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THE EFFECT OF SODIUM α-ACETONYLBENZYL-4-HYDROXY CUMARIN (SODIUM WARFARIN) AND OF ACUTE BLOOD LOSS ON LOCALIZED INFLAMMATION IN THE LIVER INDUCED BY IMPLANTED SURGICAL GUT

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ABSTRACT

(1) The effect of sodium warfarin (warficide) on the organization of surgical gut in the liver of rats was investigated.

(2) Low doses of warficide which caused reduction of prothrombin level but no loss of plasma proteins due to haemorrhage revealed depressing effect on granulation tissue which was assumed to be specific. This depression did not interfere with the normal cytomorphological aspects of fibroblasts.

(3) The administration of high doses of warficide was accompanied by severe haemorrhage. Possible relationship between the decreased tissue response and loss of blood and/or plasma proteins was investigated and acute protein depletion was found to be equally efficient in suppressing the granulation response.

Various possible factors active in the production of fibrosis in the liver have previously been investigated in model experiments (Ungar 1953, Ungar and Feldman 1953, Ungar and Ginsburg 1955, Ungar et al. 1956). We have used the tissue response around implanted surgical gut in the liver of white rats as indication of the formation of connective tissue. By this method the effects of various diets, cortisone, and trypsin on the development of connective tissue in the liver have been evaluated. We found that the intravenous administration of trypsin to rats caused a marked suppression of granulation tissue formation in the liver which was accompanied by fibrinolysis. It was considered of interest to determine whether the same effect on granulation tissue would be caused by interfering with blood coagulation by means of anticoagulants.

In the present paper we describe the action of sodium warfarin on localized inflammation in the liver.

MATERIALS AND METHODS

Male white rats bred at the Hebrew University weighing 200—300 g were kept on a diet of Purina laboratory chow and tap water ad libitum.

The technique of implantation of surgical gut into the liver is described elsewhere (Ungar and Feldman 1953). 3/0 plain surgical gut was used, manufactured

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by Davies & Geck (preserved in xylene). The gut was washed in absolute alcohol and transferred to normal saline prior to use.

Sodium warfarin (warficide) (S. B. Penick & Co., New York) coated on sand was dissolved in distilled water to make 0.5 mg/ml and 2.0 mg/ml solutions which were administered intramuscularly.

Prothrombin determinations were made by the method of Quick.

Clotting time was measured by the method of Lee and White.

Plasma proteins were measured by the biuret method.

The rats were sacrificed on the fourth day following the implantation of gut. Slices of the liver containing the gut were fixed with Zenker's acetic acid solution. Paraffin sections were stained with haematoxylin-eosin. Laidlaw's silver impregnation counterstained with Van Gieson and occasionally Weigert's fibrin stain was used.

EXPERIMENTAL

Untreated controls

As shown previously, the tissue response to implanted surgical gut in the liver reaches its maximal development on the fourth day following implantation, and subsequently begins to regress (Ungar and Feldman 1953). In every case the gut was found to be surrounded by a zone of neutrophiles mingled with remnants of necrotic liver cells and an outer ring of granulation tissue composed of young fibroblasts, a lesser amount of histiocytes, moderate numbers of argyrophile fibres, and capillaries (Figure 1).

The effect of administration of warficide

The daily administration of 3—4 mg/k.b.w. into rats in which a surgical gut was implanted into a lobe of the liver caused reduction of prothrombin levels to 0—10% of normal. This reduction was accompanied by severe internal haemorrhage. As a result plasma protein levels in all animals were reduced to 3—4 g/% (6 g/% in controls) at the end of the experiments.

In view of the possibility that acute blood loss might have interfered with the tissue response, the dose of warficide which would lower prothrombin level without causing apparent haematoma or internal haemorrhages was determined. The dose of 1—2 mg/k.b.w. daily was found to lower prothrombin level to 30—40% of normal within 24 hours without causing untoward effects.

7 out of 9 rats survived to the end of the experiment. The first injection was given 18 hours after the implantation of gut. The total dose injected in 3 separate portions amounted to 3—4 mg/k.b.w. Blood coagulation time measured 24 hours after the operation and at the time of sacrifice was 20—25 minutes in all animals. Prothrombin levels in all rats were 35—40% of normal throughout. The plasma protein concentrations in all the rats were within the normal range of 5.4—6.0 g/%. Neither

internal haemorrhage nor haematoma developed. Scattered petechial haemorrhages were seen on the peritoneal surface of the intestines. No pallor of internal organs was seen as in the high-dose group. Blood vessels were moderately congested. No bleeding could be seen around or between abdominal muscles.

Histological findings

Marked depression of granulation tissue formation was found in all the animals. The gut was surrounded by a ring of granulation tissue of average width 56—90 μ with a minimum of 20 μ and a maximum of 120 μ (150—200 μ in controls). No qualitative changes in the connective tissue components could be found. In some cases the amount of macrophages was greater than in the high-dose group (Figure 2).

This tissue response was found to be identical with that observed in 7 of 9 rats receiving the higher dose of warficide (a total amount of 12—16 mg/k.b.w.). In the remaining two animals no cellular reaction was found.

The effect of bleeding and plasmapheresis

In order to determine the possible effect of acute blood loss on tissue response in the liver, 2 additional groups of animals were subjected to repeated bleeding and plasmapheresis following the implantation of gut.

Assuming the blood volume of the rats to be 10% of body weight, a total of 30% of the blood was withdrawn in 3 lots at 18, 42, 66, and 90 hours following the implantation of gut. No serious effect on the condition of the animals was observed during the withdrawals. At the end of the experiment plasma protein levels ranged between 4.5 and 5.5 g/%. In 3 additional rats a total of 10—12 ml of blood was withdrawn in 3 portions after the implantation of gut. 9 volumes of blood were mixed with 1 volume of 1.34% sodium oxalate. The mixture was centrifuged at 2000 r.p.m., the red cells washed twice with phosphate saline buffer m/15, pH = 7.4, warmed to 37° in a water bath and re-injected into the tail vein. In both experiments suppression of granulation tissue was observed.

Histological findings

At the end of 4 days the gut was found to be surrounded by a ring of granulation tissue which measured on the average 70 μ with a minimum of 50 μ and a maximum of 100 μ (150—200 μ in controls). No qualitative changes could be demonstrated in the components of the granulation tissue. However, there was a greater number of macrophages as compared with controls (Figure 3).

DISCUSSION

Following the implantation of surgical gut into the liver of white rats the adjacent tissue responded with the development of granulation tissue. The response was maximal on the fourth day before absorption of the gut was observed (Ungar and Feldman 1953).

The administration of warficide in a dose which lowered prothrombin levels to 30—40% of normal, but which caused neither reduction of plasma proteins nor untoward effects, resulted in considerable depression of the tissue response, the width of the ring of the granulation tissue being about a quarter to one half of that observed in controls. The same effect was produced in animals receiving high doses of warficide (10—12 mg/k.b.w.) which were accompanied by severe blood loss. Repeated bleeding or plasmapheresis in two series of animals produced similar effects adjacent to the implanted gut. These findings show that the depressing effect of acute blood loss is probably due to depletion of plasma proteins and not to the accompanying hypoxia.

It is concluded that warficide has a depressing action on granulation tissue in rats which may be enhanced by the simultaneous effect of acute depletion of plasma proteins due to bleeding.

No qualitative differences were observed in the cellular and fibrillar components of the granulations in treated and untreated animals. This finding is in distinct contrast to experiments reported previously, using the same methods in which the effects of cortisone and trypsin were tested (Ungar and Ginsburg 1955, Ungar et al. 1956). In the experiments with cortisone and trypsin the fibroblast nuclei appeared distorted and hyperchromatic, and the capillaries were found to be reduced in number. Only in experiments with cortisone did the reticulum fibres appear thicker, increased in number and frequently agglomerated (Ungar et al. 1956). These observations suggest that while cortisone and trypsin may act directly on the proliferating as well as on the already formed fibroblasts, warficide depressed their formation but did not affect their morphology.

Since only one stage in the tissue response to implanted absorbable material in the liver was studied, we are unable to decide whether this depressing effect applies to the entire process of connective tissue organization or represents only a lag of the response which may be compensated for at a later stage.

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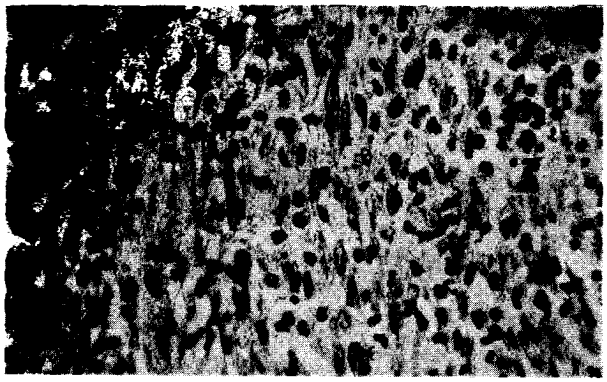

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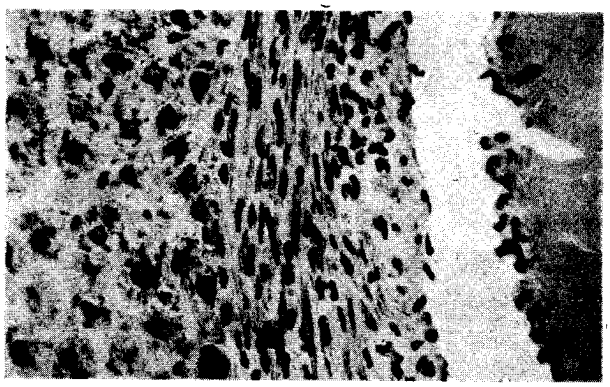

(3)

PLATE I

(1) Granulation tissue 4 days after implantation of surgical gut into the liver of normal rats (the right margin closes with leucocytic layer adjacent to the gut). — (2) Tissue response in liver following the administration of low dose of warficide. Note the narrow ring of granulation tissue as compared with controls. — (3) Tissue response in the liver following plasmapheresis.