Salivary and Dental Plaque Composition in Disabled Children Who Require Home Care: A Cross-sectional Investigation

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Aims: The purpose of this study was to compare salivary and dental plaque (DP) composition between disabled children who require home care (DCHC) and a control group (CG) and to correlate it with oral and systemic health factors. Materials and Methods: This cross-sectional study included 15 DCHC and 15 healthy children (aged between 4 and 10 years). The caregivers answered a questionnaire on disease diagnosis, medical history, dental history, and oral hygiene routine. In addition to clinical examination, saliva and DP samples were collected and analyzed using attenuated total reflection-Fourier transform infrared spectroscopy. Data were collected between January and December 2019. Student’s t and Kendall correlation tests were used. Results: Calculus (46.7%), bleeding on toothbrushing (53.3%), and gingival hyperplasia (40.0%) were prevalent in DCHC. The saliva of DCHC presented a higher amount of lipids and collagen and a lower amount of carbohydrates than that of the CG (P < 0.05). DP components were similar in DCHC and CG. Conclusion: DCHC presented oral comorbidities and changes in salivary composition, compared with the CG.

Keywords: Dental plaque, disabled children, home care services, oral hygiene, saliva

INTRODUCTION

It is estimated that 13 million children between 0 and 14 years of age have severe disabilities and require special health care.¹ Some of them are considered highly complex as they present a high degree of functional dependence, which makes caregivers responsible for feeding, mobility, medication administration, and especially the maintenance of body and oral hygiene.² Home care services are an alternative for monitoring and promoting health care, adapting to the needs of these patients and their family members.³ Oral health in home care aims to prevent and monitor oral problems by undertaking regular clinical examinations and offering anticipatory instructions and curative treatment when required to contribute to the establishment of general health and motivate caregivers regarding oral hygiene.²,³ Data on the oral health of disabled children who require home care (DCHC) are scarce, even with the confirmed impact of deficiency in oral health on systemic health.⁴ Molecular biology has indicated an association between pathogens in dental plaque (DP) and the incidence of pneumonia in hospitalized and immunocompromised individuals.⁵ Issues with oral health may cause systemic complications and have been associated with cardiovascular diseases, endocarditis, changes in glycemic levels, and respiratory infections. They may also aggravate general health conditions and increase comorbidities in patients.⁶ Oral health is obtained and maintained by factors that extrapolate the functional condition of teeth and...
stomatognathic structures, and the clinical status of oral health is related to the quality of life and levels of biological and psychosocial functions in children. Systemic diseases are common in DCHC and may present a similar frequency to oral problems; it can also induce changes in the microbiota, protein concentration, inorganic compound levels, and salivary flow. DP is known to play a direct role in the demineralization–remineralization process and the composition of oral microflora. Children with carious lesions have DP with lower concentrations of inorganic components. Moreover, the microorganisms composing DP have been shown to influence the occurrence of nosocomial pneumonia in lung disease patients, as they serve as a pathogen reservoir that may colonize the respiratory system. DP accumulation and an increased number of microorganisms in saliva are associated with a low salivary secretion rate. Saliva is used for disease diagnosis, prognosis, and follow-up. Tests using this type of fluid are considered non-invasive, practical, and low-cost and are used for the diagnosis of systemic syndromes and disorders. Moreover, salivary biomarkers have been studied for the diagnosis and prognosis of oral diseases such as periodontitis. DCHC may present specific salivary and DP changes when compared with a control group (CG) of patients without complex medical issues, and such changes may be correlated to the oral and systemic conditions of these patients. In this context, this study hypothesizes that DCHC present high indexes of oral problems and molecular changes in saliva and DP when compared with a CG and that there is a correlation between the variables related to oral health and those related to salivary and plaque composition. Therefore, this study aimed to assess the oral health of DCHC, compare salivary and DP composition between DCHC and a CG, and correlate oral and general health factors of DCHC with salivary and DP composition.

**Materials and Methods**

**Study design and ethical aspects**

This was a cross-sectional observational study involving DCHC (n = 15) and children (n = 15) in the CG. Data on salivary and DP composition were collected from the subjects to compare between groups and to establish a correlation between salivary and plaque composition and oral and general health status. The project was approved by the Human Research Ethics Committee of the Federal University of Uberlândia (Approval protocol: 63903416.0.0000.5152). The data were collected only after obtaining informed consent from the children’s caregivers. The present study is in accordance with the STROBE checklist of items that should be included in reports of cross-sectional studies.

**Study participants**

The study included children in high medical complexity care, presenting with medically diagnosed issues with the nervous system and requiring invasive mechanical ventilation, feeding by gastrostomy, and regular assistance by a multi-professional team of the public home care service. These children were aged between 4 and 10 years (n = 15). The public home care service consists of multidisciplinary teams (physicians, nurses, nutritionists, physiotherapists, physical education professionals, social workers, nursing technicians, and administrative assistants) who care for hospitalized patients. The teams were complemented by dental professionals through an extension project linked to the Federal University of Uberlândia. Additionally, patients (n = 15) who attended the Pediatric Dentistry Prevention Clinic of the School of Dentistry of the Federal University of Uberlândia, MG, Brazil were included in this study. These patients had an average age and sex ratio similar to those of the DCHC, did not use medications regularly, were not diagnosed with general or oral health morbidities, and were free from medical and dental issues (dental caries, visible dental biofilm, calculus, gingival hyperplasia, and periodontal disease). Data were collected between January and December 2019.

The sample size used in the present analysis agrees with that used in previous studies focussing on oral diseases that assessed the salivary samples of non-disabled patients or those with medical complexity. For the sample size calculation, utilizing a confidence interval of 95%, power of 80%, and a 1:1 ratio group, the sample should consist of 12 individuals in each group. Because there was an intention to evaluate multiple salivary and plaque components, 20% of the sample size was added, resulting in 15 individuals per group. The data considered for calculation were the mean and standard deviation of the salivary protein concentration evaluated by Bezerra Júnior et al.

**Saliva and dental plaque collection**

Patients provided unstimulated saliva, which was collected between 2 and 5 p.m. to avoid the influence of circadian rhythm. Moreover, the collection occurred at least 2 h after the last meal or oral hygiene practices. Saliva was collected using an individual sterile rectal probe on the floor of the tongue in the oral cavity. The probe was coupled to an electric and portable low-power secretion aspirator (Aspiramax MA 520, NS,
São Paulo, Brazil), so that one end was in contact with the oral cavity of the patient and the other end was inside a 15-mL Falcon tube to prevent contamination of the collected sample. During saliva collection and transport, the 15-mL Falcon tubes were cooled. The collection occurred until 2 mL of fluid was obtained without surpassing 15 min of collection time per patient. The samples were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was stored in a freezer at −80°C until required for processing.

DP was collected from the buccal surfaces of permanent first molars and deciduous second molars using a dental explorer, and the collected material was transferred to Eppendorf tubes with deionized water and stored in a freezer at −80°C until required for processing.

To analyze saliva and DP, attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy was applied to regions with an infrared spectrum between 4000 and 400 cm⁻¹. ATR-FTIR allowed the identification of C-H, O-H, and N-H protein functional groups with high accuracy, lipid composition, and glycidic compounds. A portion of 2 μL of saliva or DP was applied to the ATR-FTIR crystal and dried for 2 min and 30 s, after which the sample spectra were recorded. All analyses were performed in triplicate. Thirty-two scans were performed to obtain the profile of each sample with a resolution of 4 cm⁻¹.

**Medical and Dental History and Clinical Oral Examination**

A single researcher collected data regarding medical history, dental history, and oral hygiene routine through the report of caregivers. Data on the following variables were collected regarding medical history: dental history, frequency of oral hygiene, method used for oral hygiene, type of dentifrice used, and frequency of dental floss use were assessed.

Oral hygiene was assessed using the Simplified Oral Hygiene Index (OHI-S). The OHI-S scores, with mean results ranging from 0 to 3, were categorized. Means between 0 and 1.5 indicated good oral hygiene, those between 1.6 and 2.5 indicated regular oral hygiene, and those >2.6 indicated poor oral hygiene. Such assessments occurred before the dental professional (Surgeon Dentist) performed oral hygiene in the children.

After obtaining the OHI-S, the dental professional performed oral hygiene with a manual toothbrush, fluoridated dentifrice, and gauze. Other clinical assessments involved the variables of decayed, missing, and filled teeth index (DMFT) for permanent dentition, and for the deciduous dentition (dmft), the presence or absence of calculus, gingival hyperplasia, intrinsic stains, gingival bleeding during toothbrushing, mucosal changes, and changes in the dental morphology were assessed. An oral mirror #5 (Golgran, São Caetano do Sul, SP, Brazil) and a World Health Organization (WHO) probe (Golgran) were used, both previously sterilized. It is important to highlight that, for all patients, the invasive mechanical ventilation was via tracheostomy tube, allowing access to the oral cavity. Also, all dental procedures were performed at home, with the patient in a lying down position, using regular instrumentals for operative dentistry, surgery, or periodontics.

Two examiners performed the OHI-S and DMFT/dmft indexes and assessed the other clinical oral characteristics at home, aided by ambient light and a flashlight (kappa intra- and inter-examiner >0.85). All participants and caregivers were instructed on oral hygiene care according to the age group, and the oral health problems mentioned were treated in the extension project in home pediatric dentistry. It is important to highlight that in the CG, all children presented good oral hygiene according to the OHI-S, absence of caries, a minimum of two manual toothbrushes a day, and used fluoridated dentifrice.

**Statistical Analysis**

The data were computed in spreadsheets, and levels were attributed to scores for all variables studied to allow a quantitative analysis using the statistical software SPSS, version 22.0. After verifying normal data distribution, Student’s t-test was used to compare salivary and DP composition between the CG and DCHC, and the Kendall correlation test was used to verify potential associations between the variables of salivary and DP composition with oral health and medications in DCHC. The spectra obtained in the analysis of saliva and DP in FTIR were normalized using the vector method and, later, the baseline was corrected. The mean profile and band areas were obtained with the Opus 6.5 software (Bruker Optics, Reinstetten, Germany), and the original data were plotted using Origin Pro 9.0 software (OriginLab, Northampton, MA, USA) for a detailed analysis.

**Results**

Regarding age, eight (53.3%) children were in the age group of 4–6 years and seven (46.7%) were in the age group of 7–10 years, with a prevalence of males (n = 10; 66.7%) in both groups (control or DCHC). In DCHC, the most frequent medication in use was antiepileptics (n = 10; 66.7%). Table 1 presents the type of medication used, oral hygiene habits, and oral health conditions in DCHC.

Regarding the oral hygiene habits of DCHC, it was observed that most children performed oral hygiene
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once a day or more (n = 10; 66.7%) and used a manual toothbrush (n = 12; 80.0%). Only 6.7% (n = 1) of the children in high complexity care had used dental floss, and 12 (80.0%) children used fluoridated dentifrice with more than 1000 ppm of fluoride. A large proportion of the children (n = 11; 73.4%) had never undergone dental follow-up.

The OHI-S classification showed that nine children (60.0%) presented with regular or poor oral hygiene. As for oral disease, caries was found to be infrequent (dmft 0.08 ± 0.10 and DMFT 0.28 ± 0.08). Regarding oral clinical findings, calculus (n = 7, 46.7%), bleeding on toothbrushing (n = 8, 53.3%), and gingival hyperplasia (n = 6, 40.0%) were the most prevalent findings. Table 2 shows the molecular component values for each representative vibrational mode assessed using ATR-FTIR in saliva and DP samples. Figures 1 and 2 show the salivary and DP infrared spectra (CG and DCHC), respectively.

Regarding salivary composition, it was shown that patients in high complexity care presented a higher level of lipids and collagen and a lower amount of carbohydrates compared with CG (P < 0.05). The other salivary molecular components detected did not show a statistically significant difference between both the groups (P > 0.05). No significant statistical differences were observed in DP composition between DCHC and CG (P > 0.05) either. Table 3 shows the correlation between the variables. Collagen in saliva showed a weak positive correlation with the use of antiepileptics (P < 0.05). The other independent variables, salivary and DP components, did not present significant correlations.

**Table 1: Absolute and relative frequencies of the variables related to general characteristics in DCHC, oral hygiene habits, and oral changes found (n = 15)**

| Total | N  | %   |
|-------|----|-----|
| Type of medication |    |     |
| Antiepileptic | 10 | 66.7|
| Vitamin | 8  | 53.3|
| Laxative | 4  | 26.7|
| Anti-ulcerative | 7  | 46.7|
| Bronchodilator | 3  | 20.0|
| Anxiolytic | 3  | 20.0|
| Antihypertensive | 2  | 13.3|
| Muscle relaxant | 3  | 20.0|
| Antibiotic | 2  | 13.3|
| Anti-inflammatory | 1  | 6.7 |
| Toothbrushing frequency |    |     |
| Never | 1  | 6.7 |
| Less than one toothbrushing per day | 2  | 13.3|
| One toothbrushing per day | 3  | 20.0|
| More than one toothbrushing per day | 7  | 46.7|
| Toothbrushing methods |    |     |
| Manual toothbrush | 12 | 80.0|
| Gauze | 2  | 13.3|
| Finger brush | 1  | 6.7 |
| Dental floss | 1  | 6.7 |
| Use of dentifrice |    |     |
| Adult fluoridated toothpaste | 12 | 80.0|
| Low fluoride concentration | 1  | 6.7 |
| No dentifrice | 2  | 13.3|
| Dental follow-up |    |     |
| Never | 11 | 73.4|
| Once a year | 4  | 26.6|
| Once a week | 0  | 0.0 |
| Oral hygiene classification (OHI-S) |    |     |
| Good | 6  | 40.0|
| Regular | 5  | 33.4|
| Poor | 4  | 26.7|
| Oral clinical findings |    |     |
| Calculus | 7  | 46.7|
| Bleeding on toothbrushing | 8  | 53.3|
| Gingival hyperplasia | 6  | 40.0|
| Mucosal change | 1  | 6.7 |
| Dental morphology change | 1  | 6.7 |
| Intrinsic stains | 2  | 13.3|

**Discussion**

The analysis of salivary and DP components becomes important when there is a need to establish relationships between the general and oral health status of individuals.[12,16] Saliva can reflect systemic metabolite concentrations and is used for diagnosing several diseases.[12] DP defines the oral microbiota and works in the demineralization–remineralization process of dental structures.[8,17] Although the salivary and DP components did not correlate with the presence of oral and medical variables, which could be related to a wide range of systemic and oral health problems and disabilities, we showed here that DCHC exhibited higher levels of lipids and collagen and a lower level of carbohydrates than children in the CG; however, the DP composition was similar. Interestingly, these DCHC presented higher levels of oral changes such as calculus, bleeding on toothbrushing, and gingival hyperplasia associated with a lack of regular dental follow-up.

The authors used the health patients’ group to provide a baseline of saliva and DP components and to verify which components have been varying according to the baseline found for the same age. Indeed, it is important to point out that our hypothesis suggested substantial differences in molecular composition of saliva and
Table 2: Salivary and bacterial plaque composition of children with medical complexity who require in-home medical care and children in the control group assessed by ATR-FTIR

| Vibrational mode (cm⁻¹) | Molecular group          | CG (n=15) area (a.u.) | DCHC (n=15) area (a.u.) | P-value      |
|------------------------|--------------------------|-----------------------|-------------------------|--------------|
| Saliva                 |                          |                       |                         |              |
| 2986                   | Lipids                   | 0.00 (0.000)*         | 0.12 (0.001)            | <0.0001*     |
| 2960                   | Lipids                   | 0.06 (0.02)           | 0.17 (0.02)             | 0.0320*      |
| 2919                   | Lipids                   | 0.07 (0.07)           | 0.15 (0.11)             | 0.0027*      |
| 1641                   | Proteins (Amide I)       | 6.03 (0.76)           | 5.58 (0.77)             | 0.0769       |
| 1551                   | Proteins (Amide II)      | 1.39 (0.86)           | 1.82 (0.64)             | 0.0686       |
| 1454                   | Collagen                 | 0.26 (0.07)           | 0.23 (0.08)             | 0.2125       |
| 1401                   | Collagen                 | 0.43 (0.17)           | 0.31 (0.12)             | 0.0144*      |
| 1312                   | Proteins (Amide III)     | 0.01 (0.03)           | 0.01 (0.02)             | 0.1197       |
| 1242                   | Collagen                 | 0.25 (0.05)           | 0.10 (0.06)             | 0.0258*      |
| 1076                   | Carbohydrates            | 3.52 (0.54)           | 2.74 (0.73)             | 0.0510       |
| 986                    | Carbohydrates            | 0.15 (0.09)           | 0.04 (0.06)             | 0.0001*      |

Bacterial plaque

|                |                          |                       |                         |              |
|----------------|--------------------------|-----------------------|-------------------------|--------------|
| 2990          | Lipids                   | 0.04 (0.02)           | 0.03 (0.03)             | 0.422        |
| 2946          | Lipids                   | 0.29 (0.12)           | 0.23 (0.15)             | 0.327        |
| 2864          | Lipids                   | 0.05 (0.02)           | 0.06 (0.03)             | 0.424        |
| 1764          | Proteins                | 0.02 (0.02)           | 0.01 (0.01)             | 0.283        |
| 1723          | Proteins                | 2.72 (0.24)           | 2.76 (0.88)             | 0.902        |
| 1586          | Proteins (Amide II)     | 1.60 (0.56)           | 1.49 (0.62)             | 0.649        |
| 1479          | Collagen                | 0.17 (0.08)           | 0.13 (0.05)             | 0.167        |
| 1426          | Collagen                | 0.26 (0.10)           | 0.20 (0.10)             | 0.212        |
| 1335          | Proteins (Amide III)    | 0.03 (0.01)           | 0.02 (0.01)             | 0.522        |
| 1272          | Collagen                | 0.35 (0.10)           | 0.27 (0.15)             | 0.183        |
| 1182          | Carbohydrates           | 0.04 (0.02)           | 0.03 (0.03)             | 0.406        |

CG = control group, DCHC = disabled children who require home care
*Values represent the mean (standard deviation) of each group
*Statistically significant differences between groups, Student’s t-test, P <0.05.

Figure 1: Wavelength absorption range (a.u.) obtained by attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy, which characterizes each molecular group found in the saliva of the control group for healthy children (CG) and disabled children who require home care (DCHC). (2986) (2960) (2919) lipids, (2325) CO₂, (1641) proteins—amide I, (1551) proteins—amide II, (1454) (1401) collagen, (1312) proteins—amide III, (1242) collagen, (1076) (986) carbohydrates
However, DP components were similar in DCHC and CG. In DP perspective, the disability, lifestyle, medication, and personal habits were not sufficient to change this parameter. As expected, DCHC presented oral comorbidities and changes in salivary composition, compared with healthy children. The data obtained are important to guide future studies focusing on a more specific dental care and dental education for disabled patients. With this background related to salivary chances, novel studies with applications of salivary parameters for personalized dentistry can be customized for different clinical applications in DCHC population. A sentence has been added to the discussion section.

The low caries experience in DCHC was expected because these children were fed by gastrostomy tubes, and this may have been reflected in the reduction of salivary carbohydrates in these children [18]. DCHC also presented a high prevalence of gingival bleeding and hyperplasia, and the increase in lipids observed in the saliva samples of DCHC may be associated with periodontal disease. In the literature, lipids are linked to oxidative stress and inflammatory mediation [19], which reinforce their potential relationship with the development of pathologies in the periodontal region. Regarding DP composition, there were no changes associated with periodontal problems, but the high levels of supragingival plaque and calculus are related.
to progressive periodontal disease.\textsuperscript{[20]} In the DCHC group, a significant portion of the sample presented calculus and OHI-S, indicating moderate or poor oral hygiene.

The high prevalence of calculus and gingivitis (bleeding on toothbrushing) observed in DCHC is concerning because this change is associated with chronic obstructive pulmonary disease.\textsuperscript{[21]} Moreover, the periodontal problem and DP in DCHC may resonate systemically, increasing the risk of aspiration pneumonia, especially in children with enteral nutrition and respiratory problems.\textsuperscript{[22,23]} The control of supragingival and subgingival plaque in this group forms the basis for the treatment of periodontal disease and may contribute to reducing systemic comorbidities.

Regarding gender, there is a high association between males and the risk of major morbidities in the first years of life, especially in low-weight premature babies.\textsuperscript{[24]} Changes in the nervous system linked to chromosome X contribute to a higher prevalence of brain disorders in males as recessive gender-linked inheritance predominantly affects males.\textsuperscript{[25]} These factors could explain the higher rate of male DCHC observed in this study. This study of DCHC diagnosed with issues with the nervous system exposes the challenging work of dentists, who need to educate and care for children presenting with nerve impairment. In addition, respiratory support and gastrostomy use may compromise the self-care of these children, making caregivers determinant factors for achieving good oral health.\textsuperscript{[26]} Dental professionals must make an effort to educate the family, especially caregivers, regarding habits that interfere with the general and oral health of the child.

Considering the complexity of the systemic condition and daily demands of patients in home care, caregivers often find it difficult to perform tasks such as oral hygiene and diet control.\textsuperscript{[27]} Education about oral hygiene is a preventive tool in public health and, when instructed and followed up by an oral health professional, prevents the appearance of diseases, painful processes, and tooth loss.\textsuperscript{[28]} Therefore, it is the responsibility of oral health professionals to motivate caregivers and educate them on the importance of oral health and how it may interfere with systemic health. Therefore, the presence of a dental professional in the multidisciplinary care team of public home care services is clearly important and necessary.

Patients in the present study also presented a high frequency of use of antiepileptics. The use of antiepileptics is associated with excessive gingival growth, linked to the abnormal activity of fibroblasts and consequently the excessive production of collagen in tissue. However, the literature does not report the effect of antiepileptics on salivary collagen concentration.\textsuperscript{[29]} This study demonstrated a reduction in salivary collagen concentration in DCHC using antiepileptics.

Dental home care services are available and find purpose in the context of family care; however, coverage is still limited.\textsuperscript{[30]} Promoting dental home care services for DCHC requires the use of strategies that provide a trust bond and mutual responsibilities among dentists, patients, and their family members, thus obtaining cooperation in the improvement of oral health. Dental home care services for DCHC must be included in the multi-professional team, which should establish effective and close communication, produce knowledge, and maximize the success of patient and family care.\textsuperscript{[30]} Moreover, this type of assistance reverses the access route to oral health, in which the professional is active and goes to the patient, having the opportunity to promote health education and establish preventive actions in the family environment.\textsuperscript{[31]}

**Conclusion**

This study shows the following:

1. Children with medical complexity in home medical care presented higher levels of oral changes such as calculus, bleeding on toothbrushing, and gingival hyperplasia;
2. These children with medical complexity exhibited higher levels of lipids and collagen and a lower level of carbohydrates compared with children in the CG, even though the DP composition was similar; and
3. Salivary collagen was correlated with the use of antiepileptic drugs.

**Future scope/clinical significance**

Despite the limitations of the cross-sectional study, important data were obtained: the DCHC presented specific salivary changes when compared with a CG of healthy patients, and such changes may be correlated to the oral and systemic conditions of these patients. The present study considered a population group with a clinical vulnerability and that often lacks dental assistance, and the information presented may be used for future planning and oral health approach. Future prospective studies evaluating this vulnerable population are necessary to contribute with the
improvement of the oral and general health and, consequently, the quality of life of these patients.

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CONFLICTS OF INTEREST
There are no conflicts of interest.

AUTHORS’ CONTRIBUTIONS
Development of protocol: KF, GTC, RSS, APT, data collection: KF, GTC, APT, methodology: KF, GTC, LCS, WHTS, AMH, RSS, APT, analysis and interpretation of data: KF, GTC, LCS, AMH, RSS, APT, manuscript: KF, GTC, APT, final approval of manuscript: KF, GTC, LCS, WHTS, AMH, RSS, APT, project administration: APT.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT
This study followed the STROBE Statement (Checklist for reporting cross-sectional studies) guidelines and was based on the fundamentals of ethical research practice. Informed consent was obtained from all patients’ legal guardians to include Disabled Children Who Require Home Care in the experiments.

PATIENT DECLARATION OF CONSENT
Not applicable.

DATA AVAILABILITY STATEMENT
Data availability statement is available on request from Prof. Dra. Ana P. Turroni (apturrioni@ufu.br).

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