CYTOPLASMIC FRACTIONS OF THE LIVER DIFFERING IN ANTIGENIC PROPERTIES AND IN ALCOHOL DEHYDROGENASE ACTIVITY

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Immune and autoimmune antibodies against fractions of various tissues have been shown to be capable of inhibiting the activity of some enzymes [1, 4, 5, 7, 8].

In this connection it is interesting to study correlation between the antigenic properties of the liver and activity of enzymes specific for that organ and, in particular, the alcohol dehydrogenase (ADH), which is mainly synthesized in the liver. For this purpose two fractions of liver hyaloplasm obtained by fractionation with rivanol, which differed in their ADH activity, were studied. ADH activity and the antigenic properties of these fractions were compared.

EXPERIMENTAL METHOD

Saline extracts (10%) of human and rat liver were centrifuged at 104,000g (60 min) and treated with 0.4% rivanol solution [2]. The supernatant – fraction 1 (F1), and the residue – fraction 2 (F2), were tested for the presence of ADH [6] and also of organ-specific antigens, for which purpose immune sera were obtained against unfractionated liver extract and F1. Sera were obtained by repeated alternate intravenous and intraperitoneal injections of the antigenic preparations into rabbits. During a course of immunization a rabbit received from 132 to 145 mg protein depending on the batch of the antigenic preparation.

The sera were tested by the passive hemagglutination (PHT) and passive hemagglutination inhibition (PHIT) tests [3]. As a first step, the sera were inhibited in dilutions of 1:5-1:10 to abolish the reaction against nonspecific antigens, with 20% brain or thymus extract and also with serum. The concentration of the inhibiting mixture in the immune serum was 1 mg/ml.

EXPERIMENTAL RESULTS

The results of a comparative investigation of ADH activity and of the immunologic characteristics of the unfractionated preparations of human and rat liver, and of fractions F1 and F2 obtained from them, are given in Tables 1 and 2.

Maximal ADH activity was found in F1. The difference between the ADH activity of the initial preparations and F1 and also of F1 and F2 is statistically significant ($P < 0.01$).

It will be clear from Table 2 that immune sera against preparations of human and rat liver, after inhibition of their activity by F2, continued to react with F1 and lost their activity only after inhibition by F1 preparations. For comparison, the results of investigation of serum against unfractionated rat liver extract are also given in Table 2; they show that F1 and F2 were able to inhibit the reaction of the serum selectively with the corresponding fraction. The results of these investigations thus indicate the nonidentity of the antigenic components present in F1 and F2.

ADH activity and antigenic activity of human liver F1 were compared after heating to different temperatures and also after treatment with ether and chloroform. The treated extracts were recentrifuged at 1000g (15 min) and, after determination of their protein content, their enzymic and immunologic activity was studied.

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TABLE 1. ADH Activity of Human and Rat Liver in Fractions Separated by the Rivanol Fractionation Method (M ± m)

Preparation of liver	No. of experiments	Protein content in preparation, mg/ml	ADH activity	
			μ moles NADH/min/mg protein	%
Human:				
initial	14	18,73±4,00	0,0177±0,005	100
F-1	14	9,05±2,80	0,0268±0,0026	150,8
F-2	7	1,00±0,27	0,0056±0,001	31,6
Rat:				
initial	5	14,78±1,60	0,007±0,0019	100
F-1	5	2,27±0,58	0,026±0,0066	371
F 2	5	1,73±0,80	0,005±0,0009	71,4

TABLE 2. Comparative Immunologic Characteristics of Fractions of Human and Rat Liver Obtained by Treatment with Rivanol

Immune serum	Antibody titer with antigenic preparations			
	F-1	F-2	serum	brain
Against human F1:				
Initial				
inhibited:	1:20 480	1:5 120	<1:20	<1:20
F-1 of liver*	1:20	1:320	<1:20	<1:20
F-2	1:10 240	<1:20	<1:20	<1:20
Against rat F1:				
Initial				
inhibited:	1:320	<1:40	<1:40	<1:40
F-1	<1:40	<1:40	<1:40	<1:40
F-2	1:320	<1:40	<1:40	<1:40
Against unfractionated extract of rat liver:				
Initial				
inhibited:	1:640	1:640	<1:40	<1:40
F-1	<1:40	<1:640	<1:40	<1:40
F-2	1:320	<1:40	<1:40	<1:40

*Of the same species as the liver F1 preparations used for immunization.

TABLE 3. ADH Activity of Human F1 after Physical or Chemical Treatment (M ± m)

Treatment	F-1	Number of preparations tested	Mean protein concentration in preparations, mg/ml	ADH activity	
				μmoles NADH/mg protein/min	%
Heating					
45 °C	Before treatment	4	10,7±1,9	0,005±0,0003	100
(15 min)	After treatment	8	10,8±1,7	0,006±0,0016	120
50 °C	Before treatment	7	14,7±4,9	0,013±0,005	100
(15 min)	After treatment	11	5,8±2,2	0,015±0,0038	115,4
56 °C	Before treatment	5	15,3±7,2	0,0196±0,0067	100
(15 min)	After treatment	6	4,7±2,5	0,022±0,0059	117,8
60 °C	Before treatment	7	12,1±5,0	0,0156±0,0056	100
(15 min)	After treatment	7	3,6±0,8	0,0167±0,0043	106
Ether	Before treatment	4	15,75±2,96	0,023±0,0075	100
	After treatment	4	7,05±2,17	0,021±0,0078	93,5
Chloroform	Before treatment	6	7,47±1,40	0,0094±0,0022	100
	After treatment	6	2,00±0,25	0,0201±0,0085	224

As the results in Table 3 show, treatment by heating to 45-60°C and treatment with ether had no significant effect on ADH activity.

Treatment with chloroform caused some increase in ADH activity ($P < 0.1$). Similar fractions were studied by the PHIT with serum against human liver F1; the serum was inhibited beforehand to abolish the nonspecific reaction, as described above, and also with preparations of fraction F2.

TABLE 4. Antigenic Activity of Human F1 After Physical or Chemical Treatment (M ± m)

Inhibition of PHT by F1 preparations	Number of experiments	Residual activity of immune serum
Initial	8	0,12±0,038
Heated for 15 min to:		
45 °C	4	0
50 °C	7	2,86±0,98
56—60 °C	6	2,5±1,6
Treated with ether	6	3,1±1,4
Treated with chloroform	6	6,3±0,55
Uninhibited serum	8	7,3±0,5

The results of these investigations are shown in Table 4 as mean values of the difference between the number of successive double dilutions of serum, starting with 1:20, in which a PHT was obtained before and after inhibition by F1 preparations (residual activity). As Table 4 shows, the original F1 and F1 heated to 45°C possessed the strongest inhibiting activity. Other types of treatment reduced the inhibitory activity of F1 to some degree; treatment with chloroform had the strongest action.

It was thus established that ADH activity is present chiefly in F1. F1 and F2 differ in their antigenic properties. The proteins responsible for ADH activity in F1 differ from proteins of the organ-specific antigenic components.

These investigations provide an approach to the study of conformation relations between the liver ADH and proteins carrying the organ-specific antigenic "label," and also to the study of the mechanism of action of antiliver antibodies on changes in ADH activity.

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