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The Concentration of Radioactivity From Labeled Epinephrine and Its Precursors in The Dog Adrenal Medulla[1,2,3]

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SUMMARY

Twenty-six dogs were injected with ^{14}C labeled epinephrine and its precursors and two different phenylethylamines. One dog was given an ^{125}I labeled phenylethylamine. Blood samples were withdrawn at frequent intervals for the first 6 hours, the urine collected and the dog sacrificed at 6 or 24 hours. Tissue samples were obtained from 16 organs of each dog and the concentration of radioactivity determined and expressed as counts per minute per milligram of tissue. The average adrenal medulla:plasma ratio at 6 hours rose from 20 after 3,4-dihydroxyphenylalanine (dopa) to 740 after 3,4-dihydroxyphenylethylamine (dopamine) and then fell to 128 after norepinephrine and 126 after epinephrine. Similar ratios at 24 hours could not be obtained because of low plasma activity. However, activity concentrations in the adrenal medulla (cpm/mg) were tyrosine 34, dopa 110, dopamine 422, norepinephrine 401, and epinephrine 243. Activity concentrations in the adrenal medulla 6 hours after the administration of ^{14}C labeled *p*-tyramine and phenylethylamine were low. Rapid deiodination of *p*-iodophenylethylamine ^{125}I precluded any distribution studies. After dopamine the average adrenal medulla:kidney ratios were 72 and 170 after 6 and 24 hours, respectively. Similarly, adrenal medulla:liver ratios were 90 and 112. The plasma concentration of ^{14}C radioactivity from dopamine fell more rapidly and to lower levels after the first hour than after any other compounds, a more rapid urinary excretion being also found. The concentration of radioactivity from labeled dopamine in the adrenal medulla is higher than the concentrations of ^{131}I in metastases from thyroid cancer. These data suggest that similar studies after administration of labeled dopamine or similar compounds in patients with chromaffin tumors would be of interest.

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INTRODUCTION

The generally accepted schematic presentation of the precursor metabolic pathway leading to the formation of norepinephrine and epinephrine is presented in Figure 1 (1). In a previous publication[1] we demonstrated that after the injection of DL-dopa-2-^{14}C[2] into mice with melanomas, the ^{14}C radioactivity concentration 24 hrs after injection was higher in the adrenal gland than in any other tissue, reaching adrenal:plasma ratios as high as 60.

Studies have been published on the concentration of stable epinephrine in cytoplasmic particles of the bovine adrenal medulla (2), radioactivity from DL-dopa-2-^{14}C in the rat adrenal medulla (3) and specific activity of norepinephrine and epinephrine in the rat adrenal medulla following administration of ^{14}C-labeled DL-phenylalanine, DL-tyrosine, DL-dopa, DL-epinephrine, phenylethylamine and tyramine (4). These, as well as many other studies, were designed primarily to elucidate the biosynthetic pathway and metabolism of norepinephrine and epinephrine.

Hempel and Deimel (5) suggested that labeled dopa could be therapeutically useful in tumors of chromaffin origin. We report here a study in the dog of the tissue distribution of radioactivity from the administration of ^{14}C-labeled epinephrine and its precursors and related phenylethylamines, as a prelude to a study in humans with chromaffin tumors.

MATERIALS AND METHODS

Labeled compounds. The ^{14}C-labeled compounds used in this study were obtained from New England Nuclear Corp. and were as follows:

	Spec. Act. (mC/mM)
1. DL-Tyrosine-3-^{14}C	2–10
2. DL-Dopa-2-^{14}C Hydrobromide	1–5
3. Dopamine-1-^{14}C Hydrobromide	2–10
4. DL-Norepinephrine-7-^{14}C	10–20
5. DL-Epinephrine-7-^{14}C	10–20
6. Phenylethylamine-1-^{14}C Hydrobromide	1–5
7. *p*-Tyramine-1-^{14}C Hydrobromide	1–5

The *p*-iodophenylethylamine-^{125}I was synthesized by Dr. R. E. Counsell and Mr. T. Smith in the radioisotope laboratory of the College of Pharmacy, University of Michigan. Figure 2 presents the structures and site of labeling of the radiolabeled phenylethylamines used in this study.

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[1]Meier, D. A., Beierwaltes, W. H., and Counsell, R. E.: Radioactivity from Labeled Precursors of Melanin in Mice and Hamsters with Melanomas. *Cancer Res.*, admitted for publication.

[2]Abbreviations used in this paper are as follows:
dopa—3,4-dihydroxyphenylalanine
dopamine—3,4-dihydroxyphenylethylamine

BIOSYNTHETIC PATHWAY FOR CATECHOLAMINES

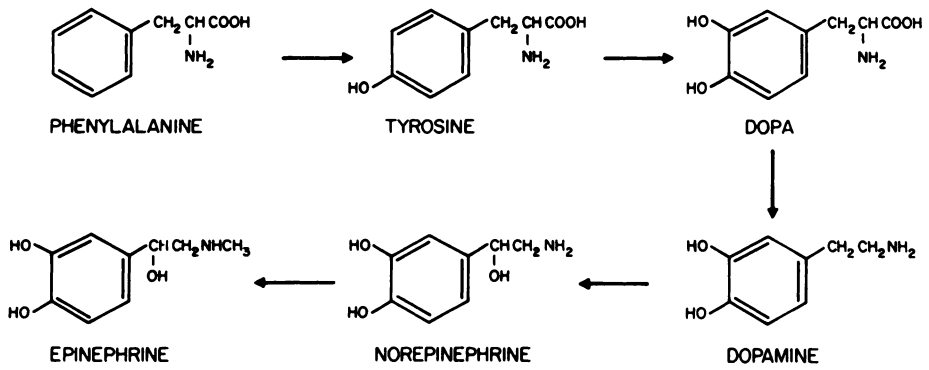

Fig. 1

OTHER RADIOLABELED PHENYLETHYLAMINES

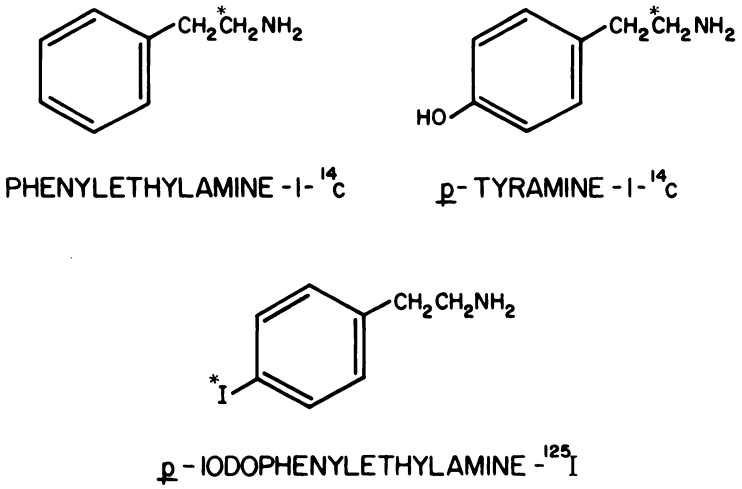

Fig. 2

Dogs and tissue specimens. A total of 26 adult female mongrel dogs weighing 10-17kg were studied. The dogs were anesthetized by injecting 25 mg of sodium pentobarbital (50 mg/ml)/kg of body weight into a front leg vein. An intracath was then inserted in the front leg vein for injecting the labeled compound and continuing anesthesia as needed. A second intracath was inserted into a hind leg vein for withdrawing serial blood samples. No heparin was used, flow being insured by small injections of normal saline every 15-20 min. Urine samples were obtained with an indwelling Foley catheter. Approximately 90-95 μC of the labeled compounds were injected in a volume of 15-20 ml of normal saline over a period of 2 min, except for norepinephrine and epinephrine which were injected over a period of 5-10 min. A small volume was kept for a reference standard. Tachycardia was observed with norepinephrine and epinephrine injections, the effect lasting no more than 10 min. Blood samples were then withdrawn into a heparinized syringe at appropriate frequent intervals for the initial 6 hr as shown in Figure 4. The dog was sacrificed at 6 or 24 hr using Lethal Solution.[1] Immediately thereafter, the whole adrenal glands were removed, weighed and frozen in dry ice. After partial thawing, most of the medulla was scooped from the cortex and weighed. (The average sample weight was 8 mg.) The 15 other tissue samples were also dissected free, cleaned of fat and foreign material and weighed.

[1]Lethal Solution is manufactured by Haver-Lockhart Laboratories, Kansas City, Missouri.

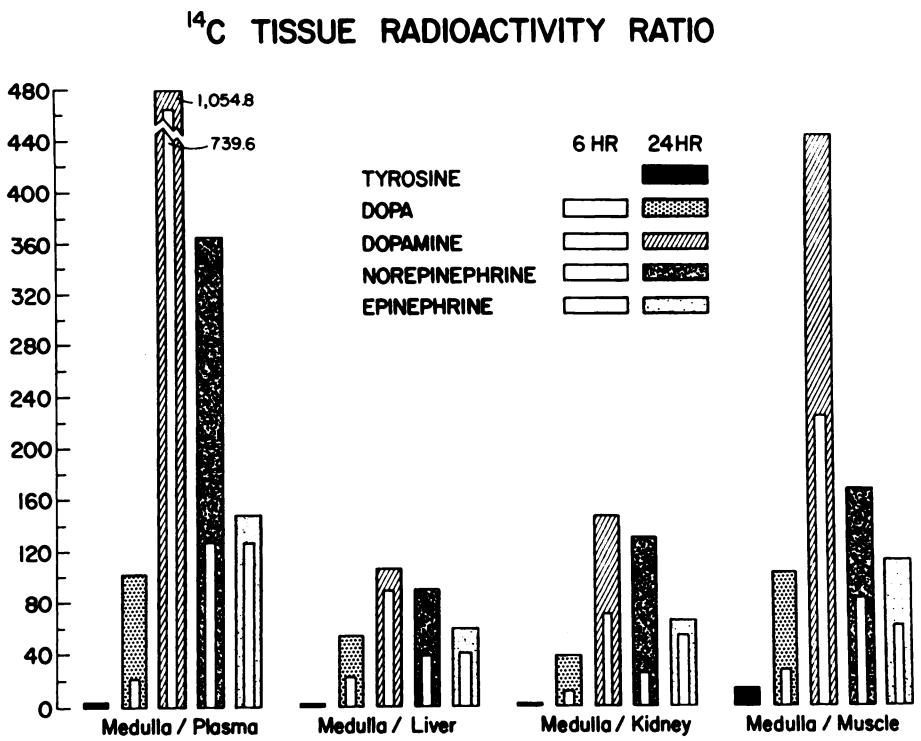

Fig. 3

All specimens were then placed in a counting vial and digested overnight in 0.3 ml of 10% NaOH. They were dissolved by heating in near boiling water for 30 sec and after cooling, 4 drops of 30% H_2O_2 were added. Then 10 ml of the liquid scintillation system of Frenkel *et al* (6) were added and the samples counted. Quenching was corrected for by the use of an internal standard. All results are expressed in dpm/mg with no correction for dog weight.

RESULTS

Tissue distribution

Table I presents the tissue concentration distribution of radioactivity in dpm/mg in 8 selected tissues (mean and range). Significant differences between the adrenal medula and all other tissues are seen with dopa, dopamine, norepinephrine and epinephrine. Limited data is available for tyramine and phenylethylamine, but it tends to show much smaller differences. One dog was injected with *p*-iodophenylethylamine and sacrificed at 24 hr; however, over 90% of the activity was found in the thyroid and in the urine, indicating a rapid deiodination, presumably in the liver.

Figure 3 presents these data in terms of adrenal medulla: tissue and plasma ratios at 6 and 24 hr. In general these ratios tended to be higher at 24 hr even though adrenal medulla concentrations were equal or less. The adrenal medula: plasma ratios were the highest obtained. At 6 hr they rose from 20 after dopa to 740 after dopamine and then fell to 128 after norepinephrine and 126 after epinephrine. Similar sequences of rank were generally observed in the adrenal medulla: liver, kidney and muscle ratios at 6 and 24 hr. Adrenal medula:plasma ratios at 24 hr were much higher, but these were largely due to very low plasma counts. The adrenal medulla:cortex ratios are more irregular and less striking presumably due to difficulties in separating all the medullary tissue from the cortex.

In general, dopamine had the highest adrenal medulla concentrations and adrenal medulla:tissue and plasma ratios. The dopamine concentration in the adrenal medulla at 6 hr represented approximately 0.01% of the administered dose. The maximum ratio of organ to plasma achieved by any of the other 15 tissues was that of heart muscle, reaching 14 at 6 hr with dopamine and 18 after norepinephrine and epinephrine.

Disappearance from plasma

Figure 4 presents the disappearance of radioactivity with time from plasma (dpm/mg). The plasma concentration of ^{14}C radioactivity from dopamine after the first hour fell more rapidly and to lower levels than after any other compound. This striking fall during the first 1.5 hr after injection suggests the possibility that a higher adrenal medulla concentration of radioactivity may have been reached some time before the first time interval tested, 6 hr after injection.

TABLE I

TISSUE ^{14}C CONCENTRATIONS AT 6 AND 24 HOURS AFTER INJECTION (CPM/MG, MEAN AND RANGE)[1]

Organ	D,L- -3-^{14}C Tyrosine	D,L- -2-^{14}C Dopa		-1-^{14}C Dopamine		D,L- -2-^{14}C Norepinephrine		D,L- -2-^{14}C Epinephrine		-1-^{14}C Tyramine	-1-^{14}C Phenylethylamine
	24 Hrs	6 Hrs	24 Hrs	6 Hrs	24 Hrs	6 Hrs	24 Hrs	6 Hrs	24 Hrs	6 Hrs	6 Hrs
	(2 dogs)	(3 dogs)	(4 dogs)	(2 dogs)	(3 dogs)	(2 dogs)	(3 dogs)	(2 dogs)	(2 dogs)	(1 dog)	(2 dogs)
Adrenal medulla	28.3-38.0	104.6-146.6	58.5-135.6	508.6-970.7	249.0-731.2	331.3-612.4	301.0-586.3	441.3-569.8	246.6-248.1	133.8	0.06-2.2
	33.2	124.0	110.5	739.6	421.9	471.9	401.4	505.6	247.4		1.13
Adrenal cortex	22.8-32.1	6.0-7.1	3.2-6.2	24.1-75.7	8.8-24.3	23.7-28.6	20.4-35.8	11.0-62.5	9.6-19.2	4.4	0.06-2.2
	27.4	6.8	4.5	49.9	18.9	26.2	28.6	36.8	14.4		1.13
Heart	3.2-6.4	2.8-7.2	0.7-2.0	8.6-14.8	3.5-7.5	51.8-68.2	14.4-33.5	15.7-104.1	3.4-28.8	2.5	1.1
	4.8	5.1	1.1	11.7	5.4	60.0	21.0	59.9	16.1		
Lung	5.7-18.7	2.7-5.5	0.5-1.8	2.3-2.5	0.4-3.7	8.6	3.1-7.9	2.2-6.3	0.54-1.4	3.7	0.01-1.1
	12.2	4.1	1.1	2.4	1.5	8.6	4.8	4.2	0.97		0.56
Kidney	14.0-39.7	4.6-18.6	1.7-4.6	6.8-14.2	1.1-5.2	13.0-24.7	1.4-4.2	9.1-10.2	3.1-4.6	9.3	0.15-2.4
	26.8	11.5	2.9	10.5	2.9	18.8	3.1	9.6	3.8		1.28
Spleen	7.5-22.9	4.5-12.6	0.7-2.1	24.0-26.0	6.0-22.6	60.9-94.0	15.9-19.8	22.8-62.8	16.1-23.7	4.0	1.8
	15.2	7.8	1.4	25.0	15.0	77.4	17.7	42.8	19.9		
Liver	11.6-23.3	4.4-7.0	1.0-3.6	5.1-11.8	1.8-6.8	11.5-12.9	3.8-5.5	12.7-13.0	3.2-5.2	4.3	0.05-1.87
	17.4	5.9	2.1	8.45	4.0	12.2	4.5	12.8	4.2		0.96
Muscle	2.0-3.6	3.9-8.8	0.4-2.6	2.7-3.9	0.6-1.3	3.4-8.2	1.5-3.1	2.3-14.4	1.6-2.8	3.2	0.03-1.0
	2.8	7.0	1.1	3.3	0.95	5.8	2.4	8.4	2.2		0.52

[1]Other tissues studied and not listed were pancreas, uterus, ovary, urinary bladder, parotid gland, stomach, small bowel and sympathetic ganglis.

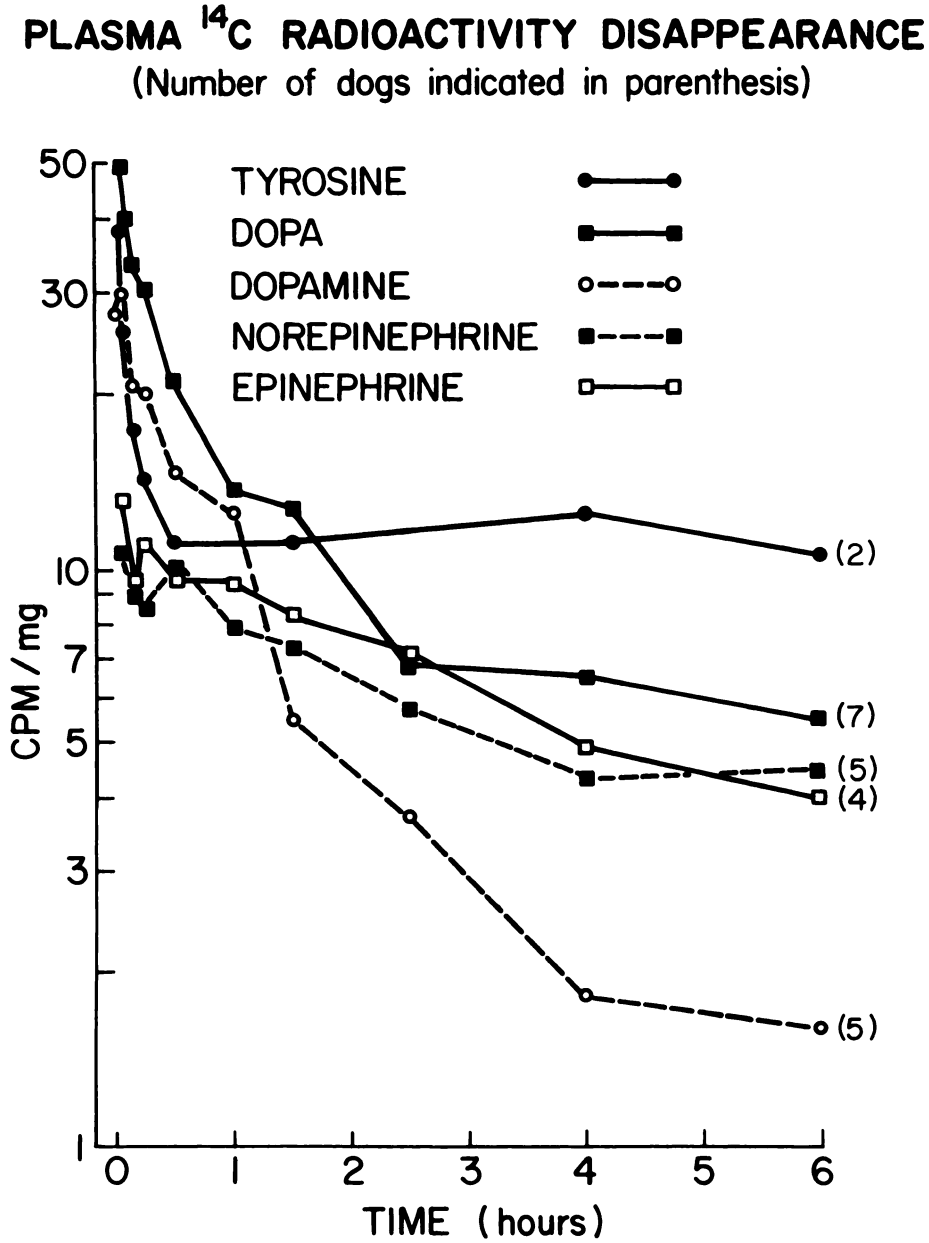

Fig. 4. Time intervals of measurements within the first hour were as follows: dopa and dopamine—3, 5, 10, 15 and 30 min Tyrosine, norepinephrine and epinephrine—5, 10, 15 and 30 min Blood samples for tyrosine at 2.5 hr were not drawn.

Appearance in urine

Figure 5 presents cumulative urinary excretion of radioactivity in percent of administered dose at 6 and 24 hr. Urinary excretion studies showed that 95% of ^{14}C radioactivity from dopamine was excreted within the first 6 hr and approximately 100% of the radioactivity was accounted for in 24 hr. Figures for the same intervals after dopa were 55% and 83%. Improper collection of urine specimens did not allow us to calculate the excretion of norepinephrine.

DISCUSSION

Pheochromocytomas and neuroblastomas are active secretors of norepinephrine, epinephrine, their precursors and their metabolites, producing them in amounts considerably higher than the normal adrenal medulla. Thus, not only should they show a higher uptake of precursors, but in addition, since some of

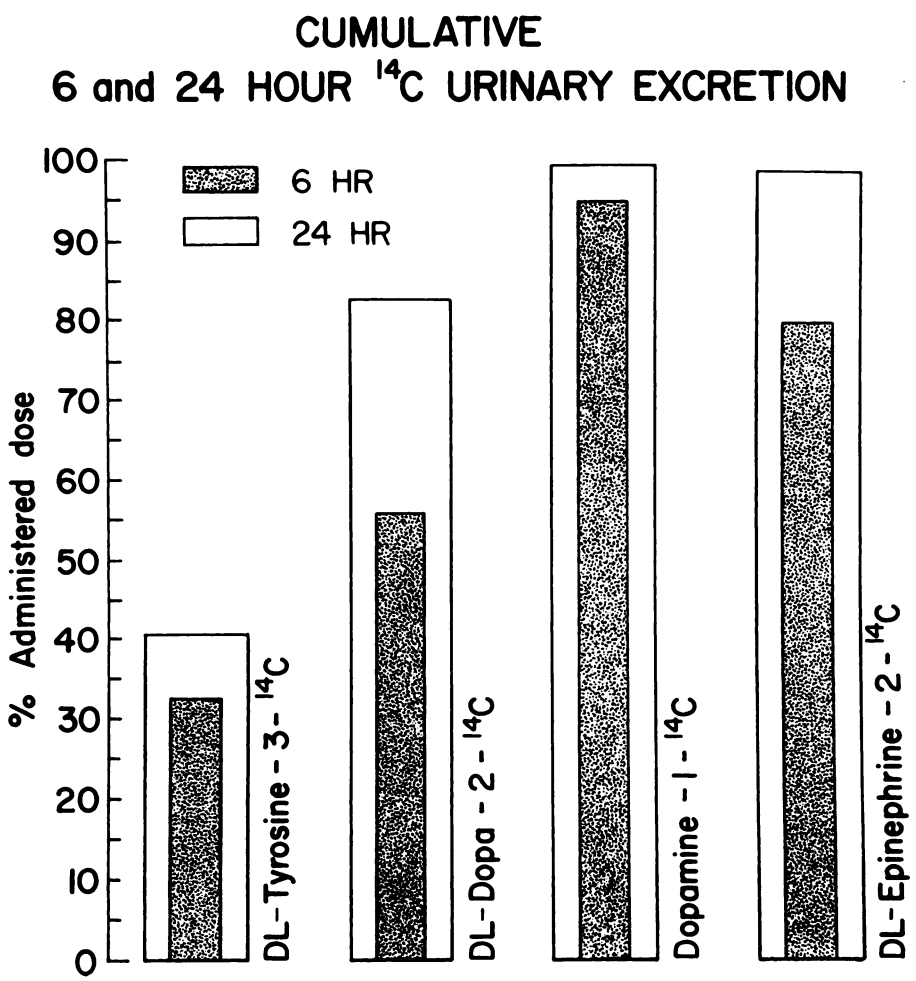

Fig. 5

these tumors have a slow turnover rate of catecholamines (7, 8), we would also expect an administered precursor to remain longer in them.

Our data show that in the two time periods studied, dopamine (optically inactive) had the highest adrenal medulla concentration and plasma ratios. However, since only the racemic forms of dopa, norepinephrine, and epinephrine were used, it is possible that their L forms would have achieved concentrations at least as high as dopamine.

Phenylethylamine-1-^{14}C and *p*-tyramine-1-^{14}C were administered, not as precursors, but based on the premise that they would displace norepinephrine stoichiometrically (9). As shown in Table I, the results were not as expected. Tyramine showed the highest concentration, similar to dopa, but still well below that of dopamine.

At shorter time periods, higher concentrations and ratios may be reached, since the plasma levels fall rapidly within the first hour. Prolongation of the infusion time might also enhance the uptake of these compounds. We are presently studying these two possibilities in patients undergoing bilateral adrenalectomy for carcinoma of the breast.

Studies of ^{131}I concentration in thyroid carcinoma metastases at necropsy have shown values up to 0.275% of the administered dose per gram of tissue (10, 11). With dopamine at 6 hr we found a maximum concentration of about 0.01% of the administered dose in the adrenal medulla. However, since the whole adrenal medulla in the dog is in general not heavier than 20 mg, the percent uptake per mg then becomes about 2 times higher than that reported for thyroid carcinoma metastases. In addition, the ratios to plasma are also much higher than those achieved by ^{131}I.

We are now developing a method to label dopamine and other related compounds with a gamma-emitting radionuclide. Two important points must be kept in mind in this effort. The first point is to label a site which will be stable and not give rise to ionic radionuclides *in vivo*. We have already observed deiodination, presumably in the liver or perhaps elsewhere, when we injected *p*-iodophenylethylamine-^{125}I with over 90% of the activity being found in the thyroid and in the urine. The second point is that the labeled compound should behave as the unlabeled compound. These considerations are being evaluated experimentally using the dog as the model.

With the rapid progress in radiochemistry and our understanding of precursor and metabolic pathways, it is probable that the development of diagnostic localization and perhaps therapeutic techniques with radionuclide-labeled compounds might be aided by this approach.

ACKNOWLEDGMENT

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