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Effect of Papain-Induced Emphysema on Permeability of Rat Lung to Drugs[1] (38937)

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Inhalation or intratracheal administration of the proteolytic enzyme papain in rats results in lung damage that morphologically and functionally resembles emphysema (1–6). For example, lesions in papain-damaged lungs include destruction of alveolar septae with subsequent air space enlargement and damage to connective tissue fibers. Functionally, there is an increase in residual air capacity.

Although considerable research has dealt with the pathogenesis of this disease model, little work has been done to investigate the effects of papain-induced damage on lung permeability. The present study in the rat describes quantitatively the pulmonary absorption of four nonvolatile drugs from the papain-damaged lung. The drugs include an acid, a base, a neutral molecule, and a quaternary ammonium compound. Comparison of altered absorption rates with lipoid solubilities of the compounds provides information concerning changes in membrane permeability.

Materials and Methods. Exposure of rats to papain aerosol. Male Charles River-derived rats weighing 150–250 g were randomly divided into two groups: papain-treated and control. Animals from the papain-treated group were exposed to an aerosol of 3% papain solution, while controls breathed an aerosol of distilled water. Exposure to aerosol was for 1 hr, three times a week, for 2 wk. Two identical aerosol chambers were constructed so that experimental and control exposures could be accomplished simultaneously. The chamber consisted of a 65-liter cylindrical plastic container 56 cm high and 38 cm in diameter. An air-jet nebulizer (DeVilbiss no. 42) was inserted into the side of the chamber 10 cm from the top, and an exhaust port was placed on the opposite side 2.5 cm from the bottom. Polyethylene tubing connected the exhaust port to a fume hood. A cylindrical metal animal container 15 cm high and 36 cm in diameter was fashioned so that it contained eight wedge-shaped animal compartments of equal size. A circular piece of hardware cloth was wired across one end of the animal container to form a floor; and a similar piece of hardware cloth was attached to the top by springs so that it could be easily removed for insertion or removal of animals. When the animal container was lowered into the aerosol chamber, it remained 5 cm above the bottom. A shallow aluminum pan placed under the animal container served to catch excreta. A close-fitting plastic lid completed the aerosol chamber. Aerosol was generated at a rate of 0.273 ml/min (SE $\pm$ 0.008) by compressed breathing air delivered to the nebulizer at a flow rate of 10 liters/min. The nebulizer was kept filled with fluid from a reservoir located above the aerosol chamber.

Assessment of lung damage. For histologic assessment of lung damage, sections of lung tissue from papain-treated and control animals were examined by light microscopy. To obtain the tissues, animals were anesthetized with pentobarbital (50 mg/kg) 20–24 hr after the last exposure to aerosol, the trachea was cannulated with PE 240 tubing, and the lungs were inflated under a pressure of 12–13 cm H_2O with 10% formalin in phosphate

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buffer (pH 7.4). After fixation, tissue sections (6 μm) were cut from paraffin blocks and stained with hematoxylin and eosin.

A quantitative assessment of the degree of lung damage was obtained by using an air space counting technique adapted from the system described by Dunnill (7) and Palecek *et al.* (2) as modified by Giles *et al.* (3). Using a projection microscope at a magnification of 290×, a slide was projected onto a grid screen consisting of 30 0.5-cm cross marks located 1.5 cm apart in 5 × 6 rows. Ten fields were chosen at random from each slide, and each field was examined in the following manner. If the cross marks fell on alveolar septae, blood vessels, or connective tissue, there was no score. If, however, a cross mark fell on an empty space, a score of one was given. For each slide, there was a maximum of 300 points possible. Slides of lung tissue from three control and seven papain-treated rats were examined in a single-blind manner by this technique and the scores expressed as percentage air spaces. A higher score (greater percentage air spaces) for lungs from papain-treated rats as compared to controls would be characteristic of emphysema, indicating an enlargement of air spaces, alveolar septal wall destruction, or both.

As an additional means of assessing lung damage, wet and dry tissue weights were measured in rats that had been exposed to aerosol for periods of 1 or 2 wk. Following removal of the lungs from an anesthetized animal, the tissue was blotted on moistened filter paper, minced into tared weighing vials, and then dried at 100° to constant weight.

Procedure for absorption studies. To investigate the effect of papain-induced lung damage on lung permeability, drug absorption rates were measured according to a method described previously (8) in rats that had been exposed intermittently for 2 wk to an aerosol of either 3% papain solution or distilled water (control). Within 20–24 hr after the last exposure to aerosol, animals were anesthetized with pentobarbital (50 mg/kg), and the trachea was exposed through a ventral midline incision in the neck. A 2.5-cm length of PE 240 tubing, which served as a tight-fitting tracheal cannula, was inserted through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage to a depth of 0.6 cm. Solutions (0.1 m*M*) of mannitol-^{14}C and *p*-aminohippuric-^{14}C acid (PAH-^{14}C) were prepared by adding radioactively labeled compound together with unlabeled compound to Krebs-Ringer phosphate solution (pH 7.4), in which Ca ion had been lowered to one-fifth the usual concentration to avoid turbidity (9). Solutions (10 m*M*) of procaine amide and procaine amide ethobromide (PAEB) were prepared by adding drug to the modified Krebs-Ringer phosphate buffer. One-tenth milliliter of drug solution was injected into the lungs through PE 20 tubing attached to a calibrated 100-μl syringe. The injection tubing was inserted through the tracheal cannula to a point 1 mm above the bifurcation of the trachea, the drug solution injected over a 1–2 sec interval, and the tubing quickly withdrawn. The incision in the skin was then closed with a wound clip after drawing the skin up close to the sides of the tracheal cannula. Body temperature was maintained at 37 ± 1° by heat from a 40-W incandescent lamp suspended above the animal.

In experiments with mannitol-^{14}C or PAH-^{14}C, at the end of an absorption period (30–90 min), the lungs and attached trachea were excised from the animal, weighed, and placed in a 15-ml Tenbroeck glass homogenizer together with sufficient distilled water to make a total weight of 6 g. After homogenization, 200-mg samples of the tissue homogenate were transferred into glass liquid scintillation counting vials, and to each vial was added 0.2 ml of 60% perchloric acid together with 0.4 ml of 30% hydrogen peroxide. The vials were then heated at 70° for 1.5 hr. To the resulting tissue digest was added 20 ml of a liquid scintillation medium of the following composition: 6 g 2,5-diphenyloxazole, 573 ml toluene, and 427 ml ethylene glycol monomethyl ether. Radioactivity was measured using a Packard model 3375 Tri-Carb liquid scintillation spectrometer equipped with automatic external standardization. Net counts per minute were corrected for quench by comparison with a standard quench correlation curve deter-

mined for the liquid scintillation medium used. When known amounts of mannitol-^{14}C were added to papain-treated or control lung tissue and the assays carried out as described above, recoveries were complete (99–100%). Tissue recoveries for PAH-^{14}C were 95% (SE ± 2 in three determinations) for papain-treated and control lungs, and results were accordingly corrected for the incomplete recovery.

In experiments with procaine amide or PAEB, the lungs and trachea were placed in a Tenbroeck glass homogenizer with sufficient distilled water to make a total fluid volume (tissue water plus distilled water) of 11 ml (8). After thorough homogenization, 1 ml of concentrated HCl and 1.2 ml of 50% trichloroacetic acid solution were added to precipitate proteins, and the sample was again homogenized. The homogenate was then centrifuged for 20 min at 600*g*. Aliquots of the resulting clear supernatant fluid were assayed for drug according to the colorimetric procedure of Bratton and Marshall (10). When known amounts of procaine amide were added to lung tissue and the assay carried out as described above, drug recovery was 97% for control lungs and 90% (SE ± 1 in three determinations) for papain-treated lungs. The recovery of PAEB was the same in control and damaged lungs (92%, SE ± 1 in five determinations). Results were corrected for the incomplete recoveries.

Statistical evaluations were made using Student's *t* test.

Drugs. D-Mannitol-1-^{14}C (sp act 51.1 mCi/mmole) and *p*-aminohippuric-1-^{14}C acid (sp act 2.52 mCi/mmole) were obtained from New England Nuclear Corp. Unlabeled D-mannitol was obtained from Difco Labs, and *p*-aminohippuric acid from Eastman Organic Chemicals. Procaine amide HCl and procaine amide ethobromide HBr were kindly provided by the Squibb Institute for Medical Research.

Results. Histologic assessment of lung dam-

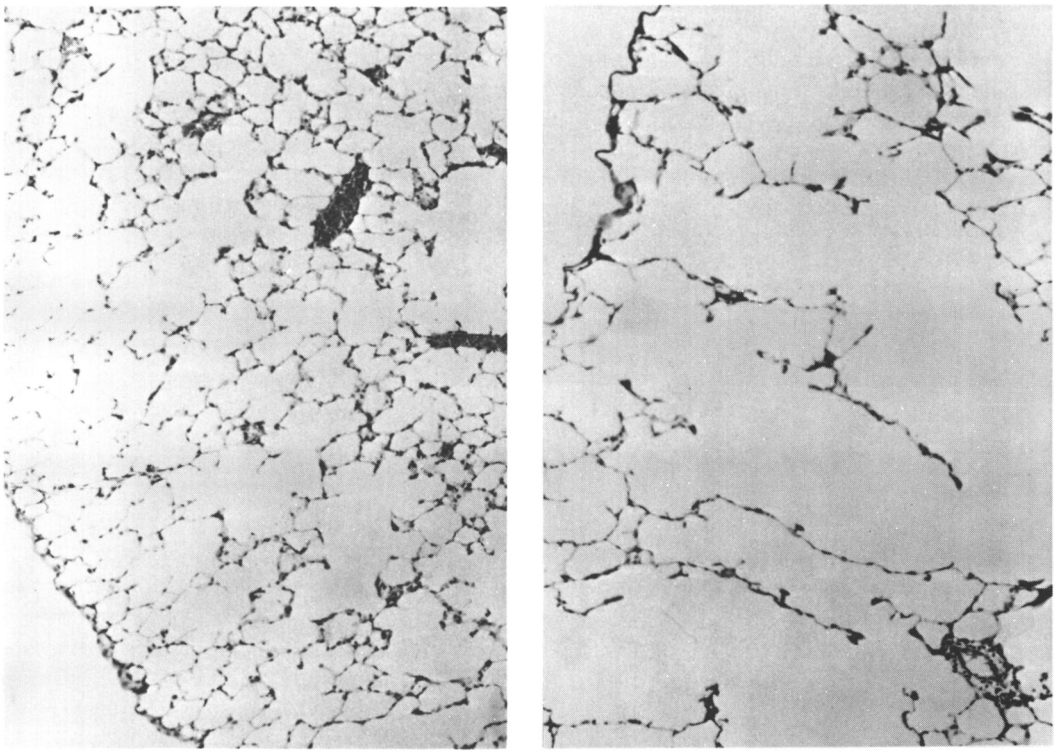

FIG. 1. Rat lung sections (6 μm thick) inflation-fixed and stained with hematoxylin and eosin (magnification 150×). Normal lung from a control rat (left) is compared with lung from a papain-treated rat (right) showing airspace enlargement after 2 wk of intermittent exposure to papain aerosol.

age. In Fig. 1 is shown a representative section of lung tissue from a control rat compared to a similar section of lung tissue at the same magnification taken from a rat treated intermittently with papain aerosol for 2 wk. There is noticeable air space enlargement, characteristic of emphysema, in the lung from the papain-treated animal. In addition, slight perivascular edema and inflammatory cell infiltration are apparent in this section.

Quantitive assessment of lung damage. According to the counting technique used, the air space count was 51% (SE ± 1) in controls and 58% (SE ± 2) in papain-treated rats. The difference was highly significant ($P < 0.001$) and indicated a moderate increase in air spaces of lungs after 2-wk of intermittent exposure to papain aerosol.

Both the dry weight and wet weight of lungs increased about twofold over the 2-wk exposure period (Fig. 2). However, since percentage of lung water remained unchanged (78% of the wet tissue weight), the weight gain was not suggestive of edema; rather, it was consistent with the inflammatory cell infiltration noted above.

Pulmonary drug absorption. Results of pulmonary absorption studies with mannitol-^{14}C, PAH-^{14}C, and PAEB in control and papain-treated rats are shown in Figs. 3–5. When the percentage of unabsorbed drug was plotted semilogarithmically against time, the data for each compound conformed to a straight line, suggesting first-order kinetics for the absorption process. Half-times for absorption and apparent first-order rate constants, calculated from the slopes of the lines, are listed in Table I. It can be seen that rats treated with papain aerosol absorbed all three drugs roughly twice as rapdly as rats treated with distilled water aerosol (control). For example, absorption rate constants for the drugs increased 1.5- to 2.1-fold in papain-treated animals as compared to controls.

In marked contrast to the above results, the pulmonary absorption of procaine amide was similar in controls and papain-treated animals. For example in nine control rats, the 3 min absorption amounted to 59% (SE ± 2) of the dose, and in eight papain-treated rats the 3 min absorption was 57% (SE ± 3) of the dose.

Discussion. It has been demonstrated previously by several investigators (1–6) that administration of papain into the respiratory tract of rats results in lung damage morphologically resembling emphysema. Perhaps the most characteristic feature of this disease model is an enlargement of air spaces in the damaged lungs. For example, Giles *et al.* (3) reported that intermittent exposure of male rats to an aerosol of papain solution for

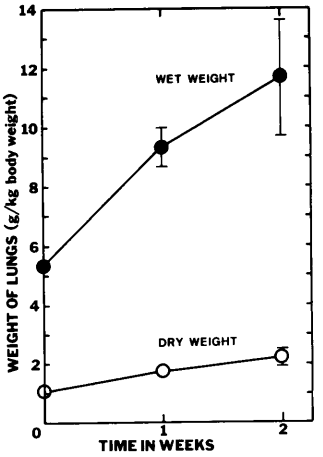

FIG. 2. Wet weight and dry weight of lungs from rats intermittently exposed to papain aerosol for 1 or 2 wk. Control values (distilled water aerosol) after 1 or 2 wk were not significantly different from 0-time values. Each point represents the mean value for 6–12 animals. Brackets indicate SE wherever large enough to be shown.

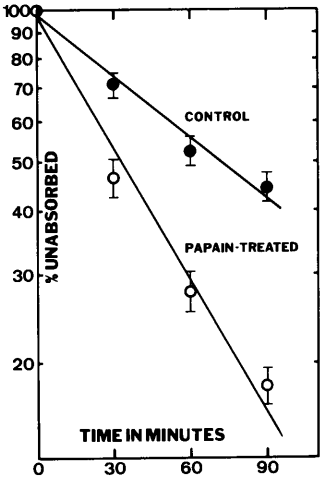

FIG. 3. Pulmonary absorption of 0.1 m*M* mannitol-^{14}C in rats intermittently exposed to papain aerosol or distilled water aerosol (control) for 2 wk. Each point represents the mean ± SE for seven animals.

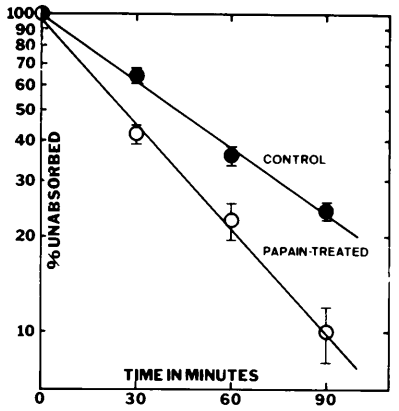

FIG. 4. Pulmonary absorption of 0.1 m*M* PAH-^{14}C in rats intermittently exposed to papain aerosol or distilled water aerosol (control) for 2 wk. Each point represents the mean ± SE for four animals.

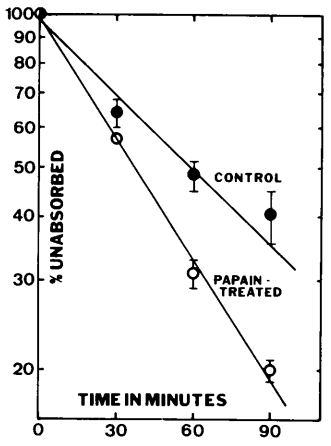

FIG. 5. Pulmonary absorption of 10 m*M* PAEB in rats intermittently exposed to papain aerosol or distilled water aerosol (control) for 2 wk. Each point represents the mean value for 4–12 animals. Brackets indicate SE wherever large enough to be shown.

4 wk resulted in a lung air space of 64% as compared to a space of 43% in controls. Results of the present investigation confirm these observations and also show that the emphysema-like lesion of increased air space may be produced after 2 wk of similar treatment.

Regarding the permeability of the papain-damaged lung to drugs, it is helpful to consider first the permeability characteristics of the normal organ. Recent work in this laboratory (8, 9) has suggested that the normal respiratory tract epithelium of the rat has permeability characteristics similar to

TABLE I. ABSORPTION OF COMPOUNDS FROM PAPAIN-DAMAGED AND CONTROL RAT LUNGS

	Rate of absorption[a]			
	Half-time (min)		Rate constant × hr^{-1}	
Compound	Control	Papain	Control	Papain
Mannitol-^{14}C	74	35[b]	0.638	1.350[b]
PAH-^{14}C	43	26[c]	0.961	1.638[c]
PAEB	62	38[c]	0.743	1.132[c]

[a] Half-time values were calculated from the slopes of the lines in Figs. 3–5. Apparent first-order rate constant is equal to 0.693 divided by the half-time in hours.

[b] Significantly different from control $P < 0.005$.

[c] Significantly different from control $P < 0.001$.

those of the classical lipoid-pore type of biologic membrane (11). For example, lipoid-insoluble substances such as mannitol, PAH, and PAEB, appear to be absorbed from the lung mainly by diffusing through aqueous pores in the pulmonary membrane. In contrast, a lipoid-soluble drug like procaine amide appears to be absorbed by diffusing through both the pores and the lipoid regions of the absorbing membrane. Since pores are generally thought to occupy only a small fraction of the total surface area of biologic membranes (12), the proportion of a lipoid-soluble drug absorbed by the pore route may be almost negligible. Accordingly, if lung damage resulted in an increased porosity (increased pore area) for the air–blood barrier, the rate of absorption of lipoid-insoluble drugs would be expected to be increased much more than that of lipoid-soluble compounds. In accord with this view, results of the present study suggest that the porosity of the pulmonary membrane is increased by treatment with papain aerosol, since, after exposure of animals to the proteolytic enzyme, mannitol-^{14}C, PAH-^{14}C, and PAEB showed about a twofold increase in absorption rate, whereas procaine amide showed no change.

The failure to find an altered absorption rate for procaine amide tends to rule out the possibility that the increased absorption of lipoid-insoluble compounds is due to a papain-induced change in lung intraluminal distribution of administered drug solution.

Any such change in distribution would be the same for all solutes and would affect all absorption rates in a like manner. Similar reasoning would tend to eliminate the possibility that the increased absorption rates for the lipoid-insoluble drugs reflected a change in membrane thickness, coating layers, or surface area. Again, any such changes would be expected to affect the absorption of lipoid-soluble and lipoid-insoluble compounds similarly.

In conclusion, in view of the similarities between papain-induced lung damage and emphysema, results of the present study could have important implications in therapeutics, toxicology, and environmental health, since enhanced systemic absorption of inhaled drugs and airborne chemicals could well occur as a consequence of this lung disease.

Summary. To investigate the effect of a papain-induced emphysema-like condition on pulmonary absorption of drugs, rats were exposed to either papain aerosol or distilled water aerosol (control) intermittently for 2 wk, and rates of drug absorption from damaged and control lungs were compared. To measure absorption rates, 0.1 ml of drug solution (0.1–10 m*M*) was administered through a tracheal cannula to anesthetized animals, and after various times lungs were assayed for unabsorbed compound. In absorption experiments with the lipoid-insoluble compounds, mannitol, *p*-aminohippuric acid, and procaine amide ethobromide, all three drugs were absorbed from the lungs about twice as rapidly in papain-treated rats as in control. In contrast, procaine amide, a relatively lipoid-soluble drug, was absorbed at the same rate in both control and papain-treated animals. The results suggest that papain-induced lung damage increases the porosity of the pulmonary epithelium.

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