Holt and Jacobs (*11*) were examined, no fluorescence bands were found, at either room temperature or at liquid-nitrogen temperature, which could be considered to correspond to the 715 mμ band found for concentrated solutions. (These crystals were described as "non-fluorescent," but we noted a weak fluorescence band at 680 mμ. It cannot belong to the crystals, whose absorption band lies at > 720 mμ; it may have been due to the presence of a small amount of colloidal material, with an absorption band at 670 mμ.)

In the cell, the fluorescence band at 720 mμ could conceivably be due to a new molecular species, such as chlorophyll *d*, having an absorption and a fluoresence band on the long-wavelength side of those of chlorophyll *a* (as suggested in the case of *Porphyridium*). However, alcoholic extracts from *Porphyridium* showed no fluorescence band at 720 mμ, at either + 20°C or − 193°C. This supports the hypothesis that the 720-mμ band is due to an aggregated form of chlorophyll *a*.

Kok (*12*) and Coleman *et al.* (*13*) have observed a "difference" band at 705 mμ in the absorption spectra of dark and illuminated cells of *Chlorella* (and some other algae). This may mean that the 720-mμ fluorescence band is correlated with an absorption band close to this position.

The great increase in intensity of the 715-mμ fluorescence band in vitro (and of the 720-mμ fluorescence band in the cell) at low temperature could be due either to an increase in the relative *concentration* of the aggregates or to an increase in their *yield of fluorescence*. The latter is not impossible if the new fluorescence band originates in a metastable level, which at room temperature is too rapidly used up by photochemical reactions, collision deactivation, and internal conversion, to emit fluorescence. At low temperatures, all these deactivation processes are slowed down. Lifetime determination, and other quantitative studies, should permit a check of this hypothesis.

The new energy level could correspond to a triplet or nπ level. The new level would lie closer to the singlet ππ excited state in the aggregate than in the monomer.

In vivo, the chlorophyll fluorescence yield increases (according to Franck, French, and Puck, *14*) and the absorption in the neighborhood of 705 mμ decreases (according to Kok, *12*) with incident light intensity, until the saturation of photosynthesis is reached. These changes can be related to changes in the concentration of aggregated chlorophyll in light. When the intensity of the incident light increases, some aggregates may break up into monomers. This would cause a decrease in absorption at 705 mμ and an increase in chlorophyll fluorescence at room temperature (if the monomeric form of chlorophyll is fluorescent at all room temperatures, while the aggregated form only becomes flourescent at low temperatures).

Possibly the drop in photosynthesis on the long-wavelength side of the chlorophyll absorption band (*15*) may be due to increased absorption of light energy by the aggregated form of chlorophyll. The absorption band leading to the new level should be located at about 700 mμ. Because of the strong selective scattering in this region (*16*), it would not be easy to identify. The most careful measurements of absorption spectra of live cells do not exclude the possibility that the red absorption band contains more than one component. To utilize the energy stabilized in the new energy level (aggregated chlorophyll), light of shorter wavelengths may be required—perhaps, to produce a doubly excited state, as suggested by Franck (*17*) (*18*).

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Immunogenetic Dissection of the T5 Bacteriophage Tail

Studies of the antigenic structure of several bacterial viruses (*1–4*) have led to the recognition of two non-cross-reacting surface antigens, which are designated the head and tail antigens, respectively, according to their apparent localization in the virus particle (*3*). Since the ability to react with neutralizing antibody appears to be an exclusive property of the phage tail (*2, 3*), which contains also the sites of attachment to host cells (*5*), specific neutralization and host adsorption afford tools for studying the structure and function of this virus organelle. The relation between the host-attachment sites and the antigenic sites of the tail has not yet been fully settled, but in some instances the sites show a close genetic association (*6*).

Several lines of evidence (*4, 7*) have suggested that the virus tail itself may be antigenically heterogeneous; however, evidence is yet to be presented that one and the same virus particle can react through different critical sites with two dissimilar neutralizing antibodies. This report is a brief description of experiments (*8*) which supply this kind of evidence for phage T5.

The principal conclusion, that this virus contains at least two distinct antigenic determinants in that portion of the virus particle which is sensitive to neutralizing antibody, rests on the following facts, some of which have been reported previously.

1) Phage T5 stocks form plaques with equal efficiency on *Escherichia coli* strains B and F (smooth derivative of FCb) (*9*).

2) Either of these hosts can adsorb the bulk (over 99 percent) of the plaque formers for both. This fact is crucial to the conclusion.

3) All T5 stocks contain readily isolated host-range mutants, designated BF^-, which adsorb onto and infect strain B but not F (*9*).

4) These mutants cross-react reciprocally with T5 in specific neutralization, but both of the homologous reactions are stronger than the cross reactions (*10*).

5) The cross-reacting antibody can be removed readily from a T5 antiserum (rabbit) by absorption with BF^-, leaving behind a large fraction of antibody (about 50 percent as measured by neutralization of T5 for strain F) which has a striking behavior against phage T5. Like the original serum, it neutralizes the infectivity for strain F in an almost exponential fashion down to 1 percent survivors and beyond; but the infectivity of the same sample for B, instead of decreasing progressively, levels off and remains at about 50 percent, while the infectivity for F vanishes. The leveling off is not due to antibody depletion, since a fresh input of T5 is neutralized just as is the first input.

6) The stable survivors infective for strain B no longer adsorb to F (the adsorption rate constant is less than 0.3 percent of that for untreated T5). They adsorb to B at about half the initial rate. When allowed to grow on B, they produce progeny that are again capable of infecting F. Hence, treatment of T5

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.

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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:

$$R_2NCS_2S_2CNR_2 \rightleftharpoons 2R_2NCS_2 \quad (1)$$

Chain carrying:

$$R_2NCS_2\cdot + R'H \rightarrow R_2NCS_2H + R'\cdot \quad (2)$$

$$R'\cdot + R_2NCS_2S_2CNR_2 \rightarrow R'S_2CNR_2 + R_2NCS_2\cdot \quad (3)$$

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

$$ZnO + R'S_2CNR_2 \rightarrow R'SZnOC(S)NR_2 \quad (4)$$

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*).

$$R'S^- + R_2NCS_2\cdot \rightarrow R'S\cdot + R_2NCS_2^- \quad (5)$$

$$2R'S\cdot \rightarrow R'SSR' \quad (6)$$

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:

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

All of these products except the oxythiocarbamate have been observed; the COS