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ALTERED METABOLISM OF [^{18}F]-6-FLUORODOPA IN THE HOODED RAT FOLLOWING INHIBITION OF CATECHOL-*O*-METHYLTRANSFERASE WITH U-0521

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Abstract—[^{18}F]-6-Fluoro-L-DOPA ([^{18}F]DOPA), a tracer for cerebral dopamine in studies utilizing positron emission tomography (PET), is rapidly metabolized by catechol-*O*-methyltransferase (COMT) in the periphery following intravenous injection to carbidopa-pretreated humans and rats. Experiments were performed to determine the effect of pretreatment with 3′,4′-dihydroxy-2-methyl-propiophenone (U-0521), a competitive inhibitor of COMT, on [^{18}F]DOPA metabolism in the carbidopa-pretreated hooded rat. U-0521 (25 mg/kg, i.p.), administered 10 min prior to the [^{18}F]DOPA, served to increase the persistence of [^{18}F]DOPA in plasma over a 2-hr period by decreasing the rate of formation of the peripheral metabolite 3-*O*-methyl-6-fluorotyrosine (Me[^{18}F]DOPA). This compound passes readily into brain and was the sole [^{18}F]DOPA metabolite observed in cortex and cerebellum. U-0521 produced a short-lasting decrease in Me[^{18}F]DOPA levels in these two tissues. In striatum, decreases in Me[^{18}F]DOPA were found to last at least 90 min. Associated with the elevated availability of [^{18}F]DOPA in plasma produced by U-0521 were 50% increases in striatal [^{18}F]dopamine ([^{18}F]DA) levels and 40% increases in the levels of [^{18}F]dihydroxyphenylacetic acid ([^{18}F]DOPAC) at times between 30 and 90 min following [^{18}F]DOPA injection. Increased decarboxylation of [^{18}F]DOPA in the striatum of U-0521-treated rats resulted in heightened radiocontrast between striatum and other cerebral tissues.

[^{18}F]-6-Fluoro-L-DOPA ([^{18}F]DOPA) is a positron-emitting compound that has been used as an experimental tracer for the study of cerebral dopamine metabolism in humans by means of positron emission tomography (PET). The accumulation of radioactivity in the striatum following intravenous [^{18}F]DOPA administration to normal humans [1] is found to be reduced in cases of Parkinson's disease [2]. A similar difference between controls and Parkinsonians is seen in subjects pretreated with the peripheral decarboxylase inhibitor, carbidopa (α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)propionic acid) [3, 4]. In the latter case, influx constants calculated for normal subjects have been shown to decrease with the age of the individual [5]. A study of the metabolism of [^{18}F]DOPA *in vivo* in carbidopa-pretreated hooded rats has shown that the greater accumulation of radioactivity in the striatum as compared with other brain areas is due to the formation and storage of fluorodopamine ([^{18}F]DA) and its metabolites [6]. In these rats, 3-*O*-methyl-6-fluorotyrosine (Me[^{18}F]DOPA) appears rapidly in plasma and is the principal labeled compound in cerebral tissues other than the striatum. A rapid accumulation of Me[^{18}F]DOPA is also observed in the plasma of human subjects given [^{18}F]DOPA after pretreatment with carbidopa [7]. Because the formation of this metabolite in the periphery may be a limiting factor in striatal [^{18}F]DA synthesis, it seemed appropriate to study the effect of catechol-*O*-methyltransferase (COMT) inhibition on [^{18}F]DOPA metabolism in the rat. 3′,4′-Dihydroxy-2-methyl-propiophenone (U-0521) was used since this compound has been characterized as a competitive inhibitor of COMT in rat red blood cells [8], has been shown to stimulate the formation of dopamine in rat striatum, and has been tested in humans as an adjuvant of DOPA therapy [9]. In contrast to *n*-butyl gallate, a COMT inhibitor that has been used in clinical trials [10], U-0521 is thought to be of relatively low toxicity.

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MATERIALS AND METHODS

Dihydroxybenzylacetic acid, L-DOPA and *S*-adenosyl-L-methionine *p*-toluenesulfonate (SAM) were from Sigma. [^{14}C-*Methyl*]-*S*-adenosyl-L-methionine (59.8 mCi/mmol) was from New England Nuclear. The syntheses of D,L-6-fluorodopa [11] and [^{18}F]-L-6-fluorodopa [12] are described elsewhere. Perchloric acid was from Baker and carbidopa was the gift of Merck Sharp and Dohme. U-0521 was provided by Upjohn. Triton X-100 was from the British Drug House. All buffers were made with water distilled over alkaline potassium permanganate.

Competitive inhibition constants (K_i) for L-DOPA and D,L-6-fluorodopa (F-DOPA) were determined using a radiochemical assay for COMT with dihydroxybenzylacetic acid (DHBA) serving as the substrate [13]. Triplicate assay tubes contained, in a final volume of 120 μl, 4 mM Mg^{2+}, 50 mM phosphate buffer at pH 7.4, DHBA at four concentrations ranging from 0.13 to 1.05 mM, and 20 μl of a rat cerebellar homogenate in 10 vol. of 0.5% Triton X-100. The pH chosen is below the reported optimum

for COMT but seemed closer to probable physiological conditions. The homogenate contained 9.6 mg protein/ml as assayed by the Bradford method [14] using bovine serum albumin as the standard. The reaction mixture contained 50,000 dpm of [^{14}C-*methyl*]-*S*-adenosyl-L-methionine per tube diluted with cold SAM to a final concentration of 60 μM. Incubation was for 30 min at 37°. The reaction was terminated by the addition of an equal volume of 1 M HCl. Methylated substrate was extracted into 5 ml toluene, and radioactivity was determined by 2-min counts in a Phillips PW-4700 liquid scintillation counter. The effects of either F-DOPA or L-DOPA on the methylation of DHBA were determined by the addition of these compounds to 1 mM final concentration. O-Methylated DOPAs were not extractable into the organic phase. The kinetics of the O-methylation of U-0521 were determined by substituting this compound for DHBA at four concentrations ranging from 5 to 90 μM. Incubation in this case was for 40 min at 37°.

For the study of the metabolism of [^{18}F]DOPA *in vivo*, male hooded rats weighing between 250 and 350 g were provided with intrajugular cannulae while under pentobarbitol anesthesia. After a recovery period of at least 1 day, control animals were pretreated with carbidopa (5 mg/kg, i.p.), followed 30 min later by [^{18}F]DOPA (500 μCi/kg, 200–300 Ci/mol) via the jugular cannula. Experimental animals also received U-0521 (25 mg/kg, i.p.) 10 min prior to [^{18}F]DOPA administration. Blood samples were taken at intervals over a 90-min period in the case of controls (N = 60) and over a 2-hr period in the case of experimental animals (N = 50). Plasma was deproteinized by the addition of an equal volume of 0.25 M perchloric acid, centrifuged for 1 hr at 10,000 *g*, and 100-μl samples of extract were analyzed by reversed phase HPLC operated isocratically at 1.0 ml/min. Control (N = 3) and experimental animals (N = 4) were killed by cervical dislocation at 30, 60 and 90 min for analysis of cerebral tissues. Striatum, vermis and parietal cortex samples were dissected on ice, sonicated briefly in 10 vol. of cold 0.1 M perchloric acid, and centrifuged for 25 min at 10,000 *g* before analysis by HPLC. Chromatographic conditions and identification of metabolites are detailed elsewhere [6].

RESULTS

The results of one experiment on the inhibition of COMT *in vitro* are illustrated in Fig. 1. Although this particular experiment might be taken to show mixed-type inhibition, the mean V_{max} values calculated from three such experiments were almost identical for the uninhibited and inhibited reactions, indicating the expected competitive inhibition. The mean K_i for L-DOPA was 1.29 ± 0.22 mM (±SEM), whereas that for F-DOPA was 0.384 ± 0.034 mM. The mean ratio of the two K_i values was 2.45 ± 0.80. The K_m for DHBA was 90 ± 10 μM, whereas that for U-0521 was 5.5 ± 0.3 μM (means of three determinations, data for U-0521 not shown).

In control animals, [^{18}F]6-fluorodopa disappeared from plasma according to a biexponential function

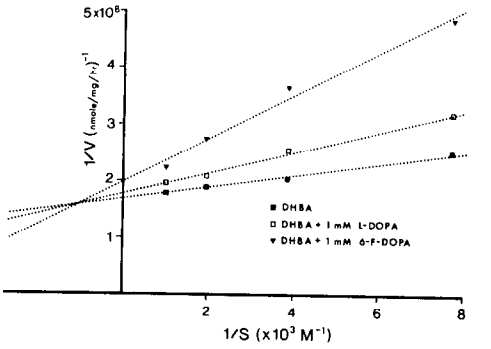

Fig. 1. Lineweaver–Burk plot of the competitive inhibition of COMT by 1 mM L-DOPA or 1 mM D,L-6-fluorodopa with DHBA serving as the substrate. Each point represents the mean of three determinations.

with half-lives of 3.3 and 34 min (Fig. 2), in close agreement with a previous report using tritiated 6-fluorodopa [15]. The extrapolated plasma [^{18}F]DOPA activity at t_0 was 1750 cpm/μl. [^{18}F]DOPA was not detectable in the plasma of control animals after 90 min.

In experimental animals receiving U-0521, the disappearance of [^{18}F]DOPA from plasma was also biexponential with half-lives of 4.7 and 38 min. The extrapolated plasma activity at t_0 was 1840 cpm/μl. At time points between 5 and 90 min, the addition of the COMT inhibitor served to double the amounts of [^{18}F]DOPA available in plasma. Calculation of the area under the curves during the first 90 min showed that the COMT inhibitor increased the availability of [^{18}F]DOPA by 46% during the first 15 min and 78% during the first 90 min.

The principal [^{18}F]DOPA metabolite in plasma was Me[^{128}F]DOPA, but traces (less than 5% of total activity) of an additional compound eluting before [^{18}F]DOPA were seen after 20 min in control animals. This compound, which may be sulfated [^{18}F]DOPA [6], appeared at 15 min in U-0521-treated animals and tended to accumulate, constituting 20% of total radioactivity in plasma at 120 min. The formation of 4-*O*-methyl-6-fluorotyrosine is a possibility, but no unknown was seen at the position where this isomer might be expected to

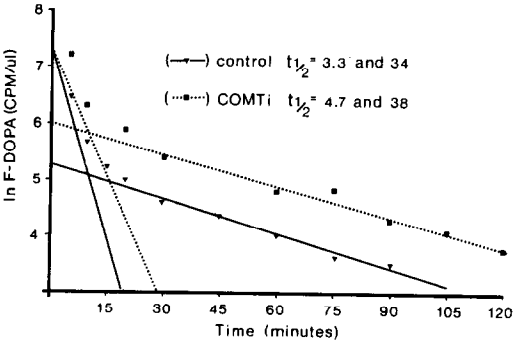

Fig. 2. Biexponential disappearance of [^{18}F]DOPA from plasma in control rats and in animals receiving 25 mg/kg, i.p., U-0521 (COMTi). Each point represents the mean of between three and eight determinations. Standard errors were less than 0.1 unit of the natural logarithm.

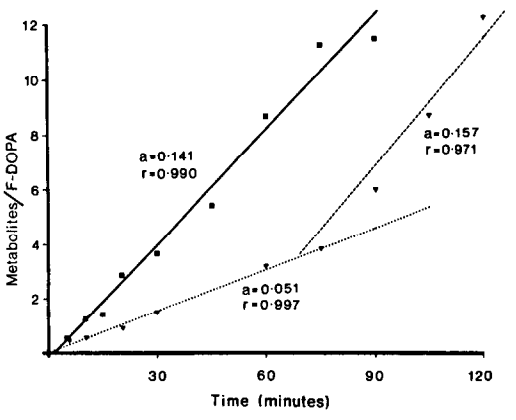

Fig. 3. Ratio of total plasma metabolites to [^{18}F]DOPA as a function of time in controls (■) and in animals receiving U-0521 (▼). In the latter case, separate coefficients of correlation and slopes were calculated for the first and last five points.

elute [16] and 6-fluoro catechols have been reported previously to be methylated predominantly in the 3-position [16, 17].

It has been shown previously that the ratio of labeled metabolites to [^{18}F]DOPA in plasma from carbidopa-pretreated humans increases in a linear manner with time [7]. This relation was seen to hold for the hooded rat (Fig. 3). When U.0521 was included, the relation was also linear during the first 75 min, but with a slope 60% less than that seen in control animals. When the ratio of methylated metabolite, rather than total metabolites, to [^{18}F]DOPA was calculated as a function of time, slopes were reduced by 10% in both experimental and control groups (data not shown).

Inhibition of COMT served to increase the relative amounts of [^{18}F]DOPA at 30 min by 90% in both cortex ($P < 0.05$, Fig. 4a) and vermis ($P < 0.025$, Fig. 4b). A 30% reduction in Me[^{18}F]DOPA was observed at 30 min ($P < 0.001$) in cortex but not in vermis. As previously reported [6], no other labeled compounds were seen in significant amounts in these regions except for an occasional appearance of traces (<5% of radioactivity) of the material eluting before [^{18}F]DOPA. In animals treated with the COMT inhibitor, small amounts of [^{18}F]dopamine ([^{18}F]DA) were also found in cortical samples from animals killed 30 min after the administration of [^{18}F]DOPA.

In the striatum, only traces of [^{18}F]DOPA were observed, with no significant increases produced by COMT inhibition (Fig. 5a) over the course of the experiment. However, the relative amounts of Me[^{18}F]DOPA were reduced in the striatum by approximately 30% at 30 ($P < 0.001$), 60 and 90 min ($P < 0.025$). Significant amounts of [^{18}F]DA were formed in the striatum of both control and U-0521-treated animals. COMT inhibition increased the proportion of this compound by 50% at all three time points studied (Fig. 5b, $P < 0.05$). Increases in [^{18}F]dihydroxyphenylacetic acid ([^{18}F]DOPAC) of 40% at 30 ($P < 0.005$) and 60 ($P < 0.05$) min were also noted in the striatum of animals treated with U-0521. There was a significant linear correlation between the amount of [^{18}F]DA and the amount of [^{18}F]DOPAC in the striatum in both control ($r = 0.90$) and experimental ($r = 0.75$) animals. In control animals, the [^{18}F]DA metabolites, [^{18}F]homovanillic acid ([^{18}F]HVA) and [^{18}F]-3-methoxytyramine ([^{18}F]3-MT), were found only at trace levels in the striatum, never collectively accounting for more than 10% of total radioactivity. No increases were noted in these two compounds in the experimental animals, and the levels of these two compounds did not correlate with the amounts of [^{18}F]DA. The metabolite ratios of the radiolabeled compounds were constant over the course of the experiment; the overall means are given in Table 1. The ratio of [^{18}F]3-MT/[^{18}F]DA was significantly less in the COMT-inhibited animals than in controls. Traces (less than 5% of total radioactivity) of the unidentified metabolite eluting before [^{18}F]DOPA were sometimes also found in striatal extracts.

The mean ^{18}F activities in striatum, cortex and vermis and the ratios of activity in striatum and vermis (S/V) are summarized in Table 2. Absolute activities in striatum were not increased significantly by COMT inhibition. The ratio of activity in striatum and vermis (S/V) was increased at all three time points, but this increase only reached significance at 30 min ($P < 0.01$).

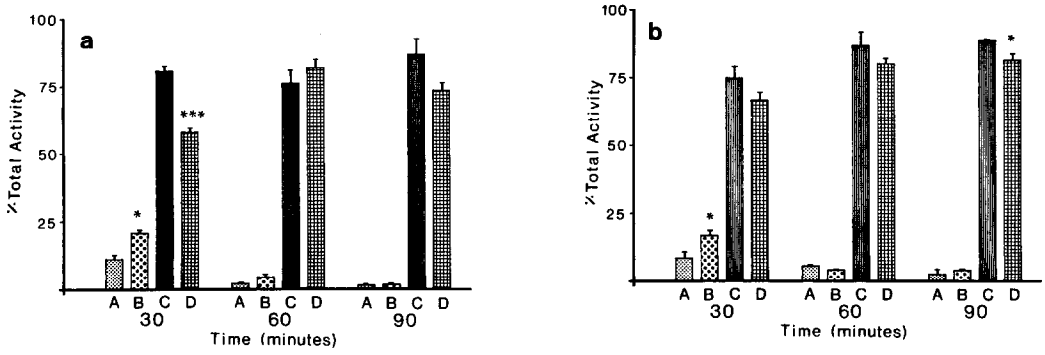

Fig. 4. Relative amounts of radiolabeled compounds in cortex (a) and vermis (b) as a function of time. A = [^{18}F]DOPA in controls (N = 3), B = [^{18}F]DOPA in animals with U-0521 (N = 4). C = Me[^{18}F]DOPA in controls, D = Me[^{18}F]DOPA in animals with U-0521. Key: (*) $P < 0.05$ and (***) $P < 0.001$ by Student's double-tailed t-test. Error bars are SEM.

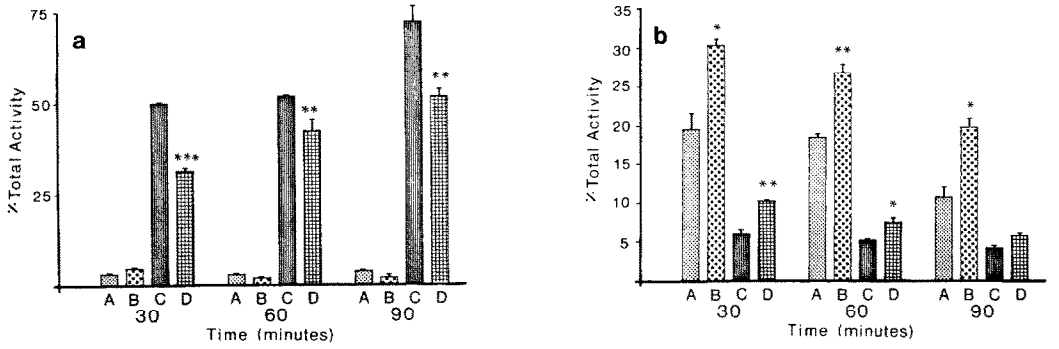

Fig. 5. Relative amounts of radiolabeled compounds in striatum at various times. (a) [^{18}F]DOPA (A and B) and Me[^{18}F]DOPA (C and D) in controls (A and C, N = 3) and in animals with U-0521 (B and D, N = 4). (b) [^{18}F]DA (A and B) and [^{18}F]DOPAC (C and D) in controls (A and C, N = 3) and in animals with U-0521 (B and D, N = 4). Key: (*) $P < 0.05$, (**) $P < 0.005$, and (***) $P < 0.001$ by Student's two-tailed t-test; means ± SEM.

Table 1. Ratios of metabolites to [^{18}F]DA in striatum

	[^{18}F]DOPAC/[^{18}F]DA	[^{18}F]HVA/[^{18}F]DA	[^{18}F]3-MT/[^{18}F]DA
Control (N = 9)	0.371 ± 0.056	0.351 ± 0.099	0.272 ± 0.072
U-0521 (N = 12)	0.305 ± 0.017	0.225 ± 0.041	0.130 ± 0.012*

Values are means ± SEM.
* $P < 0.05$, Student's two-tailed t-test.

DISCUSSION

Although 6-fluorodopa is reported to be a relatively poor substrate for COMT [16, 18], it is apparent that O-methylation proceeds rapidly *in vivo*. The COMT inhibition study indicates that D,L-6-fluorodopa is a better competitive inhibitor of COMT than is L-DOPA. This finding is not inconsistent with the reported higher K_m for F-DOPA since the fluorinated compound may compete for the active site of the enzyme while remaining a poor substrate. The K_m found for DHBA is lower than literature reports [19, 20], a difference possibly related to such factors as pH and the concentration of SAM [21]. However, this difference should not detract from the results of the competitive inhibition study. Although COMT has been reported previously to be non-stereospecific [22, 23], a recent report has shown the enzyme to have a 2- to 3-fold preference for the L-isomer of catechols [24]. This would suggest that L-fluorodopa would be an even better competitive inhibitor of COMT than is the racemic compound. The K_m determined for U-0521 (5.5 μM) is very close to its reported K_i (7.8 μM) as an inhibitor for the O-methylation of adrenaline by liver COMT [25] and indicates that U-0521 has a much higher affinity for COMT than either L-DOPA or fluorodopa.

The dose of U-0521 used in the present study (25 mg/kg) was considerably less than that required to inhibit COMT fully [9]. Higher doses were not used because of an apparent sedative effect in rats

Table 2. ^{18}F Activities in various tissues as a function of time

		^{18}F Activities (cpm/mg tissue or cpm/μl plasma)		
		30 min	60 min	90 min
Control (N = 3)	Striatum	668 ± 94	713 ± 63	434 ± 56
	Cortex	509 ± 85	415 ± 32	276 ± 12
	Vermis	557 ± 96	390 ± 31	294 ± 12
	Plasma	549 ± 54	538 ± 36	480 ± 45
	S/V	1.24 ± 0.08***	1.82 ± 0.02	1.45 ± 0.14
U-0521 (N = 4)	Striatum	737 ± 103	792 ± 64	669 ± 139
	Cortex	487 ± 65	385 ± 38	391 ± 89
	Vermis	484 ± 68	368 ± 41	366 ± 80
	Plasma	625 ± 53	570 ± 47	542 ± 44
	S/V	1.52 ± 0.02	1.97 ± 0.12	1.62 ± 0.03

Values are means ± SEM. S/V = ratio of activity in striatum and vermis.
*** $P < 0.01$, Student's two-tailed t-test.

given 100 mg/kg. Nevertheless, the dose used was sufficient to reduce markedly the rate of disappearance of [^{18}F]DOPA from plasma, an effect which is restricted to the first term of the biexponential expression. This is consistent with the interpretation that the rapid first term corresponds to a combination of O-methylation and uptake into tissue. As COMT is located intracellularly in rat liver [26], it seems probable that tissue uptake and metabolism would take place in a concerted manner. The second term of the biexponential expression, presumably related to renal elimination, was unaffected by COMT inhibition. Extrapolated plasma concentrations of [^{18}F]DOPA at t_0 were virtually the same in experimental and control groups, indicating that both groups received the same radiochemical dose.

The characterization of the effects of U-0521 on [^{18}F]DOPA metabolism in the rat may not only have significance for human PET studies but may have implications for the management of Parkinson's disease. The elimination of L-DOPA is biexponential in humans with terms similar to those found for [^{18}F]DOPA [7, 27]. The long half-life for 3-*O*-methyltyrosine in human plasma is sufficient to result in 3-*O*-methyltyrosine levels higher than those of L-DOPA in Parkinsonians under treatment [28]. In the light of evidence that 3-*O*-methyltyrosine interferes with the uptake of L-DOPA by rat striatum [29], it could be beneficial for the management of Parkinson's disease to inhibit this metabolic pathway. However, in a limited clinical trial, U-0521 was not found to potentiate L-DOPA therapy [9]. This may reflect the probable short duration of action of U-0521 *in vivo*. In the rat, for example, the effect lasted only about 1 hr. A short duration of action for U-0521 is suggested not only by the change in the rate of accumulation of Me[^{18}F]DOPA in plasma (Fig. 3), but by the lack of significant reduction in the accumulation of Me[^{18}F]DOPA in cortex and vermis at 60 and 90 min.

The striatal ratio of [^{18}F]HVA/[^{18}F]DA was not affected significantly by the U-0521, but the ratio of [^{18}F]3-MT/[^{18}F]DA was significantly lower in the COMT-inhibited animals. This might be taken to indicate some slight action of U-0521 in the striatum but, since Me[^{18}F]DOPA was reduced only slightly in cortex and vermis, the formation of methylated [^{18}F]DA metabolites is not likely to be greatly influenced by U-0521 acting in the central nervous system. An alternate explanation could be that Me[^{18}F]DOPA entering the striatum is decarboxylated to [^{18}F]3-MT, and this source contributes less in the COMT-inhibited animals since they show a greatly reduced striatal accumulation of Me[^{18}F]DOPA. Associated with this reduction is an increased synthesis of [^{18}F]DA and [^{18}F]DOPAC. It is therefore possible that U-0521 might provide a useful increase in the accumulation of radioactivity in the striatum of humans undergoing PET/[^{18}F]DOPA studies. Although the increases in S/V here reported are not dramatic and only reached significance at 30 min, the most important point is that these changes reflect major increases in striatal [^{18}F]DA synthesis. A continued search for COMT inhibitors appropriate for human use may be required, with a major criterion being the duration of action *in vivo*.

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