cytic cell and attracting it (to begin with, neutrophils) to the site of formation of MBP; hence determining the more or less substantial contribution of neutrophils to phagocytosis as an additional mechanism of protection associated with the favorable redistribution of the functional load among the cells, providing a warning of the need to mobilize extra numbers of neutrophils and also, possibly, of monocytes from the depots through the circulating blood; finally, increasing and maintaining the reserves of the pool of phagocytic cells through the appropriate influence on hematopoiesis.

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INVESTIGATION OF THE EFFECT OF CERTAIN CHLORINATED HYDROCARBONS ON THE COMPOSITION OF THE HEPATOCYTE POPULATION OF THE RAT LIVER

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Hepatocytes in the liver of albino rats poisoned by inhalation of dichloropropane and trichloropropane were investigated cytophotometrically and karyometrically. With respect to the effect on polyploidization of the hepatocyte nuclei trichloropropane was found to be more toxic than dichloropropane. The development of polyploidization is determined by the dose of the toxic agent and the exposure to it: The smaller the dose the shorter the time required for the effect to take place.

KEY WORDS: *ploidy of hepatocytes; chlorinated hydrocarbons; binuclear cells; dose; exposure.*

The action of the chlorinated hydrocarbon 1,2,3-trichloropropane (TCP) on the ploidy of rat liver hepatocytes was studied previously [2] in subacute experiments involving inhalation of TCP for 7 days. In the present investigation, besides TCP another member of this group of compounds was used, namely 1,2-dichloropropane (DCP), a compound widely used in industry and agriculture. The morphological changes arising after exposure to DCP consisted essen-

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TABLE 1. Number of Hepatocytes of Different Ploidy in Liver of Control and Experimental Rats Poisoned by Inhalation of DCP and TCP in Different Concentrations and for Different Lengths of Exposure

Group No.	Characteristics of group	Number of rats	Relative percentages of hepatocytes of different ploidy (M ± m)						
			2n	2n+2n	4n	4n+4n	8n	8n+8n	16n
1	Control	13	4,3±0,4	11,9±0,6	73,3±0,9	9,3±0,8	1,1±0,1	0,1	Single
2	Exposure to TCP for 1 week, 0.8 mg/liter	5	2,6±0,3	2,6±0,9	82,5±1,7	4,0±1,0	7,8±0,9	0,3±0,1	0,2
3*	Exposure for 1 week to DCP, 1.1 mg/liter	2	4,1±0,5	8,8±0,2	80,2±3,0	5,2±1,6	1,5±0,7	0,1	0,1
4	Exposure for 1 week to TCP, 2.16 mg/liter	7	3,0±0,3	4,3±0,5	83,1±0,9	4,6±0,5	4,4±0,8	0,4	0,2
5	Control	4	2,5±0,3	14,6±3,5	68,8±2,3	12,9±1,4	1,0±0,3	0,2	—
6*	Exposure for 20 h to DCP, 2.2 mg/liter	5	4,8±0,9	15,1±1,8	67,9±1,7	11,2±1,7	0,8±0,2	0,2	—
7*	Exposure for 3 days to DCP, 2.2 mg/liter	7	3,2±0,5	11,5±2,5	70,1±2,1	8,8±0,8	5,7±1,6	0,5±0,2	0,2
8	Control	3	4,6±0,1	9,1±0,1	76,3±0,1	8,6±0,3	1,3±0,1	0,1	Single
9	Exposure for 2 weeks to DCP: 0.1 mg/liter	4	3,3±0,7	13,2±2,4	69,6±3,8	12,2±1,8	1,6±0,3	0,1	0,1
10	0.5 mg/liter	3	4,9±0,7	19,7±5,6	68,3±5,3	5,7±1,7	1,1±0,5	0,3	—
11	Exposure for 2 weeks to TCP, 0.08 mg/liter	4	2,8±0,1	9,3±1,2	78,9±1,7	6,9±0,5	1,9±0,3	0,1	—
12	Control	4	2,2±1,0	4,6±1,0	76,8±0,9	14,0±1,3	2,2±0,3	0,2	—
13	Exposure for 3 months to DCP, 0.0017 mg/liter	5	2,5±0,6	6,7±3,0	74,2±4,1	13,4±1,5	2,9±0,7	0,2	0,1
14*	0.009 mg/liter	6	3,2±0,3	6,7±1,0	75,0±1,5	11,1±1,2	3,3±0,8	0,6±0,2	0,1
15	0.00045 mg/liter	6	1,9±0,4	4,9±1,1	76,4±2,0	13,2±1,4	3,2±0,9	0,4±0,2	—
16*	Exposure for 3 months to TCP, 0.002 mg/liter	5	2,0±0,4	5,3±1,8	73,6±4,3	13,3±2,3	4,8±1,5	0,9±0,4	0,1

*Since cytophotometric analysis in groupd 3, 6, 7, 14, and 16 showed the presence of intermediate classes and the transition classes 2n → 4n and 4n → 8n are not indicated in the table, karyometric counting for these groups is conventional.

tially of degenerative changes in the parenchymatous organs and, in particular, in the liver [11].

The relationship between the chemical structure and toxicity of certain chlorinated hydrocarbons has been examined in several investigations [3, 8]; in warm-blooded animals this is reflected in a decrease in toxicity with a decrease in the number of chlorine atoms in the molecule. Whether this relationship extends to polyploidization of hepatocytes has not hitherto been studied.

The object of the present investigation was to compare the action of various concentrations of DCP and TCP on the ploidy of hepatocytes and thus to confirm correlation between the chemical structure of chlorinated hydrocarbons and their toxicity; the effect of TCP and DCP under different experimental conditions on the ploidy of rat hepatocytes also was studied.

EXPERIMENTAL METHOD

Noninbred albino rats were poisoned with TCP and DCP by inhalation in air-tight chambers with a volume of 200 cm^3. The concentrations (see Table 1) were kept constant for 1 and 3 days, 1 and 2 weeks, 3 months. The experiments were carried out by the staff of the Laboratory of Toxicology (Head, Candidate of Medical Sciences V. R. Tsulaya) of the Institute of General Communal Hygiene. A control group of rats was provided for the experimental animals

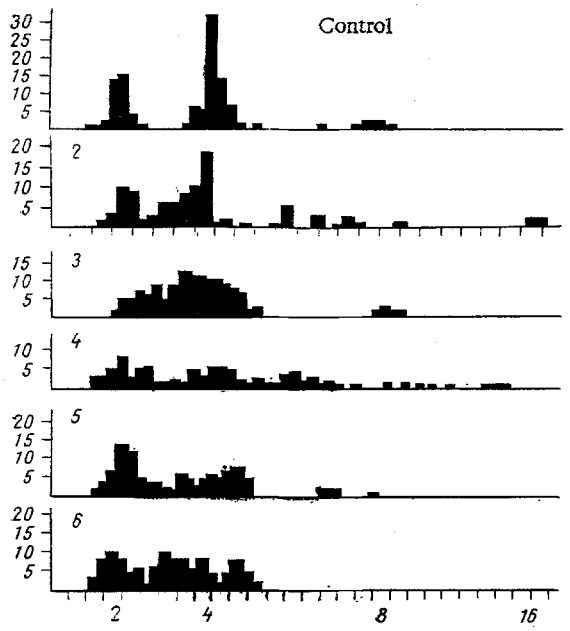

Fig. 1. Distribution of hepatocyte nuclei by ploidy classes (n): 1) control; 2-5) poisoning with DCP in a concentration of 1.1 mg/liter for 1 week, in a concentration of 2.2 mg/liter for 1 and 3 days, and in a concentration of 0.009 mg/liter for 3 months, respectively; 6) poisoning with TCP in a concentration of 0.002 mg/liter for 3 months. Optical density of DNA-fuchsin determined by single-beam probe cytophotometer in 550 nm region. Abscissa, DNA content (in ploidy units); ordinate, number of nuclei in each interval (in %).

at each time of the experiment. The control animals were kept in similar chambers supplied with atmospheric air. Altogether 24 control and 59 experimental rats were used.

Pieces of the right lateral and medial lobes of the liver were chosen for testing, for it has been shown [12] that CCl_4 gives rise to more severe morphological changes in these lobes. The method of preparing films of hepatocytes for determination of the DNA content and the conditions of photometry were described previously [2]. To illustrate the text, only a few histograms demonstrating the typical distribution for that particular experiment will be given. For all the 83 animals studied the relative percentages of mono- and binuclear cells of different degrees of ploidy was determined (from 500 to 3000 cells were counted from each rat). The films for these purposes were stained with hematoxylin–eosin and gallocyanin.

EXPERIMENTAL RESULTS

In the animals of all control groups cytophotometric analysis showed distinct grouping of the nuclei into classes of ploidy (Fig. 1, 1). The ratio between mono- and binuclear cells in the hepatocyte population of rats aged 2-4 months in groups 1, 5, and 8 (Table 1) differed from that in the rats of group 12. In the latter, despite some increase in the number of mononuclear cells of higher ploidy, the number of binuclear cells with two diploid nuclei was significantly ($P < 0.001$) reduced. These differences may have been attributable to variations in the ratio between the numbers of cells of different ploidy in the liver of the different groups of rats, as has been shown, for example, for different strains of mice [5]. On the other hand, keeping animals in small chambers for 3 months has some effect on the processes of growth in the liver. It will be noted that no morphological changes were found in the liver of these rats.

On morphological and histochemical investigations of the liver of the experimental animals poisoned for 1 and 2 weeks with DCP and TCP the severest changes were observed in the centrolobular regions of the hepatic lobules [3]. After an exposure of 3 months the same changes were found in the liver of the rats of groups 14 and 16, although they were less marked.

Analysis of the histograms of the DNA content in the hepatocytes of rats exposed to DCP for 1 week showed that hepatocytes of intermediate ploidy ($2n \rightarrow 4n$ and $4n \rightarrow 8n$) were present only after poisoning with DCP in a concentration of 1.1 mg/liter (Fig. 1, 2). In all cases except that last it was thus possible to determine the ratio between cells of different ploidy on the basis of the size of the nuclei and their number in the cell. A significant (compared with the control) increase in the number of mononuclear hepatocytes with a nucleus of higher ploidy (8n and 16n) with a decrease in the number of binuclear cells was observed in response to the action of DCP in a dose of 2.16 mg/liter. The same effect was produced by TCP in a concentration of 0.8 mg/liter after the same length of exposure. Gerhard et al. [10], who studied polyploidization of the liver after poisoning of mice with CCl_4, also ob-

served a similar decrease in the number of binuclear cells, leading to polyploidization of the nuclei, in the first 60 h after poisoning.

The counts showed that with DCP in a concentration of 1.1 mg/liter (Table 1) definite changes were already observed in the ratio between the numbers of cells of different ploidy. The number of binuclear cells with diploid nuclei ($P < 0.001$) and of tetraploid cells ($P < 0.05$) was reduced statistically significantly, and the number of mononuclear tetraploid cells was increased ($P < 0.05$; Table 1). With different daily concentrations of DCP, the response of the liver to exposure thus developed in phases: With a concentration of 1.1 mg/liter nuclei of intermediate ploidy appeared, evidence most likely of proliferative processes. Definite changes also were observed in the distribution of the hepatocytes by ploidy classes. On the 7th day after poisoning with DCP in a concentration of 2.16 mg/liter and with TCP in a concentration of 0.8 mg/liter there were no proliferating cells, as is shown by the discreteness of the histograms. The passage of a wave of proliferation was accompanied by the appearance of nuclei of higher ploidy.

Since TCP in a concentration of 0.8 mg/liter and DCP in a concentration 2.7 times higher (2.16 mg/liter) cause appropriate changes in the ploidy of hepatocytes, whereas comparable concentrations of DCP (1.1 mg/liter) and TCP (0.8 mg/liter) caused different degrees of polyploidization, this confirms that the toxicity of chlorinated hydrocarbons depends on their chemical structure.

A series of experiments (Table 1, groups Nos. 6, 7, 10, 11, 13-16) was carried out to discover whether the changes taking place in the liver depend on the dose of the compound only or on exposure as well. A cytophotometric investigation of the hepatocytes in animals poisoned for 1 (group 6) and 3 days (group 7) with DCP in a concentration of 2.2 mg/liter showed the presence of a considerable number of nuclei of intermediate ploidy ($2n \rightarrow 4n$ and $4n \rightarrow 8n$; Fig. 1, 3 and 4). Whereas after poisoning for 1 day the percentage of mono- and binuclear cells in the population still remained unchanged, by the third day there was a tendency toward an increase in the number of polyploid (8n, 8n + 8n, and 16n) cells (Table 1). For one rat, poisoned for 3 days, the changes in the distribution of the hepatocytes were the same as those for animals exposed for 7 days to inhalation of DCP and TCP. In the case of DCP, polyploidization, as the result of passage of a wave of mitosis, evidently ends between the third and seventh days of exposure.

If the animals received doses of DCP and TCP adequate in total but spread over a long period of time (Table 1, groups 9, 10, and 11), the results of the karyometric and cytophotometric investigations in the experimental groups were indistinguishable from those in the control. However, with a further decrease in the daily and total concentrations and an increase in the period of exposure to 3 months, nuclei of an intermediate ploidy class (mainly from $2n \rightarrow 4n$) were found in two of the four groups of experimental animals studied (Table 1, groups 14 and 16, Fig. 1, 5, 6), although the ratio between the numbers of mono- and binuclear cells in the hepatocyte population of these rats still remained unchanged (Table 1).

Differences between the results obtained after single and repeated exposures have been described by many workers who attribute them to the rhythm and strength of action of the noxious agent [4, 6, 7, 9, 13]. Tsirel'nikov et al. [7], who studied the action of CCl_4, administered in various ways, on the liver of mice and rats, suggested that repeated exposure inhibits DNA synthesis in the hepatocytes, whereas during longer exposures to the poison changes take place in the DNA-synthesizing system which abolish the effect of repeated doses. It may be this dependence which explains the results obtained when studying polyploidization in relation to the duration of exposure.

For compensatory polyploidization of the liver to develop in response to poisoning the following conditions are thus essential: 1) an adequate dose of the substance and 2) an increase in the exposure to offset a decrease in the strength of action of the poison.

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COMPARATIVE STUDY OF THE ACTION OF ETHIMIZOLE AND HYDROCORTISONE ON PROLIFERATIVE ACTIVITY AND PROTEIN SYNTHESIS IN EPITHELIAL CELLS OF THE TONGUE AND LIVER

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Unlike hydrocortisone, ethimizole stimulated mitotic activity of the epithelial cells of the tongue and liver 6 h after its administration. The decrease in the number of mitoses in the hepatocytes after 12 h was due to the action of both substances on DNA synthesis and not to a disturbance of the entry of the cells into mitosis. Stimulation of protein synthesis was detected by autoradiographic and biochemical methods following the action of hydrocortisone and ethimizole at the maximum of inhibition of mitosis.

KEY WORDS: *hydrocortisone; ethimizole; proliferative activity; protein synthesis.*

Glucocorticoids are known to inhibit tissue proliferation, including in the epithelium of the tongue [3] and of the intact and regenerating liver [2, 4, 8-11], considerably in rats. However, in most investigations no account was taken of the fact that the chemical structure of hydrocortisone and cortisone does not correspond to that of corticosterone, the principal corticosteroid produced by the rat adrenals [6], and that administration of exogenous hormones depresses the output of endogenous corticosteroids by the negative feedback principle [7]. It was therefore decided to make a comparative study of the action of hydrocortisone and ethimizole* (a substance which stimulates the pituitary—adrenal system 1 h after administration [5]) on proliferative activity of the epithelial cells of the tongue and liver.

Besides the marked antimitotic action of the corticosteroids, their other action, stimulation of protein synthesis [12], is well known. No reference could be found in the acces-

*Ethimizole is an original preparation obtained at the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR (Leningrad) under the direction of Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov and Corresponding Member of the Academy of Medical Sciences of the USSR N. N. Khromov-Borisov. (It is an alkylamide of inidazoledicarboxylic acid — Translator.)

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