SERUM AND LIVER ENZYME ACTIVITY AT VARIOUS PERIODS OF EXPERIMENTAL MECHANICAL JAUNDICE AND DEVELOPMENT OF BILIARY CIRRHOSIS OF THE LIVER

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Activity of alanine aminotransferase, aspartate aminotransferase, ornithine carbamoyltransferase, glutamine synthetase, and glutamine transferase in the blood serum and liver tissues at various times after division of the common bile duct was studied in experiments on rats with experimental mechanical jaundice. During the first few hours after the operation, activity of the enzymes in the serum rises, but that in the liver falls, in association with disturbance of the ultrastructure of the hepatocytes. In the late stages, activity of the enzymes in the liver falls still lower. Changes in enzyme activity in the blood serum are varied in direction.

The object of the investigation described below was to study enzyme activity at various times after the production of mechanical jaundice and during the development of biliary cirrhosis of the liver.

EXPERIMENTAL METHOD

Experiments were carried out on 340 male albino rats weighing 250-300 g. Mechanical jaundice was produced by division of the common bile duct between two ligatures at the point where it enters the duodenum. Operations on the animals were performed under hexobarbital anesthesia. Tests were made 1, 2, 4, 8, 24, and 48 h, 1 and 2 weeks, and 1 month after division of the common bile duct. Normal animals and rats undergoing laparotomy under the same conditions (anesthesia, etc.) as for division of the bile duct acted as the controls. Activity of alanine and aspartate aminotransferases (ALT and AST), sorbitol dehydrogenase (SD), and ornithine carbamoyltransferase (OCT) in the blood serum was determined by methods described previously [1, 3, 4, 7], glutamine transferase (GT) activity was determined by the method described by Trush [10], and glutamine synthetase (GS) activity was determined by Kennan's method [11]. These methods were adapted for determination of enzyme activity in liver tissue homogenates.

Activity of SD, ALT and AST, GT, and GS was expressed in micromoles, and activity of OCT in micrograms of substrate converted by 1 ml or 1 g liver tissue during incubation for 1 h at 37°.

To study the structure of the liver cells, besides general stains (hematoxylineosin, picrofuchsin by Van Gieson's method, silver impregnation of connective tissue by Gomori's method), investigations with the UÉMV-100V electron microscope were carried out 2, 4, 6, and 8 h after division of the ducts. The liver was fixed with glutaraldehyde and osmium tetroxide and shadow-cast with uranyl acetate by the usual methods. Sections were cut on Sjöstrand and LKB ultramicrotomes.

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TABLE 1. Activity of Enzymes in Liver of Various Groups of Animals

Group of animals	Statistical index	Activity of enzyme					
		ALT	AST	SD	OCT	GT	GS
Healthy	n	60	60	20	27	21	10
	M	1 728	1 505	84,9	5 505	3 450	125
	$\pm m$	55	61	1,9	116	86	6
Laparotomy	n	43	43	15	15	15	5
	M	1 757	1 538	85	5 400	3 400	128
	$\pm m$	69	48,6	2,2	150	164	3
Mechanical jaundice							
1 h	n	11	11	4	8	8	6
	M	1 621	1 419	78	5 225	3 272	118
	$\pm m$	80	85	3,2	64	124	7
	P	0,42	0,43	0,06	0,05	0,28	0,49
2 h	n	20	20	10	14	9	7
	M	1 516	1 368	78	4 970	2 712	114
	$\pm m$	78	42	2,2	95	168	11
	P	0,04	0,06	0,007	0,001	0,001	0,43
4 h	n	25	25	5	9	11	7
	M	1 450	1 308	74	4 705	2 716	97
	$\pm m$	59	62	2,4	120	167	17
	P	0,001	0,05	0,001	0,001	0,001	0,05
8 h	n	15	15	4	4	8	8
	M	1 454	1 038	64	4 670	2 536	95
	$\pm m$	64	96,5	2,9	80	175	9
	P	0,001	<0,001	<0,001	<0,001	<0,001	0,002
24 h	n	26	26	6	6	11	3
	M	1 283	1 035	66	4 500	1 884	90
	$\pm m$	46	82	4,8	23	109	5
	P	<0,001	<0,001	<0,001	<0,001	<0,001	0,001
48 h	n	12	12	5	7	4	
	M	1 180	936	66	4 400	1 770	—
	$\pm m$	78	88	2,4	23	125	
	P	<0,001	<0,001	<0,001	<0,001	<0,001	
1 week	n	11	11	8	10	4	4
	M	518	927	51,6	4 360	1 180	54
	$\pm m$	45	95	4,7	19	134	7
	P	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
2 weeks	n	10	10	4	6		
	M	461	907	45	3 500	—	—
	$\pm m$	19	78	3,6	31		
	P	<0,001	<0,001	<0,001	<0,001		
1 month	n	11	11	9	6	4	4
	M	354	682	39	3 000	970	38
	$\pm m$	67	95	2,4	32	185	4
	P	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001

Note. For units of activity in Tables 1 and 2, see section "Experimental Method."

EXPERIMENTAL RESULTS

During the first few hours after disturbance of the drainage of bile, enzyme activity both in the liver tissue and in the blood serum was altered. The direction of the changes in enzyme activity in the liver and serum differed, depending on the time of observation (Tables 1 and 2). The longer the period after division of the bile duct, the greater the decrease in activity of all investigated enzymes in the liver. As was shown previously [2], during prolonged interference with the drainage of bile, cirrhosis of the liver develops. To correspond to the marked cirrhotic changes observed morphologically in the liver tissue one month after division of the bile duct, activity of all the enzymes was lower. For instance, compared with the control

TABLE 2. Activity of Enzymes in Blood Serum of Various Groups of Animals

Group of animals	Statistical index	Activity of enzymes				
		ALT	AST	SD	OCT	GT
Healthy	n	60	60	20	27	21
	M	0,24	0,74	0,27	4,35	3,6
	$\pm m$	0,08	0,08	0,02	1,1	0,6
Laparotomy	n	43	43	15	15	15
	M	0,29	0,99	0,26	4	3,7
	$\pm m$	0,01	0,09	0,024	0,95	0,4
Mechanical jaundice						
1 h	n	11	11	4	4	8
	M	0,88	1,38	1,14	6,1	7,8
	$\pm m$	0,28	0,21	0,23	1,2	1,4
	P	0,04	0,007	0,002	0,2	0,01
2 h	n	20	20	10	10	9
	M	4,6	3,59	1,53	11,3	10
	$\pm m$	0,74	0,64	0,15	2,7	1,2
	P	<0,001	<0,001	<0,001	<0,05	<0,001
4 h	n	25	25	5	9	11
	M	4,89	4,44	3,16	16,5	16
	$\pm m$	0,58	0,33	0,14	1,1	2,6
	P	<0,001	<0,001	<0,001	<0,001	<0,001
8 h	n	15	15	4		8
	M	6,45	5,6	2,29	—	7
	$\pm m$	1,5	1,75	0,26		0,6
	P	<0,001	<0,001	<0,001		<0,01
1 day	n	26	26	6	6	4
	M	6,72	6,4	0,84	16,7	11
	$\pm m$	1,12	0,64	0,11	3,16	1,4
	P	<0,001	<0,001	<0,001	<0,001	<0,001
2 days	n	12	12	5	7	4
	M	4,66	4,7	0,56	14	10
	$\pm m$	1,8	1,1	0,06	1,8	0,7
	P	<0,001	<0,001	<0,05	<0,001	<0,001
1 week	n	11	11	8	10	4
	M	1,09	2,68	0,37	7,0	6
	$\pm m$	0,26	0,097	0,09	1,5	0,9
	P	<0,001	<0,001	>0,05	>0,05	0,05
2 weeks	n	10	10	4	6	
	M	0,73	3,17	0,27	9,0	—
	$\pm m$	0,2	1,2	0,08	1,4	
	P	0,02	<0,001	>0,05	0,02	
1 month	n	11	11	9	6	4
	M	0,13	3,5	0,28	9,6	5
	$\pm m$	0,06	1,0	0,04	0,97	1,3
	P	0,28	<0,001	>0,05	<0,05	0,38

(laparotomized animals), ALT activity was lower by 77%, AST by 51%, SD by 53%, OCT by 46%, GT by 68%, and GS by 68%. Changes in activity of the individual enzymes in the blood serum at different times after the operation varied. During the first few hours the activity of all the enzymes rose. After 24 h, however, SD activity was lower than at previous times, and it subsequently approached normal values. Activity of the other enzymes after 24 h showed a greater increase, but later it began to fall gradually, although one month after the operation AST and OCT activity was still considerably above normal. As during cirrhosis of the liver produced by prolonged administration of CCl_4 [5], the development of biliary cirrhosis was accompanied by a characteristic dynamics of the ratio between the aminotransferases: ALT activity fell more sharply than AST.

A study of the ultrastructure of the hepatocytes 2 h after division of the duct showed swelling of the mitochondria, a decrease in the number of cristae contained in them, and an increase in the number of electron-dense granules in different areas of the cytoplasm, most commonly in the perinuclear zone. With an increase in the period after operation, vacuolation of the endoplasmic reticulum increased in intensity around the nuclei, and some displacement and grouping of the mitochondria were observed (Fig. 1). Signs of a change in the energy balance of the cells continued to progress: more mitochondria with a reduced number of cristae were seen. Partial degranulation of the endoplasmic membranes took place, and most of the ribosomes were located in the cytoplasmic matrix, outside the membranes. The number of electron-dense vacuoles in the nucleus and at the periphery of the hepatocytes was increased. Osmiophilic bilirubin-containing particles were concentrated in the dilated cisterns of the endoplasmic reticulum (Fig. 2).

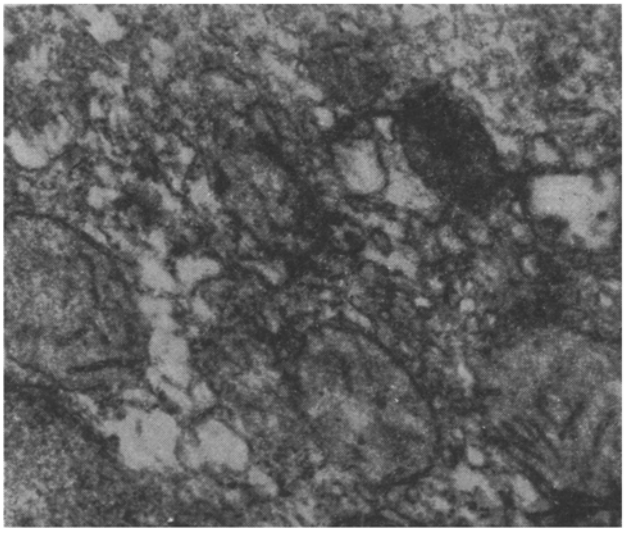

Fig. 1

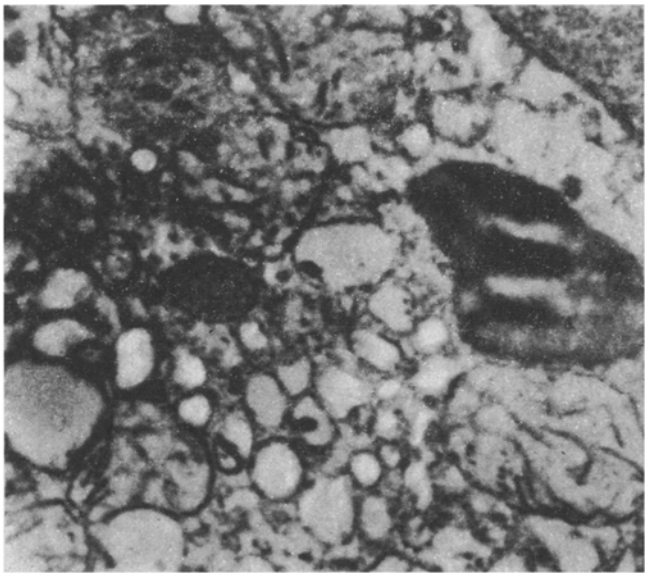

Fig. 2

Fig. 1. Hepatocyte, 4 h after beginning of experiment. Vacuolation and partial degranulation of endoplasmic reticulum. Slight swelling of mitochondria (20,000×).

Fig. 2. Hepatocyte, 8 h after beginning of experiment. Increased vacuolation of endoplasmic reticulum. Increase in number of granules of bile pigments in its vacuoles. Lipoid masses appearing in perinuclear zone of cytoplasm, around swollen and deformed mitochondria (25,000×).

Consequently, changes in enzyme activity during the first few hours of mechanical jaundice are evidently connected with metabolic disorders arising in the liver following interruption to the drainage of bile. The development of degenerative changes in the liver and of cirrhosis produces a characteristic dynamics of the changes in enzyme activity in the serum and liver tissue at different times after obstruction of the duct. Bile acids, which accumulate in large quantities during the first few hours [2] and produce morphological and enzymic changes [6, 8, 9] play an important role in the pathogenesis of these disturbances.

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