

Relaxant Effects of Quercetin and Rutin on Human Isolated Bronchus

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Received April 20, 2012; revised May 10, 2012; accepted May 18, 2012

ABSTRACT

Increasing epidemiological evidence supports the view, that quercetin has protective roles in a multitude of disease states in human who have a high intake of polyphenols. To investigate the ability of quercetin and its rutoside, rutin, to modulate the relaxation of human airways smooth muscle and to determine the mechanism (s) of such relaxation, isolated human bronchus rings were suspended in individual organ baths, precontracted with acetylcholine or with histamine and the relaxing effects of quercetin and rutin were determined by measurement of isometric tension. Quercetin induced concentration-dependent relaxant responses on acetylcholine or histamine precontracted human bronchial rings and with almost equal effectiveness. In terms of potency (pD_2) and efficacy (E_{max}), quercetin is more potent than rutin on relaxant responses of human bronchus. K^+ and Ca^{2+} concentration-dependent contraction curves were inhibited after incubation with increasing concentrations of quercetin. Quercetin potentiated in a concentration-dependent manner the relaxant effects of isoprenaline or sodium nitroprusside. Rutin had no effect on K^+ -induced contraction and on relaxant activity of isoprenaline or sodium nitroprusside. Our results suggest that the bronchodilator effects of quercetin are modulated by an increase in cyclic nucleotide levels as well as an alteration in availability of Ca^{2+} to the contractile machinery.

Keywords: Calcium Channels; Cyclic Nucleotide; Human Bronchus; Quercetin; Relaxant Effects; Rutin

1. Introduction

Several epidemiological studies have shown a beneficial link between dietary flavonols intake and reduced risk of chronic disease. The beneficial effects of flavonoids on human health have been attributed to their antioxidant and anti-inflammatory properties [1]. Among flavonols, quercetin is the most abundant in the human diet, accounting for about 60% of the total intake [2]. Quercetin is present mostly in the form of glycosides in plant foods such as vegetables and fruits. Almost 180 different glycosides of quercetin have been described in nature, with rutin (quercetin-3-*O*-rutinoside) being one of the most common [3]. Based on its polyphenol structure (**Figure 1**), quercetin exerts many beneficial health effects, including improvement of cardiovascular health, reducing risk for cancer, protection against osteoporosis [2,4].

Most of these properties are linked to its strong antioxidant action but quercetin also modulates the expression of specific enzymes [5,6]. However, few studies have investigated the relationship between quercetin consumption and obstructive lung disease. In one epidemic-

ological report with over 13,000 participants, it is found that dietary intake of flavonols (a subclass of flavonoids including quercetin and kaempferol) was positively associated with pulmonary function and inversely associated with chronic cough and breathlessness in Dutch Chronic obstructive pulmonary disease (COPD) patients [7,8]. Furthermore, intake of polyphenol-containing fruit was inversely correlated with COPD mortality in three European countries [9]. In addition to their antioxidant properties, quercetin show several interesting effects, including antispasmodic effects [10-12]. A number of flavonoids have been reported to affect smooth muscle contractility in response to various agonists [13-15]. The precise mechanisms by which flavonoids exert their actions remain unclear. In the vascular smooth muscle, they reduce smooth muscle contraction, evoked by several spasmogens, with a mechanism probably due to alteration in the availability of Ca^{2+} to the contractile machinery or to their action on crucial enzyme systems of intracellular cascades [16-18]. The aim of our present study is to investigate the ability of quercetin and its rutoside, rutin,

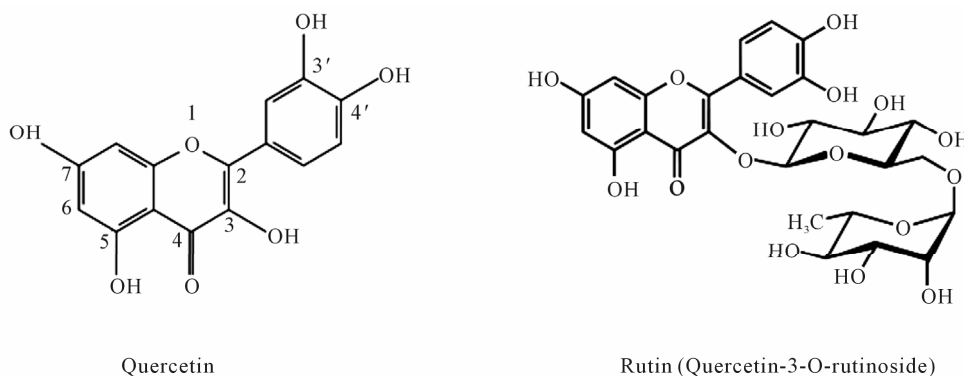


Figure 1. Chemical structure of quercetin and its rutinoside, rutin, tested.

to modulate the relaxation of human airways smooth muscle and to determine the mechanism (s) of such relaxation.

2. Material and Methods

2.1. Isolated Human Airways

Human bronchial tissues were obtained from thoracotomy specimens of patients undergoing surgery for lung carcinoma after consent of the patients. The protocol was approved by a local ethics committee. Specimens were selected from a macroscopically normal tissue at distance from the malignancy, and rapidly transferred to the laboratory in Krebs solution. After resection, segments of bronchi were carefully dissected from connective tissue and surrounding parenchyma and cut into rings. Human bronchial rings (5 - 7 mm in length \times 0.5 - 1 mm internal diameter) were either used immediately or stored overnight in a large volume of cooled (4°C) Krebs previously aerated. We found no functional differences in bronchial tissues that were studied immediately compared with those used after 24 h refrigeration.

2.2. Airway Rings Bath Technique

Bronchial human rings were placed in organ bath chamber containing 5 ml of fresh Krebs solution that was replaced continuously, and bubbled with 95% O_2 - 5% CO_2 at 37°C . The airways were mounted between stainless steel hooks under a resting tension of 2 to 2.5 g and allowed to equilibrate for 1 h. Changes in force of contraction were measured isometric with strain gauges (UF-1; Piodem, Canterbury, Kent, UK) and EMKA amplifiers (EMKA Technology, Les Ulis, France). In all experiments, the bronchial rings were first contracted maximally with acetylcholine (3 mM) and then relaxed, at the end of experiments without washout, with aminophylline (3 mM) to obtain the maximal relaxation. Before beginning experiments, the human airways were allowed to equilibrate for 1 h during which the Krebs solution was

changed every 15 min.

2.3. Effect of Quercetin or Rutin on Histamine- or Acetylcholine-Induced Contraction of Human Bronchi

Following the second equilibration period, the cumulative concentration-response curves for quercetin or for rutin were obtained on bronchial rings contracted to 50% - 60% of the maximal contraction with acetylcholine 10^{-6} M or histamine 10^{-5} M. When a steady level of contraction has been reached, quercetin and rutin were cumulatively added, with half log increments, to the bathing solution every 30 min when a steady-state effect has been reached. The maximal relaxant response elicited by aminophylline (3 mM) was taken as 100%. Only one concentration-response curve to a drug was recorded in each ring.

2.4. Effect of Quercetin or Rutin on KCl- or CaCl_2 -Induced Contraction of Human Bronchus

In order to test the potential action of quercetin and rutin as calcium antagonists, the inhibitory effect of these compounds was tested on the contraction induced by cumulative addition of KCl (3.75 to 75 mM) [19] and on the contraction directly caused by extracellular calcium (CaCl_2) added to the bath in a depolarising medium. CaCl_2 concentration-response curves were established according to Advenier, *et al.* [20]. Briefly, human bronchial rings were incubated for 1 h in Krebs solution devoid of CaCl_2 then for 15 min in CaCl_2 -free Krebs solution containing 1 mM ethylenediaminetetraacetic acid. The preparations were washed with CaCl_2 -free Krebs solution and then incubated with Krebs solution containing 30 mM KCl (the concentration of NaCl was reduced to preserve isomolality). Concentration-response curves to CaCl_2 (0.01 to 3 mM) or to KCl (3.75 to 75 mM) were then obtained in presence of quercetin, rutin or an equivalent volume of vehicle added to the bath 15 min before

addition of CaCl₂ or KCl.

2.5. Effect of Quercetin and Rutin on Concentration-Response Curves to Isoprenaline and Sodium Nitroprusside

In another series of experiments, the airway rings were pretreated for 30 min with one concentration (10⁻⁵, 3 × 10⁻⁵ or 10⁻⁴ M) of quercetin or rutin or an equivalent volume of vehicle. Concentration-response curves to isoprenaline or sodium nitroprusside were then obtained by cumulative addition on bronchial rings contracted to a level similar to the controls. At the end of the relaxant response, aminophylline (3 mM) was added to determine the maximum relaxation achievable.

2.6. Drugs

Isoprenaline, aminophylline (PCH, Paris, France); and acetylcholine (Glaxo, Paris, France), sodium nitroprusside (SNP), (Sigma, L'Isle d'Abeau, France). Quercétine and rutine (Fluka Chemikalien GmbH Buchs, Suisse). All stock solutions of the chemicals were made in distilled water excepted quercetin, and rutin in dimethyl sulfoxide (DMSO). All the stock solutions were kept frozen until use and then diluted in distilled water. No significant vehicle effect was observed.

2.7. Statistical Analysis

The relaxation responses were expressed as a percentage of the maximal relaxation produced by aminophylline (3 mM). pD₂ value represents the negative logarithm of the concentration (EC₅₀) of drug which induces a relaxation equal to 50% of its own maximal effect. EC₅₀ was determined from each relaxation curve by a logistic curve-fitting equation. E_{max} represents the maximal effect of drug expressed as a percentage of the maximal relaxation to aminophylline. For the studies on the inhibitory activity of quercetin on KCl- and CaCl₂-induced contractions, results are expressed as percentages of the maximal contraction induced by KCl (75 mM) and acetylcholine (10⁻⁶ M), respectively. All values are expressed as mean ± standard error of estimate mean value (s.e. mean). Differences between treatments were tested using analysis of variance (ANOVA) for repeated measures followed by Bonferroni-Dunn's test if required and the pD₂ values were compared by Student's *t*-test (GraphPad). *p* < 0.05 was considered to be significant.

3. Results and Discussion

Relaxation of human isolated bronchus is clearly observed in the presence of quercetin in a concentration-dependent manner (Figure 2). In a first series of experiments, a comparing of the relaxant effect of quercetin

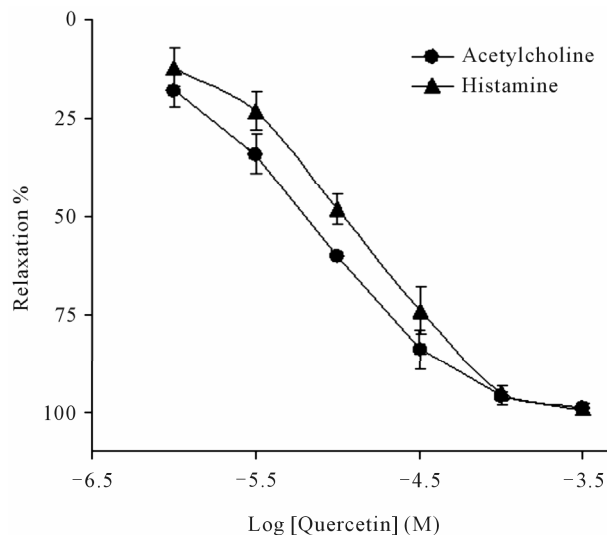


Figure 2. Concentration-effect curves of quercetin (10⁻⁶ – 3 × 10⁻⁴ M) on human bronchi rings precontracted to 50% - 60% of maximal contraction with acetylcholine (10⁻⁶ M) or histamine (10⁻⁵ M). Results are expressed as percentages of the relaxation induced by aminophylline 3 mM. Each point represents the mean value ± standard error of means (s.e.m.) from five experiments.

was performed on human bronchial rings precontracted with acetylcholine and histamine. These spasmogens were chosen because they are classically used not only in pulmonary pharmacology but are also two broncho-constricting agents involved in the contraction in human bronchial. The relaxant effect elicited by quercetin in human isolated bronchus, against the contractions induced by acetylcholine and histamine, was almost identical, with pD₂ value of 5.2 ± 0.1 and 5.0 ± 0.1, respectively. Our results apparently agree with those reported in rat aorta [11,16] and guinea-pig tracheal [21].

In addition, a concentration-dependent relaxation is observed with increasing cumulative concentrations of rutin, the quercetin-3-*O*-rutinoside, in human bronchial preparations precontracted with histamine and acetylcholine (Figures 3(a) and (b)). Compared with quercetin, the relaxation effect of rutin is weakly potentiated, with a maximum relaxation (E_{max}) of 58% ± 9% and 64% ± 3% respectively on acetylcholine- and histamine-contracted human airways. On acetylcholine- and histamine-contracted human airways respectively, quercetin was more potent than rutin with an EC₅₀ value of 6 μM and 8 μM, in comparison with EC₅₀ of rutin 10 μM and 27 μM. The hydrophilicity or the presence of bulky oligosaccharide rutinose makes rutin unable to penetrate the cell membrane and might account for its ineffectiveness, as suggested by others works [14,15].

At relatively high concentrations >3 × 10⁻⁵ M of quercetin, the contractions induced by both contractile agonists were completely abolished (Figure 2). Hence, the

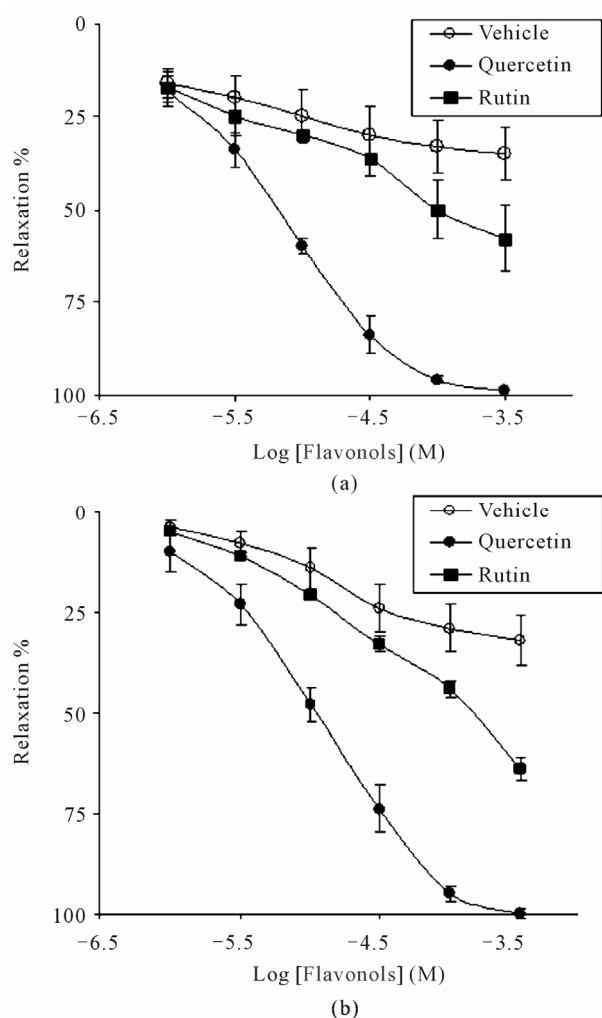


Figure 3. Cumulative concentration-relaxation curves for quercetin and rutin ($10^{-6} - 3 \times 10^{-4}$ M) in human bronchial rings precontracted to 50% - 60% of maximal contraction with acetylcholine (10^{-6} M, panel a) or histamine (10^{-5} M, panel b). Results are expressed as percentages of the relaxation induced by aminophylline 3 mM. Each point represents the mean value \pm standard error of means (s.e.m.) of five to seven experiments.

lower concentrations $\leq 3 \times 10^{-5}$ M were selected for study the mechanisms of bronchorelaxation action of this quercetin on human isolated bronchus.

In a series of experiments, the human bronchial rings were incubated with increasing KCl and calcium concentration in the absence and presence of different concentrations of quercetin or rutin. In control conditions, cumulative addition of KCl (3.75 to 75 mM) or calcium (0.01 to 3 mM) produced contractile response of the human bronchial rings in a concentration-dependant manner (**Figures 4(a)** and **(b)**). Quercetin inhibited effectively and concentration-dependently these two ways of contraction in human airways preparations (**Figures 4(a)** and **(b)**). Quercetin, 3 and 10 μ M, displaced and significantly

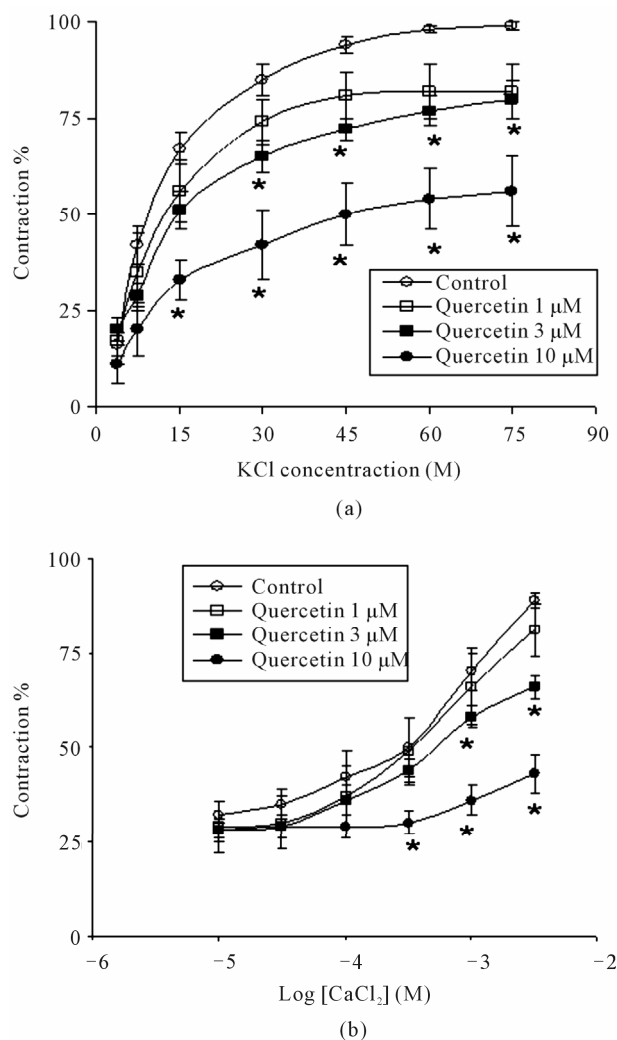


Figure 4. Concentration-response curves to KCl (a) and to CaCl_2 (b) in human isolated bronchus incubated with increasing concentrations of quercetin. Each point represents the mean value \pm standard error of means (s.e.m.) of six experiments. * $p < 0.05$.

($p < 0.05$) concentration-response curves to the right with depression of the maximal responses (**Figures 4(a)** and **(b)**). The $-\log\text{IC}_{50}$ values of quercetin against KCl (3×10^{-2} M) and CaCl_2 (10^{-3} M)-induced contractions were 7 μ M and 4 μ M, respectively. K^+ -induced contraction is result of an increase Ca^{2+} influx through L-type voltage-dependent calcium channels (VDCC). They have reported that constriction of human bronchial muscle by acetylcholine, histamine, and pollen antigen challenge, was significantly reduced by nifedipine, a selective VDCC blocker [22]. Therefore, the inhibition of K^+ -induced contraction by quercetin might be interpreted as a consequence of blockade of VDCC. In fact, quercetin and related compounds, 3-*O*-methylquercetin and apigenin, are shown to be the potent inhibitors of Ca^{2+} -up-take in vascular smooth muscle. In contrast, Duarte, *et al.* [23]

found that, in rat aorta, the vasodilator effect of quercetin is unrelated to changes in cytosolic Ca^{2+} . This latter observation suggests that the anti-spasmodic effect of quercetin may be explained by its action with a second target beyond the Ca^{2+} channels. In fact, quercetin has been reported to inhibit Ca^{2+} -sensitising mechanism in contractile proteins [10,16,17,24]. In contrast, rutin was devoid of inhibitory effect on the K^{+} -induced contraction in human bronchial rings (data not shown).

The possible involvement of cAMP and cGMP-dependent protein kinases on the relaxant effect of quercetin was examined in the presence of isoprenaline, β -adrenoceptor agonist, and sodium nitroprusside (SNP), an activator of guanylate cyclase. On acetylcholine-contracted human bronchi, quercetin (3×10^{-5} M) shifted the concentration-relaxation curves of isoprenaline to the left and significantly increased the potency (pD_2), as well as the efficacy (E_{max}) of isoprenaline (Table 1).

Furthermore, the relaxation elicited by sodium nitroprusside on acetylcholine-contracted human bronchial was significantly potentiated by quercetin 10^{-5} M in terms of potency and efficacy (Table 1). This potentiating effect of quercetin on isoprenaline-elicited relaxation might be mediated via inhibition of cAMP-phosphodiesterase (PDE). Inhibition of cAMP-phosphodiesterase has been involved in the relaxing effect of quercetin on vascular and intestinal smooth muscle [25,26]. In rat aorta high concentrations of quercetin increased cyclic AMP levels [16]. Indeed, the 3-*O*-methylquercetin, a metabolite of quercetin, potentiates the relaxing effect of forskolin and SNP on the trachea of guinea pig and significantly inhibits the activity of cAMP- and cGMP-phosphodiesterase [21]. The potentiating effect of quercetin on SNP-evoked relaxation might not be due to its inhibitory effect on PDE-V activity, since they have been reported that quercetin has only low potency of PDE-V inhibition with $\text{IC}_{50} > 100 \mu\text{M}$ [27]. However, quercetin is potent inhibitor of PDE III and IV, showing IC_{50} values of $5.6 \mu\text{M}$ and $9.9 \mu\text{M}$, respectively [27], which corresponded to the EC_{50} values producing its bronchodilator effect in the present study. Thus, our data suggested that the potentiated effect of relaxant activity of SNP by quercetin may be interpreted by increased the rate of cAMP. This subsequently activates the cAMP-dependent protein kinase and inhibits the myosin light chain kinase by phosphorylation and reducing the contraction [28]. The mechanism by which relaxation is produced by the cAMP pathway is not yet clear, but may result from the decrease in intracellular Ca^{2+} . This decrease may be due to the reduction of the influx of Ca^{2+} by increasing its accumulation in the sarcoplasmic reticulum, or extrusion through the plasma membrane [28]. However, the concentration-response curves to isoprenaline and to SNP were unaffected by rutin 10^{-4} M (data not shown).

Table 1. Potentiating effect of quercetin on both isoprenaline- and sodium nitroprusside-induced relaxation of pre-contracted bronchus to 50% - 60% of maximal contraction with acetylcholine (10^{-6} M).

	n	pD_2	$E_{\text{max}} \%$
Isoprenaline			
Control	7	8.1 ± 0.2	93 ± 4
Quercetin (10^{-5} M)	5	8.5 ± 0.1	92 ± 4
Quercetin (3×10^{-5})	7	$8.7 \pm 0.1^*$	97 ± 2
SNP			
Control	7	6.1 ± 0.2	62 ± 5
Quercetin (10^{-5} M)	7	$6.6 \pm 0.1^*$	77 ± 6

Values are expressed as mean value \pm standard error of means (S.E.M.). E_{max} are expressed as percentages of the relaxation induced by aminophylline 3 mM. The control preparations were treated with vehicle (DMSO) only. *Indicates significant differences between control and pretreated rings ($p < 0.05$).

Potential health effects of bioactive compounds depend on their bioavailability following oral administration. Although, the concentrations of quercetin reported effective in our study are higher from those observed in human plasma under various nutritional conditions [29-32], those concentrations may be achieved in the body with diet containing high flavonols dietary supplements [33]. Furthermore, the half-life of quercetin is on the order of 10 - 30 h [32,34], thus repeated consumption of foods rich in quercetin (such as onions or apples) might cause its accumulation in blood at levels similar to those found effective in the present study.

In conclusion, the data presented in this study indicate that quercetin, the most abundantly consumed bioflavonoid, is able to evoke airway smooth muscle relaxation. Although the mode of action remains to be clarified, our findings support the view that mechanisms of relaxant effects were mediated by an increase in cytosolic nucleotide cyclic levels possibly via nonselective inhibition of phosphodiesterases as well as an alteration in availability of Ca^{2+} to the contractile machinery. Like many biochemical effects have been attributed to quercetin, we can not rule out its ability to interfere with crucial enzyme systems involved in airways contraction. Moreover, the bronchorelaxing properties of quercetin observed in this study support the epidemiological data, which postulate an inverse relationship between consumption of dietary flavonoids and the incidence of obstructive lung disease.

4. Acknowledgements

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS) and

by the Algerian Agency for the Development of Research in Health (ANDRS).

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