Journal of Chromatography, 165 (1979) 103–116
Chromatographic Reviews

CHREV. 115

APPLICATIONS OF GAS CHROMATOGRAPHY IN DENTAL RESEARCH

MAURICE VINCENT STACK
Medical Research Council Dental Unit, University of Bristol Dental School, Bristol BS1 2LY (Great Britain)

(Received October 5th, 1978)

CONTENTS

1. INTRODUCTION

The overall applications of gas chromatography to dental research extend to the study of metabolic products of microorganisms commonly encountered in the oral cavity, to the monitoring of numerous drugs in the saliva, and to the analysis of dental materials, as well as to the most direct applications to the composition of teeth, soft tissues and saliva. This review will be concerned with the direct applications, but the subject of volatile sulphur compounds in the oral cavity, although a feature of bacterial metabolism, has been included since it features so strongly in the literature. About half the published papers relate to the use of gas chromatography in the analysis of volatile fatty acids and those derived from lipid components: half the remainder are accounted for by publications on volatile sulphur compounds, and the residual contributions cover amino acids, carbohydrates and miscellaneous applications.

2. MONOCARBOXYLIC ACIDS

The use of gas chromatography (GC) in dental research has been most widespread in studying monocarboxylic acids, and for the purpose of this review it may be found convenient to consider the "volatile" fatty acids separately from those for which esterification is necessary to render them separable. GC was initiated with

this type of compound, and the first application in dental research may be that in which acetic and propionic acids were compared, 25 years ago, in the blood and saliva of several sheep by Annison[1]. It was found that formic acid, which accounted for 7–14% of the total volatile fatty acids in blood, was absent in saliva. Propionic acid amounted to no more than 3% of the total in any comparison.

2.1. Volatile acids in plaque and saliva

Comprehensive studies of lower fatty acids in dental plaque have been reported by a number of workers, and it is convenient to consider lactic acid along with these, although it presents special problems in GC and has sometimes been determined by enzyme systems. Little work has been reported with non-volatile acids of the Krebs cycle, which features in a qualitative study of saliva catabolism by Andlaw[2]. Lactic acid has been determined after extraction of salt-saturated saliva with diethyl ether and reaction with diazomethane to give methyl esters by Schleiffer *et al.*[3]. "Artificial" peaks were noted during the GC of lactate: these may be due to the presence of lactide and hemi-lactide.

The contributions of lactic acid and other fatty acids to the pH of human salivary sediment during glucose catabolism have been evaluated by Sandham and Kleinberg[4]. The products were mainly acetic and propionic acids as also found in a contemporaneous study (Schleiffer *et al.*[5]). The contributions of several fatty acids to their total content in saliva and plaque were evaluated by Bloch *et al.*[6].

Volatile monocarboxylic acids (C_1–C_4) and lactic acid were determined in interproximal plaque by Gilmour *et al.*[7]. In a further study[8] median values for formate and acetate were reported as 2.3 and $8.5 \cdot 10^{-9}$ moles/plaque, respectively. Lactate ($3.2 \cdot 10^{-9}$ moles/plaque), but not propionate and *n*-butyrate, increased significantly after a sucrose rinse. In earlier work a major source of error in GC determinations of volatile fatty acids has been found to be "peak ghosting": a means of controlling this was devised by Geddes and Gilmour[9]. All the titrated acids of outer regions of plaque were accounted for as lactic, acetic, and propionic acids, but only half the quantity in the case of the inner surface in contact with the teeth[10].

The concentrations of L-(+)- to D-(−)-lactate, determined enzymatically, were similar in some plaques, but in others the ratio was nearly 4:1 (ref. 11). In starved plaque the L–D ratio was about 1:2, this being reversed after exposure to sucrose, after which there was an eightfold increase in the L-form and a fivefold increase in the D-form; meanwhile the concentration of volatile fatty acids was halved[12].

2.2. Volatile acids in dental tissues

Volatile acids have been determined quantitatively in carious dentine[13,14] and carious enamel[15]. Formic, acetic, propionic, butyric (*n*- and iso-), and valeric (*n*- and iso-) acids were estimated as the free acids or as their methyl esters, and lactic acid as the trimethylsilyl derivative. Total volatile acids varied from 0.05–0.3% and lactic acid from 0.2–0.7%. A simple apparatus for separating the volatile acids by vacuum distillation was designed for these analyses by Tyler and Dibdin[16].

Lactic acid accounted for one-third of total organic acids present in plaque fluid from 8 monkeys (*M. fascicularis*) 1 h after a 10% sucrose application but was absent in similar samples after overnight fasting[17]. Acetic, propionic, and *n*-butyric acids were present on the 1–3 μmoles/μl scale, with smaller quantities of isobutyric,

caproic and succinic acids in fluid from starved plaque. However, after sucrose exposure, the *n*- and isobutyric acids, the *n*- and isovaleric acids together, and caproic acid, each accounted for about 1 μmole/μl, but 4 μmoles/μl succinic acid were present; most of the organic acids present were accounted for as acetic acid (16 μmoles/μl) and lactic acid (14 μmoles/μl). Other groups of 8 monkeys received supplements of pyridoxine, phytate, and invert sugar in place of sucrose; inter-group differences in acid concentrations were observed in 5 of the 9 acids identified, but only after sucrose application.

Isobutyrate and isovalerate had previously been detected not only in plaque but also in calculus, carious dentine, and "white spot" enamel[15]. These acids together with formate, acetate, propionate, and *n*-butyrate, were present in all the specimens examined.

2.3. Lipid fatty acids in dental tissues

The fatty acid composition of human dentine was found to be similar to that of most other tissues when allowance was made for the 30% hydrolysis of the original esters[18]. Some 9% of the fatty acids comprised those containing 8–12 carbon atoms, which are not often mentioned in studies covering this field. As much as one-fifth of the total fatty acids in human enamel comprised the unsaturated linoleic ($C_{18:2}$) and linoleic ($C_{18:3}$) acids, compared with only 7% of the dentine fatty acids[19].

The most abundant fatty acids (palmitate, stearate, and oleate) were determined in the neutral lipids and lipophilic proteins of developing bovine enamel matrix by Fincham *et al.*[20], and their relationship to the well-known lipophilia of the matrix was explored.

In a comparative study of tooth lipid fatty acids by Das and Harris[21] it was shown that the total concentration varied from 0.12 to 0.23% among 16 species of animal. Palmitic, stearic, and oleic acids accounted for 69–96% of these totals, but minor contents of three other acids were present in all samples. Similar findings emerged from a study of a number of fossil teeth recovered after 0.1 to 230 million years. The above fatty acids accounted for 75–99% of the total fatty acid content of 0.4–0.9%. In this study[22] three of the eight samples contained an unidentified component(s), and the oldest at least two. Thus, it was shown that in general the fatty acid content and composition of fossil teeth was surprisingly similar to that of contemporary teeth; even the unsaturated fatty acids were comparable.

Most investigators have studied the effect of dietary changes on teeth in the rat but there are several reports which concern other species. The modifications induced in rabbit molars by diets containing lard, butter, and cottonseed oil were described biochemically by Das and co-workers[23,24]. It was concluded that the levels of linoleic acid in the teeth matched those in the diets, it was highest in the diet rich in cottonseed oil, in which 72% of the total fatty acid present was unsaturated. With lard prominent in the diet, the enamel was found to contain remarkably high levels of eicosapentaenoic acid ($C_{20:5}$, up to 2%). Acids up to $C_{24:0}$ were analysed.

Earlier work had shown the relative quantities of various fatty acids in the enamel and dentine of rats deficient in essential fatty acids[25,26] following the investigation of the composition of normal rat tissues[27,28]. A major component of the deficient rat tissues was found to be a docosahexaenoic acid.

The effect on caries and on fatty acid composition of rat teeth has also been

studied in relation to the restoration of a cariogenic diet[29]. Modifications in dietary carbohydrates and in the types of oils and fats included in rat diets have also been shown to affect fatty acid composition of the teeth[30,31].

Although research has been concentrated on the effects of deficiency in the quality of diet and in essential fatty acids, there is some evidence on the response of animals to several isocaloric diets differing in protein:carbohydrate ratio[32]; another diet tested had a higher fat:carbohydrate ratio with protein content similar to one of the above. A high protein diet led to a preponderance of saturated fats in the teeth, a high fat diet resulted in more linoleic and the docasapentaenoic acid already noted. For all diets in which balance was lacking there was an increase in the proportion of rat tooth lipids. Simple restriction in the quantity of diet resulted in a higher ratio of oleic acid to total fatty acids in restricted-diet rats than in controls, yet the ratio did not rise with age as it did in the controls[33].

These acids have also been studied in rat teeth affected by dental caries. The fatty acid composition of carious molar enamel and dentine was, in addition, found to include some acids with odd numbers of carbon atoms ($C_{17:0}$, $C_{19:0}$, $C_{21:0}$) as well as those with even numbers (C_{20}, C_{22}, $C_{22:1}$), which are not found in sound molars; these were thought to have arisen from bacterial lipids, or through reactions between bacteria and tooth lipids[34].

2.4. Lipid fatty acids in dental pulp

Fatty acids present in the lipids of rat, rabbit, and bovine dental pulp have been found[35] to consist mainly of palmitic, stearic, oleic, and arachidonic acids. From all three species it was possible to identify 10–14% docosapolyenoic acids (as many as six isomers). At least 28% polyunsaturated fatty acids, including linoleic acid, were present. Fatty acid profiles of the phospholipid fraction of rabbit and bovine dental pulp were later compared by Larson and Ellingson[36]. In the total lipid fraction the content of the polyunsaturated fatty acids exceeded 25%, of which 10–15% comprised linoleic and linolenic acids. However, less than 10% of the "polyunsaturates" have been found by other workers in lipids of the calcified dental tissues, which contain little, if any, arachidonic and docosapolyenoic acids. Changes in fatty acid composition of rat incisor pulp tissue following whole body X-ray exposure have been reported by Hashimoto[37].

2.5. Lipid fatty acids in saliva and salivary glands

Several investigators have followed the nature of the fatty acids in lipids of salivary glands and their secretions. The incorporation of ^{14}C-labelled palmitic acid was used to follow the secretory mechanism in rats[38]. The lipid content of human parotid saliva was found to be nearly 7 mg/100 ml, of which one-quarter consisted of polar lipids[39]. The fatty acid composition of the nonpolar fraction was richer in $C_{18:0}$ and poorer in $C_{20:4\omega6}$ fatty acids. Rather high free fatty acid levels were found, and it was thought that this was due, in part, to the lipase activity known to be a feature of saliva: however, methyl esters were shown to be true components.

Changes in gland lipid composition have been induced by altering diets supplied to rats. Essential fatty acid deficiency affected the lipids of submandibular glands[40]. It was suggested that a deficiency of dihomo-γ-linolenic acid, present to the extent of 2–3% in parotid gland lipids of control rats[41] might result in low prosta-

glandin levels, since arachidonic acid ($C_{20:4\omega6}$) and dihomo-γ-linolenic acid ($C_{20:3\omega6}$) —both of which were decreased— are prostaglandin precursors. With several levels of vitamin E supplementation, however, the changes in $C_{20:3\omega9}$ and $C_{20:3\omega6}$ acids in rat salivary glands were in the opposite sense[42]. Choline deficiency has been seen to lead to an increase in oleic acid content of rat submandibular gland fatty acids and a deficiency in the content of $C_{20:3}$ and $C_{20:4}$ fatty acids[43,44].

Comprehensive studies of lipid fatty acids in human and rat saliva and plasma have been fully described by Kakudo *et al.*[45,46]. In human subjects, parotid levels were about six times those of other salivary glands, in keeping with their secretion rates. There was an inverse relationship between excretion ratio (plasma concentration:saliva concentration) and molecular weight. There was also a direct proportion between fatty acid excretion in saliva and in plasma, and it was noted that the essential fatty acids had low excretion ratios.

Free fatty aldehydes and alcohols of rat submaxillary glands have been shown to be similar to those of brain tissues, but there were differences in the alkoxy lipids[47].

2.6. Lipid fatty acids in gingival and palatal tissue

The lipid pattern and fatty acid composition of human palatal epithelium have been investigated[48] following a similar study of rat palatal epithelium. In the experimental modifications, the effects of essential fatty acid deficiency and liver cirrhosis (induced by carbon tetrachloride) were followed. The composition of rat palatal tissue lipids has been determined after gamma-ray doses of up to 1200 rads by Rabinowitz and Beidemann[50]. At this level there were no changes in the contents of protein and nucleic acid material but cholesterol increased, and the lipid content was doubled.

Previously[51], the polar and neutral lipids of pig gingiva had been shown to have a free fatty acid content of 20%. This study was extended[52] to demonstrate the presence of similar contents of free fatty acids and triglycerides in the epithelium and connective tissue fractions. Incorporation of ^{14}C from labelled sodium acetate into the fatty acids of the gingiva of scorbutic guinea pigs has been followed by Otake *et al.*[53]. The C_{18} fatty acids were the most affected by vitamin C deficiency, saturated stearic acid production being stimulated, that of unsaturated C_{18} acids being inhibited.

Simple chromatograms were obtained when phenylacetic acid, present as a metabolite in the saliva of subjects with phenylketonuria, was determined as the *n*-propyl ester[54]. A wide range of organic acids, including this and three other phenyl derivatives, was identified in normal saliva using GC combined with mass spectrometry (MS)[55]. In addition to several fatty acids, lactic acid among them, there were identifications of 2-hydroxyisocaproic and succinic acids: the other phenyl derivatives were 3-phenyllactic acid, 4-hydroxyphenylacetic acid, and 3-(4-hydroxyphenyl)-propionic acid. Cholesterol and a commonly used food antioxidant, 2,6-di-(*tert.*-butyl)-cresol, were also found to be present.

3. SULPHUR COMPOUNDS

A review of the malodorous products arising from the putrefaction of saliva implicates chiefly hydrogen sulphide and methyl mercaptan, with dimethyl sulphide and dimethyl disulphide being of less importance[56]. These are present in the mouth as well as in the atmosphere (head space) of a container in which saliva is incubated

in vitro. Most of the volatile sulphur-containing products of incubation of heat-inactivated salivary filtrate with dental plaque and sulphur-containing amino acids were accounted for by these acids[57].

The mercaptan and the monosulphide were apparent even before incubation when the saliva was obtained from mouths in which periodontal disease or ulcerative stomatitis was present[58]; other volatile organic compounds detected in the head space above incubated saliva were acetone, acetaldehyde, ethanol, propanol and diacyl. It was possible to determine the most malodorous compounds, methyl mercaptan and hydrogen sulphide, which accounted for 90% of the volatile sulphur content, directly in the atmosphere of the oral cavity[59]; analysis of the minor component, dimethyl monosulphide was also reported.

Phenol and indole were demonstrated in ether extracts of saliva that had been incubated for five days[60]. These and other volatile metabolites (*p*-cresol, skatole and dimethyl disulphide) were detected in the head space above incubated saliva samples by Zelson *et al.*[61]. However, with greater severity of periodontal disease there were increasing concentrations of dimethyl disulphide and a nitrogenous aromatic compound not then identified in the GC–MS system used. Using a sulphur-specific flame photometric detector coupled to the gas chromatograph, a relationship was shown between volatile sulphur compound profiles and adverse oral environments, with particular reference to periodontal disease.

Gingival crevice fluid samples have been incubated with cysteine and sterile salivary fluid in order to examine the potential of the system for generating hydrogen sulphide[62]. Concentrations of the metabolite were determined by GC combined with flame photometry in the head space above the incubated samples. Four gram-negative microorganisms tested were found to produce the characteristic volatile sulphur compounds on incubation with sterile salivary filtrate and cysteine or methionine[63]. They were not found in a system incorporating eight gram-positive organisms, including *Streptococcus mutans*, *S. sanguis*, *S. salivarius* and *S. faecalis*.

Collagenase-positive (CP+) strains of *Bacteroides melaninogenicus* could be distinguished from collagenase-negative (CP—) strains by their much greater production of volatile sulphur compounds when grown on a trypticase-yeast medium[64]. This was related to the collagenolysis evident in periodontitis and the associated elevated production of such compounds.

The above observations might suggest that thiocyanate would be a suitable source of volatile sulphur compounds, since this ion is present at relatively high concentrations in saliva. Using ^{35}S-labelled compounds[65,66], thiocyanate appeared inert; when labelled thiosulphate was used the two sulphur atoms were metabolized differently: one ($S \cdot {}^{35}SO_3^{2-}$) was recovered as sulphate, but the other ($^{35}S \cdot SO_3^{2-}$) was largely converted to hydrogen sulphide and further to sulphate together with at least four polythionates, as well as being incorporated into protein.

A number of workers have followed the effect of attempts to reduce volatile sulphur compound production from the oral cavity. Ascorbic acid solutions were able to eliminate the methyl mercaptan, together with the very much smaller quantity of dimethyl disulphide, but the content of dimethyl monosulphide was halved[67]. Evidence for the normal absence of ascorbic acid in saliva has been obtained, using test samples derivatized with trimethylsilyl acetamide, by Feller *et al.*[68]. Methyl mercaptan production in the oral cavity was markedly reduced by toothbrushing or

mastication, with removal of deposits from the teeth and, perhaps more importantly, from the tongue[69]. In another study this reduction has been found to depend on the elimination of periodontal pockets when the disease state was present[70]. Much earlier, the deodorization of the breath was studied following the development of objective means of measurement of odour[71]. It has recently been suggested[72] that the GC analysis of mouth odour be used as a diagnostic aid in subjects who may have periodontal disease.

A reduction in the formation of volatile sulphur compounds in the head space above incubated saliva from volunteers who provided samples after using a commercially available mouthrinse, containing a quaternary ammonium compound, has also been reported[73,74]. Mouth odour can also be suppressed by treating with metronidazole, active against the genera *Fusobacterium* and *Bacteroides*[75].

The subjective organoleptic perception of objectionable concentrations of methyl mercaptan and hydrogen sulphide rivals the sensitivity of the amplified detecting systems used in GC. A significant association between the results of using the two methods was demonstrated in two separate studies[76]. Limits of 15 ppb* for methyl mercaptan and 7 ppb for hydrogen sulphide for detection by GC were claimed recently[77]; the former value lies somewhat below the accepted objectionable threshold (25 ppb) for methyl mercaptan. The mean value for this compound was one-quarter that of hydrogen sulphide in a considerable test series.

Further chemical analyses of sulphur-containing compounds relevant to saliva have been reported. Thiol, disulphide, and total sulphur have been quantitated[78] and methionine, methionine sulphoxide, and methionine sulphone, also their products of oral microbial metabolism, have been separated chromatographically[79].

There is a peak in oral production of volatile sulphur compounds after the overnight stagnation evident in half the population. In addition, other such peaks have been noted in women during the menstrual cycle, the general trend being an increase by factors of 2–4 around the middle of the cycle and during menstruation; there were also increases during the proliferative and luteal phases which were considered to be related to progesterone and oestrogen levels[80].

4. AMINO ACIDS

Protein loss during development of human enamel has been followed by GC of the *n*-butyl esters of trifluoracetyl-derivatized hydrolysates by Tyler and Stack[81]. Under the conditions used it was possible to separate 11 amino acids: the monoacylated proline ester was found to separate well from the diacylated hydroxyproline ester. The same derivatives were prepared in identifying N^G-methylated arginine among the amino acids of permanent teeth[82]. Guanidine-methylated arginine was found to be partially bound. Other N-methylated basic amino acids compounds significant in biological methylation, cell multiplication and growth regulation, have since been identified[83]. Two dimethyl-L-arginines and monomethyl-L-lysine were identified in human teeth, the arginine derivatives being present in equal proportions to the extent of half the unsubstituted arginine. However, the dimethyl-L-arginines,

* Throughout this article the American billion (10^9) is meant.

which were distinguished by thin-layer chromatography and by the automatic amino acid analyzer, did not separate by GC.

A remarkable use of amino acid separation by GC was that in which the D–L ratios of aspartic acid were determined in order to assess age in mineralized tissue in terms of racemization[84]. This method was applied to enamel[85] and to dentine, which provided less variable data[86]. As much as 96% of the variation in D–L ratio was due to age alone, when dentine specimens were used. It has been found that D-aspartyl residues increase at a rate of the order of 0.1% per year, some uncertainty being introduced by temperature variation. No detectable racemization (*i.e.* less than 1%) of valine, leucine, alanine, proline, phenylalanine, and glutamic acid was observed in specimens from donors aged between 11 and 65.

Phenylthiohydantoin derivatives were prepared for amino-terminal analysis by GC when a phosphorylated polypeptide (E_4) was isolated from bovine embryonic dental enamel[87]. This peptide was partially sequenced (mol.wt. 5000–6000) and found to contain O-phosphoserine residues, all three of which were linked to glutamic acid and tyrosine. It was considered that the presence of glutamic acid, tyrosine and leucine adjacent to or near O-phosphoserine residues may be of importance in calcium-binding and mineralization.

Proline, glycine, and glutamic acid were present in similar amounts, representing about 60% of the total amino acids, in the extractable (non-membrane) fraction of monkey parotid zymogen granules[88]. The N(O)-heptafluorobutyryl amino acid isoamyl esters were used in the separations.

In a survey of contamination in relation to amino acid analysis by Rash *et al.*[89] the characteristic amino acids associated with salivary contamination were determined. Arginine and histidine were considered unique to salivary contamination, and high concentrations of proline and glutamic acid were observed.

Applications of GC to amino acid determination in fossil bones and teeth have been reported by Wyckoff[90], but results have not been encountered.

5. CARBOHYDRATES

There have been several identifications of carbohydrates in salivary glycoproteins by GC. A major glycoprotein from the submandibular gland of *Dasypus novemcinctus mexicanus* Peters (the nine-banded armadillo) was considered to be the simplest known mucus glycoprotein. Using trimethylsilyl derivatization it was shown to contain N-acetylneuraminic acid as the only sialic acid type by combined GC–MS[91].

N-acetylneuraminic acid, fucose, neutral hexoses, and N-acetylhexosamines were methanolysed and separated by GC in another study of purified salivary glycoproteins[92,93]. The samples were taken from *in vitro* cultures of *Cercopithecus aethiops* (grivets) salivary gland tissue. Fucose was not detected, but this was not taken to be conclusive evidence.

Carbohydrate analyses have been reported for the proteins freshly adsorbed to tooth surfaces[94] and to solids with established surface characteristics[95]. In the plaques formed on glass bead and plastic films (*e.g.* polyethylene terephthalate) less glucose and mannose was formed than in pellicle formed within 2 h, but the hexosamine content was twice as high. Hydrolysis, acetylation, and formation of trimethylsilyl derivatives showed that glucose accounted for more than half the carbohydrate

in natural pellicle; sialic acid was not detected. The high glucose value found in experimental salivary pellicle by Sønju *et al.*[95] was confirmed in a study by Mayhall and Butler[96] where a glucose:galactose ratio of about 1:1 was noted by analysis using alditol acetates.

A remarkably constant basic structure, having galactose, glucosamine and galactosamine in the ratios 4:3:1, was observed in all the glycoproteins examined in a study of human mixed saliva[97–99]. Additional sugar residues were superimposed such that an additional galactosamine residue conferred specificity characterizing blood group A, whereas a galactose residue distinguished those with blood group B specificity. More fucose was associated with blood group H glycoprotein, less in glycoproteins of the fourth group distinguished, showing none of the above three specificities.

Differentiation between carbohydrate polymers has been reported using pyrolysis GC. A sample isolated from developing enamel by procedures suitable for preparing chondroitin sulphate was compared with a commercial sample which was subjected to the same manipulations, and the identity demonstrated[100].

The release of N-acetyl-β-glucosamine from the phenyl aminide substrate by a purified fraction from human parotid saliva was verified by GC of the trimethylsilyl derivatives[101]. It was demonstrated that the transglycosylation activity evident in a similar preparation containing the *exo*-type of β-N-acetylglucosaminidase prepared from *Aspergillus oryzae* was absent in the purified saliva fraction.

6. MISCELLANEOUS

GC has been applied to the analysis of several inorganic components of dental interest. Fluoride has been determined as the trimethylsilyl derivative in enamel biopsies[102]. Less than 1 ng F per sample could be detected and four biopsies per minute could be obtained. Interdental variations in F content of enamel were greater than intradental variations. The method has also been used in estimating the fluoride concentration of milk[103]; human milk was not found to differ in F content according to whether mothers resided in high- and low-fluoride areas. Nitrates and nitrites have been determined in saliva[104], typical ranges being 5–15 ppm and 1–10 ppm, respectively. Nitrate was converted to nitrobenzene, and nitrite found by difference after conversion to nitrate by hydrogen peroxide.

A novel pathway for secondary metabolism of salivary organisms was noted by Wishnok and Tannenbaum[105] when secondary amines were present in saliva. Formation of cyanamides by reaction with salivary thiocyanate was proposed. For example, morpholine was converted to 1-cyanomorpholine. Other secondary amines form similar compounds. These authors have also reported an unknown salivary morpholine metabolite[106].

Although this review is not concerned with the monitoring of drugs in saliva, the analysis of chlorhexidine by GC provides a suitable example[107]. In tooth pellicles discolored by chlorhexidine, analyses showed the presence of furfural and hydroxymethylfurfural, which were absent in pellicles from the mouths of subjects not exposed to chlorhexidine[108].

In considering the identification of organic compounds in the atmosphere of the oral cavity other than sulphur compounds the distinction is not made here

between those derived from the oral cavity and those present because of interactions in the lungs. Using condensation in a liquid air trap, or a pre-column, several authors[71,109–110] found methanol and ethanol in all breath samples and two of these found acetone also[71,110]. Using a direct method[111], as many as ten peaks appeared, and in addition to the above three compounds there was a tentative identification of acetaldehyde. Hydrocarbons have been found in the expired air of vitamin E-deficient rats by Dillard *et al.*[112]. They have also been generated by pyrolysis GC of enamel and dentine at elevated temperatures[113].

Pyrolysis at lower temperatures has been employed for a GC estimation of the range of mineralization in dentine samples from protein-deficient rats, the method depending upon quantitation of the most prominent product(s) derived from the proline and hydroxyproline present in the collagen[100]. The logarithm of the chromatogram peak area representing these components was observed in another study to vary directly with the specific gravity of separated fractions of developing enamel, which contain proteins rich in proline. The fractionation of these proteins was also monitored by pyrolysis GC analysis[100].

7. SUMMARY

Studies of monocarboxylic acids having a wide range of chain length feature in half the papers reviewed. Shorter-chain acids have been analysed in relation to carbohydrate metabolic products at the tooth surface. Lipid changes following dietary modifications have been followed by determining the longer-chain acids. Substantial use of GC has also been made in studying the volatile sulphur-containing products of oral anaerobes, such compounds being unacceptable at very low concentrations in the breath. Less important applications are noted for amino acid and carbohydrate analysis in the dental context.

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