were carried out under isometric conditions, the effect of contracture and of other factors changing the length of the muscle, and at the same time, changing the degree of activity of the muscle spindles, was ruled out. Metabolites are a possible cause of the change in the functional state of the receptors in the working muscle and in warm-blooded animals; it may be that it is the same substances which also influence neuromuscular synapses [2, 3, 8]. Dependence of the activity of muscle receptors on the state of metabolism in the muscle is also demonstrated indirectly by the functional difference observed between the muscle spindles of the fast (plantaris) and slow (soleus) muscles.

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EFFECT OF THE PRINCIPAL BLOOD COMPONENTS ON LUNG SURFACTANT

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The action of the principal blood components on surface activity of substrates containing lung surfactant was investigated. Mixing blood hemolysate, serum, albumin, and fibrinogen with extracts and washings from the lungs, application as a monolayer, or their injection into the hypophase of a monolayer of washings increased the surface tension (ST) of these substrates. Hemoglobin, serum lipids, and cholesterol had the opposite action. Contact of all these blood components, exhibiting opposite effects on ST of medium containing surfactant, with bubbles from the lungs during determination of their coefficient of stability (CS), by Pattle's method, led to an increase in CS.

KEY WORDS: lung surfactant; endogenous substances; blood components.

Of the endogenous substances with which alveolar surfactant can come into contact, with a change in its activity, the most important are the blood components. The incomplete and highly contradictory data given by different workers on interaction between lung surface-active substances (surfactants) with the principal blood components are difficult to compare, for many were based on methods of investigation which differed in principle or in their essential details [6-9, 11, 13]. The solution to this problem is highly relevant to the explanation of mechanisms of the changes in surfactant arising in pulmonary edeman, inflammatory lesion of the lungs, alveolar proteinosis, the respiratory distress syndrome in newborn infants and adults, and so on. It was there-

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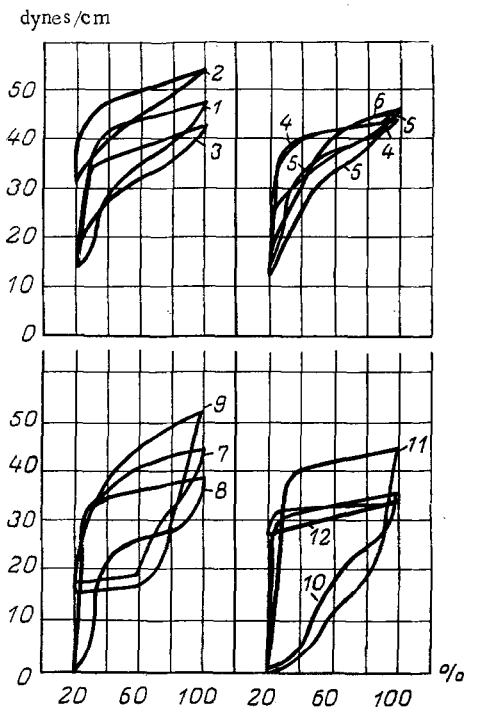

Fig. 1. Hysteresis loop of surface tension of adsorption layers of lung extract (1), solutions of 10 ml serum (2), 160 mg hemoglobin (3), 10 ml plasma (4), 100 mg gammaglobulin (5), and 900 mg albumin (6) in 90 ml physiological saline and monolayers of serum lipids (7), cholesterol (8), lecithin (9), and palmitic (10), stearic (11), and oleic (12) acids. Abscissa, surface tension (in dynes/cm); ordinate, surface area of test substrate (in % of initial area).

fore decided to continue the study of the effect of blood components on lung surfactant by the use of a combined approach.

EXPERIMENTAL METHOD

Experiments were carried out on 15 sexually mature rabbits and 100 noninbred albino rats weighing 180-350 g. The surface activity (SA) of the surfactant was tested in an extract of the lungs (1 g tissue to 100 ml physiological saline) and in washings obtained by injecting physiological saline into the trachea and withdrawing it up to a volume of 90 ml, i.e., the volume of the cuvette in the surface tension scales designed by Byul' [1]. To obtain a monomolecular film of the test substance it was diluted 1:2 with isopropyl alcohol and applied to the surface of the hypophase with a micropipet in drops measuring 0.015 ml. The static, maximal, and minimal surface tension (γ_{st}, γ_{max}, γ_{min}) and the index of stability ($IS = 2(\gamma_{max} - \gamma_{min})/(\gamma_{max} + \gamma_{min})$) were determined. The shape and area of the hysteresis loop also were considered as indices of SA. The effects of hemolysate, freshly prepared solution of hemoglobin, and serum from rabbit blood, solutions of dried plasma, albumin, γ-globulin, and fibrinogen from human blood, lipids extracted from rabbit serum, and cholesterol on the surfactant were studied. Different variants of contact between the above-mentioned substances and adsorption film and monolayers of surfactant were tested by injecting them into the hypophase or applying them on the surface. The coefficient of stability (CS) of bubbles expressed from a piece of lung in physiological saline [3], with and without addition of the test substances, also was determined by Pattle's method.

EXPERIMENTAL RESULTS

Comparison of the hysteresis loop of adsorption layers of lung extract and solutions of some of the principal blood components (Fig. 1: 1-6) showed that all possessed definite SA; the loop for plasma was much

TABLE 1. Changes in SA of Lung Extract and Washings when Mixed with Blood Components (M ± m)

Series No.	Substrate tested	Number of experiments	Surface tension, dynes/cm			IS
			γ_{st}	γ_{max}	γ_{min}	
1	Control	10	37,8±1,9	43,7±1,6	10,3±1,4	1,25±0,06
2	Hemolysate 0,2 ml blood	6	38,7±1,9	45,3±2,5	15,5±0,5*	0,96±0,05*
3	Hemoglobin 160 mg	9	34,5±1,1	42,2±1,6	11,1±0,2	1,15±0,03
4	Serum, 0,1 mg	8	37,7±1,5	43,1±1,7	12,7±0,7	1,08±0,03*
5	» 0,5 mg	9	41,4±1,1	46,7±1,5	16,0±0,8*	0,97±0,04*
6	» 1,0 mg	8	43,1±1,5	47,3±1,5	17,4±1,0*	0,92±0,05*
7	Control	10	40,0±0,5	47,3±0,7	13,2±0,6	1,13±0,02*
8	Hemoglobin, 260 mg + serum, 1.0 ml	10	43,2±0,6*	49,5±0,7	18,4±1,2*	0,91±0,04*
9	Control	30	36,1±0,9	43,2±0,7	9,8±0,8	1,26±0,05
10	Plasma, 1.0 ml	10	39,7±0,9*	47,1±0,7*	13,3±0,8*	1,12±0,04*
11	Albumin, 900 mg	10	43,8±0,7*	46,1±0,7*	20,7±1,2*	0,76±0,04*
12	Gamma-globulin, 900 mg	10	38,5±0,6*	44,3±0,5	13,5±1,0*	1,09±0,06*
13	Fibrinogen 100 mg	10	38,1±1,1	42,8±0,4	14,5±0,9*	0,99±0,04*
14	Control	4	40,5±0,6	48,0±0,7	14,1±0,6	1,08±0,04
15	Lipids, 0.5 ml serum	12	39,1±0,2	44,9±0,7*	8,2±0,5*	1,37±0,04*
16	Cholesterol, 2 mg	8	38,8±0,2*	44,6±0,4*	5,8±0,3*	1,53±0,02*
17	Control	6	50,1±1,1	57,3±1,2	20,2±1,5	0,96±0,04
18	Hemoglobin, 160 mg	6	44,8±2,3	57,3±0,8	12,6±2,1*	1,26±0,09*
19	Control	6	48,2±0,9	53,1±1,4	18,2±0,4	0,96±0,02
20	Serum, 0.5 ml	6	51,7±0,6*	56,7±1,0	23,5±0,7*	0,71±0,03*

Legend. Control series 1, 7, and 14) extracts of rabbit lungs, 9) extracts of rat lungs, 17 and 19) washings of rat lungs. Asterisk indicates significant differences from control at $P < 0.05$–0.001.

TABLE 2. Changes in SA of Monolayer of Lung Washings after Injection of Blood Components Into Hypophase, and of Adsorption Layer of Washings when Used as Hypophase for Blood Components

Expt. No.	Substrate tested	Surface tension, dynes/cm			IS
		γ_{st}	γ_{max}	γ_{min}	
1	Control	40,0	48,6	12,4	1,19
	Hemoglobin	25,9	36,7	8,1	1,28
2	Control	40,0	47,6	19,4	0,84
	Serum	40,0	49,7	23,8	0,70
3	Control	40,0	56,2	16,2	1,10
	Albumin	38,3	45,9	16,2	0,96
4	Control	40,0	56,2	16,2	1,10
	Fibrinogen	34,0	49,1	15,1	1,06
5	Control	43,2	47,0	18,4	0,87
	Hemoglobin	38,9	43,7	14,0	1,03
6	Control	37,3	41,0	9,7	1,24
	Serum	35,1	40,5	13,5	1,00
7	Control	32,9	36,2	13,0	0,94
	Albumin	35,6	41,0	18,9	0,74
8	Control	32,9	36,2	13,0	0,94
	Fibrinogen	38,3	43,2	20,5	0,71
9	Control	37,3	41,0	9,7	1,24
	Serum lipids	23,8	32,4	0,5	1,94
10	Control	40,0	44,8	15,7	0,96
	Cholesterol	20,0	28,6	0,0	2,00

Legend. Control in experiments Nos. 1-4) monolayer of lung washings in physiological saline, adjusted to $\gamma_{st} = 40$ dynes/cm; experiment) monolayer of same number of drops of washings on hypophase containing test substance in same quantity as in Table 1. Control in experiments Nos. 5-10) lung washings; experiment) application of 10 drops of test substance to surface of washings.

wider, however, than those usually given [4, 5]. Quantitative comparison of SA of monolayers of lipid blood components, no reference to which could be found in the literature, revealed the very high but unequal ability of most of these substances to affect surface tension (Fig. 1: 7-12). Very small quantities of cholesterol (1 or 2 drops of a 0.05% solution of it in isopropyl alcohol) brought γ_{min} down to zero. Palmitic and, in particular, stearic acids had the highest activity, whereas oleic acid had very low activity.

Mixing blood hemolysates with lung surfactant significantly lowered SA of the latter (Table 1). The addition of a significantly larger quantity of hemoglobin than was contained in the hemolysate to the extract did not change the mean values of SA of the extract. In experiments with the highest (over 11 dynes/cm) initial values of γ_{min} of the extract hemoglobin lowered this index. Blood serum, like serum to which a large quantity of hemoglobin was added, lowered SA of the extract. The addition of hemoglobin to the lung washings (Table 1, series Nos. 17-18), the use of hemoglobin solution as hypophase for the monolayer of lung surfactant, and also application of a monolayer of hemoglobin above washings (Table 2, experiments Nos. 1 and 5) led to an increase in surfactant activity. CS of the bubbles on the addition of hemoglobin to the medium also increased (0.85 ± 0.002 and 0.92 ± 0.006; $P < 0.001$). No information on these properties of hemoglobin could be found in the literature. In all similar variants of the experiments with serum (Table 1, series Nos. 19-20 and Table 2, experiments Nos 2 and 6) it had the opposite effect to hemoglobin on SA of lung extracts and washings. This shows that during contact of surfactant with blood penetrating into the alveoli, the action of the components of the serum on it is predominantly inactivating.

It was found that blood plasma, albumin, gamma-globulin, and fibrinogen, mixed with the extract (Table 1, series Nos. 9-13), and also albumin and fibrinogen, introduced into the hypophase or applied as a monolayer above washings (Table 2, experiments Nos. 3, 4, 7, 8) lowered activity of the surfactant. Meanwhile CS of bubbles after the addition of serum, albumin, and fibrinogen to physiological saline in all cases was significantly ($P < 0.001$) increased (0.93 ± 0.006; 0.95 ± 0.01; 0.97 ± 0.008) compared with the control (0.85 ± 0.002). The biophysical significance of the opposite direction of results obtained by Pattle's method compared with the combination of methods of direct determination of the action of these substances on lung surfactant is not yet clear.

Lipids and cholesterol, extracted from serum, when tested by all methods exhibited the ability to increase the activity of lung surfactant considerably (see Table 1, series Nos. 14-16; Table 2, experiments Nos. 9 and 10). CS of bubbles expressed from a piece of lung into physiological saline to which blood serum had been added was considerably increased when the lipid concentration in the serum was increased more than twofold, and the cholesterol concentration increased almost fourfold (0.91 ± 0.008 and 0.97 ± 0.004, $P < 0.001$). In other words, Pattle's method also indicated an increase in SA of surfactant under the influence of lipids and cholesterol.

The results are thus evidence that during contact of alveolar surfactant with blood or its filtrate the effect of the plasma proteins on SA is predominantly inhibitory, although hemoglobin, total lipids, and cholesterol have the opposite action. This conclusion corresponds in its details to data obtained by some workers [2, 5, 8, 9] but not by others [5, 6, 10-13]. The whole range of methods used in the present investigation was not, however, used by the authors cited.

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