Biochemical Pharmacology, Vol. 30, No. 23, pp. 3187–3193, 1981.
Printed in Great Britain.

0006-2952/81/233187-07 $02.00/0

MECHANISMS INFLUENCING THE DISPOSITION OF SEROTONIN IN MOUSE LUNG

TALMAGE R. BOSIN* and PATRICIA D. LAHR
Section of Pharmacology, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405, U.S.A.

(*Received* 15 *September* 1980; *accepted* 21 *April* 1981)

Abstract—Serotonin (5-HT) disposition in the intact mouse lung and its modification by animal age, concentration of injected 5-HT, method of killing the animal, and select pharmacological agents were investigated. Animal age (16–270 days) did not influence the lung levels of [^{3}H]5-HT but did influence the percentage of the dose accumulated by the lung. The effect of injected 5-HT concentration on the pulmonary accumulation of [^{3}H]5-HT indicated a dose-dependent, non-saturable accumulation. The method of killing the animal was found to be a major source of pulmonary [^{3}H]5-HT level variation, and a method involving ether asphyxiation/decapitation was developed. Following intravenous administration, [^{3}H]5-HT was accumulated and retained unchanged by lung and represented greater than 97 per cent of the total lung radioactivity. Imipramine, fluoxetine, cocaine and chlorpromazine were shown to inhibit the pulmonary uptake of [^{3}H]5-HT, while 5,6-dihydroxytryptamine lowered and clorgyline elevated lung levels of the amine. Reserpine treatment resulted in a time-dependent increase followed by a decrease in lung [^{3}H]5-HT.

It has been known for some time that the lungs are capable of removing vasoactive substances from the blood. As early as 1925, Starling and Verney [1] reported that it was impossible to maintain adequate circulation through an isolated kidney using defibrinated blood without including the lungs in the perfusion circuit. This was due to the presence of a potent vasoconstrictor substance in blood which was later identified [2, 3] as serotonin (5-hydroxytryptamine, 5-HT). More recently this ability of the lungs to remove 5-HT from the pulmonary circulation has been investigated in detail [4–15] and has established the pulmonary vasculature as an important site in regulating the arterial concentration of 5-HT. Since the entire cardiac output passes through the pulmonary microcirculation, the lung is uniquely situated to influence the arterial concentration of 5-HT. This role of the lung may be important in maintaining the fluidity of the pulmonary circulation by removing and/or metabolizing 5-HT released from platelets during microembolization of blood clots or platelet aggregates, an action which may be crucial in the prevention of 5-HT-induced platelet aggregation [16]. In addition, the control of circulating 5-HT levels by the lung and its function therein has been the subject of considerable speculation, which has included such pathological states as anaphylaxis [17–19], delayed-type hypersensitivity [20], and pulmonary hypertension [21].

Recent research using isolated perfused lungs from a variety of species has confirmed the rapid uptake of 5-HT from the pulmonary circulation [4–14]. This process has been shown to be a Na^+-dependent, saturable process which is susceptible to various metabolic inhibitors and which represents the rate-limiting step in the pulmonary disposition of 5-HT. Once taken up, 5-HT is rapidly metabolized to 5-hydroxyindole-3-acetic acid (5-HIAA) with no retention of the parent amine. Autoradiographic [22, 23] and histofluorescent [6] studies have indicated the pulmonary capillary endothelial cells to be the site of uptake and metabolism of circulating 5-HT.

Drugs which have known effects on 5-HT uptake, storage, release and metabolism have been studied using the isolated perfused lung technique [4, 5, 7–9, 12]. Imipramine, cocaine, and chlorpromazine have been found to be inhibitors of 5-HT uptake into rat and rabbit lung and recently imipramine and cocaine have been shown [15] to reduce 5-HT uptake *in vivo*. Pretreatment of animals with reserpine, 6-hydroxydopamine, iproniazid or pargyline failed to alter 5-HT removal by the isolated perfused lung, although metabolism of 5-HT was markedly reduced following monoamine oxidase inhibition.

In contrast to the data obtained using the isolated perfused lung system, the *in vivo* disposition study of Axelrod and Inscoe [24] demonstrated the presence of significant amounts of unchanged 5-HT in the mouse lung 1 week after administration. This long-term retention of 5-HT has been confirmed in mouse and rat lung [25] and has been shown not to result from platelet entrapment. In addition, 5-HIAA has been shown to be the sole metabolite which is present in small quantities in lungs obtained from animals 15 min to 1 week after [^{3}H]5-HT administration. Furthermore, treatment of animals with reserpine resulted in a marked decrease in lung [^{3}H]5-HT levels, while guanethidine treatment was without effect.

In light of the importance of the pulmonary vasculature in controlling circulating levels of 5-HT and

* Author to whom correspondence should be sent.

the lack of information concerning the mechanisms responsible for the pulmonary disposition of 5-HT in the intact animal, the present study was undertaken.

MATERIALS AND METHODS

Materials

Weanling, male, Swiss–Webster mice, weighing 15–16 g, juveniles weighing 20–25 g, and retired breeders weighing 35–40 g were purchased from the Laboratory Supply Co. Inc. (Indianapolis, IN). All animals were fed food and water *ad lib.* and were maintained on a 12:12 light/dark cycle. 5-Hydroxytryptamine binoxalate, 5-hydroxyindole-3-acetic acid, and 5,6-dihydroxytryptamine creatinine sulfate hydrate were purchased from the Regis Chemical Co. (Morton Grove, IL). Reserpine, pentobarbital, chlorpromazine hydrochloride, and Grade I porcine intestinal heparin were purchased from the Sigma Chemical Co. (St. Louis, MO). Ketamine hydrochloride was purchased from Bristol Laboratories (Syracuse, NY). The following radiochemicals were purchased from the New England Nuclear Corp. (Boston, MA): 5-hydroxytryptamine binoxalate-[1,2-^{3}H(N)] (sp. act. 27.0 Ci/mmole) and 5-hydroxytryptamine binoxalate [2-^{14}C] (sp. act. 51.5 mCi/mmole). The purity of all labeled compounds was determined by thin-layer chromatography on 250 μm silica gel G plates purchased from Analtech, Inc. (Newark, DE), using a solvent system composed of acetone–2-propanol–water–ammonium hydroxide (50:40:7:3). The thin-layer plates were scanned on a Packard model 7200 radiochromatogram scanner. The following drugs were gifts: clorgyline, May & Baker, Ltd. (Dagenham U.K.), fluoxetine, Eli Lilly & Co. (Indianapolis, IN), and imipramine, Ciba-Geigy Corp. (Summit, NJ).

Methods

Animal treatments. A solution containing 0.2 μCi [^{3}H]5-HT and 75 pmoles of 5-HT binoxalate/μl was injected (1 μl/g) into the tail vein of mice. The mice were loosely restrained under an inverted 100 ml blackened beaker with the tail protruding from the lip of the beaker. The animals were decapitated at various times following administration, and the lungs were quickly removed, rinsed in ice-cold saline, blotted dry, and frozen at −80° for analysis. Blood was collected from the decapitation site in glass tubes previously rinsed with heparinized saline (1800 units/ml) and dried. Hematocrits were routinely determined and were comparable to values previously reported [26]. Plasma samples were prepared by centrifuging the samples for 0.5 hr at 2000 rpm in a Beckman J-6B centrifuge cooled to 4°. A 100 μl aliquot of each plasma sample was transferred to a 125 × 15 mm glass tube and stored at −80° for analysis. In experiments designed to investigate the effect of increasing 5-HT binoxalate concentration on lung [^{3}H]5-HT levels, animals were injected i.v. (1 μl/g) with solutions containing 0.2 μCi[^{3}H]5-HT and 0.1–100 pmoles of 5-HT binoxalate/μl. The experiments, designed to investigate the influence of the method of sacrifice on lung [^{3}H]5-HT, employed the following treatment schedules: ketamine hydrochloride and sodium pentobarbital anesthesia were achieved by the i.p. injection of 200 mg/kg and 60 mg/kg, respectively, 10 min prior to the i.v. administration of [^{3}H]5-HT. Asphyxiation of animals with chloroform, diethyl ether, or carbon dioxide was accomplished by placing the animal in a warmed (37°) dessicator saturated with the specific asphyxiant. Animals were kept in the dessicator until all breathing stopped which corresponded to 1.5, 1.1 and 0.5 min for chloroform, diethyl ether, and carbon dioxide respectively.

Drug treatments. Groups (8–10) of male, Swiss–Webster mice (20–25 g) were pretreated with the following drugs (dose, pretreatment time): clorgyline hydrochloride (20 mg/kg, 2 hr), fluoxetine hydrochloride (10 mg/kg, 24 and 0.5 hr), reserpine (5 mg/kg, 24 hr), imipramine hydrochloride (20 mg/kg, 0.5 hr), cocaine hydrochloride (20 mg/kg, 0.5 hr), chlorpromazine hydrochloride (20 mg/kg, 0.5 hr), and 5,6-dihydroxytryptamine creatinine sulfate hydrate (68.6 mg/kg, 24 hr). The [^{3}H]5-HT was injected i.v. (1 μl/g) as a solution containing 0.2 μCi [^{3}H]5-HT and 75 pmoles of 5-HT binoxalate/μl, and animals were killed by ether asphyxiation/decapitation 15 and 120 min after administration. Control animals were injected with 0.9% saline solution. The reserpine solution was prepared as previously reported [27].

Analytical procedures. Lung tissue was weighed and homogenized (Brinkman Polytron homogenizer) in 4 ml of cold acidified butanol (0.85 ml of 12 N HCl/liter) containing 100 μl of 10% EDTA, 50 μl of ascorbic acid solution (200 mg/ml, prepared fresh daily) and 5.0 nCi of 5-hydroxytryptamine binoxalate[2-^{14}C]. Plasma samples (100 μl) were handled in the same manner. The resulting homogenate was centrifuged for 10 min at 2000 rpm, and the supernatant fraction was transferred to 13-ml glass-stoppered tubes containing 7 ml of *n*-heptane and 0.2 ml of 0.1 N HCl. The tubes were shaken for 10 min, centrifuged for 10 min at 2000 rpm, and an 8.5-ml aliquot of the organic phase was transferred to clean 13-ml glass-stoppered tubes containing 0.2 ml of 0.1 N NaOH. The remaining organic phase was removed by aspiration, discarded, and a 50-μl sample of the acid extract was taken for liquid scintillation counting of [^{3}H]5-HT. The recovery of [^{3}H]5-HT was 90–95 per cent and was corrected by means of the [^{14}C]5-HT internal standard. The tubes containing the transferred organic phase were shaken and centrifuged, and the organic phase was removed by aspiration and discarded. A 50-μl aliquot of the alkaline extract was taken for liquid scintillation counting of [^{3}H]5-HIAA. The recovery of [^{3}H]5-HIAA using this extraction procedure was 60 per cent and the data are corrected. All the samples were counted in a Beckman LS-8000 liquid scintillation counter programmed to count either single or dual-labeled (^{3}H/^{14}C) samples. The liquid scintillation fluid was prepared by dissolving 5 g of PPO/POPOP* (98:2) and 100 g of napthalene in a liter of *p*-dioxane. A calibration curve was prepared by add-

* PPO = 2,5-diphenylopazole; and POPOP = 1,4-bis-[2-(4-methyl-5-phenylopazolyl)] benzene.

ing known amounts of [^{3}H]5-HT and 5 nCi or [^{14}C]5-HT to "blank" lung tissue and analyzing the samples (*vide supra*). Plots of [^{3}H]5-HT/[^{14}C]5-HT cpm versus concentration of [^{3}H]5-HT (nCi) were linear in the range for lung samples in these experiments. The specificity of the extraction procedure was confirmed by thin-layer chromatography using a solvent system composed of acetone–2-propanol–water–ammonium hydroxide (50:40:7:3) for [^{3}H]5-HT, and chloroform–methanol–acetic acid (60:35:5) for [^{3}H]5-HIAA. Each thin-layer chromatogram yielded a single radioactive component possessing an R_f identical to that of authentic material.

Statistical analysis. Results are expressed as means ± S.E.M. (nCi/g) and were analyzed statistically by the Student's *t*-test with two-tailed probability values; the level of significance was taken to be $P < 0.05$.

RESULTS

Factors influencing lung levels of [^{3}H]5-*HT*

The initial studies were focused on examining three factors capable of influencing lung [^{3}H]5-HT levels and potentially responsible for the high animal-to-animal variation encountered when working with intact animals: age of animal, concentration of injected 5-HT, and method of killing the animal. When [^{3}H]5-HT was administered to weanling mice (21–25 days old), juvenile mice (32–33 days old) and retired breeders (>270 days old), no significant difference in lung levels of [^{3}H]5-HT was observed; however, the percentage of the [^{3}H]5-HT dose accumulated by the lung was shown to decrease significantly with age (Table 1).

In an effort to further define the factors influencing the pulmonary disposition of 5-HT, the effect of the injected 5-HT binoxalate concentration on the pulmonary disposition of [^{3}H]5-HT was investigated to determine if the pulmonary uptake mechanism was dose-dependent and saturable under the experimental conditions. Animals were injected with a constant amount of [^{3}H]5-HT contained in differing amounts of 5-HT binoxalate. Concentrations of 5-HT binoxalate greater than 100 pmoles/μl could not be safely employed without the risk of encountering hemodynamic changes [28]. The results are shown in Table 2 and indicate that the uptake of 5-HT into lung is linearly related to concentration and does not appear to be saturated. Since the [^{3}H]5-HT accumulated in lung remains constant with decreasing [^{3}H]5-HT specific activity, this indicates that uptake is dose-dependent whereas the percentage of the dose taken up by the lung is constant.

Table 1. Effect of age on lung [^{3}H]5-HT levels*

Age of animal (days)	*N*	[^{3}H]5-HT (nCi/g)	[^{3}H]5-HT (% Dose)†
21–25	8	829 ± 56	3.50 ± 0.24
30–33	9	810 ± 56	2.76 ± 0.16
>270	6	785 ± 74	2.05 ± 0.16

* Male, Swiss–Webster mice were injected i.v. (1 μl/g) with a solution containing 0.2 μCi [^{3}H]5-HT and 75 pmoles of 5-HT binoxalate/μl, and decapitated 10 min after administration. Values are means ± S.E.M.

† $P < 0.05$ between groups.

Table 2. Effect of administered 5-HT concentrations on lung [^{3}H]5-HT levels*

5-HT conc (μM)	*N*	Specific activity (μCi/nmole)	[^{3}H]5-HT (nCi/g)
0.1	7	900	862 ± 70
1	7	90	810 ± 141
10	8	9	882 ± 72
100	8	0.9	781 ± 41

* Male, Swiss–Webster mice (20–25 g) were injected i.v. (1 μl/g) with solutions containing 0.2 μCi [^{3}H]5-HT and 0.1–100 pmoles of 5-HT binoxalate/μl, and decapitated 10 min after administration. Values are means ± S.E.M. Differences between the groups were not significant by Student's *t*-test.

Lastly, the method of killing the animal was examined as a factor influencing the lungs levels of [^{3}H]5-HT. Earlier work [25] had found a 3-fold higher level of [^{3}H]5-HT in animals killed by cervical dislocation versus decapitation, and others [29] have reported similar effects on endogenous 5-HT levels in lung. Several methods of sacrifice which would potentially suppress cardiovascular function and the release of [^{3}H]5-HT from neuronal and non-neuronal sites during sacrifice were investigated. These data appear in Table 3. Both ketamine anesthesia/decapitation and carbon dioxide asphyxiation/decapitation lowered the pulmonary levels of [^{3}H]5-HT. The method of sacrifice that produced the lowest variation without affecting the pulmonary [^{3}H]5-HT levels was ether asphyxiation/decapitation, which consisted of placing the animal in an ether-saturated dessicator (37°); anesthesia was achieved in 10 sec and death in 70 ± 10 sec. This method was used in all subsequent experiments.

Time-course of [^{3}H]5-*HT and* [^{3}H]5-*HIAA in lung and plasma*

The time-course of lung and plasma levels of [^{3}H]5-HT and [^{3}H]5-HIAA was investigated and is presented in Fig. 1. At all time points from 5 to 240 min after the administration of [^{3}H]5-HT, the lung levels of [^{3}H]5-HT exceeded plasma levels by factors ranging from 5.2 to 4.8 respectively. During the same period, lung/plasma [^{3}H]5-HIAA ratios were 1.5 to 1.8 respectively. In addition, the percentage of the total lung radioactivity represented by unchanged [^{3}H]5-HT ranged from 97 per cent at 5 min to 98 per cent at 240 min, indicating a long-term retention of unchanged [^{3}H]5-HT by the lung. The slope calculated by regression analysis of the lung points between 60 and 240 min gave a half-life of 209 min. Between 30 and 60 min following administration of [^{3}H]5-HT there was an apparent redistribution into the lung which was not statistically significant.

Effect of drugs on lung [^{3}H]5-*HT levels*

In order to characterize the mechanism responsible for the uptake, storage and metabolism of

Table 3. Effect of method of sacrifice on lung [^{3}H]5-HT levels*

Method of sacrifice	N	[^{3}H]5-HT (nCi/g)
Decapitation	9	765 ± 71
Ketamine anesthesia/decapitation	7	465 ± 17†
Pentobarbital anesthesia/decapitation	8	816 ± 45
Ether asphyxiation/decapitation	8	807 ± 27
Chloroform asphyxiation/decapitation	7	827 ± 66
Carbon dioxide asphyxiation/decapitation	7	523 ± 15†

* Male, Swiss–Webster mice (20–25 g) were injected i.v. (1 μl/g) with a solution containing 0.2 μCi [^{3}H]5-HT and 75 pmoles of 5-HT binoxalate/μl, and killed 10 min after administration. Values are means ± S.E.M.

† $P < 0.01$ by Student's t-test, compared with decapitation.

[^{3}H]5-HT from the pulmonary vasculature, the effects of select pharmacological agents on these processes were evaluated. Animals were pretreated with doses of the specific drug known to produce a pharmacological effect [30–33], injected i.v. with [^{3}H]5-HT, and killed 15 and 120 min after administration. The lung and plasma levels of [^{3}H]5-HT and [^{3}H]5-HIAA were determined and these data are presented in Table 4. Drugs such as imipramine, cocaine and chlorpromazine which are known to block the membrane transport of 5-HT into platelets, nerve terminals and brain produced significant decreases in lung levels of [^{3}H]5-HT and [^{3}H]5-HIAA at both the 15- and 120-min time points. In addition, fluoxetine which is a specific inhibitor of 5-HT uptake into platelets [30] and serotonergic nerve terminals in brain [34] also decreased lung and plasma levels of [^{3}H]5-HIAA at both time points. Lung and plasma [^{3}H]5-HT/[^{3}H]5-HIAA ratios following imipramine treatment were 24 and 5.6 at 15 min and 53 and 11 at 120 min respectively. Ratios for other uptake inhibitors fell within this range. Pretreatment of animals with the serotonin neurotoxin, 5,6-dihydroxytryptamine, resulted in significantly lower lung [^{3}H]5-HT levels at both time points. Lung and plasma [^{3}H]5-HT/[^{3}H]5-HIAA ratios and [^{3}H]5-HIAA levels were similar to those of control animals.

The data obtained from animals pretreated with clorgyline and reserpine are of particular interest. Clorgyline which is a selective inhibitor of type A monoamine oxidase [35] produced a significant increase in lung [^{3}H]5-HT and decrease in lung [^{3}H]5-HIAA at both time points while the plasma levels of [^{3}H]5-HT were elevated at 15 min but decreased below controls at 120 min. Lung and plasma [^{3}H]5-HT/[^{3}H]5-HIAA ratios were approximately 70 and 16, respectively, at both time points, reflecting the inhibition of monoamine oxidase.

When animals were pretreated with reserpine, lung [^{3}H]5-HT and [^{3}H]5-HIAA were elevated at 15 min to 134 and 198 per cent of control levels, respectively, while plasma [^{3}H]5-HT was significantly reduced and the plasma [^{3}H]5-HIAA elevated at the 15-min time point. In contrast, at 120 min the lung levels of [^{3}H]5-HT were markedly decreased and the plasma levels were elevated to the corresponding lung levels. These dramatic changes are reflected in the lung and plasma [^{3}H]5-HT/[^{3}H]5-HIAA ratios which were 23 and 3.5 at 15 min and 20 and 37 at 120 min respectively.

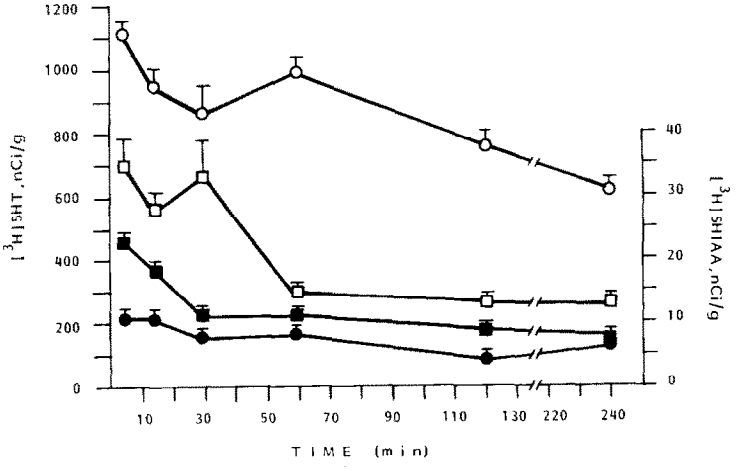

Fig. 1. Time-course of lung and plasma levels of [^{3}H]5-HT and [^{3}H]5-HIAA after i.v. administration of [^{3}H]5-HT to groups of eight mice. Key: (○) lung [^{3}H]5-HT; (●) plasma [^{3}H]5-HT; (□) lung [^{3}H]5-HIAA; and (■) plasma [^{3}H]5-HIAA. Values are means ± S.E.M.

Table 4. Effect of drugs on lung and plasma levels or [^{3}H]5-HT and [^{3}H]5-HIAA*

Drug	N	Time (min)	[^{3}H]5-HT (nCi/g) Lung	[^{3}H]5-HT (nCi/g) Plasma	[^{3}H]5-HIAA (nCi/g) Lung	[^{3}H]5-HIAA (nCi/g) Plasma
Control	20	15	817 ± 25	169 ± 10	23.5 ± 1.6	21.3 ± 1.0
	8	120	645 ± 19	125 ± 8	14.8 ± 0.9	6.4 ± 0.3
Imipramine	7	15	445 ± 30†	144 ± 15	17.9 ± 0.8‡	25.9 ± 1.1‡
	7	120	439 ± 24†	65 ± 5†	8.3 ± 0.8†	6.2 ± 0.6
Cocaine	7	15	529 ± 19†	149 ± 20	13.6 ± 1.1†	18.1 ± 1.7
	7	120	373 ± 21†	97 ± 11	9.9 ± 0.7†	8.4 ± 0.8§
Chlorpromazine	7	15	572 ± 32†	120 ± 6†	15.3 ± 1.1†	19.4 ± 1.6
	7	120	548 ± 29§	84 ± 9‡	13.3 ± 1.1	9.6 ± 0.8‡
Fluoxetine	7	15	335 ± 19†	128 ± 7‡	13.7 ± 0.7†	15.6 ± 1.6‡
	8	120	281 ± 24†	51 ± 2†	6.6 ± 0.7†	5.1 ± 0.1‡
5,6-Dihydroxy-tryptamine	7	15	685 ± 46§	116 ± 5†	21.0 ± 1.2	20.7 ± 1.8
	8	120	511 ± 54§	106 ± 14	14.3 ± 1.4	6.2 ± 1.1
Clorgyline	8	15	934 ± 50§	223 ± 24§	13.6 ± 0.4†	13.3 ± 0.8†
	8	120	724 ± 27§	80 ± 3†	10.1 ± 0.9‡	5.5 ± 0.4
Reserpine	6	15	1093 ± 70‡	117 ± 20§	46.7 ± 3.5†	33.4 ± 2.9†
	7	120	457 ± 25†	430 ± 19†	23.4 ± 3.6§	11.5 ± 1.4‡

* Male, Swiss–Webster mice (20–25 g) were pretreated with drugs as described in Methods. They were injected i.v. (1 μl/g) with a solution containing 0.2 μCi [^{3}H]5-HT and 75 pmoles of 5-HT binoxalate/μl, and killed 15 and 120 min after administration. Values are means ± S.E.M.

† $P < 0.01$, compared with control.

‡ $P < 0.01$, compared with control.

§ $P < 0.05$, compared with control.

DISCUSSION

The *in vivo* method described in this paper is particularly suited for the study of the mechanisms and factors responsible for the pulmonary disposition of 5-HT. The experimental design employed in this study departs significantly from the isolated perfused lung technique used to document the mechanisms involved in the pulmonary uptake and metabolism of 5-HT and the results may, in part, reflect this difference.

Various factors have been implicated as influencing the pulmonary concentration of both endogenous and exogenous compounds. One such factor is animal age, which has been shown to directly correlate with endogenous levels of 5-HT in rat lung [36]. When animal age was investigated for its influence on the functional aspects of the pulmonary vasculature as reflected in lung [^{3}H]5-HT levels, no difference was noted; however, the percentage of the [^{3}H]5-HT dose accumulated in the lung was found to be inversely related to animal age (Table 1). Thus, the percentage of the [^{3}H]5-HT dose accumulated in lung appears to be directly related to the endogenous 5-HT levels in lung.

Experiments employing isolated perfused lungs of rabbits have shown the pulmonary uptake of 5-HT to be a dose-dependent, saturable process over a concentration range of 1–20 μM [5, 6, 37]. Although a direct comparison to the isolated perfused lung is not possible, the influence of injected 5-HT concentration on the pulmonary uptake of [^{3}H]5-HT was examined. When animals were injected with varying amounts of 5-HT and a constant amount of [^{3}H]5-HT, no difference in the pulmonary levels of [^{3}H]5-HT was observed (Table 2). The data show that, under these conditions, the uptake of [^{3}H]5-HT is a non-saturable and dose-dependent process in which a constant percentage of the dose is taken up by the lung.

Lastly, the method of restraint used during the intravenous administration of 5-HT and the method of killing the animal were evaluated for their influence on pulmonary [^{3}H]5-HT levels. Fowler *et al.* [38] demonstrated a significant difference in the tissue distribution of [^{11}C]aliphatic amines depending on the method of restraint used for intravenous administration. This difference was attributed to a redistribution of the cardiac output as a result of sympathetic discharge associated with injection stress. Similarly, we found that the intravenous administration of [^{3}H]5-HT to animals tightly enclosed in a rodent restrainer resulted in highly variable levels of [^{3}H]5-HT in the lung. In contrast, when animals were injected in a less stressful manner as described in Methods, this variation was reduced.

The method of sacrifice has been shown to influence dramatically the lung levels of endogenous 5-HT [29] and exogenous [^{3}H]5-HT [25] and represents a major factor influencing lung 5-HT levels. Buckpitt *et al.* [25] found that lungs from animals killed by cervical dislocation had [^{3}H]5-HT levels three times greater than those killed by decapitation. This difference was attributed to the release of [^{3}H]5-HT from neuronal and non-neuronal storage sites coupled with continued circulation of the blood through the lungs, thus allowing for a greater removal of the amine. Several methods of sacrifice were investigated in an attempt to overcome this problem (Table 3) and of these methods only ketamine anesthesia/decapitation and carbon dioxide asphyxiation/decapitation altered the lung levels of [^{3}H]5-HT. Ketamine is known to competitively inhibit the neuronal uptake of 5-HT [39] and the lowered pulmonary accumulation of [^{3}H]5-HT in the presence of ketamine suggests a similar effect on the endothelial cell uptake

mechanism. The decreased [^{3}H]5-HT levels following carbon dioxide asphyxiation/decapitation most likely resulted from the severe respiratory acidosis produced by the carbon dioxide which causes the release of [^{3}H]5-HT from the lung in response to the lowered pH. The killing of animals by ether asphyxiation/decapitation produced lung [^{3}H]5-HT levels similar to those obtained using other methods of sacrifice but with a markedly reduced standard error of the mean. Waalkes and Coburn [40] have reported similar results for endogenous levels of mouse lung 5-HT using this method.

Several studies [24, 25, 29] have demonstrated the long-term retention ($T_{\frac{1}{2}} > 100$ hr) of 5-HT by lung and have shown this retention not to result from platelet or mast cell uptake. Examination of the early time-course of [^{3}H]5-HT and its metabolite [^{3}H]5-HIAA in lung and plasma reported herein confirm the avid uptake and retention of [^{3}H]5-HT by lung. At times ranging from 5 to 240 min after administration, the lung [^{3}H]5-HT levels exceeded the plasma levels by a factor of five and represented greater than 97 per cent of the lung radioactivity. The 209 min half-life of lung [^{3}H]5-HT calculated between 60 and 240 min differs from that previously determined [25] between 12 and 120 hr and results from the multiphasic nature of the [^{3}H]5-HT decay curve. These data confirm other reports [24, 25, 29] that in the intact animal significant amounts of 5-HT are taken up by the lung and retained unchanged for long periods of time.

The mechanisms responsible for the pulmonary uptake, storage and metabolism of circulating 5-HT were examined by the use of select pharmacological agents (Table 4). Drugs such as imipramine, cocaine and chlorpromazine, which inhibit 5-HT uptake into platelets and brain vesicles [41] and which also inhibit 5-HT uptake in the isolated perfused lung [5] and *in vivo* [5], are effective inhibitors of pulmonary [^{3}H]5-HT accumulation in the intact animal. In the intact animal it is possible that some of the effects observed with these drugs may result from altered hemodynamics. In addition, fluoxetine which is a specific inhibitor of 5-HT uptake into platelets [30] and brain synaptosomes [34] was a potent inhibitor of [^{3}H]5-HT uptake into the mouse lung.

In 1971, Baumgarten *et al.* [42] described the selective degeneration of indoleamine nerve terminals following the administration of the chemical neurotoxin 5,6-dihydroxytryptamine. More recently, Donelson *et al.* [32] reported prolonged, reduced levels of 5-HT and norepinephrine following the intraperitoneal administration of 5,6-dihydroxytryptamine to rats. When animals were pretreated with 5,6-dihydroxytryptamine, a significant decrease in lung levels of [^{3}H]5-HT was observed. These data suggest that some of the administered [^{3}H]5-HT is taken up by pulmonary neuronal sites which are susceptible to the neurotoxic effects of 5,6-dihydroxytryptamine.

The data obtained from animals pretreated with clorgyline and reserpine are of particular interest in attempting to define the mechanisms responsible for the pulmonary disposition of 5-HT. Clorgyline is a selective inhibitor of rat lung type A monoamine oxidase [43], the form of the enzyme responsible for the metabolism of 5-HT [44]. When animals were pretreated with clorgyline, lung [^{3}H]5-HT levels were significantly elevated and [^{3}H]5-HIAA levels were decreased at both time points. These effects of clorgyline inhibition of monoamine oxidase are reflected in the high lung [^{3}H]5-HT/[^{3}H]5-HIAA ratio. Similarly, inhibition of monoamine oxidase in the perfused rabbit lung has been reported [45] to produce a 4-fold increase in lung 5-HT.

Reserpine has been shown to be an effective depletor of biogenic amines present in both neuronal and non-neuronal sites [46]. Furthermore, reserpine has been found to deplete endogenous lung 5-HT [29] and exogenous [^{3}H]5-HT [25]. When rats were pretreated with reserpine and their lungs subsequently removed and perfused, no effect on 5-HT clearance was observed [5]. The data (Table 4) show that, in reserpine-pretreated animals, lung [^{3}H]5-HT levels were significantly affected. At the 15 min time point lung [^{3}H]5-HT and both lung and plasma [^{3}H]5-HIAA were significantly elevated, while plasma [^{3}H]5-HT was reduced relative to control animals. In contrast, at 120 min the lung [^{3}H]5-HT levels were lower and the plasma levels were higher than control values. These data are consistent with the view that the lowered endogenous levels of lung 5-HT produced by reserpine pretreatment initially increase the removal of [^{3}H]5-HT from the pulmonary vasculature into a cytoplasmic "pool"; however, in the absence of functional storage sites, the [^{3}H]5-HT becomes rapidly inactivated by monoaminase oxidase, subsequently resulting in lower lung levels of [^{3}H]5-HT. In this respect, the lung differs from brain [47, 48] in which reserpine had no effect on the initial *in vivo* uptake of 5-HT. The plasma levels of [^{3}H]5-HT in reserpine-pretreated animals, on the other hand, are initally low due to the marked uptake of the amine by lung and other tissues; however, in the absence of functional storage of [^{3}H]5-HT, the plasma and tissue levels of [^{3}H]5-HT in time equilibrate and at 120 min the [^{3}H]5-HT levels are similar to those found in lung. The results obtained with reserpine suggest that agents (drugs, dietary, environmental) which alter endogenous levels of 5-HT may potentially affect the ability of the lungs to effectively control the concentration of circulating 5-HT.

Acknowledgements—This work was carried out under a grant-in-aid from the American Heart Association, Indiana Affiliate, Inc., and a Young Investigator Pulmonary Research Grant (LH 19573) from the National Institute of Heart, Lung and Blood.

REFERENCES

1. E. Starling and E. Verney, *Proc. R. Soc. B* **97**, 321 (1925).
2. M. M. Rapport, A. A. Green and I. H. Page, *Science* **108**, 329 (1948).
3. M. M. Rapport, A. A. Green and I. H. Page, *J. biol. Chem.* **176**, 1243 (1948).
4. V. A. Alabaster and Y. S. Bakhle, *Br. J. Pharmac.* **40**, 468 (1970).
5. A. F. Junod, *J. Pharmac. exp. Ther.* **183**, 341 (1972).
6. Y. Iwasawa, C. N. Gillis and G. Aghajanian, *J. Pharmac. exp. Ther.* **186**, 498 (1973).

7. Y. Iwasawa and C. N. Gillis, *J. Pharmac. exp. Ther.* **188**, 386 (1974).
8. R. D. Pickett, M. W. Anderson, T. C. Orton and T. E. Eling, *J. Pharmac. exp. Ther.* **194**, 545 (1975).
9. C. N. Gillis and J. A. Roth, *Br. J. Pharmac.* **59**, 585 (1977).
10. Y. S. Bakhle and M. B. H. Youdim, *Br. J. Pharmac.* **65**, 147 (1979).
11. Y. S. Bakhle, J. Hartiala, H. Toivonen and P. Uotila, *Br. J. Pharmac.* **65**, 495 (1979).
12. A. G. E. Wilson, R. D. Pickett, T. E. Eling and M. W. Anderson, *Drug Metab. Dispos.* **7**, 420 (1979).
13. R. A. Roth, K. B. Wallace, R. H. Alper and M. D. Bailie, *Biochem. Pharmac.* **28**, 2349 (1979).
14. D. A. Wiersma and R. A. Roth, *J. Pharmac. exp. Ther.* **212**, 97 (1980).
15. J. D. Catravas and C. N. Gillis, *J. Pharmac. exp. Ther.* **213**, 120 (1980).
16. H. Steinberg and A. Fisher, in *Serotonin in Health and Disease* (Ed. W. B. Essman), Vol. V, pp.69–98. Spectrum Press, New York (1979).
17. T. R. Waalkes, H. Weissbach, J. Bozicevich and S. Udenfriend, *J. clin. Invest.* **36**, 1115 (1957).
18. M. A. Fink, *Proc. Soc. exp. Biol. Med.* **92**, 673 (1956).
19. M. D. Gershon and L. L. Ross, *Proc. Soc. exp. Biol. Med.* **115**, 367 (1961).
20. R. K. Gershon, P. W. Askenase and M. D. Gershon, *J. exp. Med.* **142**, 732 (1975).
21. A. P. Fishman, *Circulation Res.* **35**, 657 (1974).
22. J. M. Strum and A. F. Junod, *J. Cell Biol.* **54**, 456 (1972).
23. S. A. M. Cross, V. Alabaster, Y. Bakhle and J. Vane, *Histochemistry* **39**, 83 (1974).
24. J. Axlerod and J. Inscoe, *J. Pharmac. exp. Ther.* **141**, 161 (1963).
25. A. R. Buckpitt, R. P. Maickel and T. R. Bosin, *Biochem. Pharmac.* **27**, 569 (1978).
26. J. J. Friedman, *Am. J. Physiol.* **196**, 420 (1959).
27. A. Pletscher, P. A. Shore and B. B. Brodie, *J. Pharmac. exp. Ther.* **116**, 84 (1956).
28. S. Garattini and L. Valzelli, *Serotonin*, pp. 169–89. Elsevier, Amsterdam (1965).
29. C. Sadavongvivad, *Br. J. Pharmac.* **38**, 353 (1970).
30. J. S. Horng and D. T. Wong, *Biochem. Pharmac.* **25**, 865 (1976).
31. A. E. Felner and P. C. Waldmeier, *Biochem. Pharmac.* **28**, 995 (1979).
32. A. C. Donelson, T. R. Bosin, E. Campaigne, R. B. Rogers and R. P. Maichel, *Biochem. Pharmac.* **28**, 3339 (1979).
33. C. E. Barnes and L. G. Otherington, *Drug Dosage in Laboratory Animals: A Handbook*, pp. 15–70. University of California Press, Berkeley (1973).
34. D. T. Wong, F. P. Bymaster, J. S. Horng and B. B. Molloy, *Life Sci.* **15**, 471 (1974).
35. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
36. G. Bertaccini, *Ric. Sci.* **28**, 2661 (1958).
37. V. A. Albaster, in *Metabolic Functions of the Lung* (Eds. Y. S. Bakhle and J. R. Vane), pp. 3–20 Marcel Dekker, New York (1977).
38. J. S. Fowler, B. M. Gallagher, R. R. MacGregor and A. P. Wolf, *J. Pharmac. exp. Ther.* **198**, 133 (1976).
39. A. J. Azzaro and D. J. Smith, *Neuropharmacology* **16**, 349 (1977).
40. T. P. Waalkes and H. Coburn, *J. Allergy* **31**, 151 (1960).
41. D. L. Murphy and I. J. Kopin, in *Metabolic Pathways* (Ed. D. M. Greenberg), Vol. VI, pp. 503–50. Academic Press, New York (1972).
42. H. G. Baumgarten, A. Bjorklund, L. Lachenmayer, A. Nobin and U. Stenevi, *Acta physiol. scand.* (Suppl.) **373**, 1 (1971).
43. H. C. Kung and A. G. E. Wilson, *Life Sci.* **24**, 425 (1979).
44. C. J. Fowler, B. A. Callingham, T. J. Mantle and F. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
45. C. N. Gillis and Y. Iwasawa, *J. appl. Physiol.* **33**, 404 (1972).
46. E. F. Domino, in *Drills' Pharmacology* (Ed. J. R. DiPalma), pp. 484–5, McGraw-Hill, New York (1971).
47. D. Palaic, I. H. Page and P. A. Khairallah, *J. Neurochem.* **14**, 63 (1967).
48. K. J. Blackburn, P. C. French and R. J. Merrills, *Life Sci.* **6**, 1653 (1967).