[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).

---

*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

THE ROCKEFELLER INSTITUTE

Communicated by Theodore Shedlovsky, July 24, 1963

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

complete Freund's adjuvant. Subsequent intradermal injections of 0.4 mg of bacteriophage were given weekly in the skin of the back.

Isolation of antibodies: Except as noted, the antibodies were obtained from the sera of individual guinea pigs. Anti-DNP antibodies were isolated as described previously.[8] Purified antibodies directed against f2 phage were isolated using the method of Singer *et al.*[16] The same method was employed to purify the antibodies to rabbit γ-globulin. The following procedure was used to purify antibodies to f1 phage: immune precipitates formed near equivalence were washed three times with phosphate buffer, pH 7.0, $\Gamma/2$, 0.2. They were then treated with glycine sulfate buffer, pH 2.4, $\Gamma/2$, 0.35 to dissociate the complexes, and the preparations were centrifuged for 90 min at 78,000 $\times$ g to remove the phage. The supernate was neutralized with phosphate buffer, pH 7.0, $\Gamma/2$, 1.0, and dialyzed against 0.15 N NaCl. After centrifugation to remove the small amount of precipitate formed, the preparation was concentrated by ultrafiltration.

Tests for the purity of the antibody preparations included immunoelectrophoresis and double diffusion in agar which were performed as previously described.[4]

Reduction and alkylation of the purified antibodies and separation of L and H chains: Reduction and alkylation followed the description of Edelman and Poulik.[2] $^1/_2$ % protein solutions were reduced for 2 hr at room temperature in phosphate buffer, pH 7.0, $\Gamma/2$, 0.04 made 0.1 M in 2-mercaptoethanol. The reaction mixtures were made 0.2 M in iodoacetamide, and after 10 min at room temperature they were dialyzed against 0.5 M propionic acid.

Separation of the chains followed the procedure of Fleischman *et al.*[17] Five to 10 mg of reduced alkylated antibody was filtered through 100 $\times$ 1.5 cm or 100 $\times$ 1 cm columns of Sephadex G-100 in 0.5 N propionic acid. The sample was applied in 1.0–1.5 ml volumes, the flow rate was 4.0–8.0 ml per hour, and 2.0 ml fractions were collected. The eluate was monitored by measuring absorbancy at 280 mμ. The purity of the fractions was tested by starch gel electrophoresis in urea.[2] Selected fractions obtained by gel filtration were pooled while still in propionic acid and dialyzed at 4°C against several changes of phosphate buffer, pH 7.0, $\Gamma/2$, 0.04 made 0.15 N in NaCl. In most of the mixing experiments, the L and H chain fractions were pooled in the ratio of the yield of absorbancy units given by the gel filtration patterns. When L or H chain fractions of two different antibody preparations were mixed, all of the dissociation and separation procedures were carried out simultaneously.

For clarity in presentation, the original specificity of the antibodies from which the chains are derived is denoted in parentheses next to the designation of the chain type, e.g., L(f1) or H(f1). Homologous mixtures are denoted: L(f1) + H(f1). Hybrid mixtures are denoted: L(f1) + H(f2) or L(f2) + H(f1).

Assay of bacteriophage neutralization: This assay method has been described in detail.[18] The assumption is that, in antibody excess, phage inactivation follows the relationship: $-\ln (p/p_0) = Kct$. p_0 is the plaque count at zero time; p is the plaque count at t min; c is the antibody concentration in units of absorbancy at 280 mμ, and K, the rate constant, is expressed per minute per unit absorbancy at 280 mμ. f1 and f2 phage neutralization was found to obey this relation over several decades of neutralization (see Fig. 3). Phage concentrations and dilutions were chosen so that at any point in the determination at least 200 plaques of residual phage could be counted in each replicate plate. This high plaque number diminishes the sampling error.

Equilibrium dialysis: One to 2.5 mg of the protein in 2.5 ml of 0.15 N NaCl were placed in one chamber of the apparatus. To the chamber on the opposite side of the membrane (Visking 23/32 casing) 2.5 ml of 0.15 N NaCl was added. 250 μl of 3.9 $\times$ 10^{-5} M C^{14} dinitrophenol (specific activity 1μc/μM) was added to each chamber and the apparatus was placed at 4°C for 48 hr without stirring. 100 μl aliquots from each compartment were counted in a liquid scintillation counter, and 24 hr later additional 100 μl aliquots were counted to ensure that equilibrium had been reached. The fluid from the compartment containing only hapten was replaced by 2.5 ml of 0.15 N NaCl and after 72 hr at 4°C, 250 μl aliquots from each chamber were counted. This procedure was repeated one more time. The data were plotted as difference in counts per minute across the membrane against the ratio between this difference and the counts per minute on the side containing only hapten. After extrapolation to infinite hapten concentration, it was calculated that approximately 0.5 sites were occupied per mole of native antibody.

Results.—The purified antibodies directed against f1 bacteriophage and f2 bacteriophage were free of contaminating serum proteins. This was shown by im-

munoelectrophoretic analyses using rabbit antisera against whole guinea pig serum and against isolated guinea pig γ-globulin (Fig. 1). The immunologic and starch gel electrophoretic analyses indicated that the antibodies were of the 7S γ class.

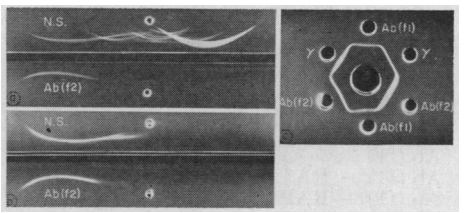

FIG. 1.—Immunoelectrophoresis and double diffusion in agar of preparations of purified guinea pig antibodies. (*a*) Immunoelectrophoresis using rabbit antiserum directed against guinea pig serum; (*b*) immunoelectrophoresis using rabbit antiserum directed against guinea pig γ-globulin; (*c*) double diffusion in agar. Central well contained rabbit antiserum directed against guinea pig serum. γ = guinea pig γ-globulin; Ab (f1) = antibodies against f1 phage; Ab (f2) = antibodies against f2 phage; N.S. = normal guinea pig serum.

Two patterns of separation of the chains of partially reduced alkylated antibodies were obtained after filtration. The first type (Fig. 2*A*) showed two incompletely resolved peaks followed by a well-resolved peak. The second type (Fig. 2*B*) showed no resolution of the first two peaks. Both types of patterns were found for different antibody preparations directed against f1 phage and f2 phage. Depending on the pattern obtained, the H chain fraction was taken either to be the second peak (Fig. 2*A*) or the trailing portion of the first peak (Fig. 2*B*). The L chain fraction consisted of the major portion of the well-resolved and most retarded peak in each case. As shown by starch gel electrophoresis in urea (Fig. 2*C*), this fraction appeared to be free of H chains and of undissociated material. The H chain fractions contained material of higher molecular weight which was also present in earlier fractions, regardless of the type of separation pattern.

Phage neutralization by anti-f1 antibodies, by the separated chain fractions, and by mixtures of the chain fractions is illustrated in Figure 3. Reduction and alkylation of the antibodies caused slight diminution of phage-neutralizing activity. A

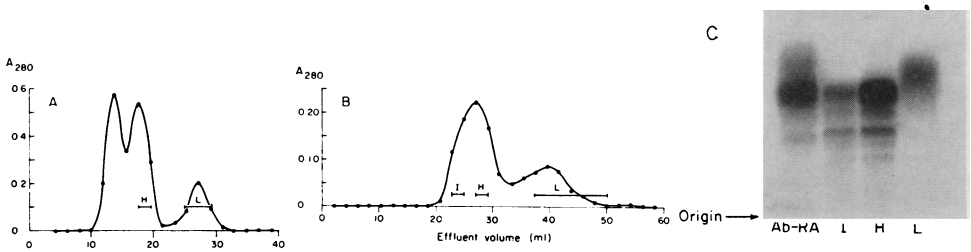

FIG. 2.—Separation of H and L chain fractions from reduced alkylated antibodies by gel filtration on Sephadex G-100 in 0.5 *N* propionic acid. (*A*) Reduced alkylated anti-f1 antibodies; (*B*) reduced alkylated anti-f2 antibodies; (*C*) starch gel electrophoresis of fractions from Fig. 2*B*. I = first fraction; H = H chain fraction; L = L chain fraction; Ab-RA = reduced alkylated antibodies; A_{280} = absorbancy at 280 mμ.

greater loss of neutralizing activity occurred when partially reduced alkylated antibody was exposed to 0.5 *N* propionic acid for the same time and at the same temperature as the sample that was subjected to gel filtration. L(f1) chains showed little or no neutralizing activity wheras H(f1) chain fractions showed low levels of activity. The mixture of chains showed enhancement of activity of about fourfold over that expected if there were no interaction.

Results of a similar experiment in which homologous and hybrid mixtures of chains from anti-f1 and anti-f2 antibodies were compared are given in Table 1. At the levels tested, there was no cross-reactivity of the two antibody systems or of their separated chain fractions. In both systems, reduction and alkylation led to a drop in neutralizing activity. Reduced alkylated antibodies exposed to propionic acid showed a marked drop in activity. Both H(f1) and H(f2) chain fractions had some residual activity, whereas the L chains were minimally active in both cases.

TABLE 1

RECONSTITUTION OF ACTIVITY OF ANTIPHAGE ANTIBODIES

Sample	K (per minute per unit absorbancy at 280 mμ) f1 neutralization	f2 neutralization
Ab (f1)	5,300	0
Ab (f1) – RA*	1,900	0
Ab (f1) – RAP*	190	0
H (f1)	19	0
L (f1)	0.9	0
Ab (f2)	0	230
Ab (f2) – RA	0	140
Ab (f2) – RAP	0	58
H (f2)	0	9.3
L (f2)	0	1.9
H(f1) + L(f1)	53 (9.6)†	0
H(f1) + L(f2)	16 (9.6)	0.4
H(f2) + L(f1)	0.2	11 (4.6)
H(f2) + L(f2)	0	14 (4.6)

* Abbreviations: Ab = antibody (Ab (f1) and Ab (f2) were isolated from two different guinea pigs); RA = reduced alkylated; RAP = reduced and alkylated and exposed to propionic acid for the same length of time as the separated fractions.

† Numbers in parentheses refer to activity expected from the amount of H chain fraction present in the mixture.

Homologous mixtures of H(f1) and L(f1) fractions showed a fivefold enhancement of activity over that expected for the amount of H(f1) chains present in the mixture. H(f1) + L(f2) hybrid mixtures showed a twofold enhancement, whereas H(f2) + L(f1) hybrids were inactive against f1 bacteriophage. In the f2 system, H(f2) + L(f2) mixtures showed a threefold enhancement of activity; hybrid H(f2) + L(f1) mixtures showed a twofold enhancement. H(f2) + L(f2) and H(f1) + L(f1) mixtures were active only against the homologous antigens.

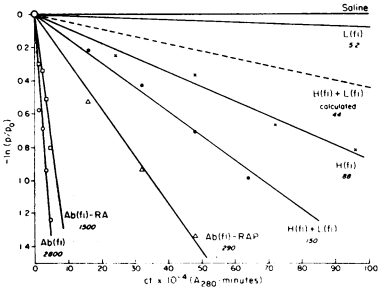

FIG. 3.—Phage neutralization by anti-f1 antibodies, chain fractions, and mixtures. Ab (f1) = antibody against f1 phage isolated from pooled sera of two animals; Ab (f1) RA = reduced alkylated antibody; Ab (f1) RAP = reduced alkylated antibody exposed to 0.5 *N* propionic acid; H (f1) = H chain fraction; L (f1) = L chain fraction. Numbers in italics refer to K, the rate constant expressed in dimensions of per minute per unit absorbancy at 280 mμ (see *Materials and Methods*). The measured points determining the line for L (f1) fall outside the graphed region and are not shown.

In a separate experiment, H(f2) + L(f2) mixtures were found to be ten times as effective in neutralization as mixtures of H(f2) with L chains from purified guinea pig antibodies directed against rabbit γ-globulin. Hybrid mixtures of H(f2) chain fractions with L chains of the nonspecific γ-globulin from the same animal were only one seventh as effective as homologous H(f2) + L(f2) mixtures.

Results consistent with those shown in Figure 3 and Table 1 were found upon measuring the phage-neutralizing activity of each tube obtained by gel filtration of reduced alkylated anti-f1 antibodies (Fig. 4). The highest activity was found in the material from the first peak. Less activity was found in the H chain fraction, and another small peak of activity was found in the region between the

H and L chain fractions. No activity was seen in the L chain fraction itself. The high activity in material from the first peak was correlated with the presence of incompletely dissociated antibodies as shown by starch gel electrophoresis. The region between the H and L fractions would be expected to contain both types of chains and therefore also the reconstituted activity.

In several other experiments phage neutralization was observed when reconstitution was attempted using antibodies of completely unrelated specificities as starting material. For example, L(f1) chains from certain preparations were found to neutralize f2 phage, and this neutralization was enhanced when either L(f1) + H(f1) or L(f1) + H(f2) mixtures were used. Furthermore, the mixture H (DNP) + L (DNP) showed measurable amounts of phage neutralization (Table 2), although the neutralization by Ab (DNP) and the H (DNP) and L (DNP) chains was at much lower levels. It should be stressed, however, that the neutralization of f2 phage by mixtures of chains from apparently unrelated antibodies was at lower levels than that of mixtures of the chains of homologous antibodies. Moreover, H chain fractions from nonspecific guinea pig γ-globulin showed low levels of neutralization ($K = 0.3$).

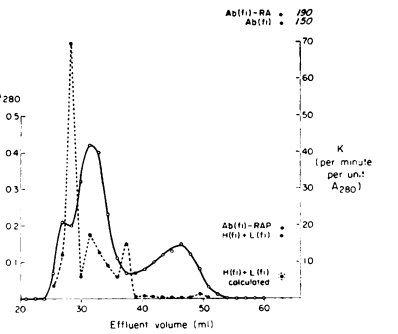

FIG. 4.—Activity in chain fractions of anti-f1 antibodies (Sephadex G-100 in 0.5 N propionic acid). O——O absorbancy at 280 mμ; ●——● activity in terms of K. On right are given the K values of the untreated antibodies Ab (f1), the reduced alkylated antibodies Ab (f1) RA, and the chain mixture H(f1) + L(f1). The antibodies were isolated from pooled sera of four animals.

TABLE 2

f2 PHAGE NEUTRALIZATION BY MIXTURES OF CHAINS FROM ANTI-f2 AND ANTI-DNP ANTIBODIES

Sample	K (per minute per unit absorbancy at 280 mμ)	
Ab (f2)	860	
Ab (f2) − RAP*	3.2	
H (f2)	3.6	
L (f2)	0.17	
Ab (DNP)	0.05	
H (DNP)	0.13	
L (DNP)	0.13	
H(f2) + L(f2)	29.0	(1.8)†
H(f2) + L(f2) saline‡	6.5	(1.8)
H(f2) + L(DNP)	9.6	(1.9)
H(DNP) + L(f2)	0.08	(0.15)
H(DNP) + L(DNP)	3.1	(0.13)

* RAP = reduced and alkylated; exposed to 0.5 N propionic acid.

† Numbers in parentheses refer to activity expected from the amount of H chain fraction present in the mixture.

‡ Fractions were mixed after dialysis against 0.15 N NaCl + phosphate buffer pH 7.0, $\Gamma/2$, 0.04.

TABLE 3

BINDING OF C^{14} DINITROPHENOL BY ANTI-DNP ANTIBODIES AND THEIR POLYPEPTIDE CHAINS

Sample	cpm bound†
Ab	900
Ab − P*	790
Ab − RA*	710
Ab − RAP*	480
H(DNP)	380
L(DNP)	<200
H(DNP) + L(DNP)	580‡

* Abbreviations: P = exposed to 0.5 N propionic acid; RA = reduced and alkylated; RAP = reduced and alkylated and then exposed to 0.5 N propionic acid; Ab = antibody.

† cpm bound = counts per minute calculated to be bound per unit absorbancy of protein at 280 mμ after extrapolation to infinite hapten concentration.

‡ The maximal value expected if the contributions of H and L chains were only additive is 340 cpm per unit absorbancy at 280 mμ. The ratio of absorbancies of H chains to L chains in the mixture was 4:1.

As shown in Table 2, the mixture of H(f2) and L(f2) chains after dialysis against saline was less effective in phage neutralization than mixture in propionic acid followed by dialysis. Otherwise, the results were similar to those in Table 1.

Reconstitution of antibody activity in a system measuring binding directly was observed using purified anti-DNP antibodies. The results of an equilibrium dialysis experiment in which C^{14} dinitrophenol was the hapten are given in Table 3. Reduction and alkylation of the antibody or exposure of untreated antibody to 0.5 *N* propionic acid resulted in slight diminution of binding activity. As in the experiments utilizing antiphage antibodies, exposure of the reduced alkylated antibody to propionic acid resulted in a considerable drop in activity. The H chain fraction retained some binding affinity as did the L chain fraction. The H (DNP) + L (DNP) mixture showed an increase in affinity of 1.7 times that expected if the H (DNP) fraction and L (DNP) fraction were binding without interaction. The H (DNP) + L (DNP) mixture showed 64 per cent of the binding of untreated antibody. The native antibodies, chain fractions, and mixtures all had association constants of the same order of magnitude (approximately 10^6 l/mole).

Discussion.—The aim of the present study was to determine whether chain interaction influences the activity and specificity of antibodies. For this purpose, we chose three unrelated systems of purified guinea pig antibodies: anti-f1 phage, anti-f2 phage, and anti-DNP. The antibodies, their separated L and H chains, and homologous and hybrid mixtures of the chains were tested for activity by assay of phage neutralization and in some cases by equilibrium dialysis. Before discussing the implications of the experimental results, we wish to stress that the two assay methods may not test the same functions of antibodies. The assay of phage neutralization is a kinetic test which measures binding, but in addition may depend upon functions of the antibody molecule other than binding. Equilibrium dialysis measures only the binding of hapten to proteins under conditions of thermodynamic equilibrium. This method has the advantage of testing binding directly, whereas the neutralization assay has the advantage of measuring an enormous range of activities with great sensitivity.

As measured by both methods, specific antibody activity was partially restored by recombination of homologous H and L chain fractions, and these results strongly support the chain interaction hypothesis. Although only about 1–6 per cent of the original activity of native antiphage antibodies was recovered, the mixtures of H and L chain fractions were 2–10 times more active than any of the unmixed fractions. The level of activity reconstituted in the mixtures was approximately the same as the amount of activity remaining in reduced alkylated antibodies that had been exposed to 0.5 *N* propionic acid.

Considerably greater reconstitution was obtained in the DNP system, as measured by equilibrium dialysis. Up to 60 per cent of the original hapten-binding capacity was restored by mixing the L and H chain fractions. The difference in the degree of reconstitution of the antihapten antibodies and antiphage antibodies may result from differences in the heterogeneity of their polypeptide chains.[8] It may depend also upon the more stringent requirements of the phage assay methods, as emphasized above.

In the experiments on the antiphage antibodies, the purity of the L chain fractions was indicated by starch gel electrophoresis, as well as by the fact that they had no activity. On the other hand, starch gel electrophoresis of the H chain fractions showed the presence of small amounts of incompletely dissociated material. This material may be responsible for the residual activity found in H chain fractions,

in view of the relatively high activities found in material from the first peak of the filtration pattern shown in Figure 4. Although this material may still contain interchain disulfide bonds, the mixing experiments indicate that interchain disulfide bonds are not absolutely required for partial reconstitution of activity.

Homologous mixtures of chains (e.g., L(f1) + H(f1) using f1 phage as test antigen) were consistently more effective in reconstituting activity than hybrid mixtures (e.g., L(f1) + H(f2) or L(f2) + H(f1)). Most of the hybrid mixtures showed reconstitution only when H chains from antibodies originally directed against the test antigen were used. This was also observed by Franěk and Nezlin[10] who concluded that the antibody specificity is determined by the H chain but the presence of the L chain is required for the formation of a fully active combining site. This conclusion would be fully warranted if L chains of different origin were equally effective in reconstituting specific activity. The experiments revealed, however, that homologous L chains are more effective than heterologous L chains. This indicates that L chains also contribute to the specificity, a conclusion consistent with both the heterogeneity and the structural differences found among L chains of antibodies of different specificities.[1, 8]

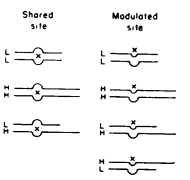

FIG. 5.—Hypothetical models of chain interaction (× marks the vicinity of one antibody combining site).

The activity and specificity of antibodies thus appears to be a complex function of the structure and interaction of both H and L chains. There are two different modes by which an active site might be generated through chain interaction, as illustrated diagramatically in Figure 5. Both types of chains might contribute amino acid residues directly to the site (shared site). On the other hand, interaction of one chain with another might result in formation of a stable binding region on only one of the chains (modulated site). In addition, both modes may operate together but to a different extent in different antibodies.

The diagrams shown in Figure 5 are based on the assumption that the 7S antibody molecule consists of two L and two H chains.[3–6] If the molecule consists of four L and two H chains, the same basic mechanisms of interaction could occur. The present experimental data exclude the possibility of shared or modulated sites exclusively between two H chains. Among the remaining possibilities, the most probable would seem to be shared or modulated interaction between L and H chains.

The type of experiment described here may not permit one to draw definite conclusions about the relative contributions of the L and H chains to the specificity. For example, a modulated site on an H chain[17] might have been generated by interaction with one particular type of L chain. Stabilization of the active conformation may then arise from intrachain interactions (including disulfide bonds). Subsequent experimental removal of the L chain would not necessarily destroy the active conformation completely. Since L chains have certain common structural features,[3, 4] interaction of the H chains with L chains of different origin might lead to different degrees of reconstitution.

Specific combining activity can result from interaction of more than one type of L chain with H chains derived from an antibody. This suggests that specific combining activity may arise from a variety of different chains. Several of the present observations indicate that such complex interactions are possible. Signifi-

cant levels of f2 phage neutralization were obtained with L (DNP) + H (DNP) mixtures, although the native anti-DNP antibody showed no neutralizing activity. In some experiments, L(f1) chains neutralized f2 phage, and the neutralization was enhanced by mixing L(f1) chains with either H (f1) or H(f2) chain fractions. In contrast, none of the chain fractions or mixtures from anti-f2 antibodies neutralized f1 phage. H chains from normal γ-globulins were also found to neutralize f2 phage. In all of these experiments the neutralizing activity was lower than that of homologous mixtures of chains from anti-f2 antibodies.

These observations suggest that γ-globulins and antibodies originally showing no specificity for certain test antigens can yield chains which interact to form complexes capable of binding to those antigens. Classical notions of immunologic specificity imply that the test antigen and the immunogen have the same or closely related structures. It is not known whether the noncross-reactive antigens used in the present study share any structural features. Moreover, the test antigen may alter the conformation of interacting L and H chains derived from antibodies originally unrelated to the test antigen. Alternatively, among any given set of L and H chains, a great variety of interactions may be possible, and some may be capable of binding selectively to certain antigens. Experiments to decide among the various possibilities are in progress.

Summary.—Dissociation of purified guinea pig antibodies against f1 phage, f2 phage, and the dinitrophenyl group into L and H polypeptide chains led to a marked drop in the activity of the separated fractions. Mixture of the L and H chain fractions of the same antibody preparation resulted in partial reconstitution of activity as measured by phage neutralization or equilibrium dialysis. Reconstitution of activity against a given antigen was also found using mixtures of H chains from antibodies originally directed against that antigen and L chains from unrelated antibodies or γ-globulin. In every case, however, the reconstitution of activity was greatest when both L and H chains from the homologous antibodies were mixed. Mixtures of L chain fractions from homologous antibodies and H chain fractions from unrelated antibodies usually showed no activity. In some cases, however, activity was observed when L and H chains from antibodies originally unrelated to the test antigen were mixed.

The results support the chain interaction hypothesis of antibody activity and suggest that both H and L chains contribute to immunologic specificity.

* Supported by USPHS grant A-4256 from the National Institute of Arthritis and Metabolic Diseases.

[1] Edelman, G. M., and B. Benacerraf, these PROCEEDINGS, **48**, 1035 (1962).

[2] Edelman, G. M., and M. D. Poulik, *J. Exptl. Med.*, **113**, 861 (1961).

[3] Edelman, G. M., and J. A. Gally, *J. Exptl. Med.*, **116**, 207 (1962).

[4] Olins, D. E., and G. M. Edelman, *J. Exptl. Med.*, **116**, 635 (1962).

[5] Edelman, G. M., in *Abstracts of Papers*, **2A**, 143rd meeting, American Chemical Society, Cincinnati, Ohio, January, 1963.

[6] Porter, R. R., in *Abstracts of Papers*, **1A**, 143rd meeting, American Chemical Society, Cincinnati, Ohio, January, 1963.

[7] Edelman, G. M., B. Benacerraf, and Z. Ovary, *J. Exptl. Med.*, **118**, 229 (1963).

[8] Edelman, G. M., B. Benacerraf, Z. Ovary, and M. D. Poulik, these PROCEEDINGS, **47**, 1751 (1961).

[9] Edelman, G. M., *Ann. N. Y. Acad. Sci.*, **101**, 246 (1962).

[10] Franěk, F., and R. S. Nezlin, *Folia Microbiologica*, **8**, 128 (1963).

[11] Franěk, F., and R. S. Nezlin, *Biokhimia*, **28**, 193 (1963).
[12] Zinder, N. D., R. Valentine, M. Roger, and W. Stoeckenius, *Virology*, in press (1963).
[13] Loeb, T., and N. Zinder, these PROCEEDINGS, **47**, 282 (1961).
[14] Kunkel, H. G., *Methods Biochem. Anal.*, **1**, 141 (1954).
[15] Farah, F. S., M. Kern, and H. N. Eisen, *J. Exptl. Med.*, **112**, 1195 (1960).
[16] Singer, S. J., J. E. Fothergill, and J. R. Shainoff, *J. Am. Chem. Soc.*, **82**, 565 (1960).
[17] Fleischman, J. B., R. H. Pain, and R. R. Porter, *Arch. Biochem. Biophys.*, Suppl. 1, 174 (1962).
[18] Adams, M. H., *Bacteriophages* (New York: Interscience Publishers, 1959), p. 463.

THE ULTRAVIOLET PHOTOCHEMISTRY OF DEOXYURIDYLYL (3′→5′) DEOXYURIDINE

BY C. W. HELLEINER,* M. L. PEARSON, AND H. E. JOHNS

ONTARIO CANCER INSTITUTE, AND DEPARTMENT OF MEDICAL BIOPHYSICS, UNIVERSITY OF TORONTO

Communicated by M. Delbrück, July 19, 1963

The work of Wacker *et al.*[1] and Smith[2] has shown that, when uracil is irradiated with ultraviolet light (UVL), it is converted into two major photoproducts, viz., 5-hydro-6-hydroxyuracil (the hydrate) and dimers in which two uracil moieties are linked by a cyclobutane ring connecting their 5 and 6 carbon atoms. Grossman[3] has shown that, when polyuridylic acid is irradiated with UVL, its coding properties are altered when it is used as a messenger in an *in vitro* polypeptide synthesizing system. In this system it is not clear whether the observed effects are due to the production of uracil hydration products or dimers, or both. Investigating this question with polyuridylic acid is not easy. We have therefore studied the photochemistry of the model compound, deoxyuridylyl (3′→5′) deoxyuridine (dUpU), which we assumed would have similar photochemistry to UpU but which is much easier to synthesize. Wierzchowski and Shugar[4] investigated the photolysis of uracil dinucleotides and found effects which can now be understood in terms of the formation of intramolecular dimers as well as hydration products of uracil.

In the present paper the photoproducts produced on the irradiation of radioactive dUpU were separated by paper chromatography. Four major photoproducts were found, and the rates of production of these have been studied as a function of wavelength.

Materials and Methods.—Irradiations were carried out with a high intensity UV monochromator to be described in detail elsewhere. The instrument used as dispersing elements a water preprism between the source (BH6, Hg arc) and entrance slit, and a blazed grating (blaze wavelength 200 mμ) with dimensions 25.6 × 20.6 cm. The entrance and exit slits were 15 cm high and 0.6 cm wide, and the dispersion was 4 mμ per cm at the exit slit. Light from the exit slit was collected by an $f/1.2$ mirror and focused at the center of a stoppered quartz cuvette.

dUpU, labeled with P^{32} at an initial specific activity of approximately 10 mc/mmole, was prepared by condensation of 3′-O-acetyldeoxyuridine-5′-phosphate (made by a combination of the methods of Tener[5] and Smrt and Sorm[6]) with 5′-O-trityldeoxyuridine[6] in the presence of dicyclohexylcarbodiimide (Gilham and