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Nebulization of a bovine surfactant in cystic fibrosis: a pilot study

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Nebulization of a bovine surfactant in cystic fibrosis: a pilot study. M. Griese, P. Bufler, J. Teller, D. Reinhardt. ©ERS Journals Ltd 1997.

ABSTRACT: Cystic fibrosis (CF) is a lethal disorder which results in excessive airway secretions and in chronic inflammation of the airways. *In vitro* and *in vivo* studies have shown that a lack of surfactant results in the closure of the small airways. In this pilot study, we aimed to determine whether surfactant administered by aerosol might improve lung function on a short-term basis in patients with CF.

In a randomized, crossover double-blind pilot study, 120 mg of a lipid-extracted bovine surfactant (Alveofact) or placebo was aerosolized to five young adult patients with CF over a period of 30 min for five consecutive days. The sample size had the power of 90% to detect an increase in forced expiratory volume in one second (FEV_1) of 15% ($p<0.05$).

Jet nebulization of surfactant produced particles of which more than 75% were the respirable range (<5 μm). The inhalations were well tolerated. No changes in serum antibody titres against the surfactant proteins-B and -C (SP-B/SP-C) were observed. No differences in FEV_1 and forced vital capacity were found before, and 30 or 90 min after, the inhalation.

This pilot study shows no acute or short-term benefits of surfactant inhalation in young adults with cystic fibrosis. However, a beneficial effect of exogenous surfactant cannot be excluded before other reasons for a lack of effect, such as insufficient quantity delivered, inhomogeneous distribution or inhibition of the surfactant in the lungs, have been completely ruled out.

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Treatment of lung disease in cystic fibrosis (CF) is based mainly on the clearance of pathological airway secretions, antimicrobial therapy and anti-inflammatory approaches to halt the destruction of the small airways, which severely limits the expiratory airflow.

Pulmonary surfactant is necessary to prevent the alveoli from collapsing at end-expiration. Several synthetic and natural surfactant preparations have recently been successfully introduced to treat neonatal and acute respiratory distress syndrome (ARDS) [1, 2]. Increasing evidence suggests that surfactant is needed not only in the most terminal parts of the airways but also in the narrow section through which air is conducted to the alveoli [3–6]. *In vitro* and *in vivo* studies have shown that a lack of surfactant results in the closure of the small cylindrical airways. In addition to this deficiency, the presence of phospholipases, albumin or fibrinogen in the airways with inflammatory reactions, severely disrupts the functional ability of surfactant to keep the conducting airways open [5, 7]. The biological surface activity of surfactant, which was isolated from bronchoalveolar lavages of patients with CF, was found to be severely impaired, levels of the major phospholipid, phosphatidylcholine, and of surfactant protein-A (SP-A) being reduced [8, 9].

Surfactant administered by aerosolization has previously been shown to result in a significant improvement in lung function in animal models of injury [10, 11] and in adult asthmatics with an acute attack [12]. In patients with ARDS, natural lipid-extracted surfactants may be effective [13, 14], whereas an artificial surfactant was ineffective [15]. Asthmatic children with chronic airflow obstruction also showed no benefit [16].

In this pilot study, we aimed to determine whether surfactant aerosolization produces a short-term improvement in lung function in patients with CF and airflow obstruction especially in the small airways.

Materials and methods

Chemicals

The surfactant used was a lipid-extracted, natural surfactant preparation (Alveofact; Thomae, Biberach, Germany) now routinely used for the treatment of infant (neonatal) respiratory distress syndrome (IRDS) [17]. Apart from phospholipids, it contains about 1.7% SP-B [7]. Human SP-B-dimer (in 1-propanol/phosphate-buffered saline (1:1), 4 $mg \cdot mL^{-1}$) was used as the standard and was a gift from W. Seeger (Gießen, Germany). The monoclonal antibody against SP-B was a gift from Y. Suzuki (Kyoto, Japan) [18].

Lung function measurements

Lung volumes were determined by body plethysmography and the He-dilution technique, CO transfer

factor by the single breath technique, and forced expiratory variables by pneumotachographic measurements (Masterlab V 4.2; Jäger, Würzburg, Germany). The tests were performed according to the guidelines of the American Thoracic Society/European Respiratory Society (1993) [19].

Nebulization of surfactant

The surfactant (Alveofact) was adjusted to the phospholipid concentrations indicated and a final NaCl concentration of 0.9%. A 2.4 mL volume was placed in the blinded reservoir of the jet nebulizer (IS-2; Pari, Starnberg, Germany), which was connected to a Pari IS-2 compressor. The nebulizer was activated during the inspiratory cycle in the *in vitro* studies by an electronic trigger impulse from the respiratory cycle machine, and in the *in vivo* studies by the patients themselves.

In the *in vitro* studies, Alveofact, at 50 mg phospholipids·mL^{-1}, was nebulized and the material recovered on a filter after 20 min and after an additional 10 min, during which time the surfactant suspension was nebulized to dryness. The recovered material was lipid-extracted. Phospholipid composition was determined by high performance thin-layer chromatography, and comparison was made with control surfactant (20 min at room temperature).

The respiratory cycle machine (Pari, Starnberg, Germany) emulates tidal breathing and was operated at a minute ventilation of 5 L, using a respiratory frequency of 10 breaths·min^{-1} and a tidal volume of 500 mL. The nebulizer output was trapped on fibre glass filters or particle size was determined by laser light scattering in Master Sizer X 1.2 (Malvern Instruments, Herrsching, Germany). Alternatively, nebulizer output was delivered *via* a mouthpiece to the seated patient. The aerosol was generated and delivered solely during inspiration. At end-inspiration the breath was held briefly for 2–3 s. All inhalations by the patients were monitored to ensure compliance and to identify adverse effects.

Subjects

Five male patients with CF (aged 26±3 yrs, range 19–34 yrs) were studied. All had a positive test for sweat chloride and all were in a stable clinical condition, showing no change in antibiotic, bronchodilator, or corticosteroid therapy in the previous 4 weeks and no hospitalization for respiratory infection within the previous 6 weeks. All patients had *Pseudomonas aeruginosa*, and one patient also had *Burkholderia cepacia* in his sputum. The sputum was obtained by drying the mucus membranes of the mouth before expectoration in order to protect it from saliva. The study protocol was approved by the local Ethics Committee.

Study design

In the first part of the study, the maximum dose of surfactant, that could be delivered during a reasonable period of nebulization, and the properties of the surfactant were determined *in vitro*. In the second part of this study, the immediate (30 and 90 min) and the short-term effects (5 days) of surfactant nebulization on lung function were assessed in a study of randomized, double-blind, placebo-controlled, and crossover design. The evaluation of safety included physical examination, routine serum chemistry and haematological tests, urine analysis, the determination of hydrophobic surfactant protein antibodies and pulmonary function tests, before and after the study. Each patient included was routinely seen on an out-patient basis and had a stable clinical condition and lung function parameters, at 6 and 3 months and immediately before the start of the study.

After randomization, a basal lung function test was performed and either placebo (0.9% NaCl) or surfactant (2.4 mL Alveofact (120 mg surfactant) in 0.9% NaCl) was administered as described above. The nebulization took approximately 20–25 min. Before, and 30 min and 90 min after starting each nebulization, lung function tests were performed. All subjects were studied at the same time of day. After 5 days of daily consecutive inhalations and after a washout period of 3–5 days, treatment was crossed over and the other compound was administered in the same way for another 5 days.

Biochemical and biophysical analysis

Sputum was collected three times a day throughout the study period and was weighted. Aliquots of sputum from each day were lipid-extracted [20], the lower phase was washed using the method of FOLCH *et al*. [21], and phospholipid content [22], and phospholipid species composition were determined [23]. Other aliquots were used to determine the SP-B content by a solid phase enzyme-linked immunosorbent assay (ELISA) [24]. The presence of serum antibodies directed against the surfactant proteins SP-B or SP-C was screened in serum samples taken before, and 2 weeks and 3 months after the study, using polyclonal antibodies which had previously been raised in rats against the hydrophobic proteins of the surfactant preparation (Alveofact) [25]. Surface tension was measured in a pulsating bubble surfactometer (Electronetics, Amerherst, NY, USA) at a phospholipid concentration of 3 mg·mL^{-1} in 0.9% saline with 3 mM $CaCl_2$.

Statistical analysis

Based on a standard deviation of 9% for FVC and 7% for FEV_1 on repeated lung function testing during the previous 6 months, the sample size necessary to detect an increase in FEV_1 by 15% and in FVC by 20% was calculated before starting the study. Setting a power of 90% and a significance level of 5%, it was found that a minimum of five patients would be required in each group to demonstrate changes of this magnitude. To decrease variability between different subjects in a control and treatment group, a crossover design was chosen [26]. Data are presented as mean±SEM for n independent determinations. Comparisons were made by the t-test, and a p-value of less than 0.05 was set as level of significance.

Results

In vitro *studies of surfactant nebulization*

Jet nebulization of the surfactant with the IS-2 nebulizer resulted in a particle spectrum with more than 75%

Table 1. – Effect of jet nebulization on phospholipid composition and surface activity of a bovine, lipid-extracted surfactant (Alveofact)

	Control	Jet nebulization 20 min	Jet nebulization 30 min
Lysophosphatidylcholine	2.8±0.3	3.3±0.4	3.9±0.2*
% of total	(n=6)	(n=4)	(n=4)
γ_{ads} mN·m^{-1}	52.2±4.9	45.0±9.4	58.5±13.3
	(n=5)	(n=4)	(n=4)
γ_{min} mN·m^{-1}	16.9±1.8	16.8±3.3	15.4±5.1
	(n=5)	(n=4)	(n=4)

The relative content of lysophosphatidylcholine was significantly elevated after 30 min (*p<0.05). No significant changes were observed for the other phospholipids. Surface active properties measured at 3 mg·mL^{-1} in a pulsating bubble surfactometer remained unchanged. Data are presented as mean±SEM from (n) experiments. γ_{ads}: surface tension after absorption; γ_{min}: minimal surface tension after 3 min.

of the particles in the respirable size range, *e.g.* <5 μm, and 20% were <2 μm. Mass median diameter was 3.4 μm, and the geometric standard deviation 1.7 μm. Foaming, which might interfere with nebulization, did not develop. Mass output increased linearly with the surfactant concentration (3, 6, 12, 25, 50, 100 mg·mL^{-1}) used, whereas the relative recovery decreased (from about 35 to 18%). Most of the losses were due to dried surfactant being trapped against the walls of the nebulizer. From these data, it can be estimated that from 2.4 mL of a 50 mg·mL^{-1} surfactant suspension about 20 mg will be delivered to the mouth. Although some degradation of phosphatidylcholine occurred towards the end of the nebulization procedure, as indicated by an increased fraction of lysophosphatidylcholine (table 1), there was no loss of surface activity during nebulization (table 1). On the basis of these *in vitro* data, 50 mg·mL^{-1} was selected as the optimal concentration for usage in further studies.

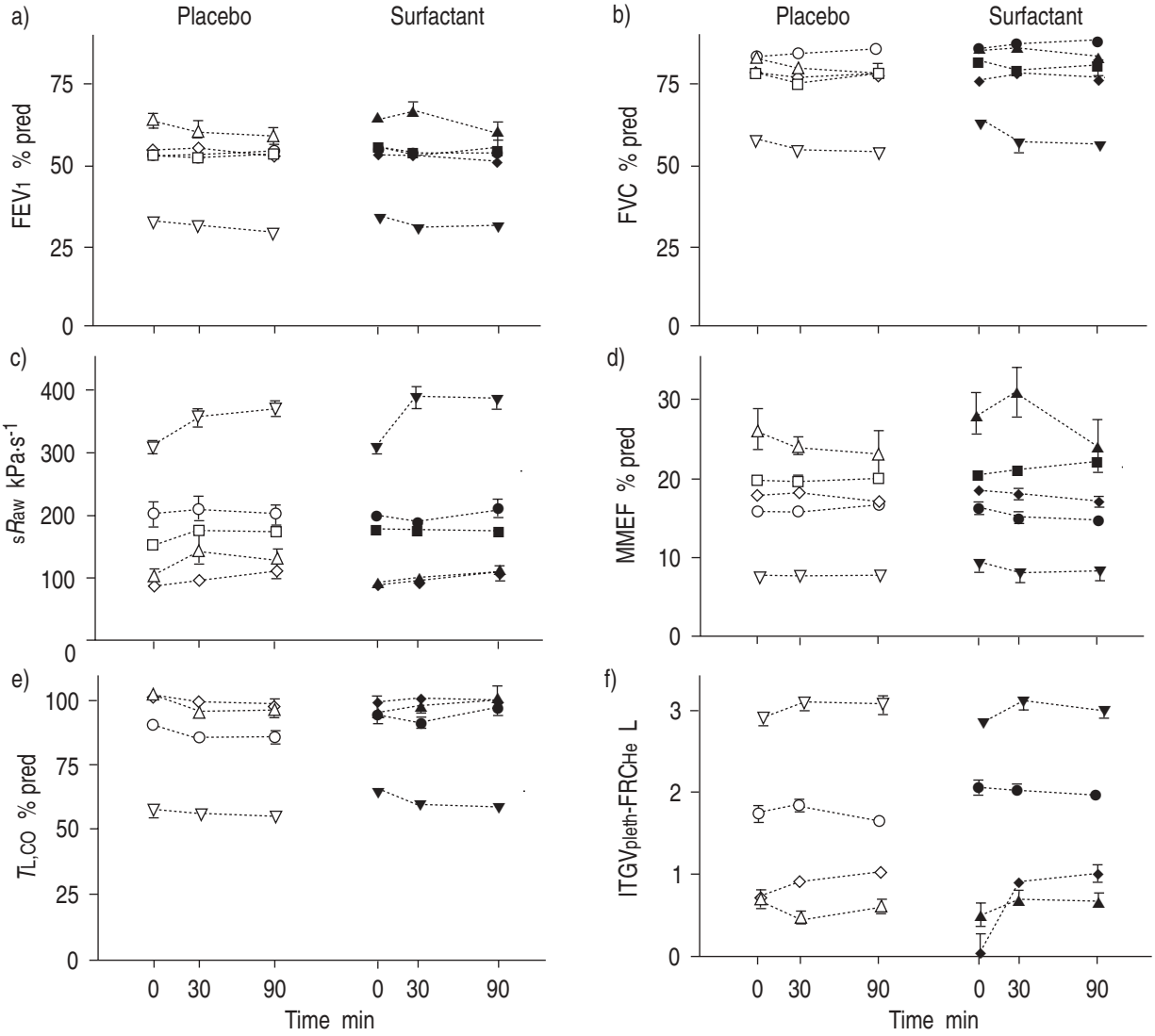

Fig. 1. – Effect of jet nebulization of a bovine, lipid-extracted natural surfactant (Alveofact, 120 mg) on lung function in five patients with cystic fibrosis (CF). The patients inhaled placebo or surfactant in a study of double-blind design, on five consecutive days. After a washout period, the treatment was crossed over. a) Forced expiratory volume in one second (FEV_1); b) forced vital capacity (FVC); c) specific airway resistance ($_sR_{aw}$); d) maximum midexpiratory flow rate between 75–25% of expired vital capacity (MMEF); e) transfer factor of the lung for carbon monoxide ($T_{L,CO}$); f) trapped gas, *i.e.* intrathoracic gas volume measured in the body plethysmograph ($ITGV_{pleth}$) minus functional residual capacity measured by helium dilution (FRC_{He}). Data are presented as mean±SEM for individual patients over the 5 days. Lung function tests were performed before and 30 and 90 min after the inhalations. % pred: percentage of predicted value.

Nebulization of surfactant in patients with CF

The inhalation of surfactant was well tolerated by patients who showed no adverse effects that could be attributed to the aerosolized administration of surfactant. One patient developed an exacerbation of the pulmonary infection 2 weeks after the completion of the surfactant inhalation period, this being treated with intravenous antibiotics. Whilst healthy normal adults do not usually have measurable serum antibody titres against the hydrophobic surfactant proteins (mainly against SP-B) (<1:10) [25], all but one of the CF patients had elevated levels before therapy (1:20–1:80). No significant changes in serum antibody titres were observed in the patients studied, when assessed at 2 weeks and at 3 months after surfactant therapy.

Aerosolized surfactant had no significant acute effect on FEV_1 and FVC. This was observed on each day of aerosolization (fig. 1a and b). Moreover, there was no cumulative effect on these parameters during the short-term treatment period of five consecutive days.

Additional secondary variables, which included: mean midexpiratory flow (MMEF), specific airway resistance (fig. 1c and d) and transfer factor of the lungs for carbon monoxide, and trapped gas (*i.e.* the difference between intrathoracic gas volume as measured by the body plethysmograph and functional residual capacity as measured by the helium dilution technique ($ITGV_{pleth}$-FRC_{He})), (fig. 1e and f), and O_2 saturation were not significantly altered by the inhalation of surfactant. In contrast, the inhalation of two puffs of salbutamol (200 μg) resulted, in all patients, in a small but significant increase of FEV_1 from 2.0 to 2.3 $L \cdot s^{-1}$, and a fall of specific airway resistance from 196 to 170% of predicted ($p<0.05$).

Sputum volume did not change with surfactant therapy. SP-B could not be determined in all the sputum samples due to nonspecific interferences. In those samples where reliable measurements could be made, the amount of SP-B recovered during the period of surfactant administration was increased in comparison to the placebo period. This indicates that some of the surfactant components delivered were expectorated later in association with the sputum (fig. 2).

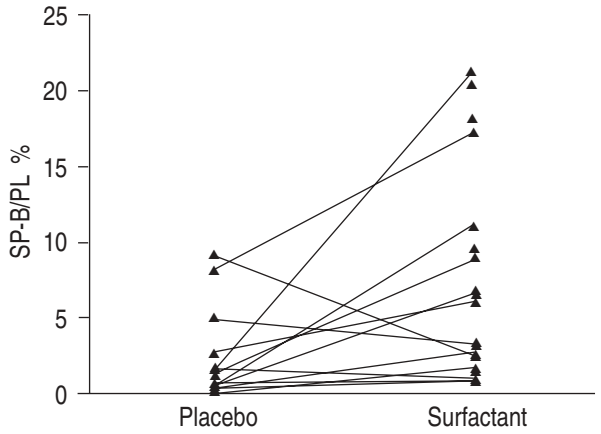

Fig. 2. – Surfactant protein-B (SP-B) recovered in sputum from five patients with cystic fibrosis was expressed as a percentage of non-changing phospholipid (PL) mass. Values for individual subjects are connected by lines. During surfactant treatment, significantly more SP-B was recovered ($p<0.001$).

Discussion

We first investigated whether the jet nebulization of the lipid-extracted, natural surfactant preparation was feasible. Although a small increase in the content of lysophosphatidylcholine was noted after nebulization to dryness, the functional activity of the material was not impaired as suggested from experiments performed in the pulsating bubble surfactometer. This was in contrast to sonication by ultrasound, which led to a loss of biophysical activity [27]. Seventy five per cent of the particles were in a range which allowed them to be inhaled into the alveolar space. Delivery was only during inspiration, and an optimized surfactant concentration allowed the inhalation of the maximum amounts. However, intrapulmonary delivery was not directly assessed with a radiotracer, because this particular inhalation device has already been shown to result in about 19% intrapulmonary delivery in normal persons [28]. In adults with CF, a similar total pulmonary deposition is expected, the distribution, however, being uneven with a decreased aerosol entry to poorly ventilated regions [29].

Our hypothesis on the beneficial effects of aerolized exogenous surfactant in CF was based on a substantial amount of *in vitro* and *in vivo* data, as summarized in the introduction. The results of this pilot study clearly demonstrate no significant acute or short-term effect in these patients. The conditions used were optimized to deliver the maximum surfactant by the current state-of-the-art jet nebulization technology.

Potential reasons for our failure to detect the expected effect on lung function include the following. Firstly, the inadequate delivery of the aerosol. This possibility, however, can be ruled out because measurements of the surfactant aerosol generated demonstrate that the majority of the particles produced were in the respirable range. Various other studies, *e.g.* on inhaled antibiotics or radiolabelled drugs, have clearly demonstrated intrapulmonary delivery of reasonable amounts of these aerosols.

Secondly, the distribution of the drug was inhomogeneous. This is very likely [11, 29]. However, surfactant would have been expected to be deposited in relatively well-ventilated areas, to stabilize those airways which are patent only with the help of somewhat forced respirations, and thus improve FEV_1 and FVC.

Thirdly, the dose of the surfactant administered may have been too small. Significant improvements in FEV_1 and FVC were observed in acute asthma with a much smaller nebulized dose (10 mg) than used in this study (120 mg). Recently, Oetomo *et al.* [16] observed no effects in asthmatic children with chronic airflow limitation when the same surfactant as that used in the present study was administered. Surfactant delivered by aerosol has been effective in animal experiments with severe ARDS [10, 11]. Although the amounts were some orders of magnitude smaller than those given by bolus, they were still somewhat higher than those used in human studies, when expressed per kilogram body weight (BW). In rabbits, about 4.9 $mg \cdot kg\ BW^{-1}$ delivered by aerosol was superior to the 100 $mg \cdot kg\ BW^{-1}$ delivered by bolus [11]. Similarly, 2 $mg \cdot kg\ BW^{-1}$, significantly improved lung function in premature lambs with respiratory distress syndrome [30]. We estimated the delivery to the

lungs in the present study to be in the range of 0.1–0.3 mg·kg BW^{-1}.

A fourth potential explanation as to why there was no effect on the lung function might involve the inhibition of the exogenous surfactant by inactivators, which might be present in the lung lavages of patients with CF. This cannot be excluded, but appears less likely, as in preliminary experiments we did not find increased inhibitory activity in the protein fraction of CF bronchoalveolar lavages compared to controls (own unpublished observations). In addition, the surfactant used was a preparation with a high resistance to inhibition, possibly due to its relatively high content of SP-B [7] when compared to other lipid-extracted surfactant preparations available for clinical usage. All currently available preparations lack SP-A, which might substantially improve the quality of the surfactant with respect to low surface tension and resistance to inhibition.

Fifthly, the case could be argued that we had set too high an improvement of lung function as being clinically relevant. However, we felt that a consistent difference in FEV_1 between placebo and surfactant treatment of about 15% would be adequate, comparable to what may be expected with β-agonists. The power of the present study is high, the chance of not detecting the difference being only 10%. Considering the case that only an improvement is of relevance, the power of such a one-sided hypothesis would be even greater. Lowering this power to 80% at the same sample size would be sufficient to detect 12% differences. To find smaller differences, the sample size would have to be increased.

Finally, the hypothesized relative surfactant deficiency in the small airways of patients with CF might be of no relevance for the clinical conditions observed, including overinflation, decreased expiratory flows or reduced FEV_1. Although the above-mentioned studies on the pathophysiology of the small airway closure and the demonstration of impaired surfactant in CF patients argue for a role of surfactant in maintaining the patency of the airways, this hypothesis has not yet been verified. Possibly, this may be easier in younger patients with CF, who have less severe lung injury. Further studies are needed to define more clearly the reasons for the result obtained in this trial.

The current investigation did not address other potential targets of surfactant therapy in CF. These include an improvement of surface and transport properties of CF mucus [31], and an enhancement of bronchotracheal mucociliary clearance [32]. In this study, no changes in the amount of sputum recovered were observed. Finally, pulmonary surfactant participates in the nonspecific first-line host defence reactions and may modulate the function of immune cells in the lungs [33].

Our pilot study, under the conditions of maximal surfactant administration in a realistic setting, clearly shows that nebulization of 120 mg surfactant during inspiration does not alter forced expiratory volume in one second and forced vital capacity in adult patients with cystic fibrosis. Furthermore, daily delivery for five consecutive days did not improve lung function. Potential reasons for this failure include: insufficient amount of surfactant delivered; inhomogeneous distribution; or an inhibition of exogenous surfactant in the lungs of patients with cystic fibrosis. Thus, future studies with greater technological capacities may achieve a substantially higher lung delivery, and the use of a superior surfactant preparation, possibly containing recombinant surfactant protein A, may improve lung function in cystic fibrosis.

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