Leaching of organic additives from dentures in vivo

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Samples of saliva were collected from subjects with dentures. These samples were collected both before the dentures were replaced and 1 week after the subjects had received their new dentures. Dibutylphthalate and phenyl benzoate were detected in the saliva samples with a gas-chromatography and a gas-chromatography/mass-spectrometry technique. We also quantified the dibutylphthalate in the saliva. In addition, in an in vitro study, we identified biphenyl leached from heat-cured denture base polymer plates. Our study suggests that subjects with dentures have higher contents of the above organic substances in saliva than subjects without dentures and that organic additives leach from new heat-cured dentures. □ *Chemical analysis; gas chromatography/mass spectrometry; saliva*

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Denture base polymers contain many components besides the basic polymer. These additives may leach and cause adverse reactions in patients, reactions that may be toxic or allergenic, while some remain unexplained (1).

Diffusion and leaching characteristics of methyl methacrylate monomer (MMA) from polymethylmethacrylate (PMMA) acrylic resins have been studied in vitro (2–4). Methacrylic acid and benzoic acid leached from denture base acrylic resins have also been identified (4). However, little is known about the leaching behavior of resins under in vivo conditions, although a gas-liquid chromatography assay for the estimation of MMA from autopolymerized denture base materials in whole saliva has been published (5).

Phthalate esters are used extensively as plasticizers in various dental and biomedical devices (6). In denture base materials the plasticizer can increase the solubility of the polymer beads mixed in the monomer and decrease the brittleness of the polymer by lowering its glass transition temperature (7). The plasticizer content is particularly high in prosthodontic soft lining materials, materials most often used for limited periods of time (8).

Some denture base materials for permanent use contain plasticizers (9), but the leachability of plasticizers from such materials is unknown.

The aim of the present study was to quantify plasticizers (phthalate esters) present in saliva collected in vivo and by use of gas chromatography and gas chromatography/mass spectrometry also to identify other organic additives leached from dentures.

Materials and methods

The denture-wearing subjects included in this study were to receive new dentures at the Department of Prosthodontics at the School of Dentistry, University of Bergen, Norway. Demographic, special, and general anamnestic information was collected for each subject. Eleven subjects, seven women and four men with a mean age of 67.8 years, participated in the study (Table 1). Nine of these subjects had maxillary and mandibular complete dentures, one a complete maxillary denture, and one a removable partial maxillary denture (Table 1). New dentures were made using a heat-cured denture base material (Vertex 5 RS, batch 9004033 (powder) and batch 8910305 (liquid); Dentimex, Zeist,

Table 1. Data from questionnaires and gas-chromatographic and gas-chromatographic/mass-spectrometric studies

	Subject no.										
	1	2	3	4	5	6	7	8	9	10	11
Age (years)/sex (x = 67.8 years)	65/F	65/F	72/M	70/M	60/F	55/M	60/F	70/F	80/M	75/F	74/F
Types of dentures*	CMx/ CMd	CMx/	CMx/ CMd	CMx/ CMd	CMx/ CMd	CMx/ CMd	CMx/ CMd	RMx/-	CMx/ CMd	CMx/ CMd	CMx/ CMd
Use of dentures†											
Max. dent.	D/N	D/N	D	D/N	D/N	D/N	D/N	D	D	D/N	D/N
Mand. dent.	D/N	/N	D	D/N	D/N	D/N	D/N	—	D	D/N	D
Age of dentures (years)											
Max. dent.	20	14	40	20	30	19	10	15	30	20	20
Mand. dent.	20	—	40	1	25	19	10	—	30	20	20
Mucosal condition‡	3	2	2	2	3	2	2	1	2	2	—
Area of dentures											
Max. dent.	Large	Large	Medium	Medium	Medium	Large	Large	Small	Large	Medium	—
Mand. dent.	Medium	—	Medium	Medium	Medium	Small	Large	—	Small	Small	—
No. of peaks in chromatograms											
Old dentures	17	30	18	16	33	22	17	17	29	44	39
New dentures	29	30	29	17	31	20	63	23	23	34	57
DBP (μg/ml) in new dentures	1.5	0.1	0	0	7.5	0	0.1	0.1	0	0.1	3.0

* CMx = complete maxillary denture; CMd = complete mandibular denture; RMx = removable partial maxillary denture.
† D/N = day and night; D = day; N = night.
‡ Criteria in accordance with Lindquist et al., 1975 (26).

The Netherlands). The packed flasks were polymerized in boiling water. The materials used in the old dentures could not be established in any of the cases, only that they were of the methacrylate type, presumably of heat-cured type. To identify the substances found in the saliva samples, 26 × 26 × 3-mm reference plates were made by the same dental technician who made the dentures and who used the same materials and procedures. These plates were placed in separate glass vessels containing redistilled ethanol (80 ml), covered by aluminum foil, and subjected to agitation (KS 10, Edmund Bühler, Tübingen, Germany), for 20 h at 37 °C. To obtain saliva from subjects without dentures, saliva were collected from 11 dental students (mean age, 22.9 years).

Saliva sampling

We collected unstimulated saliva from each subject for 5 min, during which time the subject kept her/his mouth closed. Saliva samples were collected while the subjects still used their old dentures (sample A) and also after they had used their new dentures for more than 1 week (sample B). Both samples were obtained immediately after the dentures had been removed from the mouth. The saliva samples were collected in glass beakers covered with an aluminum foil and kept at −20 °C until they were analyzed. The beakers had been rinsed in glass-distilled ethylacetate (868, Merck, E. Merck, Darmstadt, Germany) and were heated to 100 °C for at least 12 h before they were used.

Processing of the saliva samples

One milliliter saliva was extracted three times in 15-ml tubes by mixing with 1 ml distilled ethylacetate (10), containing 2 μg/ml diethylphthalate (822323, Merck-Schuchardt, Schuchardt, Hohenbrunn bei München, Germany) as an internal standard. These extracts were then transferred to screw-capped glass vials (2783/2 Heigar & Co. A/S, Oslo, Norway) and evaporated to 100–200 μl at 60 °C. After being cooled the evaporated samples were transferred to sample vials (02-MTV, Vials PK A 100, Cromacol Limited, London, UK) with inner vials (2-CV, Vials PK A 100, Cromacol Limited). A 3-mm hole was punched in the rubber septum (11/A C4, Crimpcaps PK A 100, Cromacol Limited) and covered with an aluminum foil to avoid contamination of the analyzing equipment by the needle/septum system (Fig. 1). A recovery test was performed both on saliva and on Ringer solution with known added amounts of dibutylphthalate (DBP) and subjected to two extraction sequences. DBP was not detected from the second extraction sequence.

Analytical procedure

A gas chromatograph (GC) (Perkin Elmer Autosystem Gas Chromatograph, Perkin-Elmer Corp., Norwalk, Conn., USA) was used. The instrument was equipped with a flame ionization detector and a fused silica column 25 m long and 0.32 mm in diameter coated with a 0.52-μm-thick film (Hewlett-Packard Ultra 2 WCOT, Hewlett-Packard Co., Avondale, Pa., USA). Peak areas and retention times were recorded with an integrator (Perkin-Elmer, Model 1020 Personal Integrator).

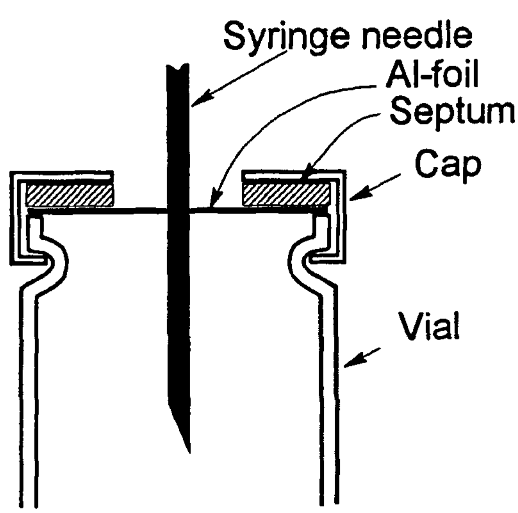

Fig. 1. Arrangement of sample vial during gas-chromatographic analyses to avoid contamination from the needle/septum system.

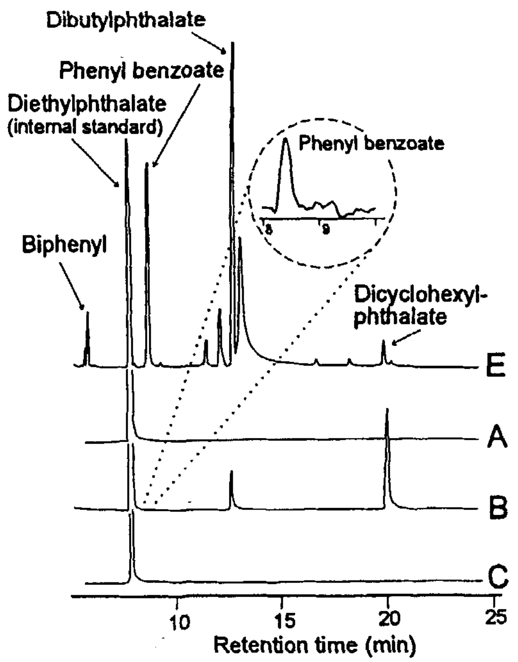

Fig. 2. Gas-chromatographic/mass-spectrometric (GC/MS) chromatograms from saliva in subjects with old dentures (A) and new dentures (B) and from the control group (C). In addition, a GC/MS chromatogram from the in vitro ethanol sample from reference plate of Vertex 5 RS (E).

A gas-chromatography/mass-spectrometry (GC/MS) system (HP 5970 MSD) with an autosampler (HP 7673) was used to identify the compounds separated by the GC. The fused silica columns we used were the same as those described for gas chromatography. The quantitative detection limit of DBP was established by different solutions of DBP (minimum, 0.01 μg/ml) in ethylacetate and found to be 0.1 μg/ml. The median absolute difference between the duplicate analyses was 0.004 μg/ml.

Presentation of results and statistics

The concentrations of DBP and dicyclohexylphthalate (DCHP) were calculated as μg/ml saliva, on the basis of the mean of duplicate analyses of each sample, and related to a standard curve of the internal standard. The other substances were qualitatively analyzed.

The number of peaks in the chromatograms was counted by the chromatography software, indicating the number of organic substances in the samples that could be separated by GC. The denture area was classified as small, medium, or large (11). Mann–Whitney two-sample test and Wilcoxon test were used to test for statistical significance. A significance level of $p < 0.05$ was chosen.

Results

The individual characteristics of the 11 denture-wearing subjects are presented in Table

Table 2. Summary of data from gas-chromatographic and gas-chromatographic/mass-spectrometric studies

	With old dentures (A samples)	With new dentures (B samples)	Control group (without dentures)
Dibutylphthalate (μg/ml), median (range)	0*	0.1 (0–7.5)	0*
Dicyclohexylphthalate (μg/ml), median (range)	0.1 (0–0.5)	0.1 (0.1–23.4)	0*
Phenyl benzoate	ND†	Detected in two subjects	ND†
No. of peaks in chromatograms (retention time, 5 to 35 min), median (range)	22 (15–46)	29 (11–63)	15 (8–31)

* All values below quantitative detection limit.
† ND = not detected.

1. DBP and phenyl benzoate (PB) were consistently found in the saliva of denture-wearing subjects with new dentures (Fig. 2). Neither of these substances was found in the control group or in subjects with old dentures. The concentration of DBP among subjects with new dentures (B samples) was significantly higher than that of subjects with old ones (A samples) ($p = 0.022$). The difference between these two groups with regard to DCHP was not statistically significant ($p = 0.760$). Phenyl benzoate was detected in two subjects with new dentures (Subject 7 and Subject 11) but not in the group with old dentures or in the control group (Table 2). BP was detected in samples from Vertex 5 RS after leaching in ethanol (Fig. 2). The number of peaks recorded from the chromatograms was highest with the new dentures and was significantly different from the control group ($p = 0.005$) but not from the group of subjects with old dentures ($p = 0.221$).

Discussion

Heat-cured denture base materials consist of prepolymerized poly(methyl methacrylate) beads (PMMA), a monomer system comprising one or more oligo- or poly-functional methacrylate or acrylate monomers, an initiator system such as benzoyl peroxide (BPO), and additives such as plasticizers (phthalates), stabilizers, and antioxidants (12). After mixing and doughing, heat-cured materials are polymerized by the free radical-initiated mechanism (12).

Much emphasis has been placed on the detection and analysis of leached MMA, whereas little is known about leaching of the additives. Previous studies using high-performance liquid chromatography (HPLC) have shown that in addition to MMA, methacrylic acid (MA) and benzoic acid (BA) from cold-cured resins, but not from heat-cured resins, can be quantified in artificial saliva (4). Results obtained with saliva from subjects with cold-cured resin devices in vivo demonstrated levels of MMA ranging from 1 to 45 μg/ml by GC (5). Dental soft liners release considerable levels of plasticizers, both under in vivo and in vitro conditions (8, 13). These materials are, however, considered temporary.

The technique described allowed quantification in saliva of DBP and DCHP and qualitative detection of PB and DP. Reagents and equipment used had to be monitored for contamination, and special precautions were taken to avoid contamination during injection into the GC instruments. The quantitative detection limit of DBP was set to 0.1 μg/ml in saliva. Peaks could be identified below this limit, but on the basis of the variation between analyses at low levels, we chose not to quantify low-level values.

The higher levels of DBP found in saliva obtained from subjects with new dentures were the most consistent finding in this study. Subjects with new dentures also had more peaks in the chromatograms of saliva than those with old dentures, but this difference was not significant. Although the peak intensities were low and could not be identified, it is likely that these peaks represent substances associated with the dentures. The finding that DCHP did not differ among the three groups suggests that this substance constitutes a general pollutant in man. For example, DCHP is commonly used as a plasticizer in food-packaging products (14). The large variation between individuals is consistent with saliva analysis of metal ions leached from partial dentures (11) and leached diethylhexylphthalate in blood from patients receiving hemodialysis (15).

The present analyses of saliva by GC and GC/MS indicate that DBP and PB leach from new dentures made from heat-cured acrylic resin. In contrast to another study, the salivary content of DBP from heat-processed dentures was quantified (4). The substances analyzed most likely originate from the dentures, as supported by the results from the ethanol extraction of acrylic specimens (Fig. 2). Detected DCHP may be a result of deliberate addition or it may be a pollutant in the production process. The PB is probably a degradation product from the initiator, benzoylperoxide, as suggested by Rathbun et al. (16).

Leaching is diffusion of small molecules from the bulk and the surface of a material, from which they may enter the physiologic milieu (17). When the solubility of the additive in the solvent phase is very low, as is the case with DBP, leaching occurs at a constant rate (18). Saliva represents a dynamic fluid environment in subjects with dentures, and the transfer rate can be assumed to be proportional to the surface concentration and surface area (17). It has been suggested that small amounts of MMA, below the detection limit of the GC, would continue to leach for 30 years (2). Dentures are intended for permanent use, and the potential biologic effects of substances released should be considered. Substances leaching from dentures into saliva are conveyed to the oral mucosa and/or to the gastrointestinal tract. Phthalates are metabolized by partial hydrolysis initially (19), and in vitro studies have shown that they affect the rat liver by changing the permeability of the mitochondrial membranes (20). Harsanyi et al. (21) have reported that polymer discs containing DBP resulted in epithelial changes in the hamster cheek pouch model. Calculations based on the flow of resting saliva in edentulous patients (22) indicate that Subject 5, who had the highest value of DBP, received a daily dose in saliva of about 3 mg DBP. On the basis of information from the questionnaire, this subject had a history of previous use of dental adhesives. However, the daily dose of DBP in Subject 5 is 20 times lower than the Acceptable Daily Intake value (23).

Less information is available for PB. The substance has been detected as one of the cytotoxic factors in a commercial BIS-GMA composite resin (16). Biologic activity (mutagenicity) has been shown in vitro with biphenyl (24), but the effects of low levels remain unknown. On the basis of these findings chemotoxicity cannot be ruled out as an etiologic factor in denture stomatitis (25).

In conclusion, this study has demonstrated that additives to denture base polymers are leached to saliva from new heat-processed dentures under normal clinical conditions. It is possible to quantify the salivary content of DBP in subjects wearing new heat-processed dentures with a GC and GC/MS method.

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