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Research Article

METABOLISM OF NORADRENALINE IN THE HUMAN *

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It is now generally accepted that noradrenaline is the neurohormone of the sympathetic nerves (1–4) and is released as such at the nerve endings. However, it is not understood how the body metabolizes noradrenaline. This paper describes a method for separating and identifying the various metabolic products of noradrenaline in the human and discusses the import of this procedure as a tool for studying those clinical entities which represent an aberration in the physiology of the sympathetic nervous system.

As early as 1895 Cybulski (5) showed that extracts of the adrenal medulla, when injected intravenously, increased the pressor activity of the urine. Later Holtz, Credner, Kroneberg and Shümann (6, 7) and von Euler and Hellner (8) demonstrated that this pressor activity was due to adrenaline, noradrenaline, and, to a far lesser extent, hydroxytyramine. Subsequently, von Euler, Luft and Sundin (9) showed that by infusing adrenaline, 0.4 to 1.7 per cent of the infused adrenaline appeared in the urine, and, in similar experiments performed with noradrenaline (10), 1.5 to 3.3 per cent of the infused noradrenaline was excreted in the urine. This therefore means that over 95 per cent of the adrenaline and noradrenaline is metabolized following release; consequently, the metabolic products which appear in the urine must be in a relatively biologically inactive form.

The first of these relatively inactive metabolic products was demonstrated in 1940 by Richter (11). He showed that by ingesting large quantities of adrenaline, a conjugate of adrenaline could be isolated from the urine. He concluded that this conjugate was a sulfate. Beyer and Shapiro (12) performed somewhat similar experiments but concluded that the conjugate was a glucuronide. Dodgson, Garton and Williams in 1947 (13) fed rabbits d-adrenaline and isolated from the urine of these rabbits a conjugate of adrenaline. Clark and Drell (14), repeating this work, showed that rabbits metabolized dl- and l-adrenaline in a similar manner and that the conjugate was a glucuronide.

Then in 1952 and 1953 Schayer, Smiley, Kaplan and Kennedy (15–17) published their papers on the metabolism of adrenaline. They not only showed something about the cleavage of adrenaline but pointed out that there were at least five or six urinary catabolites of adrenaline. In 1957 Armstrong, McMillan and Shaw (18, 19) identified 3-methoxy-4-hydroxymandelic acid as a major urinary metabolite of adrenaline in the human. Subsequently, Axelrod and associates (20, 21) isolated metadrenaline (3-O-methyladrenaline) and normetadrenaline (3-O-methylnoradrenaline) from rat urine and metadrenaline from human urine. Also in 1957 von Euler (22) demonstrated 3,4-dihydroxymandelic acid in human urine, and in 1958 LaBrosse, Axelrod and Sjoerdsma (23) isolated normetadrenaline from the urine of pheochromocytoma patients. Goodall, Rosen and Kirshner (24, 25) infused normal male subjects with labeled dl-adrenaline-2-C^{14} and separated and identified a number of the urinary catabolites of adrenaline. Hitherto, with the exception of 3-methoxy-4-hydroxymandelic acid, none of the metabolic products of noradrenaline in the normal human have been separated or identified.

MATERIALS

A. dl-Noradrenaline-2-C^{14}, specific activity 20 mc. per mM, from Volk Radiochemical Company, Chicago, Ill. Prepared by the method of Howton, Mead and Clark (26).

B. Amberlite IRC-50, X E-64 from Rohm and Haas Company, Philadelphia, Pa.

C. Dowex 1-2 X, 200–400 mesh from Dow Chemical Company, Midlands, Mich.

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METHODS

A. Infusion of dl-noradrenaline-2-C¹⁴. Six healthy adult males between the ages of 20 and 35 years were infused with 9.5 μc. (80 μg. of free base) of sterile dl-noradrenaline-2-C¹⁴. The noradrenaline was diluted with 250 ml. of physiological saline and infused over a period of one hour. Urine was collected after the infusion period, at one hour intervals for the next five hours, and then every six hours for the next 18 hours, thereby covering a total collection period of 24 hours. The urine specimens were refrigerated immediately after collection and stored at −15°.

Aliquots of each hourly specimen were pooled to give a six hour sample, and aliquots of each six hour sample were pooled to give a 24 hour sample. The radioactivity in all specimens was determined with a thin window geiger tube (18 cpm background) and corrected to infinite thinness by reference to a previously prepared absorption curve.

B. Separation of urinary metabolic products of noradrenaline. The principle behind this method is the absorption of the basic catabolites on Amberlite IRC-50, the absorption of acidic catabolites on Dowex 1-2X and the passage of the neutral catabolites through both resins.

An aliquot of urine (5 to 10 ml.) containing 15,000 to 30,000 cpm is passed through a 3 × 1 cm. column of Amberlite IRC-50 prepared as previously described (27). The column is then washed with 15 ml. of water. The effluent and wash are combined and saved. The IRC-50 column is then eluted with 20 ml. of 0.5 N acetic acid. The eluate is evaporated to dryness and the residue dissolved in 0.5 ml. of water. This solution is then chromatographed for 15 to 18 hours on Whatman No. 1 filter paper using *n*-butanol saturated with N HCl as the solvent. After drying, the paper is cut into 1 cm. strips; each of the strips is eluted with water and an aliquot plated out for the determination of radioactivity. Three radioactive peaks are found.

The effluent and wash from the IRC-50 column is then placed on a 10 × 1 cm. column of Dowex-1 acetate buffered at pH 6.1. This column is then washed with 30 ml. of water; again the effluent and wash are collected and assayed for radioactivity.

The Dowex-1 column is then attached to an automatic fraction collector and eluted with 0.05 M ammonium acetate, pH 4.8. Three ml. fractions are collected. The course of the fractionation is followed by assaying the radioactivity of every third sample. When the radioactivity of the eluate returns to background (usually after 75 to 80 ml.) the elution is continued with 50 ml. of 0.3 M ammonium acetate, pH 4.8. This is again repeated with 100 ml. of 1.0 M and with 150 ml. of 3.0 M ammonium acetate, pH 4.8. Each elution yielded one radioactive peak except the 3.0 M buffer which yielded two peaks.

RESULTS

A. IRC-50 elution

Three radioactive peaks were eluted from the paper chromatograms. They represent basic compounds. One peak was chromatographically identical to normetadrenaline and another peak to free noradrenaline. The third peak has not yet been

TABLE I

Urinary excretion of noradrenaline and its metabolic products in the 24 hours following an infusion of dl-noradrenaline-2-C¹⁴ *

Subjects	% Infused dose recovered	IRC-50 fractions				Dowex-1 fractions					Dowex effluent	% Recovery urinary-C¹⁴
		Total	Nor†	NMA‡	Unk§	0.05 M eluate NMAX‖	0.30 M eluate Unk	1.0 M eluate MOMA¶	3.0 M(A) eluate DOMA** +Unk	3.0 M(B) eluate DOMA +Unk		
M. A.	62	10	4	3	2	17	9	30	8	16	3	93
S. N.	69	8	2	2	3	20	10	33	12	12	4	99
L. O.	64	12	4	3	3	21	7	38	11	9	5	103
B. O.	72	9	4	3	2	22	13	30	10	11	4	99
W. E.	65	11	3	4	2	20	10	30	12	14	5	102
M. R.	67	9	4	2	2	16	8	29	12	13	3	90
Average	67	10	4	3	2	19	10	32	11	13	4	98
S. D.††	4	2	1	1	1	2	2	3	2	2	1	5
S. E.‡‡	2	1	1	1	1	1	1	1	1	1	1	2

* Figures are expressed as per cent of total urinary radioactivity except Column 1, which is the per cent of the infused dose recovered in the urine.
† Nor, noradrenaline.
‡ NMA, normetadrenaline.
§ Unk, unknown.
‖ NMAX, normetadrenaline conjugate.
¶ MOMA, 3-methoxy-4-hydroxymandelic acid.
** DOMA, 3,4-dihydroxymandelic acid.
†† S. D., standard deviation.
‡‡ S. E., standard error of the mean.

identified and represents only 2 per cent of the total radioactivity found in the 24 hour urine sample (see Table I).

B. Dowex-1 elution

1. 0.05 M eluate. This fraction is an acid labile conjugate of normetadrenaline. After heating for 90 minutes with 0.1 N hydrochloric acid at 100°, 82 per cent of the radioactivity originally present is recovered as normetadrenaline. The normetadrenaline was isolated from the hydrolysate by absorption on IRC-50 and identified by paper chromatography using two different solvent systems, *i.e.,* butanol saturated with N HCl and isopropanol-ammonia-water (8–1–1). Because of its ready hydrolysis in dilute acid and since it is hydrolyzed to about 70 per cent by Mylase P® (Nutritional Biochemicals), an enzyme preparation containing arylsulfatase, the conjugate is probably a sulphate ester. The conjugate is not hydrolyzed after a 24 hour incubation with either bacterial (Sigma) or animal (Warner-Chilcott) β-glucuronidase preparations.

2. 0.3 M eluate. The radioactivity in this fraction appears to be due to a single compound since only one radioactive area can be detected by paper chromatography using butanol-N HCl and isopropanol-ammonia-water as solvents. The compound, which has not yet been identified, represents 10 per cent of the total radioactivity in the 24 hour urine (see Table I).

3. 1.0 M eluate. The radioactivity in this fraction is chromatographically identical with 3-methoxy-4-hydroxymandelic acid (see Table I). Again two different solvent systems were used for identification, *i.e.,* butanol-N HCl and isopropanol-ammonia-water.

4. 3.0 M eluate. Two rather diffuse peaks are obtained from the Dowex column in the 3.0 M elution. Upon chromatographing these peaks in butanol-N HCl, butanol-acetic acid-water (4–1–1) and in isopropanol-ammonia-water, two radioactive areas are obtained from each of these fractions. One of the radioactive areas in each of these fractions corresponds to 3,4-dihydroxymandelic acid; the other radioactive areas have not been identified. Approximately 30 to 50 per cent of the radioactivity eluted from the filter paper is found in the fraction tentatively identified as 3,4-dihydroxymandelic acid.

C. General results

It should be noted that only 4 per cent of the total radioactivity found in the 24 hour urine passed through both columns, and too, there was no detectable radioactivity left on either the IRC-50 or the Dowex-1 column.

When dl-noradrenaline-2-C^{14} is infused, 67 ± 4 per cent of the radioactivity is recovered from the urine within a period of 24 hours (see Table 1). Figure 1 shows, in six subjects, the average hourly excretion of radioactivity. These findings compare well with the 73 ± 2 per cent recovery of radioactivity in the adrenaline infusion experiments previously reported from this laboratory (24) and the adrenaline infusion experiments of Resnick and Elmadjian (28). Table I presents the data on the excretion of noradrenaline and the catabolites of noradrenaline over a 24 hour period following an infusion of dl-noradrenaline-2-C^{14}. From Table I it is apparent that approximately 85 per cent of the radioactivity noted in the urine can be separated on Dowex-1 and about 10 per cent on IRC-50. The identified noradrenaline catabolites eluted from the Dowex-1 are: normetadrenaline conjugate; 3-methoxy-4-hydroxymandelic acid; 3,4-dihydroxymandelic acid. Of these products, 3-methoxy-4-hydroxymandelic acid is the largest single fraction, representing about 32 per cent of the total radioactivity. The second largest fraction is the normetadrenaline conjugate represent-

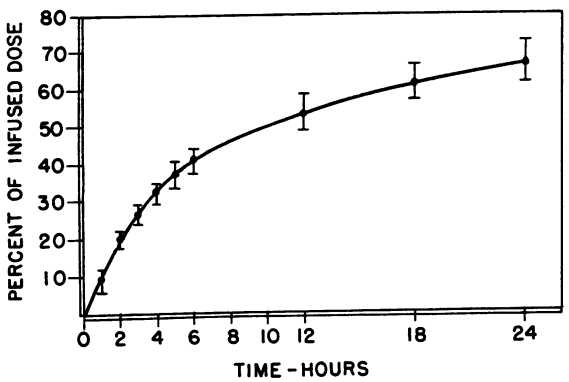

FIG. 1. EXCRETION OF RADIOACTIVITY IN THE URINE AFTER INFUSIONS OF NORADRENALINE-2-C^{14}

The points represent the averages of six subjects; the vertical lines represent the range of variations.

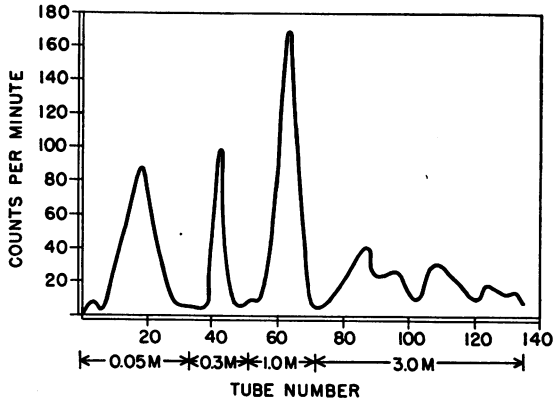

FIG. 2. SEPARATION OF URINARY METABOLITES OF NORADRENALINE-2-C^{14} ON DOWEX-1

The concentration at the bottom of the graph refers to the ammonium acetate buffers.

ing 19 per cent; the remaining 35 per cent eluted from the Dowex column is divided about equally among the other three peaks (0.3 M and 3.0 M eluates) which include 3,4-dihydroxymandelic acid and two or three unidentified compounds. Figure 2 graphically represents the radioactivity in the noradrenaline catabolites eluted from Dowex-1 from a 24 hour urine sample.

Ten per cent of the total radioactivity is absorbed on IRC-50. Of this radioactivity, 4 per cent is due to unchanged noradrenaline, 3 per cent to free normetadrenaline and 2 per cent to an unidentified compound. Previous work (10) on the excretion of noradrenaline in urine following an infusion showed 1.5 to 3.3 per cent of the infused dose appearing in the urine as free noradrenaline and/or conjugates of noradrenaline.

Table II shows the hourly excretion pattern of noradrenaline and its metabolic products. Though this table represents the pattern from a single subject, this pattern is similar to that of the five other subjects. From Table II it is also apparent that the noradrenaline is rapidly metabolized by the body. This is seen by the rapid disappearance of free noradrenaline from the urine after the infusion period. Approximately 16 per cent of the radioactivity noted in the urine during the infusion period is free noradrenaline. This decreases to 3 per cent during the second hour and 1 per cent during the third and fourth hours; thereafter only small amounts of radioactive noradrenaline can be detected. The normetadrenaline follows a similar pattern as does the unidentified

TABLE II

*Excretion of noradrenaline and its metabolic products during the 24 hour period following an infusion of dl-noradrenaline-2-C^{14} **

Time in hours	% Infused dose recovered	IRC-50 fractions				Dowex-1 fractions					Dowex effluent	% Recovery urinary-C^{14}
		Total	Nor†	NMA	Unk	0.05 M eluate NMAX	0.30 M eluate Unk	1.0 M eluate MOMA	3.0 M(A) eluate DOMA +Unk	3.0 M(B) eluate DOMA +Unk		
1	11	38	16	12	8	10	2	33	4	8	4	86
2	10	9	3	4	1	15	7	41	11	9	5	98
3	7	3	1	1	0.4	24	12	37	8	19	3	107
4	6	3	1	1	0.4	26	11	30	8	21	4	102
5	5	2	0.7	0.7	0.5	21	8	29	12	22	4	97
6	4	2	0.9	0.7	0	26	7	22	19	17	4	98
6–12	10	2	Trace	0	0	33	15	16	12	17	3	98
12–18	7	1	0	0	0	33	11	18	13	20	3	99
18–24	5	2	0	0	0	19	8	16	12	15	11	83
Calculated 24 hour aliquot	65	9	4	3	2	22	9	28	9	15	4	96
24 hour aliquot experimental	65	11	3	4	2	20	10	30	12	14	5	103

* Figures are expressed as per cent of total urinary radioactivity except Column 1, which is the per cent of the infused dose recovered in the urine.

† For abbreviations, see Table I.

TABLE III

*Excretion of noradrenaline and its metabolic products during the infusion period and at the end of six hours and at the end of 24 hours following the infusion of dl-noradrenaline-2-C^{14} **

Time	% Infused dose recovered	IRC-50 fractions				Dowex-1 fractions					Dowex effluent	% Recovery urinary-C^{14}
		Total	Nor†	NMA	Unk	0.05 M eluate NMAX	0.30 M eluate Unk	1.0 M eluate MOMA	3.0 M(A) eluate DOMA +Unk	3.0 M(B) eluate DOMA +Unk		
Infusion	10±2	37±3	17±3	10±3	8±3	9±1	2±4	32±3	4±1	6±1	3±1	93±7
6 hours	41±4	12±1	5±1	4±1	2±1	17±2	8±2	36±5	9±1	11±2	5±1	98±6
24 hours	67±4	10±2	4±1	3±1	2±1	19±2	10±2	32±3	11±2	13±4	4±1	98±5

* Figures are expressed as per cent of total urinary radioactivity except Column 1, which is the per cent of the infused dose recovered in the urine.

† For abbreviations, see Table I.

compound eluted from the IRC-50. The amounts of these later catabolites were less than that of free noradrenaline.

From the Dowex-1 elution shown in Table II, it is apparent that 3-methoxy-4-hydroxymandelic acid represents the largest single catabolite and that the radioactivity in this fraction is initially high (33 per cent, increasing to 41 per cent in the second hour and gradually decreasing during the remaining 22 hours). On the other hand, the radioactivity in the normetadrenaline conjugate fraction gradually increased from an initial 10 per cent during the infusion period to 33 per cent during the 12 to 18 hour collection period. It should be noted that the 18 to 24 hour sample had to be concentrated four times to obtain sufficient radioactivity for assay purposes. Because of the large amount of salt present in this concentrated sample, a distorted fractionation picture was obtained, thereby leading one to believe that most of the radioactivity in the Dowex-1 effluent of the 18 to 24 hour sample is probably due to the normetadrenaline conjugate.

The unknown catabolite resulting from the 0.3 M eluate appears to increase for two hours following the infusion and then plateaus at a constant excretion rate. A somewhat similar pattern is noted for each of the 3.0 M eluates.

A comparison was made between the 24 hour distribution of C^{14} calculated from each hourly sample and that actually obtained by measuring the distribution of radioactivity in a 24 hour aliquot (see Table II). Good agreement was obtained which indicates the accuracy of this procedure.

Table III compares the distribution of radioactivity in the various catabolites of noradrenaline at the end of the infusion period, at the end of six hours, and at the end of 24 hours. Total recoveries were better in the six and 24 hour periods than in the infusion period. A marked difference can be seen in the distribution of radioactivity at the end of the infusion period and at the end of six and 24 hours. There was only a slight difference between the six and 24 hour sample.

A total of 10 radioactive fractions are obtained by using a combination of ion exchange and filter paper chromatography to separate the metabolic products of noradrenaline-2-C^{14}. Five of these fractions have been identified. Of the remaining five, at least three are distinctly different compounds. These are: unidentified radioactive peak obtained upon chromatographing the IRC-50 eluate, the material eluted from the Dowex-1 column with 0.3 M ammonium acetate, and the unidentified compound eluted with 3.0 M ammonium acetate. Although dihydroxymandelic acid and an unidentified radioactive area are found upon chromatographing each of the peaks obtained in the 3.0 M elute, it is not certain that these unidentified compounds are different from each other. Because of the small amount of radioactivity in the Dowex effluent, an unequivocal chromatogram of this fraction has not yet been obtained. The work of Booth, Emerson, Jones and DeEds (29) suggests that one of these compounds may be *m*-hydroxymandelic acid, and it is quite possible that one may find glycine or glucuronidate conjugates of the previously identified compounds among the unidentified fractions.

DISCUSSION

After an infusion of dl-noradrenaline-2-C^{14}, 67 ± 4 per cent of the infused radioactivity is recovered from the urine in 24 hours (see Figure 1). The fact that the urinary excretion of radioactive catabolites continues for longer than 24 hours, and since only small amounts of free noradrenaline appear in the urine after the infusion, would imply that the noradrenaline *per se* rapidly disappears from the blood stream but that the metabolic products are gradually released into the blood stream for renal clearance. The fact that the radioactivity in the blood during and especially after the infusion was very low supports this concept.

Why the radioactive noradrenaline rapidly disappears from the urine and why the metabolic products continue to be excreted over a 24 hour period is not yet clear. However, it is quite possible that the tissue cells pick up the noradrenaline, store it as a complex (30, 31) and gradually release it for metabolism, thereby permitting the metabolic products to gradually appear in the general circulation for renal clearance. As an alternate explanation, the noradrenaline could be metabolized at the time of release and the metabolic products could diffuse into the tissue spaces or cells from which they could gradually diffuse back into the blood stream and be excreted by the kidney. Either of these processes would account for the rapid disappearance of free noradrenaline and the gradual excretion of the metabolic products. The fact that small amounts of free radioactive noradrenaline are excreted over the first 12 hours (see Table II) would indicate that at least some of the free noradrenaline is stored in the tissues for subsequent release and metabolism. Obviously, how noradrenaline is precisely metabolized cannot be shown until tissue studies are made following an injection of labeled noradrenaline. Such work is currently underway.

From the data in Table III, about 55 per cent of the metabolic products of noradrenaline appear in the urine as O-methylated compounds, namely normetadrenaline, normetadrenaline conjugate and 3-methoxy-4-hydroxymandelic acid. If one assumes that the compounds, other than the normetadrenaline conjugate, which are absorbed on the Dowex-1 column arise from oxidative deamination, then 65 per cent of the metabolic products are acidic compounds which probably result from the action of monoamine oxidase upon noradrenaline and normetadrenaline.

The major products of noradrenaline metabolism in man are 3-methoxy-4-hydroxymandelic acid and a conjugate of normetadrenaline. The former compound results from the action of the enzymes amine oxidase, aldehyde dehydrogenase and catechol-O-methyl transferase; the latter compound from the action of catechol-*o*-methyl transferase and the enzymes involved in the conjugation. Similarly, the major products of adrenaline metabolism in man (24, 25) are 3-methoxy-4-hydroxymandelic acid and a conjugate of metadrenaline.

The conclusion (32) that O-methylation precedes oxidative deamination and that the former process is the principal mechanism for the initial inactivation of adrenaline is not sufficiently supported by evidence. By an analysis of the 24 or 48 hour urine specimen one cannot determine the sequence of reaction in the formation of 3-methoxy-4-hydroxymandelic acid from adrenaline or noradrenaline. Data (33) on the metabolic products of dl-adrenaline-2-C^{14} which appear in the urine immediately after an infusion of adrenaline-2-C^{14} in normal subjects and subjects treated with an amine oxidase inhibitor (Marsilid®) show that, in the subjects who received Marsilid®, the radioactivity in the urinary adrenaline was twice that of the untreated subjects; the radioactivity in the metadrenaline and conjugated metadrenaline fraction was increased 65 per cent above the untreated subjects. These data suggest that, during the short period of time when adrenaline and noradrenaline have physiological activity, amine oxidase activity is of considerable importance in the inactivation of these hormones. The presence of significant amounts of 3,4-dihydroxymandelic acid in the urine and the recent reports by Pekkarinen, Castren and Iisolo (34) and Shore, Prockop, Spector and Brodie (35) that the noradrenaline levels in brain and other tissues were significantly elevated after treatment with an amine oxidase inhibitor (iproniazed, Marsilid®), also support this concept. The present evidence shows that oxidative deamination and O-methylation are the major alternative pathways for the metabolism of adrenaline and noradrenaline, and strongly sug-

gests that each contributes significantly in the initial inactivation of adrenaline and noradrenaline during the period of physiological activity.

The results reported here for dl-noradrenaline are not necessarily applicable to the natural occurring l-isomer. However, from the demonstration by Schayer and co-workers (15, 16) that both l-adrenaline and dl-adrenaline were metabolized similarly by the rat, and from the observations that both the l and d isomers of adrenaline serve almost equally well as substrates for amine oxidase (36) and catechol-O-methyl transferase (37), one is led to believe that the quantitative results obtained with dl-noradrenaline would be in very close agreement with those of the l-isomer.

Noradrenaline is the neurohormone of the sympathetic nerves (1–4). In view of this, the metabolic products of noradrenaline would represent a barometer of the functioning of the sympathetic nervous system. There are a number of clinical conditions which result from an aberration in the physiology of the sympathetic nervous system, *i.e.*, chronic postural hypotension, certain forms of neurogenic hypertension, diabetic neuropathy, sympathetic nerve tumor, and so forth. Therefore, the procedure herein described for separating and identifying the metabolic products of noradrenaline could be a useful tool for studying these conditions as well as evaluating their therapy.

SUMMARY

Six adult males were infused with labeled noradrenaline and the urinary catabolites were separated and identified. A procedure is herein described which utilizes a combination of filter paper and ion exchange chromatography to separate the urinary metabolic products of labeled noradrenaline. Sixty-seven ± 4 per cent of the infused radioactivity was recovered in the 24 hour urine. The total radioactivity which appeared in the urine was distributed among the various catabolites of noradrenaline as follows: noradrenaline, 4 ± 1 per cent; normetadrenaline, 3± 1 per cent; normetadrenaline conjugate, 19 ± 2 per cent; 3-methoxy-4-hydroxymandelic acid, 32 ± 3 per cent; 3,4-dihyroxymandelic acid, and five unidentified fractions, 40 per cent. The 3,4-dihydroxymandelic acid has been tentatively identified as part of two of the unidentified fractions.

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