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MORPHOMETRIC ANALYSIS OF THE TOXIC ACTION OF ETHANOL OF INTRAPHASE NUCLEAR CHROMATIN OF RAT HEPATOCYTES

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Alcohol-induced liver damage occupies an important place in the organic pathology of patients with alcoholism, but many biological aspects of this urgent medical problem have received little study [11, 13]. This applies in particular to the mechanism of action of ethanol at levels of structural and functional organization of the hepatocytes whose study has become possible only in recent years thanks to the appearance of computer-based high-resolution scanning photometric systems [2]. Among objects at this level, the most interesting in our opinion is the supramolecular organization of the interphase nuclear chromatin (INC) of the hepatocytes, structural modification of which is regarded as an evolutionarily consolidated method of genome regulation [12], damage to which is linked with the negative effect of pathogenic factors in the normal course of the life cycle of the cell [10]. However, instruments used for this purpose permit the accurate recording only of a set of quantitative parameters of structure of INC, whereas the degree to which the latter reflect relationships that exist objectively, and in fact determine the behavior of this complex and little-studied system, although hidden from direct observation, under conditions of exposure to ethanol is still unknown.

TABLE 1. Effect of Ethanol on DNA and RNA Content and Structural Parameters of Interphase Nuclear Chromatin of Rat Hepatocytes (in % of control, taken as 100%; M ± m, n = 10)

Morphometric parameter	Dose of ethanol, g/kg			
	4	6	8	10
DNA (nucleus)	110±1,6	81±1,8}	66±4,4	52±8,4
RNA (cytoplasm)	109±1,4	87±3,7	73±2,1	63±2,1
Diameter of chromatin granules (Ag)	118±6,8	116±5,4	118±5,5	124±6,9
Steepness of optical profile of granule (∇)	92±2,8	131±3,4	162±3,6	192±5,1
Integral optical density (mass) of granule (DgAg)	124±8,3	85±6,2	100±7,1*	118±6,9
Optical density of granule (Dg)	102±3,6*	71±2,4	83±3,4	100±3,2*
Height of granule (Hg)	120±7,2	86±5,4	89±6,3*	78±7,3
Distance between granules (M)	124±6,8	115±6,2*	134±7,1	121±7,0
Contrast of nucleus (K)	107±3,1*	116±4,0	95±2,3*	62±4,2
Optical density of pale regions of nucleus (D min)	93±2,1	63±3,7	82±3,3	105±2,3*

Legend. Asterisk indicates that differences are not significant; in all other cases $p \leq 0.05$ compared with control.

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TABLE 2. Matrix of Ceofficients of Correlation between Morphometric Parameters of Rat Hepatocyte Nuclei

	DNA	RNA	Ag	▽	DgAg	Dg	Hg	M	K	Dmin
DNA	1,0									
RNA	0,99	1,0								
Ag	0,65	0,65	1,0							
▽	—0,99	—0,99	—0,70	1,0						
Dg Ag	0,08	0,07	—0,44	0,05	1,0					
Dg	0,48	0,46	—0,17	—0,40	0,77	1,0				
Hg	0,91	0,91	0,57	—0,86	0,40	0,63	1,0			
M	—0,43	—0,43	0,03	0,48	0,30	—0,24	—0,09	1,0		
K	0,70	0,72	0,92	—0,76	—0,53	—0,26	0,52	—0,19	1,0	
D min	—0,01	—0,03	—0,61	0,09	0,75	0,88 0,15	—0,19	—0,71		1,0

In connection with the facts described above, it was decided to use a different but essential approach to the solution of this problem, namely factor analysis of multidimensional systems [7].

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 g. Daily for 1 month animals of the control group (n = 10) received 2 ml of water by gastric tube. Rats of the experimental groups (n = 40) were given ethanol under similar conditions in doses of 4, 6, 8, and 10 g/kg body weight (0.33-0.8 LD_{50}) in the form of a 50% solution. At the end of the experiment the rats were decapitated and the liver removed and fixed in Carnoy's fluid. The DNA content in the nucleus and the RNA content in the cytoplasm of the hepatocytes were determined quantitatively on paired (control—experiment) paraffin sections (5-7 μ), stained with fuchsin—sulfurous acid by Feulgen's method and with gallocyanin and chrome alum by Einarson's method [9], and with the use of an SMP-01 microspectrophotometer ("Opton," West Germany). The structure of INC of the hepatocytes was analyzed on an SMP-05 scanning microspectrophotometer ("Opton" using an algorithm of structural analysis developed by the authors and programmed for the PDP 12/20 computer (DEC, USA). The numerical results were analyzed by computer by the chief component method [1].

EXPERIMENTAL RESULTS

Ethanol in a dose of 4 g/kg increased, whereas in doses from 6 to 10 g/kg it reduced, the DNA and RNA content, probably by inhibiting their synthesis [14], and it also led to significant structural disturbances of INC (Table 1). Factor analysis showed that the strongest positive correlation was present between DNA, RNA, Hg, and K, and also DgAg, Dg, and Dmin (Table 2), confirming data on correlation between DNA and RNA synthesis and chromatin structure [8]. Strongest negative correlation was observed between DNA, RNA, and ▽, as the writers showed previously [4]. It wil be clear from Table 3 that the first two chief components (CC) explain about 86% of the total composition of intrinsic factors. Positive correlation of the first CC with the DNA and RNA content is understandable: the higher the functional activity of the cell, the higher the levels of DNA and RNA synthesis [10]. With an increase in the load on the cell, the contrast of its nucleus increases, and with it the euchromatin fraction, and

TABLE 3. Matrix of Coefficients of Correlation of CC with Morphometric Parameters of Rat Hepatocyte Nuclei

Parameter	First CC	Second CC
DNA	0,98	—0,18
RNA	0,98	0,16
Ag	0,78	—0,50
▽	—0,99	—0,08
DgAg	—0,08	0,89
Dg	0,33	0,93
Hg	0,87	0,38
M	—0,38	—0,11
K	0,82	—0,57
D min	—0,17	0,95
Dispersion (informativeness), %	52,4	33,5

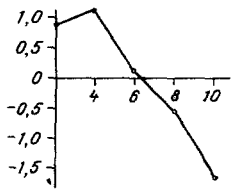

Fig. 1. Index of evaluation of the morphological state of the hepatocytes in rats with chronic alcohol poisoning. Abscissa, dose of ethanol (in g/kg/day); ordinate, value of index (in conventional units).

this is objectively confirmed by the positive correlation of the first CC with the parameters Hg and K. On exhaustion of the compensatory powers of the hepatocytes, an increase takes place in the gradient parameter ∇ [3], hence its negative correlation with the first CC. A similar examination of the character of correlation of the first CC with all the features allows it to be confidently interpreted as a factor of abundance of the compensatory-adaptive reactions of the cells. The second CC correlated positively with DgAg, Dg, and Dmin and negatively with Ag and K, evidence of an increase in the heterochromatin fraction, an increase in the optical density of the pale regions, and a reduction in contrast of the nucleus. These changes correspond to the picture of hyperchromic pycnotic nuclei, characteristic of cells completing their life cycle by apoptosis, so that the second CC can be interpreted as a factor of the harmful (toxic) action of ethanol.

As an integral (in the form of a single number) characteristic of the state of the hepatocytes, a special index was calculated with the aid of an inverse factor model, by the equation:

$$f = \frac{1}{5.2}(0.98y1 + 0.98y2 + 0.73y3 - 0.99y4 - 0.08y5 + 0.33y6 + 0.87y7 - 0.38y8 + 0.82y9 - 0.17y10),$$

where y_i is the normalized and centered value of the corresponding parameter. As Fig. 1 shows, with a dose of 4 g/kg there was an increase, but with an increase in the dose of ethanol, a progressive decrease in the value of the index, in agreement with our data on exhaustion of the compensatory and adaptive powers of the liver and death of some of the animals after a dose of 8 g/kg and, in particular, of 10 k/kg of ethanol [6].

On the basis of these results INC of the hepatocytes can thus be regarded both as the possible first target for the toxic action of ethanol, and as the initial step in the formation of the whole spectrum of protective and compensatory reactions maintaining the homeostasis of these cells (and through them, to a large extent, the reactivity of the body as a whole) under the conditions of alcohol intoxication. The complex structural modification of INC observed under these circumstances can be reduced basically to interaction between two simultaneously occurring antigonistic processes, namely the injurious action of ethanol and reparative regeneration of the hepatocytes. The most probable explanation of this, at first glance, contradictory conclusion may be the concept of spatial separation of these processes at the hepatocyte population level, in which the ratio between the number of cells damaged by ethanol and the number of cells in a state of active reparative regeneration undergoes a regular change depending on the dose of ethanol. This assumption is in agreement with the known views on the "mosaic" pattern of damage to the hepatocytes by the action of various pathogenic factors [10] and it can be regarded as a special case of manifestation of the general biological rule of intermittent activity of functioning structures [5].

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APOPTOSIS AND RENEWAL OF ENTEROCYTES IN EXPERIMENTAL ATROPHY OF THE SMALL INTESTINAL MUCOSA

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The steady state of renewing tissue is maintained by equilibrium between the formation of new cells and loss of cells entering the system. Cells completing their life cycle may not only be cast off, but may also undergo self-destruction in situ. This process of "programmed cell death" [10], called apoptosis to distinguish it from necrosis, has been ascribed in recent years an important role in the regulation of the size of renewing tissues, in response to the use of cytotoxic agents and hormones [2, 5, 10]. Apoptosis has been described in normal developing tissues, in embryogenesis, metamorphosis, and endocrine-dependent tissue atrophy, in the majority of growing tumors, during irradiation with small doses, and during the action of radiomimetic agents [5, 8, 9, 11, 12]. The rapid development of atrophy when the blood supply is disturbed [13] and when stem cells are damaged by preparations disturbing DNA synthesis [4, 5], and also during involution of hyperplastic organs [7], is explained by apoptosis.

These "acute" atrophies can also undergo rapid regression after removal of the noxious agents, as a result of intensified cell division.

EXPERIMENTAL METHOD

The aim of this investigation was to assess the link between production and death of cells by apoptosis in "acute" atrophy of the mucosa caused by the S phase-specific agent hydroxyurea (HU). Female (CBA × C57Bl)F_1 mice weighing 20-22 g were used. The animals were irradiated in a dose of 200 rads 24 h before the beginning of the experiments to stimulate their stem cells [3]. Next they were given six intraperitoneal injections, each of 0.25 g/kg of HU in physiological saline. Depending on the intervals between injections the animals were divided into four groups: 1) interval 7 h; 2) 12 h; 3) 16.5 h; 4) 19 h. The mice were killed 6, 30, 78, and 126 h after the last injection of HU. The animals received an intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 28.5 Ci/mmole) 1 h before sacrifice. Segments of the small intestine were separated at a distance of 3 cm from the pylorus and fixed in 10% formalin. The state of the architectonics of the mucosa was assessed in histological sections, the height of the villi, dimensions of the enterocytes of the crypts and villi, and the depth of the crypts were measured with an ocular micrometer, and the mitotic index (MI), labeling index (LI), and apoptosis index (AI) were calculated as percentage of the number of cells in the generative zone.

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