Purine Nucleoside Phosphorylase Is Associated with Centrioles and Basal Bodies

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ABSTRACT We have localized a fraction of the enzyme, purine nucleoside phosphorylase (PNP), to the centrioles and basal bodies of mammalian, avian, and protozoan cells. Two completely independent methods were used, one based on the ultrastructural cytochemistry of the enzyme activity and one based on immunofluorescence microscopy using an antibody raised in rabbit against purified human PNP. PNP catalyzes the reversible conversion of purine nucleosides and inorganic phosphate to the corresponding purine bases and ribose-1-phosphate. Its partial localization to centrioles and basal bodies raises the possibility that purine compounds are involved in centriole replication and/or in the regulation of microtubule assembly in vivo.

No centriolar PNP could be detected in primary skin fibroblast from two infants with severe immunodeficiency disease associated with the absence of soluble PNP. This raises the possibility that defects in centriole function may contribute to the impaired division and maturation of T lymphoid precursor in this inherited disorder.

Initially, the immunofluorescence analyses were complicated by a residual centriole-binding antibody that persisted in immunoglobulins from immune animals after complete removal of anti-PNP by affinity chromatography. Binding was abolished by exposure of cells to sodium periodate, indicating that this (and possibly other) "spontaneous" anticentriole antibodies in rabbit serum may be directed against carbohydrates.

It has become clear from ultrastructural studies that the majority of microtubules in animal cells grow from distinct regions known as microtubule-organizing centers (MTOCs). These structures, which consist of centrioles and their associated satellite substance, basal bodies, and kinetochores, may be the key to understanding the organization and assembly of cytoplasmic, mitotic spindle, and ciliary microtubules. Nevertheless, little is known of their biochemical composition or function (10, 38).

We report here that an enzyme, purine nucleoside phosphorylase (PNP; purine nucleoside; orthophosphate ribosyltransferase, EC 2.4.2.1) is associated with centrioles and their derivatives, the basal bodies of ciliated cells. This enzyme, which catalyzes the reversible reaction of purine nucleosides (guanosine, inosine, and xanthosine, and their deoxyhomologues) with inorganic phosphate to form free bases and (deoxy)ribose-1-phosphate, has been measured previously in soluble extracts of cells ranging from bacteria to man. Its forward reaction (nucleoside or deoxynucleoside to base) can provide substrates to hypoxanthine/guanine phosphoribosyltransferase and may thus contribute to purine ribonucleotide synthesis by the so-called preformed or salvage pathway. Its back reaction (base to nucleoside or deoxynucleoside) is probably unimportant in vivo because of the absence from animal cells of significant amounts of ribose-1-phosphate or deoxyribose-1-phosphate (14, 18, 23, 27). The localization of this enzyme to centrioles and basal bodies suggests a role for purine metabolism in the function of MTOCs and their associated microtubules.

The inherited absence of cytoplasmic PNP is associated with severe immune deficiency disease characterized by a lack of T cell function (13, 14). We show here that centriolar PNP is also missing from the fibroblasts of these patients. Defects in microtubule function secondary to this deficiency in the immobilized fraction of PNP may constitute an element in the pathogenesis of the disease.

Finally we report the removal by sodium metaperiodate

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treatment of a centriolar, probably carbohydrate, antigen that binds an apparently "spontaneous" anticentriole antibody in rabbit serum.

A preliminary account of some of this work was recently published (24).

MATERIALS AND METHODS

Cells

Suspensions of human peripheral blood leukocytes, containing polymorphonuclear leukocytes (PMN), T and B lymphocytes, and monocytes, with some contaminating erythrocytes, were obtained by 45 min sedimentation of whole heparinized human blood with 1.5% pyrogen-free dextran, followed by washing and suspension of cells in Hanks' medium pH 7.2 with 1% bovine serum albumin (BSA). The cells were used in suspension for cytochemical studies or after cytocentrifugation (Shandon cytofuge; 3 min at 100 rpm) onto cover slips for immunofluorescence.

Mitogen-stimulated human lymphocytes were prepared by centrifuging 5-ml portions of fresh, heparinized human blood over 4 ml of sterile Hypaque/Ficoll medium (leukocyte separation medium; Litton Bionetics Inc., Kensington, Md.) for 20 min at ~500 rpm, collecting the mononuclear cells from the interface, washing once in RPMI 1640 medium with 20% heat-inactivated fetal calf serum, and plating at 10^6 cells/ml into 24-well Linbro trays (Flow Laboratories, Hamden, Conn.) with 5 μg/ml phytohemagglutinin (PHA). The blasts were harvested 2–4 d after initiation of mitogenesis. They were studied in suspension or after cytocentrifugation onto glass cover slips.

Rabbit PMN were obtained from peritoneal exudates by the method of Becker (3). They were handled as described for human leukocytes.

PtK_2 rat kangaroo epithelial cells and mouse 3T3 fibroblasts were purchased from the American Type Culture Collection (Rockville, Md.) and grown in RPMI 1640 medium with 20% fetal calf serum. They were grown on 15-mm glass cover slips for immunofluorescence studies.

Several lines of normal human primary skin fibroblasts were obtained as gifts from Drs. H. Malech, Department of Medicine, and M. J. Mahoney, Department of Human Genetics, Yale Medical School. Most of our studies were with fibroblast line 86. Two skin fibroblast cell lines from PNP-deficient patients were also studied. The first, designated PNP-deficient 1 (PNP_1^-) was the generous gift of Dr. I. Fox, Department of Medicine, University of Michigan Medical School. Blood hemolysate from the patient lacked PNP activity and his T cell function was severely impaired (I. Fox, personal communication). The other, designated PNP-deficient 2 (PNP_2^-), was obtained from the Human Genetic Mutant Cell Repository, Camden, N. J. The line was submitted by Dr. G. Griscelli, Centre d'Etudes de Biologie Prénatale, Paris, through the courtesy of Dr. R. Hirschhorn, Department of Medicine, New York University Medical School. Its repository number is GM-2757. These cells were from the severely immunodeficient patient described by Virelizier et al. (39). Fibroblasts were grown as described above for PtK_2 and 3T3 cells.

Mouse tracheal epithelium was obtained by dissection of tracheas from ether-killed C57 black mice, insertion of a hypodermic needle into the lumen at one end of the tissue, and repeated withdrawal and expulsion of Hanks' medium with 1% BSA through the tisue to dislodge and collect ciliated cells. The cells were either studied in suspension or collected onto glass cover slips by cytocentrifugation. Chick tracheal cells from 15- to 20-d embryos were also studied by cytochemical approaches: in this case we simply dissected and cleaned the tracheas, slit them lengthwise, and cut them into a fine mince.

Tetrahymena thermophila strain BIII (previously *T. pyriformis*) were grown axenically at 25°C in slanted tubes. The liquid medium consisted of 2% wt/vol protease-peptone (Difco Laboratories, Detroit, Mich.), 0.2% glucose, 0.1% yeast extract (Difco Laboratories), and 0.003% Sequestrene (CIBA-GEIGY Corporation, Summit, N. J.; 13% Fe/EDTA), pH 6.8.

PNP Antibody

Human PNP was purified to homogeneity from outdated donor erythrocytes by affinity chromatography (26) and was used to produce antibodies in rabits as described by Osborne et al. (28). Immunoglobulins were isolated from pooled rabbit sera by the method of Harboe and Ingild (15). Specificity of the antibody was confirmed by Ouchterlony diffusion and rocket immunoelectrophoresis. 1 ml of immunoglobulin solution neutralized 115 enzyme units of PNP activity (1 enzyme unit = 1 mol uric acid formed/min at 37°C).

Immobilized PNP was prepared by linking Sepharose-6B to purified PNP with trichloro-*s*-triazine using the procedure of Findlay et al. (11). This material was used to adsorb anti-PNP immunoglobulins to give a control antiserum that did not react immunologically with PNP in Ouchterlony double diffusion gels, and did not neutralize PNP activity. The corresponding positive antiserum used here was passed over a column of Sepharose-6B, which was not linked to PNP. The final protein concentrations of column-treated immunoglobulins were 4.1 mg/ml (anti-PNP serum) and 3.9 mg/ml (anti-PNP-depleted serum). This represented a 10-fold protein dilution of the initial purified immunoglobulin preparation.

Pure anti-PNP was obtained by elution from the affinity columns with 0.1 M glycine-HCl buffer pH 3 and concentration by pressure dialysis. Its final protein concentration was ~0.1 mg/ml. This material reacted with purified PNP in Ouchterlony gels. 1 mg of protein neutralized 16.1 enzyme units of PNP.

Immunofluorescence Labeling

Cell monolayers on glass cover-slips were incubated for 1 min at 37°C in either Hanks' medium with BSA or tissue culture medium containing 0.5% Triton X-100. They were drained and transferred for 5 min to methanol in a −10°C freezer. Subsequently the cover slips were rinsed in phosphate-buffered saline (PBS) at room temperature and incubated for 10 min at 37°C with either PBS (in experiments with pure anti-PNP) or with sodium metaperiodate ($NaIO_4$; 1 or 5 mM in PBS: in experiments with anti-PNP in the gamma globulin fraction). The cover slips were then rinsed exhaustively in 1% BSA in Hanks' medium and labeled for 30 min at 37°C with one of the following: 1:10 dilution of column-purified positive and negative anti-PNP immunoglobulins; a 1:100 dilution of original immunoglobulins; or a 1:5 dilution of affinity-purified anti-PNP. Finally they were incubated with either fluorescein- or rhodamine-conjugated $F(Ab)_2$ fragments of goat anti-rabbit IgG. Whole IgG could also be used but the $F(Ab)_2$ fragment reduced background staining resulting from Fc receptor activity in leukocytes. A fluorescent DNA stain, Hoechst 33662, (2 μg/ml) was included in the second antibody solution. The cover slips were mounted in 50% glycerol, and patterns of fluorescence distribution resulting from Hoechst and from fluorescein or rhodamine conjugates were optically separated using specific filter combinations in a Zeiss Photomicroscope III as before (4). Cells were photographed with Kodak Tri-X-Pan film.

Cytochemical Procedures

PNP was detected from the release of inorganic phosphate (P_i) and its precipitation with lead during the enzyme-mediated conversion of ribose-1-phosphate and hypoxanthine to inosine and P_i. The procedure was essentially that of Rubio (34). In particular the fixation, rinse, and reaction buffers developed by Rubio for PNP assay were used without modification. However cell suspensions or finely minced tissue rather than large tissue chunks were used, and so sectioning before addition of substrates was unnecessary in our work. We also varied the time of fixation (2.5–10 min depending on the cell or tissue) and the concentration of fixative (0.25 or 0.5% glutaraldehyde). The standard reaction solution used here contained 4 mM hypoxanthine (or other base) and 10 mM ribose-1-phosphate (or other sugar phosphate) in the Tris-maleate-sucrose buffer described by Rubio (34). Control reaction mixtures lacking purine base were included in each experiment. After incubation with substrates (30 or 60 min at room temperature), cells were rinsed, postfixed with osmium tetroxide, stained with buffered uranyl acetate, dehydrated through a graded series of ethanols, and embedded in Epon. Thin sections were observed without further staining using a JEOL 100 CX/ASID electron microscope.

Characterization of PNP Levels in Normal and Mutant Fibroblasts

Fibroblast extracts were prepared by scraping the cells from each of 4 confluent 100-mm culture dishes, concentrating them in 0.5 ml of PBS followed by lysis by five sequential cycles of freezing (acetone and dry ice) and thawing (37°C). The resulting supernatants were tested for PNP enzyme activity from the rate of conversion of inosine to urate at 30°C in the presence of excess xanthine oxidase (19). The amount of PNP protein was measured by the enzyme-linked immunosorbent assay (ELISA) developed by Osborne and Scott (29). Results were normalized to total cell protein.

RESULTS

The Distribution of PNP: Ultrastructural Cytochemistry

As described by Rubio (34), PNP can be localized from the deposition of lead phosphate during PNP-catalyzed synthesis of inosine from hypoxanthine and ribose-1-phosphate. Initial ultrastructural studies on human peripheral blood leukocytes

(PMN, lymphocytes, monocytes), PHA-transformed human lymphocytes, and rabbit PMN revealed the expected pattern of diffusely distributed reaction product throughout the cytoplasm of all leukocyte types and in erythrocytes. The intensity of staining varied between cells, but variability was not obviously linked to particular classes of leukocytes.

In addition to the general cytoplasmic reaction, a densely stained region was frequently seen adjacent to the nucleus (Fig. 1). In all cases, this staining was associated with the centriole (Fig. 2*A*). Reaction product was concentrated both within and over the centriole and onto the pericentriolar satellites where present in the sections. Centriolar staining was observed in all classes of leukocytes examined (PMN, T and B lymphocytes, monocytes). In PHA-transformed populations, the spindle poles of mitotic cells were also stained for PNP. In strong contrast with centrioles, kinetochores did not stain for PNP. The reaction product was excluded from mitochondria and granules, and nuclear staining was only erratically observed.

Centriolar PNP was not restricted to leukocytes. Staining was also observed on the centrioles of normal human fibroblasts. In confluent primary fibroblasts, stained centrioles were associated with primary cilia.

A variety of experiments indicate the selectivity and specificity of the reaction. Thus the same distribution of stain was observed when ribose-1-phosphate was replaced by deoxyribose-1-phosphate. This is consistent with the known substrate specificity of PNP (18). No staining was seen when hypoxanthine was omitted (Fig. 2*B*) or was replaced by uracil. Similarly omission of ribose-1-phosphate yielded no reaction product. However, replacement of ribose-1-phosphate with ribose-5-phosphate (which is not a substrate for PNP [18]) produced an entirely new staining pattern: centrioles were unlabeled but endoplasmic reticulum and nuclear membranes were intensely stained (Fig. 3). This pattern was unchanged in the absence of

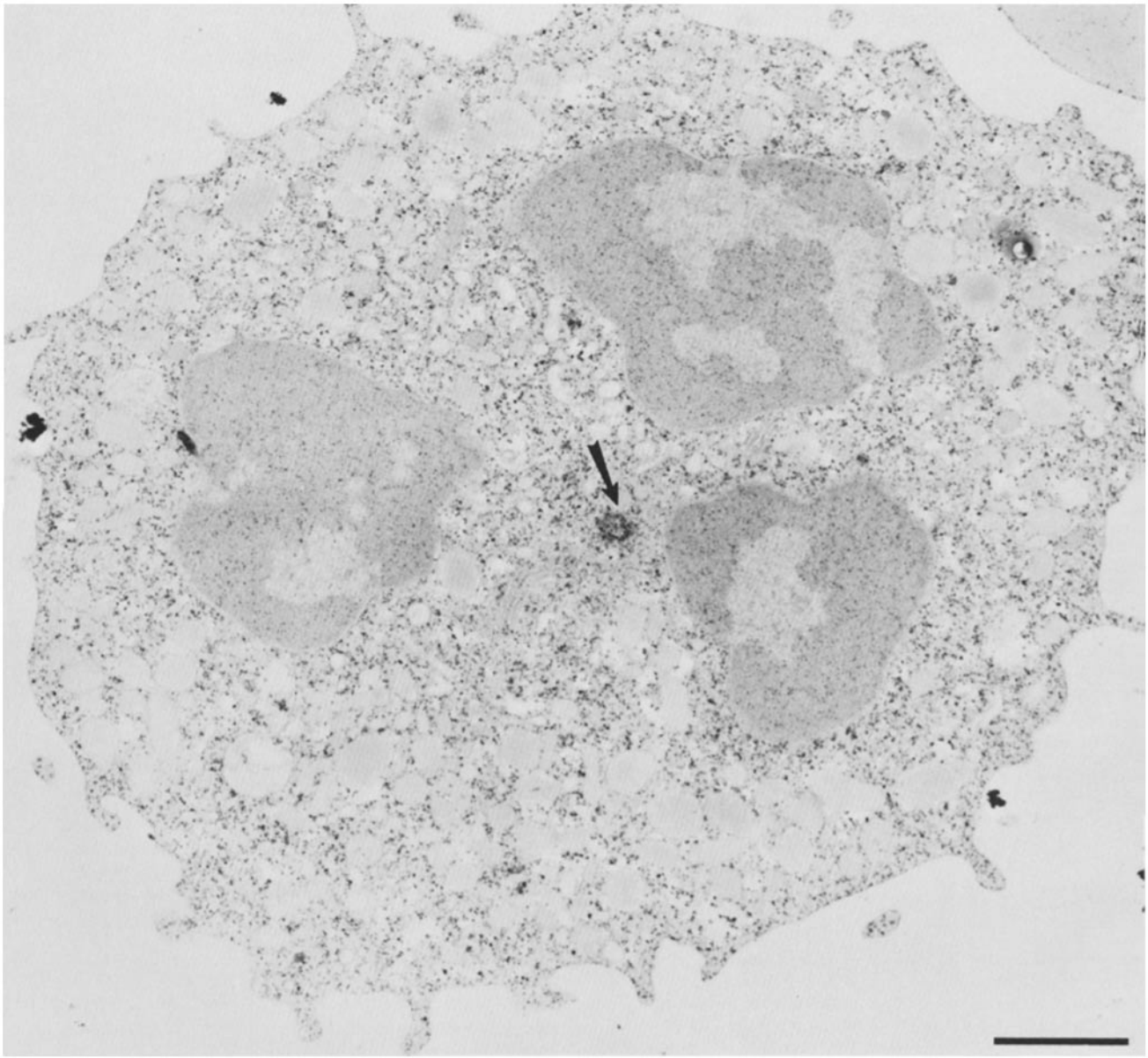

FIGURE 1 Localization of PNP in a human leukocyte. A mixed blood leukocyte suspension was fixed with 0.5% glutaraldehyde for 5 min and incubated for 30 min with hypoxanthine and ribose-1-phosphate as described in Materials and Methods. A typical PMN from this population shows lead phosphate precipitate distributed randomly throughout the cytoplasm with a dense accumulation of precipitate over the centriole (arrow). Cytoplasmic granules, Golgi stacks, and mitochondria are all unreactive. Bar, 1 μm.

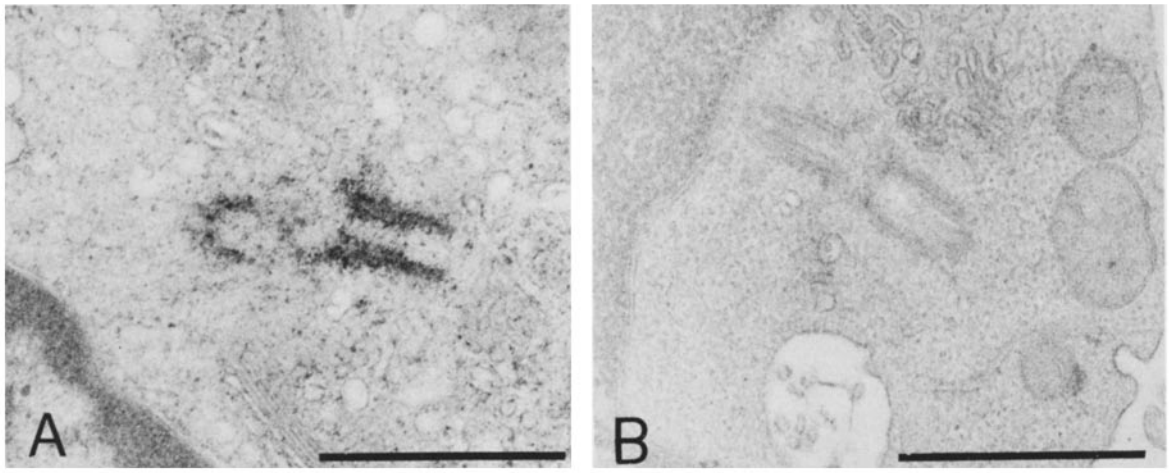

FIGURE 2 The typical appearance of centrioles in leukocytes incubated with ribose-1-phosphate with (*A*) or without (*B*) hypoxanthine. The cells were treated as in Fig. 1. Reaction product coats the inner core as well as the outer surface and satellites of the centriole exposed to hypoxanthine. No lead precipitate is formed in the absence of purine base. Bar, 1 μm.

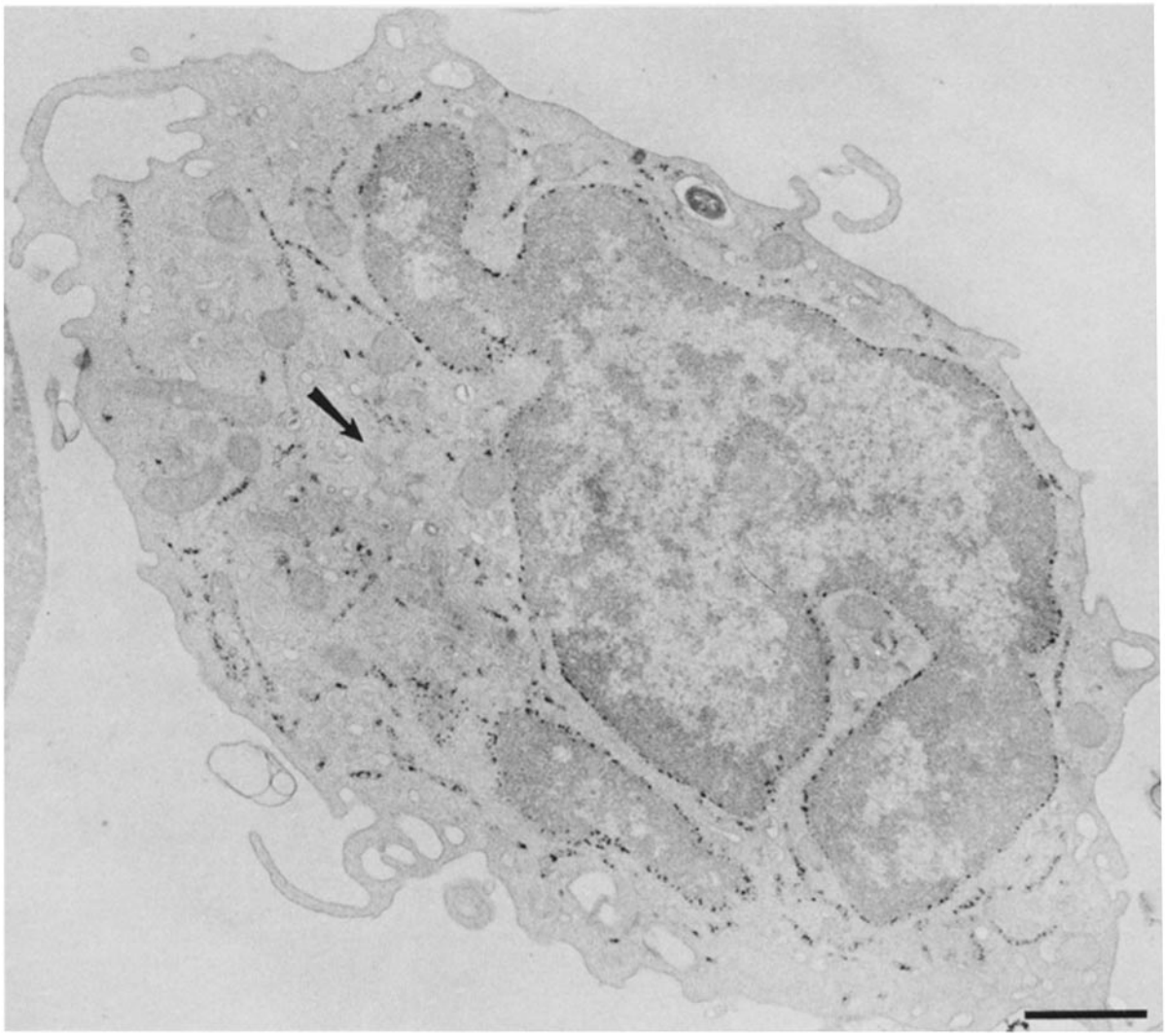

FIGURE 3 Localization of ribose-5-phosphatase activity in human leukocytes. A mixed leukocyte suspension was fixed with 0.5% glutaraldehyde for 5 min and incubated with ribose-5-phosphate and hypoxanthine for 30 min. Under these conditions, lead precipitate lies within the endoplasmic reticulum and nuclear membranes. Centrioles (arrows) are completely unreactive. The cell illustrated here is probably a monocyte. Bar, 1 μm.

hypoxanthine, indicating an underlying ribose-5-phosphatase activity, unrelated to purine metabolism. Finally, incubation of cells for 1 min at 37°C with 0.5% saponin before fixation and processing caused extensive depletion of cytoplasmic reaction product but did not prevent centriolar staining. These data are summarized in Table I.

The Distribution of PNP: Analyses of Ciliated Cells

The basal bodies that determine the growth and function of ciliary and flagellar microtubules are directly related to centrioles. Indeed they generally appear to develop by the proliferation of centrioles followed by migration to the cell surface where additional structural components, such as rootlets, basal feet, and basal plates are added (10). We therefore postulated that staining resulting from PNP might also be localized in basal bodies. This hypothesis was tested in mouse and chick tracheal epithelium cells and in *Tetrahymena.*

The typical appearance of basal bodies in ciliated mouse epithelium stained for PNP is shown in Figs. 4*A* and 5*A*. The ciliary shafts are weakly reactive, with increased reactivity in the most basal regions. The general cytoplasm shows a light and uniform distribution of stain. The basal bodies are stained intensely on both their inner and outer surfaces. This staining extends to the basal plate and basal feet. Essentially no staining is observed in the absence of purine base (Fig. 4*B*). As in leukocytes, the distribution of stain was consistent from cell to cell but the intensity of the reaction varied, even between adjacent cells.

Similarly the basal bodies of most chick tracheal cells were strongly reactive for PNP (e.g., Fig. 5*B*). In contrast, the cytoplasm was lightly labeled, and the ciliary shafts were either lightly labeled (near the base) or unlabeled.

PNP was also localized to basal bodies in a protozoan. Fig. 6 shows equivalent views from the buccal region of *Tetrahymena* incubated with ribose-1-phosphate with or without hypoxanthine. No staining is seen without hypoxanthine (Fig. 6*B*). In the presence of purine base (Fig. 6*A*), reaction product is dispersed lightly through the entire cytoplasm. In addition, intense staining is associated with the closely packed basal bodies (*BB*) of the buccal cavity that support the oral cilia (*OC*) as well as the more widely spaced basal bodies that support the somatic cilia (*SC*). The cilia themselves are minimally stained. In *Tetrahymena* two additional reactive structures were noted. First, reaction product typically occurred along the sets of parallel microtubules that run in the ridges of the ribbed wall of the buccal cavity. These ridges (*R*) are visible immediately above the basal bodies of the oral ciliature in Fig. 6*A*. Second, reaction product was seen in a proportion of the unusual tubular cristae of *Tetrahymena* mitochondria. As noted above reaction product was not seen in the mitochondria of any of the higher animal cells studied.

TABLE I

Distribution of Lead Phosphate Precipitate on Leukocytes Incubated under Various Conditions

Treatment	Presence or absence of stain		
	Cytoplasm	Centriole	Other organelles
Hypoxanthine + ribose-1-phosphate	+	+	−*
Hypoxanthine + deoxyribose-1-phosphate	+	+	−*
Ribose-1-phosphate only	−	−	−
Hypoxanthine	−	−	−
Uracil + ribose-1-phosphate	−	−	−
Hypoxanthine + ribose-5-phosphate	−	−	+‡
Ribose-5-phosphate only	−	−	+‡
Saponin; hypoxanthine + ribose-1-phosphate	−§	+	−

This summary was developed from analyses with human leukocytes. However, identical results were obtained in less complete analyses of rabbit PMN, cultured mammalian cells, mouse and chick tracheal cells, and *Tetrahymena.*

* Nuclear reaction product was seen in some experiments.

‡ ER, nuclear membrane.

§ Cytoplasmic label after saponin varied from reduced to absent.

The Distribution of PNP: Immunofluorescence Studies

An antibody prepared against the soluble PNP purified from human erythrocytes provided an entirely independent route to confirm and extend the study of MTOC-associated PNP. It was found that centrioles of human blood PMN, lymphocytes, and monocytes were intensely stained by a 1:100 dilution of rabbit immunoglobulins containing a specific anti-PNP antibody. An identical centriole labeling was obtained with a 1:10 dilution of anti-PNP immunoglobulins after passage through a column of Sepharose-6B (Fig. 7*A*). The same antibody labeled centrioles of human primary fibroblasts (see below: Fig. 8), 3T3 mouse fibroblasts, rabbit peritoneal PMN and macrophages, and PtK_2 rat kangaroo kidney epithelial cells. This staining depended on prior Triton or saponin extraction of cytosolic enzyme that otherwise masked the centriolar component. Very little variation in staining intensity was seen between cells of the same type. However, lymphocyte centrioles were stained more brilliantly than similarly treated fibroblast centrioles.

The cells illustrated in Fig. 7*A* were exposed to $NaIO_4$ for 10 min before incubation with immunoglobulins containing anti-PNP. Cells from the same $NaIO_4$-treated population, but incubated with immunoglobulins that were depleted of anti-PNP by passage through an affinity column of Sepharose-6B-PNP, are shown in Fig. 7*B*. No centriolar fluorescence is visible. Thus the centriolar label is specific for PNP and does not reflect the activity of other "spontaneous" anticentriole antibodies found in the sera of some of our rabbits and in sera obtained by other investigators (7).

On the other hand the specificity of centriole labeling with whole immunoglobulins is absolutely dependent on $NaIO_4$ treatment. Anti-PNP-depleted immunoglobulins showed a weak but persistent centriolar fluorescence on cells reacted without $NaIO_4$. This labeling required the presence of rabbit immunoglobulin and was not observed on cells labeled with second antibody alone. It could not be suppressed by addition of various proteins or heterologous sera. Its complete suppression by $NaIO_4$, which attacks sugars, indicates the presence of a spontaneous anticarbohydrate antibody in our rabbit immunoglobulins. This antibody recognizes a component of centrioles.

To further establish the centriolar location of PNP, we purified anti-PNP from total immunoglobulins, which included the apparent anticarbohydrate antibody, by elution from the

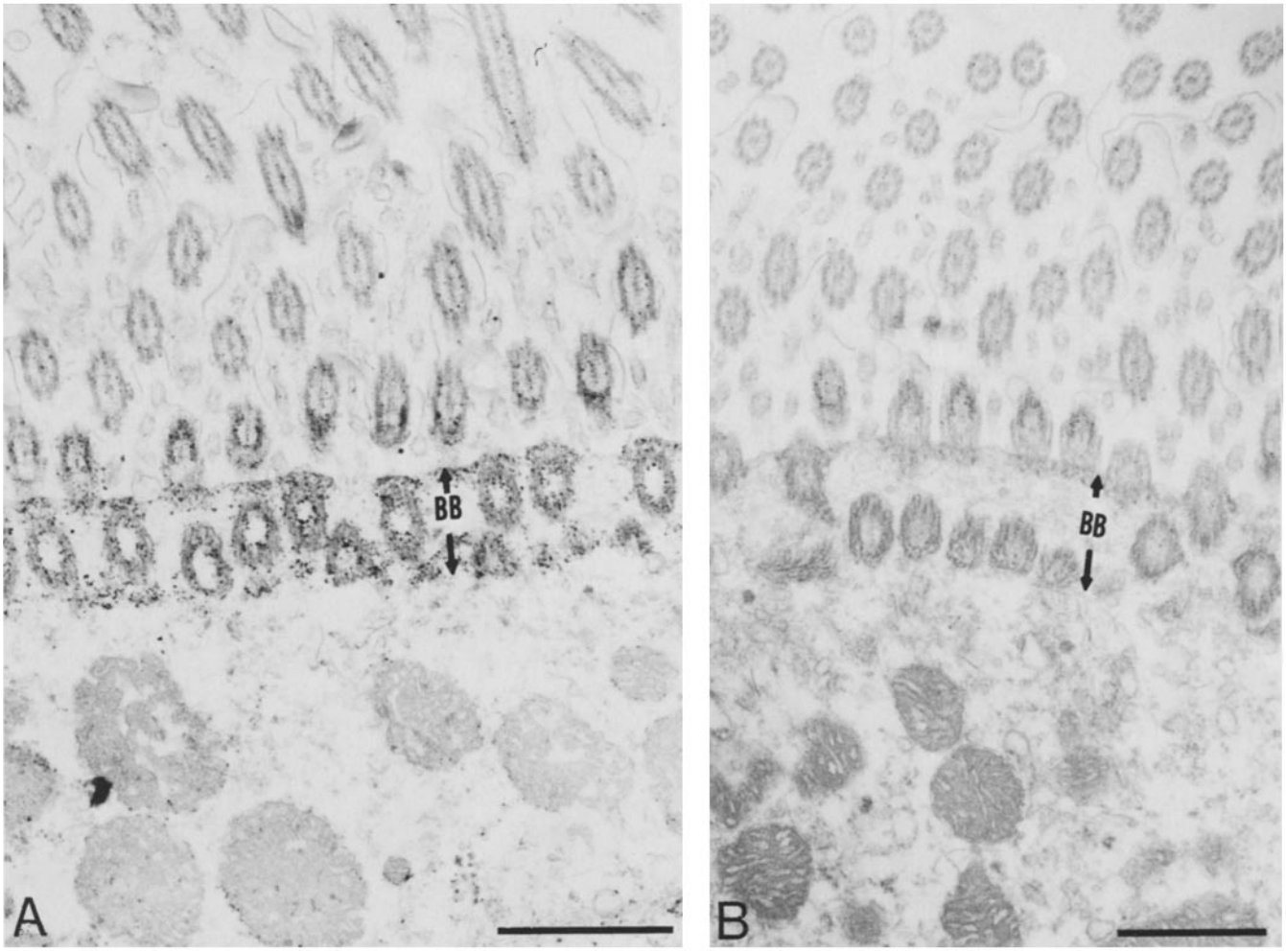

FIGURE 4 Localization of PNP in mouse ciliated epithelium. Ciliated cells collected by tracheal lavage from freshly killed mice were fixed with 0.25% glutaraldehyde for 5 min and incubated with ribose-1-phosphate with or without hypoxanthine for 60 min. Essentially no lead phosphate reaction product is seen in the absence of purine base (*B*). In the presence of hypoxanthine (*A*), cells show a light distribution of stain throughout the cytosol and an intense accumulation of stain over the basal bodies (*BB*). Mitochondria lack reaction product. Similarly, little or no reaction product is observed away from the most basal regions of the ciliary shafts. Bar, 1 μm.

affinity column. The pure antibody bound to leukocyte and fibroblast centrioles and spindle poles (Fig. 8*A*, and *B*). Its binding was completely eliminated by convential adsorption against purified PNP: $NaIO_4$ pretreatment of the cells was not required. It did not recognize the centrioles of PNP-deficient fibroblasts (Fig. 8*C*; see below).

The localization of PNP to basal bodies was readily confirmed by use of anti-PNP immunoglobulins in fixed, $NaIO_4$-treated mouse tracheal epithelium. As shown in Fig. 9, the entire region underlying the cilia reacts with anti-PNP. In contrast no accumulation of fluorescence is seen with antibody-depleted immunoglobulin (Fig. 10). As with centrioles, basal bodies not exposed to $NaIO_4$ showed a substantially reduced but persistent label even in anti-PNP-depleted immunoglobulins. This presumably reflects the accumulation of carbohydrate in the basal body as well as the centriolar region of cells.

Analyses in PNP-deficient Fibroblasts

The mutant fibroblasts used here were from patients whose erythrocytes completely lacked PNP and who expressed essentially no T cell function.

Our characterization of these two lines and a typical normal human fibroblast line are summarized in Table II. The activities and amount of PNP in the normal line is within the range measured for many normal cell lines by Osborne and Scott (27). One mutant line, designated PNP_2^-, had no detectable enzyme protein or enzyme activity. The other line, PNP_1^-, had ~20% of normal enzyme activity and amount.

By immunofluorescence assay neither PNP_1^- nor PNP_2^- cells showed detectable centriolar staining provided anti-PNP was applied to $NaIO_4$-treated cells or purified anti-PNP immunoglobulin was used (illustrated for PNP_2^- in Fig. 8*C*). Thus residual, immobile centriolar enzymes did not persist in cells lacking soluble PNP.

By ultrastructural cytochemistry neither centrioles nor cytoplasm of PNP_2^- showed reaction product attributable to PNP (Fig. 11*C*). In the same experiment, both cytoplasm and centrioles of normal cells were stained (Fig. 11*A*). Consistent with their biochemical and immunochemical characterization (Table II), PNP_1^- cells showed a small, residual cytoplasmic staining (Fig. 11*B*). However, no centriole label was seen in PNP_1^- cells.

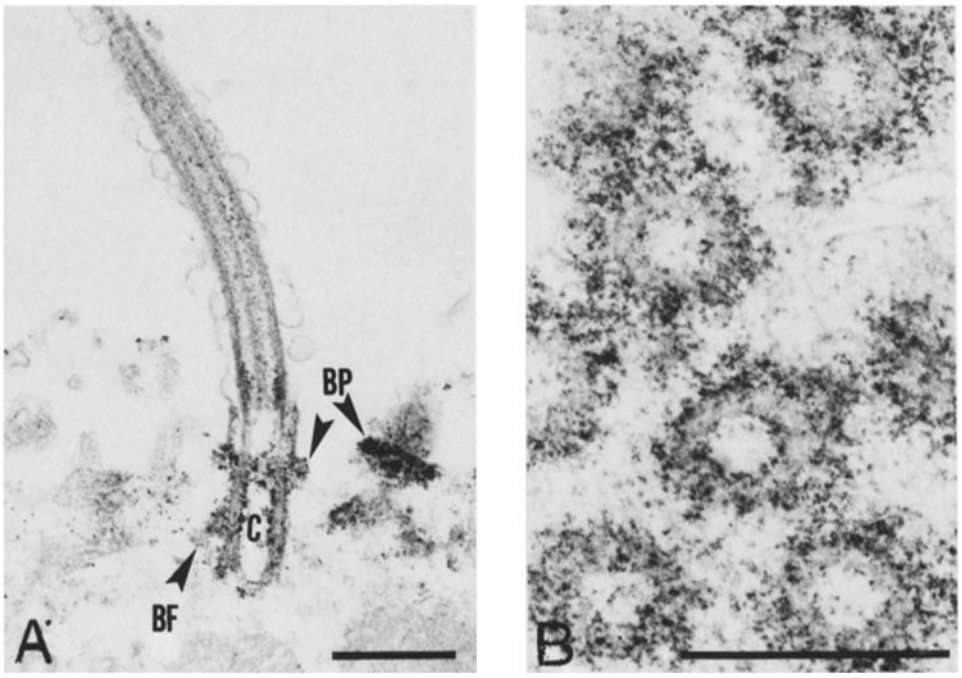

FIGURE 5 The localization of PNP within basal bodies. A longitudinal section of a basal body cilium from a mouse bronchial epithelial cell (*A*) shows PNP reaction product associated with the inner core (*C*), the basal foot (*BF*), and the basal plate (*BP*). Reaction product is also dispersed in the most basal region of the cilium. The grazing section across a group of basal bodies from chick bronchial epithelium cells (*B*) emphasizes the presence of reaction product on both the inner and outer sides of the basal body. The cells were treated as described in Fig. 4. Bar, 0.5 μm.

DISCUSSION

The morphology, function, and replication of centrioles and basal bodies was reviewed recently by Dustin (10). It is generally believed that these organelles, or closely associated structures (the satellite material surrounding centrioles; the basal plate of cilia), determine the organization of microtubules into cytoplasmic networks, mitotic spindles, cilia, and flagella. Attempts to isolate centrioles and basal bodies, however, have met with only limited success (e.g., 33, 37). Thus the replication of centrioles and the maturation of basal bodies has been elegantly described at the ultrastructural level (2, 20) but has not been studied biochemically; an incompletely characterized ATPase appears to be the only enzyme that has been localized to basal bodies (1); and the protein composition of pericentriolar material is unknown except for the recent demonstration in a highly specialized cnidarian sperm of pericentriolar actin (21). Interpretations of morphological and functional changes after RNase digestion suggest the presence of basal-body-associated and centriolar RNA (9, 15, 30, 32) that may be involved in formation of the mitotic spindle (17, 31). Immunocytochemical approaches have revealed a host of different anticentriolar antibodies (8, 33) that, as yet, have not further elucidated structure, biochemistry, or function.

We have used two completely independent methods, one based on enzyme activity and one based on antibody localization, to show that an enzyme of purine metabolism, PNP, is associated with centrioles and basal bodies. This association can be demonstrated in species as widely separated as man and *Tetrahymena*. The centriolar localization is found whether cytoplasmic PNP is detergent extracted (for immunoassay) or not (for cytochemistry) before enzyme localization and whether fixation is by use of aldehyde or methanol. The enzyme is synthesized by the cells and not obtained by incorporation of residual enzyme present in serum: thus fibroblasts lacking soluble PNP were grown in the same tissue culture medium as normal cells and showed no evidence of centriolar enzyme. We believe that the centriolar enzyme is the same as the enzyme found at lower activity in the cytoplasm of these cells: first, and most importantly, a single human gene defect leading to loss of soluble PNP also removes centriolar PNP; second, antibody raised against soluble enzyme recognizes the immobilized, detergent-resistant centriolar enzyme. We suppose that the soluble and immobilized enzyme proteins may prove to show different posttranslational modification. For example, the centriolar enzyme could be glycosylated, whereas the cytoplasmic form was recently shown to be a nonglycosylated protein (36).

The association of PNP with centrioles was noted in passing by Borgers and co-workers (5). In describing the distribution of PNP in human leukocytes these investigators wrote "the reaction product was confined to the cytosol. With the exception of centrioles, the subcellular organelles. . . were devoid of the precipitate." These authors went on to propose cytosolic PNP as a possible histochemical marker for T cells. However, we have observed no consistent patterns of PNP staining intensity linked to particular classes of leukocytes. Indeed, significantly different densities of reaction product are commonly seen between cells of the same type.

Although the occurrence of centriole and basal-body-associated PNP over a broad phylogenetic range encourages belief in its importance, the role of this enzyme is yet to be defined. Two possibilities seem obvious. PNP may be involved in centriole replication, either promoting the formation of daughter centrioles or protecting centrioles against potentially toxic purine nucleosides. In particular, PNP may provide substrates

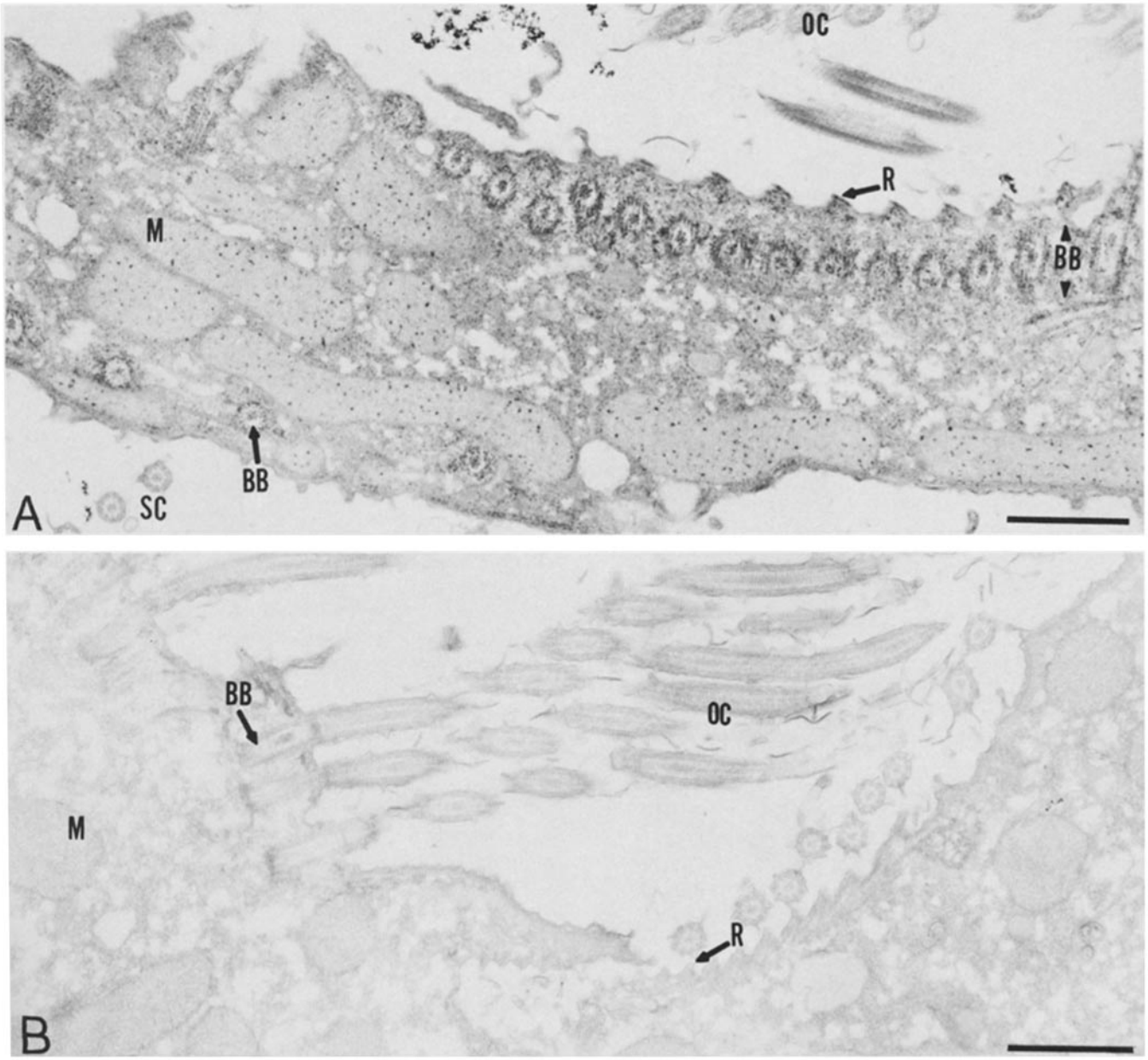

FIGURE 6 Localization of PNP in *Tetrahymena*. The organisms were fixed (0.25% glutaraldehyde, 5 min) and incubated for 60 min with ribose-1-phosphate with or without hypoxanthine. The sections illustrated are from the buccal cavity. No lead precipitate is formed in the absence of hypoxanthine (*B*). In the presence of hypoxanthine (*A*), the general cytoplasm is lightly stained. In addition, the basal bodies (*BB*) that support both the oral cilia (*OC*) and somatic cilia (*SC*) are intensely stained. In *Tetrahymena*, reaction product is also associated with mitochondria (*M*) and with microtubules that run in the ridges of the ribbed wall (*R*) of the buccal cavity. Bar, 1 μm.

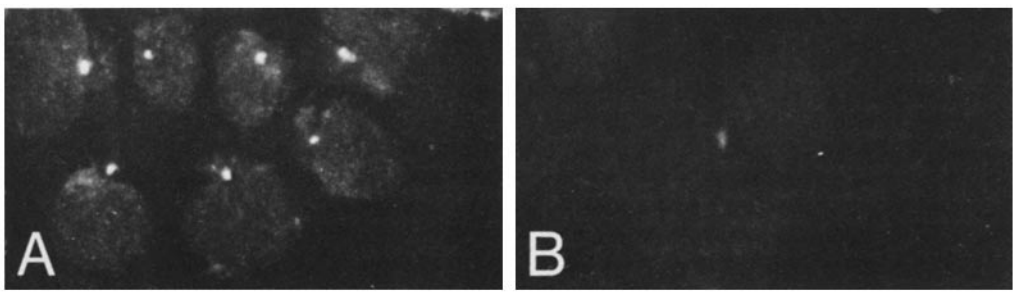

FIGURE 7 Immunofluorescence localization of PNP. Lymphocytes were extracted with Triton X-100, fixed in methanol, and incubated for 10 min at 37°C with 5 mM $NaIO_4$. The cells were then labeled with 1:10 dilution of rabbit immunoglobulins that had passed over a column of Sepharose-6B alone (anti-PNP-positive immunoglobulins) or over a Sepharose-6B-PNP affinity column (anti-PNP-depleted immunoglobulins). Centrioles are intensely stained by anti-PNP immunoglobulins (*A*). Centriole fluorescence is at background levels in cells treated with anti-PNP-depleted immunoglobulins (*B*). × 750.

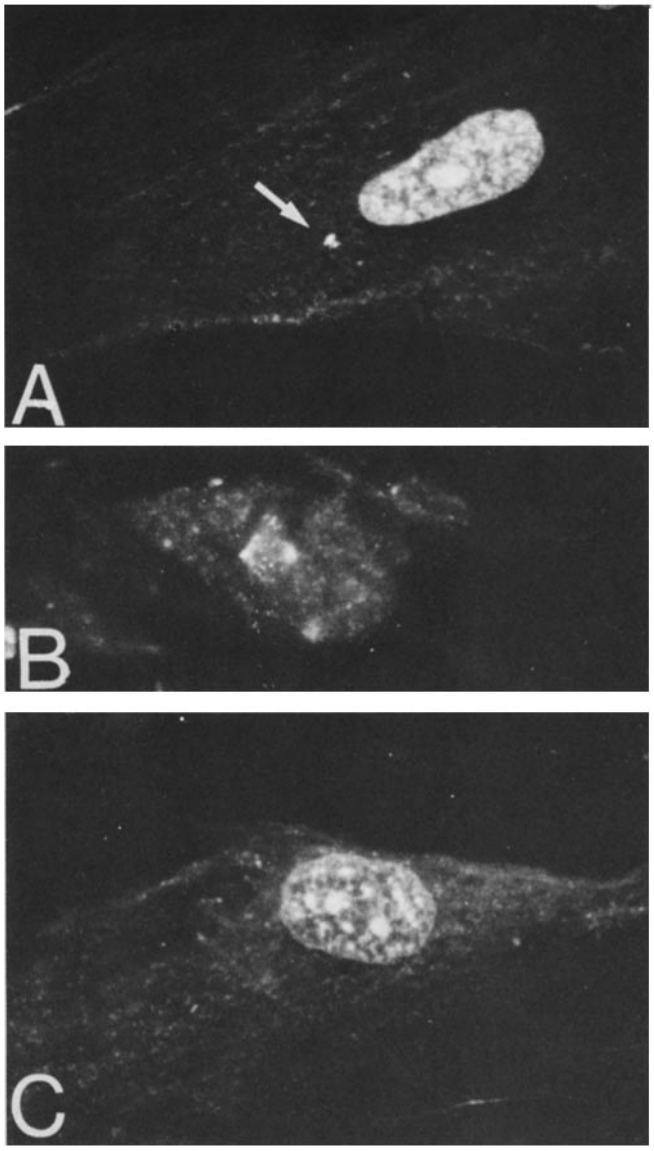

FIGURE 8 Immunofluorescence localization of PNP using purified anti-PNP IgG. Fixed primary human fibroblasts were incubated with affinity-purified anti-PNP IgG without prior $NaIO_4$ treatment. Normal cells (line 86) showed fluorescence attributable to anti-PNP labeling at centrioles (arrow, *A*) and at the spindle poles of mitotic cells (*B*). No fluorescence was associated with the centrioles of PNP-deficient (PNP_2^-) cells (*C*). Note that the nuclear fluorescence in *A* and *C* is seen when fibroblasts are incubated with rhodamine-conjugated F(ab)$_2$ antirabbit IgG alone and so does not indicate a nuclear localization of PNP. (*A* and *C*) × 650; (*B*) × 830.

for the replication of the RNA that is reportedly associated with centrioles and basal bodies. Alternatively, the substrates or products of PNP may influence the assembly and disassembly of microtubules about centrioles. In particular PNP localized within the centriole or ciliary base may be involved in the metabolism of guanosine generated from the breakdown of GTP during microtubule disassembly (4, 22, 40).

The discovery in man of severe immune disorders linked to the absence of PNP adds urgency to analyses of the possible role(s) of centriole and basal-body-associated PNP. In PNP deficiency, T lymphoid precursors are profoundly defective in their ability to divide and differentiate. Consequently T-cell-mediated immune function is almost completely absent in affected children. The underlying cause of this T cell dysfunction has been explained by several hypotheses centered on the toxicity of increased levels of various purine compounds (reviewed in references 12, 14, 27, 35). In most cases, DNA synthesis is considered the primary target. A current, popular hypothesis suggests that excess deoxy GTP (dGTP) is the major toxic metabolite. This nucleotide is synthesized by kinase action from deoxyguanosine that accumulates in PNP-deficient cells. It inhibits ribonucleotide reductase and may thus starve cells of the deoxynucleotide precursors of DNA synthesis. It is proposed that the greater susceptibility of T than B cells to growth inhibition resulting from PNP deficiency stems from the greater activity of deoxynucleoside kinase in T cells.

This hypothesis is entirely plausible. However it is also possible that the absence of immobilized PNP from T cell precursors impairs centriole replication or centriole-dependent microtubule functions, such as mitosis, thus compromising the generation of mature T cells. PNP-depleted fibroblasts, which do divide, may be less dependent on this role of centriolar PNP. This proposal is not without precedent. Abnormal cytoskeleton and membrane properties have already been suggested in lymphocytes from patients with two other forms of severe immune dysfunction (6, 25).

In the course of these studies we obtained evidence for the presence of a carbohydrate antigen in association with centrioles and basal bodies. As described above, we were frustrated during the initial studies with anti-PNP antibody by the inability to remove all centriole or basal body labeling by removing anti-PNP from the mixed rabbit immunoglobulins of immune rabbits. Subsequently, it was found that incubation of cells with $NaIO_4$ eliminates fluorescence labeling due to anti-PNP-depleted immunoglobulins while preserving fluorescence due to immunoglobulin containing anti-PNP. $NaIO_4$ oxidizes sugars with adjacent *cis* hydroxyl groups. Thus its removal of "spontaneous" antibody reaction is most readily explained by the presence in our rabbit immunoglobulins of an antibody that recognizes centriole-associated carbohydrates. Of course, the target is not defined: obvious possibilities are centriole-associated glycoproteins or glycolipids or even nucleic acids. The origin of the carbohydrate antibody is also not defined. It may have been elicited against a centriolar or basal body antigen from an endogenous or exogenous (protozoan infection?) source. However, an immune response to an infectious agent whose surface coat contained a carbohydrate antigen that resembled a centriole carbohydrate seems a more probable route.

In summary, we have localized a specific enzyme, PNP, to the centrioles and basal bodies of mammalian, avian, and protozoan cells. We propose a role for this PNP in the metabolism of guanine nucleotides which in turn may affect either the replication of centrioles or the regulation of microtubule assembly from sites adjacent to centrioles and basal bodies. We also propose that a particular dependence of T lymphoid precursors on centriolar PNP for the replication of centrioles or the assembly of microtubules from centrioles regions may contribute to the failure of T cell development in PNP deficiency.

We find that at least one "nonspecific" antibody to centrioles is probably directed against carbohydrate, as indicated by the elimination of its binding site by $NaIO_4$. This observation is of practical importance for the elimination of interfering antigen-antibody reactions in immunological procedures. In addition, we conclude that centrioles and basal bodies contain carbohydrate antigens.

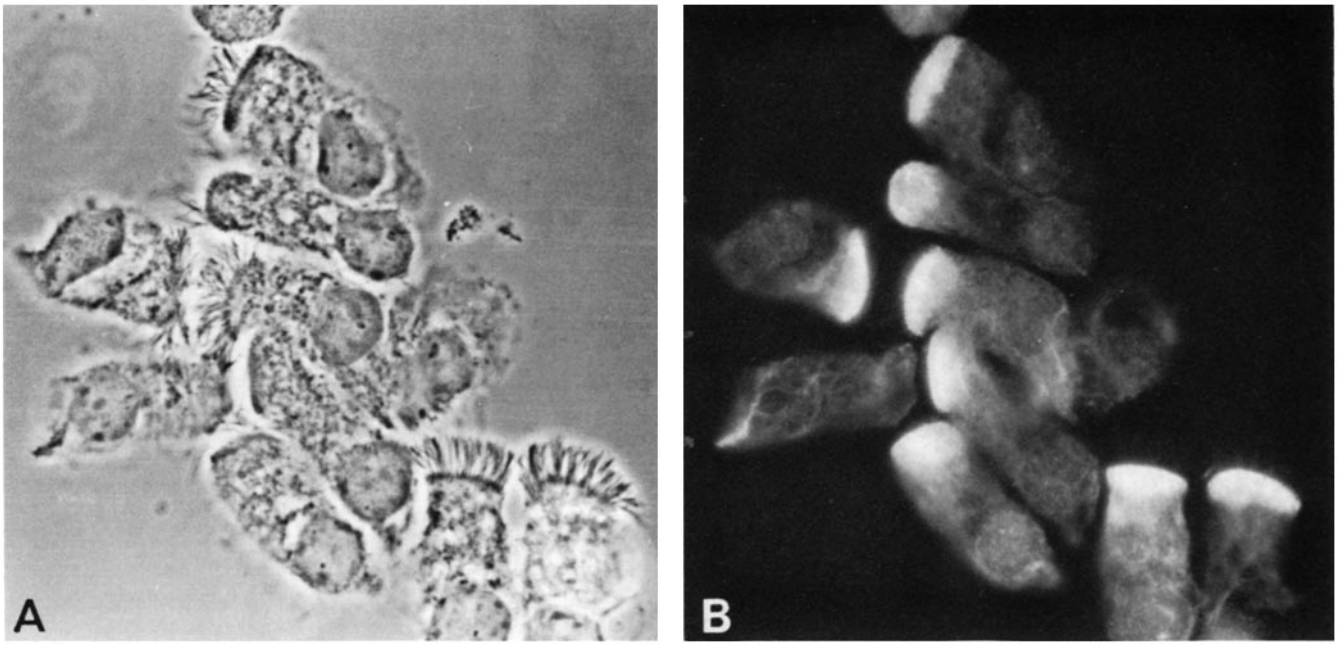

FIGURE 9 Immunofluorescence localization of PNP in ciliated cells. Mouse tracheal epithelium was fixed, treated with $NaIO_4$ (1.0 mM; 10 min) and incubated with anti-PNP. The basal body region is intensely labeled. The general cytoplasm shows a weak fluorescence, perhaps because of remaining cytosolic PNP. Cilia are visible by phase-contrast microscopy (*A*) but cannot be seen by fluorescence microscopy (*B*) after anti-PNP labeling. × 650.

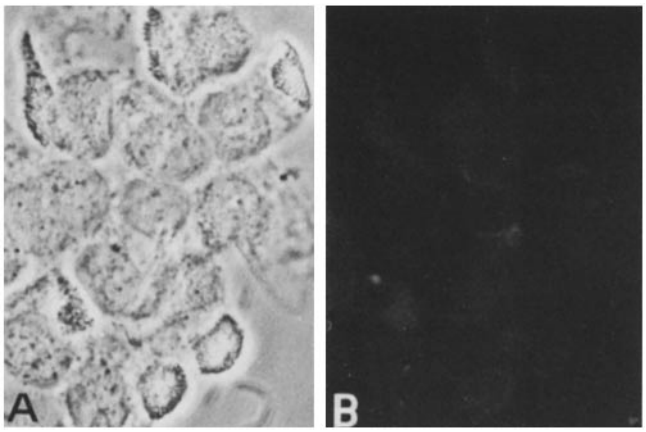

FIGURE 10 The distribution of fluorescence in ciliated cells incubated with anti-PNP-depleted immunoglobulins. The $NaIO_4$-treated (1.0 mM; 10 min) mouse tracheal cells show essentially no fluorescence labeling after incubation with anti-PNP-depleted rabbit immunoglobulins. × 640.

TABLE II

The Activity and Amount of PNP in Normal and Mutant Human Primary Fibroblasts

	PNP activity
	μmol inosine phosphorylyzed/min/g protein
A. Kinetic analysis	
Normal	10.7
PNP_1^-	2.0
PNP_2^-	<0.04*
	Enzyme protein
	μmol inosine phosphorylyzed/min/g protein
B. ELISA	
Normal	11.0
PNP_1^-	2.3
PNP_2^-	<0.02*

Results are the average of two separate determinations.
* Limit of assay detection.

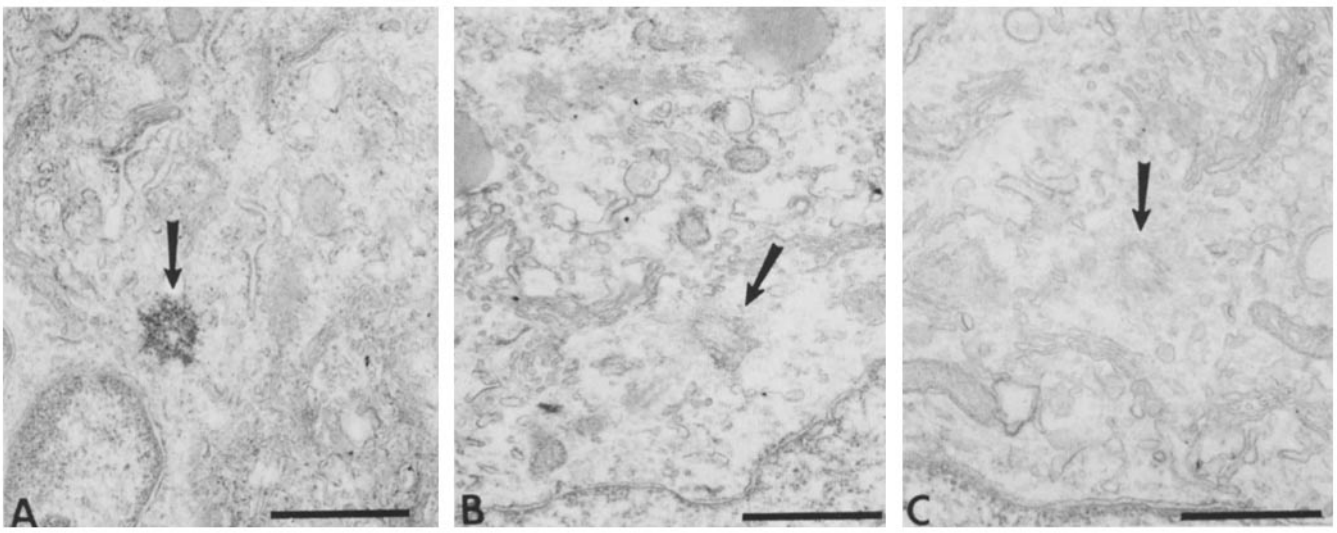

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FIGURE 11 The typical appearance of PNP reaction product in the centriole region (arrows) is shown for three primary fibroblast cell lines. Cells were fixed with 0.5% glutaraldehyde for 5 min and incubated with hypoxanthine and ribose-1-phosphate for 60 min. Line 86, a normal human cell line (*A*), demonstrates a strongly stained centriole surrounded by finely stained cytoplasm. Line PNP_1^-, which had ~20% of normal levels of PNP activity, showed sparse cytoplasmic labeling without detectable centriolar labeling (*B*). No reaction product was found in either cytoplasm or centrioles of line PNP_2^-, a severely PNP-deficient fibroblast line (*C*). Bar, 1 μm.