Chromatographic Analysis and Sulfhydryl Sensitivity of Antileptospira Agglutinins in Rabbit and Human Sera.* (30654)

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Antibody activity for various antigens has been found chiefly in the γG (7S) and the γM (19S) fractions of globulin. The molecular type of antibody which predominates appears to depend to some extent on the chemical nature of the antigen involved. Protein antigens such as serum albumin and bacterial flagella induce the early formation of γM antibody followed by γG which soon comprises the major portion of the antibody(1). Other antibodies, notably those produced in response to the somatic antigens of Gram-negative bacilli(1,2,3) and the infectious mononucleosis antibody that reacts with the beef thermostable antigen(4), are predominantly or exclusively in the γM globulin.

Since only limited information is available regarding the antigenic composition of leptospirae and the relation of antigenic components to various serologic reactions involving these organisms(5), it seemed of interest to determine the types of globulin associated with the agglutinins in antileptospira sera.

Materials and methods. Rabbits were immunized by 4 intravenous injections, each consisting of 1 ml of live culture of leptospirae, given during a period of 6 days. Three of the sera from patients with leptospirosis had been preserved in the frozen state for 6 to 7 years without apparent reduction in agglutinin titer. Serum from one patient, 812, was examined after 6 weeks refrigeration without being frozen. Antibodies were determined by the microscopic agglutination test employing live cultures in Korthof's medium(6). Chromatographic separation of fractions from 5 ml samples of serum was accomplished as previously described(3, 4) or, when the amount of serum was limited, 1 ml of serum was placed on a column containing 1 g of DEAE cellulose and from 8 to 20 4-ml fractions were eluted with each of the 4 buffers. Individual fractions were screened for agglutinins and the positive fractions eluted with each buffer were pooled and concentrated with the aid of carbowax(3). Sensitivity of antibody to disulfide bond reduction was determined by mixing equal volumes of diluted serum and 0.2 M 2-mercaptoethanol (ME), allowing the mixture to stand at room temperature for 16 to 18 hours, and testing for agglutinins without further treatment. A control consisting of equal parts of serum dilution and saline was included in each instance.

Results. Human sera. The data obtained on analysis of serum specimens from 4 patients with leptospirosis are shown in Table I. Each serum reacted with at least 4 serotypes. If one assumes that the type showing the highest agglutinin titer was the type responsible for the infection, it appears that patients 328 and 812 were *Leptospira canicola* infections, that patient 336 was infected with *L. pomona,* and that *L. grippotyphosa* was the infecting type in patient 406. Although the differences between titers for *L. sejroe* and the presumed infecting type was only one tube, it is unlikely that all 4 patients had multiple infections, especially since neither *L. sejroe* nor a closely related type has been isolated from man in the U. S.

After treatment with ME, agglutinins for *L. icterohemorrhagiae, L. canicola, L. ballum, L. pomona, L. autumnalis,* and *L. grippotyphosa* were either no longer detectable in a 1:100 dilution of serum or the titers were at least 16-fold lower than those of the controls, indicating that the predominant agglutinins were macroglobulins even as late as 2 months (patient 336) after the onset of disease. The agglutinin titers for *L. sejroe,* on the other hand, were reduced only 2- and 4-fold by ME, suggesting that the agglutinins for this type consistently occurred to a greater degree in the γG, ME-resistant globulin.

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TABLE I. Mercaptoethanol Sensitivity and Chromatographic Distribution of Agglutinins for Leptospira in the Sera of Patients.

Patient	Time after onset	Type of leptospira	Titer of serum: Control	Titer of serum: Treated with ME	% of total agglutinins in eluate*: .01 M	% of total agglutinins in eluate*: .1 and .3 M
328	2 weeks	*icterohemorrhagiae*	1,600	<100	<1	93
		canicola	6,400	400	1	91
		sejroe	3,200	800	7	79
		ballum	1,600	<100	<1	92
	4 "	*icterohemorrhagiae*	400	<100	<1	90
		canicola	3,200	200	1	95
		sejroe	3,200	800	20	60
		ballum	800	<100	4	63
812	4 "	*icterohemorrhagiae*	1,600	<100	4	93
		canicola	25,600	800	1	96
		sejroe	12,800	6,400	55	37
		ballum	1,600	<100	<1	99
336	3 "	*icterohemorrhagiae*	800	<100	1	95
		pomona	3,200	100	1	96
		autumnalis A	1,600	<100	<1	98
		autumnalis AB	400	<100	<1	99
		sejroe	1,600	800	15	78
	2 mo	*icterohemorrhagiae*	400	<100	1	95
		pomona	3,200	200	<1	99
		autumnalis A	800	<100	<1	91
		autumnalis AB	400	<100	<1	99
		sejroe	1,600	800	49	39
406	2 weeks	*canicola*	800	<100	3	70
		grippotyphosa	25,600	800	<1	48
		sejroe	12,800	6,400	62	22
		ballum	400	<100	11	43
	5 "	*canicola*	200	<100	3	90
		grippotyphosa	12,800	100	<1	97
		sejroe	6,400	3,200	90	9
		ballum	100	<100	15	83

* Stepwise elution from DEAE cellulose with buffers of .01, .05, .1 and .3 M phosphate.

The chromatographic distribution of the leptospira agglutinins is shown in the right hand columns of Table I. The titer of the pooled, concentrated fractions eluted with each of the 4 buffers of increasing molar concentrations was determined. The reciprocal of the titer multiplied by the volume of the solution was taken as a quantitative estimate of the relative amount of antibody present. It was then possible to calculate the percentage of the total detectable antibody eluted with each buffer. In every instance these results confirm the impression gained by the use of ME. No more than 15% of the agglutinins for any type except *L. sejroe* were eluted with 0.01 M phosphate, indicating that the major portion of the agglutinating activity was in the γM globulin. Among these types, only *L. ballum* showed a suggestion of an increase in the proportion of the agglutinin eluted with 0.01 M buffer in the later serum specimens. The agglutinins for *L. sejroe*, however, showed a distinctly different chromatographic distribution in that from 7 to 62% of the agglutinating activity of the earliest sera was found in the eluate obtained with 0.01 M buffer. This percentage increased with time in each of the 3 patients from whom paired serum specimens were examined.

Rabbit immune sera. The sera obtained from 2 immunized rabbits were analyzed by the same procedure as that employed for the study of human sera. The results are shown in Table II. The serum of rabbit 70, immunized with *L. pomona*, showed only a slight degree of reaction with heterologous types and, in contrast to patient 336 (Table I), no reaction with *L. sejroe*. The agglutinins in the serum sample obtained 2 days

TABLE II. Mercaptoethanol Sensitivity and Chromatographic Distribution of Agglutinins for Leptospira in Sera of Immunized Rabbits.

Rabbit	Time after last injection	Type of leptospira	Titer of serum		% of total agglutinins in eluate*	
			Control	Treated with ME	.01 M	.1 and .3 M
70†	2 days	*icterohemorrhagiae*	200	<100	<1	>99
		pomona	51,200	<100	1	99
		autumnalis AB	200	<100	<1	>99
	16 "	*icterohemorrhagiae*	<100	<100	—	—
		pomona	12,800	1,600	14	84
		autumnalis AB	<100	<100	—	—
19‡	2 "	*sejroe*	25,600	<100	<1	>99
		hebdomadis	1,600	<100	<1	>99
	16 "	*sejroe*	12,800	12,800	52	45
		hebdomadis	400	<100	2	98
	44 "	*sejroe*	12,800	12,800	80	17
		hebdomadis	400	<100	36	60

* Stepwise elution from DEAE cellulose with buffers of .01, .05, .1 and .3 M phosphate.
† Immunized with *Leptospira pomona.*
‡ " " *Leptospira sejroe.*

after the last injection were completely inactivated by ME and were present almost exclusively in the chromatographic eluate obtained with the higher ionic strength buffers. Two weeks later the agglutinating activity was markedly reduced but not completely destroyed by ME and the first eluate contained a significant portion of the agglutinins.

The 2-day serum from rabbit 19, immunized with *L. sejroe,* was comparable to the corresponding serum from rabbit 70 with respect to ME sensitivity and chromatographic distribution of agglutinins. The increase in γG antibody with time, however, was more rapid, 52% of the homologous agglutinins appearing in the 0.01 M eluate of the 16-day serum and 80% on the 44th day. The agglutinins for *L. hebdomadis,* the only cross-reacting type, remained predominantly in the high molecular weight globulin fraction.

Discussion. The antigenic composition of leptospirae is complex(5). There is evidence to suggest that these organisms contain a somatic antigenic component which is genus specific, perhaps a lipopolysaccharide, and which is involved in complement fixation and erythrocyte sensitization but not in agglutination(7). Antibodies to this antigen were probably not detected in the studies reported here. A second antigenic component, located more peripherally, is involved in agglutination(7). The tendency for agglutinins reacting with all the leptospira serotypes studied, with the exception of *L. sejroe,* to remain in the γM fraction of serum for as long as 2 months after the initial contact with antigen suggests that this antigenic component also may be similar in chemical composition to the somatic antigens of the Gram-negative intestinal bacilli.

The relative amounts of different types of antibody that appear to be present in a serum will be influenced by the serologic reaction used to assay the antibody. For example, the γM rabbit anti-sheep cell antibody is known to possess greater hemolytic activity in proportion to agglutinating ability than does the γG antibody(8). A similar hemolytic superiority for the γM has been shown by passive hemolysis in studies with fractions of rabbit anti-typhoid O serum(9). On the other hand, γG is more efficient in fixing complement than is γM(10,11). Since agglutination was the only method of antibody assay used in the work reported here, the data reflect the antibody distribution in the antileptospira sera only insofar as agglutinating properties are concerned.

The fact that agglutinins reacting with *L. sejroe* showed a distinctly greater tendency to appear in the chromatographic fractions eluted with 0.01 M buffer than agglutinins reacting with other types tested suggests that the *L. sejroe* antigens involved are chemically

different from the others. This difference was noted not only in the analysis of sera from patients who were presumably infected with a type other than *L. sejroe* but also in the serum of the rabbit immunized with this type.

Certain strains of *L. sejroe* are known to be strongly cross-reactive when tested against sera from patients(12). Since *L. sejroe* reacted with all the patients' sera tested, one might have expected a reaction with the rabbit anti-*L. pomona* serum. The relative specificity of the rabbit anti-*L. sejroe* serum is also remarkable considering that the same strain was used in the agglutination tests and for immunizing the rabbit. No explanation for these seemingly paradoxical findings is apparent.

Summary. Chromatography on DEAE cellulose and susceptibility to inactivation by 2-mercaptoethanol indicate that the major portion of the agglutinins in patients' sera for 7 types of leptospirae are 19S macroglobulins even at 4 to 8 weeks following the onset of symptoms. A distinctly greater proportion of the agglutinins for *L. sejroe* was found in the 7S fraction of patients' sera. The agglutinins produced in rabbits following the injection of *L. pomona* and *L. sejroe* were initially almost entirely in the 19S fraction of serum but appeared also in the 7S fraction as time progressed. *L. sejroe* showed a greater tendency to elicit the production of 7S agglutinins in the rabbit than did *L. pomona.*

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Differences in Sensitivity of Human Blood Cells to Fresh Rabbit Serum.* (30655)

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Previous work(1) has shown that 50% fresh rabbit serum had little or no cytocidal effect on the survival of normal human lymphocytes. In this study, the cytocidal effects of fresh rabbit serum were determined on normal granulocytes, monocytes and erythrocytes and on the blood cells from patients with chronic and acute granulocytic leukemia.

Methods. Heparinized blood from normal individuals or leukemic patients was allowed to sediment for 30 or more minutes at 37°C. The plasma and leukocytes were removed and

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