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METABOLISM IN THE HUMAN OF 3, 4-DIHYDROXYMANDELIC ACID, ONE OF THE METABOLITES OF NORADRENALINE AND ADRENALINE*

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Abstract—Five normal human subjects were used. Three subjects were infused with 250 μc of 3,4-dihydroxymandelic acid 2-^{14}C (DOMA) (sp. act., 17 mc/m-mole) over a period of 1 hr and two were injected i.v. over 1 min. Urine was collected at 10-min intervals for 1 hr after the 1-min injection and hourly after the 1-hr infusion for 6 hr, and then at 12 hr and at 24 hr. By using a special procedure and system herein described, the various metabolic products of DOMA were separated and identified and their radioactivity was measured. Within 24 hr after a 1-hr infusion of DOMA, 95·3 $\pm$ 1·2 per cent of the total radioactivity was recovered and 72·3 $\pm$ 0·6 per cent after a 1-min injection of DOMA. Results after the 1-hr infusion indicate that 17·7 $\pm$ 2·5 per cent of the infused (circulating) DOMA is recovered as 3-methoxy-4-hydroxymandelic acid, 7·7 $\pm$ 1·4 per cent as 3,4-dihydroxybenzoic acid and 2·0 $\pm$ 0·7 per cent as vanillic acid acid in 24 hr; 3-methoxy-4-hydroxyphenylacetic acid was present in trace amounts. Several unknown metabolites of DOMA were isolated, but not identified. Radioactive protocatechuic aldehyde was found in the urine, but was shown to be a degradation product of DOMA-2-^{14}C rather than a metabolic product.

3,4-DIHYDROXYMANDELIC acid (DOMA, DHMA) has been shown by Goodall *et al* to be the deaminated metabolic product of both adrenaline and noradrenaline.[1–4] The quantity occurring normally in the urine is approximately 100 μg per 24 hr; Weil-Malherbe[5,6] found this to be 97 $\pm$ 15·5 μg per 24 hr, which was in good agreement with the 99·9 μg found by Wada[7] and by Wada and Watanabe;[8] other investigators have found amounts ranging from 528 to 920 μg per 24 hr.[9–11] Since patients with pheochromocytoma release increased amounts of catecholamines,[12–20] it follows that the amount of DOMA excreted by these patients is also increased.[6,8,9] With the exception of 3-methoxy-4- hydroxymandelic acid (MOMA, VMA), very little is known about the other metabolic products of DOMA. Therefore, the purpose of these studies is to separate, identify and quantitate the various metabolites of DOMA and to determine their relative rate of appearance in the urine.

METHODS

General. A total of 5 normal healthy subjects were infused with 250 μc of 3,4-dihydroxymandelic acid-2-^{14}C (sp. act., 17 mc/m-mole) (DOMA-2-^{14}C).† Two subjects were injected for 1 min via the antecubital vein with 250 μc of DOMA-2-^{14}C mixed in

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† Mallinckrodt, Nuclear Research Chemicals, Inc., Orlando, Fla.

10 ml of physiological saline. Urine was collected with an indwelling catheter 0–2 min, 2–5 min, 5–10 min, and then at 10-min intervals for the next 50 min; then at 6 hr, 12 hr and 24 hr.

The second group (3 subjects) was infused with 250 μc DOMA-2-^{14}C in 200 ml of physiological saline at a steady rate for a period of 1 hr. Urine was collected at hourly intervals after infusion for 6 hr and then at 12 hr and 24 hr. The urine samples were immediately frozen and stored at − 20° until assayed.

Procedure for separating, identifying and measuring the DOMA-2-^{14}C metabolites. The method for isolating, identifying and quantitating DOMA and its metabolic products in urine has not been described; however, the procedure is rather similar to that used previously in following the metabolic products of 3-hydroxytyramine[21] and noradrenaline.[22]

An aliquot of urine containing 100,000 dpm, to which was added carrier compounds of protocatechuic aldehyde, 3-methoxy-4-hydroxymandelic acid (MOMA), 3-methoxy-4-hydroxyphenylacetic acid (HVA), 3,4-dihydroxymandelic acid (DOMA), 3,4-dihydroxyphenylacetic acid (DOPAC), vanillic acid (VA) and 3,4-dihydroxybenzoic acid (protocatechuic acid, DOBA), was placed on a 1 × 35 cm column of Dowex 1-X2* acetate anion exchange resin. The column was placed on an automatic fraction collecting system and eluted with 300 ml of glass-distilled water, followed by a variable gradient elution consisting of four series-connected chambers. The first chamber contained water; the second, 1·5 M ammonium acetate, pH 4·8; the third, water; and the fourth, 6 M ammonium acetate, pH 4·8. Each chamber contained 275 ml of solution. The flow rate was adjusted to approximately 1 ml/min.

The eluate was passed through a quartz cell flow (1·0 ml volume) of a Beckman DB-G spectrophotometer and the optical density was measured at 279 mμ. The output of the DB-G spectrophotometer was recorded on one channel of a dual pen recorder. After passing through the DB-G spectrophotometer, the eluate entered a 10-ml flow cell and the radioactivity was counted with a Packard model 3041 flow monitor† equipped with an analog, ratemeter output. This output was recorded on the other channel of the dual recorder. From the flow cell, the eluate was then passed to an automatic fraction collector and fractionated. From the fraction collector, an impulse was relayed to an event marker on the recorder so as to indicate the change of each fraction by the fraction collector. Those fractions comprising a single radioactivity peak were pooled and assayed for total radioactivity. Figures 1 and 2 represent typical tracings obtained by this method after a 1-hr infusion and a 1-min i.v. injection of DOMA-2-^{14}C.

The recovery of the total radioactivity placed on the Dowex 1-X2 column was approximately 100 per cent. The peaks containing specific free phenolic acids, such as MOMA, VA, DOMA and DOBA, were each confirmed by paper chromatography using three different solvent systems, i.e. *n*-butanol–acetic acid–H_2O (4:1:1); benzene–propionic acid–H_2O (8:2:2) and isopropanol–5% NH_3 (8:2).

RESULTS

Results of the 1-min injection and the 1-hr infusion of DOMA-2-^{14}C are summarized in Table 1. The principal metabolic products of DOMA are MOMA, DOPAC,

* Dowex 1-X2, 200–400 mesh; BioRad Laboratories, Richmond, Calif.

† Packard Instrument Corp., Downers Grove, Ill.

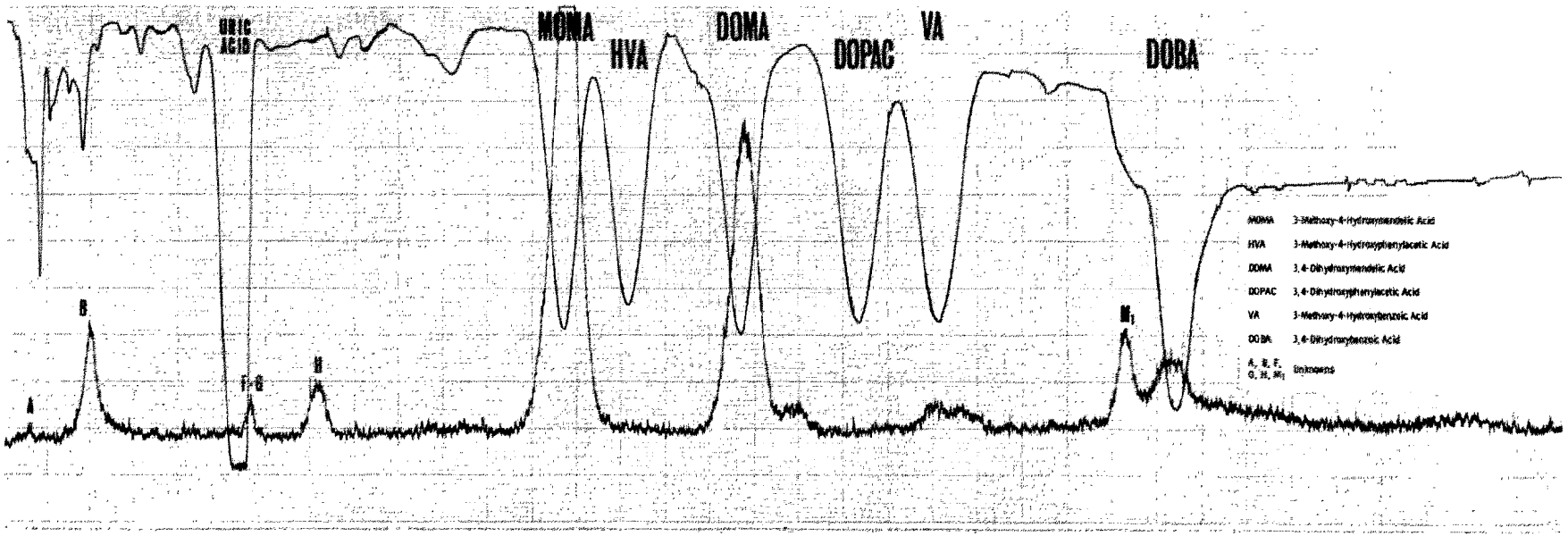

FIG. 1. Tracing of the 4–5 hr urinary collection period of metabolic products of 3,4-dihydroxymandelic acid (DOMA) after 1-hr i.v. infusion of 3, 4-dihydroxymandelic acid 2-^{14}C. The solid line, with base at the top, represents optical density and is plotted against the radioactivity.

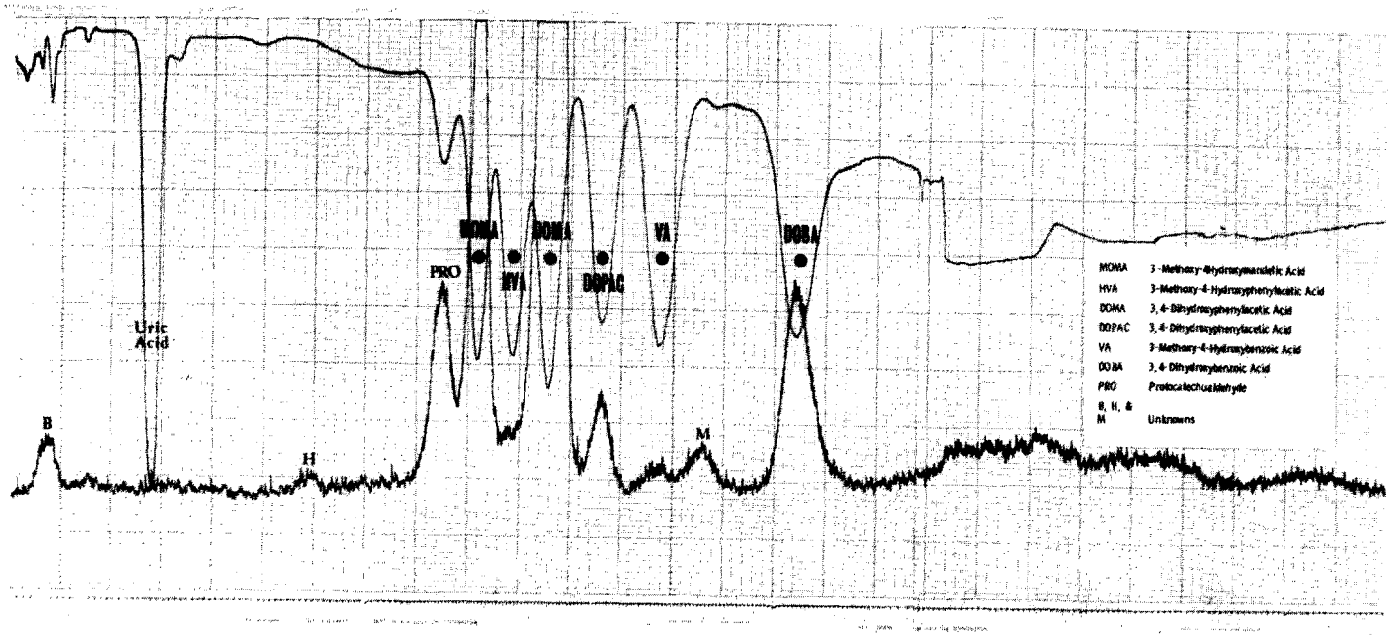

FIG. 2. Tracing of the 50–60 min collection period of the metabolic products of 3,4-dihydroxymandelic acid (DOMA) after a 1-min i.v. injection of 3,4-dihydroxymandelic acid 2-^{14}C. The solid line, with base at the top, represents optical density and is plotted against the radioactivity.

TABLE 1. EXCRETION PATTERN OF THE METABOLITES OF 3, 4-DIHYDROXYMANDELIC ACID-2-^{14}C (DOMA) AFTER A 1-hr i.v. INFUSION OF DOMA-2-^{14}C AND A 1-min i.v. INJECTION OF DOMA-2-^{14}C.*

1-hr infusion

Period	A	B	F	G	H	MOMA	DOMA	HVA	UNK	VA	M·1	DOBA	Col residue	Total recovered
0–1 hr	0·08 ± 0·04	0·33 ± 0·04	0·25 ± 0·08	Trace	0·41 ± 0·17	4·80 ± 1·16	25·34 ± 0·50	Trace	0·66 ± 0·12	0·61 ± 0·17	0·50 ± 0·12	3·56 ± 0·17	3·77 ± 0·29	41·50 ± 5·20
1–2 hr	0·06 ± 0·03	0·31 ± 0·09	0·25 ± 0·19	Trace	0·22 ± 0·06	5·23 ± 0·59	16·68 ± 2·19	Trace	1·00 ± 0·34	0·63 ± 0·22	0·59 ± 0·03	2·38 ± 0·85	3·60 ± 0·19	31·30 ± 2·50
2–3 hr	0·09 ± 0·08	0·20 ± 0·08	0·11 ± 0·07	0·26 ± 0·20	0·19 ± 0·07	3·12 ± 0·24	2·77 ± 1·23		0·34 ± 0·09	0·29 ± 0·24	0·24 ± 0·09	0·76 ± 0·24	1·50 ± 0·24	10·10 ± 2·30
3–4 hr	0·07 ± 0·03	0·14 ± 0·01	0·07 ± 0·01	0·20 ± 0·06	0·16 ± 0·03	1·52 ± 0·20	0·92 ± 0·18		0·21 ± 0·01	0·22 ± 0·03	0·19 ± 0·04	0·39 ± 0·03	1·39 ± 0·21	5·50 ± 0·40
4–5 hr	0·02 ± 0·01	0·09 ± 0·02	0·02 ± 0·01	0·06 ± 0·04	0·10 ± 0·03	1·02 ± 0·10	0·59 ± 0·16		0·09 ± 0·02	0·11 ± 0·02	0·12 ± 0·03	0·23 ± 0·03	0·44 ± 0·06	2·90 ± 0·40
5–6 hr	0·01 ± 0·01	0·07 ± 0·01	0·02 ± 0·01	0·01 ± 0·02	0·06 ± 0·03	0·68 ± 0·08	0·47 ± 0·03		0·04 ± 0·00	0·04 ± 0·01	0·07 ± 0·02	0·13 ± 0·04	0·28 ± 0·08	1·90 ± 0·30
6–12 hr	0·02 ± 0·01	0·12 ± 0·06	0·06 ± 0·03		0·16 ± 0·04	1·23 ± 0·14	0·87 ± 0·28		0·01 ± 0·01	0·08 ± 0·02	0·13 ± 0·03	0·25 ± 0·04	0·56 ± 0·07	3·50 ± 2·70
12–24 hr	Trace	Trace			0·01 ± 0·01	0·13 ± 0·02	0·05 ± 0·01		Trace	Trace	Trace	0·03 ± 0·01	0·06 ± 0·01	0·30 ± 0·08
Total 24 hr recovery	0·35 ± 0·21	1·27 ± 0·30	0·78 ± 0·38	0·53 ± 0·32	1·31 ± 0·43	17·73 ± 2·53	47·69 ± 4·58	Trace	2·36 ± 0·59	1·99 ± 0·69	1·84 ± 0·36	77·3 ± 1·40	11·60 ± 1·15	95·26 ± 1 22

1-min i.v. injection

0–2 min														
2–5 min	0·06 ± 0·04	0·07 ± 0·04	0·11 ± 0·10	0·07 ± 0·05	0·05 ± 0·04	0·27 ± 0·02	0·68 ± 0·34		0·02 ± 0·02	0·08 ± 0·04	0·05 ± 0·02	0·41 ± 0·03	0·70 ± 0·12	2·60 ± 0·10
5–10 min	0·32 ± 0·31	0·21 ± 0·07	0·38 ± 0·31	0·62 ± 0·55	0·18 ± 0·17	1·54 ± 0·49	7·83 ± 1·86		0·07 ± 0·06	0·27 ± 0·11	0·21 ± 0·03	1·17 ± 0·04	0·99 ± 0 01	14·10 ± 4·10
10–20 min	0·09 ± 0·08	0·19 ± 0·09	0·09 ± 0·08	0·16 ± 0·15	Trace	1·40 ± 0·40	8·35 ± 1·37		0·31 ± 0·11	0·27 ± 0·01	0·21 ± 0·04	1·00 ± 0·11	1·09 ± 0·08	13·30 ± 1·10
20–30 min	0·05 ± 0·04	0·12 ± 0·06	0·06 ± 0·03	0·09 ± 0·06	Trace	1·09 ± 0·07	5·45 ± 0·73	Trace	0·30 ± 0·05	0·18 ± 0·08	0·19 ± 0·06	0·61 ± 0·02	1·07 ± 0·37	9·20 ± 1·00
30–40 min	0·11 ± 0·10	0·04 ± 0·01	0·03 ± 0·02	0·08 ± 0·05	Trace	0·93 ± 0·05	4·42 ± 0·64	0·19 ± 0·04	0·22 ± 0·04	0·15 ± 0·10	0·11 ± 0·02	0·49 ± 0·02	0·76 ± 0·30	7·60 ± 0·20
40–50 min	0·07 ± 0·06	0·03 ± 0·01	0·05 ± 0·03	0·06 ± 0·05	0·03 ± 0·02	0·77 ± 0·01	3·23 ± 0·32	0·08 ± 0·02	0·25 ± 0·03	0·11 ± 0·06	0·09 ± 0·01	0·36 ± 0·01	0·59 ± 0·13	5·70 ± 0·60
50–60 min	0·05 ± 0·01	0·04 ± 0·31	0·05 ± 0·04	0·07 ± 0·05	0·04 ± 0·01	0·79 ± 0·03	2·51 ± 0·41	0·06 ± 0·05	0·18 ± 0·03	0·90 ± 0·04	0·09 ± 0·01	0·32 ± 0·02	0·58 ± 0·12	4·90 ± 0·70
1–6 hr	0·13 ± 0·01	Trace	0·01 ± 0·01	0.01 ± 0·01	0·33 ± 0·07	3·52 ± 0·49	7·62 ± 0·24	0·01 ± 0·01	0·39 ± 0·04	0·21 ± 0·11	0·34 ± 0·09	1·02 ± 0·09	0·57 ± 0·43	14·30 ± 3·60
6–12 hr	Trace	0·02 ± 0·01	Trace		0·06 ± 0·01	0·37 ± 0·04	0·31 ± 0·02	Trace	0·01 ± 0·01	0·01 ± 0·01	0·02 ± 0·01	0·09 ± 0·02	0·02 ± 0·01	0·90 ± 0·30
12–24 hr	Trace	Trace	Trace		0·02 ± 0·01	0·26 ± 0·01	0·10 ± 0·01		Trace	Trace	Trace	0·04 ± 0·01	0·07 ± 0·02	0·50 ± 0·00
Total 24-hr recovery	0·88 ± 0·64	0·73 ± 0·29	0·78 ± 0·62	1·16 ± 0·35	0·71 ± 0·32	10·94 ± 1·61	40·41 ± 5·95	0·34 ± 0·11	1·75 ± 0·39	1·76 ± 0·55	1·31 ± 0·28	5·51 ± 0·37	6·44 ± 1·59	72·27 ± 057

*All figures are expressed as per cent of radioactivity infused or injected; MOMA = 3-methoxy-4-hydroxymandelic acid; DOMA = 3, 4-dihydroxmandelic acid; HVA=3-methoxy-4-hydroxyphenylacetic acid; UNK=unknown; VA=vanillic acid; DOBA=3, 4-dihydroxybenzoic acid; A, B, F, G, H and M1= unknowns.

VA, DOBA and several unidentified compounds. The amount of radioactivity recovered as DOMA gradually decreases from the infusion period (and 10 min after injection in the 1-min injection experiment) such that the amount found in the last 12-hr period represents less than 1 per cent of that found in the first 6 hr (see Table 1 and Figs. 3 and 4).

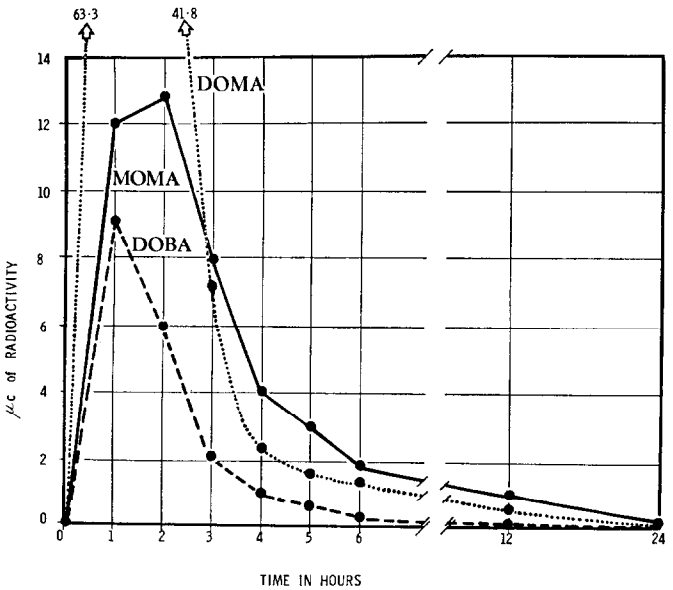

FIG. 3. A comparison of the recovery of the infused radioactivity as 3-methoxy-4-hydroxymandelic acid (MOMA) and 3,4-dihydroxybenzoic acid (DOBA) as plotted against the recovery of the radioactive 3,4-dihydroxymandelic acid (DOMA) from the beginning of the 1-hr infusion of 250 μc of DOMA-2-^{14}C to 24 hr after the infusion. Figures are expressed in μc recovered.

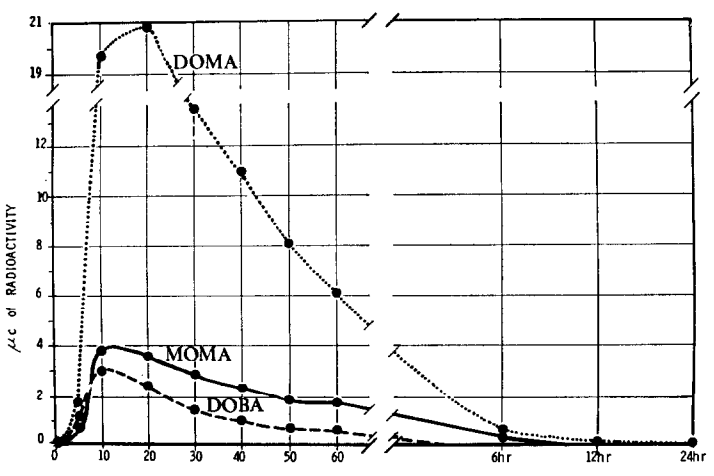

FIG. 4. A comparison of the recovery of the infused radioactivity as 3-methoxy-4-hydroxymandelic acid (MOMA) and 3,4-dihydroxybenzoic acid (DOBA) as plotted against the recovery of the radioactive 3,4-dihydroxymandelic acid (DOMA) from the beginning of the 1-min i.v. injection of 250 μc DOMA-2-^{14}C to 6 hr after the injection. Figures are expressed in μc and collections were made at 10-min intervals after injection.

The principal single metabolic product of DOMA is MOMA (see Table 1 and Figs. 3 and 4). Next in terms of recovered radioactivity, are DOBA, an unknown and VA. This unknown is eluted at the same position as carrier DOPAC (see Fig. 2), but has not been identified as DOPAC. After a 1-hr infusion of DOMA-2-^{14}C, 17·7 $\pm$ 2·5 per cent of the infused DOMA is recovered as MOMA in 24 hr, 7·7 $\pm$ 1·4 per cent as DOBA, 2·4 $\pm$ 0·6 per cent as an unknown and 2·0 $\pm$ 0·7 per cent as vanillic acid. There are small amounts of HVA recovered after the 1 min injection, but only trace amounts after the 1-hr infusion. Unidentified metabolic products of DOMA are described in Table 1 as peaks A, B, F, G, H and M1. The amount of radioactivity recovered in 24 hr after a 1-hr infusion, represented by these unidentified metabolic products, varies from 0·35 $\pm$ 0·2 per cent for peak A to 1·84 $\pm$ 0·4 per cent for peak M1. Therefore, at most, they do not represent a very large percentage of the recovered radioactivity.

Peak A is commonly found in the early periods after the 1-min injection and the 1-hr infusion; 1 hr after the 1-min injection of DOMA, this peak can be found only in trace amounts. Peaks F and G follow a pattern of excretion somewhat similar to that seen for peak A, except that G only appears during the 2–3, 3–4, 4–5 and 5–6 hr collection period after the 1-hr infusion; after the 1-min injection, peak G is found in all of the early collection periods up to 6 hr after injection.

Peak B is particularly interesting. It is volatile and disappears rapidly from an acid urine. By gas chromatography, the volatile component has been tentatively identified as radioactive CO_2. This information is of some importance, since it apparently represents the splitting of the ring structure. Peak B, in some elutions, comes off the column with another closely associated peak rather than as a single peak (see Figs. 1 and 2).

Peak M1 is consistently present throughout all collection periods and has been tentatively identified as a free acid. It represents 1·8 $\pm$ 0·4 per cent of the radioactivity recovered in 24 hr after a 1-hr infusion of 250 μc DOMA-2-^{14}C.

Protocatechuic aldehyde is found in varying quantities in all urine samples (see Figs. 1 and 2). However, this compound is a degradation product of DOMA rather than a metabolic product. This was demonstrated by placing some DOMA-2-^{14}C into urine and in physiological saline for various periods up to 6 months and fractionating the urine and physiological saline as described under Methods. The results show that for the first few days the urine and physiological saline show only radioactive DOMA, but thereafter there is a gradual increase in the amount of radioactivity recovered in the protocatechuic aldehyde fraction with a proportional loss of radioactivity found in the DOMA fraction; no other fractions or degradation products were detectable.

DISCUSSION

DOMA is the deaminated metabolic product of adrenaline, noradrenaline and hydroxytyramine, and appears as such in the urine.[1,4,21] It is also known that DOMA is *O*-methylated to form MOMA, the principal metabolite of adrenaline and noradrenaline. There is some question as to how much MOMA is formed via the DOMA pathway and how much via metadrenaline (metanephrine) or via the normetadrenaline (normetanephrine) pathway (Fig. 5). Hitherto, it was not known what percentage of DOMA was converted to MOMA and how much was converted to other metabolic

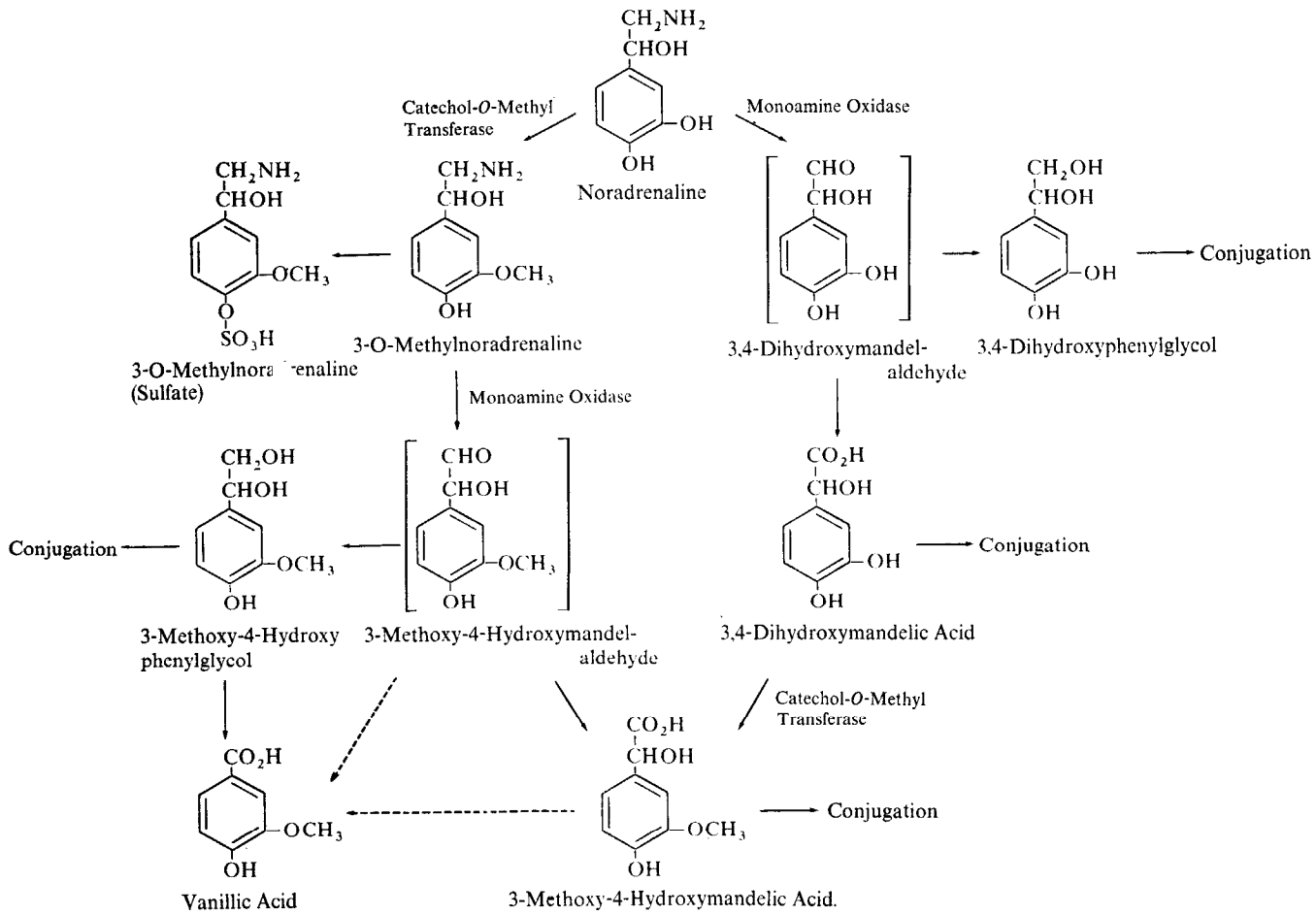

FIG. 5. Alternative pathways for the metabolism of noradrenaline.

products of DOMA; the rate of this conversion was also unknown. These experiments have endeavored to answer some of these questions as they relate to circulating DOMA.

It is of interest that 95·3 ± 1·2 per cent of the total radioactivity was recovered within 24 hr after a 1-hr infusion of DOMA-2-^{14}C, whereas only 72·3 ± 0·6 per cent was recovered after a 1-min injection of DOMA. This difference is reflected in the recovery of each of the metabolic products. Why this difference should occur cannot be adequately explained, but it could be related to a larger percentage of the DOMA being bound when DOMA occurs in greater concentration in the circulation, as was the case after the 1-min infusion, i.e. 250 μc in 1 min versus 250 μc in 1 hr (4·2 μc/min).

Within 24 hr after a 1-hr infusion of DOMA, 17·7 ± 2·5 per cent of circulating DOMA is *O*-methylated to MOMA. The conversion is rather rapid, as is indicated in Table 1 and in Figs. 3 and 4. Further, there are at least 10 other compounds found in the urine which take their origin from DOMA. Although some of these compounds have not been identified, others have been, i.e. DOBA, VA and HVA (see Table 1 and Figs. 1 and 2). Previous experiments indicate that these phenolic acids are conjugated and appear in the urine.[21] In these experiments, however, the conjugates are found in the urine in such small quantities that they are difficult to separate and identify; the conjugates for the most part were eluted after DOBA (see Figs. 1 and 2), and are collectively grouped under column residue (Table 1). The DOBA is the second largest metabolite of DOMA. It is found throughout the collection periods and represents 7·7 ± 1·4 per cent of the radioactivity recovered in 24 hr after a 1-hr infusion of DOMA-2-C^{14} (see Table 1 and Fig. 3); after a 1-min injection of DOMA-2-^{14}C, 5·5 ± 0·4 per cent of the radioactivity is recovered as DOBA in 24 hr.

VA is consistently present in all the collection periods. It represents 2·0 ± 0·7 per cent of the total radioactivity recovered in the urine 24 hr after a 1-hr infusion of DOMA-2-^{14}C; only trace amounts of HVA were recovered. MOMA, DOPAC, HVA and VA have been shown to be metabolic products of both adrenaline and noradrenaline as well as of hydroxytyramine (dopamine);[4,21–32] however, DOBA has not. Since these metabolic products are also derived from DOMA, it would seem that, in part, they must take their origin from adrenaline, noradrenaline and dopamine via the DOMA pathway (see Fig. 5).

Appreciable amounts of radioactive protocatechuic aldehyde were found in the urine, but this was shown to be a degradation product of DOMA rather than a metabolic product.

The question of where DOMA is formed is considerably more difficult to answer. Since in the human adrenaline is normally released directly into the general circulation, it would seem that the deamination of adrenaline to form DOMA occurs at some distal focus which is rich in monoamine oxidase, such as the liver.[33–39] Noradrenaline, on the other hand, is principally released at the sympathetic nerve endings and, since monoamine oxidase is present in the sympathetic nerves,[29,40–52] it follows that the deamination of noradrenaline to form DOMA probably occurs within close proximity to the nerve endings. Whether formed from adrenaline or from noradrenaline, these experiments demonstrate that circulating DOMA is not only *O*-methylated to form MOMA, but that it is also converted to other metabolic products, i.e. DOBA, VA, and several unidentified compounds,

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