SPECIFICITY IN THE COMBINATION OF Fd FRAGMENTS WITH L CHAINS TO FORM HAPTEN-BINDING SITES*

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The separated heavy (H) and light (L) polypeptide chains of the γG-immunoglobulin[1] molecule can recombine to form a unit having physical properties similar to the original globulin molecule (1, 2). In the case of specifically purified antibody against either the *p*-azobenzenearsonate or the *p*-azobenzoate group, we have shown (3) that the recombined H and L chains result in a good recovery of antibody activity when both chains were derived from antibody from the same rabbit but not when the chains were derived from different rabbits even though both sera were directed against the same haptenic group (unless perhaps the chains are derived from related rabbits). There is also a specificity in the combination such that, in a mixture of hapten-specific and nonspecific H and L chains, there is preferential combination of those chains which combine to give good hapten-binding activity (4).

Little is known concerning the interactions involved in this combination of the chains or the basis of the specificity of recombination.

In the work reported here we have separated the L chains and Fd[1] fragments of specifically purified antihapten antibody and have found that the Fd fragments, like the H chains from which they are derived, have only low hapten-binding activity even though they are more soluble than H chain. Furthermore, the Fd fragments will recombine with the L chains either from intact antibody or from the Fab[1] fragment to form a unit similar to the Fab fragment by immunoelectrophoretic and sedimentation criteria and these recombinants show good recovery of hapten-binding activity.

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[1] The following abbreviations are used: The 7S γ-globulin is designated by IgG or γG-immunoglobulin as suggested by the memorandum on "Nomenclature for human immunoglobulins" in the *Bull. World Health Organ.*, 1964, **30**, 447. The γG-immunoglobulin *fraction* of an antiserum, for example, of anti-Xp serum, is designated as IgG_{Fr} (anti-Xp); of normal rabbit serum as IgG_{Fr} (NRS). The corresponding specifically purified *antibody* is designated as IgG_{Ab} (anti-Xp). The antibody-depleted IgG-fraction of an anti-Xp antiserum is designated as IgG_{Fr} (depleted anti-Xp).

Xp stands for *p*-azobenzoate. Anti-Xp refer to the corresponding antibody or serum.

An important observation was that Fd fragments derived from the Fab fragments in either fraction I or fraction II, when combined with L chains from fraction I (or from fraction II or even from the whole antibody) give good hapten-binding activity. The L chains (from all three sources) will also combine effectively with H chains from the whole antibody. However, neither these Fd fragments nor L chains gave good antibody activity when mixed with L or H chains, respectively, from a pool of antibody from a different group of rabbits in spite of the fact that all were of the same haptenic specificity and combination did take place to form Fab type fragments.

Thus, the structural features of the H chain which are required for the specific and preferential combination with L chains to give good hapten-binding sites are on the Fd portion of the H chain and do not involve the Fc portion of the chain to any important extent.

Materials and Methods

Specific Antibody and Antibody-depleted γG-Immunoglobulin.[1]—The γG-immunoglobulin fraction of serum was prepared by a sodium sulfate fractionation procedure similar to that of Kekwick (5) but using successive sodium sulfate concentrations of 18, 14, and 12½%. This procedure yields a product containing essentially pure γG-immunoglobulin as judged by ultracentrifugal and immunoelectrophoretic analyses.

Antiserum from rabbits injected repeatedly with a *p*-azobenzoate–bovine-γ-globulin conjugate was used as a source of specific antibody (6).

Specifically purified anti-*p*-azobenzoate antibody ($IgG_{Ab}(anti\text{-}X_p)$)[1] was isolated from the γG-immunoglobulin fraction of anti-Xp serum by means of a solid adsorbent consisting of a conjugate of an insoluble polymer of rabbit serum albumin and *p*-azobenzoate (Xp-poly-RSA) as previously described (4, 7). The antibody-depleted γG-immunoglobulin resulting after the specific adsorption was found to be essentially free of anti-Xp antibody by both radioimmunoelectrophoresis and by hapten-binding measurements. This is referred to as IgG_{Fr} (depleted anti-Xp).[1]

Reduction and Alkylation of γG-Immunoglobulin.—γG-Immunoglobulin was reduced with 0.2 M mercaptoethanol and alkylated with an equivalent amount of iodoacetamide, all at pH 8 (8). The preparations were then dialyzed against cold saline followed by dialysis against the solution dictated by the subsequent procedure.

Papain Digestion of γG-Immunoglobulin and Chromatography.—γG-Immunoglobulin, either before or after reduction and alkylation, was digested with papain under conditions similar to those described by Porter (9). Some digests were examined in the analytical ultracentrifuge and only 3.5S material was found. Digests were fractionated on carboxymethylcellulose (CMC) according to Porter (9) but employing a different gradient (10).

Fractionation and Concentration of Solutions of L Chains, H Chains, and Fd fragments.—Separation of H and L chains as well as L chains and Fd fragments was carried out in the cold on Sephadex G-100 equilibrated with 1 M propionic acid (3, 8). The eluates were alternately dialyzed against 40 volumes of cold distilled water and pervaporated in the cold until the desired concentration was achieved. The concentrated solution was finally dialyzed against 0.01 M propionic acid. Solutions containing several mg of protein per ml were obtained in this manner.

Protein Concentration.—The absorbancy (A) of the protein solutions at 280 mμ in 1 M propionic acid was used as a basis for calculating protein concentrations. The value of $A^{1\%}_{1cm}$

for γG-immunoglobulin was taken as 13.5; for Fab fragments, 14.0; for Fd fragments, 14.4; for H chains, 13.7; and for L chains, 11.8 (9, 11). The absorbancy of each was found to be essentially the same in 0.01 N HCl, in 1 M propionic acid, and in borate buffer. The absorbancy of H chains in the latter solvent was not checked. Molar concentrations were calculated taking the respective molecular weight values as 150,000, 40,700, 21,600, 55,000, and 20,000 (12, 13).

Equilibrium Dialysis.—Hapten-binding measurements were made by equilibrium dialysis using I^{125}-labeled *p*-iodobenzoate (14).

Radioimmunoelectrophoresis.—This was carried out according to the procedure of Onoue, Yagi, and Pressman (15) using goat antibodies to rabbit H and L chains.

Disc Electrophoresis.—Disc electrophoresis was carried out on polyacrylamide gel using the "alternative procedure" described by Davis (16) and stabilizing the sample solution with 40% sucrose rather than gel.

RESULTS AND DISCUSSION

Separation of Fd Fragments and L Chains Derived From the Fab Fragments of Papain Fraction I and Fraction II.—(17) Preliminary experiments showed that γG-immunoglobulin which had been reduced and alkylated, in the manner of Fleischman *et al.* (8), yielded Fab fragments when digested by papain which are similar to the Fab fragments ordinarily obtained from a papain digest of intact γG-immunoglobulin (9). These digests (like digests of intact γG-immunoglobulin) could be fractionated on a carboxymethylcellulose column into three fractions, fractions I, II, and III (10). When fractions I and II were individually concentrated, dialyzed against 1 M propionic acid, and put through Sephadex G-100, fractions containing Fd fragments and L chains were obtained.

Starting with this procedure, we have studied the interactions among the Fd fragments and the L chains from papain fractions I and II and the H and L chains obtained from whole antibody by the procedure of Fleischman *et al.* (3, 8).

100 mg of specifically purified IgG_{Ab} (anti-Xp)[1] was reduced and alkylated and then dialyzed thoroughly, first against cold saline and then against 0.05 M phosphate buffer at pH 7.0. The reduced and alkylated globulin was then digested with papain (9) for 2½ hr. When examined in the ultracentrifuge, the digest showed a single symmetrical peak of sedimentation constant 3.5S. The digest was dialyzed against cold water and then 0.01 M acetate buffer of pH 5.5 during which time crystalline "reduced Fc fragment" appeared. The entire suspension was fractionated on a CMC column and the protein elution curve was the same with respect to conductivity as that ordinarily obtained from a digest of globulin (Fig. 1).

Fractions I, II, and III were individually concentrated by pervaporation with alternate dialysis against borate buffer: saline (1:9) (18), and finally fractions I and II were dialyzed against cold 1 M propionic acid. They were placed on essentially identical columns of Sephadex G-100 and fractionated. The elution patterns are shown as the inserts in Fig. 1.

Although relatively large columns were used, the second peak was not completely separated from the first. The second peak appeared at the same elution volume as did the L chains derived from reduced and alkylated, but undigested, globulin previously fractionated on these same columns. On this basis the second peak was tentatively identified as the L chain fraction (17) and the material in the first peak as Fd fragments (17).

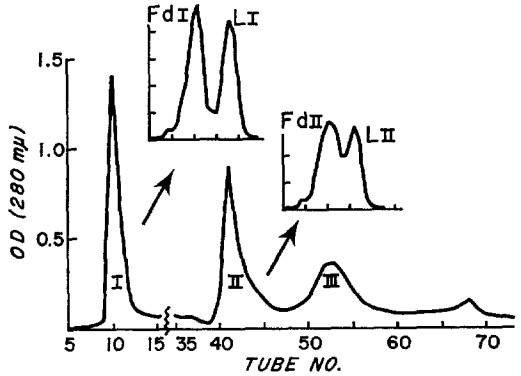

FIG. 1. Fractionation of papain digest of reduced and alkylated anti-*p*-azobenzoate rabbit antibody. The inserts represent further fractionation of peaks I and II on Sephadex G-100 in 1 M propionic acid to yield Fd fragments and L chains.

The ratio of the total absorbancy units in the first peak to those in the second peak, in both runs, was 60:40, close to the value of 57:43 calculated for the ratio for Fd fragments to L chains on the basis of the $A^{1\%}_{1\,cm}$ and molecular weights values of Fd fragments and L chains given in the Materials and Methods section.[2]

Selected tubes from each peak were pooled and concentrated by alternate pervaporation and dialysis against 0.01 M propionic acid. In view of the above

[2] This pattern resembles the pattern reported by Fleischman, Porter, and Press (17) for the separation on Sephadex G-75 of Fd fragments and L chains from Fab fragments, reduced and alkylated after digestion of whole rabbit IgG immunoglobulin rather than from fragments obtained by digestion of reduced and alkylated IgG immunoglobulin as carried out here. In the present work a shoulder preceded the first peak but was very small (Fig. 1). Fleischman *et al.* obtained a considerably larger shoulder. We have found that in several runs in which the starting material was the whole IgG fraction rather than specifically purified antibody, considerably more material was eluted in the position of this shoulder and occasionally appeared as a partially resolved peak. In such runs, two peaks preceded the L chain fraction. Here also, the ratio of total absorbancy units in these two peaks to those in the L chain peak was 60:40. The material in these peaks was identified as Fd fragments, essentially free of L chains, by immunoelectrophoresis using specific goat anti-rabbit H and L chain antiserum. The reason why the whole IgG fraction gives a large amount of aggregated material compared to specifically purified antibody is being investigated.

consideration and the characterizations presented below, we have designated the fragments as Fd_I and Fd_{II} and the L chains as L_I and L_{II} with reference to the papain fraction from which they were derived. The antihapten specificity is indicated by incorporating the term Xp, e.g., Fd_I (Xp).

Hapten-Binding Activity of Recombined Fragments and Chains of IgG_{Ab} (Anti-Xp).—Fd fragments and L chains, in 0.01 M propionic acid, were mixed in equimolar amounts and dialyzed for 16 hr against cold 1 M propionic acid, then saline, and finally borate buffer. The volume of the outer solutions and the number of changes of each employed were sufficient to assure equilibration with the desired acid or buffer solution. The hapten-binding activity of these solutions along with several reference and control preparations was then meas-

TABLE I

Hapten-Binding Activity of Subunits of IgG_{Ab} (Anti-Xp) in Various Combinations‡*

L chains	Heavy chains and derivatives §									
	FdI $(Xp)_A$		FdII $(Xp)_A$		H $(Xp)_A$		H $(Xp)_B$		H (N)	
LI $(Xp)_A$	*a*	70	*g*	63	*m*	78	*q*	9	*u*	3
LII $(Xp)_A$	*b*	74	*h*	74	*n*	86	*r*	13	*v*	2
L $(Xp)_A$	*c*	72	*i*	71	*o*	82	*s*	11		
L $(Xp)_B$	*d*	13	*j*	6	*p*	9	*t*	78		
L (N)	*e*	11	*k*	4						
None	*f*	9	*l*	7						

* Relative to the anti-Xp antibody preparation from which the Fd fragments or H chains were derived.

‡ The concentration of each subunit was 13.3×10^{-6} M based on the absorbancy of each solution and the molecular weight and $A^{1\%}_{1cm}$ values in the Materials and Methods section.

§ Relative binding activity of related preparations were: papain digest, 84; FabI, 72; and FabII, 76.

ured by equilibrium dialysis. The results are in Table I. There are two points relative to the question of hapten-binding activity by H chains. First, the individual Fd_I and Fd_{II} fragments show binding activity (combinations *f* and *l*, Table I) less than 10% of whole anti-Xp antibody, similar to that observed for H chains (3). The second point is that when either Fd_I or Fd_{II} fragments are mixed with either L_I or L_{II} chains, the hapten-binding activity of the original anti-Xp antibody is largely reconstituted (Table I, combinations *a*, *b*, *g*, *h*). Thus, it appears that the low hapten-binding activity of H chain is not due to low solubility (3, 19) because the Fd fragments are more soluble than H chains and the separated Fd fragment and L chain do recombine to give good antibody activity. The Fc portion of the H chain is not involved in the antibody site reconstitution.

Haber (20) reduced Fab fragments of rabbit anti-RNase antibody in 6 M

guanidine and after careful reoxidation recovered antibody activity as well as material that sedimented at 3.2S, similar to the starting material. The L chains and the Fd fragments must have been dissociated and recombined during this procedure.

A very important observation is that the subunits of the Fab_I and Fab_{II} types of fragments derived from the corresponding different types of antibody molecules have the capacity to cross-combine to form active Fab fragments with competent binding sites. Thus Fd_I from Fab_I will combine with L_{II} chains from Fab_{II} to give the composite (Fd_I-L_{II}) with good binding activity. Likewise, the composite (Fd_{II}-L_I) has good binding activity. Thus the composites from the two types of antibody molecules have antibody activity although heretofore these molecules have appeared to be quite different on the following bases: (*a*) chromatography of their papain digests yields Fab fragments of different elution properties (9); (*b*) the intact molecules separate on chromatography on CMC (21); and (*c*) the Fab_I and Fab_{II} fragments have been shown to have different amino acid compositions (22). Nonetheless, these differences are not of importance here since they apparently are not in features which are concerned with the binding site nor with the regions on the Fd fragment and L chains which are responsible for correct alignment of the two parts of the molecule to give a competent site.

This reconstitution also shows a specificity of combination just as the H and L chain combination does (3). L chains isolated from the same pool of anti-Xp antiserum as the Fd_I and Fd_{II} fragments (pool A) but without papain digestion (designated $L(Xp)_A$), did show a good recovery of hapten-binding activity with these Fd fragments, 71 or 72% (combinations *c* and *i*), while L chains isolated from a different pool of anti-Xp antiserum (pool B) and also without involving papain digestion (designated $L(Xp)_B$) did not bring about a good recovery of activity, only 13 and 6% (combinations *d* and *j*).

Similarly, $L_I(Xp)_A$ or $L_{II}(Xp)_A$ chains, when mixed with $H(Xp)_A$ chains, from the same pool of anti-Xp antiserum, both led to good hapten-binding activity, 78 and 86% (combinations *m* and *n*). However, when $H(Xp)_B$ chains, from a different anti-Xp antiserum, were used, hapten-binding activity was not recovered, 9 and 13% (combinations *q* and *r*) even though the $H(Xp)_B$ chains were functional since they did lead to good activity when mixed with L chains from the same antiserum (combination *i*).

Demonstrations of the Recombination of Fd Fragments and L Chains.—Recombination of Fd fragments and L chains was shown by immunoelectrophoresis, sedimentation analysis, and electrophoresis in polyacrylamide gel (disc electrophoresis).

In immunoelectrophoresis, recombination was shown by the fact that mixtures of Fd fragments and L chains or H chains and L chains migrated as units with mobilities different from those of the components.

Immunoelectrophoresis was carried out on the preparations with which hapten-binding measurements had been made. The results are in Fig. 2. Fd_I fragments migrate at about the same rate as do Fd_{II} fragments (compare patterns *1* and *2*) and L_I and L_{II} chains both migrate at about the same rate (compare *3* and *4*). The combinations of various Fd fragments and L chains (*6*, *7*, *8*, and *9*) are intermediate in their mobility with respect to the separated Fd fragments (*1*, *2*) and L chains (*3*, *4*) and are similar to the original Fab_I and

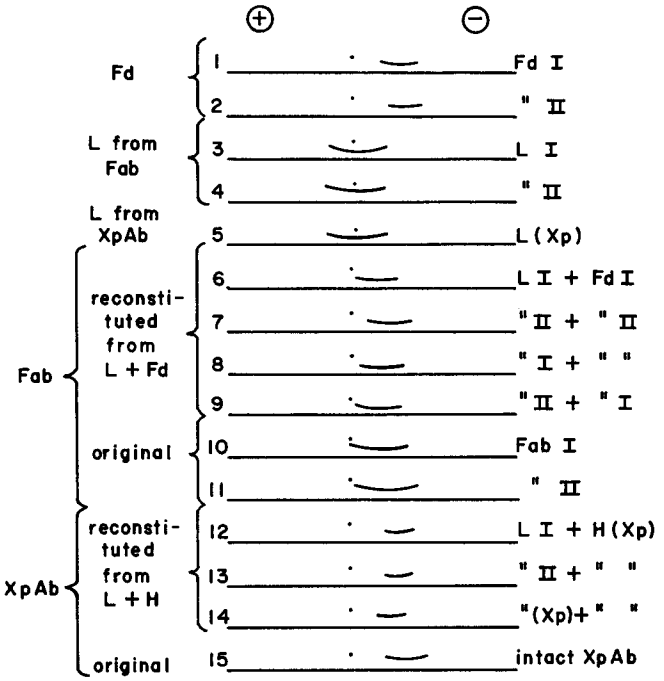

FIG. 2. Immunoelectrophoretic patterns of various subunits of anti-*p*-azobenzoate rabbit antibody. The starting well on each slide is indicated by a dot.

Fab_{II} fragments (*10* and *11*). This indicates that recombination has occurred.

The mobility of the L_I and L_{II} chains is similar to that of L chains prepared directly from IgG_{Ab} (anti-Xp)[1] (compare *3*, *4*, and *5*) and when combined with H chains of IgG_{Ab} (anti-Xp)[1] give similar patterns (compare *12*, *13* and *14*), all of which resemble the patterns of intact IgG_{Ab} (anti-Xp)[1] (*15*).

The ultracentrifugal behavior of Fd_{II} fragments, L_{II} chains, and a mixture of the two was examined. The mixture consisted of 1.6 mg (0.074) μmole) of Fd_{II} fragments and 2.0 mg (0.10 μmole) of L_{II} chains (a 35% molar excess of the latter) initially in 0.01 M propionic acid and then dialyzed against borate buffer with alternate pervaporation in the cold to a concentration of 3 mg per

ml. Individual solutions of Fd_{II} fragments and L_{II} chains were similarly treated. Each of the preparations was centrifuged in a synthetic boundary cell and the sedimentation constants determined. For the Fd_{II} fragments, there was a single symmetrical peak with a sedimentation coefficient of 2.8S in borate buffer at 20°C. The L_{II} chain peak was also symmetrical with a sedimentation coefficient of 3.2S in borate buffer at 20°C.

The Fd_{II} fragments and L_{II} chains, when mixed, appeared to combine in the ratio of 1 to 1 since the mixture sedimented as a single boundary with a sedi-

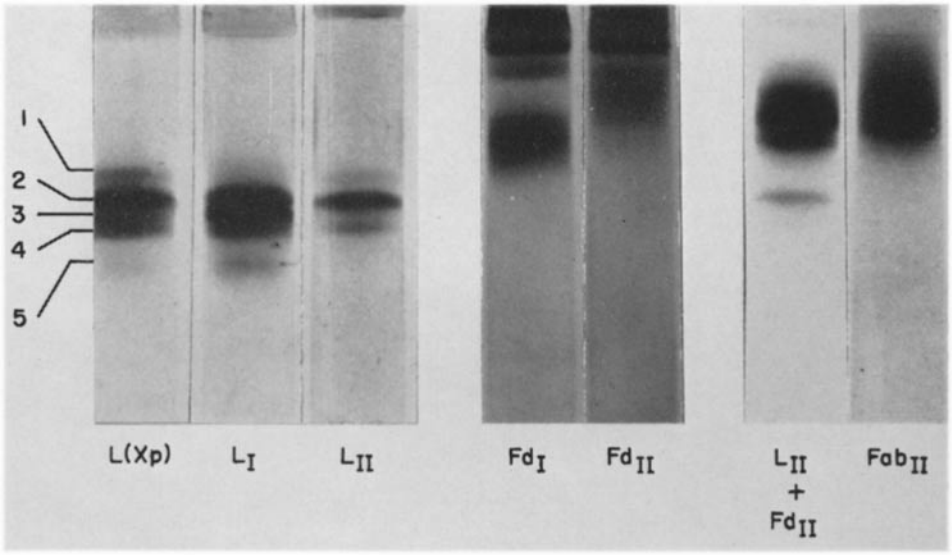

FIG. 3. Disc electrophoretic patterns of preparations of various subunits of anti-*p*-azobenzoate antibody from a single rabbit. Note differences between L_I and L_{II} and between Fd_I and Fd_{II}. The patterns of Fab_{II} and of Fab, reconstituted from L_{II} and Fd_{II}, are similar except for the band in the latter due to the presence of excess L_{II}.

mentation coefficient of 3.4S, which is essentially that of Fab fragments. There was no evidence of uncombined Fd_{II} fragments, i.e., a 2.8S component. The presence of the excess L_{II} chains was masked in the boundary of the L chain-Fd fragment recombinants because of the small difference in sedimentation coefficients (3.2 and 3.4).

Disc electrophoresis analyses of the subunits and of the mixtures, including the solutions examined in the ultracentrifuge, were also carried out. Electrophoretic behaviors consistent with those obtained in immunoelectrophoresis were obtained and some of the disc patterns are shown on Fig. 3. The L_I and L_{II} chains as well as the L(Xp) chains from the same Xp-antibody preparation

all have similar mobilities but are heterogeneous. The L(Xp) pattern shows five discrete bands (Fig. 3, 1–5) and five bands also appear in the L_I pattern but in very different relative amounts. The L_{II} pattern shows only three bands and these appear to correspond to bands 1, 2, and 4 of the L(Xp) pattern. Again the relative amounts of each are very different from those in the L(Xp) pattern. Thus, the L chain array derived from the Fab_I fragments is different from that from the Fab_{II} fragments, but nevertheless, all of these chains combine with either Fd_I or Fd_{II} fragments or with H(Xp) chains, from the same antisera, to give good binding activity.

TABLE II

Effect of Various Treatments on the Hapten-Binding Activity of Anti-Xp Antibody

Pattern No.	Treatment	Relative binding activity*	
		Antibody alone	Antibody plus 2 parts depleted globulin
1	Untreated	100	100
2	Propionic acid only	102	—
3	Papain digestion	86	93
4	" " , propionic acid	97	102
5	" " , reduce and alkylate	92	96
6	" " , " " " , propionic acid	86	63
7	Reduce and alkylate	100	96
8	" " " , propionic acid	93	75
9	" " " , papain digestion	95	96
10	" " " , " " , propionic acid	86	78

* Values are binding relative to that of the untreated anti-Xp antibody taken as 100. The free hapten concentration was 17.4×10^{-6} M. Bindings were calculated on the basis of a protein concentration equivalent to 1.0 mg of untreated antibody per ml.

The disc electrophoresis patterns of Fd_I and Fd_{II} fragments also differ. Fd_I shows a fast, broad band, a slower, lighter staining band, and an intense band which hardly enters the gel and which may be aggregated material. Fd_{II} shows three bands, all slower than the broad band of Fd_I, plus the band of aggregated material. The slowest band in Fd_I and Fd_{II} (not considering the aggregate) may represent a fragment common to both preparations.

The disc electrophoresis pattern of the mixture of Fd_{II} fragments and 35% mole excess L_{II} chains shows a fast band followed by a broad zone consisting of three different bands. The three bands correspond to the recombined Fd_{II}-L_{II} units and they have a mobility similar to the original Fab_{II} fragments and intermediate to that of the L_{II} chains and the Fd_{II} fragments alone. This obser-

vation indicates that recombination of Fd_{II} fragments and L_{II} chains occurred. The fast band represents the darkest band of the excess L_{II} chains in the mixture (compare with L_{II}).

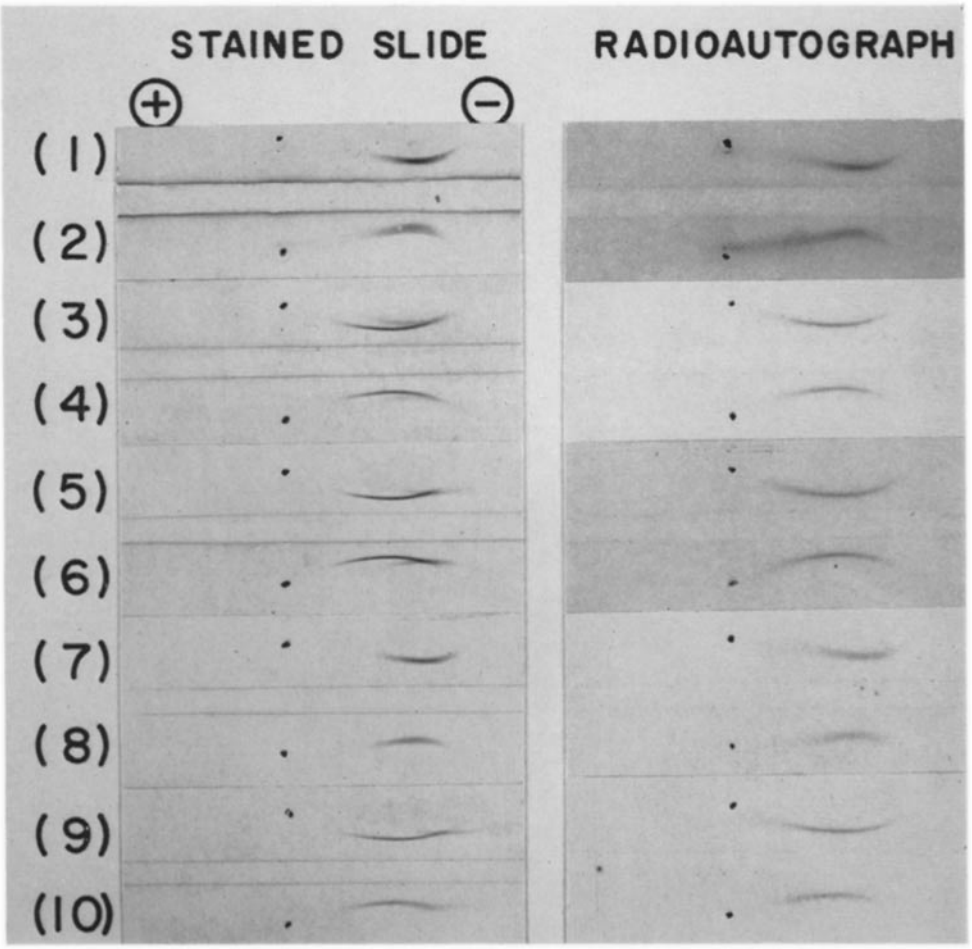

FIG. 4. Radioimmunoelectrophoretic patterns and radioautographs of preparation in Table II. The starting well on each slide is indicated by a dot.

The Hapten-Binding Activity of Antibody After Reduction and Alkylation, Papain Digestion, and Exposure to Propionic Acid in Various Order.—These experiments demonstrated that the order of treatment has little effect on the products formed.

One portion of IgG_{Ab} (anti-Xp) was reduced and alkylated and then dialyzed

against 0.1 M phosphate buffer of pH 7 containing 0.002 M versene. Then cysteine and papain were added and digestion carried out for 1 hr. A second portion was similarly treated but in the opposite order; in this case, papain digestion was followed by dialysis against water and then against 0.5 M tris buffer at pH 8.0 before reduction and subsequent alkylation. After each step, two samples of the antibody solutions were taken. One was dialyzed directly against borate buffer and the other dialyzed first against 1 M propionic acid and then against borate buffer. The hapten-binding activity of each sample was then determined by equilibrium dialysis.

The results are in Table II ("antibody alone" column) and show that no appreciable loss of hapten-binding activity occurs after any of these treatments. These high recoveries of activity are in agreement with those obtained above in which the Fd fragments and L chains were actually separated and then mixed.

That recombination occurred in those preparations whose components had been dissociated by propionic acid was shown by the immunoelectrophoretic patterns of these materials and their radioautographs (Fig. 4). The patterns on Fig. 4 are designated the same as on Table II.

The radioautographs show the antigen-binding activity by the intact antibody, *1*, after its papain digestion, *3*, and subsequent reduction and alkylation, *5*, or after its reduction and alkylation, *7*, followed by papain digestion, *9*. Re duction and alkylation has no effect on the electrophoretic behavior of the globulin (compare *1* and *7*) but papain digestion of native globulin results in two arcs, *3*; one for Fab fragments, the other for Fc fragments. The Fab arc is identified by the presence of antigen-binding activity which is also clearly shown on patterns *5* and *9* while the Fc arc is identified by the absence of antigen-binding on the radioautographs of these patterns. Exposure to 1 M propionic acid does not alter the positions of any of these Fab arcs, *4*, *6*, *10*. It is to be noted that the pattern (as well as hapten-binding activity) found for Fab fragments, *3*, *4*, is not altered by their being reduced and alkylated, *5*, *6*, nor is it different from when they are derived from the IgG-immunoglobulin *after* its reduction and alkylation, *9*, *10*.

Exposure to 1 M propionic acid of either intact antibody, *2*, or reduced and alkylated globulin, *8*, does not alter either the electrophoretic mobility or the antigen-binding activity compared to that not exposed to the propionic acid (compare with *1* and *7*).

Preferential Combination of Fd Fragments and L Chains to Give Effective Haplen-Binding Sites.—There is a preferential combination of those Fd fragments and L chains which form good hapten-binding sites. Even the presence of a twofold excess of nonspecific Fd fragments and L chains causes only a small decrease in the recovery of activity from recombined Fd fragments and L chains from specifically purified antibody. IgG_{Ab} (anti-Xp)[1] was mixed with

IgG_{F_T} (depleted anti-Xp),[1] both from the same antiserum and in the ratio of 1:2, and this mixture was carried through the above procedures. The results are in the last column of Table II. There was essentially no loss of activity except in the cases of those preparations which were both reduced and alkylated and exposed to propionic acid; whether or not papain digestion was involved made little difference (compare *6* and *10* with *8*). This loss appears to have been due to the formation of some ineffective recombinants during the dissociation and association (in the propionic acid) of the various specific and nonspecific L chains and H chains (or Fd fragments) derived from the mixture of globulins. However, recombination was not a random process whether Fd fragments (*6* or *10*) or H chains, *8*, were involved. If it were random, the remaining activity would have been much lower (one-third). As in the case of combination of F' and L chains, *4*, there is a preferential combination of the Fd fragments and L chains which give good hapten-binding activity.

That recombination occurred in the samples of patterns *6*, *8*, and *10* (Fig. 4) was shown by the immunoelectrophoretic patterns of these materials and their radioautographs. The patterns were the same as the patterns with IgG_{Ab} (anti-Xp) described above.

SUMMARY

In the work reported here we have shown that light chains and Fd fragments can be separated completely in propionic acid and then recombined to form Fab fragments with antibody activity. This experiment indicates that in the recombination a correct alignment of the Fd fragments and the L chains occurs to give a competent antibody site, just as occurs with the recombination of separated heavy and light chains of the antibody; thus the Fc fragment is not required for correct alignment.

Fd fragments of antibody alone show very low binding activity toward the specific hapten.

As is the case for the combination of heavy and light chains, the combination of Fd fragments and light chains also requires that both components come from antibody from the same rabbit in order to give binding sites. When they are derived from different rabbits producing antibody against the same antigen, they still give Fab fragments as shown by immunoelectrophoresis but do not have competent binding sites.

An important observation is that the subunits of the papain digest fractions, Fab_I and Fab_{II}, have the capacity to cross-combine to form active Fab fragments with competent binding sites. Fd_I from Fab_I combines with L_{II} chains from Fab_{II} to give the composite (Fd_I-L_{II}) with good binding activity. Likewise, the composite (Fd_{II}-L_I) has good binding activity. The composites from the two types of antibody molecules yielding different Fab fragments have antibody activity although heretofore these molecules have appeared to be different on the bases of chromatography and amino acid analysis.

There is also a preferential combination of the Fd fragments to combine with the correct L fragments to give binding sites since this combination takes preference over the combination of Fd fragments of antibody with light chains of normal globulin (or of light chains of antibody with Fd fragments of normal globulin).

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