EFFECT OF A HEPATOTROPIC POISON (CCl_4) ON METABOLISM OF HOMOLOGOUS IMMUNE GLOBULINS IN A RECIPIENT

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The character of distribution of exogenous homologous antibodies differs considerably in rabbits poisoned with CCl_4 and in control animals. Administration of CCl_4 also delays the catabolism of homologous antibodies, because of a sharp decrease in the catabolic function of the liver relative to these proteins when damaged by a hepatotropic poison.

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The concentration of immune globulins, as of other plasma proteins, in the serum reflects equilibrium between the velocity of their synthesis, the velocity of catabolism, and the character of distribution in the organs and tissues [15]. In recent years much information has been obtained concerning the synthesis of the various plasma proteins and their distribution in the body. However, too little is still known concerning the site and mechanism of catabolism of the immune globulins.

Previous investigations yield results indicating that the liver cells play an active part in the catabolism of immune proteins [2]. Other workers have also described the important role of the liver in degradation of immune globulins and antibodies [8, 10].

The object of the present investigation was to study the distribution and catabolism of homologous antibodies after toxic injury to the liver caused by carbon tetrachloride (CCl_4).

EXPERIMENTAL METHOD

Experiments were carried out on 50 chinchilla rabbits weighing 2.5-3 kg, of which 25 were control. CCl_4 was injected subcutaneously in doses of 0.3 ml/kg body weight for two weeks [1]. Homologous immune serum was obtained from donor rabbits immunized and reimmunized with human serum γ-globulin. On the 7th day after reimmunization blood was taken from the rabbits and the absolute titer of antibodies determined by the method of Heidelberger and Kendall [12], with subsequent determination of protein in the precipitate by the method of Lowry and co-workers [13] and the antibody titer in the passive hemagglutination reaction (PHR) [6]. Recipient rabbits were immunized passively with serum containing 1000 μg antibody nitrogen/ml (titer of antibodies in PHR 1:262 144). Serum was injected into control rabbits and rabbits poisoned with CCl_4 intravenously in a dose of 1 ml/kg body weight. Blood was taken from the marginal vein of the ear of the rabbits 15 min and 6, 24, 48, and 120 h after injection of the homologous immune serum, and the animals were then perfused intravitally through the inferior vena cava with 0.15M sodium chloride solution under pentobarbital anesthesia (pentobarbital sodium in physiological saline was injected intraperitoneally in a dose of 40 mg/kg body weight). Immediately after the end of perfusion, the lungs, liver, kidneys, spleen, and popliteal lymph glands were removed from the rabbits. Extracts from the parenchymatous organs were obtained by the method of Kaplanskii and co-workers [3], and extracts from the lymph glands by the method of Demlin and co-workers [9]. The antibody concentration in the blood serum and organ extracts was determined by the PHR and the complement fixation reaction (CFR) at 50% hemolysis, the antibody titer in the latter case being expressed in units of fixed complement (CFU) [4]. Statistical analysis of the results was carried out by the use of Student's t criterion, differences being regarded as significant when $P < 0.05$.

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TABLE 1. Antibody Concentration in Blood Serum and Organs of Intact Rabbits and Rabbits Poisoned with CCl_4 at Various Times after Passive Immunization (mean data)

Organ or tissue	Group of animals	Time after passive immunization									
		15 min		6 h		24 h		48 h		120 h	
		PHR	CFR	PHR	CFR	PHR	CFR	PHR	CFR	PHR	CFR
Blood serum	Control	1:7 131	10,28	1:7 131	10,25	1:3 566	5,33	1:3 566	3,85	1: 676	2,69
	Experimental	1:8 192	10,48	1:8 192	10,46	0:8 192	10,45	1:6 209	7,66	1:1 782	3,39
	P	<0,5	>0,5	<0,5	<0,5	<0,001	<0,001	<0,05	<0,001	<0,01	<0,02
Lung	Control	1:5	1,65	1:32	2,57	1:24	1,57	1:14	1,30	1:8	1,24
	Experimental	1:4	1,24	1:18	2,26	1:9	1,19	1:5	1,00	0	0,38
	P	<0,5	<0,2	<0,1	<0,2	<0,1	<0,5	<0,001	<0,1	<0,001	<0,01
Liver	Control	1:6	1,56	1:147	5,74	1:64	3,53	1:74	3,50	1:7	1,42
	Experimental	1:8	0,75	1:18	1,37	1:18	1,23	1:9	1,09	1:9	0,46
	P	<0,2	<0,01	<0,001	<0,001	<0,001	<0,001	<0,01	<0,001	>0,5	<0,001
Kidney	Control	1:5	1,08	1:21	2,29	1:16	1,70	1:21	1,83	1:6	1,35
	Experimental	1:16	1,80	1:32	2,78	1:49	3,13	1:32	2,77	1:32	2,48
	P	<0,02	<0,01	<0,2	=0,2	<0,01	<0,001	<0,05	<0,001	<0,001	<0,001
Spleen	Control	1:21	1,79	1:147	5,48	1:74	2,84	1:56	2,93	1:9	1,01
	Experimental	1:28	1,97	1:223	6,21	0:64	3,20	1:32	3,08	1:28	2,70
	P	<0,5	<0,5	<0,1	<0,1	>0,5	<0,01	<0,5	>0,5	<0,1	<0,001
Lymph gland	Control	1:18	2,06	1:85	5,48	1:49	2,49	1:32	2,34	1:16	1,26
	Experimental	1:9	1,73	1:49	4,68	1:28	2,33	1:14	2,19	1:8	1,02
	P	<0,01	<0,1	<0,05	<0,01	<0,5	0,5	<0,001	<0,5	<0,1	<0,2

Note. At each time of the investigation 5 control rabbits and 5 poisoned with CCl_4 were used.

EXPERIMENTAL RESULTS

The results are given in Table 1.

The concentration of homologous antibodies in the serum of rabbits poisoned with CCl_4 remained at a constant level (titer 1:8192) for 24 h after passive immunization, whereas in the control animals during the same period of observation the antibody titer fell by 2.3 times to 1:3566 ($P < 0.001$). The blood antibody titer of the experimental animals remained higher than that of the controls 48 h (1:6209 and 1:3566 respectively; $P < 0.05$) and 120 h after injection of homologous serum (1:1782 and 1:676 respectively; $P < 0.01$). The dynamics of changes in concentration of immune globulins in the blood of rabbits poisoned with CCl_4 determined by the CFR at 50% hemolysis was similar (Table 1). Hence, in animals poisoned with CCl_4, marked delay in the elimination of antibodies from the blood stream was observed.

In most investigated organs from the experimental rabbits, just as in the controls, the antibody titer reached a maximum 6 h after passive immunization, but the character of distribution of antibodies in the body differed significantly in the poisoned rabbits from the controls. The organs of rabbits poisoned with CCl_4 can be arranged in the following descending order of antibody titer: spleen > lymph > kidney > lung > liver. In the control rabbits, on the other hand, the order of distribution of immune globulins in the organs was as follows: liver > spleen = lymph gland > lung > kidney (Table 1).

The titer of immune globulins in the liver tissue was the most interesting feature, because it is this organ which is selectively damaged by CCl_4. Carbon tetrachloride causes marked degenerative changes and definite necrosis of the liver cells [1, 5, 7]. At most times of observation the antibody concentration in the liver of the poisoned rabbits was found to be much below that in the controls. In particular, 6 h after injection of homologous immune serum the antibody titer in the liver of the experimental rabbits was one-eighth of that in animals not receiving injections of the poison (1:18 and 1:147 respectively; $P < 0.001$).

The titer of antibodies in the PHR and in the CFR at 50% hemolysis was much higher in the kidneys of rabbits poisoned with CCl_4 at all times of investigation than in the intact animals. For instance, 15 min after passive immunization the antibody titer in the kidney tissue was 1:16 (1:5 in the control; $P < 0.02$), after 24 h it was three times higher than the antibody level in the kidneys of the intact rabbits (1:49 and 1:16 respectively; $P < 0.01$), and at the end of the 5th day it was 5.3 times higher (1:32 and 1:6; $P < 0.001$).

The change in titer of exogenous homologous antibodies in the spleen, lymph glands, and lungs of the rabbits poisoned with CCl_4 was negligible compared with the controls, and in most cases the difference between the results obtained was not statistically significant (Table 1).

Hence, slowing of the metabolism of homologous antibodies takes place in rabbits poisoned with CCl_4. In particular, the first phage of metabolism of immune proteins (from the moment of passive immunization until establishment of dynamic equilibrium between plasma and tissues), whose duration in intact rabbits is 24 h [11], is lengthened in animals poisoned with CCl_4 to 3.5 days. The cause of the delay in catabolism of antibodies in rabbits poisoned with CCl_4, as the results obtained demonstrate, is a marked decrease in the ability of the liver, poisoned by the hepatotropic compound CCl_4, to absorb exogenous homologous immune globulins from the blood stream and to decompose them.

The increase in accumulation of immune proteins in the kidney tissues of the poisoned rabbits compared with the controls can evidently be regarded as the stage preceding their breakdown in this organ into fragments of low molecular weight, subsequently eliminated in the urine. This suggestion is confirmed by results [14, 15] demonstrating participation of the cells of the renal glomeruli in the catabolism of immune globulins.

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