VISUALIZATION OF CENTRIOLES AND BASAL BODIES BY FLUORESCENT STAINING WITH NONIMMUNE RABBIT SERA

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ABSTRACT

Several sera from nonimmunized rabbits have been found which stain centrioles and basal bodies by indirect immunofluorescence in a wide variety of cell types. So far, ~10% of the rabbit sera that we have examined gave strong positive staining of centrioles and basal bodies. Cytoplasmic networks, mitotic spindles, and ciliary axonemes, however, remain unstained. This specific fluorescent staining of centrioles and basal bodies could not be abolished by absorption of sera with purified brain tubulin. This technique is superior to previous methods for the visualization of basal bodies and centrioles at the light microscopic level and should be useful for rapid and convenient detection of these organelles in large populations of cells.

KEY WORDS centrioles · basal bodies · indirect immunofluorescence

Although centrioles and basal bodies are readily identified by electron microscopy, they are difficult to distinguish at the light microscopic level from the multitude of small granules and vesicles around them in the cytoplasm. This has prevented studies of their distribution in large populations of cells. Attempts to improve the visualization of these structures at the light microscopic level have only been partially successful. These have included the staining of centrioles with iron hematoxylin and crystal violet (13), examination of cells by phase-contrast microscopy after Colcemid treatment (2) or alcohol/detergent fixation (12), and the visualization of basal bodies in ciliates by silver staining methods (9).

Recently, we have discovered several sera collected from rabbits before immunization which give fluorescent staining of centrioles (3). In this report, we present a more detailed investigation of the staining of centrioles and basal bodies with two of these sera.

MATERIALS AND METHODS

Cells

Secondary cultures of mouse embryo fibroblasts, rat C6 glial cells, and mouse neuroblastoma cell line N_2A-A_4 were grown in alpha minimal essential medium supplemented with 10% fetal calf serum. Pig kidney cells (CCL 33, American Type Culture Collection, Rockville, Md.) were grown in Eagle's minimal essential medium supplemented with Earle's salts and 5% newborn calf serum.

For primary cultures of embryonic brain, rat embryos were removed at 14 days gestation, and the tips of the developing cerebral hemispheres were excised, dissociated with a Pasteur pipette, and passed through a metal sieve to obtain a single cell suspension. Cultures were seeded at 5×10^5 cells/ml on collagen-coated glass cover slips (1).

For ciliated cells, tracheae were removed from adult chickens and placed in 50% glycerol in phosphate-buffered saline (PBS), pH 7.0, for 24 h at 4°C (5). A

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suspension of ciliated cells was prepared by cutting the tracheae in half and scraping the inside with a glass cover slip to remove the tracheal epithelium.

Sera

The two nonimmune sera (designated Z-3 and Z-7) used in this study were obtained from female New Zealand white rabbits ~2 kg in weight. The antiserum to tubulin and preimmune serum from the same rabbit were characterized previously (4). For immunofluorescent staining, all sera were used at a dilution of 1:30 in PBS. In some experiments, sera were absorbed with electrophoretically purified tubulin before use (3).

Immunofluorescence

Cells grown on glass cover slips were stained as previously described (4). Ciliated cells were washed twice by centrifugation in PBS containing 10% glycerol and resuspended in diluted serum for 40 min at room temperature. Cells were then washed three times and resuspended for 30 min at room temperature in fluorescein conjugated goat IgG to rabbit IgG (Hyland Diagnostics Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) diluted 1:5 in PBS. Cells were washed three times, resuspended in PBS containing 50% glycerol, pH 7.8, and mounted on glass slides. Cells were examined with a Zeiss Photomicroscope II equipped with epifluorescence optics.

RESULTS

In primary cultures of embryonic rat brain, fibroblasts, neurons and glial cells could be distinguished. When these cultures were treated with sera from nonimmunized rabbits Z-3 and Z-7, fluorescent staining was observed in almost all cells (Figs. 1 and 2). Fig. 1 shows a number of cells from a culture treated with Z-7 serum (Fig. 1 *a*) and part of a single cell at higher magnification (Fig. 1 *b*). In most cells, a pair of fluorescent granules, whose position coincides with that occupied by the centrioles, can be distinguished in the perinuclear region. These fluorescent granules are 0.6–0.9 μm long, which is only slightly greater than the value of 0.3–0.7 μm obtained from the more precise measurements of centriole length in mammalian cells obtained by electron microscopy (6). We believe that these two fluorescent granules are the two centrioles or the two pairs of centrioles of the interphase cell. A single neuron from a similar culture treated with Z-3 serum is seen in Fig. 2. Again, the centrioles appear as two

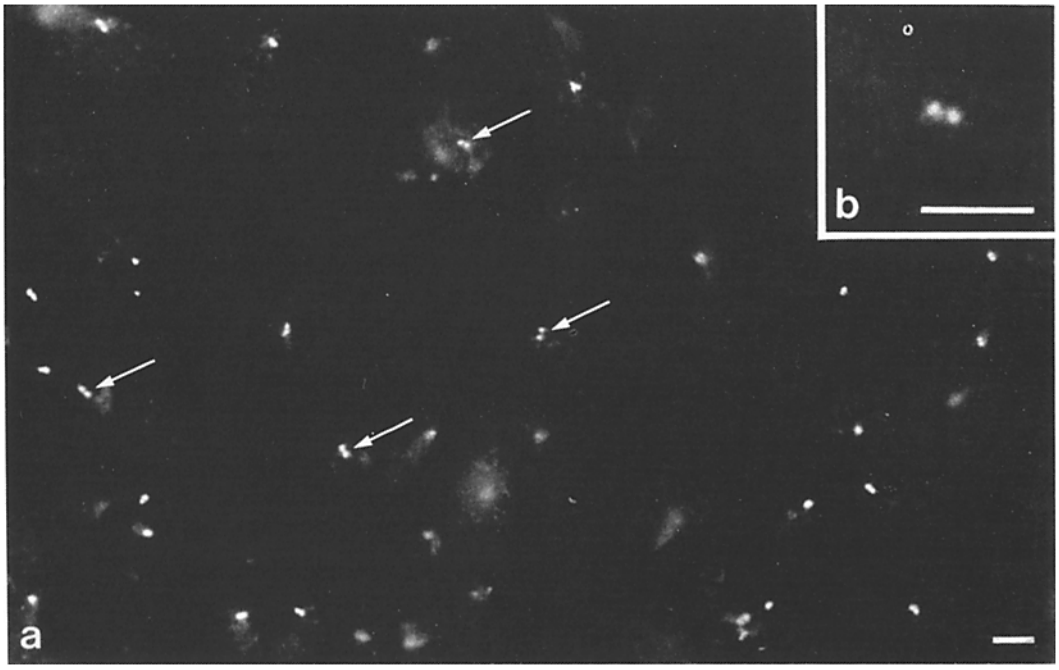

FIGURE 1 Immunofluorescent staining of cells from embryonic rat brain cultures treated with Z-7 serum. In most cells, the centrioles can be distinguished as a pair of brightly fluorescent granules (*a*) (arrows). A pair of centrioles is seen at higher magnification (b). Bars, 5 μm. (*a*) × 1,070. (*b*) ×2,950.

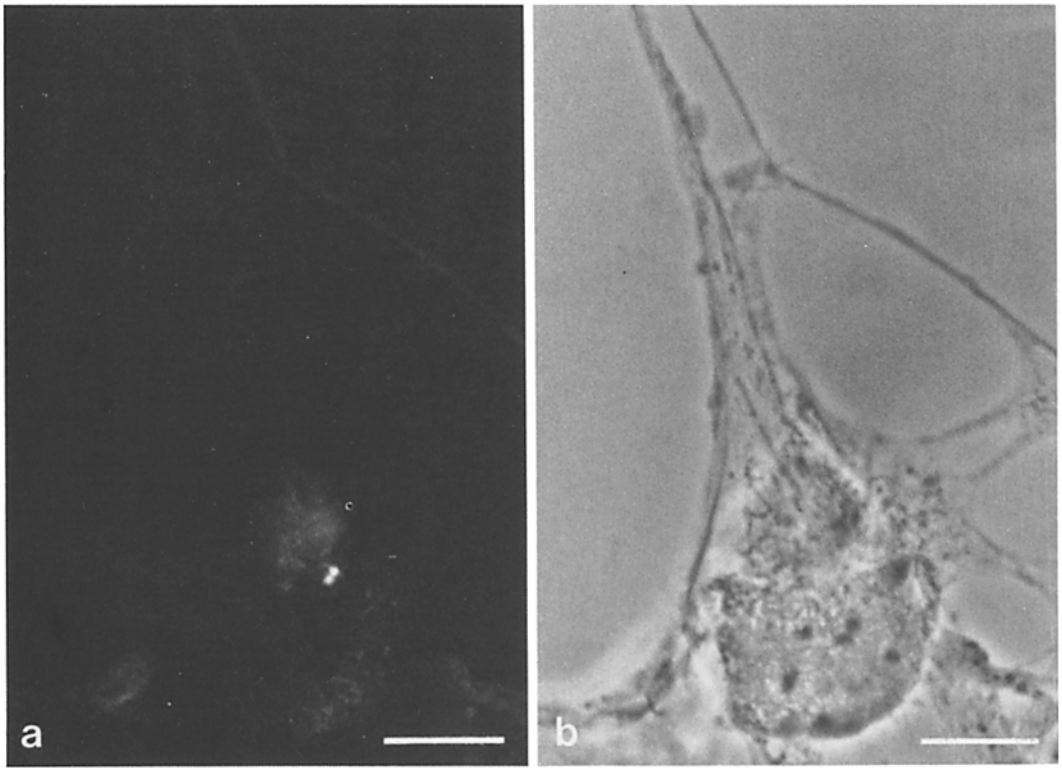

FIGURE 2 Fluorescence and phase-contrast micrographs of the same neuron from embryonic rat brain cultures treated with Z-3 serum. The centrioles appear as a pair of fluorescent granules (*a*). Phase-contrast microscopy (b) shows that they are located near the nucleus. Bars, 10 μm. (*a* and *b*) × 1,575.

brightly fluorescent, closely apposed granules (Fig. 2*a*) which by phase-contrast microscopy can be seen to reside near the nucleus (Fig. 2*b*). Similar staining of centrioles was observed when the purified IgG fraction from these sera was used.

To study the relationship of this centriole staining to other microtubule-containing structures, secondary cultures of mouse embryo fibroblasts were examined with these two centriole staining sera (Fig. 3*a–c*) and with anti tubulin serum (Fig. 3*d* and *e*). In fibroblasts treated with anti tubulin serum (Fig. 3*e*), the centrioles are clearly stained and the cytoplasmic network of microtubules is well defined. Several of the microtubules can be seen to emanate from the centriole-containing region. Preimmune serum from this particular rabbit did not stain centrioles, mitotic spindles or cytoplasmic microtubules in these cells, In contrast, in fibroblasts treated with Z-7 serum, only the paired centrioles are visible as bright fluorescent granules, and no other structures are stained (Fig. 3*a*). In some cells, presumably in the G2 phase of the cell cycle when the centrioles have replicated, two pairs of granules can be seen (Fig. 3*b*).

During the process of cell division, the intracellular network of microtubules breaks down and the mitotic spindle is formed. When a mitotic cell is treated with anti-tubulin serum, the fibers of the spindle are brightly stained (Fig. 3*d*), but the centrioles at the spindle poles are obscured by this staining and cannot be distinguished. When a similar mitotic cell is treated with Z-7 serum, a brightly staining centriole can be seen at each pole of the spindle (Fig. 3*c*), but the rest of the spindle remains unstained.

In addition to the cultures described above, several continuous cell lines, which included pig kidney cells, rat glial cells, and mouse neuroblastoma cells, were examined and similar staining of centrioles was observed. Staining of centrioles has also been noted with several other sera from nonimmunized rabbits in addition to the two

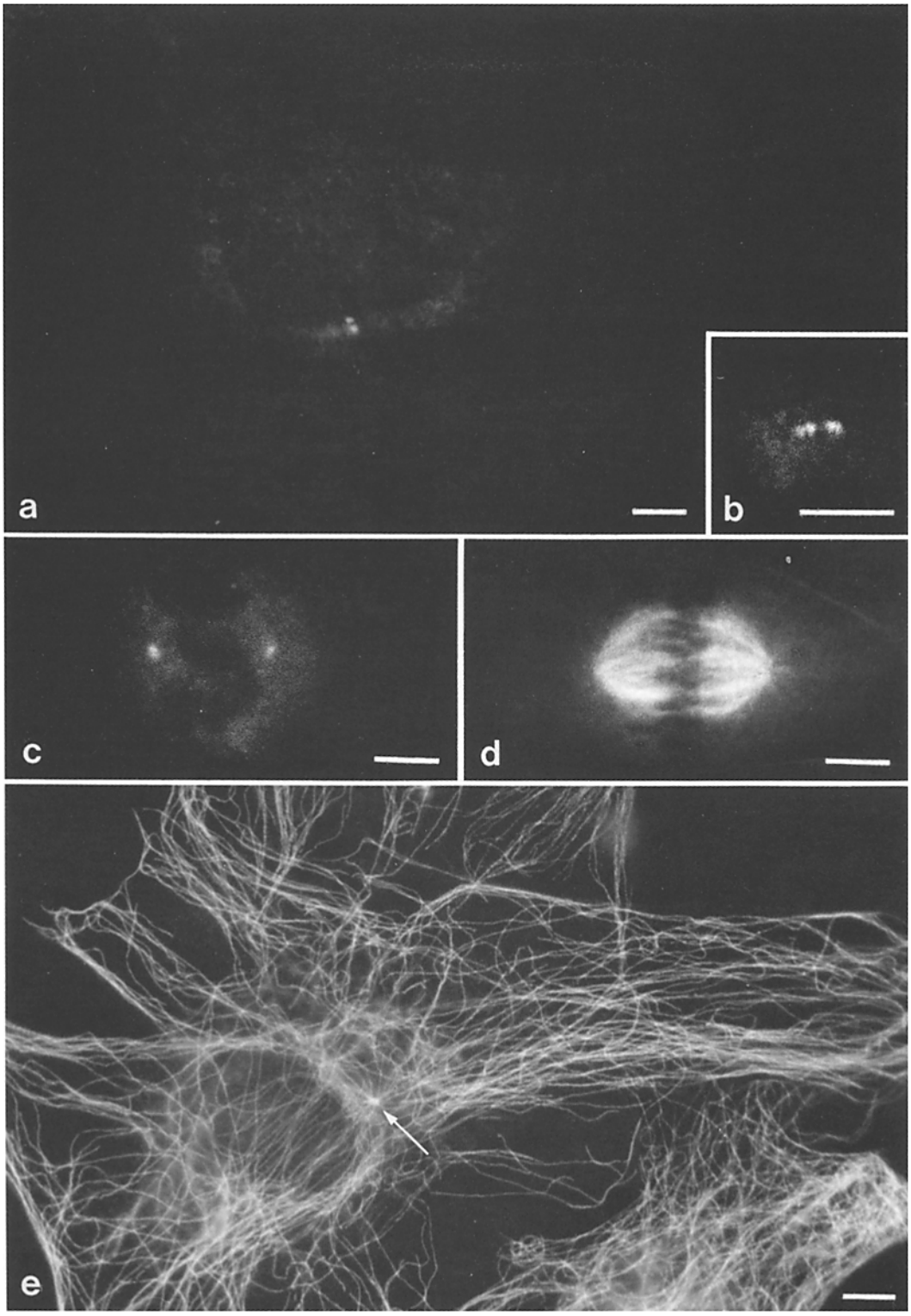

FIGURE 3 Immunofluorescent staining of mouse embryo fibroblasts with Z-3 serum (*a–c*) and anti-tubulin serum (*d* and *e*). With anti-tubulin serum, the centrioles (arrow) as well as the cytoplasmic network of microtubules are clearly stained (*e*). In fibroblasts stained with Z-3 serum, only the pair of fluorescent centrioles are visible (*a*). In some fibroblasts the centrioles appear as two pairs of fluorescent granules (*b*). In mitotic cells, anti-tubulin serum stains the spindle fibers very brightly (*d*) thereby obscuring the centrioles, whereas Z-3 serum stains only the centrioles at the poles of the spindle (c). Bars, 5 μm. (*a*) ×1,565. (*b*) × 2,780. (*c*) × 1,880. (*d*) × 1,880. (*e*) × 1,565.

included in this study. So far, a total of 35 sera from nonimmunized rabbits have been examined for their ability to stain centrioles. Of these sera, 5 stained centrioles clearly, 10 were negative, whereas 20 of the sera showed some centriole staining activity, but this was often obscured by high background staining.

When either Z-7 or Z-3 serum was absorbed with electrophoretically purified tubulin before treatment of mouse embryo fibroblasts, no diminution in the staining of the centrioles was noted (Fig. 4*a*), indicating that the staining was not due to the presence of antibodies which react with tubulin. When anti-tubulin serum was used after absorption with the same quantity of purified tubulin, the staining of cytoplasmic microtubules, mitotic spindles and centrioles in mouse embryo fibroblasts was abolished (Fig. 4*b*). Cells examined after incubation with the fluorescein conjugate alone showed no specific fluorescent staining.

Basal bodies, which have the same basic structure as centrioles, are also visualized with these two sera from nonimmunized rabbits. In ciliated cells isolated from chicken tracheal epithelium and treated with Z-3 serum, a row of fluorescent staining granules is seen at the apical end of the cell (Fig. 5*a*). When the same cell is examined by phase-contrast microscopy (Fig. 5*b*), it can be seen that this staining is at the basal ends of the cilia in the region occupied by the basal bodies. The axonemes of these cilia, however, remain unstained. Thus, binding sites responsible for the staining of centrioles and basal bodies are not shared by the axonemes. When ciliated cells were treated with anti-tubulin serum, the axonemes as well as the basal bodies fluoresced very strongly. This can be seen by comparing phase and fluorescence micrographs of the same cell (Fig. 5*c* and *d*). That the basal bodies are indeed staining with the anti-tubulin serum can be better seen in the small number of cells which become deciliated but still retain a row of brightly stained basal bodies at their apical ends (not shown).

DISCUSSION

We have demonstrated that sera from some nonimmunized rabbits will specifically stain centrioles and basal bodies in many different mammalian and avian cell types. Other microtubule-containing structures such as ciliary axonomes, mitotic spindles, and the cytoplasmic network of microtubules remain unstained.

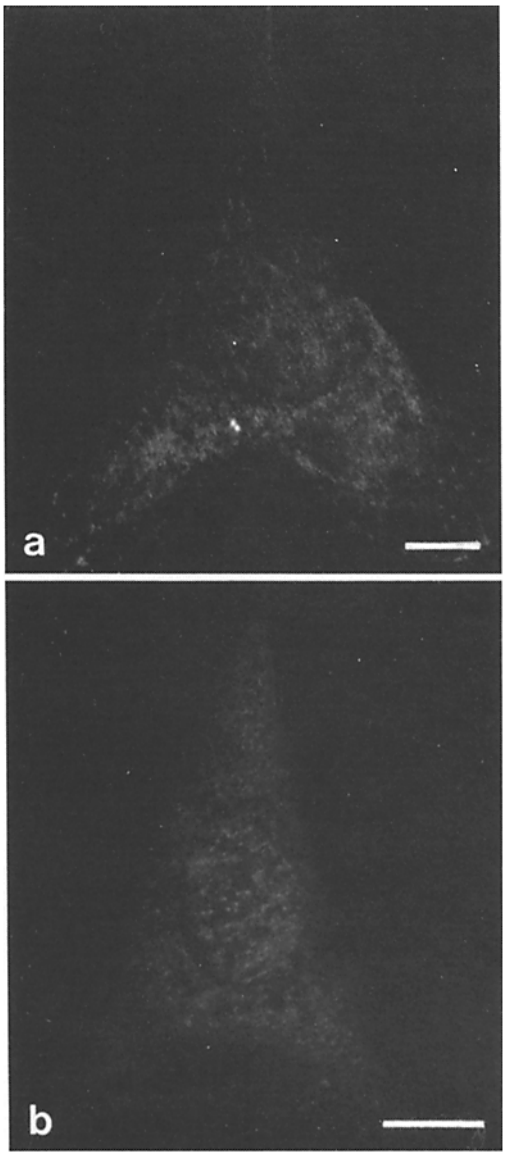

FIGURE 4 Immunofluorescent staining of mouse embryo fibroblasts with centriole staining serum Z-7 and anti-tubulin serum after absorption of both sera with purified brain tubulin. Absorption of anti-tubulin serum with tubulin abolishes the staining of centrioles and the cytoplasmic network of microtubules (compare with Fig. 3*e*) (*b*). In contrast, absorption of Z-7 serum with tubulin does not reduce the staining of the centrioles (*a*). Bars, 10 μm. (*a*) × 1,300. (*b*) × 970.

Similar immunofluorescent staining of centrioles and basal bodies in different cell types has been reported recently by Nenci and Marchetti

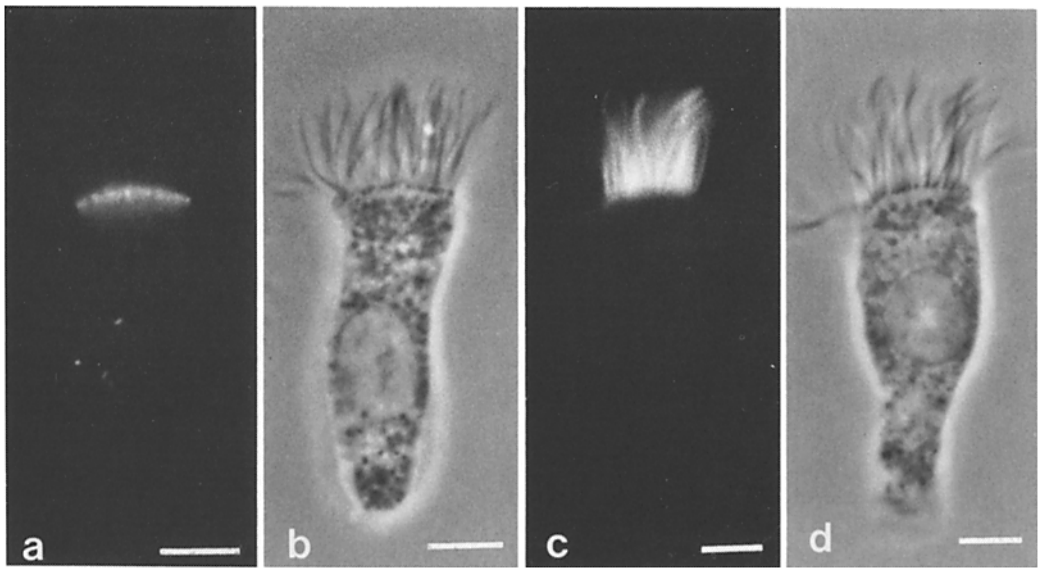

FIGURE 5 Fluorescence and phase-contrast micrographs of two ciliated chicken tracheal epithelial cells treated with centriole-staining serum Z-3 (*a* and *b*) and anti-tubulin serum (*c* and *d*). Z-3 serum stains only the basal bodies (*a*) which are seen at the basal ends of the cilia (*b*). Anti-tubulin serum, on the other hand, stains basal bodies as well as the axonemes of the cilia (*c* and *d*). Bars, 5 μm. (*a* and *b*) × 2,045. (*c* and *d*) × 1,630.

(10) using specific antisera to various steroids. They conclude that this staining demonstrates the presence of steroids in centrioles and basal bodies. It is not clear, however, that preimmune sera from the same animals, which could account for such staining, were used as controls in these studies.

Since the fluorescein conjugated secondary reagent used is specific for rabbit IgG, we conclude that it is IgG binding to centrioles and basal bodies that is being detected. We do not know the identity of the molecules in the basal bodies and centrioles to which IgG from our nonimmune rabbit sera are binding. It is clear, though, that the IgG is not binding to tubulin since the staining of centrioles and basal bodies could not be eliminated by absorption of these sera with purified brain tubulin. Whether this staining is due to nonimmune but nonetheless specific attachment of rabbit IgG to some component of the centriole, or whether these sera contain antibodies which react with some as yet unidentified antigen in centrioles and basal bodies, is presently under investigation. It is also not clear whether this binding is to the centriole or basal body itself or to material closely associated with these structures. It is surprising that this binding is absent from axonemes, which like centrioles and basal bodies, contain cold- and colchicine-resistant microtubules. We hope that by characterizing this binding reaction more completely, we will obtain more information about the molecules responsible for this interaction.

So far, ~10% of the nonimmune rabbit sera we have examined stain centrioles and basal bodies clearly and distinctly. It is of interest to note that several recent reports have documented the staining of 100 Å filaments (7, 11) and vinblastine-induced tubulin paracrystals (8) in cells with nonimmune rabbit sera, but this is the first report of centriole staining by sera from nonimmunized animals. The technique described in this paper is relatively simple and extremely specific for the localization of centrioles and basal bodies at the light microscopic level and lends itself well to the visualization of these organelles in large populations of cells.

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