CHANGES IN ANTIOXIDATIVE ACTIVITY OF CELLS AND FUNCTIONS OF THE NUCLEUS BY THE ACTION OF SUBTOXIC DOSES OF PHENOL–PYRIDINE MIXTURES FROM COAL TAR

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The action of phenol-pyridine mixtures found in the water-soluble part of the heavy fraction of coal tar in the following concentrations were studied in two series of experiments, on pure cultures of paramecia and on albino rats: phenol and its homologs 54 μg/ml, pyridine compounds 30 μg/ml. A 1:4 solution of the hydrocarbons inhibited reproductions of the paramecia and caused karyotropic disturbances (fragmentation), with the appearance of mutant forms on the 18th-22nd day after the beginning of the experiment. After intratracheal injection of the solution into albino rats in doses of 0.25 ml twice a week, gradual disturbance of the native state of DNA in the nuclei of the small alveolar cells, irregular distribution of RNA in the cytoplasm and a progressive decrease in the antioxidative activity of the lung tissue lipids were observed.

Workers who have investigated phenol derivatives report inhibition of oxidative phosphorylation reactions by these compounds [4, 12] and their ability to bind with histones [2] and to inhibit mitotic activity of cells [1, 6, 7]. The question of the site and character of the cytotoxic action of phenols occuring in a natural complex with other compounds has not yet been settled.

In the investigation described below the effects of hydrocarbons of the phenol and pyridine groups, when administered in subtoxic concentrations over long periods, were studied at the cellular and subcellular levels.

EXPERIMENTAL METHOD

The water-soluable fraction was isolated from a standard sample of the heavy fraction of coal tar and investigated by thin-layer chromatography [10] followed by spectral analysis of the eluates. The liquid, obtained in a volume of 100 ml per gram of original product, contained: phenol up to 32 μg/ml, cresols 12 μg/ml, xylenols 10 μg/ml, and pyridine bases 30 μg/ml. This phenol-pyridine solution (PPS) was used for two series of experiments. The experiments of series I were carried out on pure cultures of paramecia grown from a local clone on Losina-Losinski medium with paramecia. An intact culture served as the control. Cytological observations were made daily and cytochemical tests once every 2 days.

The experiments of series II were carried out on 70 male albino rats weighing 180-190 g (the control group contained 20 rats). The PPS was injected intratracheally in a dose of 0.25 ml twice a week. Once every 6 days a pair of animals was decapitated, and the alveolar cells, their nuclei, the state of the antioxidative activity of the lung tissues and the presence of metabolytes of phenol and pyridines in the lungs were investigated in films and sections. In both series of experiments in order to detect karyological changes in the cells and to determine the state of DNA and RNA, methods of luminescence-cytochemistry and, in particular, metachromatic staining with acridine orange in a concentration of 10^{-4} M in citrate

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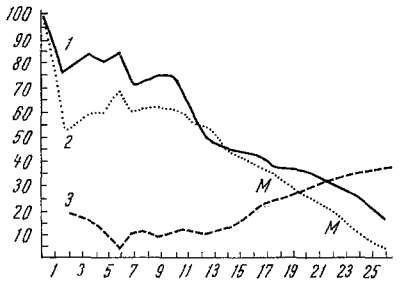

Fig. 1

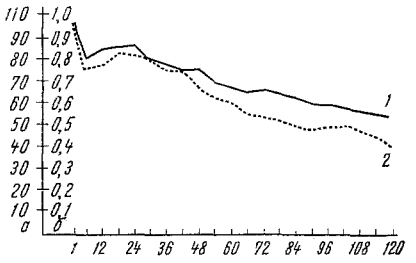

Fig. 2

Fig. 1. Effect of phenol-pyridine mixture from coal tar on biological activity of paramecia. 1) Number of cells with unchanged luminescence metachromasia of DNA-RNA; 2) total number of cells in culture; 3) number of cells with deformed nucleus (in control 0%). Abscissa, duration of experiment (in days); ordinate, percent of control.

Fig. 2. State of DNA of alveolar cells and antioxidative activity of lung tissue lipids after intratracheal injection of phenol-pyridine mixtures; 1) intensity of luminescence of DNA determined from optical density of its absorption; 2) antioxidative activity of lung tissue lipids (in percent of control). Abscissa, duration of experiment (in days), ordinate: a) percent of control, b) optical density of filter at λ = 530 nm.

buffer mixture, pH 4.3 [8, 9], were used. To confirm the DNA changes found in the cell nuclei, the luminescence-cytochemical variant of the Feulgen reaction with auramine was used [5]. A method of photometric interpolation was used to estimate the state of DNA and RNA in the cells quantitatively: it was judged from the level of absorption of luminescence, the reciprocal of which is the intensity of luminescence, which is proportional to the quantity of absorbed dye. To carry out this measurement freshly frozen sections were cut to a thickness not exceeding 10 μ, fluorochromed, and examined under the microscope with the use of a scale of membrane filters (MF) of increasing optical density. These filters were graduated on a type SF-4 spectrometer relative to λ_{max} of the radiation: 530 nm for DNA and 640 nm for RNA. The number of cells whose luminescence was not absorbed was counted in each specimen in 8-10 fields of vision after each successive change of MF. The level of absorption achieved could be compared with the level of intensity of luminescence if single cells whose luminescence had not yet been absorbed were still present in a field of vision. Preparations containing hydrolyzed nucleic acids served as the control, and fluorochroming at pH 2.0 was used for differentiation from acid mucopolysaccharides. The antioxidative activity of the lung tissue lipids was determined by the iodometric method [3] and the peroxide numbers were expressed as percentages of the control. Phenol metabolites were extracted from the lung tissue homogenate with 5% NaOH solution, and after chromatography the eluates were dissolved in ethanol for spectrophotometry. Pyridine compounds were extracted from the tissue with cyclohexane and the spectral characteristics of the eluates were photographed in the same solvent. The compound was identified from the UV absorption spectra. All numerical results were subjected to statistical analysis and differences for which $P < 0.05$ were considered to be significant.

EXPERIMENTAL RESULTS

Series I. On the 2nd day after addition of the PPS to a culture of paramecia the number of cells was reduced by 47 ± 3%, their movements were retarded, the luminescence metachromasia of the nucleic acids was reduced, and up to 20 ± 5% of individuals with deformed nucei was counted in the population. During the next four days signs of repair were observed: the number of cells increased to 70 ± 3% of the initial level and the shape of the nucleus improved. However, on the 7th day from the beginning of the experiment the number of individuals with weak DNA-RNA luminescence began to appear, and this was followed by a progressive decrease in the number of paramecia and a simultaneuos increase in the number of disturbances of the nucleus; deformation and fragmentation. Between the 18th and 22nd days mutant forms were found in several cultures, consisting of cells with a reduced anterior end to the number of 3-5 per

population. Mutants transplanted into fresh medium and cells with a fragmented nucleus did not reproduce. Control cultures developed normally. The dynamics of the changes observed are illustrated in Fig. 1.

Series II. After the first two injections of PPS (6th day from the beginning of the experiment) the intensity of DNA luminescence in the nuclei of the small alveolar cells was reduced to 0.8 with respect to optical density of absorption and the antioxidative activity of the lung tissue lipids was reduced by 25%. The state of the antioxidative activity improved slightly from the 12th to the 18th day (77-83%), and during this period the luminescence metachromasia of nucleic acids in the cells increased. The level attained was stable on the 24th day after the beginning of the experiment also. At this time orthodiphenols, phenol-quinones, and methylpyridines were formed in extracts from the lungs. In the period after the 24th day, unchanged phenols began to appear in the extracts (up tp 3 μg/g fresh lung tissues by the end of the experiment), while the intensity of DNA luminescence in the nuclei of the alveolar cells fell steadily, andby the end of the experiment it was down to 0.55 with respect to optical density of absorption. Microscopic examination of sections stained by Carazzi's method showed focal changes in the alveolar epithelium in the lungs: a decrease in volume of the cells and predominance of elongated forms. Luminescence-cytochemical tests revealed an irregular distribution of RNA in the cytoplasm of these cells, with a decrease in RNA luminescence to 0.6-0.5 with respect to optical density of absorption. The antioxidative activity of the lung tissue, which was 83-82% in the first 18-24 days, subsequently began to fall rapidly, to reach 40% on the 120th day. The development of these changes is illustrated in Fig. 2.

Notwithstanding the great biological difference between the experimental objects, it will be noted that the results of the two series of experiments are to some extent similar. This suggests that the action of the phenol–pyridine group of hydrocarbons is basically similar. A significant feature is the disturbance of nucleic acid metabolism. Evidence of this is given by the karyomorphic changes and the appearance of mutants in the paramecia, the decrease in the luminescence metachromasia of DNA-RNA in these cells, and the inhibition of reproduction, the decrease in the intensity of DNA luminescence in the nuclei of the alveolar cells of the rats' lungs, and the disturbance of the distribution of RNA in the cytoplasm with a decrease in the intensity of its emission after staining with acridine orange. The direct karyotropic action of phenol–pyridine mixtures on the cells cannot be ruled out, but the effect could also be secondary: first, because the processes of detoxication take place at the distant approaches to the nucleus, and second, the state of oxidative and antioxidative reactions in the cell plays an important role in the mechanism under analysis, especially in tissues of the warm-blooded animal. Since they penetrate readily into protein-lipid structures of the intracellular membranes, phenols suppress enzymic oxidation, with the consequent stimulation of free-radical reactions and depression of the level of the tissue bioantioxidants – a phenomenon observed in the experiments of series II. The quinone groups formed during metabolism of the hydrocarbons and also the methylpyridines possess the properties of free-radical compounds and inhibit mitotic activity of cells [11].

In the experiments on animals described above these changes contributed to increasingly severe disturbances of intracellular functions together with the karyotropic manifestations already described. Some improvement in the state of the cells observed in the experiments of series II between the 12th and 24th days can be regarded as mobilization of adaptation mechanisms, including induction of synthesis of detoxication enzymes. The continued entry of hydrocarbons into the lung tissues and accumulation of their metabolites in them led to an early decrease in antioxidative activity, followed by a progressive decrease in nuclear cell synthesis.

During the experiment no clinical disturbances affecting the health of the experimental animals could be observed.

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