2. F. E. Vishnevetskii, A. A. Yushchenko, and V. V. Anokhina, "A method of determining antileprosy activity of drugs," Author's Certificate 1157457 (USSR).
3. T. A. Demchenko, "Determination of the macrophagal transformation phenomenon of mononuclear cells in cultures of blood leukocytes in order to evaluate the immunologic status of an organism," Technical Recommendations [in Russian], Leningrad (1980).
4. K. A. Kolesov, Vest. Dermatol., No. 10, 55 (1968).
5. A. A. Yushchenko and F. E. Vishnevetskii, Byull. Éksp. Biol. Med., No. 3, 376 (1987).
6. E. J. Ambrose, S. R. Khanolkar, N. H. Antia, et al., 11th International Leprosy Congress, Abstracts, Mexico City (1978), pp. 96-97.
7. A. N. Band, A. Bhattarja, and G. P. Talwar, Int. J. Leprosy, 54, No. 1, 71 (1986).
8. J. Baohong and S. K. Noordeen, Tropical Disease Research: A Global Partnership (UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases), Geneva (1987), pp. 113-124.
9. A. M. Dhople and K. J. Green, IRCS Med. Sci., 14, 807 (1986).
10. D. V. Freire-Maia, Int. J. Leprosy, 53, No. 1, 141 (1985).
11. I. Nath, H. K. Prasad, M. Sathish, et al., Antimicrob. Agents Chemother., 21, No. 1, 26 (1982).
12. H. K. Prasad and R. C. Hastings, J. Clin. Microbiol., 21, 861 (1985).
13. D. S. Ridley and W. H. Jopling, Int. J. Leprosy, 34, 255 (1966).
14. H. Saito, H. Tomioka, K. Sato, and T. Watanabe, Infect. Immun., 51, 157 (1986).
15. F. E. Vishnevetskii (F.E. Vishnevetsky), A. A. Yushchenko (A. A. Yuscenko), and V. V. Anokhina, 12th International Leprosy Congress, Abstracts, New Delhi (1984), p. X/440.

ACCUMULATION OF BIOLOGICAL AND NONBIOLOGICAL CORPUSCULAR PARTICLES IN MAMMALIAN LUNGS

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UDC 612.112.3+612.21/.019.08

KEY WORDS: lungs; capillary lumen; accumulation of particles

The phenomenon of selective phagocytosis of autologous erythrocytes, treated with glutaraldehyde solution, by leukocytes located in the capillaries of the lungs has been described [3]. It is natural to suggest that this phenomenon reflects the presence of a special function, characteristic of the mammalian lung, connected with the elimination of foreign particles circulating in the blood stream. The investigation described below was undertaken to study this function.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 260-300 g. All painful procedures and removal of the animals from the experiment were performed under ether anesthesia. Altogether 36 rats were divided into nine groups. Animals of group 1 received an intravenous injection of 1 ml of a 1% suspension of yeast cells, used for making dough, suspended in isotonic salt solution. Animals of group 2 received an injection of a suspension of staphylococci ("Zhaev" strain) into the blood stream, in a dose of 10^9 microbial cells to 1 ml of isotonic salt solution; animals of group 3 received an injection of 0.5 ml of commercial black ink, free from preservatives and other coarse particles [1]. Animals of groups 4, 5, and 6 received an injection of 1 ml of polyacrolein microspheres 3 μ in diameter into the blood stream. The microspheres were labeled with a fluorochrome (pyronine). In group 4, the microspheres were

Department of Pathological Anatomy, Second Department of Internal Medicine, I. M. Sechenov First Moscow Medical Institute. Laboratory of Electron Microscopy, Research Institute of Physical and Chemical Medicine, Ministry of Health of the RSFSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 741-744, December, 1988. Original article submitted December 18, 1987.

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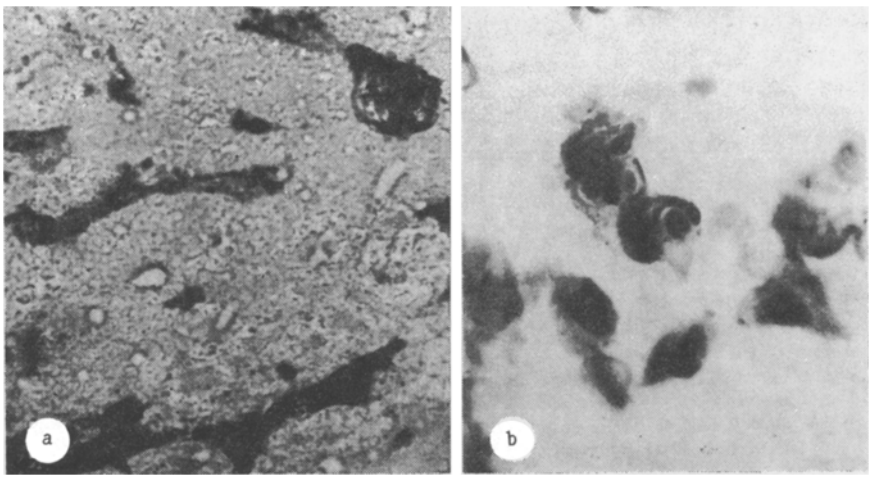

Fig. 1. Morphology of accumulation of colloidal black ink solution in stellate reticuloendotheliocytes of the liver and of yeast cells in the lungs. a) Liver tissue after injection of black ink into blood stream, stellate reticuloendotheliocytes clearly distinguished by black color of cytoplasm (accumulation of ink particles); b) yeast cells in substance of alveolar septa of lungs (arrows). Hematoxylin and eosin. Magnification: a) 600×, b) 900×.

incubated for 4 h before injection in distilled water, in group 5 they were incubated in rat blood serum, and in group 6, in a 1% solution of bovine serum albumin (BSA) in distilled water. The animals of groups 7, 8, and 9 were injected with a suspension of polyacrolein microspheres 0.3 μ in diameter. The microspheres were treated in the same way as in groups 4, 5, and 6. The animals were removed from the experiment 1 h after the injections. Frozen sections through the lungs, liver, and spleen, fixed in 10% neutral formalin solution, were examined in the ML-2 luminescence microscope. In addition, scrapings were taken from the cut surface of the organs mentioned, stained, and also examined in the ML-2 microscope (animals of groups 4-9). Additionally, material was taken from unfixed lungs for electron-microscopic investigation, prefixed in Karnovsky's solution [2] and postfixed in a buffered 1% solution of osmium tetroxide. The material was imbedded in water-soluble Durcupan or in an Epon-Araldite mixture, and dehydrated in acetones of increasing strength. Ultrathin sections were studied and photographed in the Hitachi-300 electron microscope.

EXPERIMENTAL RESULTS

The liver, spleen, and visible lymph nodes 1 h after injection of ink into the blood stream of the rats were stained black. The lung tissue was pale gray. No traces of ink were found in the blood serum during this period. Histologically, clumps of dye could be seen in the stellate reticuloendotheliocytes of the liver (Fig. 1a) and macrophages in the spleen also contained ink in their cytoplasm. There were few such cells in the lungs (one or two per field of vision of the microscope under magnification of 40) and they were located at the junction of several capillaries; a few clumps of dye were observed in the cytoplasm of these cells — three or four, of small size.

One hour after injection of the yeast suspension into the blood stream histological examination showed that most yeast cells were located in the lungs. In each field of vision of the microscope ×100 objective) three or four intrapulmonary concentrations of yeast cells were found, each consisting of three to five cells (Fig. 1b). A few reticuloendothelial (Kupffer) cells were seen in the liver (one or two in five fields of vision), with one or, less frequently, two yeast corpuscles in their cytoplasm. They were seen equally rarely in the spleen. Electron-microscopic investigation of the lungs showed that yeast cells were located in the cytoplasm of the leukocytes, more especially neutrophilic granulocytes (Fig. 2a) and macrophages. The number of leukocytes in the lung capillaries was much greater than in intact animals (in an ultrathin section, under magnification of 4000, three or four cells were seen in the area of the screen in rats into which yeast cells were injected, the leukocytes being

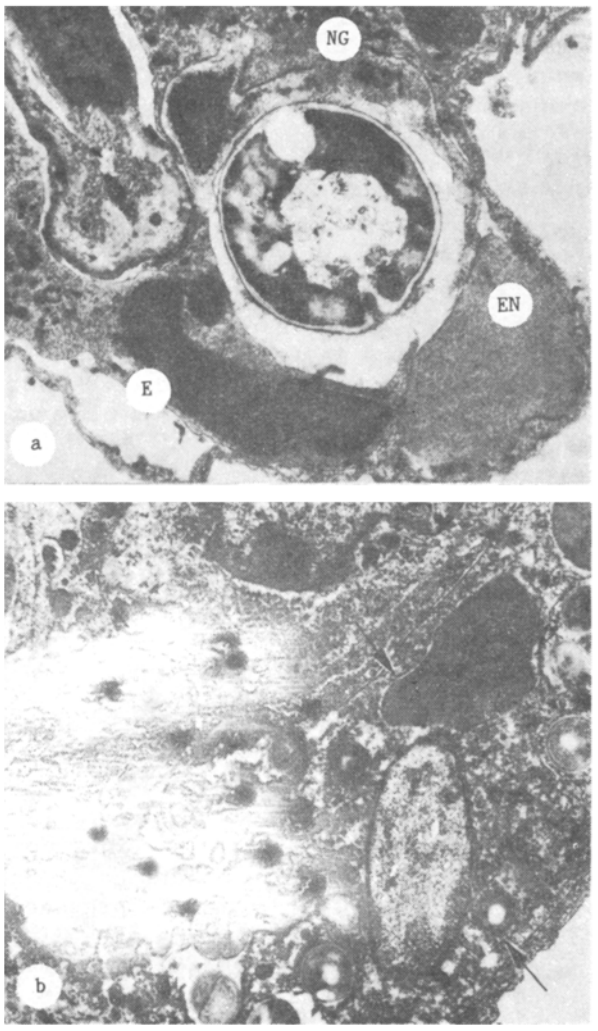

Fig. 2

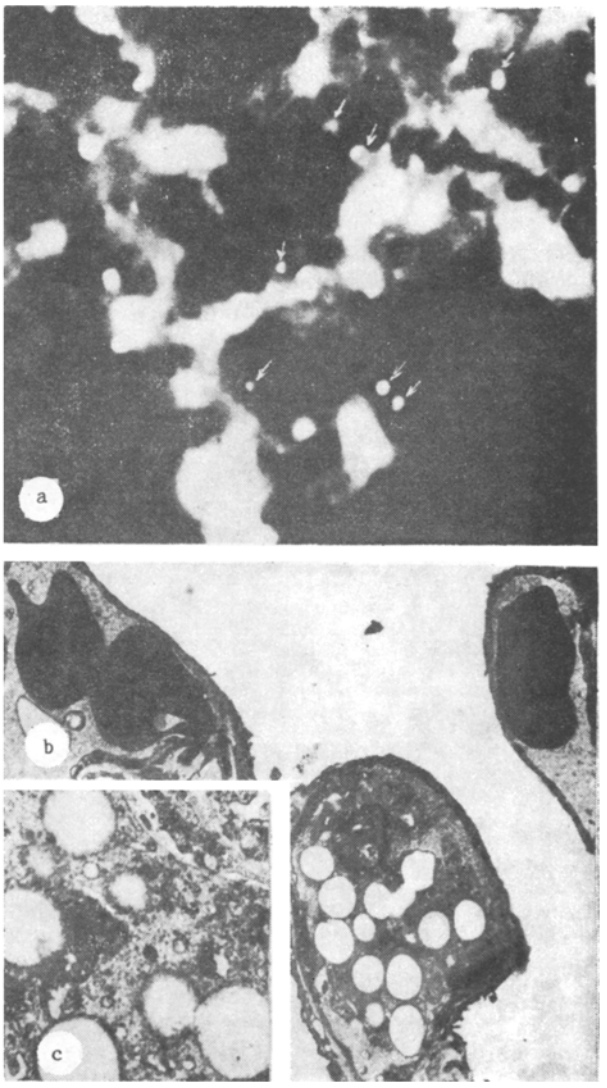

Fig. 3

Fig. 2. Accumulation of biological corpuscular objects (yeast cells and cocci) in the lungs. a) Yeast cell in cytoplasm of neutrophilic granulocyte (NG) located in capillary lumen in alveolar septum. E) Erythrocyte, EN) endotheliocyte; b) staphylococci in cytoplasm of neutrophilic granulocyte. Arrows indicate boundaries of capillary lumen. Magnification: a) 10,000×. b) 8000 ×.

Fig. 3. Accumulation of polyacrolein microspheres in rat lungs. a) Luminescence of individual accumulated microspheres in lung tissue against the background of autoluminescence; b) microspheres in cytoplasm of a neutrophilic granulocyte located in a lung capillary; c) microspheres in phagosome of neutrophilic granulocyte (arrow). a) Native frozen section, 250 ×; b) 5000 ×, c) 22,000 ×.

located in the capillary lumen; in intact animals, however, there was only one leukocyte to every 2 or 3 times the area of the screen).

After injection of a staphylococcal suspension into the blood stream the picture observed was similar to that after injection of yeast. Leukocytes, each containing 3-5-7 phagocytosed cocci were seen in the lung capillaries, and they were especially clearly identifiable on electron-microscopic study of the material (Fig. 2b). Light-optical investigation of material from the liver revealed single stellate reticuloendotheliocytes, each containing two or three phagocytosed cocci.

On the basis of these results it was postulated that corpuscular material accumulates selectively in the lungs, and the effectiveness of this accumulation is limited by the size of the particles. Besides large particles which become mechanically lodged in the lung capil-

laries, corpuscles similar in size to molds and bacteria, i.e., with a diameter of 1-3 μ, also are retained. This hypothesis was tested with the aid of polyacrolein microspheres of two classes: 1) 3 μ in diameter, and 2) 0.3 μ in diameter. Light and luminescence microscopy clearly demonstrated retention of the 3-μ microspheres in the lung tissue (Fig. 3a). Single-microspheres were seen in the liver and spleen. Electron-microscopic investigation revealed microspheres 3 μ in diameter, located in the cytoplasm of neutrophils present in the lumen of the lung capillaries. These particles were separated from the cytoplasm by the cell membrane, and in some places phagosome formation was noted (Fig. 3b, c). The largest number of cells which had ingested particles and the largest number of particles per cell were found after injection of microspheres preincubated in a solution of BSA (5-7 particles per cell; Fig. 3b, c), when 3-5 phagocytes were found in a field of vision of the light microscope with a ×40 objective.

When particles with a diameter of 0.3 μ were studied under light and luminescence microscopes, no accumulation of these particles could be found in the internal organs examined because of the autofluorescence of the tissue structures. Meanwhile in films of scrapings from the surface of the lungs particles of this kind were found in the form of luminescent "stars," but they were extracellular (evidently they were circulating in the blood stream). Electron-microscopic investigation revealed no reliable evidence of phagocytosis of particles 0.3 μ in diameter.

At this stage of the work it was thus shown that after injection of corpuscular objects of biological or nonbiological origin, measuring 2 μ or more, into the blood stream marked accumulation of the particles takes place in the lungs of healthy rats. Particles accumulate as a result of their phagocytosis by leukocytes (neutrophilic granulocytes and macrophages), located in the lumen of the lung capillaries. The intensity of accumulation of these particles in the lungs is much greater than in the liver and spleen, whereas after injection of colloidal carbon (ink) solution into the blood stream, the particles are retained chiefly by the liver and spleen. Two mechanisms of this retention of leukocytes containing phagocytosed corpuscular objects, in the lung capillaries can be postulated. The first may be connected with an increase in volume of the leukocytes after carrying out phagocytosis of the particles, so that these cells are "stuck" in the lumen of the lung capillaries. The second mechanism may be connected with expression of specialized receptors, resposible for interaction with the surface of the endotheliocytes of the lung capillaries, on the surface of the phagocytes. This last explanation appears to be more likely because, even in the presence of marked accumulation of particles of this diameter in the lungs no external signs of a disturbance of the pulmonary circulation were found, and this could not be the case with mechanical blocking of the microcirculatory pathways. Then phenomenon is of great interest for pathologists, for it may lie at the basis of development of hematogenous pneumonic foci in bacteriemia. Evidence of species-specific differences in the cellular mechanisms of realization of this phenomenon has been obtained [4].

LITERATURE CITED

1. O. V. Alekseev and A. M. Chernukh, On Problems of the Microcirculation: Structure and Function [in Russian], Moscow (1977), p. 132.
2. B. S. Weakley, Electron Microscopy for Beginners [Russian translation], Moscow (1975).
3. P. Aulenbacher, N. Paweletz, E. Friedrich, and R. Suss, Virchows Arch. Abt. A. Path. Anat., 44, 57 (1983).
4. A. Warner and J. Brain, Am. J. Physiol., 250, R728 (1986).