EFFECT OF LEUKOCYTIC FACTORS ON CUTANEOUS BLOOD VESSELS

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Experiments on rabbits and rats have shown that intradermal injection of homologous fragmented leukocytes and leukocytic lysosomes causes disturbances of permeability and integrity of the blood vessels, leading to the development of hemorrhages of nonthrombohemorrhagic character.

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Participation of polymorphonuclear leukocytes in the development of disturbances of permeability of blood vessels has been demonstrated experimentally [1, 4, 6, 7]. The suggestion has been made that leukocytic factors may have a possible role in the late phase of disturbances of permeability in inflammation [1, 6, 7]. An important role is also ascribed to polymorphonuclear leukocytes in the pathogenesis of the Sanarelli–Schwartzmann and Arthus phenomena, which develop in a manner similar to that of hemorrhagic inflammation [4, 8].

The object of the present investigation was to continue the study of the effect of leukocytes on blood vessels.

Lysosomes and fragmented leukocytes were obtained as described previously [1]. Six specimens of leukocytic preparations made at different times, but by the same method, were used in the investigation. The criterion of action of the leukocytic factors on blood vessels was their ability to disturb the permeability of cutaneous blood vessels as shown by the passage of the dye Evans' blue into the skin when injected intravenously in a dose of 20 mg/kg body weight of a 1% solution [3]. Vascular permeability was studied in 14 chinchilla rabbits (weight 2.5-3.5 kg) and 25 Wistar rats (weight 200-250 g) of both sexes. The animals were sacrificed 1 h after injection of the dye and an additional inspection of the inner aspect of the skin was made to assess disturbances of permeability. To determine the duration of changes in vascular permeability the dye was injected 72, 64, 48, 40, 24, 12, 9, 6, 3, 1, 0.5, and 0.25 h after intradermal injection of the leukocytic preparation [2]. Tests of the leukocytic preparations on three rabbits and five rats were carried out after intravenous injection of heparin (3 units/ml blood every 4 h for 16 h). The quantity of the lysosome material injected into the rabbits, expressed as its protein content determined by Lowry's method [5], was 3×10^{-7}–3×10^{-2}, and that of fragmented leukocytes 4×10^{-6}–1×10^{-1} mg; the corresponding values for rats were 2×10^{-7}–2×10^{-2} and 3×10^{-5}–1×10^{-1} mg. Intradermal injection of the homologous preparation in 0.1 ml physiological saline was given into the shaved skin of the abdominal wall. An intradermal injection of 0.1 ml physiological saline acted as control.

EXPERIMENTAL RESULTS

Intradermal injection of fragmented lysosomes and leukocytes caused disturbances of vascular permeability in the skin observable for 0.25-6 h after the injection, and equal in severity at all times of the investigation. Neither partial nor complete recovery of normal permeability of the vessel wall was observed during this period. The degree of disturbance of permeability was directly dependent on the dose of the preparation.

Later observations (up to 72 h) on all experimental rabbits revealed the development of extensive hemorrhages, 0.4-3 cm in diameter, in the regions of intradermal injection of lysosomes and leukocytes. The severity of the hemorrhages depended on the dose of leukocytic preparations given, and where identical quantities of protein were injected, they were greater in the case of lysosomes than of fragmented leukocytes.

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After injection of large doses of the preparations, the hemorrhages spread to the subcutaneous areolar tissue and muscles, with the formation of blood clots. Marked hyperemia of all vessels was observed. The caliber of the vessles was sharply increased. The hemorrhages were largest 9-12 h after intradermal injection of the preparation. In some cases (3-6 h after injection of lysosomes and leukocytes), disturbances of permeability and petechial hemorrhages were observed simultaneously. At the latest observations (48-72 h after injection) the hemorrhages had begun to disappear and had become brownish in color. The hemorrhages in rats were less severe than in rabbits.

The threshold doses, i.e., those evoking slight hemorrhages, for rabbits were 3×10^{-6} mg lysosome protein and 4×10^{-5} mg protein of fragmented leukocytes, and for rats 2×10^{-6} and 3×10^{-5} mg respectively. When expressed as the initial number of leukocytes, this meant that vascular disturbances appeared in the rabbits and rats after intradermal injection of the equivalent of 1200-12,000 leukocytes.

Intravenous injection of heparin into the rabbits and rats did not affect the character of the disturbances produced by leukocytic preparations. In the zones of intradermal injection of lysosome and leukocytes, a disturbance of permeability was observed 0.25-6 h after injection, and this was slightly more pronounced than in animals not receiving heparin. Hemorrhages developed in the heparinized rabbits and rats in the same way as in the control animals.

The experiments confirmed the results of investigations described in the literature and of previous work by the present authors, indicating an increase in vascular permeability under the influence of leukocytic factors. Leukocytes evidently do not participate in the development of the earliest manifestations of acute inflammation. Manifestation of their activity requires the accumulation of leukocytes in the tissues, with their destruction and liberation of substances, especially lysosome enzymes. The view can therefore be accepted that leukocytic factors participate in the pathogenesis of late manifestations of permeability disturbance in inflammation.

The data for the development of hemorrhages are new. There are reports in the literature that polymorphonuclear leukocytes participate in the pathogenesis of thrombohemorrhagic phenomena [4, 8]. The present investigation showed that hemorrhages are also produced in animals receiving heparin. This means that the hemorrhages observed cannot be regarded as the result of primary thrombosis of very small vessels. Under the influence of leukocytic factors injury apparently takes place to the vessel wall, expressed initially by an increase in permeability, and later by disturbance of the integrity of the vascular walls with the consequent development of hemorrhages. There was grounds for asserting that the activity of fragmented leukocytic lysosomes and leukocytes is due to liberation of hydrolytic enzymes. This is confirmed by the results of preliminary experiments showing that extensive hemorrhages develop in rabbits at the site of intradermal injections of 0.3 units of acid phosphatases (Reanal, Hungary). It is interesting to note that the permeability disturbances and hemorrhages observed after injections of acid phosphatase were similar in character to those found after administration of leukocytic preparations. There is thus an apparent contradiction between the observed hemorrhagic activity of leukocytic substances and the absence of hemorrhages in acute inflammatory reactions of the skin. This may evidently be attributed to the fact that in ordinary acute inflammation the local concentration of lysosomal enzymes of leukocytic origin is too low to cause the development of disturbances of integrity of the blood vessels, followed by hemorrhages.

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