

MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE SYNOVIAL MICROCIRCULATORY
BED IN ANTIGEN-INDUCED ARTHRITIS

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In inflammation of a joint, whether clinical or experimental permeability of the microcirculatory bed (MCB) of the synovial membrane (SM), usually measured by means of radioisotope methods [3-5, 9], is considerably increased [1, 2, 6-8]. During morphological investigations, several ultrastructural characteristics are regarded as features of increased permeability, although their significance is not completely clear. These include an increase in the number of pinocytotic vesicles in endotheliocytes of microvessels, widening of the spaces at junctions between endothelial cells, changes in the thickness and structure of the basement membrane, etc. However, the study of the dynamics of development of ultrastructural features of damage to MCB of SM, and its comparison with the results of a parallel investigation of its function, has not hitherto been undertaken. In the present investigation, the aim was to determine more precise ultrastructural criteria of increased permeability of MCB.

EXPERIMENTAL METHOD

Experiments were carried out on 56 male chinchilla rabbits weighing 1200-1600 g. The animals were immunized by intradermal injection of 5 mg of bovine serum albumin in 0.5 ml of Freund's complete adjuvant in the interscapular region. On the 15th day after immunization a reacting dose of bovine albumin (0.5 mg) in 0.1 ml of physiological saline was injected into the knee joint. To ensure sterility the solution was filtered before injection through a Millex TM (USA) filter (0.22 μ). Physiological saline was injected into the joint of 10 control animals. Antigen in a dose of 0.5 mg was injected intradermally in the interscapular region of the rabbits 24 h before injection of the reacting dose in order to detect an immune response in the form of hyperemia around the injection site. The rabbits were killed under barbitol anesthesia on the 1st, 3rd, 7th, 30th, and 60th days after injection of the reacting dose. Pieces of SM of the affected and contralateral knee joints were studied by the usual histological and electron-microscopic methods. Radioisotope investigation of permeability of SM was carried out 1 h before sacrifice on a "Gamma" gamma-camera (Hungary) with ^{99m}Tc per-technetate, which was injected in a dose of 74 mBq into the auricular vein of rabbits immobilized in the supine position. The investigation was repeated at intervals of 1 min for 20 min, with polaroid pictures obtained after 2 and 10 min (Fig. 1). The experimental data were analyzed by computer distinguishing of zones of interest corresponding to the regions of the affected and contralateral knee joints, and with plotting of functional curves and their mathematical analysis. The total number of counts and their mean number per unit area of matrix were determined. The ratio of the number of counts in the inflamed and contralateral joints (coefficient of absorption - CA) was calculated on the basis of total (CA_t) and average (CA_a) counts. These coefficients in the control animals were very close to unity. To assess the presence of potentiation of a particular feature of ultrastructural damage to MCB of SM, five capillaries and five postcapillary venules in the subcutaneous layer of SM were examined in each rabbit. At each time from five to 14 rabbits were investigated. The presence and intensity of the feature were assessed visually directly under the electron microscope by two observers. The features were regarded as positive if the following conditions were satisfied: a) edematous thickening of the endothelium (4) - swelling of the endotheliocytes with translucent cytoplasm; b) thickening of the endothelium due to hyperplasia - an increase

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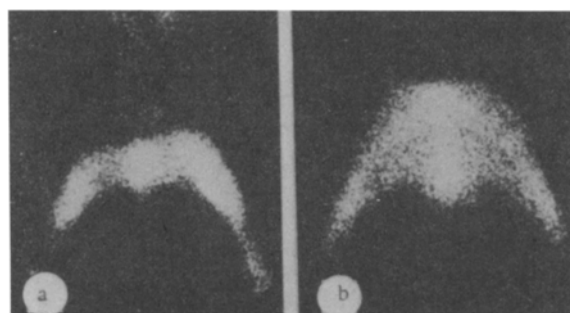


Fig. 1. Accumulation of ^{99m}Tc pertechnetate in inflamed knee joint (arrow) on 7th day of antigen-induced arthritis (a) and distribution of radionuclide in knee joints of control animal (b).

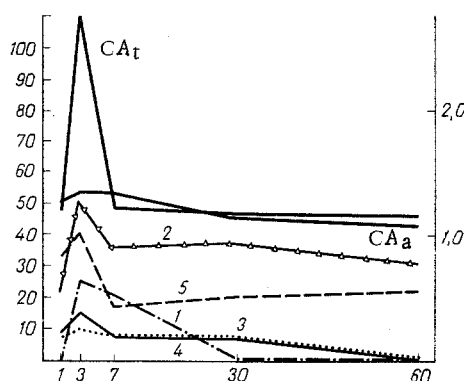


Fig. 2. Comparison of absorption of ^{99m}Tc pertechnetate in affected joints and frequency of discovery of ultrastructural features of damage to MCB of SM at different times of antigen-induced arthritis.

TABLE 1. Absorption of ^{99m}Tc Pertechnetate in Affected Joints at Different Times of Antigen-Induced Arthritis

Parameter	Time of antigen-induced arthritis, days				
	1	3	7	30	60
CA_t	1.20 ± 0.02	2.74 ± 0.53	1.21 ± 0.02	1.15 ± 0.02	1.15 ± 0.02
CA_a	1.25 ± 0.04	1.33 ± 0.15	1.33 ± 0.06	1.13 ± 0.03	1.07 ± 0.02

in the number of organelles outside the perinuclear cytoplasm of the endotheliocytes; c) intensification of pinocytosis (2) — the pinocytotic vesicles filled the greater part of the cytoplasm of the endotheliocytes; d) widening of the periendothelial space (1) — wide slits between the basal cytolemma of the endotheliocyte and the lamina densa of the basement membrane; e) thickening of loosening of the structure of the basement membrane (these features are usually combined) — thickening by not less than 50% compared with normal; f) stratification of the basement membrane — the number of layers exceeding two; g) widening of spaces at junctions between endotheliocytes (5) — this may take place over the whole area of the junction or only in a limited part (lacunar); h) fibrous thickening of the adventitia — a distinct increase in the weight of collagen fibrils in it; i) plasma inhibition of the vascular wall — the appearance of a precipitate analogous to that in the lumen of the vessel, in the zone of the basement membrane and adventitia; j) passage of leukocytes through the vessel wall (3) — leukocytes among endotheliocytes, in the subendothelial space, in the adventitia.

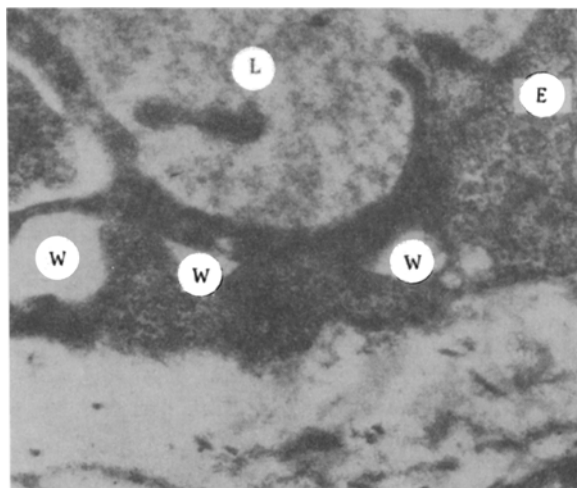


Fig. 3. Lacunar widenings (W) of intercellular spaces at junction of endotheliocytes (E) of a venule on 3rd day of antigen-induced arthritis. L) Lumen of venule. 45,300 \times .

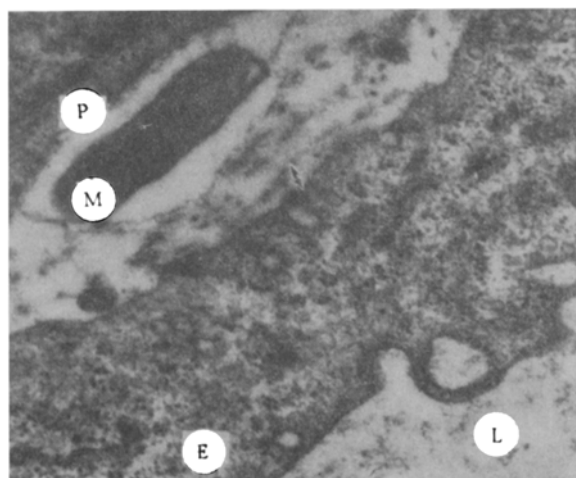


Fig. 4. Widening of periendothelial space (arrow) and myelin figure in space between endotheliocyte (E) of capillary and pericyte (P) on 7th day of arthritis. L) Capillary lumen. 37,500 \times .

EXPERIMENTAL RESULTS

The results of the scintigraphic investigations are given in Table 1.

The time course of the values of CA_t and CA_a , and also the frequency of discovery of the most characteristic features of damage to MCB at different times of antigen-induced arthritis, expressed as a percentage of the number of vessels recorded on examination in the electron microscope, are shown in Fig. 2. The frequency of occurrence of some of the ultrastructural features of damage to MCB, namely edematous thickening of the endothelium, widening of the spaces at junctions between endotheliocytes (Fig. 3), widening of the periendothelial space (Fig. 4), intensification of pinocytosis in the endotheliocytes, and passage of leukocytes through the vascular wall, showed a definite parallel with the scintigraphic parameters, reflecting increased permeability of SM. Other ultrastructural features (thickening of the endothelium due to hyperplasia of organelles, an increase in the number of cytoplasmic fibrils in the endotheliocytes, stratification of the basement membranes, and fibrous thickening of the adventitia) were present to only a mild degree at the early stages of arthritis. The frequency of their occurrence increased toward the later stages of observation, when values of CA_t and CA_a decreased. These two groups of features thus characterize different phases of

inflammation. Features included in group 1, because of the parallel observed with the scintigraphic parameters, can be regarded as reliable ultrastructural evidence of increased permeability of MCB.

With respect to both the 1st and 2nd groups of features significant differences were found in the frequency of their occurrence between the early (until the 7th day inclusive) and the later stages of observation, which reflect the exudative and proliferative stages, respectively, of inflammation. Features such as edematous thickening of the endothelium and widening of the periendothelial space were found significantly more often in the microvessels in the earlier stages of arthritis, whereas thickening of the endothelium due to hyperplasia of organelles, stratification of the basement membrane, and fibrous thickening of the adventitia were found more often in the later stages ($P < 0.01$). Widening of the spaces at the junctions between endotheliocytes were found significantly more often in the early stages of the experiment in the venules. Destructive changes in organelles of endotheliocytes (swelling of mitochondria, widening of cisterns of the rough endoplasmic reticulum and perinuclear space) were not characteristic of either the exudative or the proliferative stages of antigen-induced arthritis. Nevertheless, at the peak of exudation (the 3rd day after intra-articular injection of the reacting dose) these phenomena were observed more constantly than at other times, which evidently reflects functional overloading of the endotheliocytes at the height of the exudative stage of inflammation. Fenestrated endotheliocytes were seen at all times of the experiment, but because of the considerable variability of their number in different animals it was impossible to draw any definite conclusions.

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