PHARMACOLOGICAL EFFECTS OF CEFCLIDIN ON SMOOTH MUSCLE *IN VITRO*

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The spasmolytic effects of cefclidin (CFCL), a novel antibacterial agent and cefazolin (CEZ) were examined in a variety of isolated tissue preparations.

The tissue preparations used comprised guinea-pig ileum, guinea-pig trachea, rat phrenic nerve-diaphragm, rat vas deferens, rat uterus and pregnant rat uterus.

In the preparations studied neither CFCL or CEZ caused any notable inhibition of the contractile responses induced by either agonists or electrical stimulation.

Key words : cefclidin, general, pharmacology, smooth muscle, *in vitro*.

Introduction

Cefclidin (CFCL) is a new parenteral cephalosporin synthesized by Eisai Co. Ltd, Japan. It has a broad antibacterial spectrum and potent activity against gram-negative bacteria including *Pseudomonas aeruginosa*. CFCL also showed high resistance to hydrolysis by and low affinity for various types of β-lactamases.

This paper describes the results of the pharmacological studies with CFCL in comparison with cefazoin (CEZ). CFCL and CEZ were each evaluated at two concentrations using a battery of standard in vitro preparations to investigate the effects on smooth muscle.

Materials and Methods

1. Materials

1) Animals

Male Dunkin-Hartley guinea-pigs (254 - 402 g) and male and female Wistar rats (180 - 340 g) were housed appropriately in air-conditioned, temperature and humidity controlled rooms. The lighting schedule was 12 : 12 light/dark.

The animals were fed an appropriate laboratory diet. Tap water was available ad libitum.

2) Test compounds

CFCL and CEZ were dissolved in physiological saline solution at different concentrations.

The other test materials used were cefazolin (CEZ) (Fujisawa Pharmaceutical Co., Ltd.), acetylcholine chloride, acetyl-β-methacholine chloride, noradrenaline, oxytocin (Sigma Chemical Company Limited): histamine phosphate and barium chloride (BDH Chemicals Limited). The materials were dissolved in a medium compatible with the test procedure.

2. Methods

1) Isolated guinea-pig ileum

Four male guinea-pigs were used for this experiment which was based essentially on the method of Magnus[1]. The guinea-pigs were sacrificed by cervical dislocation and a portion of terminal ileum approximately 2cm in length was placed in a 15 ml organ bath containing Tyrode's solution at 32°C oxygenated with 95% O_2 and 5% CO_2. The ileum was attached by a cotton thread at a tension of 0.5 g to an isotonic transducer coupled to an amplifier and recorder to facilitate measurement of isotonic contractions.

Following an equilibration period of at least 10 minutes, vehicle and two concentrations of CFCL and CEZ were added to the organ bath. Viability of each tissue was checked before addition of the test

articles by adding acetylcholine chloride to the organ bath.

Four tissue preparations from separate animals were used for this experiment.

2) Effects on acetylcholine, histamine and barium chloride responses in the isolated guinea-pig ileum

Twelve male guinea-pigs were used for this experiment. The guinea-pigs were killed by cervical dislocation and the terminal ileum immediately removed and placed in Krebs solution. A section of ileum approximately 2 cm in length was suspended in a 15 ml organ bath containing Krebs solution at 32°C aerated with 95% O_2 and 5% CO_2. The ileum was attached by a cotton thread at a tension of 0.5 g to an isotonic transducer coupled to an amplifier and recorder to facilitate measurement of isotonic contractions.

Following an equilibrium period of at least 10 minutes, reproducible submaximal contractions to either acetylcholine, histamine or barium chloride were obtained. The vehicle and two concentrations of CFCL and CEZ were then administered and 2 minutes later the response of the ileum to a submaximal concentration of the appropriate agonist was again determined. This procedure was repeated for each agonist in 4 tissue preparations (one agonist per tissue) removed from separate guinea-pig.

3) Isolated guinea-pig trachea

Four male guinea-pigs were used for this experiment which was performed according to the method of Costillo and De Beer[2]. The animals were killed by cervical dislocation and the trachea immediately removed. The trachea was cut transversely to obtain a number of rings of which one was mounted in a 15 ml organ bath containing Krebs solution at 37°C aerated with 95% O_2 and 5 % CO_2. The tracheal ring was attached by a cotton thread to a Dynamometer UFI isometric strain gauge coupled to an amplifier and a recorder to facilitate measurement of tension of the tracheal muscle.

The tissue was allowed to equilibrate for approximtely 20 minutes before any drug additions.

Methacholine (7 μg/ml) was added to the organ bath to cause a sustained contraction. Vehicle and two concentrations of CFCL or CEZ were then added to the organ bath. Each concentration was allowed a 3-minute contact time before the tissue was washed and methachloine was added to produce a sustained increase in tension before the next concentration was added.

This experimental procedure was repeated in 4 tissue preparations.

4) Isolated rat phrenic nerve diaphragm

Four male Wistar rats were used for this experiment. The method was essentially as described by Bulbring[3] Rats were killed by a sharp blow to the head. The phrenic nerve and diaphragm were carefully removed from the animal and mounted in a 120 ml organ bath on a purpose-designed, bipolar electrode for direct nerve stimulation. Tyrode's solution containing double the standard amount of glucose was used as a bathing fluid, maintained at 37°C and aerated with 95% O_2, 5% CO_2.

Electrically-induced isometric muscle contractions were recorded. The phrenic nerve was continuously stimulated using a Grass S88 stimulator at a rate of 0.2 Hz using rectangular wave pulses of 0.5 msec duration. A supramaximal voltage was applied to ensure a reproducible contraction.

Once the contractions were of a uniform amplitude, vehicle or two concentrations of CFCL and CEZ were added to the bath and allowed a contact time of 3 minutes. The Tyrode's solution was then changed at 4 minute intervals until the response returned to pre-dose values.

5) Isolated rat vas deferens

Four male Wistar rats were used for this experiment. the method used was based on that of Hart et al[4]. The rats were killed by cervical deslocation and the vas deferens carefully removed and mounted (under 0.5 g tension) in a 15 ml organ bath. The tissue was bathed with Krebs Henseleit solution maintained at 37°C and aerated with 95 % O_2 and 5% CO_2. Bipolar concentric electrodes were used for field stimulation.

A dose-response curve to increasing concentrations of noradrenaline was obtained and a dose producing sub-maximal contractions was selected.

This dose was administered at 3-minute intervals until consistent results were obtained. The tissue was then stimulated transmurally, using a Grass S88 stimulator, for 5 seconds at a frequency which produced a similar sized contraction as that caused by the agonist. Electrical stimulations and exogenous noradrenaline administrations were then alternated using a 3-minute time cycle until the contractions were of a uniform size. All responses were recorded using an isometric transducer coupled with a suitable chart recorder.

The effects of addition to the bathing fluid of successive administrantions of vehicle and 2 concentrations of CFCL and CEZ on the responses to transmural stimulation and exogenous noradrenaline were recorded.

6) Isolated rat uterus

Four female Wistar rats were used for this experiment which was based on the method of De Jalon *et al*[5]. Twenty-four hours before used, the rats were dosed with stilboestrol (100 μg/kg, s.c.). On the day of the experiment a vaginal smear was taken from each rat and examined microscopically to confirm the oestrus state.

Rats in oestrus were killed by cervical dislocation followed by exsanguination. One uterine horn was removed from each animal and suspended in a 15 ml organ bath in De Jalons solution at 37°C aerated with 95% O_2 and 5% CO_2. The tissue was mounted under a tension 0.5 g and contractions were recorded by means of an isotonic transducer. Following an 8-minute equilibrarion period acetylcholine chloride was added to each tissue to check viability. The effects of vehicle and two concentrations of CFCL and CEZ were examined in each tissue preparation.

7) Isolated pregnant rat uterus

Four time-mated Wistar rats were used for this experiment.

On the 19th day of gestation 4 female pregnant Wistar rats were sacrificed by cervical dislocation. The right uterine horn was dissected out and each foetus immediately removed and killed using ether. Strips of uterine wall approximate-ly 2 cm in length were suspended in a 15 ml organ bath containing Ringer-Locke solution at 37°C aerated with 95% O_2 and 5% CO_2. The tissue was attached by a cotton thread at a tension of 0.5 g to an isotonic transducer, coupled to an amplifier and recorder, to facilitate measurement of the contractions.

Following an equilibration period of at least 15 minutes reproducible submaximal contractions to oxytocin were obtained. The effect of vehicle and two doses of CFCL and CEZ were then examined for their effect on contractions induced by a submaximal dose of oxytocin.

Each dose of drug was allowed a 2-minute contact period before oxytocin was administered. Thirty seconds after oxytocin was added the tissue was washed with fresh Ringer-Locke solution. A period of at least 5 minutes was allowed between successive doses.

Results

1) Isolated guinea-pig ileum (Figure 1)

Neither CFCL nor CEZ (2 x 10^{-5}M and 2 x 10^{-4} M) produced any effect on the resting tone of isolated segments of guinea-pig ileum.

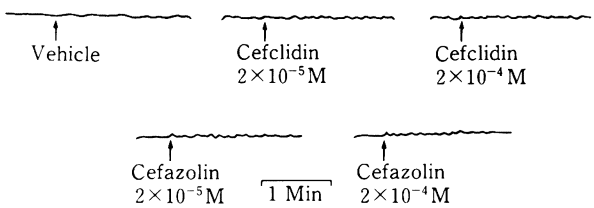
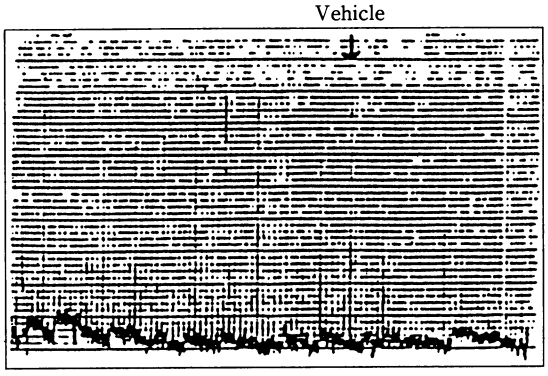


Fig. 1. Effect of cefclidin and cefazolin on the isolated guinea-pig ileum

Table 1. A summary of the effects of cefclidin and cefazolin on the amplitude of contraction of the isolated guinea-pig ileum to submaximal concentrations of various spasmogens

Treatment	Concentration (M)	Mean % change in amplitude of contraction (± SD)		
		Ach	Histamine	$BaCl_2$
Vehicle	–	−3.43 ±14.58	−0.25 ±5.98	−3.15 ±10.92
Cefclidin	2×10^{-5}	−5.25 ±9.76	8.22 ±20.78	3.18 ±11.42
Cefclidin	2×10^{-4}	3.23 ±16.56	31.35* ±24.22	13.98 ±36.41
Cefazolin	2×10^{-5}	12.23 ±9.28	5.55 ±6.65	6.40 ±28.70
Cefazolin	2×10^{-4}	4.88 ±13.46	−1.10 ±5.69	12.40 ±7.95

Significance of difference from vehicle-treated group using analysis of variance * : $p<0.05$



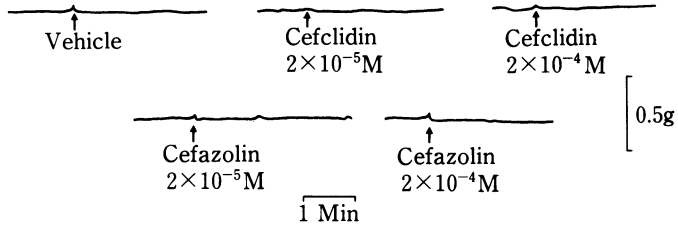
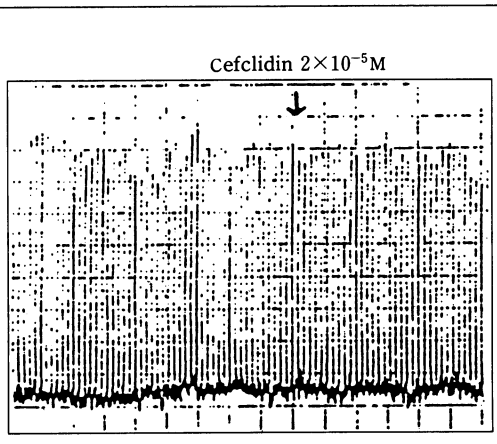
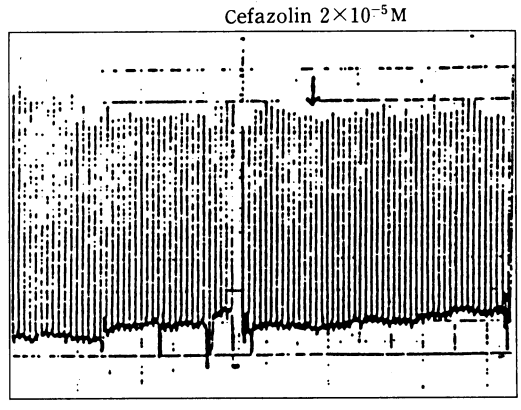
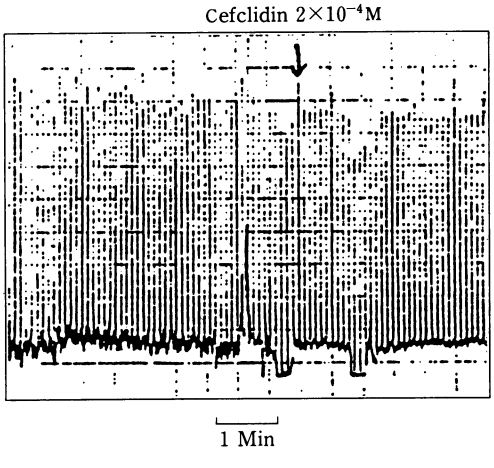


Fig. 2. Effect of cefclidin and cefazolin on MeCh-induced tone in the isolated guinea-pig trachea







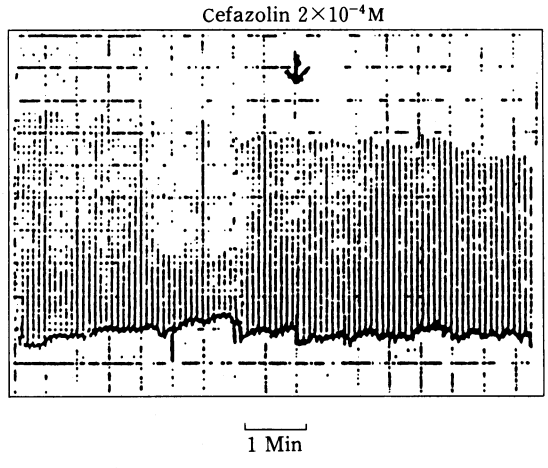


Fig. 3. Effects of cefclidin and cefazolin on the isolated rat phrenic nerve diaphragm preparation

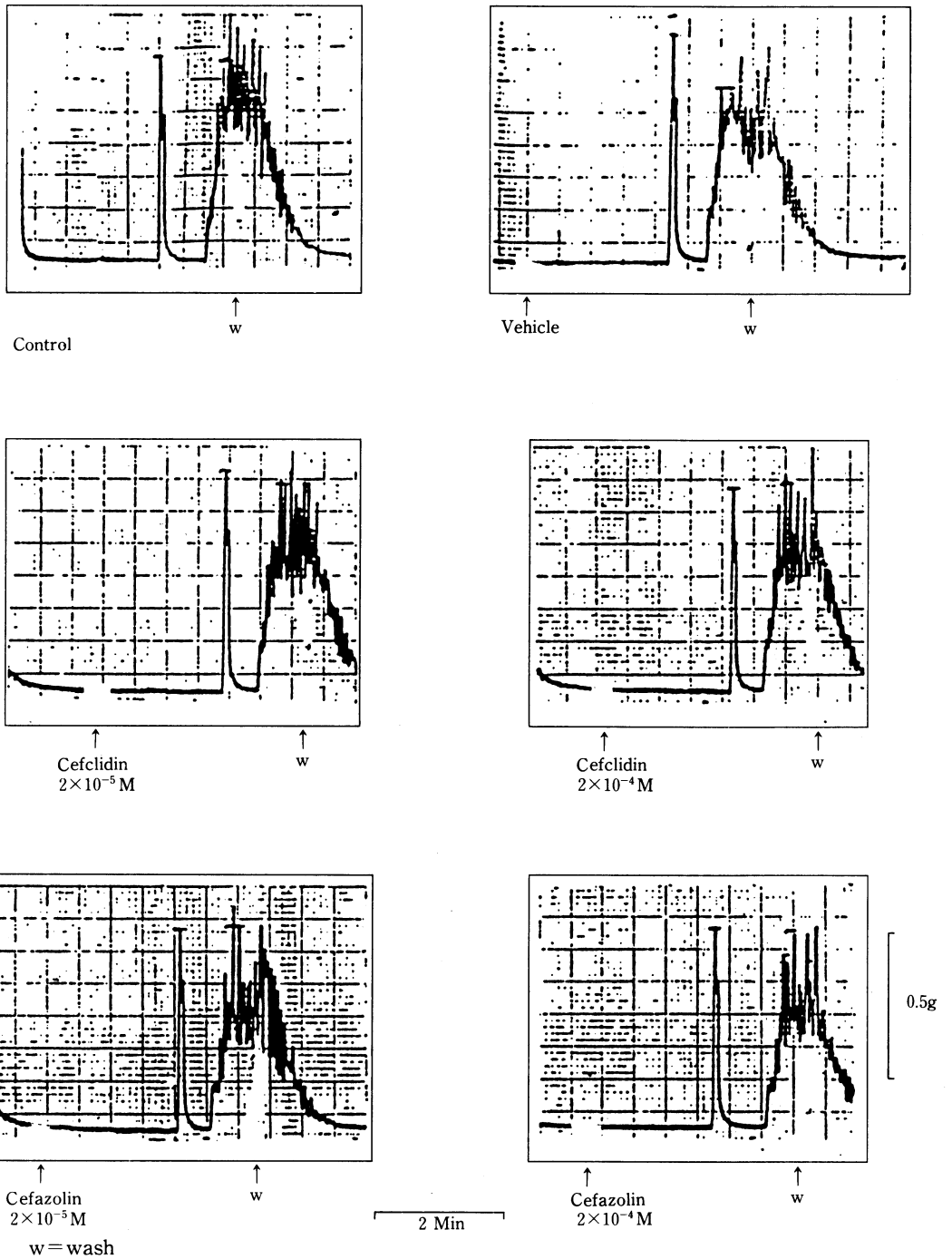


Fig. 4. Effects of cefclidin and cefazolin on the responses to electrical stimulation and noradrenaline of the isolated rat vas deferens

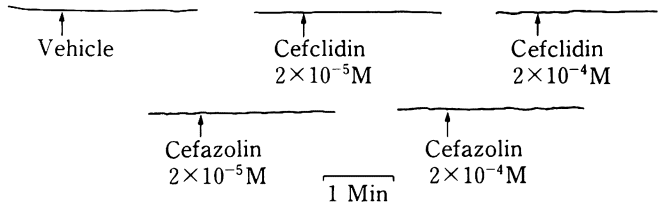


Fig. 5. Effect of cefclidin and cefazolin on the isolated rat uterus (oestrus)

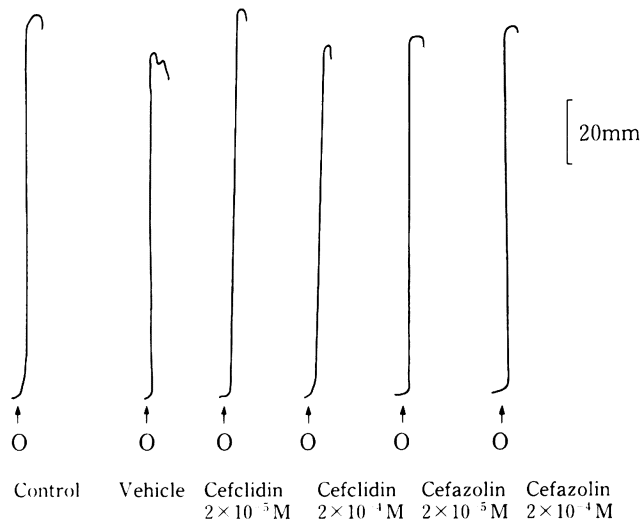


Fig. 6. Effect of cefclidin and cefazolin on oxytocin-induced contractions of the isolated pregnant rat uterus
O = Oxytocin

2) Effects on acetylcholine, histamine and barium chloride responses in the isolated guinea-pig ileum (Table 1)

CFCL and CEZ (2 x 10^{-5}M and 2 x 10^{-4}M) did not modify acetylcholine and barium chloride-induced contractions of the ileum. At a final bath concentration of 2 x 10^{-4}M, CFCL was found to enhance histamine-induced contractions of the ileum and this was statistically significant. CEZ had no effect on histamine-induced contrations.

3) Isolated guinea-pig trachea (Figure 2)

No effect on the methacholine-induced contractions of isolated tracheal rings were observed after CFCL and CEZ (2 x 10^{-5}M and 2 x 10^{-4}M) administration.

4) Isolated rat phrenic nerve diaphragm (Figure 3)

Neither CFCL nor CEZ (2 x 10^{-5}M and 2 x 10^{-4}M) had any notable effect on contractions of the rat diaphragm elicited by stimulation of the phrenic nerve.

5) Isolated rat vas deferens (Figure 4)

CFCL and CEZ (2 x 10^{-5}M and 2 x 10^{-4}M) had no effect on contractions of the isolated vas deferens preparation induced by transmural stimu-

lation or exogenous noradrenaline.

6) Isolated rat uterus (Figure 5)

The test compounds CFCL and CEZ at concentrations of 2 x 10^{-5}M and 2 × 10^{-4}M, had no effect on the basal tone of the isolated rat uterus in oestrus.

7) Isolated pregnant rat uterus (Figure 6)

Neither CFCL nor CEZ (2 x 10^{-5}M and 2 x 10^{-4} M) caused any statistically significant modification of the contractions induced by oxytocin in this preparation.

Discussion

The spasmolytic effects of CFCL and CEZ, which was used for comparative purposes, were examined in a variety of standard isolated tissue preparations.

The preparations selected allowed the examination of effects is a range of tissues and against a variety of different receptor-mediated contractions. Effects on skeletal as well as smooth muscle were investigated.

Neither CFCL nor CEZ produced any inhibition of the contractile responses of the skeletal and smooth muscle preparation studied whether stimulated by the addition of selected agonists or by neuronal stimulation.

The apparent facilitated response to histamine of the guinea-pig ileum following the highest dose of CFCL (2 x 10^{-4}M) is not considered to be of any major pharmacological significance.

In conclusion neither CFCL nor CEZ demonstrated any in vitro spasmolytic activity in the preparations examined.

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Cefclidinの一般薬理作用：摘出平滑筋への影響

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Cefclidin(CFCL)の一般薬理試験の一環として，摘出平滑筋に対する影響について，cefazoline（CEZ）と比較検討した。

CFCLは2×10^{-4}Mで平滑筋に対する直接作用を示さなかった。CFCLは2×10^{-4}Mでhistamine（回腸）による収縮反応を増強した以外は，acethylcholine（回腸），methacholine（気管），barium（回腸），外因性noradrenaline（輸精管）の反応に影響しなかった。また，妊娠・非妊娠子宮の静止張力あるいはoxytocin収縮に影響しなかった。さらに横隔膜神経－筋標本での電気刺激時の反応にも変化はなかった。

一方，CEZはいずれの反応系に対しても影響が認められなかった。

以上，CFCLは高濃度でhistamineの反応を増強した以外は，各種収縮剤あるいは電気刺激で誘発した収縮反応に対して顕著な抑制作用を示さなかった。

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