DYNAMICS OF FUNCTIONAL AND ULTRASTRUCTURAL CHANGES IN THE LIVER CELLS DURING THE DEVELOPMENT OF ALLYL ALCOHOL NECROSIS

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The dynamics of changes in the ultrastructure and functional state of the liver cells during the development of necrosis induced by oral administration of a 1% solution of allyl alcohol (1 ml/100 g body weight) was studied in experiments on male albino rats. At various times after poisoning of the animals (1, 2, 4, 8, and 48 h) the activity of organelle-specific enzymes (succinate dehydrogenase, glucose-6-phosphatase, acid phosphatase, acid deoxyribonuclease, acid ribonuclease) was studied in homogenates and subcellular fractions of the liver isolated on the differential ultracentrifuge. A parallel electron-microscopic examination of the liver tissue was carried out. The results show that allyl alcohol induces early changes in the structure of all types of organelles, leading to the formation of necrosis. Changes in the ultrastructure of the liver cells were preceded or accompanied by severe disturbances of the functional integrity of the organelles, manifested by changes in activity of the organelle-specific enzymes and in their solubilization into the cell hyaloplasm.

The object of this investigation was to study the dynamics of functional and ultrastructural changes in the subcellular structures of the liver in experimental poisoning with allyl alcohol, producing focal necrosis of the liver [1, 5-7].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 200-250 g receiving a 1% aqueous solution of allyl alcohol by mouth (through a tube) in a dose of 1 ml/100 g body weight. In the experiments of series I the rats were decapitated 1, 2, 4, and 8 h after administration of a single dose of the poison; activity of the enzymes was determined in liver homogenate and in the supernatant. The experiments of series II were carried out 24 h after 2 doses (at an interval of 24 h) of allyl alcohol; activity of the enzymes was determined in homogenates and subcellular fractions of the liver isolated on the differential ultracentrifuge [3].

To quantify the functional state of the organelles the ultramicromethods developed by Pokrovskii [3, 4] were used to study the activity of the following marker enzymes: succinate dehydrogenase (1.3.9.9), acid phosphatase (3.1.3.2), acid deoxyribonuclease (3.1.4.5), acid ribonuclease (2.7.7.16), and glucose-6-phosphatase (3.1.3.9).

Samples of liver tissue for electron-microscopic investigation were fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4, for 2 h. After rinsing they were postfixed in 1% osmium tetroxide solution and embedded in a mixture of p-butyl and p-methyl methacrylates or in Épon 812. Sections were cut on the LKB-8810 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in the FEM-6C electron microscope. Acid phosphatase was detected electron-histochemically by a modified Gomori's method.

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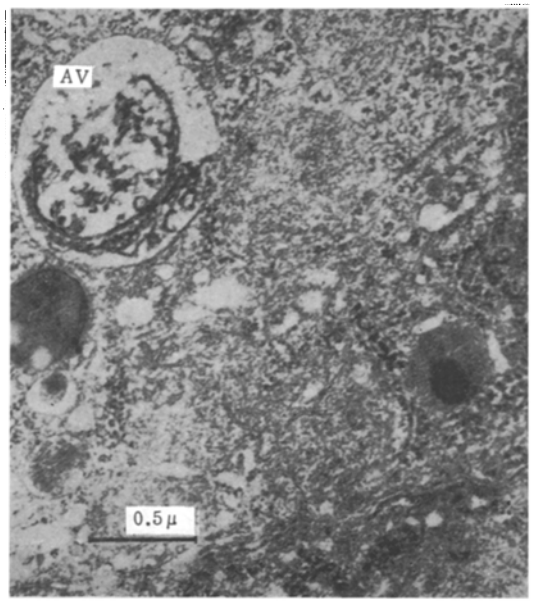
TABLE 1. Activity of Organelle-Specific Enzymes in the Liver During the Development of Allyl Alcohol Necrosis (percent of normal; M ± δ)

Duration of experiment (in h)	Material tested	No. of animals	Succinate dehydrogenase	Glucose-6-phosphatase	DNAase	RNAase	Acid phosphatase
1	Homogenate	10	92,4±4,6*	88,7±15,2	108,4±10,9	108,9±8,1	100,7±7,8
	Supernatant	10	0	0	13,2±2,6*	15,0±3,3*	—
			(0)	(0)	(3,8±2,0)	(6,8±2,6)	
2	Homogenate	10	64,8±15,2*	99,5±15,2	112,9±11,3	104,0±8,1	90,2±17,8
	Supernatant	10	3,6±2,7	0,9±0,9	12,8±3,4*	15,5±2,3*	—
4	Homogenate	10	77,5±5,1*	92,2±8,1	92,5±8,1	104,6±8,2	97,9±18,3
	Supernatant	10	2,9±2,8	5,2±4,2*	14,0±3,5*	15,1±6,6*	—
8	Homogenate	5	68,0±14,2*	92,7±12,8	94,8±10,1	97,7±10,3	110,9±1,8*
	Supernatant	5	8,5±14,3	6,6±2,5*	19,9±3,9*	19,9±4,3*	—
24 (after administration of 2 doses of allyl alcohol)	Homogenate	8	54,6±12,0*	63,1±10,4*	122,5±25,8	122,9±20,7*	132,7±17,9*
	Nuclei	8	—	—	140,2±53,9	130,9±25,4*	—
	Mitochondria	8	65,7±20,5*	—	109,4±33,7	93,4±20,7	—
	Lysosomes	8	73,0±27,0*	—	128,1±29,5	119,8±37,3	180,1±49,5*
	Microsomes	8	—	65,0±20,9*	—	—	—
	Supernatant	8	8,8±1,4*	7,8±3,8*	9,9,±8,2*	10,9±8,3*	9,4±5,6*

Note. Enzyme activity in supernatant expressed as a percentage of activity in homogenate.
Unsedimented enzyme activity under normal conditions shown in parentheses.
Changes significant relative to the control group ($P \leq 0.05$) marked by an asterisk.



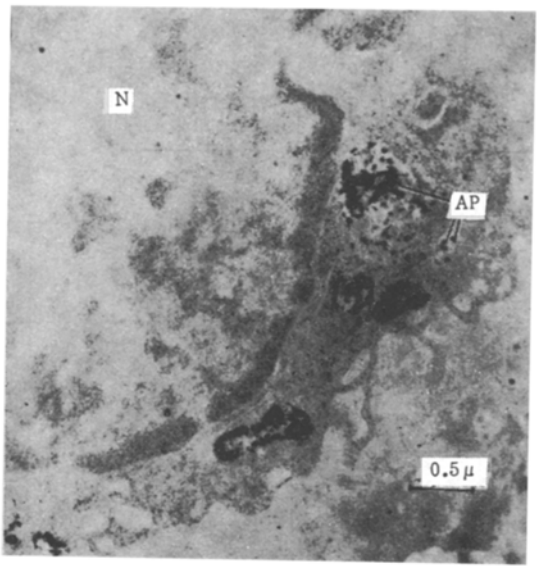


Fig. 1 Fig. 2

Fig. 1. Autophagous vacuole (AV) in cytoplasm of a hepatocyte from the rat liver 4 h after adminstration of allyl alcohol (47,200×).

Fig. 2. Part of a hepatocyte from a rat liver 1 h after receiving allyl alcohol. Acid phosphatase (AP) revealed electron-histochemically as granules of lead phosphate in lysosomes and in the cytoplasm outside them; the nucleus of a hepatocyte (N) can be seen (26,800×).

EXPERIMENTAL RESULTS

The electron-optical investigation of the liver tissue showed no evidence of necrosis in the ultrastructure of the cells during the first hour of action of allyl alcohol. Numerous mitochondria of different shapes were seen in the hepatocytes, some of them with a clearly defined osmiophilic matrix. The endoplasmic reticulum (ER) was indistinguishable from normal, and partial dilatation of the tubules was observed only in individual hepatocytes. The ultrastructure of the nuclei and nucleoli was not significantly changed. Few lysosomes were present in the hepatocytes and Kupffer cells.

The first signs of necrobiosis were observed 4 h after administration of the poison: single phagosomes and autophagous vacuoles of very large size appeared in the cytoplasm of the hepatocytes and Kupffer cells (Fig. 1). The matrix of the mitochondria varied in its electron density. Changes in the ultrastructure of individual nuclei were observed: margination of the nuclear material; the contents of some nuclei became gray and homogeneous.

Evidence of necrosis of all types of organelles of the liver cells was seen on the electron micrographs 8 h after the action of allyl alcohol. Margination of the nuclear material was observed in some hepatocyte nuclei while in others pycnosis or, frequently, karyolysis was seen. Under the influence of allyl alcohol, destruction of the mitochondria took place, and often only faint traces of their outer membrane could be seen. The ER was fragmented and swollen and formed twisted and spherical shapes. In the destroyed cytoplasm there was an increased number of large lipid granules and a few lysosomes. Because of destruction of the outer membrane of the hepatocytes some cells were indistinguishable. Infiltration of the liver cells with neutrophils was often observed, leading to enzymic breakdown of the necrotic substrate.

The study of the activity of the organelle-specific enzymes showed that the change in ultrastructure of the liver cells during the development of necrosis was preceded or accompanied by severe disturbances of the functional integrity of organelles of all types. Table 1 shows that 1 h after administration of the poison the activity of mitochondrial succinate dehydrogenase in the liver homogenate was significantly lowered, and after 2 h its mean value was 68% of normal. By this time the binding of the enzyme in the organelles was disturbed, as shown by an increase in its unsedimented activity. Changes in the functional state of the ER were no less severe. Judging from the glucose-6-phosphate activity the structural changes in the ergastoplasm were combined with inactivation of the enzyme and its liberation into the supernatant.

The action of allyl alcohol in producing necrosis is evidently linked with its ability to cause labilization of the lysosomal membranes. The results show that 1 h after poisoning of the animals there was a significant liberation of acid ribonuclease and deoxyribonuclease into the supernatant. The electron-histochemical study showed that some acid phosphatase lay outside the lysosomes where it was revealed as electron-dense granules of lead phosphate (Fig. 2). It is interesting to note that the degree of solubilization of the hydrolases remained about the same throughout the experiment. The results of the study of the activity of the lysosomal enzymes in the subcellular fractions of the liver show that allyl alcohol activates these enzymes within the lysosomes and also produces a redistribution of activity on account of an increase in activity in the nuclear fraction, with which the autophagous vacuoles and phagosomes, containing hydrolytic enzymes in the active state, are evidently sedimented.

Allyl alcohol thus induces early and severe changes in the enzyme patterns of the subcellular structures and, in accordance with Pokrovskii's observations [2], these must be regarded as evocative of necrosis.

The results of the present experiments suggest that the intimate mechanism of the necrotic action of allyl alcohol is connected with the early irreversible damage to the functional and morphological integrity of the organelles of the liver cells, primarily the mitochondria and lysosomes, as a result of which the cells die and foci of necrosis are formed in the liver.

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