

SEROLOGICAL REACTIONS OF MOUSE PLASMA FRACTIONS

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THE fractionation of mouse plasma into its protein constituents was undertaken in an attempt to isolate the γ -globulin as an immunologically specific preparation. Since there was no established precedence in the literature, the technique of Cohn and his co-workers¹ was utilized.

MATERIALS AND METHODS

*Gamma Globulin.**—About 200 ml. of mouse plasma was pooled and the cold ethanol Method 6 of Cohn¹ was applied. Separation into the five major fractions, namely, I, II + III, IV-1, IV-4, and V, appeared routine. For the preparation of γ -globulin, subfractionation of Fraction II + III was attempted by the standard procedure of Oncley and his associates,² Method 9. However, γ -globulin did not precipitate as expected with a low concentration of zinc ions under the conditions of 20 per cent ethanol, 0.005 ionic strength, pH 7.2, -5° C., and 1 per cent protein concentration. This was due, at least in part, to the fact that mouse plasma contains little or no lipoprotein; human plasma contains β -lipoprotein as a major component of Fraction II + III.

With the absence of contaminating lipoprotein it was presumed that mouse γ -globulin was insoluble in the presence of zinc ions. Zinc glycinate to produce a zinc concentration of 5 mM. gave good precipitate formation. Hence, this precipitate was dissolved in a small volume of EDTA solution, dialyzed to free the mixture of Zn-EDTA complex, and the dialyzed material lyophilized as γ -globulin. This material was used for immunization.

Since subsequent studies showed that this material was not serologically homogeneous, each of the fractions of mouse serum was examined electrophoretically to learn more of the distribution of proteins which had been obtained. The Spinco Model R, Series D, electrophoretic cell was used at 2.5 Ma. for 18 hours at room temperature. The fractions were dialyzed against pH 7.6, 0.1 μ phosphate buffer, and S and S 2043 A-mgl paper strips, 2.0 cm. wide by 30.5 cm. long, were used. The analysis showed a band with the mobility of γ -globulin

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in the fraction corresponding to Fraction IV-4 of human plasma. The remainder of the IV-4 was then dissolved to give a solution containing 19 per cent protein. A total of 1 ml. of this solution was placed on 4 strips, 4 cm. wide and 31 cm. long, of Whatman 31 double thickness paper. Electrophoresis was performed at 2° C. for 17 hours, at 40 Ma. in pH 7.6, 0.1 μ phosphate buffer. A control strip was stained and the 4 large strips were cut so that the area corresponding to γ -globulin on the control strip was isolated. The clipped portions were eluted in 0.15 M NaCl solution, and the eluate further dialyzed against a large volume of 0.15 M NaCl solution. Twenty-five milliliters, containing 0.065 per cent protein, were obtained and used for subsequent serological studies.

Antiserum.—The rabbits of each of two groups were immunized according to the following dosage schedule with γ -globulin as prepared above: Week 1—4 successive intravenous injections of 0.5 mg. protein each; Week 2—4 of 1.0 mg.; Week 3—4 of 1.5 mg.; Week 4—4 of 2.0 mg.; Week 5—4 of 2.0 mg.; and Week 6—4 of 2.0 mg. The first group of rabbits received the γ -globulin as a 0.1 per cent saline solution (s.s.). The second group received an alum-precipitate prepared by adding 2.5 ml. of 1 per cent solution of potassium alum to 75 mg. of γ -globulin in 50 ml. s.s. One-tenth normal NaOH was added to maximum turbidity and the suspension adjusted to make a 0.1 per cent protein concentration.

Following the prescribed course of immunization, the rabbits of each group were bled on successive days, and the expressed serum was pooled and stored in the frozen state.

Antibody Titrations.—The antiserum from each group of rabbits was titrated by the interfacial ring technique, the interfacial zones were read after 2 hr. of incubation at 37° C. and the precipitates read after 24 hr. at 4° C.

Gel Diffusion.—The classical method of Ouchterlony³ was applied to the antisera.

Adsorption and Cross-Reactions.—Exhaustive adsorptions were carried out on the antiserum prepared against the alum-precipitated γ -globulin with the use of all the plasma fractions, according to the technique described by Kabat and Mayer.⁴ Following the treatment, the sera were tested against each of the other plasma proteins at a concentration of 0.5 per cent by the interfacial ring technique.

Exhaustive adsorption of the antiserum prepared against the alum-precipitated γ -globulin was carried out with the γ -globulin subfraction obtained from IV-4 and the serum so treated was tested against the other plasma proteins at a concentration of 0.5 per cent. Conversely, adsorption was carried out with each plasma fraction followed by testing against a 0.5 per cent solution of the γ -globulin from IV-4.

SEROLOGICAL RESULTS

The antiserum produced against the alum-precipitated γ -globulin gave reactions with aqueous antigen in a dilution of 1:100,000, while that prepared against the aqueous solution gave reactions with the homologous antigen in a

dilution of 1:10,000. Both antisera tested by the Ouchterlony technique showed a multiplicity of lines not only against the γ -globulin subfraction but also against the original plasma and other plasma fractions.

The results of the adsorption experiments which are found in Table I indicate that exhaustive adsorption with γ -globulin, the homologous antigen, removed essentially all antibody to fractions I, IV-1, IV-4, and V. Complete adsorption of all antibody was not effected by Fraction I, IV-1, IV-4, or V.

TABLE IA. CROSS-REACTIVITY OF MOUSE PLASMA FRACTIONS AFTER EXHAUSTIVE ADSORPTION (INTERFACIAL RING TECHNIQUE)

ANTISERUM ADSORBED; FINAL DILU- TION 1:2	γ -GLOBULIN FROM II + III				I				IV-1			
	V	I	IV-1	IV-4	V	γ	IV-1	IV-4	V	γ	I	IV-4
1:10 ³	±	++	±	—	+++	+++	++	++	+	++++	++++	+++
1:10 ⁴	±	±	—	—	++	++	++	+	+	+++	++++	+
1:10 ⁵	±	—	—	—	++	++	+	+	±	+++	±	±
1:10 ⁶	—	—	—	—	++	++	+	+	±	+	—	—
1:10 ⁷	—	—	—	—	++	++	+	+	±	+	—	—
1:10 ⁸	—	—	—	—	++	++	+	+	±	+	—	—

TABLE IB

ANTISERUM ADSORBED; FINAL DILU- TION 1:2	IV-4				V			
	V	γ	I	IV-1	γ	I	IV-1	IV-4
1:10 ³	±	+++	++++	++	++++	++++	++	+++
1:10 ⁴	±	+++	+++	+	++++	++++	+	+
1:10 ⁵	±	+	±	±	±	++	±	—
1:10 ⁶	±	±	±	±	±	±	±	—
1:10 ⁷	—	±	—	—	±	±	—	—
1:10 ⁸	—	±	—	—	±	±	—	—

TABLE IIA. REACTIONS AFTER ADSORPTION OF ANTISERUM WITH γ -GLOBULIN FROM IV-4

INTERFACIAL RING TECHNIQUE	
TEST ANTIGEN 0.5%	ANTISERUM (1:4) REACTION
IV-1	++++
γ (II + III)	++++
IV-4	++++
I	++++
V	+

TABLE IIB. ADSORPTION OF ANTISERUM WITH PLASMA FRACTIONS

INTERFACIAL RING TECHNIQUE		
ADSORBING ANTIGEN	TEST ANTIGEN	ANTISERUM (1:2.5) REACTION
γ (II + III)	γ -globulin	—
I	from	—
IV-1	IV-4	—
IV-4		—
V	0.5%	±

Exhaustive adsorption with γ -globulin from IV-4 did not remove antibody completely for reactions against all the other fractions including IV-4 occurred, as can be seen from Table II. Conversely, exhaustive adsorption of the antiserum with each individual fraction removed all the antibody to the γ -globulin from IV-4.

DISCUSSION

There has been no recent literature which specifically is concerned with fractionation of mouse plasma. The present study has shown that the classical techniques of Cohn and his co-workers do not produce fractions which are serologically homogeneous. Extensive modification of the fractionation techniques would be required to achieve this result. Clausen and Heremans⁵ have also indicated that salt fractionation procedure is not a good basis for the isolation of the immunoelectrophoretically pure mouse serum proteins. In addition, our own studies by the cellulose acetate immunoelectrophoresis technique disclosed that all the fractions studied contained common contaminating antigens, and that no fraction was composed of but a single antigenically distinct protein. Thus, it is apparent that the Cohn techniques of fractionation with respect to mouse plasma do not result in proteins with serological homogeneity.

SUMMARY

Gamma globulin was prepared from mouse plasma by subfractionation of Cohn fraction II + III obtained by the cold ethanol fractionation technique. Serological studies indicated that this preparation gave cross-reactions with other plasma proteins, indicating contamination. It is suggested that the difficulty in isolating serologically specific γ -globulin may be the result of a low concentration of lipoprotein in mouse plasma. The γ -globulin recovered from Fraction IV-4 was not serologically homogeneous, either.

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