THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 183, No. 2

Printed in U.S.A.

UPTAKE, METABOLISM AND EFFLUX OF ^{14}C-5-HYDROXYTRYPTAMINE IN ISOLATED PERFUSED RAT LUNGS[1]

ALAIN F. JUNOD[2]

Cardiovascular Research Institute, University of California at San Francisco, San Francisco, California

Accepted for publication May 12, 1972

ABSTRACT

JUNOD, ALAIN F.: Uptake, metabolism and efflux of ^{14}C-5-hydroxytryptamine in isolated perfused rat lungs. J. Pharmacol. Exp. Ther. **183:** 341–355, 1972.

The uptake, metabolism and efflux of ^{14}C-5-hydroxytryptamine (5-HT) were studied in isolated rat lungs, artificially ventilated and perfused *via* the pulmonary artery with Krebs' medium containing 5 mM glucose and 4.5% bovine serum albumin. Infusion of 0.11 to 17 μM ^{14}C-5-HT resulted in concentration of radioactivity in the lungs. The uptake was a saturable process with the same K_m and V_{max} whether the main product found in the lungs was 5-hydroxyindoleacetic acid (control conditions) or 5-HT (treatment with iproniazid, an inhibitor of monoamine oxidase). This finding indicates that the rate-limiting step in the uptake of ^{14}C-5-HT was its intracellular transport, not its subsequent metabolism. Once taken up, ^{14}C-5-HT was located in the soluble fraction of lung homogenate. Cocaine, imipramine, chlorpromazine and cold markedly inhibited the pulmonary uptake of ^{14}C-5-HT; anoxia, norepinephrine and tryptamine had only a partial inhibitor effect. Pretreatment with reserpine or perfusion with glucose-free medium had no effect on the uptake. Changes in the ionic composition of the medium were also studied: the absence of Na^+ resulted in nearly complete inhibition of concentration of ^{14}C-5-HT in the lungs, and lowered Na^+, as well as increased K^+, caused a decrease in the affinity of the transport system for ^{14}C-5-HT. Ouabain at 10^{-3} M had only a partial inhibitory effect. The efflux of ^{14}C-5-HT from the lungs was accelerated by cocaine, imipramine or chlorpromazine, by the absence of extracellular Na^+ and by low extracellular Na^+. These results indicate that the mechanisms of uptake of 5-HT by perfused lungs, platelets and brain are similar and compatible with the model of Na^+-dependent transport; however, the lungs, unlike the other cellular systems, do not store 5-HT, but metabolize it rapidly.

In the past few years, several reviews have stressed the function of the lungs as a metabolic organ, capable of activating or inactivating various hormones and mediators (Heinemann and Fishman, 1969; Said, 1968; Vane, 1969). One of the substances removed from the pulmonary cir-

Received for publication June 14, 1971.
[1] This work was supported by Program Project Grant HE 06285 from the National Heart and Lung Institute.

Send reprint requests to: Alain F. Junod, Cardiovascular Research Institute, University of California Medical Center, San Francisco, Calif. 94122.

[2] Recipient of Public Health Service International Post-Doctoral Fellowship 5 FO5 TW 1464 and of the R. C. Kirkwood Memorial Fellowship of the San Francisco Bay Area Heart Research Committee.

culation is 5-hydroxytryptamine (5-HT), or serotonin. The first conclusive report on the disappearance of 5-HT from the pulmonary circulation (Gaddum *et al.*, 1953) has since been confirmed several times (Eiseman *et al.*, 1964; Davis and Wang, 1965). Thomas and Vane (1967), studying the dog lung *in vivo*, and Alabaster and Bakhle (1970), studying the rat lung *in vitro*, found that approximately 95% of the 5-HT infused was inactivated during its passage through the pulmonary circulation. However, the techniques used, generally based on bioassay, allowed only partial and indirect studies on the pulmonary uptake and metabolism of 5-HT. The present experiments were undertaken, therefore, to investigate the uptake, metabolism and disposal of 5-HT in isolated perfused rat lungs over a large range of concentrations. To characterize the nature of the uptake of 5-HT by the lungs, the effect of drugs known to be inhibitors of 5-HT uptake in platelets and brain and the effects of temperature, Na^+, K^+, ouabain and deletion of glucose were also studied. In view of the similarities found between the uptake of 5-HT by the lungs and the uptake of 5-HT and norepinephrine (NE) by other cellular systems in which a Na^+-dependent transport has been demonstrated (Bogdanski *et al.*, 1968; Bogdanski and Brodie, 1969; Tissari *et al.*, 1969; Sneddon, 1969), studies on the effects of ions, Na^+ in particular, were undertaken to determine whether the uptake of 5-HT by the lung is also Na^+-dependent.

Methods

Media and solution used. The standard perfusion medium was Krebs-Ringer-bicarbonate buffer (Umbreit *et al.*, 1964), containing 5 mM glucose and 4.5% bovine serum albumin, Cohn fraction V. Its composition (millimolar concentration) was: NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; KH_2PO_4, 1.19; $MgSO_4$, 1.19; and $NaHCO_3$, 25. The pH of the albumin solution was adjusted by the addition of 1 N NaOH so that the final Na^+ concentration of the standard medium was 161 mM. The medium, pH 7.4, was equilibrated with 5% CO_2 and 95% oxygen. In some experiments, NaCl was replaced by isotonic amounts of sucrose (0.25 M) or LiCl, and $NaHCO_3$ by Tris (25 mM), the pH of the final solution being adjusted to 7.4 by adding 1 N HCl. Na^+-free albumin was obtained by bringing the pH of a 20% albumin solution to 4.8 to 5.0 with 1 N HCl and dialyzing the solution repeatedly against twice-distilled water at 4°C. The only Na^+ contamination was the presence of NaCl in the stock solution of ^{14}C-5-HT. Since the stock solution was diluted 300 times before use, the final Na^+ concentration during the infusion of ^{14}C-5-HT in a "Na^+-free" medium was about 0.5 mM. To obtain a K^+-free medium, KH_2PO_4 and KCl were replaced by the equivalent Na^+ salts in equimolar amount. To obtain a K^+-rich medium, KCl was added in suitable amount, and the sucrose or NaCl concentration was decreased accordingly.

5-Hydroxytryptamine-3′-^{14}C creatinine sulfate (56 and 58 mc/mmol, Amersham-Searle, Arlington Heights, Ill.) was dissolved in saline solution containing 0.1% ascorbic acid and kept at −20°C. Solutions for infusion were made up of 0.2 to 2.0 μc of ^{14}C-5-HT in a known volume of perfusion medium; unlabeled 5-HT was added when high concentrations were desired. 5-HT creatinine sulfate, ouabain, *l*-norepinephrine bitartrate, reserpine, cocaine HCl and tryptamine HCl were obtained from commercial sources. Imipramine HCl was donated by Geigy Pharmaceuticals (Ardsley, N.Y.), iproniazid phosphate by Hoffmann-La Roche Inc. (Nutley, N.J.) and chlorpromazine HCl by Smith Kline and French Laboratories (Philadelphia, Pa.). Unless otherwise specified, the drugs, when used, were added to the perfusion medium.

Perfusion. Male Sprague-Dawley rats, weighing 250 to 300 g, were used. After anesthesia with Na pentobarbital, 50 mg/kg injected i.p., tracheostomy was performed and the rat was artificially ventilated. The tidal volume was 2.5 ml, the rate of breathing was 60/min and the end-expiratory pressure was +2 cm H_2O. The gas mixture used was 5% CO_2 and 95% oxygen. The thorax was opened and a loose ligature was placed around the pulmonary artery and the aorta. A cannula was then inserted into the pulmonary artery through the right ventricle and secured by tying the ligature. Immediately afterward, the left ventricle was cut and a cannula was inserted for the collection of effluent. The lungs were removed and placed in a closed chamber, kept at 35°C and saturated with water vapor. The whole procedure usually took less than three minutes and was accomplished without interruption of ventilation and circulation. A peristaltic pump assured a perfusion flow of 10 ml/min. A constant infusion-withdrawal pump was used to infuse the solution containing ^{14}C-5-HT at the rate of 1 ml/min at a point just before the intake of the peristaltic pump and to withdraw at the same rate a representative sample of the solution between the peristaltic pump and the pulmonary artery. Therefore, during the infusion period, the flow through the pulmonary circulation was reduced to 9 ml/min. The lungs were perfused with standard medium or with medium of different

ionic composition for 10 minutes (equilibration period); ^{14}C-5-HT was then infused with the medium for one to eight minutes. The effluent and the representative sample of the inflow were collected during the infusion period. After the infusion was completed, the lungs were dissected, blotted dry, weighed and homogenized at 4°C; 60 to 90 seconds elapsed between the end of the perfusion and the homogenization.

Analytical procedures. The lungs were homogenized in 6 ml of methanol–acetone (1:1). Part of the homogenate (0.1–0.2 ml) was taken for counting radioactivity. It was evaporated, taken up with 0.2 ml of water and dissolved in 1 or 1.5 ml of NCS solubilizer. The rest of the homogenate was centrifuged at 10,000 × *g* for 30 minutes at 4°C. A sample of the supernatant fluid was evaporated under a stream of nitrogen and the dry residue was taken up with 0.2 ml of 95% ethanol and centrifuged for 10 minutes at 1,000 × *g* to remove the turbidity. A sample of the supernatant fluid was then analyzed by thin-layer chromatography.

Samples of the inflow and the effluent were also dissolved in NCS solubilizer for counting radioactivity. A second portion was extracted with 2 volumes of methanol–acetone (1:1) and centrifuged at 10,000 × *g* for 30 minutes at 4°C. The supernatant fluid, concentrated under a stream of nitrogen when necessary, was submitted to chromatographic analysis.

Ten milliliters of scintillation fluid [7 g of 2,5-diphenyloxazole (PPO) and 0.1 g of *p*-bis(*o*-methylstyryl)-benzene (bis-MSB) per liter of toluene] were added to the samples of lung homogenate, inflow and effluent. Radioactivity was measured in a Packard scintillation counter. Counting efficiency and quenching were monitored by the addition of an internal standard and results were corrected accordingly.

The separation of 5-HT from its metabolites was carried out by thin-layer chromatography on glass plates precoated with Silica-Gel F 254. The samples were spotted with a standard carrier mixture containing 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), N-acetyl serotonin, melatonin, 5-hydroxytryptophol, 5-methoxyindoleacetic acid and 5-methoxytryptophol. The spots were detected with an ultraviolet lamp and scraped and the material was collected in scintillation vials containing 10 ml of a scintillation mixture (7 g of PPO, 1 g of bis-MSB and 40 g of Cab-O-Sil per liter of toluene). Since it soon became obvious that, beside 5-HT, the only important compound present was 5-HIAA, only the spots corresponding to these substances were specifically scraped; the radioactivity of the remaining part of the chromatogram was measured to make sure that no significant amount of radioactivity remained undetected. The solvent system most often used was methylacetate–isopropanol–ammonia (45:35:20) (Stahl and Kaldewey, 1961); a second system, chloroform–methanol–glacial acetic acid (97:7:1) (Klein and Notides, 1969) was used only occasionally, together with the first, to confirm the identification of 5-HT and 5-HIAA. No additional quenching of the radioactivity could be detected with the added Cab-O-Sil silica gel in these samples.

Subcellular location of ^{14}C-5-HT. After perfusion with standard medium containing 5 × 10^{-4} M iproniazid, an inhibitor of monoamine oxidase (MAO), and the subsequent infusion of 1.1 μM ^{14}C-5-HT for three minutes, the lungs were homogenized in 10 volumes of 0.25 M sucrose at 4°C. The subcellular fractions were then separated by differential centrifugation according to Thoa *et al.* (1969). The pellets corresponding to the different subcellular fractions and the final supernatant fluid were dissolved in NCS solubilizer, and the radioactivity of each fraction was calculated, after correction for quenching using internal standards.

Efflux of ^{14}C-5-HT from the lungs. The efflux of ^{14}C-5-HT was determined as follows. After perfusion for 10 minutes with standard medium containing 5 × 10^{-4} M iproniazid, the pulmonary circulation was infused for a three minute period with 1.1 μM ^{14}C-5-HT, during which a representative sample of the inflow and the effluent were collected. The lungs were then perfused for an additional 12-minute period with the standard medium (control experiments), with standard medium containing the test drug or with medium of different ionic composition, and samples of the effluent were collected for 15 seconds at selected times (1, 2, 4, 8 and 12 minutes).

The uptake of ^{14}C-5-HT by the lungs was calculated from the difference in the radioactivity of an aliquot (0.2 ml) of the inflow and of the effluent during the infusion period. The efflux of ^{14}C-5-HT measured during the subsequent period from an aliquot (0.2 ml) of the collected samples was expressed as a percentage of that uptake. This procedure was considered satisfactory since the gradient of radioactivity between inflow and effluent during the infusion of ^{14}C-5-HT was the same in all the series of experiments. This estimated value of uptake could therefore be taken as a reference to which the radioactivity released during the subsequent period could be related. The points obtained were analyzed as a function of time and were found to follow a single exponential function (r = 0.99 in all cases). Thus it was possible to calculate the amount of radioactivity released from the lungs during the 12-minute period by integrating the exponential function over this period.

Results

The weight of the lungs after perfusion, 1.65 ± 0.04 g (mean ± S.E. of 38 experiments), was comparable to the weight of the lungs immediately after excision, which indicates that no significant pulmonary edema had developed. Perfusions for as long as 90 minutes under the same conditions did not result in gain in weight of the lungs or in increase in pulmonary arterial pressure. Preliminary experiments had showed that the presence of bovine serum albumin in the perfusion medium was required to prevent pulmonary edema. With other plasma expanders, such as low molecular weight dextran or polyvinylpyrrolidone, both at 6%, pulmonary edema occurred within 5 to 10 minutes.

The recovery of ^{14}C in the supernatant after methanol–acetone extraction and centrifugation was 70% for the lung tissue and 93% for the inflow or effluent. The percentage of recovery of 5-HT from various tissues after one single extraction is generally reported to be of this order of magnitude (Gal *et al.*, 1964; Jonsson and Lewander, 1970; Tyce *et al.*, 1968). Binding to protein is commonly assumed to be responsible for the incomplete recovery. The measurements of uptake did not have to be corrected for that incomplete recovery since they were calculated directly from the measurement of the radioactivity of the lung homogenate. The radioactivity recovered in the lung extract was assumed, however, to be representative of the total radioactivity taken up by the lungs. This assumption is supported by the fact that the same percentage was recovered when the main product found in the lungs was either 5-HT or 5-HIAA.

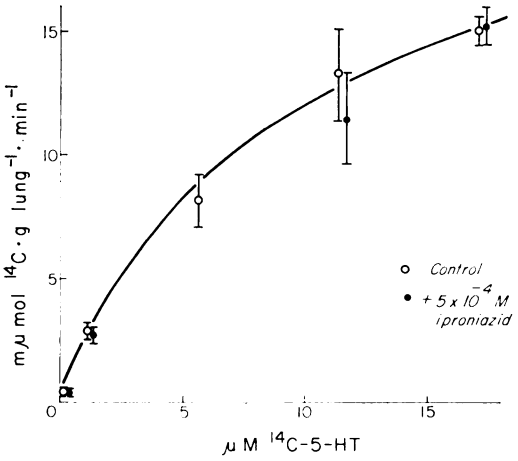

FIG. 1. Relationship between the concentration of ^{14}C-5-HT in the perfusion medium (micromolar concentration) and the rate of uptake of ^{14}C by the lungs (millimicromoles·gram $lung^{-1} \cdot min^{-1}$) under control conditions and in the presence of 5×10^{-4} M iproniazid. ^{14}C-5-HT was infused for three minutes after a 10-minute equilibration period. Iproniazid was present in the perfusion medium during the duration of the experiment. Each point represents the means of at least four experiments ± S.E.

The accumulation of radioactivity in the lungs after a three-minute infusion of 0.11 to 17 μM of ^{14}C-5-HT under control conditions and in the presence of iproniazid at 5×10^{-4} M is shown in figure 1. The relationship between the concentration of 5-HT in the perfusion medium and the accumulation of radioactivity in the lungs suggests a saturable process. Table 1 shows the distribution of the radioactivity in the lungs and in the effluent. Under the control conditions, the lung was able to metabolize almost all the ^{14}C-5-HT taken up, since only 4 to 8% of the radioactivity was accounted for by 5-HT, whereas 5-HIAA accounted for 80% or more. No attempt was made to characterize further the remaining radioactivity. Control experiments showed that the same degree of oxidation of 5-HT was found in extracts of lungs homogenized immediately after the end of the perfusion and that the procedures of homogenization and extraction did not result in the breakdown of the ^{14}C-5-HT added to a lung homogenate.

In the effluent, 80 to 97% of the radioactivity recovered was accounted for by 5-HT, depending on the uptake. 5-HIAA accounted for about half the remaining radioactivity. The fact that up to 20% of the radioactivity recovered in the effluent was not attributable to 5-HT can be explained by the early released of metabolites of 5-HT from the lungs. Since the effluent, when the largest fraction was taken up, contained approximately 50% of the radioactivity infused (table 2), the measured uptake of ^{14}C-5-HT was underestimated by 10% at most.

When iproniazid was added to the perfusion medium, MAO was nearly completely blocked. In these experiments (table 1), 5-HT accounted for more than 95% of the radioactivity in the lungs and for 93 to 100% of the radioactivity recovered in the effluent. This finding indicates that the radioactivity in the lungs and the effluent that could not be attributed to 5-HT or 5-HIAA resulted from the action of MAO and could be a glucurono- or sulfo-conjugate of 5-HIAA. More important, the uptake of ^{14}C-5-HT in the pres-

TABLE 1

Uptake and metabolism of ^{14}C-5-HT in the lungs under control conditions and after the addition of iproniazid

After a 10-minute equilibration period, lungs were perfused for three minutes with ^{14}C-5-HT. In some experiments, iproniazid (5×10^{-4} M) was added to the perfusion medium. Numbers in parentheses refer to the number of experiments. Results are expressed as means ± S.E.

^{14}C-5-HT Conc.	Perfusion Medium	Lung			Effluent Recovered as 5-HT
		Uptake	Recovered as 5-HT	Recovered as 5-HIAA	
μM		*mμmol/g wet weight*	*% of uptake*	*% of uptake*	*% of effluent radioactivity*
0.11	Control (4)	1.14 ± 0.08	4	84	80
	+ Iproniazid (4)	1.09 ± 0.17	95	2	93
1.11	Control (8)	8.69 ± 0.94	7	83	80
	+ Iproniazid (5)	8.25. ± 0.86	97	2	98
11	Control (5)	39.63 ± 5.5	8	80	97
	+ Iproniazid (5)	34.20 ± 5.7	96	1	100

ence of iproniazid was the same as that under the control conditions, which establishes that the metabolism of 5-HT had no effect on its rate of entry and that its transport was the rate-limiting step for the uptake of 5-HT.

The rate of uptake of ^{14}C-5-HT after a one-minute infusion of 0.11 to 11 μM ^{14}C-5-HT did not differ significantly from the rate obtained after a three-minute infusion (table 2). The infusion of 1.1 μM ^{14}C-5-HT for eight minutes, however, resulted in a 32% decrease in the rate of uptake, suggesting that saturation occurred as a function of time. The values obtained after a three-minute infusion could be considered, therefore, as values of initial rate of uptake, and the Michaelis-Menten equation could be used to study the relationship between the pulmonary uptake and the concentration in the perfusion medium of ^{14}C-5-HT and to calculate V_{max} and K_m. The values were: 19 mμmol/g of lung per min and 5.9 μM, respectively, for the control experiments and 19 mμmol/g of lung per min and 6.2 μM, respectively, for experiments in which iproniazid was added to the perfusion medium. The percentage of unaltered ^{14}C-5-HT in the effluent was 41.5 to 79%, depending on the concentration of ^{14}C-HT in the perfusion medium (table 2). The inactivation of ^{14}C-5-HT by the lungs was thus 20.1 to 58.5%.

Attempts were made to study by differential centrifugation the subcellular distribution of ^{14}C-5-HT, once it had been taken up, under the

TABLE 2

Rate of uptake of ^{14}C-5-HT during a one- and three-minute infusion of ^{14}C-5-HT and calculated percentage of unaltered ^{14}C-5-HT in the effluent

After a 10-minute equilibration period, lungs were perfused for one or three minutes with different concentrations of ^{14}C-5-HT. Numbers in parentheses represent the number of experiments. Results are expressed as means ± S.E.

^{14}C-5-HT Conc.	1-Min Infusion; Rate of Uptake by Lung	3-Min Infusion		
		Rate of uptake by lung	^{14}C effluent/ ^{14}C inflow	^{14}C-5-HT effluent/ ^{14}C-5-HT inflow
μM	*mμmol/g wet weight/min*	*mμmol/g wet weight/min*	%	%
0.11	0.37 ± 0.08 (3)	0.38 ± 0.03 (4)	52.1 ± 5.3	41.5
1.1	3.51 ± 0.61 (4)	2.90 ± 0.31 (8)	56.1 ± 4.3	44.8
11	13.12 ± 1.02 (4)	13.21 ± 1.80 (8)	82.8 ± 1.6	79.9

protective action of iproniazid (table 3). The final supernatant fluid contained the major part of the radioactivity; in two of these experiments, the supernatant fluid obtained after centrifugation at 30,000 × *g* for 120 minutes was centrifuged at 105,000 × *g* for 120 minutes; no difference in the radioactive content of the two supernatant fluids could be detected, which indicates that ^{14}C-5-HT was in the soluble fraction. About one-third of the radioactivity was in the 1,000 × *g* pellet, probably composed of unbroken cells and nuclei. Only small amounts of radioactivity

TABLE 3

Subcellular distribution of ^{14}C-5-HT

After a 10-minute equilibration period, lungs were perfused for three minutes with 1.1 μM ^{14}C-5-HT. Iproniazid (5×10^{-4} M) was present during the entire perfusion period, which was followed by homogenization in 0.25 M sucrose and differential centrifugation. Results are expressed as means of four determinations ± S.D.

	^{14}C-5-HT Taken Up
	%
1,000 × *g* pellet	31.4 ± 3.8
9,000 × *g* pellet	5.9 ± 0.9
30,000 × *g* pellet	8.3 ± 0.7
30,000 × *g* supernatant fluid	54.4 ± 4.6

were found in the two other pellets, the composition of which therefore was not investigated.

In the subsequent experiments, since the pulmonary uptake of ^{14}C-5-HT was similar with and without blockade of MAO, the uptake was calculated from the radioactivity measured in the lungs without inhibition of MAO. This procedure was judged advantageous because it could also provide information on the effect of various drugs or conditions on the metabolism of 5-HT in the lungs. In addition, the values of uptake so obtained were independent of a possible primary effect of drugs on the release of 5-HT.

The effects of various experimental conditions and of drugs on the metabolism and uptake of 5-HT in the lungs are shown in table 4. Cold (perfusion at 4°C) markedly inhibited the uptake of 5-HT. Chromatographic analysis of the lung extract revealed the presence of a third peak, other than 5-HT and 5-HIAA, but not identified with certainty, accounting for 18% of the radioactivity. Anoxia produced by perfusing the circulation with a medium equilibrated with nitrogen and ventilating the lungs with nitrogen inhibited partially, but significantly, the uptake of 5-HT. The oxidation of 5-HT was only moderately reduced; it is likely, however, that exposure of lung tissue to oxygen took place between the end of the perfusion and the beginning of the homogenization. Attempts at perfusing the lungs with 10^{-3} M dinitrophenol were unsuccessful since gross pulmonary edema developed in all but two experiments. In these experiments, the uptake was 6.1 and 8.9 mμmol/g of lung per 3 min. Perfusion with a glucose-free medium did not result in any significant change in uptake or metabolism. Administration of cocaine, imipramine and chlorpromazine at 10^{-4} M during the entire perfusion period, also markedly inhibited the uptake of 5-HT, both when iproniazid was present and when it was not. The percentage of radioactivity recovered as 5-HT in the lungs was increased in the experiments done without iproniazid. This increase could result from the inhibitory effect of these drugs on MAO, an effect which has been reported for cocaine (Philpot, 1940) and imipramine (Pulver *et al.*, 1960). A more likely explanation, however, is that they inhibited the pulmonary uptake of 5-HT, thus preventing the transport of the amine into the cell. In such event, the ^{14}C-5-HT remaining in the extravascular space would account for the increased percentage of ^{14}C-5-HT found in the lungs. The interstitial space for 5-HT (expressed as milliliters per gram of lung), calculated from these percentages, the values for the pulmonary uptake and the substrate concentration, was equal to 25 to 33%, compared with the 40% reported for pulmonary interstitial space in stud-

TABLE 4

Effect of various drugs and conditions on the uptake and metabolism of ^{14}C-5-HT

After a 10-minute equilibration period, lungs were perfused for three minutes with 1.1 μM ^{14}C-5-HT. Drugs were present during the entire perfusion period, except for reserpine (5 mg/kg) which was administered i.p. 48 and 24 hours before the experiment. Iproniazid concentration was 5×10^{-4} M. Results are expressed as means ± S.E.

	N[a]	^{14}C-5-HT Uptake by Lung	% as 5-HT	% as 5-HIAA
		mμmol/g wet weight		
Control	8	8.69 ± 0.90	7	83
Cold (4°C)	4	1.19 ± 0.18[b]	18	58
Anoxia	6	5.70 ± 0.05[b]	24	68
Glucose-free medium	4	7.62 ± 0.50	6	90
Cocaine, 10^{-4} M	4	0.76 ± 0.06[b]	41	55
Cocaine, 10^{-4} M + iproniazid	3	0.64 ± 0.02[b]	99	
Imipramine, 10^{-4} M	4	0.69 ± 0.02[b]	53	40
Imipramine, 10^{-4} M + iproniazid	3	0.79 ± 0.04[b]	98	
Chlorpromazine, 10^{-4} M	4	1.07 ± 0.24[b]	25	72
Chlorpromazine, 10^{-4} M + iproniazid	3	1.72 ± 0.22[b]	98	
Norepinephrine, 10^{-5} M	5	7.69 ± 0.68	9	86
Norepinephrine, 10^{-4} M	4	5.53 ± 0.65[b]	8	88
Tryptamine, 10^{-5} M	4	5.81 ± 0.26[b]	5	91
Reserpine	4	7.30 ± 0.25	9	87

[a] *N*, number of experiments.

[b] Significantly different from control values ($P < .05$).

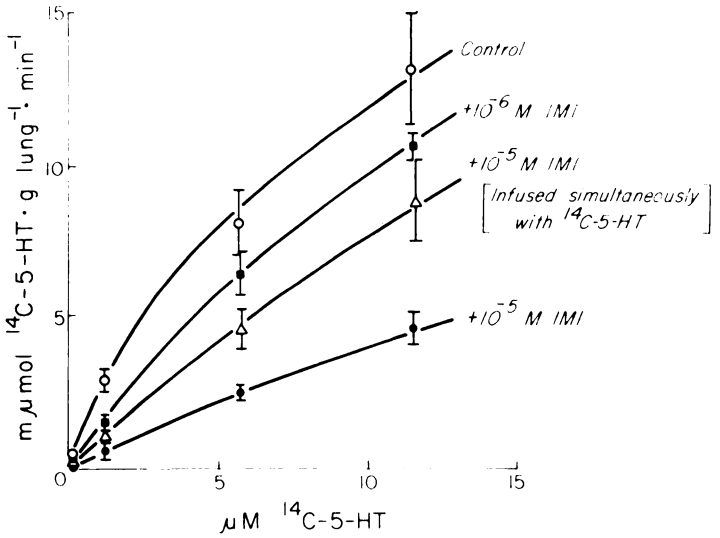

FIG. 2. Effect of imipramine (IMI) on the rate of uptake of ^{14}C-5-HT by the lungs. On the ordinate is the rate of uptake of ^{14}C-5-HT by the lungs (millimicromoles·gram wet weight^{-1}·min^{-1}); on the absicssa is the concentration of ^{14}C-5-HT in the perfusion medium (micromolar concentration). ^{14}C-5-HT was infused for three minutes. Imipramine was present in the perfusion medium during the entire perfusion (■——■, 10^{-6} M; ●——●, 10^{-5}M) or was administered simultaneously with ^{14}C-5-HT (△——△, 10^{-5} M). Means of at least four experiments ± S.E.

ies with other methods (Weibel and Knight, 1964; Mejia, 1968). In the experiments carried out with chlorpromazine and imipramine, chromatograms of the lung extracts showed the presence of new, strong fluorescent spots.

As shown in figure 2, the effect of imipramine on the uptake of ^{14}C-5-HT depended on the concentration and on the mode of administration of the drug. Kinetic analysis indicated that the inhibition of uptake was of the competitive type, whether imipramine was present during the entire perfusion period or administered simultaneously with ^{14}C-5-HT.

The results of the experiments with NE, with tryptamine and with reserpine are also given in table 4. Only at a concentration approximately 100 times greater than that of 5-HT did NE inhibit partially and significantly the uptake of 5-HT. A lower concentration of tryptamine elicited the same effect. Pretreatment of rats with reserpine (5 mg/kg i.p., 48 and 24 hours before the experiment) did not result in a significant decrease in 5-HT uptake.

Table 5 shows the effects of ouabain (10^{-4} and 10^{-3} M) and of K^+-free and Na^+-free media on the uptake and metabolism of ^{14}C-5-HT by the lungs. Ouabain at 10^{-3} M resulted in significant, but partial, inhibition of the uptake of ^{14}C-5-HT,

TABLE 5

Effects of ouabain and of absence of extracellular Na^+ and K^+ on the uptake and metabolism of ^{14}C-5-HT

After a 10-minute equilibration period (15 minutes when ouabain was tested), the lungs were infused with 1.1 μM ^{14}C-5-HT for three minutes. The lungs were exposed to ouabain and to changes in the ionic composition of the medium during the entire experiment. Results are expressed as means ± S.E.

Perfusion Medium	N^a	Uptake by Lung	% Recovered from Lung	
			As 5-HT	As 5-HIAA
		mμmol/g wet weight		
Control	8	8.69 ± 0.94	7	83
Ouabain, 10^{-4} M	4	7.71 ± 0.74		
Ouabain, 10^{-3} M	5	5.68 ± 0.37[b]	9	86
K^+-free medium	5	6.40 ± 0.68	10	84
Ouabain, 10^{-3} M, in K^+-free medium	4	5.20 ± 0.57[b]	8	89
Na^+-free medium (Na^+ replaced by Li^+)	4	2.10 ± 0.13[b]	9	86
Na^+-free medium (Na^+ replaced by sucrose)	4	1.30 ± 0.07[b]	33	61

[a] N, number of experiments.
[b] Significantly different from control value ($P < .05$).

without affecting its metabolism. In the absence of K^+, the values did not differ significantly from control values. The combination of 10^{-3} M oua-

bain and K^+-free medium did not result in a significant potentiation of the effect of ouabain. Absence of extracellular Na^+ markedly inhibited the uptake of ^{14}C-5-HT, and substitution of sucrose or of LiCl for NaCl resulted in a comparable degree of inhibition of uptake. The percentage of radioactivity recovered in the lungs as 5-HT rose to 33% when the inhibition was maximal, an effect already observed under similar degrees of drug-induced inhibition of 5-HT uptake and probably related to the amount of ^{14}C-5-HT remaining in the interstitial space. In the subsequent experiments on the effects of various Na^+ concentrations, sucrose was substituted for NaCl.

The effect of lowering the Na^+ concentration of the perfusion medium on the uptake of ^{14}C-5-HT after a three-minute infusion of ^{14}C-5-HT at various concentrations is illustrated in figure 3. The kinetic analysis of the data indicated that the effect of the decrease in the Na^+ concentration was to increase the K_m of the reaction, but not to modify significantly V_{max}. The effects of K^+ (6 and 28 mM) were also tested, in the presence of the same concentration of Na^+ (18 mM). This low Na^+ concentration might sensitize the system to the action of K^+, so that the K^+ ion could have an effect even at 28 mM, without an increase in the tonicity of the medium. K^+ at 28 mM produced an inhibition of the uptake of ^{14}C-5-HT, compatible with an increase in the K_m or a decrease in the affinity of the transport system for 5-HT. In these experiments, however, there was some pulmonary edema since the lungs weighed 1.96 g, 20% more than the lungs perfused with 18 mM Na^+ and 6 mM K^+. Nevertheless, the effect of 28 mM K^+ was statistically significant ($P < .005$) when the values of uptake were corrected for the increased weight of the lungs. A higher K^+ concentration had an even greater effect on the development of pulmonary edema. After perfusion with a medium containing 100 mM K^+ and 28 mM Na^+, the weight of the lungs was 3.7 g (mean of four experiments); the uptake, after a three-minute infusion of 1.1 μM ^{14}C-5-HT, was 1.01 ± 0.06 mμmol/g of lung (mean ± S.E.). This result suggests that the degree of inhibition was related to the K^+ concentration. Because the lungs were edematous, these results should be interpreted with caution.

The relationship between the extracellular Na^+ concentration and the uptake of ^{14}C-5-HT after

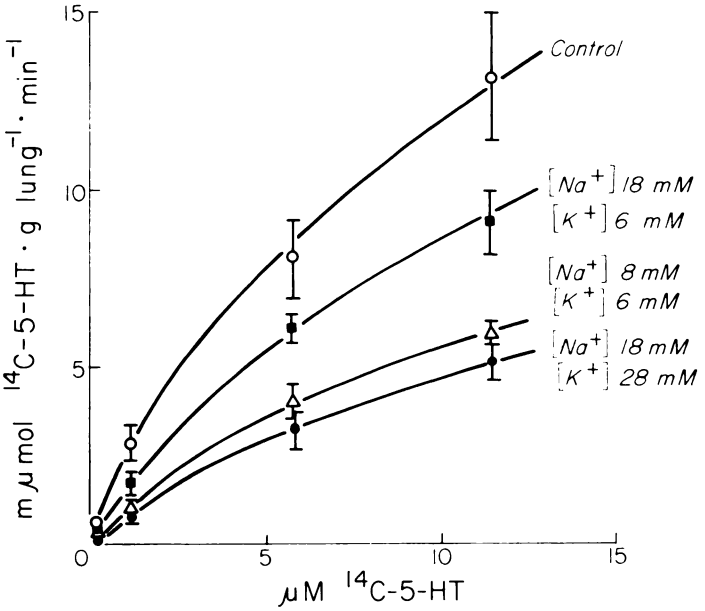

FIG. 3. Effects of decreasing the Na^+ concentration and of increasing the K^+ concentration on the rate of uptake of ^{14}C-5-HT by the lungs after a three-minute infusion of different concentrations of ^{14}C-5-HT. On the ordinate is the rate of uptake of ^{14}C-5-HT (millimicromoles·gram lung^{-1}·min^{-1}); on the abscissa is the concentration of ^{14}C-5-HT in the perfusion medium (micromolar concentration). Na^+ was replaced by sucrose and K^+ was replaced by Na^+. Means of at least four experiments ± S.E.

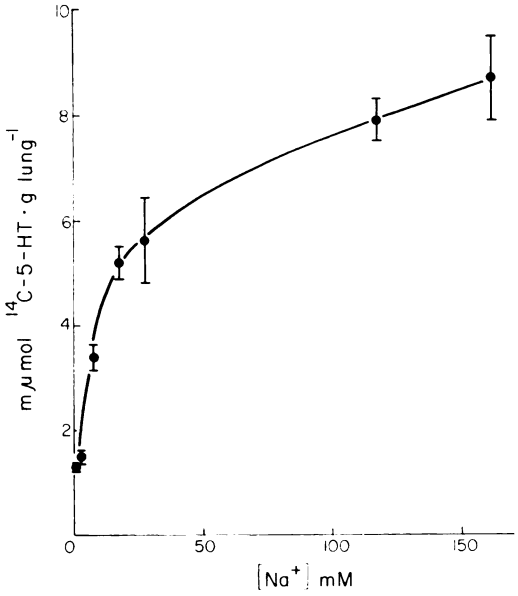

FIG. 4. Relationship between the extracellular Na^+ concentration (millimolar concentration) and the uptake of ^{14}C-5-HT by the lungs after a three-minute infusion of 1.1 μM ^{14}C-5-HT (millimicromoles·gram lung^{-1}). Na^+ was replaced by sucrose. Means of at least four experiments ± S.E.

a three-minute infusion of 1.1 μM ^{14}C-5-HT was hyperbolic (fig. 4). A straight line was obtained when the reciprocal of ^{14}C-5-HT uptake was plotted against the reciprocal of Na^+ concentration.

The efflux of ^{14}C-5-HT was measured under various conditions (table 6). These experiments were not designed to analyze precisely the different components or systems involved in the loss of ^{14}C-5-HT from the lungs over a long period, but it may be of interest to note that the efflux of ^{14}C-5-HT could be described as a single exponential function and that no fast component of efflux (washout of interstitial space) could be detected one minute after the end of infusion of ^{14}C-5-HT. Except for the experiments with 2 μM chlorpromazine, the values for all effluxes were significantly higher than those obtained under the control conditions ($P < .05$). Thus, the efflux of 5-HT from the lungs was accelerated by the addition to the perfusion medium of unlabeled 5-HT (11 μM), imipramine (2 and 20 μM), cocaine (1 μM) and chlorplomazine (20 μM). The efflux of 5-HIAA or other metabolites of 5-HT was faster than the efflux of 5-HT.

TABLE 6

Efflux of ^{14}C-5-HT and ^{14}C-5-HT metabolites

After a 10-minute equilibration period, lungs were infused with 1.1 μM ^{14}C-5-HT for three minutes. After the infusion was completed, the effluent was collected for 15 seconds at different times over a 12-minute period during which the lungs were exposed to the test drugs. Iproniazid, 5×10^{-4} M, was present during the entire perfusion period in all experiments except those in which the efflux of ^{14}C-5-HT metabolites was studied (first row). The lung uptake was calculated from the measured difference in radioactive content of the inflow and the effluent collected during the infusion period; the radioactive content of the effluent measured subsequently was expressed as percentage of that uptake. The last column represents the total loss of ^{14}C during the 12-minute period, calculated by integrating the values of efflux over this 12-minute period. Numbers in parentheses are the number of experiments. Results are expressed as means ± S.E.

Perfusion Medium	Efflux of ^{14}C as % of Pulmonary Uptake Lost per Min					% ^{14}C Uptake Lost in 12 Min
	1 min	2 min	4 min	8 min	12 min	
Control (without iproniazid) (3)	9.9 ± 1.1[a]	7.5 ± 1.1[a]	5.7 ± 0.4[a]	3.2 ± 0.5	2.1 ± 0.3	60
Control (with iproniazid) (4)	4.2 ± 0.6	3.8 ± 0.9	3.2 ± 0.9	2.1 ± 0.6	1.8 ± 0.5	34
+ 11 μM 5-HT (4)	12.5 ± 1.3[a]	11.3 ± 1.1[a]	8.1 ± 0.6[a]	4.2 ± 0.3[a]	2.1 ± 0.2	81
+ 2 μM imipramine (4)	7.3 ± 1.1[a]	7.0 ± 1.1[a]	6.3 ± 1.0[a]	5.1 ± 1.0[a]	3.7 ± 1.0	68
+ 20 μM imipramine (4)	10.0 ± 1.9[a]	9.3 ± 2.1[a]	7.7 ± 1.1[a]	4.7 ± 0.2[a]	3.0 ± 0.5	76
+ 1 μM cocaine (4)	6.2 ± 0.5[a]	6.0 ± 0.6[a]	5.2 ± 0.4[a]	4.1 ± 0.3[a]	3.1 ± 0.2	57
+ 2 μM chlorpromazine (3)	4.0 ± 0.6	3.4 ± 0.1	3.2 ± 0.1	2.7 ± 0.1	2.3 ± 0.1	36
+ 20 μM chlorpromazine (4)	6.5 ± 1.1[a]	6.5 ± 0.9[a]	5.7 ± 0.9[a]	4.8 ± 0.7[a]	3.4 ± 0.5	63

[a] Significantly different from control values (with iproniazid) ($P < .05$).

TABLE 7

Effects of ouabain and of changes in the ionic composition of the medium on the efflux of ^{14}C-5-HT from the lungs

After a 10-minute equilibration period, lungs were infused with 1.1 μM ^{14}C-5-HT for three minutes. After the infusion was completed, the effluent was collected for 15 seconds at different times over a period of 12 minutes during which the lungs were exposed to ouabain or to changes in ionic composition of the medium. Sucrose was used to replace Na^+; the K^+ concentration was increased by the addition of KCl. Iproniazid (5×10^{-4} M) was present during the entire experiment to prevent conversion of 5-HT to 5-HIAA. The lung uptake was calculated from the measured difference in radioactive content of the inflow and the effluent collected during the infusion period; the radioactive content of the effluent measured subsequently was expressed as percentage of that uptake. The last column represents the total loss of ^{14}C-5-HT during the 12-minute period, calculated by integrating the values of efflux over this 12-minute period. Results are expressed as means ± S.E.

Perfusion Medium	N[a]	Efflux of ^{14}C-5-HT as % of Pulmonary Uptake Lost per Min					% of ^{14}C-5-HT Uptake Lost in 12 Min
		1 min	2 min	4 min	8 min	12 min	
Control (standard medium)	4	4.2 ± 0.6	3.8 ± 0.9	3.2 ± 0.9	2.1 ± 0.6	1.8 ± 0.5	34
Ouabain, 10^{-3} M, in K^+-free medium	4	3.6 ± 0.6	3.4 ± 0.5	3.2 ± 0.5	2.9 ± 0.4	2.7 ± 0.4	37
Na^+-free medium	4	21.8 ± 4.1[b]	18.9 ± 4.2[b]	7.3 ± 0.8[b]	1.9 ± 0.3	0.8 ± 0.3	92
Na^+, 18 mM and K^+, 6 mM	4	10.3 ± 1.3[b]	9.7 ± 1.0[b]	7.4 ± 0.3[b]	4.7 ± 0.2[b]	2.9 ± 2.2	77
Na^+, 18 mM and K^+, 28 mM	4	8.8 ± 1.3[b]	7.4 ± 1.2[b]	4.8 ± 0.6	2.9 ± 0.1	1.9 ± 0.1	55

[a] *N*, number of experiments.

[b] Significantly different from control values ($P < .05$).

The efflux of ^{14}C-5-HT was also studied under different ionic conditions and under the effect of ouabain as shown in table 7. Perfusion of K^+-free medium and 10^{-3} M ouabain in combination had no significant effect on the rate of efflux of ^{14}C-5-HT, whereas replacement of Na^+ by sucrose resulted in the rapid efflux of ^{14}C-5-HT. Perfusion with a medium containing 18 mM Na^+ and 6 mM K^+ had an intermediate effect, and raising the K^+ concentration to 28 mM at the same concentration of Na^+ (18 mM) resulted in a decrease in the rate of efflux.

Discussion

The purpose of these experiments was to study specifically the uptake of ^{14}C-5-HT from the pulmonary circulation. Perfusion of isolated lungs seemed to be the most suitable procedure since it permitted examination of the dynamics of a rapidly occurring phenomenon while respecting the physiological barriers and avoided the problems of diffusion and artifacts created by the use of tissue slices. The difficulty of measuring lung uptake deserves a few comments. Ideally, the amount of radioactivity remaining in the interstitial space should be subtracted and the amount of "backflow" radioactivity released from the lungs to the perfusion fluid should be added to the radioactivity counted in the lungs. The interstitial 5-HT space was estimated to be about 0.25 to 0.30 ml/g of wet lung. This value can be considered as negligible when the uptake is maximal, but becomes proportionately more important when the uptake is low. The amount of backflow radioactivity released during the infusion period could be measured in the absence of inhibition of MAO since it corresponded to the percentage of effluent radioactivity which was not recovered as 5-HT; it could be estimated, at most, as 10% of the radioactivity infused (tables 1 and 2). In the presence of iproniazid, however, the amount of backflow of ^{14}C during the infusion period could not be measured but can be expected to be less than in the absence of iproniazid (table 6). Thus, these two effects tend to counterbalance each other, but not equally under different conditions of uptake. For the sake of simplicity, I decided not to correct for these two factors. The reported values for K_m and V_{max}, therefore, are estimates and give only an order of magnitude.

Another problem originates from the possible presence of pulmonary edema. Although I have no evidence that pulmonary edema *per se* affects the uptake of ^{14}C-5-HT by the lungs, it would certainly affect the calculation of the uptake if expressed per gram of wet weight of tissue as in this study. For that reason, in the present experiments, lungs that became edamatous were discarded. In addition, pulmonary edema by increasing the interstitial space might have other effects, such as prolongation of equilibration period and slowing of the washout of radioactivity from the interstitial space. In the present study on the efflux of ^{14}C-5-HT from the lungs, no fast component attributable to the washout of radioactivity from the interstitial space could be detected one minute after the end of the infusion of ^{14}C-5-HT. In the studies reported by Alabaster and Bakhle (1970), however, an initial phase of rapid washout of radioactivity could be followed over a period of several minutes (from their fig. 6). This difference could explain the discrepancy between the percentage of removal of 5-HT over a three- to five-minute infusion reported by these investigators (95%) and that found in the present study at similar concentrations (about 60%). Differences in the experimental conditions and the strain of animals used could also contribute to these discordant results.

The deaminative oxidation of 5-HT in the lungs is extremely rapid, since a minimal amount of 5-HT was recovered from the lungs at the end of the three-minute periods of infusion at all concentrations used (0.11–17 μM or 0.02–3 μg/ml). In fact, as suggested by the experiments in which iproniazid was added to the perfusion medium, MAO is probably the only enzyme directly involved in the degradation of 5-HT. The metabolic pattern was the same when other substrates for MAO (tryptamine, NE) were present in large concentrations in the perfusion medium, and, if taken up by the lungs, could have competed with 5-HT. Although the MAO activity in the lungs is reported to be moderate (Pletscher *et al.*, 1966), it is clearly not a limiting factor in the breakdown of 5-HT.

That the uptake of 5-HT in the lungs did not take place in platelets, mast cells or adrenergic fibers can be inferred only from the metabolic pattern since under normal conditions these structures are able to store 5-HT without metabolizing it rapidly (Paasonen, 1965; Furano and Greene, 1964; Thoa *et al.*, 1969). The absence of significant change in the uptake and metabolism of 5-HT in the lungs of animals pretreated with reserpine supports that inference. Finally, the information gained from the experiments on the subcellular distribution of ^{14}C-5-HT confirms that 5-HT probably exists in a free state in the cellular cytoplasm, where it would be exposed to the action of MAO. The coherence of these results makes it unlikely that the subcellular fractionation studies were affected by artifacts resulting in the redistribution of ^{14}C-5-HT during the procedure.

Comparison with tissues that do not store 5-HT, such as intestinal smooth muscle (Weiss and Rosecrans, 1971) and liver (Tyce *et al.*, 1968), indicates that the lungs metabolize 5-HT in the same fashion as intestinal smooth muscle, but do not give rise to as many metabolites as does liver. Because practically all the venous blood passes through the pulmonary circulation, the lung is potentially the best suited organ to dispose of large amounts of 5-HT. Since the concentration of 5-HT in plasma is usually extremely low, the lungs probably have a minor role in the overall metabolism of 5-HT. Yet they may be essential when large amounts of 5-HT are present in the venous blood (carcinoid syndrome) or are released locally (after pulmonary embolism, for example) (Thomas *et al.*, 1964). Considering the striking effects of 5-HT on smooth muscle and some neuroreceptors, it is significant that the lung quickly converts 5-HT into the physiologically inactive 5-HIAA. In view of the present results it is difficult to understand why a few investigators (Sadavongvivad, 1970; Bulat and Supek, 1967) found an increase in the 5-HT content of the lungs after its i.v. injection, unless their measurements were influenced by the uptake of 5-HT in platelets or in some other structure irrigated by the bronchial circulation and capable of storing 5-HT.

The uptake of ^{14}C-5-HT in the lungs is in many respects similar to that reported for platelets (Stacey, 1961), brain (Blackburn *et al.*, 1967; Ross and Renyi, 1967; Shaskan and Snyder, 1970) and sympathetic nerve endings (Thoa *et al.*, 1969). Drugs such as imipramine, cocaine and chlorpromazine, known to block the membrane transport of 5-HT in platelets,

nerve endings and brain, have the same effect in the lungs. The presence in chromatograms of the lung extracts of a new and strong fluorescent spot suggests furthermore that imipramine and chlorpromazine are also taken up by the lungs. Previous *in vivo* studies (Sjöstrand *et al.*, 1965; Dingell *et al.*, 1964) have shown that the lungs are capable of retaining large amounts of these compounds, after i.v. administration, and a recent publication has confirmed that finding in the isolated perfused lung of the rabbit (Rosenblom and Bass, 1970) and of the rat (Junod, 1972). Imipramine seems to act by competitive inhibition. This fact, coupled with the known accumulation of the drug in the lungs and its effect on the efflux of ^{14}C-5-HT, suggests that imipramine might share, at least in part, the same transport system as 5-HT. However, recent studies on the mechanism of accumulation of ^{14}C-imipramine in isolated perfused rat lung (Junod, 1972) have failed to support that hypothesis.

In the present study, uptake of 5-HT was only partially inhibited by compounds chemically or functionally related (tryptamine and NE). The absence of strong competition between 5-HT and NE indicates that the uptake probably did not take place in sympathetic nerve endings, as suggested for the guinea-pig vas deferens (Thoa *et al.*, 1969). The results of the subcellular fractionation studies substantiate that conclusion, and Hughes *et al.* (1969) found no evidence for a neuronal uptake of NE in perfused rat lungs.

Studies on the effects of metabolic inhibitors on the uptake of 5-HT were limited because the perparation tended to deteriorate too rapidly to give acceptable results. The results obtained indicate that, in contrast to the brain which shows significant response to inhibition of oxidative metabolism and production of adenosine triphosphate (Blackburn *et al.*, 1967), the lungs were not very sensitive to anoxia and to dinitrophenol. The lack of effect on the uptake of 5-HT may be explained by the relative short exposure of the lungs to these adverse conditions and by the possibility that the cells were able to derive enough energy from anaerobic glycolysis. Stacey (1961), Born and Gillson (1959) and Weissbach and Redfield (1960) also reported that anoxia and some metabolic inhibitors had little or no effect on the uptake of 5-HT by platelets. An important finding was the absence of effect of a glucose-free medium. The lack of significant change does not absolutely rule out glucose as the main substrate required for active 5-HT uptake since the period of exposure to glucose-free medium was short and since endogenous glucose could have been formed and utilized. However, it indicates that the effect of changes in the ionic composition of the medium on the uptake of 5-HT by the lungs are not secondary to a primary effect on glucose transport.

Although free in the cytoplasm rather than being stored in granules or vesicles, 5-HT is not released from the lungs as rapidly as would be expected if only a simple diffusion process were involved. Under control conditions (perfusion with standard medium and iproniazid), the half-life of the ^{14}C-5-HT in the lungs was well over 12 minutes (table 6). Addition of unlabeled 5-HT at a concentration of 11 μM produced a marked increase in the release of radioactive 5-HT. Such an effect can be attributed to the displacement of ^{14}C-5-HT from binding sites, to a reduced uptake or to a counterflow phenomenon. Confirmation of the third hypothesis would require the demonstration of an efflux of ^{14}C-5-HT against a concentration gradient, which would in turn require the use of compounds capable of inhibiting the uptake of 5-HT. Unfortunately, all the compounds tested so far have produced an increase in the rate of efflux of ^{14}C-5-HT from the lungs. Chlorpromazine was the least efficient compound, since a concentration of 20 μM was required to bring about approximately the same effect as 1 μM cocaine or 2 μM imipramine. Only at concentrations equal to or greater than 10^{-4} M were imipramine and chlorpromazine shown to affect the permeability of the membrane of platelets to 5-HT (Bartholini *et al.*, 1961; Ahtee and Paasonen, 1968). These substances are thought to affect primarily the uptake of biogenic amines, and the release of 5-HT from brain slices after treatment with imipramine has been explained as a result of inhibition of the uptake mechanism (Carlsson *et al.*, 1969). The same explanation could be invoked here, particularly since the experiments on the efflux of ^{14}C-5-HT were conducted before a steady state was achieved.

The increased rate of efflux of ^{14}C-5-HT could also be accounted for by the displacement of 5-HT from its binding sites or by the counter-

flow phenomenon (Alvarado, 1965). This would be substantiated by demonstrating an intracellular movement of cocaine, imipramine or chlorpromazine. Chlorpromazine has been shown to accumulate in the particulate fraction of platelets that contain most of the platelets 5-HT (Solatunturi and Ahtee, 1968). However, since the mechanisms of uptake of 5-HT and imipramine are different (Junod, 1972), the effect of imipramine is unlikely to be due to a counterflow phenomenon.

The effects of Na^+, K^+ and ouabain on the transport of 5-HT in isolated perfused rat lungs appear to be qualitatively similar to those reported for the transport of 5-HT and NE in other cellular systems by several investigators (Bogdanski *et al.*, 1968; Bogdanski and Brodie, 1969; Tissari *et al.*, 1969; Sneddon, 1969), who invoked the participation of a Na^+-coupled transport mechanism. The transport of solute according to that concept is mediated by a carrier, common for Na^+ and the solute (Crane, 1965; Schultz and Curran, 1970). The affinity of the carrier for the solute is increased when Na^+ is present in high concentration, but is decreased when K^+ is present in high concentration; K^+ probably competes with Na^+ for the same site on the carrier, thus changing the affinity of the carrier for the solute. The movement of solute therefore depends primarily on the concentration gradients of Na^+ and of K^+ across the cell membrane: a high Na^+ concentration and a low K^+ concentration in the extracellular phase favor the binding of the solute to the carrier, whereas a low Na^+ concentration and a high K^+ concentration in the intracellular phase favor the release of the solute from the carrier. The transport of the solute against a concentration gradient can be explained by the changes in the affinity of the carrier system on the two sides of the membrane, without invoking a direct, energy-yielding reaction.

The results presented in this study are compatible with this model. The uptake of 5-HT was dependent on the extracellular concentration of Na^+ and was markedly inhibited when Na^+ was omitted. Lowering the extracellular Na^+ concentration resulted in a decreased affinity of the transport system for 5-HT. Since the relationship between the reciprocals of the Na^+ concentration and of the uptake of 5-HT was linear, the existence of a carrier transporting one Na^+ ion with one molecule of 5-HT can be postulated (Vidaver, 1964). Finally, K^+ at 28 mM inhibited the uptake of 5-HT, possibly by competing with Na^+.

Ouabain and K^+-free medium should act indirectly on the uptake of 5-HT by blocking the Na^+ pump, thereby abolishing the concentration gradients of Na^+ and K^+ across the cell membrane. In the present study, treatment with ouabain or K^+-free medium had only a partial effect on the uptake. It might be that the abolition of the ionic concentration gradients follows the blockade of the Na^+-K^+ activated adenosine triphosphatase (ATPase) with some delay and that more time is required to see an ouabain-induced effect. This explanation is supported by the observation that the degree of ouabain-sensitive inhibition of NE transport in slices of rat heart was dependent on the duration of exposure to ouabain (Bogdanski and Brodie, 1969) and that in synaptosomes of rabbit brain the blockade of the Na^+-K^+ activated ATPase preceded the effect on the transport of 5-HT and NE (Tissari *et al.*, 1969). The analysis of this problem is made more difficult by the known insensitivity of the rat to cardiac glycosides (Repke *et al.*, 1965). Therefore, it is proper to delay the interpretation of these findings until measurement can be made of Na^+-K^+ activated ATPase and of Na^+ and K^+ concentrations in the cells responsible for the uptake of 5-HT by the lung. The measurement of these variables in such a heterogeneous preparation was thought to be of very limited value.

The studies on the efflux of amine also yielded results similar to those reported by Bogdanski and Brodie (1969). Since the lungs apparently do not store 5-HT, the problems of accumulation in granules or vesicles and of release from these subcellular structures do not exist. Bogdanski and Brodie measured the efflux of NE across the membranes of both the vesicles and the cells of rat heart slices. It is of interest therefore to note that in both studies, in spite of these differences, absence or low concentration of Na^+ increased the efflux of amines, ouabain had little or no effect and K^+, at 26 to 28 mM, had an inhibitory effect on the efflux.

Since, under normal conditions, most of the 5-HT taken up by the lungs is rapidly metabolized, the Na^+-dependent transport of 5-HT in perfused rat lungs seems to be involved in the disposal of an unwanted product. Such a phenomenon is unusual; in most cellular systems

where a Na^+-dependent transport has been demonstrated, with the exception of the kidney, the solute involved is a substrate necessary for the metabolic requirements of the cell or the body (sugar, amino acid) or a mediator (NE, 5-HT).

The lung is made up of many cellular populations; among them, platelets, mast cells and adrenergic nerve endings are known to take up 5-HT or NE. For the reasons developed here, however, it is difficult to conclude that platelets, mast cells or nerve endings are responsible for the uptake of 5-HT by perfused rat lung. Recent radioautographic studies by light and electron microscopy (Strum and Junod, 1972) indicated that this uptake takes place essentially in endothelial cells. Hughes *et al.* (1969) have suggested that endothelial cells also take up NE. Since no strong competition seems to exist between 5-HT and NE, two specific transport systems for these two biogenic amines could exist in the same cell.

Acknowledgment. The technical assistance of Mrs Lynne S. Angelo is gratefully acknowledged.

References

AHTEE, L. AND PAASONEN, M. K.: Potentiation of 5-hydroxytryptamine release from platelets by desmethylation of chlorpromazine and related agents. Acta Pharmacol. Toxicol. **26:** 213–221, 1968.

ALABASTER, V. A. AND BAKHLE, Y. S.: Removal of 5-hydroxytryptamine by rat isolated lung. Brit. J. Pharmacol. **40:** 468–482, 1970.

ALVARADO, F.: The relationship between Na^+ and the active transport of arbutin in the small intestine. Biochim. Biophys. Acta **109:** 478–494, 1965.

BARTHOLINI, G., PLETSCHER, A. AND GEY, K. F.: Diminution of 5-hydroxytryptamine in thrombocytes in vitro by chlorpromazine and related compounds. Experientia (Basel) **17:** 541–542, 1961.

BLACKBURN, K. J., FRENCH, P. C. AND MERRILLS, R. J.: 5-Hydroxytryptamine uptake by rat brain in vitro. Life Sci. **6:** 1653–1663, 1967.

BOGDANSKI, D. F. AND BRODIE, B. B.: The effect of inorganic ions on the storage and uptake of H^3-norepinephrine by rat heart slices. J. Pharmacol. Exp. Ther. **165:** 181–189, 1969.

BOGDANSKI, D. F., TISSARI, A. AND BRODIE, B. B.: Role of sodium, potassium, ouabain and reserpine on the uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci. **7:** 419–428, 1968.

BORN, G. V. R. AND GILLSON, R. E.: Studies on the uptake 5-hydroxytryptamine by blood platelets. J. Physiol. (London) **146:** 472–491, 1959.

BULAT, M. AND SUPEK, Z.: The penetration of 5-hydroxytryptamine through the blood-brain barrier. J. Neurochem. **14:** 265–271, 1967.

CARLSSON, A., JONASON, J. AND LINDQVIST, M.: On the mechanism of 5-hydroxytryptamine release by thymoleptics. J. Pharm. Pharmacol. **21:** 769–773, 1969.

CRANE, R. K.: Na^+-dependent transport in the intestine and other animal tissues. Fed. Proc. **24:** 1000–1005, 1965.

DAVIS, R. B. AND WANG, Y.: Rapid pulmonary removal of 5-hydroxytryptamine in the intact dog. Proc. Soc. Exp. Biol. Med. **118:** 797–800, 1965.

DINGELL, J. V., SULSER, F. AND GILLETTE, J. R.: Species differences in the metabolism of imipramine and desmethylimipramine (DMI). J. Pharmacol. Exp. Ther. **143:** 14–22, 1964.

EISEMAN, B., BRYANT, L. AND WALTUCH, T.: Metabolism of vasomotor agents by the isolated perfused lung. J. Thorac. Cardiovasc. Surg. **48:** 798–806, 1964.

FURANO, A. V. AND GREENE, N. M.: The uptake of biogenic amines by mast cells of the rat. J. Physiol. (London) **170:** 263–271, 1964.

GADDUM, J. H., HEBB, C. O., SILVER, A. AND SWAN, A. A. B.: 5-Hydroxytryptamine: Pharmacological action and destruction in perfused lungs. Quart. J. Exp. Physiol. **38:** 255–262, 1953.

GAL, E. M., MORGAN, M., CHATTERJEE, S. K. AND MARSHALL, F. D., JR.: Hydroxylation of tryptophan by brain tissue in vivo and related aspects of 5-hydroxytryptamine metabolism. Biochem. Pharmacol. **13:** 1639–1653, 1964.

HEINEMANN, H. O. AND FISHMAN, A. P.: Nonrespiratory functions of mammalian lung. Physiol. Rev. **49:** 1–47, 1969.

HUGHES, J., GILLIS, C. N. AND BLOOM, F. E.: The uptake and disposition of *dl*-norepinephrine in perfused rat lung. J. Pharmacol. Exp. Ther. **169:** 237–248, 1969.

JONSSON, J. AND LEWANDER, T.: A method for the simultaneous determination of 5-hydroxy-3-indole-acetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) in brain tissue and cerebrospinal fluid. Acta Physiol. Scand. **78:** 43–51, 1970.

JUNOD, A. F.: Accumulation of ^{14}C-imipramine in isolated perfused rat lungs. J. Pharmacol. Exp. Ther. **183:** 182–187, 1972.

KLEIN, D. C. AND NOTIDES, A.: Thin-layer chromatographic separation of pineal gland derivatives of serotonin-^{14}C. Anal. Biochem. **30:** 480–483, 1969.

MEJIA, R. H.: Regional hematocrit ratio and interstitial fluid volume in the normal rat. Experientia (Basel) **24:** 43–44, 1968.

PAASONEN, M. K.: Release of 5-hydroxytryptamine from blood platelets. J. Pharm. Pharmacol. **17:** 681–697, 1965.

PHILPOT, F. J.: The inhibition of adrenalin oxidation by local anesthetics. J. Physiol. (London) **97:** 301–307, 1940.

PLETSCHER, A., GEY, K. F. AND BURKARD, W. P.: *In* Handbook of Experimental Pharmacology, Vol. 19, pp. 593–735, Springer-Verlag, Berlin, 1966.

PULVER, R., EXER, B. AND HERRMANN, B.: Einige Wirkungen des N-(α-dimethylamino-propyl)-imidobenzyl-HCl und seiner Metabolite auf den Stoffwechsel von Neurohormonen. Arzneimittel-Forschung **10:** 530–533, 1960.

REPKE, K., EST, M. AND PORTIUS, H. J.: Ueber die Ursache der Speciesunterschiede in der Digitalisempfindlichkeit. Biochem. Pharmacol. **14:** 1785–1802, 1965.

ROSENBLOM, P. M. AND BASS, A. D.: A lung perfusion preparation for the study of drug metabolism. J. Appl. Physiol. **29:** 138–144, 1970.

ROSS, S. B. AND RENYI, A. L.: Accumulation of tritiated 5-hydroxytryptamine in brain slices. Life Sci. **6:** 1407–1415, 1967.

SADAVONGVIVAD, C.: Pharmacological significance of biogenic amines in the lungs: 5-Hydroxytryptamine. Brit. J. Pharmacol. **38:** 353–365, 1970.

SAID, S. I.: The lung as a metabolic organ. N. Engl. J. Med. **279:** 1330–1334, 1968.

SCHULTZ, S. G. AND CURRAN, P. F.: Coupled transport of sodium and organic solute. Physiol. Rev. **50:** 637–718, 1970.

SHASKAN, G. AND SNYDER, S. S.: Kinetics of serotonin accumulation into slices from rat brain: Relationship to catecholamine uptake. J. Pharmacol. Exp. Ther. **175:** 404–418, 1970.

SJÖSTRAND, S. E., CASSANO, G. B. AND HANSON, E.: The distribution of ^{35}S-chlorpromazine in mice studied by whole body autoradiography. Arch. Int. Pharmacodyn. Thér. **156:** 34–47, 1965.

SNEDDON, J. M.: Sodium-dependent accumulation of 5-hydroxytryptamine by rat blood platelets. Brit. J. Pharmacol. **37:** 680–688, 1969.

SOLATUNTURI, E. AND AHTEE, L.: Subcellular distribution of some phenothiazines in blood platelets of rabbit. J. Pharm. Pharmacol. **20:** 289–292, 1968.

STACEY, R. S.: Uptake of 5-hydroxytryptamine by platelets. Brit. J. Pharmacol. **16:** 284–295, 1961.

STAHL, E. AND KALDEWEY, H.: Spurenanalyse physiologisch aktiver, einfacher Indolderivate. Hoppe-Seylers Z. Physiol. Chem. **323:** 182–191, 1961.

STRUM, J. AND JUNOD, A. F.: Autoradiographic demonstration of ^{3}H-5-hydroxytryptamine uptake by pulmonary endothelial cells. J. Cell. Biol., in press.

THOA, N. B., ECCLESTON, D. AND AXELROD, J.: The accumulation of C^{14}-serotonin in the guinea-pig vas deferens. J. Pharmacol. Exp. Ther. **169:** 68–73, 1969.

THOMAS, D. P., STEIN, M., TANABE, G., REGE, V. AND WESSLER, S.: Mechanism of bronchoconstriction produced by thromboemboli in dogs. Amer. J. Physiol. **206:** 1207–1212, 1964.

THOMAS, D. P. AND VANE, J. R.: 5-Hydroxytryptamine in the circulation of the dog. Nature (London) **216:** 335–338, 1967.

TISSARI, A. H., SCHÖNHÖFFER, P. S., BOGDANSKI, D. F. AND BRODIE, B. B.: Mechanisms of biogenic amine transport. II. Relationship between sodium and the mechanism of ouabain blockade of the accumulation of serotonin and norepinephrine by synaptosomes. Mol. Pharmacol. **5:** 593–604, 1969.

TYCE, M., FLOCK, E. V. AND OWEN, C. A., JR.: Uptake and metabolism of 5-hydroxytryptamine by the isolated perfused rat liver. Amer. J. Physiol. **215:** 611–619, 1968.

UMBREIT, W. W., BURRIS, R. H. AND STAUFFER, T. F.: Manometric Techniques. Burgess Publishing Co., Minneapolis, 1964.

VANE, J. R.: The release and fate of vaso-active hormones in the circulation. Brit. J. Pharmacol. **35:** 209–242, 1969.

VIDAVER, G. A.: Transport of glycine by pigeon red cells. Biochemistry **3:** 662–667, 1964.

WEIBEL, E. R. AND KNIGHT, B. W.: A morphometric study on the thickness of the pulmonary air-blood barrier. J. Cell Biol. **21:** 367–384, 1964.

WEISS, G. B. AND ROSECRANS, J. A.: Analysis of 5-hydroxytryptamine-^{14}C uptake and metabolism in intestinal smooth muscle. Eur. J. Pharmacol. **13:** 192–207, 1971.

WEISSBACH, H. AND REDFIELD, B. G.: Factors affecting the uptake of 5-hydroxytryptamine by human platelets in an inorganic medium. J. Biol. Chem. **235:** 3287–3291, 1960.