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ORIGINAL ARTICLE

Saliva in relation to dental erosion before and after radiotherapy

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Abstract

Objective. Low saliva flow and abnormal saliva composition are common conditions after radiotherapy for oral cavity and pharyngeal cancer. Both conditions increase the susceptibility to dental caries and erosion, which may be further accelerated by changes in food preferences. The aim of this study was to determine changes in saliva flow and susceptibility to erosive challenges in pharyngeal cancer patients before and after radiotherapy to the head and neck. ***Materials and methods:*** The erosive potential of sucking acidic candies with and without calcium was determined in nine patients (50–68 years) before and after receiving a radiation dose of 66 Gy to the head and neck area. The erosive potential was evaluated from saliva degree of saturation with respect to hydroxyapatite (HAp) and by dissolution of HAp in candy-stimulated saliva. ***Results.*** Sucking acidic candies increased saliva flow rates ~ 17-fold before as well as after radiotherapy ($p < 0.001$). However, significantly lower unstimulated ($p < 0.05$) and stimulated ($p < 0.01$) saliva flow rates were obtained after radiotherapy. Also, saliva became more under-saturated with respect to HAp during ($p < 0.01$) and in a period after sucking the candies ($p < 0.01$). HAp dissolution was significantly lower with the candy containing calcium compared with the control candy, both before and after radiotherapy ($p < 0.001$ and $p < 0.05$). ***Conclusions.*** Radiotherapy to the head and neck area significantly reduced saliva flow and altered saliva composition in a way that may increase the susceptibility to dental disease. However, saliva could be stimulated by acidic candies, which could be made nearly non-erosive even in irradiated patients.

Key Words: *dry mouth, oral cancer, longitudinal clinical study, saliva stimulation, erosive potential, human, food modification*

Introduction

Pharyngeal and oral cavity cancers are life-threatening diseases for which surgery and radiotherapy remain the two main treatment options [1]. However, radiotherapy to head and neck often leads to dry mouth, reduced saliva flow rates and abnormal saliva composition [2–4]. Dreizen et al. [5] showed that saliva flow rates underwent a marked reduction during 6 weeks of radiotherapy to the head and neck area and that this reduction was accompanied by a highly increased susceptibility to dental caries. Dental caries could, however, be significantly reduced if caries preventive measures were introduced [5]. In this context, the susceptibility to dental erosion, which is the loss of dental hard tissue by chemical processes not involving bacteria [6], also seems to be increased in patients suffering from low saliva flow rates [7,8]. Like with dental caries [9], low flow rates and reduced remineralization seems to be the main causes of increased dental erosion among these patients. Low saliva flow rates may also lead to changed food preferences [10,11] and dry mouth patients are often recommended sugar-free, but acidic products in the form of candies, tablets, jellies and chewing gum to relieve symptoms of dry mouth. However, such foodstuffs with saliva-stimulating effects are likely to have erosive potential due to their acidic content [12] and this potential is accelerated in patients who have received radiotherapy to head and neck [8]. So far no prospective data are available about the effect of radiotherapy to the head and neck on susceptibility to dental erosion and no prospective data exist on the possibility to reduce the erosive

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potential of acidic foodstuffs for dry mouth relief. Therefore, the aim of this study was to determine changes in saliva flow and susceptibility to erosive challenges in pharyngeal cancer patients before and after radiotherapy to the head and neck.

Materials and methods

Study group and design

The study group included 10 cancer patients who all received radiotherapy to the head and neck area against pharyngeal cancer in the tonsil area. The group comprised eight men and two women with a mean age of 58 years (50–68), a mean weight of 78 kg (50–93) and a mean height of 177 cm (162–186). Three patients were irradiated on the right side of their head and neck, five patients on the left side and two patients were irradiated on both sides. Unilaterally irradiated patients were treated with a 4 MV photon linear accelerator by angled wedge fields and bilaterally irradiated patients by parallel opposed compensated fields. The field covered the diseased tonsillar fossae and the ipsilateral neck lymph node regions. All patients had the submandibular and the ipsilateral parotid glands included in the target field where they received 66 Gy. The first part of the study was conducted 1–3 weeks before radiotherapy and the second part at least 3 months after completion of radiotherapy. At the second visit, i. e. after radiotherapy, all patients showed no signs of remaining or recurring cancer. Prior to the experiments all participants gave informed consent to the protocol, which was approved by the Ethical Committee of Copenhagen, Denmark (No: 03-001/03).

Interview and saliva collection

All experiments were conducted between 9 am and noon-time at a dental clinic at the Department of Oral Medicine, University of Copenhagen, Denmark. The patients neither ate nor drank and were not allowed to smoke or perform oral hygiene for 1 h before the study. Prior to saliva collection each patient was interviewed about smoking habits and asked about daily symptoms of dry mouth according to the UKU side-effect rating scale item 3.3 [13]. The saliva collection procedure, at both visits, i.e. before and after radiotherapy, was identical. Each visit comprised two separate saliva collection series, which were separated by a 30 min pause. To ensure that no patient suffered from thirst, the pause included the intake of one or two glasses of water. The first saliva collection series was a combined sequence of 19 min, which started with collection of unstimulated whole saliva for 5 min (unstimulated), candy-stimulated whole saliva for 4 min (stimulated) and post-stimulated whole saliva for 10 min (post-stimulated). The stimulated and the post-stimulated periods were further sub-divided into two separate collections, resulting in a total of five collections (one unstimulated, two stimulated and two post-stimulated). After stimulation all remaining candy (on average ~ 1 g) was removed prior to the post-stimulated collection. Following the pause with intake of water the second series did not include collection of unstimulated saliva. Instead stimulated saliva was collected for 5 min in response to sucking a candy without calcium (control) whereafter a candy with calcium was sucked for another 5 min (modified). The candies used for all three collections had a weight of ~ 4.5 g, contained ~ 1% of tartaric acid and had a taste of rhubarb and strawberry. The calcium content in the modified candy was obtained by adding 16.5 g of calcium lactate to each kilo of candy giving rise to ~ 9.7 mg of calcium per piece of modified candy. To avoid loss of CO_2 and changes in pH all saliva collections were performed under paraffin oil as previously described [14] and flow rates were determined in g/min, which is almost equivalent to mL/min [15]. After saliva collection, samples were stored on ice in individual closed syringes until pH and P_{CO_2} was determined on a blood gas analyser within 30 min [16]. Thereafter aliquots were made and stored at −80°C for further chemical analyses.

Saliva analyses

Salivary concentrations of sodium and potassium were determined by atomic emission spectroscopy (AES) and calcium by atomic absorption spectroscopy (AAS). Diluents were based on type 1 water (ASTM D1193-91) containing concentrated analytical grade HNO_3 at a concentration of 1.0%. For AES 0.1% CsCl was added to the diluent and for AAS 0.1% $SrCl_2$ and 0.1% KCl were added. Single and multi-element commercial quality control standards (Alfa Aesar™, Karlsruhe, Germany) were included per 10 saliva samples to detect interferences and drift. Salivary chloride and total phosphate were determined by colorimetric methods; chloride by the mercury–iron–TPTZ reaction [17] and total phosphate by the molybdenum reaction [18]. The erosive potential of saliva was determined by dissolution of synthetic hydroxyapatite crystals (Ca/P = 1.66) directly in the collected saliva [19]. Briefly, candy-stimulated saliva was depleted of its CO_2 content by vacuum, agitation and acidification after which the pH was adjusted to the pH obtained in response to sucking the candy. HAp crystals were then added to the saliva with continuous pH recordings for 5 min. If the saliva pH was constant the candy was assessed as non-erosive. In case the pH increased, dissolution was assumed and then the magnitude of erosion was quantified by back titration. The saliva degree of saturation with respect to HAp was determined from measurements of pH, calcium, phosphorus and ionic strength and the critical pH was iteratively estimated [12].

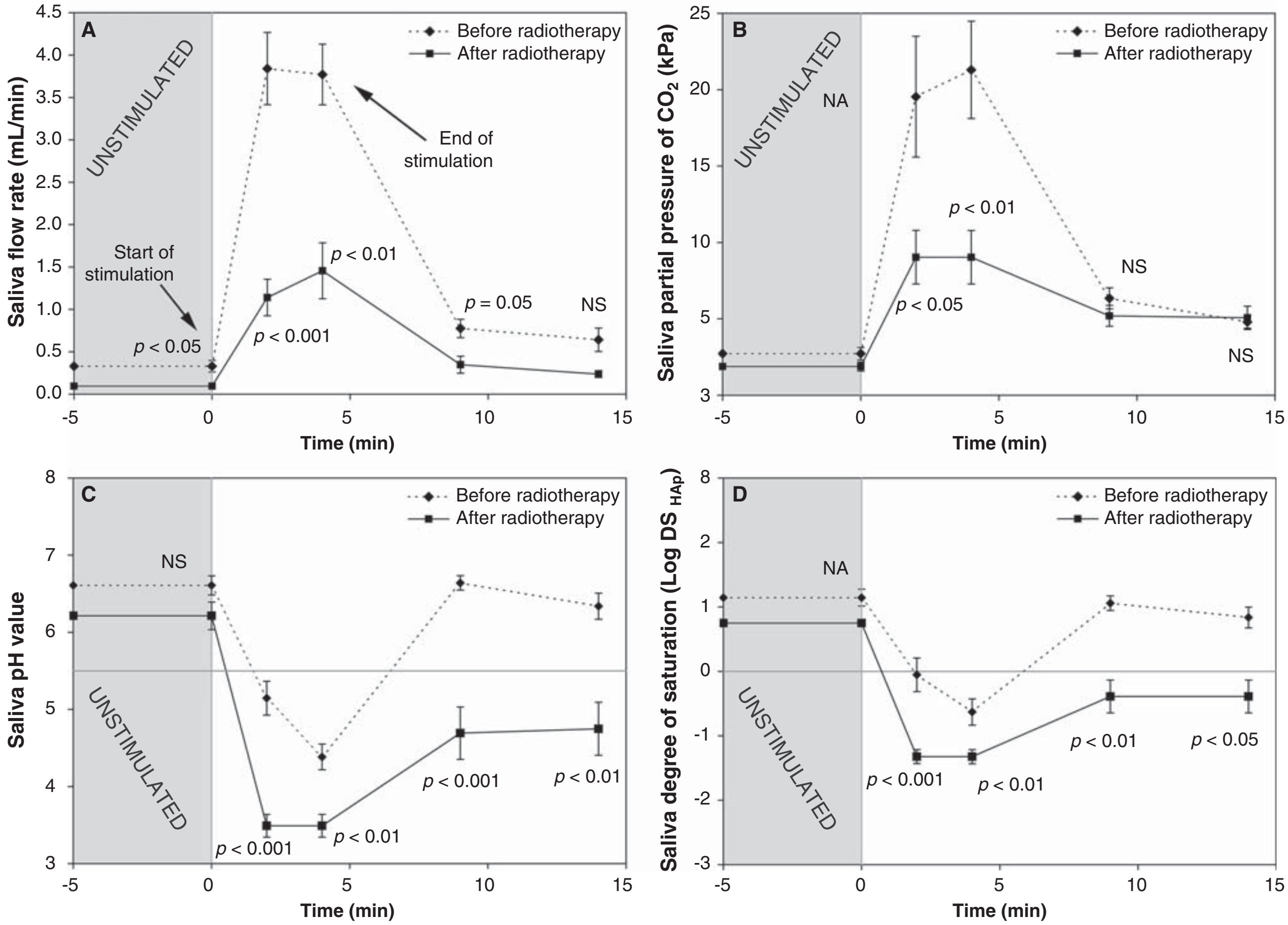

Figure 1. (A) The saliva flow rate, (B) the saliva partial pressure of carbon dioxide (P_{CO2}), (C) the saliva pH and (D) the linearized estimate for the saliva degree of saturation with respect to hydroxyapatite (Log DS_{HAp}) before, during and after sucking acidic candy in pharyngeal cancer patients. Dotted lines represent the values obtained before radiotherapy and continuous lines the values obtained after radiation therapy. Only patients completing both visits ($n = 9$) were included. The horizontal line in (C) indicates the average critical pH of 5.5 for human saliva. The horizontal line in (D) indicates saturation, below the line saliva was under-saturated with respect to hydroxyapatite and dissolution likely to occur, above the line saliva was supersaturated and dissolution not likely to occur. Identical values at time points −5 and 0 min were used to plot the unstimulated period, assuming constant flow and composition. Because saliva was stimulated differently in the following collections, stimulated and post-stimulated data points are placed corresponding to the end of each collection interval, not at the mid-point of the interval. Vertical bars indicate SEM, NS states non-significant and NA non-accessible due to missing data caused by insufficient sample volume.

Statistics

Statistical analyses were done with Excel and with the R statistical software (www.r-project.org). In the text, the results are given as mean ± SD. In the figures, the results are given as mean values ± SEM (Figure 1) or ± SD (Figure 2) and straight lines were used to connect the data points in Figure 1. Results on saliva flow and composition were analysed by a paired *t*-test and results on dry mouth and erosive potential by the Fisher's exact test. The level of significance was set at $p < 0.05$.

Results

Two patients had never smoked, whereas eight patients had a history of smoking; however, at the time of the radiotherapy only three patients remained smokers. In addition, three patients suffered from mild symptoms of dry mouth before treatment. After radiotherapy saliva secretion was nearly abolished in one patient who received a bilateral treatment and therefore this patient did not participate in the post-treatment session. Among the remaining nine patients, three suffered from mild symptoms of dry mouth, three from moderate symptoms and three from severe symptoms of dry mouth, which was a major increase compared with pre-treatment symptoms ($p < 0.01$).

Figure 1 shows the first saliva collection series that included collection of unstimulated, candy-stimulated and post-stimulated whole saliva. Figure 1A shows the saliva flow rates, Figure 1B the saliva partial pressure of carbon dioxide, Figure 1C the saliva pH and Figure 1D the saliva degree of saturation with respect to hydroxy-apatite before and after radiotherapy. As shown in Figure 1A, the unstimulated saliva flow rate, which on average was normal prior to radiotherapy (0.33 ±

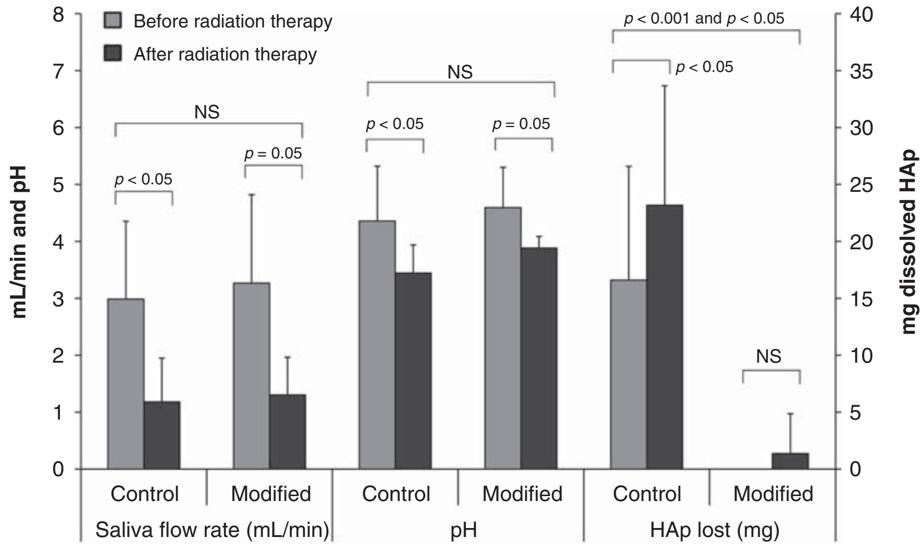

Figure 2. Saliva flow, pH and erosive potential obtained from sucking acidic candy with (modified) and without (control) calcium in pharyngeal cancer patients ($n = 9$) before (light grey columns) and after (dark grey columns) radiotherapy to the head and neck area. Only patients completing both visits ($n = 9$) were included. The erosive potential was determined by dissolution of pure hydroxyapatite crystals directly in whole saliva. The p-values with large brackets show the level of significance obtained between the control candy and calcium modified candy and p-values with small brackets show the level of significance for values obtained before and after radiotherapy. NS states non-significant and please note the use of two different scales in the figure.

0.20 ml/min), became significantly reduced following radiotherapy. This was also the case for the stimulated saliva flow rate and the first post-stimulated saliva flow rate. In spite of this reduction, the proportional increase in flow rate upon sucking acidic candies compared with unstimulated was nearly the same before and after radiotherapy and ~ 17-fold ($p < 0.001$) compared with unstimulated saliva. As shown in Figure 1B, no difference was obtained in the saliva partial pressure of carbon dioxide during the unstimulated state or after stimulation. Both before and after radiotherapy the partial pressure increased significantly in response to sucking the candies ($p < 0.01$); however, significantly less after radiotherapy ($p < 0.05$).

Figure 1C shows the saliva pH in response to sucking the acidic candy before and after radiotherapy. At the unstimulated measurements the saliva pH was somewhat lower after radiotherapy, although not significant. However, both the stimulated saliva pH ($p < 0.001$ and $p < 0.01$) and the post-stimulated saliva pH ($p < 0.001$ and $p < 0.01$) were significantly lower after, compared to before the radiotherapy. Figure 1D shows that the saliva at the unstimulated state was supersaturated with respect to hydroxyapatite before and after radiotherapy. While sucking the candy all samples became under-saturated both before and after the radiotherapy. However, following radiotherapy saliva was significantly more under-saturated with respect to hydroxyapatite than before radiotherapy ($p < 0.001$– < 0.05). Following radiotherapy, saliva also failed to reach saturation, even 10 min after the acidic saliva stimulant was removed from the mouth, which was not the case before radiotherapy, where saliva reached saturation within 5 min after removal of the stimulant.

Figure 2 shows effects obtained on saliva flow and composition from calcium addition to acidic candies before and after radiotherapy. As shown, radiotherapy significantly decreased saliva flow rates and pH values. However, neither before nor after radiotherapy did calcium addition significantly change the saliva flow rate or pH values obtained in response to sucking the candies. Although more hydroxyapatite was lost after radiotherapy with both candies the effect of calcium addition was far more pronounced than the effect of radiotherapy. Thus, a calcium addition to the candies induced a major decrease in the loss of hydroxyapatite and this difference was present before as well as after radiotherapy. Before radiotherapy eight of nine patients experienced loss of hydroxyapatite with the control candy, whereas no dissolution occurred with the calcium modified candy. Following radiotherapy, all patients experienced loss of hydroxyapatite in the control candy, whereas only one patient experienced dissolution of hydroxyapatite with the modified candy.

Discussion

Eight of 10 patients had a history of smoking, indicating that this habit was closely related to pharyngeal cancer in the tonsil area within the study group. With surgery and radiotherapy as the two main treatment options [1], dry mouth, reduced saliva flow rates and abnormal saliva composition still remain a definitive consequence of this disease [2]. However, the effect of radiotherapy on saliva flow and composition, with special reference to dental erosion, has not been described in detail in longitudinal studies. Because cancer in general is considered a life-threatening disease it is likely that the diagnosis in itself may reduce the mental ability of many cancer patients to participate in longitudinal research projects such as this one. In the present study we were able to recruit 10 patients and perform a full pre- and post-treatment examination of saliva flow and composition in nine patients who all suffered from dry mouth after radiotherapy. In all patients the complaint of dry mouth following radiotherapy was accompanied by a reduction in the unstimulated saliva flow rate. Thus, from a total of nine patients, eight suffered from sub-normal unstimulated saliva flow (≤ 0.20 ml/min) and of these eight patients five could be diagnosed with hyposalivation (≤ 0.10 ml/min) after radiotherapy.

With respect to dental caries, even sub-normal saliva flow rates are often related to an accelerated caries activity [9]. With respect to dental erosion, hyposalivation has been related to an increased prevalence [7]. In the present study, the reduction in saliva flow rates after radiotherapy was accompanied by major changes in saliva composition. Thus, the partial pressure of carbon dioxide became nearly 3-times lower in the stimulated saliva after radiotherapy. Because the bicarbonate buffer system at low pH values exists mainly in the form of carbon dioxide, this change indicates that the bicarbonate concentration in the stimulated saliva was much lower after radiotherapy. A decreased saliva bicarbonate concentration is critical during intake of acidic foodstuffs, because bicarbonate is the most important buffer system in stimulated human whole saliva [14] where it also can act as a phase buffer [12] increasing its buffer capacity significantly [20]. The finding of low CO_2 values also in unstimulated samples, however, indicates that CO_2 equilibration between saliva and the airways could have been increased after radiotherapy. Thus, low flow rates resulting in a very thin film of whole saliva will increase CO_2 equilibration with the inspired and expired air. Therefore, the stimulated saliva CO_2 values before and after radiotherapy cannot be compared directly and may not be fully indicative of the bicarbonate concentration. Nonetheless, it seems reasonable to assume that the much lower saliva pH and saturation after radiotherapy was due to a combination of slow oral clearance of the acid in combination with low buffering capacity of the saliva. Thus, the lack of saturation with respect to HAp in saliva 10 min after the erosive challenge clearly emphasizes how the development of dental disease will be strongly accelerated after radiotherapy.

Thus, the changes obtained in saliva flow rate and composition due to radiotherapy show an increased risk of dental diseases such as dental caries and dental erosion for the radiated patients. Here, saliva stimulants may become helpful both to relieve symptoms of dry mouth and subsequently to obtain a more effective oral clearance. However, to be safe to use for irradiated dry mouth patients it is important that the acid content of the stimulants has no demineralizing effect on the teeth. It has previously been shown that selected commercially available acidic saliva stimulating tablets (Xerodent™) are a non-erosive alternative for dry mouth patients [4]. However, these tablets are less acidic than the present product and, therefore, stimulate less saliva secretion. It has also been shown that acidic saliva stimulants can be made non-erosive by modification with calcium in healthy and dry mouth patients [19,21]. The present study confirms that it is possible to modify acidic saliva stimulants by addition of calcium so that they have significantly reduced erosive potential even in cancer patients irradiated on head and neck area. It should, however, be noted that the sequence of sucking the candies was so that the modified candy was sucked after the control candy. This approach could have caused a higher bicarbonate concentration in the modified samples due to the effect of duration of stimulation on saliva bicarbonate secretion [22]. Nonetheless, the fixed sequence was preferred compared to the risk of active ingredients from the modified candy being transferred to the control collection. Thus, the limited flow of saliva and slow clearance rates in irradiated patients may give rise to very high concentrations of an active ingredient of a candy or lozenge carried in the oral environment. When the ingredient is tooth protecting like fluoride [4] and calcium [21] the effect on dental health is often very high compared to a control, as shown in the present study.

Apart from saliva flow, saliva inorganic composition and candy or lozenges carrying tooth protective ingredients, other substances in saliva also protect against acid erosion. Thus, a very interesting finding in the present study was that the modified candies were non-erosive with respect to dissolution of HAp crystals even in saliva samples that were under-saturated with respect to HAp. Before radiotherapy the saliva pH was lower than the corresponding calculated critical pH with respect to HAp in half of the samples but without dissolution of HAp. A similar trend was seen after radiotherapy when the saliva pH was lower than the calculated critical pH in all the samples, while dissolution of HAp crystals only took place in one of the saliva samples. The lack of dissolution, in spite of under-saturation, was most likely due to the presence of the saliva proteins and their pellicle-forming properties [19,23]. In support of this, the sample in which dissolution occurred was also the sample with the lowest protein concentration (data not shown). It is reasonable to assume that a longer observation time in the dissolution experiment would have resulted in some dissolution, since most samples were under-saturated. However, the present observation time of 5 min corresponded to the time the candy was present in the mouth. For similar cases of a limited exposure, this study shows that theoretical under-saturation does not necessarily indicate that something will happen, only that it can happen. Therefore, clinical testing, including HAp crystal dissolution in human saliva, is clearly recommended for obtaining a realistic evaluation of saliva stimulants.

In conclusion, saliva flow may be drastically reduced and the composition changed in patients who have undergone radiotherapy to the head and neck area. As a consequence this patient group can suffer from sub-normal flow rates or hyposalivation, which increases the risk of dental diseases. Saliva stimulants having significantly reduced erosive potential should be recommended by health professionals for dry mouth relief.

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Declaration of interest: The first author Thorbjorg Jensdottir is part time employed with Ice Medico Ltd, Reykjavik, Iceland, which produces non-erosive saliva stimulants based in part on the results presented in the present paper. The remaining authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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