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Reovirus Serotypes 1 and 3 Differ in Their In Vitro Association with Microtubules

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Utilizing negative-stain electron microscopy in which similar concentrations of reovirus types 1 and 3 are incubated with a carbon support film containing chick brain, rabbit brain, or HeLa cell microtubules, 81% of the type 1 and 56% of type 3 exhibited an association with the apparent "edge" of the microtubule. This implies that there is a high level of specific affinity for type 1 but not for type 3 to microtubules, since it has previously been determined that only 50% of randomly associated particles would be associated with the edge. The high edge binding of reovirus type 1 is virtually independent of the origin of microtubule, or of whether microtubules or virus has been initially adhered to the support film. On the other hand, reovirus type 1-specific antiserum reduced the edge binding of reovirus type 1 to 45%, whereas type 3-specific antiserum caused no loss (within the variability of the assay) of the edge binding of reovirus type 1 to microtubules (76% edge bound). High edge binding of reovirus type 1 to microtubules is correlated with the presence of type 1 σ1 polypeptide. This minor outer capsid polypeptide is encoded in the S1 double-stranded RNA segment and is the viral hemagglutinin and neutralization antigen. Recombinant reovirus clones containing the S1 double-stranded RNA segment of type 1 (80 and 802) show about 85% edge binding, as compared to a value of 42% for clones with the S1 gene of type 3 (204). Electron microscopy of purified reovirus types 1 and 3 by negative staining reveals that type 1 and 802 capsomers are distinctly visualized, whereas those of type 3 and 204 appear diffuse. Thus, the greater in vitro binding of type 1 to microtubules may reflect an increased accessibility of certain of its outer capsomers, and thereby, σ1 polypeptides to microtubules. Examination of thin sections of reovirus type 1- and 3-infected cells at 24 to 48 h postinfection at 31°C showed that about eight times as many viral factories in type 1-infected cells exhibited an extensive association of virus particles with microtubules, as compared to viral factories of type 3-infected cells. Thus, both in vivo and in vitro there appears to be a greater specificity for the association of reovirus type 1 particles with microtubules, as compared to reovirus type 3 particles.

Reovirus is an icosahedral animal virus that has a diameter of approximately 76 nm (11). It is unique in that it contains 10 segments of double-stranded RNA (dsRNA) (18) separated into three size classes: three large (2.7×10^6 daltons), three medium (1.4×10^6 daltons), and four small (0.8×10^6 daltons) (8). These ten segments are completely transcribed into 10 mRNA species (2), which in turn are translated into 10 primary virus-specific polypeptides (3). All 10 dsRNA segments have been found to be necessary for infectivity, resulting in progeny with the same amount and type of dsRNA. In the virion, the dsRNA is surrounded by two protein shells; an outer capsid and an internal core. The outer capsid is easily digested by chymotrypsin (17, 20) to reveal the core which has a diameter of 52 nm and contains 12 hollow spikes protruding from its surface (11). The reoviral polypeptides have recently been renamed, based on biochemical (12) and genetic (14) studies. The core polypeptides have been designated λ1, λ2, λ3, σ2, and μ2, whereas the outer capsid polypeptides are designated σ1, σ3, and μ1C. σ1 is a minor component of the outer capsid encoded for by the S1 gene, one of the small dsRNA segments. The σ1 polypeptide is biologically important in that it is the hemagglutinin (28) as well as the determinant of specificity of neutralization (27) and cell tropism (26). In this

paper, we describe yet another specificity controlled by σ1, the in vitro binding of reovirus to microtubules.

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MATERIALS AND METHODS

Virus. Reovirus serotypes 1 (Lang strain), 2 (Jones strain), and 3 (Dearing strain) were obtained as lysates of infected-mouse L cells and purified on CsCl gradients as previously described (15). A band was obtained at a density of 1.36 g/cm^3 which contained reovirus as determined by electron microscopy. The bands were concentrated with Aquacide II and dialyzed overnight against 0.05 M Tris at pH 8. The final concentrations of virus types 1, 2, and 3 obtained were 1×10^{13}, 2×10^{11}, and 3×10^{12} particles per ml, respectively. The concentration was determined by measuring the absorbancy at 260 nm and applying the relationship 1 optical density (OD) unit at 260 nm = 2.1×10^{12} particles (20). Virus samples were diluted to equal virus concentrations for electron microscopy by using 0.01 M Tris (pH 7.9).

Reovirus recombinant clones were prepared as previously described (16). These clones were obtained as lysates and purified in the same manner as the reovirus serotypes. The origin of the dsRNA segments for the recombinant clones employed are shown in Table 1. The final concentrations (in particles per milliliter) of the recombinants obtained were: clone 80, 1×10^{12}; clone 802, 3×10^{11}; and clone 204, 1×10^{13}.

Adenovirus type 5, provided by B. Miles (Worcester Foundation), was purified from infected HEp-2 cells by an unpublished procedure described to us by J. Weber (Sherbrooke University Medical School, Quebec, Canada). The method is similar to that described previously (1), except that sodium deoxycholate (0.2%, wt/vol) was used to break open the pellet of infected cells, and the Freon 113 extraction was omitted. Purity of the virus was established by electron microscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described earlier (9). Adenovirus was stored at −70°C in small portions (100 μl) and was used immediately after thawing. The concentrated adenovirus samples were diluted fivefold, using 0.01 M Tris (pH 7.9), to about 1×10^{12} particles per ml before use.

Microtubules. Three types of microtubules were employed; chick brain, rabbit brain, and HeLa cell. They were prepared by two cycles of polymerization as previously described (24, 25) and stored in the unpolymerized form at −70°C. The microtubules were polymerized by incubating 200 μl of a solution containing 1 mg of microtubule protein per ml at 37°C. For electron microscopy, microtubules were diluted 1:2 with PM buffer [1 mM $MgSO_4 \cdot 7H_2O$, 2 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid, and 100 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid), pH 6.9] containing 0.1 mM GTP.

Virus-specific antisera. Antisera against reovirus serotypes 1 and 3 were obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md., catalog no. V-701-511-570 (anti-I) and V-703-501-570 (anti-III). The antisera were diluted 1:60 with 0.01 M Tris-hydrochloride, pH 7.9. Carbon-coated grids coated with virus were incubated with antisera for 4 min before incubation with microtubules, using a procedure similar to that described earlier (29). Type 1- and 3-specific antisera covered the homologous virus with a fuzzy coat (Fig. 3) as has been previously described for T4 phage capsid protein (29).

TABLE 1. *Origin of genome segments for recombinant clones 80, 802, 204*[a]

Location of genome segment (polypeptide)[b]	Reovirus serotype		
	Clone 80	Clone 802	Clone 204
Outer capsid			
M2 (μ1C)	3	3	1
S1 (σ1)	1	1	3
S4 (σ3)	3	3	1
Core			
L1 (λ3)	1	1	3
L2 (λ2)	3	3	1
L3 (λ1)	1	3	1
M1 (μ2)	3	3	1
S2 (σ2)	1	3	1
Nonstructural			
M3 (μNS)	3	3	1
S3 (σNS)	3	3	1

[a] Each genome segment represents one class of dsRNA. Assignments of segment type of the several different clones are taken from Sharpe et al. (16).

[b] Entries in parentheses indicate polypeptide coded for by the genome segment, as taken from McCrae and Joklik (12) and Mustoe et al. (14).

Electron microscopy: (i) binding assays: Two methods were used for making specimens on carbon support films (9). Method A consisted of floating grids carbon side down onto a drop of microtubules, washing with distilled water, floating the grid on a drop of virus, fixing with 5% glutaraldehyde in PM buffer with 0.1 mM GTP, and negative staining as described earlier (9). Method B was essentially the same as method A, except the grids were coated with virus first, and then with microtubules.

(ii) Thin-section electron microscopy. Mouse L-cell monolayers (T-25 flasks) were infected with reovirus at a multiplicity of 8 to 10 PFU/cell at 31°C. About 0.2 ml of virus stock was adsorbed to the L-cell monolayer for 2 h at 31°C. The cells were washed once with 3 ml of Dulbecco modified Eagle minimum essential medium (Grand Island Biological Co.) plus 5% fetal bovine serum (Sterile Systems, Inc.); then 5 ml of fresh medium was added, and the infected cells were further incubated for 24 to 48 h at 31°C. The medium was aspirated, and the cells were prepared as follows. A 2.5-ml amount of 2.5% glutaraldehyde in PM buffer with 1 mM GTP was added to the monolayer. Then the cells were scraped off with a rubber policeman and pelleted at 1,500 × *g* for 5 min in an International PRJ centrifuge at 10°C. The superna-

tant was removed, and 2.5 ml of 2.5% glutaraldehyde in PM buffer with 1 mM GTP was added. Pellets were incubated at 22°C for 2 to 6 h and stored at 4°C before further processing. The PM-buffered glutaraldehyde fixative was used, since it enhances visualization of microtubules in cultured cells by about threefold (10). Post-fixation of pellets in 1% OsO_4, dehydration through increasing concentrations of ethanol, embedding in Spurr's Epoxy Resin or Epon 812, cutting of thin sections on a Sorvall Porter-Blum MT-2B ultramicrotome, and staining with 2% aqueous uranyl acetate and Reynolds lead citrate have been described in detail (10).

(iii) Examination of specimens. A Philips EM 301 electron microscope was employed to examine the specimens. Liquid nitrogen was used to reduce electron beam-induced contamination, and a 50-μm silver objective aperture was used to enhance the contrast. Grids were examined at an accelerating voltage of 80 kV.

RESULTS

In vitro binding assays of viruses and microtubules. The results of the in vitro binding of reovirus types 1, 2, and 3 or adenovirus to chick brain, rabbit brain, and HeLa cell microtubules are shown in Table 2. These results are averages of at least three trials employing fresh virus and microtubules. Similar results were obtained for both methods A and B, indicating that the binding is independent of whether microtubules are initially adhered to a carbon support film before exposure to microtubules or vice versa.

To fully understand the results presented in Table 2, it is important to illustrate the experimental system we used. Figure 1 contains several micrographs which exemplify the in vitro binding system we used. These or the subsequent micrographs should not be considered as representative of the final results we obtained; however, they do illustrate the different types of association between the virus and microtubules. Some virus particles are associated with the "edge" of the microtubules (straight arrows), whereas others are located on top of or under the microtubule (curved arrows). A statistical treatment of the significance of this edge-binding assay is provided in the Discussion.

Adenovirus type 5 was employed as a control, and we note in confirmation of previously reported results (9) that on the average more than 87% of the adenovirus was bound to the edge of chick brain microtubules. A similarly high percentage, i.e., 77% of the particles, was bound to the edge of HeLa cell microtubules. Since this is the first time HeLa cell microtubules have been isolated, and since HeLa cell microtubules have been implicated in the vectorial transport of adenovirus to the HeLa cell nucleus (5), it is of interest to note the high degree of edge binding seen for the HeLa cell microtubules with adenovirus. For chick brain microtubules, the binding site has been shown to be the high-molecular-weight microtubule-associated proteins (MAPs) (24), which are on the outer surface of the microtubule (7, 13). HeLa cell microtubules could thus be expected to have some similar surface projections. Comparison of sections of embedded HeLa cell and rabbit brain microtubules appears to confirm this expectation (Fig. 2).

Averaging the data in Table 2 for methods A and B, we observed that 56 and 61% of the reovirus type 3 particles were edge bound to microtubules derived from chick brain or HeLa cells, respectively. Since a randomly associated particle would show 50% edge binding (random means values derived by tracing back microtubules onto the same micrograph and counting the number of particles associated with the edge and with the central regions [9]), this implies

TABLE 2. *Binding of reovirus serotypes 1, 2, and 3 or adenovirus to chick brain, rabbit brain, and HeLa cell microtubules*

Virus	Fraction of particles edge bound[a]					
	Chick brain MT		HeLa cell MT		Rabbit brain MT	
	Method A	Method B	Method A	Method B	Method A	Method B
Reovirus type 1	0.81 (230)	0.81 (180)	0.78 (156)	0.82 (217)	0.79 (218)	0.84 (230)
Reovirus type 2	0.39 (260)	0.45 (161)	ND	ND	ND	0.53 (137)
Reovirus type 3	0.55 (250)	0.57 (240)	0.56 (155)	0.66 (210)	ND	ND
Adenovirus	0.91 (105)	0.84 (150)	0.70 (227)	0.85 (304)	ND	ND

[a] Each fraction is expressed as the ratio of number of particles associated ± 4 nm within the edge of a microtubule (edge bound) to the total number of particles associated with the microtubule (edge and top). If random association occurred, it has previously been calculated that the fraction edge bound would be 0.50 (9). Fractions are averages of three or more experiments, each performed with freshly prepared virus and microtubules. Values in parentheses indicate average number of particles counted for each experiment. MT, Microtubules; ND, not determined.

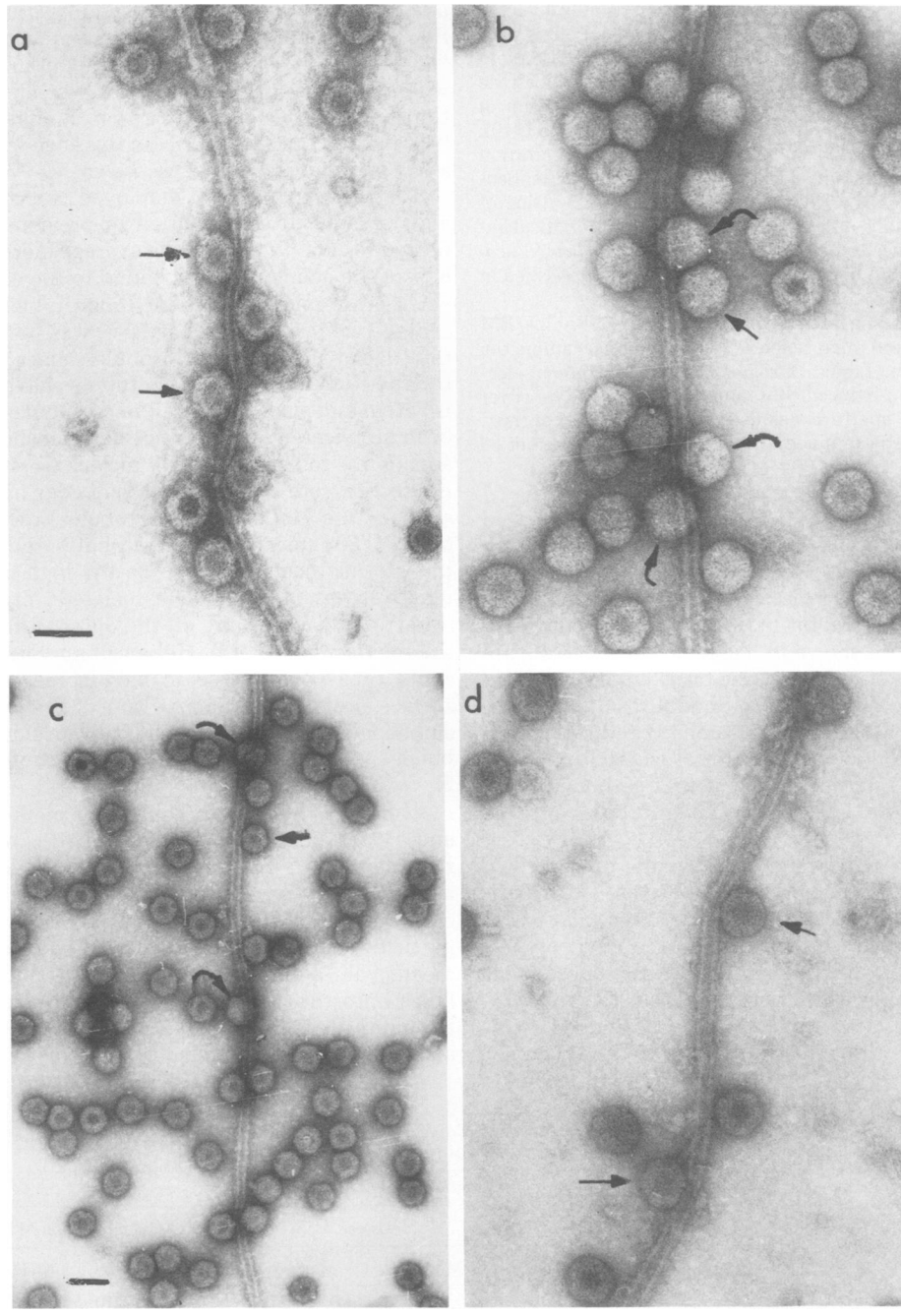

FIG. 1. *Electron micrographs of (a) reovirus type 1 with rabbit brain microtubules; (b) reovirus type 3 with HeLa microtubules; (c) reovirus clone 204 with rabbit brain microtubules; (d) reovirus clone 802 with chick brain microtubules. Straight arrows point to particles associated with the edge of microtubules; curved arrows indicate representative particles lying on or under microtubules. In this and subsequent figures, the preparations were fixed with 5% glutaraldehyde and stained with 2% uranyl acetate, except where noted differently. Magnification: ×81,000 for a, b, and d; and ×48,000 for c. Bar represents 0.1 μm in all micrographs.*

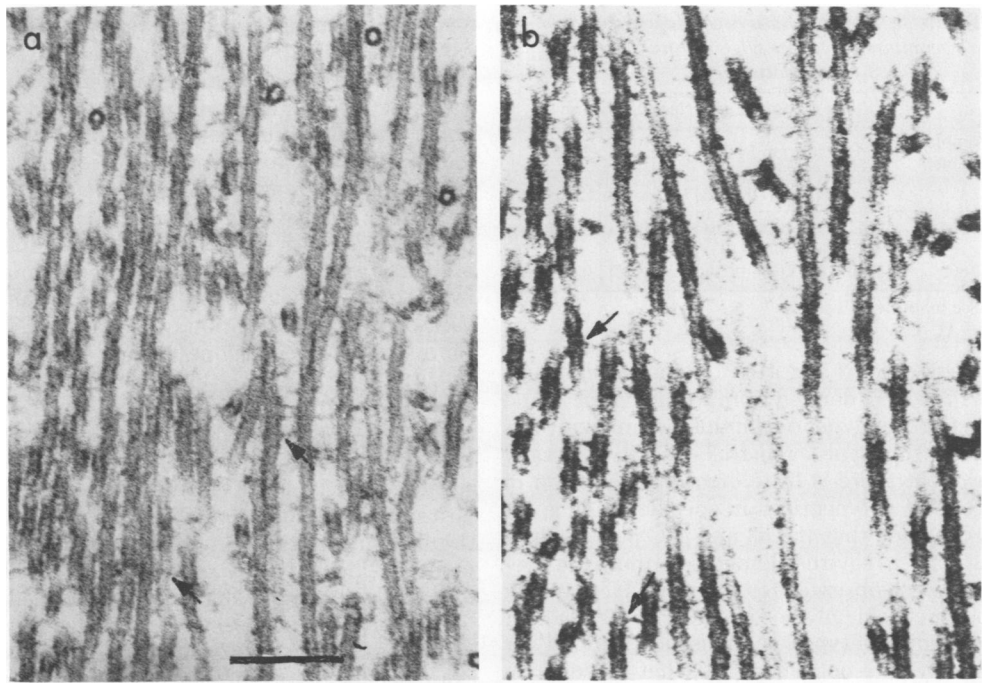

FIG. 2. *Thin-section electron micrographs of microtubules from (a) HeLa cell and (b) rabbit brain. Details of the procedure have been provided earlier (10). Arrows point to projections. Magnification: ×150,000.*

that there is largely random association of reovirus type 3 to microtubules.

In contrast to these results, a high level of edge binding was found to occur between reovirus type 1 and all three types of microtubules. An average of approximately 81% (Table 2) of all virus associated with chick brain, rabbit brain, or HeLa cell microtubules was edge bound.

Reovirus type 2 also bound randomly to brain microtubules, because less than or close to 50% of the particles were edgebound.

Binding assays with reovirus recombinants and microtubules. Since any interaction between microtubule side arms, i.e., MAPs, and reovirus must involve the outer shell of the virus, only the three outer capsid polypeptides (σ1, σ3, and μ1C) could participate in binding. The fact that σ1 polypeptide is the hemagglutinin, and thus a polypeptide involved in specific interactions between reovirus and host components, suggested that this capsid polypeptide may be the one responsible for interacting with microtubules. The availability of recombinant clones of reovirus types 1 and 3 enabled us to test whether a high level of edge binding could be correlated with the presence of a single capsid polypeptide (σ1) derived from a reovirus which does exhibit a high level of edge binding (type 1).

To test this hypothesis, three reovirus recombinant clones, 80, 802, and 204, were incubated with either chick brain, rabbit brain, or HeLa cell microtubules in our in vitro assay system. Microtubule and virus concentrations were kept constant in all experiments, so that the total number of particles counted per standard field was the same for each preparation. As seen in Fig. 1c and d, the viruses derived from recombinant clones show a pattern of microtubule associations similar to the reovirus serotypes. The results of the binding assay are shown in Table 3. Clone 204 has an S1 genome segment derived from reovirus type 3. On the average, 42% of the clone 204 virus particles are associated with the edge of the microtubules. By contrast, clone 80 shows a high edge binding (81%) for chick brain microtubules. Clone 80 has S1, S2, L1, and L3 genome segments derived from reovirus type 1. Like clone 80, clone 802 also shows a high edge binding to microtubules (89%). Clone 802 contains only the S1 and L1 genes from reovirus type 1. The two genes of type 1 that these two clones thus have in common are S1 and L1. Since the L1 gene codes for an interior core polypeptide, λ3 (12), the high edge binding of these two recombinant clones, 80 and 802, is most probably attributable to the σ1 outer capsid polypeptide from reovirus type 1. Furthermore, since two of the outer capsid

TABLE 3. *Binding assays employing reovirus recombinant clones 80, 802, and 204 with microtubules*[a]

Recombinant clone	Fraction of particles edge bound			
	Chick brain MT		HeLa cell MT	
	Method A	Method B	Method A	Method B
204	0.44 (197)	0.38 (220)	0.40 (150)	0.46 (138)
80	0.86 (96)	0.79 (105)	ND	ND
802	0.80 (180)	0.86 (220)	0.89 (181)	0.88 (200)

[a] See footnote to Table 2.

polypeptides (σ3 and μ1C) for the recombinant clone 204 were derived from reovirus type 1, and since the σ1 polypeptide of this recombinant was derived from type 3 reovirus, where we already know that there is little or no participation of the capsid polypeptides in specific binding, we can conclude that the σ3 and μ1C polypeptides from type 1 reovirus cannot determine the specific binding observed for this virus. Hence, only σ1 outer capsid polypeptide determines the high edge binding of type 1 reovirus either directly or indirectly. We note, however, that the data so far available do not tell us whether the effect of σ1 is caused by specific affinity of type 1 σ1 for microtubules or whether the presence of type 1 σ1 alters the outer capsid of the virus so that it can specifically bind to the edge of microtubules (see Fig. 4).

Binding assays with reovirus types 1 and 3, recombinant clones, antisera, and microtubules. The results of the binding assays employing virus-specific antibody with reovirus serotypes 1 and 3 or recombinant clones 80, 802, and 204 with microtubules are shown in Table 4. Only method B could be applied to these experiments, since in method A the additional step of incubation with antisera caused the microtubules on the grid to become masked by excess antisera.

Reovirus type 1 treated with type 1-specific antisera revealed the deposition of fibrillar material around the outer capsid of the virus (Fig. 3). When such antibody-treated reovirus type 1 particles were mixed with microtubules, we no longer observed high edge binding (Table 4). On the average, approximately 49% of the virus counted was now edge bound to chick brain microtubules. When HeLa cells microtubules were incubated with antibody-treated type 1 virus, only 34% of the particles were edge bound. Both of these values are in the range of 50%, which is equivalent to a random association of virus with microtubules. Thus, loss of the type 1-specific binding to microtubules occurred as a result of the use of virus specific antibody.

When reovirus type 1 was incubated with type 3-specific antisera (Fig. 3b), we found that for rabbit brain microtubules approximately 77% of the reovirus type 1 was edge bound. A similar value of 78% edge bound was obtained when chick brain microtubules were employed. Thus, very little if any loss of the specific binding of reovirus type 1 was seen when type 3 antibody was used. Further controls, using reovirus type 3 or clone 204 with either type 1 or type 3 specific antisera (Fig. 3c and d), revealed no change in microtubule association from the 50% or random value seen without antibody incubation. Thus, the use of antibody alone cannot enhance the reovirus type 3 association with microtubules. In conclusion, it appears that edge binding of reovirus type 1 to microtubules is inhibited by the binding of specific antibody to its outer surface. Based on our results with recombinant viruses, we presume that this is attributable to the blocking of the σ1 polypeptides. However, monospecific σ1 type 1 antibody is required before this point can be definitely established.

Negative-stain electron microscopy of reovirus capsid ultrastructure. Virions were examined by high-resolution negative-stain electron microscopy. It was noted that the overall capsomer morphology of reovirus type 1 was clearly revealed (Fig. 4a and b). This occurred with either uranyl acetate (pH 4.2) or sodium-phosphotungstate (pH 7.2) as the negative stain. In contrast, for reovirus type 3, only a quadrant of the capsomers was occasionally visible on the outer surface; the remainder appeared diffuse (Fig. 4c and d). Recombinant clones 802 and 204 showed capsomer morphologies corresponding to type 1 and 3, respectively (data not shown). Thus, the ability to clearly visualize the capso-

TABLE 4. *Binding assays employing reovirus types 1 and 3 and recombinants 204 and 802 with microtubules and virus-specific antibody*

Virus and antibody type[a]	Fraction of particles edge bound[b]		
	Chick brain MT	HeLa cell MT	Rabbit brain MT
Reovirus type 1, Ab 1	0.49 (213)	0.34 (110)	ND
Reovirus type 1, Ab 3	0.78 (142)	ND	0.77 (155)
Reovirus type 3, Ab 1	0.44 (135)	0.46 (134)	0.39 (147)
Reovirus type 3, Ab 3	0.44 (305)	0.36 (205)	ND
Clone 204, Ab 1	0.51 (250)	0.40 (280)	ND
Clone 204, Ab 3	0.54 (300)	ND	0.41 (146)
Clone 802, Ab 1	0.40 (200)	ND	0.40 (216)
Clone 802, Ab 3	ND	0.77 (184)	0.79 (162)

[a] Ab, Antibody.

[b] Values in parentheses indicate the average number of particles counted for each experiment. Method B was used in all of these assays.

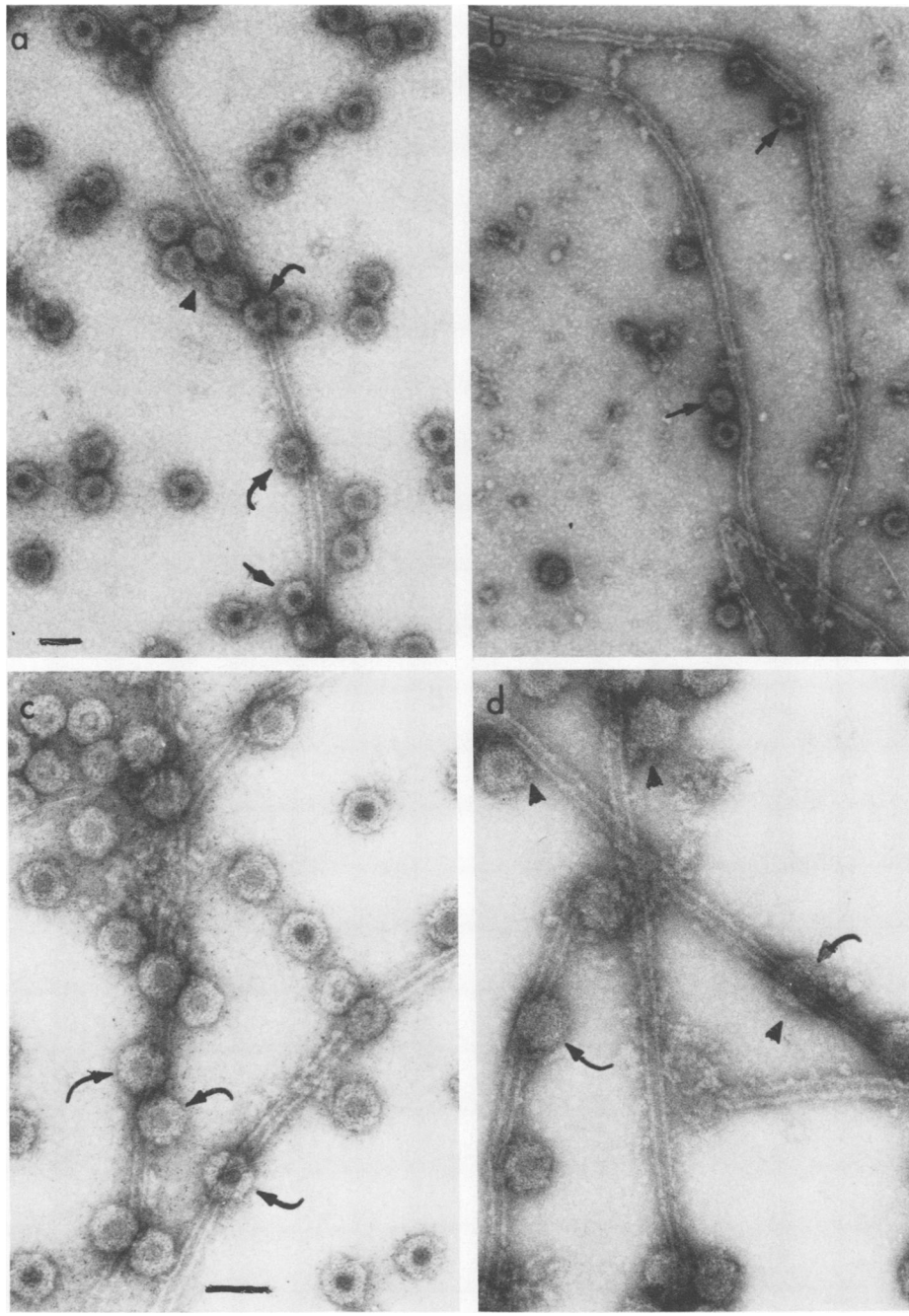

FIG. 3. *Electron micrographs of (a) reovirus type 1 with type 1 antibody and chick brain microtubules; (b) type 1 with type 3 antibody and chick brain microtubules; (c) reovirus type 3 with type 3 antibody; (d) reovirus 204 with type 1 antibody and rabbit brain microtubules. All were prepared by method B. The antibody (fibrillar mass) can be seen around the outer capsid of the virus particles (arrowheads). Arrows and curved arrows point to particles as described in the legend to Fig. 1. Magnification: ×48,000 for a and b; ×81,000 for c and d.*

mer structure follows the σ1 phenotype. This may correlate with our earlier results, where type 1 and 802 reovirus bind with high specificity to microtubules, because it suggests that σ1 polypeptides of type 1 are more readily accessible to binding with microtubules.

Thin-section electron microscopy of reovirus-infected cells. Since we had found that

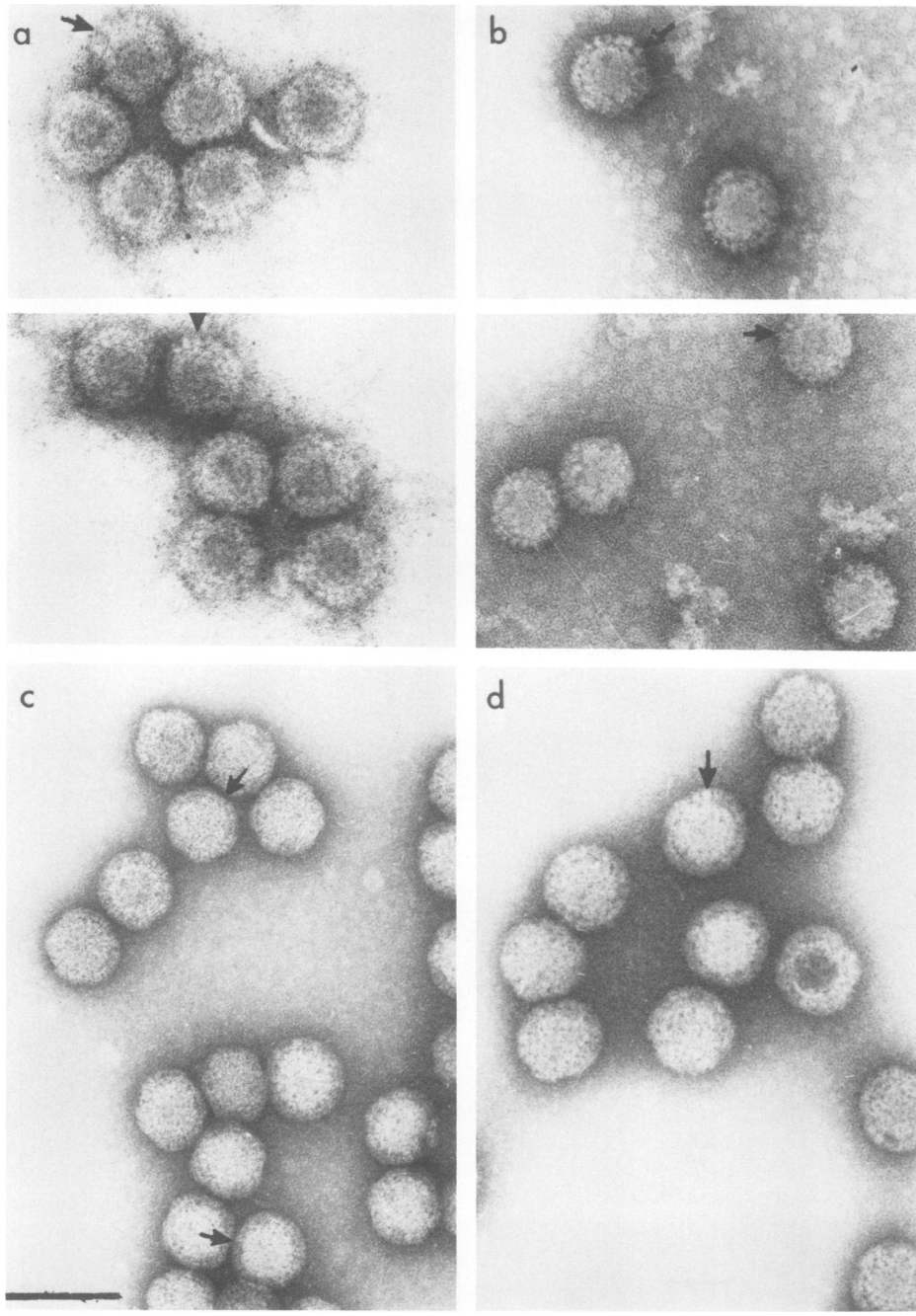

FIG. 4. *Capsomer structure of reovirus type 1 (a, b), or reovirus type 3 (c, d), using uranyl acetate (a, c) or sodium phosphotungstate (b, d). Magnification of all particles is about ×170,000. Arrows point to capsomers.*

reovirus type 1 exhibited a higher specificity for in vitro binding to microtubules than reovirus type 3 particles, we asked in vivo if there were any differences that could be correlated with the differences in vitro. Thin sections of reovirus type 1- and 3-infected cells were examined at 0, 6, and 24 to 48 h postinfection at 31°C. At neither of the early times was any dramatic difference observed between the virus types; i.e., there was a partial coating of microtubules by

fibrillar material of unknown origin at 6 h, and a few virus particles were associated with these coated regions. These results are similar to those reported by Dales et al. (6). However, at 24 to 48 h, when virus production was predominantly occurring in viral factories, there was a dramatic difference in the association of the two reovirus serotypes with microtubules. The reovirus type 1 particles were clustered in ordered, crystalline arrays and were extensively associated with microtubules (Fig. 5a–c, f); for reovirus type 3 particles, although there was an occasional factory which showed a similar association with microtubules, most of the particles were in factories that exhibited particles in a less-ordered array and showed no microtubule inclusion (Fig. 5d and e). Quantitation of these data is provided in Table 5. These results show that in vivo, as well as in vitro, there is a striking qualitative difference in the association of microtubules with reovirus type 1, as compared to reovirus type 3. This difference was further supported by preliminary experiments in which colchicine (10 μg/ml), an agent that disrupts microtubules, was added to cells at the time of infection, and virus yield was assayed at different times postinfection. We observed that both at 24 and 36 h postinfection there was a two to threefold decrease in the yield of reovirus type 1, as compared to reovirus type 3 (the reovirus type 3 yield was about 50 to 60% the yield of untreated cells) (U. Ray, umpublished data). The colchicine inhibition, however, was not absolute, since at later times, i.e., 72 to 96 h, there was no observed decrease in yield for either reovirus type 1 or 3 (U. Ray, unpublished data).

In summary, the above results, both from thin-section electron microscopy and preliminary colchicine treatment studies, suggest that, in vivo as well as in vitro, there appears to be a higher degree of specificity in the association of reovirus type 1 with microtubules than that of reovirus type 3.

DISCUSSION

The results presented in this paper demonstrate quantitative and qualitative differences in the association of reovirus types 1 and 3 with microtubules both in vivo and in vitro. Previously, it was known from the work of Spendlove et al. (21, 22), Dales et al. (6), and Silverstein et al. (19) that reovirus types 1 and 3, respectively, could associate with microtubules in vivo; but, because of marked differences in experimental techniques, these workers did not discern any differences in the association of reovirus with microtubules that could be attributed to differences in viral serotypes. We previously reported (9, 24) that reovirus type 3 shows a low degree of edge binding to microtubules. The data of this paper confirm our earlier finding and show the new and unexpected finding that reovirus type 1 shows a high degree of edge binding. These differences in the specificity of binding of the reovirus serotypes observed in our negative-staining assay can be correlated with other differences between the viruses. Thus, reovirus type 1 shows a high degree of edge binding, clearly discernible capsomers (by negative staining), and a high percentage of viral factories containing highly organized microtubules at 24 to 48 h of infection. In contrast, reovirus type 3 shows a low degree of edge binding, its capsomers are difficult to identify in negatively stained virus, and very few of the viral factories contain microtubules at 24 to 48 h of infection.

What is the molecular basis for these differences in behavior of reovirus types 1 and 3? Our experiments with recombinant clones of reovirus types 1 and 3 indicate that virions containing the σ1 polypeptide of type 1 reovirus show a high degree of edge binding and a clear capsomer structure; conversely, virions containing the σ1 of type 3 show a low degree of edge binding and blurred-looking capsomers. The presence of σ1 of type 1 clearly alters the capsomer in a manner that results in a high degree of edge-binding. Furthermore, our experiments showing that only antibodies to type 1 reovirus affect the degree of edge binding strongly suggest that a high degree of edge binding can occur only if the outer capsid polypeptides are directly accessible to the microtubule. Based on these considerations, we believe it is likely that the presence of the σ1 polypeptide of type 1 is responsible for highly organized microtubules being present in a high percentage of the viral factories. The presence of the type 3 σ1 polypeptide leads to fewer factories that contain microtubules. Suitable in vivo experiments with recombinant viruses are in progress to test this possibility.

Consideration of these new results, in light of our earlier observations that only microtubules containing MAPs show a high degree of edge binding of adenovirus (24), raises the interesting possibility that the two reovirus serotypes interact differently with microtubules in vitro and in vivo by virtue of a specific interaction of σ1 of type 1 with microtubule side arms. Although this interpretation of our data is appealing because of its simplicity, other more complicated interpretations cannot be ruled out right now. For example, it is also possible that σ1 of type 1 acts by altering the entire capsomer to a configuration which can interact with MAPs and

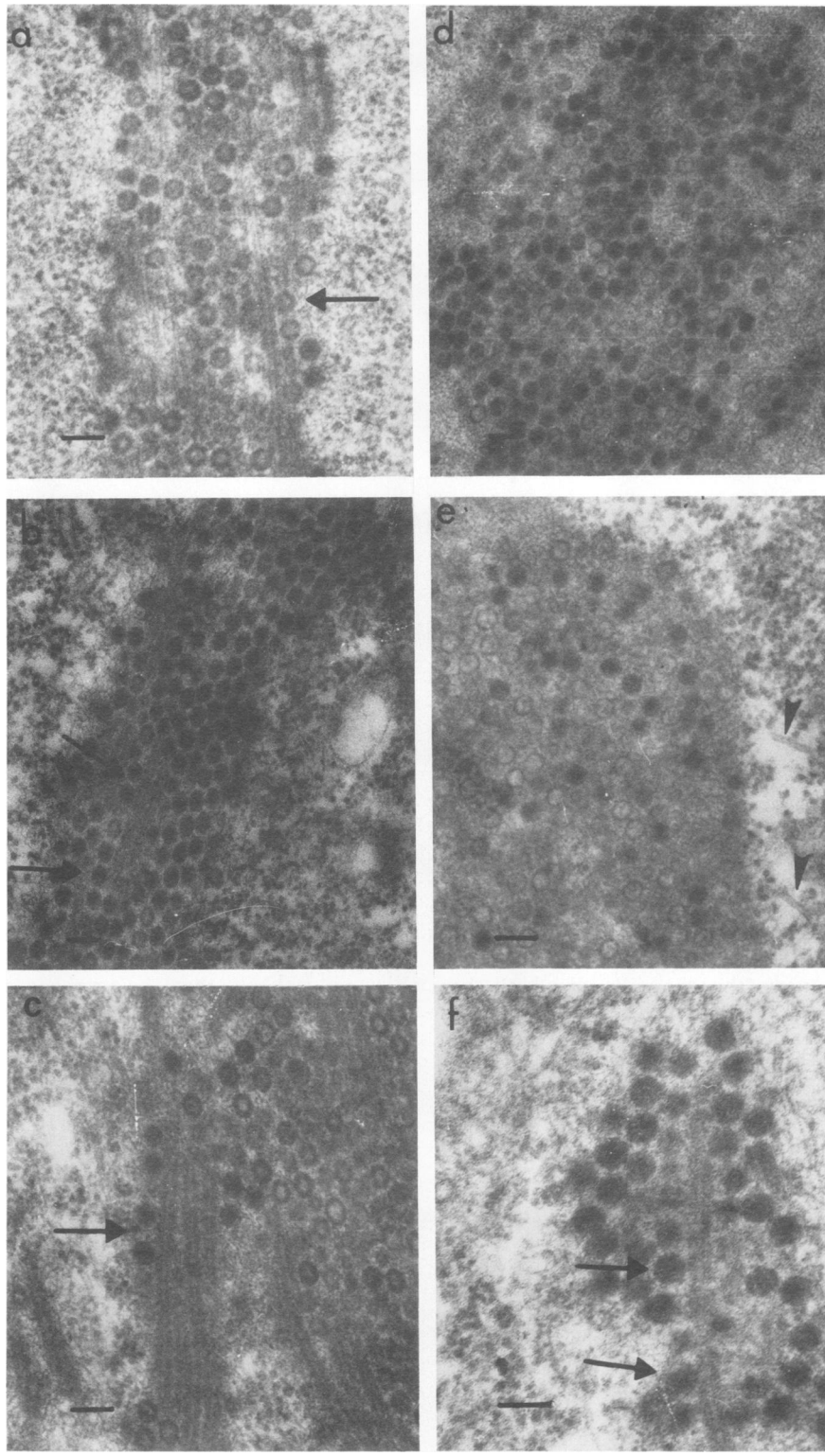

FIG. 5. *Thin sections of reovirus type 1- (a through c, f) and type 3- (d, e) infected cells. Arrows point to microtubule-virus associations. Note (i) closeness of association between reovirus type I and microtubules, as in f, (ii) empty-looking virus particles in a and e which may be assembly intermediates, and (iii) microtubules outside reovirus type 3 factory (arrowheads) in e, indicating that microtubule fixation is not a problem. Magnification: ×45,000 for b and d; ×60,000 for a, c, and e; ×75,000 for f.*

TABLE 5. *Thin sections of reovirus-infected cells exhibiting factories associated with microtubules*[a]

Expt	Reovirus serotype	Factories with microtubules/total[b]
1	1	29/43 (67%)
	3	2/41 (5%)
2	1	21/27 (78%)
	3	5/46 (11%)

[a] Thin sections of reovirus-infected cells at 24 h postinfection were examined at ×16,000 to ×21,000 magnification. At least four separate grids and two separate embedded blocks were examined for each point. Every thin section on the grid was analyzed. Of all cell sections examined, about 40% had factories. The range was one to three factories per cell section, whereas 68% of those sections containing factories were found to have only one factory per cell section. Sometimes factories had not yet appeared at 24 h, but were seen by 48 h. This may reflect small variations in the experimental conditions, such as multiplicity of infection. However, when the factories were seen, the same differences regarding microtubule association were observed.

[b] Fraction is expressed as numbers counted. Percent is given in parentheses.

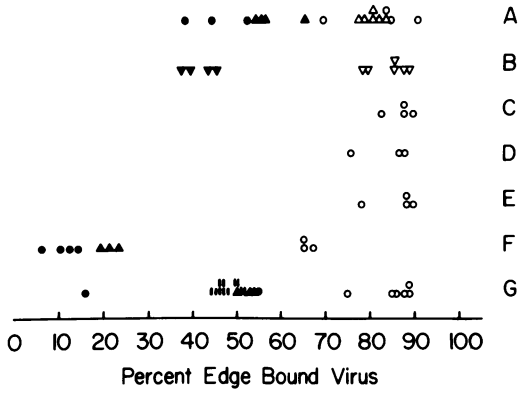

FIG. 6. *Distribution of percent edge binding for various particles for seven separate sets of data. Data sets A and B are from Tables 2 and 3, respectively, of this paper; sets C through E are from Tables 1 to 3, respectively, of Weatherbee et al. (24); and sets F and G are from Tables 1 and 3, respectively, of Luftig and Weihing (9). Symbols: ○, reovirus type 1; △, adenovirus; ▽, recombinant clones 80 and 802; ●, reovirus type 3; ▲, coliphage f1, polystyrene latex particles, and reovirus type 2; ▼, recombinant clone 204; ■, controls derived by tracing (9).*

hence would be expected to show a high degree of edge binding. This interpretation is consistent with our observation of differences in the clarity with which one can observe the capsomers of negatively stained reovirus types 1 and 3.

Implicit in the above discussion is the assumption that a high degree of edge binding means a higher physical affinity of the virus for the microtubules, and to the side arms formed by the MAPs in particular. We have previously provided some evidence that this assumption is correct (24), but it should be pointed out that technical difficulties have so far prevented us from actually measuring the affinity of the various particles to microtubules.

Regardless of whether we eventually show that particles showing a high degree of edge binding contain a receptor that binds specifically and with high affinity to MAPs, we would like to point out that the phenomenon of edge binding provides a useful, empirical way to distinguish the interactions of various particles with microtubules. We have summarized the present data and relevant data from previously published papers in Fig. 6.

This assay clearly distinguishes particles showing a high degree of edge binding, such as reovirus type 1 from particles showing a lower degree of edge binding such as reovirus type 3. In particular, we see that adenovirus, reovirus type 1, and reovirus recombinant clones 80 and 802 fall naturally into one group of particles showing a high degree of edge binding (mean, 83%; standard deviation, 7.2%), whereas reovirus types 2 and 3, reovirus recombinant clone 204, coliphage f1, and latex spheres fall into another group of particles sharing a lower degree of edge-binding (mean, 41%; standard deviation, 15.8%). Using the Student's t test, we calculate that the probability that the means of the two groups are the same is <0.005, and therefore we are quite confident that the negative-staining assay provides a useful, empirical method for comparing the interactions of different particles with microtubules.

Our data and the earlier observations on association of reovirus with microtubules in vivo (6, 19, 21) indicate that there is a potential for involvement of microtubules in reovirus infection. In addition, our data indicate that there are significant differences in the details of this involvement. We plan to examine the kinetics of virion growth in greater detail so that we can determine the role that microtubules play at different times of the reovirus infection.

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