with the non-cross-reacting antibody may be said to produce a phenotypic modification of host range: approximately 50 percent of the initial particles, which could adsorb both to B and to F, become incapable of adsorbing to F and resemble BF^- mutants for their very next host interactions. The evidence is thus clear that these serum-induced BF^- phenocopies have reacted with one or more antibody molecules in a region, presumably the tail, which is critical for host interactions. It remains to be determined whether they differ initially, before serum treatment, from the remainder of the virus particles that become inactive for B.

7) The serum-induced BF^- phenocopies are immune to a fresh input of the same absorbed serum, but they are neutralized readily by unabsorbed serum at a comparable concentration. Hence, they contain, in the critical region, unreacted antigenic sites which react specifically with cross-reacting antibody. Topographically, these unreacted sites must be separated from the reacted sites at least by a distance sufficient to allow further antibody attachment.

Thus, at least 50 percent, and probably more, of the B-infective particles in a T5 stock contain at least two spatially separated and serologically distinguishable antigenic sites in the critical region for host interactions. The experiments reveal nothing further as yet about the manner in which these sites are associated in the virus particle; they may or may not prove to reside on separable subunits. Mutation to BF^- leads to the loss of one kind of antigenic determinant not only from the critical region but from the entire antibody-reactive surface of the virus particle. The exclusion from the critical region follows, by definition, from the failure of the non-cross-reacting antibody to neutralize BF^- mutants. The exclusion from the entire reactive surface follows from the inability of BF^- mutants to remove this antibody from a T5 antiserum.

The tight correlation between the host-interacting and the antibody-binding activities both of T5 and of the BF^- mutants suggests a close relation, possibly identity, between the surface structures responsible for these activities in a given virus particle. Further analysis will be needed, however, before this correlation can be interpreted with confidence. Nevertheless, we can now interpret, or approach from a stronger vantage point, such puzzling phenomena as the dependence of the serum-survivor assay on the assay host, the dependence also of the shape of the neutralization curve on the assay host, and the dependence of these host effects on the particular antiserum employed.

Finally, the demonstration of an antibody that can react with critical sites on a virus particle without neutralizing its infectivity for all hosts means that caution must henceforth be exercised in deciding the locus of action of a "non-neutralizing" antibody.

FRANK LANNI

Department of Bacteriology,
Emory University, Atlanta, Georgia

References and Notes

1. P. M. Rountree, *Australian J. Exptl. Biol. Med. Sci.* 30, 567 (1952).
2. R. I. DeMars *et al.*, *Ann. inst. Pasteur* 84, 113 (1953).
3. F. Lanni and Y. T. Lanni, *Cold Spring Harbor Symposia Quant. Biol.* 18, 159 (1953).
4. A. R. Fodor and M. H. Adams, *J. Immunol.* 74, 228 (1955); A. R. Fodor, *ibid.* 79, 227 (1957).
5. T. F. Anderson, *Am. Naturalist* 86, 91 (1952).
6. G. Streisinger, *Virology* 2, 377 (1956).
7. Y. Tanami and Y. Miyajima, *J. Bacteriol.* 72, 721 (1956); G. Streisinger, *Virology* 2, 388 (1956).
8. This study was supported in part by a research grant (E-857) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. The principal experiments herein reported were described at the 41st annual meeting of the American Association of Immunologists, Chicago, April 1957, but were not included in the covering abstract.
9. F. Lanni and Y. T. Lanni, *Bacteriol. Proc. Soc. Am. Bacteriologists* 1956, 51 (1956).
10. ———, *Federation Proc.* 16, 421 (1957).

19 May 1958

Vulcanization with Tetramethylthiuram Disulfide

Three principal hypotheses have been advanced in efforts to explain the efficiency with which rubber is vulcanized by tetramethylthiuram disulfide (TMTD) in the presence of zinc oxide: (i) formation of carbon-to-carbon crosslinks, in analogy with vulcanization by tertiary peroxides (*1, 2*) or as a consequence of radical addition to the rubber (*2, 3*); (ii) "copolymerization" of disulfide biradicals with olefin double bonds (*4, 5*); (iii) oxidation of a mercaptan formed by attack of a disulfide radical derived from TMTD on the hydrocarbon (*6*).

An outline of a fourth mechanism is described in this report (*7*). Only known reactions, or those for which a closely analogous reaction is known, are required. The mechanism involves the same final step as that of Bielstein and Scheele (*6*). The preceding steps are different, being derived from a symmetrical dissociation of the reagent instead of the unsymmetrical dissociation postulated by these authors.

Table 1. Stoichiometric relations in TMTD vulcanization.

Ratio	Predicted	Found	Reference
$R_2NCS_2^-$ formed/ TMTD taken	4/3	1.32	(*15*)
S combined/ crosslinks	2/1	1.9	(*16*)
S combined/ TMTD taken	2/3	0.61	(*13*)
ZnO required/ TMTD taken	1/1	1.1 –1.2	(*17*)
H_2O formed/ TMTD taken	1/3	0.33–0.50	(*18*)
Crosslinks formed/ TMTD taken	1/3	0.26 0.28	(*16, 19*) (*20*)

The reaction occurs in three stages. The first is a radical reaction of TMTD with the olefin:

Initiation:

$$\mathrm{R_2NCS_2S_2CNR_2 \rightleftharpoons 2R_2NCS_2} \qquad (1)$$

Chain carrying:

$$\mathrm{R_2NCS_2\cdot + R'H \rightarrow R_2NCS_2H + R'\cdot} \qquad (2)$$

$$\mathrm{R'\cdot + R_2NCS_2\,S_2CNR_2 \rightarrow R'S_2CNR_2 + R_2NCS_2\cdot} \qquad (3)$$

where R′H is rubber hydrocarbon and R is methyl.

Evidence for free radical activity of TMTD exists in its effects on vinyl polymerization (*8*). The stoichiometry of reactions 1 to 3 follows directly from the experiments of Moore (*9*) with benzothiazyl disulfide. (An alternative set of chain-carrying reactions involving addition at the double bonds is not completely excluded by the available evidence, but reactions 2 and 3 are presently preferred on general grounds.)

The second stage is hydrolysis of the dithiocarbamate ester. In this view the hitherto mysterious role of zinc oxide in vulcanization is seen to result simply from the solubility of basic zinc salts in rubber. Although zinc oxide per se does not react directly with the intermediate sulfur compound, we may write the reaction formally as

$$\mathrm{ZnO + R'S_2CNR_2 \rightarrow R'SZnOC(S)NR_2} \qquad (4)$$

to illustrate the essential features of this stage: (i) the carbon-sulfur bond of the vulcanizing reagent is broken by hydrolysis and (ii) with a sufficient excess of basic salt, the rubber hydrocarbon-sulfur reaction product is present as mercaptide.

Finally, the mercaptide is oxidized by TMTD (*5, 10*).

$$\mathrm{R'S^- + R_2NCS_2\cdot \rightarrow R'S\cdot + R_2NCS_2^-} \qquad (5)$$

$$\mathrm{2R'S\cdot \rightarrow R'SSR'} \qquad (6)$$

As with reaction 4, reaction 5 is a formal representation of the oxidation step. The actual mechanism may involve radical exchange in the presence of base (*11*) rather than the free radical oxidation implied by Eq. 5.

The over-all equation is:

$$\mathrm{2R'H + 3R_2NCS_2S_2CNR_2 + 3ZnO \rightarrow R'SSR' + 2Zn(S_2CNR_2)_2 + Zn(OCSNR_2)_2 + H_2O} \qquad (7)$$

All of these products except the oxythiocarbamate have been observed; the COS detected by Craig, Diller, and Rowe (*12*) may be derived from it. Experimentally determined stoichiometric ratios are summarized in Table 1, for comparison with Eq. 7.

No attempt is made at present to specify the structure of the crosslinks formed, which may depend on temperature. The instability of olefinic disulfides at curing temperatures is well known (*13, 14*).

E. M. Bevilacqua
United States Rubber Company,
Wayne, New Jersey

References and Notes

1. E. H. Farmer, *Trans. Faraday Soc.* 38, 356 (1942); G. Gee, *J. Polymer Sci.* 2, 451 (1947).
2. N. Bergem, *Vulcanization* (A/S Askim Gummivarefabrik, Askim, Norway, 1947).
3. A. Jarrijon, *Rev. gén. caoutchouc* 20, 155, 177 (1943).
4. D. Craig, *J. Polymer Sci.* 20, 197 (1956).
5. D. Craig *et al.*, *ibid.* 8, 321 (1952).
6. G. Bielstein and W. Scheele, *Kolloid-Z.* 147, 152 (1956).
7. A detailed discussion of this mechanism is in preparation.
8. T. E. Ferington and A. V. Tobolsky, *J. Am. Chem. Soc.* 77, 4510 (1955).
9. C. G. Moore, *J. Chem. Soc.* 1952, 4232 (1952).
10. D. M. French and E. J. Hart, unpublished.
11. E. R. Bertozzi, F. O. Davis, E. M. Fettes, *J. Polymer Sci.* 19, 17 (1956).
12. D. Craig, D. Diller, E. H. Rowe, *ibid.* 28, 435 (1958).
13. B. C. Barton, unpublished.
14. See review by L. C. Bateman, R. W. Glazebrook, C. G. Moore, R. W. Saville, *Proc. Rubber Technol. Conf., 3rd Conf., London* (1954), p. 298.
15. W. Scheele, *Kolloid-Z.* 146, 14 (1956).
16. E. M. Bevilacqua, *J. Polymer Sci.* 28, 651 (1958).
17. This stoichiometry, originally observed by B. C. Barton (1944), has been confirmed over a range of TMTD concentrations.
18. Determined in the system squalene-TMTD-zinc oxide by direct titration of water with Karl Fischer reagent.
19. At 150°C. It should be noted that the maximum efficiency of crosslinking will be obtained only under mild conditions. At normal "curing" temperatures (130° to 160°C) in the absence of added fatty acid or zinc soap, TMTD may be wasted in side reactions. The concentration of so called "sulfide" sulfur in a rubber-TMTD-zinc oxide mixture after vulcanization is a direct measure of the side reactions, as is a low ratio of sulfur combined to TMTD taken.
20. M. L. Studebaker, *Rubber Chem. and Technol.* 30, 1400 (1957).

16 May 1958

Hydrothermal Recrystallization of Molybdenum Trioxide

Whisker-like crystal growth in metals has been the object of several investigations, especially within the past few years (*1*). Not until very recently has whisker growth in an oxide system been reported (*2*).

In this investigation, whisker-like crystals were grown in the system MoO_3–SiO_2 under hydrothermal conditions. Homogeneous MoO_3–SiO_2 solid mixtures were prepared by "flash-drying" a solution of ammonium heptamolybdate in ammonia-stabilized Ludox (*3*). This was accomplished by dropping the solution, a drop at a time, into a platinum crucible that was heated to a dull red.

It was found that whisker growth could be carried out by placing small ampules containing the MoO_3–SiO_2 solid, together with capillary tubes filled with water, directly into a controlled-temperature furnace. A temperature of 375°C, a steam pressure of 5 atm, and a hydrothermal treatment time of 72 hours produced abundant whisker growth from a 3 weight percent MoO_3–97 weight percent SiO_2 substrate.

A photomicrograph of a MoO_3–SiO_2 solid which was subjected to hydrothermal treatment is shown in Fig. 1*A*. An electron micrograph of a sample of these whiskers is shown in Fig. 1*B*. Their size was observed to vary widely: diameters of 5 to 50 μ and lengths up to about 5 mm were typical.

Physical property measurements were not undertaken as a part of this investigation. However, it was qualitatively observed that MoO_3 whiskers could be bent over a large radius of curvature with no apparent permanent deformation. If the bending were continued, whiskers tended to split longitudinally rather than break along the shorter dimension.

Samples of the whiskers were separated from the MoO_3–SiO_2 substrate and subjected to x-ray diffraction, infrared spectrometer, and arc-emission spectrographic analysis. As a result of these analyses, it was concluded that the whiskers consisted of high-purity MoO_3.

In one series of experiments, films of SiO_2 were prepared by grinding flash-dried Ludox silica sol with enough water to form a paste and spreading the resulting paste on platinum slides. Similar films of MoO_3 were prepared by spreading MoO_3–H_2O paste on the slides. In one of these experiments a film of SiO_2, about 1 mm thick and 2 cm long, was laid down in such a way as to cover one half of a slide. On the other half of the slide a MoO_3 film of similar dimensions was laid down. The two films were in contact. The films were rapidly dried in a furnace at 480°C, and the slide supporting the films was subjected to hydrothermal conditions (400°C and 4.85 atm steam pressure) for 96 hours. The slide was then examined with the aid of a microscope. Whiskers were observed over the entire length of the SiO_2 film. No whiskers were observed on the MoO_3 film.

In a similar experiment but with the SiO_2 and MoO_3 films separated by a 2-mm gap, no whiskers were observed on either the SiO_2 or MoO_3 films.

These data indicate that the migration of molybdena in the growth of MoO_3 whiskers occurs via the solid phase rather than by a vapor-phase process. The silica substrate is believed to play a unique part in the molybdena transport and crystal-growth process. This is suggested by the fact that whiskers were observed on the silica substrate but not on the molybdena substrate in the film migration study.

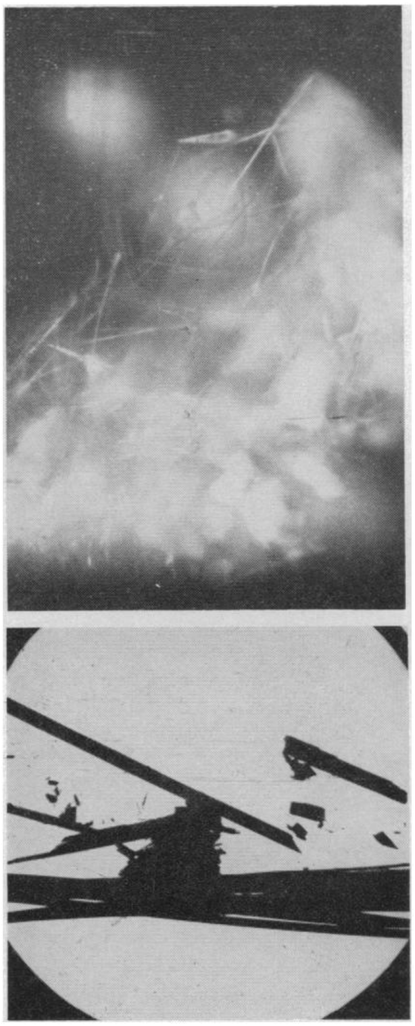

Fig. 1. (Top) Photomicrograph of MoO_3 whiskers grown from a MoO_3–SiO_2 substrate (about × 80). (Bottom) Electron micrograph (2800X) of MoO_3 whiskers (about × 2200).

James L. Callahan*
Ralph H. Petrucci
Charles A. Brown†
Department of Chemistry, Western Reserve University, Cleveland, Ohio

References and Notes

1. S. S. Brenner, *Acta Met.* 4, 62 (1956); K. G. Compton, A. Mendizza, S. M. Arnold, *Corrosion* 7, 327 (1951); J. D. Eshelby, *J. Appl. Phys.* 24, 176 (1953); R. M. Fisher, L. S. Darken, K. G. Carroll, *Acta Met.* 2, 386 (1954); C. Herring and J. K. Galt, *Phys. Rev.* 85, 1060 (1952); S. E. Koonce and S. M. Arnold, *J. Appl. Phys.* 25, 135 (1954); M. O. Peach, *ibid.* 23, 1401 (1952); E. F. Riebling and W. W. Webb, *Science* 126, 309 (1957).
2. W. W. Webb and W. D. Forgang, *J. Appl. Phys.* 28, 1449 (1957).
3. Ludox is an experimental silica sol obtained from E. I. duPont de Nemours and Company.

* Present address: Sohio Research Laboratory, Cleveland, Ohio.

† Present address: Advanced Phosphor Development Laboratory, General Electric Company, Cleveland, Ohio.

7 May 1958