Biophys. J. **3**, 309 (1963)] between sedimentation coefficient (determined here by sucrose density-gradient centrifugation) and molecular weight.

8. Supported by NIH (grant AI-01725, contract PH43-64-928, and a fellowship). Sister S. Lacy is a predoctoral fellow and M. Green is a Research Career Awardee of NIH. We thank Mrs. C. Boudreau and Mrs. C. Self for technical assistance.

2 July 1965

Rabbit 19S Antibodies with Allotypic Specificities of the a-Locus Group

Abstract. *Activity of bacteriophage T4 neutralizing antiserums obtained 8 days after a primary immunization of rabbits with purified phage T4 is due to antibodies of the 19S (IgM) type. This neutralizing activity could be partially inhibited by treatment with excess antiserum directed against the a-locus group allotypic specificity carried by the whole rabbit serum.*

Todd (*1*) presented evidence that rabbit 19*S* macroglobulin—IgM (*2*)—carried allotypic specificities controlled at both the *a* and *b* loci (*3*). The 7*S* γ-globulin (IgG) has specificities of both locus groups; these group specificities are distributed in such a way that the "light" chains (L or B) of IgG have specificities only of the *b*-group (A4, A5, A6) and the "heavy" chains (H or A) of IgG probably have specificities only of the *a*-group (A1, A2, A3) (*4*). Such a separation of group specificities is not surprising, as the two loci, *a* and *b*, are not closely linked (*5*). One might expect IgM to have the *b*-group specificities since L or B chains are probably common to the different types of rabbit immunoglobulins as seems to be the case with human and horse immunoglobulins (*6*). But since the *a*-group specificities are carried by the A or H chain (*4*), a portion of which is not common in antigenicity (*7*) between IgG and IgM, it is surprising that the two types of rabbit immunoglobulins both have the *a*-group allotypy. Feinstein *et al.* (*8*) confirmed the existence of *b*-group specificities on IgM, but could come to no conclusions about the existence of *a*-group specificities. One of us (*9*) found some evidence for the *b*-group specificity but failed to find *a*-group specificity on a single sample of IgM. We now have confirmed and extended the findings of Todd (*1*) by showing that bacteriophage T4-neutralizing antibodies of the 19*S* type have allotypic specificities of the *a*-group.

Antiserum directed against allotypic specificities were prepared (*10*) and were shown by ring test to be monospecific for the particular allotypic activity. Bacteriophage-neutralizing antiserums were prepared by the single injection of about 10^{10} bacteriophage-T4 plaque-forming units per rabbit; 8 days later the rabbits were bled from the marginal vein of the ear. The serums were stored frozen until used. High-titer neutralizing antiserum to T4 was obtained by continuing the immunization for 3 months, about 10^{10} phage being injected at monthly intervals; serums were obtained 10 days after the final injection. Phage-neutralizing activity was assayed by the kinetic method (*11*), and results were recorded as first-order rate constants of neutralization. After more than 90 percent phage neutralization had occurred phage survival was generally not linear (if the log of the number surviving was plotted against time); the values of neutralization constants given were obtained from the initial, linear region in these cases. The neutralizing activity of IgM against T4 was destroyed by reduction of the globulin with 0.1*M* or 0.2*M* 2-mercaptoethanol (1 hour at 37°C) before the addition of bacteriophage and the start of the neutralization assay (*12*). Residual activity was determined in the presence of the reducing agent; no effect of the mercaptoethanol was noted with high-titer (7*S*) antiserum to T4 assayed in the above manner.

Sucrose-gradient sedimentation was performed as described by Vaerman *et al.* (*12*), except that 5 percent sodium chloride was replaced by 0.1*M* phosphate, *p*H 7.5. Samples were centrifuged in the cold at 35,000 rev/min (Spinco Model L, SW-39 head) for 14 or 16 hours. The β-galactosidase activity of a crude extract of induced *Escherichia coli* K12 was used as a sedimentation-velocity marker; the enzyme, as detected by its activity with *o*-nitrophenyl-β-D-galactoside, has a reported sedimentation value of about 16*S* (*14*).

The effect of the antiserum with allotypic specificity on the neutralizing activity of the antiserum to T4 was assayed by addition of 0.005 ml of 8-day antiserum to T4 to 1 ml of either normal rabbit serum or rabbit antiserum with allotypic specificity. This mixture was incubated at 37°C for ½ or 1 hour and then was held in the refrigerator overnight. The next day the total mixture was brought to 37°C, and the phage was added (about 2×10^5 plaque-forming units). Phage survival was followed up to 750 minutes, and the first-order rate constant of neutralization was calculated. The results of such assays are given in Table 1. The homologous antiserum to allotypic specificity significantly inhibited the activity of the antiserum to phage in every case, the inhibition being from 40 to 90 percent. The one antiserum to *b*-group tried (antiserum to A4 with serum A002) also resulted in a large inhibition (78 percent) of the antiserum to T4.

The effect was specific (Table 1); antiserum directed against an allotypic specificity lacking in the antiserum to T4 gave no comparable inhibition. The neutralizing values in such cases were within the experimental error of that found when antiserum to T4 was mixed with normal rabbit serum, although some small effect could not be ruled out by these experiments. Also (Table 1) the addition of a normal serum with the A1 allotypic specificity to the mixture of antiserum to A1 and A1-negative antiserum to T4 was without effect. This control was included to test for possible indirect effects of the reaction of antiserum to allotypic specificity and IgG (that could not react with T4) such that 19*S* antibody would be inhibited. However, no such indirect action was observed. No explanation is attempted for the variability of the degree of the inhibition by antiserum directed against allotypic specificity on the 19*S* neutralizing antibodies. However, the final three values in Table 1 were obtained with rabbits immunized with a fresh purified lysate of phage T4, and this same lysate was used to assay these three antiserums for activity against T4.

To show that the phage neutralizing antibody of these early antiserums was actually 19*S* antibody (IgM), use was made of several properties of IgM. Thus the early antiserums were assayed with and without mercaptoethanol treatment (*12*). By this assay all antiserums to T4 were 80 to 90 percent, or more, sensitive to the reducing agent, as expected of IgM antibody. Sucrose-gradient sedimentation separations were carried out on serums A002 and A003 as a direct measurement of the 19*S* antibody of each serum. Again the phage-neutralizing activity of both serums was only in the 19*S* peak—slightly faster in sedimentation velocity than that of β-galactosidase and far

Table 1. Effect of antiserum directed against allotypic specificity on phage-neutralizing activity of antiserums obtained from rabbits 8 days after a single injection of bacteriophage T4. Five microliters of antiserum to T4 were added to 1 ml of either normal rabbit serum or antiserum to the indicated allotypic specificity. This mixture was incubated ½ or 1 hour at 37°C and then at 4°C overnight before activity against T4 was assayed.

Serum	Allotypic formula	Antiserum	K_{T4} (min^{-1})	Inhibition (%)
A002	A *a*2*b*4	None*	1.3	
A002	A *a*2*b*4	A2	0.33	75
A002	A *a*2*b*4	A1	1.1	15
A002	A *a*2*b*4	A4	0.26	80
A003	A *a*1*b*4	None*	1.6	
A003	A *a*1*b*4	A1	0.96	40
A003	A *a*1*b*4	A3	1.8	−12
A006	A *a*3*b*4	None*	1.6	
A006	A *a*3*b*4	A3	0.66	59
A006	A *a*3*b*4	A1	1.8	−12
A006	A *a*3*b*4	A1†	1.6	0
A010	A *a*1*b*4.5	None*	1.3‡	
A010	A *a*3*b*4.5	A1	0.1	92
A010	A *a*1*b*4.5	A2	1.1	15
A012	A *a*2*b*4	None*	0.94‡	
A012	A *a*2*b*4	A2	0.1	89
A012	A *a*2*b*4	A1	0.85	10
A013	A *a*1*b*4	None*	0.79‡	
A013	A *a*1*b*4	A1	0.1	87
A013	A *a*1*b*4	A2	0.74	6

* Normal rabbit serum used instead of antiserum. † Normal A1$^+$ serum (5 mμ) was also added to antiserum to A1 to test for indirect effects. ‡ These values are corrected for some T4-neutralizing activity present in the normal serum used for these assays. This correction was about 0.2 K units (0.2 min^{-1}) and was subtracted from each of the final K values of the three serums to yield the values given here.

removed from the 7*S* position as marked by the phage-neutralizing activity of a high-titer antiserum to T4.

Another characteristic of IgG and IgM is the difference in antigenicity of the two immunoglobulins. Antiserum directed against the papain digest fragments I and I′ (or Fab) of IgG will react with both IgM and IgG, whereas antiserum directed against the papain fragment III (Fc) of IgG will react only with IgG (*7*). Furthermore, either antiserum to fragment I or antiserum to fragment III added in excess to high-titer (IgG) phage-neutralizing antiserum will inhibit the neutralizing activity (*15*). The addition of antiserum to fragment I to the first three 8-day antiserums to T4 followed by incubation at 37° and at 4°C resulted in the loss of 56 to 90 percent of the T4-neutralizing activity; antiserum to fragment III inhibited these antiserums to T4 less than 10 percent in two cases and 20 percent in the third case, and these inhibitions with antiserum to fragment III could easily be explained by nonspecific effects in view of the large amount of precipitation.

Thus antiserum directed against the *a*-group specificity of early antiserums against bacteriophage inhibits phage-neutralizing activity. This inhibition is apparently due to a direct action on IgM—at least that particular IgM represented by 19*S* antibodies to T4. Also, the phage-neutralizing activity of such 8-day antiserum was mainly IgM by all criteria tested; the amount of 7*S* antibody (IgG) which could be present would not be enough to modify the conclusions that the foregoing effects were due to action on IgM.

The chemical nature of the *a*-group allotypic specificities is not known at present; the specificities could be due either to variations in the protein primary structure or to variations in nonprotein moieties—perhaps carbohydrate—added to the polypeptide chains of either IgG or IgM protein. But if the *a*-group specificities are due to variations in the primary structure of the polypeptide chains, the suggestion by Todd (*1*) and again by Cohen and Porter (*6*) that the primary heavy chain actually is at least two polypeptide chains, with at least one portion common between IgG and IgM, must be very seriously considered. It should be pointed out that the two polypeptide chains might fuse to a single chain after their independent synthesis. Again, a mechanism such as suggested by Smithies *et al.* (*16*) would allow as another possibility the formation of a single polypeptide chain with its primary structure controlled by nonadjacent genetic elements. It should be possible before too long to choose from these or other suggestions to form a clearer concept of the biosynthesis of the immunoglobulins.

G. W. Stemke
R. J. Fischer
Department of Biology, Division of the Natural Sciences, and *Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania*

References and Notes

1. C. W. Todd, *Biochem. Biophys. Research Commun.* **11**, 170 (1963).
2. R. Ceppellini *et al.*, *Bull. World Health Organ.* **30**, 447 (1964).
3. S. Dray, S. Dubiski, A. Kelus, E. S. Lennox, J. Oudin, *Nature* **195**, 785 (1962).
4. A. S. Kellus, *Biochem. J.* **88**, 4P (1963); G. W. Stemke, *Science* **145**, 403 (1964).
5. S. Dubiski, J. Rapacz, A. Dubiski, *Acta Genet. Statist. Med.* **12**, 136 (1962). S. Dray, G. O. Young, L. Gerald, *J. Immunol.* **91**, 403 (1963).
6. S. Cohen, and R. R. Porter, *Adv. Immunol.* **4**, 287 (1964).
7. G. J. Thorbecke, and E. C. Franklin, *J. Immunol.* **87**, 753 (1961).
8. A. Feinstein, P. G. H. Gell, A. Kelus, *Nature* **200**, 653 (1963).
9. G. W. Stemke, thesis, University of Illinois, Urbana, 1963.
10. J. Oudin, *J. Exp. Med.* **112**, 107 (1960).
11. M. H. Adams, *Bacteriophages* (Interscience, New York, 1959), pp. 463–464.
12. J. P. Vaerman, J. F. Heremans, C. Vaerman, *J. Immunol.* **91**, 7 (1963).
13. D. C. Bauer, and A. B. Stravitsky, *Proc. Natl. Acad. Sci. U.S.* **47**, 1667 (1961); J. W. Uhr and M. S. Finkelstein, *J. Exp. Med.* **117**, 457 (1963).
14. A. S. L. Hu, R. G. Wolfe, F. J. Reithel, *Arch. Biochem. Biophys.* **81**, 500 (1959).
15. G. W. Stemke, unpublished.
16. O. Smithies, G. E. Connell, G. H. Dixon, *Nature* **196**, 232 (1962); O. Smithies, *Science* **149**, 151 (1965).
17. Supported in part by a NSF summer fellowship for graduate teaching assistants to RJF.

26 July 1965

Rotational Symmetry in Foot-and-Mouth Disease Virus and Models

Abstract. *With small, animal viruses for which electron-microscope images show little penetration by negative stain, the rotation technique for structure determination is useful. Foot-and-mouth disease virus resembles a 32-unit model when rotational images of virus and models simulating virus in negative stain are compared.*

The smaller animal viruses do not show the same resolution of morphological subunits in negatively stained preparations for electron microscopy as some other viruses of the same general size do. For example, foot-and-mouth disease virus (FMDV) and poliomyelitis virus (*1*) are not as easily penetrated by phosphotungstic acid as turnip yellow mosaic virus (*2*) and ϕX174 virus (*3*) are. To determine the probable arrangement of morphological subunits in FMDV, the rotation technique of Markham (*4*) was applied to images of highly purified virus as well as to models constructed to simulate the condition of virus in negative stain.

Foot-and-mouth disease virus, strain A119, was produced in tissue cultures of bovine kidney and purified (*5*). Virus was deposited by loop-drop on formvar films lightly coated with carbon. The grids were stained with 1 percent potassium phosphotungstate (*6*) and examined in an electron microscope (RCA-EMU-3G) with a double condenser. Selected micrographs of individual virus particles (Fig. 1) indicate that morphological units are visi-