EFFECT OF 1,2,3-TRICHLOROPROPANE ON THE PLOIDY OF RAT LIVER CELLS

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Cytophotometric and karyometric studies of ploidy of albino rat hepatocytes showed that after inhalation of 1,2,3-trichloropropane in a concentration of 0.8 mg/liter the number of mononuclear cells with a nucleus of higher ploidy was increased and the number of binuclear cells was reduced.

KEY WORDS: ploidy of hepatocytes; 1,2,3-trichloropropane; binuclear cells.

The ploidy of the hepatocytes can be changed by several procedures injuring the liver. In particular, an increase in the ploidy of the nuclei can be observed after partial hepatectomy on the normal liver [6, 9] and in the liver when pathologically changed by the action of carbon tetrachloride [8] and potassium permanganate [7]. Polyploidization of the hepatocytes developed in rats treated with thioacetamide [13], carbon tetrachloride [17], and phenobarbital [18] and also in domestic cats after denervation of the liver [4]. Meanwhile, certain other procedures may leave the polidy of the liver cells unchanged or reduce it [5, 10].

The study of factors capable of changing cell ploidy is promising in connection with the discovery of ways of influencing regeneration in the liver.

The response of the liver to poisoning has been studied in the case of only one of the halogen-substituted hydrocarbons, CCl_4. This paper describes an investigation of another compound of this class, namely 1,2,3-trichloropropane (TCP), which is used in industry in the manufacture of certain synthetic rubbers and, because of its action, is classed among the general anesthetics. After an exposure of 2 h to TCP in a concentration of 7 mg/liter in the air, narcosis develops in 50% of mice. In a subacute experiment as a result of inhalation the animals were retarded in weight, their conditioned-reflex activity was disturbed, and degenerative changes appeared in some parenchymatous organs, notably in the liver [11].

Under the conditions of the present experiments TCP had no appreciable harmful action on the liver.

The object of this investigation was to examine the effect of TCP on the ploidy of liver cells.

EXPERIMENTAL

Male albino rats inhaled TCP for 1 week. The concentration of TCP in the chamber was 0.8 mg/liter. Another group of rats, not exposed to the action of this compound, acted as the control.

Films of hepatocytes were obtained by the method of Jacob and Bhargava [14]. The air-dried films were fixed for 30 min in Carnoy's mixture and stained by Feulgen's method with hydrolysis for 13 min in 1 N hydrochloric acid at 60°C. The optical density of the DNA-fuchsin was determined with a single-beam probe cytophotometer with square probe of regularly changing size. Measurements were made in the 540 nm region. The DNA content in the nucleus was calculated as the product of optical density and area of the probe and expressed in ploidy units. Histograms were plotted for two of the control and three of the experimental animals on the basis of counts of 100-170 nuclei from each animal.

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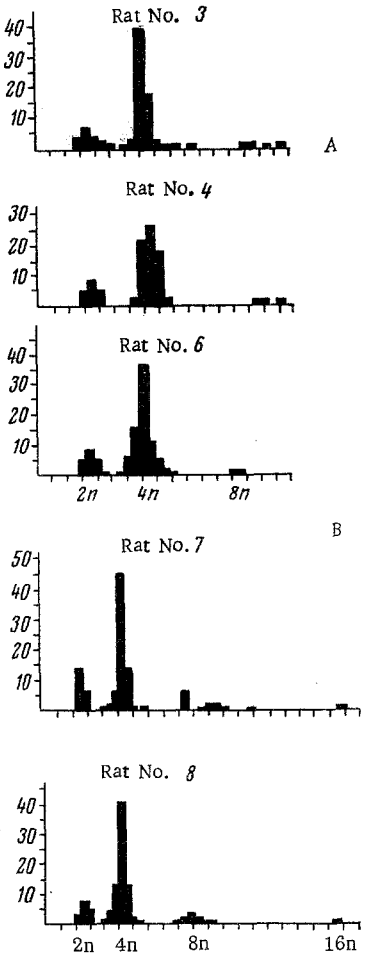

Fig. 1. Distribution of hepatocyte nuclei by ploidy classes (N) in control (A) and experimental (B) rats. Abscissa, DNA content (in ploidy units); ordinate, number of nuclei in each interval (in %).

To identify the hepatocytes when the relative percentages of mono- and binuclear cells of different ploidy the films were stained with gallocyanin by Einarson's method. In that case about 14,000 cells were counted. The ploidy index (P) was calculated by the equation [6]:

$$P = \frac{\Sigma_K \cdot P_K}{100};$$

where P_k is the percentage of cells with a particular ploidy (k).

RESULTS

The cytophotometric investigation showed that the nuclei in the liver of both intact and experimental rats were grouped into clearly defined principle classes of ploidy (Fig. 1).

However, the histograms give an idea only of the ploidy of the nuclei. The ratio between the numbers of mononuclear and binuclear cells in the population is no less important. For hepatocyte nuclei, correlation has been shown between the size of the nuclei and their content of DNA in a number of cytophotometric investigations [1, 13, 15]. This correlation and the absence of hepatocytes of intermediate ploidy make it possible to study the distribution of mono- and binuclear hepatocytes of different ploidy classes by using parameters such as the size of the nuclei and their number in the cell for this purpose. Investigations of the liver of normal rats aged 7-8 months by this method (Table 1) showed no difference as regards the ratio between the numbers of hepatocytes of different ploidy from the mean values given by Ryabinina and Benyush for the liver of Wistar rats [6].

Statistical analysis of the results of the distribution for two age groups in the group of experimental animals inhaling TCP (subgroup 1: rats aged 7-11 months Nos. 6, 7, and 8; subgroup 2: rats aged 2 weeks, Nos. 9, 10) showed that the differences within each ploidy class were not significant; this meant that the experimental and control groups of animals could be compared by taking the mean values.

The ploidy index calculated from these data, characterizing the mean ploidy of the hepatocytes, was the same in the experimental animals (P = 4.48) as in the controls (P = 4.49). These indices corresponded to the analogous indices for the regenerating and normal rat liver [6].

Meanwhile, comparison of the distribution of the hepatocytes by ploidy classes showed a significant increase in the number of mononuclear polyploid hepatocytes with a decrease in the percentage of binuclear cells. As Table 1 shows, as a result of the inhalation of TCP the percentage of mononuclear tetraploid ($P < 0.001$) and octaploid ($P < 0.005$) cells increased and cells with a ploidy of 16 n appeared. The percentage of mononuclear diploid cells was unchanged. There was a parallel decrease in the percentage of binuclear cells ($P < 0.01$ for tetraploid and $P < 0.05$ for octaploid). A decrease in the number of binuclear cells in the liver during polyploidization of the nuclei has also been observed after the action of potassium permanganate [7] and also in the early period after partial hepatectomy [6]. Presumably the increase in the number of mononuclear polyploid cells in the liver observed in the present investigation after inhalation of TCP by rats is the result of a completed wave of mitosis. In this case, just as in normal ontogeny of the liver [9, 12, 15], mitosis of the binuclear cell most likely leads to the appearance of two mononuclear cells of the same ploidy (2 n × 2 → mitosis → 4 nM + 4 n).

TABLE 1. Relative Percentage of Cells of Different Ploidy in the Liver of Intact and Experimental Rats

Group of animals	Weight of rats (in g)	Number of hepatocytes of different ploidy (in %)							Number of cells counted
		2n	2n+2n	4n	4n+4n	8n	8n+8n	16n	
Control animals									
№ 1	265	3,2	14,7	72,5	7,4	1,8	0,4	—	2000
№ 2	240	2,9	14,7	73,4	7,3	1,7	—	—	1000
№ 3	260	2,1	12,8	75,1	5,7	4,0	0,3	—	2000
№ 4	260	1,2	5,5	74,1	15,2	3,9	0,1	—	1000
№ 5	270	2,3	9,8	70,1	14,8	2,6	0,4	—	1000
								—	
$M \pm m$	259	2,4±0,4	11,5±1,7	73,0±0,9	10,1±2,0	2,8±0,5	0,2±0,1	—	
Experim. animals (inhaling TCP)									
№ 6	240	3,1	2,7	82,6	3,9	7,2	0,3	0,2	2000
№ 7	270	3,4	0,9	86,3	1,4	7,9	0,1	—	1328
№ 8	305	1,6	5,8	79,7	6,6	6,0	0,3	—	1000
№ 9	160	2,7	1,7	85,9	2,3	6,9	0,4	0,1	1000
№ 10	160	2,2	1,7	77,9	5,6	11,3	0,6	0,7	1000
$M \pm m$	227	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	

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