FIXATION OF SHEEP'S RED CELLS BY THE LYMPHATIC GLANDS OF CATS AND DOGS

N. V. Medunitsyn

From the Department of Pathological Physiology (Head — Corresponding Member AMN SSSR Prof. A. D. Ado) of the N. I. Pirogov Second Moscow State Medical Institute (Director — Docent M. G. Sirotkina)

(Received October 31, 1958. Presented by Active Member AMN SSSR N. N. Sirotinin)

From information in the literature, all substances with a molecular weight over 20,000-30,000 are absorbed exclusively through the lymphatic vessels [10]. Antigens which pass through the lymphatic glands become fixed there. The degree of fixation depends on the dispersion of the substances.

There is indirect evidence that foreign proteins are held up with difficulty by lymphatic glands [15, 17]. The smallpox virus passes freely through the lymphatic barrier into the blood stream [16].

There is no unanimity on the fixation of bacteria. Some authors [6, 7] regard the lymphatic system and, hence, the lymphatic glands as conductors of infection. The majority of workers hold the opposite opinion.

Large particles are retained with particular intensity in the lymphatic glands. During perfusion of the popliteal lymphatic glands of the dog with a suspension of isogenous red cells, 99% of them were held up [11].

The mechanism of fixation of large corpuscular antigens is one of mechanical holding up of the antigen by the reticular tissue of the sinuses of the lymphatic glands and of subsequent phagocytosis.

Bearing in mind the role of the lymphatic glands in the process of antibody formation, it may be assumed that they take part in the process of fixation of a specific component — in the reaction of combination of antigen and antibodies within the lymphatic gland itself [8, 9, 12, 13, 14].

The barrier properties of lymphatic glands depend on various conditions: on the reactive state of the body, on the pressure of the lymph entering the gland, on the quantity of antigen reaching it, and so on. In response to immunization there is a significant increase in the barrier function of the lymphatic glands [1,2,3,5].

In the present research we studied the barrier function of the lymphatic glands in relation to the fixation of foreign red cells.

EXPERIMENTAL METHOD

In this investigation we used the method of perfusion of the lymphatic vessels [4] of the popliteal lymphatic glands of dogs and cats with an antigen solution. Estimation of the antigen content of the original fluid and of the fluid after passing through the lymphatic glands enabled the barrier function of the lymphatic glands to be judged with accuracy.

As anesthetic we used a 10% solution of evipan, which was injected into the cats in a dose of 1 ml/kg intramuscularly, and into the dogs in a dose of 0.25 ml/kg intravenously. Furthermore the dogs were given a preliminary injection of a 1% solution of morphine hydrochloride in a dose of 1 ml/kg, 15-20 minutes beforehand.

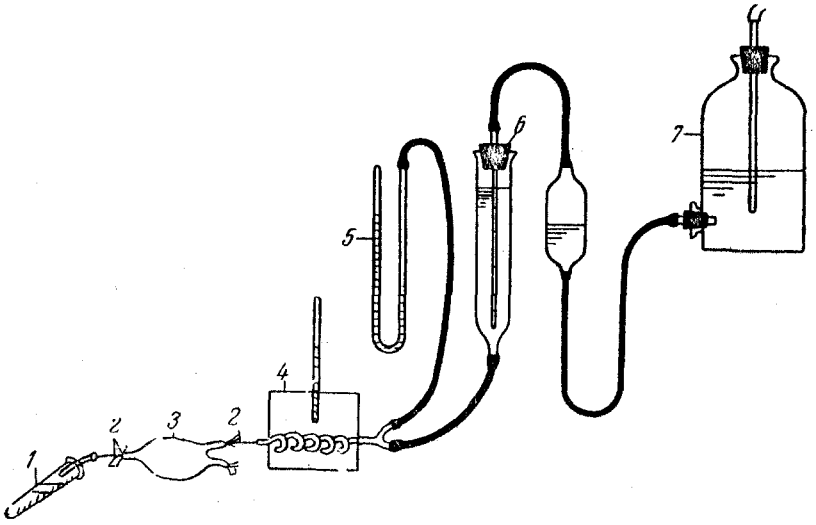

Fig. 1. Diagram of the apparatus for perfusion of a lymphatic gland. 1) Graduated test tube; 2) needles inserted into the lymphatic vessels; 3) lymphatic gland; 4) bath (temperature 38-39°); 5) water manometer; 3) measuring cylinder; 7) Marriott's bottle.

A suspension of sheep's red cells (50,000-60,000/mm^3) in Tyrode solution was used for the perfusion. In order to prevent sedimentation of the red cells, a glass tube, through which air was blown, was introduced into the closed vessel containing the fluid, preventing sedimentation of the red cells and maintaining a constant perfusion pressure (see diagram in Fig. 1).

The pressure of the perfusate was 20, 25 and 30 mm of water. At a lower pressure the percentage fixation of the sheep's red cells was very high. Corresponding to the three levels of pressure, three samples of perfusate were obtained, each of which was collected for 15 minutes. The lymphatic glands on both sides were examined.

After the experiment a solution of Chinese ink was injected through the afferent vessel into the lymphatic gland in order to reveal any possible anastomoses between the afferent and efferent lymphatic vessels.

The number of red cells in the original fluid and in the perfusate was determined by counting in a Goryaev chamber. The barrier function of the lymphatic glands was calculated as the percentage retention of sheep's red cells.

Immunization of the animals was carried out by means of five injections, every 5 days, of a 5% suspension of sheep's red cells in a dose of 1 ml/kg for the cats and 0.3 ml/kg for the dogs. In one group of animals the red cell suspension was injected subcutaneously into the lower part of the calf, and in the other group – into the lateral surface of the body. Investigation of the barrier function of the lymphatic glands was carried out on the 2nd-16th day after completion of immunization.

Altogether 35 cats and 10 dogs were used in the experiments. The number of lymphatic glands perfused was 72 (56 in cats and 16 in dogs).

EXPERIMENTAL RESULTS

The results obtained from perfusion of the lymphatic glands of the cats and dogs were of the same type.

The percentage fixation of sheep's red cells by the lymphatic glands of the unimmunized animals at a perfusion pressure of 20 cm of water varied between limits of 53.8-95.5%, at a pressure of 25 cm between 48.3-94.5%, and at a pressure of 30 cm between 36.0-93.1% (Fig. 2).

The variations in the percentage fixation of sheep's red cells by the lymphatic glands of the immunized cats, injected with the suspension in the region of the calf, were as follows: at a perfusion pressure of 20 cm water 93.1-100%, at a pressure of 25 cm 88.7-100% and at a pressure of 30 cm 78.7-100%.

Thus with an increase in the pressure of the perfusate the percentage fixation of red cells decreased. The degree of lowering of the percentage fixation in response to a change in the pressure of the perfusate was less pronounced in animals immunized in the paw than in intact cats.

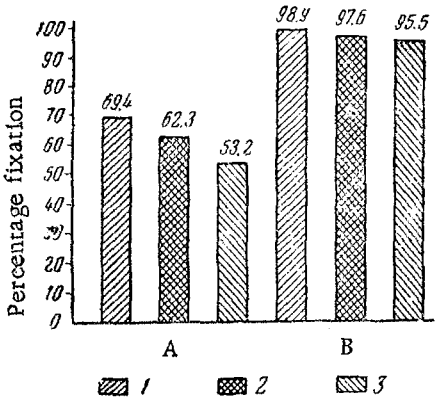

Fig. 2. Fixation of sheep's red cells by the lymphatic glands of the cat at different pressures of perfusate (in percent; mean values given. A) Unimmunized cats; B) immunized (in the region of the calf) cats. 1) At a perfusate pressure of 20 cm of water; 2) at a pressure of 25 cm of water; 3) at a pressure of 30 cm of water.

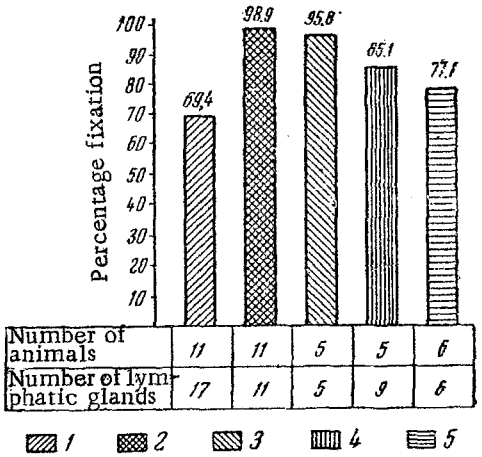

Fig. 3. Fixation of sheep's red cells by the lymphatic glands of the cat (in percent; mean values given. 1) In unimmunized animals; 2) in animals immunized with sheep's red cells (in the region of the calf on the side of immunization); 3) in the same animals, on the opposite side; 4) in animals immunized with sheep's red cells in the lateral region of the body; 5) in animals immunized with dogs' red cells in the region of the calf.

During perfusion of the lymphatic glands at constant pressure, and in some cases even at increased pressure, the percentage fixation gradually rose and the volume of perfusate obtained in each 15 minutes diminished. This phenomenon probably arose as the result of rapidly developing edema and mechanical blocking of the reticular filter of the lymphatic gland.

In Fig. 3 is shown the relationship between the percentage fixation of sheep's red cells and the site of injection of the antigen during immunization of the animals (pressure of the perfusate 20 cm of water).

After immunization of the cats with sheep's red cells in the left paw, the percentage fixation of red cells during perfusion of the lymphatic gland on the right also increased (variation between limits of 89.3-100), and after immunization of the cats in the lateral part of the body the percentage fixation varied from 71.7 to 99.3.

The results of the experiments showed that after immunization of the animals, the fixing power of the lymphatic glands, especially the regional glands, was increased. The question naturally arose of the specificity of this phenomenon.

In order to elucidate this question we immunized cats subcutaneously in the region of the paw with dogs' red cells and perfused the lymphatic glands with a suspension of sheep's red cells. The conditions of immunization and perfusion were the same as in the previous experiments. Under these circumstances the percentage fixation at a perfusate pressure of 20 cm of water was 45.9-99.

On these grounds we may postulate the existence of two components of the function of fixation of the lymphatic glands of immunized animals — a specific and a nonspecific.

After immunization of the animals, especially in the region of the calf, hypertrophy of the lymphatic glands was observed. The weight of the glands was doubled or trebled. The area of contact between the antigen and the reticular tissue of the lymphatic gland was enlarged, thereby stimulating phagocytosis and resulting in considerable antigen fixation. In some experiments, during perfusion of the lymphatic glands of immunized animals, hemolysis appeared in the perfusate, especially in the first samples (when the perfusate was allowed to stand for 24 hours in the cold). In addition hemolysis was observed after the perfusate was allowed to stand in a bath (at a temperature of 37°) and complement was added.

SUMMARY

The method of perfusion of popliteal lymph nodes by suspension of sheep red cells was employed in cats and dogs. With increased perfusate pressure a diminuition of the lymph nodes' fixating function of respective sheep red cells was noted.

In immunized animals this relationship is less pronounced. During the process of immunization in animals, the barrier function of the lymph showed a rise. When compared with the immunization effected in other areas of the body, the fixating function was appreciably higher in the animals inoculated into the region lying below the given lymph node.

LITERATURE CITED

[1] M. M. Barats, The Effect of the Functional State of the Nervous System on the Barrier Function of the Lymphatic Glands, Author's abstract of candidate's dissertation, Ufa, 1957 [In Russian].

[2] V. M. Berman, Problems of Age Changes in Immunology, pp. 7-50, Leningrad, 1947 [In Russian].

[3] V. M. Berman and E. M. Slavskaya, Reactivity Changes with Age in Infectious and Immunological Processes, pp. 56-68, Leningrad, 1955 [In Russian].

[4] Yu. I. Borodin, The Innervation of the Popliteal Lymphatic Gland. Candidate's dissertation, Novosibirsk, 1956 [In Russian].

[5] E. V. Glotova and T. N. Karpenko, Transactions of the Kirov Institute of Epidemiology and Microbiology, Collection 2, pp. 139-143, 1948 [In Russian].

[6] A. A. Kazanskii, The Role of the Lymphatic System in the Pathogenesis of Acute Empyema, Candidate's dissertation, 1946 [In Russian].

[7] Kh. Kh. Planel'es, The Chemotherapy of Bacterial Infections, vol. 5, pp. 159-171, Moscow, 1950 [In Russian].

[8] B. M. Sagalovich, Zhur. Mikrobiol., Épidemiol. i. Immunobiol., No. 1, 77-81 (1952).

[9] F. K. Forshter, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 11, 100-106 (1955).

[10] J. M. Barnes, and J. Trueta, Lancet, 1941, v. 1, p. 623-626.

[11] C. K. Drinker, M. E. Field and H. K. Ward, J. Exper. Med., 1934, v. 59, p. 393-405.

[12] L. D. Hamilton, Nature, 1956, v. 178, p. 597-599.

[13] S. Harris, T. N. Harris and M. B. Farber, J. Immunol., 1954, v. 72, p. 148-160.

[14] S. Hayes and T. Dougherty, J. Immunol., 1954, v. 73, n. 2, p. 95-99.

[15] H. Rouvière and G. Valette, Physiologie du système lymphatique, Paris, 1937.

[16] J. Yoffey and E. Sullivan, J. Exper. Med., 1939, v. 69, p. 133

[17] J. Yoffey, E. Sullivan and C. Drinker, J. Exp. Med., 1938, v. 68, p. 941-947.