The Relative Efficacies of 7S and 19S Forssman Antibody in Producing Lesions in the Guinea Pig[1] (36212)

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(Introduced by L. Ellenbogen)

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It has long been known that administration of rabbit anti-Forssman antiserum to the guinea pig produces a complement-dependent hemorrhagic lesion on intradermal injection and death from heterophile shock on intravenous injection. The investigations establishing these phenomena were performed with whole sera without separation of antibody types (1). As the majority of the complement-fixing activity of whole serum resides in the 19S fraction when examined in the sheep cell system (2), it was thought that this might also be the case in the Forssman reaction. The present investigation shows that exactly the opposite is the case; namely, that 7S antibody is far more effective in causing local Forssman vasculitis and systemic Forssman reaction.

Materials and Methods. Antibody preparation. Heated sheep erythrocyte stroma were prepared and administered to adult New Zealand rabbits as described by Mayer (3).

For separation of immunoglobulins, a 20 ml sample of serum from an individual rabbit was treated with 10 ml of saturated ammonium sulfate. The precipitated globulins were dissolved in saline, adjusted to pH 7.8 with NaOH, and dialyzed 24 hr at 4° against 3 changes of saline buffered with 0.02 *M* sodium phosphate, pH 7.5. The dialyzed globulins were then applied to a 2.5 × 90 cm column of agarose 1.5 *M* at 4°, and eluted with the phosphate-buffered saline described above. Effluent fractions were monitored by measuring absorbancy at 280 nm and revealed two well-separated peaks. The first peak, corresponding to the void volume, was used as a source of 19S antibody, the retarded peak, as 7S antibody. Fractions from peak centers were pooled and concentrated to 20 ml, using the Amicon pressure filtration cell with UM-1 or UM-20E membranes, followed, if necessary, by pressure filtration through a collodion bag (Schleicher and Scheull).

Lyophilized commercial amboceptor (Baltimore Biological Laboratories) was dissolved and then separated on the agarose column as described above.

A 7S antiserum was further resolved into its immunoglobulin classes by chromatography on diethylaminoethyl cellulose as described by Stechschulte *et al.* (4).

Biological reactions. Forssman shock was induced by intravenous injection of antibody into guinea pigs. Shock was manifested by nose twitching, labored breathing, frothy blood-tinged sputum, convulsion, and death. Forssman cutaneous vasculitis was induced by injection of 0.1 or 0.2 ml of antibody fraction intradermally in the previously shaved flank. Hemorrhagic lesions appeared in 5–10 min, were maximum in 30 min, and were usually measured at 60 min, the dimension taken being the mean of the widest and narrowest diameters.

Complement fixation reactions.[2] Hemolytic antibody was assayed according to Mayer (3). Sheep erythrocytes were sensitized with 1/1000 dilution of commercial antibody to whole sheep cells in the presence of .01 *M* ethylenediaminetetraacetic acid (EDTA) for 30 min at 30° and 30 min at 0°. They were converted to EAC4 as described by Borsos and Rapp (5). Guinea pig lung tissue was obtained from

[1] A preliminary report of these findings was presented at the 1969 annual meeting of the Federation of American Societies for Experimental Biology.

[2] Terminology conforms with the Memorandum on Nomenclature of Complement, Bull. World Health Org. **39**, 935 (1968).

TABLE I. Relative Efficacy of Fractions of Rabbit Anti-Forssman Serum in Producing SI in Lesions in Guinea Pigs.

Rabbit No.	AbH_{50}/ml[a]			Diameter of Lesion (mm)		
	Globulins[b]	19S[c]	7S[c]	Globulins[b]	19S[c]	7S[c]
3	17000	11000	1500	10	0	10
5,6 (pooled)	10000	7000	1800	8	0	10
10	3500	1300	190	10	0	11.5
11 early	12000	5000	800	13	0	14
11 late	5000	1800	500	8	0	10

[a] AbH_{50} = 50% hemolytic antibody units, determined according to Mayer (3).
[b] = Dialyzed redissolved precipitate of serum globulins prior to gel filtration (See Methods).
[c] 19S, 7S = Fractions from gel filtration column.

freshly-killed normal guinea pigs and homogenized with a glass homogenizer in ice-cold 0.25 *M* sucrose. The lung homogenate was then washed in Veronal buffer (3) and in Veronal buffer containing 0.01 *M* EDTA. Sensitization with antibody and reaction with complement components were done exactly as for sheep erythrocytes. The number of C1-fixing sites was then estimated by C1 fixation and transfer (6). Serum C4 was measured as described by Ruddy and Austen (7) using guinea pig components throughout.

Results. Forssman lesions. The 19S and 7S fractions of rabbit antiserum to sheep cell stroma were titrated against sheep erythrocytes and were also used to produce cutaneous vasculitis in guinea pigs. The results with five such antisera are shown in Table I. There is obviously a great discrepancy between the *in vitro* effect on sheep cells and the *in vivo* effect in guinea pigs; namely, high titer anti-sheep-cell 19S antibody failed to produce a lesion, while low titer 7S antibody readily produced a lesion. Similarly, in Table II we see the same discrepancy in producing heterophile shock. Identical results were obtained with a fractionated commercial antibody to whole sheep cells.

TABLE II. Relative Efficacy of Fractions of Rabbit Anti-Forssman Serum in Producing Heterophile Shock in Guinea Pigs.

Antiserum	AbH_{50}/ml	Dose (ml)	Result
Whole	3500	0.5 or 1.0	+[a]
19S	1300	1.0 or 2.0	0
7S	190	0.5	+

[a] + = Died in less than 5 min.
0 = Alive after 2 hours.

When a 7S fraction which produced cutaneous vasculitis was further resolved into immunoglobulin classes, the lesion-producing activity was found only in the fraction which passed directly through the DEAE cellulose in 0.01 *M* buffer. This fraction consists primarily of IgG (4, 8).

Attempted potentiation with vasodilators. Neither histamine (2 μg) nor serotonin (20 μg) injected intradermally increased the lesion observed with 7S antibody or created a lesion with 19S antibody.

Complement fixation in vitro. Figure 1 shows

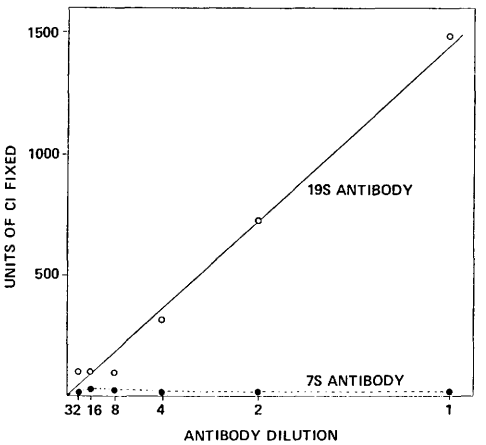

FIG. 1. C1 fixation by guinea pig lung *in vitro*: 0.5 ml aliquots of a 10% suspension of homogenized guinea pig lung were treated with dilutions of 7S and 19S Forssman antibody and then with excess C1. After washing, the residual C1 was assayed by transfer to EAC4.

that C1 fixation was roughly linear with 19S antibody concentration, while C1 fixation with 7S antibody was hardly above the control (unsensitized lung tissue).

Complement fixation in vivo. That complement consumption actually occurred *in vivo* was demonstrated in an experiment in which guinea pigs were bled for serum C4 determination before and 2 min after challenge with a lethal dose of 7S Forssman antibody or a large nonlethal dose of 19S Forssman antibody. Both antibodies caused a marked depression in serum C4; post-challenge levels ranged from 10–42% of control with 7S antibody and from 4–47% of control with 19S antibody.

Discussion. Data have been presented to show that complement-fixing ability of rabbit Forssman antibody as measured in the hemolytic system *in vitro* does not reflect its ability to cause complement-dependent lesions *in vivo*. Indeed, IgG seems to be entirely responsible for Forssman shock and Forssman cutaneous vasculitis, IgM showing very little ability to produce these reactions, although the majority of the *in vitro* hemolytic activity of whole serum lies in its IgM fraction. (These observatons contrast two *activities* of IgG and IgM, and do not compare them on a *weight* basis.)

These results are in accord with those of other investigators, such as Sell and Spooner (9), who found that 7S γ-globulin-fixed complement was more cytolytic to dog kidney cells, and Frank and Gaither (10), who showed that complement fixation proceeds more extensively when mediated by 7S antibody. The coadministration of permeability-enhancing vasoactive amines did not alter our findings, suggesting that the difference between IgG and IgM cannot be ascribed to the latter's poorer penetration of tissue.

Measured *in vitro,* guinea pig tissue behaves as sheep erythrocytes, fixing C1 better when sensitized with 19S antibody; C4 was consumed on administration of 19S antibody *in vivo*. Thus it must be postulated that much of the consumption of C4 and C1 seen in nonlethal challenge with 19S antibody and *in vitro* is not biologically relevant to production of Forssman shock.

Summary. The ability of rabbit antiserum to heated sheep erythrocyte stroma to cause Forssman shock and cutaneous vasculitis in the guinea pig resides in its IgG fraction and not in its IgM fraction, although the latter fixes complement with guinea pig tissue both *in vitro* and *in vivo*.

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