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THE FATE OF C^{14}-DIHYDROXYPHENYLALANINE (C^{14}-DOPA) IN THE WHOLE MOUSE[1]

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ABSTRACT

WURTMAN, R. J., C. CHOU AND C. ROSE: The fate of C^{14}-dihydroxyphenylalanine (C^{14}-dopa) in the whole mouse. J. Pharmacol. Exp. Ther. **174:** 351–356, 1970. The metabolic fate of i.p. administered C^{14}-DL-dopa was examined in the whole mouse. The amino acid was rapidly destroyed at an initial rate (7.3% of injected dose per min) that was maintained for almost 10 minutes. The level of C^{14}-catecholamine in the whole carcass reached a peak 20 minutes after injection; almost all of this material was C^{14}-dopamine. Less than 0.1% of the C^{14}-catechols present in the body 20 or 60 minutes after C^{14}-DL-dopa administration was found in the brain. At all doses studied (0.5–150 mg/kg), more than half of the C^{14}-dopa was metabolized during the first 20 minutes after its administration to C^{14}-methoxydopa and C^{14}-homovanillic acid. If the metabolism of the circulating amino acid in humans is similar to that of mice, it can be calculated that the amount of methionine needed to O-methylate the doses of L-dopa commonly used in parkinsonian patients may be greater than the average daily methionine intake.

There is now considerable evidence that large oral doses of the levo isomer of dihydroxyphenylalanine (L-dopa) can be of considerable benefit in the treatment of Parkinson's disease (Cotzias *et al.*, 1969). Dopa is synthesized *in vivo* within chromaffin cells and certain neurons by the hydroxylation of tyrosine (Nagatsu *et al.*, 1964; Wurtman, 1966). Little of the endogenous amino acid can usually be found in the blood or the tissues (Anton and Sayre, 1964), suggesting that it is rapidly metabolized, probably by decarboxylation. Hence exogenous dopa, which enters the tissues by uptake from the bloodstream should probably be considered a drug, and the physiologic disposition of this material might be expected to differ significantly from that of the endogenous amino acid.

We have examined the fate of C^{14}-dopa after its i.p. administration to whole mice.[2] It will be shown that a relatively small fraction of a single dose of administered dopa is available to the brain for conversion to catecholamines and that most of the C^{14}-amino acid is rapidly destroyed in the periphery by O-methylation and decarboxylation.

METHODS. *Animals.* Swiss type CD-1 male mice, obtained from Charles River Breeding Laboratories (Wilmington, Mass.), weighing 30 to 40 g, were housed five per cage and given access *ad libitum* to Big Red Laboratory Chow and water. Animals were exposed between 9 A.M. and 9 P.M. to approximately 50 footcandles of fluorescent light emitted by Vita-Lite (Duro-Test Corp., North Bergen, N.J.), a source whose spectrum simulates that of sunlight. All injections were made between 10 A.M. and noon.

3,4-DL-Dihydroxyphenylalanine-2-C^{14} (New England Nuclear Corporation, Boston, Mass.; 4.5 $\mu c/\mu mol$) was mixed with 0.5 N HAc containing various quantities of nonradioactive L-dopa and administered i.p. in a volume of 0.5 ml; control animals received only the 0.5 N HAc. Animals were killed by neck fracture and homogenized in 9 volumes of cold 0.4 N perchloric acid, with a Waring Blendor. In some experiments, the brain was homogenized separately.

Assay of C^{14}-dopa and its metabolites. Aliquots of the whole homogenate were centrifuged in the cold at 10,000 × *g*, and triplicate 20-ml portions

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[2] Preliminary reports of these observations have appeared before (Rose *et al.*, 1970; Wurtman, 1970).

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of the supernatant fluid were passed over prepared alumina columns at pH 8.6 (Whitby *et al.*, 1961). C^{14}-catechols were eluted in 6 ml of 0.1 N hydrochloric acid; the radioactivity in a 1-ml aliquot of the eluate was counted in a liquid scintillation spectrophotometer, and the remaining 5 ml were pooled with the two other eluates from the same animal and passed over a 4.5-cm Dowex 50W-4X column (Bio-Rad Laboratories, Richmond, Calif.; 200–400 mesh; hydrogen form) at pH 2.0 (Carlsson and Waldeck, 1964). After washing with 5 ml of water, the C^{14}-dopa was eluted in 10 ml of phosphate buffer (0.1 M; pH 6.5). The column was then washed again with 5 ml of water and with 4 ml of 1 N HCl; the C^{14}-norepinephrine and C^{14}-dopamine were eluted with 7 ml of 1 N HCl and 8 ml of 2 N HCl, respectively. In some experiments, both of the C^{14}-catecholamines were eluted together with 10 ml of 2 N HCl. The major O-methylated metabolites of C^{14}-dopa present in the alumina column effluents were assayed by Dowex chromatography, with the procedure described above. The Dowex effluent [the "homovanillic acid (HVA)" fraction] contained acidic and neutral O-methylated metabolites, largely HVA; the fraction eluted with pH 6.5 phosphate buffer contained the O-methylated amino acid 4-hydroxy-3-methoxyphenylalanine (methoxydopa). The fraction eluded by 2 N HCl would have contained such C^{14}-O-methylated catecholamines as C^{14}-methoxydopamine; however, little or no radioactivity was generally found in this material.

The recoveries of the C^{14}-dopa, C^{14}-dopamine and H^3-norepinephrine over alumina and Dowex columns were determined by adding known amounts of the labeled catechols to whole homogenates of uninjected mice. Recoveries over alumina averaged 79, 84 and 85%, respectively. Recoveries over Dowex were generally on the order of 85 to 95% for all three compounds. However, different batches of Dowex yielded recoveries which, on occasion, varied considerably from these averages. Hence, Dowex recoveries were measured routinely as parts of all experiments, and all data were corrected for these recoveries.

The recoveries of HVA and methoxydopa over Dowex were determined by measuring the absorbance at 280 mμ of the unlabeled compounds in standard solutions before and after column chromatography. Essentially all of the HVA appeared in the Dowex effluent, whereas the methoxydopa behaved like dopa. The methoxydopa standard was kindly provided by Dr. S. Archer of the Sterling-Winthrop Research Institute (Rensselaer, N.Y.). Its identity was confirmed by Dr. Phillip Issenberg, of the Massachusetts Institute of Technology, with mass spectroscopy. The identities of the major radioactive metabolites of C^{14}-dopa in each alumina or Dowex fraction were confirmed by paper chromatography, as described below. Data are analyzed as mean ± S.E.M. and analyzed by Student's *t* test.

RESULTS. *Disappearance of C^{14}-dopa in the whole mouse.* Groups of 3 to 6 animals received C^{14}-dopa (0.5 μc, 0.5–0.6 mg/kg i.p.) and were killed after 5, 10, 20 or 60 minutes. The labeled amino acid disappeared almost linearly for approximately 10 minutes at an initial rate of 7.3% of injected dose per min (fig. 1). Subsequently, its rate of disappearance declined, so that 60 minutes after its administration, 14.0 ± 1.1% of the injected dose still remained as unchanged C^{14}-dopa (fig. 1).

The C^{14}-catecholamine content of the whole carcass reached a peak level of 22.0% of the injected dose 20 minutes after C^{14}-dopa administration and fell to 14.0% in the next 40 minutes (fig. 1). Since D-dopa is apparently not decarboxylated (Lovenberg *et al.*, 1962), the proportion of precursor C^{14}-DL-dopa present as C^{14}-catecholamines at various times after injection should probably be corrected by multiplying by two the percentage of C^{14}-DL-dopa converted. In no experiment was it possible to demonstrate unequivocally that a significant fraction of the C^{14}-catecholamine present in the whole mouse carcass was C^{14}-norepinephrine; hence the C^{14}-catecholamine is indicated in figure 1 as C^{14}-dopamine.

Composition of C^{14}-labeled metabolites of C^{14}-dopa in the whole mouse. The major C^{14}-metabolites present in the whole mouse 20 minutes after C^{14}-dopa administration were examined in animals given larger amounts of radioactive material (5 μc, 5–6 mg/kg) than those used in the above experiments. Aliquots of alumina eluates and effluents were cochromatographed with reference standards with ascending paper systems (butanol–acetic acid–water, 8:2:2 and isopropanol–ammonia, 8:2); other aliquots were passed over Dowex columns, and the identities of C^{14}-metabolites in the various Dowex fractions were similarly confirmed by paper chromatography. The R_f values for HVA and methoxydopa in butanol–acetic acid–water were 0.82 and 0.35, respectively; their corresponding values in isopropanol–ammonia were 0.35 and 0.19.

Twenty minutes after its administration, approximately 41% of the injected C^{14}-dopa was

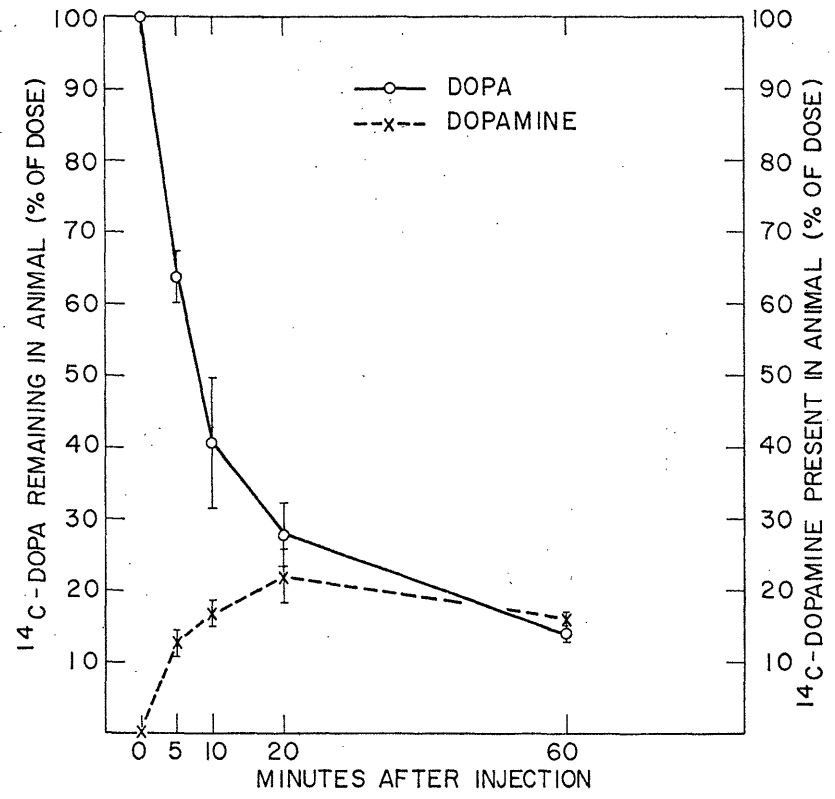

FIG. 1. Disappearance of C^{14}-dopa and appearance of C^{14}-dopamine in the whole mouse. Groups of three to six animals received C^{14}-dopa (0.5 μc, 0.5–0.6 mg/kg i.p.) and were killed after 5, 10, 20 or 60 minutes. Animals were homogenized in 9 volumes of 0.4 N $HClO_4$, and the C^{14}-dopa and C^{14}-dopamine present in the supernatants after centrifugation at 10,000 × g were assayed by column chromatography. Vertical bars indicate S.E.M.

present in the whole carcass as a catechol (table 1); of this, 21% had the chromatographic properties of dopa on paper and Dowex, whereas 19% was a catecholamine. About 1% of the radioactive material in the alumina eluate behaved chromatographically as a catechol acid or alcohol. Its R_f in butanol–acetic acid–water (0.60) was similar to those of dihydroxyphenyl acetic acid (0.69), dihydroxyphenethanol (0.61) or dihydroxyphenyl glycol (0.51), but differed from those of dopa (0.21) or dopamine (0.28). The major metabolite of C^{14}-dopa present in the whole carcass was C^{14}-methoxydopa: this compound accounted for at least 30% of the total radioactivity, whereas HVA represented an additional 20% (table 2). Sixty minutes after C^{14}-dopa administration, this ratio reversed.

The 10,000 × g pellets from whole mouse homogenates were purified and assayed for C^{14}-protein by the method of Sokoloff and Kaufman (1961). No evidence was obtained that significant amounts of C^{14}-dopa were incorporated into protein.

Relation between dose of C^{14}-dopa and its rate of metabolism. Isotopically labeled C^{14}-DL-dopa was diluted with various amounts of unlabeled L-dopa so that mice injected with 0.5 μc received a total of 22, 522 or 5022 μg (0.5–0.6, 12–15 or 120–150 mg/kg) of the amino acid. Animals were killed 20 minutes later, and their homogenates were assayed for C^{14}-dopa and C^{14}-catecholamines.

Increasing the dose of C^{14}-dopa from 22 to 522 μg did not significantly modify the rate of disappearance of the amino acid, but it did decrease the percentage present in the carcass as C^{14}-catecholamines ($P < .01$; table 2). Further increasing the dose of C^{14}-dopa to 5022 μg decreased both the percentage of the amino acid that was metabolized ($P < .01$) and the percentage present in the whole carcass as C^{14}-catecholamines ($P < .01$). A 10-fold increase in the dose of C^{14}-dopa produced a 7-fold increase in the C^{14}-catecholamine content of the whole carcass.

Distribution of C^{14}-dopa between the brain and the carcass. Groups of mice received 2.5 μc (2.5–3.0 mg/kg i.p.) of C^{14}-dopa and were killed

TABLE 1

Identity of radioactive metabolites in whole mice 20 minutes after administration of C^{14}-dopa

Six mice received 5 μc (5–6 mg/kg) of C^{14}-dopa i.p. Data are presented as average and range (in parentheses) for each metabolite.

	Percentage of Metabolite
	%
Alumina eluate	41 (32–47)
Dopa	21 (18–27)
Catecholamines	19 (15–21)
Unidentified compounds	1 (0–3)
Alumina effluent	59 (53–68)
"HVA" [a]	20 (18–26)
Methoxydopa	30 (24–33)
Unidentified compounds[b]	9 (6–15)

[a] This material had chromatographic properties similar to authentic HVA on Dowex columns and on two ascending paper systems. However, it might have included neutral O-methylated metabolites of C^{14}-dopa as well as HVA.

[b] About one-half of this material was present in the first aqueous wash from the Dowex column and was probably composed largely of HVA.

after 20 or 60 minutes. The brain was separated from the rest of the carcass, and both were assayed for total C^{14}-catechols and for C^{14}-dopa. The carcass was also assayed for C^{14}-catecholamines; however, too little catecholamine radioactivity was present in the brain to allow its measurement. (The limit of resolution in these experiments would be about 100 ng/g.)

At both times tested, less than 1/1000 of the total radioactivity, the C^{14}-catechols or the C^{14}-dopa present in the whole animal was localized within the brain (table 3). The total counts in whole carcass and in brain declined by about 20% between the 20th and 60th minute after C^{14}-dopa injection; this fall was significant in carcass ($P < .05$), but not in brain. It seems probable that much of this fall represented loss of C^{14}-D-dopa into the urine inasmuch as this isomer is excreted to a considerably larger extent than the natural L-form (Shaw *et al.*, 1957; Sourkes, 1961). The rates at which C^{14}-dopa disappeared from the brain and the carcass were similar and were greater than the decrease in total radioactivity.

DISCUSSION. These data provide several bases

TABLE 2

Relation between dose of C^{14}-dopa and its rate of metabolism

Groups of four mice received C^{14}-dopa and were killed 20 minutes later.

Dose	C^{14}-Dopa Metabolized	C^{14}-Dopa Present as C^{14}-Catecholamine	Dopa Metabolized	Amount of Catecholamines Formed from C^{14}-Dopa Present in Whole Animals
μg	%	%	μg	μg
22	80.7 ± 3.0	11.7 ± 0.1	17.8 ± 0.7	2.6 ± 0.1
522	86.5 ± 1.9	6.3 ± 1.2[a]	451.5 ± 9.9[a]	33.0 ± 6.8[a]
5022	64.1 ± 2.0[a]	4.7 ± 0.1[a]	3219.1 ± 100.4[a]	235.5 ± 16.0[a]

[a] Differs from 22-μg dose, $P < .01$.

TABLE 3

Distribution of C^{14}-dopa between brain and carcass

Groups of mice were killed 20 or 60 minutes after receiving C^{14}-dopa (2.5 μc i.p.).

	Time after Injection	Radioactivity	Catechols	Dopa	Catecholamines
	min	*cpm* × 10^3	%	%	%
Carcass	20	2420 ± 90	55.9 ± 0.91	27.9 ± 0.37	26.1 ± 2.3
	60	1960 ± 120[a]	36.6 ± 2.19[b]	14.1 ± 1.13[b]	20.4 ± 1.5
Brain	20	2.35 ± 0.18	56.1 ± 7.22	14.6 ± 5.75	
	60	1.72 ± 0.26	27.4 ± 3.40[b]	8.5 ± 1.91	

[a] Differs from 20-minute value, $P < .05$.

[b] Differs from 20-minute value, $P < .01$.

for explaining the relatively large doses of L-dopa generally found necessary for therapeutic relief in Parkinson's disease. Dopa entering the body *via* the portal circulation is metabolized very rapidly (fig. 1), and only a tiny fraction of this material is available to the brain for conversion to catecholamines (table 3). The inability of brain to accumulate circulating dopa should be contrasted with the ease with which it concentrates tyrosine: 20 or 60 minutes after C^{14}-dopa injection, less than $\frac{1}{1000}$ of the total C^{14}-amino acid present in the body is localized within the brain (table 3). In contrast, about 2.2% of the tyrosine present in the normal young rat is found in the brain (Shoemaker and Wurtman, 1970), and this proportion can be quadrupled under conditions of protein malnutrition. The failure of brain to take up large amounts of dopa from the blood probably results in part from the ability of brain capillaries to concentrate and then to decarboxylate the amino acid (Owman and Rosengren, 1967). This protective mechanism would be expected to have little or no effect on endogenous brain dopa, which derives not from the circulating amino acid but from *in situ* biosynthesis.

The fate of injected C^{14}-dopa depends upon the amount administered (table 2), as well as the amount of time that has elapsed since its administration. Our data suggest that the decarboxylation of dopa begins to be saturated at lower doses than the overall metabolism of the amino acid. Thus, the disappearance of C^{14}-dopa proceeded at least as rapidly when animals were given 522 μg of the amino acid as when they received 22 μg, but the percentage of the radioactivity present as C^{14}-catecholamines (largely dopamine) was decreased by almost half with the larger dose. It is possible that these differences are artefactual and result from our use of C^{14}-DL-dopa instead of the pure L-isomer. Thus, if the decarboxylase catalyzes the formation of dopamine from L-dopa more effectively than from the D-amino acid, the likelihood that a given molecule of C^{14}-DL-dopa will be converted to C^{14}-dopamine will decrease as the amount of L-dopa injected increases. If, as has been suggested before, no D-dopa is decarboxylated (Lovenberg *et al.*, 1962), the dose-related decrease in C^{14}-catecholamine formation observed in our studies would not simply reflect use of the racemic amino acid. Dopa is an excellent substrate for catechol-O-methyl transferase (Axelrod and Tomchick, 1958). This enzyme catalyzes the O-methylation of D-epinephrine as well as of L-epinephrine (Axelrod and Tomchick, 1958), and thus would not be expected to display stereospecificity in acting on dopa.[3]

The extent to which C^{14}-dopa was rapidly metabolized by O-methylation was surprising. Bartholini and Pletscher (1968) had observed 10:1 ratios of C^{14}-L-methoxydopa to C^{14}-L-dopa in brain 60 minutes after rats received the labeled amino acid but had attributed this to the fact that the O-methylated metabolite is a poor substrate for dopa decarboxylase (Ferrini and Glässer, 1964). The extensive O-methylation of dopa suggests that the chronic daily administration of 6 to 8 g of the amino acid to human subjects might, in the presence of a normal methionine intake (*i.e.*, about 1–2 g/day) (Rose *et al.*, 1955; Rose and Wixon, 1955), lead eventually to a relative deficiency of methionine or *S*-adenosylmethionine in the body. This likelihood is further supported by the observation (Calne *et al.*, 1969) that considerably more than 50% of the urinary metabolites of L-dopa are O-methylated in men receiving the amino acid chronically. It also suggests the converse, *i.e.*, that the physiologic effects of a given dose of L-dopa might be potentiated if methionine intake were lowered or if tissue *S*-adenosylmethionine levels were depressed pharmacologically. Studies are in progress to examine the effects of acute and chronic L-dopa administration on methionine metabolism, and to determine whether chronic L-dopa treatment might modify the fate of injected C^{14}-dopa, perhaps by altering tissue *S*-adenosylmethionine levels.

Our failure to demonstrate significant quantities of C^{14}-norepinephrine in bodies of mice given C^{14}-dopa is consistent with the findings of Constantinidis *et al.* (1968) and of Butcher and Engel (1969), who found no elevation in brain norepinephrine levels among rats given doses of L-dopa sufficient to increase brain dopamine content. It is also compatible with the observation of Calne *et al.* (1969) that norepinephrine metabolites in human urine do not show a major increase in subjects given L-dopa. However,

[3] Preliminary studies on the fate of H^3-L-dopa in whole mice indicate that the proportion of administered material that is O-methylated within the first 20 minutes of injection is similar to that of C^{14}-DL-dopa (table 1).

other investigators (*cf.* Bartholini and Pletscher, 1968; Persson and Waldeck, 1968) have identified significant amounts of isotopically labeled norepinephrine in brains of rats and mice given much larger amounts of radioactivity than our experimental animals. It seems likely that the proportion of a given dose of dopa that is converted to norepinephrine is considerably smaller than the fraction converted to dopamine. This may explain in part the welcome failure of most human subjects treated with L-dopa to develop serious hypertension. The failure of C^{14}-dopa to be incorporated into new proteins is compatible with the absence of a triplet codon for this amino acid (Nirenberg *et al.*, 1966).

CONCLUSION. The metabolic fate of C^{14}-DL-dopa was examined in whole mice. Almost 60% of the C^{14}-amino acid was destroyed in the first 10 minutes after its i.p. injection. C^{14}-catecholamine levels in the whole carcass reached a peak 20 minutes after injection. Almost all of this material was C^{14}-dopamine. Essentially none of the amino acid was incorporated into protein. A surprisingly large fraction of the C^{14}-amino acid was metabolized by O-methylation during the first 20 minutes after injection; only a small proportion (*i.e.*, about 0.1%) was present in the brain as C^{14}-dopa or C^{14}-dopamine. These data suggest that inhibition of the O-methylation of dopa might provide a useful approach for increasing the proportion of administered material that is converted to brain catecholamines.

REFERENCES

ANTON, A. H. AND SAYRE, D. F.: The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. J. Pharmacol. Exp. Ther. **145:** 326–336, 1964.

AXELROD, J. AND TOMCHICK, R.: Enzymatic O-methylation of epinephrine and other catechols. J. Biol. Chem. **233:** 702–705, 1958.

BARTHOLINI, G. AND PLETSCHER, A.: Cerebral accumulation and metabolism of C^{14}-dopa after selective inhibition of peripheral decarboxylase. J. Pharmacol. Exp. Ther. **161:** 14–20, 1968.

BUTCHER, L. L. AND ENGEL, J.: Behavioral and biochemical effects of L-dopa after peripheral decarboxylase inhibition. Brain Res. **15:** 233–242, 1969.

CALNE, D. B., KAROUM, F., RUTHVEN, C. R. J. AND SANDLER, M.: The metabolism of orally administered L-dopa in Parkinsonism. Brit. J. Pharmacol. **37:** 57–68, 1969.

CARLSSON, A. AND WALDECK, B.: A method for the fluorometric determination of methoxytyramine in tissues and the occurrence of this amine in brain. Scand. J. Clin. Lab. Invest. **16:** 133–138, 1964.

CONSTANTINIDIS, J., BARTHOLINI, G., TISSOT, R. AND PLETSCHER, A.: Accumulation of dopamine in the parenchyma after decarboxylase inhibition in the capillaries of brain. Experientia (Basel) **24:** 130–131, 1968.

COTZIAS, G. C., PAPAVASILIOU, P. S. AND GELLENE, R.: Modification of Parkinsonism—Chronic treatment with L-DOPA. N. Engl. J. Med. **280:** 337–345, 1969.

FERRINI, R. AND GLÄSSER, A.: *In-vitro* decarboxylation of new phenylalanine derivatives. Biochem. Pharmacol. **13:** 798–800, 1964.

LOVENBERG, W., WEISSBACH, H. AND UDENFRIEND, S.: Aromatic L-amino acid decarboxylase. J. Biol. Chem. **237:** 89–93, 1962.

NAGATSU, T., LEVITT, M. AND UDENFRIEND, S.: Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem. **239:** 2910–2917, 1964.

NIRENBERG, M., CASKEY, T., MARSHALL, R., BRIMACOMBE, R., KELLOGG, D., DOCTOR, B., HATFIELD, D., LEVIN, J., ROTTMAN, F., PESTKA, S., WILCOX, M. AND ANDERSON, F.: The RNA code and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. **31:** 11, 1966.

OWMAN, C. AND ROSENGREN, E.: Dopamine formation in brain capillaries—An enzymatic blood-brain barrier mechanism. J. Neurochem. **14:** 547–550, 1967.

PERSSON, T. AND WALDECK, B.: The use of ^{3}H-DOPA for studying cerebral catecholamine metabolism. Acta Pharmacol. Toxicol. **26:** 363–372, 1968.

ROSE, C. M., CHOU, C. AND WURTMAN, R. J.: The metabolism of ^{14}C-DOPA in the whole mouse. Fed. Proc. **29:** 511, 1970.

ROSE, W. C., COON, M. J., LOCKHART, H. B. AND LAMBERT, G. F.: The amino acid requirements of man. XI. The threonine and methionine requirements. J. Biol. Chem. **215:** 101–110, 1955.

ROSE, W. C. AND WIXON, R. L.: The amino acid requirements of man. XIII. The sparing effect of cystine on methionine requirements. J. Biol. Chem. **216:** 763–773, 1955.

SHAW, K. N. F., MCMILLIAN, A. AND ARMSTRONG, M. D.: The metabolism of 3,4-dihydroxyphenylalanine. J. Biol. Chem. **226:** 255–266, 1957.

SHOEMAKER, W. J. AND WURTMAN, R. J.: Effect of perinatal undernutrition on development of brain catecholamines in the rat. Fed. Proc. **29:** 496, 1970.

SOKOLOFF, L. AND KAUFMAN, S.: Thyroxine stimulation of amino acid incorporation into protein. J. Biol. Chem. **236:** 795–799, 1961.

SOURKES, T. L.: Formation of dopamine *in vivo:* Relation to the function of the basal ganglia. Rev. Can. Biol. **20:** 187–196, 1961.

WHITBY, L. H., AXELROD, J. AND WEIL-MALHERBE, H.: The fate of H^{3}-norepinephrine in animals. J. Pharmacol. Exp. Ther. **132:** 193–201, 1961.

WURTMAN, R. J.: Catecholamines, Little, Brown and Company, Boston, 1966.

WURTMAN, R. J.: Catecholamines and neurologic disease. N. Engl. J. Med. **282:** 45–46, 1970.