Brit. J. Pharmacol. (1966), **26**, 385–392.

THE EFFECT OF DRUGS ON THE HOMOVANILLIC ACID CONTENT OF THE CORPUS STRIATUM OF SOME RODENTS

BY

A. V. JUORIO, D. F. SHARMAN AND T. TRAJKOV

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received October* 14, 1965)

The presence of homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid), an acid metabolite of dopamine (3,4-dihydroxyphenylethylamine), in the mammalian brain, particularly in the corpus striatum, has been demonstrated in a number of species (Andén, Roos & Werdinius, 1963a; Sharman, 1963a; Bernheimer, 1964; Pletscher, Bartholini, Bruderer, Burkard & Gey, 1964). Juorio & Vogt (1965) reported that the striatal region of the rat brain contained very little homovanillic acid and that the level of this acid did not change after the administration of reserpine. In contrast, treatment with reserpine increases the homovanillic acid content of the brain of the rabbit (Andén, Roos & Werdinius, 1964) and of the cat (Sharman, 1963b). It was of interest to examine the response to reserpine in the brains of other small mammals and to reinvestigate the metabolism of the endogenous dopamine in the rat brain.

METHODS

Materials

These were: L-cysteine hydrochloride (Roche); Dowex 1×2 anion exchange resin 100–200 and 200–400 mesh (Dow Chemical Co.); homovanillic acid (California Corporation for Biochemical Research); hydrochloric acid (Microanalytical Reagent quality); and potassium ferricyanide (Analytical Reagent quality, recrystallized from water). Other reagents were of Analytical Reagent quality. Glass distilled water was used throughout.

The following drugs were used: reserpine (Serpasil, Ciba), either manufacturer's ampoules or a solution prepared from Serpasil powder dissolved in 10% ascorbic acid in water; tetrabenazine, dissolved in a little glacial acetic acid and the solution diluted with 0.9% saline; chlorpromazine hydrochloride, dissolved in 0.9% saline; and DL-3,4-dihydroxyphenylalanine (DL-dopa, L. Light), a suspension prepared in 0.9% saline.

The animals used were guinea-pigs, coypu, albino rats from three colonies and *Meriones* (the gerbil). Rats and *Meriones* were stunned and decapitated; guinea-pigs and coypu were anaesthetized with chloroform and bled. The brain was rapidly removed and the required part dissected out and chilled on ice. When the extraction was not carried out immediately the tissues were stored at −17° C. They did not usually remain at this temperature for longer than 1 hr. The tissue excised was selected for reproducibility of the dissection and varied in the different species as follows: guinea-pig, caudate nucleus and a small amount of the underlying striatal tissue; coypu, caudate nucleus; rat, a block of tissue including the striatum and most of the orbital cortex; and *Meriones*, as for the rat, but with some parietal cortex.

Extraction of homovanillic acid

The tissue was homogenized in 2 ml. of 1 N-acetic acid for each g of tissue, using a glass homogenizer. The homogenate was diluted with water to a volume of 5 to 10 ml. 1 N-sodium hydroxide (1 ml./g tissue) was added and the homogenate was mixed thoroughly. After centrifugation the clear supernatant fluid was ready for extraction on a column of anion exchange resin. A small column of Dowex 1 × 2 anion exchange resin was set up as shown in Fig. 1. The resin was washed with 10 ml. of 2 N-hydrochloric acid, followed by 10 ml. of water. It was then converted to the acetate form by means of 10 ml. of 1 N-acetic acid. After washing with a further 15 ml. of water, the column was ready for the passage of the tissue extract. Frequently a small air bubble was trapped just above the resin, preventing the flow of liquid. This was easily removed with a thin glass rod. The tissue extract was filtered into the reservoir above the resin column and the filter paper was washed with water to bring the final volume of liquid in the reservoir to 10 to 20 ml. When all of the extract had passed through the column, the reservoir and column were carefully washed with 10 to 15 ml. of water. Homovanillic acid was eluted with 4 to 4.5 ml. of 0.1 N-hydrochloric acid.

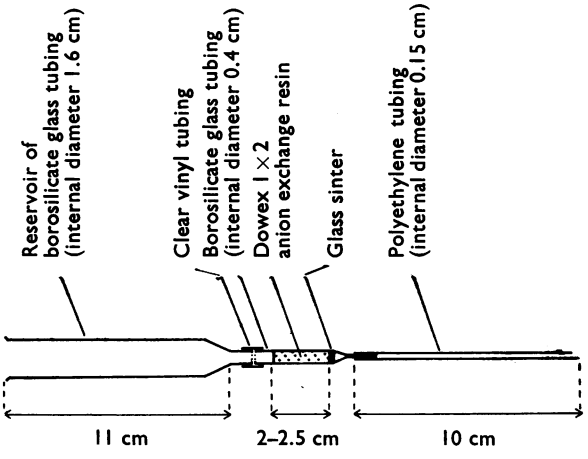

Fig. 1. Diagram of the apparatus used to extract homovanillic acid.

Fluorimetry

A slight modification of the method described by Andén *et al.* (1963a) for the estimation of homovanillic acid was used. A portion of the eluate from the resin column was diluted to 1 ml. with water. 1 ml. of a freshly prepared 5 N-ammonium hydroxide solution, containing 20 μg/ml. of potassium ferricyanide, was then added with mixing. After 4 min the reaction was stopped by the addition of 0.2 ml. of a freshly prepared 0.1% solution of cysteine hydrochloride. A blank determination was made for each eluate by reversing the order of the addition of the last two solutions. The fluorescence developed from authentic homovanillic acid in the presence of the eluate was also determined.

The fluorescence was measured either with an Aminco-Bowman Spectrophotofluorometer or with a Locarte filter fluorimeter. In the former instrument the wavelength of the activating light was 310 mμ (uncorrected instrumental value). In some experiments a wavelength of 320 mμ was used for convenience in standardizing the instrument. The fluorescence was measured at a wavelength of 430 mμ (uncorrected instrumental value) with a Corning 5113 filter in the fluorescence light path. The activating light of the Locarte fluorimeter was passed through two Chance OX7 filters, and the fluorescence light was filtered through a combination of a Corning 5113 (half standard thickness) filter and a Corning 3389 filter. It was found that the results obtained with the filter fluorimeter were somewhat higher than those obtained using the spectrophotofluorimeter unless the method involved the paper chromatographic separation described below. This was due to the

presence of some fluorescing material, not visible in the non-oxidized blank, but also seen in extracts of brain tissue which did not contain homovanillic acid. The results calculated from measurements made with the filter fluorimeter were corrected for this tissue fluorescence. The recovery of authentic homovanillic acid added to tissue homogenates was determined in five series of experiments by three different workers. The mean recoveries ranged from 57 to 71% with an overall mean recovery of 64% (n=67). The standard error of a single observation was ±15.6%.

Limitations of the method

The method was developed so that drugs could be quickly screened for their effect on the homovanillic acid content of the brain, and in common with many other simple methods, there are certain limitations to its use. Many acidic substances are adsorbed on to the anion exchange resin under the conditions used. The adsorption of homovanillic acid depends on the nature of other anions present in the extract. For example, the adsorption of homovanillic acid proceeds adequately in the presence of 0.2 M-acetate at *p*H 5.2. A concentration of chloride up to 0.02 M in a solution of 0.14 M-acetate (*p*H 5.2) did not prevent the retention of homovanillic acid, but concentrations of chloride above 0.02 M reduced the recovery, 0.1 M-chloride resulting in a loss of 70%. Homovanillic acid is completely eluted by 8 ml. of 1.0 N-acetic acid or 4 ml. of 0.1 N-hydrochloric acid. These observations indicate that the hydrogen ion contributes to the elution of homovanillic acid from the column. Experiments showed that some derivatives of phenylacetic acid could be separated from the corresponding mandelic acid derivatives, the former being eluted with a more dilute solution of acetic acid. It was found, however, that the development of the fluorescence from homovanillic acid was irregular in the presence of acetic acid. With hydrochloric acid the results are reproducible, but there is a small decrease in the intensity of the fluorescence derived from homovanillic acid. A number of acidic substances, in addition to homovanillic acid, are eluted from the column with 0.1 N-hydrochloric acid. 5-Hydroxyindolylacetic acid was not eluted from the column by 0.1 N-hydrochloric acid, but the eluate would contain any 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxymandelic acid and 4-hydroxy-3-methoxymandelic acid. 3,4-Dihydroxyphenylacetic acid is known to occur in striatal tissue (Andén *et al.*, 1963b). When 3,4-dihydroxyphenylacetic acid was present in solutions in a concentration equal to that of homovanillic acid a reduction of 10 to 20% in the fluorescence was observed. Because the eluting acid and possibly other substances present in the eluate are likely to bring about some reduction in the fluorescence, all estimations were calculated against the fluorescence developed from a known amount of homovanillic acid added to a portion of the eluate. In practice, any quenching other than that by the 0.1 N-hydrochloric acid has rarely been seen. The development of a fluorescent compound from homovanillic acid by oxidation with potassium ferricyanide in ammonium hydroxide solution, under the conditions described here, is reasonably specific for this acid. Some other derivatives of 4-hydroxy-3-methoxyphenylethane give rise to a small fluorescence. Of these, only 4-hydroxy-3-methoxymandelic acid is likely to be extracted by this method. This acid has not been detected in brain tissue (Andén *et al.*, 1964 ; Sharman, unpublished).

Identification of homovanillic acid in the tissues

Tissue extracts, deproteinized with zinc sulphate and sodium hydroxide, or eluates from resin columns were extracted with ethyl acetate. The acids in these extracts were separated by paper chromatography on alkali-washed, hardened filter paper, using a benzene : propionic acid : water mixture as the developing solvent (for details see Sharman, 1963a). Consecutive strips were cut from the whole chromatogram and eluted with water. The fluorescence reaction described above and that described by Sharman (1963a) have been applied to these eluates. The positions of materials on the chromatograms giving rise to a fluorescence were compared with the position of authentic homovanillic acid used as a marker. This was visualized by means of the reaction with diazotized *p*-nitroaniline to give a blue-grey colour. The procedures applied to identify homovanillic acid were also used in some experiments to confirm the estimates made with the quicker but less specific method using the anion exchange resin. For identification, it was frequently necessary to pool the tissues from two to twenty animals.

Estimation of dopamine

The dopamine content of rat striatal tissue was estimated fluorimetrically after acetylation of the amine in an eluate from a column of cation exchange resin (Dowex 50 × 8) and condensation with ethylenediamine to form a fluorescent derivative (Laverty & Sharman, 1965a).

RESULTS

Rat

Groups of rats, obtained from three different sources, were given reserpine, 2 mg/kg. One of these colonies (A) was the same source as the rats used by Juorio & Vogt (1965). The results are given in Table 1. Observations made 4 hr after injection of the drug,

TABLE 1

THE EFFECT OF DRUGS ON THE CONCENTRATION OF HOMOVANILLIC ACID IN THE CORPUS STRIATUM OF THE RAT

Drugs were given subcutaneously in a single dose : reserpine, 2 mg/kg ; chlorpromazine, 10 mg/kg ; and tetrabenazine, 50 mg/kg. The results are expressed as the means and standard errors of the concentration of homovanillic acid in μg/g fresh tissue and are corrected for recovery. The number of experiments is given in parentheses. Homovanillic acid was estimated after extraction on an ion exchange column. Significance of difference from control value : $*P<0.01$; $\dagger P<0.05$

Drug	Duration of experiment (hr)	Homovanillic acid content (μg/g) for rat colony A	B	C
Control		0·23±0·05 (14)	0·23±0·03 (7)	0·23±0·02 (12)
Reserpine	4	0·30±0·08 (7)	0·43±0·05† (8)	0·46±0·05* (8)
	6	0·60±0·02* (3)		
	8	0·43±0·05* (7)		0·44±0·04* (6)
	16			0·36±0·03* (6)
Chlorpromazine	3	0·75±0·12* (4)		1·65 ; 0·94 (2)
Tetrabenazine	1·5			0·77±0·06* (6)

the time interval used by Juorio & Vogt (1965), show that, in contrast to animals from colony A, rats from colonies B and C responded with an increase in homovanillic acid in the striatum. The increase seen with rats from colony B was only significant at the 5% level but, in a further series of similar experiments in which the homovanillic acid was estimated after paper chromatography, the effect of reserpine on rats of the same colony was highly significant (Table 2). The results given in Table 2 also confirm that

TABLE 2

THE EFFECT OF DRUGS ON THE CONCENTRATION OF DOPAMINE AND HOMOVANILLIC ACID IN THE CORPUS STRIATUM OF THE RAT

Results are expressed as means and standard errors of the concentration in μg/g fresh tissue and are corrected for recovery. Homovanillic acid estimations were made after paper chromatographic separation of extracts of pooled tissues. Dopamine estimations were usually made on a portion of the homogenate used for the estimation of homovanillic acid. The number of experiments is given in parentheses. I.p.=intraperitoneal ; s.c.=subcutaneous. Significance of difference from control values : $*P<0.01$; $\dagger P<0.05$

Colony	Drug	Dose (mg/kg)	Duration of treatment (hr)	Concentration (μg/g) of Homovanillic acid	Dopamine
A	Control			0·17±0·02 (5)	3·0 ±0·3 (8)
A	Reserpine	2 s.c.	4	0·19±0·04 (4)	0·9 (1)
A	Reserpine	5 i.p.	4	0·10 ; 0·17 (2)	0·6 ; 0·6 (2)
A	Dopa	200 i.p.	4	3·30 ; 3·30 (2)	3·2 ±0·4 (6)
B	Control			0·20±0·05 (4)	3·0 ±0·05 (4)
B	Reserpine	2 s.c.	4	0·56±0·03† (4)	
B	Tetrabenazine	50 s.c.	1·5		0·09±0·01* (4)

rats from colony A did not show an increase in homovanillic acid 4 hr after the administration of reserpine, even when a larger dose (5 mg/kg) was used. However, rats from colony A did show an increase in homovanillic acid after 6 and after 8 hr. There was no difference between rats from colonies A and C in the response to chlorpromazine (10 mg/kg); a clear increase in homovanillic acid was seen 3 hr after the injection (Table 1). The effect of tetrabenazine was only tested on rats of colony C. The homovanillic acid content of the striatum was increased after 90 min (Table 1).

Homovanillic acid was identified by scanning the whole chromatograms, developed from extracts of the brains of untreated rats (colonies A and C), of rats treated with reserpine (colony A) and of rats given chlorpromazine (colony C). Fig. 2 shows the distribution of material giving rise to fluorescence on chromatograms of extracts from the brains of normal and reserpine-treated rats of colony A. In addition to confirming the presence of homovanillic acid in these tissues, Fig. 2 shows that there is, in normal animals, another fluorescing acidic substance. This fluorescence was seen in alkaline solution, even without the oxidation with ferric chloride, which was used in these experiments to develop the fluorescence from homovanillic acid. The amount of this substance was much reduced after treatment of the animals with reserpine.

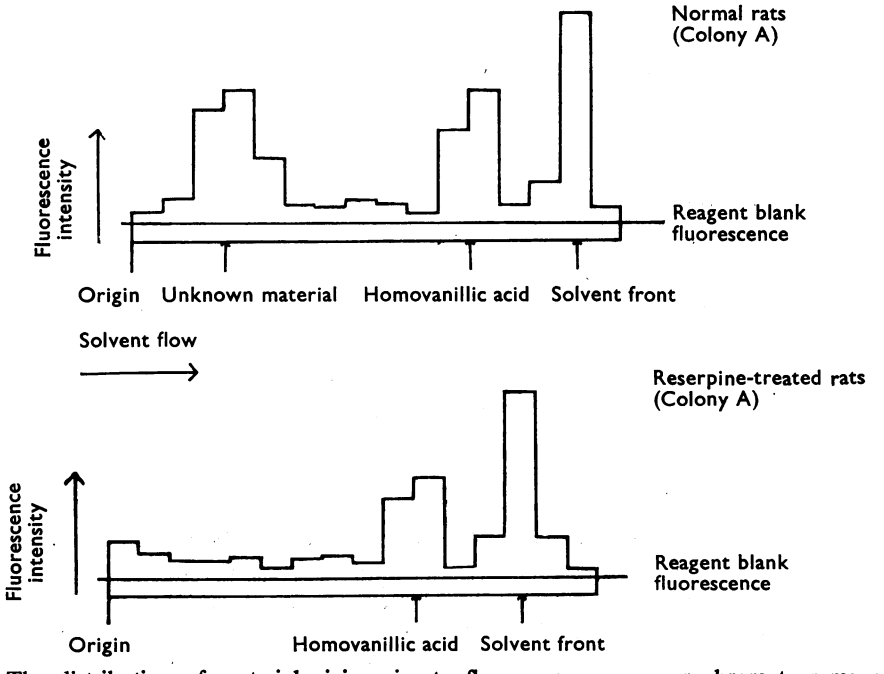

Fig. 2. The distribution of material giving rise to fluorescence on paper chromatograms of acids extracted from the brain of the rat. Fluorescence was developed as described by Sharman (1963a).

Guinea-pig, coypu and Meriones

The effects of reserpine, chlorpromazine and tetrabenazine on the content of homovanillic acid in the striatal tissues of the guinea-pig and the coypu are illustrated in Table 3, which also shows the effects of the first two drugs in *Meriones*. In all instances

the drug treatment resulted in an increase in the content of homovanillic acid. The identity of the homovanillic acid in the brains of normal animals was established in all three species.

TABLE 3

THE EFFECT OF DRUGS ON THE CONCENTRATION OF HOMOVANILLIC ACID IN THE CORPUS STRIATUM OF THREE RODENTS

Results (means and standard errors) are expressed as μg/g fresh tissue and are corrected for recovery. §Homovanillic acid estimations made after paper chromatographic separation. S.c.=subcutaneous ; i.p.=intraperitoneal. Significance of difference from control values : *$P<0.01$; †$P<0.05$

Species	Drug	Dose (mg/kg)	Duration of treatment (hr)	No. of observations	Homovanillic acid content (μg/g)
Guinea-pig	—		—	48	2·68±0·20
	—		—	5	2·65±0·60§
	Reserpine, s.c.	2	2	11	5·36±0·82*
			4	9	6·33±1·02*
			8	11	4·43±0·73*
			16	5	3·41±0·45†
	Chlorpromazine, s.c.	10	2	4	4·78±0·65*
			4	5	4·62±0·35*
			8	5	3·21±0·55
			16	5	1·78±0·21
	Tetrabenazine, s.c.	20	1	11	5·23±0·69*
			2	13	5·78±0·56*
			4	5	5·85±0·48*
			8	5	3·96±0·31†
Coypu	—		—	6	4·02±0·35
	Reserpine, i.p.	4	4	6	7·27±0·87*
	Chlorpromazine, i.p.	10	4	2	11·78 ; 8·58
			8	2	7·45 ; 7·60
	Tetrabenazine, i.p.	20	4	2	10·10 ; 5·42
Meriones	—		—	18	0·54±0·09
	Reserpine, s.c.	2	4	12	1·22±0·26*
			8	5	0·49±0·26
	Chlorpromazine, s.c.	10	4	9	1·71±0·24*

DISCUSSION

The results show that homovanillic acid is a normal metabolite of dopamine in the brains of several rodents. The concentration of this acid is much lower in the striatum of the rat than in other species which have been studied, and this difference is not a result of a greater amount of non-striatal tissue in the piece of brain excised, of which at least one-third was striatal tissue. Significant amounts of homovanillic acid were not detected in cortical tissues. In spite of the large fall in the concentration of dopamine which occurred after reserpine, the increase in homovanillic acid was absent or very small, corresponding at most to about 10% of the fall in the dopamine concentration. This contrasts with the rabbit (Andén *et al.*, 1964) and the cat (Laverty & Sharman, 1965b), in which, 2 hr after the injection of reserpine, the homovanillic acid corresponded to approximately 55% and 33% of the missing dopamine. This suggested that the rat either metabolized dopamine through an alternative pathway or that the homovanillic

acid was more rapidly removed from the brain in this species. That the rat brain is capable of building up high concentrations of homovanillic acid was illustrated by the large increase produced by the administration of DL-dopa (Table 2) and confirms in the rat the observations of Carlsson & Hillarp (1962) on the rabbit. Bertler, Falck & Rosengren (1963) have reported that this treatment results in the formation of dopamine in cells in the walls of the blood-vessels in the brain as well as in nervous tissue, and it is not clear what contribution to the total homovanillic acid content estimated is made by the metabolism of the dopamine in the walls of the cerebral blood-vessels. This objection is, however, not valid for the large rise in homovanillic acid seen after chlorpromazine, which presumably occurs exclusively in nervous tissue. Preliminary experiments on pooled brains of reserpine-treated rats did not reveal any increases in 3-methoxytyramine, 4-hydroxy-3-methoxyphenylethanol or 3,4-dihydroxyphenylacetic acid, known metabolites of dopamine. Their formation would be an alternative reason why, in this species, reserpine causes such a small and delayed increase in the homovanillic acid content of the striatum. When tetrabenazine, which depletes dopamine more rapidly than reserpine, is injected into rats there is a large increase in homovanillic acid. This might be due to the saturation of a mechanism which removes the homovanillic acid from nervous tissue. If such a mechanism were more efficient in the rat than in other species, this would explain the low concentration of homovanillic acid in the striatum of normal and reserpine-treated rats.

In the guinea-pig the time courses of the increases of homovanillic acid after reserpine or chlorpromazine were three times shorter than those observed by Andén *et al.* (1964) in the rabbit. In general, the administration of reserpine or chlorpromazine to mammals results in an increase of the content of homovanillic acid in the striatal tissue, but it is apparent that even within a single species quantitive and temporal differences in this response can occur.

SUMMARY

1. The effect of some tranquillizing drugs on the homovanillic acid content of the striatum has been studied in rat, guinea-pig, coypu and *Meriones*. The administration of reserpine or chlorpromazine produced an increase in the concentration of homovanillic acid in all four species. Tetrabenazine was examined in the first three species ; it also increased the homovanillic acid content of the brain.

2. In the rat the normal level of this acid is low and the increase afer reserpine is small and may be delayed. It is suggested that, in this species, there is a more efficient mechanism for removing homovanillic acid from the brain than in other species.

We would like to thank Dr M. Vogt for her advice and encouragement. We are grateful to Dr. D. R. Maxwell (May & Baker) for the chlorpromazine and Dr J. M. O'Brien (Ciba Laboratories, Horsham, Sussex) for the reserpine. A. V. J. was a Riker Fellow and T. T. a British Council Scholar.

REFERENCES

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

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1963b). 3,4-Dihydroxyphenylacetic acid in rabbit corpus striatum normally and after reserpine treatment. *Life Sciences*, **2**, 319–325.

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 Sciences*, **3**, 149–158.

BERNHEIMER, H. (1964). Distribution of homovanillic acid in the human brain. *Nature* (*Lond.*), **204**, 587–588.

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

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

JUORIO, A. V. & VOGT, M. (1965). The effect of prenylamine on the metabolism of catechol amines and 5-hydroxytryptamine in brain and adrenal medulla. *Brit. J. Pharmacol.*, **24**, 566–573.

LAVERTY, R. & SHARMAN, D. F. (1965a). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Brit. J. Pharmacol.*, **24**, 538–548.

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

PLETSCHER, A., BARTHOLINI, G., BRUDERER, H., BURKARD, W. P. & GEY, K. F. (1964). Chlorinated arylalkylamines affecting the cerebral metabolism of 5-hydroxytryptamine. *J. Pharmacol. exp. Ther.*, **145**, 344–350.

SHARMAN, D. F. (1963a). A fluorimetric method for the estimation of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) and its identification in brain tissue. *Brit. J. Pharmacol.*, **20**, 204–213.

SHARMAN, D. F. (1963b). Homovanillic acid in the caudate nucleus. *J. Physiol.* (*Lond.*), **169**, 95–96*P*.