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CHANGES IN SURFACTANT SYSTEM OF THE LUNGS DURING AND AFTER STARVATION

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UDC 612.212.014.462.8:612.391-092.9

KEY WORDS: Surfactant system; starvation; vitamin A.

Data in the literature [4, 6, 11] are evidence that starvation can cause a decrease in lung surfactant activity. However, they do not give the final answer to the question of the functional integrity of the surfactant system during starvation or of the possibility of its restoration.

The object of this investigation was to study the state of the lung surfactant system by a combination of physical and morphological methods in rats after starvation, to determine the possibility of its restoration, and to attempt to accelerate this process by means of vitamin A, which has the property of stimulating maturation of the surfactant system [9].

EXPERIMENTAL METHOD

Experiments were carried out on 76 noninbred male albino rats weighing 190-230 g. Of these animals 51 were completely deprived of food for 4-5 days, but allowed free access to water. At that stage 21 rats (series I) were killed by cutting the throat. Nine animals (series II) were killed in the same way two weeks later, and another 10 rats (series III) four weeks after resumption of normal feeding. The remaining 11 starved animals (series IV) were given additional vitamin A (0.02 ml of a 3.44% oily solution of retinol acetate, 100,000 i.u./ml) by mouth daily; 25 intact rats served as the control (series V).

The lung index was calculated:

$$LI = \frac{\text{weight of lungs}}{\text{weight of rat}} \times 100$$

To characterize the surfactant system the following were investigated; 1) the surface tension (ST) of lung washings and extracts on surface scales [2]; 2) the coefficient of stability (CS) of bubbles expressed from pieces of the lungs [1, 3]; 3) luminescence in UV light of cryostat sections of the lungs stained with rhodamine 6G [1, 7]; 4) the number of birefringent structures in frozen unstained sections of fixed lungs [8, 10].

Alveolar washings were obtained by repeated injection of 7-8 ml physiological saline from the syringe into the trachea, followed by its aspiration until enough had been collected to fill the cuvette of the surface scales (100 ml). An extract was prepared from the washed, homogenized lungs (1 g tissue to 100 ml physiological saline). Minimal and maximal values of ST were determined and the stability index (SI) calculated by the formula:

$$SI = 2\,\frac{ST_{max} - ST_{min}}{ST_{max} \times ST_{min}}$$

The state of ST of the extract is an over-all index of surfactant activity of the lung tissues; ST of the washings, CS of the bubbles, and luminescence microscopy mainly characterize the state of the alveolar lining. By po-

Department of Pathological Physiology, Karaganda Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 6, pp. 678-680, June, 1980. Original article submitted September 5, 1979.

0007-4888/80/8906-0759$07.50

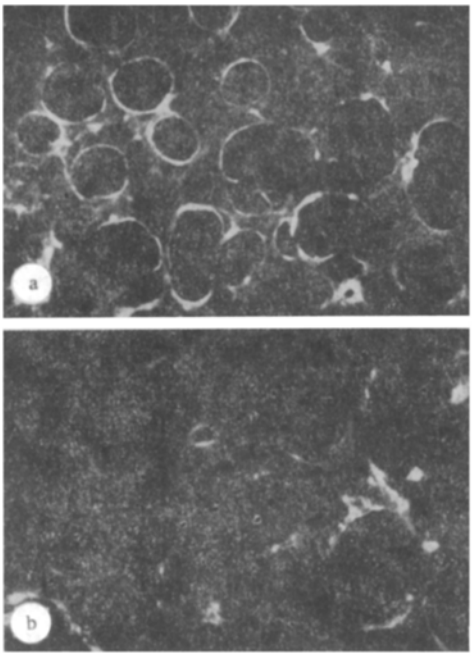

Fig. 1

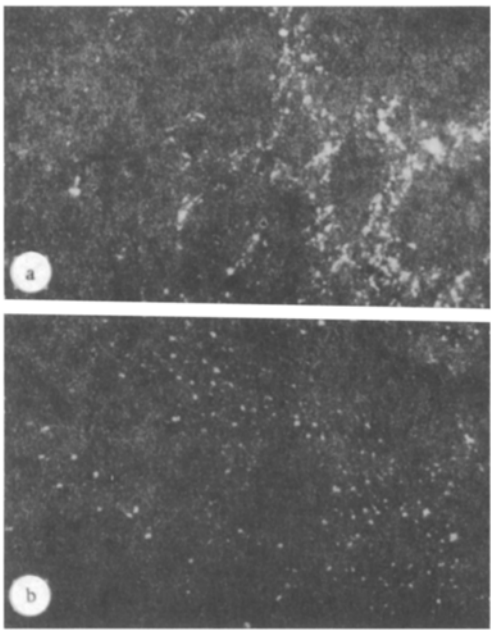

Fig. 2

Fig. 1. Luminescence microscopy of the lungs: a) Normal; b) after starvation. Stained with rhodamine 6G, 80×.

Fig. 2. Polarization microscopy of the lungs: a) Normal, b) after starvation, 100×.

larization microscopy surfactant can be detected in granular pneumocytes, where it is formed. This relatively simple but very informative method of studying surfactant has been used only infrequently [4, 8, 10]. It has not been described in the Soviet literature. To detect birefringent phospholipids the lungs were fixed for 24 h at room temperature in formol–calcium solution, pH 7.2. Frozen sections 25-30 μ thick were mounted unstained in gum arabic and examined in polarized light. For this purpose, a system of polarizer and analyzer was introduced into an ordinary microscope with a constant source of light. For quantitative estimation of the various test structures, 10 lung sections from animals of each series were analyzed at 520 nm in a cytospectrophotometer based on the FÉU-31 instrument. In each section 10 fields of the same, empirically chosen, area were examined photometrically and the results expressed in relative units (based on readings of the instrument).

EXPERIMENTAL RESULTS

After starvation for 4-5 days the weight of the rats decreased by 40-50 g. Animals which died were discarded from the experiment. The lungs of the starved rats appeared rather denser and pinker than those of the intact animals, but the small increase in LI was not significant. Nearly all parameters of the washings and extracts of the lungs from the starved animals of series I were significantly higher than in intact rats, and SI of both substrates was significantly reduced (Table 1). CS of the lung bubbles also was significantly reduced (0.88 ± 0.01 and 0.92 ± 0.01, respectively: $P < 0.05$) evidence of an increased tendency of the alveoli to collapse in expiration. Investigation of lung sections from the starved animals of series I in UV light revealed a considerable decrease in the number of luminescent alveoli with the appearance of clear gold-orange rings; they became thin and discontinuous (Fig. 1). On polarization microscopy the alveolar septa of the lungs of the control animals consisted mainly of areas with many large, intensively birefringent structures of phospholipid granules (Fig. 2a). In the starved rats of series I, the number and size of the birefringent granules were considerably reduced (Fig. 2b), and this was reliably confirmed by photometry.

Two weeks after resumption of normal feeding (series II) the over-all indices of the state of the surfactant system (ST and SI of the extracts) were practically indistinguishable from those of intact animals and, on the contrary, they showed much higher activity than in the starved rats in the experiments of series I (Table 1). Meanwhile, the surface activity (SA) of the washings, which was the same as after starvation, was significantly below normal. The intensity of birefringence also was significantly lower than in the healthy animals.

TABLE 1. Changes in Surface Tension Indices of Washings and Extracts and Polarization Microscopy of Lungs of Animals after Starvation and Recovery after Feeding (M ± m)

Series of experiments	Number of rats	LI	ST, dynes/cm						SI		Intensity of birefringence
			static		maximal		minimal				
			W	E	W	E	W	E	W	E	
I. Starvation	10	0,65±0,04	48,9±0,68	37,1±1,44	56,6±0,52	46,2±1,12	21,8±0,64	15,3±1,13	0,88±0,02	1,02±0,04	22,0±1,10
$P_{I,V}$			<0,02		<0,02	<0,05	<0,001	<0,001	<0,001	<0,001	<0,001
II. Starvation plus feeding for 2 weeks	6	0,64±0,04	50,0±0,78	34,2±0,97	56,7±0,27	44,9±0,90	20,7±0,39	11,6±0,92	0,93±0,02	1,18±0,05	37,4±1,28
$P_{II,V}$			<0,01		<0,001		<0,001		<0,01		<0,01
$P_{II,I}$								<0,01		<0,02	<0,001
III. Starvation plus feeding for 4 weeks	7	0,61±0,04	48,2±0,51	35,3±0,73	55,3±0,44	43,9±0,85	19,6±0,62	10,8±0,62	0,96±0,02	1,21±0,03	41,5±1,32
$P_{III,V}$			<0,05				<0,05				
$P_{III,I}$								<0,01		<0,01	<0,001
IV. Starvation plus feeding for 2 weeks plus addition of vitamin A	8	0,59±0,03	46,8±0,54	32,6±0,33	54,0±0,45	42,4±0,83	18,3±0,19	10,2±0,69	0,99±0,01	1,23±0,03	43,6±1,35
$P_{IV,V}$			<0,01	<0,02	<0,02		<0,01		<0,02		<0,001
$P_{IV,II}$											
V. Control (intact rats)	10	0,59±0,02	46,7±0,48	34,9±0,76	54,2±0,63	43,0±0,63	18,1±0,31	10,5±0,49	1,0±0,01	1,22±0,04	42,7±1,43

Legend. Lungs of 35 rats data for which are not included in the Table, were used for determination of CS and for morphological investigation. W) Washings, E) extract.

In the animals of series III, 4 weeks after the beginning of feeding all the main parameters of SA of the extracts and all the quantitative results of polarization microscopy were significantly higher than their values in the starved rats and were indistinguishable from normal (Table 1).

In animals which were fed for only two weeks, but which received vitamin A at the same time (series IV) the indices of SA of both the washings and extracts and also the intensity of birefringence were indistinguishable from normal (series V), and ST was in fact statistically significantly reduced (Table 1). Evidence of the more active recovery process of the surfactant system in animals receiving vitamin A is given by the significantly higher levels of SA of the washings and the higher intensity of birefringence compared with animals of series II, and also by the distinct tendency for these indices to increase, which was noted during investigation of the extracts.

These experiments thus confirmed the conclusions that during starvation the total content of surfactant in the lungs falls [5, 6], and that the cellular part of the surfactant system [4, 6] and the alveolar lining [6] are exhausted. The decrease in CS of the lung bubbles after starvation is in agreement with data showing that under these conditions the antiatelectatic function of the surfactant system is depressed and the P/v hysteresis loop is altered [4, 5], although this was not found by other workers [6, 11, 12]. PA of the tissue surfactant of the extract was almost completely restored two weeks after resumption of normal feeding, despite the fact that its content in the cells was below normal. By the fourth week the tissue reserves of surfactant were indistinguishable from their original levels. Restoration of SA of the alveolar lining took place more slowly. A large dose of vitamin A considerably accelerated the normalization of the surfactant system during feeding. This fact may be of practical interest to the clinician.

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