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URINARY EXCRETION OF ADRENALINE METABOLITES IN MAN DURING INTERVALS OF 2 MINUTES, 5 MINUTES, AND 10 MINUTES AFTER INTRAVENOUS INJECTION OF ADRENALINE*

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Abstract—Six subjects were injected i.v. within 1 min with 4·8 μc (34 μg) of DL-adrenaline-2-^{14}C. The urine was collected via an indwelling catheter at 2 min and 5 min after injection and thereafter at 10-min intervals for 1 hr, and continued for 6 and 24 hr. Urinary adrenaline and its metabolic products were separated and identified by means of column fractionation and paper chromatography. The radioactivity of each metabolite was measured by liquid scintillation. The basic metabolites, including adrenaline, are metadrenaline and an unknown. The acidic metabolites are: 3-methoxy-4-hydroxyphenylglycol; 3,4-dihydroxyphenylglycol; sulfate conjugate of metadrenaline; glucuronic acid conjugate of metadrenaline; 3-methoxy-4-hydroxymandelic acid; 3,4-dihydroxymandelic acid; 3-methoxy-4-hydroxyphenylacetic acid; 3,4-dihydroxyphenylacetic acid; 3-methoxy-4-hydroxybenzoic acid; 3-methoxy-4-hydroxyphenylglycol sulfate; 3,4-dihydroxyphenylglycol sulfate and several unknowns. No radioactivity was recovered during the 0 to 2-min post injection period. However, the highest percentage of recovered adrenaline and 3,4-dihydroxymandelic acid appeared in the 2 to 5-min postinjection period. The greatest amount and the highest percentage of radioactivity recovered as metadrenaline appeared 5 to 10 min after injection. In terms of total radioactivity the amounts of adrenaline, metadrenaline, and 3,4-dihydroxymandelic acid so rapidly decline that by 30 min after injection these compounds each represent less than 3·5 per cent. Concomitant with this decline there is a rapid increase in the radioactivity of 3-methoxy-4-hydroxymandelic acid such that by 20 min after injection it represents more than 50 per cent of the total radioactivity found in the urine; after 40 min this metabolite begins to decrease. There is a gradual increase in the metadrenaline sulfate; in the 6 to 24 hr period it represents 53·0 $\pm$ 1·6 per cent of the total radioactivity. The importance of understanding the early formation of these adrenaline metabolites is emphasized, and the probable mechanisms involved in the metabolism and inactivation of circulating adrenaline are discussed.

ADRENALINE is now generally accepted as being the principal hormone of the human adrenal medulla; i.e. the adrenal gland contains 220–840 μg of adrenaline and 44–100 μg of noradrenaline per gram of tissue.[1–3] Adrenaline may also arise from other chromaffin tissue but, relative to that arising from the adrenal gland, the amount is quite small;[1] after a bilateral adrenalectomy there is an approximate 80 per cent fall in the adrenaline output with little or no change in the noradrenaline output.[4] Furthermore, the output of adrenaline is greatly increased under various stressful conditions

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including centrifugation,[5] muscle excercise,[6] thermal burn,[7] and trauma,[8] and appears to be associated with anxiety.[5, 9, 10] Experimentally both hypoglycemia[11] and insulin shock[12] produced a marked increase in adrenaline outflow.

In man, adrenaline is released from the adrenal medulla into the inferior vena cava either directly or via the renal vein. This means that the adrenaline rapidly reaches the heart, passes through the pulmonary field, and is distributed throughout the body. In these experiments the adrenaline has been introduced into the antecubital vein rather than directly into the inferior vena cava. Therefore, the most salient difference between the normal release of adrenaline from the adrenal medulla and that injected into the antecubital vein is one of position. Other than this it would appear that in these experiments there is actually very little difference between the exogenous release of adrenaline and the normal endogenous release of adrenaline. In brief, these experiments were designed to as nearly as possible simulate a normal transient release of adrenaline into the general circulation, thereby providing more accurate and detailed information regarding the rate of appearance and excretion of the various metabolic products as they appear in the urine.

METHODS

Injection of DL-*adrenaline*-2-^{14}C. Six women between the ages of 20 and 30 years were injected with 4·8 μc (34 μg) of DL-adrenaline-2-^{14}C (specific activity 25 mc/mmole); Nuclear Research Chemicals, Inc., Orlando, Fla.. The purity of the injected dose as determined by column and paper chromatography was found to be at least 95% adrenaline. The labeled adrenaline was mixed with 10 ml distilled water and injected into the antecubital vein over a period of 1 min. Urine was collected by means of an indwelling catheter (Foley) for the first 2 min (0 to 2 min) after beginning the injection, then 2 to 5 min, and 5 to 10 min (see Table 1). At the end of 10 min urine was collected at 10-min intervals for the next 50 min. Further, collections were made during the next five hr and the subsequent 18 hr for a total of 24 hr. The urine specimens were immediately frozen and stored at $-20°$ until assayed.

Separation of the urinary catabolites of adrenaline. Procedures for the separation and quantitation of the urinary catabolites of adrenaline have been previously described;[13] however, in these experiments modifications have been made to provide improved resolution and purity of the radioactive peaks. An aliquot of urine containing 100,000–150,000 dpm was placed on a 1 × 5 cm column of Amberlite IRC-50 resin (CG-50, type II, Rohn & Haas Co., Philadelphia, Pa.). The column was washed with 30 ml water. The effluent and water wash were combined and saved. The Amberlite column was then eluted with 40 ml 0·5 N acetic acid.

The column effluent (wash) and eluate were each assayed for total radioactivity as follows. Into each of two 20-dram counting vials was placed 1·0 ml of sample, to which was added 15·0 ml of previously prepared liquid containing 15 g naphthalene, 50 mg POPOP, 2 g PPO, 500 ml absolute ethanol, and 500 ml reagent grade toluene. Counting time, using a Packard liquid scintillation spectrometer, was 20 min per vial.

An aliquot of the eluate, containing 500–1,500 dpm, was evaporated to about 1 ml and chromatographed for 24 hr on Whatman 1 paper; *n*-butanol saturated with N HCl as the solvent was used. After drying, the paper was cut into 1-cm strips. Each strip was placed in a 20-dram counting vial filled with scintillation liquid and its radioactivity measured with a Tri-Carb liquid scintillation spectrometer. Three

TABLE 1. EXCRETION PATTERN OF THE METABOLITES OF INTRAVENOUSLY INJECTED DL-ADRENALINE DURING A 60-MINUTE POSTINJECTION PERIOD AND FOR 6 AND 24 HOURS AFTER INJECTION.*

Period of collection after infusion	IRC-50 Fractions				Dowex fractions												Neutral fract.	
	IRC-50 Eluate	Adr.	Metadr.	Unk.	PA	MDC I	MDC II	P IB unk.	PB unk.	MOMA	DOMA	HOMO VA	DOPAC	VA	MOPEG SO_4	DOPEG SO_4	Column residue	Dowex effluent	Recovery of injected ^{14}C
0–2 min																			
2–5 min	26·4 ± 7·6	8·8 ± 7·1	15·0 ± 1·7	1·1 ± 0·7	4·2 ± 0·6	6·9 ± 2·0	3·1 ± 0·3	3·3 ± 0·8	6·4 ± 3·1	8·0 ± 4·3	6·0 ± 2·2	3·0 ± 0·4	3·5 ± 0·9	3·5 ± 0·5	3·4 ± 1·6	2·7 ± 1·1	5·2 ± 1·9	9·6 ± 1·3	2·2 ± 0·6
5–10 min	31·3 ± 2·1	7·6 ± 2·1	20·6 ± 3·4	1·3 ± 0·9	5·7 ± 0·8	5·3 ± 1·2	1·5 ± 0·6	2·9 ± 1·0	4·4 ± 0·6	21·2 ± 5·2	4·8 ± 0·6	1·4 ± 0·6	2·9 ± 1·6	2·5 ± 0·6	2·9 ± 1·4	2·0 ± 0·1	4·4 ± 1·6	6·1 ± 0·8	4·0 ± 0·3
10–20 min	15·2 ± 3·6	3·3 ± 1·7	9·5 ± 1·1	1·3 ± 1·0	1·5 ± 0·7	4·1 ± 1·6	1·1 ± 0·5	1·9 ± 0·5	2·5 ± 1·3	53·3 ± 4·3	3·4 ± 1·7	2·3 ± 1·1	1·2 ± 0·6	2·3 ± 0·6	3·8 ± 1·9	2·6 ± 0·8	2·6 ± 0·4	1·7 ± 0·4	4·0 ± 0·8
20–30 min	7·4 ± 2·5	1·3 ± 0·6	4·5 ± 2·1	1·0 ± 1·0	2·0 ± 0·7	4·3 ± 1·5	0·9 ± 0·7	2·7 ± 0·4	3·1 ± 2·1	54·0 ± 3·0	2·2 ± 0·4	1·4 ± 0·6	1·7 ± 0·4	2·8 ± 0·6	5·4 ± 3·9	2·2 ± 0·8	2·9 ± 1·0	1·2 ± 0·3	3·6 ± 0·5
30–40 min	5·8 ± 1·7	1·0 ± 0·5	3·4 ± 0·3	0·9 ± 0·9	1·4 ± 0·6	11·8 ± 1·2	1·0 ± 0·5	3·4 ± 1·0	1·5 ± 0·9	51·1 ± 4·2	1·7 ± 0·5	0·9 ± 0·5	1·9 ± 0·9	3·5 ± 0·6	6·3 ± 2·9	2·4 ± 1·1	2·6 ± 0·9	1·5 ± 0·5	2·8 ± 0·4
40–50 min	5·0 ± 1·1	0·7 ± 0·3	2·9 ± 0·5	0·8 ± 0·7	1·8 ± 1·0	14·3 ± 1·4	1·1 ± 0·1	4·4 ± 1·3	1·5 ± 0·6	47·4 ± 5·6	2·9 ± 1·5	1·3 ± 1·1	2·7 ± 0·6	3·0 ± 0·6	6·7 ± 3·0	2·4 ± 0·7	2·4 ± 0·8	1·1 ± 0·8	2·5 ± 0·9
50–60 min	5·6 ± 2·1	1·0 ± 0·6	2·9 ± 0·6	1·1 ± 0·9	1·9 ± 0·8	15·7 ± 1·6	1·4 ± 0·8	4·9 ± 1·0	2·3 ± 1·0	44·6 ± 5·0	0·8 ± 0·4	1·2 ± 1·2	2·8 ± 0·7	3·1 ± 0·6	6·9 ± 3·3	3·1 ± 1·0	2·6 ± 2·1	1·2 ± 0·4	2·4 ± 0·3
1–6 hr	4·1 ± 0·5	0·7 ± 0·4	1·6 ± 0·1	1·1 ± 0·7	1·2 ± 0·2	32·0 ± 7·3	1·4 ± 0·5	6·7 ± 1·5	1·8 ± 1·0	33·0 ± 7·6	0·9 ± 0·5	0·5 ± 0·5	2·6 ± 0·6	1·4 ± 0·4	8·1 ± 2·7	1·7 ± 0·6	1·7 ± 1·0	1·1 ± 0·3	31·3 ± 4·8
6–24 hr	4·0 ± 1·1	1·1 ± 1·0	1·2 ± 0·4	1·1 ± 0·7	1·5 ± 0·8	53·0 ± 1·6	3·0 ± 1·3	2·8 ± 2·4	0·6 ± 0·4	19·0 ± 3·7	1·7 ± 0·8	0·8 ± 0·4	1·6 ± 1·0	1·0 ± 0·4	5·0 ± 2·7	2·2 ± 1·7	2·4 ± 0·8	1·1 ± 0·3	26·6 ± 1·1
0–60 min pool	13·5 ± 3·0	3·8 ± 1·9	7·9 ± 2·4	1·0 ± 0·8	2·4 ± 0·4	9·1 ± 1·4	1·4 ± 0·8	3·3 ± 0·8	3·3 ± 1·3	41·0 ± 3·3	2·8 ± 0·5	1·7 ± 0·8	2·4 ± 0·4	2·9 ± 0·3	5·0 ± 2·4	2·5 ± 0·4	3·3 ± 0·9	3·0 ± 0·3	21·0 ± 3·8
0–6 hr pool	7·9 ± 0·5	1·7 ± 0·3	4·3 ± 0·7	1·1 ± 0·4	1·6 ± 0·2	23·0 ± 0·5	1·4 ± 0·9	5·1 ± 1·1	2·4 ± 1·2	36·0 ± 5·6	1·6 ± 0·4	0·9 ± 0·7	2·1 ± 0·7	2·1 ± 0·4	6·8 ± 2·5	2·0 ± 0·7	2·3 ± 1·0	1·9 ± 0·2	46·1 ± 4·8
0–24 hr pool	8·3 ± 3·4	2·0 ± 0·7	4·5 ± 2·2	1·0 ± 0·9	1·6 ± 0·3	33·0 ± 0·6	1·3 ± 0·5	4·4 ± 1·1	1·7 ± 0·8	30·2 ± 5·3	1·6 ± 0·7	0·8 ± 0·5	1·7 ± 0·6	1·8 ± 0·4	6·3 ± 2·4	2·1 ± 0·6	2·2 ± 0·7	2·3 ± 0·6	77·2 ± 7·6

* Figures are expressed as per cent of the recovered radioactivity ± S.D. Adr., adrenaline; metadr., metadrenaline; unk., unknown; PA contains 3,4-dihydroxyphenyl glycol and 3-methoxy-4-hydroxyphenylglycol; MDC I, metadrenaline sulfate; MDC II, metadrenaline glucuronide; MOMA, 3-methoxy-4-hydroxymandelic acid; DOMA, 3,4-dihydroxymandelic acid; HOMO VA, 3-methoxy-4-hydroxyphenylacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VA, vanillic acid; MOPEG-SO_4, 3-methoxy-4-hydroxyphenylglycol sulfate; DOPEG-SO_4, 3,4-dihydroxy-phenylglycol sulfate.

radioactive peaks were obtained, corresponding to adrenaline, metadrenaline, and an unknown compound. The percentage of radioactive adrenaline, metadrenaline, and the unknown substance in each sample was calculated, and when interpreted in terms of the total radioactivity recovered in the eluate of the Amberlite column, gave information as to the total amount of radioactivity of each compound.

An aliquot of the IRC-50 effluent containing 70,000–80,000 dpm was placed on 0·9 × 45-cm Dowex-1-acetate column (200–400 mesh; Calbiochem, Los Angeles, Calif.). The column was placed on an automatic fraction collector and eluted with 75 ml distilled water. The column was then attached to an automatic gradient elution system consisting of four series-connected cylinders, each of which contained 275 ml of solution; the first contained distilled water, the second 1·5 M ammonium acetate buffer (pH 4·8), the third distilled water, and the fourth 6 M ammonium acetate buffer (pH 4·8). During the elution the flow rate was maintained at a rate somewhat less than 0·5 ml/min; 5-ml fractions were collected. Throughout the course of the elution alternate fractions were assayed for radioactivity in an automatic low background planchet counter. Fractions constituting single radioactive peak were pooled

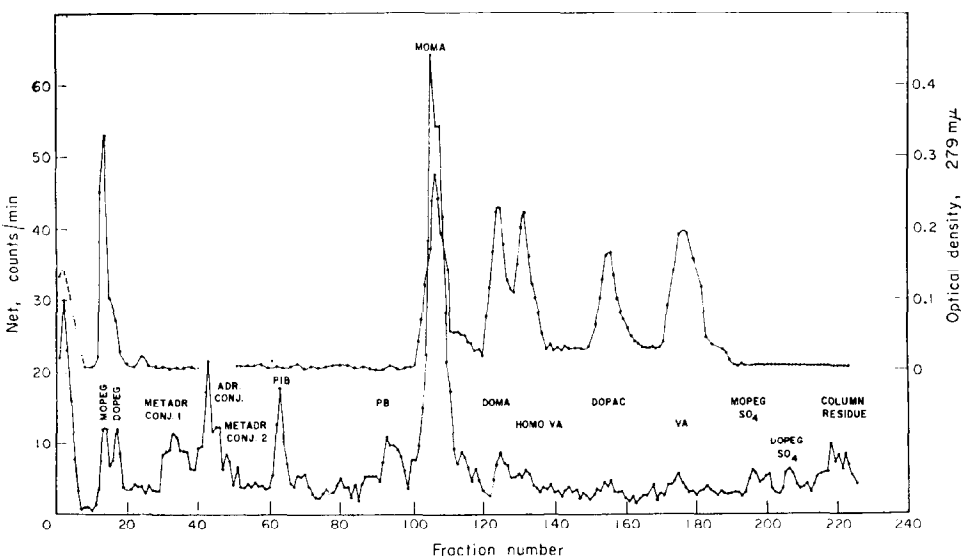

FIG. 1. A representative Dowex-1 acetate elution pattern. Carrier compounds which were added to the column are shown in the upper curve; from left to right these are: MOPEG, 3-methoxy-4-hydroxyphenylglycol; MOMA, 3-methoxy-4-hydroxymandelic acid; DOMA, 3,4-dihydroxymandelic acid; HOMO VA, 3-methoxy-4-hydroxyphenylacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VA, vanillic acid. Background was 3 ± 1 cpm.

and the radioactivity of the pooled samples measured by liquid scintillation. A representative elution pattern is shown in Fig. 1; Fig. 2 shows the alternative pathways for the metabolism of adrenaline.

The data obtained over the various collection periods up to 24 hr were compiled and the results calculated on a General Precision LGP-30 digital computer.

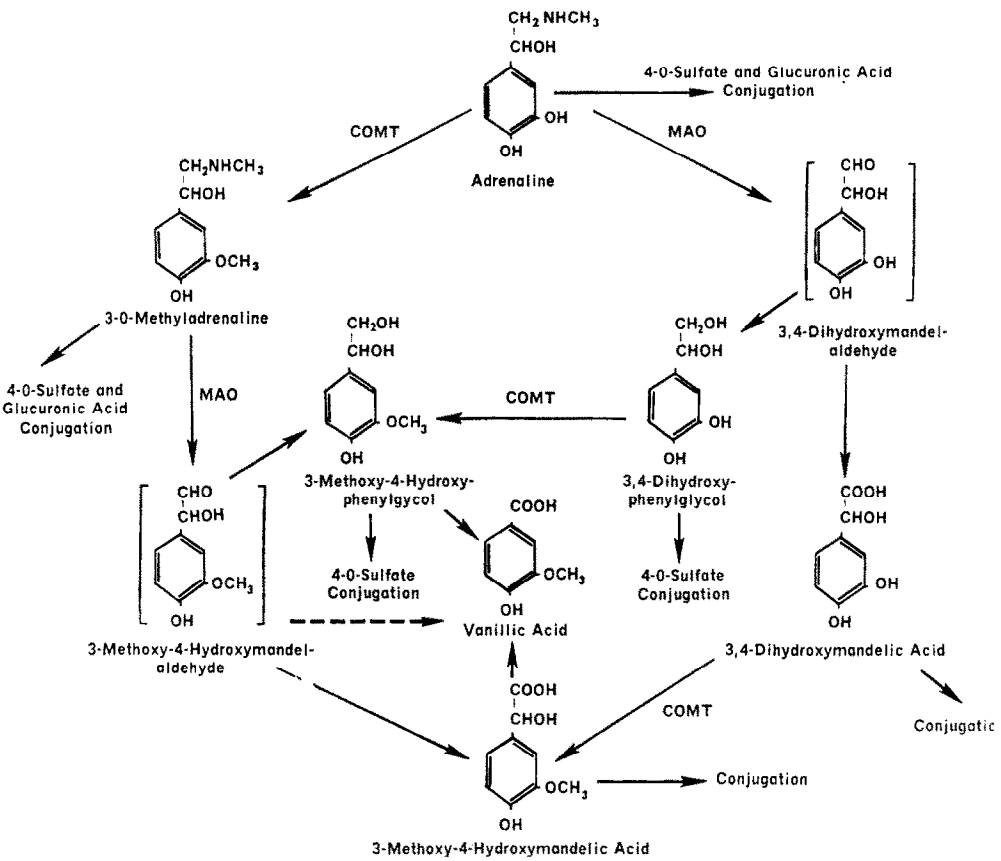

FIG. 2. Alternative pathways for the metabolism of adrenaline.

RESULTS

IRC-50 *eluate* (*basic catabolites*)

From the examination of the data in Table 1 it is clear that at least 2 min are required before adrenaline or its metabolic products appear in the urine. During the 2 to 5 min interval, radioactive adrenaline appears in the urine, but the largest single metabolite is metadrenaline (3-O-methyladrenaline, metanephrine). The amount excreted continues to increase and reaches a maximum during the 5 to 10 min-period (Fig. 3). The excretion of free adrenaline falls to trace amounts after 20 min (Fig. 4).

Dowex-1 *elution* (*acidic catabolites*)

The sequence in which the catabolites are eluted from the Dowex-1 column is shown in Table 1 and Fig. 1. The neutral compounds pass through both Amberlite and Dowex and are herein represented as the first peaks eluted from the Dowex column (see Table 1, PA and Dowex effluent).

Each radioactive peak was chromatographed on Whatman 3MM paper in either, or in both, *n*-butanol : acetic acid : water (8 : 2 : 2) (solvent A), or benzene : propionic acid : water (2 : 2 : 1) (solvent B) to determine its purity. Where possible, authentic carrier compounds were used to assist in the separation and identification.

Dowex effluent. This represents an unknown neutral or very slightly acidic compound.

Peak A (MOPEG and DOPEG). Chromatography of this peak in solvent system A yields two distinct radioactive peaks having R_F values of 0·50–0·55 and 0·70–0·73. The peak (R_F 0·70–0·73) when compared to an authentic sample was found to be

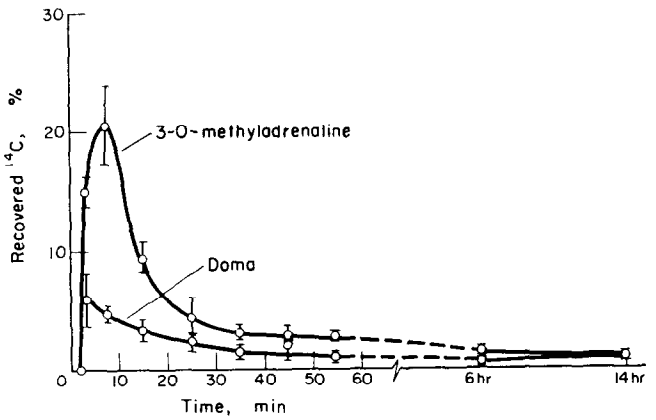

FIG. 3. A comparative excretion pattern of metadrenaline and 3,4-dihydroxymandelic acid after a 1-min i.v. injection of DL-adrenaline-2-^{14}C.

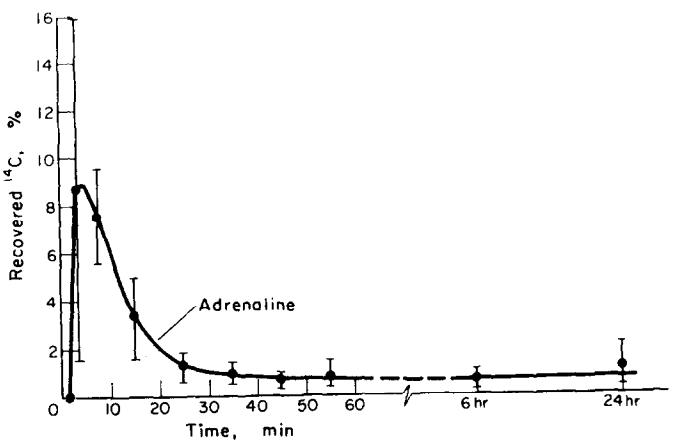

FIG. 4. Excretion of adrenaline after a 1-min i.v. injection of DL-adrenaline-2-^{14}C.

identical with 3-methoxy-4-hydroxyphenylglycol (MOPEG). The peak (R_F 0·50–0·55) has been tentatively identified as 3,4-dihydroxyphenylglycol (DOPEG). Both of these compounds have been described previously.[14, 15] It was noted, however, that during the initial 10-min period after injection nearly all the radioactivity in this peak could be accounted for as MOPEG. At the end of 60 min the radioactivity was nearly equally divided between MOPEG and DOPEG, and at the end of 6 hr most of the radioactivity was represented as MOPEG. This fraction represents only a small percentage of the total radioactivity; the greatest percentage was in the 5 to 10-min collection period where it represents 5·7 $\pm$ 0·8 per cent of the total radioactivity.

Metadrenaline conjugate (*MDC I*). In the urine specimens collected after 20 min, the radioactivity in this peak, when chromatographed in solvent A, moved as one distinct peak (R_F 0·60–0·64). Previous work has shown this compound to be the sulfate conjugate of metadrenaline.[16] The percentage of total radioactivity in this fraction greatly increases, so that by 60 min it represents 15·7 ± 1·6 per cent and in the 6 to 24-hr period it represents 53·0 ± 1·6 per cent (Table 1 and Fig. 5). During

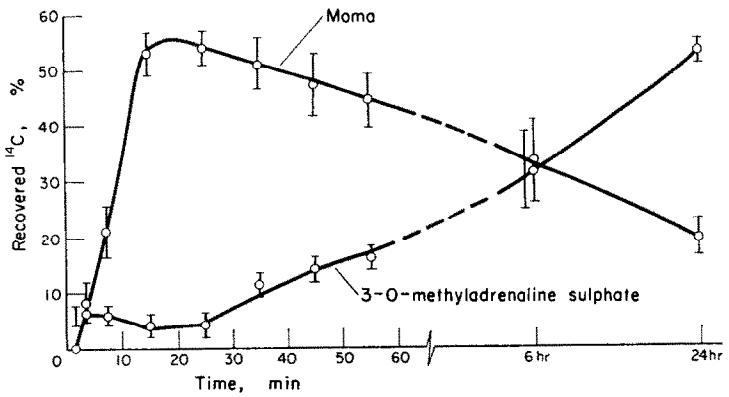

FIG. 5. A comparative excretion pattern of 3-O-methyladrenaline sulfate and 3-methoxy-4-hydroxy-mandelic acid after a 1-min i.v. injection of DL-adrenaline-2-^{14}C.

the first 20 min after injection a significant amount of the radioactivity recovered in this peak was due to the appearance of an adrenaline conjugate, which appears to be a sulfate conjugate since it is easily hydrolyzed either by heating in dilute mineral acids or by the sulfatase preparation, Mylase P (Nutritional Biochemicals, Co. Cleveland, Ohio). This adrenaline conjugate is excreted in a maximal amount during the 2 to 5-min period (6·9 ± 2·0 per cent of total radioactivity) and thereafter rapidly disappears.

Metadrenaline conjugate (*MDC II*). This peak contains a compound which has been tentatively identified as the glucuronic acid conjugate of metadrenaline. Incubation of whole urine with β-glucuronidase leads to the disappearance of most of the radioactivity normally found in this peak. The amount of this compound recovered is small throughout the 24-hr collection, indicating that it is not an important product of adrenaline metabolism. Similar results have been obtained from studies of noradrenaline and normetadrenaline metabolism,[13, 17] implying that, at least in man, conjugation by glucuronic acid is not an important biological means for the inactivation of circulating catecholamines.

P. 1B. This is an unidentified compound which is also a metabolite of noradrenaline-2-^{14}C and normetadrenaline-1-^{14}C.[13, 17] The amount recovered after an i.v. injection of normetadrenaline-1-^{14}C is two to three times greater than when either adrenaline-2-^{14}C or noradrenaline-2-^{14}C is injected, indicating that it is probably a 3-O-methylated derivative and that it retains the number one carbon atom of the side chain. When subjects are pretreated with a monoamine oxidase inhibitor (iproniazid) prior to

injection of either adrenaline of normetadrenaline, less than half the normal amount of this compound is excreted, which implies that it may be a deaminated metabolite.[13 18]

PB. The identity of the compounds found in this peak has not been definitely established. Chromatography of the material in this peak in isopropanol–5% NH_3 yields two peaks with R_F values 0·47 and 0·75. In solvent B the R_F values are 0·05 and 0·57.

MOMA. The radioactivity in this peak represents chromatographically pure 3-methoxy-4-hydroxymandelic acid. This compound is one of the major urinary products of adrenaline and noradrenaline metabolism.[16, 17, 19] During the 2 to 5-min period MOMA represents only 8 ± 4 per cent of the total urinary radioactivity, but thereafter it increases sharply to a maximal amount during the 10 to 30-min interval (54·0 ± 3·0 per cent) and then gradually decreases throughout the remainder of the collection (Table 1 and Fig. 5).

DOMA. This compound is chromatographically identical with 3,4-dihydroxymandelic acid and represents at least 90 per cent of the radioactivity recovered in this peak. With the exception of the 2 to 5-min and 5 to 10 min period in which DOMA is excreted in maximal amounts, i.e. 6·0 ± 2·2 per cent and 4·8 ± 0·6 per cent, respectively, the radioactivity recovered in this peak does not contribute greatly to the total urinary radioactivity. It should not be concluded, however, that this compound represents an unimportant product of adrenaline metabolism. To the contrary, a significant proportion of the intravenously injected adrenaline is deaminated to DOMA[20] which may be rapidly O-methylated to MOMA. Therefore, only small amounts of DOMA would be expected to appear in the urine.[17]

HOMA VA and DOPAC. The radioactivity recovered in these peaks is chromatographically identical with 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid. A significant proportion of the starting radioactivity was retained when the material from each peak was crystallized with authentic carrier compounds to obtain constant specific activity (unpublished results). These compounds appear predominantly during the 2 to 5-min collection, but small amounts may be detected throughout the 24-hr period. Their origin is not clear, but they have been described as metabolic products of dopa and dopamine, precursors of adrenaline;[21] dopamine is not only a precursor of noradrenaline but possibly a metabolic product.[22] Also, the adrenaline molecule may be chemically rearranged to form 3,4-dihydroxyphenylacetaldehyde,[23] a precursor of DOPAC.

VA. This peak contains vanillic acid (3-methoxy-4-hydroxybenzoic acid) and has been described previously as a metabolic product of adrenaline and noradrenaline.[24, 25] Though consistently present, the maximal amount found in any single collection period does not exceed 3·5 per cent of the total radioactivity.

MOPEG-SO_4. The radioactivity in this peak was found to be chromatographically identical with 3-methoxy-4-hydroxyphenylglycol sulfate.[15] After mild hydrolysis, 80–90% of the radioactivity may be recovered as MOPEG. The amount of this compound excreted increases gradually from 3·4 ± 1·6 per cent at the end of 5 min to a maximum of 8·1 ± 2·7 per cent at the end of 6 hr.

DOPEG-SO_4. This metabolite is presumably 3,4-dihydroxyphenylglycol sulfate. After hydrolysis of the material in this peak in 0·1 N HCl, 40–60% of the radioactivity was recovered as a very slightly acidic compound with an R_F 0·48–0·54 (solvent A), which was identical with the compound identified in peak A as DOPEG.

Column residue. The radioactivity contained in the remainder of the Dowex-1 eluate was found to be divided between several compounds which have not been identified. In terms of total radioactivity the column residue does not exceed 5·2 ± 1·9 per cent during any single collection period and is never less than 3 per cent.

DISCUSSION

It has been postulated that circulating catecholamines are largely inactivated by the enzyme O-methyl transferase and that the enzyme monoamine oxidase metabolizes these amines in the tissue.[26, 27] Since in man adrenaline is principally released from the adrenal gland into the general circulation, and noradrenaline is principally released in the tissue at the sympathetic nerve endings,[1, 21, 28–30] the implication is that adrenaline is largely inactivated by catechol-O-methyl transferase and noradrenaline by monoamine oxidase. Although this postulation as a general statement has validity, nevertheless it is not precisely correct, since catechol-O-methyl transferase and momoamine oxidase activity vary greatly from one tissue to another and from one species of animal to another.[26, 31–36] In these experiments, shortly after the i.v. injection of adrenaline, a large amount of radioactivity appears in the metadrenaline fraction. The radioactivity of the metadrenaline fraction rapidly decreases (Fig. 3); however, there is a concomitant increase of radioactivity in the conjugate of metadrenaline and 3-methoxy-4-hydroxymandelic acid(Fig. 5, Table 1). The metadrenaline conjugate fraction continues to increase so that in the 6 to 24-hr period it represents 53·0 ± 1·6 per cent of the total radioactivity, thereby implying that at least with circulating adrenaline, inactivation by O-methylation and conjugation are indeed important.

When one considers O-methylation and conjugation as a means of inactivating circulating adrenaline, one must naturally consider the role of the liver in such inactivation, since the liver contains large quantities of both catechol-O-methyl transferase and monoamine oxidase and especially the former.[26, 31, 32, 34, 36, 37] It would certainly seem that hepatic tissue was uniquely suitable for O-methylating and deaminating circulating catecholamines and their metabolic products. And too, the liver is anatomically well situated to handle circulating adrenaline, since the adrenaline is released into the venous system just proximal to the heart and approximately one third of the cardiac output passes through the liver;[38–41] further, adrenaline increases the circulation through the liver[38, 41, 42] and increases the cardiac output. The early experiments of Philpot and Cantoni demonstrated the remarkable efficiency of the liver in inactivating circulating adrenaline.[43] More recently, O-methyl transferase inhibitors were used to show that O-methylation was mainly responsible for the inactivation of intraportally absorbed catecholamines,[44] and hepatic conjugation, at least in the human, was found to be important in the inactivation of circulating catecholamines.[45]

Binding, conjugation, and diffusion as a means of inactivating adrenaline have been demonstrated; however, metabolism of adrenaline is one of the more important means of inactivation. As has been stated, the metabolism of catecholamines depends largely upon the enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) (Fig. 2). Monoamine oxidase is found in the mitochondrial fraction of the cell,[46] whereas catechol-O-methyl transferase is localized in the soluble fraction.[47] The localization of these enzymes in different parts of the cell could possibly

contribute to a difference in the relative amount of hormone that is initially metabolized by catechol-O-methyl transferase or monoamine oxidase. For years it has been known that there was distinct difference in the physiological and pharmacological action of the D and L isomers of adrenaline. In these experiments DL-adrenaline was injected because of the very limited availability of the labeled L-adrenaline-^{14}C of high specific activity and because of the dangers associated with injecting intravenously large amounts of L-adrenaline. Further, it has been demonstrated that catechol-O-methyl transferase does not possess a stereospecificity to either the D or L isomer,[47] and that L-adrenaline is only slightly better as a substrate for monoamine oxidase than is D-adrenaline.[35] Seemingly very little difference has been found in the amounts of the metabolic products formed when either D or L--adrenaline is injected.[48, 49] However, it has been demonstrated that a significant difference in the uptake of the D and L isomers of adrenaline does exist in isolated nerve granules.[50] Therefore, in this study of the metabolism of injected DL-adrenaline, the data presented in Table 1 may be closely representative of the metabolic fate of the majority of the intravenously injected hormone, but not precisely representative of the rate at which the metabolic products are normally formed from the naturally occurring L-isomer.[49]

Even though no radioactivity was found in the urine during the 0 to 2-min period, the plasma levels of radioactivity have been found to be high;[51, 52] therefore, this delay in appearance of radioactivity in the urine probably represents a renal or ureteral lag. The maximal excretion of adrenaline occurs in the 2 to 5- and 5 to 10-min period (Fig. 4 and Table 1); thereafter, only small to trace amounts of adrenaline were recovered, indicating that the injected adrenaline was rapidly bound or otherwise inactivated with a termination of its pharmacological properties. However, the fact that adrenaline may appear in the urine in trace amounts for as long as 24 hr indicates that not all the adrenaline is immediately metabolized but that some of it must be bound or stored and subsequently released. Of the metabolites which appear between 2 and 5 and 5 and 10 min, metadrenaline and DOMA are the most important since these represent the initial fate of the hormone.

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