EFFECT OF INHALATION OF SUBTOXIC DOSES OF PYRIDINE ON THE STATE OF THE SURFACTANT OF THE LUNG ALVEOLI AND ON LIPID FORMATION OF THE PNEUMOCYTES

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Inhalation of pyridine in a concentration of 0.015 mg/liter by experimental rats causes inhibition of lipid formation in the pneumocytes during the first 6 days of the experiment, with a decrease in protein synthesis by the cells and in the content of phospholipids in the surfactant. Later (12th-24th day) signs of adaptation appear, followed by compensation of the toxic effects on the high level of phospholipid formation by the pneumocytes and of the lipid content of the surfactant. After 36 days of the experiment the features of decompensation gradually increase in severity, with a sharp decrease in the phospholipid content in the alveolar cells and in the surface-active lining of the alveoli.

Exposure to factors producing changes in the surface-active lining of the alveoli of the lungs, including the inhalation of toxic substances, has received little study [4, 5].

In the investigation described below the effect of inhalation of a chemical product in a subtoxic concentration on the state of the surfactant was studied in chronic experiments.

EXPERIMENTAL METHOD

Experiments were carried out on 45 female albino rats of the same age and weight, 10 of which were used as the control. Daily except at weekends 35 rats were placed for 5 h in an inhalation chamber where they were exposed to pyridine in a concentration of 0.015 mg/liter. This compound is highly volatile and lipotropic, and at the same time its nitrogen atom with a double bond endows it with some degree of hydrophilicity. During the first 6 days of the experiment 2 rats were sacrificed on alternate days (by decapitation) and later 2 rats were sacrificed each week. To study regeneration, 10 rats were left intact. To assess the state of the surfactant in the experimental animals their phospholipid content was determined by extraction from the washed lungs with chloroform and methanol–chloroform in the ratio of 1:1 [3]. After centrifugation part of the extract was concentrated by evaporation and investigated by luminescence chromatography in a thin layer with development by rhodamine and coriphosphine [11]. The test substances were identified by their R_f values, using lecithin as the standard. Quantitative analysis was carried out by calculation from the area of the stains determined by planimetry [8]. The statistically significant background (control) value was taken as 1. To confirm the results of the chromatographic analysis, the presence and quantity of lecithin extracted from the surfactant were determined on the SF-4A spectrophotometer from the character of its ultraviolet absorption. The study of the components of the surfactant was completed by observation of the content of phospholipids in the large alveolar cell. To detect phospholipid granules and to count them the sections were treated with 3,4-benzpyrene [6]. Sections were cut in the same areas of the lungs from all lobes, and the number of granules in 100 pneumocytes was counted and compared with the control values, the results being expressed as a percentage. The character of granulation was also noted. The correlation between lipid formation and protein synthesis in the cells was determined at the transcription level, with reference to RNA detected by a luminescence-cytochemical method by staining

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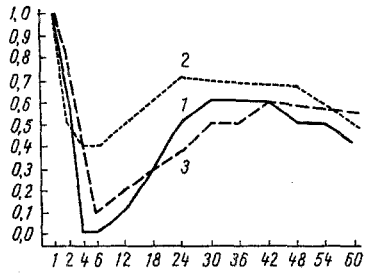

Fig. 1

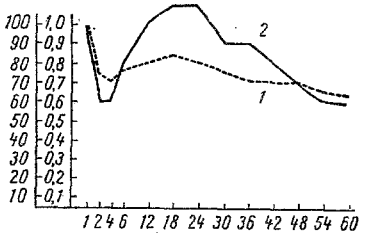

Fig. 2

Fig. 1. Content of phospholipids in surfactant during inhalation of pyridine: 1) sphingomyelin, 2) cephalin, 3) lecithin. Abscissa, days of experiment; ordinate, quantity of phospholipids calculated from chromatogram (control 1).

Fig. 2. State of phospholipid formation and RNA content in pneumocytes during inhalation of pyridine: 1) intensity of luminescence of RNA determined from optical density of its absorption; 2) content of phospholipid granules (in percent of control). Abscissa, days of experiment; ordinate: left) phospholipid formation (in percent); right) optical density of filter at λ = 640 nm.

with acridine orange. The RNA content was estimated from the optical density of absorption of its luminescence as measured with filters at λ_{max} = 640 nm [1]. Only results for which the difference was significant ($P < 0.05$) are given.

EXPERIMENTAL RESULTS

On the 2nd day after the experiment began a decrease in the sphingomyelin content to 0.5 of the control value, lecithin to 0.6, and cephalin to 0.8 was observed in the composition of the phospholipids of the surfactant (Fig. 1). On examination of the sections under the microscope this time the number of grains of phospholipids in the alveolar cells was reduced to 60% of the control and the intensity of luminescence of RNA was weakened to 0.75 determined from the optical density of absorption (Fig. 2). During the next 2 days the content of phospholipids in extracts from the surface-active layer of the alveoli continued to fall, and on the 6th day after the experiment began it was 0.1 for sphingomyelin and 0.4 for cephalin. Lecithin at this time (4th-6th days) could not be found on the chromatograms or by spectrophotometry of the extracts.

The level of lipid formation in the cells on the 4th day remained the same as before but the intensity of luminescence of RNA was reduced by 5%. On the 6th day an increase in the phospholipid content in the pneumocytes to 80% and an increase in the intensity of luminescence of RNA to 0.75 on the basis of the optical density of absorption were found. This time interval (the 6th day and later) was the critical period for the state of the surfactant, for it was followed by an increase in the phospholipid content. On the investigation of chromatograms and eluates and on spectrophotometry of the washings (with chloroform) from the surface of the alveoli on the 12th day lecithin was found to have appeared and the content of sphingomyelin and cephalin was increased. At the same time the phospholipid formation in the alveolar cells reached the level observed in the intact animals. The subsequent weeks were characterized by a continued rise in the phospholipid content in the surface-active lining of the alveoli, while the content of lipid granules in the cells was higher than that observed in the control animals. At the same time changes also took place in the state of the RNA and the intensity of the luminescence increased in the period from the 12th to the 18th day to 0.85 as determined from the absorption density, but later it began to fall slowly to 0.7 (36th-48th days of the experiment), and ultimately to 0.65 by the end of the experiment.

The state of the surface-active layer of the alveoli after inhalation of pyridine for 24 days was characterized by relative stability for cephalin, the content of which did not begin to fall until after the 48th day. The lecithin level in the surfactant, on the other hand, began to fall earlier – after the 42nd day. The content of sphingomyelin rose from the 18th to the 42nd day of the experiment, after which a tendency was observed for it to fall slowly until the end of the experiment.

Considerable changes in phospholipid production in the pneumocytes took place after the first 24 days of the experiment. In the following week granule formation fell to 90%, at which level it remained until the 36th day, after which it fell fairly rapidly to reach 60% by the end of the experiment.

The following interpretation can be given for these results. The inhaled pyridine, entering the lungs, easily passed through the lipoprotein film of surfactant because of its physicochemical properties (lipotropism combined with relative hydrophilicity) and penetrated rapidly through the plasmalemma into the interior of the alveolar cells. On the first days of inhalation the subtoxic doses of this compound were too much for the detoxicating powers of the microsomal system of the pneumocytes, with resulting spread of toxic effects to other enzyme-carrying structures and, in particular, the endoplasmic reticulum. This was immediately reflected in metabolic functions, for the components of the smooth and rough reticulum, like the microsomes themselves, play an important role in phospholipid synthesis [7, 9, 10]. Inhibition of activity of the lipid metabolism enzymes of the large and small alveolar cells characterized the stage of a sharp decrease in the content of choline-containing phospholipids in the surfactant (2nd-6th days of the experiment) and of a marked decrease in the phospholipid content in the pneumocytes during the first 4 days of the experiment. The comparatively high level of cephalin in the lipids of the surfactant can be explained by the special nature of its metabolism: the cephalin precursor – dimethylethanolamine – is renewed much quicker in the tissues than choline [2].

The next stages in the change in composition of the surfactant and in lipid formation by the pneumocytes are regarded as evidence of adaptation to the toxic effect and of ability to maintain that state (compensation). During continuation of exposure to the toxic substance they are followed by decompensation of functions.

The phenomena of adaptation to continued inhalation of pyridine and relative stabilization of the surface-active layer of the alveoli are connected primarily with increased functions of the microsomal system of the pneumocytes, for this system is responsible for detoxication and it plays an important role in the synthesis of phospholipids. These processes were reflected in the facts observed in the experiment between the 6th and 30th days: a rapid increase in lipid formation in the alveolar cells and an increase in the phospholipid content in the surfactant.

The phenomena distinguishing the subsequent state of the alveolar tissue deserve attention. As the experiment showed, during this short period (24th-42nd days) average levels of the phospholipid content in the surface-active layer and a decrease in the lipid-synthetic activity of the pneumocytes were observed. Since both phenomena are closely connected with the functions of the endoplasmic reticulum of the cytoplasm, their character can evidently be explained by insufficiency of the process of formation of new membranes of the reticulum in the alveolar cells damaged by exposure to the poison. Changes in RNA observed in the experiment confirm this conclusion. Consequently, for the mechanism of compensation of the toxic effects to be maintained at a sufficiently high level interaction must take place between factors including those stimulating the protein-synthetic activity of the cells. In the experiment described above, with continued inhalation of pyridine this interaction was insufficient.

In the group of experimental animals left for observation on the recovery period, improvement of the metabolic activity of the alveolar cells was observed by the end of the first week after the inhalation had stopped. Phospholipid formation in the pneumocytes rose to 75%, and the content of lecithin and cephalin in the surfactant increased to 0.6. By the end of the 2nd week the intensity of luminescence of RNA had increased to 0.8 as determined from the optical density of absorption, the cephalin level in the surfactant had reached 0.8, the level of the other phospholipids 0.75, and lipid formation in the cells had increased to 80%.

Final restoration of the normal functions of the alveolar cells of the lungs tested occurred in these animals by the end of the first month.

No clinical signs of disease were observed in any of the experimental animals during the period of the experiment.

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