Br. J. Pharmac. Chemother. (1968), 33, 457–471.

SOME STUDIES OF THE EFFECTS OF CHLORPROMAZINE, RESERPINE AND DIHYDROXYPHENYLALANINE ON THE CONCENTRATIONS OF HOMOVANILLIC ACID, 3,4-DIHYDROXYPHENYLACETIC ACID AND 5-HYDROXYINDOL-3-YLACETIC ACID IN VENTRICULAR CEREBROSPINAL FLUID OF THE DOG USING THE TECHNIQUE OF SERIAL SAMPLING OF THE CEREBROSPINAL FLUID

BY

H. C. GULDBERG* AND CELIA M. YATES

From the M.R.C. Unit for Research in Brain Metabolism, Department of Pharmacology, University of Edinburgh Medical School, Edinburgh

(*Received January* 2, 1968)

The development and application of a technique of serial sampling of lateral ventricular cerebrospinal fluid (C.S.F.) in the dog to a study of the concentrations of homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the fluid have been described (Ashcroft, Crawford, Dow & Guldberg, 1968). Using these methods, the effects of L-dopa (3,4-dihydroxyphenylalanine), chlorpromazine and reserpine on the concentrations of the same phenolic acids in the C.S.F. have been investigated in order to determine whether the changes in the acid concentrations were comparable with those reported to occur in the brain.

An increase in the brain concentrations of HVA and DOPAC following administration of L-dopa to animals has been reported (Rosengren, 1960; Carlsson & Hillarp, 1962; Andén, Roos & Werdinius, 1963; Laverty & Sharman, 1965a; Juorio, Sharman & Trajkov, 1966), the increase of DOPAC concentration preceding that of the HVA concentration in the rabbit corpus striatum (Andén *et al.*, 1963). Chlorpromazine has been found to increase the concentration of HVA in the basal ganglia of various species (Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965a; Juorio *et al.*, 1966; Sharman, 1966). Andén *et al.* (1964) also demonstrated a rise in the concentration of DOPAC after chlorpromazine in the rabbit corpus striatum, the maximum rise occurring at about the same time as that of the HVA concentration. Chlorpromazine did not significantly affect the concentration of 5-HIAA in the brain (Gey & Pletscher, 1964). Reserpine has been found to increase the concentration of 5-HIAA (Ashcroft & Sharman,

* Present address: Department of Pharmacology, University of Bergen, Bergen, Norway.

1962 ; Roos & Werdinius, 1962 ; Laverty & Sharman, 1965a) and DOPAC (Andén *et al.*, 1964) as well as that of HVA (Andén *et al.*, 1964 ; Laverty & Sharman, 1965a ; Juorio *et al.*, 1966 ; Sharman, 1966). The trend in the effect of reserpine treatment on the dopamine metabolites was similar to that of dopa administration, in that the increase in the DOPAC concentration preceded that of the HVA (Andén *et al.*, 1964).

METHODS

Animals

Beagle dogs, of about 10 kg body weight, with chronically implanted guide-tubes for the repeated sampling of lateral ventricular C.S.F. were used as described in the preceding paper (Ashcroft *et al.*, 1968). All the experiments were performed, under thiopentone anaesthesia, on animals which had been starved since noon on the previous day. Endotracheal intubation was performed in the experiments in which dopa was administered because this substance invariably caused some vomiting which persisted for about half an hour after the administration of the drug. For the period of the experiment the dogs were kept in a room with a thermostatically controlled environmental temperature of 24° C. The rectal temperature of the animals was measured from time to time with a clinical thermometer. At the completion of the experiment the animals were returned to a heated kennel because two of the drugs used, chlorpromazine and reserpine, might otherwise cause fatal hypothermia.

The sampling of C.S.F. was carried out as previously described (Ashcroft *et al.*, 1968). Samples of 0.5 ml. of C.S.F. were withdrawn for analysis at intervals of 0.5 hr or 1 hr over periods of 4–6 hr. The first sample of C.S.F. constituted the control and as soon as this had been withdrawn, the drug was given. The subsequent samples were then taken at intervals of 0.5 or 1 hr timed from the start of the administration of the drug.

In some experiments blood samples (5–10 ml.) were withdrawn from a leg vein into a sterile disposable syringe, transferred to a centrifuge tube containing heparin (10 u./ml. of blood) and centrifuged at 3,000 rev/min. The separated plasma was then analysed.

An interval of at least 2 weeks elapsed between experiments on any one animal.

Drug treatments

Anaesthetic. In all the experiments, except for two on the conscious dog, the dogs were anaesthetized with thiopentone sodium (Pentothal, Abbott Lab.) intravenously. An attempt was made to keep the degree of anaesthesia as uniform as possible throughout the experiments and the plane of anaesthesia, as judged by clinical signs, the same in each experiment. In the experiments with dopa administration, the anaesthetic requirements were considerably higher than in untreated dogs while in the experiments in which chlorpromazine and reserpine were studied, only small amounts of the anaesthetic were required to maintain anaesthesia after the drug had been given.

L-dopa (L-β-(3,4-dihydroxyphenyl)-alanine). L-dopa (Koch-Light Laboratories, 25 mg/kg body weight) was administered as a freshly prepared 5 mg/ml. solution in sterile 0.9% sodium chloride solution into a leg vein over a period of 5 min.

Samples of ventricular C.S.F. and venous blood were withdrawn at intervals of 0.5 hr for a period of 4 hr after the administration of L-dopa to three dogs.

In an additional experiment, L-dopa was given to the conscious dog, the experimental conditions being otherwise the same.

Homovanillic acid and dopamine infusions. Homovanillic acid (A grade, Calbiochem) was administered intravenously to one dog as a solution in sterile saline. A loading dose of 2.5 mg/kg was given by rapid injection and this was followed by a slow infusion, using an intravenous " giving set ", of 2.5 mg/kg over the subsequent hour. Samples of C.S.F. and venous blood were withdrawn at 0.5 hourly intervals for a total period of 3.5 hr.

In another single experiment, dopamine (3-hydroxytyramine hydrochloride, Calbiochem) was administered as a solution in sterile saline by intravenous infusion at a constant rate of 0.24 mg/min for 105 min. Samples of C.S.F. and venous blood were taken at intervals of 0.5 hr for a period of 3.5 hr.

Chlorpromazine. Chlorpromazine ("Largactil" Inj. May and Baker) was administered to five dogs in a dose of 10 mg/kg by the intravenous route taking 5 min to inject the requisite amount. Ventricular C.S.F. was withdrawn thereafter at intervals of 1 hr for a period of 5–6 hr.

Reserpine. Reserpine ("Serpasil" Inj. Ciba) was given intravenously to three dogs in a dose of 0.5 mg/kg over a period of 5 min. Thereafter a C.S.F. sample was taken every hour for a period of 4 hr.

In two separate experiments on the same dog, reserpine was given intravenously in a dose of 1 mg/kg when the dog was anaesthetized with thiopentone, as described, and when the dog was conscious throughout the experiment.

Analytical procedures

All extractions and reactions to produce fluorophors were carried out in glass test-tubes of suitable size fitted with ground glass stoppers. Reagents were of Analar grade or freshly distilled. Deionized distilled water was used throughout.

Estimation of HVA, DOPAC and 5-HIAA in C.S.F.

The concentrations of HVA, DOPAC and 5-HIAA in C.S.F. were estimated by the methods described in detail in the preceding paper (Ashcroft *et al.*, 1968). Samples of C.S.F. were acidified, saturated with sodium chloride and extracted twice with ethyl acetate. The phenolic acids in the pooled ethyl acetate extracts were returned into Tris buffer and portions were taken for the separate estimation of the acids by their conversion to fluorophors. A slight modification in the extraction procedure was made for the estimations on the samples from the dogs given dopa. Some mechanical carry-over of dopa from the aqueous phase to the ethyl acetate extract might occur and appreciable amounts of dopa so transferred would interfere with the fluorometric estimation of DOPAC. To make this possibility less likely, the ethyl acetate extract was washed with 2 ml. of 0.01N hydrochloric acid, which had been saturated with sodium chloride, by shaking for 5 min and centrifuging at 2,500 rev/min for 5 min. The aqueous phase was taken off and discarded.

Estimations of dopa and dopamine in C.S.F.

After extraction of the phenolic acids, the C.S.F. was assayed for dopa and dopamine when this was relevant. The procedure was the same as described below for the estimation of these substances in plasma.

Estimations of HVA, catechol acids, dopa and dopamine in plasma

Plasma was obtained by centrifugation of the heparinized whole blood. To 1.0–2.0 ml. of plasma treated with sodium edetate (100 μg/ml.) was added 0.1 vol. of 4 N perchloric acid (PCA). After thorough shaking, the mixture was chilled at 4° C for 10 min and then centrifuged at 3,000 rev/min for 7 min to effect the protein precipitation. The supernatant was removed and its pH adjusted to about 4 (1 drop of 0.02% bromophenol blue, internal indicator) by the dropwise addition with shaking of 5N and 1N potassium carbonate. The sample was stored at −20° C for at least 30 min, then thawed and centrifuged as before. The precipitate was washed with 0.5 ml. of deionized distilled water, at 4° C, the wash fluid, after centrifugation, being added to the previously obtained supernatant solution. HVA and catechol acids were extracted from the supernatant solution as already described. After removal of the acids, the aqueous phase was freed from traces of ethyl acetate by evaporation *in vacuo* for a short time and its pH adjusted to 4 as before and the samples chilled and centrifuged. The precipitates were washed with 1.0 ml. chilled water and centrifuged. The bulked supernatants were left overnight at −20° C in order to obtain a clear solution after centrifuging at 3,000 rev/min for 10 min the following morning. Portions of 1.0 or 1.5 ml were taken for the spectrophotofluorimetric estimation of dopa.

Spectrophotofluorometric estimations

Dopa. A freshly prepared mixture (0.5 ml.) of ethylenediamine and 4M ammonium chloride (1:1.3 v/v) was added to the portion of the extract and the mixture heated with shaking in a water bath for 20 min at 50° C in the dark. The solution was cooled and the fluorescence measured in an Aminco-Bowman spectrophotofluorimeter set at activation 360 mμ and fluorescence 470 mμ which were the wavelengths for optimal fluorescence of the dopa derivative. The lower limit of sensitivity of the method using pure solutions was 30–70 mμg. Quenching of the fluorescence derived from dopa added to plasma extracts occurred to varying degrees (0%–40%) and had to be checked in each batch of analyses.

Recovery of 400 mμg of dopa added to dopa-free plasma was 74%, when checked in one experiment. L-tyrosine (50 or 100 μg) added to plasma did not contribute to the fluorescence readings at dopa wavelengths. Dopamine (100 mμg) added just before the ethylenediamine did not contribute to the fluorescence readings at dopa wavelengths.

Dopamine. The amine in the neutralized aqueous phase was acetylated, extracted into methylene dichloride and the acetylated dopamine measured spectrophotofluorimetrically according to the method described by Laverty & Sharman (1965b).

HVA and catechol acids. The spectrophotofluorimetric estimations were carried out as described for C.S.F. (Ashcroft *et al.*, 1968). In these experiments the recoveries of 400 mμg of HVA and DOPAC added to plasma were 80%, 70% and 60% for HVA and 60%, 50% and 45% for DOPAC. Quoted plasma estimates have been corrected for recoveries.

Qualitative chromatography of extracts of C.S.F. from dogs loaded with dopa. Samples (0.5 ml.) of C.S.F. were collected at 0.5 hourly intervals over a period of 4 hr from untreated dogs and from dogs treated with L-dopa (25 mg/kg). The samples from each dog were pooled and processed in parallel for the identification of phenolic acids as previously described (Ashcroft *et al.*, 1968).

In addition, the pooled C.S.F. samples were examined for the presence of dopa and catecholamines. After the extraction of the phenolic acids into ethyl acetate, the aqueous phases were neutralized as already described, acetylated according to the procedure of Laverty & Sharman (1965b) and extracted with methylene dichloride to remove any acetylated amines. The aqueous and the organic phases were then treated separately.

The aqueous phase was evacuated by the use of a mechanical pump to remove traces of methylene dichloride and then acidified to a pH less than 2 with concentrated hydrochloric acid and the acetylated dopa was extracted into ethyl acetate by shaking three times for 4 min each time with 2 vol. of the organic solvent. The ethyl acetate extracts were separated by centrifugation, pooled and dried with anhydrous sodium sulphate. The ethyl acetate extract was evaporated to dryness under a nitrogen stream and the residue dissolved in a few drops of distilled methanol and applied to thin layer plates of silica gel H (Merck). Marker substances, both in pure solution and added in solution to control C.S.F. extracts, were also applied. The chromatograms were equilibrated for 1 hr and then developed for 1.5 hr in the organic phase of a mixture of chloroform:acetic acid:water (2:2:1 v/v). Acetylated dopa was visualized by spraying the developed chromatogram with a freshly prepared solution of 1 vol. ethylenediamine in 5 vol. of 1M ammonium chloride, and heating the chromatograms, covered with a clean glass plate, at 60° C for 30 min. The chromatograms were then examined under ultraviolet light.

The methylene dichloride extracts were evaporated to dryness under a stream of nitrogen. The residue was taken up in a few drops of methanol and applied to silica gel H plates along with the appropriate acetyl derivatives of 5-hydroxytryptamine, dopamine, noradrenaline, adrenaline, normetanephrine, metanephrine and methoxydopamine as marker substances. The chromatogram was equilibrated for 30 min and developed for 45 min twice in the same direction using chloroform:methanol, 95:5 by vol., the chromatograms being dried and equilibrated between the two developments in the solvent. The amines were visualized using the ethylenediamine reagent as described above.

RESULTS

L-*dopa administration*

Quantitative studies. Following its intravenous administration, L-dopa, which was not normally detectable in plasma (<100 mμg/ml.), was present in high concentrations and showed an exponential clearance from the plasma (Table 1). As with plasma, dopa was

TABLE 1

CHANGE IN PLASMA CONCENTRATIONS OF HOMOVANILLIC ACID CATECHOL ACIDS AND DOPA FOLLOWING INTRAVENOUS ADMINISTRATION OF L-DOPA (25 MG/KG)

L-Dopa was administered intravenously at 0 time to three dogs and venous blood withdrawn at 0·5 hourly intervals for 3·5 hr. The concentrations of acids are expressed as alterations (mμg/ml.) from the "control concentration" at 0 time. The "control concentration" (mμg/ml.) is given in brackets for each dog. e.f., Experimental failure; n.d., not done.

	Increase in concentrations (mμg/ml. plasma) of acids in plasma								
	HVA			Catechol acids			DOPA		
Time (hr)	Dog No. I	Dog No. II	Dog No. III	Dog No. I	Dog No. II	Dog No. III	Dog No. I	Dog No. II	Dog No. III
0	0 (<25)	0 (<25)	0 (<25)	0 (<30)	0 (<30)	0 (<30)	0 (<100)	0 (<100)	0 (<100)
0·5	300	100	400	200	60	120	16,000	12,500	11,000
1	350	250	570	40	410	50	9,000	7,500	6,500
1·5	400	600	e.f.	30	e.f.	30	5,000	5,000	4,000
2	700	700	800	40	210	90	2,250	2,000	2,300
2·5	350	250	530	100	200	85	1,100	1,200	1,000
3	e.f.	e.f.	n.d.	e.f.	e.f.	65	500	e.f.	e.f.
3·5	330	210	n.d.	50	60	n.d.	300	500	n.d.

not normally detectable in C.S.F. (<75 mμg/ml.) but after administration of dopa it appeared in the C.S.F. with peak concentrations in the 0.5–1 hr samples suggesting a rapid transfer from blood to C.S.F. (Table 2). At any time throughout a 4 hr experiment the plasma concentration was always higher than the corresponding C.S.F. concentration. There was a marked rise in the concentrations of both HVA and DOPAC in the lateral ventricular C.S.F. following the dopa treatment (Table 2). The maximum increase in concentration of HVA, of the order of 1,400–2,120 mμg/ml. of C.S.F. in the different dogs, amounted to 35–150% over the control values. Although the absolute rise in the DOPAC concentration was smaller, 540–840 mμg/ml. of C.S.F., the rise of 250–350% in relation to the control concentration was much higher than with the HVA. The time course for the rise in the concentrations was different for the two acids, the peak concentration of DOPAC occurring at 1.5–2 hr while that of HVA occurred at 2.5–3 hr, at which time the DOPAC concentration had fallen. The concentrations of 5-HIAA in the C.S.F. were not significantly altered at any time following the dopa administration (Table 2).

Because the plasma concentration of HVA and of the catechol acids were at all times much lower than the corresponding C.S.F. concentration, an active transfer process must be involved if the acids in C.S.F. were derived from the blood. The peak rise in plasma HVA occurred at 2 hr, which was, in fact, 0.5–1 hr earlier than that for C.S.F. and could be compatible with the equilibration lag were there a transfer from blood to C.S.F. The plasma catechol acids showed an earlier peak at 0.5–1 hr and a delayed peak at 2–2.5 hr. The term " catechol acids " is used because we have not identified the plasma acids fully

TABLE 2

CHANGE IN C.S.F. CONCENTRATIONS OF HOMOVANILLIC ACID, 3,4-DIHYDROXYPHENYL-ACETIC ACID, 5-HYDROXYINDOL-3-YLACETIC ACID AND DOPA FOLLOWING INTRAVENOUS ADMINISTRATION OF L-DOPA (25 MG/KG)

L-dopa was administered to three dogs and ventricular C.S.F. as well as venous blood (Table 1), withdrawn at 0·5 hourly intervals for 4 hr. The concentrations of acids are expressed as alterations (mμg/ml.) from the "control concentration" at 0 time. In brackets is given the "control concentration" (mμg/ml.) for each acid for each dog. e.f., Experimental failure; n.d., not done.

	Change in concentrations (mμg/ml. C.S.F.) of acids in ventricular C.S.F.											
	HVA Dog No.			DOPAC Dog No.			5HIAA Dog No.			DOPA Dog No.		
Time (hr)	I	II	III	I	II	III	I	II	III	I	II	III
0	0 (3000)	0 (1550)	0 (1520)	0 (200)	0 (180)	0 (210)	0 (340)	0 (340)	0 (200)	0 (<75)	0 (<75)	0 (<75)
0·5	25	190	280	70	−50	10	0	0	0	1750	1000	2000
1	210	950	480	540	320	360	−30	10	20	1500	2600	1750
1·5	450..	1200	880	720	550	540	−20	−10	20	1100	2000	1250
2	700	e.f.	1400	840	e.f.	470	−20	e.f.	20	600	e.f.	1000
2·5	1090	2120	1700	570	140	330	0	0	0	400	500	500
3	1400	1090	1840	330	−50	340	0	−20	0	75	n.d.	e.f.
3·5	1100	1100	1540	190	−70	150	−20	−20	−10	−75	n.d.	n.d.
4	1100	990	1470	100	−70	160	−20	−30	−10	n.d.	n.d.	n.d.

and both DOPAC and 3,4-dihydroxymandelic acid would be estimated by the spectrophotofluorimetric method used.

Effect of HVA given intravenously on HVA concentrations in lateral ventricular C.S.F.

When HVA was administered intravenously to obtain and maintain plasma levels much higher than those found after administration of dopa, there was no significant rise in the concentrations of HVA in the C.S.F. (Table 3). Thus the possibility of a significant transfer of HVA from peripheral blood to C.S.F. can be excluded.

TABLE 3

EFFECT OF INTRAVENOUS ADMINISTRATION OF HOMOVANILLIC ACID

HVA was administered intravenously to a dog under light thiopentone anaesthesia in a dose of 2·5 mg/kg injected at 0 time followed by an infusion of 2·5 mg/kg over the subsequent hour.. Samples of C.S.F. were taken from the lateral ventricle and of blood from a leg vein at intervals of 0·5 hr after the initial dose of HVA. e.f., Experimental failure; n.d., not done.

Time (hr)	Concentration (mμg/ml.) in C.S.F. HVA	5HIAA	In plasma. HVA
0	2880	320	<25
0·5	3060	330	4000
1	2770	310	3750
1·5	2620	310	2250
2	2380	280	2050
2·5	2470	280	e.f.
3	2430	260	200
3·5	n.d.	n.d.	150

Effect of dopamine given intravenously on acid concentrations in lateral ventricular C.S.F.

Similarly, it can be seen from Table 4 that dopamine did not cross the blood–C.S.F. barrier and hence it is unlikely that dopamine formed in the periphery contributes to the rise in HVA and DOPAC concentrations of C.S.F. following administration of dopa.

TABLE 4

EFFECT OF INTRAVENOUS ADMINISTRATION OF DOPAMINE

Dopamine was infused intravenously to a dog under light thiopentone anaesthesia at a constant rate of 240 μg/min to a total amount of 25 mg (weight of dog 10 kg). Samples of C.S.F. were taken from the lateral ventricle and of blood from leg vein at intervals of 0·5 hr after the start of the dopamine infusion. n.d., Not done.

Time (hr)	Concentration (mμg/ml.) in C.S.F.				Concentration in plasma		
	HVA	DOPAC	5HIAA	Dopamine	HVA	Dopamine	DOPAC
0	2480	200	210	<50	<25	<50	<30
0·5	2430	150	220	<50	80	100	<30
1	2430	150	e.f.	<50	130	200	<30
1·5	2370	130	210	<50	250	200	<30
2	2290	140	220	<50	360	150	<30
2·5	2530	160	240	<50	n.d.	<50	n.d.
3	2430	160	240	<50	n.d.	n.d.	n.d.
3·5	2560	170	240	<50	70	<50	<30

Qualitative studies: results from thin layer chromatography of C.S.F. extracts

Phenolic acids. Thin-layer chromatography of extracts from ventricular C.S.F. from untreated dogs had given evidence for the presence of HVA, DOPAC and 5-HIAA in the C.S.F. (Ashcroft *et al.*, 1968). The chief purpose of investigating the C.S.F. after administration of dopa was to try to find evidence of the formation of other phenolic acids in these conditions, particularly in view of the fact that if such acids were present they might interfere with the estimations of HVA and DOPAC.

Figure 1 is a photograph of a thin-layer chromatogram of C.S.F. extracts developed in chloroform : acetic acid : water (2 : 2 : 1 by vol.) and then treated with the ethylenediamine

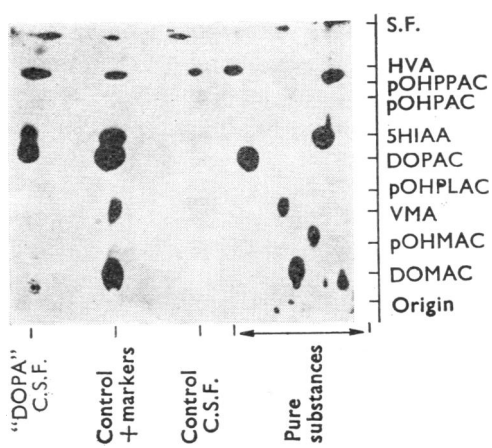

Fig. 1. Thin-layer chromatogram of an ethyl acetate extract of C.S.F. on silica gel H. Solvent system-organic phase of chloroform : acetic acid : water (2 : 2 : 1 by vol.) "Multiple chromatography" (see text). Homovanillic acid (HVA), *p*-hydroxyphenylpyruvic acid (pOHPPAC), *p*-hydroxyphenylacetic acid (pOHPAC), 5-hydroxyindol-3-ylacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), *p*-hydroxyphenyllactic acid (pOHPLAC), vanillinmandelic acid (VMA), p-hydroxymandelic acid (pOHMAC) and 3,4-dihydroxymandelic acid (DOMAC).

reagent as described previously (Ashcroft *et al.*, 1968). This reagent was used essentially to demonstrate any catechol acids. The spots on the far left are from an extract of ventricular C.S.F. from a dog given L-dopa (25 mg/kg). Next to that are chromatograms from two C.S.F. extracts from untreated dogs, one extract containing marker substances. On exposure to ultraviolet light, intense yellow fluorescent spots were noted in the DOPAC positions, the intensity being much greater in the C.S.F. extract from the "dopa-treated" animal. Although ethylenediamine is not a reagent generally used for the detec-

tion of HVA and 5-HIAA, these acids can, in fact, be detected. HVA gave a blue fluorescence in the alkaline conditions and 5-HIAA gave an orange-green fluorescence. Further evidence for the presence of these two acids was obtained as described previously (Ashcroft *et al.*, 1968). Using our technique we were unable to detect any other phenolic acids in the C.S.F. extracts from dogs treated with dopa. Particularly relevant to the present study was the absence of detectable amounts of 4-hydroxy-3-methoxy-mandelic acid or of 3,4-dihydroxymandelic acid, possible metabolites of noradrenaline.

Dopa. Acetylated dopa on the thin-layer chromatograms was visualized using the ethylenediamine reagent, the mixture giving a yellow fluorescence in ultraviolet light. No acetylated dopa was found in chromatograms of samples of ventricular C.S.F. from control dogs, while in samples from dopa-loaded dogs a spot corresponding in position to acetylated dopa was obtained.

Catecholamines. With the ethylenediamine reagent applied to the thin-layer chromatogram, the authentic catecholamines showed up as pink to yellow spots which fluoresced strongly yellow under the ultraviolet light. The 3-*O*-methyl derivatives of the catecholamines showed as absorbing areas under ultraviolet light. The plates were then sprayed with diazotised *p*-nitroaniline (Ashcroft *et al.*, 1968) to visualize the 3-*O*-methyl derivatives of the catecholamines as brown spots. There was no trace of any catecholamines or of the 3-*O*-methyl derivatives of the catecholamines in ventricular C.S.F. from control dogs.

Effect of chlorpromazine administration

Following the intravenous administration of chlorpromazine (10 mg/kg) the concentration of HVA in ventricular C.S.F. showed an increase which was evident within 1 hr and maximal at 4 hr (Table 5). Although the time-course for the rise in concentration was fairly similar in all the dogs, there was a considerable difference in the magnitude of the effect.

TABLE 5

EFFECT OF INTRAVENOUS ADMINISTRATION OF CHLORPROMAZINE (10 MG/KG)

Chlorpromazine (10 mg/kg) was administered intravenously at 0 time to five dogs under light thiopentone anaesthesia. Body temperature of the dogs was maintained within 1°–2° F. Samples of ventricular C.S.F. were collected at hourly intervals. The concentrations of acids are expressed as changes (mμg/ml.) from the "control concentration" at zero time. In brackets is given the "control concentration" (mμg/ml.) for each acid for each dog. e.f., Experimental failure; n.d., not done.

	Change in concentration (mμg/ml.) in ventricular C.S.F.														
	HVA. Dog No.					DOPAC. Dog No.					5-HIAA. Dog No.				
Time (hr)	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
0	0 (2510)	0 (2240)	0 (2240)	0 (1400)	0 (3120)	0 (260)	0 (150)	0 (180)	0 (180)	0 (350)	0 (250)	0 (280)	0 (280)	0 (200)	0 (310)
1	150	260	+40	100	660	5	−10	−30	50	30	10	40	80	150	50
2	1330	320	480	180	1000	10	−20	−30	60	140	0	30	60	180	105
3	1500	380	480	320	1200	40	10	0	110	120	−10	0	20	180	30
4	1790	750	760	850	1400	40	−20	−30	10	40	10	20	20	120	40
5	1580	480	480	280	1130	30	0	−30	10	40	10	0	30	50	10
6	n.d.	n.d.	120	280	1180	n.d.	n.d.	−30	10	n.d.	n.d.	n.d.	e.f.	n.d.	−10

The DOPAC concentration showed an increase to about 50% above the initial concentration, at 3 hr in dogs Nos. IV and V (Table 5) and a less marked rise in dog No. I. In contrast, in dogs II and III there was no definite alteration from the initial (0 time) concentration.

In all the dogs except dog No. 1, there was a rise in the concentration of 5-HIAA in the lateral ventricular C.S.F. although the magnitude of the rise varied greatly. The peak concentration occurred at 1–2 hr after the chlorpromazine injection (Table 5). It is likely that the observed increases, although small in some cases, were genuine because in control samples removed at the same time intervals the variations in concentration from sample to sample were rarely found to exceed 10% of the initial concentration and any such variation was almost always in the direction of a decrease from the initial concentration (Ashcroft *et al.*, 1968).

Effect of reserpine administration

After the intravenous administration of reserpine (0.5 mg/kg) to the dogs there was a marked rise, of about 300 mμg/ml., in the concentration of DOPAC in the ventricular C.S.F. during the first hour. The increase was considerably reduced within the next hour and within 4 hr the concentration had returned almost to the control level (Table 6).

TABLE 6

EFFECT OF INTRAVENOUS ADMINISTRATION OF RESERPINE (0·5 MG/KG)

Reserpine (0·5 mg/kg) was administered intravenously at 0 time to three dogs under light thiopentone anaesthesia. Samples of ventricular C.S.F. were collected at hourly intervals. The concentrations of acids are expressed as changes (mμg/ml.) from the " control concentration " at zero time. In brackets is given the " control concentration " (mμg/ml.) for each acid for each dog

	Change in concentration (mμg/ml.) in ventricular C.S.F.								
	HVA Dog No.			DOPAC Dog No.			5-HIAA Dog No.		
Time (hr)	I	II	III	I	II	III	I	II	III
0	0 (2100)	0 (1950)	0 (2840)	0 (200)	0 (190)	0 (220)	0 (290)	0 (280)	0 (240)
1	−110	−110	−190	320	300	290	40	−20	60
2	150	140	50	50	40	90	80	100	90
3	880	590	480	60	40	100	20	130	110
4	210	510	370	0	50	20	20	100	60

The concentration of HVA on the other hand, showed a small decrease at 1 hr after the reserpine. Thereafter an increase in concentration was evident, reaching a maximum 3 hr after the injection of reserpine.

The time course for the rise in 5-HIAA concentrations was intermediate between that for DOPAC and for HVA and the magnitude of the rise was relatively small, in the region of 100 μg/ml.

Effect of thiopentone anaesthesia

We investigated the possible interaction between the anaesthetic and reserpine or L-dopa when the drugs were administered to the conscious and the anaesthetized dog. The changes in the concentrations of HVA, DOPAC, 5-HIAA and dopa in the C.S.F. of the conscious and the anaesthetized dog following administration of L-dopa were not

markedly different in the two situations (Table 7). The increases in the dopa concentrations in the C.S.F. of the anaesthetized and unanaesthetized dog, 30 min after administration of dopa, were estimated as 1,400 and 2,440 mμg/ml., respectively. Thus the apparent discrepancy for the rise in the C.S.F. dopa concentration at 1 hr between the anaesthetized and conscious dog (Table 7) can be explained by the fact that in the conscious dog the peak increase in the dopa concentration occurred less than 1 hr after the administration of L-dopa, whereas in the anaesthetized dog the maximal rise occurred at 1 hr or later.

TABLE 7

EFFECT OF THIOPENTONE ANAESTHESIA ON THE CHANGES IN THE CONCENTRATIONS OF PHENOLIC ACIDS IN THE C.S.F. AFTER L-DOPA ADMINISTRATION

L-Dopa (25 mg/kg) was administered intravenously to the dog (*a*) under light thiopentone anaesthesia and (*b*) conscious. n.d., Not done.

	Concentration (mμg/ml.) of acids in ventricular C.S.F. of the dog							
	HVA		DOPAC		5-HIAA		DOPA	
Time (hr)	With anaesthetic	Without anaesthetic	With anaesthetic	Without anaesthetic	With anaesthetic	Without anaesthetic	With anaesthetic	Without anaesthetic
0	1550	1870	180	200	240	270	<75	<75
1	2500	2400	500	840	250	280	2600	1100
2	n.d.	3870	n.d.	390	n.d.	n.d.	n.d.	300
3	2640	2250	130	260	220	200	500	175
4	2600	1800	110	260	220	180	—	100

The effect of reserpine (1 mg/kg) given intravenously, on the C.S.F. acid concentration showed a similar pattern in that in the conscious dog the maximum increases in acid concentrations tended to occur 1 hr earlier than in the anaesthetized dog (Table 8).

TABLE 8

EFFECT OF THIOPENTONE ANAESTHESIA ON THE CHANGES IN THE CONCENTRATIONS OF PHENOLIC ACIDS IN THE C.S.F. AFTER RESERPINE ADMINISTRATION

Reserpine (1 mg/kg) was administered intravenously to the dog (*a*) under light thiopentone anaesthesia and (*b*) conscious. n.d., Not done.

	Concentration (mμg/ml.) of acids in ventricular C.S.F.					
	HVA		DOPAC		5-HIAA	
Time (hr)	With anaesthetic	Without anaesthetic	With anaesthetic	Without anaesthetic	With anaesthetic	Without anaesthetic
0	1920	1810	210	180	210	220
1	2080	2160	480	380	240	360
2	2170	2200	500	300	280	460
3	n.d.	2250	420	230	400	420
4	2340	2200	380	n.d.	360	n.d.
5	2370	1740	380	250	340	330

DISCUSSION

The results reported in this paper of the effects of L-dopa, chlorpromazine and reserpine on the concentrations of HVA, DOPAC and 5-HIAA in the ventricular C.S.F. of the dog, using the technique of serial sampling of C.S.F., can be compared with the effects of these drugs on the brain concentrations of the acids reported in the literature. We realize that such a comparison is limited by such problems as species variation, dose

dependence, regional brain distribution of acids and different methods of assaying the acids. With regard to the metabolism of biogenic amines in the brain and its relationship to concentrations of metabolites in the C.S.F., little work has been reported, and this is an attempt to elucidate this relationship.

Following its administration, L-dopa is converted to dopamine in the brain (Carlsson, Lindqvist, Magnusson & Waldeck, 1958). The rate of accumulation in various areas in the brain of newly formed dopamine, after the administration of D,L-dopa labelled with Carbon-14, was found to parallel the regional distribution of the endogenous dopamine (Pletscher & Gey, 1962). Thus the highest amount of dopamine formed from D,L-dopa was found in the corpus striatum about half an hour after the injection of the amino-acid (Bertler & Rosengren, 1959). An increase in the concentrations of HVA and DOPAC, the acid metabolites of dopamine, was demonstrated in the corpus striatum of the rabbit (Andén *et al.*, 1963) while Laverty & Sharman (1965a) found a significant rise in the HVA concentration of the caudate nucleus of the dog following the administration of a relatively small dose of L-dopa (15 mg/kg). The results of our investigations showed that a significant rise occurred in the concentration of DOPAC as well as HVA in the ventricular C.S.F. of the dog following intravenous administration of L-dopa. We obtained results similar to those of Andén *et al.* (1963), showing that the rise in the DOPAC concentration preceded that of the HVA concentration and that the DOPAC concentration began to decline while that of HVA was still rising (Fig. 2). The concentration of

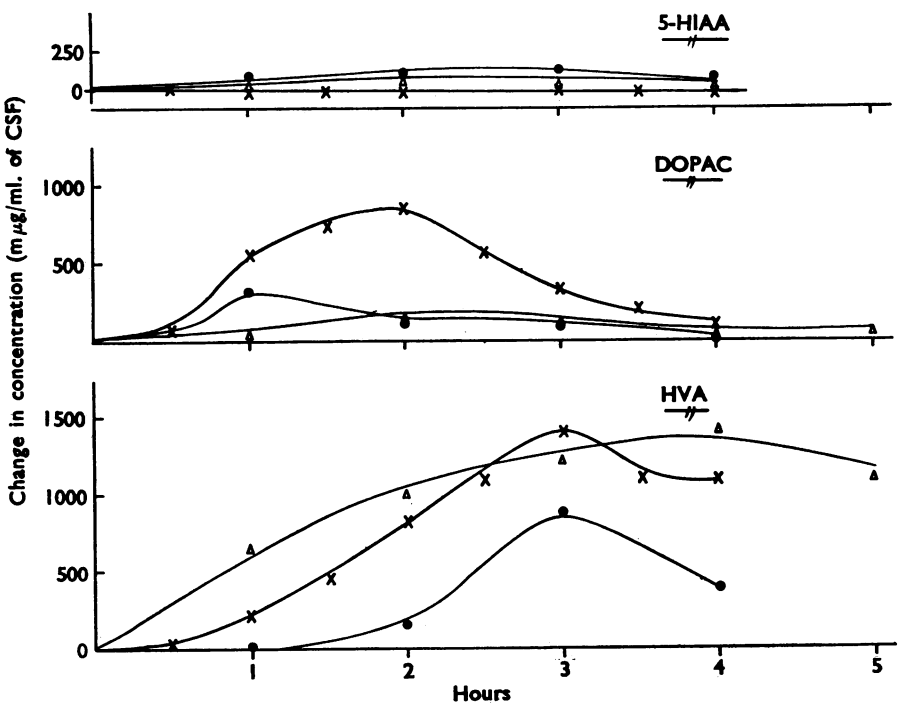

Fig. 2. Changes in concentrations of homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the ventricular C.S.F. of a dog receiving, during different experiments, L-dopa, (× ——×, 25 mg/kg), chlorpromazine (△——△, 10 mg/kg) or reserpine (●——●, 0.5 mg/kg). The drugs were given intravenously at zero time.

5-HIAA in C.S.F. was not altered at any time following the administration of dopa. The peak concentration in the C.S.F. of the precursor amino-acid, L-dopa, preceded that of the acid metabolites from dopamine.

The pattern of metabolic effects on brain amines induced by administration of L-dopa to animals can be explained by an increased synthesis of dopamine. The free dopamine so formed will presumably be metabolized by monoamine oxidase (MAO) leading to an increase in the concentration of the deaminated product DOPAC. In the striatum, where dopamine is thought to have a physiological role, the increase in stored dopamine after administration of dopa may lead to an increased extraneuronal release of dopamine. The amine released extraneuronally may be primarily metabolized by catechol-*O*-methyltransferase (COMT) and this will be reflected by an increased HVA concentration. On the other hand, the rise in HVA concentration secondary to that of the DOPAC concentration could possibly also be accounted for by the conversion of DOPAC to HVA, although there are no published studies showing that this conversion occurs *in vivo* in the brain.

We considered the possibility that after dopa administration the acid metabolites in C.S.F. were derived from the blood. The time course of the rise in the concentration of HVA in plasma, with the peak occurring 0.5 hr before that in C.S.F. would be compatible with such a transfer from blood to C.S.F. On the other hand, the plasma concentrations were always considerably lower than those of C.S.F. and one would have to postulate an active transfer from blood to C.S.F. After infusing HVA intravenously to maintain the plasma concentration for a longer time at a higher level than that found after dopa administration, however, there was no significant rise in the C.S.F. concentration and hence transfer from blood does not seem to contribute to any important extent to the HVA in the C.S.F. It has been demonstrated that DOPAC does not penetrate from blood to brain (Carlsson & Hillarp, 1962) and it seems probable that this is also the case from blood to C.S.F. Furthermore, we obtained evidence that when dopamine was infused intravenously, it did not penetrate into ventricular C.S.F., so it is likely that the phenolic acids in the C.S.F. were not derived from dopamine formed outside the central nervous system. There is evidence that the catecholamines given systemically do not penetrate the blood-brain barrier (Weil-Malherbe, Whitby & Axelrod, 1961).

The source of the phenolic acids in the C.S.F. is unknown. We have presented some evidence suggesting that the changes in HVA and DOPAC concentrations in C.S.F. following administration of L-dopa are similar to those reported for the brain. It is likely that areas of the brain adjacent to the lateral ventricles, for example parts of the corpus striatum, are most important in this respect. By the use of fluorescence microscopy for the localization of catecholamines in the brain, it has been shown (Bertler, Falck & Rosengren, 1963) that following dopa administration to rabbits, large amounts of dopa and later dopamine can be found in areas adjacent to the choroid plexus and in the small blood vessels supplying brain tissues. The source of the acids in C.S.F. could conceivably be from such sites under the conditions of dopa administration. Such sites would not, however, contribute significantly to the changes in phenolic acid concentrations following treatment of the animals with chlorpromazine or reserpine.

Chlorpromazine has been reported not to alter the concentrations of the rat brain dopamine or brain 5-hydroxytryptamine (Holzer & Hornykiewicz, 1959 ; Gey & Pletscher, 1964) although Laverty & Sharman (1965a) reported a significant decrease of dopamine in

the caudate nucleus of the cat. Our observation of a marked rise in the HVA concentrations in the C.S.F. of the dog after chlorpromazine, 10 mg/kg, occurring soon after the administration and persisting for several hours and of a much smaller but simultaneous rise in the DOPAC concentration, resemble the results of Andén *et al.* (1964) for the time course of the changes in concentrations of the acids in the corpus striatum of the rabbit after chlorpromazine. It should be noted that there was considerable variation between dogs in the intensity of effect of the chlorpromazine on the acid concentrations in the C.S.F., this variation being more marked in the case of DOPAC. The significant rise in the 5-HIAA concentration of C.S.F. shown to occur in some dogs was surprising because brain 5-HIAA concentrations have been reported to be unaffected by chlorpromazine (Gey & Pletscher, 1964), although there are no published data relating specifically to dog brain.

The mechanism by which chlorpromazine causes HVA and DOPAC to accumulate in the brain at about the same time, although the rise in the concentration of DOPAC is relatively small, is not known. It has been suggested that chlorpromazine enhances the formation of dopamine through a feedback mechanism resulting from blocking of the dopamine receptors (Carlsson & Lindqvist, 1963 ; da Prada & Pletscher, 1966) and that the effect on the phenolic acids might be the result of this.

It is well established that reserpine depletes the brain amines. Reserpine (0.5 mg/kg), like chlorpromazine, was found to produce an increase of the concentrations of both DOPAC and HVA, as well as 5-HIAA, in the C.S.F. There were, however, differences in the pattern of metabolic effects of reserpine and chlorpromazine. Reserpine, like administration of L-dopa, produced a rise in the DOPAC concentration earlier than that of HVA, and the concentrations of DOPAC were decreasing from their peaks at a time when the concentrations of HVA were still rising. The time courses of the effects of reserpine on the concentrations of these acids in the C.S.F. are again similar to those reported by Andén *et al.* (1964) to occur in the rabbit corpus striatum. Reserpine produced a more marked increase in the concentrations of DOPAC and 5-HIAA than did chlorpromazine.

Reserpine releases brain amines from their stores and the initial rise in the DOPAC concentration could be caused by the increase in unbound dopamine released into the cytoplasm being metabolized by intraneuronal MAO. It is much more difficult to explain the delayed rise in the HVA concentration and the fact that the time-course of the rise in concentration of 5-HIAA parallels that of HVA rather than that of DOPAC. Most of the evidence points against reserpine affecting the rate of synthesis of the amines or the extraneuronal release ; mechanisms which can conceivably lead to changes in the HVA concentration. Andén *et al.* (1964) suggested that the rise in HVA concentration of corpus striatum resulted from conversion of DOPAC to HVA. According to these workers the HVA concentration did not return to a normal level until about 12 hours after the reserpine treatment despite the fact hat most of the amine was depleted several hours before that. The prolonged elevation of the HVA concentration following reserpine treatment therefore seems to result from a diminution in the removal of the acid from brain, possibly by saturation of the normal mechanisms or more probably from an inhibitory action exerted on the mechanisms by reserpine itself. The finding that the changes in the concentrations of 5-HIAA in the C.S.F. parallel those of HVA rather than the DOPAC

concentrations is surprising because both 5-HIAA and DOPAC are deaminated products. It may, of course, be related to varying rates of access of the metabolites from brain to C.S.F. but there is a major difference in the inactivation of 5-hydroxytryptamine and dopamine because the COMT system is not involved in the metabolism of the former and it is possible that physiologically MAO has a different degree of importance in the inactivation of the two amines.

The use of C.S.F. for investigative work lends itself to attractive possibilities for studies on the metabolism of cerebral amines. In the larger laboratory animals C.S.F. can be obtained from various sites with relative ease; in the living animal a dynamic picture of the amine metabolism can be obtained; the animals can be used repeatedly and can also act as their own controls in drug studies. The anaesthetic given to the dogs did not seem to affect the results significantly. In man it offers an almost unique approach to the study of brain metabolism. It is, of course, true that the amines themselves are not found in appreciable concentrations in the C.S.F. but perhaps the C.S.F. should be considered as an excretory system for metabolic end-products of cerebral amine metabolism. We do not at present know its relative importance in the mechanism of excretion as compared, for instance, with the possibility of a direct transfer from brain to blood. We have presented some evidence that changes in the phenolic acid concentrations in ventricular C.S.F. of the dog, using the technique of serial sampling, reflect changes reported to occur in the brain following the administration of drugs. We are at present studying the relationship between C.S.F. and brain more directly by comparing C.S.F. and brain concentrations of these phenolic acids in the dog and using, in addition, serial sampling of ventricular C.S.F. as a simple screening technique for drug actions.

SUMMARY

1. The effects of some drugs on the concentrations of phenolic acids derived from dopamine and 5-hydroxytryptamine metabolism were studied in the ventricular cerebrospinal fluid (C.S.F.) of dogs using the technique of serial sampling of C.S.F.

2. Homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) were estimated in samples of C.S.F. withdrawn at intervals of 0.5 or 1 hr for periods of 4–6 hr following the intravenous administration to the dogs of L-dopa, chlorpromazine or reserpine.

3. The changes in concentrations of the acids in the C.S.F. were as follows. Following the administration of L-dopa (25 mg/kg) there was a marked rise in the concentrations of DOPAC and HVA in the C.S.F. The rise in DOPAC preceded that of HVA and declined from its peak concentration at a time when HVA was still rising to reach its peak concentration about 2 hr later than that of DOPAC. The concentration of 5-HIAA was unaffected. Chlorpromazine (10 mg/kg) caused a marked rise in the concentration of HVA, becoming evident within 1 hr and a relatively small rise, with considerable variation between dogs, in the concentrations of the DOPAC and 5-HIAA. Reserpine (0.5 mg/kg) caused an increase of the DOPAC concentration preceding that of HVA while the time-course for the rise in the 5-HIAA concentration parallelled that of HVA rather than that of DOPAC.

4. Evidence was presented for the fact that the phenolic acids in C.S.F. were not derived from peripheral blood.

5. The changes in the C.S.F. concentrations of HVA, DOPAC and 5-HIAA following the administration of L-dopa, chlorpromazine or reserpine were compared with changes in the brain concentrations of these acids reported in the literature to occur after the administration of these drugs. There was considerable evidence to support the view that the changes in the acid concentrations in C.S.F. reflected changes in the brain.

6. It seems that C.S.F. plays a part in the removal of metabolites from the brain. Examination of C.S.F. enables one to study some parameters of cerebral amine metabolism without having always to resort to the more laborious acute experiments for brain analysis. Attention has been drawn to the advantages of such C.S.F. analyses as have been illustrated in this paper.

We thank Professor W. L. M. Perry, Dr. G. W. Ashcroft and Dr. T. B. B. Crawford for their invaluable help and advice and Mr. R. C. Dow for his skilled technical assistance in this work.

REFERENCES

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1963). On the occurrence of homovanillic acid in brain and cerebrospinal fluid and its determination by a fluorimetric method. *Life Sci.*, **2**, 448–458.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci.*, **3**, 149–158.

ASHCROFT, G. W. & SHARMAN, D. F. (1962). Drug-induced changes in the concentration of 5-OR indolyl compounds in cerebrospinal fluid and caudate nucleus. *Br. J. Pharmac. Chemother.*, **19**, 153–160.

ASHCROFT, G. W., CRAWFORD, T. B. B., DOW, R. C., & GULDBERG, H. C. (1968). Homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindol-3-yl-acetic acid in serial samples of cerebrospinal fluid from the lateral ventricle of the dog. *Br. J. Pharmac. Chemother.*, **33**, 441–456.

BERTLER, A. & ROSENGREN, E. (1959). Occurrence and distribution of catecholamines in brain. *Acta physiol. scand.*, **47**, 350–361.

BERTLER, A., FALCK, B. & ROSENGREN, E. (1963). The direct demonstration of a barrier mechanism in the brain capillaries. *Acta pharmac. tox.*, **20**, 317–321.

CARLSSON, A. & HILLARP, N.-A. (1962). Formation of phenolic acids in brain after administration of 3,4-dihydroxyphenylalanine. *Acta physiol. scand.*, **55**, 95–100.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140–144.

CARLSSON, A., LINDQVIST, M., MAGNUSSON, T. & WALDECK, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science, N.Y.*, **127**, 471.

DA PRADA, M. & PLETSCHER, A. (1966). On the mechanism of chlorpromazine-induced changes of cerebral homovanillic acid levels. *J. Pharm. Pharmac.*, **18**, 628–630.

GEY, K. F. & PLETSCHER, A. (1964). Effects of chlorpromazine on the metabolism of *d,l*-2-C-14-dopa in the rat. *J. Pharmac. exp. Ther.*, **145**, 337–343.

HOLZER, G. & HORNYKIEWICZ, O. (1959). Über den Dopamine- (Hydroxytyramine-) Stoffwechsel in Gehirn der Ratte. *Arch. exp. Path. Pharmak.*, **237**, 27–33.

JUORIO, A. V., SHARMAN, D. F. & TRAJKOV, T. (1966). The effect of drugs on the homovanillic acid content of the corpus striatum of some rodents. *Br. J. Pharmac. Chemother.*, **26**, 385–392.

LAVERTY, R. & SHARMAN, D. F. (1965a). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759–772.

LAVERTY, R. & SHARMAN, D. F. (1965b). The estimation of small quantities of 3,4-dihydroxyphenyl ethylamine in tissues. *Br. J. Pharmac. Chemother.*, **24**, 538–548.

PLETSCHER, A. & GEY, K. F. (1962). Topographical differences in the cerebral metabolism of D,L-2-C^{14} 3,4-dihydroxyphenylalanine. *Experientia*, **18**, 512–513.

ROOS, B.-E. & WERDINIUS, B. (1962). Effect of reserpine on the level of 5-hydroxyindoleacetic acid in brain. *Life Sci.*, **1**, 105–107.

ROSENGREN, E. (1960). On the role of monoamine oxidase for the inactivation of dopamine in brain. *Acta physiol. scand.*, **49**, 370–375.

SHARMAN, D. F. (1966). Changes in the metabolism of 3,4-dihydroxyphenylethylamine (dopamine) in the striatum of the mouse induced by drugs. *Br. J. Pharmac. Chemother.*, **28**, 153–163.

WEIL-MALHERBE, H., WHITBY, L. G. & AXELROD, J. (1961). The uptake of circulating [3H] norepinephrine by the pituitary gland and various areas of the brain. *J. Neurochem.*, **8**, 55–66.