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POLYPLOIDIZATION OF HEPATOCYTES IN RATS FOLLOWING EXPOSURE TO CCl_4 UNDER DIFFERENT CONDITIONS

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A karyometric study was made of hepatocyte polyploidization in albino rats during continuous and interrupted poisoning with CCl_4. Polyploidization, as a response to exposure to chlorinated hydrocarbons, was shown to depend on the toxicity of the poison, which depends on the number of chlorine atoms in the molecule, and on the character of exposure (mode of administration, dose, regime).

KEY WORDS: karyometry; ploidy of hepatocytes; chlorinated hydrocarbons; CCl_4.

Studies of the effects of various chlorinated hydrocarbons (di- and trichloropropanes – DCP and TCP) [1, 2] and investigations of the action of CCl_4 on the rat liver [10] have shown that the response of the hepatocytes depends on the concentration of the harmful agent and the duration of exposure to it. Since it is assumed that the number of DNA-synthesizing hepatocytes depends on the rhythm of exposure to CCl_4 [8], the effect of this factor on polyploidization under the conditions of action of the hepatotoxin on rats must also be taken into account. The object of the investigation described below was to determine how polyploidization of the hepatocytes depends on the character of entry of CCl_4 into the body (continuous or interrupted exposure). Both the total (including intervals between exposures in the case of interrupted administration) period of exposure and the duration of exposure proper were estimated.

EXPERIMENTAL METHOD

The DNA content in the hepatocytes was studied in control (20) and experimental (51) noninbred male albino rats made to inhale CCl_4 continuously or interruptedly, and divided into 13 groups depending on the experimental conditions. In airtight chambers (capacity 200 liters) containing the experimental animals constant concentrations of CCl_4 (5, 100, and 300 mg/m^3) were maintained. Interrupted exposure was studied by two programs: inhalation of CCl_4 in a concentration of 300 mg/m^3 for 4 h with intervals of 20 and 8 h between exposures. The data on the length of exposure in the case of continuous and interrupted programs are given in Table 1. Rats kept in airtight chambers ventilated with atmospheric air for the same length of time as the experimental animals served as the control.

Films of hepatocytes for karyometric analysis were obtained by the method described previously [1], fixed in Carnoy's mixture, and stained with hematoxylin-eosin and gallocyanin. Karyometric analysis for each animal was based on determination of 500-2000 cells.

EXPERIMENTAL RESULTS

During constant inhalation of CCl_4 for 1, 3, and 7 days changes in polyploidization began after poisoning for 24 h (group 2). An increase in the length of exposure led to aggravation of the changes. After exposure for 3 days (group 3) the number of binuclear cells with diploid nuclei was significantly reduced ($P < 0.02$) and the number of tetraploid hepatocytes was increased ($P < 0.001$). After exposure for 7 days (group 4) the number of binuclear tetraploid hepatocytes was reduced even more ($P < 0.001$) whereas the number of mononuclear

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TABLE 1. Relative Numbers of Hepatocytes in Different Ploidy Classes in Control and Experimental Rats after Continuous and Interrupted Inhalation of CCl_4

Group	Experimental conditions	CCl_4 concentration, mg/m³, or program of administration	No. of rats	Relative percentages of hepatocytes with different ploidy (M ± m)						
				2n	2n+2n	4n	4n+4n	8n	8n+8n	16 n
1	Control		20	6,4±0,92	16,4±1,69	66,9±1,95	9,0±0,64	1,2±0,33	0,1±0,01	Single
	Continuous exposure									
2	1 day	300	4	3,7±0,53	12,3±1,88	67,7±3,23	13,0±2,87	2,7±1,26	0,6±0,34	»
3	3 »	300	4	1,4±0,30	9,1±2,50	77,3±1,54	10,0±2,11	1,9±0,51	0,3±0,11	»
4	7 »	300	4	3,8±1,82	7,2±1,91	66,2±1,68	12,8±2,67	8,5±1,88	1,3±0,56	0,2±0,06
5	9 »	100	5	2,7±0,90	7,3±1,90	71,6±2,96	10,5±0,62	6,6±1,16	1,3±0,49	Single
6	19 »	100	4	1,9±0,59	5,9±2,36	71,1±1,38	9,5±0,76	10,4±2,92	1,2±0,48	»
7	3. »	100	6	4,9±0,76	3,6±0,76	75,5±2,62	7,0±1,68	7,6±2,46	0,9±0,14	0,5±0,14
	Interrupted exposure:									
8	2 days (8 h)	4 h CCl_4, 20 h interrupt.	4	3,3±0,48	14,0±3,40	71,0±3,10	9,9±0,50	1,6±0,70	0,2±0,03	Single
9	6 days (24 h)	The same	4	1,9±0,80	11,4±2,60	75,0±2,55	8,3±1,40	2,9±0,81	0,5±0,03	»
10	1 day (8 h)	4 h CCl_4, 8 h interrupt.	4	3,8±1,21	17,3±4,10	66,3±4,30	9,8±1,20	2,8±1,40	Single	»
11	3 days (24 h)	The same	4	3,8±1,60	10,2±2,07	73,8±2,90	10,2±2,10	1,9±0,71	0,1±0,07	»
12	6 days (48 h)	» »	4	2,3±0,50	9,6±2,60	74,0±2,30	12,1±2,80	1,7±0,50	0,3±0,01	»
13	9 days (72 h)	» »	4	3,0±0,60	8,4±2,20	75,8±2,30	9,5±1,50	3,0±0,87	0,2±0,10	0,1

Legend. Duration of actual exposure to CCl_4 during interrupted administration shown in parentheses.

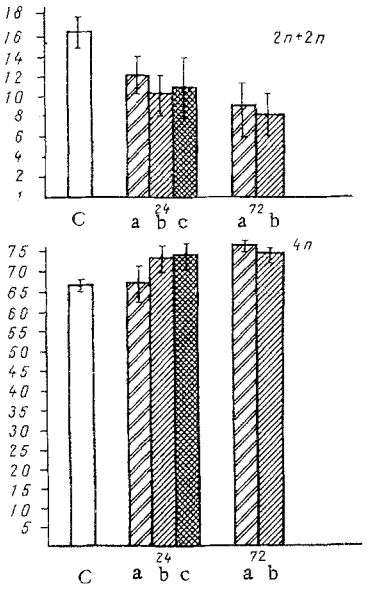

Fig. 1. Relative numbers of tetraploid (mono- and binuclear) hepatocytes following exposure to CCl_4 under different conditions (300 mg/m^3). Abscissa, actual exposure to CCl_4 (in h); ordinate, % of hepatocytes of different ploidy values. C) Control; a) continuous exposure; b) exposure for 4 h, interruption for 8 h; c) exposure for 4 h, interruption for 20 h.

($P > 0.001$) and binuclear cells ($P < 0.02$) with octaploid nuclei was increased. A significant number of nuclei with a ploidy of 16n appeared.

Morphological investigation of the liver of the animals showed that after exposure to CCl_4 for only 24 h fatty degeneration of the liver, with the appearance of tiny droplets, had developed in the zone of cells around the central veins. By the end of the 3rd day of exposure, besides fatty degeneration, vacuolar degeneration also had begun to develop in many hepatocytes in the centrilobular regions. After 7 days of poisoning the depth and extent of the degenerative changes were increased. Signs of necrobiosis were intensified. Disorganization of the trabecular structure of the organ was observed. The zone of fatty and vacuolar degeneration extended almost throughout the lobule.

A dynamic investigation after exposure to CCl_4 in a concentration of 100 mg/m^3 for 9, 19, and 33 days (groups 5, 6, and 7) also showed an increase in the degree of polyploidization of hepatocytes in the experimental groups of rats. After exposure to CCl_4 for 9 and 19 days the number of tetraploid hepatocytes was significantly reduced ($P < 0.001$) and the number of mononuclear ($P > 0.001$ for 9 days' and $P > 0.002$ for 19 days' exposure) and binuclear ($P < 0.02$) cells with octaploid nuclei was increased. After poisoning for 33 days, besides a significant decrease in the number of binuclear cells with diploid nuclei ($P < 0.001$) the number not only of mononuclear ($P < 0.01$) and binuclear ($P > 0.001$) hepatocytes with octaploid nuclei, but also the number of mononuclear tetraploid cells ($P < 0.01$) and of hepatocytes with a ploidy of 16n were increased. Centrilobular necrosis was observed in the hepatic lobules.

The ratio between the numbers of hepatocytes in the different ploidy classes after exposure for 9 and 19 days to 100 mg/m^3 CCl_4 was similar to that following exposure to CCl_4 in a concentration of 300 mg/m^3 for shorter periods, namely 3-7 days (Table 1, Fig. 1). Consequently CCl_4, like other compounds from the chlorinated hydrocarbon group, such as DCP and TCP [2], causes polyploidization of the liver in accordance with a concentration versus time rule. Polyploidization following chronic inhalation of DCP, TCP, and CCl_4 does not lead to complete disappearance of binuclear cells, as is observed after exposure to sublethal doses of CCl_4 [7].

If the liver was investigated after exposure to CCl_4 for 4 h followed by an interruption of 20 h, the character of distribution of the hepatocytes among ploidy classes 48 h later was still unchanged, although signs of fatty and vacuolar degeneration could be seen in the liver. If exposure by this program was prolonged

to 6 days (group 9) the pathological changes in the liver progressed. The number of tetraploid mononuclear hepatocytes was increased ($P < 0.01$).

If the intervals between exposures were shortened from 20 to 8 h (groups 10, 11, 12, and 13) morphological changes and polyploidization developed after shorter periods of exposure. For instance, a significant increase in the number of tetraploid mononuclear hepatocytes took place on the 6th and 9th days of exposure ($P < 0.02$); the number of binuclear cells under these circumstances was reduced on the 3rd, 6th ($P < 0.02$), and 9th ($P < 0.002$) days of exposure. If the interval between exposures was reduced, the degree of polyploidization of the liver increased, so that the effect observed on the 6th day of exposure on the 4–20 h program corresponded to that on the 3rd day on the 4–8 h program (Table 1, Fig. 1).

Comparison of the reaction of the hepatocytes to the different conditions of exposure, allowing for the total period and the actual time of exposure to CCl_4 (Fig. 1) revealed no significant difference both after continuous exposure to 300 mg/m^3 CCl_4 for 24 h (group 2) and after poisoning for 24 h on the 4–20 h program (group 9) and 4–8 h program (group 11), and also after continuous poisoning for 72 h (group 3) and interrupted poisoning on the 4–8 h program for the same period (group 13). Consequently, the development of hepatocyte polyploidization depends not only on the conditions of exposure, but also on the total dose of the hepatotropic poison received during the actual exposure time. Dose dependence may be disturbed in the case of minimal and maximal durations of exposure. In fact, since polyploidization following exposure to hepatotoxins arises in response to structural or functional changes [3, 4, 6], a definite length of exposure is essential for its development (under our experimental conditions 24 h with a dose of CCl_4 of 300 mg/m^3). Meanwhile, after short exposures to sublethal doses or chronic exposure to low concentrations polyploidization increased only up to a certain level. The reason is that during the development of liver damage the organ may become resistant to the effects both of subsequent doses of CCl_4 and of other toxic substances, which can be attributed to their effect on the enzymes concerned in metabolism of foreign substances in the hepatocytes [5, 9, 11, 12].

Comparison of our own data with those of other workers on the action of CCl_4 on the liver with the results of a study of certain chlorinated hydrocarbons (DCP and TCP [2]) indicates that polyploidization of the liver, as a manifestation of a compensatory and repair reaction, depends on the degree of hepatotoxicity of the substance and the character of exposure, including the mode of administration, dose (i.e., concentration of the substance and the actual time of exposure), and also the program of administration (once only, chronic, continuous, or interrupted with different intervals between exposures). Dependence of the degree of polyploidization on toxicity of chlorinated hydrocarbons, demonstrated by the writers in the case of DCP and TCP and associated with the number of chlorine atoms in the molecule, is valid for CCl_4 also. For instance, whereas exposure to DCP for 2 weeks in a concentration of 100 mg/m^3 [2] did not cause polyploidization of the tissue, exposure to CCl_4 in the same concentration led to the onset of polyploidization as early as on the 9th day (group 7, Table 1). The differences in the intensity of polyploidization discovered by different workers under different experimental conditions can thus be explained.

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