COMPARISON OF PHYSICAL METHODS OF INVESTIGATION OF THE LUNG SURFACTANT SYSTEM DURING EXPOSURE TO ACUTE HYPOXIA

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The basic physical methods of investigating the lung surfactant system (LSS) were suggested by Pattle in 1955 [10] and later by Clements in 1957 [9], but until now different workers have used different substrates for this purpose, and this makes comparison of the results difficult. Some workers [5] use mainly lung extract, others [11] prefer bronchoalveolar washings (BAW), a third group [7] studies the extracellular component of LSS in BAW, and determines the combined properties of the cellular and extracellular components of LSS in lung extract, and a fourth group [6] also study the extracellular component in washings and the cellular component in lung extract after BAW. There is no general agreement on the best concentration of the extract. A 0.1% extract [4], a 1% extract [7], and more concentrated, purified solutions [5] have been used. There are modifications [8] of Clements' original method of investigation of LSS and of interpretation of the hysteresis loop of the surfactant [12].

In the present investigation an attempt was made to compare investigation of LSS by different methods and in different substrates. The action of acute hypoxia, which leads to a decrease in LSS activity [4, 7] was studied.

EXPERIMENTAL

Experiments were carried out on 32 noninbred albino rats weighing 180-250 g (reared in Frunze, altitude 720 m above sea level). The remaining 24 rats were "lifted" in a pressure chamber to an "altitude of 6000 m for 6 h and then decapitated in groups of six animals. Rats of group 2 were killed immediately after descent, animals of groups 3, 4, and 5 were killed on the 1st, 3rd, and 5th days respectively after exposure in the pressure chamber. BAW were prepared from the left lung in the proportion of 100 ml physiological saline to 1 g of lung tissue. An extract after washing (EAW) was then prepared from the same lung. The lung was homogenized and the suspension centrifuged and diluted with physiological saline at the rate of 100 ml to 1 g of original mass. A 1% lung extract (LE) was then prepared from the unwashed three lobes of the right lung by the same method. To obtain a 0.1% LE, 10 ml of 1% LE was treated with 90 ml of physiological saline. The brightness of luminescence (BL) of the lipid lining of the alveoli was determined in frozen sections of the apical lobe of the right lung by the method in [2]. Air bubbles were obtained from the same lobe by Pattle's method and studied by means of the apparatus described in [1]. To determine surfactant activity of the washings monolayer (WM) by the method in [8], 1 ml of BAW was mixed with 2 ml of isopropyl alcohol and the mixture layered in drops weighing 0.01 g on the surface of physiological saline in a cuvette with an area of 10^{-2} m^2, lowering the surface tension (ST) of the subphase to 40 mN/m. ST was measured on an automatic balance of the writers' own design, which is an improved version of the balance in [3], printing out the results on an automatic N-306 xy printer. Static (ST_{stat}), minimal (ST_{min}), and maximal (ST_{max}) values of ST were recorded and used to calculate a stability index (SI) by the method in [9]. The shape index of the hysteresis loop ρ was calculated from graphs of surface activity of the washings and extracts by the method in [12], using the equation:

$$\rho = \frac{1}{k} \sum^{k} (1.0 - v \text{ compression} \,/\, v \text{ expansion}.$$

Surface activity of the lung surfactant was judged by the value of the parameters ST_{min}, SI, and the index ρ. To estimate surface activity of WM, the number of drops necessary to reduce ST of the subphase to an

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TABLE 1. Parameters of Surface Activity of LSS during Exposure to Acute Hypoxia (M ± m)

Experimental conditions	ST_{min}						SI					ρ		KC	BL
	BAW	WM	Number of drops	EAW	1 % LE	0,1 % LE	BAW	WM	EAW	1 % LE	0,1 % LE	BAW	EAW		
Control	16,2±0,8	19,2±0,6	41,6±3,2	22,5±0,7	19,4±1,3	21,2±0,5	0,95±0,04	0,84±0,03	0,71±0,03	0,81±0,05	0,73±0,02	0,21±0,01	0,11±0,01	0,92±0,03	16,1±0,8
In pressure chamber for 6 h	23,0*±1,8	20,8±0,5	69,3*±5,6	26,3*±1,6	24,7*±0,9	25,0*±0,8	0,77*±0,03	0,78±0,03	0,63*±0,02	0,64*±0,02	0,64*±0,02	0,16*±0,02	0,13±0,01	0,84±0,04	8,6*±0,6
Disadaptation, days															
1-st	18,8*±1,0	21,2±0,9	106,8*±8,3	22,2±0,6	21,3±0,3	23,2±1,4	0,88±0,02	0,75*±0,03	0,75±0 03	0,80±0,01	0,75±0,04	0,23±0,01	0,13±0,01	0,84±0,03	9,4*±0,5
3-rd	19,2*±0,7	20,7*±0,3	71,2*±5,7	20,4*±0,6	21,8±1,0	22,0±1,2	0,87±0,03	0,85±0,03	0,76±0,03	0,75±0,06	0,73±0,04	0,20±0,01	0,15*±0,01	0,94±0,02	10,7*±0,8
5-th	17,2±0,8	21,2±2,0	51,2±4,1	23,0±0,6	21,7±1,4	22,6±1,7	0,93±0,02	0,82±0,04	0,68±0,02	0,81±0,05	0,75±0,07	0,20±0,01	0,12±0,01	0,96±0,02	15,4±1,1

Legend. *P < 0.05 compared with control.

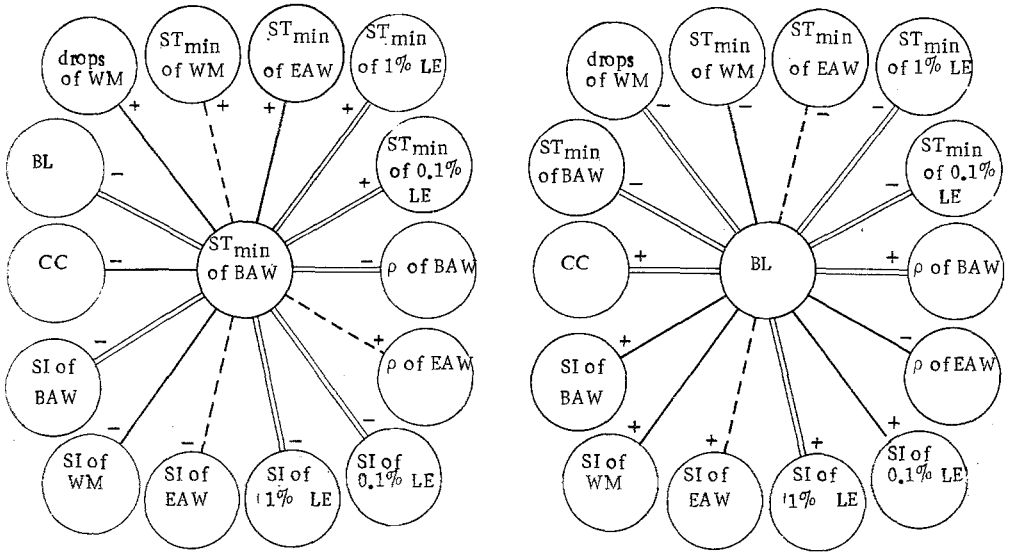

Fig. 1. Scheme of correlations between parameters of surface activity of various substrates and values of ST_{min} of washings and BL of lipid lining of alveoli. Double line – strong correlation, single line – moderately strong correlation, broken line – weak correlation.

assigned level also was taken into account. High values of SI and ρ and low ST_{min} are evidence of high surface activity of LSS. The parameters of ST_{max} and ST_{stat} are less informative as measures of surface activity.

EXPERIMENTAL RESULTS

Parameters of surface activity in different substrates from control animals differed. Surface activity was highest in BAW, rather lower in WM, and lowest of all in EAW. The ratio between SI in these substrates was 1.00:0.85:0.75, and the ratio between ST_{min} was 0.72:0.85:1.00, respectively. Values of SI of the 1% LE were about 1.1 times higher than for the 0.1% LE, whereas values of ST_{min} were 1.1 times lower (difference not significant; $P > 0.05$).

The shape of the hysteresis loop also depended on the substrate and its concentration. A wide hysteresis loop is characteristic of BAW ($\rho = 0.21 \pm 0.02$). A narrower loop is given by WM ($\rho = 0.14 \pm 0.01$). The narrowest hysteresis loop is found in EAW ($\rho = 0.11 \pm 0.01$). The hysteresis loop is significantly narrower in 0.1% LE than in 1% LE ($\rho = 0.11 \pm 0.01$ and 0.15 ± 0.01 respectively; $P < 0.02$). The lipid lining is uniform in thickness over the whole perimeter of the alveoli and gives bright luminescence after staining with Rhodamine 6G.

Exposure to acute hypoxia in a pressure chamber for 6 h led to a marked decrease in surface activity of all the substrates tested and also to a sharp reduction in BL of the lipid lining of the alveoli (Table 1). However, the degree of the change differed in different substrates. The decrease in all the parameters, including the index ρ, was most marked in BAW. In other substrates changes in the parameters of the surface activity were less marked, and the index ρ in general was unchanged. A significant increase was observed in the number of drops in WM required to reduce ST of the subphase to 40 mN/m, but changes in SI and ST_{min} were not significant. The decrease in the contraction coefficient (CC) of the air bubbles was not statistically significant ($P > 0.05$). This indicates that Pattle's method of determining surface activity of LSS is less sensitive than the method using a balance of Wilhelmy type.

The period of disadaptation after exposure of the animals in a pressure chamber was characterized by normalization of surface activity of the extracts and washings, and also of BL of the lipid lining of the alveoli. Times of normalization of the parameters of surface activity differed in different substrates. ST_{min} of BAW was back to normal on the 5th day of disadaptation, but on the 1st and 3rd days the values differed significantly from the control. The stability index of BAW and the index ρ regained their original levels on the very first day. The number of drops in WM required to reduce ST of the subphase to the assigned level differed significantly from the original value on the 1st and 3rd days of disadaptation, and this parameter returned to normal

on the 5th day. Surface activity of EAW was back to normal on the 1st day, and on the 3rd day it was actually higher than the control. Evidence of this is given by changes in the value of ST_{min} and the index ρ. This trend reflects the accumulation of intracellular surfactant. SI of 1% LE and 0.1% LE was restored on the 1st day of disadaptation. This can be explained, in our view, on the grounds that surface activity of the lung extract is due to the sum of the cellular and extracellular components of LSS. The changes in these components do not coincide in time, and on the 3rd day they are actually opposite. The value of BL of the lipid lining of the alveoli was significantly below the control level on the 1st and 3rd days of disadaptation, and on the 5th day BL regained its original value.

To assess correlation between the parameters of surface activity of the various substrates, values of ST_{min} of BAW and BL of the lipid lining of the alveoli, which possess the largest number of strong and moderately strong correlations (Fig. 1), were chosen as initial data. Strong correlation between ST_{min} and SI of BAW, 1% LE, and 0.1% LE, and also between BL of the lipid lining and CC of the air bubbles is due, in our opinion, to predominance of the extracellular component of LSS in these substrates. A certain quantity of cellular surfactant is present only in 1% LE and 0.1% LE. Strong correlations of the index ρ of BAW and moderately strong correlations of ρ of EAW and values of ST_{min} of BAW and BL are evidence that this index can be used as a secondary parameter to assess the surface activity of LSS. Moderately strong or weak correlation between parameters of surface activity of BAW and WM is probably due to qualitative differences in their structure. The most likely candidates are changes in the physicochemical properties of the surfactant in WM. Weak or moderately strong correlation between parameters of BAW and EAW can be explained on the grounds that the surface activity of these substrates is due to different components of LSS: extracellular and intracellular respectively. Shifts of surface activity of LSS are adequately reflected in both 1% and 0.1% LE. This confirms the view [4] that 0.1% LE can be used if the volume of substrate is limited.

The value of parameters of surface activity of LSS under both normal and pathological conditions thus depends on the substrate and its concentration. To compare the surface activity of different substrates it is therefore necessary to use correcting factors (the ratio of SI in BAW, WM, and EAW is 1.00:0.85:0.75, and the ratio of ST_{min} in them is 0.72:0.85:1.00, respectively; the ratio of SI of 1% LE and 0.1% LE is 1.1:1.0, whereas that of ST_{min} between them is 0.9:1.0).

Acute hypoxia in a pressure chamber causes a marked decrease in LSS activity. The recovery times of the parameters of surface activity during disadaptation differ for different substrates. Parameters due to the presence of cellular surfactant (EAW, 1% LE, 0.1% LE) in the substrate return to normal first. Parameters reflecting changes mainly of extracellular surfactant in the substrate return to normal later.

To assess the surface activity of LSS it is permissible to use any of the substrates studied, depending on the possibility of obtaining material. Cellular and extracellular components of LSS should be studied separately in experiments, using BAW and EAW. For clinical purposes, the use of BAW or WM is indicated. In the latter case the volume of substrate can be as little as 2-5 ml. During investigation of surface activity in lung biopsy material, it is better to use 0.1% LE. Pattle's method should be used only if a balance of Wilhelmy type is not available. Morphological confirmation of changes in LSS is best obtained by quantitative investigation of BL of the lipid lining of the alveoli in sections stained with Rhodamine 6G.

LITERATURE CITED

1. A. A. Arbuzov and G. V. Belov, Arkh. Patol., No. 4, 80 (1980).
2. A. A. Arbuzov and G. V. Belov, in: Lung Surfactants under Normal and Pathological Conditions [in Russian], Kiev (1983), p. 68.
3. A. I. Arbuzov and A. A. Arbuzov, in: Lung Surfactants under Normal and Pathological Conditions [in Russian], Kiev (1983), p. 163.
4. V. A. Berezovskii and V. Yu. Gorchakov, Surface Active Substances of the Lung [in Russian], Kiev (1982).
5. A. A. Birkun, E. N. Nesterov, and G. V. Kobozev, The Surfactant of the Lungs [in Russian], Kiev (1981).
6. V. T. Lyamtsev and A. A. Arbuzov, Byull. Eksp. Biol. Med., No. 11, 612 (1981).
7. I. A. Serebrovskaya, É. V. Byul', and V. V. Shishkanov, in: Lung Surfactants under Normal and Pathological Conditions [in Russian], Kiev (1983), p. 108.
8. M. Abrams. J. Appl. Physiol., 21, 718 (1966).
9. J. Clements, Proc. Soc. Exp. Biol. (New York), 95, 170 (1957).
10. R. E. Pattle, Nature, 175, 1125 (1955).
11. R. Ramires, B. Schwartz, and A. Dowell, Arch. Intern. Med., 127, 395 (1971).
12. H. Williams, R. Rhoades, and W. Adams, Arch. Intern. Med., 128, 101 (1971).