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PULMONARY DISPOSITION OF SEROTONIN IN THE INTACT ANIMAL*

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Abstract—Trace doses of [^{3}H]serotonin ([^{3}H]5-HT) administered intravenously to mice accumulated rapidly in the lungs and were subsequently retained unchanged for long periods of time. Although maximal [^{3}H]5-HT levels achieved in animals killed by stunning were approximately three times higher than after decapitation, the plasma decay curves, beginning 12 hr after administration, showed a similar first-order monophasic decay with half-lives ($T_{1/2}$) of 108–120 hr). Examination of lung extracts obtained from mice sacrificed at time points ranging from 15 min to 1 week after [^{3}H]5-HT injection revealed the presence of small quantities of a single metabolite, 5-hydroxyindole-3-acetic acid. No difference in [^{3}H]5-HT levels was observed in platelet "rich" and platelet "poor" plasma fractions when the number of platelets in the two fractions varied from 7.5 to 1, respectively. Reserpine was shown to markedly deplete pulmonary [^{3}H]5-HT levels, while guanethidine was without effect.

There have been many studies on the localization of endogenous serotonin (5-HT), but little information is available on the uptake and storage of exogenously administered physiological amounts of the amine by various tissues. In 1956, Erspamer [1] observed that 5-HT was taken up and retained by platelets for several hours after subcutaneous administration, while the gastrointestinal tract, which is known to contain large amounts of 5-HT [2], failed to selectively accumulate the amine. Several years later, Axelrod and Inscoe [3] investigated the fate of intravenously administered 5-HT in the mouse and found the amine to be taken up and retained unchanged by the lung, spleen and adrenal gland for periods longer than a week. In addition, they noted that pretreatment of the mice with tryptamine, reserpine, cocaine or imipramine blocked the uptake of 5-HT by the lung, while chlorpromazine had no significant effect on the process.

Earlier work had shown that the lung may well act as a "filter" system toward vasoconstrictor substances such as 5-HT. As early as 1925, Starling and Verney [4] reported that the use of a heart–lung preparation was required to permit the use of defibrinated blood in studies of the isolated kidney. In the absence of the heart–lung "filter," the vessels of the kidney constrict so firmly that blood flow becomes virtually zero. These authors concluded that "...defibrinated blood becomes 'detoxicated' in the heart–lung preparation, presumably in the lungs" [4]. Some 28 years later, Gaddum *et al.* [5] demonstrated that this "filter" action of the pulmonary circulation involved the inactivation and/or removal of a specific vasoconstrictor substance, 5-HT. The potent vasoconstriction and bronchoconstriction elicited by injected 5-HT [6] have promoted considerable speculation as to the role this amine might play in such diverse disease states as anaphylaxis [7], pulmonary embolism [8] and pulmonary vascular hypertension [9].

Recent research using the isolated perfused lung of the rat and rabbit has shown that 5-HT undergoes very rapid uptake from the capillary blood supply to the lung [10–14]. This uptake process was found to be a Na^+-dependent, saturable process, susceptible to inhibitors such as cocaine, imipramine and chlorpromazine. In the isolated perfused lung preparation, 5-HT was rapidly and completely metabolized; autoradiographic studies suggest the capillary endothelial cells as the major, if not only, site of metabolism [15, 16].

The present study was designed to examine the fate of tracer doses of 5-HT in the lungs of an intact animal; the results indicate significant uptake and retention.

MATERIALS AND METHODS

Materials

5-Hydroxytryptamine binoxalate, 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-methoxytryptamine were purchased from Regis Chemical Co., Chicago, IL. *N*-acetyl-5-hydroxytryptamine, 5-hydroxytryptophol and 5-hydroxy-*N*-ω-methyltryptamine were obtained from Aldrich Chemical Co., Milwaukee, WI. The following radiochemicals were obtained from New England Nuclear Corp., Boston, MA: 5-hydroxytryptamine bionoxalate [1,2-^{3}H (N)] with specific activities ranging from 4.25 to 5.70 Ci/m-mole, 5-hyd-

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roxytryptamine binoxalate[2-^{14}C] with a specific activity of 48.5 mCi/m-mole and 5-hydroxyindole-3-acetic-carboxyl[^{14}C]acid with a specific activity of 22.1 mCi/m-mole. The purity of all labeled compounds was determined by thin-layer chromatography (t.l.c.) on 250 μ Silica gel G plates (Analtech Inc., Newark, DE), using at least two of the following solvent systems: (A) acetone–isopropanol–water–ammonium hydroxide (50:40:7:3); (B) methyl acetate–isopropanol–ammoniumhydroxide(45:35:20); and (C) chloroform–methanol–acetic acid (60:35:5). The thin-layer plates were subsequently scanned on a Packard model 7200 radiochromatogram scanner.

Although [^{3}H]5-HT has the advantage of having relatively high specific activity, it is unstable and certain precautions must be taken. On two occasions, samples of [^{3}H]5-HT obtained from the supplier were grossly impure and required purification by ascending paper chromatography in butanol–acetic acid–water (60:15:25) on Whatman No. 1 chromatography paper prior to use. Once pure, samples of [^{3}H]5-HT could be stored under nitrogen, in the dark, in an ethanol–water solution (98:2) for 4 months without detectable degradation. Lung samples containing [^{3}H]5-HT and [^{3}H]5-HIAA could not be stored in the freezer, since radiolysis of radiolabeled compounds in lung tissue was extensive. In all the studies reported in this paper, the quantitative analyses of [^{3}H]5-HT levels in the lung were performed immediately after animal sacrifice.

Male Swiss–Webster mice, weighing 20–30 g, and male Sprague–Dawley rats, weighing 300–400 g, were purchased from Murphy Breeding Laboratories, Plainfield, IN. Guanethidine hydrochloride and reserpine (Serpasil) were obtained from Ciba–Geigy Pharmaceuticals, Summit, NJ.

Methods

Extraction of [^{3}H]5-HT and metabolites. All procedures, with the exception of sample concentration, were carried out at 0–4°. Lung tissue was weighed and homogenized in 4 ml of cold methanol–acetone (1:1, spectral grade) to which was added 100 μl of 10% EDTA (aqueous), 50 μl of 200 mg/ml aqueous ascorbic acid solution (prepared fresh daily) and 100 μl of carrier mixture (prepared fresh daily). In experiments on the metabolism of 5-HT, this carrier was a methanolic solution (0.6 mg/ml) of 5-HT, 5-methoxytryptamine (5-MT), 5-HIAA, 5-hydroxytryptophol (5-HTOL), 5-hydroxy-*N*-ω-methyltryptamine (5-HNMT) and *N*-acetyl-5-hydroxytryptamine (NAS), while in the quantitative studies only 5-HT and 5-HIAA were used. The tissue was homogenized for 1 min, using a Sorvall Omni-Mixer equipped with a micro attachment. The homogenate was then centrifuged at 4° for 10 min at 3500 rev/min and a 3-ml aliquot of the supernatant solution was transferred to a 15-ml conical tube. The protein precipitate was resuspended in 4 ml of cold methanol–acetone (1:1) by vortexing for 0.5 min. The extract was centrifuged and a 3.5-ml aliquot was added to the conical tube. The combined methanol–acetone extracts were reduced to dryness under N_2 in a water bath at 30°. The samples were removed from the water bath as soon as they were dry. Methanol (100 μl) and pyridine (20 μl) were added to each sample and the extract was dissolved by vortexing at high speed for 2 min.

Isolation and identification of 5-HT metabolites in the lung. 5-Hydroxytryptamine binoxalate[1,2-^{3}H (N)] was injected into the tail vein of mice (n = 10–20) at a dose of 37 ng/g (0.8 μCi/g). The mice were killed by cervical dislocation or decapitation at various times after injection. In experiments where blood was collected, the animals were decapitated, and the lungs were quickly removed, rinsed in ice-cold saline, and blotted dry. Standards were prepared by adding [^{3}H]5-HT to lungs from untreated mice. All samples were then carried through the extraction procedure (*vide supra*). A 20-μl aliquot of the final extract was streaked on a 5- to 10-mm section on each of three t.l.c. plates. All samples were developed for 12 cm in a N_2 atmosphere, in the dark, using the solvent systems previously described. The plates were allowed to dry and the reference compounds were visualized under 254 nm u.v. light. Each sample lane (4-mm sections) was then scraped into a scintillation vial using an Isolab t.l.c. plate scraper (Isolab, Akron, OH). All samples were counted twice for 10 min in either a Packard model 2425 or a 3375 liquid scintillation counter. The scintillation fluid was prepared by dissolving 14 g BBOT (2,5-bis[5-(tert-butylbenzoxazolyl)]thiophene) and 280 g napthalene in 2 l. of toluene and 1.4 l. of 2-methoxyethanol. Counting efficiency was determined with [^{3}H]toluene and Silica gel from a blank t.l.c. plate. The R_f values of the labeled indoles were determined from the d.p.m. vs distance plots and compared with authentic standards [17].

Two-dimensional chromatographic identification of the metabolite. Two mice each received 30 μCi [^{3}H]5-HT intravenously. They were sacrificed 12 hr after injection and the lungs were prepared as previously described, with the exception that 0.002 μCi [^{14}C]5-HIAA was added to the lung homogenate. A portion of the extract was spotted and the sample was chromatographed bidirectionally; development (12 cm) in solvent system A was followed by development (12 cm) in solvent system C. The lane containing 5-HIAA, located by visualization under 254 nm u.v. light, was scraped in 4-mm sections into scintillation vials and counted. Similarly, 5-HIAA was identified by two-dimensional chromatography of mouse lungs (n = 2–6) obtained from animals sacrificed at various times (0.25 to 168 hr) after [^{3}H]5-HT injection.

Quantitative determination of [^{3}H]5-HT levels in the lung. The extraction procedure for the quantitative determination of 5-HT was the same as that previously described with the exception that 0.005 μCi [^{14}C]5-HT was added to each sample before homogenization and the carrier mixture consisted of only 5-HT and 5-HIAA. All chromatography was done in solvent system A. After the samples had been developed, the 5-HT and 5-HIAA areas were located, marked and scraped to serve as blanks. A calibration curve was established for each set of experiments by adding known amounts of [^{3}H]5-HT to "blank" lung tissue and analyzing the samples (*vide supra*). Plots of c.p.m. [^{3}H]/c.p.m. ^{14}C vs concentration of [^{3}H]5-HT (ng) were linear in the range found for lung samples in these experiments.

Analysis of [^3H]5-HT in plasma fractions. The blood from five mice was drained into polyethylene tubes rinsed with heparinized saline (1800 units/ml). Plasma was separated into platelet "rich" and platelet "poor" fractions utilizing the method of Shore *et al.* [18]. All samples were centrifuged for 60 min at 600 r.p.m. (150 *g*) in an IEC model PR-2 centrifuge cooled to 4°. A 100-μl aliquot of each plasma fraction was transferred to a glass-stoppered 13-ml centrifuge tube; methanol–acetone, EDTA, ascorbate, carrier and [^{14}C]5-HT were added (*vide supra*) and the contents were shaken for 5 min. The [^{3}H]5-HT was analyzed as previously described.

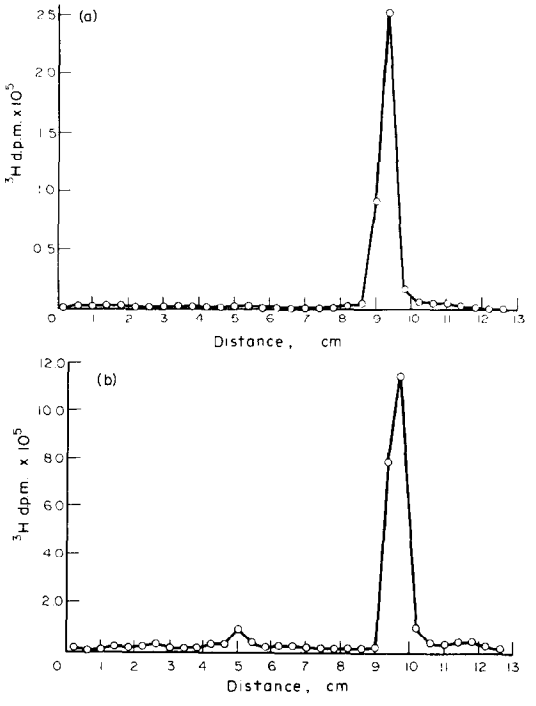

Fig. 1. Chromatographic profile of [^{3}H]d.p.m. as a function of distance. Panel A: chromatographic profile for a sample prepared by adding [^{3}H]5-HT to an untreated mouse lung. Panel B: chromatographic profile for a lung sample prepared from a mouse sacrificed 48 hr after the intravenous injection of [^{3}H]5-HT. Radioactive indoles were extracted as described in Methods. The samples were chromatographed for 12 cm on Silica gel G thin-layer plates in methyl acetate–isopropanol–NH_4OH (45:35:20).

Platelet counts taken on representative samples showed a 7.5 to 1 ratio of the number of platelets in the platelet rich to the platelet poor plasma fraction.

Drug studies. Drugs were prepared in normal saline in doses calculated as the free base: reserpine, 1.7 mg/kg, and guanethidine, 5 mg/kg. Eleven hr after the intravenous injection of [^{3}H]5-HT, animals were intravenously given saline (control) or drug and were sacrificed by decapitation 12, 48 and 72 hr after [^{3}H]5-HT administration.

RESULTS

Metabolism

When [^{3}H]5-HT was added to lung tissue from untreated mice, followed by extraction and chromatography in the three solvent systems as described, it chromatographed as a single peak (Fig. 1A). Thin-layer chromatographic profiles of radioactive indoles extracted from lungs of animals sacrificed from 0.25 to 168 hr after [^{3}H]5-HT injection revealed only two areas of radioactivity. In all three solvent systems, one area (containing greater than 90 per cent of the total activity) co-chromatographed with authentic 5-HT; the remaining activity co-chromatographed in a single band with 5-HIAA (Fig. 1B). The presence of 5-HIAA in the lung was confirmed using bidimensional thin-layer chromatography of lung extract to which [^{14}C]5-HIAA had been added as described in Methods (Fig. 2).

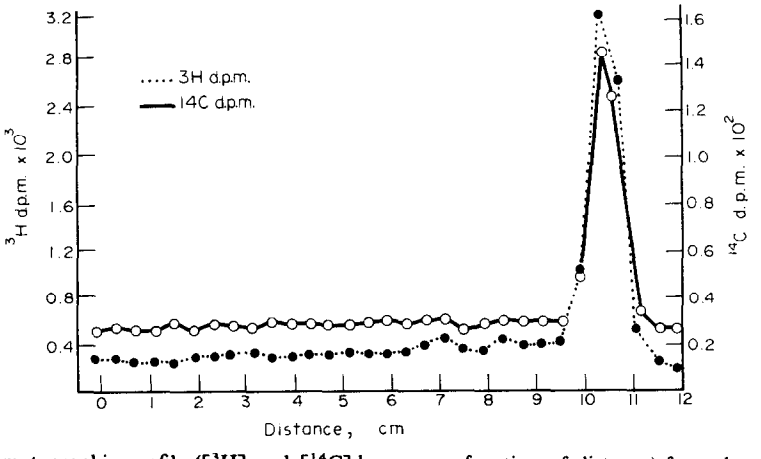

Fig. 2. Chromatographic profile ([^{3}H] and [^{14}C]d.p.m. as a function of distance) for a lung sample of [^{3}H]5-HIAA prepared from two mice sacrificed 12 hr after the intravenous injection of [^{3}H]5-HT. Radioactive indoles were extracted as described in Methods except that 0.002 μCi [^{14}C]5-HIAA was added to the lung extract. The sample was chromatographed bidimensionally on a Silica gel G plate. Chromatography in acetone–isopropanol–NH_4OH–H_2O (50:40:7:3) was followed by chromatography in chloroform–methanol–acetic acid (60:35:5). The lane parallel to the direction of travel in the second solvent system was scraped in 4-mm sections.

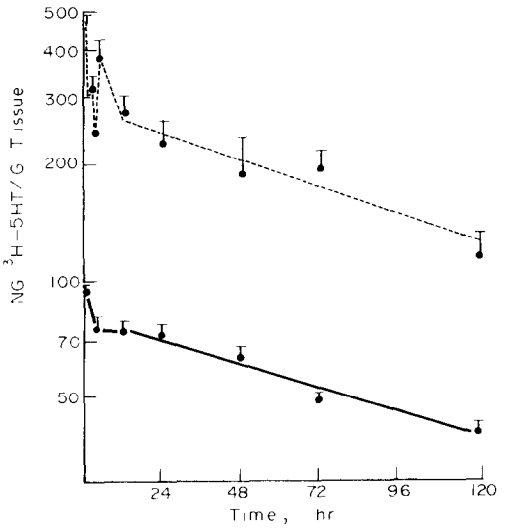

Fig. 3. Plot of the levels of [^{3}H]5-HT in the lung (ng/g of tissue ± S.E.) vs time. Nine to twenty mice were given [^{3}H]5-HT intravenously and were sacrificed by either cervical dislocation (-----) or by decapitation (——) at time points between 0.25 and 120 hr after injection. [^{3}H]5-HT levels were determined as described in Methods.

[^{3}H]5-HT disposition in the lung

The levels of unchanged [^{3}H]5-HT in the lung depend upon the method of animal sacrifice (Fig. 3). Lungs from animals sacrificed by cervical dislocation had levels of [^{3}H]5-HT three times greater than those of lungs taken from animals killed by decapitation. At the earliest time point studied (15 min), the lungs of animals killed by cervical dislocation contained nearly 10 per cent of the injected dose of [^{3}H]5-HT, while lungs from animals killed by decapitation contained only 3–4 per cent of the injected dose. The levels of labeled amine in the lung fluctuated in the first 12 hr after which they appeared to decay by first-order kinetics. The slopes calculated by regression analysis of the points between 12 and 120 hr gave half-lives of 108 and 120 hr for the animals killed by cervical dislocation and decapitation, respectively; these values were not significantly different ($P < 0.05$). Since decapitation resulted in decreased variability in lung [^{3}H]5-HT levels and eliminated the continued circulation of the blood to the lungs, it was chosen as the method of animal sacrifice in all subsequent experiments.

Table 1. Levels of [^{3}H]5-HT in platelet rich and platelet poor plasma fractions

	[^{3}H]5-HT levels* (ng/ml)	
Time (hr)	Platelet rich	Platelet poor
0.25	32.0	29.8
4	25.3	26.7
24	25.8	24.3
48	21.2	20.1
72	20.0	19.0
120	22.0	19.8

* Each value was obtained from pooled blood from four to five animals and was determined in duplicate.

Table 2. Levels of [^{3}H]5-HT in mouse lung after drug treatment*

	[^{3}H]5-HT (ng/g)		
Treatment	m2 hr	48 hr	72 hr
Control	73 ± 6.7	74 ± 6.7	54 ± 5.3
Reserpine	51 ± 3.1†	2 ± 0.45†	ND‡
Control	74 ± 4.3	64 ± 4.1	49 ± 2.9
Guanethidine	74 ± 3.4	67 ± 5.0	50 ± 3.7

* Each value represents the mean ± S. E. M. for ten animals.
† Significantly different from control values, $P < 0.05$.
‡ Not determined.

In order to determine whether the prolonged storage of 5-HT in the lung was due to the uptake and retention of the labeled amine by platelets, studies were undertaken to quantitate [^{3}H]5-HT levels in platelet rich and platelet poor plasma fractions. The results (Table 1) show no difference in the levels of [^{3}H]5-HT found in these fractions, yet platelet counts showed a 7.5 to 1 ratio in the two fractions.

Drug studies

In an attempt to further characterize the long-term storage of injected [^{3}H]5-HT, reserpine and guanethidine were administered as described in Methods. Ten animals were sacrificed at each time point. Reserpine produced a significant ($P < 0.05$) decrease in the levels of [^{3}H]5-HT in the lung at each time point studied, while levels of [^{3}H]5-HT were unaffected by guanethidine (Table 2).

DISCUSSION

The precise mechanism(s) by which the pulmonary vascular beds influence the circulating levels of vasoactive substances (such as 5-HT) are still not clearly understood. The lungs may well act as a "filter" for such substances much in the manner that the liver removes drugs or toxic materials from the general circulation. Such pulmonary mechanisms for vasoactive amine inactivation have been implicated in a diversity of pathological conditions including anaphylaxis [7, 19, 20], delayed-type hypersensitivity [21], pulmonary hypertension [9] and pulmonary embolism [8].

Serotonin has been identified as the mediator of anaphylaxis in mice [19, 20] and rabbits [7], and these effects have been correlated with enterochromaffin cell 5-HT and platelet 5-HT respectively. In these studies [19, 20], it was shown that pretreatment of mice with reserpine or 5-HT antagonists protected mice against lethal anaphylactic shock, while 5-HT administration was shown to mimic anaphylaxis.

It has also been suggested that in mice 5-HT plays an important role in the production of the lesions of delayed-type hypersensitivity [21]. This is accomplished by acting on endothelial cells of the post-capillary venules and permitting the egress of macrophages or their precursors from the blood into the sites of delayed inflammation.

In a review of dietary pulmonary hypertension, Fishman [9] has suggested a role for 5-HT in the pathogenesis of this disease. He proposed that subtle injury to the pulmonary endothelial cells and/or to the mechanisms involved the 5-HT inactivation might result in failure to inactivate circulating 5-HT which in the minute capillary vessels might cause platelet aggregation with the release of additional 5-HT and ultimate obstruction of the vessels. A similar proposal has been put forth to describe small pulmonary emboli [8].

The work described in this paper was directed at examining the pulmonary disposition and metabolism of 5-HT in mice. Thin-layer chromatographic identification of the radioactive indoles present in the lung revealed the presence of only 5-HT and 5-HIAA. This is in agreement with the work of other investigators using the isolated perfused lung system [10–14]. No trace of 5-HT-*O*-glucuronide, reported to be present in mouse lung 4 hr after the i.v. administration of [^{14}C]5-HTP [22], could be found. Small quantities of [^{3}H]5-HIAA were identified in lung samples obtained during the entire time period studied and ranged from 14.7 ng/g at 15 min to 2.5 ng/g at 120 hr. This suggests that a portion of 5-HT which undergoes storage in the lung is slowly released from the storage sites and metabolized by monoamine oxidase.

Studies reported in this paper support and extend the findings of Axelrod and Inscoe [3] and are in contrast to the data obtained using the isolated perfused lung. Examination of earlier time points may show that a substantial percentage of an injected dose of [^{3}H]5-HT is taken up and metabolized by the lung in the first few passes through the pulmonary circulation; however, a significant portion of this [^{3}H]5-HT is stored for long periods of time ($T_{1/2}$ > 100 hr). Preliminary experiments in rats at a dose of 37 ng/g indicated the level of [^{3}H]5-HT at 120 hr to be approximately half that found in the mouse at the same time point [23].

The difference in the [^{3}H]5-HT levels found in the lungs as a function of the method of sacrifice possibly reflects a release of amine from other storage sites. In animals sacrificed by cervical dislocation, the blood presumably continues to circulate through the lungs; any [^{3}H]5-HT present in the blood during this period is subject to removal by the lungs. In contrast, animals killed by decapitation showed lower levels of [^{3}H]5-HT, possibly because blood does not continue to circulate through this tissue after the animal is killed. The lung level of [^{3}H]5-HT in mice killed by cervical dislocation may vary as a function of the time it takes to remove the lungs from the animal, which may be the reason for the higher S. E. as a per cent of the mean seen in those animals killed by cervical dislocation, in contrast to those killed by decapitation.

The uptake and storage of 5-HT in the mouse lung do not appear to be the result of retention by platelets, since no difference in the [^{3}H]5-HT levels in platelet rich and platelet poor plasma fractions could be demonstrated (Table 1). Preliminary experiments in the rat also revealed no difference in [^{3}H]5-HT levels in platelet rich and platelet poor fractions [23]. In addition, the calculated half-life of [^{3}H]5-HT in mouse lung ($T_{1/2}$ 108–120 hr) is far greater than the reported half-life of 5-HT in platelets ($T_{1/2}$ 24–72 hr) [24, 25]. The levels of [^{3}H]5-HT present in the platelet rich and platelet poor fractions appear to simply be a function of the plasma half-life of [^{3}H]5-HT ($T_{1/2}$ > 242 hr).

In an attempt to further characterize the long-term storage of [^{3}H]5-HT by the mouse lung, the effects of reserpine and guanethidine on this process were investigated. Reserpine is an effective depletor of biogenic amines in both neuronal and non-neuronal sites [26]. It depletes endogenous 5-HT in the lung [27] and, therefore, it is not surprising that it dramatically lowers [^{3}H]5-HT levels in the lung (Table 2). Since mast cells, on the other hand, have been shown to be resistant to the releasing actions of reserpine [28, 29], these results would suggest that the long-term storage of [^{3}H]5-HT in the lung is not the result of storage in mast cells.

Guanethidine has been shown to be an effective depletor of brain norepinephrine and 5-HT when injected intracisternally, while at doses sufficient to lower intestinal norepinephrine by 50 per cent, 5-HT in the gut was unaffected, suggesting that the nature of 5-HT storage by brain and intestine might be different [30]. Guanethidine, when given at the same doses reported in the earlier work [30], produced no decrease in the lung levels of [^{3}H]5-HT. The failure of guanethidine to produce a decrease in [^{3}H]5-HT in the lung cannot be attributed to its inability to reach the lung in sufficient concentration; previous studies [31] have demonstrated significant levels of guanethidine in the lung. The nature of [^{3}H]5-HT storage in the lung, therefore, appears to resemble that in the intestine.

In conclusion, we have shown that tracer doses of injected 5-HT are taken up by the lung and retained by this organ for periods of time in excess of 1 week. This occurs in both mice and rats; mast cells and platelets appear to play an insignificant role in this process. Reserpine was able to markedly reduce the levels of [^{3}H]5-HT found in the lung and guanethidine was without an effect. The only detectable metabolite found in the lung 0.25 to 168 hr after [^{3}H]5-HT injection was 5-HIAA.

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