Ann. Rev. Pharmacol. Toxicol. 1986. 26:183–200

PHARMACOLOGICAL ASPECTS OF METABOLIC PROCESSES IN THE PULMONARY MICROCIRCULATION

C. Norman Gillis

Departments of Anesthesiology and Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

INTRODUCTION

Lung pharmacology is concerned with both therapeutic and toxic effects of drugs on lung cells and cellular processes. Increasingly in the last several years, those interested in this field have also focused their attention on actions of lung on drugs and toxins reaching it via the air space or blood—the pharmacokinetic (metabolic or nonrespiratory) function of lung (1, 2). These occur in many cells of the lung; however, particularly intimate contact of the delicate alveolar-capillary unit with both the external environment and blood makes this site a frequent focus of injury due to environmental or vascular insults, such as hyperoxia, xenobiotic toxicity, septicemia, and adult respiratory distress in man (1, 2, 2a). This, coupled with the intense metabolic activity known to occur in cells of the alveolar-capillary membrane, has generated much effort to understand mechanisms and the significance of lung metabolic functions.

In carrying out these functions, the lung acts as a highly efficient "biochemical filter" for central venous blood. This reflects primarily two actions. First, the pulmonary circulation is interposed between the right and the left heart, and therefore all circulating blood passes through the lungs about once per minute. Secondly, within the lung, blood interacts with the very large metabolically active endothelial surface, which is now recognized as the site of many pharmacokinetic functions (1–4). Alveolar epithelium is an equally active site for degradation or activation of many xenobiotic substances (2), especially those whose route of access is via the airway.

0362-1642/86/0415-0183$02.00

Prominent among pharmacokinetic functions is removal[1], biosynthesis, and release of several vasoactive hormones that have significant effects on cardiovascular regulation, both in normal and disease states. These substances include biogenic amines, prostaglandins, leukotrienes, and peptides. Thus, aortic blood can differ markedly from central venous blood in its content of substances that profoundly alter the physiological status of the cardiovascular and other systems. An informative review of xenobiotic uptake and metabolism was recently published (2). Accordingly, this topic will be given only limited consideration. The main purpose of this chapter is to consider recent developments concerning pharmacokinetic processes that affect endogenous "substrates."

PHARMACOKINETIC LUNG FUNCTIONS

Recent review articles (2, 5, 6, 7) provide detailed information on mechanisms whereby the lung removes endogenous substances from pulmonary blood. Important features of these metabolic functions include the following. (*a*) Endogenous substrates for lung metabolism have profound physiologic effects, either per se or by modulating other effector mechanisms. Many are involved in critical regulatory processes, including homeostasis, capillary permeability change, endothelial interaction with platelets and leukocytes, and the inflammatory process. (*b*) Removal frequently results in loss of biological activity, but usually not in binding of unchanged substrate (in contrast to many xenobiotics). (*c*) There is considerable specificity of removal, even among close chemical congeners. (*d*) Good evidence now links many of the processes to the microvascular endothelium, although there are other sites of removal. Major classes of endogenous substrate for removal include biogenic amines, prostaglandins, peptides, and adenine nucleotides.

Biogenic Amines

Removal of the biogenic amines [norepinephrine (NE), and 5-hydroxytryptamine (5-HT)] in mammalian lungs is achieved by a carrier-mediated, temperature- and drug-sensitive transport process, followed by intracellular degradation by monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) in the case of NE (7). Removal of biogenic amines has characteristics of both neuronal and extraneuronal NE uptake (7), occurs rapidly, and can effectively reduce pulmonary arterial blood concentration of these amines in a single transpulmonary passage. While they cannot account for this rapid

[1] The term *removal* is used throughout this chapter to connote a net reduction in concentration of drug or vasoactive substance in lung effluent (whether in vitro or in vivo) compared to that in central venous blood or pulmonary arterial outflow.

removal, other processes such as uptake into platelets, adrenergic nerve endings (8), and neuroepithelial cells are important for pulmonary retention of biogenic amines over the longer period (9). It was recently shown that NE removed by perfused rat lung, in which endothelial and other sites of MAO and COMT were blocked, enters three kinetically defined pools identified (10) as vascular, extracellular, and a "slowly effluxing pool." The latter is blocked by cocaine and thus may reflect adrenergic nerve endings. Interestingly, entry into a slowly effluxing pool is also characteristic of xenobiotics (2), although the corresponding morphological site is the macrophage (2) rather than adrenergic nerves. Although removal of 5-HT involves a transport phenomenon, the nature of the carrier or its binding site for the amine is unknown. There may also be a subcellular site for 5-HT removal, since high affinity, temperature-sensitive binding of 5-HT to purified mitochondrial preparations has been reported (11). The latter binding sites may be serotonin receptors that subserve some as yet unknown action, presumably in mitochondria (11), although high affinity binding sites per se need not necessarily be coupled with a physiological function. Transport of biogenic amines from blood is the rate-limiting step in overall removal (5–7) and is blocked by a variety of drugs, including inhibitors of both adrenergic neuronal and non-neuronal uptake systems, as well as certain adrenergic blocking agents (7). Some structural specificity for 5-HT and NE (12) transport is evident, although this topic has been relatively little studied. N-acetyl-5-methoxytryptamine (melatonin) is not taken up by lung (13), and alpha-methyl serotonin has a lower affinity for removal than serotonin itself (14).

Serotonin congeners that inhibit uptake of the parent biogenic amine by rat lung slices have been studied (15). The indole ring system and an amine side chain were essential for activity. Structures with increased ionization of the indole hydroxyl group had greater inhibitory activity. However, it is unclear whether, in the lung slice, 5-HT removal occurs into endothelial cells (i.e. the normal vascular route) or whether other cells are equally involved. In contrast to the biogenic amines, inactivation of phenylethylamine (12), mescaline (16), and octopamine (12) reflects solely deamination by isoenzymes of monoamine oxidase. A possible role for lung in regulating circulating concentrations of mescaline and perhaps other psychotomimetic amines seems to merit consideration. Serotonin and NE are found in the lung (17, 18) and thus de novo biosynthesis of these amines seems probable. The rich adrenergic innervation of the lung suggests that neuronal regulation of metabolic functions is possible, either by causing local change in amine concentration, or by influencing hemodynamic factors such as blood flow.

Several studies suggest that altered biogenic amine removal might be linked to cardiovascular regulation. A surprisingly high incidence of primary pulmonary hypertension was reported among patients using the anorectic drug ami-

norex (19), which also decreases 5-HT removal by rat lung (20). Some of these patients were successfully treated by use of a serotonin antagonist, supporting the likelihood of increased "free" 5-HT (consequent to diminished removal) as the mechanism. Severe systemic hypertension after cardiopulmonary bypass (CPB) in man has been linked to increased circulating NE concentrations (21). We recently reported that lung NE removal was decreased after prolonged (3–4 hr) CPB in dogs (22), a finding similar to our observation with another substrate, prostaglandin E_1 (23). Also, patients with pulmonary hypertension had no net transpulmonary NE gradient (24, 25). These studies suggest it would be profitable to explore further the link between pulmonary vascular disease and experimentally or disease-induced change in lung metabolic functions (24, 25).

Prostaglandins

Prostaglandins of the E and F series also are extensively removed by mammalian lungs (26–28). A carrier-mediated, energy-requiring process has been implicated (29–31), although there is also rapid degradation by 15-hydroxyprostaglandin-dehydrogenase (PGDH) and other enzymes (29–31). Although prostaglandin removal is drug-sensitive, both in vivo (32) and in vitro (29–31, 33, 34), the rate-limiting step in the process is unclear. Studies with perfused lung (29–31, 33, 34) are consistent with an endothelial site, but large vessel endothelial cells in culture do not transport prostaglandins. This negative finding might reflect either the relatively small number of cells used or the absence of this property in cells from large vessels.

To define, adequately, the rate-limiting step in postaglandin removal one needs inhibitors that act specifically either on transport or enzymatic degradation. Unfortunately, available antagonists affect both processes simultaneously. Studies have been reported of structural requirements among sulfasalazine analogues for inhibition of $PGF_{2\alpha}$ degradation by NAD^+-dependent PGDH in preparations of bovine lung and human placenta (35). Requirements for optimal inhibition were two aromatic rings and acetyl and hydroxyl moieties at positions 1 and 2 in the salicyl C-ring system. Further study of structural requirements for prostaglandin transport and enzymatic degradation by PGDH are essential to define more clearly the mechanism of prostaglandin removal and the consequences of its inhibition.

Specificity is also evident in prostaglandin removal since thromboxane and prostacyclin pass through the pulmonary circulation unchanged—although they are taken up by skeletal muscle (36). There is therefore a parallel between PGI_2 and epinephrine (37), both of which are taken up by skeletal muscle but escape significant degradation in the lung.

Peptides

Both bradykinin and angiotensin I (AI) are substrates for kininase II or angiotensin converting enzyme (ACE). This membrane-bound enzyme hydrolyzes peptidyl dipeptide bonds from the carboxyl terminal of bradykinin and AI, resulting in biological inactivation of the former and conversion of the latter to angiotensin II. Angiotensin II is not normally metabolized by the lung, which is consistent with its role as a "circulating" hormone (38). However in pathophysiological states, including edema, it may be hydrolyzed, perhaps because it is exposed to normally intracellular angiotensinases (28).

Several other biologically relevant peptides also escape degradation by the lung. Thus, vasoactive intestinal polypeptide (VIP), found in autonomic nerves of airway and vascular smooth muscle cells (5, 28), bombasin, which occurs in endocrine cells of fetal lung (28), and oxytocin all escape lung inactivation (1, 28). Substance P is reported to be hydrolyzed by cultured endothelial cells (39) but not by intact lung (28).

An aminopeptidase in human vascular endothelial cells hydrolyzes leu-enkephalins (40). Degradation of both leu^5- and met^5-enkephalin by perfused lung was established by studies that employed rat colon bioassay (43) and high performance liquid chromatographic techniques (41) to identify metabolites formed. Over 95% of the initial enkephalin radioactivity was recovered in the perfusion medium. This is reminiscent of earlier studies with bradykinin (42) and indicates that neither parent compound nor the metabolites are stored in the tissue. The inhibition of ACE by captopril, or of aminopeptidase by bestatin, diminished met-enkephalen metabolism (43). Interestingly, inhibition of enkephalinase with thiorphan did not affect metabolism. Thus, at least in rat lung, both leu^5- and met^5-enkephalin are metabolized by two enzymes—ACE and an aminopeptidase, perhaps the recently described peptidyl dipeptidase (44), which is distinct from ACE. These observations suggest a role for lung metabolism in modulating physiological and pharmacological functions of these opiate peptides, including control of arterial concentrations of enkephalins released into the circulation in, for example, canine endotoxin shock (44a). In fact, increased rat pulmonary vascular resistance, in response to leu-enkephalin, was enhanced by a combination of captopril and bestatin (44b).

BENZ-PHE-ALA-PRO Since the introduction of hipp-his-leu (45) to measure ACE activity, these synthetic substrates have attracted much interest because they are technically easier to use than the natural peptides. One such compound is benzoyl-phenylalanyl-alanyl-proline (BPAP), synthesized and first used to measure ACE of endothelial cells in culture (54). BPAP has found application in the measurement of lung ACE both in this laboratory (46–50) and others (51–53). The attractiveness of tritiated BPAP to measure ACE lies in the fact

that it is rapidly hydrolyzed (46, 48) by ACE to yield tritiated benz-phe, which is easily separated from the parent compound (54). Also, BPAP lacks significant cardiovascular effects (46, 48, 49) and thus does not alter perfusion characteristics of the lung during its use. Hydrolysis of BPAP in vivo is inhibited, in a dose-dependent manner, by bradykinin or captopril (46, 48). The apparent K_m for hydrolysis in vivo is about 9 μM (49), which is close to that reported with cultured cells (54).

INSULIN REMOVAL Purified hog lung ACE cleaves dipeptides from the B-chain of insulin (55). Also, insulin is an effective inhibitor of both plasma and lung ACE (55). Recently, it was reported that aortic (56) and pulmonary arterial (56a) endothelial cells in culture transport insulin by an energy-dependent, receptor-mediated process. Transport in the cultured cell was relatively slow (15% in 2 hr, when aortic cells were incubated with 1 ng insulin/ml). Binding of insulin could be the first step in the process leading to transfer of the hormone from blood to target cells. Lung binding may reflect merely the large endothelial surface area present. On the other hand, it is tempting to imagine the lung exerting some regulatory role in the physiological function of insulin.

NATRIURETIC ATRIAL FACTOR The natriuretic atrial factor (NAF), a potent vasodilator of pre-contracted vessels (57), binds specifically to high affinity sites on membrane preparations of rat mesenteric and renal vessels (58). However, the capacity of these sites is low, an observation consistent with only modest uptake of NAF by large pulmonary vessels and small systemic vessels (57). Yet we observed 67 ± 4% single-pass removal of 3-[^{125}I]-iodotyrosyl ^{28} NAF by rabbit lung in situ (M. Turrin & C. N. Gillis, unpublished observations). The nature of this avid removal is unknown. However, the lung is ideally suited to contribute to the regulation of systemic physiological actions of NAF released from the right heart.

ADENINE NUCLEOTIDES Adenine nucleotides are substrates for 5'-nucleotidase and ATPase that degrade ADP to AMP and then to adenosine (6). Adenosine itself is a substrate for carrier-mediated transport into endothelial cells (59–61) and is an important mediator of regional blood flow. Thus, knowledge of its pharmacokinetics in the pulmonary (and extrapulmonary) vascular bed is of considerable interest. Aortic (61) and pulmonary arterial (62) endothelial cells or isolated perfused lungs (63, 64) remove adenosine from culture or perfusion medium, respectively. The biological significance of this property, in vivo, has been questioned, since red blood cell transport of adenosine appears to have a potentially greater role in removal of blood-borne nucleotide than pulmonary vascular endothelium (59, 60). Much less is known

about abluminal endothelial transport of adenosine. It has been suggested (65) that endothelium plays an integral role in modifying interstitial concentrations of adenosine, an important link between tissue metabolism and vascular smooth muscle activity.

XENOBIOTICS In addition to the endogenous substrates mentioned, many xenobiotics are also removed during their transpulmonary passage. Many of these compounds are lipophilic basic amines (e.g. propranolol, lidocaine) and their removal by lung reflects both high lipid solubility and specific binding sites (2, 67).

Because of the avid removal and subsequent release of unchanged xenobiotics, including important drugs in clinical use such as beta-blocking agents, local anesthetics, and antihypertensives, it has been suggested that the lung could act as a "capacitor," preventing sudden increases in systemic levels of these compounds (66, 67). If so, previously bound drug might be displaced by a second agent, with consequent liberation of relatively large (and potentially toxic) concentrations of the first drug [e.g. propranolol (66,67)] into the coronary, cerebral, and other critical vascular beds. Recent reports of competition between bupivacaine and serotonin (68) and of Fentanyl uptake by the lung (69) raise similar possibilities. In general, however, this apparently critical area of clinical pharmacology has not received the systematic study it merits.

ENDOTHELIAL BIOSYNTHESIS OF VASODILATORS AND PULMONARY HEMODYNAMICS

Endothelial cells in culture synthesize many vasoactive substances, both spontaneously and in response to pharmacological challenge. Whether similar synthetic properties are present (or are biologically important) in vivo is uncertain. Nevertheless, it is appropriate to emphasize that release of substances by lung could have significant effects not only on systemic vessels "downstream" from the lungs but also on pulmonary vasculature per se. Two specific examples merit further consideration.

Prostacyclin

It was recently reported that release of prostacyclin (PGI_2) by cultured aortic endothelial cells was greatly enhanced by exposure to shear stress, either pulsatile or nonpulsatile (70). Bovine pulmonary arterial endothelial cells and perfused rat lung (71) release more PGI_2 in response to shear stress or altered flow, respectively. Studies of this type could shed light on the role of the lung in modifying events downstream, including cardiac function, pulmonary and peripheral vascular tone as well as platelet aggregation. Furthermore, the pattern of pulse wave and shear stress might affect metabolic processes in the

lung by influencing synthesis of PGI_2—or that of other mediators, including EDRF (see below). For example, PGI_2 is reported to depress 5-HT clearance (72) and thromboxane apparently can diminish PGE_2 inactivation by rat lung (73). Therefore, the possibility exists that such interactions may represent a method of indirect control over lung metabolic functions.

Endothelial-Derived Relaxing Factor (EDRF)

Some vasoactive hormones, including acetylcholine and bradykinin, are thought to dilate vascular smooth muscle by releasing a diffusible vasodilator substance (74). This mechanism clearly occurs in large diameter systemic or pulmonary blood vessels, in which it has been demonstrated that there is a basal release of the compound. It is much less certain whether this also applies to precapillary resistance vessels. Also in question is the chemical identity of EDRF, although it is not a product of cyclo-oxygenase activity (74). It seems reasonable to question whether basal release of EDRF, especially if it occurs in lung microvasculature, contributes to the normally low resistance of the pulmonary circuit. Might hypoxic vasoconstriction occur, therefore, because such release, or de novo synthesis, is reduced? If so, can we then suggest that the pathophysiology of acute respiratory failure [secondary to loss of hypoxic vasoconstriction (2a)] might also involve impaired release of EDRF? Indeed, this suggestion has been offered in discussing the fact that bradykinin constricts, rather than dilates, canine pulmonary vessels from which the endothelium was physically removed (76). Certain drugs that inhibit EDRF synthesis and release may increase pulmonary vascular resistance by a similar mechanism. The opposite drug action, namely local promotion of EDRF synthesis and release, could represent a unique approach to the pharmacotherapy of increased pulmonary vascular resistance. Indeed, when the structure of EDRF is elucidated, it is likely that analogs of the molecule will be attractive candidates for therapy of both systemic and pulmonary hypertension.

FUNCTIONAL ASPECTS OF ACE INHIBITION

Interest in peptide hydrolysis by lung ACE increased with development of a new class of antihypertensive drugs that inhibit the enzyme. These drugs also have effects other than ACE inhibition, including release of arachidonic acid derivatives (77)—presumably via local or systemic elevation of kinin concentration. Nevertheless, there is good correlation between inhibition of lung, vascular, central nervous system, or renal ACE in vitro and the antihypertensive effects of these drugs (78, 79). Recently we found (47) that pulmonary ACE activity in conscious rabbits was depressed for over six days after a single i.v. dose of captopril (2 mg/kg). During this period, systemic arterial pressure correlated closely with inhibition of pulmonary ACE. In

contrast, there was an early, pronounced fall in plasma ACE; however, 24 hours later, when plasma ACE activity had returned to control levels, systemic blood pressure was at its nadir. This hypotensive effect could involve decreased arterial AII or increased arterial bradykinin (secondary to inhibition of pulmonary ACE) as well as additional mechanisms.

Lung Removal of ACE Inhibitors

Uptake and binding of ACE inhibitors has been studied with a view to defining the location and function of ACE in the lung as well as the pharmacological actions of these drugs. ^{3}H-Captopril binds with high affinity to a single site in membrane preparations of rat lung and choroid plexus in vitro (80). Binding parallels ACE activity, determined in the same preparation. Furthermore, binding of ^{3}H-captopril and ACE activity were inhibited in a parallel, dose-dependent manner by several other carboxylate ACE inhibitors, suggesting that under the conditions of this assay in vitro, captopril binds selectively, only to ACE (80).

Whether this is also true in vivo was recently studied in this laboratory. We found (81) that 40% of a bolus injection of captopril (10 nmoles/kg) was removed by lungs of anesthetized rabbits. Uptake was saturable, decreasing to 6% when 70 nmole of captopril were given, and was specific since the drug inhibited hydrolysis of BPAP in vivo, but captopril failed to affect the uptake of 5-HT given in the same bolus. This study (81) suggested that lung removal of captopril in vivo was due primarily to binding of the drug to endothelial ACE, as was also found in vitro (80).

Because of the specificity evident in the binding of captopril to ACE, we recently began to study lung removal (i.e. specific binding) of another ACE inhibitor in preparation for the design of techniques for "in-line" measurement of lung metabolic functions (see below). We used a new ACE inhibitor, (N-[1-(S)-carboxy-(4-OH-3-^{125}I-phenyl)-ethyl-L-ala-L-pro) or CPAP, kindly supplied by Drs. Ryan and Chung of the University of Miami. With rabbit lungs perfused in situ, we found (82) that this compound was rapidly taken up (67%) in a single pass. The process was inhibited in dose-dependent fashion by unlabelled CPAP or by captopril. This compound was also shown (82) to be equipotent with captopril in inhibiting hydrolysis of BPAP by rabbit lungs. In a similar study (83), the same compound was used to determine that hypoxia had no direct effect on the activity of ACE in the rabbit lung.

In-line Measurement of Lung Metabolic Functions

The fact that ^{125}I-CPAP is photon emitting allowed us to begin development of a system for measurement of its removal in-line. Such a system offers the hope of virtually instantaneous measurement of a process that could provide data about an enzyme present on the microvascular surface of lung as well as other

organs. Furthermore, if successful, this method could allow study of modifications during experimental changes imposed in the lung. In the system developed (84), we used a bolus injection, double indicator dilution technique. The bolus contained ^{125}I-CPAP and also ^{99m}Tc-sulfur colloid, as an intravascular reference. Lung effluent, sampled from the carotid artery of an anesthetized rabbit, is passed through a flow cell, in which these photon-emitting isotopes are detected by a sodium iodide crystal, the output of which was directed to counting equipment that allowed separation of the two isotopes on the basis of their photopeak energies. Output was passed through a buffered interface to a microcomputer. Custom software was used to record radioactivity in the arterial outflow as a continuous function of time, for about 25 seconds after administration of the bolus injection. Data were stored in the computer and used to calculate cardiac output, mean transit times, and volumes of distribution of both isotopes.

Cardiac outputs calculated with this system compared closely with those determined simultaneously by direct Fick method, and the slope relating both values was not significantly different from 1. Also, instantaneous and integral removal curves are available at the same time. Figure 1 shows the fractional concentration curves so obtained after the administration of 0.1 μg (left) and 10.0 μg (right) of CPAP/kg to an anesthetized rabbit. It can be seen that (*a*) the integral removal was about 45% in this animal after 0.1 μg CPAP and (*b*) that removal was reduced to zero by use of 10 μg cold CPAP/kg. Thus, in-line determination of CPAP removal reveals similar behavior to experiments in situ. The fact that removal is apparently somewhat lower in the in-line system may be due to the much higher blood flow in the intact, anesthetized animal.

ALTERED ENDOTHELIAL REMOVAL FUNCTION AND LUNG INJURY

This topic has been extensively reviewed during the last three years (2, 85–87) and the interested reader is referred to these papers for more detailed discussion. Endothelial cells of the lung are often early sites of injury caused by a variety of substances, including oxygen, bleomycin, and monocrotaline, and experimental or disease-associated conditions, such as radiation injury (85–87). The mechanism of such injury is uncertain, but it has now been reported that normobaric hyperoxia decreases fluidity in the hydrophobic core of the plasma membrane in cultured pulmonary endothelial cells (87a). Such effects, coupled with the close association of lung removal functions with the microvascular endothelium, led to the proposal that acute lung injury might modify the latter process (85). If so, it was reasoned, measurement of metabolic functions may provide early reflection of injury to the pulmonary endothelium.

There is now considerable support for this hypothesis from experiments in a

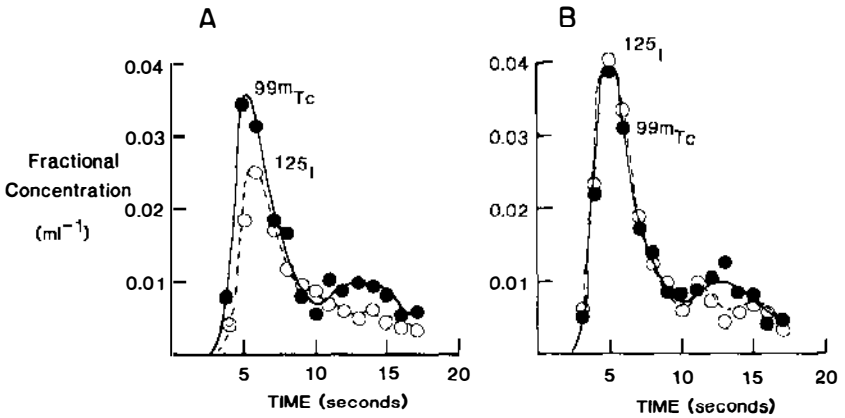

Figure 1 Indicator dilution data obtained, in-line, from an anesthetized rabbit. Panel *A* presents the fractional concentration curves obtained after rapid i.v. injection of a bolus containing ^{99m}Tc-sulfur colloid as intravascular reference and 0.1 μg (total dose) of the ACE inhibitor, CPAP (see text). Integral removal was 45%. Panel *B* shows corresponding data after injection of bolus containing 10 μg of CPAP per kg. Notice that the two curves are coincident, implying that removal was zero in the presence of excess cold CPAP.

number of animal models of lung injury, including each of the models mentioned above (85–87). In virtually all cases, the predominant effect of acute injury is to lower removal of the substrate tested. The most frequently employed substrates for removal were 5-HT, prostaglandins, and synthetic substrates for ACE. Furthermore, in many instances altered removal precedes morphological or clinical evidence of injury (85, 88). Studies designed to evaluate this link in man revealed that 5-HT (89, 90) or PGE_1 (91) removal is relatively well preserved after short periods of cardiopulmonary bypass. In contrast, patients with adult respiratory distress syndrome (ARDS) had significantly reduced single-pass removal of ^{3}H-PGE_1 (91), ^{14}C-5-HT (91, 91a); those suffering from emphysema also showed diminished ^{3}H-propranolol removal (92). The transpulmonary gradient for endogenous NE (25) and prostaglandin $F_{2\alpha}$ (93) is decreased in adults with primary pulmonary hypertension. The NE gradient was zero in children with pulmonary hypertension secondary to congenital heart disease; however, surgical correction of the underlying lesion caused prompt recovery of NE removal (24).

INTERPRETATION OF REDUCED REMOVAL IN THE INJURED LUNG

Experimental and disease-associated lung injury (see above) is often associated with altered blood flow rate and nonuniform perfusion of the lung that may alter

quantitative measurements of removal (86). Thus it is critically important to distinguish altered removal due to hemodynamic changes from that due to intrinsic endothelial injury. There is presently no fully accepted method for reliably achieving this separation in vivo. However, Rickaby et al (34, 94–96) used the rapid injection multiple indicator dilution method in a promising approach to this difficult problem. Reasoning that data from this type of experiment contain information about both convective (hemodynamic) and endothelial transport (kinetic) processes in the lung, they developed a mathematical formulation (97, 98) for lung removal processes, assuming that Michaelis Menten kinetics governs the interaction between substrate and transport (or surface enzyme) site. In this method, the injected bolus contains an intravascular reference indicator, trace amounts of radiolabeled substrate, and varying amounts of unlabeled substrate. The total amount of substrate is adjusted to provide, in a single traverse of the lung circulation, a profile of intracapillary concentrations such that the kinetics of the unidirectional removal process ranges from first order to nearly zero order.

Several studies indicate that this technique can separate altered endothelial cell functions (i.e. due to injury) from those produced by changes in lung perfusion both in vitro (34, 95, 99) and in vivo (32, 49, 50). First, varying flow rate in isolated perfused dog lung did not change the calculated apparent V_{max} (96), which indirectly reflects the number of transport sites. Also the apparent K_m was unchanged, unless flow rates less than 50% of normal were used. Varying the pH of perfusion medium over the range 7.2–7.8 did not change 5-HT kinetic parameters (100). There was fair agreement in calculated values for the apparent V_{max} and K_m for 5-HT removal, determined simultaneously in the same lung preparation, after steady infusion and bolus injections of the amine (101). Embolization of lungs with large (550 μm) glass beads, which reduced vascular volume and surface area, significantly reduced V_{max} for 5-HT removal but did not alter the K_m (94). Similarly, the apparent V_{max} for PGE_1 is reduced after embolization (102), probably again as a result of decreased surface area.

This model (34, 95), originally proposed for transport functions, seems equally applicable to substances removed only by enzymatic processes (48–50, 102a, 103). Thus, similar estimates have been reported for apparent K_m and V_{max} for BPAP hydrolysis whether studied by indicator dilution techniques (48–50, 59, 103) or by steady-state techniques in isolated perfused lungs (48, 102a, 99). The apparent kinetics for BPAP removal were unaffected by large changes in pulmonary perfusion. However, V_{max} was significantly reduced at high transpulmonary pressures (and lung volumes), presumably because alveolar vessels were compressed in this state (104) and thus reduced perfused surface area.

Although the apparent kinetics for lung metabolic functions appears to be largely independant of flow, less is known about the predictive value of the model in analyzing the mechanism of drug inhibition. Imipramine, which antagonizes 5-HT removal, doubled the K_m for the process and reduced V_{max} (94). Under the usual conditions for analysis in vitro, these are changes consistent with a mixed inhibitor mechanism. However, since imipramine is taken up by lung tissue (67), thereby creating uncertainty about its local concentration in relation to the 5-HT transport site (105), the precise nature of its inhibitory effect on the chemical kinetics of the amine transport remains to be elucidated. In particular, although increases in apparent K_m are suggestive of competition for carrier receptor sites, a literal interpretation of the estimated organ value of K_m as a function only of chemical affinity is an oversimplification. Nevertheless, this model has provided a useful basis on which to begin efforts to separate endothelial functional change due to injury from effects associated with strictly hemodynamic factors. Dawson et al reported (106) in a later study that 5-HT removal was lowered if surface area was sufficiently reduced, but was unaffected by oleic acid injury. This is consistent with data in patients showing that, unless lung microvascular surface area was decreased by atelectasis, 5-HT removal was unaffected by cardiopulmonary bypass (90). Thus it seems that relatively small quantitative reduction in 5-HT removal, for example, may actually reflect a functional loss of major portions of perfused endothelial surface [see also reference (86)].

As an alternative experimental approach, removal can be measured during steady infusions of a substrate (48, 101, 107, 108). In this case, the transpulmonary gradient can be determined directly and will measure the rate of removal if return of unchanged material to the vascular space is sufficiently slow. Since steady infusion appears impractical for clinical application, the bolus injection technique seems a more realistic means to measure lung metabolic functions in vivo. Certainly there is a need to expand greatly our understanding of the quantitative interpretation of data derived by the latter technique. Nevertheless, currently available data amply justify continued use of the bolus injection method to study microvascular and endothelial metabolic function in vivo as well as in the clinical setting.

ACKNOWLEDGMENTS

I am grateful to Dr. Bruce R. Pitt for helpful discussions during the preparation of this paper. Original investigations in the author's laboratory were supported, in part, by Grants HL 13315, HL 27207, and HL 07410 from the National Heart, Lung, and Blood Institute.

Literature Cited

1. Bakhle, Y. S., Vane, J. R. 1974. Pharmacokinetic function of the pulmonary circulation. *Physiol. Rev.* 54:1007–54
2. Bend, J. R., Serabjit-Singh, C. J., Philpot, R. M. 1985. The pulmonary uptake, accumulation and metabolism of xenobiotics. *Ann. Rev. Pharmacol.* 25:97–125

2a. Rinaldi, J. E., Rogers, R. M. 1982. Adult respiratory distress syndrome: changing concepts of lung injury and repair. *N. Eng. J. Med.* 306:900–9

3. Gillis, C. N., Roth, J. A. 1976. Pulmonary disposition of circulation vasoactive hormones. *Biochem. Pharmacol.* 25:2547–53
4. Junod, A. F. 1977. Metabolism of vasoactive agents in lung. *Am. Rev. Respir. Dis.* 115:51–57
5. Said, S. I. 1982. Metabolic functions of the pulmonary circulation. *Circ. Res.* 50:325–33
6. Ryan, U. S., Ryan, J. W. 1984. Cell biology of pulmonary endothelium. *Circulation* 70(Suppl. 3):46–62
7. Gillis, C. N., Pitt, B. R. 1982. The fate of circulating amines within the pulmonary circulation. *Ann. Rev. Physiol.* 44:269–81
8. Bosin, T. R., Lahr, P. D. 1981. Mechanisms influencing the disposition of serotonin in mouse lung. *Biochem. Pharmacol.* 30:3187–93
9. Gillis, C. N. 1985. Peripheral metabolism of serotonin. In *Serotonin and the Cardiovascular System,* ed. P. M. Vanhoutte, pp. 27–36. New York: Raven
10. Metting, P. J., Levin, J. A., Samuels, J. T. 1985. Compartmental analysis of the efflux of 1-[^{3}H]norepinephrine from isolated perfused rat lung. *J. Appl. Physiol.* 58:244–50
11. Das, D. K., Steinberg, M. 1985. Specific binding of serotonin in rat lung. *Am. J. Physiol.* 248:E58–E63
12. Gillis, C. N., Roth, J. A. 1977. The fate of biogenic monoamines in perfused lung. *Br. J. Pharmacol.* 59:585–90
13. Kopin, I. J., Pare, C. M. B., Axelrod, J., Weissbach, H. 1961. The fate of melatonin in animals. *J. Biol. Chem.* 236:3072–75
14. Alabaster, V. A. 1977. Inactivation of endogenous amines in the lung. In *Metabolic Functions of the Lung: Lung Biology in Health and Disease,* ed. C. Lenfant, 4:3–31. New York: Dekker
15. Mais, D. E., Bosin, T. R. 1981. Structural specificity of the inhibition of serotonin accumulation in rat lung slices. *Res. Commun. Chem. Pathol. Pharmacol.* 34:241–49
16. Roth, R. A., Roth, J. A., Gillis, C. N. 1977. Disposition of ^{14}C-mescaline by rabbit lung. *J. Pharmacol. Exp. Ther.* 200:394–401
17. Sadavongvivad, C. 1970. Pharmacological significance of biogenic amines in the lungs: 5-hydroxytryptamine. *Br. J. Pharmacol.* 38:353–65
18. Aviado, D. M., Sadavongvivad, C. 1970. Pharmacological significance of biogenic amines in the lungs: noradrenaline and dopamine. *Br. J. Pharmacol.* 38:374–85
19. Steinberg, H., Fisher, A. 1977. Serotonin in the lung. In *Serotonin in Health and Disease,* ed. W. B. Essman, pp. 69–109. New York: Spectrum
20. Seiler, K. U., Wassermann, O., Wensky, H. 1976. On the role of serotonin in the pathogenesis of pulmonary hypertension induced by anorectic drugs: an experimental study in the isolated perfused rat lung. *Clin. Exp. Pharmacol. Physiol.* 3:323–30
21. Wallach, R., Karp, R. B., Reves, J. G., Oparil, S., Smith, L. R., James, T. N. 1980. Pathogenesis of paroxysmal hypertension developing during and after coronary bypass surgery: a study of hemodynamic and humoral factors. *Am. J. Cardiol.* 46:559–65
22. Pitt, B. R., Gillis, C. N., Hammond, G. L. 1984. Depression of pulmonary metabolic function by cardiopulmonary bypass procedures increases levels of circulating norepinephrine. *Ann. Thorac. Surg.* 38:508–13
23. Pitt, B. R., Hammond, G. L., Gillis, C. N. 1982. Depressed pulmonary removal of ^{3}H-prostaglandin E_1 after prolonged cardiopulmonary bypass. *J. Appl. Physiol.* 52:887–92
24. Gewitz, M., Pitt, B. R., Laks, H., Hammond, G. L., Talner, N., Gillis, C. N. 1982. Reversible effect of pulmonary hypertension on extraction of endogenous catecholamines by the pulmonary vasculature. *Pediatr. Pharmacol.* 2:57–63
25. Sole, M. J., Drobac, M., Schwartz, L., Hussain, M. N., Vaughan-Neil, E. F. 1979. The extraction of circulating catecholamines by the lungs in normal man and in patients with pulmonary hypertension. *Circulation* 60:160–63
26. Ferreira, S. H., Vane, J. R. 1967. Prostaglandins: their disappearance from and

release into the circulation. *Nature* 216:868–73
27. Hammond, G. L., Cronau, L. H., Whittaker, D., Gillis, C. N. 1977. Fate of prostaglandins E_1 and A_1 in the human pulmonary circulation. *Surgery* 81:716–22
28. Said, S. I. 1982. Pulmonary metabolism of prostaglandins and vasoactive peptides. *Ann. Rev. Physiol.* 44:257–68
29. Anderson, M. W., Eling, T. E. 1976. Prostaglandin removal and metabolism by isolated perfused rat lung. *Prostaglandins* 11:645–77
30. Bito, L. Z., Baroody, R. A., Reitz, M. E. 1977. Dependence of pulmonary prostaglandin metabolism on carrier-mediated transport processes. *Am. J. Physiol.* 232:E382–87
31. Bito, L. Z., Baroody, R. A. 1975. Inhibition of pulmonary prostaglandin metabolism by inhibitors of prostaglandin biotransport (probenecid and bromocresol green). *Prostaglandins* 10:633–39
32. Pitt, B. R., Forder, J. R., Gillis, C. N. 1983. Drug-induced impairment of pulmonary ^3H prostaglandin E_1 removal in vivo. *J. Pharmacol. Exp. Ther.* 227: 531–37
33. Hook, R., Gillis, C. N. 1975. Removal and metabolism of prostaglandin E_1 by rabbit lung. *Prostaglandins* 9:193–201
34. Linehan, J. H., Dawson, C. A. 1979. A kinetic model of prostaglandin metabolism in the lung. *J. Appl. Physiol.* 47:404–11
35. Berry, C. N., Hoult, J. R. S., Peers, S. H., Agback, H. 1983. Inhibition of prostaglandin 15-hydroxydehydrogenase by sulphasalazine and a novel series of potent analogues. *Biochem. Pharmacol.* 32:2863–71
36. Dusting, G. J., Moncada, S., Vane, J. R. 1978. Recirculation of prostacyclin in the dog. *Br. J. Pharmacol.* 64:315–20
37. Pitt, B. R., Hammond, G. L., Gillis, C. N. 1982. Comparison of pulmonary and extrapulmonary extraction of biogenic amines. *J. Appl. Physiol.* 52:1545–51
38. Vane, J. R. 1969. The release and fate of vaso-active hormones in the circulation. *Br. J. Pharmacol.* 35:209–42
39. Johnson, A. R., Erdos, E. G. 1977. Metabolism of vasoactive peptides by human endothelial cells in culture. Angiotensin I converting enzyme (kininase II) and angiotensinase. *J. Clin. Invest.* 59:684–95
40. Erdos, E. G., Johnson, A. R., Boyden, N. T. 1978. Hydrolysis of enkephalin by cultured human endothelial cells and by purified peptidyl dipeptidase. *Biochem. Pharmacol.* 27:843–48
41. Gillespie, M. N., Krechniak, J. W., Crooks, P. A., Altiere, R. J., Olson, J. W. 1985. Pulmonary metabolism of exogenous enkephalins in isolated perfused rat lungs. *J. Pharmacol. Exp. Ther.* 232:675–81
42. Ryan, J. W. 1982. Processing of endogenous polypeptides by the lung. *Ann. Rev. Physiol.* 44:241–55
43. Manwaring, D., Mullane, K. 1984. Disappearance of enkephalins in the isolated perfused rat lung. *Life Sci.* 35:1787–94
44. Benuck, M., Berg, M. J., Marks, N. 1981. A distinct peptidyl dipeptidase that degrades enkephalin: exceptionally high activity in rabbit kidney. *Life Sci.* 28:2643–50
44a. Evans, S. F., Medbank, S., Hinds, C. J., Tomlin, S. J., Varley, J. G., Rees, L. H. 1984. Plasma levels and biochemical characterization of circulating met-enkephalin in canine endotoxic shock. *Life Sci.* 34:1481–86
44b. Crooks, P. A., Bowdy, B. D., Reinsel, C. N., Iwamoto, E. T., Gillespie, M. N. 1984. Structure activity evidence against opiate receptor involvement in leu-enkephalin-induced pulmonary vasoconstriction. *Biochem. Pharmacol.* 33:4095–98
45. Cushman, D. W., Cheung, H. S. 1971. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit lung. *Biochem. Pharmacol.* 20: 1637–48
46. Catravas, J. D., Gillis, C. N. 1981. Metabolism of ^3H-benzoyl-phenylalanyl-alanyl-proline by pulmonary angiotensin converting enzyme *in vivo:* Effects of bradykinin, SQ14225, or acute hypoxia. *J. Pharmacol. Exp. Ther.* 217:263–70
47. Chen, X., Pitt, B. R., Moalli, R., Gillis, C. N. 1984. Correlation between lung and plasma angiotensin converting enzyme and the hypotensive effect of captopril in conscious rabbits. *J. Pharmacol. Exp. Ther.* 229:649–53
48. Ashton, J. H., Pitt, B. R., Gillis, C. N. 1985. Apparent kinetics of angiotensin converting enzyme: hydrolysis of [^3H] benzoyl-phenylalanyl-alanyl-proline in the isolated perfused lung. *J. Pharmacol. Exp. Ther.* 232:602–7
49. Howell, R. E., Moalli, R., Gillis, C. N. 1984. Analysis of rabbit pulmonary angiotensin converting enzyme kinetics in vivo. *J. Pharmacol. Exp. Ther.* 228: 154–60
50. Moalli, R., Man, P., Hasan, F. M., Faruggia, R., Teplitz, C., Gillis, C. N.

1984. Pulmonary metabolic functions in rabbits given *E. coli* endotoxin. *Fed. Proc.* 43:405
51. Ryan, J. W. 1983. Assay of peptidase and protease enzymes *in vivo*. *Biochem. Pharmacol.* 32:2127–37
52. Rubin, D. B., Mason, R. J., Bodds, L. J. 1982. Angiotensin converting enzyme substrates hydrolysed by fibroblasts and vascular endothelial cells. *Exp. Lung Res.* 3:137–45
53. Jackson, R. M., Pisarello, J. B. 1984. Hypoxia pre-adaptation prevents oxygen-induced depression of lung angiotensin converting enzyme. *Am. Rev. Respir. Dis.* 130:424–28
54. Ryan, U. S., Clements, E., Habliston, D., Ryan, J. W. 1978. Isolation and culture of pulmonary endothelial cells. *Tissue Cell.* 10:535–54
55. Igic, R., Erdos, E. G., Yeh, H. S. J., Sorrells, K., Nakajima, T. 1972. Angiotensin I converting enzyme of the lung. *Circ. Res. 31(3)II*51–61
56. King, G. L., Johnson, S. M. 1985. Receptor mediated transport of insulin accross endothelial cells. *Science* 227: 1583–86
56a. Dernovsek, K. D., Bar, R. S. 1985. Processing of cell-bound insulin by capillary and macrovascular endothelial cells in culture. *Am. J. Physiol.* 248:E244–51
57. Maack, T., Camargo, M. J. F., Laragh, J. F., Atlas, S. A. 1985. Atrial natriuretic factor: structure and function. *Kidney Int.* 27:607–16
58. Schiffrin, E. L., Chartier, L., Thibault, G., St-Louis, J., Cantin, M., Genest, J. 1985. Vascular and adrenal receptors for atrial natriuretic factor in the rat. *Circ. Res.* 56:801–7
59. Catravas, J. D., White, R. E. 1984. Kinetics of pulmonary angiotensin converting enzyme and 5'-nucleotidase in vivo. *J. Appl. Physiol.* 57:1173–81
60. Catravas, J. D. 1984. Removal of adenosine from the rabbit pulmonary circulation in vivo and in situ. *Circ. Res.* 54:603–11
61. Pearson, J. D., Carleton, J. S., Hutchings, A., Gordon, J. L. 1978. Uptake and metabolism of adenosine by pig aortic endothelial and smooth muscle cells in culture. *Biochem. J.* 170:265–71
62. Dieterle, Y., Ody, C., Ehrensberger, A., Stalder, H.,
Iism and uptake of adenosine triphosphate and adenosine by porcine aortic and pulmonary endothelial cells and fibroblasts in culture. *Circ. Res.* 42:869–76
63. Hellewell, P. G., Pearson, J. D. 1983. Metabolism of circulating adenosine by the porcine isolated perfused lung. *Circ. Res.* 53:1–7
64. Bakhle, Y. S., Chelliah, R. 1983. Metabolism and uptake of adenosine in rat isolated lung and its inhibition. *Br. J. Pharmacol.* 79:509–15
65. Bassingthwaighte, J. B., Wang, C. Y., King, R. B. 1985. Modeling adenosine metabolism by endothelial cells. *Fed. Proc.* 44:1263
66. Dollery, C. T., Junod, A. F. 1976. Concentration of +/− propranolol in isolated perfused lungs of rat. *Br. J. Pharmacol.* 57:67–71
67. Junod, A. F. 1975. Mechanisms of drug accumulation in the lung. In *Lung Metabolism*, ed. A. F. Junod, R. de Haller, pp. 219–31. New York: Academic
68. Rothstein, P., Cole, J., Pitt, B. R. 1984. Pulmonary extraction of bupivacaine is dose-dependent. *Anesthesiology* 61: A236
69. Roerig, D., Bunke, S., Dawson, C. A., Kotrly, K., Kampine, J. P. 1985. Inhibition of Fentanyl uptake in the isolated perfused rat lung by propranolol. *Fed. Proc.* 44:1758
70. Frangos, J. A., Eskin, S. G., McIntire, L. V., Ives, C. L. 1985. Flow effects on prostacyclin production by cultured human endothelial cells. *Science* 227:1477–79
71. Grondelle, A., Worthen, G. S., Ellis, D., Mathias, M. M., Murphy, R. C., et al. 1984. Altering hydrodynamic variables influences PGI_2 production by isolated lungs and endothelial cells. *J. Appl. Physiol.* 57:388–95
72. Utsunomiya, T., Krausz, M. M., Shepro, D., Hechtman, H. B. 1981. Prostaglandin control of plasma and plalelet 5-hydroxytryptamine in normal and embolized animals. *Am. J. Physiol.* 241:H766–71
73. Boura, A. L. A., Murphy, R. D. 1978. Thromboxane B_2 inhibits prostaglandin E_2 inactivation by the rat isolated perfused lung. *Clin. Exp. Pharmacol. Physiol.* 5:387–92
74. Furchgott, R. F. 1984. Role of endothelium in the responses of vascular smooth muscle to drugs. *Ann. Rev. Pharmacol.* 24:175–97
75. Deleted in proof
76. Chand, N., Altura, B. M. 1981. Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular disease. *Science* 213:1376–79
77. McGiff, J. C. 1981. Interactions of prostaglandins with the kallikrein-kinin and

renin-angiotensin systems. *Clin. Sci.* 59:105–16

78. Cohen, M. L., Kurz, K. D. 1982. Angiotensin converting enzyme inhibition in tissues from spontaneously hypertensive rats after treatment with captopril or MK421. *J. Pharmacol. Exp. Ther.* 220:63–69
79. deOliveiraSalgado, M. C., Krieger, E. M. 1983. Acute changes in the renin-angiotensin system modify bradykinin and angiotensin reactivity and metabolism in conscious rats. *Hypertension* 5:V172–76 (Suppl.)
80. Strittmatter, S. M., Kapiloff, M. S., Snyder, S. H. 1983. ^{3}H-Captopril binding to membrane associated angiotensin converting enzyme. *Biochem. Biophys. Res. Commun.* 112:1027–33
81. Howell, R. E., Moalli, R., Gillis, C. N. 1985. Captopril removal by rabbit lung *in vivo*. *Biochem. Pharmacol.* 34:2371–75
82. Turrin, M., Pitt, B. R., Ryan, J. W., Chung, A., Clark, M. B., Gillis, C. N. 1985. Uptake of RAC (N-[1-(S)-carboxy - (4 - OH - 3- ^{125}I - phenyl - ethyl-L-ala-L-pro), an inhibitor of angiotensin converting enzyme (ACE) by rabbit lungs, *in situ*. *Fed. Proc.* 44:1890
83. Mason, G. R., Effros, R. M., Huszcuk, A., Chung, A., Ryan, J. W. 1985. Pulmonary vascular uptake of an angiotensin converting enzyme inhibitor is uninfluenced by hypoxia. *Fed. Proc.* 44:1757
84. Pitt, B. R., Woodford, M., Gillis, C. N. 1985. In-line measurement of pulmonary metabolic function in vivo. *Fed. Proc.* 44:1890
85. Gillis, C. N., Catravas, J. D. 1982. Altered removal of vasoactive substances in the injured lung: Detection of lung microvascular injury. *Ann. NY Acad. Sci.* 384:458–74
86. Gillis, C. N., Pitt, B. R. 1986. The pulmonary microcirculation and metabolic functions of the lung. In *Current Topics in Pulmonary Pharmacology and Toxicology*, ed. M. Hollinger. Philadelphia: Praeger. In press
87. Block, E. R. 1984. Early metabolic changes in response to lung injury: extrapolation from animals to humans. *J. Toxicol. Environ. Health* 13:369–86

87a. Block, E. R., Parel, J. M., Angelides, K. J., Sheridan, N. P., Garg, L. C. 1986. Hyperoxia reduces plasma membrane fluidity: a mechanism for endothelial cell dysfunction. *J. Appl. Physiol.* In press

88. Dobuler, K. J., Catravas, J. D., Gillis, C. N. 1982. Early detection of oxygen-induced lung injury in conscious rabbits: Reduced activity of angiotensin converting enzyme and removal of 5-hydroxytryptamine in vivo. *Am. Rev. Respir. Dis.* 126:534–39
89. Gillis, C. N., Cronau, L. H., Mandel, S., Hammond, G. L. 1979. Indicator dilution measurement of 5-hydroxytryptamine clearance by human-lung. *J. Appl. Physiol.* 46:1178–83
90. Dargent, F., Neidhart, P., Bachmann, M., Suter, P. M., Junod, A. F. 1985. Simultaneous measurement of serotonin and propanolol pulmonary extraction in patients after extracorporeal circulation and surgery. *Am. Rev. Respir. Dis.* 131:242–45
91. Gillis, C. N., Pitt, B. R., Hammond, G. L. 1983. Depressed pulmonary removal of prostaglandin E_1 in patients with respiratory failure. *Circulation* 68:401

91a. Morel, D. R., Dargent, F., Bachman, M., Suter, P. M., Jurod, A. F. 1985. Pulmonary extraction of serotonin and propanolol in patients with adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 132:479–84

92. Pang, J. A., Butland, R. J. A., Brooks, N., Cattell, M., Geddes, D. M. 1982. Impaired lung uptake of propranolol in human pulmonary emphysema. *Am. Rev. Respir. Dis.* 125:194–98
93. Jose, P., Niederhauser, U., Piper, P. J., Robinson, C., Smith, A. P. 1976. Degradation of prostaglandin $F_{2\alpha}$ in the human pulmonary circulation. *Thorax* 31:713–19
94. Rickaby, D. A., Dawson, C. A., Linehan, J. H. 1984. Influence of embolism and imipramine on kinetics of serotonin uptake by dog lung. *J. Appl. Physiol.* 56:1170–77
95. Rickaby, D. A., Linehan, J. H., Bronikowski, T. A., Dawson, C. A. 1981. Kinetics of serotonin uptake in dog lung. *J. Appl. Physiol.* 51:405–14
96. Rickaby, D. A., Dawson, C. A., Linehan, J. H. 1982. Influence of blood and plasma flow rate on kinetics of serotonin uptake by lungs. *J. Appl. Physiol.* 53: 677–84
97. Bronikowski, T. A., Linehan, J. H., Dawson, C. A. 1980. A mathematical analysis of perfusion heterogeneity on indicator extraction. *Math. Biosci.* 52:27–51
98. Bronikowski, T. A., Dawson, C. A., Linehan, J. H., Rickaby, D. A. 1982. A mathematical model of indicator extraction by the pulmonary endothelium via saturation kinetics. *Math. Biosci.* 61: 237–66
99. Linehan, J. H., Dawson, C. A., Wagner-

Weber, V. M. 1981. Prostaglandin E_1 uptake by isolated cat lungs perfused with physiological salt solutions. *J. Appl. Physiol.* 50:428–34

100. Rickaby, D. A., Dawson, C. A., Linehan, J. H. 1984. Kinetics of serotonin uptake by the dog lung is pH independent in the physiological range. *Proc. Soc. Exp. Biol. Med.* 175:361–65
101. Malcorps, C. M., Dawson, C. A., Linehan, J. H., Bronikowski, T. A., Rickaby, D. A., et al. 1984. Lung serotonin uptake kinetics from indicator dilution and constant infusion methods. *J. Appl. Physiol.* 57:720–30
102. Pitt, B. R., Moalli, R., Gillis, C. N. 1984. Apparent kinetics of pulmonary removal of prostaglandin E_1 (PGE_1) after microembolization in anesthetized rabbits. *Am. Rev. Respir. Dis.* 129:A350
102a. Moalli, R., Howell, T. E., Gillis, C.N. 1985. Kinetics of captopril- and enalapril-induced inhibition of pulmonary angiotensin converting enzyme in vivo. *J. Pharmacol. Exp. Ther.* 234:372–77
103. Pitt, B. R., Lister, G. 1984. Kinetics of pulmonary angiotensin-converting enzyme activity in conscious developing lambs. *J. Appl. Physiol.* 57:1158–66
104. Moalli, R., Ashton, J. H., Pitt, B. R., Gillis, C. N. 1983. Effect of airway pressure (P_{aw}) on pulmonary angiotensin converting enzyme (ACE) activity in isolated perfused rabbit lung. *Fed. Proc.* 42: 593
105. Straus, O. H., Goldstein, A. 1943. Zone behavior of enzymes. *J. Gen. Physiol.* 26:559–85
106. Dawson, C. A., Christensen, C. W., Rickaby, D. A., Linehan, J. H., Johnston, M. R. 1985. Lung damage and pulmonary uptake of serotonin in intact dogs. *J. Appl. Physiol.* 58:1761–66
107. Iwasawa, Y., Gillis, C. N. 1974. Pharmacological analysis of norepinephrine and 5-hydroxytryptamine uptake from the pulmonary circulation: Differentiation of uptake uptake sites for each amine. *J. Pharmacol. Exp. Ther.* 188:386–93
108. Block, E. R., Fisher, A. B. 1977. Depression of serotonin clearance by rat lungs during oxygen exposure. *J. Appl. Physiol.* 42:33–38