Complement Fixation on Cell Surfaces by 19S and 7S Antibodies

Abstract. *The mechanism of complement fixation on cell surfaces by whole antiserums, and by 19S and 7S fractions has been studied with a new complement-fixation test. This test is based on the fixation and transfer of the activated first component of complement (C'1a). We have concluded that a single molecule of 19S antibody in combination with antigen at the cell surface is sufficient to bind one molecule of C'1a. For 7S antibodies at least two molecules in close proximity at the cell surface are required to fix one molecule of C'1a.*

We have reported that a single molecule of Forssman hemolysin of the 19*S* immunoglobulin (IgM) type is sufficient to sensitize a sheep erythrocyte to the lytic action of guinea pig complement (C') (*1*). Furthermore, each sensitized site on the cell surface consisting of antigen and one molecule of 19*S* hemolysin is capable of binding one molecule of C'1a (the activated form of the first component of complement) (*2*). These findings were obtained, in part, with a new C' fixation test designated the C'1a fixation and transfer test (C'1a FT). This test is based on the ability of C'1a to react with hemolysin in combination with antigen on the cell surface and then to transfer quantitatively to appropriate recipient cells (*1*). The recipient cells are referred to as EAC'4, for they consist of sheep erythrocytes (E) sensitized with Forssman antibody (A) and the fourth component of C' (C'4). The number of molecules of C'1a transferred to EAC'4 is calculated on the basis of the one-hit theory of immune hemolysis (*3*).

We now report the application of the C'1a FT test to several antigen–antibody systems including the Forssman system. It was found that 19*S* and 7*S* antibodies do not fix C' by the same mechanism. Experimental details and the preparation of reagents have been reported (*1, 3, 4*).

The relation between antibody concentration and the amount of C'1a fixed was determined for the following antigens and corresponding antibodies (*5*): (i) sheep erythrocytes and rabbit antibody to boiled stromata of sheep erythrocytes (Forssman), (ii) *Salmonella typhosa* (0901) and rabbit antibody to *S. typhosa*, (iii) *S. typhosa* "O" somatic antigen and rabbit antibody to this antigen, (iv) *Escherichia coli* (B7086) and rabbit antibody to *E. coli*, (v) human erythrocytes containing I and human serum containing I "cold agglutinin," and (vi) human erythrocytes and human antibody to A substance. Whole antiserum, fractions containing 19*S* antibodies, and fractions containing 7*S* antibodies were tested. All three of these reagents, however, were not available in all cases.

The results of C'1a FT tests were analyzed by plotting the logarithm of the number of C'1a molecules fixed as a function of the logarithm of relative antibody concentration (Fig. 1). The slopes of the lines shown in Fig. 1 are a measure of the number of antibody molecules required to establish one site on the cell surface capable of binding one molecule of C'1a. For whole serum and for the fraction containing 19*S* antibody molecules, the slope is close to unity. For the fraction containing 7*S* molecules, on the other hand, the slopes of the lines in Fig. 1 are 2.1 for the *Salmonella* system and 2.6 for the Forssman system. The results for all of the systems tested are summarized in Table 1. For whole serums and for the 19*S* fractions, the slopes are close to unity. For the 7*S* fractions, the slopes are somewhat greater than 2. These results mean that a single molecule of 19*S* antibody in combination with antigen at the cell surface is sufficient to establish a site capable of binding C'1a. The slope of 1 obtained with whole antiserum to Forssman antigen and with whole antiserum to *Salmonella* means that C'1a fixation by these reagents is due mainly to 19*S* antibody molecules, despite the fact that both serums contained 7*S* molecules capable of fixing C'. The slopes obtained with 7*S* anti-

Table 1. Slopes of dose-response curves plotted in Fig. 1.

Antigen	Source of antiserum	Reagents		
		Whole antiserums	19*S* fractions	7*S* fractions
Forssman	Rabbit	1.1	1.0	2.6
S. typhosa	Rabbit	0.96	0.96	2.1
"O" antigen*	Rabbit	1.2	N.A.†	N.A.
E. coli	Rabbit	0.95	N.A.	N.A.
I	Human	.90	1	N.A.
A substance	Human	N.A.	1.1	2.3

* This antigen from *S. typhosa* was adsorbed on human group O erythrocytes. † N.A., reagent not available.

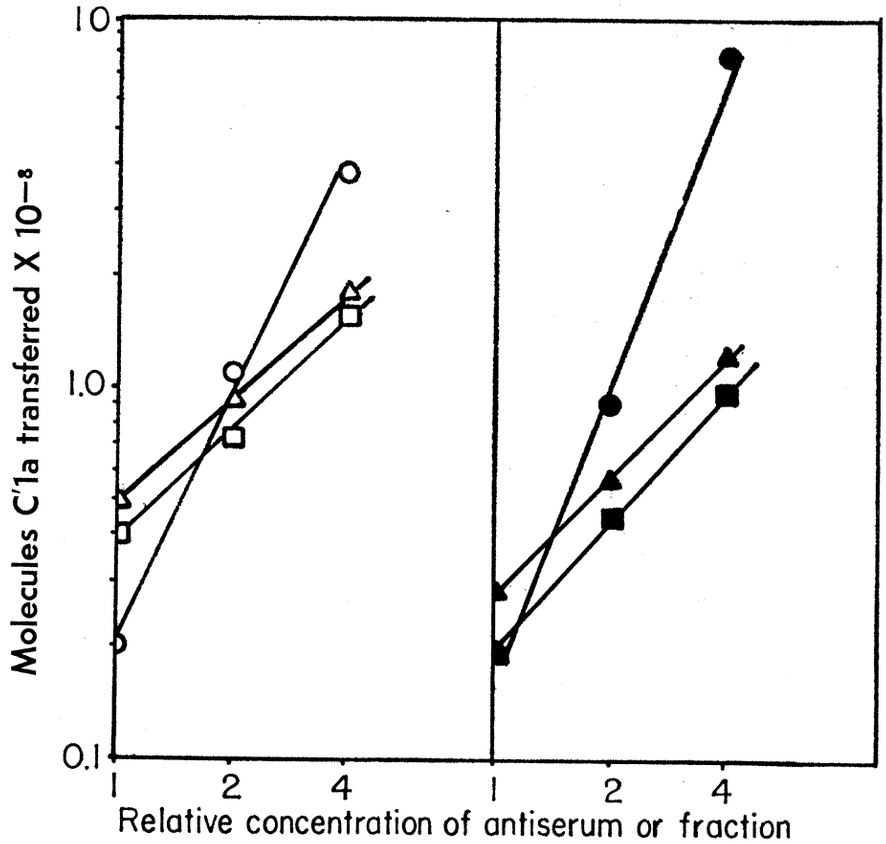

Fig. 1. Dose-response curves for whole serum (squares), 19*S* fraction (triangles), and 7*S* fraction (circles). Open symbols: rabbit antiserum to *S. typhosa*; solid symbols: rabbit antiserum to boiled stromata of sheep erythrocytes.

bodies indicate that at least two 7*S* molecules in close proximity on the cell surface are required to fix a C′1a molecule. Furthermore, the generation of such doublets must be a random process, that is, the presence of a 7*S* molecule at a particular site on the cell surface does not influence the probability of doublet formation. If the process of doublet formation were directed rather than random, the slope of the 7*S* dose-response curve would approach unity. Thus, under conditions where the number of antibody-binding sites is relatively large, one would expect only a small proportion of the 7*S* antibody molecules to form doublets and, therefore, to be capable of binding C′1a. Since, on the other hand, each molecule of 19*S* antibody is capable of binding one molecule of C′1a, whole antiserum will behave like the 19*S* fraction, provided that the number of antibody binding sites is large. This result has been obtained even with antiserums in which the ratio of the number of molecules of 7*S* antibody to the number of molecules of 19*S* antibody was more than 100. It can be predicted from these results that the number of C′1a molecules fixed by a given amount of 19*S* antibody should be independent of cell concentration, provided that the number of antibody binding sites is not limiting and that the antibody in combination with antigen has a low tendency to dissociate. Under similar conditions, the number of C′1a binding sites generated by a given amount of 7*S* antibody will decrease with increasing cell concentration. Experimental results have been obtained which are in agreement with these predictions.

Earlier evidence indicated that relatively few sensitized sites on the cell surface were required to initiate the lytic process. Rapp presented evidence that fewer than ten molecules of 19*S* hemolysin per cell were required for sensitization (*3*, p. 145). On the basis of probability this observation was at variance with the conclusion of Weinrach *et al.* that two 19*S* antibody molecules in close proximity on the cell surface are required for sensitization (*6*). Weinrach *et al.* also proposed that four 7*S* antibody molecules are required to establish a potentially lytic site on the cell surface. The experiments on which their conclusions were based, however, suffered because the extent of lysis as a function of antibody concentration was not independent of complement concentration (*1*). More recently Humphrey and Dourmashkin have shown that as few as two 19*S* antibody molecules suffice to sensitize an erythrocyte (*7*). They presented evidence that several hundred molecules of 7*S* antibody are required to sensitize an erythrocyte to the lytic action of C′. Statistical considerations led them to postulate that either a single molecule of 19*S* antibody or two molecules of 7*S* antibody are sufficient to initiate the lytic process. Based on the evidence given in this report, we propose that on cell surfaces a single molecule of 19*S* antibody suffices to fix C′ while doublets are required for 7*S* antibodies.

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References and Notes

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2. In support of this conclusion we have determined (unpublished) on a weight basis the ratio of the number of molecules of 19*S* antibody to the number of C′1a molecules bound. This ratio is close to unity for Forssman antibody and for antibody to human A substance, the two systems for which weight determinations were available.
3. M. M. Mayer, in E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Sprinfigeld, Ill., ed. 2, 1961).
4. H. J. Rapp and T. Borsos, *J. Immunol.* **91**, 826 (1963); T. Borsos and H. J. Rapp, *ibid.*, p. 851.
5. We are indebted to the following individuals for supplying some of the reagents: Dr. L. Muschel (*S. typhosa* and rabbit antiserum to *S. typhosa*), Dr. W. Rosse (human I "cold agglutinin" and *S. typhosa* somatic antigen and its corresponding rabbit antibody), and Dr. T. Ishizaka and Dr. K. Ishizaka (IgG and IgM fractions of human antiserum to A substance).
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Dulcin and Saccharin Taste in Squirrel Monkeys, Rats, and Men

Abstract. *In a taste-preference comparison of sweetening agents, men reacted positively to two nonnutritive sweeteners, dulcin and sodium saccharin; rats preferred only saccharin and squirrel monkeys, only dulcin.*

The mechanisms regulating taste preferences may also be mechanisms of reinforcement in the conditioning situation (*1*).

Sodium saccharin is reported by humans to be about 675 times as sweet as sucrose, and dulcin is about 200 times as sweet as sucrose. These are the sweetest substances known to man (*2*). Sodium saccharin has been used as an incentive for rats in the conditioning situation; dulcin has not been so used. The increasing importance of the squirrel monkey as a laboratory animal warrants systematic study of preferences for sweeteners on this animal and comparison of the resulting data with that from similar work on other laboratory subjects.

Preferences for dulcin and sodium saccharin in four male squirrel monkeys (*Saimiri sciureus*) were studied and compared to data obtained from five male albino rats and three male humans. Three additional male squirrel monkeys provided information about the reinforcing properties of dulcin.

The Richter two-bottle preference test (*3*) was used to determine taste functions in the animals. Two drinking tubes were suspended from the wall of the animals' cages; one contained distilled water and the other a sapid solution made with distilled water. Intake was recorded every 24 hours at which time the contents were reversed to compensate for position preferences. Every 48 hours the concentration of the sapid solution was increased to provide an ascending concentration series.

Information on the reinforcing properties of dulcin for squirrel monkeys in a lever-pressing situation was collected from three squirrel monkeys. The animals were trained to press a bar, on a

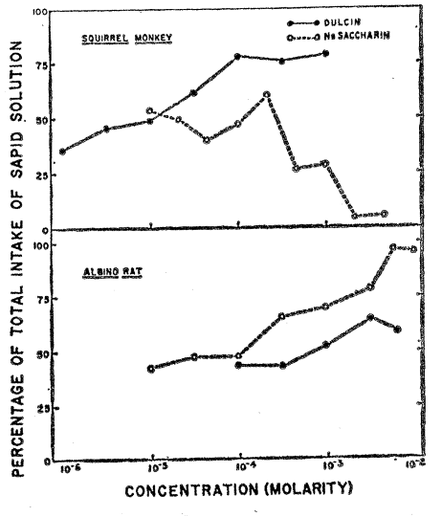

Fig. 1. Averaged preference-aversion curves for dulcin and saccharin for four squirrel monkeys and five albino rats.