overwhelmingly dominates the sulfide mineralogy and primary sulfate minerals are absent. At 21°N, however, we see only the very top of the system, which may not be representative of the whole and may be geologically ephemeral.

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20. The assertion that the system is rock-dominated is based on the observation that the fluid contains no Mg (*8*). This means that each kilogram of seawater need react with only 60 g of rock (*24*). This is equivalent to a maximum water/rock ratio of about 16. A lower limit for the water/rock ratio can be obtained from heat transport considerations, where it is assumed that the total heat-flow anomaly is transported by circulating seawater. Taking 2 km as the average depth of seawater circulation, area of new crust as 2.94 km^2/year (*26*), and density as about 2.7 g/cm^3, a total of 15.87×10^{12} kg of basalt is produced annually. If the seawater is heated to an average temperature of only 350°C, then 1.36×10^{14} kg of seawater is required to dissipate the heat-flow anomaly (*24*) for a water/rock ratio of 8.5. For a maximum temperature of 400°C the water required is 1.13×10^{14} kg for a water/rock ratio of 7.12. These ratios represent a lower limit because the fluid need not react chemically with the full mass of rock from which it extracts heat. Thus, the effective water/rock ratio, defined as the ratio of the mass of water to the mass of rock altered by the water, is between 16:1 and 7:1. At temperature of 350°C and below, metals will not be effectively transported.
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Cytochrome P-450: Localization in Rabbit Lung

Abstract. *Cytochrome P-450-dependent monooxygenase systems, which metabolize endogenous as well as foreign compounds, are found in hepatic and several extrahepatic tissues of mammals, including humans. A form of cytochrome P-450 is localized in the nonciliated bronchiolar epithelial cells (Clara cells) of the small airways of rabbit lung. The apparent high concentration of the cytochrome in this pulmonary cell type compared to liver may be an important determinant in the susceptibility of the lung to a number of toxic chemicals that undergo metabolic activation.*

Cytochrome P-450-dependent monooxygenase systems provide a major pathway in a number of tissues for the oxidative metabolism of many chemicals present in the environment. These enzyme systems are bound to the endoplasmic reticulum and are composed of a flavoprotein (NADPH-cytochrome P-450 reductase; E.C. 1.6.2.4) and a family of hemoproteins called cytochrome P-450. Several forms of the cytochrome that have overlapping, but different, substrate specificities have been purified and characterized (*1, 2*). The monooxygenase systems generally produce metabolites that are less toxic than the parent compounds, which include a number of pesticides, drugs, and direct-acting carcinogens and mutagens. However, many procarcinogens, promutagens, and other toxic substances are activated by this metabolic process and are converted from relatively nontoxic compounds to extremely toxic metabolites.

Cytochrome P-450-dependent metabolism is required for the activation of several pulmonary toxins that produce different deleterious effects. For example, activation of 4-methyl-benzaldehyde results in the destruction of pulmonary cytochrome P-450 (*3*), activation of benzo[*a*]pyrene is involved in pulmonary carcinogenesis (*4*), and activation of 4-ipomeanol ("lung edema factor" isolated from mold-infected sweet potatoes) leads to pulmonary edema, congestion, and hemorrhage (*5*). Histopathologic and autoradiographic studies in rat and mouse (*6, 7*) have shown that the toxicity and covalent binding of 4-ipomeanol are both associated with the nonciliated bronchiolar epithelial cell (Clara cell) of the small airways of the lung. These findings suggest that the Clara cell is the site of activation of this toxin. In addition, the Clara cell contains large amounts of endoplasmic reticulum relative to other pulmonary cell types (*8*).

By using antibodies against a form of cytochrome P-450, we have demonstrated directly the presence of this enzyme in the nonciliated bronchiolar epithelium of rabbit lung. The cytochrome, $P\text{-}450_I$ (*9*), was purified to apparent homogeneity, as determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, by the method of Wolf *et al.* (*10*). Antibodies were elicited in goats by intermittent injections of the purified protein. The specificity of the antibody was demonstrated by the Ouchterlony technique of double immunodiffusion with purified enzymes and microsomal preparations (*11*). The antibody was used with the techniques of immunofluorescence (*12*) and antibody peroxidase-antiperoxidase (PAP) (*13*) in the study reported here. Sections of tissue containing small airways were examined.

Figure 1 shows that incubation of rabbit pulmonary tissue with the specific antiserum (1000-fold dilution), but not with the normal goat serum, resulted in most of the fluorescein isothiocyanate-conjugated rabbit antiserum to goat immunoglobulin G being bound in the Clara cell. No fluorescent staining was observed in the ciliated bronchiolar epithelium or in the alveolar epithelium. Similar results were obtained by the PAP method (Fig. 2). Reaction product was observed in the luminal pole of the cell cytoplasm with the greatest intensity at the perimeter of the cytoplasm when either method was used. Endogenous peroxidase activity was reduced by exposing the tissue to hydrogen peroxide (3 percent) prior to treatment with the antiserum when the PAP method was used. Further dilution of the antiserum (2500-fold) resulted in a decreased intensity of staining but did not alter the location of the reaction.

Our results suggest that cytochrome $P\text{-}450_I$ is an important factor in the pulmonary-specific effects of some toxic chemicals even though this enzyme is present in the livers of untreated rabbits at ap-

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proximately the same microsomal concentration as found in the lung (*11*). It appears that P-450$_I$ is highly concentrated in the Clara cells of the lung as compared to hepatocytes; immunofluorescence in liver sections, stained as described above, was not localized in specific areas but was weakly dispersed throughout the tissue (data not shown). This may explain why intracellular concentrations of active metabolites from compounds such as 4-ipomeanol and CMF [2-(*N*-ethylcarbamoylhydroxymethyl)furan], known substrates for cytochrome P-450$_I$ (*14*), reach toxic levels in the lung but not in the liver. In addition, there may be significantly less competition in the lung between activation and deactivation pathways catalyzed by different forms of cytochrome P-450. Approximately 50 percent of the pulmonary cytochrome P-450, but only about 10 percent of the hepatic enzyme, is P-450$_I$ (*11*). Cytochrome P-450$_{II}$ (*9*), for which 4-ipomeanol and CMF are marginal substrates (*14*), constitutes most of the remaining pulmonary cytochrome P-450 (*9*), whereas there appear to be at least five forms of the cytochrome in rabbit liver (*2*).

Thus we have determined that a major form of rabbit pulmonary cytochrome P-450 is present in the Clara cell of the lung in apparently high concentrations relative to that found in other cell types. This distribution is in contrast to the diffuse localization of the same form of the cytochrome in the liver—a difference that may be important to the susceptibility of the lung to certain toxic chemicals that have little or no effect on the liver.

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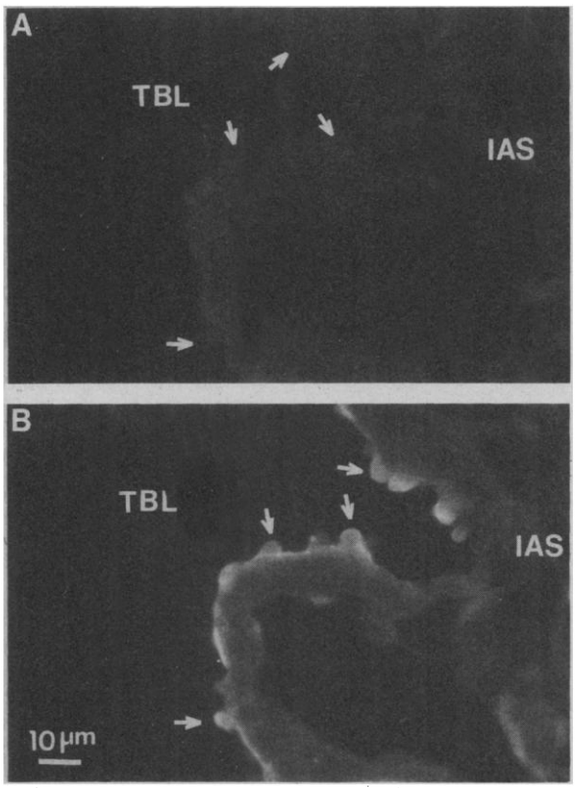

Fig. 1. Localization of cytochrome P-450$_I$ in the apices of nonciliated epithelial cells of the terminal bronchiole as shown by immunofluorescence. Pulmonary tissue from a male New Zealand White rabbit was fixed by infusion with paraformaldehyde (1 percent) in phosphate buffer (100 m*M*), *p*H 7.4, prior to being embedded in paraffin. Serial sections (6 μm) were cut. (A) A section on which normal goat serum was substituted for the specific antiserum. (B) A section that was reacted with the specific antiserum (diluted 1000-fold in phosphate-buffered saline) for 48 hours at 4°C prior to reaction with fluorescein-labeled antiserum to goat IgG. The apices of the Clara cell (shown by the arrows) project into the bronchiolar lumen (*TBL*). The interalveolar septa (*IAS*) do not show reaction product.

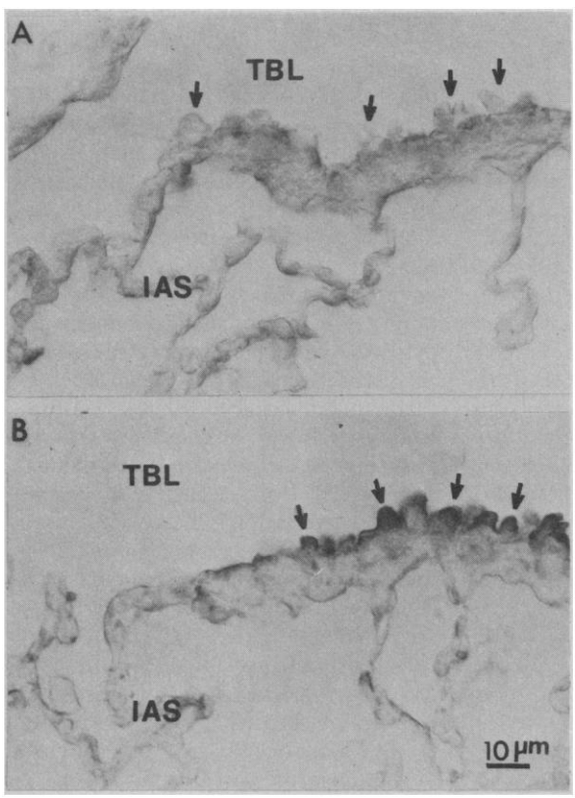

Fig. 2. Localization of cytochrome P-450$_I$ in the apices of nonciliated epithelial cells of the terminal bronchiole as shown by immunoperoxidase. The preparation of the tissue was as described for Fig. 1. (A) Control, treated with normal serum. (B) The reaction product associated with the specific antiserum (1000-fold dilution). The apices of the Clara cell (shown by the arrows) project into the bronchiolar lumen (*TBL*). The interalveolar septa (*IAS*) do not show any reaction product. Peroxidase activity was demonstrated by reaction with 3′,3′-diaminobenzidine followed by reaction with osmium tetroxide.

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