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METABOLISM OF 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC) IN THE HUMAN*

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Abstract—Five subjects were infused i.v. with 75 μc of 3,4-dihydroxyphenylacetic acid-2-^{14}C (DOPAC) and urine collected at various intervals for 24 hr. The radioactive metabolic products in the urine were separated and identified. The results indicate that DOPAC is rapidly *O*-methylated to 3-methoxy-4-hydroxyphenylacetic acid (HVA). Within 24 hr after an i.v. infusion of DOPAC-2-^{14}C 40 per cent of the radioactivity was recovered in the urine as HVA. Whereas HVA was the principal metabolic product, approximately 5 per cent of the DOPAC was metabolized to the conjugate of HVA. 3,4-Dihydroxybenzoic acid (DOBA) and trace amounts of vanillic acid were found, plus several unidentified metabolites.

THE PRESENCE of 3,4-dihydroxyphenylacetic acid (DOPAC) in the urine was first demonstrated by von Euler[1] and later confirmed by others.[2–4] DOPAC was shown by Goodall and Alton[5, 6] to be a minor metabolite of both adrenaline (epinephrine) and noradrenaline (norepinephrine). Further, it has been shown to be a major metabolite of both 3,4-dihydroxyphenylalanine (DOPA)[7–10] and 3-hydroxytyramine (dopamine).[11–13] Also, it has been found in tissues;[14, 15] of particular significance is its occurrence in the corpus striatum,[16, 17] a structure rich in dopamine.[18–23]

Werdinius[15] showed that DOPAC was rapidly eliminated from the blood and excreted in the urine. Further, it is well established that DOPAC is *O*-methylated to 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA). However, little is known about the other metabolites of DOPAC or how the human metabolizes circulating DOPAC.

METHODS

General. A total of 5 normal, healthy males between 25–35 yrs were infused i.v. with 75 μc of 3,4-dihydroxyphenylacetic acid-2-^{14}C (DOPAC-2-^{14}C)† (sp.act. 5 mc/m-mole). Three subjects were infused at a steady rate for a period of 1 hr with DOPAC-2-^{14}C mixed in 250 ml of physiological saline. The urine was collected via an indwelling catheter at hourly intervals for 6 hr and then at 12 and 24 hr.

The second group of 2 subjects was injected over a 1-min period via the antecubital

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† Source of material: Dowex-1-X2, 200–400 mesh, Bio Rad Laboratories, Richmond, Calif.; 3,4-dihydroxyphenylacetic acid-2-^{14}C, Mallinckrodt, Nuclear Research Chemicals, Inc., Orlando, Fla.; Mylase P (from *Aspergillus oryzae*), Mann Research Laboratories, New York, N.Y.; Packard Instrument Corp., Downers Grove, Ill.

vein with 75 μc of DOPAC-2-^{14}C mixed in 10 ml of physiological saline. In this latter group the urine was collected via an indwelling catheter at 0–2, 2–5, 5–10 min and at 10 min intervals for 1 hr; then at 6, 12 and 24 hr. Each urine sample was stored at −20°C until assayed.

Procedure for separating, identifying and measuring the DOPAC-2-^{14}C metabolites. The method for separating, identifying and measuring DOPAC-2-^{14}C and its metabolites in urine is rather similar to that used in following the metabolites of hydroxytyramine, noradrenaline and 3,4-dihydroxymandelic acid.[11, 24, 25]

An aliquot of urine containing 100,000 dpm was placed on 1 × 35 cm column of Dowex-1-X2 acetate anion exchange resin. To the aliquot of urine was added carrier compounds of 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). The column was placed on an automatic fraction collection system and eluted by a variable gradient elution consisting of four series-connected chambers, the first of which 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 approx. 1 ml/min.

The eluate was passed through a quartz-flow cell (1·0 ml vol.) 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 monitored 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 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. Figure 1 represents a typical tracing obtained by this method from a 2–3-hr collection period following a 1-hr infusion of DOPAC-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 VA, HVA, DOPAC 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). The peaks containing the conjugated HVA or DOPAC were confirmed by hydrolysis of the conjugate in 2 N H_2SO_4, under nitrogen for 60 min at 100°, followed by extraction of the free acid into ether. The ether extract was then chromatographed in each of the previously described solvent systems.

RESULTS

The results of the 1-min and 1-hr infusion of DOPAC-2-^{14}C are summarized in Tables 1 and 2 and Figs. 2 and 3. The metabolic products of DOPAC are 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA), 3,4-dihydroxybenzoic acid (DOBA), the conjugate of HVA, and trace amounts of VA and the conjugate of

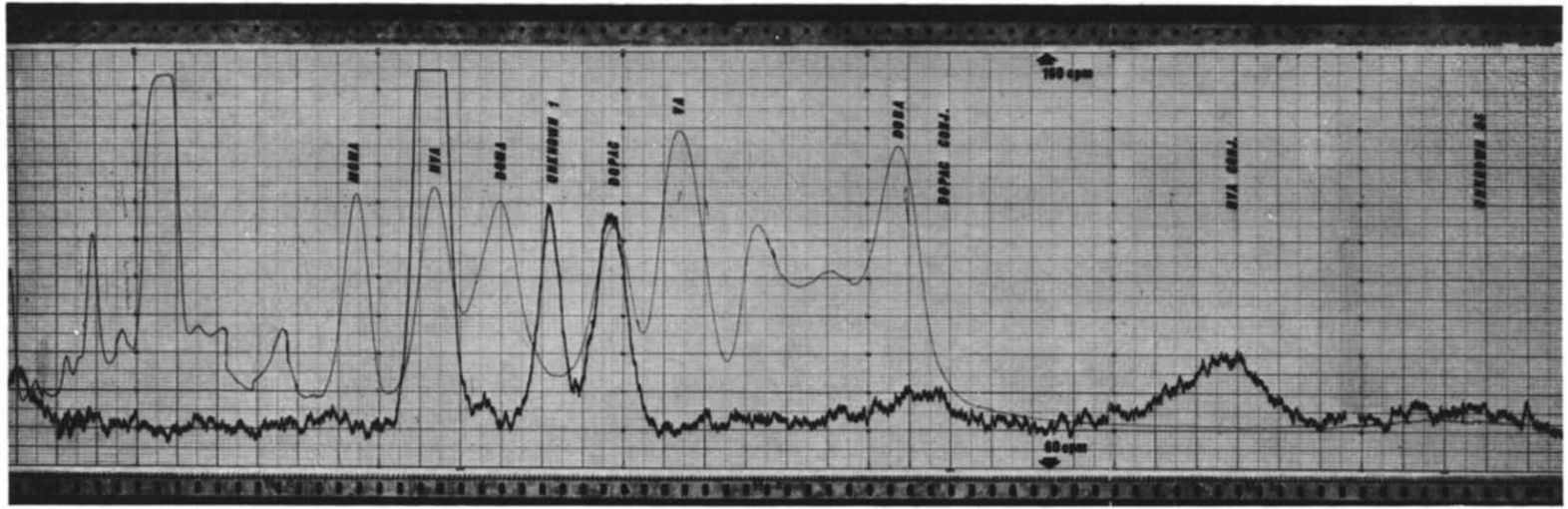

FIG. 1. Typical Dowex-1 elution pattern. MOMA, 3-methoxy-4-hydroxymandelic acid; HVA, homovanillic acid, 3-methoxy-4-hydroxyphenylacetic acid; DOMA, 3,4-dihydroxymandelic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VA, vanillic acid; DOBA, 3,4-dihydroxybenzoic acid; DOPAC conj., 3,4-dihydroxyphenylacetic acid conjugate; HVA conj., 3-methoxy-4-hydroxyphenylacetic acid conjugate.

TABLE 1. EXCRETION PATTERN OF 3,4-DIHYDROXYPHENYLACETIC ACID AND METABOLITES AFTER A 1-min. INJECTION

Period of urine collection	% of the infused dose recovered	Dowex-1 fractions*						
		HVA	Unk.-1	DOPAC	VA	DOBA	HVA conj.	D_3 unk.
0–2 min	trace	0·01 14·6 ± 1·4 0·86	trace 3·8 ± 0·7 trace	0·03 39·2 ± 3·0 3·05		0·01 16·2 ± 0·8 0·15	trace 4·1 ± 0·8 0·44	trace
2–5 min	5·5 ± 3·4	15·7 ± 1·6 3·31	0·6 ± 0·7 0·23	55·4 ± 0·3 10·66		9·2 ± 5·0 0·28	8·0 ± 3·8 0·15	trace 0·42
5–10 min	15·4 ± 1·8	21·5 ± 6·7 2·87	1·5 ± 1·6 0·17	69·2 ± 6·8 4·98		1·8 ± 0·3	1·0 ± 0·2 0·28	2·7 ± 0·4 0·78
10–20 min	9·4 ± 4·3	30·5 ± 6·1 4·13	1·8 ± 1·4 0·19	53·0 ± 6·0 2·19			3·0 ± 0·4 0·30	8·3 ± 0·8 0·77
20–30 min	8·0 ± 0·5	51·6 ± 7·5 4·05	2·4 ± 0·5 0·25	27·4 ± 11·0 0·91			3·8 ± 1·3 0·45	9·6 ± 1·0 1·02
30–40 min	7·2 ± 0·1	56·3 ± 6·1 3·46	3·5 ± 0·2 0·19	12·6 ± 5·9 0·75			6·3 ± 0·4 0·40	14·2 ± 2·4 0·80
40–50 min	5·7 ± 0·7	60·7 ± 7·5 3·32	3·4 ± 0·3 0·21	13·1 ± 5·4			7·0 ± 0·2 0·38	14·1 ± 3·9 0·75
50–60 min	5·0 ± 0·2	66·4 ± 4·8 16·19	4·2 ± 0·2 2·12	trace 3·60			7·6 ± 0·3 2·86	15·0 ± 3·0 3·27
1–6 hr	29·5 ± 8·4	54·9 ± 3·3 1·39	7·5 ± 2·2 0·40	12·2 ± 2·3 0·09			9·7 ± 0·5 0·41	11·1 ± 2·7 0·19
6–12 hr	2·6 ± 0·9	53·5 ± 1·0 0·21	15·4 ± 1·4 0·28	3·6 ± 0·5			15·7 ± 3·0 0·13	7·5 ± 2·6
12–24 hr	0·7 ± 0·4	30·2 ± 1·2	39·9 ± 1·8	trace			18·6 ± 4·1	trace
Total 24 hr	89·1	39·8	4·2	26·3	trace	0·8	5·8	8·0

* Dowex-1 fractions are expressed as: radioactivity of fraction as per cent of total radioactivity excreted during each collection period (second line) ± S.D.; radioactivity recovered as per cent of total radioactivity infused (first line). HVA, homovanillic acid, 3-methoxy-4-hydroxyphenylacetic acid; Unk., unknown; DOPAC, 3,4-dihydroxyphenylacetic acid; VA, vanillic acid, 3-methoxy-4-hydroxybenzoic acid; DOBA, 3,4-dihydroxybenzoic acid; DOPAC conj., 3,4-dihydroxyphenylacetic acid conjugate; HVA conj., 3-methoxy-4-hydroxyphenylacetic acid conjugate.

TABLE 2. EXCRETION PATTERN OF 3,4-DIHYDROXYPHENYLACETIC ACID AND METABOLITES AFTER A 1-hr INFUSION

Period of urine collection	% of the infused dose recovered	Dowex-1 fractions*						
		HVA	Unk.-1	DOPAC	VA	DOBA	HVA conj.	D_3 unk.
Infusion	47·0 ± 4·2	20·54 43·7 ± 5·1 6·65	trace 0·43	25·47 54·2 ± 1·5 2·99		0·89 1·9 ± 0·8 0·83	1·27 2·7 ± 0·5 0·81	3·05 6·5 ± 0·4 1·39
0–1 hr	11·9 ± 0·2	55·9 ± 4·7 6·10	3·6 ± 0·5 0·58	25·1 ± 1·7 1·28		7·0 ± 2·2 0·73	6·8 ± 1·4 0·93	11·7 ± 0·3 1·31
1–2 hr	10·0 ± 2·8	61·0 ± 6·8 2·95	5·8 ± 0·5 0·38	12·8 ± 1·1 0·40		7·3 ± 4·0 0·24	9·3 ± 1·7 0·46	13·1 ± 0·9 0·68
2–3 hr	4·7 ± 1·0	62·8 ± 6·9 2·31	8·2 ± 2·0 0·33	8·5 ± 1·1 0·18		5·1 ± 0·1 0·09	9·9 ± 3·8 0·46	14·4 ± 1·9 0·38
3–4 hr	3·6 ± 0·6	64·3 ± 3·7 0·55	9·1 ± 1·1 0·13	4·9 ± 1·1 0·03		2·4 ± 0·1 0·03	12·7 ± 2·1 0·12	10·7 ± 1·3 0·09
4–5 hr	1·0 ± 0·4	55·4 ± 5·0 0·27	12·7 ± 0·9 0·08	3·2 ± 0·6 0·01		2·7 ± 0·2	12·5 ± 2·0 0·07	9·1 ± 3·8 0·04
5–6 hr	0·5 ± 0·2	53·5 ± 8·5 0·76	15·6 ± 2·9 0·24	2·6 ± 2·7 0·02			15·1 ± 2·1 0·16	7·6 ± 3·1 0·10
6–12 hr	1·4 ± 0·5	54·5 ± 17·9	17·1 ± 5·3	1·4 ± 1·4			11·7 ± 3·1	7·1 ± 3·0
12–24 hr	0·5 ± 0·2							
Total 24 hr	80·6	40·1	2·1	30·4	trace	2·8	4·3	7·0

* Dowex 1 fractions are expressed as: radioactivity of fraction as per cent of total radioactivity excreted during each collection period (second line) ± S.D.; radioactivity recovered as per cent of total radioactivity infused (first line). HVA, homovanillic acid, 3-methoxy-4-hydroxyphenylacetic acid; Unk., unknown; DOPAC, 3,4-dihydroxyphenylacetic acid; VA, vanillic acid, 3-methoxy-4-hydroxybenzoic acid; DOBA, 3,4-dihydroxybenzoic acid; DOPAC conj., 3,4-dihydroxyphenylacetic acid conjugate; HVA conj., 3-methoxy-4-hydroxyphenylacetic acid conjugate.

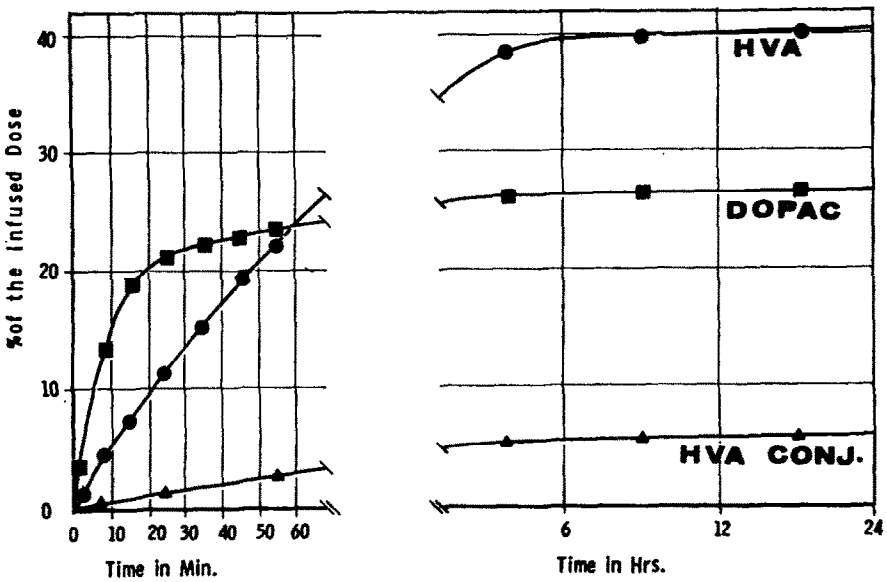

FIG. 2. Accumulative recovery of HVA, HVA conjugate and DOPAC after a 1-min injection of DOPAC-2-^{14}C.

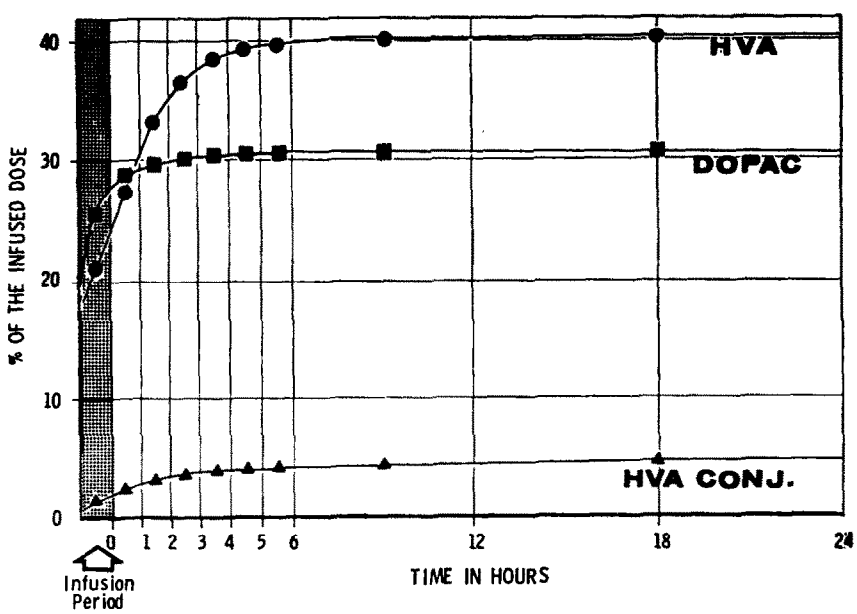

FIG. 3. Accumulative recovery of HVA, HVA conjugate and DOPAC after a 1-hr infusion of DOPAC-2-^{14}C.

DOPAC. HVA, the major metabolite of infused DOPAC, represented about 40 per cent of the radioactivity recovered in the 24-hr urine collection from both the 1-min and the 1-hr infusion. (See Figs. 2 and 3.)

From Table 1 it can be seen that most of the radioactivity recovered in the urine during the infusion period and for 20 min following the 1-min injection is represented by DOPAC-2-^{14}C. This decreases slowly over the following 12-hr period to trace amounts. Concomitantly, there is a rapid increase in the total radioactivity recovered as HVA, such that 30–40 min following a 1-min injection or immediately following the 1-hr infusion, the radioactivity recovered in the urine as HVA represented more than 50 per cent of the total radioactivity. There is also a gradual increase in the radioactivity recovered as the conjugate of 3-methoxy-4-hydroxyphenylacetic acid, such that during the 6–12-hr period this compound represented over 15 per cent of the urinary metabolites. The conjugate of DOPAC was found only in trace amounts. These conjugates appear to be the sulfate since they are hydrolyzed when incubated with the sulfatase enzyme, Mylase P.

DOBA and unknown 1 are consistently present and gradually increase following the 1-hr infusion of DOPAC-2-^{14}C, such that DOBA represented 7·3 per cent of the radioactivity recovered during the 1–2-hr period and unknown 1 represented 17·1 per cent of the radioactivity recovered during the 6–12-hr period. (See Table 2.) VA was recovered in trace to small amounts throughout the collection period, as were several unknown peaks.

DISCUSSION

DOPAC is a minor metabolite of adrenaline and noradrenaline[5, 6] and a major metabolite of DOPA and dopamine.[7–13] Its origin from dopamine seems particularly significant since in recent years much attention has been focused on dopamine as a possible neurohormone with transmitter functions in the central nervous system.[11, 19, 20, 22, 23, 26–36] The fact that both dopamine and DOPAC have been found in relatively high concentration in the same structures, i.e. corpus striatum, emphasizes the possible importance of DOPAC as a metabolite.

The fact that DOPAC, as well as HVA, is found in the nervous system suggests that a direct deamination may very well be the important step in the metabolism of DOPA and dopamine and that the DOPAC formed is then *O*-methylated to HVA. The latter step could occur both locally and distantly. However, from these studies it is apparent that circulating DOPAC is readily *O*-methylated to homovanillic acid.

Whereas, the principal metabolite of circulating DOPAC is HVA, these studies clearly indicate that HVA is not the only metabolic product. Approximately 5 per cent of the infused DOPAC appears as conjugated HVA. Further, DOBA is formed as well as trace amounts of VA plus several unidentified metabolites.

REFERENCES

1. CHR. VON EULER, U. S. VON EULER and I. FLODING, *Acta physiol. scand.* **33**, suppl. 118 32 (1955).
2. M. D. ARMSTRONG, K. N. F. SHAW and P. E. WALL, *J. biol. Chem.* **218**, 293 (1956).
3. H. WEIL-MALHERBE and A. D. BONE, *J. clin. Path.* **10**, 138 (1957).
4 B. D. DRUJAN, N. ALVAREZ and J. M. DIAZ BORGES, *Analyt. Biochem.* **15**
5. McC. GOODALL and H. ALTON, *Biochem. Pharmac.* **14**, 1595 (1965).
6. McC. GOODALL and H. ALTON, *Tex. Rep. Biol. Med.* **26**, 107 (1968).
7. K. H. LIN, *J. med. Ass. Formosa* **47**, 85 (1948).

8. J. PELLERIN and A. D'IORIO, *Can. J. Biochem. Physiol.* **33**, 1055 (1955).
9. F. DE EDS, A. N. BOOTH and F. T. JONES, *J. biol. Chem.* **225**, 615 (1957).
10. K. N. F. SHAW, A. MCMILLAN and M. D. ARMSTRONG, *J. biol. Chem.* **226**, 255 (1957).
11. MCC. GOODALL and H. ALTON, *Biochem. Pharmac.* **17**, 905 (1968).
12. M. GOLDSTEIN, A. J. FRIEDHOFF and C. SIMMONS, *Biochim. biophys. Acta* **33**, 572 (1959).
13. C. M. WILLIAMS, A. A. BABUSCIO and R. WATSON, *Am. J. Physiol.* **199**, 722 (1960).
14. U. S. VON EULER, I. FLODING and F. LISHAJKO, *Acta Soc. Med. upsal.* **64**, 217 (1959).
15. B. WERDINIUS, *Acta pharmac. tox.* **25**, 9 (1967).
16. E. ROSENGREN, *Acta physiol. scand.* **49**, 370 (1960).
17. N. -E. ANDEN, B. E. ROOS and B. WERCINIUS, *Life Sci.* **2**, 319 (1963a).
18. K. A. MONTAGU, *Nature, Lond.* **180**, 244 (1957).
19. A. CARLSSON, M. LINDQUIST, T. MAGNUSSON and B. WALDECK, *Science, N.Y.* **127**, 471 (1958).
20. I. SANO, T. GAMO, Y. KAKIMOTO, K. TANIGUCHI, M. TAKESADA and K. NISHINUMA, *Biochim. biophys. Acta* **32**, 586 (1959).
21. Å. BERTLER and A. ROSENGREN, *Experentia* **15**, 10 (1959a).
22. Å. BERTLER, in *Biochemical and Neurophysiological Correlations of Centrally Acting Drugs* (Eds. E. TRABUCCHI, R. PAOLETTI and N. CANAL), **2**, 51 Pergamon, Oxford (1964).
23. O. HORNYKIEWICZ, *W. Klin. Wschr.* **75** 309 (1963).
24. MCC. GOODALL, W. R. HARLAN, JR. and H. ALTON, *Circulation* **36**, 489 (1968).
25. MCC. GOODALL and H. ALTON, *Biochem. Pharmac.* **18**, 295 (1969).
26. H. J. SCHÜMANN, *exp. Path Pharmak.* **236**, 474 (1959).
27. R. LAVERTY and D. F. SHARMAN, *Br. J. Pharmac.* **24**, 538 (1965).
28. A. CARLSSON, *Pharmac. Rev.* **11**, 490 (1959).
29. H. EHRINGER and O. HORNYKIEWICZ, *Klin. Wschr.* **38**, 1236 (1960).
30. R. G. WIEGAND and J. E. PERRY, *Biochem. Pharmac.* **7**, 181 (1961).
31. P. HOLTZ, K. STOCK and E. WESTERMANN, *Arch. exp. Path. Pharmak.* **246**, 133 (1963).
32. J. HAGGENDAL and T. MALMFORS, *Acta physiol. scand.* **59**, 295 (1963).
33. N. E. ANDEN, A. CARLSSON, A. DAHLSTRÖM, K. FUXE, N. Å. HILLARP and K. LARSSON, *Life Sci.* **3**, 523 (1964).
34. L. J. POIRIER and T. L. SOURKES, *Brain* **88**, 181 (1965).
35. C. HEYMANS and A. DE SCHAEPDRYVER, *Scr. varia, Pontificiae Acad. Sci.* 761 (1965)
36. O. HORNYKIEWICZ, *Pharmac. Rev.* **18**, 925 (1966).