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CONDITIONS AND PARAMETERS DOMINATING DISPLACEMENT THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The parameters dominating thin-layer displacement chromatography are investigated. The length of zones is influenced by the amount of substance displaced, but neither the size of the sample spot nor the length of displacement development influences the size of a zone after of the displacement train has been fully developed. The steady state can be achieved after displacement over a distance that depends on the concentration of the displacer in the carrier solvent.

Examples of the use of carrier displacement thin-layer chromatography are presented. Space requirements for displacement thin-layer chromatography and carrier displacement thin-layer chromatography are given.

INTRODUCTION

In their recent publications, Horváth and co-workers[1–5] discussed the theory and application of displacement chromatography on high-performance stationary phases. The substances that have been separated by displacement development include phenolic compounds[1], basic amino acids and dipeptides[2], polymyxins[3], corticosteroids[4], and guanosine nucleotides. The separations were carried out on octadecyl silica[1,2,5], octyl silica[3], siliceous cation exchanger[2] and silica[4]. The amount of components separated ranged from several milligrams to several hundred milligrams, when these preparative-scale separations were performed with the columns generally employed for analytical purposes.

Displacement thin-layer chromatography (TLC) was also initiated by Horváth when he suggested scouting for displacers TLC in the case of corticosteroids[4]. Later on, the same system was applied for finding the optimal conditions for displacement chromatography of phenylalkylamines[6]. The use of displacement chromatography on planar stationary phases offers additional unique possibilities for exploration, as the displacement separation of some coloured substances can be directly observed[6,7]. The planar method also offers the advantage that several samples can be separated in parallel, and a multicomponent dye mixture can be applied which brackets the individual components in the displacement train[6,7]. Direct visual evaluation and the opportunity for two-dimensional development[7], facilitate the separation of sub-

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stances with similar chemical structures. The development steps in high-performance displacement chromatography (HPDC) were examined[1] on the basis of publications by Tiselius[8] and Helfferich and Knein[9]. The procedure consists of four consecutive steps as the feed components, the displacer, the regenerant, and the carrier are fed into the column[1–3].

Because thin-layer plates are disposable, regeneration is generally omitted from the procedure. Sample feed is a discrete operation prior to development, and the separated bands are rarely evaluated on-line.

In column HPDC, the procedure takes place in a closed column of stationary phase, which has been extensively prewashed and equilibrated with the carrier solvent. By contrast, displacement TLC is carried out on an open sorbent layer and the flow of the solvent front encounters dry stationary phase. Therefore, the constant evaporation and condensation of the carrier solvent must be taken into consideration[10], in addition to the fact that even with a single-component carrier solvent there is a multiple solvent front moving ahead[11,12]. These factors strongly suggest that the individual component zones may be misshapen, in contrast to the zones of components in HPDC, which are determined by the intersection of the operating line and adsorption isotherms of the components.

The localization of the displaced zones has never been discussed from the point of view of their restriction to a well-defined volume of the column effluent and of the extent of deformation of the displacer front. These questions specifically arise in displacement TLC, as the space for the displaced zone is limited between the carrier front an displacer front. The deformation of the displacer front can be easily observed on an open planar stationary phase.

EXPERIMENTAL

Materials

Silica gel F_{254} TLC plates, pre-coated on glass, 20 × 20 cm, were purchased from Merck (Darmstadt, F.R.G.).

Triethanolamine, chloroform and acetone were supplied by Reanal (Budapest, Hungary). Benzoic acid, phenylacetic acid, benzylamine, phenylethylamine and tyramine were purchased from Fluka (Buchs, Switzerland). Deprenyl [N-methyl-N-propargyl-(2-phenyl-1-methyl)ethylammonium chloride, Jumex®], amphetamine and methamphetamine were kindly contributed by Chinoin (Budapest, Hungary). The dye mixture marked "Test Substance II" was a kind gift of Dr. Jänchen (Camag, Muttenz, Switzerland). The displaced components of "Test Substance II" were the elements called "Sudanschwarz".

Apparatus

All-glass developing chambers with covers, purchased from Desaga (Heidelberg, F.R.G.), were used for displacement TLC. Glass syringes, bought from Hamilton (Reno, NV, U.S.A.), were used for spotting the samples and the dye mixture.

The ^{14}C-labelled deprenyl and its degradation products were detected either by using X-ray film (Mamoray RP-3, Agfa Gevaert, Belgium) or with a Berthold TLC linear analyzer LB 282 (Berthold, Vienna, Austria). The exposure time for the X-ray film was 22 h; the film was developed in the usual way.

RESULTS

Fig. 1 shows plots of the length of the displaced zones *vs.* the amount of sample in the case of deprenyl hydrochloride when silica gel plates, chloroform and triethanolamine were used as sorbent, carrier solvent, and displacer, respectively.

Fig. 2 demonstrates that the length of the displaced zones does not depend on the length of the sample spots. The radiolabelled deprenyl (^{14}C-labelled at the 2-position of the ethyl-2-phenyl radical) was spotted in 1, 2, 5, 10, 15, 20, 25 and 30 mm lengths, but the displaced zones remained very sharp.

Fig. 3 depicts the results of a separation of deprenyl and benzylamine (both hydrochlorides). The picture indicates that even the 30-mm long sample strip gave the same separation and length of displaced zone. However, the increase in the amount of sample led to a corresponding enlargement of the length of the displaced zone. In this case, 500 μg of substance were spotted in zones 5, 10, 15, 20, 25 and 30 mm in length (right side) and 50, 100, 200, 500 and 1000 μg of substance were spotted in zones 5 mm in length (left side).

Fig. 4 demonstrates the relation between the sample load and length of the displacement zone. The larger the sample load, the longer is the zone (Fig. 4a). If the carrier solvent is changed from chloroform–acetone 999:1 to 4:1 and 1:1, the same amount of sample results in a longer zone. The total zone of the displaced benzylamine hydrochloride consists of two parts: one reaches over the displacer zone and remains behind the tentative front line of the displacer. This is depicted in Fig. 4b. In contrast, zones of benzylamine free base can be observed ahead of the displacer

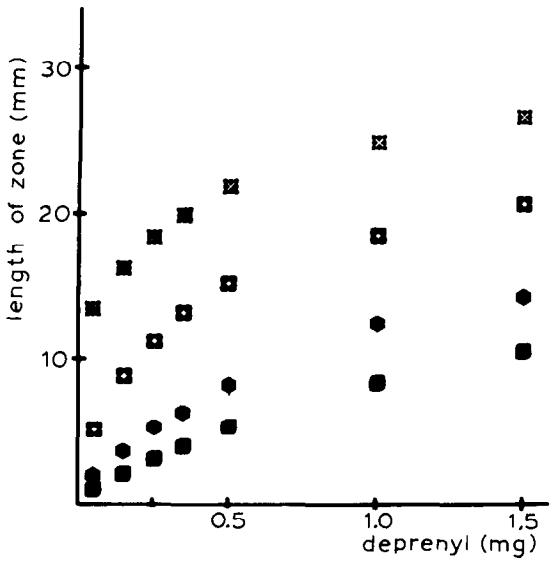

Fig. 1. Length of zone *vs.* sample load for displacement TLC of deprenyl. The points were determined by displacement with 1, 2, 3 and 5% triethanolamine in chloroform (from top to bottom); the stationary phase was silica gel.

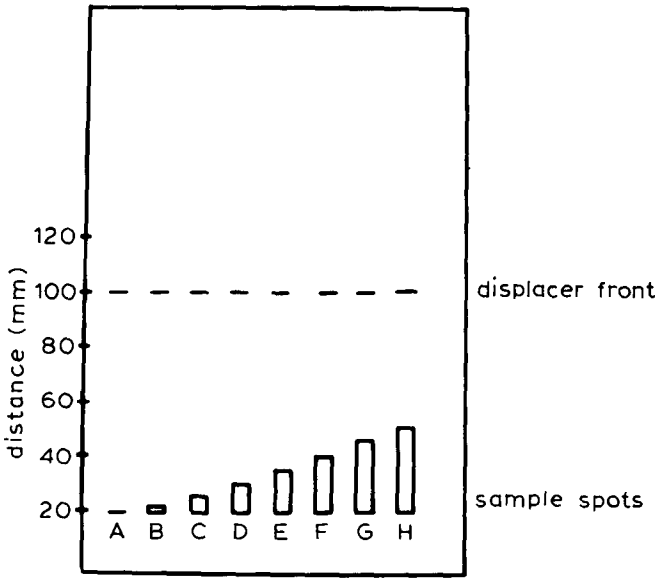

Fig. 2. Displacement chromatography of radiolabelled deprenyl from various sample spots. Detection by autoradiography.

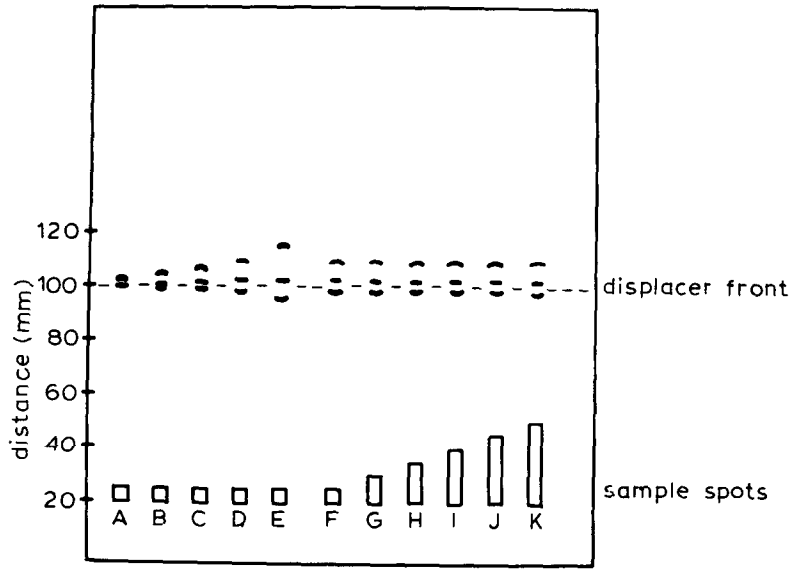

Fig. 3. Effect of sample load and size of sample spots on the length of the displaced zones. Increasing amounts of deprenyl and benzylamine were spotted at A, B, C, D and E (50, 100, 250, 500 and 1000 μg, respectively); 500 μg of deprenyl and 500 μg of benzylamine were spotted in zones F, G, H, I, J and K measuring 5, 10, 15, 20, 25, 30 mm in length, respectively.

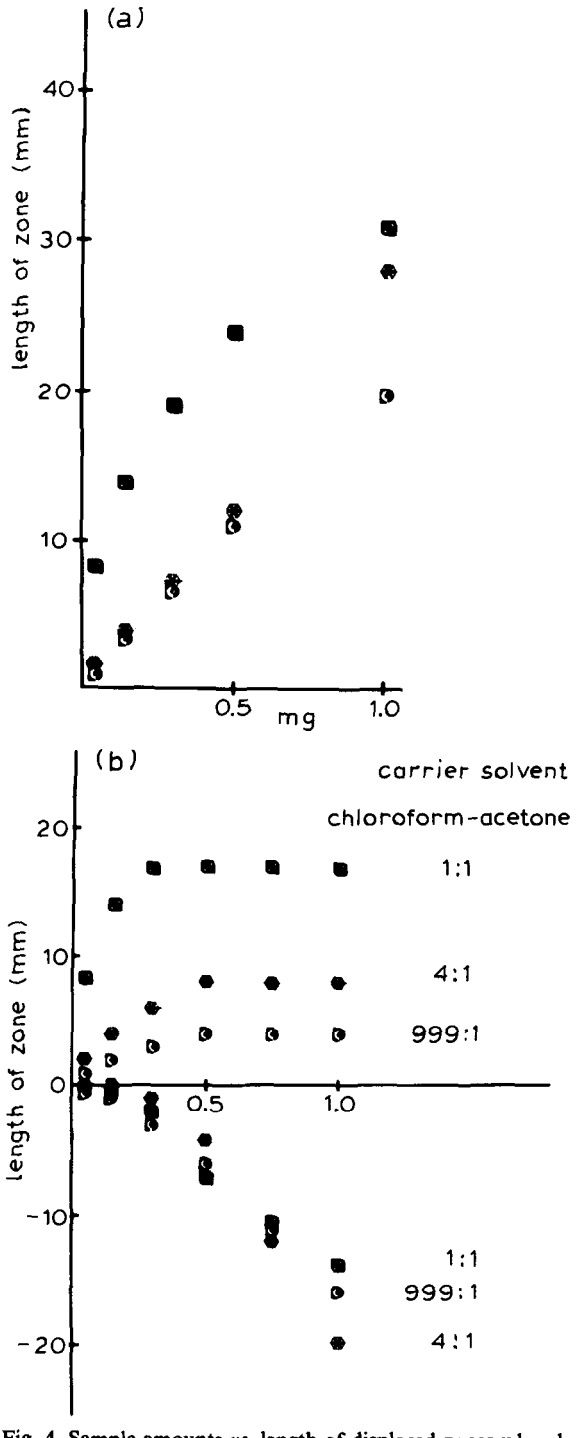

Fig. 4. Sample amounts *vs.* length of displaced zones when benzylamine hydrochloride was displaced in various carrier solvents, containing 5% triethanolamine as displacer. The stationary phase was silica gel. (a) The total length of the displaced zones. (b) The zones over and under the "tentative" displacer front line.

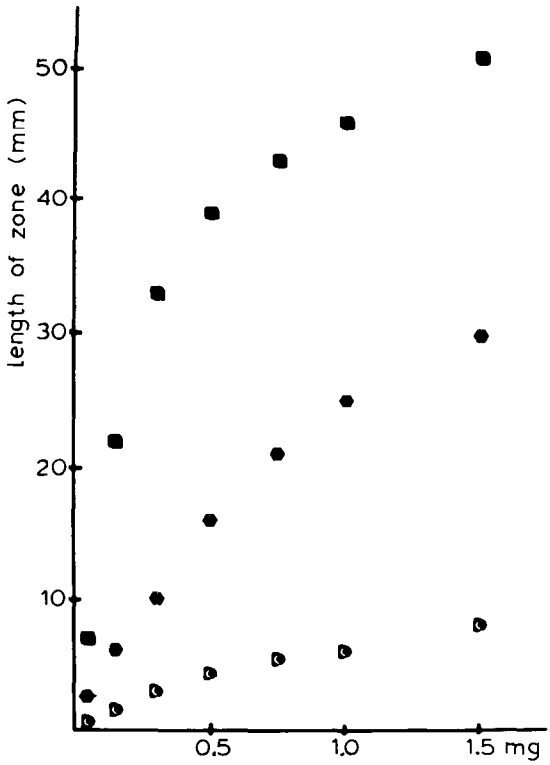

Fig. 5. Sample amounts *vs.* length of displaced zone when benzylamine free base was displaced in various carriers (symbols and explanations are shown in Fig. 4b and legend).

front line. The length of the displacement zones of benzylamine free base is given in Fig. 5.

Fig. 6 shows the relation between the displacement distance and the length of the displaced zone for silica gel as stationary phase, chloroform as carrier, and 2.0, 3.5 and 5.0% triethanolamine in chloroform as displacer and 0.8 mg of deprenyl hydrochloride as the sample. In the case of the 5.0% displacer, the totally developed displacement train can be measured after the displacer front has run 10 mm over the spots of sample. However, the 2.0% displacer needs a considerably longer distance to generate the steady state of displacement chromatography. This displacement distance is *ca.* 32 mm, and throughout the displacement process the length of zone does not change.

Figs. 7 and 8 illustrate the fact that the displacement train is always a complete system, where the positions of individual components are determined by the components of the displacement train. If one or more parts are changed, the new elements of the displacement train can interfere with the original order of the components. In Fig. 7 the outdated deprenyl sample contains an impurity (degradation product of radiolysis), which appears quite near to deprenyl (left side). The substances can be separated if the displacement train contains a new member (*e.g.* phenylethylamine) that is less displaceable than deprenyl itself, but more than the degradation product.

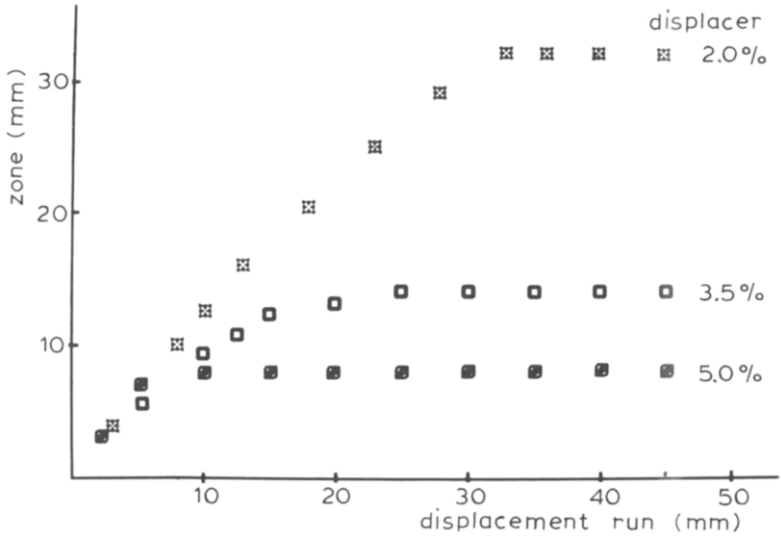

Fig. 6. Zone lengths *vs.* displacement distance, indicating that various concentrations of the displacer require different displacement distances to reach the steady state.

Similarly, the separation of metabolites of deprenyl can be improved if phenylethylamine is added to the system. Phenylethylamine was streaked on the left side of the plate; chromatography on the right side was performed without the phenylethylamine spacer (Fig. 8).

DISCUSSION

TLC accomplishes the separation of several samples in parallel. Thus, many samples can be compared directly, and the analysis time can be shortened considerably.

Fig. 1 shows plots of the length of zones *vs.* sample size when silica gel is used

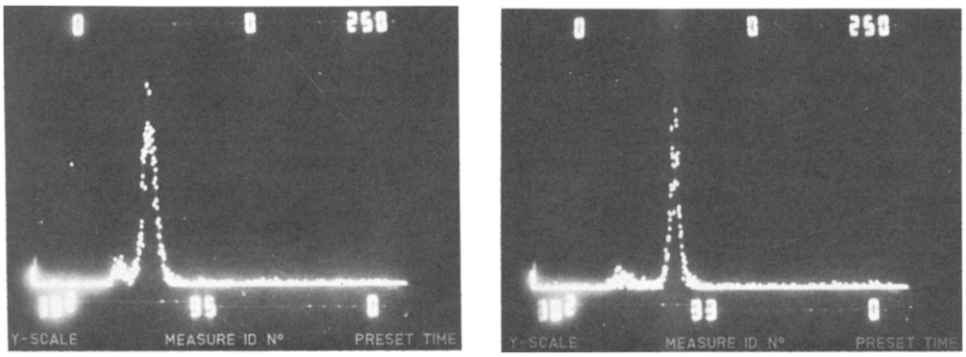

Fig. 7. Separation of deprenyl from its radiolysis products. A silica gel plate and 5% triethanolamine in chloroform were used as sorbent and displacer, respectively. Phenylethylamine hydrochloride was streaked as carrier substance for the separation shown on the right side. The chromatograms were monitored by a Berthold TLC scanner.

Fig. 8. Displacement TLC of deprenyl metabolites. The carrier substance was phenylethylamine, applied on the left side. Detection by autoradiography; other conditions as in the legend to Fig. 7.

as the stationary phase and different amounts of displacer (triethanolamine) in chloroform carrier are added.

The length of the displaced zones does not depend on the original length of the applied zone of the component, *i.e.* on the size of the sample spot. This is very important when samples of biological origin are tested. Even samples with zones 30 mm in length can be concentrated into sharp zones when the displacement train has been fully developed. Reaching this stage, *i.e.* to fulfill the condition of the totally developed displacement train, requires only a short running distance, as is suggested in our earlier publication[10] and demonstrated in Fig. 3. The analysis of 150, 100, 80, 60, 40, 20 and 15 mm zones resulted in the same chromatographic pattern, whereas a development distance shorter than 15 mm does not give adequate separation. The very short distance needed for the total development of the displacing process ensures that either concentration or extension of the component zones takes place.

The change of carrier influences the length of the displaced zone, which can also be influenced by the sorbent, the concentration of displacer and the amount of substance to be displaced. If the concentration of displacer is decreased longer displacement zones are generated, but changes in both the carrier and the displacer may cause slight alteration in the development of the displacement train. The carrier solvent permits the formation of the displacement train ahead of the displacer front, and a decrease in the solubility of the compound in the carrier may decrease the length of the displacement zone. The shortening consists of two parts (Fig. 4b): the zone ahead of displacer front line is shortened substantially, and the deformation of the displacer front is also changed. If the substance (*e.g.* benzylamine free base) is very soluble in the carrier solvent, the deformation of the displacer zone is generally avoided (Fig. 5). The evaluation of all these experiments is greatly facilitated by the use of carrier substances with a bright colour.

A decrease in the concentration of displacer causes a substantial increase in the distance necessary for the full development of the displacement train. This is demonstrated in Fig. 6, which depicts and gives the formation of the fully developed displacement zones as a function of the displacement distance.

The displacement procedure is determined by the amount of individual com-

ponents, the stationary phase, the carrier, and the displacer (including its concentration in the carrier solvent), but neither the original size of the sample spot nor the size of the displacement distance can influence the length of zones when the steady state of the fully developed displacement train has been reached.

Similarly, the order of samples plays no part in the sequence and length of the displaced zones, as we have shown by recent experiments with phenylalkylamines[10].

The separated zones overlap partially[1–5], and this is serious, because displacement chromatography is generally applied to the separation of very similar or closely related subtances. Both problems —the partial overlap and the similarity of the consecutive zones— can be alleviated by the use of carrier displacement chromatography in which certain substances are interposed between the zones. It is essential for the purposes of carrier displacement that these substances, wedged between neighbouring zones, should differ from them in one or several charcteristics. The components of Sudanschwarz are black and lipophilic. They somehow take their place in the displacement train and thereby not only allow the chromatogram to be visualized[6,7,10]. The essentially different nature of the phenylalkylamines and the lipophilic dye components make it easy to remove them by a simple extraction if the final purpose of the separation is preparative.

The amount of carrier substances to be applied depends on the purpose of the analysis, *i.e.* in the case of visualization, trace amounts of dyes may be enough. In some cases, the carrier substance should be applied in larger amounts than the substance to be separated. If a radiolabelled compound and its metabolites are to be visualized, a large excess of carrier substance can be applied (Figs. 7 and 8).

In the case of carrier displacement chromatography, the substance to be displaced is under the quadrupole effect of the stationary phase, the carrier solvent, the displacer and the carrier substances (Fig. 9). When the space required for displacement chromatography is calculated, this fact should be considered.

For displacement chromatography, the equation is:

$$z_{\text{total}} = z_{\text{start}} + z_{\text{zone}} + z_{\text{carrier solvent}}$$

and for carrier displacement chromatography it is:

$$z_{\text{total}} = z_{\text{start}} + z_{\text{zone}} + z_{\text{carrier solvent}} + z_{\text{carrier substance}}$$

where z_{total} is the space required, z_{start} is the distance of the sample spots from the

TABLE I

Sample components to be isolated or analysed	*Carrier substances to be used*	*Reason for use*
Colourless	Coloured	Visual observation
UV-adsorbing	Not UV-adsorbing	UV observation
Radiolabelled	Not labelled	Identification of metabolites
Polar	Lipophilic	Isolation
Non-reactive	Reactive	Isolation

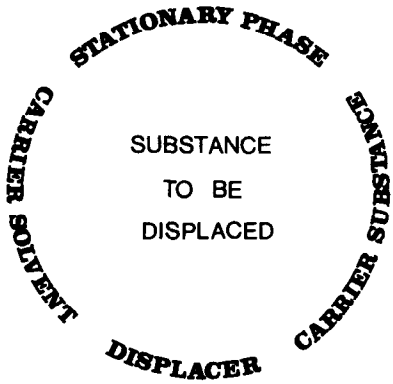

Fig. 9. Factors determining conditions of displacement chromatography. Generally, when several substances are to be displaced and several carrier substances are applied, their effect must be totaled.

bottom of the plate, z_{zone} is the sum of the displaced zones, $z_{carrier\ substance}$ is the length of zone of the carrier substance, and $z_{carrier\ solvent}$ is the space between the top of the displaced zones and the carrier solvent front.

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