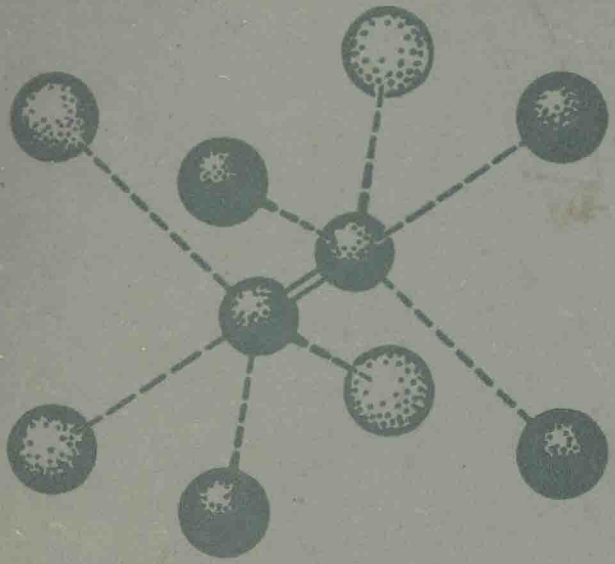

Molecular Structure and Biological Specificity

EDITED BY
LINUS PAULING and
HARVEY A. ITANO

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Molecular Complementariness in Antigen-Antibody Systems

David Pressman

Roswell Park Memorial Institute, Buffalo, N. Y.

In many examples of biological specificity there appears to be a close complementariness of fit of the active site of the receptor molecule about its substrate, so that enough of the various weak, short-range forces, such as the van der Waals forces, charge interaction, hydrogen bond, dipole interaction and so forth, can be effective in holding molecules together (Pauling, Campbell and Pressman, 1943; Pressman, 1953).

Specificity arises from the fact that only certain configurations can fit the site; other configurations are blocked sterically so that they cannot fit.

The study of hapten-antibody interactions permits the determination in a particular specific system of just how close the fit is, and what forces are acting. This is so because antibodies can be formed against simple chemical substances of known configuration, and the contour of the antibody's specific site can then be determined more or less precisely, or felt out, by the interaction of that site with substances of known configuration.

The studies of hapten-antibody interactions discussed here stem from the voluminous pioneer studies of Landsteiner (1945) on chemically altered proteins and the antibodies dervied therefrom.

The extent of combination of a hapten with an antibody which is directed against it can be determined either by partition of the hapten between an antibody solution and a control solution, as was first carried out by Dr. Haurowitz, (1933) our next speaker, and by Marrack and Smith (1932), or by the ability of the hapten to combine with the antibody and inhibit the precipitation of that antibody by the homologous antigen, as in the extensive studies by Landsteiner.

The studies which I shall be reporting here were carried out initially under Dr. Linus Pauling's direction at the California Institute of Technology and subsequently at the Sloan-Kettering Institute and now at the Roswell Park Memorial Institute. Some of the work reported here, as yet unpublished, was done by Dr. Nisonoff, who is now at Roswell Park with me.

An antibody acts as though it were formed against the antigen as a template. It is important to realize that the orientation of the hapten with respect to the antigen surface during antibody formation is a prime factor. Fig. 1 shows three such orientations for a hapten, *p*(*p'*-azobenzeneazo) benzoate, attached

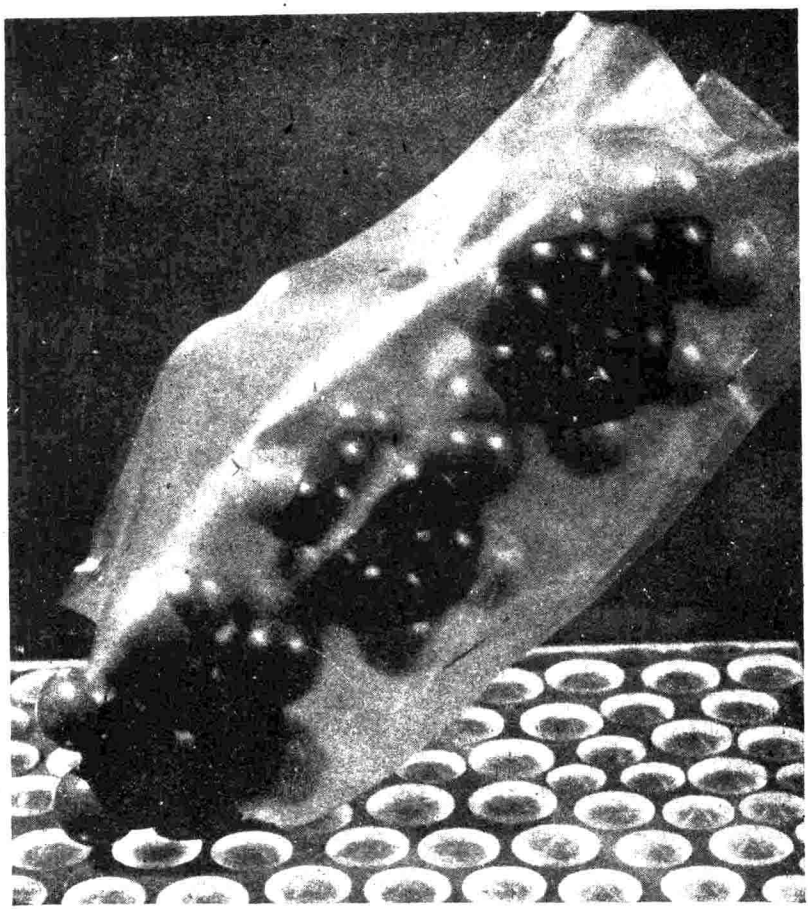


Fig. 1a

FIG. 1. Possible orientation of hapten with respect to antigen during antibody formation. a. Extended from surface of antigen. b. Lying flat on surface. c. Lying perpendicular to surface.

to the tyrosine of an antigen. In the first case (Fig. 1a), the hapten extends normal to the surface of the protein and an antibody formed against this portion of the antigen might have a long invagination, about 12 Å long, to accommodate the hapten. There are two other possible orientations, each with the hapten lying along the surface of the protein. One is with the faces of the benzene ring lying flat on the surface of the antigen (Fig. 1b) and the other is with the benzene rings lying perpendicular to the antigen surface (Fig. 1c). In the first case, an antibody formed against the haptenic portion of the anti-

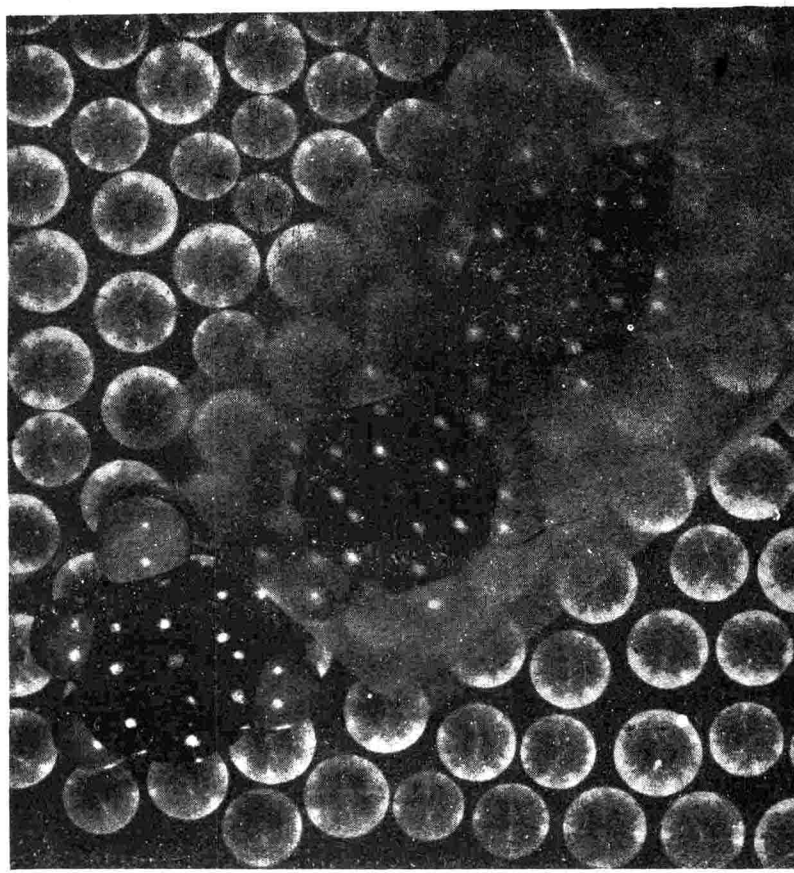


Fig. 1b

gen would have a long invagination to accommodate the hapten. The fit would be all around the hapten but perhaps not very close in view of the length of the invagination, ca. 12 Å. In the second case, the antibody would be formed against the face of the hapten group, and in the third, the antibody would have a slit trench type of anti-hapten region with one side open to accommodate the hapten. Evidence is available that indicates that these three types of antibody do exist.

Another point to bear in mind is that the antibody is quite heterogeneous. An antiserum formed against even a simple hapten contains a mosaic of different antibody molecules, each having specificity directed toward particular portions of the hapten. Thus Landsteiner and van der Scheer prepared antiserum against a hapten containing two different groupings, 5-azoisophthalyl-

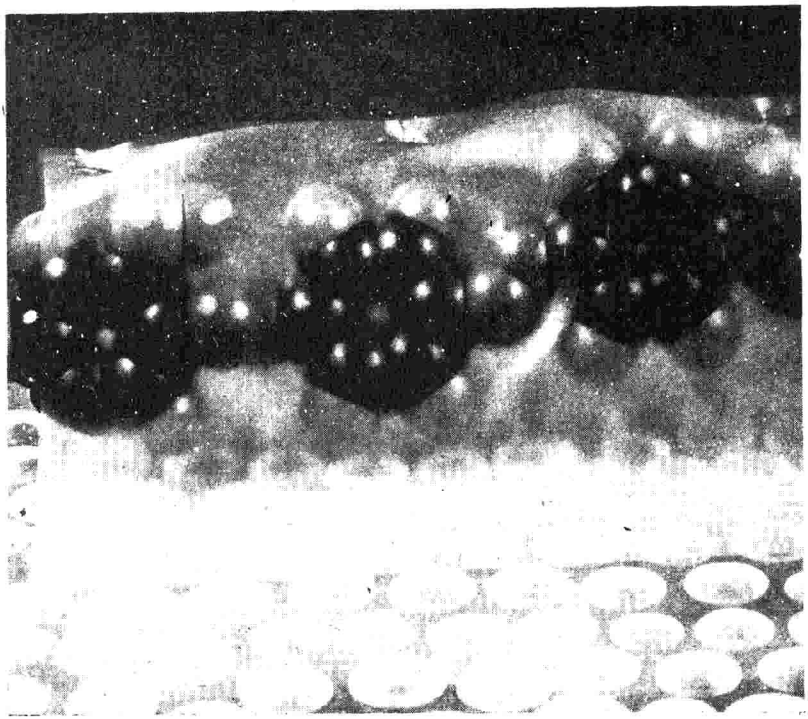
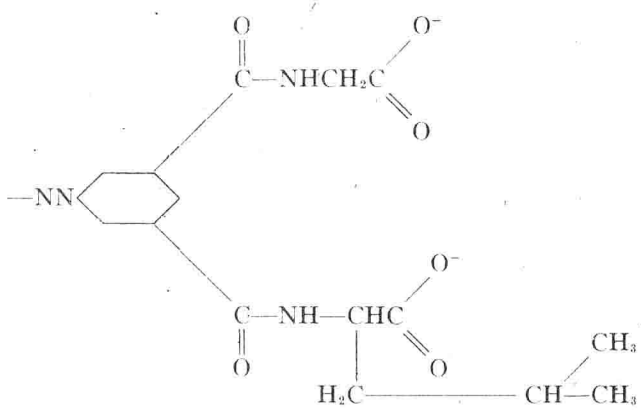


Fig. 1c

glycine-leucine, which contains 1 glycinate residue and 1 leucinate residue (1932).



Among the antibodies formed, there could be found antibodies with specificities directed against either one or the other of the two groups as well as

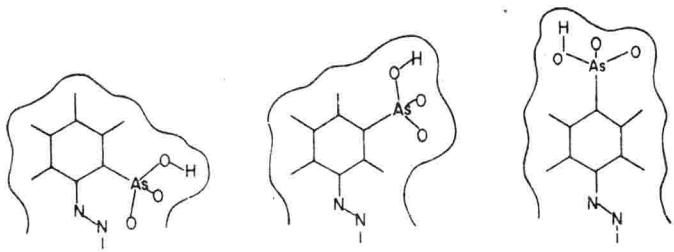


FIG. 2. Van der Waals outline of *ortho*, *meta* and *para*-azobenzenearsonates

antibodies directed against both groups. Thus, even though both determinants are present in the antigen in close juxtaposition and in equal quantities, individual antibodies are formed against individual parts.

For an example of an antibody formed against a simple substance, let us look first at antibodies formed against the azobenzenearsonate ion (Pressman and Siegel, 1953). Fig. 2 shows the *para*, *meta* and *ortho* azobenzenearsonates. The outer line is the van der Waals outline of the molecule.

When antibodies are formed against any one of these, they act as though they were formed against the van der Waals outline of the hapten as a template. They can also combine with an unsubstituted benzenearsonate ion. Substituents on the benzenearsonate decrease the extent of combination when they interfere sterically.

Thus, for antibodies to the *p*-azobenzenearsonate group, substituents in the *ortho* or *meta* positions decrease combining power generally. If we place a substituent in the *para* position, we might expect an increased combination of the hapten with the antibody, because the antibody would have a region which has been formed against the azo group and this region can also accommodate some other substituent.

In the case of antibody to the *meta*-azobenzenearsonate, there would be steric effects observed for substituents in all positions except the *meta* position. In the case of antibody to the *ortho*-azobenzenearsonate, there would be observed steric effects of substituents which are in the *meta* or *para* position. Substituents in the *ortho* position, however, would fit in the region of the antibody which was formed to accommodate the azo group.

The results of studies obtained from these three systems are shown in Fig. 3. Values are listed for the relative combining constant, K_0', which is the combining constant of the substituted benzenearsonate relative to the combining constant of the unsubstituted benzenearsonate in these various systems (Pauling, Pressman and Grossberg, 1944). The various substituents used are listed, and it can be seen that with the antibody against the *ortho*-azobenzenearsonate we get the best combination with the *ortho* substituted compounds, interme-

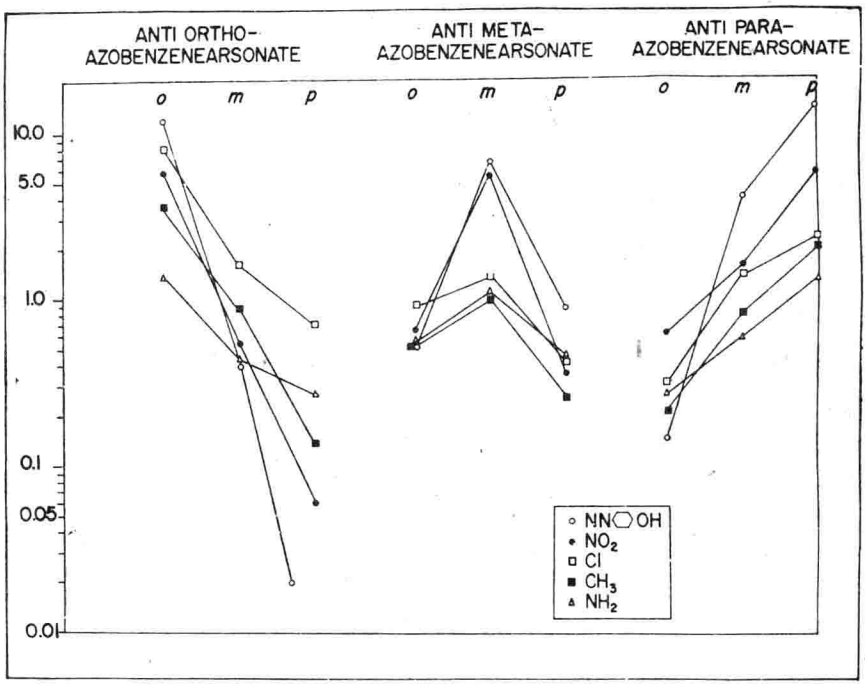


FIG. 3. Effect of position of substituent on K_0' value of hapten

diate combination with the *meta*, and least combination with the *para*, indicating steric interference of the substituent in the *meta* and *para* positions.

In the anti-*meta*-azobenzenearsonate system, we find the greatest combination with haptens with the substituent in the *meta* position, where there is no steric interference, and less combination when the substituents are in the *ortho* or *para* position. Similarly, with the antibodies specific to the *para*-azobenzenearsonate system, greatest combination takes place with *para* substituted compounds.

In order to determine more precisely how closely antibodies fit around the hapten group, we have carried out studies with the *ortho*, *meta*, and *para*-azobenzoate ions. We prepared the *o*-, *m*-, and *p*(*p*-hydroxybenzeneazo)benzoates and all of the monochlor derivatives (except one) of these compounds with the chlorine in the benzoate ring. We then measured the interaction of the chlor-substituted haptens with the antibody against the *ortho*, *meta*, and *para*-azobenzoate ions (Pressman, Siegel and Hall, 1954).

The results are shown in Fig. 4. Here I have indicated the van der Waals outline of the injected hapten by the dotted lines and the van der Waals outline of the chlor-substituted hapten by the solid lines.

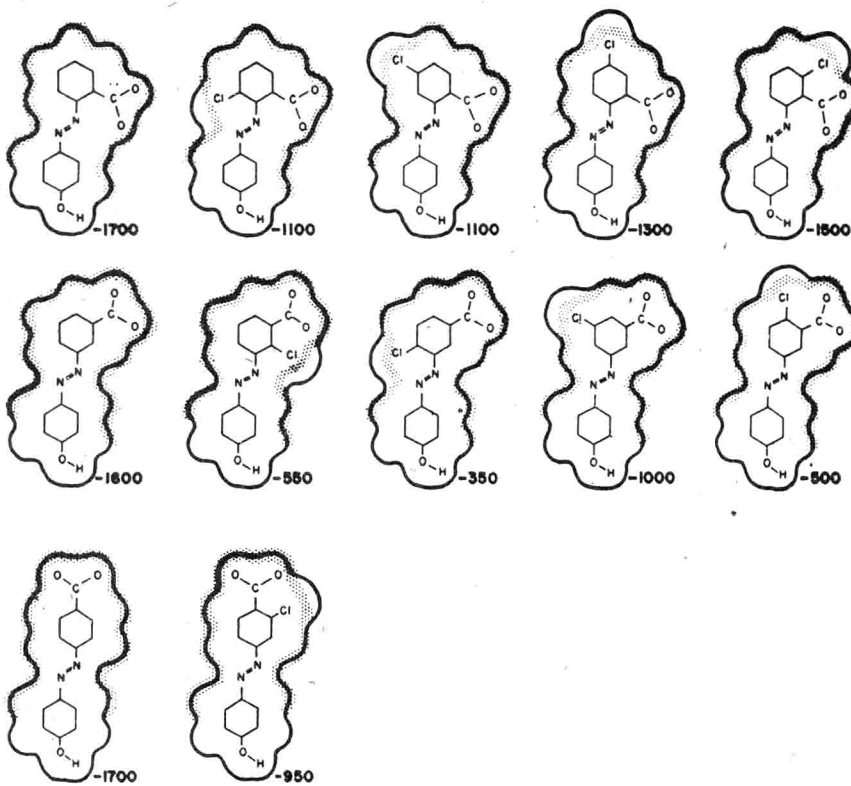


FIG. 4. Van der Waals outline of inhibiting hapten superimposed on outline of an injected hapten group. Numerical values are for ΔF(relative). (Reproduced from the Journal of the American Chemical Society, *76*, 6339 (1954) with permission of the Editor.)

There are listed the free energies of combination of these substances with homologous antisera relative to the combination of the unsubstituted benzoate ion. You can see that, depending on which position the chlorine occupies, there is a steric interaction which contributes to the decreased interaction of these substances with the specific antibodies.

The free energies involved when the chlorine is in the various positions are summarized in Fig. 5.

In the case of the antibody against the *meta*-azobenzoate group, we find that there is a large effect of a chlorine in either of the *ortho* positions. There are two possible reasons for this large effect. There may be a steric effect of the chlorine in interfering with the combination of the antibody or there may be an effect of the *o*-chlorine to tilt the carboxylate ion out of the plane of the

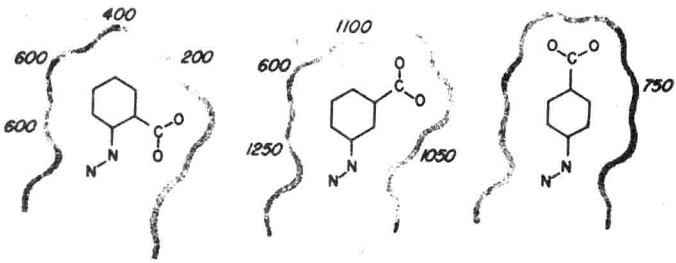


FIG. 5. Effect of chlorosubstituent in indicated position on the free energy of combination of (*p'*-hydroxyphenylazo)benzoates with antibody. (Reproduced from the Journal of the American Chemical Society, *76*, 6339 (1954) with permission of the Editor.)

benzene ring so that the carboxylate group no longer fits the antibody site as it did in the original hapten.

In correlation with this tilt effect, we find that antibodies against the *ortho*-azobenzoate ion show very low interference with substituents in the *ortho* position. This is presumably due to the fact that the carboxylate in the hapten against which the antibody was formed is already tilted out of the plane of the benzene ring, so that the antibody formed against this nonplanar carboxylate can accommodate a chlor-substituent in the *ortho* position.

So far, I have discussed substituents which exhibit a steric interference on the combination of hapten with antibody. What happens if a substituent is placed on the hapten in the position occupied by the azo group of the injected hapten (position of attachment of hapten with antigen)? Since the antibody was formed against the azo group, it can accommodate other substituents in this position. Indeed, a substituent in this position almost always increases the combining power of the hapten.

In the case where no hydrogen bonds are formed, one might well expect the greatest interaction to take place with those radicals which have the greatest van der Waals interaction, and this is actually the case.

Fig. 6 shows the increase of combining power with van der Waals attraction in several systems for the case where the substituent is in the position of attachment of the hapten group to the antigen. It can be seen that for each system, the order for strength of combination is methyl < chlorine < bromine < iodine, which is in the order of the polarizabilities of the groups and indicates an attraction of these groups for the part of the antibody directed toward the azo group.

The order holds throughout except in the *ortho*-azobenzoate system, where there is the problem of tilt. As the substituent becomes larger, there is a tendency toward decreased combination due to increased tilt of the carboxyl on the one hand and a tendency toward increased combination due to polariza-

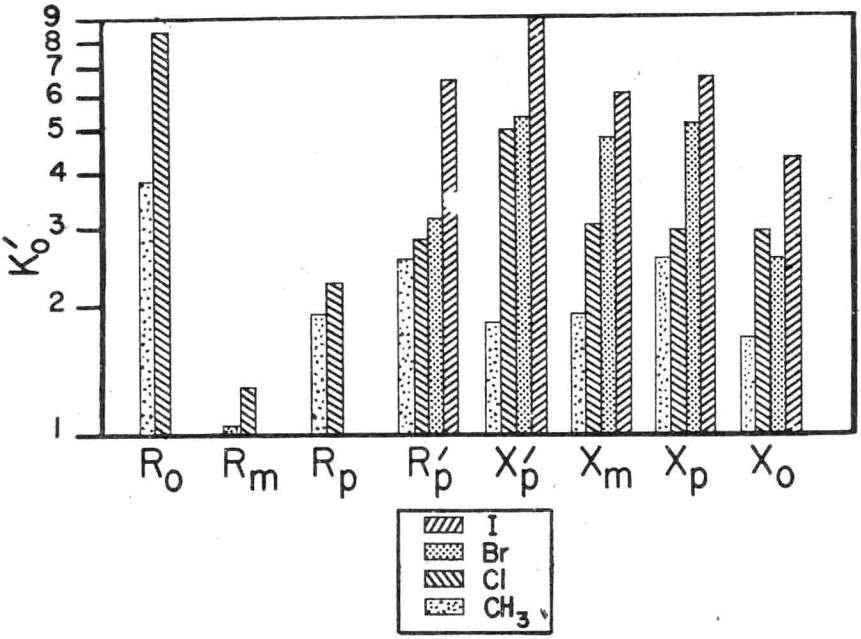


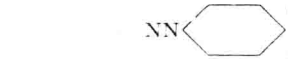
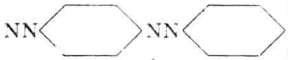
FIG. 6. Effects of van der Waals attraction for several systems where substituent is in position of attachment.

bility on the other. The order observed in the anti-*ortho*-azobenzoate system is $CH_3 < Br < Cl < I$. Apparently, the increased tilt due to the bromine over that induced by chlorine due to size is not compensated for by the greater polarizability of bromine, while for iodine the greater polarizability wins out.

Table 1 is a summary of some other *para* systems investigated (Pressman and Siegel, 1953; Pressman, Grossberg and Pauling, 1943; Pressman and Siegel, 1953a; Pressman and Siegel, 1953b). Antisera were prepared against the various haptens indicated. The asterisk represents the homologous charged group as indicated. The values are the relative combining constants of the homologous hapten with antibody. The benzene derivative has the value of 1 in all cases. A *para*-methyl substituent increases the constant in all cases. The methyl group fits into the part of the antibody site directed toward the azo group in the position of attachment and thus, exhibits a greater van der Waals attraction than the hydrogen of the benzene derivative without any steric effect. The relative constants for the *meta*-methyl, *ortho*-methyl and *alpha*-naphthyl derivatives are an indication of the tightness of fit of antibody around the hapten group. For a tightly fitting antibody we would expect the constant to be decreased; the tighter the fit, the greater the decrease (taking into account also the tilt of the carboxylate out of the plane of the benzene

TABLE 1

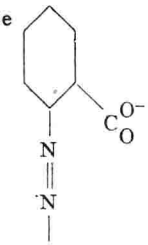
Closeness of fit in various *para* systems

System	Hapten *			K_0'			
		1.0	1.45	0.86	1.05	2.0	
		1.0	2.7	1.1	1.0	2.9	3.9
		1.0	1.9	0.78	0.21	0.52	6.0
		1.0	1.8	.21	.03	.03	1.98
		1.0	3.0	.66	.08	.18	10

ring). It can be seen that the fit is much closer in the last three systems than in the first two. The fit around the positive charged hapten and the azobenzene-azobenzenearsonate hapten is loose enough along one side so that the extra benzene ring of the naphthyl or the *ortho* or *para*-methyl groups can be accommodated. This is taken as an indication that the antibodies formed here are of the slit trench type. The antibody presumably was formed against one side of the hapten leaving the other side free.

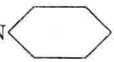
Table 2 shows how closely anti-*ortho*-azobenzoate antibody fits around the 4 position of the benzoate ion. The combining constants of the antibody with the *para*-fluoro, -chloro, -bromo and -iodobenzoates are listed. The hapten can fit into the antibody site in only one way. Fluorine, which is not much larger than hydrogen, decreases the relative constant to a value of 0.6. Chlorine decreases it somewhat more, and bromine still more. The differences are significant. Iodine shows a slightly increased combining constant over that of bromine and is about the same as that of chlorine. This order is an example of balance between steric effect and van der Waals interaction. As the size increases there is a greater steric interaction, but frequently the van der Waals attraction increases in a compensating manner, as occurs here with iodine.

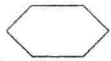
TABLE 2

Closeness of fit about para position in the  system

Hapten	K
H	1.00
F	0.6
Cl	.5
Br	.4
I	.5

TABLE 3

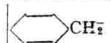
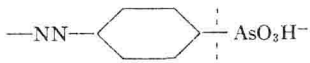
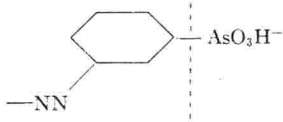
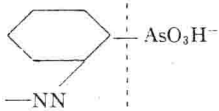
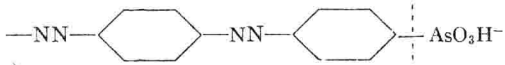
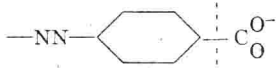
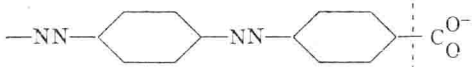
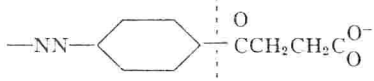
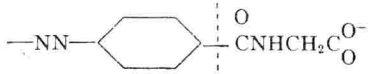
Importance of van der Waals attraction in the —NN  AsO_3H^- system

Hapten	K_0'
$^-O\ AsO_3H^-$	0
$CH_3\ AsO_3H^-$	0
 AsO_3H^-	1.0
 $CH_2\ AsO_3H^-$	0

The relative position of components of a haptenic group is important. This is shown in Table 3, where we have relative constants for the interaction of various substances with anti-*para*-azobenzenearsonate antibodies. It can be seen that it is very important for the benzene ring to be right next to the arsonate group, since separating the benzene from the arsonate by the CH_2 group, as in the case of benzylarsonate, decreases the combining constant markedly. This indicates that the fit of the antibody around the hapten is so close that the displacement of the ring essentially destroys combining power. The benzene ring is very important here as is shown by the fact that neither the arsonate ion itself nor methylarsonate combines with the antibody. This relation is not unique for the benzenearsonate system but holds also for several other systems,

TABLE 4

Van der Waals attraction due to benzene ring

System	Hapten		
		H_3C^-	
		K_0	
	1.00	0	
	1.00	0	0
	1.00	0	0
	1.00	0	0.05
	1.00	0	0
	1.00	0	0
	1.00	0.12	
	1.00	.01	

as is shown by Table 4 (Pressman, Bryden and Pauling, 1948; Pressman, Maynard, Grossberg and Pauling, 1943; Pressman and Siegel, 1953a; Pressman and Siegel, 1953b; Pressman, Siegel and Hall, 1954; Pauling, Pressman and Grossberg, 1944).

In the several systems listed, the benzene derivative gave good combination; the methyl derivative gave essentially none, except in the case of the benzoyl propionate system and the phenyl-trimethylammonium ion system, where there was a slight combination with the methyl derivative. Moving the benzene ring over by one methylene group also decreased combination, except for the phenyl-trimethylammonium ion system. The fit around the latter