Carbohydrate Components of Supragingival Salivary Calculus.* (27498)

I. D. MANDEL, B. HAMPAR AND S. A. ELLISON (Introduced by B. C. Seegal)
Division of Stomatology, School of Dental and Oral Surgery, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York City

Histochemical examinations of several ectopic calcifications (urinary calculus(1), submaxillary gland calculus(2), supragingival salivary calculus(3), pulp stones(4), etc.(5) disclosed several common features, including the presence in each of carbohydrate-containing matrix constituents. In view of these similarities, it has been suggested that there is a common mechanism of formation(3,6). Urinary and submaxillary duct calculi have been compared and were found to be identical with regard to the amino-acids and carbohydrates which they contained(2,7). It appeared of interest to determine the composition of the most common ectopic calcification, supragingival salivary calculus, and to compare it with these other accretions. Additional interest in the composition of supragingival salivary calculus stems from its probable role in the pathogenesis of periodontal disease.

Methods. Supragingival calculus was collected from the lingual surfaces of lower anterior teeth (submaxillary calculus) or from the buccal surfaces of upper molar teeth (parotid calculus). Calculus was stored at –20°C in water containing thymol crystals. Pools of submaxillary or parotid calculus were washed with tap water and distilled water, ground in a mortar and dried *in vacuo* over P_2O_5. Weighed samples were placed in acid-washed dialysis bags and decalcified by dialysis against changes of 5% disodium-ethylenediamine-tetraacetate, adjusted to pH 7.8. A few samples were decalcified by dialysis against 0.01N HCl or 0.2M formate buffer, pH 4.2. When decalcification was complete, the contents of the dialysis bag were dialyzed against water, and then centrifuged in the cold. The supernatant, containing soluble non-dialyzable materials, was lyophilized, dried over P_2O_5 and weighed. The sediment was similarly dried and weighed. In some instances the entire content of the dialysis bag was lyophilized and the water soluble materials subsequently extracted. The ratio of soluble to insoluble substances was not affected by the procedure used at this step.

In some instances the dialyzates were retained for carbohydrate analysis. The solutions were acidified with 1N HCl, the EDTA extracted with chloroform, and the aqueous layer containing the carbohydrates concentrated by flash evaporation.

Analytical methods. Neutral sugars (hexose, pentose and methylpentose). Samples were hydrolyzed in 0.5-1.0N HCl for 2-4 hours. Alternatively, in some instances, Dowex 50-0.05N HCl(8) or Permutite Q(9) were employed. Subsequently, the hydrolysates were dialyzed against water and the dialyzates concentrated and passed through columns of Amberlite MB-3. The sugars were eluted from the column with water, assayed and identified. Hexoses and methyl pentose were determined by the primary cysteine reaction(10) and pentose by the orcinol reaction(11). The sugars were identified chromographically using propanol:ethyl acetate: H_2O (7:1:2), quadruple descent(12) and ethyl acetate : pyridine : H_2O (3.6 : 1 : 1.15) (13).

Deoxypentose. Deoxypentose was identified in the soluble matrix and estimated both by the thiobarbituric acid method of Waravdekar and Saslaw(14) and by a dipenylamine reaction(15). In the former case the sample was hydrolyzed by refluxing in 0.1N HCl, and the test performed on the material then recovered by dialysis. In the latter instance, the matrix was extracted with perchloric acid and the extract tested(15).

Sugar acids. 1. Sialic acid: samples of soluble and insoluble matrix were hydrolyzed and chromotographed on Dowex 2-X8 as described by Svennerholm(16) and assayed with thiobarbituric acid (Warren 17).

* This work was supported in part by grants from Bristol-Myers Co., and by a Research Contract between Columbia University and the Office of Naval Research.

TABLE I. Matrix Recovery from Salivary Calculus.

	Submaxillary area*		Parotid area†	
	EDTA[a]	Acid[c]	EDTA[b]	Acid[c]
Total matrix (% of sample wt)				
Avg	11.2	9.3	11.2	9.8
Range	6–17		9.1–13	
Insoluble matrix (% of total matrix)				
Avg	74	92	70	94.5
Range	73–83.7		61.8–77.5	
Soluble matrix (% of total matrix)				
Avg	26	8	30	5.5
Range	16.3–27		22.5–38.2	

* Collected from lingual surfaces of lower anterior teeth.

† Collected from buccal surfaces of upper molar teeth.

[a] At least 6 samples. [b] 2 samples. [c] 1 sample.

2. Hexuronic acids: samples were hydrolyzed in 0.1N and 0.5N HCl, and with Dowex 50-0.05N HCl. The hydrolytic products were chromatographed on Dowex 2-X8 (acetate form), eluted with 1N and 2N acetic acid and assayed with the carbazole reaction (10).

Amino-sugars. Samples were hydrolyzed in 2N and 4N HCl for 2-4 hours, chromatographed on Dowex 50 and analyzed by a modified Elson-Morgan reaction(18).

Nitrogen was measured by micro-Kjeldahl reaction, and ash was measured gravimetrically after ignition of the sample in acid.

Results. Both a soluble and an insoluble matrix fraction were consistently recovered after decalcification (Table I). Although total matrix recovery was only slightly higher when decalcification was done using EDTA rather than acid, the soluble fraction was considerably larger when EDTA was employed. Hexose, constituting 0.3% of the sample weight, and a trace of methyl-pentose were recovered from the EDTA dialyzate after decalcifying both submaxillary and parotid calculus. No hexosamine was detected in this fraction.

The findings with respect to the composition of the matrix are summarized in Table II. The soluble matrix differed clearly from the insoluble matrix. Hexuronic acids could not be demonstrated. Both tests for deoxyribose showed that insoluble matrix contained small amounts (0.05-0.1%) of this carbohydrate.

Typical chromatographs of hydrolyzed matrix samples are shown in Fig. 1. The same neutral sugars were found in the soluble and insoluble matrix fractions. Comparison with standards in the 2 solvent systems enabled the identification of galactose, glucose, mannose, arabinose, xylose, fucose, and rhamnose. An additional, unidentified sugar with the Rf of deoxyribose in propanol:ethyl acetate:water, but having a higher Rf than deoxyribose in ethyl acetate:pyridine:water was also detected.

Discussion. The proportion of organic to inorganic elements, determined from the amount of matrix recovered, indicates that supragingival salivary calculus is intermediate in composition between urinary calculus (3% organic)(1) and salivary gland calculus (18%)(2). The liberation of a soluble, nondialyzable, matrix fraction as a consequence of decalcification was also observed in the case of urinary stones(1). This observation of a soluble matrix fraction may be relevant in interpreting histochemical data, since specimens demineralized in EDTA will have lost a portion of their matrix.

TABLE II. The Percentage Composition of Soluble, Insoluble and Total Calculus Matrix.*

	Soluble	Insoluble	Total†
Hexose[a]	13.4 (8 –21)	6.1 (3 –9.7)	7.9
Methylpentose[a]	3.3 (1.8– 6)	2.2 (1 –3.8)	2.5
Hexosamine[a]	.2 (0 – .5)	2.2 (1.7–3)	1.7
Pentose[b]	2	.9	1.2
Sialic acid[b]	.3	.2	.2
Ash[c]	13.4	4.4	6.7
Nitrogen[b]	2.7	10	8.2

* Calculus collected from lingual surfaces of lower anterior teeth was decalcified in EDTA.

† Calculated on the basis that total matrix consists of 75% insoluble and 25% soluble matrix. *Cf.* Table I.

[a] Based on minimum of 6 samples analyzed.

[b] 2 samples.

[c] 1 sample.

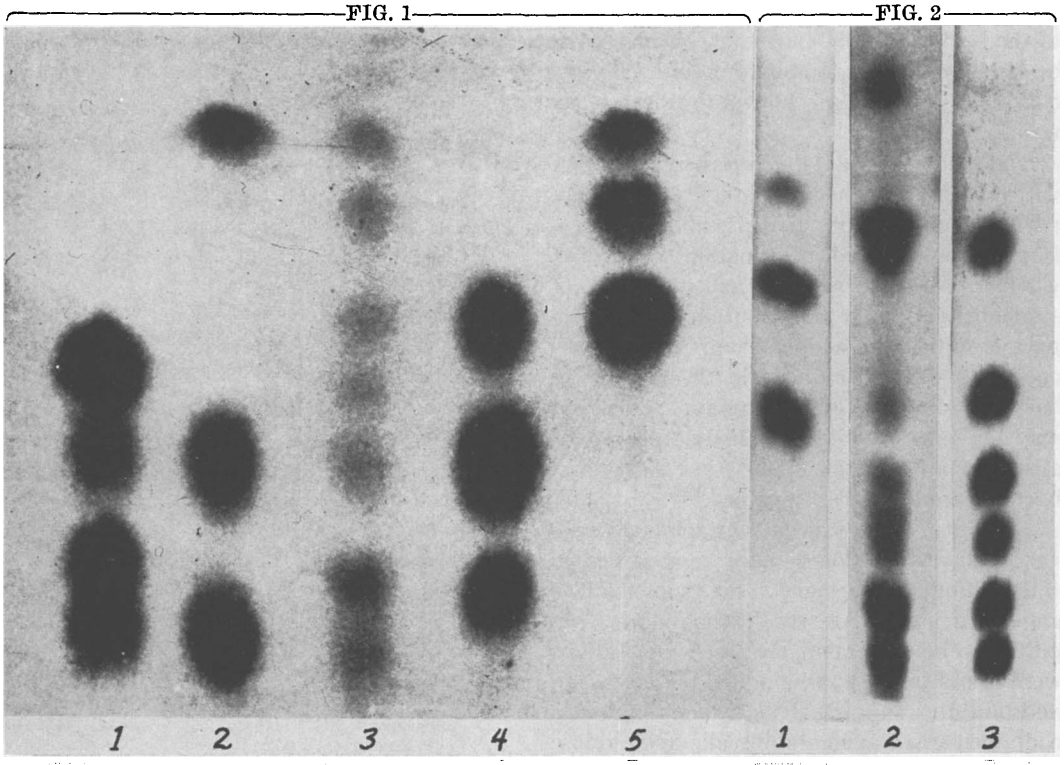

FIG. 1. (Left) Chromatograph of total calculus matrix hydrolyzed with Permutite Q. Solvent was propanol:ethly acetate: H_2O (7:1:2), quadruple descent. Developer was aniline hydrogen phthlate. 1– Standard: galactose, glucose, mannose, xylose. 2– Standard: galactose, mannose. 3– Calculus: galactose, glucose, mannose or arabinose, xylose, fucose or ribose, rhamnose, deoxyribose (?). 4– Standard: glucose, arabinose, fucose. 5– Standard: ribose, rhamnose, deoxyribose.

FIG. 2. (Right) Chromatograph of soluble calculus matrix hydrolyzed with Dowex 50 + 0.1N HCl. Solvent was ethyl acetate:pyridine:H_2O (3.6:1:1.5), triple descent. Developer was silver nitrate. 1– Standard: fucose, ribose, deoxyribose. 2– Calculus: galactose, glucose, mannose, arabinose, fucose or xylose, rhamnose, unknown. 3– Standard: galactose, glucose, mannose, arabinose, xylose, rhamnose.

The differences between soluble and insoluble calculus matrix reported above, parallel the findings in the urinary calculi(19). In both instances the soluble material contained more hexose, less hexosamine, and yielded more ash than did the insoluble fraction. All the carbohydrates found in urinary and submaxillary gland calculus were present in supragingival salivary calculus. Hexuronic acids, absent from urinary and submaxillary gland calculus, were not found in the material we analyzed. However, sialic acid, arabinose, and xylose, which had not been reported in the other ectopic calcifications, were detected in supragingival calculus.

There is little doubt that a portion of the matrix of supragingival salivary calculus is derived from bacteria and a portion from saliva(3). It is interesting to note in this connection that we have identified glucose, galactose, mannose, fucose, hexosamine, and sialic acid, in both parotid(12) and submaxillary saliva, and xylose and rhamnose in submaxillary saliva only. Arabinose, not found in submaxillary saliva or parotid saliva, has been found in the cell walls of certain oral bacteria which may be present in oral calculus, *i.e.*, *Leptotrichia, Nocardia,* and *Corynebacteria* (20). The small amount of deoxypentose found in supragingival calculus might come from the bacterial nucleic acid.

It is apparent from the data in Table II that about 20% of the matrix might consist of substances not yet identified. In view of

previous findings(21), and the contribution of the bacteria to the matrix, these might well be lipid, or lipid in combination with polysaccharide or protein, as is known to exist in bacterial cell walls(22).

The analytical data given here, together with earlier histochemical studies, lend support to the possibility that a similar mechanism may be involved in formation of calculus, whether it be urinary, submaxillary gland, or supragingival. The contribution of the bacteria is difficult to assess. Supragingival calculus can form in animals in the absence of bacteria(23); bacteria themselves, however, may become calcified(24,25). Apparently, although not essential to the process, the oral bacteria are ordinarily involved.

Summary. Analysis of supragingival salivary calculus disclosed many similarities in composition to urinary and submaxillary gland calculus. All the carbohydrates reported to be present in the latter concretions were found in the supragingival material. In addition the supragingival salivary calculus contained small amounts of sialic acid, xylose and arabinose. From the nature of the carbohydrate components it would appear that both saliva and bacteria contribute to the composition of supragingival salivary calculus.

1. Boyce, W. H., Sulkin, N. M., *J. Clin. Invest.*, 1956, v35, 1067.

2. Harrill, J. A., King, J. S., Jr., Boyce, W. H., *The Laryngoscope*, 1959, v69, 481.

3. Mandel, I. D., Levy, B., Wasserman, B., *J. Periodont.*, 1957, v28, 132.

4. Johnson, P. L., Bevelander, G., *J. Dental Res.*, 1956, v35, 714.

5. Rubin, P. S., Howard, J. R., *Metabolic Interrelations*, 2nd Conf. Josiah Macy Jr. Found., N. Y., 1950, v2, 155.

6. Sobel, A. E., *Ann. N. Y. Acad. Sci.*, 1955, v60, 713.

7. King, J. S., Jr., Boyce, W. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 183.

8. Anastassiadis, P. A., Common, R. H., *Can. J. Biochem. and Physiol.*, 1958, v36, 413.

9. Glegg, R. E., Eidinger, D., LeBlond, C., *Science*, 1953, v118, 614.

10. Dische, Z., *Methods of Biochemical Analysis*, Interscience, N. Y., 1955, v2, p327-340.

11. Albaum, H. G., Umbreit, W. W., *J. Biol. Chem.*, 1947, v167, 369.

12. Mandel, I. D., Hampar, B., Thompson, R. H., Jr., Ellison, S. A., *Arch. Oral Biol.*, 1961, v3, 278.

13. Colombo, P., Corbetta, D., Pirotta, A., Ruffini, G., Sartori, A., *J. Chromatography*, 1960, v3, 343.

14. Waravdekar, V. S., Saslaw, G. D., *Biochim. Biophys. Acta*, 1957, v24, 429.

15. Burton, K., *Biochem. J.*, 1956, v62, 315.

16. Svennerholm, L., *Acta Chem. Scand.*, 1958, v12, 547.

17. Warren, L., *J. Biol. Chem.*, 1959, v234, 1972.

18. Schiffman, G., Howe, C., Kabat, G. A., *J.A.C.S.*, 1958, v80, 6662.

19. King, J. S., Jr., Boyce, W. H., *Arch. Biochem. Biophys.*, 1959, v82, 455.

20. Davis, G. H. G., Baird-Parker, A. C., *Nature*, 1959, v183, 1206.

21. Mandel, I. D., Levy, B., *Oral Surg., Oral Med., Oral Path.*, 1953, v10, 874.

22. Zilliken, F., *Fed. Proc.*, 1959, v18, 966.

23. Fitzgerald, R. J., McDaniel, E. G., *Arch. Oral Biol.*, 1960, v2, 239.

24. Ennever, J., *J. Perio.*, 1960, v31, 304.

25. Rizzo, A. A., Martin, G. R., Scott, D. B., Mergenhagen, S. E., *Science*, 1962, v135, 439.

Received March 26, 1962. P.S.E.B.M., 1962, v110.

Presence of Acetylsalicylic Acid in Plasma Following Oral Ingestion of Aspirin. (27499)

JACK R. LEONARDS

Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio

Recent investigations have questioned the assumption that aspirin is hydrolyzed in the intestinal tract prior to absorption. Although in several studies aspirin has been noted to be rapidly converted to salicylic acid following its oral ingestion(1,2), the presence of acetylsalicylic acid has been demonstrable in blood for up to an hour(3,4,5). In experi-