RADIOMETRIC AND HISTOAUTORADIOGRAPHIC INVESTIGATION OF THE CAPILLARY CIRCULATION IN TUBERCULOSIS

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Data are described which confirm the fluctuating character of permeability of the tissue-blood barriers of parenchymatous organs associated with different types of course of tuberculosis: during progression and in the period of treatment. Differences in the character of incorporation of albumin-I^{131} into the region of specific lesions and into distant areas, and its distribution in the lymphatic system are described.

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During the development of tuberculosis, disturbances of vascular permeability are systemic in character [2, 3], Within 6 h of injection of an attenuated culture of Mycobacterium tuberculosis into animals, changes occur in the ultrastructures of the air-blood barrier which indicate a disturbance of permeability of the capillary wall [5]. The fluctuating course of tuberculosis is accompanied by marked fluctuations in permeability of the tissue-blood barriers [3, 6]. However, the permeability of the vascular wall and cell membranes in tuberculous inflammation has not yet been adequately studied.

This paper gives data characterizing permeability of the blood and lymphatic capillaries to radioactive compounds of low and high molecular weight.

EXPERIMENTAL METHOD

Experiments were carried out on 64 guinea pigs weighing 350-500 g, infected by subcutaneous injection of a virulent culture of Mycobacterium tuberculosis strain H_{37} Rv in a dose of 0.0001 mg. The animals were divided into six groups: animals with tuberculosis (1.5 and 3 months after infection), animals with tuberculosis treated for 1.5, 3, and 6 months with antibiotics, and healthy control animals. A morbidity index was calculated by the scheme adopted at the Central Research Institute of Tuberculosis. Indicators of permeability used in the investigation were of low ($Na_2HP^{32}O_4$) and high molecular weight (human albumin I^{131}), and they were injected intravenously in a dose of 0.1 μCi/g body weight. Incorporation of the isotopes into tissues of the lungs, liver, spleen, adrenals, and striated muscles was determined radiometrically, using the B-2 radiometer and BFL-25 end-type counter. The coefficient of permeability was calculated as the ratio between activity of 1 g tissue and the activity of 1 ml blood. Microautoradiographs with albumin-I^{131} were prepared by the usual method [4, 7]. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

Permeability of the vascular wall to P^{32} and albumin-I^{131} differed in animals with tuberculosis (1.5 months after infection) whose morbidity index was 14. Penetration of radioalbumins into the tissues either was not significantly different from the control or was very slightly reduced (Fig. 1). Permeability in the spleen was increased, due to the more extensive lesions in its tissue.

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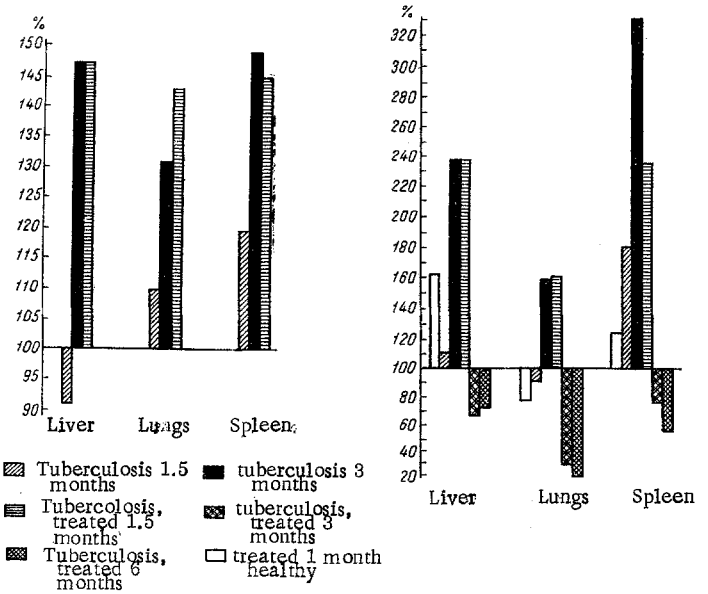

Fig. 1. Coefficients of permeability of vascular walls to P^{32} and albumin-I^{131}. 1) Striated muscles; 2) adrenals; 3) lungs; 4) liver; 5) spleen. Coefficients of permeability of healthy animals taken as 100. I) Tuberculosis for 1.5 months; II) tuberculosis for 3 months; III) treatment for 1.5 months; IV) treatment for 3 months; V) for 6 months.

Radioactive phosphate participates in many metabolic processes, so that the coefficient of its permeability reflects mainly the requirement of a particular organ for phosphorous compounds. Despite the decrease in permeability of the tissue-blood barrier (TBB) of the adrenals (relative to penetration of radioalbumin), the penetration of P^{32} into the adrenal tissue was increased, reflecting a high intensity of phosphate metabolism characteristic of a state of stress of the endocrine organ at this period of the disease.

During development of the disease (3 months after infection), the foci of tuberculous inflammation increased in size, fused together, and liquified, and when the morbidity index was 20-22, the uptake of both isotopes into the tissues was increased. Permeability of the vascular walls of all organs was increased ($P < 0.01$), except for striated muscles (Fig. 1). The concentration gradient of radiophosphorus between blood and tissues characterizes the increased metabolism in the organs affected by tuberculosis, and in tissues where no inflammatory changes were present (striated muscles).

Treatment of the guinea pigs began one month after infection. As a result, after antibiotic therapy for 1.5 months the morbidity index fell to 8. However, the tissue requirement of phosphorus compounds and the permeability of their TBB remained high (identical with that for the terminal period of the disease). Phosphate metabolism in muscle tissue, on the other hand, was reduced in intensity, although their vascular wall was highly permeable to radioalbumin (Fig. 1). The permeability was sharply reduced 3 and 6 months after the beginning of treatment (morbidity index 4-1).

Radiometric measurements reflect the degree of accumulation of isotope in the organs and blood and the concentration gradient between blood and tissue can be determined from them.

Local disturbances of permeability can be demonstrated only by histoautoradiography. Irregularity of the distribution of radioalbumin was found on autoradiographs of the lungs, liver, and spleen of the healthy animals, as a result of differences in the level of function and metabolism in different parts of the tissue.

Irregularity of distribution of the isotope was also found in the organs of animals with tuberculosis, irrespective of treatment. The highest density of isotope on the autoradiographs of these animals was found above groups of lymphocytes around the vessels and bronchi, and around the interlobular vessels of the liver. These findings were less marked in healthy animals (Fig. 2A, B). If, on the other hand, the formation of a specific lesion had begun in these areas, the number of silver grains on the autoradiograph was sharply reduced, and sometimes it did not exceed the background value.

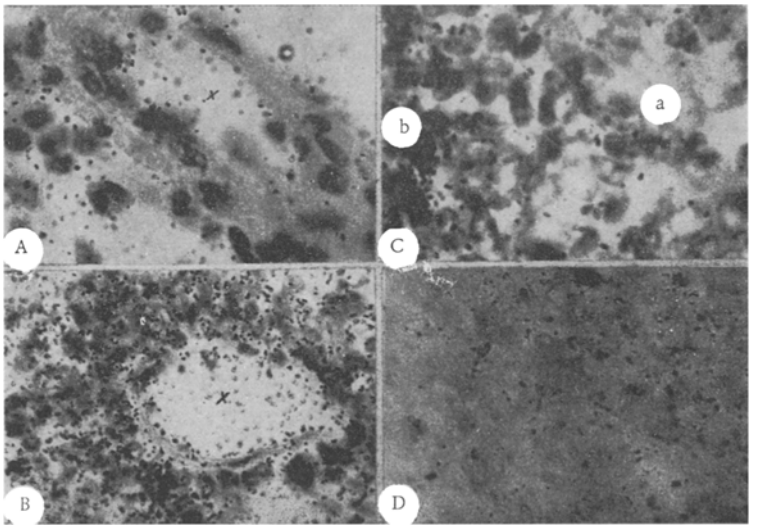

Fig. 2. Zonal character of distribution of albumin-I^{131} in different parts of lung tissue (A, B, C) and zone of caseous necrosis in the liver (D). A) Perivascular space of healthy lung; B) same area in animal after treatment for 1.5 months (x marks blood vessel); C) a) zone of epithelioid cells, b) collections of lymphocytes. Hematoxylineosin. A, 1200 ×; B) 770 ×; C) 1200 ×; D) 480 ×.

A high concentration of albumin-I^{131} was found in the zone of necrobiosis, and in the course of its homogenization and caseation the density of silver grains on the autoradiograph fell sharply (Fig. 2D). Solitary silver grains were seen above collections of epithelioid cells, and the surrounding barrier of lymphocytes and plasma cells was rich in radioactive compounds (Fig. 2C).

It can thus be concluded from the facts described above that experimental tuberculosis in guinea pigs is accompanied by periods of rise and fall in the TBB permeability of organs affected by the specific process. The increase in their permeability in the terminal period of the disease (3 months after infection) and 1.5 months after the beginning of treatment probably takes place through different mechanisms, as the different values for permeability of the vascular walls of the intact tissues (striated muscles) suggest.

Having regard to data in the literature concerning the dynamics of specific antigens and antibodies (at the same times of observation) it can be postulated that at the moment of the animal's death effects produced on the vascular wall are mainly toxic in character, while during the period of treatment the allergic component is predominant [1].

The investigation using P^{32} only approximately reflects the state of TBB permeability in tuberculosis, and when this method is used, a control is therefore necessary, using compounds of high molecular weight. Penetration of radiophosphorus through the vascular wall reflects principally metabolic processes in the tissues.

The histoautoradiographic investigation showed that zones differing in the state of permeability of their capillary walls are present in organs affected by tuberculosis.

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