acteristic magnification 4 times). With this magnification an alveolus with the wide capillaries surrounding it is placed inside the frame of the camera (Fig. 3). The quantitative parameters of the microcirculatory network of the alveoli (n = 68) are given below.

The diameter of the wide capillaries was 34.8 ± 7 μ, the length of the wide capillaries 663 ± 18 μ, and the area of the wide capillaries 22,300 ± 900 μ^2; the length of the narrow capillaries was 600 ± 34 μ and their area 4815 ± 242 μ^2; the area of an alveolus was 17,700 ± 800 μ^2:

$$\frac{S_{nc}+S_{wc}}{S_a} - 1{,}65 \pm 0{,}05, \quad \frac{S_{wc}}{S_a} - 1.36 \pm 0{,}04,$$

$$\frac{S_{nc}}{S_a} - 0.28 \pm 0.01.$$

The method of studying the pulmonary microcirculation described above thus enabled: 1) trauma associated with fixation of the lungs to be abolished; 2) the capillary circulation to be studied in a wide area of the lungs, with the chest closed, and without disturbance of physiological excursions of the chest wall; 3) photographs of sufficiently high quality for studying quantitative parameters of the pulmonary microcirculatory system by the Leitz ASM semiautomatic image analysis system to be obtained, thereby increasing the accuracy of the measurements and speeding up analysis of the experimental results.

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MORPHOLOGICAL HETEROGENEITY AND FUNCTIONAL STATUS OF ALVEOLAR MACROPHAGES OBTAINED BY BRONCHOALVEOLAR LAVAGE DURING DEVELOPMENT OF TUBERCULOUS INFLAMMATION IN GUINEA PIGS

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KEY WORDS: bronchoalveolar washings; alveolar macrophages; electron microscopy; proteinases; tuberculosis.

Alveolar macrophages (AM) are a factor of antimicrobial resistance of animals and man, they determine the development of chronic inflammation, and participate in the maintenance of structural homeostasis of lung tissue in health and disease. It is from this standpoint that in recent years the character of the macrophagal response in several diseases, including tuberculosis, has been assessed, and the possibility of its use for diagnostic and prognostic purposes has been studied [2-5]. Good prospects from this point of view are provided by the method of bronchoalveolar lavage, by means of which a virtually pure population of

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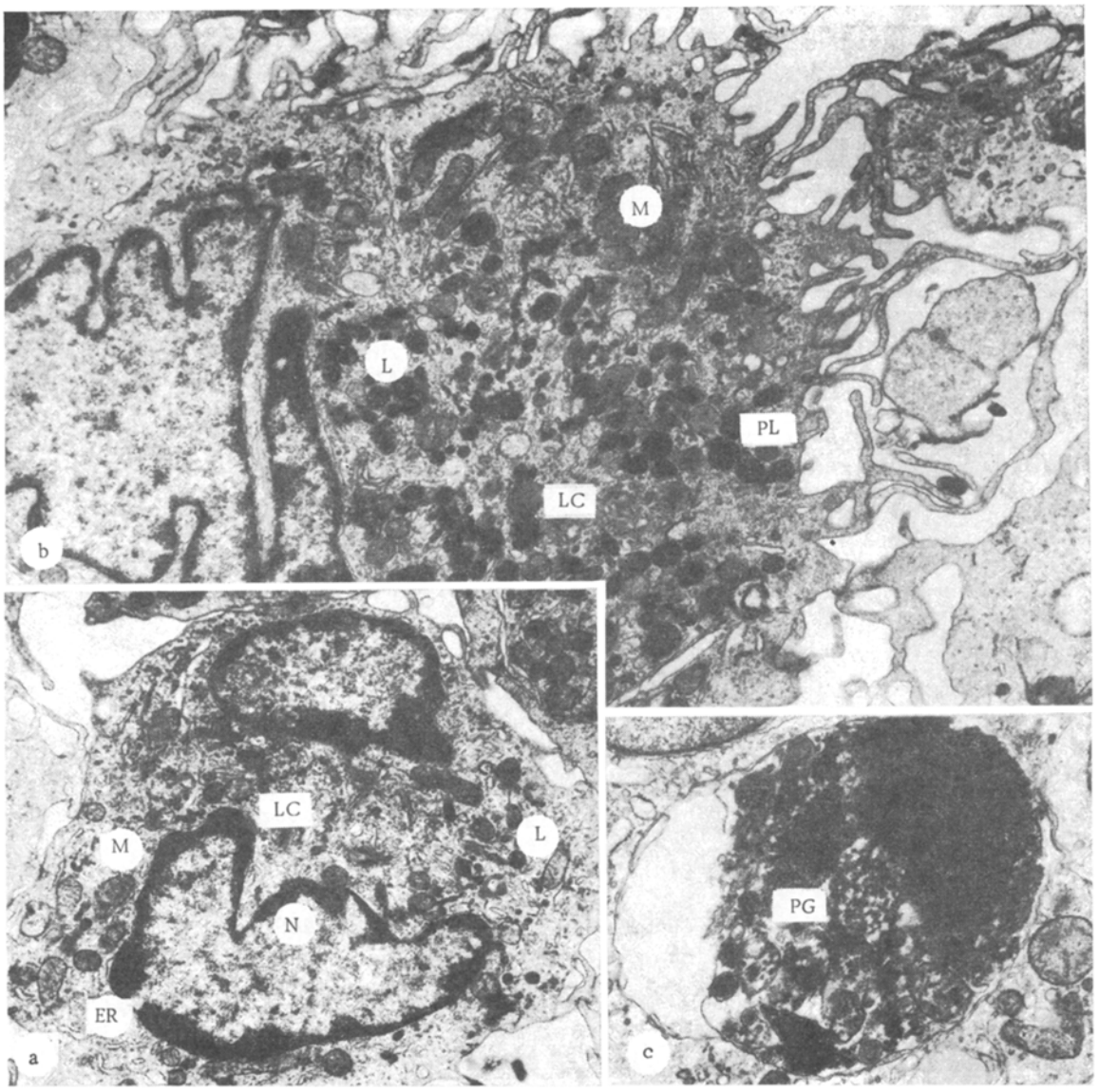

Fig. 1. Ultrastructural features of AM in early period of development of tuberculous infection (7 days). a) Biosynthesized macrophage with developed endoplasmic reticulum and vesicles of lamellar complex (16,500 ×); b) phagocytic macrophage with numerous mitochondria, lysosomes, and phagolysosomes (13,200 ×); c) fragment of cytoplasma of phagocytic macrophage, phagosome contains disintegrated neutrophil (32,400). Here and in Figs. 2 and 3: N) nucleus; LC) lamellar complex; ER) endoplasmic reticulum; L) lysosome; PL) phagolysosome; PG) phagosome; M) mitochondrion; V) vesicles.

macrophages can be isolated from a large surface area of the lung [1, 7, 10]. Several laboratory animals and, in particular, guinea pigs, are highly susceptible to the development of lung infection and are widely used to obtain a stable model of progressive tuberculous inflammation. Depending on the severity and character of the macrophagal reaction in spontaneous tuberculosis three principal stages can be distinguished in these animals: stage 1) entry of cells into the alveoli (7-10 days after infection); stage 2) the formation of multiple granulomatous formations (1-2 months); stage 3) destruction changes in the lung tissue and the formation of confluent necrotic foci (3-4 months).

The aim of this investigation was to study the morphological and physiological state of AM isolated from bronchoalveolar washings (BAW) at the above-mentioned stages of development of progressive tuberculous inflammation in guinea pigs.

TABLE 1. Functional Status of AM during Development of Generalized Tuberculosis in Guinea Pigs (M ± m)

Time of observation, days	Number of animals	Number of AM in BAW ($\times 10^6$)	Viability of AM, %	Protein concentration, mg/10^6 cells	Proteolytic activity		
					cathepsin D, units/mg	cathepsin B, nmoles/mg	macrophagal elastase, nmoles/mg
Control (intact animals)	57	0,75±0,02	96,5±0,38	0,6±0,02	3,4±0,31	10,9±0,17	3,9±0,8
7	30	0,93±0,01	94,3±0,26	0,53±0,06	3,7±0,08	11,7±0,26	8,6±0,09
p		<0,001	<0,001	<0,002		<0,02	<0,001
30	31	0,32±0,004	93,3±0,14	0,31±0,05	4,99±0,7	22,0±3,8	6,2±2,6
p		<0,001	<0,001	<0,001	<0,002	<0,002	—
60	29	0,89±0,06	90,7±0,3	0,61±0,04	2,21±0,16	11,9±0,61	1,95±0,8
p		<0,05	<0,001	—	<0,001	—	—
90	8	0,98	89,0	0,56	1,3	0	0

Legend. p) Significance of differences compared with control (only significant differences are shown). Because of the small number of animals at the last time it was impossible to carry out a statistical analysis of the results.

EXPERIMENTAL METHOD

Experiments were carried out on 200 guinea pigs weighing 210-300 g, infected subcutaneously with a virulent culture of *Mycobacterium tuberculosis* strain $H_{37}RV$, in a dose of 0.001 mg. BAW were obtained by Myrvik's method 7 days and 1, 2, and 3 months after infection, and also from 69 intact animals (control group). The number of AM in the total volume of BAW was counted in a Goryaev's chamber, and their viability was determined with the aid of 1% trypan blue solution. Morphological heterogeneity was assessed at the electron-microscopic level. For this purpose the cell residue obtained after centrifugation of BAW for 10 min at 1500 rpm was fixed for 40 min in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and post-fixed for 30-40 min with 1% OsO_4. Dehydration was carried out in alcohols of increasing concentration and propylene oxide, and the material was embedded in Epon—Araldite by the usual method. Ultrathin sections were studied in the IEM 100B electron microscope. The functional status of AM was obtained by passing the cell residue through a Ficoll density gradient; the cells were disintegrated by successive freezing and thawing 3 times. The purity of the fraction and degree of disintegration of AM were verified in the electron microscope. The protein concentration in the AM lysate was determined by Lowry's method [9] and elastase-like activity was measured as degradation of the synthetic substrate N, tert-butyloxycarbonyl-L-alanine paranitrophenyl ester [11]. Cathepsin D activity was determined as breakdown of hemoglobin [6], and cathepsin B activity as hydrolysis of the synthetic substrate N,α-benzyl-D,L-arginine paranitranilide [6].

Siliconized glassware was used at all stages of the work with cells.

EXPERIMENTAL RESULTS

Unlike the control animals, in which the basis of the AM population consisted of mature, mainly phagocytic cells, the early period of development of tuberculous infection in the guinea pigs (7 days) was characterized by the appearance of numerous monocytes and young macrophages with ultrastructural features of active biosynthesis and growth of the lysosomal apparatus, in BAW (Fig. 1a).

Most of the mature macrophages were phagocytic cells with numerous mitochondria, lysosomal inclusions, and phagosomes (Fig. 1b). The latter could contain fragments of destructively changed erythrocytes and neutrophils (Fig. 1c). Despite the increase in phagocytic activity of AM, activity of their cathepsins, which digest phagocytic material, remained at the control level (Table 1) because of an inflow of functionally inactive cells into the population. The appearance of numerous biosynthesizing forms among AM coincided with a significant increase in activity of metallodependent elastase in the population (Table 1).

The beginning of the second stage of development of tuberculous inflammation (1 month) was characterized by a further increase in the phagocytic function of AM and by predominance of cells with a developed lysosomal apparatus and with numerous phagolysosomes. A moderate increase in intracellular cathepsin D activity and a sharp rise in the cathepsin B content (Table 1) were observed in the population, evidence of the high digestive power of phagocytes.

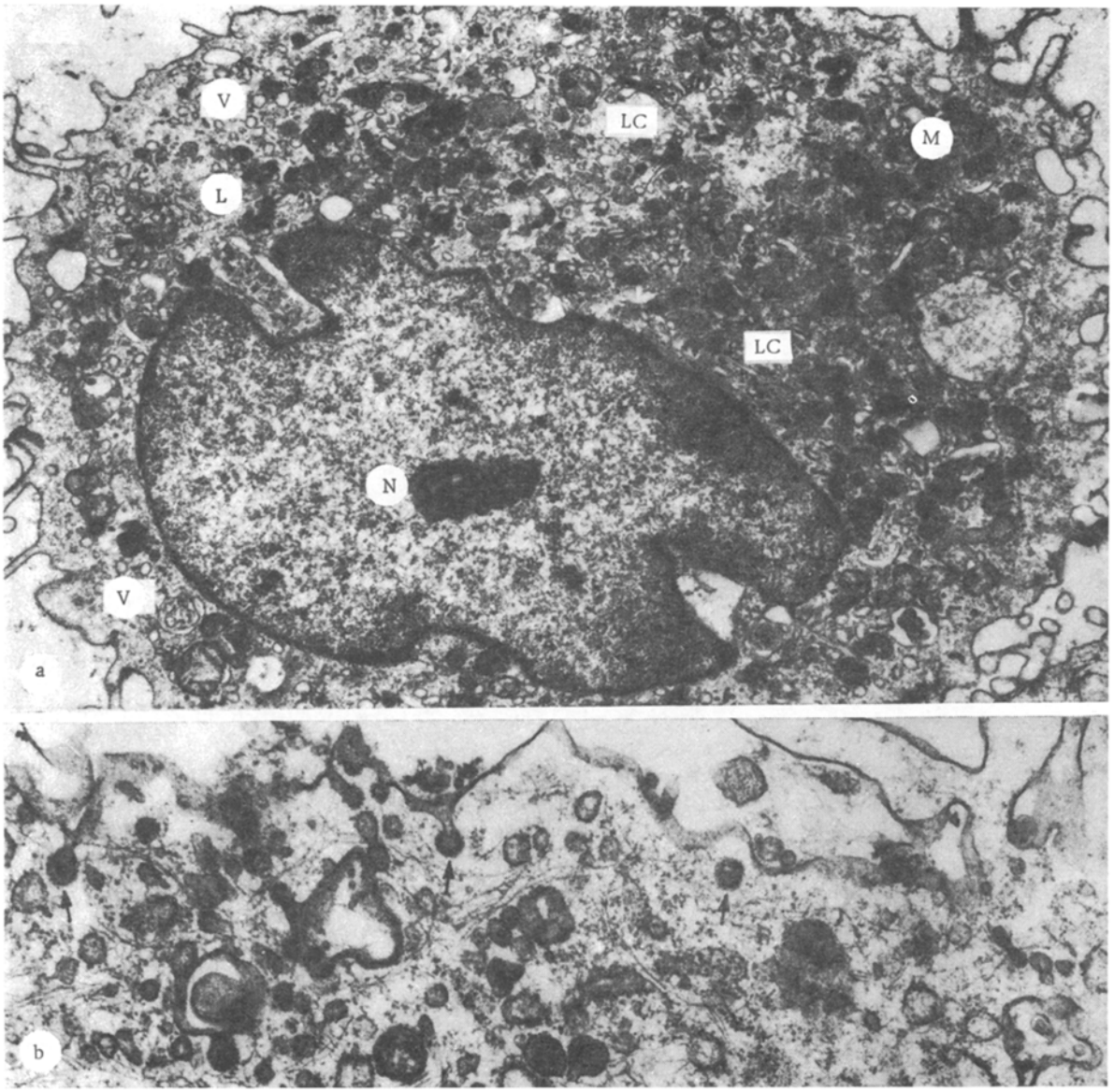

Fig. 2. Ultrastructure of secreting AM at height of development of tuberculous infection (1 month). a) General view of cell; it contains many small vesicles, distributed throughout the cytoplasm (14,600 ×); b) fragment of peripheral area of cytoplasm, coated vesicles (arrows) are in direct contact with plasmalemma (42,000 ×).

In this period the incidence of AM and of epithelioid cells with ultrastructural features of secretion (Fig. 2a), with a considerable number of small vesicles and coated vesicles with electron-transparent, less frequently electron-dense contents, often in direct contact with the plasmalemma (Fig. 2b), was increased four-fivefold among mature forms of cells. The function of the secretory AM and epithelioid cells is connected with release of mediators of inflammation, and of factors drawing monocytes, lymphocytes, and neutrophils into the focus [2, 8].

An inflow of numerous monocytes was again observed in the guinea pig's lungs 2 months after infection, whereas there were fewer typical biosynthetizing forms than in the early period of development of the infection. The viability of the mature AM was depressed and the incidence of activated phagocytic and secretory cells in the population was reduced. Macrophages with few lysosomal inclusions, containing many phagosomes, some of them large, were noted. The functional status of the population was characterized by a marked reduction in activity of both cathepsins and of elastase in the cells (Table 1). The reduction of the proteolytic activity of AM in this period of development of infection was evidently accompanied by preservation of the ingestive capacity of the phagocytes. This led to slowing of diges-

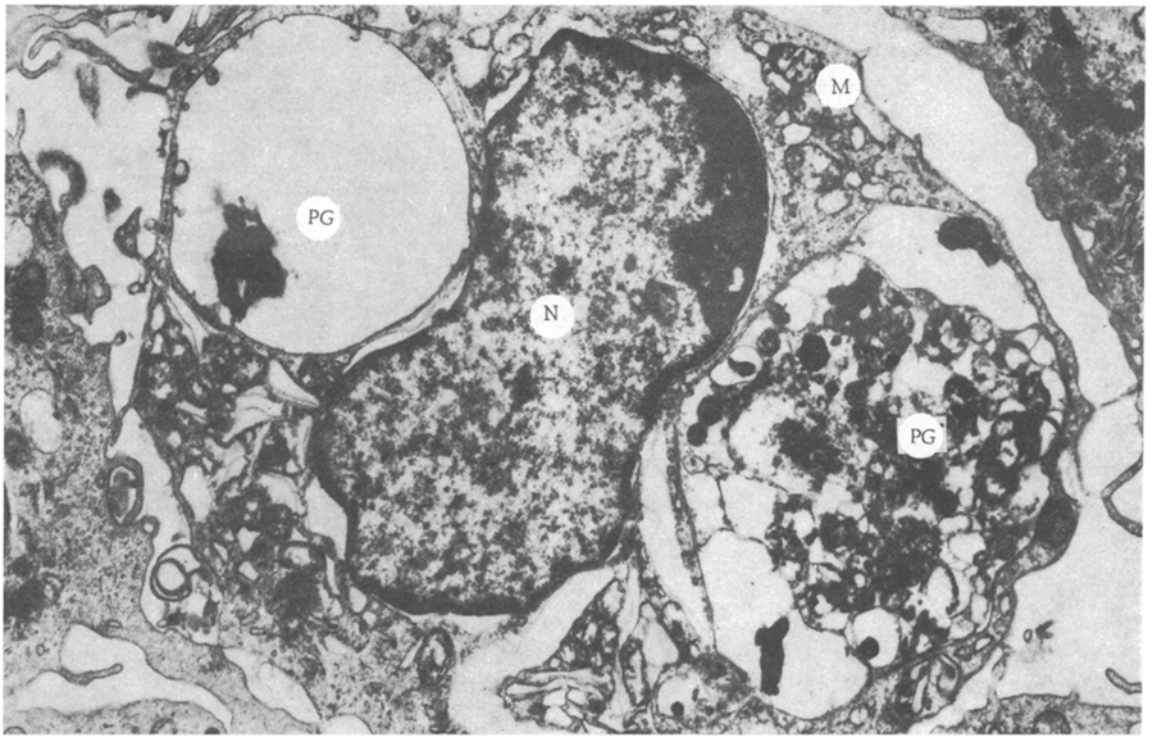

Fig. 3. Ultrastructure of AM with marked dysfunction in terminal period of development of tuberculous infection (3 months). AM contains large phagosomes but lysosomal apparatus is undeveloped (22, 500 ×).

tion and to accumulation of phagocytosed material in the cells, and it reflected the beginning of developing dysfunction of the phagocytes.

In the third, final stage of tuberculous inflammation the number of typical biosynthesizing forms of AM decreased further and the viability and functional activity of the mature cells were reduced, and all these phenomena were observed at all levels of investigation. Most AM contained an average number of mitochondria, elements of the lamellar complex, and lysosome-like inclusions. In cases when many phagosomes were found in the cytoplasm of the macrophages, this was accompanied by underdevelopment of the lysosomal apparatus (Fig. 3). Proteolytic enzyme activity fell to minimal values by this stage of the investigation (Table 1).

In guinea pigs with progressive tuberculous inflammation the morphological heterogeneity of the mature macrophages was enhanced and their functional status changed. In the early period of development of the infection an increased inflow of monocytes and young macrophages, building up their own lysosomal apparatus and increasing the proteolytic activity of the phagocytes, are observed in the early period of development of infection. Functional activity of the macrophages reaches a maximum 1 month after infection, when the BAW contain large numbers of actively phagocytic and secreting forms, and typical epithelioid cells appear. After 2 months of development of specific inflammation the digestive power of AM begins to decline, whereas their ingestive power remains at its previous level. This leads to gradual accumulation of phagocytosed material in the cells and reflects the initial stages of development of phagocyte dysfunction. Further progression of the tuberculous infection is accompanied by inhibition of the biosynthetic processes of maturation in the young AM and by a marked decline in the viability and functional activity of the mature forms.

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MORPHOLOGICAL EVALUATION OF RECONSTRUCTIVE OPERATIONS ON THE BRONCHI USING EXPERIMENTAL MICROSURGICAL TECHNIQUES

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KEY WORDS: microsurgical techniques; reconstructive operation on the bronchi; morphology of bronchial anastomosis.

Of all reconstructive operations on the bronchi 5-10% are undertaken on patients with cancer and 70% on patients with benign tumors [1, 5, 7, 11, 12]; suture failure, and granulations and cicatricial stenosis of the anastomoses develop in 5-10% of patients [4, 8-10]. As a rule, interrupted sutures passing through all layers of the bronchial wall are used in the formation of bronchial anastomoses. A high-precision method of formation of the bronchial anastomosis by a microsurgical technique must create the most favorable conditions for healing by first intention. The aim of the present investigation was accordingly a morphological comparison of the processes of healing of a bronchial anastomosis formed by a microsurgical technique and by the traditional method with through-and-through sutures.

EXPERIMENTAL METHOD

Experiments were carried out on 30 mongrel dogs. An anastomosis between the left main and lower-lobe bronchi was formed after upper bilobectomy, with circular resection of the main bronchus. The anastomosis in 18 dogs of the experimental series was formed by a method developed by ourselves: after removal of the upper and cardiac lobes of the left lung, in order to create an excess of mucous membrane, the mucosa was separated, under an OM-2 operating microscope with magnification of 6 times, from the extreme cartilaginous half-ring of the main bronchus with a razor blade, fixed in a holder, and half the circumference of the extreme cartilaginous half-ring was excised. A similar manipulation was performed on the lower lobe bronchus. Next, without the operating microscope, three external Lavsan thread 3/0 external sutures were applied to the posterior half-circumference of the anastomosis, between the mucous membrane and cartilage or muscular layer of the membranous part. Under the operating microscope with magnification of 6 times, a continuous suture of 7/0 thread was applied to the mucous membrane. Next, external sutures were applied to the anterior half-circumference of the anastomosis with 3/0 thread, not including the mucous membrane. On 12 dogs of the control series the anastomosis was formed by interrupted sutures passing through all layers of the bronchial wall. In the postoperative period bronchoscopy was performed on all the animals at various times — from 2 days to 1 month. The animals were removed from the experiment 2-5, 7-8, 14-16, and 28-30 days after the operation. At autopsy, the state of the anastomosis was assessed macroscopically, after which the region of anastomosis of the bronchus was removed en bloc and fixed in 10% buffered formalin by Lillie's method. Pieces of tissue were excised from different parts of the anastomosis and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, with picrofuchsine-fuchselin, by the PAS reaction, and by Gomori's impregnation method. Pieces of mucosa from the anastomosis measuring 0.3 × 0.3 cm were processed by Rostovshchikov's method for scanning electron microscopy [6] and examined in the Hitachi S 500 microscope.

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