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Adenovirus Binds to Rat Brain Microtubules In Vitro

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We have found by negative staining electron microscopy that when similar concentrations of adenovirus and reovirus (viruses of about the same diameter, 75 to 80 nm, and density, 1.34 to 1.36 g/cm^3) were incubated with a carbon support film containing microtubules, 72% of adenovirus on the grid, but only 32% (equivalent to random association) of reovirus, were associated with microtubules. Similar concentrations of both larger and smaller particles, such as polystyrene latex spheres and coliphage f2, also exhibited a low degree of interaction, viz., 17 to 37%, with microtubules. Moreover, 90% of microtubule-associated adenovirus binds to within ±4 nm of the edge of microtubules, but lower fractions (again equivalent to a random association) of the other particles bind to the edge of the microtubules. The mechanism behind this phenomenon, which we denote as "edge binding," is presently obscure; however, it provides us with a second, albeit empirical, method to distinguish between the microtubular association of adenovirus and other particles. We found that edge binding of adenovirus also occurred when adenovirus was initially placed on the carbon support film and then incubated with microtubules and when adenovirus and microtubules were mixed prior to placement on the support. In contrast, reovirus or the other particles prepared by similar techniques exhibited a random amount of edge binding. The binding of adenovirus appears to involve the hexon capsomers of the virion since (i) high resolution electron micrographs showed that the edge of the virus was in contact with the edge of the microtubules, and (ii) adenovirions briefly treated with formamide to remove pentons and fibers bind as efficiently as intact virions. Core structures, which were obtained by further formamide degradation of the virion, do not associate with microtubules. These observations support the hypothesis of Dales and Chardonnet (1973) that the transport of adenovirions within infected cells is mediated by interaction with microtubules.

Viruses that replicate in animal cells can do so either in the nucleus, viz., adenovirus (10) and simian virus 40 (1), or in the cytoplasm, viz., reovirus (18) and vaccinia (11). For those viruses in the first category, such as adenovirus type 5, some motility mechanism must be invoked to account for the transfer of genetic material from the inner edge of the cell membrane to the nucleus. Based upon electron microscopy observations of adenovirus-infected cells (4), as well as on motility-inhibitor studies during adenovirus infection (6), it was suggested by Dales and Chardonnet (6) that cytoplasmic microtubules may be involved in adenovirus transport. In support of this model we have found that adenovirus type 5 binds specifically to reassociated rat brain microtubules in vitro. We chose rat brain microtubules because conditions for their isolation in high purity and their polymerization in vitro are well known, but it has proven difficult to isolate microtubules from HeLa cells using similar conditions (Weihing and Luftig, unpublished observations).

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MATERIALS AND METHODS

Rat brain microtubules. Rat brain microtubules were isolated by in vitro assembly methods (19, 21). Approximately 35 g of fresh rat brains were homogenized at 0 C in a volume of polymerization solution buffer [1 mM $MgSO_4$, 2 mM ethylene glycol bis(β-aminoethylether) *N,N'*-tetraacetic acid, 1 mM GTP, 100 mM PIPES, pH 6.9] equal to 1.5 times the weight of the brains and centrifuged at 17,000 rpm in the SS-34 rotor of a Sorvall RC-2B centrifuge (1 h at 4 C). The supernatant solution was incubated at 37 C for 30 or 65 min to assemble the tubulin into microtubules. The microtubules were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 15 min at 25 C,

resuspended in about one-third the volume of polymerization solution buffer used to homogenize the brain, and incubated at 0 C for 30 min to depolymerize the microtubules. The suspension was then centrifuged at 40,000 rpm (Spinco SW50.1 rotor) for 1 h at 4 C to remove tubulin aggregates. The resulting supernatant solution was then incubated again at 37 C for 30 min and reassociated microtubules were collected as before. This two-cycle polymerization provided microtubules which, when analyzed by polyacrylamide gel electrophoresis according to Neville (15) (see Fig. 2a), were of high purity (3). Their

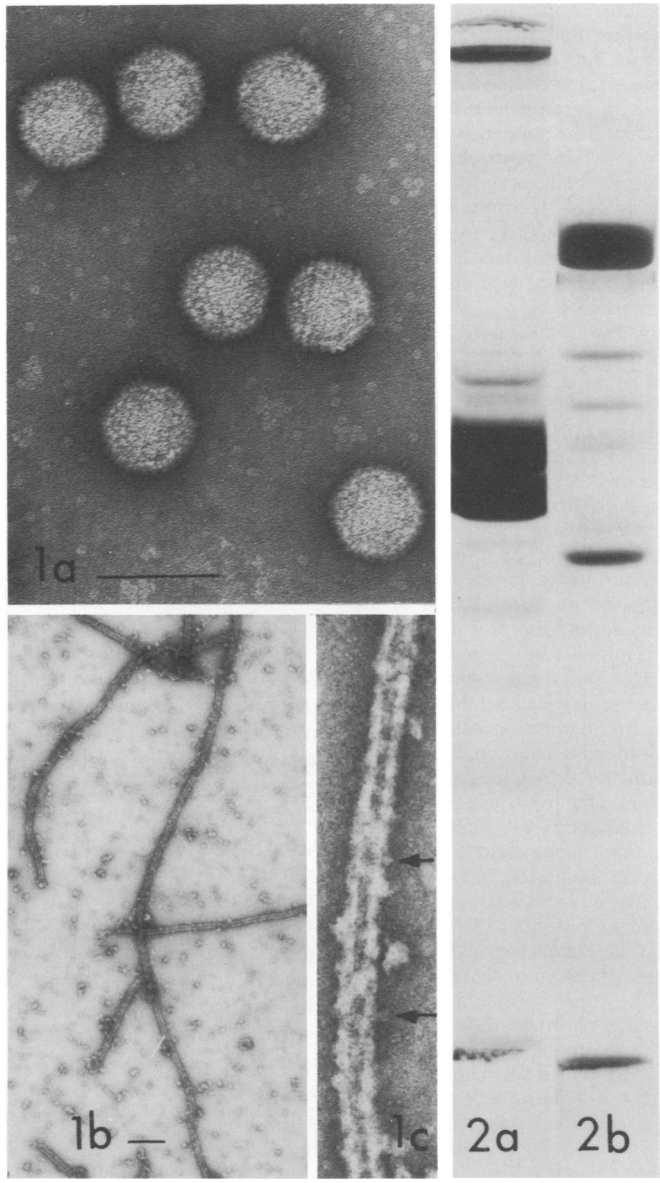

FIG. 1. *Electron micrographs of (a) adenovirus type 5 and (b) and (c) rat brain microtubules. Arrows on (c) point to fuzzy projections on microtubules. In this and subsequent figures the preparations were fixed with 5% glutaraldehyde and stained with 2% uranyl acetate. Magnification: (a) ×160,000, (b) ×48,000, and (c) ×225,000. Bar represents 0.1 μm in all micrographs.*

FIG. 2. *Polyacrylamide gel electrophoresis of (a) rat brain microtubules and (b) adenovirus type 5. The major components of the microtubules (tubulin) have apparent molecular weights of 58,000 and 53,000. The microtubules also contain the high molecular weight (>200,000) doublet observed previously (3) in similar preparations.*

appearance by negative stain electron microscopy was the same as reported by others for reassociated brain microtubules (3, 21), viz., they were 25.5 ± 1.3 nm wide (Fig. 1b), had protofilaments, and had fuzzy projections on their sides (Fig. 1c, arrows).

Adenovirus type 5. This was propagated in HeLa cells grown in spinner culture in Joklik modified Eagle minimal essential medium supplemented with 5% fetal calf serum (17). After incubation for 48 to 72 h, at 37 C, cells were harvested and disrupted. The centrifuged supernatant was extracted with fluorocarbon and then subjected to sucrose gradient centrifugation followed by two cycles of equilibrium density gradient centrifugation in CsCl (7). The virus was free of adeno-associated or other contaminating virions based upon polyacrylamide gel electrophoresis (Fig. 2b; 8) and morphological criteria (Fig. 1a). Concentrated adenovirus solutions were diluted 30-fold with 0.03 M Tris, pH 8.0, to about 5×10^{11} particles/ml prior to specimen preparation.

Electron microscopy. Preparations of all particle or microtubule suspensions were made on carbon support films at ambient temperature by the droplet procedure (12), fixed for 4 min in 5% glutaraldehyde (0.15 M sodium cacodylate, pH 7.4), touched to a surface of distilled water for 5 s, and then stained for 1 to 2 min with 2% uranyl acetate, pH 4.2. The preparations made prior to fixation were of three types. (Method A) A grid was placed on a 15-μl drop of concentrated microtubules (1 to 4 mg of protein/ml) for 2 min, touched to a surface of distilled water for about 5 s, and then floated on a 20-μl drop of an adenovirus or other particle solution (5×10^{10} to 5×10^{11} particles/ml) for 2 min. Unless otherwise indicated, dilutions of particles were made so that approximately the same number of particles would be on the grid. This involved an initial dilution based on the protein concentration of the viral solution, followed by an adjustment to account for differences in diffusion rates among the viruses. In most experiments a dilution was used so that about 150 particles were observed per electron microscope field at 16,000× magnification when viruses alone were adhered to the grid. For example, reovirus at a concentration of 8 mg of protein/ml was diluted 90-fold into 0.03 M Tris, pH 8.0. Since the molecular weight of reovirus was 100×10^6 (9), we expected the suspension to contain about 5×10^{11} particles/ml. At this concentration we observed 135 ± 16 particles per electron microscope field (n = 10 fields), after a 2-min incubation of the virus suspension. Adenovirus diluted to this concentration gave a similar value of 164 ± 30 particles. For f2 phage a dilution of 4×10^{10} particles/ml was found to give values in this range, whereas with latex particles a 10^{-3} dilution of the original stock was used. These particle concentrations (or 5- to 10-fold lower concentrations) were empirically selected: (i) so that on the average a particle would have an optimal chance to encounter a microtubule rather than the carbon support film; and (ii) to allow for enough particles to be counted per grid so that a statistically significant number could be obtained. In quantitative terms, at the lowest particle concentrations, our conditions led to an average of 1 particle diameter per equivalent 1,000 diameters of microtubule contour length. (Method B) Particles at a concentration of about 5×10^{11}/ml were allowed to adhere to a grid for 2 min. The grid was then floated on: (i) a solution of 0.03 M Tris, pH 8.0, for about 5 s; (ii) a 15-μl drop of concentrated microtubules for 5 s up to 30 min; and (iii) a solution of distilled water for about 5 s. (Method C) Twenty microliters each of a concentrated microtubule suspension and a 3×10^{12}-particle/ml solution of adenovirus were incubated at ambient temperature for 3 or 10 min; a grid was placed on the mixture for 1 min and then onto a solution of distilled water for about 5 s. Particles other than adenovirus used in this study were reovirus type 3 (obtained from A. Hay [13]); Dow polystyrene latex spheres, 0.234-μm diameter (from Dow Chemical Co.); and coliphage f2 (a gift of R. Webster, Duke University).

RESULTS

Brief outline of methods. The electron microscopy assay for interaction of virus with microtubules was described in detail above. We recapitulate with a brief outline of the three methods used to prepare grids: method A, microtubules adsorbed to the grid followed by particles; method B, particles adsorbed to the grid followed by microtubules; method C, a mixture of microtubules and particles adsorbed to the grid. The association of particles with microtubules was then measured as discussed below.

Binding of adenovirus to microtubules. When microtubules were first adsorbed to the grid and then exposed to adenovirus (method A) an average of 72% of the adenovirus particles whose association could be unequivocally determined were associated with microtubules (Table 1, Fig. 3; average over three separate experiments). This indicates that adenovirions preferentially bind to microtubules rather than to the carbon support film. In contrast, reovirus, whose diameter and diffusion coefficient are approximately the same as adenovirus (respective diameters are 76 and 70 to 80 nm; diffusion coefficients are in a ratio of 1.33:1.0, calculated by Svedberg equation and data in reference 9) exhibited only 32% of its particles in association with microtubules (Table 1; Fig. 4a). This lower percentage probably represents a nonspecific association, since both latex particles and coliphage f2, particles one would not expect to have a functionally significant association with mammalian microtubules, also exhibited a similar low fraction (17 to 37%) of bound particles (Table 1; Fig. 4c). Further evidence for such nonspecificity is provided by a control experiment where viruses were traced from prints and randomly placed on micrographs of the microtubules. In that case we

TABLE 1. *Association of adenovirus and other particles with microtubules*[a]

Particles	Experiment	% Particles bound to microtubules[b]		No. of particles per replication			
		Total	On edge	a	b	c	d
Adenovirus	1[c]	75 ± 4	65 ± 4	46	147	180	383
	2	75 ± 1	67 ± 2	148	181		
	3	67	65	172			
Reovirus	1	26 ± 10	19 ± 10	91	103	90	
	2	35	21	289			
	3	34	23	495			
Latex particles	1	37 ± 5	14 ± 4	141	129	203	
	2[d]	25	6	16			
Coliphage f2	1	17	10	135			
	2[e]	19 ± 1	12 ± 2	613	525		

[a] Each experiment was performed with a fresh particle and microtubule preparation. The averages and standard deviations are for replicated experiments with a given preparation. Counts were made either on the electron microscope screen at 77,000× or from prints at 48,000× magnification with essentially no difference in results. Particles associated with bush-like structures (Fig. 4a and 6b; thick arrows) were not counted since we were unable to decide whether the primary association was with the underlying carbon film or microtubule fragments that were present in these structures. In general, 20 to 40% of particles in all categories were associated with the bushes.

[b] In controls performed where over 100 reo- or adenoviruses were traced from prints of electron micrographs and randomly placed back on the same micrograph (48,000× magnification) we found that 32 ± 5% of the particles were associated with microtubules. This can be taken as an estimate of nonspecific association.

[c] In this experiment adenovirus was diluted to 5×10^{10} (replicate a), 10^{11} (replicate b), and 5×10^{11} (replicate c and d) particles/ml. The small standard deviations indicate the fraction of particles bound to microtubules is independent of particle concentration.

[d] This dilution was 10-fold less than the first experiment with latex particles.

[e] These data were taken from micrographs at a total magnification of 110,000×. As in the case of adenovirus, almost identical results were obtained when we counted microtubule associated particles on the electron microscope screen at a total magnification of 160,000×.

found again that only about 30% of the virus particles were associated with microtubules (Table 1, footnote *b*).

A novel feature of the interaction between adenoviruses and microtubules was that over 90% of microtubule-associated adenovirus were bound to within ±4 nm of the edge (the basis for edge binding is obscure, because microtubules are cylinders and not flat two-dimensional structures. We have presented a detailed discussion of the possible meaning of this "edge" phenomenon below) of microtubules (Table 1; Fig. 3, arrows). In contrast, reovirus, coliphage f2, and latex particles had an average of 65, 61, and 30% of their respective associated particles bound to the edge of microtubules. If one randomly traced microtubules onto micrographs of these particles (see Table 2 for a detailed explanation of this method), one found that about 50% of associated adenovirus, reovirus, and coliphage f2 particles were bound to the edge and 50% to an interior region of the microtubule. These values are consistent with a random association for reovirus and coliphage f2 with the edge of microtubules; the edge binding of adenovirus, on the other hand, is not consistent with such a random association.

We do not understand the basis of this edge binding for adenovirus; however, it provides us with an empirical, second method to distinguish between the microtubular association of adenovirus and of other particles. We further investigated the specificity of edge binding for adenovirus using methods B and C.

Alternative methods of specimen preparation for examination of edge binding to microtubules. Various viruses or latex particles were initially adsorbed to a carbon support film and subsequently exposed to a solution of microtubules (method B). This technique created a large particle excess on the support film. We now asked whether particles interacted with the edge or with all surfaces of the microtubules. We again found that almost 90% of the microtubule-associated adenoviruses were bound to the edge of microtubules (Fig. 5, arrows) and about 10% to the central region (Fig. 5a, arrow-

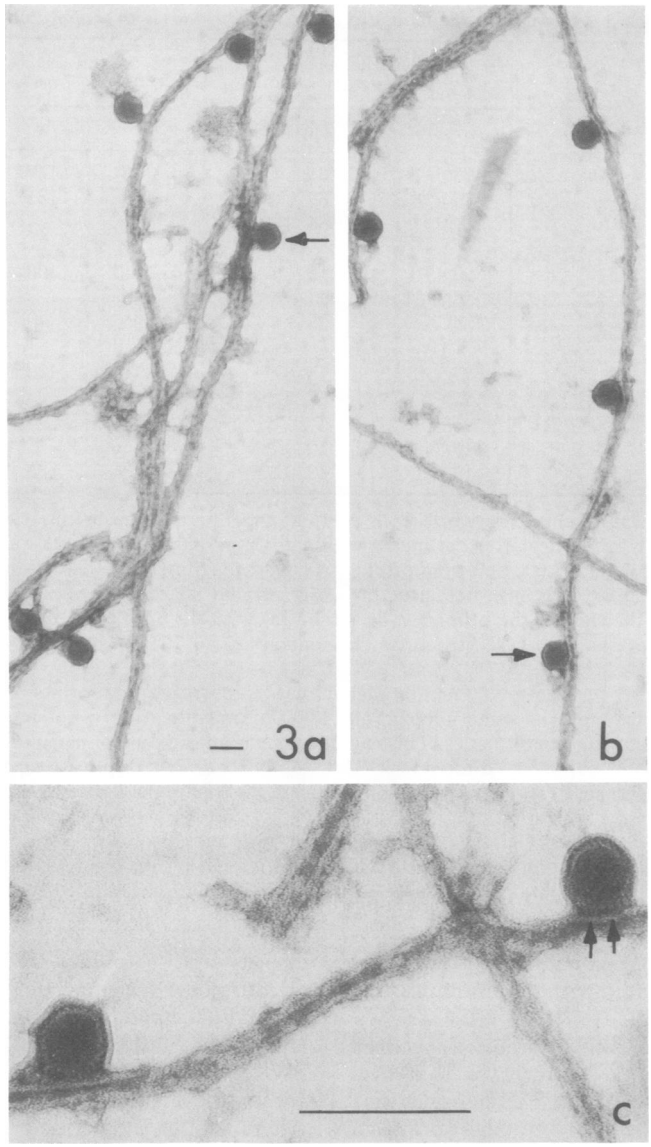

FIG. 3. *Electron micrograph of adenovirus associated with microtubules. The grid was first covered with microtubules and then treated with adenovirus. Arrows point to capsomeres at the edge of adenovirus. Magnification:* (*a and b*) ×48,000; (*c*) ×160,000.

head; Table 2, experiment column). We compared this value with control values (Table 2, control column) derived by tracing microtubules from prints, randomly placing the tracing back on the same micrograph, and counting the number of particles associated with the edge and with the central regions. This procedure showed that only 50% or less of randomly associated particles would be associated with the edge of microtubules. We conclude from this comparison that the association of adenovirus with microtubules is nonrandom and preferentially with the edge.

This result was obtained under conditions where the number of particles (of 200 counted in one experiment) not associated with microtubules was four times greater than the number associated in any way with microtubules, and thus we expected that the presence of such an excess of virus would promote nonspecific binding of virus to the central region of microtubules, especially if there were any co-precipitation of protein structures caused by drying down of the suspension on the grid. Instead, as with method A, we found that most microtubule-associated virions

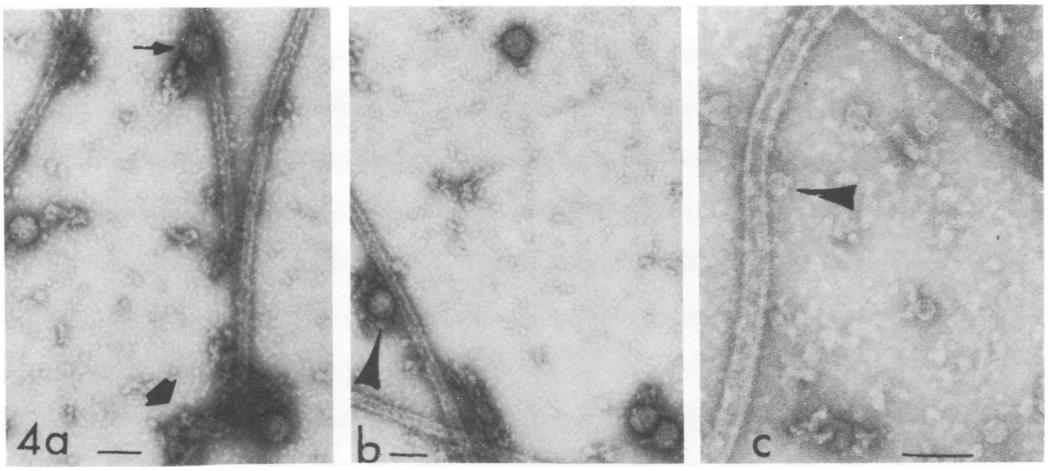

FIG. 4. *Electron micrograph of reovirus (a and b) and coliphage f2 (c) associated with microtubules. Grids were prepared as in Fig. 3. Arrows indicate representative particles lying on or under microtubules; arrowheads point to particles associated with the edge of microtubules. Magnification: (a and b) ×48,000; (c) ×110,000.*

TABLE 2. *Association of microtubules with adenovirus and other particles using alternative preparation techniques*[a]

Techniques	Dilution[b] factor of particles	No. of particles on edge of microtubules/total particles[c] associated with microtubules	
		Experiment[a]	Control[d]
Adenovirus and microtubules			
Adenovirus initially on carbon support (method B)	1.0	0.89 (114)	0.47 (70)
	1.0	0.86 (166)	0.47 (72)
	0.2	0.85 (78)	0.46 (65)
	0.1	0.88 (43)	0.46 (65)
Adenovirus incubated with microtubules (method C)[e]	1.0	0.89 (156)	0.44 (68)
	1.0	0.75 (99)	0.45 (31)
Other particles and microtubules[f]			
Reovirus	1.0	0.50 (249)	0.50 (60)
	1.0	0.54 (68)	0.52 (29)
	0.1	0.53 (32)	0.48 (31)
Latex spheres	1.0	0.16 (55)	0.33 (43)
Coliphage f2	1.0	0.55 (128)	0.51 (141)

[a] Each point was calculated using data combined from at least eight separate fields on two different grids. Parentheses give the number of particles in each experiment.

[b] A dilution factor of unity is defined as concentration which yields about 150 particles on a carbon support film at a magnification of 16,000×.

[c] Total number refers to particles associated with the edge plus those on or under the microtubules.

[d] To derive control values, microtubules traced from prints of electron micrographs were randomly placed back on the same micrograph and the number of associated particles was counted.

[e] Incubation of the first and second mixtures was for 3 min and 10 min, respectively.

[f] In this series of experiments the grid was first covered with the other particles.

were associated with the edge, indicating edge binding to the microtubules under conditions which should promote random association. Furthermore, edge binding was observed over a 10-fold range of adenovirus concentrations (Table 2). In contrast to these results, we found that latex particles, reovirions, and coliphage f2 appeared to associate randomly with microtubules (Fig. 6a,b,c, and Table 1), i.e., the same fraction of particles was associated with the edge as was observed in a control where microtubules were placed randomly over corresponding micrographs of the particles.

The third technique used to examine the binding of adenovirus to microtubules was to incubate a mixture of the two suspensions prior

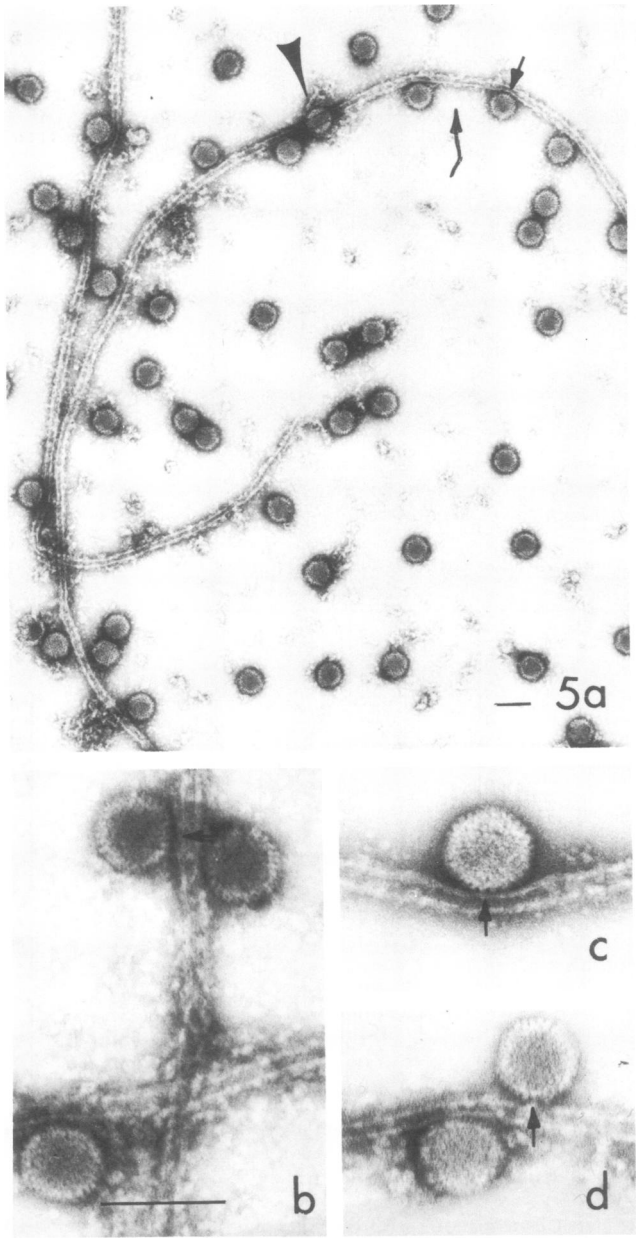

FIG. 5. *Electron micrograph of adenovirus associated with microtubules. In this preparation the grid was first treated with adenovirus. Arrows indicate regions where capsomeres are closely associated with microtubules. Magnification:* (*a*) *×48,000;* (*b, c, and d*) *×160,000.*

to adsorbing the preparation to a carbon support film (method C). As seen in Table 2, we again found a nonrandom association of adenovirus with the edge of the microtubules.

Time course of binding. To investigate the rate of binding of adenovirus to microtubules, one must estimate the number of bound virions per unit mass of microtubule as a function of time. We used the number of virions bound per centimeter of contour length as a measure of the virions bound per unit mass. To estimate the number of such specifically bound virions per centimeter of microtubule one must subtract the number of nonspecifically associated particles (Table 3, footnote *c*) from the total number of associated particles and divide this difference

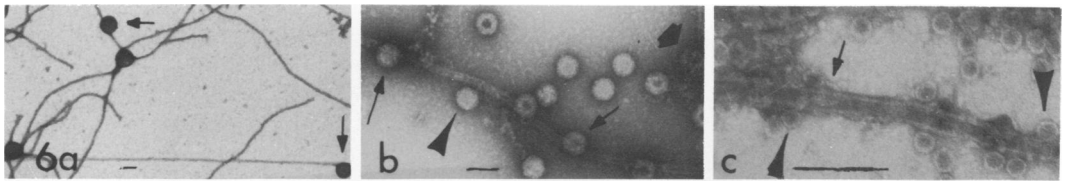

FIG. 6. *Electron micrograph of various particles associated with microtubules. A grid was first covered with (a) latex particles; (b) reovirus type 3; and (c) coliphage f2. Arrows and arrowheads point to particles as described in Fig. 4. Magnification: (a) ×20,000; (b) ×42,000, and (c) ×130,000. Note: the magnifications were adjusted so that the particles appeared to be the same size.*

TABLE 3. *Time independence of association of adenovirus with microtubules*[a]

Time of exposure to microtubules	Total particles[b] counted	Number of particles[c] specifically associated at edge of microtubules	Edge-associated[d] particles per contour length (particles/cm)
5 s	73	49	0.45
3 min	140	88	0.34
12 min	76	56	0.35
21 min	48	26	0.31
30 min	63	43	0.35

[a] In these experiments adenovirus was initially placed on the grid (method B).

[b] Total includes particles associated with the edge as well as on or under microtubules.

[c] This number is obtained by subtracting nonspecifically associated particles from the total number of associated particles. An equal number of randomly associated particles will be associated with the edge and with the top and bottom (Table 2), and therefore the number of nonspecifically associated particles is equal to the number of particles on or under the microtubule plus an equal number of particles associated with the edge. The total fraction of edge-bound particles (calculated as in the experiment column of Table 2) was 0.84, 0.81, 0.87, 0.77, and 0.84 for the above time points. These values are essentially the same as found in Table 2.

[d] The contour length was measured with a map measurer from prints at a total magnification of 48,000×. (The magnification of the negative was 16,000×.)

by the corresponding contour length of microtubules. The results of such a calculation are shown in Table 3. We found that the number of specifically bound particles per unit length of microtubules did not change significantly between 5 s and 30 min after mixing. Thus, the maximal binding which can be achieved at the particular concentrations of virus and microtubules used in this experiment is reached very quickly.

Suitable calculations from the data of column 4 of Table 3 show that on the average there is one site per 600 nm of microtubule contour length. Clearly this is a minimal estimate of site density, because in several instances we found particles as close as 150 nm together (Fig. 5a, curved arrow).

Adenovirus binds by hexons to microtubules. The high resolution micrographs of Fig. 3c, and 5b,c, and d show that in all cases the edge of the virion, which contains several hexon capsomers (16), appears in direct contact with the edge of the microtubule. This suggests that the viruses bind via hexons. This suggestion was supported by the following results where we subjected the virus to limited degradation with formamide. Formamide treatment (40%) (2 min at ambient temperature [20]) produced particles that are morphologically identical to penton-less virion particles as evidenced by the increased intraparticle staining (Fig. 7a) indicating that the treatment caused the interior of the particle to become accessible to stain. Eighty percent formamide produced viral cores (Fig. 7b). The quantitative data for percentage of edge binding indicate that removal of pentons and then hexons is associated with a stepwise randomization of edge binding (Table 4). Thus, removal of pentons may only partially randomize the binding. However, because we did not measure the composition of the particles produced by 40% formamide treatment, we cannot tell whether partial randomization of binding is a property of pure, penton-free particles, or whether 40% formamide treatment produces a mixture of particles some of which bind exclusively to the edge and the rest of which bind randomly.

DISCUSSION

We have described an in vitro system, using an electron microscopy assay, which provides strong evidence for specific binding of adenovirus to microtubules. In this assay we found that adenovirus exhibited a preference for binding to microtubules rather than to a carbon support film, and binding to within ±4 nm of the edge of the negatively stained microtubule. Control particles did not behave in this manner.

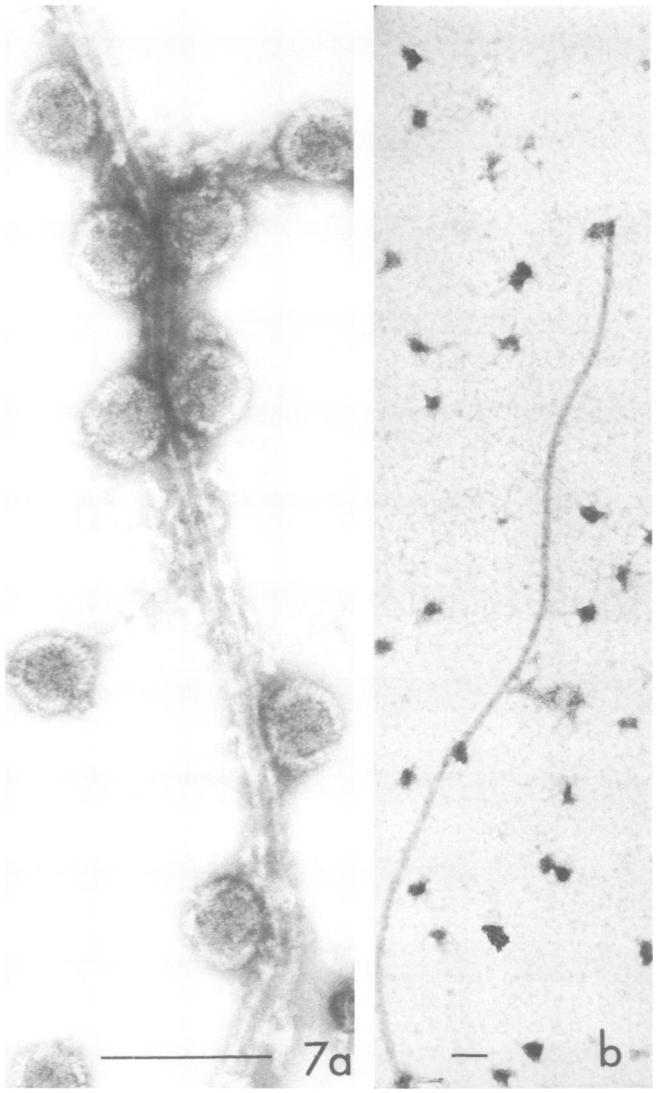

FIG. 7. *Electron micrograph of subparticles of adenovirus associated with microtubules. A grid was first covered with adenovirus subparticles obtained by exposure for 3 min at 4 C to (a) 40 and (b) 80% formamide. Magnification: (a) ×225,000; (b) ×48,000.*

The basis for edge binding of adenoviruses is obscure because microtubules are cylinders and one would expect that, if binding sites were randomly distributed on the surface of the cylinder, the adenovirus should be randomly distributed over the surface of the microtubule. Since this was not observed, we are left with at least two possible explanations: (i) binding sites are predominantly bilaterally distributed, and an exposure of the site to the virus leads to an embedding of the microtubule within the negative stain in such a way that the bound virus particles are at the periphery of the cylinder nearest the carbon support film; or (ii) binding sites are randomly distributed; however, viruses bound to the top of a microtubule fall off or down to the carbon support film near the periphery of the microtubule after air drying.

Our attempts to determine whether a significant number of adenoviruses initially bind to the tops of microtubules by utilizing a critical-point drying technique has proven technically unfeasible thus far. We found that microtubules and virus particles co-precipitated into large aggregates on the grid, preventing us from making any statistically significant evaluation of edge- versus top-associated virions. We can say qualitatively that, in nonaggregated areas of

TABLE 4. *Association of adenovirus and its subparticles with microtubules*[a]

% Formamide	Resultant particle	Total particles counted	% Particles at edge of microtubules
0	Intact virus	61	90
40	Virus without pentons	86	76
80	Nucleoprotein core	45	51

[a] In these experiments the grid was first covered with formamide-treated particles and then exposed to microtubules for 3 min (method B). Specifically, adenovirus placed on the grid for 2 min was floated on a 40 or 80% surface of formamide at 4 C for an additional 2 min, washed by touching the grid to distilled water for 5 s, exposed to microtubules, fixed, and stained as previously described.

the support film, the adenoviruses still were predominantly associated with microtubule edges (R. Luftig and R. Weihing, unpublished data). This observation would favor possibility (i) above if it can be rigorously demonstrated by a quantitative method.

The in vitro binding of adenovirus to microtubules as described above involves sites on both structures. From our present data the binding site on the adenovirus appears associated with the hexon capsomers at the edge of the virion. However, more quantitative studies using viral subparticles of defined composition will be necessary to prove this point definitively. The locus of binding on the microtubules is not yet clear because the present results do not tell us whether the virion bind to the protofilaments or to the fuzzy projections at the edge of the reassociated microtubules.

Although this is the first report describing the in vitro binding of virus to reconstituted microtubules, association of viruses with microtubules has been reported previously. Dales and Chardonnet (6) observed the association of adenovirus with tubulin paracrystals induced by vinblastine treatment of HeLa cells and provided preliminary evidence that adenovirus binds in vitro to isolated paracrystals. Mayhew and Carroll have observed apparent attachment of barley stripe mosaic virus to the microtubules in the mitotic apparatus of various cells from *Hordeum vulgare* (barley) (14). Several years ago, Dales (5) observed association (but not direct contact) of reovirus with microtubules of the mitotic apparatus of mouse L cells. However, the relation of these earlier results to our results, which show that reovirus binds randomly to microtubules in vitro, is not yet clear.

Our result in vitro is consistent with, but does not prove, the hypothesis that microtubules participate in vectorial movement of adenovirus from the cell surface to the nucleus (6). This conclusion would have been strengthened if we had used HeLa cell microtubules, but these have proven difficult to isolate. We feel that our observations with brain microtubules are significant however, because others (2, 19) have shown that polymerization of brain tubulin, e.g., from chicks, can be nucleated by microtubule fragments from sources other than brain. Such experiments imply that investigations of hybrid systems provide useful information about the naturally occurring system and suggests that regions of microtubule proteins, including binding sites, from a wide variety of sources may be preserved during evolution.

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