mechanism by which binding of the B subunit to G_{M1} leads to a mitogenic response, changes in intracellular free Ca^{2+},K^+ channel gating, and Na^+-H^+ exchange have all been implicated in the mitogenic response (*19*). Thus, cross-linking of G_{M1} by the multivalent B subunit may modulate ion channels in the plasma membrane (*20*).

Our findings also raise the possibility that other physiological processes may be triggered by an interaction with gangliosides on the cell surface. Gangliosides have been implicated as inhibitors of both mitogen-stimulated lymphocyte proliferation (*4*) and the growth of fibroblasts in response to serum and growth factors (*5*); they also mediate neuronal growth, survival, and differentiation (*6, 7*). Thus, gangliosides may play a role as membrane transducers of both positive and negative signals that regulate cell growth and differentiation.

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10. Rat thymocytes (3.9×10^6) were incubated in 0.2 ml of a solution (*21*) containing 0.5 n*M* ^{125}I-labeled CT (1120 cpm/fmol). Some samples contained CT (5 μg), B (2.5 μg), or antibodies to CT (1:10). After one hour at 37°C with constant shaking, the cells were washed three times with 3 ml of the ice-cold solution containing 0.2 percent bovine serum albumin by centrifugation at 1875*g* for 10 minutes. The washed cells were then counted for bound ^{125}I-labeled CT in a gamma counter.
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Colocalization of Band 3 with Ankyrin and Spectrin at the Basal Membrane of Intercalated Cells in the Rat Kidney

Abstract. *An immunoreactive form of the anion channel protein of erythrocytes, band 3, has been identified in the rat kidney. It is found in the intercalated cells of the distal tubule and collecting ducts. Immunostaining specific for band 3 is confined to the basolateral plasma membrane of these cells, where this protein probably mediates the transport of bicarbonate across the tubular wall. Double-immunolabeling studies demonstrate that band 3 is colocalized with immunoreactive forms of ankyrin and spectrin along the basolateral plasma membrane. The polarized distribution of band 3 may be the result of the association of its cytoplasmic domain with ankyrin, which in turn links band 3 to spectrin and the cytoskeleton. These observations help to explain how the collecting ducts of the kidney can direct the transport of bicarbonate ions, thus maintaining the acid-base balance.*

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The plasma membrane of transporting epithelial cells can be divided into apical and basolateral regions, which differ in protein composition and function. The mechanisms responsible for the placement of membrane proteins at specific sites on the cell surface are under active investigation (*1*). One way by which membrane proteins might be confined to a particular region of the cell surface could be by association with cytoskeletal proteins.

The membrane of erythrocytes provides an example in which a membrane-spanning (integral) protein, the anion channel protein (band 3), is linked by ankyrin to spectrin, which forms a fibrous meshwork on the cytoplasmic surface of the membrane (*2*). Proteins closely related to erythrocyte ankyrin and spectrin have been purified and characterized from various nucleated cell types (*3*), and immunoreactive forms of band 3 have also been described in nucleated cells (*4*), including the chicken kidney (*5*). We describe here the localization of an immunoreactive form of band 3 in the intercalated cells of the distal tubules and collecting ducts in the mammalian (rat) kidney. Band 3 is confined to the basolateral plasma membrane of these cells. This protein probably mediates reabsorption of bicarbonate at the basolateral membrane surface and thus may regulate acid-base balance. Immunoreactive forms of ankyrin are colocalized with band 3 along the basolateral surface of the intercalated cells; nonerythroid spectrin is also present at this site. Linkage of the anion channel analog via ankyrin to the spectrin-based membrane cytoskeleton may serve to maintain the polarized localization of this transport protein at the basolateral membrane.

Membrane proteins of the rat kidney were precipitated with antibodies to either rat or human erythrocyte band 3. Western blot analysis of the precipitated proteins (Fig. 1) revealed a polypeptide with an apparent molecular weight (M_r) of 120,000 migrating above rat erythrocyte band 3 (M_r ~100,000). In whole kidney membranes, band 3 migrated at M_r ~140,000. A slightly lower molecular weight (M_r ~115,000) has recently been determined for band 3 in the chicken kidney (*5*). In tissue sections of the adult rat kidney, affinity-purified antibodies to either the cytoplasmic or the membrane-spanning domain of both human and rat

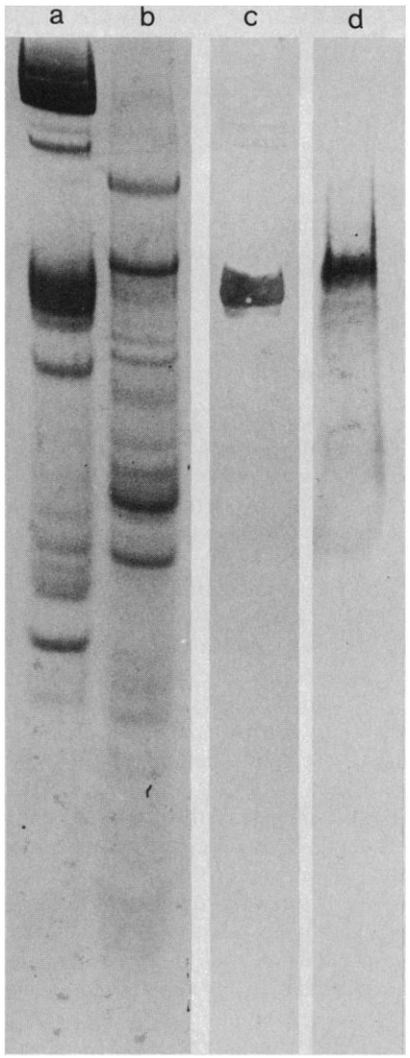

Fig. 1. Identification of an isoform of band 3 in rat kidney membranes by Western blot analysis. Kidney band 3 (M_r ~120,000) (lane d) has a slightly slower mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (10 percent) than rat erythrocyte band 3 (M_r ~100,000) (lane c). Coomassie blue–stained SDS-PAGE of human erythrocyte (lane a) and rat kidney membranes (lane b) are also shown. Rat erythrocyte membrane proteins (lane c) and immunoprecipitated rat kidney membrane proteins (lane d) were separated by SDS-PAGE, transferred to nitrocellulose paper, and labeled with antibodies to whole rat erythrocyte band 3 (for antibody preparation, see Fig. 2), by the indirect peroxidase method (*4*). Kidney membranes were obtained from homogenized rat kidneys previously depleted of erythrocytes by vascular perfusion with phosphate-buffered saline (*p*H 7.4). The membrane proteins were extracted with 4 percent Triton X-100 and 1*M* KCl in 0.4*M* tris buffer. The extract was incubated for 30 minutes at room temperature with 40 μl of undiluted antiserum to whole rat band 3 or with nonimmune serum and was then stored for 20 hours at 4°C. Immunoglobulins were precipitated with 40 μl of packed *Staphylococcus aureus* membranes (Immunoprecipitin; BRL, Neu-Isenburg, West Germany). The pellet was washed three times with phosphate-buffered saline and then boiled with sample buffer (10 minutes, 98°C) to elute the bound immunocomplexes.

band 3 bound selectively to a distinct population of epithelial cells scattered throughout the wall of the connective tubules and collecting ducts (Fig. 2b). No immunostain was seen in other parts of the nephron. Collecting ducts of the outer medulla contained the highest density of immunoreactive cells. In most of the tubular profiles, the band 3–containing cells were separated from each other by nonreactive epithelial cells. Occasionally small clusters of immunostained cells were detected. The stained cells differed from the nonreactive cells in having (i) a higher density of cytoplasm in phase-contrast microscopy and (ii) a higher density of mitochondria visualized by antibodies to mitochondrial aldehyde dehydrogenase. These features demonstrate that the antibodies to band 3 preferentially bind to the intercalated cells, a cell type found in connective tubules and collecting ducts of mammalian species including man (*6*).

In semithin sections (0.5 μm) of quick-frozen, freeze-dried, and plastic-embedded tissue, the band 3–like immunofluorescence was restricted to the basolateral cell membrane of the intercalated cells (Fig. 2b). The basal portion of the membrane was stained much more intensely than the lateral membrane, and in most cells infoldings of the basal plasma membrane were visible. The intensity in the immunofluorescence of the basal cell membrane of the intercalated cells was similar to that seen along the membrane of erythrocytes in the same tissue sections. We thus conclude that the concentration of band 3 in the basal plasma membrane of intercalated cells is comparable to that in the erythrocyte membrane.

In the chicken kidney, which is developmentally and structurally different from the mammalian kidney, band 3–like immunostain was found in certain cells of the proximal tubule where the stain appeared randomly distributed along the entire membrane of the tubular cells. Moreover, the intensity of stain was much lower than in the erythrocyte membrane (*5*).

The collecting ducts of the mammalian kidney in which the band 3–containing cells showed their highest concentration are thought to play a vital role in the control of acid-base balance (*7*), and this function appears to be mediated by the intercalated cells. Several studies reported a substantial increase in the incidence (up to 200 percent) of intercalated cells in rats subjected to bicarbonate loading, hypokalemia-induced alkalosis, or respiratory acidosis (*8*). Histochemical studies indicated a high carbonic anhydrase

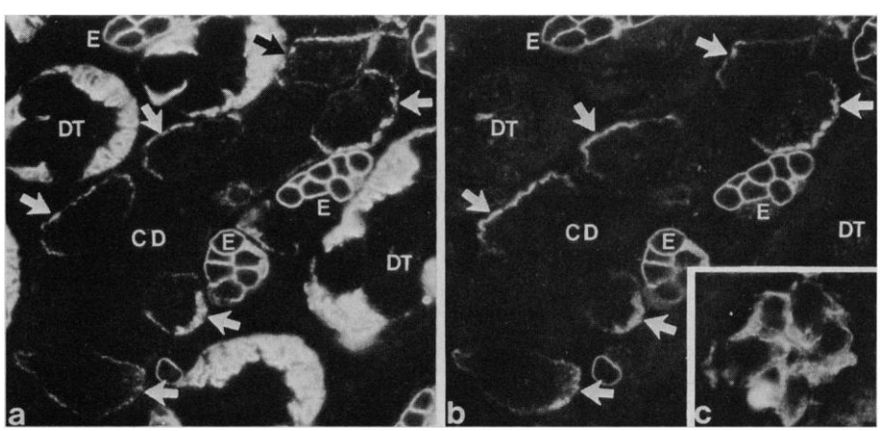

Fig. 2. Localization of ankyrin (a), band 3 (b), and nonerythroid spectrin (c) in tissue sections of the outer medulla of the rat kidney as determined by the indirect immunofluorescence technique (*12*). Tissue sections of the rat kidney were quick-frozen and processed for either cryostat sectioning (c) or freeze-drying and embedding in Epon (a and b) (*13*). Semithin sections (0.5 to 1 μm) of Epon-embedded tissue were etched with a sodium methoxide–toluene solution and were then incubated with affinity-purified antibodies to the band 3–binding, M_r ~90,000 fragment of human erythrocyte ankyrin (*12*) and photographed (a). The stain was then removed by treatment with 0.1 percent H_2SO_4 containing 0.05 percent $KMnO_4$ (10-second exposure). The sections were washed with phosphate-buffered saline and incubated with anti-rat band 3 (b). Immunostaining specific for band 3 (b) is confined to the basolateral plasma membrane (arrows) of the intercalated cells of the collecting ducts (CD) and the membrane of erythrocytes (E) in blood vessels. Ankyrin stain is found concentrated at the same sites (a) and, in addition, is also very prominent along the membranes of adjacent distal tubule cells (DT). Anti-brain spectrin (*14*) stains the entire membrane of all cell types of the collecting duct (c). Antibodies to the cytoplasmic domain of band 3 were prepared in rabbits as described (*4*). Whole band 3 of human and rat erythrocytes and the membrane-spanning domain of human band 3 were isolated as described (*14, 15*). The immunogen of rat band 3 was obtained as a result of further purification by SDS-PAGE (10 percent). Antibodies were prepared in rabbits and were affinity-purified (*16*).

activity in the intercalated cells (*9*), another sign that these cells play a potential role in the reabsorption of bicarbonate and in the secretion of hydrogen ions (under normal conditions). It is thus reasonable to propose that anion transport in intercalated cells is catalyzed by an isoform of band 3, which is concentrated along the basolateral plasma membrane of these cells. The observation that kidney band 3 reacts with antibodies to both the membrane-spanning and cytoplasmic domain of rat and human band 3 indicates a high degree of relationship between erythroid and kidney band 3.

Ankyrin and spectrin, the membrane-skeletal proteins associated with band 3 in erythrocytes, are also present in kidney epithelial cells. Antibodies to ankyrin reacted strongly with the basolateral plasma membrane of different epithelial cell types in the nephron (most intensely at basal plasmalemmal infoldings of the pars recta of distal tubules). Importantly, in the collecting ducts strong immunostaining specific for both the band 3–binding (M_r ~90,000) and spectrin-binding (M_r ~72,000) domain of human erythrocyte ankyrin was precisely colocalized with the membrane sites of band 3 in the intercalated cells (Fig. 2a). The strongest immunostain was found at the basal rather than at the lateral cell membrane. The luminal membrane of intercalated cells and the cell membrane of the principal collecting duct cells were only faintly labeled. Western blot analysis of membranes from perfused kidneys revealed isoforms of ankyrin of M_r 190,000 and 210,000. Ankyrin analogs of identical molecular weight have been described from brain (*10*). Antibodies to brain spectrin stained all tubular and ductal cells along the whole membrane (Fig. 2c), a pattern that has also been demonstrated for spectrin analogs in other types of epithelial cells (*11*).

The codistribution of kidney band 3 with ankyrin and spectrin suggests a lateral association between band 3 and the membrane cytoskeleton. The association between integral membrane proteins and the cytoskeleton may serve as a general molecular way of restricting the lateral mobility of membrane proteins and for localizing them in a nonrandom fashion at specialized regions of the cell surface. The mechanisms responsible for concentrating ankyrin and band 3 at the basolateral membrane remain to be determined. Band 3, once inserted, would be restricted in its lateral diffusion by linkage to ankyrin and spectrin. It will be important in future work to purify the kidney anion transport protein and to determine if it has anion transport characteristics similar to those of erythrocyte band 3 and if it has an ankyrin binding site.

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Tissue Plasminogen Activator Reduces Neurological Damage After Cerebral Embolism

Abstract. *Intravenous administration of tissue plasminogen activator immediately after the injection of numerous small blood clots into the carotid circulation in rabbit embolic stroke model animals caused a significant reduction in neurological damage. In vitro studies indicate that tissue plasminogen activator produced substantial lysis of clots at concentrations comparable to those expected in vivo, suggesting that this may be the mechanism of action of this drug. Drug-induced hemorrhages were not demonstrable. Tissue plasminogen activator may be of value for the immediate treatment of embolic stroke.*

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Stroke is currently the third most frequent cause of death in the United States. The three main causes of stroke are thrombosis, embolism, and hemorrhage. At present, there is no generally accepted specific therapy for thrombosis. Anticoagulation with heparin is accepted as effective in prophylaxis of cerebral embolism of cardiac origin and possibly for stroke in evolution (progressive increases in neurological deficits during the first 24 to 72 hours after the onset of ischemic strokes) (*1*). Even in these most treatable strokes, heparinization merely prevents subsequent infarction and does nothing to reverse damage produced by the initial event. Both thrombosis and embolism are potentially treatable by thrombolytic therapy. However, thrombolytic therapy with streptokinase or urokinase has been unsuccessful because intravenous administration has caused severe hemorrhagic side effects (*2*). Moreover, local injection of these drugs into the appropriate occluded artery requires catheterization, and such a procedure is so time-consuming that irreversible tissue damage may occur long before effective thrombolysis can be achieved. Consequently, it is clear that there is need for more effective methods of stroke therapy.

Tissue plasminogen activator (tPA) appears to have substantial potential for treatment of thromboembolic strokes. Studies of its efficacy in the treatment of coronary artery thrombosis (*3*) indicate that tPA may be of substantial benefit in treatment of the most common types of strokes, particularly since tPA can be given intravenously and appears to cause minimal risk of peripheral hemorrhagic complications. (*4*).

One of the main impediments to estab-