PHASES OF CELLULAR REACTION TO SUBTOXIC INHALATION OF HYDROCARBON AEROSOLS

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During prolonged (up to 60 days) inhalation of an aerosol of anthracene oil in a concentration of 100 mg/m^3 by rats, successive phases of depression, adaptation, and increasing damage were observed, as revealed by changes in sorption of neutral red and in the content of anthracene oil in the lung tissues.

Biological self-regulating systems are characterized by fluctuations of their functional activity at different levels, at the molecular and organ level, and at the level of the organism as a whole [4-6]. With an increase in the strength of a stimulus, or with predominance of activators or inhibitors of metabolic reactions, the dynamic equilibrium is disturbed, and the phases of the fluctuations become aperiodic and protracted in time [3, 7].

The effect of subtoxic factors at the cellular level was studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred female rats weighing 170-180 g. For periods of 6 h daily for 60 days the animals inhaled an aerosol of hydrocarbons from the heavy fraction of a standard coal sample (anthracene oil – AO) in a concentration of 100 mg/m^3. Changes in the functional state of the lung tissue cells were assessed in relation to the degree of vital sorption and granulation of the dye neutral red and the content of AO in the tissues. Neutral red was used in a 0.01% concentration in soda-free Ringer's solution. Sorption of neutral red was observed in vitro, and sometimes, in order to confirm the results, in vivo by Romanov's method [2]. The degree of sorption was obtained by investigation of an alcoholic extract on the SF-4A spectrophotometer, and was expressed as a percentage of sorption of the control tissue. The state of granule formation was assessed by microscopic examination of sections of the lungs, with counting of the number of cells containing granules and the number of granules in them. The content of AO and its metabolites in the lung parenchyma was determined by thin-layer chromatography by Stahl's method [8], with examination of the absorption spectrum of the eluates on the spectrophotometer. The first three rats were decapitated 24 h after the beginning of the experiment, and subsequently one or two rats were sacrificed every 2-3 h. The results were analyzed by statistical methods. Differences were regarded as significant if $P < 0.05$.

RESULTS

At no time during the inhalation were visible signs of poisoning observed in the experimental animals. Not until the end of the experiment was respiration of some rats interrupted by occasional bouts of coughing. Inhalation of the AO aerosol in a subtoxic concentration produced a marked decrease in sorption of neutral red by the parenchymatous cells of the lungs after 24 h (Fig. 1). Sorption of the dye on the fourth day was at a minimum (67%), and a marked decrease in granule formation was observed under the micro-

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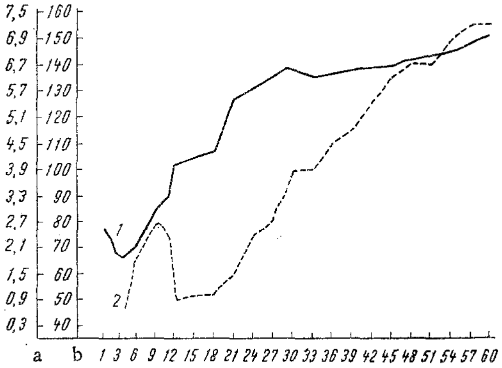

Fig. 1. State of lung tissues during inhalation of aerosol of hydrocarbons of the heavy coal fraction: 1) sorption of the dye neutral red; 2) content of anthracene oil in the tissues. Abscissa: time (in days); ordinate: a) AO content (in μg/g fresh tissue); b) content of dye (in % of control).

scope: the number of cells containing granules was 70 ± 3% per field of vision, and granules in the cytoplasm were small and few in number. At the same time, AO began to accumulate in the lungs – less than 1 μg/g fresh tissue. After the fourth day from the beginning of the experiment, a gradual increase in granule formation and in the degree of sorption of the dye was observed. On six successive days the number of cells containing granules rose to 85 ±2%, and the degree of sorption of neutral red increased to 90%. The AO content in extracts from the lung tissues began to decrease, and its metabolites of quinone character began to appear in the eluates. These figures showed substantial changes on the 12th day; sorption of neutral red was increased by 100%, the AO content in the lung tissue was increased by almost three times, and the relative proportion of phenolquinones and dihydroanthracenes was increased. This level of cell activity remained relatively stable until the 18th day, although a tendency was observed for the sorption of dye to increase, and for some decrease in the degree of granule formation to take place. Next day a rapid change occurred in the cell functions. The sorption curve of neutral red rose sharply during the next three days, to 127%, the number of granule-containing cells fell to 40 ±3%, and the AO content in extracts of lung tissues increased. The phenomena progressed in the subsequent course of the experiment. The AO content in the extracts by the end of the experiment reached 7.2 ±7.5 μg/g fresh tissue, reflecting its accumulation in the lungs. In the second half of the experiment hardly any neutral red granule formation was observed, but it was replaced by diffuse sorption of the dye. The quantity of neutral red undergoing sorption reached 150% on the 60th day.

The phenomena observed during the course of the experiment thus developed in three successive phases. The first phase was characterized by depression of cell functions from the beginning of inhalation until the fifth day. Inhalation of the hydrocarbon aerosol during this period evidently led to disorganization of the intracellular medium of parenchymatous cells of the lungs, as a result of which their enzyme activity was reduced. This was shown by a decrease in the ability of the cells to form granules of dye and by the accumulation of AO in the lung tissues.

The second phase, the initial phenomena of which were found after the fifth day of the experiment, was characterized by reactivation of the cell function (increasing granule formation and an increase in the sorption of dye up to the control level, with the appearance of AO metabolites). These features were particularly conspicuous on the 9th-12th day from the beginning of inhalation, and they were relatively stabilized for the next 6 days. During this time interval, continued inhalation of the AO aerosol was unaccompanied by its accumulation in the lung tissues, demonstrating the increased cell enzyme activity to render the foreign product harmless. This process as a whole can be described as a manifestation of the adaptive powers of the parenchymatous cells of the lungs.

In the last phase of the cell reactions to prolonged administration of AO, which began after the 18th day of inhalation, signs of injury gradually developed. The slow approximation of the curves of neutral red sorption and of the AO content in the lung tissues at a high level, demonstrated during this phase, illustrates the increasing inability of the cells to resist the toxic action. At the same time, the level of sorption of the dye showed a moderate degree of structural change in the parenchymatous cells of the lung, so that for a short period they were in a state of reversible injury.

However, this was sufficient to depress the enzyme activity of the cells, as was demonstrated during this phase.

Under the influence of inhalation of toxic hydrocarbons, primary inhibition of cell activity (the phase of inhibition) is observed, followed by signs of reactivation (the phase of adaptation). If inhalation of the toxic products continues this is followed by a phase of increasing injury.

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