tracers, it would be more effective to use them together. The cost of collection and analysis will be the critical items, and the amount of information and confidence in the data obtained can be greatly increased by the simultaneous use of methane-20 and methane-21, released at slightly different times or locations, at a relatively small increment in cost. At prices currently quoted for the sale to the public of ^{13}CO, the starting material for the synthesis of methane-21, the cost of the separated ^{13}C isotope in this experiment (about 50 g) was less than $5000; similar amounts are esimated for the cost of a kilogram of $^{12}CD_4$ if produced on an appropriate scale, since the cost of the special isotope in this case is relatively small and most of the expense would be for synthesis.

The release of 1 kg of methane-21 would, if uniformly mixed in the atmosphere, raise the mass-21/mass-16 ratio of atmospheric methane to 2×10^{-13}. Thus, the release of a few kilograms of methane-21 per year in tracer experiments would not add a measurable increment to the present detectability limit which is determined by the noise level in the mass spectrometer. Instrumental improvements can be introduced which should markedly improve the detection sensitivity, possibly by more than an order of magnitude, in which case tracer experiments on a global scale involving nonradioactive substances should become feasible. The relatively short life of methane in the atmosphere would prevent long-term isotopic contamination of atmospheric methane as a result of such tracer experiments.

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Angiotensin-Converting Enzyme: Vascular Endothelial Localization

Abstract. *Fluorescein-labeled antibody to rabbit pulmonary angiotensin-converting enzyme localized in the vascular endothelium of rabbit lung, liver, adrenal cortex, pancreas, kidney, and spleen. Epithelial cells of the renal proximal tubules were the only parenchymal cells among the organs studied that demonstrated immunoreactivity.*

Angiotensin-converting enzyme catalyzes the release of histidylleucine from the COOH-terminus of angiotensin I to yield a pressor octapeptide, angiotensin II (*1*), the vasoactive agent of the renin-angiotensin system (*2*). The same enzyme inactivates bradykinin, a vasodepressor nonapeptide, by catalyzing the cleavage of its

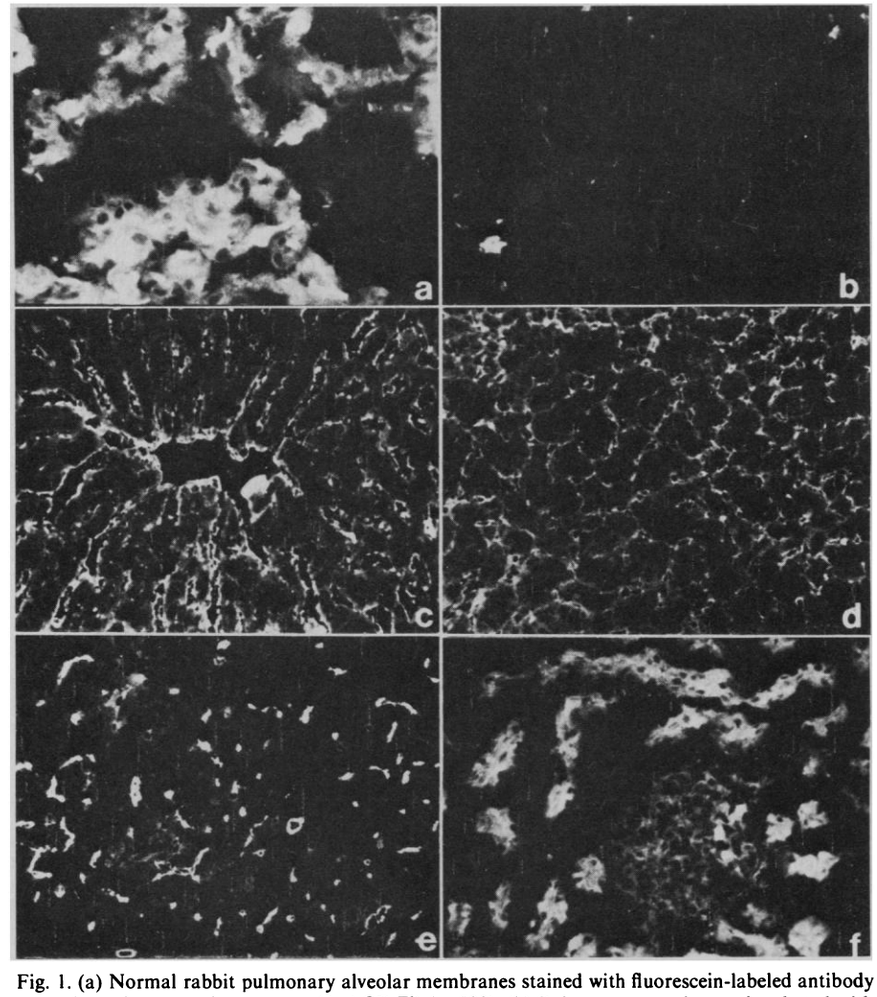

Fig. 1. (a) Normal rabbit pulmonary alveolar membranes stained with fluorescein-labeled antibody to angiotensin-converting enzyme (A-ACE-Fl) (× 500). (b) Pulmonary membranes incubated with fluorescein-labeled globulin obtained before immunization (× 500). Fluorescence appears only in occasional polymorphonuclear leukocytes and elastic fibers. (c) Vasculature of a normal liver lobule stained with A-ACE-Fl (× 100). Hepatocytes do not stain. (d) Vessel network of normal adrenal cortex stained with A-ACE-Fl (× 200). Parenchymal cells do not stain. (e) Vasculature of normal pancreas stained with A-ACE-Fl (× 100). Parenchymal cells do not stain. (f) Normal kidney showing staining both in the glomerular tuft (slight) and in the proximal tubules (prominent) (× 250).

COOH-terminal and penultimate dipeptides (*3*). Conversion of angiotensin in vivo (*4*) and converting activity in vitro (*5*) are known to occur in several organs. We have, therefore, investigated the distribution of this enzyme and found it localized in the vascular endothelium of all organs examined, including lung, liver, adrenal cortex, pancreas, kidney, and spleen.

Antibody was prepared in a goat by injecting emulsified antigen intradermally and intramuscularly at multiple sites. The emulsion consisted of 150 μg of pure rabbit lung enzyme in 1 ml of 10 m*M* tris-HCl (*p*H 7.4) and 0.15*M* NaCl homogenized with 1 ml of complete Freund's adjuvant. The purity of the enzyme was established by gel electrophoresis and equilibrium sedimentation (*6*). Five weeks after immunization test serum showed a single precipitin band by immunodiffusion against both the pure enzyme and a crude extract of rabbit lung. The immune serum possessed anticatalytic activity for the converting enzyme in vitro (*6*). The globulin fractions (12 mg of protein per milliliter) of both immune serum and serum taken before immunization from the same animal were conjugated with fluorescein isothiocyanate (*7*).

Fresh tissues taken from normal New Zealand white rabbits were snap frozen and stored at −70°C. Cryostat sections of tissue 4 μm thick, briefly fixed in acetone (30 seconds) and washed in 0.01*M* phosphate-buffered saline (PBS) (*p*H 7.2), were incubated with fluorescein-labeled goat globulin for 30 minutes at room temperature, washed again in PBS, and mounted in buffered glycerine. The stained sections were examined with a Reichert Dourex ultraviolet light microscope, with the use of a BG 12 exciter and a GG 9 barrier filter. For photography, an additional OG 4 filter was used with Tri-X or Polaroid 107 film.

The labeled immune globulin was first tested at various dilutions on tissue sections; the optimal dilution was found to be 1 : 20. In tissues stained with labeled immune globulin, fluorescence was distributed in the vasculature of the lung, liver, adrenal cortex, pancreas, kidney (Fig. 1), and spleen (not shown). Staining was localized in the endothelial cells of capillaries as well as of larger arterial and venous channels. The only parenchymal cells that stained were the epithelial luminal cells of the renal proximal tubules (Fig. 1f). Staining was also seen in the delicate intertubular capillary network. The fluorescence in the glomerular tuft was less intense and more homogeneous than it appeared elsewhere. Renal tissues were examined further with peroxidase-labeled globulin of both immune serum and serum taken before immunization (*8*). The optimal dilution of the peroxidase conjugate was 1 : 32. Positive staining was again found with immune globulin in both glomeruli and proximal tubules. Further, the localization of staining in the endothelial cells of the glomerular tuft was demonstrated (Fig. 2).

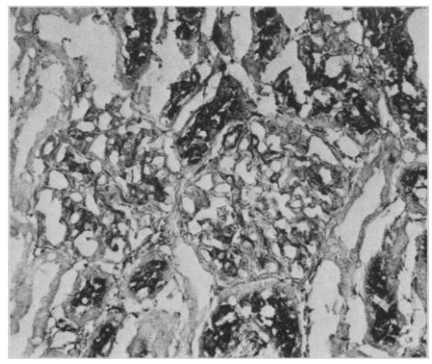

Fig. 2. Normal rabbit kidney stained with peroxidase conjugated antibody to angiotensin-converting enzyme. There is slight staining in the glomerular tuft and prominent staining in the proximal tubules (× 125).

The possibility that the immune labeled antibodies reacted nonspecifically was evaluated by performing a blocking experiment in which renal tissue was first incubated with unlabeled immune globulin, washed in PBS, and then incubated with the labeled immune globulin at a dilution of 1 : 20. No fluorescence was seen under these conditions until the unlabeled globulin was diluted to 1 : 60.

Our results indicate that angiotensin-converting enzyme is localized in vascular endothelial cells, and its presence there may provide a useful endothelial cell marker. Recent immunohistochemical evidence has indicated that pulmonary converting enzyme may be concentrated on the luminal surface of the endothelial cells (*9*). These observations suggest that converting enzyme in most organs is directly accessible to circulating blood and may, therefore, be subject to regulation by exogenous anticatalytic antibody. An immunologic agent that inhibits the generation of angiotensin II and the inactivation of bradykinin might be useful in the diagnosis and treatment of renin-dependent hypertension, as well as for regulation of other physiologic actions mediated by these peptides. The feasibility of this approach is supported by the work of others with bradykinin potentiating peptides, which inhibit angiotensin-converting enzyme (*10*), block development of renin-dependent hypertension in animal models (*11*), and lower blood pressure in renin-dependent human hypertension (*12*). Finally, the function of angiotensin-converting enzyme in the epithelium of renal proximal tubules is not known.

Note added in proof: Ward *et al.* (*13*) have found converting enzyme activity localized in the brush border membrane from proximal tubules of homogenized kidney, and Carone *et al.* (*14*) have found that bradykinin is degraded in the proximal, but not in the distal, tubule of the intact kidney. These studies suggest a role for converting enzyme in the proximal tubule and are consistent with the observations reported here on the localization of converting enzyme.

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