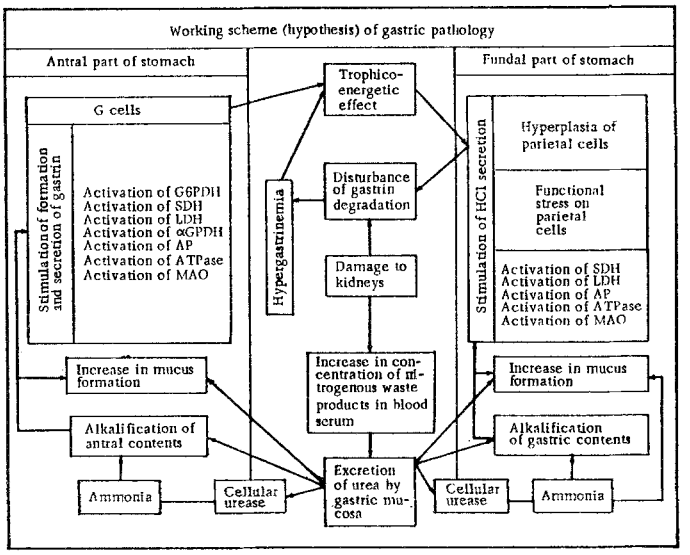

Fig. 3

gastritis against the background of previous hyperplasia of the glandular structures can evidently explain the unique "mosaic" character of the structural changes and the "paucity" of the functional parameters in patients with CRF.

LITERATURE CITED

1. L. I. Aruin, Klin. Med., No. 1, 18 (1975).
2. V. Yu. Golofeevskii, Ter. Arkh., No. 10, 13 (1976).
3. S. I. Ryabov and E. S. Ryss, Ter. Arkh., No. 3, 33 (1979).
4. V. A. Samsonov, Arkh. Patol., No. 8, 5 (1973).
5. A. M. Ugolev, The Enterin (Intestinal Hormone) System [in Russian], Leningrad (1978).
6. D. J. C. Shearman et al., Clin. Res., 15, 243 (1967).

FUNCTIONAL AND MORPHOLOGICAL FEATURES OF THE ADRENAL CORTEX OF ALBINO RATS AFTER LONG EXPOSURE TO STYRENE

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KEY WORDS: aromatic hydrocarbons; metapyrone; quantitative functional-morphological analysis; adrenocortex of albino rats.

According to some observations [5, 7] aromatic hydrocarbons (AH) have a toxic action on cells of the adrenal cortex. There have been few histochemical investigations of the chronic effect of AH on the adrenal cortex, and in none of them was a quantitative analysis made of the character of changes in the adrenal cortex in response to exposure to AH.

Considering the wide use of AH in modern industry and probability of injury to the endocrine system arising in persons working in processes associated with AH [1, 4], it was decided to study the possible mechanism of action of these compounds on the adrenal cortex, with particular reference to styrene.

The object of this investigation was to determine the mechanism of long-term action of styrene on the albino rat adrenal cortex.

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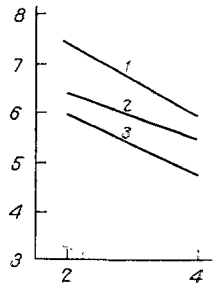

Fig. 1. Results of investigation of summation ability of the CNS (STI). Abscissa, duration of inhalation of styrene (in months); ordinate, number of pulses required to evoke reflex movement (summation number). 1) Styrene, 2) styrene + metapyrone; 3) control.

EXPERIMENTAL METHOD

Altogether 120 female albino rats weighing 140-160 g were used in three series of experiments. In series I the animals were poisoned with styrene by inhalation in a concentration of 20 MAC. In series II the animals were poisoned with styrene after physiological stabilization of their endocrine system. In this series the method developed in the Department of Pathological Anatomy, First Leningrad Medical Institute [3], was used. All the animals of series II received metapyrone in a dose of 12 mg/100 g body weight during the first 2 weeks of poisoning in order to stabilize adrenocortical function within normal physiological limits of the 11-hydroxycorticosteriod (11-HCS) level in order to prevent active participation of the adrenal cortex in the nonspecific response of the animal to chemical stress.

The animals of series III served as the control; they were kept every day in chambers similar to those used for poisoning the experimental animals, but they were supplied with pure air.

In all the animals throughout the experiment the summation-threshold index (STI) was recorded, the Quick test performed, the peripheral blood investigated, and a biochemical analysis of the blood for 11-HCS was carried out by the gel-filtration method [6].

The animals were decapitated 2 and 4 months after the beginning of poisoning. The adrenals were removed at once, weighed, frozen in isooctane cooled with liquid nitrogen, and subjected to morphometric and histoenzymologic investigation.

The dimensions of the nuclei were measured [8] in preparations stained with gallocyanin at pH 1.1.

Histochemical investigation included analysis of nonspecific metabolism (an energy-producing enzyme — glucose-6-phosphate dehydrogenase, a hydrolytic enzyme — acid phosphatase), and also of the specific enzyme of 11-HCS synthesis, i.e., 11-β-hydroxylase.

Activity of the enzymes was assessed quantitatively by a photographic cytophotometric method (the preparations were photographed on the MUF-6 instrument and this was followed by photometry of the negatives on the MF-2 photometer).

All the results of cytochemical and morphometric investigations were processed on the EC-1022 computer by a special program [2].

EXPERIMENTAL RESULTS

In the animals of series I (poisoned with styrene) deviations of the functional indices from the control level were found, whereas in the rats of series II (receiving metapyrone) the results were virtually indistinguishable from the control. Analysis of the dynamics of the physiological indices during prolonged exposure to styrene suggested that stabilization

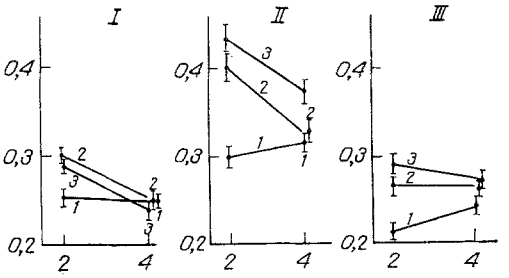

Fig. 2. Results of karyometry of zones of the adrenal cortex. I) Zona glomerulosa, II) zona fasciculata, III) zone reticularis. Abscissa, duration of inhalation of styrene (in months); ordinate, area of cell nuclei. Remainder of legend as to Fig. 1.

of adrenal function for 2 weeks at the beginning of the experiment keeps the functional parameters at a nearly normal level (Fig. 1).

In the course of 2 and 4 months a tendency was observed in the control animals for the size of the nuclei to change (Fig. 2) in all three zones of the cortex, more especially in the zona fasciculata, where this index ranged between 0.44 and 0.37. Between 2 and 4 months the nuclei became smaller, but the decrease was significant only in the zona fasciculata.

In the rats of series I 2 months after the beginning of poisoning a decrease in the size of the nuclei was observed in all three zones, but toward the end of the experiment the decrease in size of the nuclei still remained significant only in the zona fasciculata.

In the animals of series II no such marked decrease was found in the dimensions of the nuclei, but nevertheless the degree of the change was more evident in the zona fasciculata.

According to the karyometric data, stabilization of the endocrine system for 2 weeks thus abolished the effect of chronic poisoning with styrene.

The results of the cytochemical investigation of 11-β-hydroxylase, the enzyme of specific synthesis, also showed definite differences in the structural changes in the adrenal cortex (Fig. 3A).

In the control animals, activity of the enzyme in the three zones of the cortex showed relatively small fluctuations in the course of the experiment, but fell significantly toward its end.

In the rats of series I a distinct fall in 11-β-hydroxylase activity was observed in all three zones after 2 months. By the fourth month of poisoning activity of the enzyme was increased in the zona fasciculata and zona reticularis, but the degree of increase was greatest in the zona reticularis.

In series II (with physiological stabilization of the endocrine system) activity of this enzyme was close to the control level in all three zones after 2 months, and only after 4 months was the level of 11-β-hydroxylase activity in the zona reticularis significantly higher than in the control. Analysis of activity of the enzyme of specific synthesis thus confirmed the results of karyometry, showing the leveling effect of metapyrone.

Investigation of nonspecific enzymes and, in particular, of glucose-6-phosphate dehydrogenase, in the control animals showed a similar time course in all three zones with a significant increase in its activity toward the end of the experiment (Fig. 3B). During poisoning with styrene for 2 months an increase in the activity of this enzyme was observed; the greatest changes were observed in the zona glomerulosa. By the end of the experiment marked inhibition of glucose-6-phosphate dehydrogenase activity was found in all three zones.

In the animals receiving metapyrone, 2 months after the beginning of poisoning more marked activation of the enzyme was found in the zona reticularis compared with rats in the previous series. By the end of the experiment the degree of change in glucose-6-phosphate dehydrogenase activity differed in the different zones: In the zona glomerulosa it remained

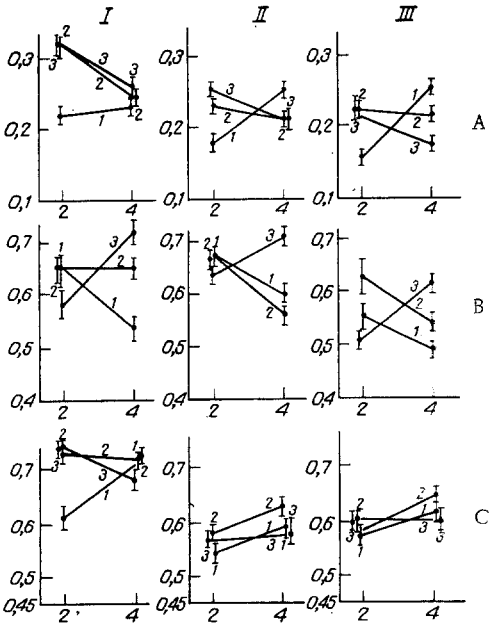

Fig. 3. Results of investigation of 11-β-hydroxylase (A), glucose-6-phosphate dehydrogenase (B), and acid phosphatase (C) activity. Abscissa, duration of inhalation of styrene (in months); ordinate optical density (at 540 nm). Remainder of legend as to Fig. 2.

at its initial level, in the zona fasciculata it was significantly lower, and in the zona reticularis the decrease was smaller than in the rats of series I.

Investigation of the state of the lysosomes (acid phosphatase activity) showed the following trend (Fig. 3C). After 2 and 4 months acid phosphatase activity in the control animals remained unchanged in the zona fasciculata and zona reticularis, and its activity was significantly reduced only in the zona glomerulosa by the end of 4 months.

During poisoning with styrene for 2 months acid phosphatase activity fell sharply in the zona glomerulosa, whereas in the zona fasciculata and zona reticularis this tendency to fall was not significant. By the end of the experiments the activity of this enzyme was virtually indistinguishable from the control.

In the animals of series II acid phosphatase activity 2 months after the beginning of poisoning remained at the control level. By the end of the experiment it showed a significant increase in all three zones.

Quantitative morphometric and enzymologic analysis of the adrenal cortex of albino rats in different versions of chronic stress thus revealed some important differences in the character of the functional and morphological changes in the adrenal cortex associated with prolonged exposure to AH.

By the use of combined quantitative cytochemical analysis it was possible to demonstrate different degrees of functional and morphological reorganization of the different zones of the adrenal cortex. This suggests that one of the decisive factors in the development of pathological processes during chronic exposure to AH is a disturbance of relations between the different structural components of the adrenal cortex which, in turn, consolidates the hormonal imbalance.

LITERATURE CITED

1. G. Z. Bakhtizina, in: The Endocrine System and Toxic External Environmental Factors [in Russian], Leningrad (1979), pp. 11-12.
2. T. B. Zhuravleva and R. A. Prochukhanov (editors), Introduction to Quantitative Enzyme Histochemistry [in Russian], Moscow (1978), pp. 183-196.

3. S. M. Ledovskaya and R. A. Prochukhanov, Probl. Endokrinol., No. 3, 103 (1976).
4. V. M. Makotchenko and V. P. Maninina-Putsenko, in: The Endocrine System and Toxic External Environmental Factors [in Russian], Leningrad (1979), pp. 136-137.
5. N. A. Minkina and E. G. Berliner, in: Current Problems in Work Hygiene and Occupational Pathology [in Russian], Moscow (1974), pp. 90-94.
6. L. V. Pavlikhina, I. Ya. Usvatova, and A. F. Bunyatyan, in: Methods of Investigation of Some Systems of Humoral Regulation [in Russian], Moscow (1967), pp. 50-59.
7. A. S. Faustov and O. A. Pankovets, in: The Endocrine System and Toxic External Environmental Factors [in Russian], Leningrad (1979), pp. 222-223.
8. M. Palcovits and S. Csapo, Z. Mikrosk. Anat. Forsch., 67, 339 (1961).