THE STRUCTURE OF ANTIGEN-ANTIBODY COMPLEXES

A STUDY BY ELECTRON MICROSCOPY

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PLATES 31 TO 35

(Received for publication, April 11, 1963)

Modern concepts of the interaction of antigen and antibody are largely based on reactions between divalent antibody and univalent antigen (1). In such systems aggregation does not occur and hence information on the first stage of antigen-antibody interaction can be obtained without the added complexity of the subsequent aggregation. The interaction between multivalent antigens and antibody has been studied extensively by microanalytical methods, and by neutralization of biologically active molecules by antibody (2). In such systems the contribution of the initially formed monomeric compounds of antigen and antibody and of the subsequent aggregation of these monomers can only be distinguished with difficulty (3, 4).

The structure of aggregates has been predicted by Marrack's lattice hypothesis (5) but has so far not been established by unequivocal and direct observations. This has been attempted by electron microscopy. Much of the earlier work, in this field, was carried out by shadow-casting techniques (6–8). The recent development of negative staining (9), widely used to elucidate the substructure of viruses, opens new possibilities in the investigation of the fine structure of antigen-antibody aggregates (10, 11).

The size of antibody molecules has been deduced from sedimentation and diffusion studies; electron microscopic studies have yielded dimensions in reasonable agreement with those obtained from hydrodynamic studies (7). The size of the combining site of antibody has been estimated by experiments in which the ability of small molecules to inhibit the combination between antigen and antibody was assessed as a function of the molecular weight of the inhibiting molecule (12–19).

We have so far referred to information on the dimensions of antibody molecules and on structure of antigen-antibody aggregates which can be obtained by electron microscopy. In addition, it should be possible to examine the antigenic relation between different entities such as different forms of viruses or membranes of host cell and membranes of viruses. It is well established that

the vast majority of antibodies show the same specificity at both combining sites of any one molecule. Repeated observations of antibody molecules linking two different structures can therefore be considered evidence of the presence of common antigen on the surface of the two different structures.

It is our aim to examine the internal arrangement of aggregates as a function of the relative concentration of antibody, to estimate size and mode of attachment of antibody, and to assess the immunlogical relation between different forms encountered in preparations of viruses such as polyoma and verruca vulgaris virus.

Materials and Methods

Immunization.—Antibodies were raised in goats and in rabbits by repeated intravenous injections. Rabbits were bled from the marginal vein of the ear; goats were bled from the carotid artery.

Verruca Vulgaris Virus.—Purified wart virus was obtained by grinding pools of five to eight common warts (verruca vulgaris) with sand and distilled water in a mortar. The homogenate was clarified by spinning at low speed in a clinical centrifuge for 5 minutes. This supernatant was then spun in four tubes for 1 hour at 34,000 *g*. The pellet in one tube was resuspended in a small amount of distilled water and checked in the electron microscope by the negative staining process. If the specimen was a suitable one, the pellets in the other three tubes were pooled and resuspended in a suitable volume of phosphate buffered saline.

Polyoma Virus.—This virus was obtained from mouse embryo cells infected 10 days previously with polyoma virus. Cells showing a distinct cytopathogenic effect were frozen and thawed three times. The suspension was clarified by spinning at low speed for 10 minutes; the resulting supernatant was centrifuged for 1 hour at 40,000 *g*. An aliquot of the pellet so obtained, was suspended in distilled water and checked in the electron microscope. The remainder was suspended in phosphate-buffered saline.

Preparation of Antigen-Antibody Complexes.—To assay the concentration of virus, the virus was mixed with a known concentration of 880 A latex particles and the number of virus particles was determined by the method of Pinteric and Taylor (20). Constant quantities of virus were mixed with varying quantities of immune sera. The volume was kept constant by the addition of phosphate saline buffer. The mixtures were kept for 1 hour at 37°C and then at +2°C over night. They were subsequently centrifuged at 2000 RPM at +2°C and were then washed twice with a volume of phosphate saline equal to the original volume of the antigen-antibody mixture.

Washed pellets of the antigen-antibody complex were suspended in a small quantity, usually 0.1 ml, of distilled water. Immediately afterwards, one drop of this suspension was mixed with an equal amount of 3 per cent phosphotungstic acid adjusted to pH 6 with potassium hydroxide. A drop of the virus-phosphotungstate mixture was placed on a 400 mesh carbon-formvar-coated grid, allowed to settle for a few seconds, and excess fluid was gently withdrawn with filter paper. As soon as this preparation was completed, the grid was placed in the electron microscope, a Siemens elmiskop I. Micrographs were taken at an initial magnification of 40,000 using the double condenser illumination.

RESULTS

The effect of antibody on the appearance and distribution of virus particles is striking. A field of virus particles not linked by antibody and of virus particles

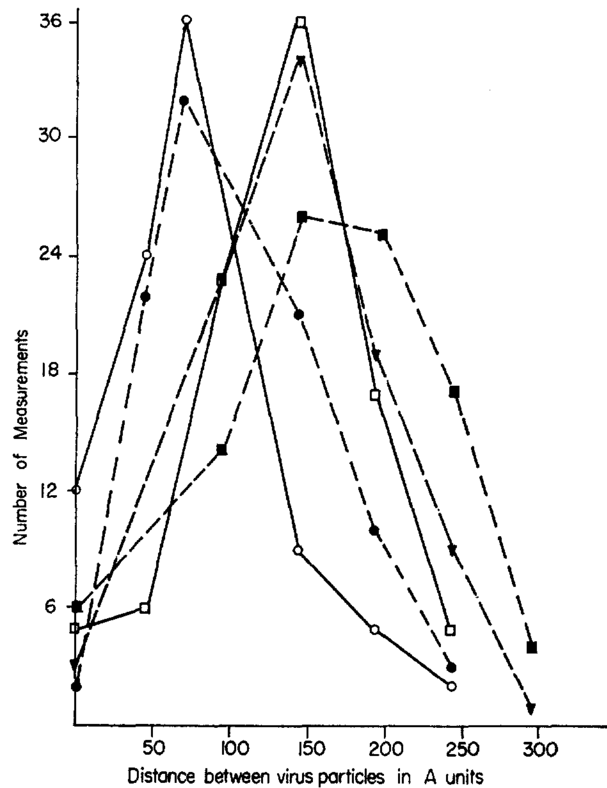
combined with antibody, shows a marked difference in the distribution of virus particles. Preparations of virus made in the absence of antibody show a fairly even distribution of particles. On the other hand, preparations of virus particles, treated with antibody, show an uneven distribution with isolated groups of particles linked by antibody (Figs. 1 and 2).

Three main types of arrangement have been observed in preparations of antibody-linked virus. At the lowest relative concentration of antibody, only about two molecules of antibody can be seen, on any one virus particle. Cross-linking of virus by antibody is not very extensive. Most of the virus particles are only linked by antibody with one, or, at most, two other particles (Figs. 3 *a* to 3 *c*). In addition, scattered small groups of 5 to 7 virus particles, sometimes in ring structures, were observed. As the concentration of antibody was increased, the number of virus particles linked with one another, through antibody, also increased. As a consequence, fairly large aggregates were formed in which several antibody molecules could be seen, connecting pairs of viruses (Fig. 5). In most of the aggregates examined, regularities of arrangement were observed in limited areas. Chains of 5 to 6 particles in linear, or circular, formation were found. Occasionally, the virus particles arranged in a ring, were linked to a virus particle at the center of the ring (Figs. 5 and 6).

If the relative concentration of antibody was further increased, a dense aura of antibody was found around some virus particles (Fig. 6). Such particles were not linked by antibody with one another. At still higher concentrations of antibody, all particles of virus showed this dense aura and antibody linking could be found only rarely (Fig. 7).

The observations in the zone of antibody excess, so far described, showed that the distance between virus particles would depend on the relative concentration of antibody. At very high concentrations of antibody, where cross-linking does not occur, the distance between particles should not depend directly on the length of the antibody. To examine this effect further, the distances between centers of 100 virus particles were measured at each of several concentrations of antibody. The diameter of the virus was subtracted from these distances to give the separation between particles. The lowest concentration of antibody was so chosen as to result in minimal aggregate formation (Figs. 3 *a* to 3 *c*, and 4); the two highest concentrations were such as to result in a dense aura of antibody round each virus particle, without any evidence of cross-linking (Fig. 7). The distribution of the measured distances varied with the concentration of antibody, increasing to a constant value (Text-fig. 1). A comparison of the mean distances and analysis of variance is given in Table I.

A comparison of all mean values, two-by-two, using a multiple comparison procedure based on the Studentized range shows that the increase in mean length is significant (5 per cent level) as the concentration is increased from 0.05 to 0.1, from 0.1 to 0.2, and from 0.2 to 0.4. The decrease in mean length



TEXT. FIG. 1. Distribution of distances between virus particles as a function of relative concentration of antibody. The amount of virus was kept constant (2×10^{11} particles) and the volume of antibody was varied (see Tables I and II). The final volume of all mixtures was 1 ml.

(*a*) ○	0.05 ml	of antibody	(Figs. 3 and 4)
(*b*) ●	0.1 ml	of antibody	(Fig. 5)
(*c*) □	0.2 ml	of antibody	(Fig. 6)
(*d*) ■	0.4 ml	of antibody	(Fig. 7)
(*e*) ▼	0.8 ml	of antibody	(Fig. 7)

Each curve is based on 100 measurements which were estimated by measuring center to center distances and then subtracting the diameter size of the virus particle.

when the concentration is increased to 0.8 is not significant (21). It follows that the distances between viruses increase with increasing amounts of antibody, in the zone of antibody excess. At the two highest concentrations of antibody, all antigenic sites are saturated, cross-linking of antibody rarely occurs, and a maximum distance of separation is observed.

In the electron microscope, antibody appears as an elongated rod-shaped molecule which is attached to the antigen by its short base. A well resolved micrograph of rabbit antibody, attached to wart virus, is shown in Fig. 8.

Goat antibody, shown in Fig. 9, has been observed in combination with polyoma virus. One hundred measurements of the width of goat and rabbit antibody were made. The maximum estimated width for the rabbit antibody was 34 A and for that of goat antibody, was 35 A. These estimates of the true width were made on the assumption that observed widths have a uniform distribution over the range of values from the minimum observable, to the "true" width. The assumption of a uniform distribution was tested. A conditional chi square test of fit was used which excluded the end intervals. For goat antibody the

TABLE I

Separation of Virus Particles as a Function of Antibody Concentration

Mixture contained 2×10^{11} virus particles and varying amounts of antibodies. The final volume was 1.0 ml. Approximately 100 measurements were made at each of the five concentrations.

Mixture	*a*	*b*	*c*	*d*	*e*
Amount of antibody in ml/ml of final mixture	0.05	0.1	0.2	0.4	0.8
Mean distance between virus particles (*A*)	82	108	132	168	149

Analysis of Variance

Source of variation	df	S.S.	M.S.	*F*
Between samples	4	408,750	102,190	33.2
Error	446	1,374,000	3,080	
Total	450			

df, degrees of freedom; S.S., sum of squares; M.S., mean square (M.S. = S.S./df); *F* — ratio (*F* = M.S. between sample/M.S. of error).

chi square on 6 df was 29.4 (*i.e.*, highly significant); for rabbit antibody the chi square on 3 df was 0.15 (*i.e.*, not significant). It follows that the assumption of uniformity of width distribution is quite reasonable for rabbit, but not for goat antibody. After correcting for unequal grouping intervals, the histogram for rabbit antibody was remarkably constant, as is indicated by the low value of chi square. The histogram for goat antibody suggested that the distribution had a higher density in the middle of the range than it had at the ends. If this is so, the estimate of the upper end of the range given above, (35.0), is likely to be an underestimate in the case of goat antibody.

The values for width are not necessarily identical with the true width of the molecule since the phosphotungstate employed in negative staining may affect the apparent width and since shrinkage during drying cannot be excluded.

However, the errors introduced by these two factors are not likely to be greater than 10 per cent (22).

The length of antibody molecules is more difficult to assess than the width. This is due to the fact that the antibody molecules may appear either as straight or as flexible, wavy rods; and that it is difficult to ascertain that the antibody molecule is attached at the observed periphery of the antigen and not above or below it. Since this cannot be unequivocally established, we attempted to assess the length of the antibody molecule by measuring the separation between virus particles linked by antibody. Such measurements can be plotted as a frequency curve as shown in Text-fig. 1 for rabbit antibody. The maximum length of separation in the zone of antibody excess was estimated for different relative concentrations of antibody assuming that the density function of the distribution could reasonably be approximated by a parabola (Table II).

TABLE II

Estimates of Maximum Length

Mixture	*a*	*b*	*c*	*d*	*e*
Amount of antibody in ml/ml of final mixture	0.05	0.1	0.2	0.4	0.8
Maximum length, *A*	241	247	256	308	286

It is clear that the observed distances between antibody linked virus particles must generally be shorter than the length of the antibody molecule; this is evident from a comparison of Fig. 3 *a* with Figs. 3 *b* and 3 *c*. Since linking between all virus particles has only been observed in the two lowest concentrations of antibody (Table II), the maximum separation obtained at these two concentrations, only, can be used to arrive at an estimate of the length of the antibody molecules. On the basis of these two sets of measurements, the maximum length of separation of virus particles and of the minimum length of rabbit antibody is found to be between 240 and 250 A.

In some rare instances individual antibody molecules which appeared to represent the full length of the molecule were measured directly, and gave values of 270 A for rabbit (Fig. 3 *a*) and 270 A, also, for goat (Fig. 10).

We have, so far, described electron micrographs for rabbit and goat antibody and the dimensions of the antibody as derived from the spacing of the aggregate as a function of the amount of antibody.

Lafferty and Oertelis (10) have described a loop of antibody in which a single antibody molecule to influenza virus was linked with both of its combining sites to a virus particle. In the interaction of antibody with polyoma and wart virus, the number of loops per virus particle is small and seems to increase with antibody concentration to a constant level. It is, however, clear that the observation of a loop need not correspond to the existence of a loop. Misinterpre-

tations could result from the superimposition of two or more antibody molecules linked with only one valency at different levels of the virus particle. The likelihood of this type of superimposition artifact increases with increasing concentration of antibody. The possibility of such erroneous interpretations is difficult to exclude but can be reduced by establishing fairly rigid criteria for the identification of a loop. We have adopted the following: both sites of attachment and the whole extent of the loop must be visible, and the course of the loop must be such as to minimize the possibility of two molecules of antibody overlying one another. If these criteria were applied, no more than three or four loops were seen in all our experimental work, one of them being shown in Fig. 10.

In the foregoing, we have confined ourselves to the characterization of antibody and of the antigen-antibody complex. However, this is not the only type of information which can be obtained by electron microscopy of antigen-antibody complexes; the study of the interaction between virus and antibody can also be utilized to elucidate the antigenic relation between unusual particles and the normal type of virus. It is well established that the majority, at least 90 per cent, of antibodies show identical specificity on both combining groups (23, 24). The presence of a common antigen can, therefore, be assumed if the divalent molecules are regularly found to link several normal virus particles with an unusual form of virus.

Whereas the absence of heteroligating antibodies can be considered, in the present context, as established, the absence of cross-linking artifacts in negative staining cannot be taken for granted. To test this condition for further work, pairs of unrelated viruses, polyoma and wart virus, were mixed with antibody to polyoma virus. Both viruses are icosahedral, have 42 subunits, but differ in diameter by 100 A (wart, diameter = 550 A; polyoma, diameter = 450 A, reference 25). Fig. 11 shows micrographs in which antibody to polyoma can be seen to combine with polyoma virus, and not with the larger wart virus. It is, therefore, clear that cross-linking that may be observed between different types of particles cannot be attributed to some artifact of preparation, but that it can be interpreted in terms of an antigen shared between two particles. It is therefore possible to use antibodies to show the presence of shared antigens between different forms of viruses, which have dimensions entirely different from each other but which have subunits which are structurally similar. If antibody is added to a mixture containing normal (icosahedral) and abnormal forms (25), molecules of antibody can be found to link the abnormal and the icosahedral forms (Figs. 12 *a* to 12 *d*). It can, therefore, be concluded that these two forms have at least one antigen in common.

DISCUSSION

The formation of aggregates of antigen and antibody was recognized with the discovery of the flocculation reaction. The structure of these aggregates became the subject of much speculation. Bordet (26) considered the precipita-

tion of mixtures of antigen and antibody a result of the changed surface properties of the antigen after it had been covered by a layer of antibody so that the particles became susceptible to the flocculating influence of electrolytes. He assumed that the "stickiness" of antigen-antibody complexes was responsible for aggregation. As soon as quantitative precipitin techniques were developed, it was realized that antibody was divalent, that antigen was multivalent, and that as a consequence, the formation of polymers was conceivable in which multivalent antigen molecules would be cross-linked by divalent molecules of antibody. This was clearly expressed in Marrack's (5) lattice hypothesis, which soon superseded the concept of Bordet, and was developed further by Heidelberger (27), and later by Pauling (28). Pauling stressed that the lattice would not have the orderliness of a solid crystal, but rather that of a glass, such as silica glass "in which each silicon atom is surrounded tetrahedrally by four oxygen atoms and each oxygen atom is bonded by two silicon atoms, but which lacks further orderliness of arrangement."

It proved very difficult to obtain evidence of an orderly arrangement within aggregates. Electron microscopy did not, at first, give enough resolution for a detailed examination of the fine structure of aggregates. In most of the early attempts to examine the structure of aggregates, reliance was placed on observed distances between antigens and a certain orderliness of arrangement was observed without resolution of the antibody attachment itself, so that no conclusions on the structure of aggregates could be drawn. However, the orderliness of the arrangement and the fact that the distances between antigen molecules were different after treatment with antibody than before, induced Easty and Mercer to conclude from their study of the interaction between ferritin and antibody, that the complex was broadly similar to what might have been expected from the lattice theory (6). Attempts to examine antigen-antibody aggregates formed between rabbit antibody and dihaptenic dye showed at high concentrations large irregularly shaped aggregates which gave no significant internal regularity, as well as smaller end-to-end and side-to-side aggregations. At very high dilutions, very long strands of end-to-end aggregated antibody were not observed and the weight averages of the particles varied between antibody alone and approximately double the length. It appeared that hapten-antibody attachment was weak and could easily be masked by non-specific attachment (7).

In the last mentioned experiments, as in most earlier work, shadowing techniques were employed. We have turned to negative staining in the hope that this technique would give us greater resolution. This was indeed found to be the case. However, there are certain disadvantages in the negative staining technique which arise out of the fact that the observed dimensions of molecules are affected by the height of the tungstate layer in relation to the height of the observed molecule. Furthermore, the structures may become distorted as a

consequence of drying and shrinking of surrounding tungstate. These difficulties may affect quantitative findings without affecting qualitative observations.

We have chosen regularly shaped viruses as antigens since they can be seen very clearly in negative staining and thus help in the identification of attached antibody molecules. At low concentrations of antibody no aggregate formation was observed. As the relative concentration of antibody was increased, the small complexes, consisting of two or three particles of virus, observed at low antibody concentration, were no longer found. In their place, were seen complexes consisting of many dozens of viruses. It was in such precipitates as this that we were able to observe areas which corresponded most closely to arrangements predictable from the lattice hypothesis. These complexes contained areas of highly regular spacing. Frequently, five or six viruses were observed cross-linked with one another in a ring structure with a virus particle in the center of the ring being linked by spokes of antibody to the surrounding ring of viruses. The appearance of this structure was reminiscent of the models proposed by Pauling for the arrangement of antigen and antibody in the equivalence zone. However, such regular areas only formed part of the aggregate and there were invariably, adjoining areas in which isolated chains or less orderly arrangements could be seen. It is clear that the electron microscopic preparation may have disrupted aggregate structure in certain portions of the aggregate. Whether these aggregates are flat sheets or three-dimensional structures cannot be decided from the electron microscopic evidence, though indirect evidence would indicate the existence of fairly irregular structures (3). In the zone of excess antibody, the symmetry of the arrangement of the aggregates becomes less and less marked as the amount of antibody increases. Finally, and at very high concentrations of antibody, aggregate formation can no longer be seen. Each virus particle is covered by a thick halo of antibody molecules radiating from the periphery and though we have occasionally observed an intermeshing between halos of neighboring virus particles, the majority of the antibody-covered viruses are clearly separated from one another. There is thus no evidence of lattice formation in extreme antibody excess.

The observation of ring structures in some of the aggregates of antigen and antibody would indicate that Goldberg's (29, 30) simplifying assumptions of the absence of cyclic structures (see also reference 31) may not be warranted.

Antibody molecules frequently appeared to consist of several (perhaps five) beads (Figs. 3 *b*, 8, 9, 12 *a*). It is difficult to interpret this observation. Two possibilities may be envisaged: several subunits may be linked by narrow chains; alternatively, a cylindrical molecule may be kinked in several places. In the latter case, the phosphotungstate might fill the spaces of the kinked molecule and thus produce the observed beaded appearance.

The dimensions of antibody have been estimated in the electron microscope

by several workers. Many have relied on the separation of antibody-linked antigens for estimates of the length of the antibody, as have Easty and Mercer (6) who have found average center-to-center distances of antibody-linked ferritin molecules to be 200 to 400 A. Hall, Nisonoff, and Slayter (7) have measured the dimensions of shadowed antibody gamma globulin and have found a weight average of 250 A and a width of 30 to 40 A. We have measured the widths of antibody in virus-antibody complexes and have determined the width to be 35 to 40 A, in good agreement with the previously mentioned values. The length of antibody was estimated from the separation of antibody-linked virus particles as 250 A, again in reasonably good agreement with the values reported by Hall and others. In several instances, where antibody molecules seem to be stretched and where we could exclude the attachment of the antibody molecule which occurred below the layer of tungstate, we have attempted direct measurements of the length of the antibody molecule and have found values of 250 to 270 A for the length of the antibody molecule. It is clear that our estimate of the width of the antibody is subject to any error which may arise from tungstate overlapping the antibody molecule and thus reducing the apparent width of the molecule. Since such events would presumably occur randomly and would depend on the quantity of tungstate employed and on the relative position of the antibody employed with respect to the supporting film of the grid, we assume that this error would not affect all our measurements and that the large number of measurements obtained would be subject to this error in terms of the distribution of width, rather than in the final estimate. It is interesting to observe that a diameter of 30 A at the base of the antibody molecule would leave a sufficient area for the longest dimension of the estimated combining site of antibody (1).

The values obtained by electron microscopy are in reasonable agreement with the dimensions calculated by Boyd (32), from data of Kabat's (33) of 251 $\times$ 43 A for unhydrated rabbit pneumococcus antibody and of 330 $\times$ 38 A for the globulin fraction of rabbit ovalbumin antiserum. This similarity of values may, however, not be relevant since hydrodynamic data are obtained by assuming the shape of the antibody to be an ellipsoid of revolution and since effects of hydration may not justify any comparison between hydrodynamic data and data obtained by electron microscopic observation.

There is indirect evidence that the two combining sites of rabbit antibody must be at a considerable distance from one another. Antibody has been shown to be divalent in reactions with haptens of relatively low molecular weight (34) as well as with macromolecules (35–37). It follows that the separation of the two combining sites must be such as to exclude steric hindrance in the combination of a macromolecular antigen with antibody already combined with a first molecule of antigen. The electron micrographs presented in this paper show that the combining sites of antibody are at the two opposite ends of the elon-

gated cylindrical molecule. The separation between the two sites is thus of the same order of magnitude as the length of the antibody molecule.

We would like to turn finally, to the use which we have attempted to make of antibody in assessing the relation between normal and aberrant forms of a virus. It has been assumed in this that both valencies of antibody are identical. This is indeed well supported by several independent investigators (23, 24, 38–40). It must, however, be stressed that while these experiments exclude the presence of more than 5 to 10 per cent of heteroligating antibody, a much smaller percentage of heteroligating antibody could, in as selective a technique as electron microscopy, lead to observations of cross-linking between antigenically unrelated forms. It is, therefore, only the regular observation of cross-linking and the cross-linking of a number of known forms that can be taken as sound evidence that an antigenic relation between two forms does exist. Of the forms shown in Figs. 12 *a* to 12 *d*, only the pear-shaped and the elongated variant of the virus (Figs. 12 *c* and 12 *d*) fulfills this criterion.

SUMMARY

Negatively stained aggregates of antigen (polyoma or verruca vulgaris virus) and antibody (from rabbit or goat) were examined in the electron microscope. The antibody molecules appeared as cylindrical rods (often, but not always, showing a beaded appearance) with a long axis of 250 to 270 A and a short axis of 35 to 40 A. The combining sites were at the opposite short ends of the antibody molecules separated by the length of 250 to 270 A of the antibody molecule.

Aggregates of antigen and antibody showed regions of orderly arrangements and frequently ring structures of five or more linked virus particles. Sometimes a virus particle in the center of these ring structures was linked to the peripheral particles. In extreme antibody excess, cross-linking was only rarely observed and virus particles were surrounded by a dense aura of antibody molecules.

The specificity of the two combining sites of most antibody molecules is identical. This was utilized to examine the antigenic relation between the normal (icosahedral) and aberrant forms of polyoma virus.

Thanks are due to Dr. R. Sheinin, Dr. D. McLeod, and Dr. M. G. Williams for the supply of polyoma and wart virus; to Dr. R. Wormleighton for statistical advice and computations; and to the Medical Research Council (MT-832), the National Cancer Institute, the National Institutes of Health (5T1 GM-506-03), the Ontario Cancer Treatment and Research Foundation, and the Banting Research Foundation for financial support.

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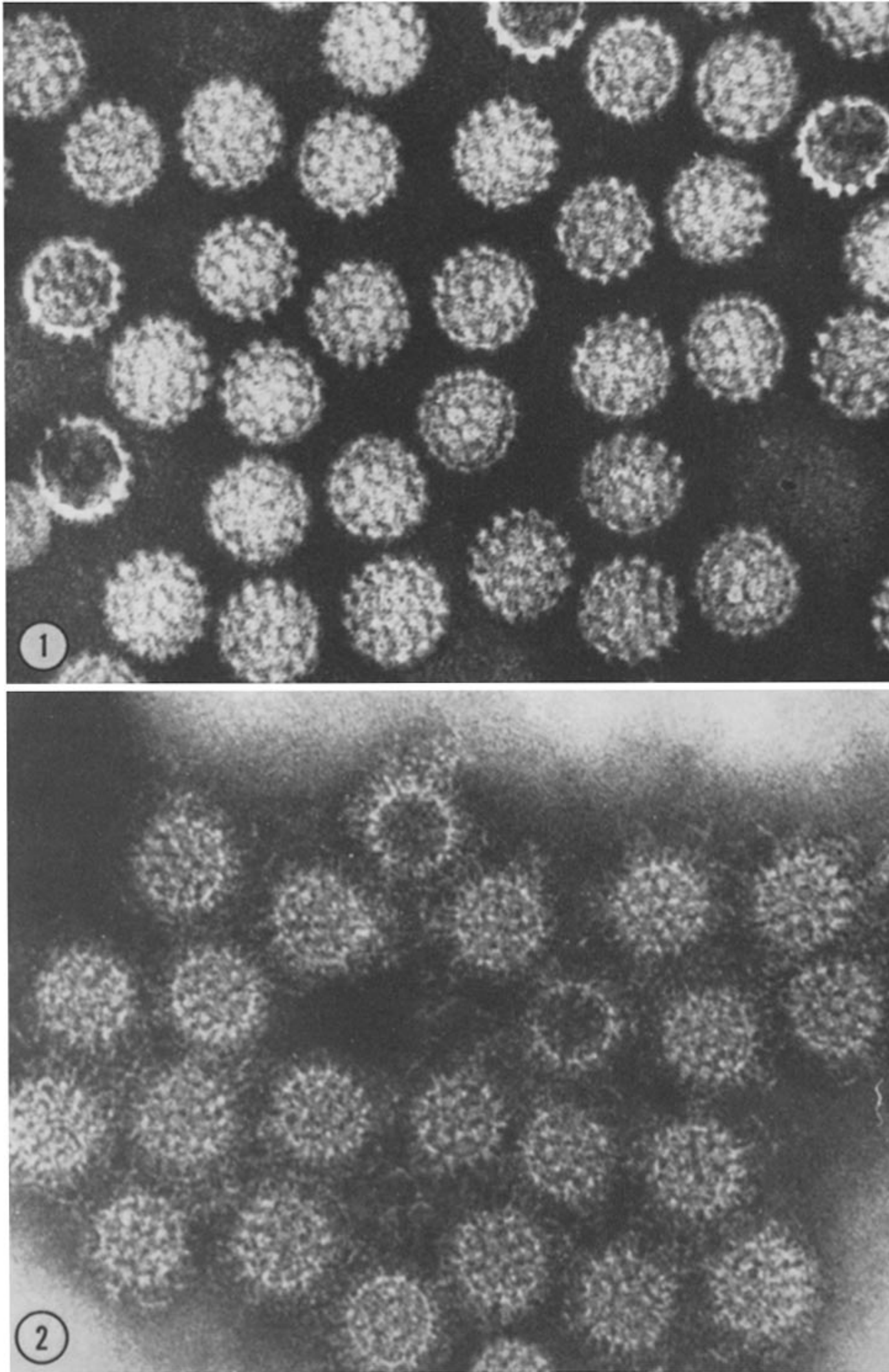
EXPLANATION OF PLATES

PLATE 31

FIG. 1. Wart virus in the absence of antibody. Negatively stained preparation of human wart virus. Particles are randomly distributed over the field and viral substructure is clearly discerned. × 300,000.

FIG. 2. Wart virus in the presence of antibody. Wart virus combined with wart virus antibody from rabbits. The substructure of the virus is obscured by the presence of antibody, each virus particle is surrounded by a halo of antibody and the particles are clumped together. × 300,000.



(Almeida *et al.*: Structure of antigen-antibody complexes)

PLATE 32

FIGS. 3 *a* to 3 *c*. Near equivalence antigen excess. Particles of polyoma virus linked by antibody raised in goat.

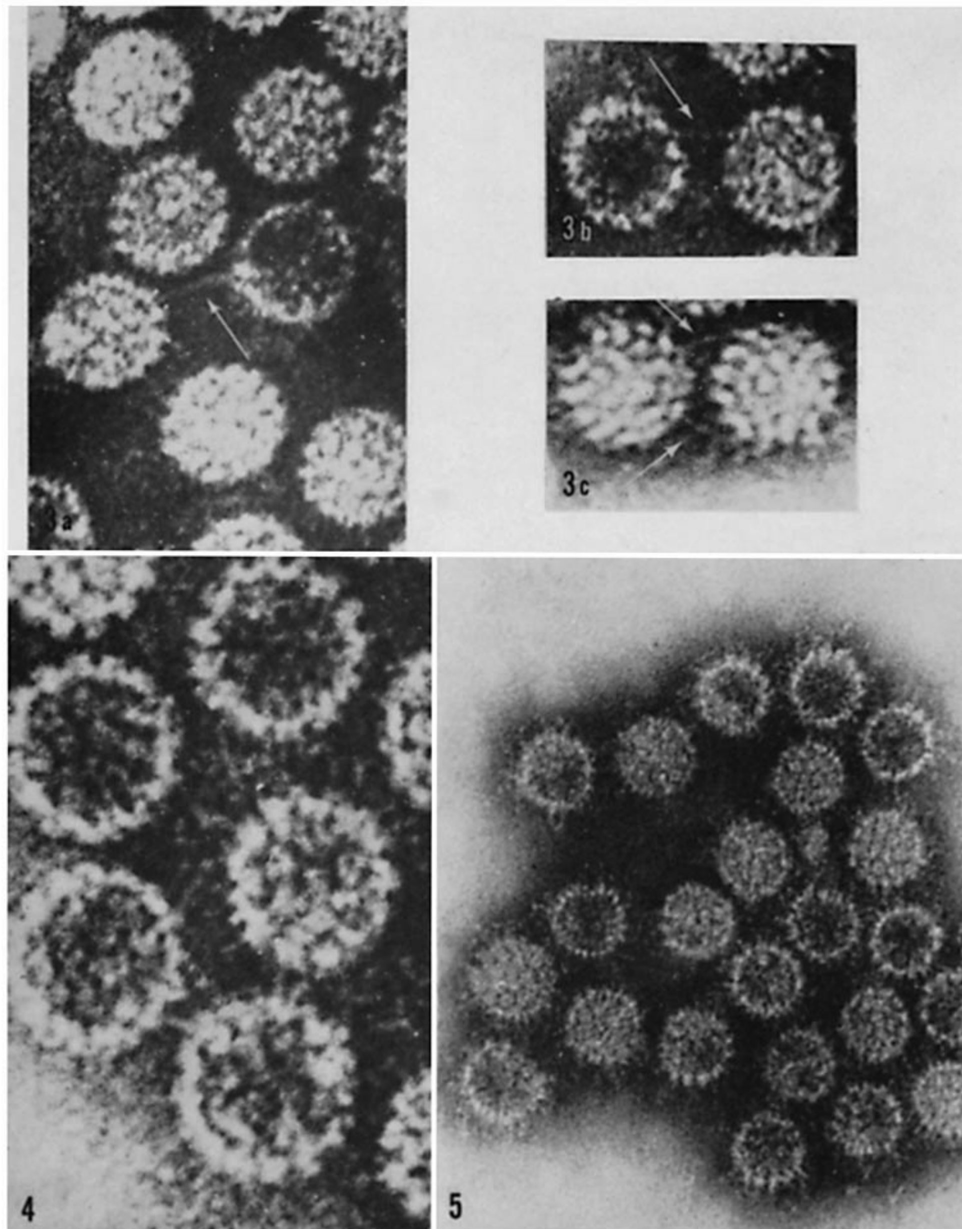
FIG. 3 *a*. A group of polyoma virus particles showing little antibody linking. The antibody molecule marked by the arrow is well delineated and measures 270 A. × 400,000.

FIG. 3 *b*. Two polyoma particles linked by a single antibody molecule. × 400,000.

FIG. 3 *c*. Two polyoma particles linked by two antibody molecules. × 400,000.

FIG. 4. Near equivalence. Aggregate of polyoma virus particles and antibody; the proportions of antigen and antibody are those given for mixture *a* in Table I and Text-fig. 1. Six particles are linked with one another by two or more antibody molecules. × 580,000.

FIG. 5. Slight antibody excess. Mixture of polyoma virus antigen and antibody of composition given for *b* in Table I and Text-fig. 1. Aggregates are larger than those shown in Fig. 4 for mixture *a*. All the particles are linked by many antibody molecules. × 200,000.

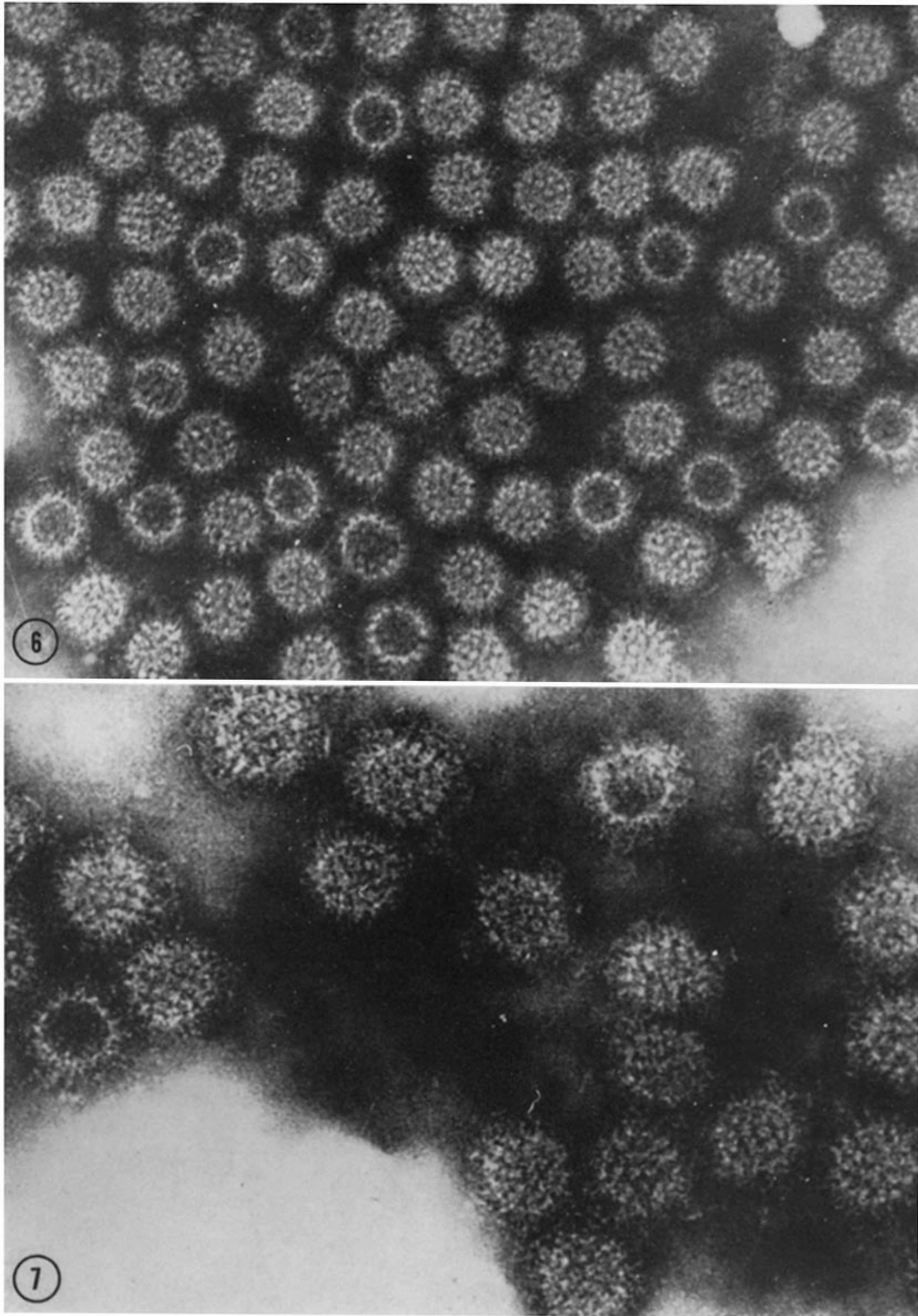


(Almeida *et al.*: Structure of antigen-antibody complexes)

PLATE 33

FIG. 6. Antibody excess. Mixture of wart virus and antibody of composition given for mixture *c* in Table I and Text-fig. 1. Very large aggregates of virus and antibody are present and although antibody is still linking most virus particles, some of the particles are, at this concentration of antibody, completely covered with antibody and are unable to connect with any other particle (through antibody). × 200,000.

FIG. 7. Extreme antibody excess. The relative composition in antibody and antigen is that of mixture *d* in Table I and Text-fig. 1. The wart virus is almost obscured by a dense aura of antibody molecules. Antibody cross-linking of particles is rare The antibody layer of two neighbouring particles has become enmeshed in a few instances. × 290,000.



(Almeida *et al.*: Structure of antigen-antibody complexes)

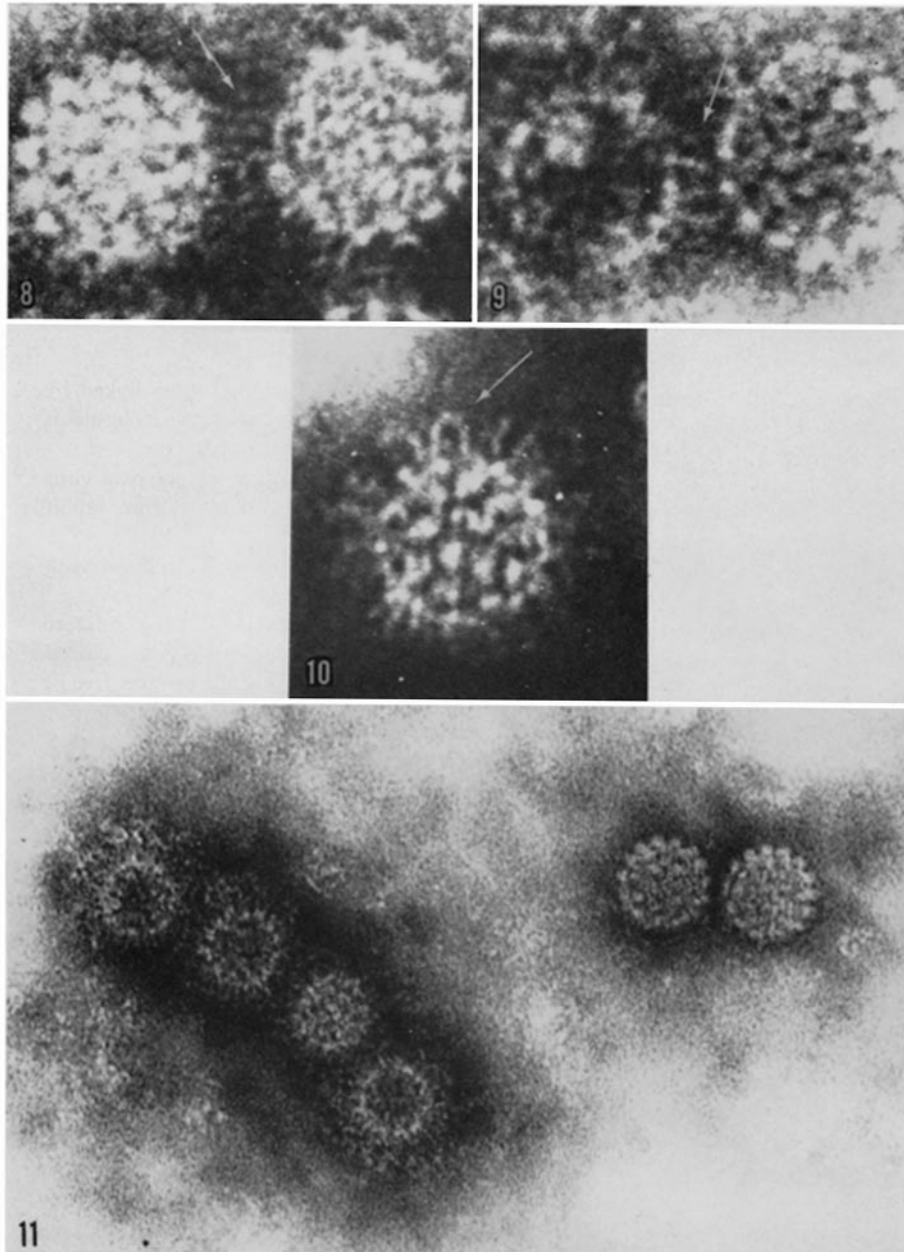
Plate 34

Fig. 8. Antibody from rabbit (see arrow). Two particles of wart virus linked by three distinct antibody molecules, the rod-like shape of the antibody molecule is clearly visible. $\times$ 600,000.

Fig. 9. Antibody from goat (indicated by arrow). Two particles of polyoma virus linked by antibody molecules. Length and width of antibody derived from rabbit and goat showed no appreciable difference. $\times$ 580,000.

Fig. 10. Loop of antibody. Antibody, indicated by an arrow, is attached with both combining sites to one and the same particle of wart virus. $\times$ 480,000.

Fig. 11. The specificity of antibody. Polyoma antiserum was added to a. mixture of wart and polyoma virus. The smaller, 450 A polyoma virus particles are coated with antibody (at left) while the larger, 550 A wart virus (at right) remains free of antibody. $\times$ 250,000.



(Almeida *et al.*: Structure of antigen-antibody complexes)

Plate 35

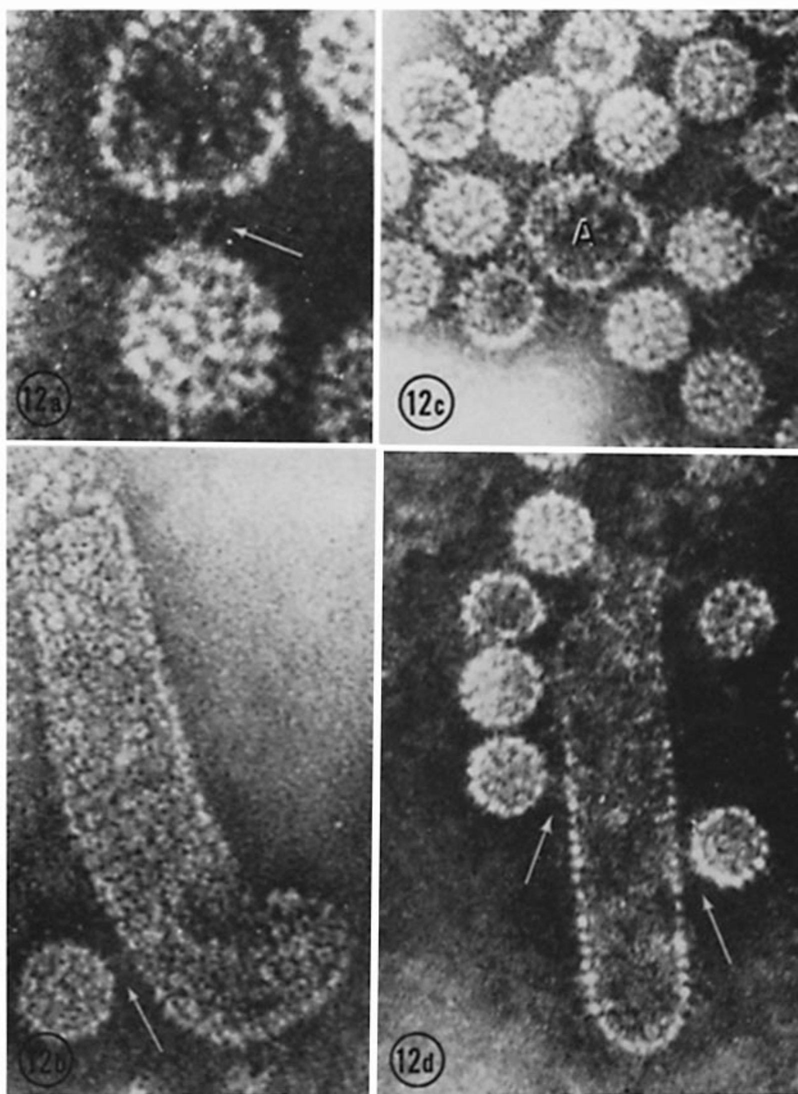
Figs. 12 *a* to 12 *d*. Antibody linking normal icosahedral with abnormal polyoma forms. A test for the presence of a common antigen.

Fig. 12 *a*. A pear-shaped particle in the upper part of the micrograph is linked by 2 antibody molecules to a normal icosahedral form in the lower part of the micrograph. The arrow indicates an antibody molecule linking normal and aberrant virus particles. × 550,000.

Fig. 12 *b*. An elongated particle linked by antibody to a normal icosahedral particle. The linking antibody molecule is indicated by an arrow × 270,000.

Fig. 12 *c*. A pear-shaped particle (*A*) linked by antibody to six surrounding normal icosahedral virus particles. × 330,000.

Fig. 12 *d*. An elongated particle linked by antibody molecules to five normal icosahedral particles. Arrows point to two such cross-linking groups of antibody molecules. × 330,000.



(Almeida *et al.*: Structure of antigen-antibody complexes)