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Studies on the Metabolism and Excretion of L-3,4-Dihydroxyphenylalanine (L-DOPA) in Human Beings by Gas Chromatography[1]

KAZUHIRO IMAI, MOTOTAKA SUGIURA, HIROAKI KUBO, ZENZO TAMURA,[2a] KAZUMI OHYA, NOBUTAKA TSUNAKAWA,[2b] KEIZO HIRAYAMA and HIROTARO NARABAYASHI[2c]

Faculty of Pharmaceutical Sciences, University of Tokyo,[2a] Pharmaceutical Research Laboratory, Research Laboratories, Daiichi Seiyaku Co., Ltd.[2b] and Department of Neurology, School of Medicine, Juntendo University[2c]

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One gram of L-DOPA was administered orally to the patients with Parkinson's disease and normal persons. Plasma DOPA and catecholamines, urinary DOPA, catecholamines and aromatic acids were determined by gas chromatography both before and after acid hydrolysis. DOPA and dopamine conjugate were detected in the plasma soon after the administration, while large personal deviations were observed both in their concentration and time courses. During 24 hr, 0.53%, 4.24%, 32.0%, and 25.9% in average of the administered L-DOPA were excreted in urine as DOPA, dopamine, DOPAC and HVA respectively in a free and conjugated form.

L-3,4-Dihydroxyphenylalanine (L-DOPA) is an effective drug for Parkinsonism when administered orally in large doses (about 3 to 10 g a day).[3]

As for the absorption, metabolism and excretion of L-DOPA in human, extensive studies have been made by measurement of L-DOPA and its metabolites. However, colorimetry[4–6] is not sensitive enough and fluorometry[7,8] is unreliable in selectivity. By isotopic method,[9,10] only the main metabolite, HVA, could be measured.

We studied the metabolisms and excretion of L-DOPA which was administered orally by 1 g to the patients with Parkinson's disease and normal persons, by gas chromatography.[11,12]

Experimental

Subjects——All the Patients had Idiopathic Parkinson's Disease, and for the Convenience the Patients were Classified into two Groups: The patients in group I were going to be treated with L-DOPA and those in group II had been treated with L-DOPA for more than 3 weeks. Group I consisted of two men and five women: the mean age was 53 years (range 34 to 60 years). Group II consisted of six men and four women:

1) A part of the work was reported in the previous communication: K. Imai, M. Sugiura, Z. Tamura, K. Hirayama, and H. Narabayashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 439 (1971).
2) Location: *a) Hongo 7-3-1, Bunkyo-ku, Tokyo; b) Narihira 5-6-9, Sumida-ku, Tokyo; c) Hongo 3-1-3, Bunkyo-ku, Tokyo.*
3) G.C. Cotzias, P.S. Papavasiliou, and R. Gellene, *New Eng. J. Med.*, **280**, 337 (1969).
4) K.N.F. Shaw, A. McMillan, and M.D. Armstrong, *J. Biol. Chem.*, **226**, 255 (1957).
5) P. Smith, *Nature*, **213**, 802 (1967).
6) C.M. Tyce, M.D. Muenter, and C.A. Owen, Jr., *Mayo Clin. Proc.*, **45**, 645 (1970).
7) T.L. Sourkes, D. Pivnicki, W.T. Brown, M.H. Wisemandistler, G.F. Murphy, I. Sankoff, and S.S. Cyr, *Psychiat. Neurol. Basel.*, **149**, 7 (1965).
8) G.H. Tyce, M.D. Muenter, and C.A. Owen, Jr., *Mayo Clin. Proc.*, **45**, 438 (1970).
9) A. Pletscher, G. Bartholini, and R. Tissot, *Brain Res.*, **4**, 106 (1967).
10) M.J. Peaston and J.R. Bianchie, *Brit. J. Med.*, **1**, 400 (1970).
11) S. Kawai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 699, 1091 (1968).
12) A part of the work was presented at the meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, November, 1970.

the mean age was 56 years (range 45 to 61 years). For the patients in the group II, the daily dose of L-DOPA had been increased over a period of 3 to 4 weeks to maxima of 3 g a day and the treatment with the drug was stopped 15 hr before the study. Details of the clinical aspect of the study will be published elsewhere.

Normal persons were men (25, 27, and 32 years respectively).

Materials——L-DOPA was the gift from Daiichi Seiyaku Co., Ltd. Dopamine hydrochloride, *dl*-norepinephrine, *l*-epinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), *dl*-3,4-dihydroxymandelic acid (DOMA), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and *dl*-4-hydroxy-3-methoxymandelic acid (VMA) were purchased from Tokyo Kasei Co., Ltd.

Method——Preparation of Plasma Samples: The blood was taken by 5 ml with a heparinized syringe before the administration, 15 min, 30 min, 45 min, 1 hr, 3 hr, and 7 hr after the oral administration of 1 g of L-DOPA. The treatment of the blood and acid hydrolysis of the plasma samples were undertaken as previously reported.[13] The samples were stored at −20° and analyzed in one month.

Preparation of Urine Samples: The urine was excreted just before the administration and discarded. The urine samples were collected 4 hr, 8 hr, and 24 hr after the administration. After the adjustment of the pH of the urine to 1 with HCl, the samples were stored at −20° until analyzed. For the determination of total quantities of metabolites, the acid hydrolysis of their conjugates was performed by heating the sample at 100° for 20 min.

Gas Chromatographic Determination of L-DOPA and Catecholamines in Plasma and Urine Samples: Extraction from biological materials and preparation of trifluoroacetyl derivatives of L-DOPA and catecholamines were the same as described in the previous paper.[11,13] The amines in the plasma samples were analyzed by gas chromatography with electron capture detector with isodrin as an internal standard, and the amines in urine samples were analyzed by that with a flame ionization detector with *n*-eicosane as an internal standard.

A Shimadzu Model GC-4AP gas chromatograph was used with glass tube sufficient for making column of 1.5 or 2.0 m in length and 4 mm in diameter. The column packing was 2% GE-XF 1105 on Chromosorb W (60—80 mesh). The gas chromatographic parameters were as follows: injection temperature, 180°; column temperature, 160—175°; detector temperature, 190—200°; nitrogen flow rate, 50—80 ml/min.

Recovery of the added amines from biological specimens were about 50% as in the previous paper[13] and those of L-DOPA added to the blood and the urine were about 20% and 40% respectively: and the corrected values are cited in the text.

Gas Chromatography of Aromatic Acids in Urine Samples[12]: Five ml of the urine samples (pH 1) was extracted twice with 10 ml of ethyl acetate after addition of 2.5 g of sodium chloride. The ethyl acetate extract was evaporated to dryness at 30—40°. The residue was dissolved in 3 ml of anhydrous *n*-butanol saturated with HCl gas and heated at 70—80° for 2 hr. The reaction mixture was evaporated under reduced pressure at 50—60°, dissolved in anhydrous chloroform containing 0.5 mg of *n*-docosane (an internal standard), added with 0.2 ml of trifluoroacetic anhydride and reacted at room temperature for 10 hr. One μl of the solution was injected to a gas chromatograph equipped with a flame ionization detector. The apparatus and conditions were almost the same as described above except that the column temperature was programed at 2°/min from 150° to 170°. Recovery of the added aromatic acids were quantitative.[12]

Result

Concentration of DOPA and Catecholamines in Plasma

DOPA was detected in plasma soon after the administration. The concentration curve with each subject differed from each other (Fig. Ia, Ib and Ic: a,b and c correspond to group I, II and that of normal persons respectively.). Acid hydrolysis of plasma at pH 1 for 20 min at 100° gave no change in the concentration of DOPA. The fact indicated the absence of it's conjugates.

As for catecholamines, dopamine was detected in plasma in a form of conjugate in much higher levels than those in the normal human plasma,[13] and norepinephrine and epinephrine were not detected (less than 20 mg/ml). The higher levels of dopamine conjugate lasted for 7 or more hours. (Fig. 2a, 2b and 2c), while free dopamine was not determined (less than 50 ng/ml).

In addition, DOPA and catecholamines were not detected in the blood cells.

13) K. Imai, M. Sugiura, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2134 (1970); **18**, 409 (1971).

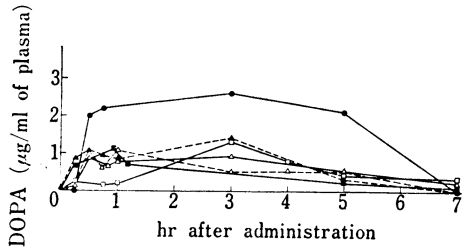

Fig. 1a. Concentration of DOPA in Plasma after the Administration of L-DOPA in Group I

—●—: M.S., —■—: A.M., —□—: K.B.,
—△—: S.T., ---▲---: H.M., ---△---: M.Y.,
---■---: S.O.

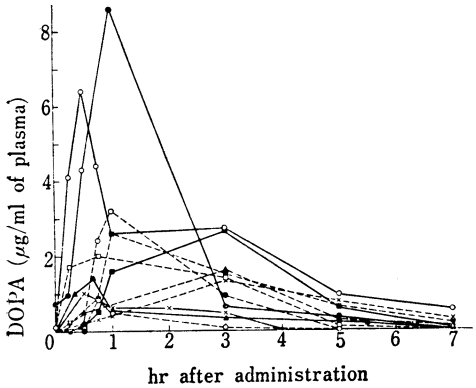

Fig. 1b. Concentration of DOPA in plasma after the Administration of L-DOPA in Group II

—●—: M.S., —■—: A.M., —○—: H.I.,
—▲—: S.T., —×—: K.H., ---●---: S.K.,
---■---: S.O., ---○---: I.O., ---▲---: H.M.,
---×---: S.F., ---□---: H.G.

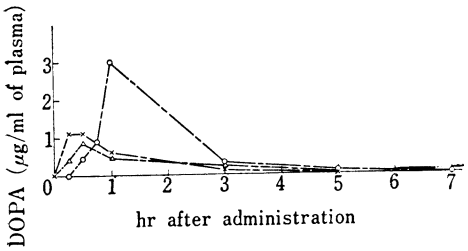

Fig. 1c. Concentration of DOPA in Plasma after the Administration of L-DOPA in Normal Persons

——○——: T.S.,
——×——: K.K.,
——△——: H.F.

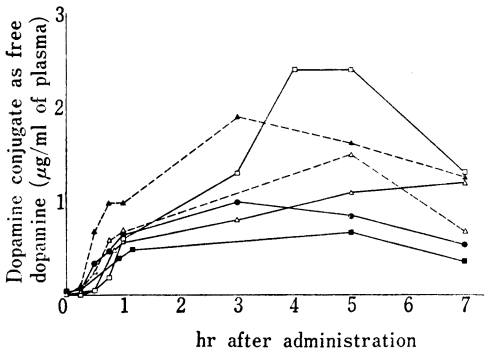

Fig. 2a. Concentration of Dopamine Conjugate in Plasma after the Administration of L-DOPA in Group I

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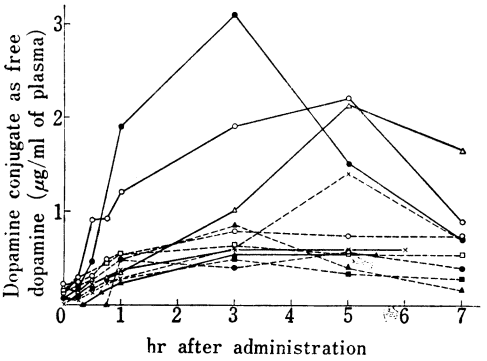

Fig. 2b. Concentration of Dopaminc Conjugate in Plasma after the Administration of L-DOPA in Group II

Each symbol was the same as in Fig. 1b.

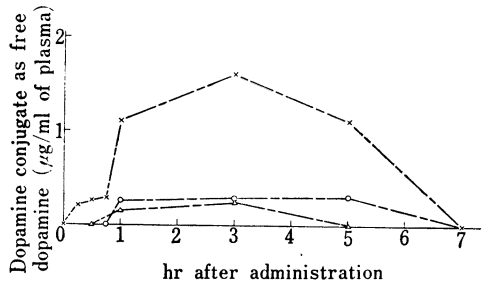

Fig. 2c. Concentration of Dopamine Conjugate in Plamma after the Administration of L-DOPA in Normal Persons

Each symbol was the same as in Fig. 1c.

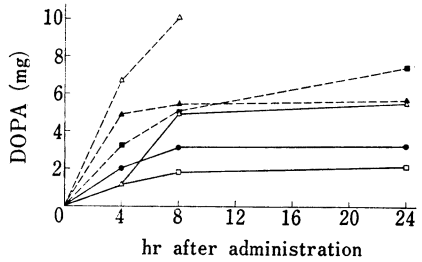

Fig. 3a. Urinary Excretion of DOPA Obtained from the Patients in Group I after L-DOPA Administration

Each symbol was the same as in Fig. 1a.

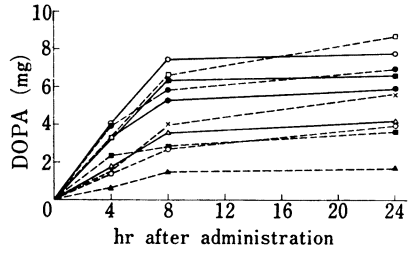

Fig. 3b. Urinary Excretion of DOPA Obtained from the Patients in Group II after L-DOPA Administration

Each symbol was the same as in Fig. 1b.

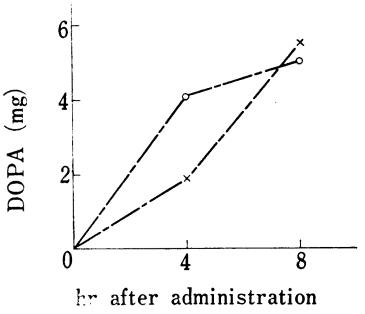

Fig. 3c. Urinary Excretion of DOPA Obtained from Normal Persons after L-DOPA Administration

Each symbol was the same as in Fig. 1c.

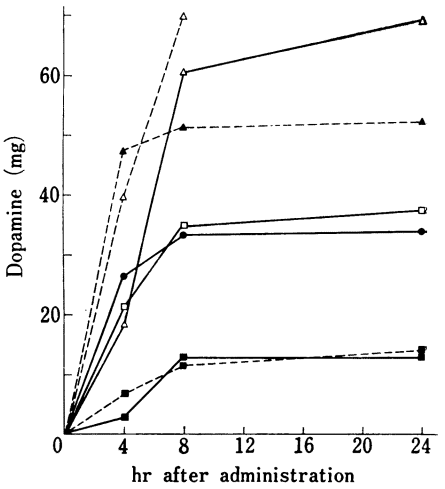

Fig. 4a. Urinary Excretion of Dopamine Obtained from the Patients in Group I after L-DOPA Administration

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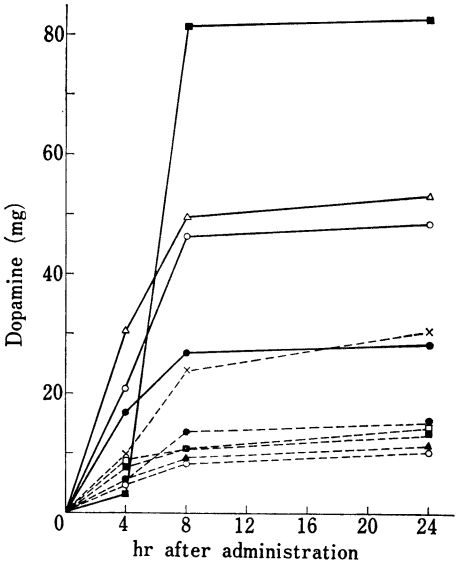

Fig. 4b. Urinary Excretion of Dopamine Obtained from the Patients in Group II after L-DOPA Administration

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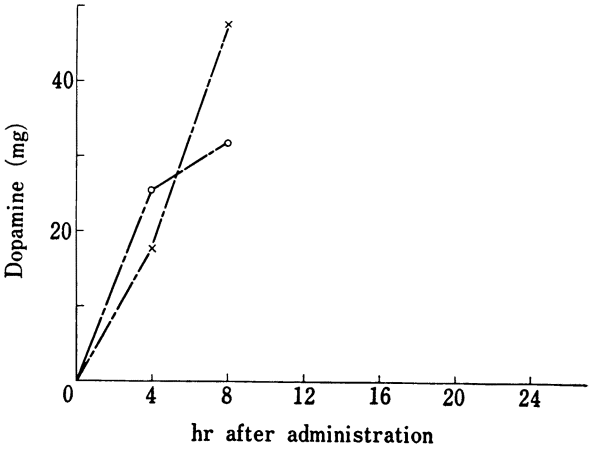

Fig. 4c. Urinary Excretion of Dopamine Obtained from Normal Persons after L-DOPA Administration

Each symbol was the same as in Fig. 1c.

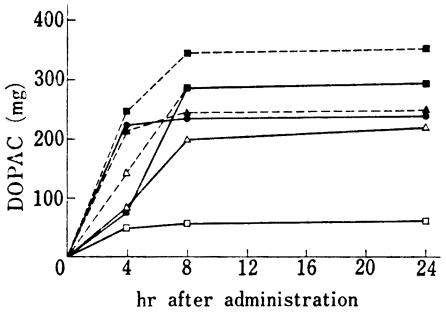

Fig. 5a. Urinary Excretion of DOPAC Obtained from the Patients in Group I after L-DOPA Administration

Each symbol was the same as in Fig. 1a.

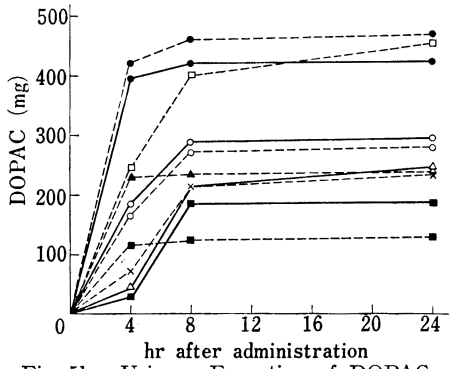

Fig. 5b. Urinary Excretion of DOPAC Obtained from the Patients in Group II after L-DOPA Administration

Each symbol was the same as in Fig. 1b.

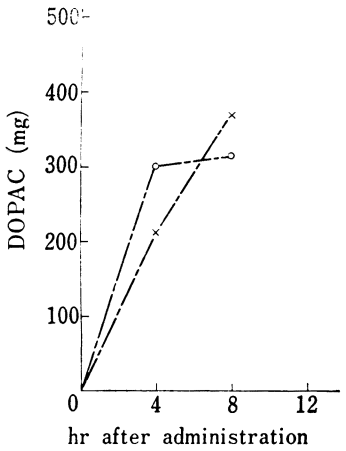

Fig. 5c. Urinary Excretion of DOPAC Obtained from Normal Persons after L-DOPA Administration

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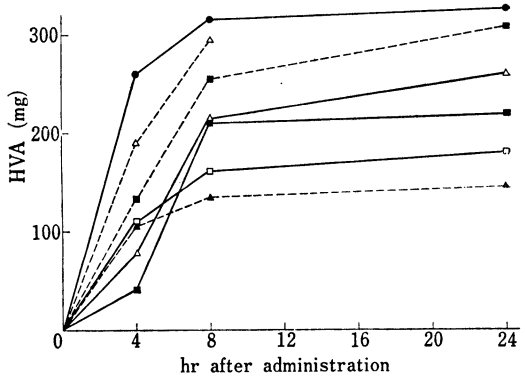

Fig. 6a. Urinary Excretion of HVA Obtained from the Patients in Group I after L-DOPA Administration

Each symbol was the same as in Fig. 1a.

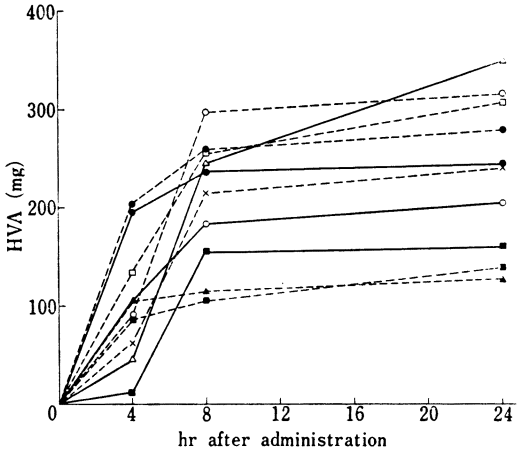

Fig. 6b. Urinary Excretion of HVA Obtained from the Patients in Group II after L-DOPA Administration

Each symbol was the same as in Fig. 1b.

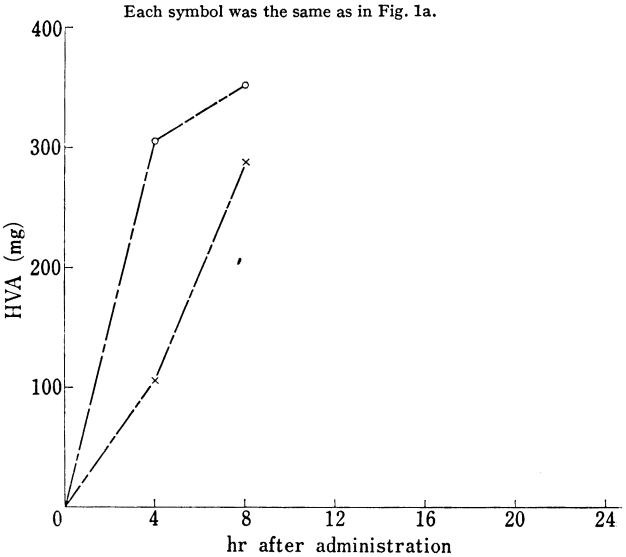

Fig. 6c. Urinary Excretion of HVA Obtained from Normal Persons after L-DOPA Administration

Each symbol was the same as in Fig. 1c.

Excretion of DOPA, Catecholamines and Aromatic Acids in Urine

Norepinephrine, epinephrine, DOMA and VMA were not detected from the urine samples (less than 40 μg/ml). The cummulative values of the total quantities of urinary metabolites were shown in Fig. 3,4,5 and 6.

During 24 hr in average, 0.53% (0.17—0.87%), 4.24% (1.35—10.6%), 32.0% (7.3—55.0%) and 25.9% (13.8—37.8%) of the administered L-DOPA were excreted as DOPA, dopamine, DOPAC and HVA respectively. The major amounts of the metabolites in 24 hr urine were excreted during 8 hr (DOPA: 83.8% (67.8—97.1%), dopamine: 87.0% (68.2—98.5%), DOPAC: 95.6% (86.5—99.7%) and HVA: 89.9% (70.2—98.5%)).

The ratio of conjugation of the metabolites varied from person to person, and 59.5% (20.6—92.9%) of DOPA, 81.2% (53.7—100%) of dopamine, 18.8% (5.9—44.7%) of DOPAC and 15.2% (0—24.9%) of HVA were acid labile conjugates.

Discussion

As shown in Fig. 1a, 1b and 1c the administered L-DOPA was absorbed quickly and found in the blood stream. Peaston, *et al.*[10] who used ^{14}C labelled DOPA reported the disappearance of DOPA from plasma within 1 hr. However, our figures show large personal deviations both in concentration and its time course of DOPA. As a whole, there seems no difference in levels of DOPA and dopamine conjugate between the three groups, in a few cases, however, distinct changes in the time courses of dopa concentration are obserbed after the DOPA-treatment for 3 weeks (M.S. and A.M. in group I to group II). The phenomena might indicate possible occurrence of increased absorption.

As for the side effect, the involuntary movement occurred especially in the case of M.S. and H.I. suggesting that the higher levels of DOPA and/or dopamine conjugate in plasma would concern with the involuntary movement.

The presence of conjugated DOPAC and HVA are not clear although the concentration of dopac and HVA slightly increased after acid hydrolysis, since there is possibility that phenylactic acid derivatives decompose to produce DOPAC and HVA after treatment with acid.[5]

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