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Angiotensin I converting enzyme in human intestine and kidney

Ultrastructural immunohistochemical localization

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Summary. The localization of immunoreactive angiotensin I-converting enzyme (ACE) has been investigated at the optical and ultrastructural level with anti-human ACE antibodies in the human kidney and small intestine. In both tissues ACE was found in blood vessels and in extravascular situation in the absorptive epithelial cells of intestinal mucosa and renal proximal tubules. Ultrastructural immunohistochemistry showed that in intestinal and renal proximal tubular cells ACE was prominent in microvilli and brush borders. In the kidney ACE was also present on the basolateral part of the plasmalemmal membrane, where it may contribute to the regulation of angiotensin II-dependant absorption processes. Intracellular positivities were also observed inside the renal vascular endothelial and proximal tubular cell in endoplasmic reticulum and nuclear envelope reflecting the synthesis and the cellular processing of ACE. The intestinal microvascular endothelium was strongly labeled suggesting that the mesenteric circulation is an important site for the production of angiotensin II. Vascular endothelial ACE was also detected in the peritubular but not glomerular capillaries of the kidney.

Introduction

The angiotensin I-converting enzyme or kininase II (E.C. 3.4.15.1.) (ACE), although it was first discovered in the blood (Skeggs et al. 1956), is present as a cellular enzyme in several vascular and extravascular localizations. Immunomorphological studies performed with anti-ACE antibodies have indeed shown that ACE is a membrane bound ectoenzyme largely distributed on the endothelial surface of the pulmonary vessels (Caldwell et al. 1976; Ryan et al. 1976). Together with the soluble ACE, the pulmonary enzyme plays an important role in the regulation of blood pressure, because it activates angiotensin I into the vasopressor peptide angiotensin II (Ng and Vane 1967; Skeggs et al. 1956). It also inactivates the vasodepressor bradykinin (Erdös 1975, 1979). Besides its vascular localization, ACE is found in abundance in several tissues, where it is bound to the cell membrane, usually in specialized cellular structures such as brush borders of renal, intestinal and choroid epithelial cells (Defendini et al. 1983; Hall et al. 1976; Ward et al. 1980; Ward and Sheridan 1983). Because of its potential importance as a model for the study of membrane bound enzymes and because limited informations were available in man on the localization at the ultrastructural level of immunoreactive ACE in epithelial and endothelial cells, we used antibodies to human ACE to describe the ultrastructural aspect of ACE in small intestine and kidney. We report the presence of ACE in intestinal microvessels and in peritubular but not in glomerular capillaries of the kidney.

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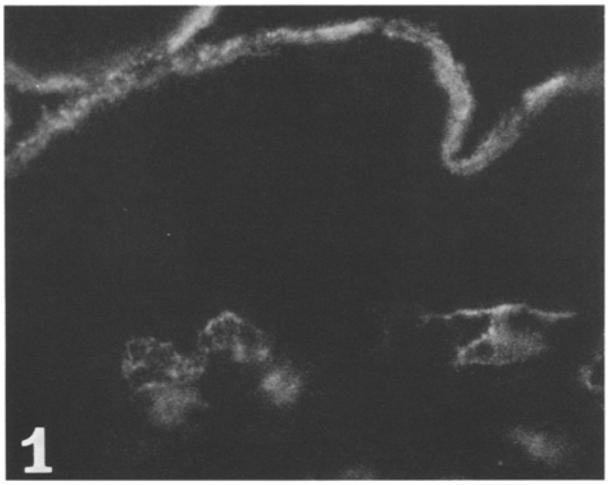
Material and methods

Antibodies against human ACE

Two different rabbit antisera have been used in this study. They were raised against human kidney ACE solubilized from renal microsomes and then purified to apparent homogeneity. Antiserum R19 was produced against ACE isolated by using a reverse immunoadsorption procedure (Weare et al. 1982). It has been previously described and was used for the development of a direct radioimmunoassay and for the immunological characterization of ACE in blood, amniotic fluid, lung and kindey extracts (Alhenc-Gelas et al. 1983; Yasui et al. 1984). The other antiserum, called Y3, was raised against ACE purified by a procedure including affinity chromatography on an inhibitor-sepharose column. The ACE inhibitor, MK 521, was kindly supplied by Dr. E.H. Cordes (Merk-Sharp and Dohme Laboratories, Rahway, NJ, USA). The purified enzyme had a specific activity of 85 nmole Hippuril-Histidil-Leucine hydrolyzed/mn/mg protein. It appeared homogeneous and migrated as a single band in SDS-polyacrylamide gel electrophoresis. Antiserum Y3, like R19, gave on immunodiffusion a single precipitation line when tested against crude kidney extracts. It equally recognized ACE from lung, blood and kidney.

Tissue specimens

Small intestine. Two specimens of human proximal jejunum were obtained during surgery from patients operated for gastric ulcers. The tissue samples were part of the intestine fragment normally removed during the gastro-intestinal anastomosis. They appeared histologically normal. One part of each specimen was quickly frozen in liquid nitrogen for immunofluorescence. The other part was immediately fixed for 24 h by immersion in 4% paraformaldehyde in phosphate buffer saline (PBS) 0.1 *M* (pH 7.4) at 4° C, rinsed for 48 h in PBS, and then processed for immunocytochemistry. Saponin (Sigma, St. Louis, USA) was added in the fixative and



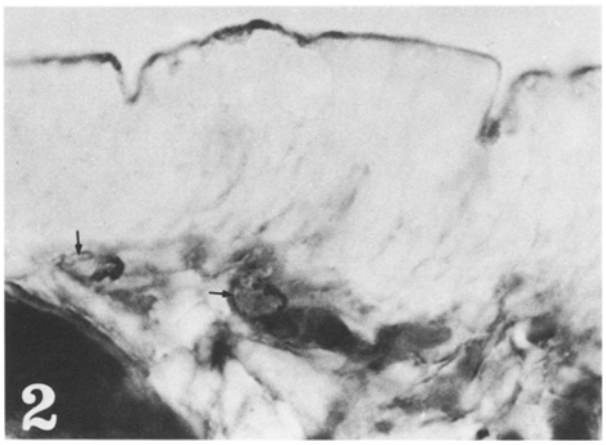


Fig. 1. Human small intestine: continuous staining at the apex of intestinal epithelial cells. Vascular labeling is observed in the lamina propria. Frozen section. Immunofluorescence using avidine-biotin technique with Y3 antibody. (× 670)

Fig. 2. Human small intestine: labeling of intestinal epithelial and vascular endothelial cells. Semi-thin section. PAP technique with Y3 antibody. (× 570)

washing solutions at a concentration of 0.05 g per 50 ml for the processing of the samples destined to ultrastructural immunocytochemistry (Bourel et al. 1981).

Kidney. Human renal tissue was obtained from the normal part of four kidneys removed for renal cancer. One part of the specimens was quickly frozen in liquid nitrogen for immunofluorescence. The other part was used for ultrastructural immunocytochemistry and fixed with 4% paraformaldehyde in 0.1 *M* cacodylate buffer (pH 7.4) as follows: in two kidneys the renal arteries were catheterized and perfused during 10 min with the fixative solution. The renal tissue was then immersed for 24 h in the same fixative; the two other kidneys were directly fixed by immersion for 24 h. Specimens were rinsed during 48 h in cacodylate buffer.

Immunofluorescence procedures

For indirect immunofluorescence, frozen sections of unfixed small intestine and kidney were incubated for 30 min at room temperature either with R19 antibody at a dilution of 1:20 in PBS or with Y3 antibody at a dilution of 1:40 to 1:100, then processed as previously described (Camilleri et al. 1984). For the avidin-biotin immunofluorescence procedure, a technique derived from that of Guesdon et al. (1979) was used. The frozen sections were incubated for 30 min at room temperature with R19 antibody at a dilution of 1:40 or with Y3 antibody at a dilution of 1:200 to 1:800, then rinsed twice in PBS. The sections were then incubated for 30 min with biotin-labeled goat anti-rabbit antibody at a dilution of 1:50 in PBS and rinsed twice. Finally the sections were incubated for 30 min, with fluorescein-conjugated avidin at a dilution of 1:100, then rinsed twice in PBS. Biotin-labeled antibody and fluorescein-conjugated avidin were purchased from Vector Laboratories (Burlingame, USA). Glycerol mounted sections were observed with a Leitz Ortoplan Ortomat UV microscope.

Ultrastructural immunocytochemistry

Small intestine. Paraformaldehyde fixed surgical specimens were soaked overnight at 4° C in 10% dimethyl-sulphoxide in PBS, and frozen in liquid nitrogen. Ten micron thick frozen slices were thawed in cold PBS and processed free-floating in the successive media at room temperature according to Sternberger (1979). The slices were first incubated for 30 min with either R19 or Y3 antibodies at a dilution of 1:200 in PBS and rinsed twice in PBS. The slices were then incubated for 10 min with normal swine serum at a dilution of 1:5 and rinsed twice in PBS. They were then incubated for 30 min with swine anti-rabbit serum (Cappel Laboratories, Westchester, PA, USA) at a dilution of 1:20 and washed twice in PBS. After incubation in rabbit PAP (Cappel Laboratories, Westchester, PA, USA) at a dilution of 1:100, and 3 rinses, the antigen-antibody reaction was fixed in 2.5% glutaraldehyde in PBS for 10 min. The slices were washed and peroxidase activity was revealed in two steps, first by incubation for 20 min in 3-3′ diaminobenzidine tetrahydrochloride (DAB), (Sigma, St. Louis, USA) at a concentration of 50 mg/100 ml in *Tris*-HCl buffer 0.2 *M* (pH 7.6), and then by incubation for 5 min in DAB at the same concentration with 200 microliters of H_2O_2 at 110 volumes (Graham and Karnovsky 1966). After fixation in 2% osmium tetroxide, the slices were dehydrated and flat embedded in Epon 812. Semi-thin sections were stained with toluidine blue. Thin sections were examined unstained with a Jeol 100 CX II electron microscope at 80 kV.

Kidney. 30 micron thick slices of fixed kidney were obtained with a tissue sectionner (Smith and Farquhar). The slices were incubated with either R19 or Y3 antibodies at dilution of 1:20, for 5 hours under continuous stirring, at room temperature. They were then washed in cacodylate buffer overnight, followed by incubation with a peroxidase labeled F(ab′)2 sheep anti-rabbit IgG antibody (Institut Pasteur Production, Paris). The sections were then rinsed for 36 h in cacodylate buffer and the peroxidase activity was revealed by incubation with 10 mg DAB (Sigma, St. Louis, USA) in 10 ml *Tris*-HCl buffer 0.2 *M* (pH 7.4) containing 0.2 ml of a 1/30 dilution of H_2O_2 (Bariety et al. 1978). After fixation in 2% osmium tetroxide, the slices were dehydrated and flat embedded in Epon 812. Thin sections were examined unstained with a Zeiss EM10 electron microscope at 60 kV.

Controls

Three control studies were performed in order to assess the specificity of the immunostaining observed in the small intestine and the kidney: *1)* The specific antisera were omitted and replaced by normal rabbit serum. *2)* Ten microliters of Y3 were incubated with 8.6 micrograms of pure human ACE in 200 microliters of 5 m*M* phosphate buffer for 48 h at 4° C in order to saturate the anti-ACE antibodies present in the serum. The saturated serum as well as a control serum treated in parallel without ACE were then tested for immunostaining. *3)* DAB alone was used to localize endogenous peroxidase activity.

Results

Small intestine

Immunofluorescence and optical immunohistochemistry. The antibodies labeled the apical side of intestinal epithelial cells

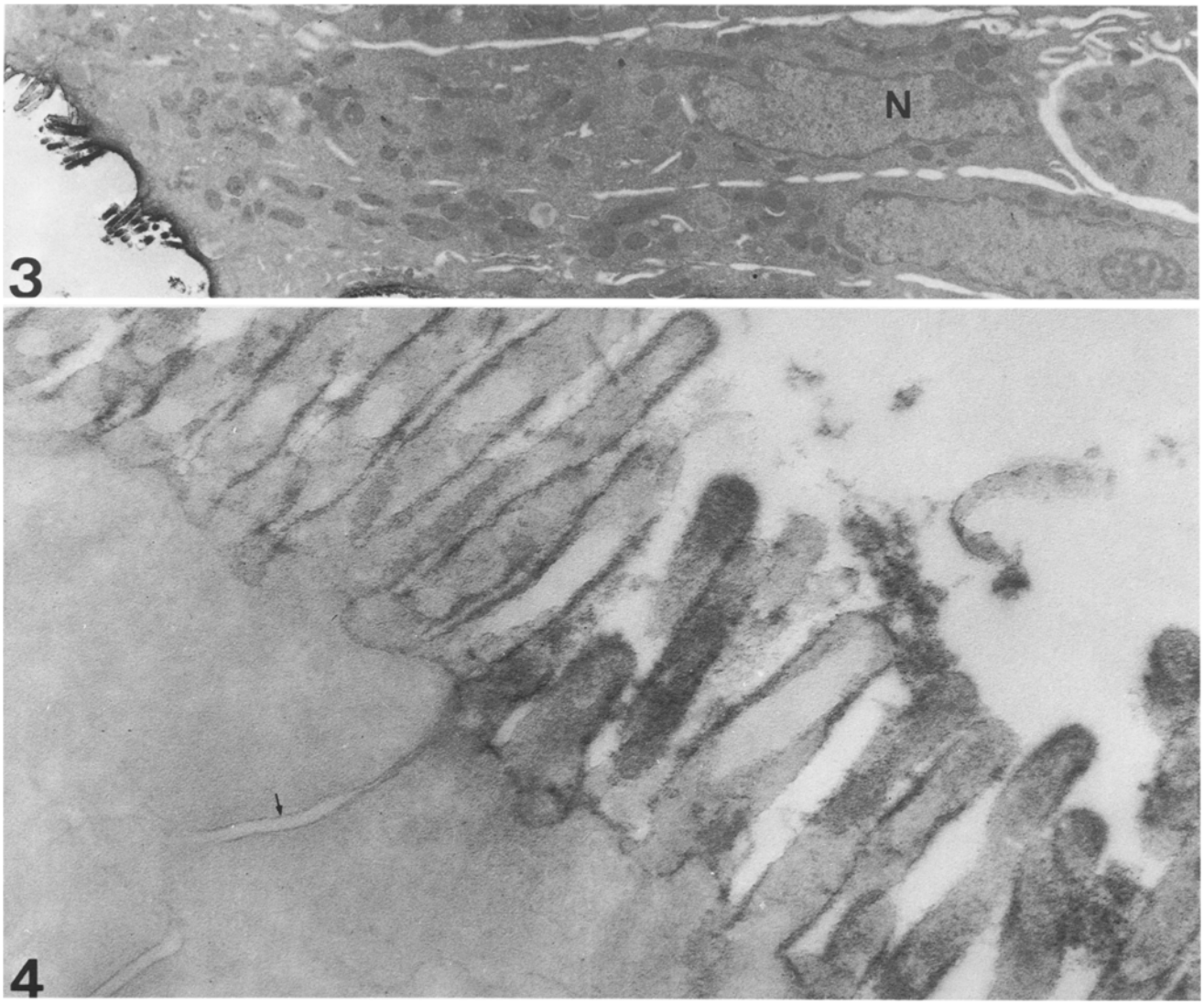


Fig. 3. Human small intestine: electron micrograph showing the labeling of microvilli at the apex of absorptive intestinal cells. No reaction product is observed in the cytoplasm and on the basolateral plasmalemmal membrane. *N*: nucleus. Immuno-electron microscopy using PAP technique with R19 antibody. No counterstaining. (× 6,650)

Fig. 4. Human small intestine: high power view showing reaction product along microvilli of epithelial cells. The lateral plasmalemmal membrane (*arrow*) is not stained. PAP technique with R19 antibody. No counterstaining. (× 66,700)

yielding a continuous strong staining along the luminal interface. No labeling was observed in the cytoplasm. Endothelial labeling was observed in numerous blood vessels in the subepithelial part of the lamina propria. The vessels in the submucosa and in the lower part of the lamina propria were only occasionally stained (Figs. 1 and 2).

Ultrastructural immunocytochemistry. In the intestinal epithelium, the antibodies labeled all absorptive intestinal cells along the microvilli (Figs. 3 and 4). The reaction product was only located to the apical plasmalemmal membrane whereas the baso-lateral sides of the plasmalemmal membrane remained unstained (Figs. 3 and 4). Positivities were never observed in other cell types including goblet and endocrine cells. In the vessels, mainly capillaries and small venules, the endothelium was strongly labeled. The labeling was restricted to the luminal side of the endothelial plasmalemmal membrane (Fig. 5), and to some plasmalemmal vesicles (Fig. 6). The abluminal side of the endothelial plasmalemmal membrane was never labeled.

Kidney

Immunofluorescence. The antibodies labeled only the proximal tubular epithelial cells (Fig. 7). The labeling was restricted to the apical side of the cell. The other structures of the nephron, including glomeruli, distal tubules and collecting ducts remained negative.

Ultrastructural immunocytochemistry. The antibodies strongly labeled the plasmalemmal membrane of the brush border in the proximal tubule (Figs. 8 and 9A). A weaker labeling was observed on the baso-lateral part of the plasmalemmal membrane (Fig. 10). The apical invagination and

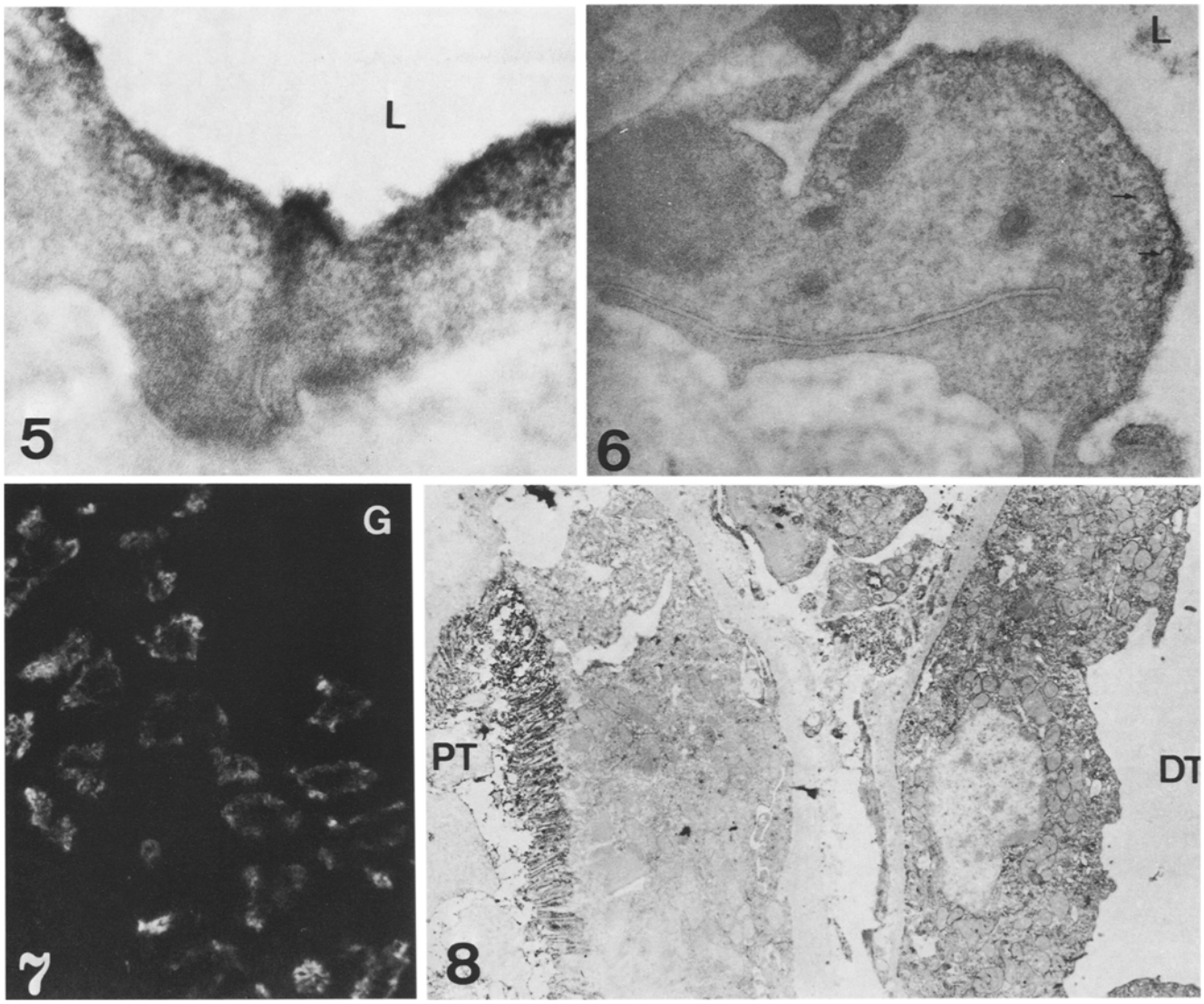


Fig. 5. Human small intestine: endothelial cell of capillary in the lamina propria: labeling of the luminal side of the plasmalemmal membrane. *L*: Lumen. PAP technique with Y3 antibody. No counterstaining. (× 66,000)

Fig. 6. Human small intestine: endothelial cell of capillary in the lamina propria: labeling of plasmalemmal vesicles (*arrows*). *L*: lumen. PAP technique with Y3 antibody. No counterstaining (× 34,200)

Fig. 7. Human kidney: labeling of the apical part of the proximal tubular epithelial cells. The glomerulus (*G*) is negative. Indirect immunofluorescence with R19 antibody. (× 670)

Fig. 8. Human kidney: low power view showing side by side negative distal tubular cells and positive brush borders of proximal tubular cells. Proximal tubule (*PT*). Distal tubule (*DT*). Indirect immunoperoxidase technique with Y3. No counterstaining. (× 2,300)

the endocytotic vesicles of the proximal tubular cells also contained a dense reaction product (Fig. 9A). In two kidneys, scattered intracellular membranes were also labeled in the proximal tubule cells. These membranes were probably part of the endoplasmic reticulum system and segment of the nuclear envelope (Fig. 11). In addition to the epithelial cells in the proximal tubule, the endothelial cells in the peritubular capillaries were also labeled. The labeling was prominent on the luminal side of the plasmalemmal membrane but was also visible in the plasmalemmal vesicles and in endocytotic vacuoles (Fig. 12). Occasionally peritubular vascular endothelial cells exhibited labeling of the nuclear envelope (Fig. 13). Glomerular capillaries were never labeled.

Controls

When specific antisera were omitted, no staining was observed in renal and intestinal endothelial or epithelial cells. DAB alone yielded a dense reaction product in red blood cells and in some macrophages in the intestinal lamina propria.

When saturated Y3 antiserum was tested by immunofluorescence in small intestine, no vascular positivity was observed and only a very weak and discontinuous labeling remained in absorptive cells. In the kidney either immunofluorescence or ultrastructural immunohistochemical techniques failed to demonstrate any positivity with this saturated antiserum (Fig. 9B).

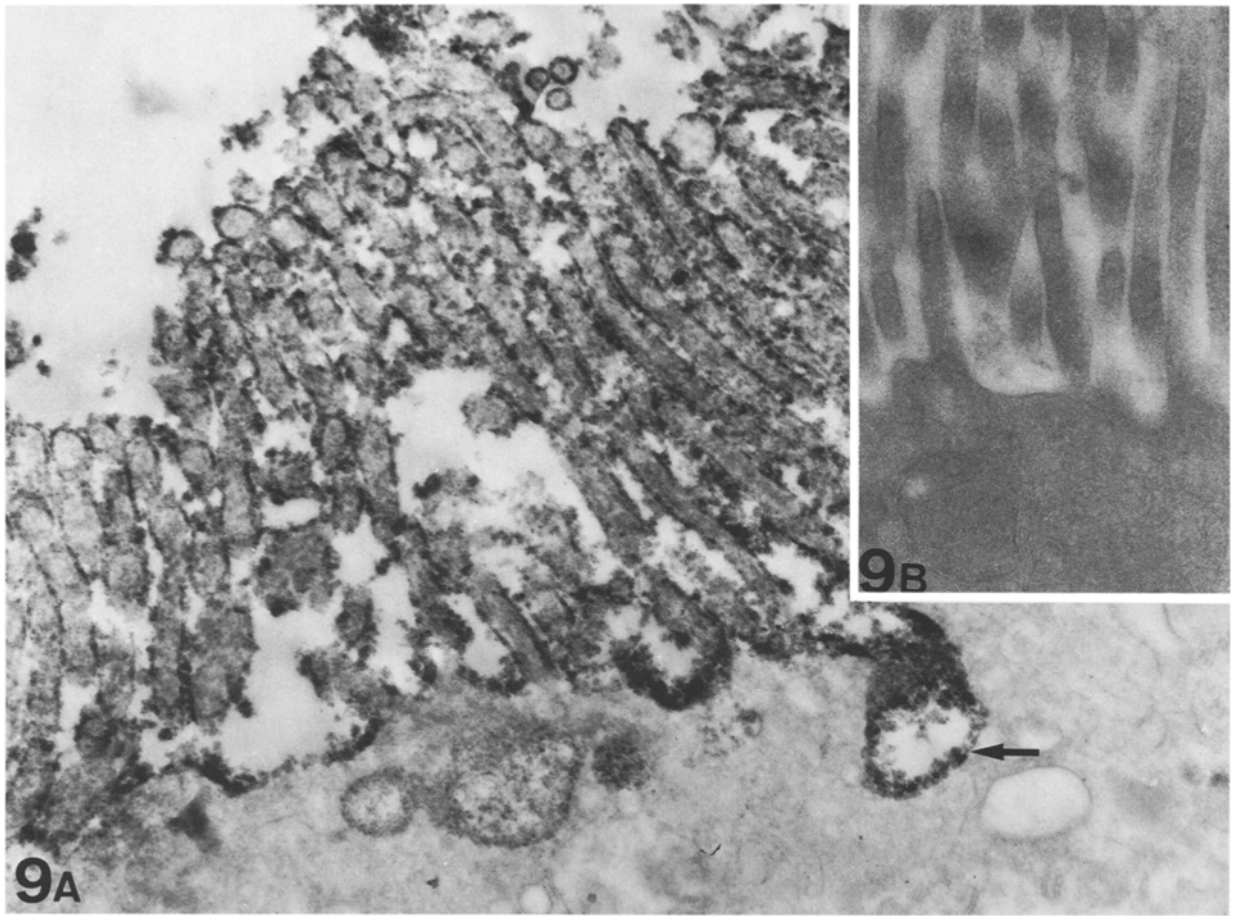


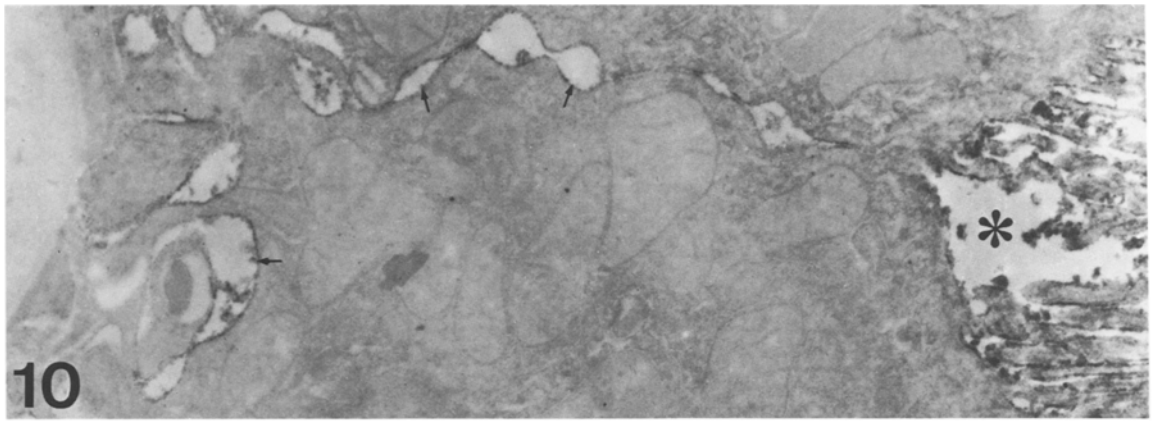
Fig. 9A. Human kidney: apex of a proximal tubular epithelial cell: thick labeling of the brush border and of the membrane of an endocytotic vesicle (*arrow*). Indirect immunoperoxidase technique with Y3 antibody. No counter staining (×25,000). **B** Human kidney: brush borders of proximal tubular cells remains negative. Indirect immunoperoxidase technique with saturated Y3. No counterstaining. (×35,000)

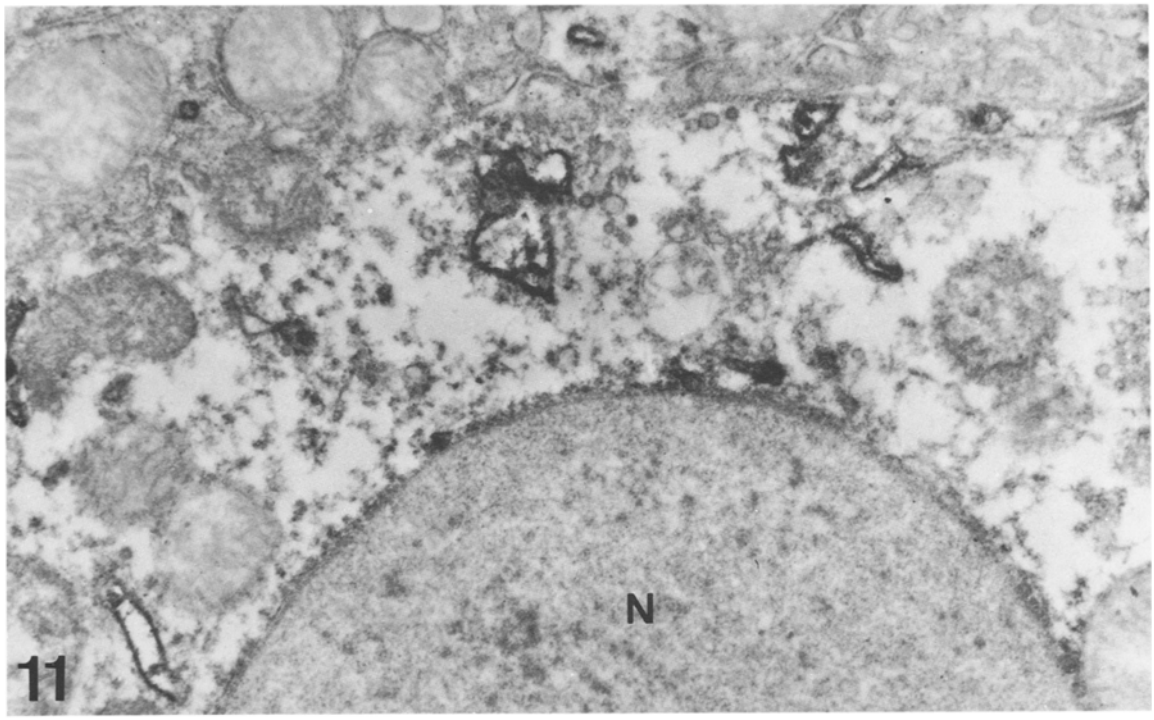
Discussion

We have localized ACE in human kidney and small intestine by immunohistochemistry and found that the enzyme was present in both tissues at two different sites. First ACE was on the plasmalemmal membrane of the absorptive epithelial cells in the intestine and proximal tubule, at sites of large surface differentiations of the membrane facing circulating fluids such as microvilli and brush borders. Second ACE was in the vascular endothelial cells on the luminal part of the plasmalemmal membrane and in plasmalemmal vesicles. The positivities for ACE were seen in the blood vessels of the lamina propria in the intestine and in the peritubular capillaries in the kidney.

Concerning the epithelial localization of ACE in human kidney, the present results are essentially in agreement with previous optical descriptions (Takada et al. 1981; Defendini et al. 1983) and with the ultrastructural study of Takada et al. (1982). In the renal proximal tubule ACE is located mainly on the brush border facing the tubule lumen, but it is also detectable on the basolateral plasmalemmal membrane. We also found positivities in several intracellular structures including endocytotic vesicles and parts of the endoplasmic reticulum and the nuclear envelope. These last localizations provide morphological evidence for the synthesis and intracellular processing of ACE in renal proximal tubular cells. Angiotensin II receptors have been identified in renal brush border and basolateral membranes (Brown and Douglas 1982, 1983). Angiotensin II is able to stimulate sodium transfert in the proximal tubule (Schuster et al. 1984). The presence of ACE as an ectoenzyme on the brush border and basolateral membrane, as well as in the peritubular capillaries, allow conversion of the angiotensin I produced by the high levels of renin present in the kidney circulation. ACE is as dipeptidylcarboxypeptidase also able to hydrolyze in vitro a wide range of peptide substrate (Erdös 1979). It can also participate together with other peptidases of the brush border in the terminal digestion and absorption of proteins and peptides (Guder and Ross 1984). ACE is able, for example, to inactivate bradykinin in the tubule lumen (Carone et al. 1982). In enterocytes, ACE was only located to the apical microvilli. No positivities were observed on the basolateral part of the plasmalemmal membrane. This may be related to differences in the structure and enzymatic content of the basolateral part of the membrane of enterocytes and tubular cells. In the gut angiotensin II stimulates water and electrolyte transport (Levens et al. 1980). It is interesting in this respect to note that ACE is present in abundance in capillaries of the subepithelial part of the mucosa. The function of brush border ACE in the gut may be related to peptide digestion as discussed above.

We found that ACE is largely distributed on the endothelium of the microvessels in the small intestine. Giv-





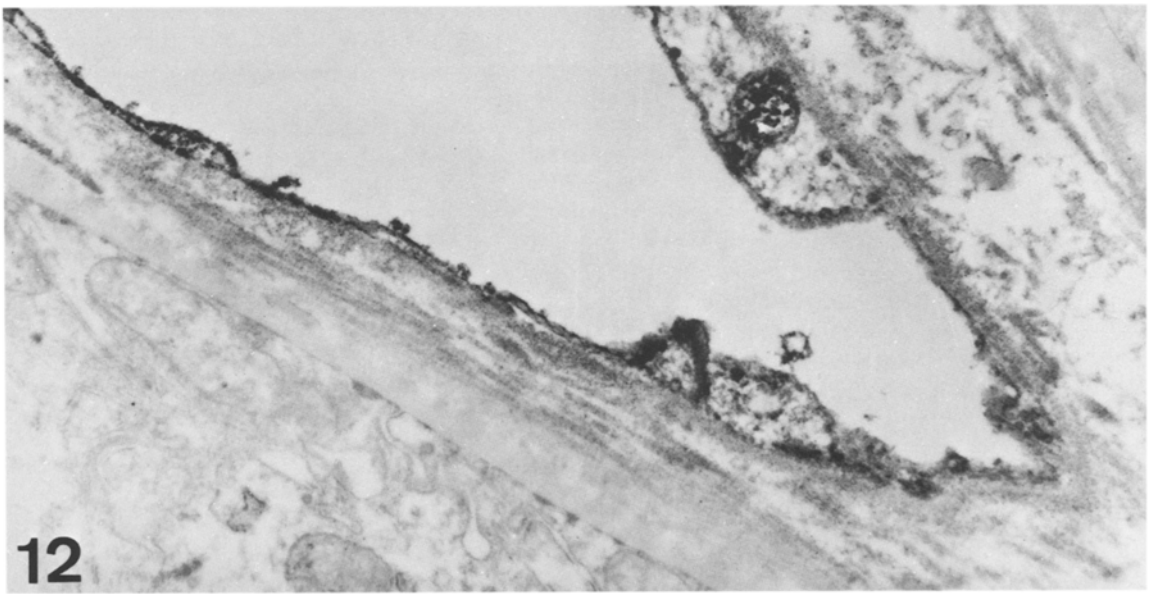


Fig. 10. Human kidney: proximal tubular epithelial cells: thin labeling of the baso-lateral plasmalemmal membrane (*arrows*). Tubular lumen (*asterisk*) and brush border are situated on the right side of the picture. Indirect immunoperoxidase technique with Y3 antibody. No counterstaining. (× 15,000)

Fig. 11. Human kidney: proximal tubular epithelial cell: labeling of intracytoplasmic vesicular membranes. *N*: nucleus. Indirect immunoperoxidase technique with R19 antibody. No counterstaining. (× 32,500)

Fig. 12. Human kidney: peritubular capillary: labeling of the luminal side of the plasmalemmal membrane and of an intracytoplasmic vacuole in an endothelial cell. Indirect immunoperoxidase technique with R19 antibody. No counterstaining (× 25,000)

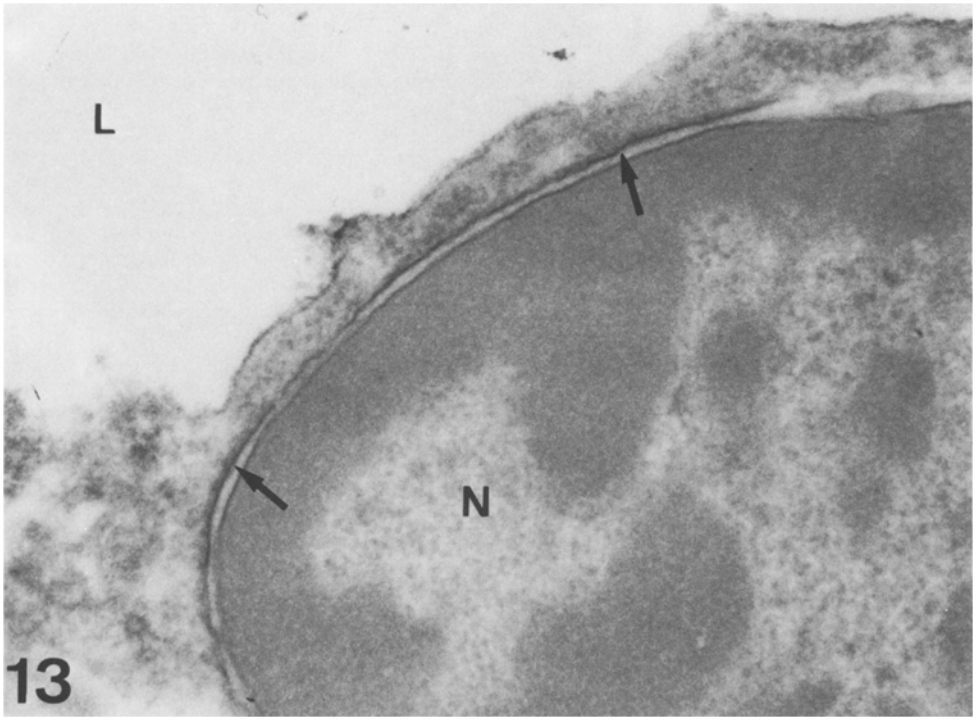


Fig. 13. Human kidney: strong labeling of the nuclear envelope (*arrow*) of an endothelial cell of a peritubular capillary. Note discontinuous labeling of the luminal side of the plasmalemmal membrane. Lumen (*L*). Nucleus (*N*). Indirect immunoperoxidase technique with Y3. No counterstaining (× 57,000)

en the large surface of the capillary network in the gut and the presence of ACE in mesenteric macrovessels (Ward and Sheridan 1982), the intestinal vascular bed appears, together with the pulmonary circulation, as a major site of vascular ACE. This is in agreement with results of earlier pharmacological experiments (Di Salvo and Montefusco 1971) suggesting that the mesenteric circulation has a high capacity to convert angiotensin I into angiotensin II. ACE is also found in renal vessels but only in the interstitial capillaries. Besides its main localization on the luminal side of the plasmalemmal membrane, ACE was also present in intracellular position on the perinuclear membrane, supporting endothelial synthesis of ACE. Contrary to the rabbit and rat kidney where immunoreactive ACE was found in the glomerular tuft (Caldwell et al. 1976; Taugner and Ganten 1982), no ACE could be detected in human glomeruli by us and others (Takada et al. 1981, 1982). This indirectly suggests that in the human species the circulating ACE rather than the endothelial enzyme plays an essential role for intrarenal conversion of angiotensin I into angiotensin II at the glomerular level. The presence of ACE in the peritubular capillaries indicates that angiotensin II can be locally formed in the peritubular vessels where it participates in the regulation of capillary and interstitial pressures at the post-glomerular level (Jensen and Steven 1977; Steven and Thorpe 1977) and in electrolyte transport as discussed above.

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