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Intestinal Decarboxylation of L-Dopa in Relation to Dose Requirement in Parkinson's Disease

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Summary. The magnitude of the decarboxylation of L-Dopa in the intestinal organs was determined by a method based on oral and intravenous administration of L-3′,4′-dihydroxyphenylalanine-1-^{14}C (carboxyl labelled L-Dopa) and analysis of the radioactivity in urine.

In 11 parkinsonian patients studied under standardized conditions 74% of L-Dopa given orally as a 0.5 g tablet was decarboxylated in the intestinal organs. Thus less than 26% of the ingested L-Dopa reached the general circulation.

The possibility that individual variations in the magnitude of intestinal decarboxylation might be at least partly responsible for the individual variations in the dose requirement of parkinsonian patients was tested. There was neither any correlation between dose requirement of L-Dopa and the intestinal decarboxylation nor between that dose and the plasma disappearance rate of L-Dopa.

It is concluded that other factors than those responsible for the peripheral metabolism of L-Dopa determine the individual dose requirement.

Key words: L-Dopa — Parkinsonism — Metabolism — Absorption — Decarboxylation.

L-Dopa is nowadays widely used for the treatment of Parkinsonism. The maintenance dose of L-Dopa giving optimal therapeutical response shows great individual variation. In a patient material treated in our hospital the dose varied between 2 and 8 g per day (Granerus *et al.*, 1972). The reason for these great variations is not known (Bianchine *et al.*, 1971).

In a preliminary report from our laboratories (Bergmark *et al.*, 1972) there was shown that a large proportion of the orally administered L-Dopa is decarboxylated already in the intestinal organs. Indirect evidence for such a decarboxylation in the human digestive tract has previously been given by Tissot *et al.* (1969). We determined the magnitude of this decarboxylation by a method based on oral and intravenous administration of L-3′,4′-dihydroxyphenylalanine-1-^{14}C and analysis of the radioactivity in urine. In the present paper this method will be described in detail. Furthermore, the possibility that individual variations in the magnitude of intestinal decarboxylation might be at least partly responsible for the variations in the dose requirement will be discussed.

Material and Methods

Material. The patient material included 12 Parkinsonian patients. In one 80 years old female patient treated with L-Dopa for 2 years, the effect of orally administered L-Dopa on the elimination rate of a tracer dose of intravenously administered L-Dopa was studied. In the remaining 11 patients the intestinal decarboxylation of L-Dopa was determined by the method to be described below. Their average age was 64 ± 6.9 years (mean ± S.D.), body weight 65 ± 13.1 kg and dose requirement of L-Dopa 3.3 ± 1.38 g per day. (Seven patients were treated with Dopastral®, 4 patients with Larodopa®). The duration of Parkinsonian symptoms varied between 4—32 years and was on an average 12 ± 7.8 years, and the duration of treatment with L-Dopa was 27 ± 4.7 months. Stereotactic operations had been performed bilaterally in one patient and unilaterally in one patient.

All the patients were also treated with anticholinergic drugs. No patient revealed symptoms of diseases in the gastro-intestinal tract but one patient (GT) had achylia (adequately treated pernicious anemia).

The material included patients with varying degrees of physical handicap caused by the Parkinsonism. In all patients the L-Dopa treatment had given significant improvement and had moved the patients at least one step between the earlier described functional disability groups (Granerus *et al.*, 1972).

One apparently healthy individual (I.E.), a 57 year old woman, was also investigated (Table 1). At the processing of the results some of the observation in the preliminary study (Bergmark *et al.*, 1972) were included (Figs. 4, 5, Table 1).

Methods. L-3′,4′-Dihydroxyphenylalanine-1-^{14}C (carboxyl labelled L-Dopa) was administered intravenously and orally. The subjects were thus studied on 2 occasions. On the first occasion the labelled compound (about 23 μCi) was given in 10 ml saline containing about 1 mg of L-Dopa as a rapid intravenous injection. An aliquot of this solution was used for determination of the exact amount of radioactivity given. Half an hour before the injection a 0.5 g tablet of L-Dopa (Larodopa®, Hoffman-La Roche) was given. Six or seven days later, the labelled compound (46 μCi) was given orally in a tablet containing 0.5 g of L-Dopa, kindly prepared by Hoffman-La Roche. The radioactivity administered was determined by analysis of two of the tablets from the same bach. Each tablet was dissolved in 500 ml of water by gently warming before analysis. The radioactivity found agreed with that stated by the company *i.e.*, 46 μCi per tablet. The purity of the labelled compound was tested by chromatography. More than 98% of the radioactivity of the solution given intravenously and of the tablet was found in a symmetrical peak emerging at the

Table 1. Cumulative recovery of ^{14}C-activity in expired air and urine after intravenous and oral administration of L-3′,4′-dihydroxyphenylalanine-1-^{14}C. The figures given within brackets are extrapolated values

Subject	Cumulative recovery of ^{14}C-activity (% of dose)				
	Expired air			Urine	
	I.v. adm. 3 h	8 h	Oral adm. 8 h	I.v. adm. 72 h	Oral adm. 72 h
Patient GT	36	(49)	70	24.8	6.1
Control TM	48	(60)	77	29.2	7.3
Control IE	52	(64)	74	32.7	8.5

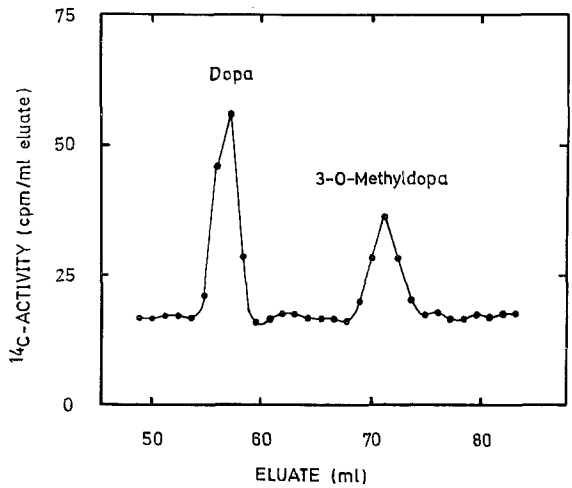

Fig. 1, Chromatographic separation of labelled dopa and 3-O-methyldopa in plasma. The blood was drawn 60 min after the intravenous injection of 25 μCi L-3′,4′-dihydroxyphenylalanine-1-^{14}C. For details of the method see text

position of Dopa in the ion-exchange chromatographic method to be described below (Fig. 1).

L-Dopa tablets were always given together with a standardized breakfast consisting of one glass of milk and 2 slices of bread and butter.

For the first 180 min after the intravenous L-Dopa administration the patients were immobilized in a recumbent position. Repeated blood samples were drawn from the antecubital vein into 10 ml tubes containing 25 mg of disodium-ethylendiaminetetraacetate (EDTA) in the period 60—180 min after the injection. The blood was immediately put in a refrigerator and centrifuged within 2 h after the collection. One aliquot (1 ml) of the plasma was used for determination of total radioactivity. To another aliquot (4 ml) 4 M perchloric acid (0.4 ml) was added. The total radioactivity was determined in an aliquot (1 ml) of the supernatant and the remainder was used for ion-exchange chromatography. When the labelled L-Dopa was administered orally, blood sampling was not performed and the patients were allowed to keep to their ordinary physical activity in order to achieve their normal gastrointestinal motility. However, in the few experiments, which included measurement of $^{14}CO_2$ in expired air (Table 1), the subjects had to be immobilized and in these experiments blood specimens were also drawn.

When not stated otherwise urine was collected for 3 days, following the administration of the labelled L-Dopa. One aliquot of the urine sample was acidified to pH 2. Both acidified and untreated urine samples were kept at −20° C for a few days before analysis.

Chromatographic Separation of Labelled Dopa and 3-O-Methyldopa. An amino acid analyzer (Biocal Instrument, model 200 BC) was used for the separation. The column dimensions was 0.9 × 55 cm. Aminex A6 (Biorad Laboratories) was used as resin. The temperature of the column was 55° C, the buffer pH 4.50, (0.2 molar citrate buffer) and the flow rate 70 ml/h. The eluate was not mixed with any reagent but collected in fractions of 1.16 ml (1 min fractions). One ml of each fraction

was used for the determination of radioactivity, Dopa (elution volume 57 ml) and 3-0-methyldopa (elution volume 71 ml) emerged as narrow, symmetrical peaks completely separate from each other (Fig. 1).

^{14}C-Activity in Solutions. The ^{14}C-activity in solutions was measured with a Packard-Tri-Carb liquid scintillation counter. One ml of the sample to be analysed was added to 10 ml Instagel®. The samples were counted for 30 min which gave a statistical error of less than $\pm$ 2% at the counting of total activity in plasma and urine and of about $\pm$ 10% for each peak (generally 5—6 fractions) at the counting of the fractions from the ion-exchange chromatography (see Fig. 1).

The efficiency was similar for plasma, urine, acid filtrate and effluent from the column and varied between 82 and 85% under the conditions used. The efficiency was determined daily by the use of internal standards (toluene-^{14}C, 4.17×10^5 dpm/ml, New England Nuclear, and a solution of L-3′-4′-dihydroxyphenylalanine-^{14}C, 1.00×10^6 dpm/ml). The efficiency was determined for each urine sample as there was slight variation due to differences in their colour.

^{14}C-Activity in Expired Air. The radioactivity appearing as $^{14}CO_2$ in the expired air was measured by an apparatus for continuous measurement of $^{14}CO_2$ (Frieseke Hoepfner Exhalationsmeßgerät FHT 50 A) at an air flow rate of 18 l/min. After the intravenous administration of the labelled compound the radioactivity was followed continously for 3 h and after the oral administration for 8 h with two short breaks for food intake after 3 and 6 h. Measurements of the activity in expired air were performed only in control subject I.E. and in the two subjects (G.T. and T.M.) previously studied (Bergmark *et al.*, 1972). The efficiency of the equipment used was measured in the following way: a continuous liberation of $^{14}CO_2$ was obtained within the breathing box by the addition of 0.2 M ^{14}C-bicarbonate (specific activity 4.50×10^3 μCi/mol) to concentrated sulphuric acid. The bicarbonate solution was delivered by a continuous in fusion pump (B. Braun, model 1830) at 2.0 ml/h to 100 ml of the acid under constant stirring. The measurement was continued for at least 2 h in order to obtain equilibrium. The air flow was kept constant at

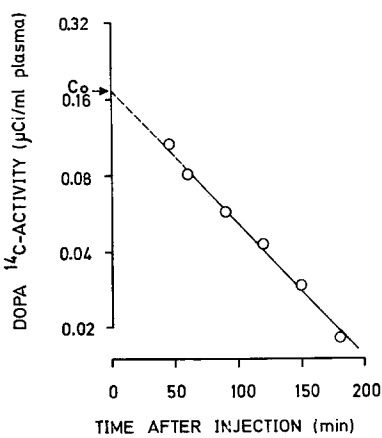

Fig. 2. The plasma disappearance curve for intravenously administered L-3′,4′-dihydroxyphenylalanine-1-^{14}C (1 mg, specific activity about $4.6 \cdot 16^6$ μCi/mol). Half an hour before the injection 0.5 g unlabelled L-Dopa was given orally

18 l/min. Under these conditions 3.50% of the total number of desintegrations of the $^{14}CO_2$ in the air passing through the detector system was recorded.

Calculation of Intestinal Decarboxylation. The decarboxylation of L-Dopa in the intestinal organs was calculated from the formula D = 1—Uo/Ui, where D is the fraction of the L-Dopa which was decarboxylated and Uo and Ui were the cumulative recoveries in urine after the oral and intravenous administration of the labelled L-Dopa, respectively.

Calculation of Plasma Disappearance Rate and Apparent Distribution Volume. The plasma disappearance curve of the intravenously administered radioactive L-Dopa appeared to be biexponential. The half-time ($t^1/_2$) for the final slope was calculated from the regression line obtained for the interval 60—180 min after the injection of the labelled compound. The apparent distribution volume D/Co was also calculated, where D is the dose in μCi/kg body weight and Co the theoretical concentration at zero time in μCi/l plasma (Fig. 2). As an injection of L-Dopa might cause nausea and vomiting, only a tracer dose (about 1 mg) was given intravenously. To mimic the conditions at the oral loading a 0.5 g tablet of L-Dopa was given orally half an hour before the intravenous injection. This gave a plasma Dopa concentration of 2 to 5 μmoles/l at the time of the injection.

Determination of unlabelled Dopa in plasma was performed by ion-exchange chromatography (Bergmark and Jagenburg, 1974).

Results

Most of the radioactivity recovered in urine appeared within 24 h both after oral and intravenous administration of the labelled L-Dopa (Fig. 3). The total recovery during 3 days was 6.2 ± 1.1% (mean ± S.D.) after oral and 24.3 ± 4.7% after intravenous administration. On the fourth day only negligible amounts (< 0.3%) of the administered activity were

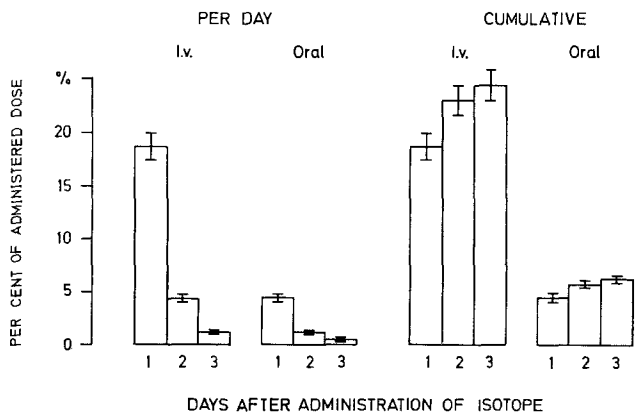

Fig. 3. Recovery of radioactivity in urine in per cent of administered dose after intravenous and oral administration of L-3′,4′-dihydroxyphenylalanine-1-^{14}C (n=11) The mean and the standard error of the mean is indicated

excreted in the urine. If the urines were alkaline up to 10% of the total activity appearing in urine could be derived from bicarbonate. All figures given refer to acidified urines. The ^{14}C-activity in urea was always negligible, less than 2% of the total urinary radioactivity.

In 3 experiments the radioactivity was measured both in urine and in expired air (Table 1): on an average 74% of the orally administered activity was recovered as $^{14}CO_2$ in the expired air within 8 h, while 7.3% was recovered in urine during 72 h. After intravenous administration 45% of the activity was recovered in expired air within 3 h. Since the decrease in activity followed an exponential function the total recovery within 8 h was assessed as 58%. In the urine the recovery within 72 h averaged 27%. Thus, 81% of the activity was accounted for after oral administration and 85% after intravenous administration. For the time from 8 to 24 h a further recovery in the expired air of about 15% was calculated from the slopes of the curves both after oral and intravenous administration. The total recovery thus amounted to almost 100%. In one subject the radioactivity in expired air, 24 h after the loading, was found to be less than 1% of the maximal $^{14}CO_2$ activity observed.

The total activity in plasma was derived mainly from Dopa, 3-O-methyldopa, and carbon dioxide-bicarbonate, together comprising about 80% of the total activity. The remaining activity might originate from conjugated Dopa and methyldopa or from 3,4-dihydroxyphenylpyruvate and other nondecarboxylated Dopa metabolites (Sharpless and McCann, 1971, Goodall and Alton, 1972). The ^{14}C-activity appearing as carbon dioxide-bicarbonate was determined from the difference between total radioactivity in untreated plasma and total radioactivity in perchloric acid filtrate corrected for the change in volume. When L-Dopa was administered orally, a greater part of the activity was recovered as carbon dioxide-bicarbonate than after intravenous administration (Fig. 4). It should be emphasized that after oral administration the first activity appearing in plasma was mainly derived from carbon dioxide-bicarbonate.

Orally administered unlabelled L-Dopa did not obviously influence the rate of elimination of an intravenously administered tracer dose of L-Dopa as far as could be judged from studies in one 80 years old patient. The half time for the elimination of Dopa from plasma was 76 min when only the tracer dose was given, and 71 min when a solution of 300 mg of L-Dopa was administered orally 30 min before the intravenous injection. This gave a Dopa concentration in plasma of 4 μmol/l at the time of the injection. The recovery of radioactivity in urine within 3 days was 26% in both experiments.

In the group of 11 patients with varying dose requirement of L-Dopa, the intestinal decarboxylation was $73.8 \pm 6.0\%$ (mean $\pm$ S.D.) under

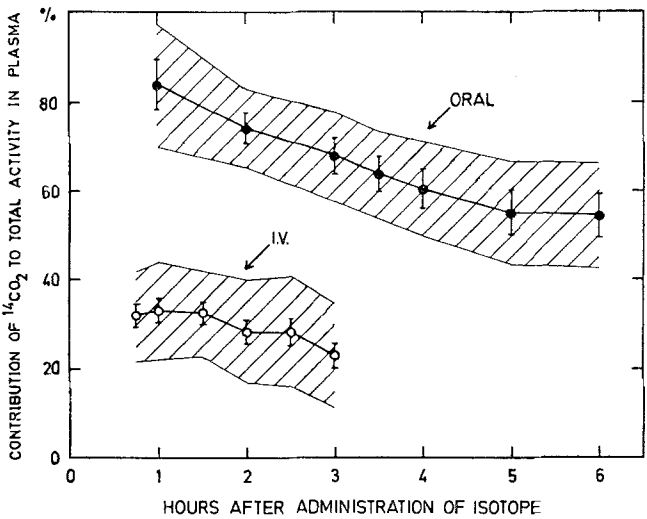

Fig. 4. Contribution of $^{14}CO_2$ to total ^{14}C-activity in plasma after intravenous ($n = 11$) and oral administration ($n = 5$) of L-3′,4′-dihydroxyphenylalanine-1-^{14}C. The vertical bars indicate $\pm$ S.E. and shadowed areas $\pm$ 1 S.D. Oral experiments include the experiments shown in Table 1 and two further experiments from a preliminary report (Bergmark *et al.*, 1972)

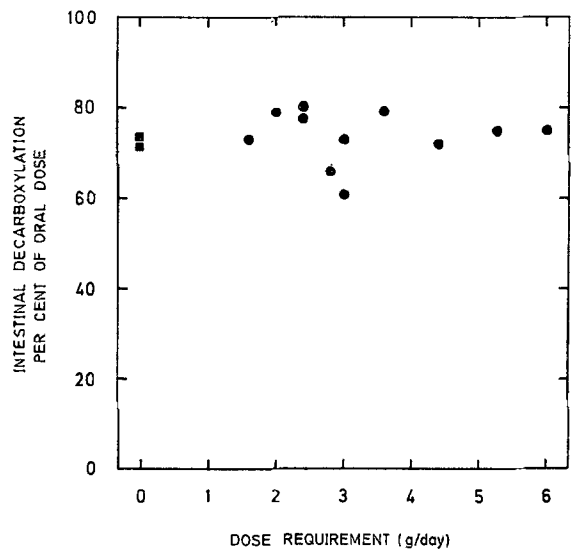

Fig. 5. Calculated intestinal L-Dopa decarboxylation under standardized conditions in relation to dose requirement. A 0.5 g tablet of L-Dopa was given together with one glass of milk and two slices of bread and butter. Parkinsonian patients treated with L-Dopa = •. Healthy controls previously not treated with L-Dopa = ▪

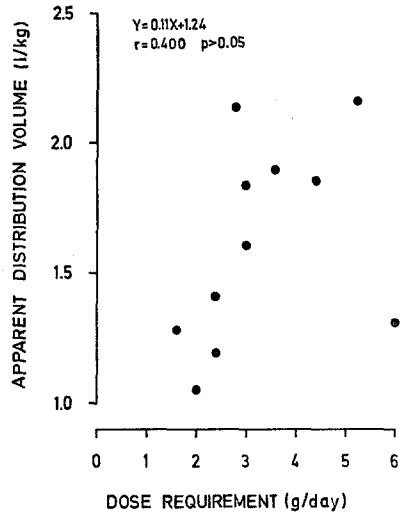

Fig. 6. Apparent distribution volume of intravenously administered L-Dopa in relation to dose requirement in 11 Parkinsonian patients

the standardized conditions used. After the intravenous administration of the labelled compound $4.0 \pm 1.4\%$ of the given dose was excreted in urine as unchanged Dopa and $4.8 \pm 1.6\%$ as 3-O-methyldopa. After the oral administration only $0.6 \pm 0.3\%$ was excreted as Dopa and $0.8 \pm 0.3\%$ as 3-O-methyldopa. After the intravenous injection the half time of L-Dopa in plasma was 55 ± 4.8 min and the apparent distribution volume 1.6 ± 0.39 l/kg body weight. There was a significant correlation neither between the dose of L-Dopa giving optimal therapeutical response and the calculated intestinal decarboxylation (Fig. 5) nor between that dose and the disappearance rate of L-Dopa from plasma or its apparent distribution volume (Fig. 6).

Discussion

Dopa is metabolised by decarboxylation, methylation or transamination (Sharpless and McCann, 1972). When carboxyl labelled L-Dopa is administered, some of the ^{14}C-activity is released as carbon dioxide which rapidly equilibrates with the carbon dioxide-bicarbonate pool and appears in the expired air. Minute amounts will be found in the urine as bicarbonate if the urine is alkaline. For the calculation of the intestinal Dopa decarboxylation the radioactivity remaining in urine after acidification was therefore used. The turnover of L-Dopa was found to be fast and this is also true for the bicarbonate pool. This was illustrated by the observation that more than 98% of the $^{14}CO_2$ appearing in expired air was recovered within 24 h.

The incorporation of $^{14}CO_2$ into urea was low, less than two per cent of the total radioactivity of the urine was found in the urea fraction. The activity of the acidified urine thus originated almost exclusively from Dopa and non-decarboxylated Dopa metabolites.

The part of the activity appearing as $^{14}CO_2$ in expired air reflects the amount of L-Dopa undergoing decarboxylation. The present results show that $^3/_4$ of the intravenously administered L-Dopa was decarboxylated, 5% was excreted in the urine as 3-O-methyldopa and 4% as unchanged Dopa.

The fact that almost all the radioactivity given was recovered from expired air and urine shows that a determination of the activity in urine allows conclusions concerning the activity in expired air. Urinary analyses are therefore sufficient for a calculation of the Dopa-decarboxylation there being an inverse relationship between the degree of decarboxylation and the ^{14}C-activity in urine.

After oral administration of L-Dopa considerably less activity was found in urine than after intravenous administration. Furthermore, a considerably greater proportion of the total radioactivity in plasma was derived from carbon dioxide-bicarbonate when the labelled compound was given orally than when it was administered intravenously. This shows that a considerable part of the orally administered L-Dopa is decarboxylated in the gastro-intestinal tract or in the liver before the L-Dopa reaches the general circulation.

The fraction of the orally administered L-Dopa that undergoes decarboxylation in the gastro-intestinal organs can be calculated with the method described here, if it is assumed that the L-Dopa reaching the general circulation after oral administration is handled in a similar way as is the intravenously administered labelled L-Dopa. The L-Dopa level in plasma did not obviously influence the disappearance rate of Dopa from plasma and the recovery of ^{14}C-activity in the urine.

The present study does not allow any conclusions concerning the site of the decarboxylating activity in the gastrointestinal organs. Rivera-Calimlim *et al.* (1971 a and b) found that rat gastric and intestinal mucosa contains L-Dopa decarboxylating activity and that L-Dopa can be metabolised in the human stomach. A high decarboxylase activity of the liver is also well known (Pletscher *et al.*, 1970).

The main part of the non-decarboxylated L-Dopa will appear in the general circulation as Dopa. By the present method the net absorption of L-Dopa, here defined as the fraction of the Dopa which is not decarboxylated in the gastrointestinal organs, can be calculated. However, it should be emphasized that lesser amounts of the absorbed L-Dopa might be methylated or transaminated already during the first passage through

the liver. The method used cannot disclose the magnitude of this methylation or transamiation.

A decrease in the decarboxylase activity by as much as 50% in the liver after chronic administration of L-Dopa has been observed in rats (Dairman *et al.*, 1971). Such a change seems unlikely in humans as the decarboxylation in the intestinal organs was of the same order of magnitude in the parkinsonian patients and in the two controls not previously treated with L-Dopa. The absorption was similar in the patient with achylia as in the other subjects. There was no correlation between the magnitude of decarboxylation in the intestinal organs and the duration of treatment, duration of disease or dose requirement. Furthermore, preliminary observations show that rather marked individual variations in the need of L-Dopa remain after treatment with peripheral decarboxylase inhibitors. The present study also indicates that individual variations in the rate of elimination of intravenously administered L-Dopa are not correlated with the dose requirement. There are therefore reasons to believe that other factors than those responsible for the peripheral metabolism of L-Dopa determine the individual dose required.

Although no significant correlation ($r = 0.40$) was obtained in the total material between the dose requirement and apparent distribution volume, we want to mention that if the observation in the patient with the highest requirement was excluded there would be a significant correlation ($r = 0.77$, $P < 0.01$) between the two variables. The apparent distribution volume of L-Dopa was found to be far above the volume of the total body water and higher than that of phenylalanine (Granerus *et al.*, 1971). This indicates that L-Dopa accumulates intracellularly to a greater extent than phenylalanine does. The factors determining the distribution of amino acids in the body are largely unknown. At present it is, therefore, difficult to evaluate the possible biological significance of a correlation between dose requirement and distribution volume of L-Dopa.

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References

Bergmark, J., Carlsson, A., Granerus, A.-K., Jagenburg, R., Magnusson, T., Svanborg, A.: Decarboxylation of orally administered L-Dopa in the human digestive tract. Naunyn-Schmiedeberg's Arch. Pharmacol. **272**, 437–440 (1972)

Bergmark, J., Jagenburg, R.: Determination of Dopa and dopaminesulphate in biological fluids. Scand. J. Lab. clin. Invest. (in press) (1974)

Bianchine, J. R., Calimlim, L. R., Morgan, J. P., Dujovne, C. A.: Metabolism and absorption of L-3,4-dihydroxyphenylalanine in patients with Parkinson's disease. Ann. N.Y. Acad. Sci. **179**, 126–139 (1971)

Dairman, W., Christenson, J. G., Udenfriend, S.: Decrease in liver aromatic L-amino decarboxylase produced by chronic administration of L-Dopa. Proc. nat. Acad. Sci. (Wash.) **68**, 2117—2120 (1971)

Goodall, Mc C., Alton, H.: Metabolism of 3,4-dihydroxyphenylalanine (L-Dopa) in human subjects. Biochem. Pharmacol. **21**, 2401—2408 (1972)

Granerus, A.-K., Jagenburg, R., Rödjer, S., Svanborg, A.: Phenylalanine absorption and metabolism in parkinsonian patients. Brit. med. J. **1971 IV**, 262—264

Granerus, A.-K., Svanborg, A., Steg, G.: Clinical analyses of factors influencing L-Dopa treatment of parkinsonian syndrome. Acta med. scand. **199**, 1—11 (1972)

Pletscher, A., Bartolini, G., Gey, K. F., Jenni, A.: Die biochemischen Grundlagen für die Behandlung des Parkinson-Syndroms mit L-Dopa. Schweiz. med. Wschr. **19**, 797—804 (1970)

Rivera-Calimlim, L., Dujovne, C. A., Morgan, L. P., Bianchine, J. R., Lasagne, L.: Absorption and metabolism of L-Dopa by the human stomach. Europ. J. clin. Invest. **1**, 313—329 (1971a)

Rivera-Calimlim, L., Morgan, J. P., Dujovne, C. A., Bianchine, J. R., Lasagne, L.: L-3,4-dihydroxyphenylalaninemetabolism by the gut in vitro. Biochem. Pharmacol. **20**, 3051—3057 (1971b)

Sharpless, N., Mc Cann, D.: Dopa and 3-O-methyldopa in crebrospinal fluid of parkinsonism patients during treatment with oral L-Dopa. Clin. chim. Acta **31**, 155—169 (1972)

Tissot, R., Bartholini, G., Pletscher, A.: Drug-induced changes of extracerebral Dopa metabolism in man. Arch. Neurol. (Chic.) **20**, 187—190 (1969)

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