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Rapid Pulmonary Removal of 5-Hydroxytryptamine in the Intact Dog.* (29974)

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Non-platelet bound 5-hydroxytryptamine (5-HT; serotonin) rapidly disappears from the circulation after injection(1,2). Monoamine oxidase activity and platelet binding *in vivo* and chemical inactivation by erythrocytes *in vitro* may occur to decrease the concentration of 5-HT prior to assay. Metabolism of 5-HT in man is primarily due to oxidative deamination by monoamine oxidase (3), which is widely distributed and particularly rich in liver, kidney, heart, and intestinal mucosa(4).

Previous studies utilizing 5-HT infusion into the liver(5) or perfusion of the isolated lung(6,7) have shown that 5-HT is removed by the liver and lung. Certain physiologic responses, however, may be altered by perfusion of the isolated lung(8). It seemed advisable, therefore, to evaluate pulmonary 5-HT removal *in vivo,* and to assess the role of β-phenylisopropylhydrazine‡ (PIH), a potent monoamine oxidase inhibitor.

Methods and materials. Adult mongrel dogs weighing from 12-15 kg were anesthetized with sodium nembutal (25 mg/kg), and both carotid arteries were cannulated with #260 polyethylene cannulae. A #6 Cournand catheter was introduced into a jugular vein and advanced so that its tip lay in the pulmonary artery, as confirmed by the characteristic pressure contour.

5-HT was used as the 3′-C^{14} creatinine sulfate§ (specific activity 11.4 mc/mM), and was dissolved in indocyanine green dye (IG),‖ final concentration 1.25 mg/ml. Six dogs were studied without pretreatment, and 4 following intravenous pretreatment with 10 mg/kg of PIH, given 2 hours before 5-HT injection. Each dog was given 2 injections of C^{14} 5-HT at approximately 30-minute intervals, the second injection being designated by the prime in Tables I and II.

Three to five ml of IG were rapidly injected into the pulmonary artery without, and with, added C^{14} 5-HT. Continuous carotid arterial blood samples were obtained thereafter for radioactivity measurement. Sample tubes were changed every 2 seconds. Five mg of EDTA (per 3-5 ml blood) was used as an anticoagulant. After thorough mixing, a 0.5 ml aliquot was added to 1.5 ml distilled water. It was frozen and thawed twice, and 20 μl of hemolysate were plated on duplicate 1.25 inch aluminum planchets by the method of Smith *et al*(9). Radioactivity was counted on an end window automatic gas flow counter having a counting efficiency of 23%. Corrections were made for the resolving time of the counter, and samples were counted at infinite thinness. Samples were counted for a minimum of 1000 counts. Samples were corrected for background, and were counted to an error of 4% or less at the 96% confidence level. Certain samples were cen-

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§ Nuclear Chicago Corp., Des Plaines, Ill.

‖ Hynson, Westcott, and Dunning, Inc., Baltimore, Md.

TABLE I. Cardiac Output and % Removal of C^{14} 5-HT in Untreated Dogs.

Dog	Dose 5-HT‡ (μg/kg)	Cardiac output (I.G.)		Cardiac output (C^{14} 5-HT)	% removal of 5-HT
1		C*	4.32		
	17.3	S†	4.09	5.24	22
1′		C			
	17.3	S	3.02	4.59	34
2		C	3.46		
	9.8	S	3.32	4.54	27
3		C	3.73		
	11.5	S	3.52	4.5	22
3′		C	4.41		
	9.7	S	4.34	5.3	18
4		C	2.26		
	12.3	S	2.55	3.48	32
5		C	2.27		
	14.4	S	2.51	4.32	42
5′		C	2.37		
	14.4	S	2.13	3.88	45
6		C	1.98		
	12.5	S	1.59	2.71	41
6′		C	1.51		
	12.5	S	1.17	2.3	49
				Mean:	33

* Control. † 5-HT. ‡ Expressed as free base.

trifuged at 4°C (3000 rpm, 30 minutes in an International PR-2 centrifuge), and the platelet poor plasma was removed and deproteinized. One dimensional descending paper chromatograms of the protein free supernatant were developed in isopropanol 20 parts: water 2 parts:ammonium hydroxide (specific gravity 0.88) 1 part, and in butanol 12 parts:water 5 parts:glacial acetic acid 3 parts. Chromatograms were scanned for radioactivity with a Nuclear Chicago C-100B Actigraph.

A Minneapolis Honeywell Visicorder was used to record the indicator dilution curve, and EKG. The densitometer used was a Waters, Model X-250 with a linear response to 40 mg/l for IG dye in blood. Calibrations for the dye curves were done for each dog by determining deflections for zero, 6 and 10 mg/ml concentrations.

Cardiac outputs with IG were determined in duplicate by the method of Stewart(10) and Hamilton(11). Cardiac output determined from the C^{14} content of blood was computed from the ratio of total counts injected to counts recovered(12). Planimetry was employed to compute the area for the curve inscribed by the amounts of radioactivity in blood samples obtained after C^{14} injection. Percent removal of 5-HT was calculated from the formula:

$$100\left(1-\frac{\text{cardiac output (dye)}}{\text{cardiac output } (C^{14}\text{ 5-HT})}\right).$$

Results. Cardiac outputs of the first 6 dogs, and per cent removal of 5-HT are shown in Table I. The amounts of 5-HT given each dog on a weight basis varied, because total radioactivity in each injection was kept relatively constant. Compared to control values cardiac output (by IG) was lowered within the first 12 seconds in 7 of 9 determinations in which 5-HT was injected simultaneously, and rose in 2. The change was small, however, and the mean difference in output in the control, and the output simultaneous with 5-HT injection was —6% (± 3%, SEM). The mean per cent removal of 5-HT was 33 ± 3%. After PIH pretreatment the change in cardiac output with 5-HT injection was —9 ± 5%, the removal of 5-HT 28 ± 4%. The mean dose of 5-HT in the 2 groups was virtually identical, 13.1 and 12.8 μg/kg respectively. Calculated cardiac outputs in the second study in Dog 3 showed values by the two methods which were virtually identical, and the removal was therefore absent. That dog had had excessive hemorrhage prior to the final study and died shortly thereafter. Exclusion of that de-

TABLE II. Cardiac Output in Dogs Given Beta Phenylisopropylhydrazine Pretreatment (10 mg/kg).

Dog	Dose 5-HT‡ (μg/kg)	Cardiac output (I.G.)		Cardiac output (C^{14} 5-HT)	% removal of 5-HT
1		C*	1.76		
	14.4	S†	1.80	2.80	36
1′		C	2.02		
	11.8	S	2.10	3.76	42
2		C	2.60		
	15.5	S	1.93	2.49	22
2′		C	1.88		
	7.8	S	1.68	2.40	30
3		C	.83		
	13.0	S	.95	1.35	30
3′		C	.84		
	13.0	S	.76	.75	0
4		C	3.41		
	13.5	S	2.84	3.74	34
4′		C	3.45		
	13.5	S	2.74	4.00	31
				Mean:	28

* Control. † 5-HT. ‡ Expressed as free base.

termination gave a mean removal after PIH of 32 ± 2%. Collection was unsatisfactory following one injection in each of 2 untreated dogs (No. 2 & 4). Chromatography of both blood and plasma specimens after C^{14} 5-HT injection revealed only a peak for 5-HT.

Discussion. Starling and Verney(13) observed that a heart-lung preparation "detoxicated" freshly defibrinated blood, so that the vasoconstricting action was lost; Gaddum *et al*(6) later confirmed their observation and quantitated the rate of 5-HT removal by the isolated perfused lung. They found that between 10 and 21% of the plasma "5-HT equivalent" was removed per circulation and as the circulation rate was increased the rate of disappearance of 5-HT rose. Our results showed a mean 33% removal of injected 5-HT in the pulmonary circulation within 12-15 seconds after injection into the pulmonary artery in the intact dog. 5-HT was removed in the first passage through the pulmonary bed. A similar prompt removal of 5-HT in the mouse after intravenous injection was shown by Axelrod and Inscoe(14), since 50% of 5-HT disappeared in the first 10 minutes after injection. In the first minute after injection the lungs removed the greatest amount of 5-HT; hepatic removal was comparatively slight(14).

Oxidative deamination of 5-HT after its perfusion through an isolated dog lung preparation has been demonstrated by Eiseman (7). In his studies an increase in 5-hydroxyindoleacetic acid concentration was observed 7 minutes after 5-HT injection. In the present study less than 20 seconds elapsed between the beginning and end of blood collection. During that time it seemed unlikely that derivatives of 5-HT would be present, and none were found on radiochromatograms. Additional evidence that pulmonary monoamine oxidase (MAO) activity, and oxidative deamination did not account for early 5-HT removal was afforded by the lack of any significant change of 5-HT removal after PIH. The dose used was found to produce essentially complete inhibition of liver MAO activity in the rat(15), and it seems likely that lung MAO activity was effectively inhibited in the present studies.

An additional observation of interest was the small change in cardiac output after injection of substantial amounts of 5-HT. The lack of immediate change in cardiac output is in accord with the earlier observations of MacCanon and Horvath, who found little variation in output during the first 10 seconds after injection of 500 μg of 5-HT (as the creatinine sulfate) in the pulmonary artery of dogs. Fifteen to twenty-five seconds after drug injection the cardiac output rose (16). They observed bradycardia within 15 seconds after 5-HT injection, which was observed in the present studies.

Summary. Simultaneous injection of indocyanine green dye and C^{14} 5-HT was done in dogs to determine the extent of pulmonary removal of 5-HT. Cardiac output determined from C^{14} data was consistently higher than by the dye method, indicating immediate removal of 5-HT in the pulmonary bed. The mean removal was 33%, and was not changed by prior administration of β-phenylisopropylhydrazine, a monoamine oxidase inhibitor.

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A Comparison of the Disposition of Intravenously-Injected, Albumin-Bound Stearic Acid-1-C^{14} and Palmitic Acid-1-C^{14} in the Rat.* (29975)

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A number of studies have demonstrated that a difference exists between the metabolism of stearic acid and other 16- and 18-carbon containing fatty acids. The following examples illustrate this point: (a) The rate of oxidation of the C^{14} of stearic acid-1-C^{14} to CO_2 in the intact rat is lower than that of palmitic acid-1-C^{14} when they are injected intravenously either as free fatty acids bound to albumin or as emulsified triglycerides(1); (b) after an intravenous injection of acetate-1-C^{14} into rats, Tove *et al*(2) found that the specific activity of stearic acid in the neutral fat fraction of the liver rose more slowly than did that of palmitic acid or those of unsaturated fatty acids; (c) Olivicrona *et al* (3) injected palmitic acid-labeled and stearic acid-labeled chylomicrons into rats and observed less recirculation of labeled glycerides and free fatty acids with the stearic acid than with the palmitic acid-labeled chylomicrons.

It has been reported that a greater proportion of lipid C^{14} is recovered in the liver as phospholipids and a smaller proportion as triglycerides when C^{14}-stearic acid is fed to rats than when either C^{14}-labeled palmitic, oleic or linoleic acid is fed(4). But since it has also been shown(5) that approximately 20% of the C^{14} of labeled stearic acid fed to rats appears in the thoracic duct lymph as phospholipids—as compared to 4% in experiments with labeled palmitic, oleic and linoleic acids—the distribution of the C^{14} among lipid fractions in the liver, after the feeding of these various fatty acids, does not necessarily establish that a preferential synthesis of phospholipids from stearic acid occurs in the liver.

To determine whether or not the preferential incorporation of stearic acid into phospholipids is restricted to incorporation during absorption from the intestines, we have in the present study injected intravenously into rats albumin-bound C^{14}-labeled stearic or palmitic acid and determined the distribution of the C^{14} in lipid fractions of liver, plasma, adipose tissue, skeletal muscle and heart at various time intervals up to 24 hours after the injections.

Experimental. Administration of labeled fatty acid. Palmitic acid-1-C^{14} (6.23 mc/mmole) and stearic acid-1-C^{14} (9.13 mc/mmole) obtained from New England Nuclear Corp., Boston, were purified on silicic acid columns(6). One hundred mg of crystalline bovine albumin (Armour Pharmaceutical Co., Kankakee, Ill.), dissolved in 0.9% NaCl, was added to 1.20 mg of the sodium salt of the labeled palmitic acid and to 1.33 mg of the sodium salt of the labeled stearic acid dissolved in isotonic saline. The final volume of each fatty acid solution was 10 ml. Sixteen male Long-Evans rats (240 to 290 g) were lightly anesthetized with ether, and 1.0 ml of the labeled palmitic or stearic acid solution was injected into a leg or tail vein of each rat. Thereafter, at intervals of 1, 4, 12 and 24 hours, a pair of rats from each group was again anesthetized and blood samples (7-10 ml) were drawn from the hearts into heparin-rinsed syringes. The following organs and tissues were then rapidly excised: liver,

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