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Absorption of Heparin and Cyanocobalamin from the Rat Lung[1] (39400)

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The respiratory tract is a frequently used site for the administration of a variety of volatile and nonvolatile therapeutic agents (1, 2). In the case of nonvolatile drugs, the intention of inhalation therapy has usually been that the compounds act locally within the lungs. However, in a few instances, nonvolatile agents have been administered into the respiratory tract with the intention that they exert a systemic action. For example, heparin, a substance that is poorly absorbed from the gastrointestinal tract and oral cavity (3-6), has been shown to produce a systemic anticoagulant effect when inhaled as an aerosol by normal human subjects (7). Moreover, cyanocobalamin (vitamin B_{12}), a substance which requires the presence of intrinsic factor for rapid intestinal absorption and which is very poorly absorbed from the gastrointestinal tract in the absence of intrinsic factor (8), has been shown to be therapeutically effective in the treatment of pernicious anemia when administered to patients by inhalation of powders or liquid aerosols containing the vitamin (9, 10).

From the work cited above, it is not clear whether absorption of inhaled heparin and cyanocobalamin occurs by diffusion or by a more complex transport process. Moreover, the studies provide no information on the rate of absorption of these substances from the respiratory tract. In the present investigation in the rat, quantitative evidence is presented that describes the mechanisms and relative rates of pulmonary absorption of heparin and cyanocobalamin.

Materials and methods. Procedure in animals. To investigate the absorption of heparin and cyanocobalamin from the lung, male Charles River-derived rats (150-200 g) were anesthetized with pentobarbital (50 mg/kg) and prepared surgically according to a method described previously (11). Briefly, 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 of heparin (0.001-1 m*M*) or cyanocobalamin (0.01-1 m*M*) 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 (12). One-tenth milliliter of 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 approximately 1 mm above the bifurcation of the trachea, the 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 and body temperature maintained at 37 ± 1° by heat from a 40-W incandescent lamp suspended above the animal.

Tissue extraction and drug assay. At the end of an absorption period (0.25-12 hr), the lungs, with attached trachea and cannula, were excised from the animal. The tissue was weighed and placed in a 15-ml Tenbroeck glass homogenizer together with washings from the tracheal cannula and sufficient distilled water to make a total weight of 4 g. After homogenization, 100-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 1-1.5 hr. To the resulting tissue digest was added 20 ml of a

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liquid scintillation medium of the following composition: 6 g of 2,5-diphenyloxazole, 573 ml of toluene, and 427 ml of 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 determined for the liquid scintillation medium used. It was assumed that no radioactivity was lost by animal expiration. When known amounts of compound were added to lung tissue and the assays carried out as described above, recoveries (± SE in three determinations) were essentially complete: heparin 99 ± 1% and cyanocobalamin 98 ± 2%.

Diffusion coefficients. For the following compounds, coefficients of free diffusion (water, 37°), as measured by the method of Schantz and Lauffer (13), have been reported previously: mannitol, sucrose, inulin, and dextran (14); erythritol (11); and urea (15). Diffusion coefficients for sulfanilic acid, *p*-aminohippuric acid (PAH), tetraethylammonium (TEA), procaine amide ethobromide cation (PAEB), and *p*-acetylaminohippuric acid (PAAH) were calculated previously (12) from the Stokes-Einstein equation (16, 17). Diffusion coefficients for heparin and cyanocobalamin were calculated in the present study from the Stokes-Einstein equation, and densities used in the calculations were determined by air displacement using a Fekrumeter (Gallard-Schlesinger Co.).

Drugs. Radioactively labeled [^{35}S]heparin, specific activity 3.23 mCi/g (64.6 mCi/mmole based on a mol wt of 20,000), and [G-^{3}H]cyanocobalamin, specific activity 1.65 Ci/mmole, were obtained from Amersham-Searle. Unlabeled heparin sodium was obtained from Fisher Scientific Co., and cyanocobalamin from Sigma Chemical Co. With the above radioactively labeled compounds, it has been shown by the manufacturer using electrophoresis that the labeled heparin has a radiochemical purity greater than 95% and the labeled cyanocobalamin a radiochemical purity greater than 98%.

Results and discussion. Results of pulmonary absorption studies with heparin and cyanocobalamin are shown in Fig. 1. When the percentage of unabsorbed radioactivity 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 the time required for 50% of a dose to be absorbed was 3 hr for cyanocobalamin and 9.2 hr for heparin.

Evidence that both compounds are absorbed by a nonsaturable transport process, such as simple diffusion, was seen when absorption rates were measured over a wide range of initial concentrations. As shown in Table II, when the concentration of cyanocobalamin was varied 100-fold, from 0.01 to 1 m*M*, the 2-hr absorption rate was directly proportional to concentration, the percentage absorption remaining essentially constant. Similarly, concentration studies with 0.001-1 m*M* heparin showed no evidence of saturation over the 1000-fold

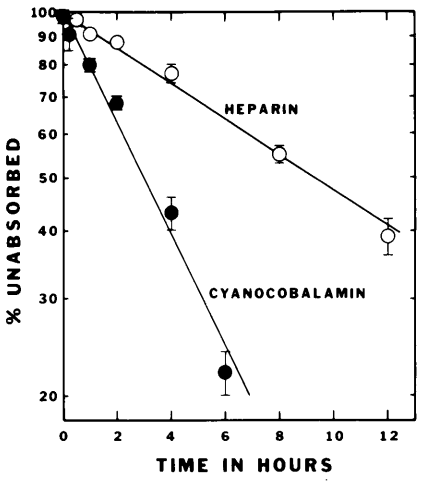

FIG. 1. Absorption of radioactivity from the rat lung after administration of [^{35}S]heparin and [^{3}H]cyanocobalamin. Krebs-Ringer phosphate solution (0.1 ml), containing a compound (0.1 m*M*), was administered intratracheally to anesthetized rats prepared with a tight-fitting tracheal cannula. Each point is the mean of three to six animals. Vertical brackets indicate SE, and absence of brackets indicates that SE was too small to be shown.

TABLE I. ABSORPTION OF RADIOACTIVITY FROM THE RAT LUNG AFTER ADMINISTRATION OF [^{3}H]CYANOCOBALAMIN AND [^{35}S]HEPARIN.

Compound	Mol wt	Diffusion coefficient (D, 37°) cm^2/sec × 10^6	Rate of absorption[a]	
			Half-time (hr)	Rate constant × hr^{-1}
Cyanocobalamin	1,355	4.33	3.0	0.231
Heparin	6,000-20,000	2.87-1.93[b]	9.2	0.0753

[a] Apparent first-order rate constant is equal to 0.693 divided by the half-time in hours. Half-time values were calculated from the curves shown in Fig. 1.

[b] Based on a molecular weight range of 6,000-20,000 (18, 20, 21).

TABLE II. EFFECT OF CONCENTRATION ON ABSORPTION OF RADIOACTIVITY FROM THE RAT LUNG AFTER ADMINISTRATION OF [^{3}H]CYANOCOBALAMIN AND [^{35}S]HEPARIN.

Compound	Time for absorption (hr)	Percentage of dose absorbed[a]			
		Concentration (mM)			
		0.001	0.01	0.1	1.0
Cyanocobalamin	2		35 ± 1	33 ± 2	39 ± 3
Heparin	8	43 ± 4		45 ± 2	46 ± 2

[a] Krebs-Ringer phosphate solution (0.1 ml), containing various concentrations of a compound, was administered intratracheally to anesthetized rats prepared with a tracheal cannula. Each absorption value is the mean of three animals ± SE.

range, since percentage absorption remained constant at 43-46% (Table II).

Previous work in this laboratory (11, 12) has suggested that the respiratory tract epithelium of the rat has permeability characteristics similar to those of the classical lipoid-pore type of biologic membrane. Lipoid-soluble compounds are absorbed more rapidly than lipoid-insoluble compounds, and the rates of absorption are generally related to the lipid/water partition coefficients of the compounds. Lipid-insoluble substances appear to be absorbed primarily by restricted diffusion through aqueous pores in the pulmonary membrane at rates inversely related to their molecular size. Moreover, a comparison of pulmonary absorption rates (apparent first-order rate constants, k) with diffusion coefficients (D, in water at 37°) for urea and some saccharides suggested that there is at least three different sizes of pores in the absorbing membrane (11). Since both cyanocobalamin and heparin are lipoid-insoluble compounds (18, 19) and would accordingly depend primarily on the pore route for their absorption, it is of interest to compare their k and D values with similar values obtained previously for certain other lipoid-insoluble molecules of widely different molecular size. In Fig. 2, k values reported previously (11, 12) for several compounds of molecular weight 60 to 75,000 are plotted semilogarithmically against D values for the same compounds. From these data, it should be possible to predict with some degree of accuracy the pulmonary absorption rate for most other lipoid-insoluble compounds that are absorbed mainly by diffusion through membrane pores. When k and D values for heparin and cyanocobalamin are plotted on this figure, it can be seen that the points for both compounds appear on or very near the drawn curve. Thus, the observed absorption rates for heparin and cyanocobalamin are about what would be predicted on the basis of their calculated diffusion coefficients.

Summary. Solutions of heparin and cyanocobalamin were administered through a tight-fitting tracheal cannula into the lungs of anesthetized rats. The times necessary for 50% absorption from the lungs were 3 hr for cyanocobalamin and 9.2 hr for heparin. The absorption process did not become saturated when drug concentrations were raised 100 to 1000-fold, suggesting that both compounds are absorbed mainly by a process of simple diffusion. A comparison of pulmonary absorption rates and diffusion coefficients of heparin and cyanocobalamin with corresponding values reported previously for other lipoid-insoluble compounds

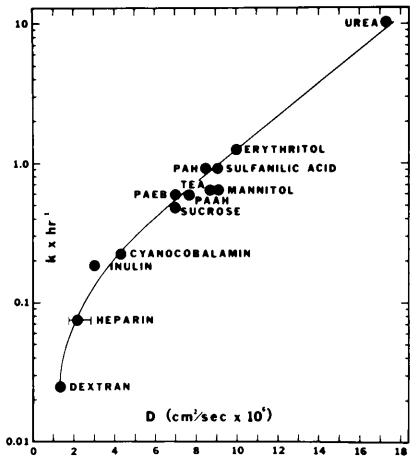

FIG. 2. Comparison of pulmonary absorption rate constants ($k \times hr^{-1}$) with diffusion coefficients (D, $cm^2/sec \times 10^6$) for various lipoid-insoluble compounds. Rate constants for all compounds were measured in this laboratory and, except for heparin and cyanocobalamin, have been reported previously (11, 12). Rate constants for the latter two drugs are taken from Table I. Diffusion coefficients for the compounds were either measured or calculated (see Materials and Methods section for details). The diffusion coefficient for heparin was calculated for a molecular weight of 13,000, and horizontal brackets indicate values calculated for a molecular weight range of 6,000–20,000 (18, 20, 21). An estimated curve of best fit has been drawn through the points.

indicated that observed absorption rates were close to the rates predicted for the two drugs based on their calculated diffusion coefficients and the view that absorption occurs mainly through membrane pores.

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