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METABOLISM OF 3-HYDROXYTYRAMINE (DOPAMINE) IN HUMAN SUBJECTS*

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Abstract—Six male subjects were infused with 104·6 μc 3-hydroxytyramine-2-C^{14} (dopamine). The hydroxytyramine was dissolved in 1000 ml physiological saline and infused at a constant rate for 4 hr. Urine was collected at the end of the infusion period and at 2-hr intervals for the first 4 hr post infusion, and then at 8 hr, 24 hr and daily for the next 4 days. The urinary hydroxytyramine and its direct metabolites as well as its products, i.e. noradrenaline and its metabolites, were separated by column fractionation and identified. A new method, herein described, was used which simultaneously monitored the optical density and measured the radioactivity of each compound as it was eluted from the column. During the infusion, 38·9 per cent of the infused dose was recovered in urine as hydroxytyramine, noradrenaline, adrenaline (trace amounts) and their respective metabolites; recovery of hydroxytyramine accounted for 9·1 per cent while 3-methoxy-4-hydroxyphenylacetic acid accounted for 53·1 per cent of the radioactivity recovered during the infusion period; noradrenaline represented 4·7 per cent during the same period. The total amount of radioactivity recovered after 5 days was 97·2 $\pm$ 3·5 per cent. Approximately 75 per cent of the infused hydroxytyramine 2-C^{14} was directly metabolized into hydroxytyramine-related metabolic products. They are 3-methoxytyramine, 3-methoxy-4-hydroxyphenylacetic acid; 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylethanol, 3-methoxy-4-hydroxyphenylethanol and their respective conjugates, as well as the conjugates of hydroxytyramine and several unknowns. Of these, 3-methoxy-4-hydroxyphenylacetic acid is by far the largest, since it represents 18·6 per cent of the infused dose recovered during the infusion period. The remaining 25 per cent of the infused hydroxytyramine was synthesized into noradrenaline and appeared in the urine as noradrenaline or metabolic products of noradrenaline, principally the latter. These experiments avoid many of the pitfalls and errors imposed by infusing exogenous labeled noradrenaline where one measures the metabolic products of circulating exogenous noradrenaline rather than the natural occurring noradrenaline. Furthermore, they provide more accurate and detailed information with regard to the rate of appearance and excretion of endogenous noradrenaline metabolites in the human.

HYDROXYTYRAMINE (3,4-dihydroxyphenylethylamine, dopamine) was first synthesized by Mannich and Jacobsohn in 1910.[1] However, hydroxytyramine was not shown to be present in mammalian tissue until 1950, when Goodall demonstrated its presence in the adrenal gland[2,3] and heart.[3,4] Since then it has been shown to occur in other mammalian tissues[5–12] and in human urine[13–15] as well as in insects[16] and plants.[17,18] The role of hydroxytyramine as a precursor to noradrenaline and adrenaline is well

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established.[19-25] In more recent years, much attention has been focused on hydroxytyramine as a possible neurohormone with transmitter function in the central nervous system.[9, 12, 26-40] Further, its relationship to Parkinsonism[41-44] and its concentration in specific areas of the brain have emphasized the possible importance of this compound as a neurohormone.

Very little is known about the metabolism of hydroxytyramine in the human. However, Shaw[45] showed that 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic could be formed from hydroxytyramine. The purpose of this paper is to delineate more precisely the metabolism and precursor–product relationship of hydroxytyramine to noradrenaline and adrenaline in normal human subjects.

METHOD

Infusion of 3-hydroxytyramine-2-C^{14} and collections of post infusion urine:

Six normal, healthy males (ages 21–35) were infused with 104·6μc (872 μg) 3-hydroxytyramine-2-C^{14}. The labeled hydroxytyramine was dissolved in 1000 ml of physiological saline and infused into the antecubital vein at a constant rate over a period of 4 hr. The subjects were kept supine during the infusion and thereafter maintained in a sedentary state until the termination of the collection periods. Urine was collected during the infusion period and at 2-hr intervals for the first 4 hr post infusion, then at the end of 8 hr, 24 hr, and at the end of the second, third, fourth and fifth days. The urine samples were immediately frozen and stored at −20°C until assayed.

The infused 3-hydroxytyramine .[3,4-dihydroxyphenyl (ethylamine-2-C^{14}) hydrochloride] was chromatographed in three solvent systems and found to be more than 96 per cent pure.

Isolation and quantitation of metabolic products of 3-hydroxytyramine

Preliminary separation of the acidic and basic metabolites. An aliquot of urine containing 2×10^6 dpm was placed on a 1·0 × 6·0 cm column of Amberlite IRC-50 cation-exchange resin.* After the effluent which contained the acidic and neutral compounds had passed through the resin, the column was washed with four 10-ml portions of glass-distilled water. The water wash was combined with the effluent and assayed for total radioactivity; 1·0 ml of sample was placed into each of two 20-ml glass counting vials to which was added 15 ml of freshly prepared scintillation fluid containing 2 g/l. PPO and 50 mg/l. POPOP in ethanol–toluene (1:1) solvent. The Amberlite column was then eluted with 40 ml of 1 N acetic acid, The eluate contained the basic compounds and was also assayed for total radioactivity.

Separation of acidic compounds. An aliquot of the Amberlite effluent and wash containing 100,000 tdpm and carrier compounds of MOMA (3-methoxy-4-hydroxymandelic acid), HVA (3-methoxy-4-hydroxyphenylacetic acid). DOMA (3,4-dihydroxymandelic acid) and DOPAC (3,4-dihydroxyphenylacetic acid) was placed on 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 glass-distilled water, followed by a variable gradient elution consisting of ammonium

* Source of Materials: Amberlite IRC-50 (CG-50, Type II), Rohm and Haas Co., Philadelphia, Pa.; Dowex-1-X2, 200–400 mesh, Bio Rad Laboratories, Richmond, Calif.; 3-hydroxytyramine-2-C^{14} HCl (3,4-dihydroxyphenylethylamine, dopamine), Nuclear-Chicago, Chicago, Ill.; Packard Instrument Corp., Downers Grove, Ill.

acetate buffers of varying molarity and acidity. The flow rate was adjusted to approximate 1 ml/min.

The course of the elution was followed by passing the column eluate through one of two types of monitoring systems. In one system, the eluate was passed through the quartz flow cell (1·0 ml volume) of a Beckman DB-G spectrophotometer, and the O.D. measured at 279 mμ. The output of the DB-G spectrophotometer was recorded on one channel of a Texas Instruments Co. Servo-Riter II dual-pen recorder; the DB-G, a dual beam instrument, was found to be necessary in the development of this monitoring system to insure long-time baseline stability. After passing through the DB-G spectrophotometer, the eluate entered a 10 ml (Packard Instrument Co.) flow cell and the radioactivity was counted; the cell was packed with 25–50 mesh pilot-B beads (scintillator) and was placed in 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 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 radioactive peak were pooled and assayed for total radioactivity.

By altering slightly the above system, it was possible to obtain a digital readout of the total radioactivity in each peak. In this latter system, the O.D. monitoring system and column operation are the same. The eluate, upon leaving the DB-G spectrophotometer, was passed into a Packard (10 ml) flow cell which was placed in a standard series 3000 Packard Tri-Carb scintillation spectrometer (model 3214). The spectrometer was instructed by a volume measuring device to digitally indicate the total counts accumulated during each preset volume collection. In this manner continuous integration was performed during the course of the column elution. Calculations of the data from this system, when compared with the first system, yielded results which, throughout the course of the elution, were well within experimental error. No evidence of quenching could be detected when samples representing less than 30 ml urine were fractionated. A typical elution pattern obtained with this system is shown in Fig. 1.

The recovery of the total radioactivity placed on the Dowex-1 column was 97 $\pm$ 6 per cent. The peaks containing specific free phenolic acids, such as MOMA, HVA, DOMA and DOPAC, were confirmed by paper chromatography of each peak in three solvent systems, i.e. *n*-butanol–*N*-acetic acid–H_2O (4:1:1); benzene–proprionic acid–H_2O (8:2:2) and isopropanol–5% NH_3 (8:2). In order to chromatograph these peaks, it was necessary first to remove the ammonium acetate. This was done by passing the peaks through a 5 $\times$ 30 cm column of Dowex 50-X8, 200–400 mesh, cation-exchange resin in the hydrogen form. The resulting acetic acid was evaporated from the effluent and the compounds were applied to Whatman No. 1 filter paper and chromatographed. The conjugates of HVA and DOPAC were identified by refluxing the ammonium acetate free peak in 3 N H_2SO_4 followed by extraction into ether and chromatography of the resulting compound with appropriate carrier compounds in the solvent systems described above. The MOMA conjugate was similarly identified after refluxing in 2 N H_2SO_4. The conjugates of 3-methoxytyramine (3-MT), normetadrenaline (NM), normadrenaline (NOR) and 3-hydroxytyramine (dopamine, 3-HT) were identified by hydrolysis of the conjugated amine in 2 N H_2SO_4 followed by ion-exchange absorption and chromatography of the free amines on IRC-50

Amberlite resin. The peak containing the normetadrenaline conjugate was also found to contain significant amounts of noradrenaline conjugate. This peak was collected and acidified to 2 N H_2SO_4 and refluxed for 30 min under nitrogen. The free noradrenaline was absorbed on acid-washed alumina at pH 8·5 while the normetadrenaline was not retained by the alumina. In this manner, the relative amounts of each compound in this peak were determined.

The peaks indicated in Table 1 as 3-methoxy-4-hydroxyphenylethanol conjugate and 3-methoxy-4-hydroxyphenylglycol conjugate have been tentatively identified on the basis of hydrolysis in 2 N H_2SO_4 and extraction in ethyl acetate at pH 6·0. Before hydrolysis no radioactivity could be extracted into ethyl acetate from an acidic or neutral medium, whereas after hydrolysis most of the radioactivity was extracted at pH 6·0 into ethyl acetate; this is a characteristic of the phenylethanol and the phenylglycol compounds. The methoxy metabolites contained in this peak are stable in a basic medium, which is in contrast to the dihydroxyphenyl compounds.

Separation of the basic compounds. An aliquot of urine containing 500,000 tdpm was placed on a 60 × 0·9 cm column of Amberlite IRC-50 resin along with carrier compounds of adrenaline, metadrenaline, normetadrenaline, noradrenaline, 3-hydroxytyramine (dopamine) and 3-methoxytyramine. The column was washed with 20 ml of 0·2 M ammonium acetate buffer, pH 6·2, and attached to one of the monitoring systems described above. The column was eluted with 0·3 M ammonium acetate buffer, pH 5·0, followed by 0·4 M buffer. Fig. 2 represents a typical elution pattern obtained with this system. The large radioactive peak at the beginning of the elution, which was washed off the column with 0·2 M ammonium acetate, contained the acidic metabolites. The basic metabolites were eluted from the column with 0·3 M and 0·4 M ammonium acetate, pH 5·0. In post-infusion urines, these basic metabolites represented 1–2 per cent of the total radioactivity placed on the column. The identity of the radioactive peaks was determined and confirmed by paper chromatography. Adrenaline and normetadrenaline elute from the column at the same point, but in these experiments further separation procedures did not yield detectable amounts of adrenaline in these urines. Most of the radioactivity was confined to the compounds normetadrenaline, noradrenaline and 3-hydroxytyramine; only trace amounts of 3-methoxytyramine were found (see Table 1).

RESULTS

During the infusion of hydroxytyramine, 38·9 per cent of the dose was recovered in urine as hydroxytyramine, noradrenaline and their respective metabolites. The recovery of hydroxytyramine, free and conjugated, accounted for 9·1 per cent while 3-methoxy-4-hydroxyphenylacetic acid, free and conjugated, accounted for the majority (53·1 per cent) of the radioactivity recovered during the infusion period (see Fig. 3). During the same period, noradrenaline and its conjugate represented a total of 4·7 per cent of the radioactivity recovered during the infusion period. This figure, however, does not include the various radioactive metabolites of adrenaline and noradrenaline, i.e. metadrenaline, normetadrenaline, normetadrenaline conjugate, MOMA, etc. (see Table 1). The individual collection periods provide information on the rate at which one might expect to find adrenaline, noradrenaline and hydroxytyramine and their respective metabolites appearing in the urine after an i.v. infusion of hydroxytyramine (see Table 1 and Figs. 3, 4 and 5).

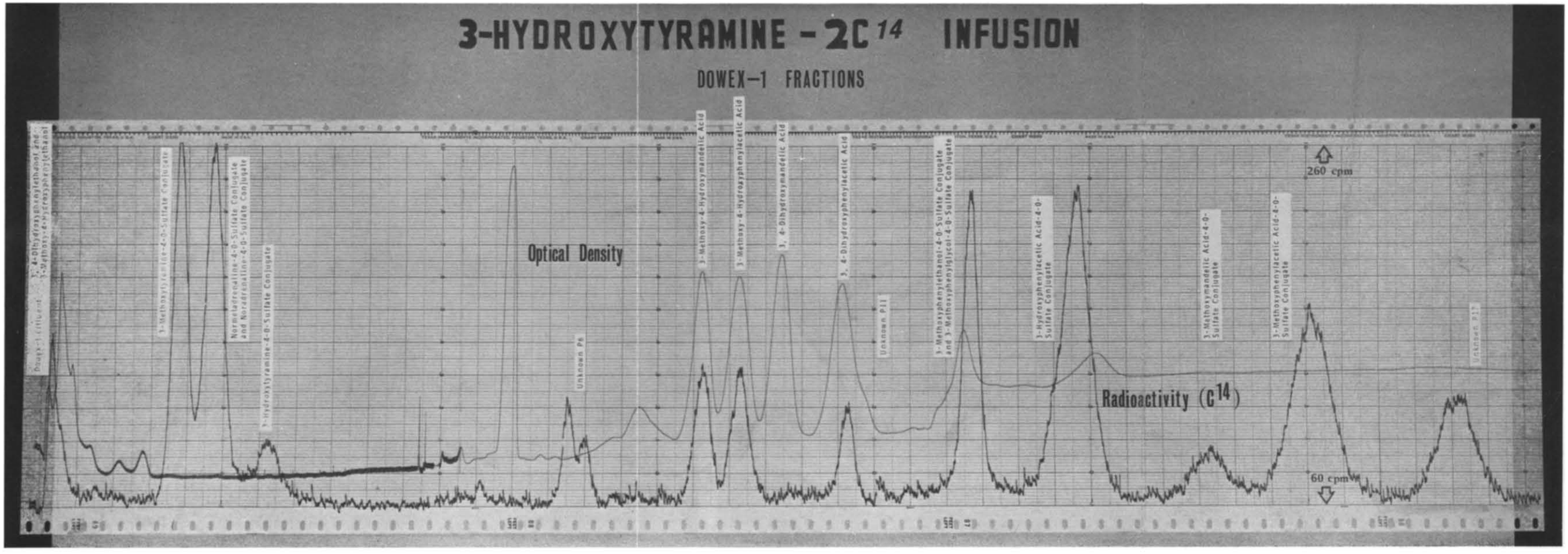

FIG. 1. A representative tracing of the acidic metabolites as they are eluted from the Dowex 1-X2 column.

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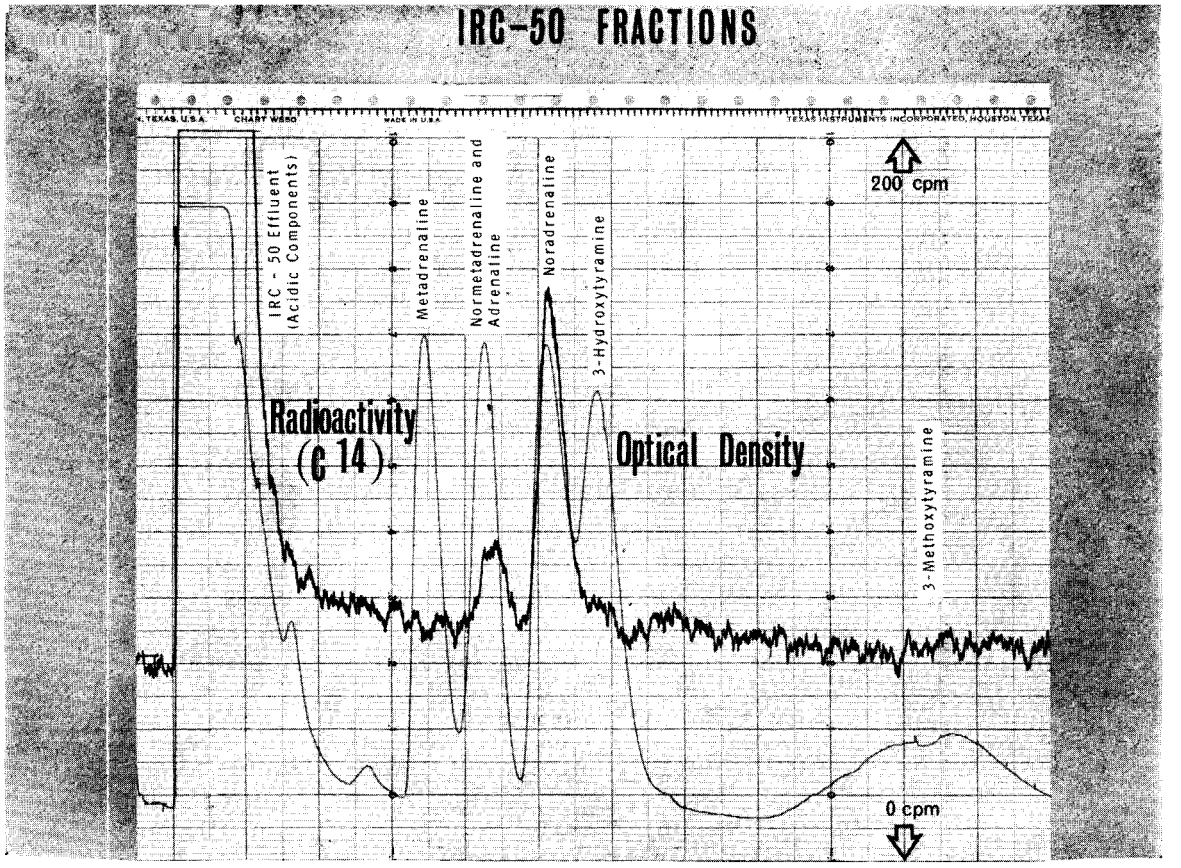

FIG. 2. 3-Hydroxytyramine-2C^{14} infusion. A representative tracing of the basic metabolites as they are eluted from the IRC-50 column. This particular tracing was the 8–24 hr collection showing the recovery of a large amount of radioactive noradrenaline relative to the small amount of radioactive hydroxytyramine.

TABLE 1. EXCRETION PATTERN OF THE METABOLITES AND BIOSYNTHETIC PRODUCTS OF I.V. INFUSED HYDROXYTYRAMINE-2-^{14}C

Period of urine collection	Per cent of the infused dose recovered	Basic metabolites—IRC-50 fractions						Acidic metabolites—Dowex-1 fractions																
		Adr	Normet	Nor	HT	Unknown	MT	Dowex-1 Effluent	(MHPE & HHPE)	MT conjugate	Nor & NM conjugates		HT Conjugate	P5B Unknown	P6 Unknown	MOMA	HVA	DOMA	P11 Unknown	DOPAC	P12 Unknown	(MHPE & MHPG conjugates)	DOPAC Conjugate	MOMA Conjugate	HVA Conjugate	P17 Unknown
Infusion period	38·9 ± 3·6		0·08 0·2 ± 0·1	1·13 2·9 ± 0·3	3·15 8·1 ± 1·5	0·43 1·1 ± 0·7	trace	0·66 1·7 ± 0·2	0·39 1·0 ± 0·4	1·40 3·6 ± 1·1	0·72 1·8 ± 0·5	1·07 2·8 ± 0·7	0·39 1·0 ± 0·3	1·13 2·9 ± 0·7	0·74 1·9 ± 0·7	0·90 2·3 ± 0·4	18·59 47·8 ± 2·9	trace	0·74 1·9 ± 1·0	0·78 2·0 ± 1·2	0·55 1·4 ± 0·5	1·17 3·0 ± 1·2	1·52 3·9 ± 0·7	0·55 1·4 ± 0·4	2·06 5·3 ± 2·0	1·67 4·3 ± 1·0
Post infusion 0–2 hr	16·5 ± 2·1		0·05 0·3 ± 0·1	0·15 0·9 ± 0·3	0·15 0·9 ± 0·4	0·03 0·2 ± 0·1	trace	0·17 1·0 ± 0·2	0·08 0·5 ± 0·3	1·32 8·0 ± 2·3	0·53 3·2 ± 0·5	1·03 6·3 ± 0·1	0·41 2·5 ± 0·5	trace	0·41 2·5 ± 1·1	1·07 6·5 ± 1·0	5·99 36·3 ± 4·2	0·25 1·5 ± 0·5	0·68 4·1 ± 1·0	0·66 4·0 ± 0·6	0·25 1·5 ± 1·3	0·46 2·8 ± 1·0	1·19 7·2 ± 2·2	0·40 2·4 ± 1·0	1·06 6·4 ± 2·1	0·99 6·0 ± 1·5
2–4 hr	9·6 ± 1·5		0·03 0·3 ± 0·1	0·06 0·6 ± 0·2	0·02 0·2 ± 0·1	trace	trace	0·09 0·9 ± 0·2	0·04 0·4 ± 0·2	1·00 10·4 ± 1·4	0·42 4·4 ± 0·4	0·75 7·8 ± 0·7	0·16 1·7 ± 0·5	trace	0·41 4·3 ± 1·3	1·13 11·8 ± 0·8	1·96 20·4 ± 1·6	0·15 1·6 ± 0·4	0·14 1·5 ± 0·4	0·42 4·4 ± 1·0	0·13 1·3 ± 0·4	0·56 5·8 ± 1·5	0·83 8·6 ± 1·9	0·37 3·8 ± 0·9	0·58 6·0 ± 0·9	0·48 5·0 ± 2·0
4–8 hr	9·1 ± 0·5		0·04 0·4 ± 0·1	0·06 0·7 ± 0·1	0·02 0·2 ± 0·1	trace	trace	0·07 0·8 ± 0·3	0·06 0·6 ± 0·2	1·30 14·3 ± 2·0	0·61 4·5 ± 0·9	0·76 8·4 ± 1·8	0·26 2·8 ± 0·3	trace	0·69 7·6 ± 0·5	1·44 15·8 ± 1·5	0·59 6·5 ± 1·4	0·11 1·2 ± 0·2	0·18 2·0 ± 0·7	0·16 1·7 ± 0·5	0·18 2·0 ± 0·6	0·65 7·1 ± 0·9	0·82 9·0 ± 1·4	0·34 3·7 ± 1·0	0·44 4·8 ± 1·8	0·60 6·6 ± 2·0
8–24 hr	13·5 ± 2·1		0·04 0·3 ± 0·1'	0·10 0·7 ± 0·1	0·01 0·1 ± 0·1	trace	trace	0·11 0·8 ± 0·3	0·12 0·9 ± 0·3	1·28 9·5 ± 2·1	0·43 3·2 ± 0·9	0·74 5·5 ± 1·6	0·31 2·3 ± 0·8	trace	1·62 12·0 ± 1·0	3·16 23·4 ± 3·7	0·37 2·7 ± 0·9	0·12 0·9 ± 0·4	0·28 2·1 ± 0·4	trace	0·34 2·5 ± 0·7	1·40 10·4 ± 1·6	1·50 11·1 ± 3·0	0·46 3·4 ± 0·3	0·64 4·7 ± 0·3	0·51 3·8 ± 1·1
24–48 hr	5·6 ± 1·0		0·03 0·5 ± 0·1	0·03 0·5 ± 0·1	trace	trace	trace	0·04 0·7 ± 0·2	0·05 1·0 ± 0·3	0·34 6·1 ± 2·1	0·07 1·2 ± 0·2	0·26 4·6 ± 0·1	trace	trace	0·85 15·1 ± 3·0	2·01 35·9 ± 3·8	0·10 1·8 ± 0·4	trace	0·13 2·3 ± 0·2	trace	0·15 2·6 ± 1·0	0·65 11·6 ± 2·1	0·47 8·3 ± 1·2	0·16 2·8 ± 1·0	0·24 4·3 ± 1·8	0·12 2·2 ± 0·6
48–72 hr	2·2 ± 0·4		0·01 0·5 ± 0·1	0·01 0·5 ± 0·1	trace	trace	trace	0·03 1·3 ± 0·5	0·02 1·0 ± 0·3	0·07 3·2 ± 0·7	trace	0·09 3·9 ± 1·0	trace	trace	0·39 17·7 ± 2·3	0·86 39·0 ± 1·6	trace		0·06 2·6 ± 0·5	trace	0·06 2·8 ± 0·7	0·26 11·7 ± 1·3	0·15 6·6 ± 1·2	0·07 3·3 ± 1·0	0·06 2·5 ± 0·5	trace
72–96 hr	1·2 ± 0·3							0·03 2·3 ± 1·0	0·01 1·0 ± 0·5	0·03 2·5 ± 0·4	trace	0·04 3·7 ± 0·4	trace	trace	0·21 17·2 ± 3·0	0·51 42·1 ± 3·5			0·03 2·6 ± 0·5		0·04 2·9 ± 0·3	0·15 12·8 ± 3·4	0·10 8·2 ± 2·1	0·04 3·0 ± 1·8	0·02 1·9 ± 0·5	trace
96–120 hr	0·6 ± 0·2							0·02 2·8 ± 1·0		0·02 2·6 ± 1·0	trace	0·02 3·8 ± 1·1			0·12 20·1 ± 4·0	0·21 34·4 ± 4·6			0·02 3·0 ± 0·8		0·02 2·5 ± 0·6	0·08 13·6 ± 4·5	0·05 8·8 ± 3·0	0·02 2·5 ± 1·1	0·03 4·1 ± 0·6	
Infusion to 24 hr	87·6 ± 3·1	trace	0·23	1·49	3·35	0·46	trace	1·09	0·69	6·30	2·41	4·47	1·53	1·13	3·88	7·70	27·50	0·63	2·03	2·02	1·44	4·24	5·85	2·10	4·77	4·26
Infusion to 120 hr	97·2 ± 3·5	trace	0·27	1·53	3·35	0·46	trace	1·21	0·78	6·76	2·57	4·78	1·53	1·13	5·44	11·28	27·60	0·63	2·26	2·02	1·70	5·38	6·61	2·38	5·11	4·38

* Figures express percent of the radioactivity recovered in each collection period ± S.D.; above this is expressed the percent of the infused dose recovered. Adr., adrenaline; Normet., normetadrenaline; Nor., noradrenaline; HT, dopamine; MT, 3-methoxytyramine; (MHPE & HHPE), tentatively identified compounds 3-methoxy-4-hydroxyphenylethanol and 3,4 dihydroxyphenylethanol; P6, unknown compound; MOMA, 3-methoxy-4-hydroxymandelic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid; DOMA, 3,4 dihydroxymandelic acid; Dopac, 3,4 dihydroxyphenylacetic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol.

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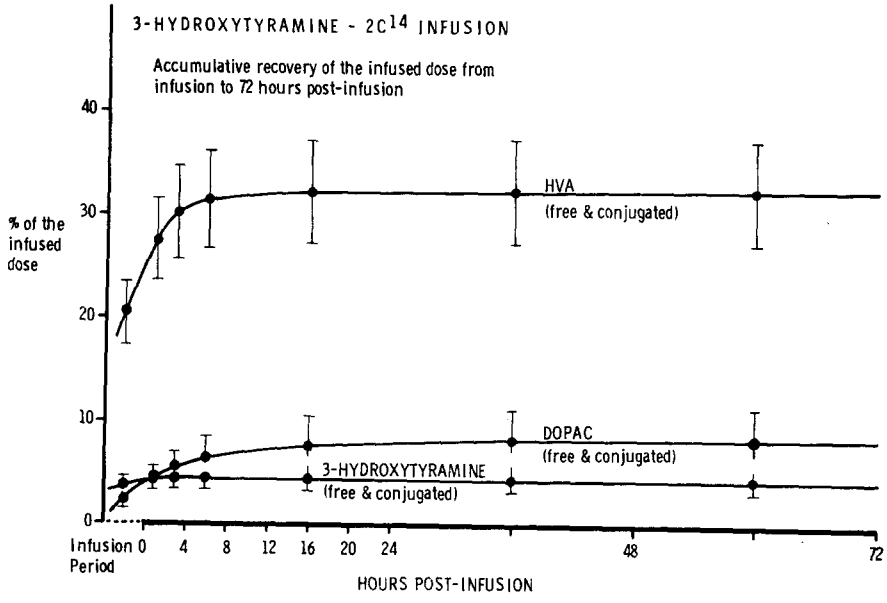

FIG. 3. A comparison of the accumulative recovery of the infused radioactivity as 3-methoxy-4-hydroxyphenylacetic acid (HVA, free and conjugated), 3,4-dihydroxyphenylacetic acid (DOPAC, free and conjugated) and 3-hydroxytyramine (free and conjugated) from the beginning of the infusion. to 72 hr post infusion.

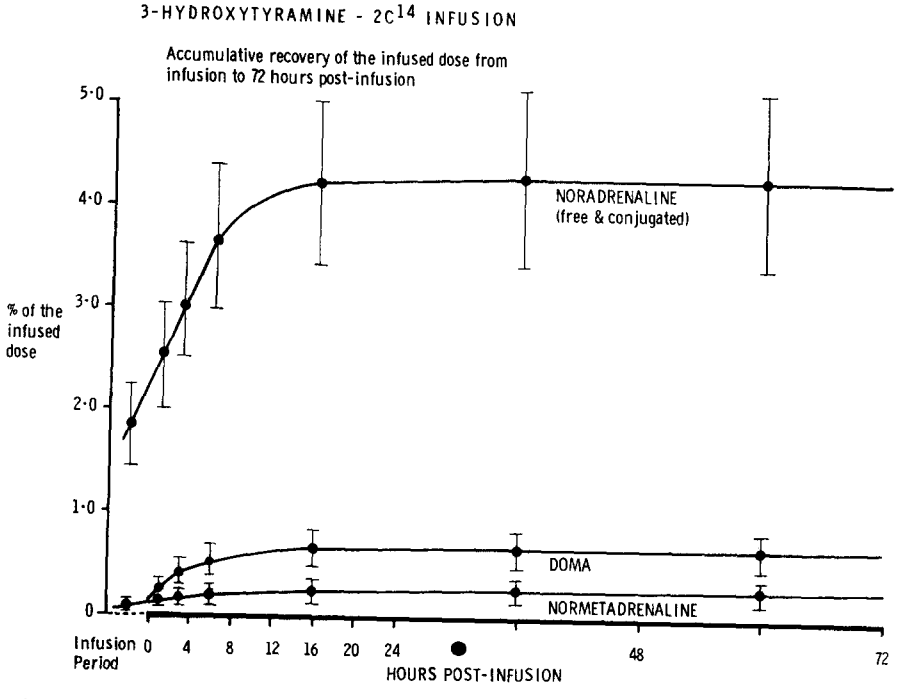

FIG. 4. A comparison of the accumulative recovery of the infused radioactivity as noradrenaline (free and conjugated), 3,4-dihydroxymandelic acid (DOMA) and normetadrenaline from the beginning of the infusion to 72 hr post infusion.

The unknown compounds indicated in Table 1 as P5B, P6, P11, P12 and P17 have not been definitely identified, but preliminary experiments seem to indicate that they are various degradation products of 3,4-dihydroxyphenylacetic acid. From these results it can be seen that much of the infused hydroxytyramine is rapidly converted to 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, and their respective conjugates (see Table 1). Fig. 3 shows this same relationship graphically as an accumulative recovery of the infused radioactivity. As can also be seen from Table 1, the excretion of radioactive noradrenaline is maximal during the infusion period and rapidly decreases until by 72 hr post infusion, none is found in the urine.

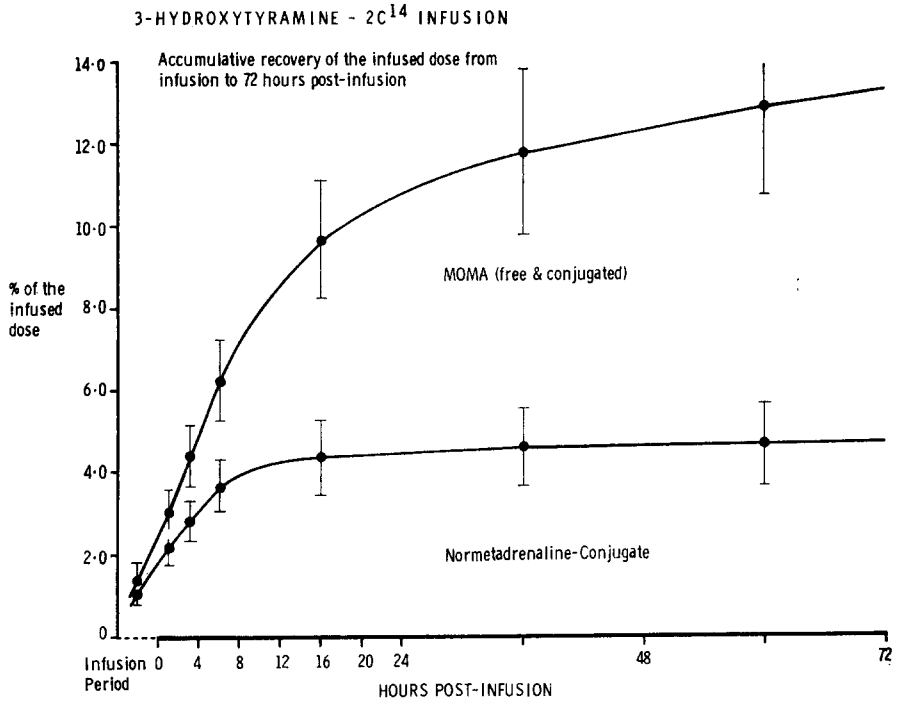

FIG. 5. A comparison of the accumulated recovery of the infused radioactivity as 3-methoxy-4-hydroxymandelic acid (free and conjugated) and normetadrenaline conjugate from the beginning of the infusion to 72 hr post infusion.

The normetadrenaline follows a somewhat similar pattern except that the quantity is considerably less. When this is illustrated as accumulative recovery (Fig. 4), very little change is noted after the 12 hr post infusion period. The conjugate of noradrenaline and normetadrenaline also reached the highest level during the infusion period and gradually decreased over the next 72–96 hr (see Table 1 and Figs. 4 and 5).

MOMA and DOMA have been clearly established as metabolic products of both adrenaline and noradrenaline;[46–49] they apparently are not derived directly from hydroxytyramine. The amount of the infused radioactivity recovered as MOMA recovered during each collection period gradually increases from the infusion period to peak at 8–24 hr post infusion and thereafter gradually decreases, but relative to the other metabolites the amount is still quite large even after 120 hr (Table 1).

Fig. 5 shows the accumulative recovery of radioactive MOMA from the infusion period to 72 hr post infusion. DOMA, on the other hand, is found in only trace amounts during the infusion period and was largest during the first 2 hr post infusion (Table 1). Although DOMA rapidly decreased so that only trace amounts could be found 48 hr post infusion, the quantities were always small, which could be explained on the basis of a rapid conversion to MOMA. The fact that MOMA was at its highest level 8–24 hr post infusion and is known to be the largest single catabolite of noradrenaline and adrenaline clearly indicates its importance as a metabolic product of endogenous noradrenaline.[46–49] The presence of DOMA in larger amounts than normetadrenaline during the first 24 hr post infusion might at first seem to indicate a greater relative importance of DOMA over normetadrenaline, but such a conclusion cannot be deduced, since the rate at which the primary and secondary metabolites are formed and excreted is not precisely known. As is known, the normetadrenaline is either converted by conjugation to normetadrenaline conjugate or deaminated.[47–51] The amount of normetadrenaline conjugate was largest in the infusion period and gradually decreased over the next 120 hr (Table 1).

The fact that no detectable amounts of adrenaline were found during the infusion period or in the periods immediately following infusion indicates that the amount of radioactive 3-hydroxytyramine incorporated into the adrenal medulla was very small as compared to that taken up by other tissues (Table 1).

DISCUSSION

3-Hydroxytyramine was first synthesized in 1910.[1] However, it was not shown to be present in mammalian tissue until 1950 when it was shown to occur in cardiac[2, 3] and adrenal tissue.[3, 4] It has now been shown to be present in various other tissues,[5–12] in human urine,[13–15] and is the immediate precursor in the biosynthesis of noradrenaline.[19–25] Recently, attention has been focused on its possible role as a neurohormone.[9, 12, 26–40] Hitherto, the precursor–product relationship of circulating hydroxytyramine has not been described.

In these experiments it is apparent that at least in the human approximately 75 per cent of the circulating infused hydroxytyramine was metabolized directly into metabolic products of hydroxytyramine, and approximately 25 per cent was synthesized into noradrenaline and metabolic products of noradrenaline (see Table 1; percent of infused dose recovered at the end of 120 hr). The fact that only trace amounts of adrenaline were detectable and that no metadrenaline was found would seem to indicate that almost all of the labeled hydroxytyramine which was not directly metabolized was incorporated into noradrenaline; very little was *N*-methylated to form adrenaline, even though the adrenals are capable of synthesizing adrenaline from hydroxytyramine via noradrenaline.[21]

The hydroxytyramine 2-C^{14} that was not directly metabolized appeared in urine either in the form of noradrenaline or metabolic products of noradrenaline (Table 1). This finding would seem to indicate that hydroxytyramine was taken up by the sympathetic system, synthesized (aliphatic hydroxylation) into endogenous noradrenaline 2-C^{14}, released and metabolized concomitantly with the naturally occurring neurohormone. In brief, these experiments avoid many of the pitfalls and errors imposed by infusing exogenous labeled noradrenaline where one measures the metabolic products of circulating exogenous noradrenaline rather than the naturally

occurring noradrenaline. Although most of the noradrenaline metabolic products have been previously identified,[46–62] these experiments provide more accurate and detailed information regarding the rate of appearance and excretion of endogenous noradrenaline and its metabolic products.

The primary metabolites of noradrenaline are DOMA and normetadrenaline; all other metabolites are secondarily formed. From the results of these experiments, it is apparent that initially both oxidative deamination and *O*-methylation take place; however, it is difficult to say which is the principal initial route of noradrenaline metabolism because the rate at which the secondary metabolites are formed cannot be determined precisely. The secondary metabolites which were separated and identified were normetadrenaline conjugate, 3-methoxy-4-hydroxymandelic acid and its 4–0 sulfate conjugate, and the conjugates of 3-methoxy-4-hydroxyphenylglycol and 3,4-dihydroxyphenylglycol. Other secondary metabolites such as 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid and their 4–0 sulfate conjugates were also separated and identified, but in these experiments their origin is principally from hydroxytyramine.

Approximately 75 per cent of the infused hydroxytyramine 2-C^{14} is directly metabolized into hydroxytyramine-related metabolic products (see Table 1 and Fig. 3). The 75 per cent recovery of metabolic products compares quite favorably with the rat experiments of Goldstein *et al.*[63] The metabolic products in the human are 3-methoxytyramine, 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylethanol, 3,4-dihydroxyphenylethanol and their respective conjugates, as well as the conjugate of hydroxytyramine and several unknowns. Of these, 3-methoxy-4-hydroxyphenylacetic acid is by far the largest in amount, since it represents 18·6 per cent of the infused dose recovered during the infusion period (see Table 1). This indicates a rapid conversion of hydroxytyramine to 3-methoxy-4-hydroxyphenylacetic acid or this is to say circulating hydroxytyramine is rapidly *O*-methylated and deaminated. The conjugates of 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid and 3-methoxytyramine are found in approximately the same amount. Although 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid are also formed from noradrenaline,[64] the amount formed from noradrenaline is small compared with that formed from hydroxytyramine.[45]

REFERENCES

1. C. MANNICH and W. JACOBSOHN, *Ber. dt. chem. Ges.* **1**, 189 (1910).
2. McC. GOODALL, *Acta chem. scand.* **4**, 550 (1950).
3. McC. GOODALL, *Acta physiol. scand.* **24**, *suppl.* 85 (1951).
4. McC. GOODALL, *Nature, Lond.* **166**, 738 (1950).
5. H. J. SCHÜMANN, *Arch. exp. Path. Pharmak.* **227**, 566 (1956).
6. U. S. von EULER and F. LISHAJKO, *Acta physiol. pharm. néerl.* **6**, 295 (1957).
7. H. DENGLER, *Arch. exp. Path. Pharmak.* **231**, 373 (1957).
8. U. S. von EULER and F. LISHAJKO, *Acta Physiol. scand.* **42**, 333 (1958).
9. H. J. SCHÜMANN, *Arch. exp. Path. Pharmak.* **236**, 474 (1959).
10. E. T. ANGELAKOS, K. FUXE and M. L. TORCHIANA, *Acta Physiol. scand.* **59**, 184 (1963).
11. Å. BERTLER, B. FALCK, C. G. GOTTFRIES, L. LJUNDGREN and E. ROSENGREN, *Acta pharmac. tox.* **21**, 283 (1964).
12. R. LAVERTY and D. F. SHARMAN, *Br. J. Pharmac. Chemother.* **24**, 538 (1965).
13. U. S. von EULER, U. HAMBERG and S. HELLNER, *Biochem. J.* **49**, 655 (1951).

14. B. D. DRUJAN, T. L. SOURKES, D. S. LAYNE and G. F. MURPHY, *Can. J. Biochem. physiol.* **37**, 1153 (1959).
15. JAN HÄGGENDAL and BENGT WERDINIUS, *Acta physiol. scand.* **66**, 223 (1966).
16. E. ÖSTLUND, *Acta physiol. scand.* **31**, *suppl.* 112 (1954).
17. S. UDENFRIEND, W. LOVENBERG and A. SJOERDSMA, *Archs Biochem. Biophys.* **85**, 487 (1959).
18. M. GUGGENHEIM, *Die Biogenen Amine*. Karger, Basel (1951).
19. H. BLASCHKO, *J. Physiol., Lond.* **13**, 96 (1939).
20. S. GURIN and A. M. DELLUVA, *J. biol. Chem.* **170**, 545 (1947).
21. McC. GOODALL and N. KIRSCHNER, *J. biol. Chem.* **226**, 213 (1957).
22. McC. GOODALL and N. KIRSCHNER, *Circulation* **17**, 366 (1958).
23. S. UDENFRIEND and J. B. WYNGARDEN, *Biochem. biophys. Acta* **20**, 48 (1956).
24. J. PELLERIN and A. D'IORIO, *Can. J. Biochem. Physiol.* **35**, 151 (1957).
25. S. SPECTOR, A. SJOERDSMA, P. ZALTMAN-NIRENBERG, M. LEVITT and S. UDENFRIEND, *Science* **139**, 1299 (1963).
26. A. CARLSSON, M. LINDQVIST, T. MAGNUSSON and B. WALDECK, *Science* **127**, 471 (1958).
27. I. SANO, T. GAMO, Y. KAKIMOTO, K. TANIGUCHI, M. TAKESADA and K. NISHINUMA, *Biochim. biophys. Acta* **32** 586 (1959).
28. K. FUXE, *Evidence for the Existence of Monoamine Neurons in the Central Nervous System*. Almqvist & Wiksells, Boktryckeri Aktiebolag, Uppsala (1965).
29. H. EHRINGER and O. HORNYKIEWICZ, *Klin. Wschr.* **38**, 1236 (1960).
30. Å. BERTLER, *Acta physiol. scand.* **51**, 97 (1961).
31. R. G. WIEGAND and J. E. PERRY, *Biochem. Pharmac.* **7**, 181 (1961).
32. P. HOLTZ, K. STOCK and E. WESTERMANN, *Arch. exp. Path. Pharmak.* **246**, 133 (1963).
33. O. HORNYKIEWICZ, *Wien. klin. Wschr.* **75**, 309 (1963).
34. J. HÄGGENDAL and T. MALMFORS, *Acta physiol. scand.* **59**, 295 (1963).
35. Å. BERTLER, *Biochemical and Neurophysiological Correlations of Centrally Acting Drugs*. Pergamon Press, Oxford, (1964).
36. N.-E. ANDÉN, A. CARLSSON, A. DAHLSTRÖM, K. FUXE, N.-Å. HILLARP and K. LARSSON, *Life Sci.* **3**, 523 (1964).
37. L. J. POIRIER, and T. L. SOURKES, *Brain* **88**, 181 (1965)
38. C. HEYMANS and A. DE SCHAEPDRYVER, *Scr. varia pont. Acad. Sci. Scripta Varia* **30**, 761 (1965).
39. O. HORNYKIEWICZ, *Pharmac. Rev.* **18**, 925 (1966).
40. L. AUSTIN, B. G. LIVETT and I. W. CHUBB *Life Sci.* **6**, part 1, 97 (1967).
41. A. BARBEAU, G. F. MURPHY and T. L. SOURKES, *Science* **133** 1706 (1961).
42. A. BARBEAU, T. L. SOURKES and G. F. MURPHY, *Monoamines et Systéme Nerveaux Centrale*· Georg, Genéve and Masson, Paris (1962).
43. H. BERNHEIMER, W. BIRKMAYER and O. HORNYKIEWICZ, *Klin. Wschr.* **41**, 465 (1963).
44. O. HORNYKIEWICZ, *Dt. med. Wschr.* **36**, 1807 (1962).
45. K. N. F. SHAW, *J. biol. Chem.* **226**, 255 (1957).
46. M. D. ARMSTRONG, A. MCMILLAN and K. N. F. SHAW, *Biochim. biophys. Acta* **25**, 422 (1957).
47. McC. GOODALL and L. ROSEN, *J. clin. Invest.* **42**, 1578 (1963).
48. McC. GOODALL and H. ALTON, *Biochem. Pharmac.* **14**, 1595 (1965).
49. J. AXELROD, *Physiol Rev.* **39**, 751 (1959).
50. E. H. LABROSSE, J. AXELROD and S. S. KETY, *Science* **128**, 593 (1958).
51. J. AXELROD, I. J. KOPIN and J. D. MANN, *Biochim. biophys. Acta* **36**, 576 (1959).
52. W. G. CLARKE, R. I. AKAWIE, R. S. POGRUND and T. A. GEISSMAN, *J. Pharmac. exp. Ther.* **101**, 6 (1951).
53. L. ROSEN and McC. GOODALL, *Proc. Soc. exp. Biol. Med.* **110**, 767 (1962).
54. J. R. CROUT, C. R. CREVELING and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **132**, 269 (1961).
55. E. IISALO, *Acta pharmac. tox.* **19**, *suppl.* 1 (1962).
56. P. IMHOF, F. HEDIGER and H. KASER, *Helv. med. Acta* **29**, 183 (1962).
57. W. DIRSCHERL, H. THOMAS and H. SCHRIEFERS, *Acta endocr. Copenh.* **39**, 385 (1962).
58. L. L. IVERSEN, *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*. Cambridge University Press (1967).
59. J. AXELROD and R. TOMCHICK, *J. biol. Chem.* **233**, 702 (1958).
60. E. H. LABROSSE and J. D. MANN, *Nature, Lond.* **185**, 50 (1960).

61. K. YOSHINAGA, C. ITOH, N. ISHIDA, T. SATO and Y. WADA, *Tohoku J. exp. Med.* **74**, 105 (1961).
62. McC. GOODALL, H. ALTON and L. ROSEN, *Biochem. Pharmac.* **13**, 703 (1964).
63. M. GOLDSTEIN, A. J. FRIEDHOFF and C. SIMMONS, *Biochem. biophys. Acta* **33**, 572 (1959).
64. McC. GOODALL and H. ALTON, *Tex. Rep. Biol. Med.* in press.