collagen fibrils, surrounded by a necrotic mass, mainly consisting of disintegrated foam cells (Fig. 4b).

Intensive collagen synthesis is thus associated with the pattern of proliferation of the intimal cells in the zone of the developing atherosclerotic lesions. Cells which have divided not only take up lipids and participate in foam cell formation, but also cause the formation of a connective-tissue stroma of the plaques. Autoradiographic investigation with the aid of ^{3}H-proline and ^{14}C-hydroxyproline showed that collagen synthesis takes place only in the intima, but in the initial stages of atherogenesis, and it is associated with the accumulation of connective-tissue cells. In the initial stages of experimental atherosclerosis SMC of synthetic phenotype are mainly involved in collagen synthesis, whereas in the progressive stages, fibroblasts are the main participants.

LITERATURE CITED

1. G. G. Avtandilov, A. G. Kuligin, M. A. Reitblat, and I. L. Telyaner, Arkh. Patol., No. 7, 64 (1983).
2. A. N. Klimov, Current Problems in the Pathogenesis of Atherosclerosis [in Russian], Leningrad (1965), pp. 26-47.
3. V. A. Nagornev, Yu. V. Bobryshev, and T. G. Babushkina, Patol. Fiziol., No. 1, 35 (1985).
4. S. Gay, L. Balleisen, K. Remberger, et al., Klin. Wochenschr., 53, 899 (1975).
5. W. H. Hauss, Path. Biol., 9, 527 (1981).
6. K. G. McCullagh and G. Balian, Nature, 258, 73 (1975).
7. K. G. McCullagh, V. C. Duance, and K. A. Bishop, J. Path., 130, 45 (1980).
8. R. W. Wissler, in: Heart Disease: a Testbook of Cardiovascular Medicine, ed. E. Braunwald, Saunders, Philadelphia (1984), pp. 1183-1204.

SURFACTANT SYSTEM AND STRUCTURE OF THE RESPIRATORY PART OF THE LUNGS DURING DISADAPTATION AFTER A SINGLE EXPOSURE TO ACUTE PRESSURE CHAMBER ANOXIA

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The lung surfactant undergoes a series of changes in the body. According to Romanova [7], it can be conventionally divided into "immature" surfactant, consisting of the lamellar bodies of large alveolocytes, "reserve" surfactant, in the form of the tubular myelin of the hypophase, "mature" surfactant, which is a phospholipid layer separating the air and liquid phase in the alveoli, and "spent" surfactant which is phagocytosed by alveolar macrophages and eliminated through the bronchi. The alveolar macrophages and Clara cells, which are responsible for catabolism of the surfactant, are considered by Nevodnik, et al. [6] to be cellular components of the "antisurfactant system of the lungs." This subdivision (despite the questionable nature of the terminology) allows a differential approach to the evaluation of the role of changes in the lung surfactant system (LSS) in pathology. Acute anoxic anoxia causes a decrease in activity [4, 8], to a degree which depends on the intensity and duration of anoxia [5]. Electron-microscopic data suggests that the decrease in LSS activity is evidently due to a decrease in the content of lamellar bodies in the large alveolocytes and to disorganization of the surfactant on the surface of the alveoli [3]. Regression of changes in LSS during disadaptation after exposure to anoxia has received little study.

The aim of this investigation was to compare changes in the surface activity of different fractions of surfactant with the structure of the lung parenchyma during disadaptation after exposure to acute pressure chamber anoxia.

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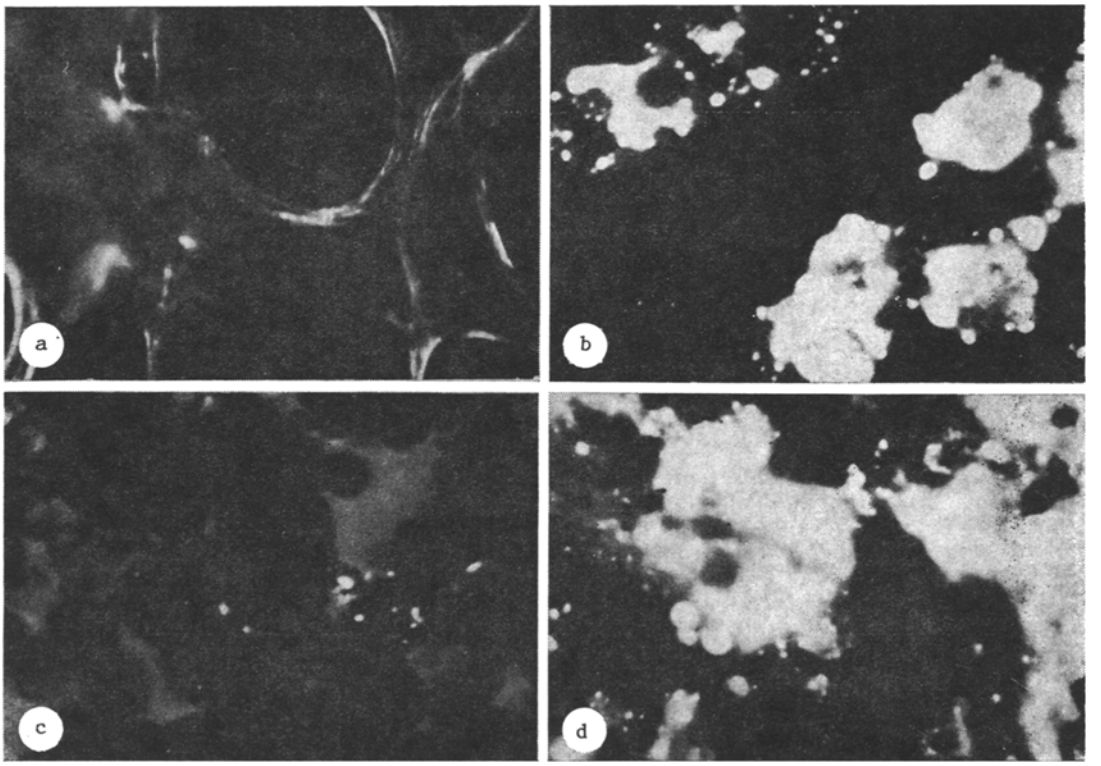

Fig. 1. Luminescence and polarization of surfactant lipids during disadaptation after exposure to acute pressure chamber anoxia. a) Weakening of luminescence of surfactant on alveolar surface (acute anoxia). Stained with Rhodamine 6G. 200×; b) Accumulation of lipid granules in alveolar macrophages (1st day of disadaptation). Stained by Berg's method. 400×; c) Content of phospholipid granules in macrophages (3rd day of disadaptation). Stained by Romhany's method. 400×; d) Increased luminescence of lipids in lung tissue cells (3rd day of disadaptation). Stained by Berg's method.

EXPERIMENTAL METHOD

Experiments were carried out on 32 noninbred albino rats weighing 180-250 g, obtained in Frunze (altitude 720 m above sea level). The control group consisted of eight animals, and the other 24 rats were "lifted" in a pressure chamber to an altitude of 6000 m for 6 h, and then decapitated six at a time immediately after descent, and on the 1st, 3rd, and 5th days of disadaptation. A 1% preparation of bronchoalveolar washings (BAW) was made from the left lung, after which a 1% extract was obtained from the same lung after washing (EAW). The surface tension (ST) was measured on improved scales of a design described previously [2]. The state of the extracellular surfactant was judged from ST of BAW, and that of the "immature" surfactant from ST of EAW. The BAW were centrifuged. Films of the residue were stained with Nile Blue and caffeine-benz(a)pyrene by Berg's method. The fraction of lipid-containing macrophages was determined in polarized light. The intensity of polarization (IP) of the phospholipids and the brightness of luminescence (BL) of lipids in the macrophages were estimated by microfluorometry. The quantity of "spent" surfactant was judged from IP and BL of the lipids in the macrophages. The upper lobe of the lung was investigated morphologically. BL of the surfactant on the surface of the alveoli of the intracellular lipids of lung tissue were determined in frozen sections by the writers' own method [1]. Lung tissue also was fixed in calcium-formol under reduced pressure (0.5 gPa) and thickened in polyvinyl alcohol. Frozen sections 3 μ thick were counterstained with a 1% solution of methylene blue at pH 7.0. Microfilming of 20 areas of the lung parenchyma was carried out on these sections. With the aid of a histoplanimeter, the bulk density of the lung tissue was determined from the photographs, and calculated as the ratio of the area of the alveolar lumen to the area of the parenchyma; the result was used as an indication of emphysema and atelectasis [10].

TABLE 1. Parameters of Activity of LSS and Bulk Density during Disadaptation after a Single Exposure to Acute Pressure Chamber Anoxia

Parameter	Control	Pressure chamber	Time of disadaptation, days		
			1	3	5
SA of washings	0,95±0,04	0,77±0,03*	0,88±0,02	0,87±0,03	0,93±0,02
ST_{min} of washings, mN/m	16,2±0,8	23,0±1,8*	18,8±1,0*	19,2±0,7*	17,2±0,8
SA of EAW	0,71±0,02	0,63±0,02*	0,75±0,03	0,76±0,03	0,68±0,01
ST_{min} of EAW, mN/m	22,5±0,7	26,3±1,6*	22,2±0,6	20,4±0,6*	24,0±0,6
Granule-containing macrophages, %	6,6±0,5	19,8±4,2*	23,5±2,9*	14,2±2,5*	9,2±1,5
IP, conventional units	1,19±0,39	1,94±0,13*	1,88±0,17*	1,30±0,25	1,42±0,39
BL of macrophages, conventional units	6,9±0,7	10,7±1,0*	13,5±1,0*	9,4±0,4	13,8±2,6*
BL of surfactant on alveolar surface, conventional units	16,1±0,8	8,6±0,6*	9,4±0,6*	10,7±0,8*	15,4±1,1
BL of intracellular lipids of lung tissue, conventional units	8,2±0,8	9,5±0,7	10,6±0,6*	10,9±0,7*	8,9±0,7
Bulk density	1,782±0,120	3,112±0,177*	1,140±0,070*	1,800±0,174	1,640±0,090

Legend. Asterisk indicates significant difference from control.

EXPERIMENTAL RESULTS

The structure of the respiratory part of the lungs in the control rats was homogeneous and corresponded to that characteristic of the species. The bulk density of the lungs was 1.782 ± 0.120. The lipid lining gave bright luminescence throughout the perimeter of the alveoli. In sections stained by Berg's method, bluish-white luminescence of the lipids was observed in the large alveolocytes, located in the substance of the alveolar septa or projecting into the lumen of the alveoli, and also in the alveolar macrophages. Cells of BAW consisted mainly of alveolar macrophages. In polarized light two populations of macrophages could be distinguished. The majority of the macrophages did not refract light, but some (6.6 ± 0.5%) contained brightly luminescent phospholipid granules. IP of the phospholipids was 1.19 ± 0.39 conventional units. On staining by Berg's method the first macrophage population gave diffuse fine-grained bluish white luminescence, while the second macrophage population contained large granules giving white luminescence. The parameters of LSS activity are given in Table 1.

Exposure to acute pressure chamber anoxia for 6 h gave rise to marked congestion of the blood vessels of the lungs, small hemorrhages, and diffuse vesicular emphysema. The presence of the latter was confirmed by a significant increase in the bulk density of the lungs. Surface activity of LSS was reduced in both BAW and EAW. The lipid lining of the alveoli gave weaker luminescence, with fragments, and individual alveoli were without a luminescent lining. BL of the surfactant on the alveolar surface was significantly reduced. BL of lipids of the lung cells showed a tendency to rise. An increase in the number of macrophages containing lipid granules was discovered cytologically, and their luminescence and polarization were more intensive than in the control.

On the 1st day of disadaptation the structure of the lungs on histological examination appeared heterogenous: against the background of parenchyma with normal aeration there were foci of emphysema and atelectasis, predominantly the latter. The bulk density of the lungs was significantly lower than in the control. Activity of LSS in BAW was moderately reduced, whereas in EAW it did not differ from the control. BL of the surfactant on the alveolar surface was depressed. BL of lipids of the lung tissue and free macrophages of BAW was significantly increased. The quantity of birefringent lipids in the macrophages also was increased. The number of lipid-containing macrophages was increased significantly.

On the 3rd day of disadaptation the structure of the respiratory part of the lungs corresponded to the control level. BL of surfactant on the alveolar surface and the parameters of LSS in BAW remained at a lower level than in the control. Parameters of LSS in EAW and BL of the intracellular lipids of the lungs were higher than initially. The number of lipid-containing macrophages was smaller than at the previous time. IP and BL of the macrophages were down to the control level.

On the 5th day of disadaptation all parameters of LSS activity were back to normal. The exception was BL of lipids of the alveolar macrophages, which was higher than initially.

A single exposure to pressure chamber anoxia thus causes the development of vesicular emphysema and a decrease in LSS activity. Emphysema is compensatory and aimed at hyperventilation of the lungs, which was recorded by physiological methods. The decrease in the content of "immature" surfactant was evidently due, as some authors have claimed [8], to a decrease in its synthesis by the large alveolocytes during anoxia. We consider that an increase in its secretion into the alveolar lumen is also important. The decrease in the quantity of "mature" surfactant was connected with its disorganization on the alveolar surface and its increased elimination through the respiratory passages on account of hyperventilation. This was confirmed in our observations by an increase in the content of "spent" surfactant in the alveolar macrophages. The decrease in LSS activity under the influence of anoxia could be the cause of the development of the atelectasis which was observed on the 1st day of disadaptation.

Restoration of the "mature" and "immature" surfactant during disadaptation followed a different time course. Activity of "immature" surfactant on the 1st day of disadaptation reached the control level, whereas on the 3rd day it actually exceeded it. This increase in the synthesis of "immature" surfactant by the large alveolocytes was demonstrated by an increase in the content of intracellular lipids in the lung tissue. The content of "mature" surfactant returned gradually to normal by the 5th day of disadaptation. "Spent" surfactant was eliminated in increased amounts via the bronchi in macrophages throughout the period of disadaptation. Using the terminology of Nevodnik, et al. [6], it can be stated that the decrease in LSS activity was accompanied by increased activity of the antisurfactant system of the lungs. Under these circumstances, the number of macrophages and the quantity of lipids phagocytosed by them were increased in the BAW. Some of these macrophages, "saturated" with lipids, remained in the alveolar lumen after normalization of the other parameters of LSS had returned to normal.

LITERATURE CITED

1. A. A. Arbuzov and G. V. Belov, The Lung surfactant under Normal and Pathological Conditions [in Russian], Kiev (1983), p. 68.
2. A. T. Arbuzov and A. A. Arbuzov, The Lung surfactant under Normal and Pathological Conditions [in Russian], Kiev (1983), p. 163.
3. L. E. Gogiashvili, Proceedings of the 3rd Transcaucasian Conference of Morphologists [in Russian], Erevan (1982), p. 56.
4. V. Yu. Gorchakov, "Changes in the surface-active properties of lung surfactant in anoxia and hyperoxia," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Kiev (1979).
5. V. T. Lyamtsev and A. A. Arbuzov, Byull. Éksp. Biol. Med., No. 11, 612 (1981).
6. V. I. Nevodnik, O. S. Kotsarov, and I. V. Belen'kii, Patol. Fiziol., No. 4, 86 (1985).
7. L. K. Romanova, A. A. Zhavoronkov, et al., Fiziol. Cheloveka, No. 6, 1006 (1977).
8. J. Costillo and F. Johnson, Lab. Invest., 21, 61 (1969).
9. E. E. Faridy, Respir. Physiol., 26, 323 (1976).
10. J. P. Rigant, P. Bergyren, and B. Robertson, J. Microscop. (Pairs), 130, 53 (1983).