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Tolerance to the Effects of Δ^9-Tetrahydrocannabinol in Mice on Intestinal Motility, Temperature and Locomotor Activity

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Abstract. The onset and duration of tolerance to three effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) given orally to mice were compared. The effects of Δ^9-THC studied were: hypothermia, the depression of intestinal motility and the effect on spontaneous locomotor activity. When mice were dosed and tested at 24 hrs intervals it was apparent that tolerance was complete to its hypothermic and locomotor depressant effects after the first doses and to depression of intestinal motility after the fourth dose. Duration of tolerance also differed so that the normal hypothermic response had returned after 12 dose-free days, but not after 5 drug-free days; the effect on locomotor activity had returned within 4 days; and, apparent partial tolerance to the depressant effect of an acute challenging dose of Δ^9-THC on intestinal motility still existed after 19 dose-free days.

It is apparent that the time of onset and the duration of tolerance to Δ^9-THC in mice showed a different pattern in the three parameters studied. It seems unlikely therefore that any one mechanism, such as metabolic tolerance, explains all the results observed and that several mechanisms should be explored to explain the phenomenon of tolerance to Δ^9-THC.

Key words: Δ^9-Tetrahydrocannabinol – Tolerance – Hypothermia – Intestinal Motility – Locomotor Activity.

Introduction

A considerable literature exists about the development of tolerance to a number of different pharmacological and behavioural measures after repeated administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) or cannabis extract to a variety of animal species (see *e.g.* McMillan *et al.*, 1971; Abel *et al.*, 1972; Davis *et al.*, 1972). Because these studies utilised a variety of preparations of cannabis, routes of administration and methods to assess tolerance, we decided to compare the rate of onset and the duration of tolerance to Δ^9-THC using three well documented effects of cannabis in rodents: the hypothermic effect (Holtzmann *et al.*, 1969; Abel, 1972), the depression of locomotor activity (Garattini, 1965; Davis *et al.*, 1972) and the depression of the passage of a charcoal meal (Dewey *et al.*, 1972; Chesher *et al.*, 1973; Anderson *et al.*, 1974).

Methods

Naive SW strain female mice used in these studies were kept at 22 ± 1°C under conditions of ambient lighting (12 hrs light, 12 hrs dark). They were allowed free access to food and water up to the commencement of experimentation except in the intestinal motility studies where animals were starved overnight (17–23 hrs) prior to use. All experiments were conducted at 22 ± 1°C.

The Δ^9-THC was dissolved in propylene glycol, stored at 2°C and suspended when required in a solution of Lissapol-Dispersol (I.C.I.) (Whittle, 1964) to produce a suspension containing in all cases 5% propylene glycol. The Δ^9-THC was administered by gavage in a dose volume of 1 ml/100 g body weight. Control mice received the vehicle. Significance levels were determined by Student's "*t*" test.

Temperature Studies. Rectal temperatures were measured with a thermistor thermometer, the probe of which was inserted approximately 2 cm into the rectum. Readings (°C) were taken immediately before and at 15 min intervals for 105 min after administration of the drug or vehicle.

Locomotor Activity Studies. Locomotor activity was measured in a circular actophotometer, 60 × 50 cm high, intersected with 4 light beams. The activity, expressed as the number of times the mice interrupted the light beams, was recorded by an automatic counter and printout. Groups of 5 mice were used and were placed into the cage when appropriate (see Results) immediately after injection. In all data reported here the activity is the total number of times the light beams were crossed in the first hour after injection.

Intestinal Motility Studies. The method used was based on that of Macht and Barba-Gose (1931), and has been described in detail by Chesher *et al.* (1973). For each mouse the distance travelled by the charcoal meal was expressed as a percentage of the total length of the intestine from pylorus to the ileo-caecal junction. The mean of this estimation for the control group was taken as 1 and the mean for the Δ^9-THC treated mice was then expressed as a percentage of the control value.

Results

Temperature Studies. A dose of 10 mg Δ^9-THC/kg produced significant hypothermia, with the maximal effect occurring 45 min after injection (Table 1). The hypothermic effect was shown to be dose-dependent (Fig. 1).

To test for the development of tolerance, groups of mice received either 10 mg Δ^9-THC/kg or the vehicle, and their rectal temperatures were recorded every 15 min for 105 min after dosing. When the Δ^9-THC-dosed mice received the same dose of the drug 24 hrs later no hypothermia was observed. To test the duration of this tolerance separate groups of mice were made tolerant by administration of 2 doses of 10 mg Δ^9-THC/kg, 24 hrs apart, and then with no intervening medication, challenged on either day 8 or day 15 with a third dose. Whilst the hypothermic response was still attenuated on day 8 (Fig. 2a), by day 15 the response was essentially that seen on day 1 (Fig. 2b).

Locomotor Activity Studies. Significant depression of locomotor activity was produced by 40 mg Δ^9-THC/kg when compared to the activity of mice treated with the vehicle (Fig. 3). Maximum depression occurred approximately 30 min after injection and the response after 80 min was no different from that of the control groups. The depressant effect of Δ^9-THC on locomotor activity was shown to be dose-dependent (Fig. 4). However a significant increase in locomotor activity was observed in mice which received the lowest dose of Δ^9-THC (1.25 mg/kg).

To test for the rate of development of tolerance and its duration, groups of five mice were dosed and locomotor activity measured according to the following schedule. Experiment I: animals dosed with either Δ^9-THC or vehicle and immediately placed in the actophotometer. Experiment II: animals dosed with either Δ^9-THC or vehicle both on day 1 and 24 hrs later on day 2 but exposed to actophotometer only on day 2. Experiment III: animals dosed on day 1 with vehicle only and placed in actophotometer. On day 2 animals dosed either with Δ^9-THC or vehicle and again placed into actophotometer. Experiment IV: animals dosed with Δ^9-THC or vehicle on days 1 and

Table 1. The effect of 10 mg Δ^9-THC/kg or the vehicle on rectal temperature in mice at various times after oral administration

Time after dosage (min)	Mean temperature (°C) ± S.E.M.		Difference in temperature (°C)[a]
	vehicle ($n = 35$)	Δ^9-THC (10 mg/kg) ($n = 35$)	
0	37.6 ± 0.1	37.8 ± 0.2	+ 0.2
15	37.8 ± 0.2	37.2 ± 0.2*	− 0.6
30	37.9 ± 0.2	36.6 ± 0.3*	− 1.3
45	37.9 ± 0.2	36.3 ± 0.3*	− 1.6
60	37.7 ± 0.2	36.6 ± 0.3*	− 1.1
75	37.7 ± 0.1	36.5 ± 0.2*	− 1.2
90	37.6 ± 0.1	36.7 ± 0.2*	− 0.9
105	37.5 ± 0.1	36.6 ± 0.2*	− 0.9
120	37.4 ± 0.1	36.8 ± 0.2*	− 0.6

* $P < 0.05$ when compared to the control.
[a] The rise (+) or fall (−) in temperature produced by Δ^9-THC.

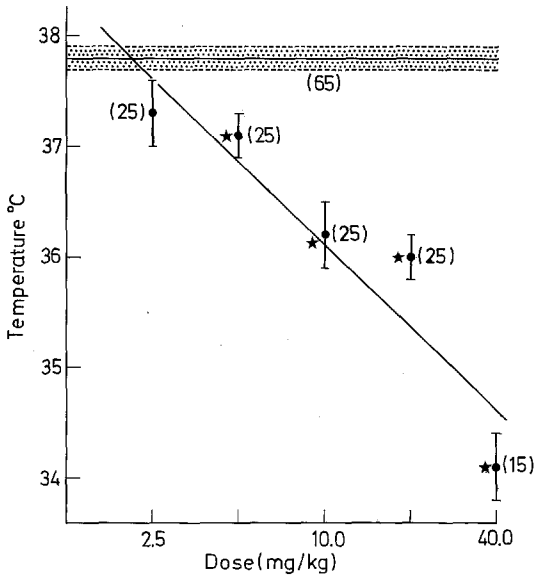

Fig. 1. The effect of Δ^9-THC on rectal temperature. Each point is the mean temperature ± S.E.M. at 1 hr after injection. The horizontal hatched area represents the mean temperature ± S.E.M. 1 hr after administration of the vehicle. The numbers in brackets are the number of animals. * $P < 0.05$ when compared to the control

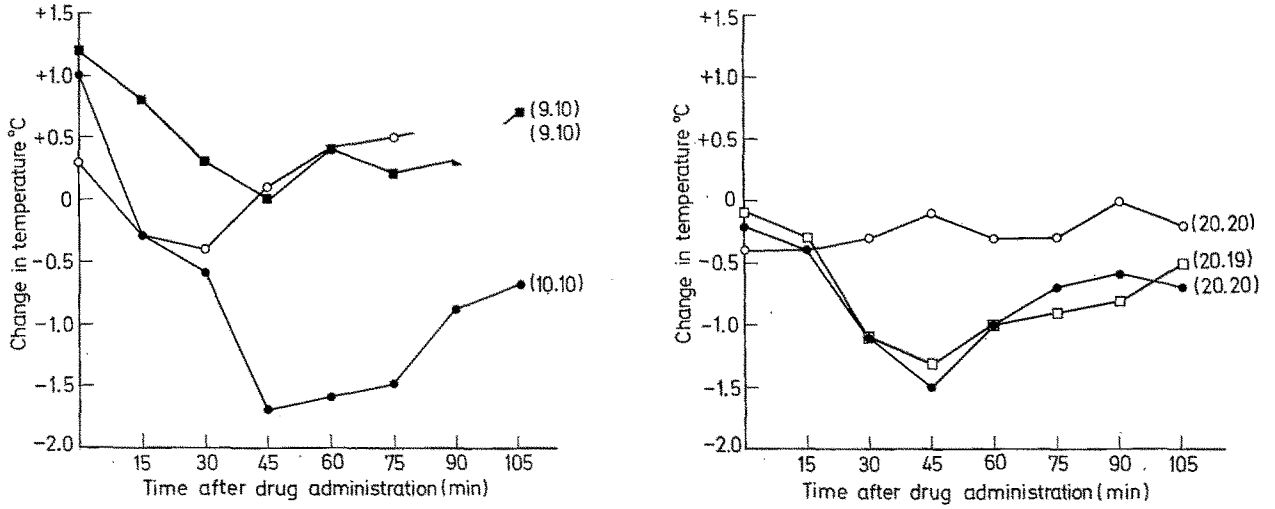

Fig. 2a and b. The onset and duration of tolerance to the hypothermic effect produced by oral administration of 10 mg Δ^9-THC/kg. Tolerance was induced in separate groups of mice by administration of two doses of Δ^9-THC 24 hrs apart (for details see text) and the duration of tolerance was tested by administration of a third challenging dose of Δ^9-THC (10 mg/kg) on either day 8 (Fig. 2a) or day 15 (Fig. 2b). The data is expressed as the differences in rectal temperatures (°C) between mice which received either Δ^9-THC or vehicle. Day 1 ●; day 2 ○; day 8 ■; day 15 □. The numbers in brackets on the graphs refer to the number of mice used in each of the treated and control groups respectively

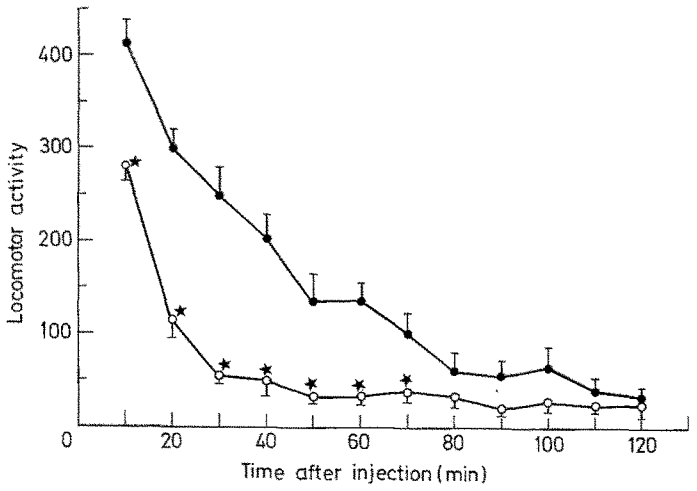

Fig. 3. Effect of 40 mg Δ^9-THC/kg on locomotor activity in groups of 5 mice at different times after dosage. Activity is expressed as the number of times the light beams of the actophotometer were crossed each 10 min after injection of either vehicle or Δ^9-THC. Each point is the mean activity ± S.E.M. ● Vehicle ($n = 12$); ○ Δ^9-THC ($n = 8$). * $P < 0.05$ when compared to the vehicle at the same time period

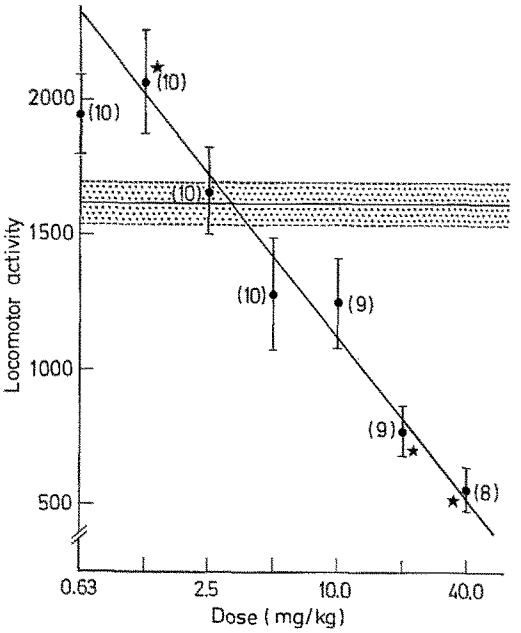

Fig. 4. The effect of Δ^9-THC on locomotor activity in groups of 5 mice. Each point is the mean number of times the light beams of the actophotometer were crossed in the first hour after dosage ± S.E.M. and the hatched area represents the mean activity ± S.E.M. of the control group. The numbers in brackets refer to the number of times the experiment was conducted. * $P < 0.05$, when compared to the mean locomotor activity of vehicle treated controls

4 and exposed to actophotometer only on day 4. The results (Table 2) confirm the depressant effect of an acute dose of 40 mg/kg Δ^9-THC in mice (Experiment I). However, the activity of mice tested immediately after the second of 2 daily doses of Δ^9-THC was not significantly different from that of controls (Experiment II). Mice tested after 3 non-treatment days had elapsed between the first and second doses of Δ^9-THC showed a depression of activity after the second dose that was significantly different from controls (Experi-

Table 2. The effect of 40 mg Δ^9-THC/kg administered orally on locomotor activity in groups of 5 mice under different dosage schedules. "*n*" is the number of times the experiment was repeated

Experiment and dose schedule (see text)		Dosage / group	Total activity in 1 hr [mean ± S.E.M. (*n*)]	Activity of Δ^9-THC as % of comparable control group
I.	Dosed and tested day 1	control	1816 ± 268 (6)[a]	35
		Δ^9-THC	642 ± 81 (5)[b]	
II.	Dosed day 1 and 2, tested day 2 only	control	2516 ± 268 (5)[c]	73
		Δ^9-THC	1832 ± 547 (5)[d]	
III.	Dosed as indicated and tested on both day 1 and day 2	group Ia	day 1 (vehicle) 3131 ± 371 (5)[e]	—
		group Ib	day 1 (vehicle) 2469 ± 407 (5)[f]	—
		group Ia	day 2 (vehicle) 2369 ± 529 (5)[g]	74
		group Ib	day 2 (Δ^9-THC) 1752 ± 724 (5)[h]	
IV.	Dosed day 1 and day 4 only tested day 4 only	control	2690 ± 233 (5)[i]	36
		Δ^9-THC	963 ± 72 (5)[j]	

[a, b] $t = 4.1932$: $P < 0.01$; [c, d] $t = 1.1229$: N.S.; [e, f] $t = 1.2020$: N.S.; [g, h] $t = 0.6881$: N.S.; [i, j] $t = 7.0816$: $P < 0.001$.

ment IV) and of the same order as that seen after a single acute dose (Experiment I).

The effect of habituation to the environment upon the response to Δ^9-THC is shown in Table 2, Experiment III. Mice which had been exposed to the actophotometer on day 1 immediately after dosage with vehicle only, did not show a significant depressive response to 40 mg Δ^9-THC/kg when tested on day 2.

Intestinal Motility Studies. A dose of 10 mg Δ^9-THC/kg (shown in previous experiments by Chesher *et al.* (1973) to depress intestinal motility) produced maximum depression of the passage of a charcoal meal 30 min after administration of the Δ^9-THC and the effect had subsided by 120 min (Fig. 5). The depressant effect of Δ^9-THC on intestinal motility was confirmed as being dose-dependent (data not shown: see Chesher *et al.*, 1973). To test for the development of tolerance, groups of mice were given repeated doses of 10 mg Δ^9-THC/kg or vehicle at 24 hrs intervals and killed 1 hr after either the first, second, third or fourth doses. Some tolerance was observed after the second dose, however after the third and fourth doses tolerance was almost complete (Fig. 6). The duration of tolerance was examined by medicating different groups of mice with 4 doses of 10 mg Δ^9-THC/kg at 24 hrs intervals and then killing after a fifth challenging dose which was given sometime between the 5 – 24 day (Fig. 6). The data suggests that by day 5 (*i.e.*, 24 hrs after the fourth and final tolerance producing dose of Δ^9-THC) the response had returned to approximately 70% of the response obtained on day 1, which means that the treated animals were now responding with less intestinal motility depression than naive animals receiving the same dose of Δ^9-THC had on day 1. Even after day 24 (*i.e.*, after 19 drug free days), the response had still not returned to that which was obtained on day1.

Discussion

Δ^9-THC has been found to induce hypothermia in mice after intraperitoneal (Holtzmann *et al.*, 1969) and intramuscular (Abel, 1972) administration. The present study confirms these observations and has shown that the effect is also produced by oral administration of this compound. Hypothermia caused by Δ^9-THC was shown to be dose-dependent, an observation which supports the findings of Holtzmann *et al.* (1969) and Abel (1972). The almost immediate development of tolerance to the hypothermic effect of Δ^9-THC in mice is similar to the findings of Lomax (1971) who used cannabis extract in rats and Abel *et al.* (1972) and Ten Ham and De Yong (1974) who used Δ^9-THC in chicks and mice respectively. The disappearance of tolerance within 15 days, but not after 8 days agrees with Lomax (1971) who found that the hypothermic response had returned 15 days after the first injection of cannabis extract to rats.

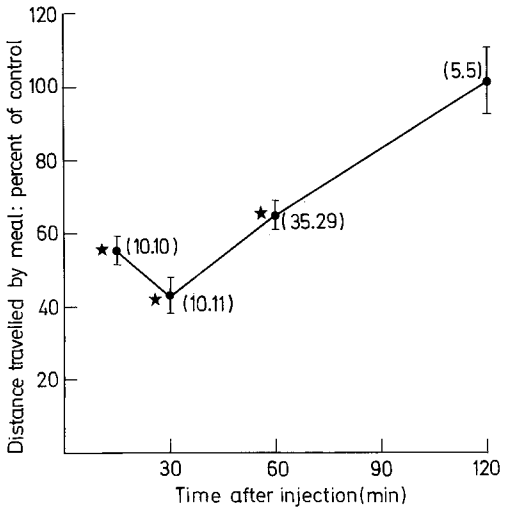

Fig. 5. The effect of Δ^9-THC (10 mg/kg) on the passage of a charcoal meal in mice at different times after dosage. The numbers in brackets refer to the number of mice used in the control and treated groups respectively. * $P < 0.05$, when compared to vehicle control treated animals

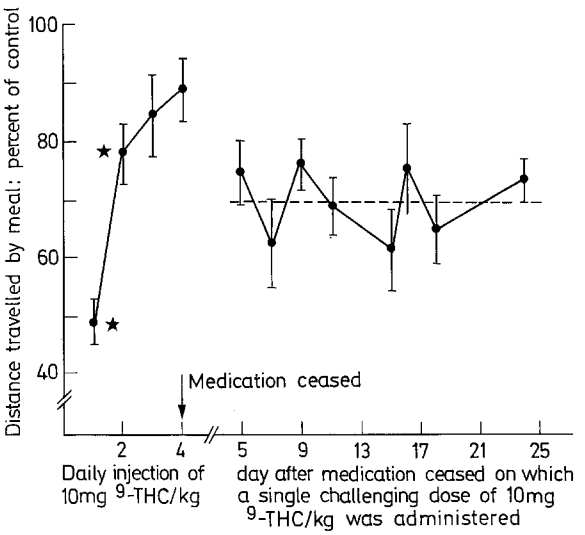

Fig. 6. The development and duration of tolerance to the depressant effect of Δ^9-THC on the passage of a charcoal meal in mice. The Δ^9-THC or vehicle was given 60 min before death. Each point is the mean ± S.E.M. The dashed line is the line of best fit estimated according to the method of least squares for the responses on days 5–24. The number of mice in each of the vehicle and Δ^9-THC treated groups was 20, except on days 15 and 16, when it was 10. For further details see the text. * $P < 0.05$ when compared to the control of the same day, which received the vehicle only. Further statistical comparisons: (a) when compared to the response on day 1: $P < 0.05$ for days 5, 9, 11, 16, 18, 24. $P > 0.05$ for days 7, 15. (b) when compared to the response on day 4: $P < 0.05$ for days 7, 9, 11, 15, 18, 24. $P > 0.05$ for days 5, 16

The finding that Δ^9-THC produced a dose-dependent depression of locomotor activity agrees with Holtzmann *et al.* (1969) and Brown (1972). At the lowest dose of Δ^9-THC studied (1.25 mg/kg) the stimulation activity confirms in mice the earlier reports of Abel (1970) and Davis *et al.* (1972) who observed stimulation after low doses of pyrahexyl and Δ^9-THC respectively to rats.

Our results show that tolerance to the depressant effect of Δ^9-THC on locomotor activity in mice developed after a single dose and are in agreement with those of Abel *et al.* (1972) who showed the same rapid development of tolerance in rats. The duration of this tolerance appeared to be less than 4 days because mice tested at this time showed a depressant response to Δ^9-THC which was similar to that observed after a single, acute dose.

The effect of habituation to the environment on the response to Δ^9-THC was particularly striking. When compared with similarly habituated control mice, Δ^9-THC failed to produce a depression in the locomotor activity of mice which had been exposed to the actophotometer for 1 hr the previous day. These results are in general agreement with Drew and Miller (1973) who showed that animals habituated to activity wheels showed a different response to Δ^9-THC when compared to animals which had not been habituated to the activity wheels, and stress the importance of vigorous control measures when testing the interaction of drugs, locomotor activity and environment. A recent report by Oliverio and Castellano (1974) that prior experience to an environment disrupts the behavioural response to morphine also supports these general findings. However, habituation to the environment cannot explain the development of tolerance in the experiments described here because, with the exception of the mice in Experiment III, all were exposed to the actophotometer only once, *i.e.*, on the day of testing.

Relatively little literature exists about the effect of cannabis on intestinal motility. Preliminary work utilised faecal boli counts in open-field experiments, and from these studies Drew *et al.* (1972) and Masur *et al.* (1971) observed that Δ^9-THC reduced defaecation in rats considered to be "high defaecators". Using the passage of a charcoal meal in mice as an index of intestinal motility, Dewey *et al.* (1972) and Chesher *et al.* (1973) found Δ^9-THC to be depressant, with the latter authors finding the effect to be dose-dependent. We report here the development of tolerance to this depressant effect in mice after 4 daily doses of 10 mg Δ^9-THC/kg. This finding confirms and extends the report of Masur *et al.* (1971) who found that daily administration of either cannabis extract or Δ^9-THC

to rats that were "high defaecators" resulted in a reduction in the number of boli eliminated towards control levels. Of importance and theoretical interest is the finding that some type of long lasting change seems to have been produced in mice which had received 4 daily doses of Δ^9-THC. The present authors have no explanation for this phenomenon. But be it either a very long lasting partial tolerance or a permanent change, the effect deserves further study.

From these studies, it is evident that the onset and duration of tolerance to Δ^9-THC varies according to the pharmacological parameter studied. For this reason it seems necessary that tolerance to Δ^9-THC can only be studied by examining several drug effects rather than an individual effect.

Furthermore, the different number of daily administrations of Δ^9-THC to produce tolerance to different pharmacological effects of cannabis when a constant dose of Δ^9-THC is used [(*e.g.* 10 mg Δ^9-THC/kg) in the temperature and intestinal motility studies] suggests that the development of tolerance to a drug such as Δ^9-THC may proceed by different mechanisms of action.

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