. The ring of granulation tissue in all treated animals was narrower than in the controls and averaged 75—130 μ in thickness (limits 60—150 μ). There were also signs of qualitative depression in the composition of granulation tissue. Fibroblasts appeared with dark nuclei, some of them tortuous, and were arranged in compact concentric rings. Mitosis was rarely seen. The numbers of histiocytes and capillaries were greatly reduced (Figure 2).

Trypsin 1 : 250

Five of ten rats survived for four days. By that time they had received 360—640 mg/kg administered in 2—4 injections, starting 6—12 hours after laparatomy. Blood coagulation time ranged between 30 and 50 minutes throughout the experiment. Fibrinolysis tests gave essentially the same results as in the preceding group.

Histological findings

The gut was surrounded by a ring of granulation tissue 80—150 μ (limits 60—190) in thickness. The tissue contained less capillaries than in untreated animals but was rich in histiocytes. The nuclei of the fibroblasts were smaller and stained darker than in controls. Reticulum fibres were developed abundantly. The cellular elements of the granulation tissue were separated by an eosinophilic amorphous material which did not stain with Weigert's fibrin stain. Varying numbers of leukocytes were present in the inner zone of the reactive focus (Figure 3).

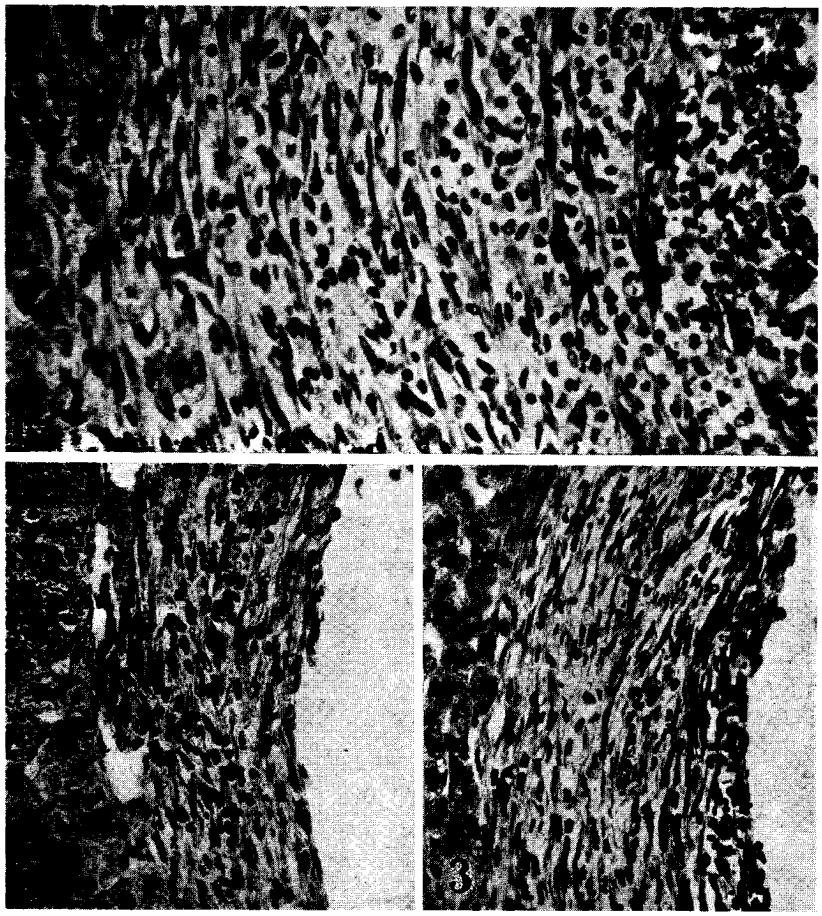

Figure 1

Granulation tissue, four days after implantation of surgical gut into liver of normal rat. (At the right margin leukocytic exudate adjacent to the gut).

Hematoxylin-eosin × 360.

Figure 2

Tissue response in liver following crystalline trypsin intravenously. Note absence of leukocytes and regression of granulation tissue.

Hematoxylin-eosin × 360.

Figure 3

Tissue response in liver following crude trypsin intravenously. Changes are similar to preceding figure.

Hematoxylin-eosin × 360.

Intramuscular trypsin in oil

During the 4 days of observation, 4 rats received a total of 13 mg/kg administered in 6 injections, and 4 others received 60 mg/kg administered in 5—6 injections. Injections were started 24 hours before laparatomy and the last one given 10 hours before death. Blood coagulation time remained normal as did prothrombin t mes and fibrinogen levels. No fibrinolysis could be detected, and all animals remained alive. The histological findings were essentially the same as in untreated controls; there was no visible suppression of granulation tissue.

DISCUSSION

Experimental investigations of the effect of trypsin on inflammatory reactions have been mainly concerned with the acute stage of edema formation (Martin et al. 1953, 1954; Beiler et al. 1955; Cohen et al. 1955; Adamkiewicz et al. 1955). There is only one study which also deals with the effect of trypsin on chronic inflammation produced by kaolin, but no histological examination is reported (Adamkiewicz et al. 1955). No studies appear to have been made of the effect of trypsin on the granulation tissue response to absorbable foreign material. The implantation of plain surgical gut into the liver has previously been shown to be a useful method for the evaluation of factors concerning the development of fibroblastic tissue and its regression in the environment of the liver (Ungar and Neuman 1952, Ungar and Feldman 1953, Ungar 1953).

Trypsin, administered intravenously, was markedly toxic and nearly half of the animals died during the course of the experiment. No lethal effect was observed following the intramuscular injection of trypsin in oil.

The tissue response following intravenous injections of crystalline or crude trypsin was similar in all animals and differed from that in untreated controls in several respects. The ring of granulation tissue surrounding the surgical gut was significantly narrower, having an average thickness of 100 μ and being in no instance thicker than 190 μ, while in the control animals it measured 250—300 μ. The number of polymorphonuclear leukocytes and blood capillaries was reduced and fibroblast nuclei appeared deformed and frequently hyperchromatic. There was no interference with the formation of reticulum fibres. No fibrin was demonstrated in any case, by Weigert's stain.

Trypsin is known for its thromboplastic and proteolytic properties; in small doses it initiates blood coagulation while in high doses it is proteolytic (Innerfield et al. 1952). Trypsin is also known to be a potent activator of plasminogen, converting it to the proteolytic enzyme plasmin (Lewis and Ferguson 1952). In our experiments animals given intravenous crystalline trypsin showed high proteolytic activity of the blood, while trypsin in oil did not have this effect.

The histological findings do not establish whether the depressive effect of trypsin on granulation tissue formation is due to its proteolytic activity, lowering the plasma fibrinogen or otherwise affecting blood coagulation or by a direct interference with organization of the inflammatory exudate. The suggestion that the apparent antiphlogistic action of trypsin in edema might be the result of hypersecretion of corticosteroids, secondary to stress, has not found experimental support (Adamkiewicz et al. 1955).

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