duplex") rather than the two strands of a single Watson-Crick helix (a "primitive duplex"). L. F. Cavalieri's work pointed to the interesting possibility of such an occurrence (*Biophys. J.*, **1**, 317 (1961). C. Shooter, R. Baldwin, and R. Inman, however, give data showing strong evidence to the effect that a primitive duplex underlies the Meselson-Stahl experiments.

[10] Longuet-Higgins, H. C., and B. H. Zimm, *J. Mol. Biol.*, **2**, 1 (1960); Ore, A., and E. Pollard, *Science*, **124**, 430 (1956).

[11] Meselson, M., and F. W. Stahl, these PROCEEDINGS, **44**, 671 (1958); Taylor, J. H., P. S. Woods, and W. L. Hughes, these PROCEEDINGS, **43**, 122 (1957).

[12] Related to a paper on "Nucleic Acids and Information Transfer" by L. F. Cavalieri and B. H. Rosenberg (preprint), and their work concerning a double-weight helix; Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963).

[13] Wilkins, M. H. F., *et al.*, *J. Mol. Biol.*, **2**, 19 and 38 (1960).

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*REPLICATIVE FRAGMENTATION IN T4 PHAGE: INHIBITION BY CHLORAMPHENICOL**†

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Reproduction of the T-even phage, known to undergo several rounds of mating in the vegetative pool inside the infected bacterial host, is an example of a semiconservative type of DNA replication which is paralleled by its fragmentation and rejoining.[1, 2] This is in contrast to a copy choice mechanism which would not lead to fragmentation of parental DNA. The parental label contribution to a single progeny phage is no more than 7 per cent, incorporated into the progeny molecule as a semiconservative fragment (or fragments). It has been proved that the parental fragment, incorporated into the progeny polynucleotide strand, is integrated, i.e., that there are no breaks in the polynucleotide chain at either end of the parental segment.[3] The system involved in the exchanges during mating provides a mechanism for repairing the integrity of the polynucleotide chain. Whether these exchanges are the exclusive affair of DNA molecules or whether a specific enzyme is involved was unknown.

This paper will demonstrate that fragmentation of the parental phage DNA, as described above, can be inhibited by chloramphenicol (CM) which, however, does not affect semiconservative replication. A catalyst, presumably an enzyme, is required for fragmentation.

Materials and Methods.—(*a*) The bacterial strain used in these experiments was *E. coli* B. The bacteriophage used was an osmotic shock resistant mutant $T_4BO_1^r$. Synthetic TCG medium (light, heavy, and radioactive), along with methods of purification, assays of bacteriophage, techniques of CsCl density gradient centrifugation, and methods for fraction collection have been previously described.[1, 3]

(*b*) The net synthesis of DNA was measured as the uptake of P^{32} into the alkali-resistant fraction obtained after fractionation of infected bacteria by the Schmidt-Thannhauser procedure.[4]

(*c*) DNA extraction from bacteriophage was performed as described before.[3] The intracellular phage DNA was extracted as follows: samples of infected bacteria,

taken at different times after infection, were chilled in ice, centrifuged, and the sediment washed once and resuspended in 0.1 *M* EDTA 0.15 *M* NaCl—pH 8.0. T4 carrier phage was added to the final concentration of 1.5×10^{12}/ml and duponol to a final concentration of 0.5%; the suspension was lysed at 37°C for approximately 3 min and then extracted with phenol, as previously described.[3]

(*d*) Sucrose gradients (5–20%) were prepared in 0.15 *M* NaCl 0.015 *M* sodium citrate, pH 7.6. Samples containing no more than 5 μg of DNA were layered over each gradient; the gradient was then overlaid with paraffin oil and centrifuged for 3 hr at 25,000 rpm. Fractions were collected in a manner similar to that used with CsCl gradient analyses.

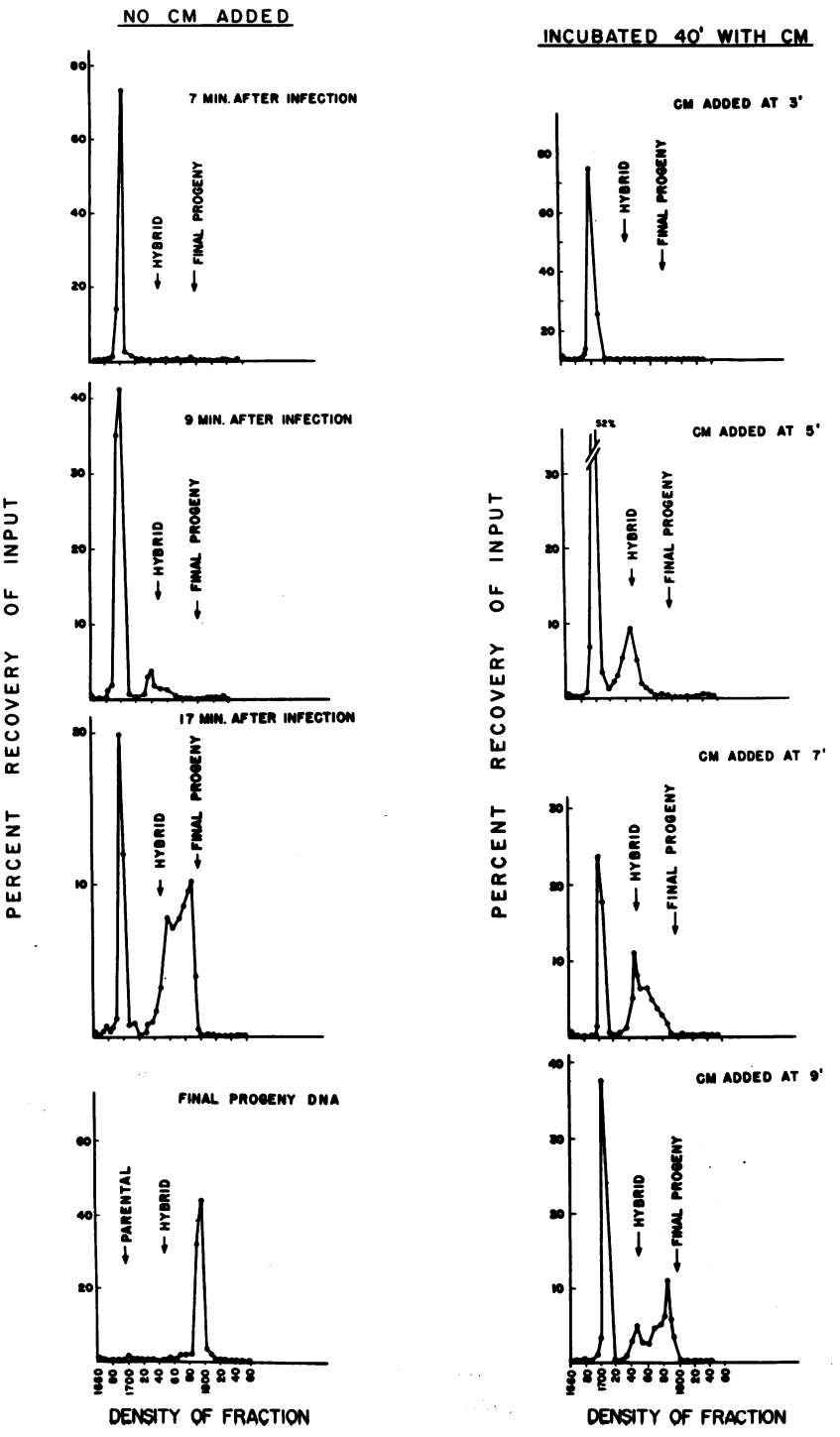
Results.—Intracellular replication of phage DNA in the presence of chloramphenicol: It has been shown that chloramphenicol (CM), if added 6 or more min after infection of bacteria with T-even phage, prevents maturation of the phage but allows production of phage DNA. DNA formed in the presence of CM is incorporated into the progeny phage if the antibiotic is withdrawn from the system.[5–7]

It has also been shown, with a double density labeling system in which light, radioactive phage was used for the infection of heavy 5-bromodeoxyuridine (5BU) labeled cold bacteria, that after infection parental DNA undergoes gradual changes of density which are associated with fragmentation. This leads to the dispersion of 5–7 per cent of the parental material in the resulting progeny phage.[3]

The alteration induced in this pattern of replication by the presence of CM is described in this section.

Heavy bacteria in heavy medium were infected with light, radioactive (specific activity 4.0 mc/mg of P) phage (m = 3 phage per bacterium).[8] Immediately after infection the bacterial suspension was separated into 5 flasks and CM, at a concentration of 100 μg/ml, was added to 4 flasks at 3, 5, 7, and 9 min, respectively, after infection. One flask was left without the addition of CM as a control. For DNA extraction samples of the bacterial suspension were taken from the control flask at 0–17 min and at 0–40 min in the flasks with CM. Infective centers, background unadsorbed phage, and unadsorbed P^{32} were estimated in all samples. In order to determine the rate of DNA synthesis, the same bacterial suspension utilized in the experiment described above, was infected with cold phage (m = 3); P^{32} was added at the time of infection and the suspension was divided into several flasks containing CM, as in the experiment just described. Samples of the bacterial suspension were taken at different times in order to estimate the amount of newly produced DNA, as already described in *Materials and Methods*. The results of both experiments are shown in Figures 1 and 2.

Integrity of the replicative molecule: As shown in Figures 1 and 2, CM inhibits the fragmentation of parental phage DNA, allowing its semiconservative replication. The resulting replicative molecule could represent a total phage genome which replicated without breaks, or its replication could be restricted to only a part of the parental DNA. In the latter case, replicative DNA should be composed of molecules smaller than the parental and, if this were so, all infected bacteria should contain some newly synthesized DNA, representing presumably partial replicas of the phage genome. This possibility was investigated in experiments in which the molecular integrity of the parental DNA in the replicative peak was analyzed by sucrose gradient centrifugation.


NO CM ADDED
INCUBATED 40' WITH CM
7 MIN. AFTER INFECTION
9 MIN. AFTER INFECTION
17 MIN. AFTER INFECTION
FINAL PROGENY DNA
CM ADDED AT 3'
CM ADDED AT 5'
CM ADDED AT 7'
CM ADDED AT 9'
HYBRID
FINAL PROGENY
PARENTAL
52%
PERCENT RECOVERY OF INPUT
DENSITY OF FRACTION

A bacterial culture was infected with T4 phage and treated with CM 7 min after infection. Forty min later intracellular DNA was extracted and fractionated in a CsCl gradient. The fractions were diluted to a volume of 0.5 ml with 0.015 *M* Na citrate–0.15 *M* NaCl (CS) and samples were withdrawn for P^{32} estimations. The pattern of replication in this experiment when CM was added 7 min after infection strongly resembled that obtained with the addition of CM 5 min after infection, as shown in Figure 1. The "peak" fraction of replicative DNA was dialyzed against CS and analyzed in a sucrose gradient. DNA extracted from the parental phage was analyzed in the same way. The results are shown in Figure 3. Since material obtained from the replicative peak and from the parental phage DNA both bonded in the sucrose gradient in the same way, these results do not support the hypothesis of partial replication of the phage genome.

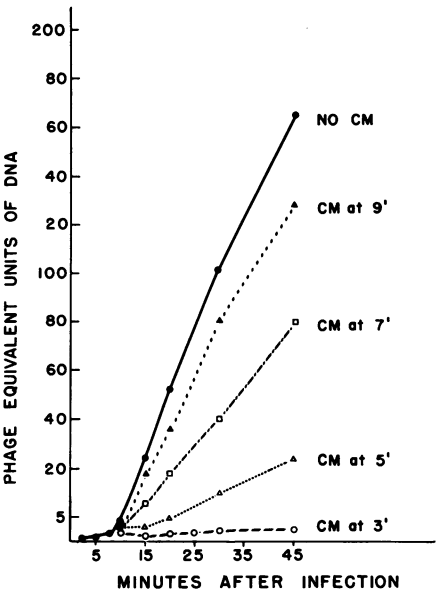


FIG. 2.—Synthesis of DNA in an infected bacterial population in the presence and absence of CM.

Lack of exchanges between DNA's of the simultaneously infecting phage: It has been shown that extensive fragmentation of parental DNA occurs in the presence of FUDR despite the fact that DNA production is very small.[3] It is important to know whether exchanges occur between parental molecules or whether they occur *pari passu* with replication. To test the possibility of exchange between simultaneously infecting parental molecules, the following experiment was performed. Light, cold bacteria were infected simultaneously with light, P^{32}-labeled (m = 1) and heavy, cold (m = 10) bacteriophage. At different times after infection, DNA was extracted from infected bacteria. (In this case, the carrier added during the extraction was a mixture of equal parts of light and heavy phage.) DNA was analyzed in a CsCl gradient. It will be noted that, when there were exchanges between parental phage, displacement of the radioactive label toward the heavy side of the gradient would occur. The results, shown in Figure 4, are presented as an integral graph in order to emphasize the low amount (not exceeding 1%) of radioactivity in the sum of the fractions between the hybrid and heavy DNA locations in the gradient.

Discussion.—Under normal conditions of infection the DNA of T4 phage replicates semiconservatively and becomes fragmented so that the final progeny contains 5–7

FIG. 1.—Intracellular replication of T4 DNA. The replication of T4 DNA in the presence of CM after a 40-min incubation at 37°C is represented on the right side of the graph. CM was added at the indicated times. The pattern of replication in the control group, which was run simultaneously, represents stages of DNA replication at the times of addition of CM. The density distribution of P^{32}-containing phage DNA, derived from the control group, is shown at the bottom of the control group. (Note: The proportions and density of the replicating peak in samples taken 17 min after infection, which are not shown on the graph, were identical to those taken at 40 min.)

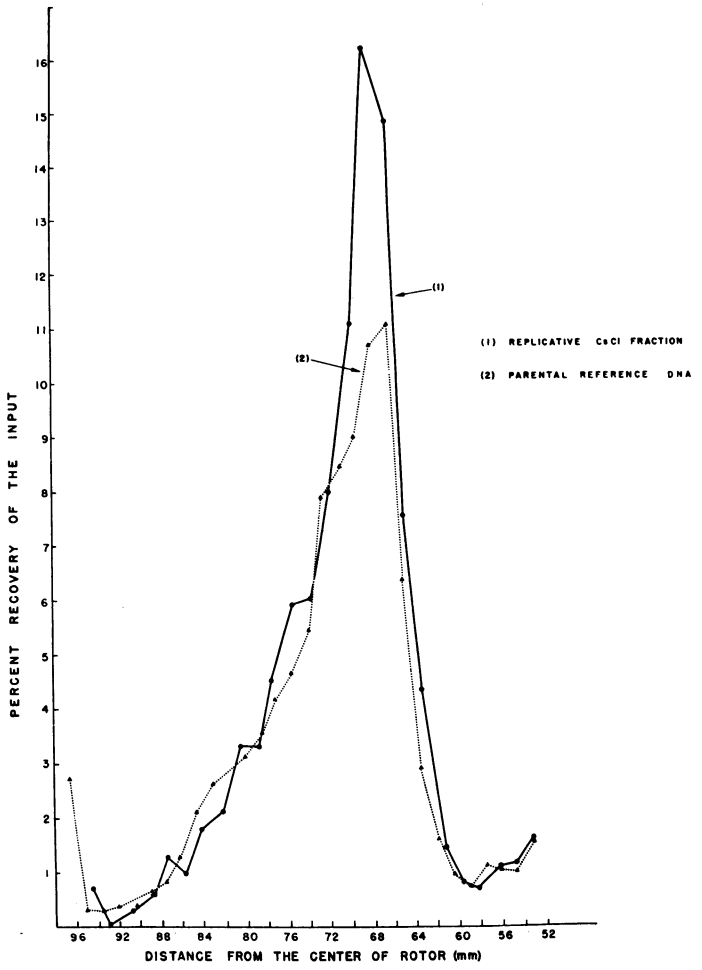


FIG. 3.—Sucrose gradient fractionation of extracted parental DNA and DNA derived from CsCl fractions corresponding to the semiconservative peak.

per cent of the parental material. Present experiments have shown that, when CM is added soon after infection, some factor responsible for the fragmentation of T4 DNA, presumably an enzyme, is affected. CM inhibits the DNA fragmentation process. It was observed that the delay of 3–5 min is necessary for the expression of the drug.

The best known activity of CM is the inhibition of protein synthesis. Therefore, in our interpretation of CM's inhibitory effect upon fragmentation of T4 we should justifiably assume that it is the *production* of the "fragmenting" enzyme that is inhibited, not its action. This enzyme should then be in duced or coded by T4 phage DNA at times later than 5 or 6 min after infection. The comparison of the degree of fragmentation achieved when the drug was added at 5, 7, or 9 min after infection (see Fig. 1) indicates that CM does not inhibit the action of the enzyme. This last statement is based upon the pattern of fragmentation observed at 7 or 9 min, the times when CM was added, when no fragmentation was yet no-

ticeable, whereas after incubation in the presence of the drug there was extensive fragmentation. The significance of this enzyme in the genetic recombination seems obvious.

The total amount of replicating DNA (the sum of the DNA of any density which can be clearly separated from the conservative parental DNA) was the same when the CM was added 5–9 min after infection. At the same time, estimation of the production of the new DNA indicates that bacteria treated with CM 5 min after infection produced only one fourth to one fifth the amount of DNA produced by bacteria which received CM 9 min after infection. This may lead to the assumption that a threshold of DNA concentration is required inside the bacteria for efficient mating of the phage DNA. However, this is an unlikely hypothesis since the equilibrium between nonreplicating and replicating DNA and its final extent of fragmentation in media containing CM is achieved within 17 min after the addition of CM and does not change, even when the total amount of the net synthesis shows a threefold increase.

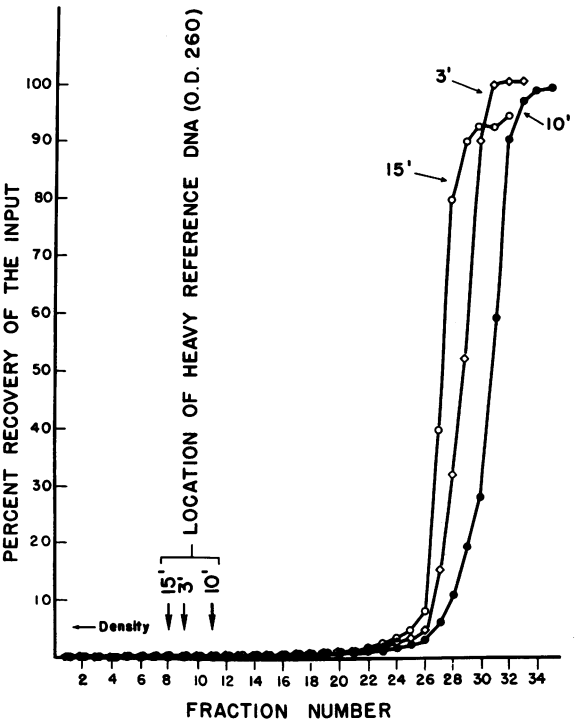


FIG. 4.—Distribution of P^{32} in intracellular DNA after simultaneous infection with light, radioactive, and heavy, cold parental phage. The light, radioactive phage was infected at a multiplicity of 1.0; the heavy, cold phage at 10.0. The arrows indicate the location of heavy DNA, as determined by optical density measurements.

Moreover, in previous experiments, where DNA synthesis was inhibited by FUDR, efficient fragmentation occurred despite the fact that there was only residual DNA synthesis. Also, in the absence of CM there was an almost final extent of fragmentation at about 17 min after infection (see Fig. 1) when only 20–35 phage equivalent units of DNA had been produced (Fig. 2). These results indicate that the lack of fragmentation is not caused by the presence of a small pool of DNA but by the absence of a specific catalyst responsible for the process.

Taking an opposite point of view, let us assume that a partial activation of DNA molecules occurs in the presence of CM (added soon after infection). This activation might be followed by the appearance of a partial replica of the activated segment of DNA and by the process of breaking off and separation of the replicating area. If this were the case, then the replicative DNA pool would have to be composed of partial replicas and be devoid of certain genes. In addition, the molecular weight of the replicating subunit would be smaller than that of the parental DNA. However, the experiments summarized in Figure 3 indicate an identical size for

both molecules. Thus, the hybrid separated in CsCl gradient represents DNA molecules that are the same size as the parental molecules. These results bear similarity to those of experiments described before[3] which indicated that parental phage DNA maintains its integrity within infected bacteria, and with the effects of CM on the molecular size of newly formed T2 DNA.[9] In the latter paper, some DNA molecules of a size larger than a one phage equivalent unit were described. It may be postulated that the enzyme responsible for fragmentation was missing in this last system. Moreover, the fact that a molecule larger than a one phage equivalent was not found in the present experiments does not eliminate the possibility that larger molecules were sheared preferentially during preparation of the DNA.

These results throw a new light on the interpretation of previously described experiments[7] in which extensive exchanges between DNA formed at different times in the presence of CM were found in the resulting progeny obtained after removal of the drug. In the present experiments the matured progeny from the DNA pool formed when CM was added 5 or 7 min after infection with bacteria (i.e., when no fragmentation had occurred) is never semiconservative but is dispersed to the level corresponding to the control progeny phage not exposed to CM during multiplication. This indicates that extensive fragmentation and exchanges occur *after* removal of the drug.

The last set of experiments eliminates the possibility that exchanges occur between simultaneously injected, nonreplicating parental molecules, since we were unable to detect any class of molecules having the altered density expected for interparental recombinants.[10] This is in contrast to the results obtained with lambda phage[11] when a class of recombinants containing double-stranded subunits of both parental phages was described. However, no DNA analysis of this phage was performed, and the presence of DNA fragments not materially associated with but incorporated into a protein coat could not be excluded.

Summary.—Chloramphenicol (CM), an inhibitor of protein synthesis, prevents DNA of T4 bacteriophage from fragmentation but permits its semiconservative replication. Apparently CM inhibits an enzyme responsible for recombination between molecules.

The semiconservative replicative moiety of DNA (in CM), analyzed in sucrose zone centrifugation, indicates similarity of size with conservative DNA.

No recombination between input parental molecules could be detected, suggesting that recombination occurs *pari passu* with replication of DNA.

* This work was supported by National Science Foundation grants GB-1 and G-23643.

† The following abbreviations or idioms will be used: CM—chloramphenicol; CS—A solution of 0.015 *M* sodium citrate in 0.15 *M* NaCl, pH 7.6; DNA—deoxyribonucleic acid; EDTA—disodium ethylenediamine tetraacetate; 5BU—5-bromodeoxyuridine; FUDR—5-fluorodeoxyuridine; "Hot"—labeled with radioactive isotope; "Cold"—not labeled with radioactive isotope; "Heavy"—substituted with heavy density marker 5BU; "Light"—not substituted with heavy density marker 5BU; Replicative DNA—A moiety of parental, labeled DNA which, due to acceptance of a new strand of different density, can be clearly separated in CsCl gradient from the conservative moiety.

[1] Kozinski, A. W., *Virology*, **13**, 124 (1961).

[2] Kozinski, A. W., and M. Bessman, *J. Mol. Biol.*, **3**, 746 (1961).

[3] Kozinski, A. W., and P. B. Kozinski, *Virology*, **20**, 213 (1963).

[4] Schmidt, G., and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945).
[5] Tomizawa, T., and S. Sunakawa, *J. Gen. Physiol.*, **39**, 553 (1956).
[6] Hershey, A. D., and N. E. Malechen, *Virology*, **3**, 207 (1957).
[7] Kozinski, A. W., and H. Uchida, *J. Mol. Biol.*, **3**, 267 (1961).
[8] Experiments in which light host and heavy phage have been used or in which there is a low multiplicity of infection resulted in the same pattern of DNA replication.
[9] Frankel, F., these PROCEEDINGS, **49**, 366 (1963).
[10] This is also true for the system in which single radioactive light phage, inactivated by UV, has been rescued by multiple infection of heavy viable phages.
[11] Meselson, M., and J. J. Weigle, these PROCEEDINGS, **47**, 857 (1961).

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*RECONSTITUTION OF IMMUNOLOGIC ACTIVITY BY INTERACTION OF POLYPEPTIDE CHAINS OF ANTIBODIES**

BY G. M. EDELMAN, D. E. OLINS, J. A. GALLY, AND N. D. ZINDER

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In a previous publication[1] it was proposed that the capacity of antibodies to combine specifically with different antigens might result from interactions of separate polypeptide chains in the antibody molecule. 7S antibody molecules contain two types of polypeptide chains:[1-4] L (light) chains with molecular weights of approximately 20,000 and H (heavy) chains with molecular weights in the neighborhood of 60,000.[5,6] L chains are contained in the active fragments produced by hydrolysis of antibodies with papain.[4-7] The L chains of antibodies of different specificities differ in their patterns of separation by starch gel electrophoresis in urea.[8] Moreover, L chains are the common structural elements[4, 9] of the various classes of γ-globulins (γ, γ_{1A}, γ_{1M}), all of which are known to contain antibodies.

These observations prompted the suggestion that "similar or different L chains interact through weak forces with each other or singly with H chains to form the combining region."[1] The present communication describes some experimental results which support this hypothesis. Most of the experiments were performed using antibacteriophage antibodies since the assay of phage neutralization measures activity over a wide range with high sensitivity. After separation of the L and H chains of specifically purified guinea pig antibodies directed against f1 bacteriophage and f2 bacteriophage, the neutralizing activity of the chains declined to low levels. Mixing the L and H chains resulted in partial restoration of activity. Confirmatory data were obtained using antibodies directed against the dinitrophenyl (DNP) group, measuring the binding of this hapten by equilibrium dialysis. Similar results on the reconstitution of equine antibodies have been reported recently by Franěk and Nezlin.[10,11]

Materials and Methods.—Proteins, antigens, and immunization procedures: Pure f1 bacteriophage was obtained as described by Zinder *et al.*[12] The methods of isolation of pure f2 bacteriophage have also been described.[13] Guinea pig γ-globulin and rabbit γ-globulin were isolated by zone electrophoresis of serum on starch.[14] Dinitrophenyl-bovine serum albumin was prepared using the method of Farah *et al.*[15] The procedure of immunizing the guinea pigs has been detailed.[8] Each animal received initial injections in the footpads of a total of 0.4 mg of bacteriophage in