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The metabolism of orally administered L-DOPA in Parkinsonism

D. B. CALNE, F. KAROUM, C. R. J. RUTHVEN AND M. SANDLER

Medical and Neurological Units, University College Hospital, London W.C.1, and Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London W.6

1. Gas-liquid chromatographic methods were used to measure urinary acidic and alcoholic metabolites of L-DOPA, which had been administered in high oral dosage to patients with postencephalitic and idiopathic Parkinsonism.

2. The output of these compounds was normal before treatment. During drug therapy, large quantities of the dopamine metabolites, homovanillic acid and dihydroxyphenylacetic acid, were excreted but traces only of 4-hydroxy-3-methoxyphenylethanol. Noradrenaline metabolites showed little change in output other than a small increase in 4-hydroxy-3-methoxymandelic acid.

3. Information was obtained about a number of minor routes of degradation which might be implicated in the therapeutic action of L-DOPA. A raised output of *m*-hydroxyphenylacetic acid pointed to *p*-dehydroxylation of dihydroxyphenylacetic acid by gut flora. Evidence of transamination as a minor metabolic pathway was obtained by finding appreciable urinary levels of 4-hydroxy-3-methoxyphenyllactic acid. A keto-acid precursor of this compound may act as competitive inhibitor of an enzyme active in the normal degradation route of tyrosine, *p*-hydroxyphenylpyruvic acid oxidase, for increased amounts of *p*-hydroxyphenyllactic acid, the major metabolic derivative of *p*-hydroxyphenylpyruvic acid, accumulated in the urine during DOPA treatment.

The amino-acid L-dihydroxyphenylalanine (L-DOPA) is now firmly established as precursor of the catecholamines in a wide variety of animal species (for review, see Sandler & Ruthven, 1969). A deficiency of its immediate decarboxylation product, dopamine, in the basal ganglia of the brain has been amply demonstrated in Parkinsonism and may be a contributory factor in its pathogenesis (see Hornykiewicz, 1966). Attempts to redress this deficiency thus appear to be a rational approach to treatment of the disease. Dopamine itself does not traverse the blood-brain barrier in appreciable quantity; however, its precursor, L-DOPA, passes across to be decarboxylated within the brain (Gey & Pletscher, 1964).

The clinical material on which the present investigation is based, was obtained during a " between patient ", " double-blind " therapeutic trial of oral L-DOPA in Parkinsonism (Calne, Stern, Laurence, Sharkey & Armitage, 1969). Although the clinical effect of the drug has now been studied in a number of trials (Calne *et al.*,

1969) the opportunity thus provided to obtain information on its further metabolism has been largely neglected, apart from some limited studies (McGeer, Boulding, Gibson & Foulkes, 1961 ; Sourkes, Pivnicki, Brown, Wiseman-Distler, Murphy, Sankoff & Saint Cyr, 1965 ; Cotzias, Papavasiliou & Gellene, 1969). Rather more is known about the metabolism of L-DOPA in the normal human subject (Guggenheim, 1913 ; Shaw, McMillan & Armstrong, 1957 ; Sourkes *et al.*, 1965 ; Pletscher, Bartholini & Tissot, 1967 ; Smith, 1967).

Hitherto, assay procedures for many of the urinary metabolites of this amino-acid have been difficult and time-consuming. Gas chromatographic methods for urinary phenolic acids and alcohols have recently become available, however (Karoum, Ruthven & Sandler, 1968 ; Karoum & Sandler, 1968 ; Karoum, Anah, Ruthven & Sandler, 1969) ; they are fast and accurate, and with their help it is possible to measure a number of different compounds on the same chromatogram. We have used these techniques to monitor the urinary excretion of some of these compounds in patients taking part in the therapeutic study described by Calne *et al.* (1969) and have obtained biochemical information which had not previously been available.

Methods

Urine specimens (24 hr), with 25 ml. of 6 N HCl as a preservative, were obtained from forty long-term patients (eighteen men and twenty-two women) with post-encephalitic Parkinsonism who were undergoing a " double-blind " therapeutic trial of oral L-DOPA (dose range 0·5–2·5 g/day in divided doses ; mean 1·3 g, for 47 days). Full details of this trial are provided elsewhere (Calne *et al.*, 1969).

Urine collections were made at four stages in the trial: (1) either before commencing placebo or L-DOPA regimes or while control subjects were on placebo (" pre-treatment ") ; (2) some days after starting oral L-DOPA (" under treatment ") ; (3) within 24–48 hr of stopping the L-DOPA regime (" end of treatment ") ; and (4) 2 weeks later (" follow-up ").

Circumstances prevented the systematic analysis of every 24 hr urine collection ; instead analyses were carried out in two series. In the " homogeneous " series, six female subjects were followed through the four stages enumerated above. In the heterogeneous series, the " pre-treatment " group consisted of three males and eleven females ; seven males and five females were " under treatment ", seven females constituted the " end of treatment " and ten females the " follow-up " groups. Some subjects were common to all groups. The full range of metabolites was not measured in every subject in the heterogeneous series ; the number of subjects from whom the mean excretion rate of each metabolite was calculated is shown in parentheses in Table 1.

Urine samples (24 hr) were also collected for analysis from four patients with idiopathic Parkinsonism who were being given 4·25 to 4·75 g L-DOPA per day, orally in divided doses, almost double the maximum dose given to any of the post-encephalitic patients.

Assay procedure

Urinary phenolic acids and alcohols were measured by isothermal gas liquid chromatography (GLC), essentially as described previously (Karoum *et al.*, 1968 ;

Karoum *et al.*, 1969), using a Pye Panchromatograph with a 7 foot 10% SE52 column at 190° C. The acids were usually run as methyl ester/trimethyl silyl ether (ME/TE) derivatives and the alcohols as their trimethyl silyl ether/esters (TE/E). Chromatographic peaks were characterized in terms of methylene unit values (Dalgliesh, Horning, Horning, Knox & Yarger, 1966) and quantified by comparing peak heights in analyses of duplicate urine samples, to one of which an internal standard was added at the start of the procedure.

In samples from patients in the " pre-treatment " or " follow-up " groups, phenolic acids were isolated from 10 ml. of salt saturated urine at pH 2·0 by extracting twice with 25 ml. of ethyl acetate. Phenolic alcohols were extracted from duplicate 10 ml. specimens of urine after incubating overnight with 0·2 ml. of a sulphatase-glucuronidase preparation (Suc d'*Helix pomatia,* Industrie Biologique Français, Gennevilliers en Seine, France) at 37° C and pH 6·3 to hydrolyse conjugated phenols. The urine sample was adjusted to pH 8, saturated with NaCl and extracted twice with 25 ml. of ethyl acetate. Twenty and 25 ml. portions of the successive extracts from each analysis were pooled, evaporated to dryness under vacuum at 40°–50° C and derivatives prepared from the dry residue.

Analysis of specimens from patients in the " under treatment " or " end of treatment " groups was modified in order to assure satisfactory quantification of the large amounts of homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) present and prevent their interference with the estimation of lesser metabolites. HVA and DOPAC were assayed following two successive extractions of 0·5 or 1 ml. urine with 25 ml. of diethyl ether AR after diluting with 10 ml. 0·01 N HCl and saturating with NaCl. Portions of the ether extracts, 20 and 25 ml. respectively, were combined and evaporated and, in this case, trimethyl silyl ether/ester derivatives were prepared. To eliminate DOPAC, which interferes with measurement of 4-hydroxy-3-methoxymandelic acid (VMA) and other phenolic acids at concentrations present, 5 ml. of urine was adjusted to pH 8·4, and 2·5 g of alumina ('Camag', MFC, Hopkin & Williams Ltd.) was added and vortex mixed ('Whirlimixer') for 1 min. After centrifuging and adjusting the pH of the supernatant to 2·0, phenolic acids were extracted with ethyl acetate, isolated and analysed as before. For the estimation of phenolic alcohols a 10 ml. portion of urine adjusted to pH 1 with 6 N HCl was extracted twice with 25 ml. of diethyl ether in order to remove phenolic acids. The extracted urine was heated at 60° C for a few minutes under vacuum to expel traces of solvent and then incubated (37° C) with the sulphatase-glucuronidase preparation after adjusting the pH to 6·3. Following incubation the pH was raised to 8·4, alumina added, mixed, centrifuged, and the phenolic alcohols isolated from the supernatant and analysed as before. The preliminary extraction of the phenolic acids is also likely to remove free alcohols. The amount of free HMPG lost in this way is probably negligible compared with the output of its conjugate for although the derivatives of free HMPG (methylene unit value 18·37) and VMA (methylene unit value 18·43) run closely to each other during GLC, there was neither an alteration in the retention time of VMA nor the presence of a shoulder on the ascending slope of its peak in any sample.

Statistical tests

Means and standard errors (S.E.) of means were calculated for the 24 hr urinary excretion values of each metabolite. *t* Tests were carried out to ascertain the signi-

ficance of differences between treatment groups as well as their relation to the dose of L-DOPA. In the homogeneous series the paired t test was used, the subjects serving as their own controls. Within each group, an examination of the degree of correlation was made for the following variables: selected metabolites, urine volume, dose of L-DOPA in the previous 24 hr and total dose to date of collection of specimen under test.

Each group was regarded as a random sample from its appropriate population and no attempt was made to match patients who appeared in more than one group. It was assumed, but not tested, that these samples were not subject to selection. The results of statistical tests on the small homogeneous series of six patients analysed during all four phases of the investigation support those found in the larger but heterogeneous series.

The " between group " analysis in the heterogeneous series was based on the logarithms of the recorded data as this transformation appeared to create greater similarity of variance within each of the groups. For the analysis of the homogeneous series, the actual readings were used as their range was smaller compared with the unmatched groups.

Differences between means and between correlation coefficients have been accepted as statistically significant if the likelihood of their occurring by chance was equal to or less than 5% ($P \leqslant 0{\cdot}05$).

Results

The results of G.L.C. analysis on the heterogeneous series expressed as mean 24 hr excretion values $\pm$ S.E. of means, together with observed ranges, are given in Table 1 and grouped according to the four collection schedules. Values are given for urinary phenolic acids and 4-hydroxy-3-methoxyphenylglycol (HMPG) and for the ratio of VMA to HMPG. A parallel but extended set of results from the homogeneous series is shown in Table 2.

The urinary excretion of metabolites in postencephalitic Parkinsonian subjects in " pre-treatment " and " follow-up " groups (Tables 1 and 2) did not differ from the normal range (Karoum *et al.*, 1969).

Compared with " pre-treatment " values, a several-hundredfold rise in HVA excretion was observed in both series of analyses while the patients were " under treatment ". It is likely that a small percentage of this high output was represented by the isomer 3-hydroxy-4-methoxyphenylacetic acid the ME/TE derivative of which cannot be separated from that of HVA under the GLC conditions used. However, the 4-hydroxy group of HVA is resistant to methylation during the 1 min exposure to diazomethane used whereas O-methylation tends to occur more readily in the 3-position. The small amount of 3,4-dimethoxyphenylacetic acid which was noted on the gas chromatogram (Fig. 1b) after subjecting the urine extract to the ME/TE procedure but not after the TE/E procedure indicates that the HVA peak was contaminated by a small amount of 3-hydroxy-4-methoxyphenylacetic acid. At the " end of treatment ", the high mean HVA concentrations fell (significantly in the homogeneous series, $P<0{\cdot}025$) to about two-thirds of the " under treatment " mean value, and had returned to normal in all subjects tested by " follow-up ".

TABLE 1. *Excretion of urinary phenolic acids and alcohols in the heterogeneous series*

Metabolite	"Pre-treatment" mg/24 hr		"Under treatment" mg/24 hr		"End of treatment" mg/24 hr		"Follow-up" mg/24 hr	
	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range
HVA	3·1± 0·33 (14)	1·6–6·0	201±38 (12)	51–460	140±66 (7)	12–505	2·4±0·41 (10)	1·0–5·1
Free DOPAC	<2·0	—	195±33 (12)	30–390	124±45 (7)	13–365	—	—
VMA	4·6±0·54 (14)	1·4–9·3	7·3±0·75 (11)	2·7–11·8	2·6±0·83 (6)	0·6–5·1	3·4±0·37 (10)	1·4–5·1
Total HMPG	2·2±0·32 (9)	0·8–3·5	1·5±0·28 (10)	0·2–3·2	1·0±0·16 (7)	0·7–1·4	1·2±0·14 (10)	0·6–2·0
VMA/HMPG ratio	2·5±0·5 (9)	0·4–4·9	6·6±1·20 (10)	2·3–14·0	2·5–0·60 (6)	0·8–3·6	3·2±0·52 (10)	0·9–6·0

Number of patients in parentheses.
HVA, 4-hydroxy-3-methoxyphenylacetic acid, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VMA, 4-hydroxy-3-methoxymandelic acid; HMPG, 4-hydroxy-3-methoxyphenylglycol.

TABLE 2. *Excretion of urinary phenolic acids and alcohols in the homogeneous series*

Metabolite	"Pre-treatment" mg/24 hr		"Under treatment" mg/24 hr		"End of treatment" mg/24 hr		"Follow-up" mg/24 hr	
	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range	Mean ±S.E. of mean	Observed range
HVA	2·7±0·27	1·8–3·8	271±48	135–460	161±73	33–505	2·9±0·53	1·7–5·1
Free DOPAC	<2·0	—	217±50	151–336	142±21	13–365	<2·0	—
VMA	4·6±0·27	3·5–5·3	6·6±0·92	3·0–9·1	2·6±0·83	0·6–5·1	3·9±0·40	2·4–4·9
Total HMPG	1·4±0·16	0·9–1·9	1·1±0·25	0·5–2·2	1·0±0·18	0·4–1·4	1·2±0·20	0·6–1·5
p-Hydroxyphenyl-acetic acid	7·4±1·52	3·2–13·9	8·8±2·18	2·1–16·7	8·5±1·79	2·1–15·6	7·5±2·30	3·0–18·5
m-Hydroxyphenyl-acetic acid	1·7–0·51	0·9–4·0	5·1±1·32	2·6–10·4	5·8±1·88	2·1–14·3	2·1±0·24	1·1–2·5
VLA	<0·1	—	27·5±16·3	2–108	12·3±10·6	0·5–66	<0·1	—
VMA/HMPG ratio	3·3±0·33	2·7–4·8	6·3±0·85	4·1–9·0	2·5±0·60	0·8–4·5	3·6±0·78	1·8–6·1

Six patients. HVA, 4-hydroxy-3-methoxyphenylacetic acid, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VMA, 4-hydroxy-3-methoxymandelic acid; HMPG, 4-hydroxy-3-methoxyphenylglycol; VLA, 4-hydroxy-3-methoxyphenyllactic acid.

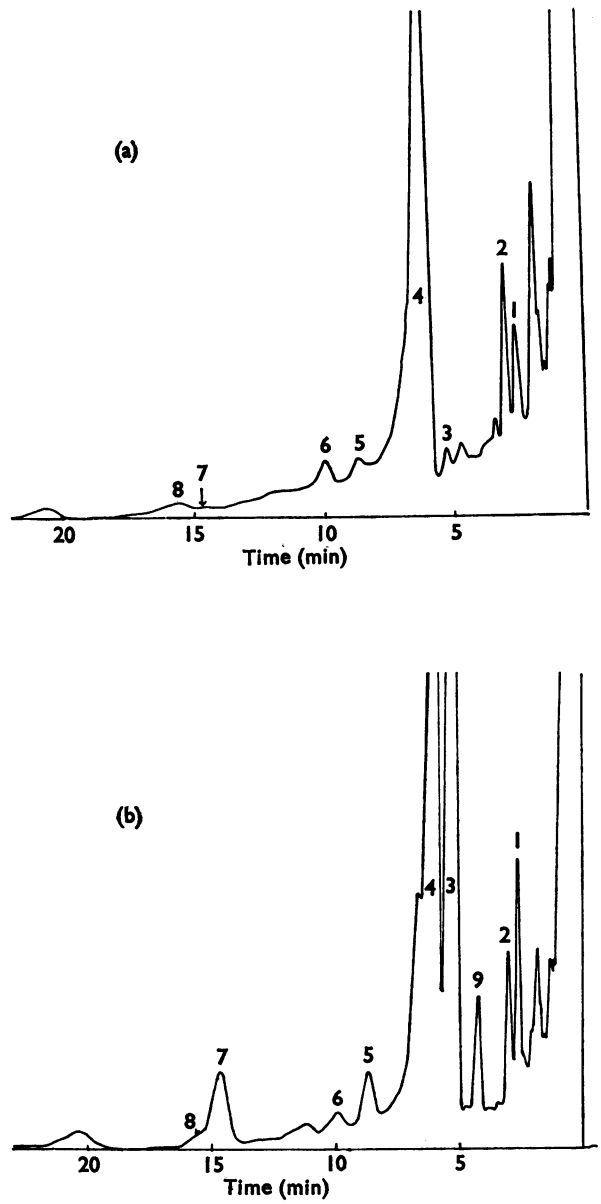

FIG. 1. (a) Gas chromatogram (7 foot, 10% SE 52 column) of methyl ester/trimethyl silyl ether derivatives of phenolic acids prepared from an ethyl acetate extract of urine (10 ml.) from a patient with postencephalitic Parkinsonism before treatment with L-DOPA. Peak identification: 1, *m*-hydroxyphenylacetic acid; 2, *p*-hydroxyphenylacetic acid; 3, homovanillic acid (HVA); 4, hippuric acid; 5, *p*-hydroxyphenyllactic acid; 6, 4-hydroxy-3-methoxymandelic acid (VMA); 7, 4-hydroxy-3-methoxyphenyllactic acid (VLA); 8, *p*-hydroxyphenylpyruvic acid. (b) Gas chromatogram (7 foot, 10% SE 52 column) of methyl ester/trimethyl silyl ether derivatives of urinary phenolic acids from the same patient as in (a) while under treatment with L-DOPA. Urine (10 ml.) at pH 8·4 was shaken with alumina to remove DOPAC before extracting at pH 2 with ethyl acetate. Peak identification: 1–8 as in (a); 9, 3,4-dimethoxyphenylacetic acid (artefact from O-methylation of 3-hydroxy-4-methoxyphenylacetic acid—see text).

There was a very similar high output of free DOPAC " under treatment " which fell considerably at the " end of treatment ", returning to normal in the " follow-up " samples. In both the " under treatment " and " end of treatment " groups, there was a strong positive correlation ($P<0.01$) between output of HVA and DOPAC. The total dose of the drug up to the time of urine sampling and the dose in the 24 hr period preceding urine collection were positively correlated with HVA and DOPAC excretion values in both the " under treatment " and " end of treatment " groups. There was also a positive correlation ($P<0.02$) between urinary HVA or DOPAC output and urine volume in the " end of treatment " group.

Although 4-hydroxy-3-methoxyphenylethanol (HMPE) excretion in the " under treatment " or " end of treatment " groups was small compared with output of HVA or DOPAC, it represents a substantial increase over the small quantities (less than 100 μg/24 hr) found in normal urine. In four subjects from the heterogeneous series " under treatment ", excretion rates of 0·5, 1, 2 and 2 mg HMPE/24 hr were recorded, whilst three others belonging to the " end of treatment " group in this series had an output of 0·25, 0·5 and 0·5 mg HMPE/24 hr. Althougth the absolute increase in urinary VMA was not as dramatic in patients " under treatment " when compared with that of HVA and DOPAC, it was still significant (heterogeneous series $P<0.02$; homogeneous series $P\simeq 0.05$).

There was a positive correlation ($P<0.02$) between HMPG and VMA output in patients in the heterogeneous series " under treatment ", although a similar relationship was not observed in any of the other groups. The mean ratio of the excretion values of VMA to HMPG in " under treatment " groups in both series was higher than in any of the other groups (Tables 1 and 2). In the homogeneous series, this rise was significant ($P<0.02$) for comparisons between " under treatment " and " pre-treatment " or " end of treatment " groups. Inspection of ratios in the heterogeneous series suggested a similar relationship.

4-Hydroxy-3-methoxyphenyllactic acid (VLA) was almost undetectable (less than 100 μg/24 hr) in urine from " pre-treatment " and " follow-up " groups but was excreted in large amount (Table 2) by patients " under treatment " (Fig. 1). 4-Hydroxy-3-methoxyphenylpyruvic acid (VPA) could not be detected in any sample.

Oral L-DOPA did not affect the excretion of *p*-hydroxyphenylacetic acid which remained within the range previously encountered by us (unpublished) in normal

TABLE 3. *Excretion of urinary phenolic acids and alcohols in high dosage* (4·25–4·75 *g* L-*DOPA per day*) *series*

Metabolite	Mean mg/24 hr	Observed range mg/24 hr
HVA	1,707	1,290–2,820
Free DOPAC	2,135	1,110–3,400
VMA	10·0	9·5–10·5
Total HMPG	4·5	2·9–7·1
p-Hydroxyphenylacetic acid	10	5–13
m-Hydroxyphenylacetic acid	14	12–16
VLA	41	32–54
HMPE	1·5	0·4–3·3
VMA/HMPG ratio	2·5	1·4–3·4

Four patients. HVA, 4-hydroxy-3-methoxyphenylacetic acid, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; VMA,4-hydroxy-3-methoxymandelic acid; HMPG, 4-hydroxy-3-methoxyphenylglycol; VLA, 4-hydroxy-3-methoxyphenyllactic acid; HMPE, 4-hydroxy-3-methoxyphenylethanol.

individuals throughout the investigation. In contrast there was a significant increase in output of *m*-hydroxyphenylacetic acid (Table 2, Fig. 1) in both " under treatment " and " end of treatment " groups when compared with " pre-treatment " ($P<0{\cdot}03$) and " follow-up " ($P<0{\cdot}05$) groups.

The range of metabolites measured in the homogeneous series was also estimated in a small group of patients with idiopathic Parkinsonism on a higher dosage of L-DOPA (Table 3). Apart from *p*-hydroxyphenylacetic acid, the value of each was greater than after treatment with the lower dosage. *m*-Hydroxyphenylacetic acid excretion was so increased as to be greater than that of its *p*-isomer ; its output is normally considerably less (Fig. 1).

The excretion of *p*-hydroxyphenyllactic acid (*p*HPLA) was measured in the homogeneous series and in the four patients on higher dosage of L-DOPA. With the exception of one atypical case, patients in the homogeneous series excreted less than 0·5 mg *p*HPLA/24 hr in the " pre-treatment " and " follow-up " periods. Whilst " under treatment ", however, four of these patients put out increased amounts of the acid, ranging from 0·9–4·8 mg/24 hr (mean 2·4 mg/24 hr). The patients on higher dosage tended to excrete slightly more, with values ranging from 1·6 to 4·7 mg/24 hr (mean 2·8 mg/24 hr). The atypical patient excreted about 2 mg *p*HPLA daily, even when not on L-DOPA, the output rising as high as 23 mg/24 hr " under treatment " and dropping to 11 mg/24 hr at the " end of treatment ". This patient also excreted by far the highest concentration of VLA whilst " under treatment ". In general, the excretion of *p*-HPLA tended to rise in parallel with VLA output.

Discussion

Since the first sighting observations on L-dihydroxyphenylalanine (L-DOPA) metabolism in man and rabbit by Guggenheim (1913), its major pathways of degradation have become well established (Sandler & Ruthven, 1969). The greater proportion is decarboxylated to dopamine which is either β-hydroxylated to noradrenaline or, in common with the other catecholamines, inactivated by two alternative routes, involving the primary action of either catechol O-methyltransferase or monoamine oxidase. Homovanillic acid (HVA) which results from the action of both enzymes on dopamine, is quantitatively the most important endogenous DOPA metabolite in normal human urine.

The " pre-treatment " data presented here which do not appear to differ from normal values (Karoum *et al.*, 1969), together with the previous finding of a normal HVA output in this disease group (Greer & Williams, 1963), argue against there being a generalized disturbance of dopamine metabolism in Parkinsonism. The claim of Barbeau, Murphy & Sourkes (1961), pointing to a contrary conclusion, has never been confirmed. Whether certain unidentified chromatographic peaks noted in " pre-treatment " and " follow-up " urine samples but not in normal urine are in any way connected with the pathogenesis of the disease is still under investigation.

Sourkes, Pivnicki, Brown, Wiseman-Distler, Murphy, Sankoff & Saint Cyr (1965) gave L-DOPA to one postencephalitic Parkinsonian subject and noted some delay in the rise in urinary HVA excretion compared with controls ; but, in general, our own findings in patients with this disease during treatment are not too dissimilar from those following L-DOPA administration to normal subjects (Shaw, McMillan & Armstrong, 1957 ; Sourkes *et al.*, 1965). There was a very large increase in urinary excretion of HVA which was equalled (Table 1), and at high dosage even exceeded

(Table 3), by 3,4-dihydroxyphenylacetic acid (DOPAC) output. This increase in relative concentration of DOPAC appears to depend to some extent on route of administration (Sourkes *et al.*, 1965).

It is of interest that children with dopamine-secreting tumours may sometimes put out as much HVA as the patients "under treatment" (Table 1). DOPAC excretion, on the other hand, although well above normal concentrations, forms a proportionately smaller part of the total metabolite output (von Studnitz, 1960; Sourkes, Denton, Murphy, Chavez & Saint Cyr, 1963). This finding implies that the site of metabolic degradation of this endogenously secreted dopamine differs from that generated from exogenously administered DOPA.

There are other points of difference from dopamine-secreting tumours. The intermediate aldehyde produced by oxidative deamination of dopamine is preferentially oxidized further to HVA rather than reduced to 4-hydroxy-3-methoxyphenylethanol (HMPE) (Breese, Chase & Kopin, 1969); during the present investigation, an even smaller proportion of HMPE was found after DOPA treatment than is observed in cases of dopamine-secreting tumour (Karoum, Anah, Ruthven & Sandler, unpublished). von Studnitz (1967) was unable to detect the excretion of the 3-hydroxy-4-methoxy isomer of HVA in patients with such tumours but there was indirect chemical evidence that small amounts were put out in the Parkinsonian subjects "under treatment". This latter finding agrees with a previous claim (Smith, 1967).

A further difference concerns the degree of β-hydroxylation of the side chain. Apart from metabolites derived from the direct metabolism of dopamine, patients with dopamine-secreting tumours almost invariably have a large output of the β-hydroxylated (noradrenaline) series of metabolites with a relatively large contribution from the aldehyde reduction product, 4-hydroxy-3-methoxyphenylglycol (HMPG). Oral DOPA treatment, however, resulted in only a comparatively small increase in 4-hydroxy-3-methoxymandelic acid (VMA) and an increased VMA/HMPG ratio. In the light of these differences, it seems likely that further metabolism of dopamine involving β-hydroxylation takes place largely within the tumour tissue of affected subjects and not at sites remote from it (Sandler & Ruthven, 1966).

The gas chromatographic techniques used have permitted accurate quantitative studies to be performed for the first time on a number of other minor metabolic routes of L-DOPA metabolism in addition to that of HMPE formation. DeEds, Booth & Jones (1957) found that DOPA administration to rabbits gave rise to an increased urinary output of *m*-hydroxyphenylacetic acid, presumably by *p*-dehydroxylation of DOPAC brought about to a large extent by gut flora (Scheline, 1968). Although Shaw *et al.* (1957) were not able to obtain any evidence for the existence of this pathway in man, Booth, Emerson, Jones & DeEds (1957) and Shaw, Gutenstein & Jepson (1961) produced evidence pointing to *p*-dehydroxylation of another catechol acid, caffeic acid, and we have now been able to show that a small but significant proportion of L-DOPA is degraded by this route. There was no evidence of *m*-dehydroxylation, however, as the output of *p*-hydroxyphenylacetic acid was unchanged.

Shaw *et al.* (1957) discussed the possible existence of an alternative pathway of DOPA metabolism via transamination, although they were not able to provide any direct evidence for it. For many years, it has been known that L-DOPA can participate in transamination reactions (Cammarata & Cohen, 1950), and Fonnum, Haavaldsen & Tangen (1964) have characterized several DOPA transaminases in

brain. The evidence now seems to point fairly strongly to an *in vivo* metabolic sequence initiated by DOPA transamination. The immediate product, 3,4-dihydroxyphenylpyruvic acid (DHPPA), is probably O-methylated to 4-hydroxy-3-methoxyphenylpyruvic acid (VPA). An alternate route to VPA would involve the initial O-methylation of DOPA to 3-O-methylDOPA, followed by transamination to VPA. Smith (1967) described an increased urinary excretion of the unstable VPA but had no evidence of its more stable reduction product 4-hydroxy-3-methoxyphenyllactic acid (VLA), after administration of L-DOPA to volunteers. Despite the sensitivity of our methods and the considerably higher dosage range of L-DOPA employed, we were unable to detect VPA in any sample. There was a considerable increase, however, of VLA, its reduction product (Weber & Zannoni, 1966; Zannoni & Weber, 1966), confirming earlier observations in patients with DOPA-secreting tumours (Gjessing, 1963; Smith, 1965). We would even postulate that its presence is indicative of a sufficient production of DHPPA or VPA to interfere with the normal metabolism of tyrosine. The major metabolite of tyrosine metabolism is *p*-hydroxyphenylpyruvic acid (*p*-HPPA) which is normally further metabolized by *p*-HPPA-oxidase (La Du, 1966), undergoing the recently described "NIH shift" (Guroff, Daly, Jerina, Renson, Witkop & Udenfriend, 1967), to 2,5-dihydroxyphenylacetic acid (homogentisic acid). Our finding of an increased output of the immediate reduction product of *p*-HPPA, *p*-HPLA, which tends to correlate with VLA output, suggests to us that DHPPA or VPA may compete with *p*-HPPA for *p*-HPPA-oxidase. If this were so, DHPPA or VPA might itself be expected to be metabolized to 2,4,5-trihydroxyphenylacetic or 2,5-dihydroxy-4-methoxyphenylacetic acid. We hope to make a careful search for compounds of this type as soon as authentic reference samples become available to us. It has been suggested that compounds with a 2,4,5-substitution pattern which appear to possess high psychotomimetic activity may be implicated in the pathogenesis of schizophrenia (Shulgin, Sargent & Naranjo, 1969).

Whilst it might seem that we have placed disproportionate stress on the existence of minor pathways of L-DOPA metabolism, it must not be forgotten that the time course of the therapeutic response to this drug (Calne *et al.*, 1969; Cotzias, Papavasiliou & Gellene, 1969) is slow. As there is indirect evidence to indicate that dopamine generation within the human central nervous system is rapid (Pletscher, Bartholini & Tissot, 1967), we cannot rule out the possibility that the clinical effect derives not from dopamine replacement but from the build-up of some minor metabolite unconnected with the main route of DOPA degradation.

The studies described in this paper were confined to a series of measurements on the acidic and alcoholic end-results of L-DOPA metabolism in the whole organism. We are well aware, however, that any therapeutic effect is likely to derive from metabolic changes localized to a small area of the brain. Pletscher *et al.* (1967) have observed appreciable amounts of labelled HVA in cerebrospinal fluid after ^{14}C-DOPA administration in man and it seems likely that metabolite concentrations in cerebrospinal fluid may mirror the metabolism of DOPA in the brain more faithfully than urinary levels. Parallel studies on amino-acid and amine excretion in these patients are in progress.

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REFERENCES

BARBEAU, A., MURPHY, C. F. & SOURKES, T. L. (1961). Excretion of dopamine in diseases of basal ganglia. *Science, N.Y.*, **133**, 1706–1707.

BOOTH, A. N., EMERSON, O. H., JONES, F. T. & DEEDS, F. (1957). Urinary metabolites of caffeic and chlorogenic acids. *J. biol. Chem.*, **229**, 51–59.

BREESE, G. R., CHASE, T. N. & KOPIN, I. J. (1969). Metabolism of some phenylethylamines and their β-hydroxylated analogs in brain. *J. Pharmac. exp. Ther.*, **165**, 9–13.

CALNE, D. B., STERN, G. M., LAURENCE, D. R., SHARKEY, J. & ARMITAGE, P. (1969). L-DOPA in postencephalitic Parkinsonism. *Lancet*, **1**, 744–747.

CAMMARATA, P. S. & COHEN, P. P. (1950). Scope of transamination reaction in animal tissues. *J. biol. Chem.*, **187**, 439–452.

COTZIAS, G. C., PAPAVASILIOU, P. S. & GELLENE, R. (1969). Modification of Parkinsonism: chronic treatment with L-DOPA. *New Engl. J. Med.*, **280**, 337–345.

DALGLIESH, C. E., HORNING, E. C., HORNING, M. G., KNOX, K. L. & YARGER, K. (1966). A gas-liquid-chromatographic procedure for separating a wide range of metabolites occurring in urine or tissue extract. *Biochem. J.*, **101**, 792–810.

DEEDS, F., BOOTH, A. N. & JONES, F. T. (1957). Methylation and dehydroxylation of phenolic compounds by rats and rabbits. *J. biol. Chem.*, **225**, 615–621.

FONNUM, F., HAAVALDSEN, R. & TANGEN, O. (1964). Transamination of aromatic amino acids in rat brain. *J. Neurochem.*, **11**, 109–118.

GEY, K. F. & PLETSCHER, A. (1964). Distribution and metabolism of DL-3,4-dihydroxy (2-^{14}C) phenylalanine in rat tissues. *Biochem. J.*, **92**, 300–308.

GJESSING, L. R. (1963). Studies of functional neural tumours V. Urinary excretion of 3-methoxy-4-hydroxy-phenyl-lactic acid. *Scand. J. clin. Lab. Invest.*, **15**, 649–653.

GREER, M. & WILLIAMS, C. M. (1963). Dopamine metabolism in Parkinson's disease. *Neurology, Minneap.*, **13**, 73–78.

GUGGENHEIM, M. (1913). Dioxyphenylalanin, eine neue Aminosäure aus Vicia faba. *Hoppe-Seyler's Z. physiol. Chem.*, **88**, 276–284.

GUROFF, G., DALY, J. W., JERINA, D. M., RENSON, J., WITKOP, B. & UDENFRIEND, S. (1967). Hydroxylation-induced migration: the NIH shift. *Science, N.Y.*, **157**, 1524–1530.

HORNYKIEWICZ, O. (1966). Dopamine (3-hydroxytyramine) and brain function. *Pharmac. Rev.*, **18**, 925–964.

KAROUM, F., ANAH, C. O., RUTHVEN, C. R. J. & SANDLER, M. (1969). Further observations on the gas chromatographic measurement of urinary phenolic and indolic metabolites. *Clinica chim. Acta*, **24**, 341–348.

KAROUM, F., RUTHVEN, C. R. J. & SANDLER, M. (1968). Gas chromatographic measurement of phenolic acids and alcohols in human urine. *Clinica chim. Acta*, **20**, 427–437.

KAROUM, F. & SANDLER, M. (1968). Gas chromatographic measurement of phenolic and indolic acids and alcohols in human urine. *Proc. Ass. clin. Biochemists*, **5**, 123–124.

LA DU, B. N. (1966). Tyrosinosis. In *The Metabolic Basis of Inherited Disease*. Ed. Stanbury, J. B., Wyngaarden, J. B. and Fredrickson, D. S., 2nd ed., pp. 295–302. New York: McGraw-Hill.

MCGEER, P. L., BOULDING, J. E., GIBSON, W. C. & FOULKES, R. G. (1961). Drug-induced extrapyramidal reactions. *J. Am. med. Ass.*, **177**, 665–670.

PLETSCHER, A., BARTHOLINI, G. & TISSOT, R. (1967). Metabolic fate of L-(^{14}C) DOPA in cerebrospinal fluid and blood plasma of humans. *Brain Res.*, **4**, 106–109.

SANDLER, M. & RUTHVEN, C. R. J. (1966). 4-Hydroxy-3-methoxyphenylglycol and other compounds in neuroblastoma. In *Recent Results in Cancer Research. II Neuroblastomas. Biochemical Studies*, ed. Bohuon, C., pp. 55–59. Berlin: Springer-Verlag.

SANDLER, M. & RUTHVEN, C. R. J. (1969). The biosynthesis and metabolism of the catecholamines. In *Progress in Medicinal Chemistry*, ed. Ellis, G. P. and West, G. B., vol. 6, pp. 200–265. London: Butterworth.

SCHELINE, R. R. (1968). Drug metabolism by intestinal microorganisms. *J. pharm. Sci.*, **57**, 2021–2037.

SHAW, K. N. F., GUTENSTEIN, M. & JEPSON, J. B. (1961). Intestinal flora and diet in relation to *m*-hydroxyphenyl acids of human urine. *Proc. 5th int. Cong. Biochem., Moscow*, vol. 9, p. 427. Oxford: Pergamon.

SHAW, K. N. F., MCMILLAN, A. & ARMSTRONG, M. D. (1957). The metabolism of 3,4-dihydroxyphenylalanine. *J. biol. Chem.*, **226**, 255–266.

SHULGIN, A. T., SARGENT, T. & NARANJO, C. (1969). Structure–activity relationships of one-ring psychotomimetics. *Nature, Lond.*, **221**, 537–541.

SMITH, P. (1965). Pathological excretion of 4-hydroxy-3-methoxyphenyllactic acid. *Nature, Lond.*, **205**, 1236.

SMITH, P. (1967). Metabolism of dihydroxyphenylalanine in human subjects. *Nature, Lond.*, **213**, 802–803.

SOURKES, T. L., DENTON, R. L., MURPHY, G. F., CHAVEZ, B. & SAINT CYR, S. (1963). The excretion of dihydroxyphenylalanine, dopamine and dihydroxyphenylacetic acid in neuroblastoma. *Pediatrics, Springfield*, **31**, 660–668.

SOURKES, T. L., PIVNICKI, D., BROWN, W. T., WISEMAN-DISTLER, M. H., MURPHY, G. F., SANKOFF, I. & SAINT CYR, S. (1965). A clinical and metabolic study of dopa (3,4-dihydroxyphenylalanine) and methyldopa in Huntington's chorea. *Psychiatria Neurol.*, **149**, 7–27.

VON STUDNITZ, W. (1960). Methodische und klinische Untersuchungen über die Ausscheidung der 3-Methoxy-4-hydroxymandelsäure im Urin. *Scand. J. clin. Lab. Invest.*, **12**, suppl. 48, 1–73.

VON STUDNITZ, W. (1967). Zur Frage des Vorkommens von 3-Hydroxy-4-methoxy-mandelsäure beim normalen und gesteigerten Katecholaminstoffwechsel. *Klin. Wschr.*, **45**, 307–308.

WEBER, W. W. & ZANNONI, V. G. (1966). Reduction of phenylpyruvic acids to phenyllactic acids in mammalian tissues. *J. biol. Chem.*, **241**, 1345–1349.

ZANNONI, V. G. & WEBER, W. W. (1966). Isolation and properties of aromatic α-keto acid reductase. *J. biol. Chem.*, **241**, 1340–1344.

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