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Effects of Two Cannabinoids Upon Abstinence Signs in Ethanol-Dependent Mice[1]

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SPRAGUE, G. L. AND A. L. CRAIGMILL. *Effects of two cannabinoids upon abstinence signs in ethanol-dependent mice.* PHARMAC. BIOCHEM. BEHAV. 9(1) 11–15, 1978.—The effects of Δ^9-tetrahydrocannabinol (THC) and nabilone, a synthetic cannabinoid, upon handling-induced convulsions and responsiveness to electric foot shock were examined during abstinence in ethanol-dependent mice. The severity of handling-induced convulsions was apparently increased by THC (10–40 mg/kg) and nabilone (2.5–10 mg/kg) but both drugs elicited similar convulsions in normal mice never exposed to ethanol. Enhanced responsiveness to electric foot shock, evident during abstinence, was suppressed by THC (10–40 mg/kg). The effects of ethanol upon the two abstinence signs were determined for comparative purposes. Ethanol (0.5–4 g/kg) reduced the severity of handling-induced convulsions and suppressed the increased responsiveness to electric foot shock. These results indicate that THC and nabilone have similar actions upon two abstinence signs in ethanol-dependent mice, and although one sign (responsiveness to electric foot shock) was clearly alleviated, another (handling-induced convulsions) was not.

Ethanol withdrawal Δ^9-Tetrahydrocannabinol Nabilone Ethanol Handled-induced convulsions Responsiveness to foot shock

THE PHARMACOLOGICAL actions exerted by ethanol and Δ^9-tetrahydrocannabinol (THC) have been shown to be similar in some respects and studies have shown that both drugs depress the central nervous system [2, 11, 25, 26]. Evidence for cross-tolerance between ethanol and THC has been demonstrated in mice and rats using both learned and unlearned behaviors [19, 20, 22]. Many drugs that are effective in the treatment of ethanol withdrawal signs in both experimental animals and in humans exhibit either cross-tolerance with ethanol or possess pharmacological properties similar to those of ethanol [12].

Clinical evidence that either THC or a derivative may indeed have therapeutic potential was presented by Thompson and Proctor [24]. They showed that an analog of THC (Pyrahexyl) clinically alleviated withdrawal signs in 84% of the cases tested (N=70). They reported signs of increased appetites, reduced irritability, reduced restlessness, and fewer sleep disturbances during withdrawal in alcoholics treated with Pyrahexyl.

The purpose of this study was to evaluate the actions of THC and nabilone upon signs of ethanol withdrawal in mice. Pharmacological effects exerted by ethanol were then compared to those of THC and also nabilone, a cannabinoid that is currently being studied for its therapeutic potential and that appears to be pharmacologically similar to Pyrahexyl [15,23].

METHOD

Animals and Drugs

Male, Swiss-Webster mice (obtained from Washington State University Lab Animal Resources, Pullman, WA), weighing 18 to 26 g were used throughout the study. Mice were housed under a regulated 12 hr light-dark cycle during the study and for at least 5 days before use. Purina Lab Chow and tap water were continuously available.

Suspensions containing THC (95% THC obtained from the National Institute of Mental Health) or nabilone (dl-3-(1,1-dimethylheptyl)- 6,$\alpha\beta$,7,8,10-10$\alpha\beta$-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran-9-one, graciously supplied by Eli Lilly and Co.) were prepared in a 1% Pluronic F68 vehicle that has been previously described [22]. Pyrazole, recrystallized with petroleum ether, and ethanol were prepared for injection in isotonic saline.

All drugs except ethanol were given intraperitoneally (IP) in a volume of 10 ml/kg. Ethanol was also given IP but the volume of injection of higher doses (e.g., 2 and 4 g/kg) was adjusted to enable the use of ethanol concentrations lower than 24% (v/v) in saline in order to produce minimal peritoneal irritation.

Induction of Physical Dependence

One method used to induce dependence upon ethanol was

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identical to that previously described by Goldstein and Pal [10] except a lower dose of pyrazole was used (40 mg/kg compared to 68 mg/kg). Briefly, this technique involved administration of ethanol vapor (8.5–9.5 mg/liter) to mice in an inhalation chamber for 72 hr. Pyrazole (40 mg/kg), a compound used to reduce the elimination of ethanol, was given at 0, 24 and 48 hr of exposure. Ethanol (1.75 g/kg at 9 hr, 0.8 g/kg at 24 and 48 hr) was given after each dose of pyrazole. The combinations of ethanol and pyrazole used were found to produce blood ethanol concentrations (BECs) which were were maintained at approximately 2.0 mg/ml over the 72 hr period. Another method for inducing physical dependence did not involve the use of pyrazole. Instead, an initial dose of ethanol (2.0 g/kg) was given to mice which were then placed in an inhalation chamber (13.5–14.5 mg/liter). The mice were removed at 5.5 hr intervals for a period of 30 min, and 0.8 g ethanol/kg was administered every 10 min until a loss of righting reflex was evident. The mice were removed from the chamber 48 hr after the first dose of ethanol was given. BECs using this technique were maintained at approximately 2 mg/ml but greater variation was evident than seen using the method which used pyrazole. Ethanol doses and treatment schedules as well as ethanol vapor concentrations used and period of exposure were determined empirically to obtain BECs comparable to those obtained using pyrazole. The treatment period was shorter than that used for the pyrazole groups because toxicity increased after 48 hr of exposure when higher ethanol vapor concentrations were used (i.e., 14.5–15.5 mg/liter).

Mice were randomly assigned to treatment groups. Ethanol dependence was induced in each group (at least seven mice/group) and the effect of one dose of ethanol, nabilone or THC upon expression of an abstinence sign was measured. A total of 616 mice were used in the study.

Ethanol Withdrawal Signs

Mice exhibited several signs characteristic of ethanol withdrawal following either the 48 or 72 hr exposure to ethanol. One of these signs was a high incidence of handling induced convulsions elicited by gently lifting each mouse by the tail [9]. Briefly, mice were given a score of 0 to 4 depending on the severity of convulsions elicited by handling, with a score of 4 given to those animals which exhibited violent tonic-clonic convulsions after being released. The score for each mouse was recorded at 1.5 hr intervals during withdrawal and the mean handling convulsion score for each group at each interval was calculated and plotted against the time of abstinence. In some cases, the mean handling convulsion score of each group during withdrawal was plotted on rectilinear graph paper and the area-under-the-curve for 6 hr of abstinence beginning at the time of drug administration was calculated and values obtained were used to construct dose-response curves for each drug given.

Another sign of ethanol withdrawal was increased responsiveness to an electric foot shock. This behavior was evaluated after individual mice were placed in a test chamber with a grid floor through which an electric shock was applied. Shocks with a 200 msec duration were delivered at 5 sec intervals by a Grass Stimulator (SD5) in increasing 10 V increments beginning at 30 V. The threshold voltage (startle threshold) at which animals exhibited a response to the shock by lifting at least one paw was determined. Mean startle threshold voltages were compared statistically using the Student's t test. Responsiveness to an electric foot shock was measured at 1.5 hr intervals during abstinence.

Ethanol (0.5–4 g/kg), nabilone (2.5–10 mg/kg), THC (10–40 mg/kg), isotonic saline or Pluronic F68 vehicle was administered at 6 and 12 or at 4 hr of abstinence, depending upon the method used to induce ethanol dependence and thus the time of occurrence of peak withdrawal signs. Nabilone (5–20 mg/kg) or THC (10–40 mg/kg) was also given to normal mice never exposed to ethanol in order to determine if either of these drugs produced convulsions similar to those elicited in mice undergoing ethanol withdrawal (eight mice used for each dose of nabilone or THC).

Assays

Blood samples (10 μl) were obtained from the tail veins of mice and BECs were determined using a modified enzymatic technique [16]. Ethanol vapor concentrations in the inhalation chambers were determined on samples withdrawn through sampling ports by gas-liquid chromatography (Varian Model 1400 Gas-Liquid Chromatograph equipped with a 6 ft $\times$ 1/4 in. glass column packed with 5% Carbowax 1540 on Chromosorb G).

RESULTS

Induction of Ethanol Dependence

Mice exposed to ethanol vapor (8.5–9.5 mg/liter) for 72 hr and given three daily injections of pyrazole (40 mg/kg; pyrazole group) had mean BECs between 2.0 and 2.6 mg/ml during the 72 hr period. In contrast, mice given ethanol at 5.5 hr intervals and exposed to 13.5–14.5 mg ethanol/liter (non-pyrazole group) showed mean BECs between 1.7 and 3.5 mg/ml over a 48 hr period. Signs of intoxication in the pyrazole groups included mild ataxia and increased motor activity and those in the non-pyrazole groups ranged from absence of intoxication to a prolonged loss of righting reflex.

Signs of Ethanol Withdrawal

Handling-induced convulsions were not observed in normal mice or in mice given only pyrazole (40 mg/kg). In addition, we found pyrazole (40 mg/kg) had no effect on responsiveness of normal mice to foot shock. Signs of withdrawal were evident after cessation of ethanol exposure in mice in the pyrazole and non-pyrazole groups. Figure 1 shows handling convulsions persisted until 15.5 and 13 hr of abstinence in the pyrazole and non-pyrazole groups, respectively. Responsiveness to foot shock (startle threshold) during withdrawal was altered in similar ways using either method to induce ethanol dependence (Fig. 1). Both groups showed an initial increase in the startle threshold followed by a significant reduction ($p<0.05$) which was of longer duration in the pyrazole than in the non-pyrazole groups. Saline or Pluronic F68 vehicle administration during abstinence produced no significant alteration in handling convulsions or responsiveness to foot shock in either pyrazole or non-pyrazole groups.

Figure 2 illustrates that ethanol and nabilone and THC administration during abstinence in ethanol dependent mice altered handling convulsions in different ways. Mean handling convulsion scores were increased by nabilone (10 mg/kg) or THC (40 mg/kg) and reduced by ethanol (4 g/kg). We administered nabilone (5–20 mg/kg) and THC (10–40 mg/kg) to normal mice never exposed to ethanol and the results are shown in Fig. 3. Both drugs elicited handling

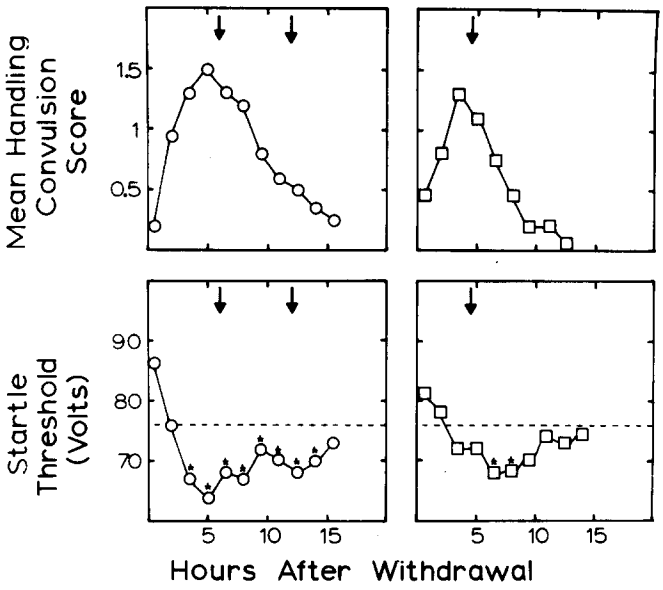

FIG. 1. Handling convulsions and startle thresholds for mice given isotonic saline (arrows) during ethanol withdrawal. Procedures used to induce ethanol dependence in the pyrazole (circles) or non-pyrazole (squares) groups are described in METHOD. Handling convulsions for individual mice were quantified as described in METHOD and mean values are shown. The mean handling convulsion score for mice given only pyrazole was 0. The mean startle thresholds for either normal mice or mice given only pyrazole (horizontal line) did not change after repeated measurement and mean values during abstinence in ethanol dependent mice which are significantly different from the normal value are indicated by * ($p<0.05$). Each group included 17–27 mice.

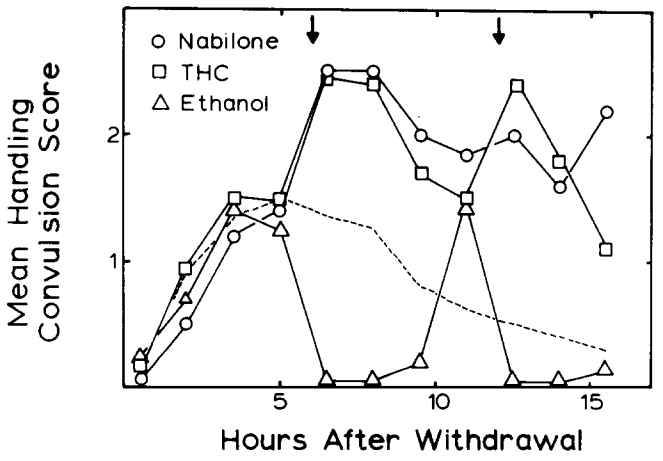

FIG. 2. Handling convulsions for mice given ethanol (4 g /kg), nabilone (10 mg/kg) or THC (40 mg/kg) at 6 and 12 hr of abstinence. Mice were made dependent upon ethanol using pyrazole as described in METHOD. Eight to 12 mice were used for each dose of each drug and the dashed line shows mean scores for mice given Pluronic F68 vehicle during withdrawal (N=53).

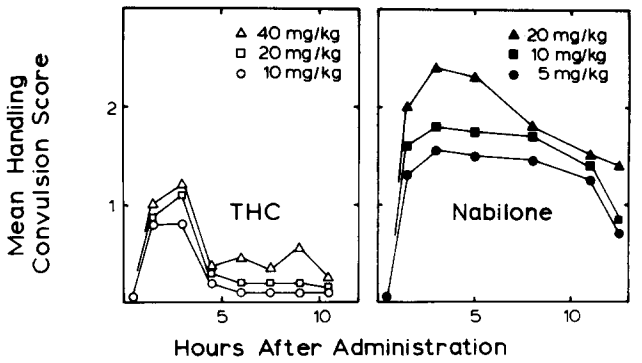

FIG. 3. Effect of nabilone or THC upon handling convulsions in normal mice never exposed to ethanol. Handling convulsions were scored according to the same procedure used for mice undergoing ethanol withdrawal. Eight mice were used for each dose of drug given. The mean handling convulsion score for normal mice given only Pluronic F68 vehicle was 0 score×hr.

convulsions in normal mice that were similar to those observed in ethanol dependent mice during abstinence.

Figure 4 shows how ethanol, nabilone and THC affected the area-under-the-curve (AUC) for handling convulsions during abstinence in the pyrazole group. Ethanol, nabilone and THC administration during abstinence produced qualitatively similar results in the non-pyrazole groups and results from the pyrazole groups are shown since all three drugs produced quantitatively similar effects which were more easily compared statistically.

Increasing doses of ethanol (0.5–4 g/kg) reduced the AUC for handling convulsions during abstinence in an apparent dose-dependent manner but only 4 g/kg produced a significant reduction ($p<0.025$) compared to the AUC for mice given saline during abstinence (6.6 score×hr). In contrast, nabilone (2.5–10 mg/kg) or THC (10–40 mg/kg) administration increased the AUC for handling convulsions during abstinence. All doses of nabilone or THC used produced significant increases ($p<0.05$) compared to the AUC for mice given Pluronic F68 vehicle during abstinence (6.9 score×hr). Nabilone and THC also increased the AUC for handling convulsions in normal mice never exposed to ethanol (Fig. 4).

Figure 5 shows the effects of ethanol, nabilone and THC on responsiveness to foot shock during abstinence in the non-pyrazole groups. Qualitatively similar results were obtained in the pyrazole groups given ethanol, nabilone or THC and data from the non-pyrazole groups are shown because less variation was obtained in the results, a factor which may or may not have been related to the absence of pyrazole. Ethanol (1–4 g/kg), nabilone (2.5–10 mg/kg) and THC (10–40 mg/kg) significantly reduced responsiveness to foot shock during abstinence ($p<0.05$) compared to mice given saline or Pluronic F68 vehicle during abstinence. Only the highest dose of each drug (4 g ethanol/kg, 102 V; 10 mg nabilone/kg, 96 V; 40 mg THC/kg, 89 V) reduced responsiveness to foot shock to levels significantly different ($p<0.05$) from values for normal mice not undergoing ethanol withdrawal. Responsiveness to foot shock during abstinence produced by ethanol (4 g/kg), nabilone (10 mg/kg) or THC (40 mg/kg) were of 3, 4.5 and 7.5 hr durations, respectively.

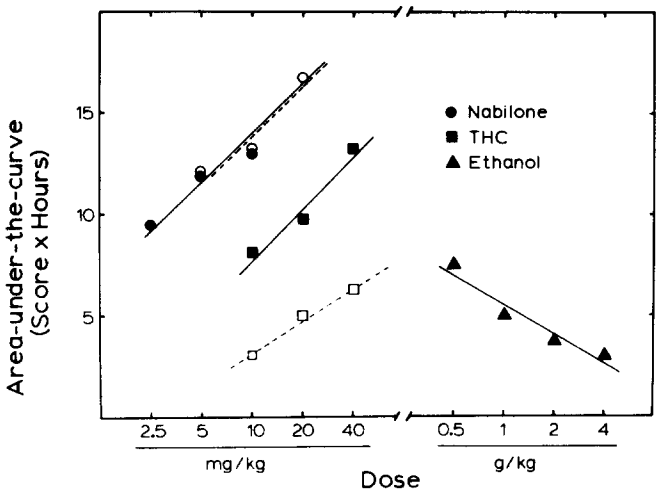

FIG. 4. Effect of ethanol, nabilone or THC in normal mice (dashed lines) and in mice undergoing ethanol withdrawal (solid lines). Schedules for drug administration and procedures to induce ethanol dependence (pyrazole group) and for calculating area-under-the-curve (AUC) are described in METHOD. Each point shows data for 8–12 mice. The AUC for ethanol dependent mice given Pluronic F68 vehicle or saline during abstinence was 6.9 and 6.6 score×hr, respectively. The AUC for normal mice given Pluronic F68 vehicle, saline or ethanol (0.5–4 g/kg) was 0 score×hr.

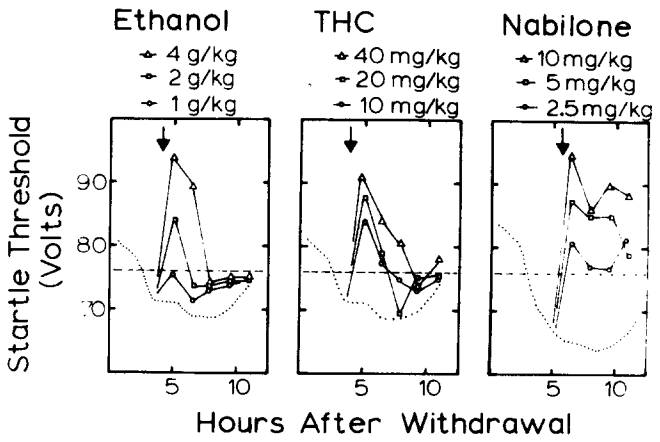

FIG. 5. Effect of ethanol, nabilone or THC administration during abstinence on responsiveness to foot shock (startle threshold) in ethanol dependent mice. Ethanol dependence was induced without using pyrazole as described in METHOD. At least eight mice were used for each dose of drug given. The dashed line shows the mean threshold for normal mice given only saline and the dotted line shows the mean threshold for ethanol dependent mice given saline (N=27) or Pluronic F68 vehicle (N=53) during abstinence.

DISCUSSION

Two methods were used to induce ethanol dependence in our study. One method, described previously [8,10], involved the use of pyrazole to stabilize BECs. We showed BECs comparable to those previously reported [8,10] but we used lower doses of pyrazole (40 compared to 68 mg/kg). We also used a method to induce ethanol dependence which did not involve the use of pyrazole. This was done to determine if prior pyrazole administration qualitatively or quantitatively altered the action of ethanol, nabilone or THC, since this possibility has been suggested because of possible metabolic alterations produced by pyrazole [14].

Handling-induced convulsions [1,8] and hyperactive startle responses [4, 6, 9, 18] have been reported in mice or rats following chronic ethanol exposure and we used handling convulsions and responsiveness to electric foot shock as measures of the severity of abstinence in ethanol-dependent mice. These two signs were noted during abstinence and were qualitatively similar using either method (pyrazole or non-pyrazole) to induce ethanol dependence. The quantitative differences in the severity of abstinence signs noted in the two groups is probably related to the total dose of ethanol given and not to the use of pyrazole. For example, the total dose (mean BEC×duration of exposure) was 1.5 times greater in the pyrazole than in the non-pyrazole groups. Therefore, if one assumes that the severity of abstinence signs are related to the total dose of ethanol administered, one would expect more severe signs in the pyrazole than in the non-pyrazole groups.

The only study dealing with the effect of a cannabinoid on ethanol signs (handling-induced convulsions) in mice was described by Blum and coworkers [1]. Mice were made dependent upon ethanol using three dialy injections of pyrazole (68 mg/kg) and 72 hr of exposure to ethanol vapor (16 or 21 mg/liter). THC (0.5–3 mg/kg) was given intravenously at 5 and 13 hr of abstinence and a biphasic action was reported. Low doses of THC (0.5–1 mg/kg) reduced and a high dose (3 mg/kg) increased the incidence and severity of handling convulsions during ethanol withdrawal.

The results of Blum and coworkers [1] are similar to ours in some respects since we showed that both nabilone and THC increased the AUC for handling convulsions during abstinence. However, we found no evidence for a biphasic action of either nabilone or THC. Nabilone was more potent than THC but the two drugs were pharmacologically similar as evidenced by parallel dose-response curves (Fig. 4). These results are similar to those reported by Goldstein [8] for promazine and chlorpromazine which intensified handling convulsions in mice undergoing ethanol withdrawal. In contrast, however, we found handling-induced convulsions similar to those seen during ethanol withdrawal were evident following nabilone or THC administration to normal mice never exposed to ethanol. Nabilone was more potent than THC and the dose-response curves for the two drugs were parallel (Fig. 4).

Although the anticonvulsant actions of THC in several experimental animals have been well documented [21] a recent study showed evidence for THC-produced behavioral convulsions in rabbits [17]. The behavioral convulsions we observed in mice following either nabilone or THC administration may be etiologically similar to those reported in rabbits [17] and they may be indicative of species or strain differences in the sensitivity to convulsant actions of THC.

Since handling convulsions were elicited in THC- and nabilone-treated mice never exposed to ethanol, evaluation of the effects of these two drugs in ethanol-dependent mice is difficult. Relationships are apparent, however, which suggest that nabilone, but not THC may reduce the severity of handling convulsions indicative of ethanol withdrawal.

The AUC for handling convulsions with time are similar in normal and ethanol-dependent mice given nabilone. Since the entire AUC can be attributed to the action of nabilone

alone, the handling convulsions attributed to ethanol withdrawal may have been reduced by nabilone. In contrast, the AUC for handling convulsions in mice given THC during withdrawal appears to equal the contribution due to ethanol withdrawal (determined in mice given Pluronic F68 vehicle during withdrawal) and that due to THC (determined in normal mice given THC).

Increased responsiveness to electric foot shock during abstinence in mice was demonstrated in our study. The enhanced responsiveness during abstinence was alleviated by ethanol, nabilone or THC in a similar manner (Fig. 5). The observed effects could have been related to analgesic actions of these drugs, but relatively large doses have been necessary to demonstrate analgesia in other studies [3, 5, 13, 23, 27]. Only the highest doses of ethanol, nabilone or THC given during abstinence in this study had significant effects upon the responsiveness of normal animals. The reduced responsiveness during abstinence elicited by ethanol, nabilone or THC was probably related to some action to suppress the symptom of ethanol withdrawal manifested as enhanced responsiveness to foot shock. These results indicate, that at least in the case of nabilone and THC, responsiveness to foot shock may be more reliable as a measure of the effect of drug administration on abstinence signs in mice.

In summary, our results using handling-induced convulsions suggest that nabilone but not THC may reduce the severity of abstinence signs in ethanol-dependent mice. In contrast, results obtained using responsiveness to foot shock, which we judged to be a more reliable indicator of the severity of abstinence, indicated that both nabilone and THC reduced the severity of abstinence in mice. The latter conclusion concerning THC is supported by a recent study done by Gildea and Bourn [7]. They found that THC protected against barbiturate withdrawal convulsions in rats. Although barbiturate and ethanol withdrawal are not identical, they are similar in many respects, and THC may act to alleviate withdrawal in dependent animals.

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