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THE CARBOHYDRATE CONSTITUENTS OF HUMAN SALIVA

by

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In discussions on factors influencing dental caries, the composition of the saliva has often been suggested to be of importance. Several theories on the possible role of the saliva have been advanced. It has often been assumed that the carbohydrate constituents are fermented to an acid, such as lactic acid, the acid subsequently attacking the intact enamel or the dental plaques. Such theories, however, are based on vague assumptions, since little is in fact known about the carbohydrate components of saliva, and still less about its possible fermentation or degradation in the oral cavity.

It is well known that the salivary secretion does not contain appreciable amounts of free polysaccharides or sugars. Almost all carbohydrate present is chemically linked to proteins, forming glycoproteins.

One type of glycoprotein has previously been isolated in small amounts from human saliva, viz. blood-group substances (12, 10, 1, 14). The hexosamine-rich mucoid fraction obtained by *Knox & Still* (11) from human saliva, in all probability also belongs to that type of substance. Furthermore, the saliva may be assumed to contain a sialic-acid-bearing glycoprotein, since *Blix* (2) has shown this kind of glycoprotein to be the main component of aqueous extracts of submaxillary glands.

The present investigation was undertaken to obtain information on the carbohydrate components of human salivary secretion, and thus to provide a firmer basis for the discussion of their possible influence on dental caries.

MATERIAL AND METHODS

Saliva was collected individually from 8 persons, apparently healthy but for the fact that all showed moderate dental caries. From 7 of them the mixed salivary secretion was collected. From the 8th (GB), only the secretion emerging from the sublingual salivary papillae was collected (this was possible because this individual was able voluntarily to induce a jet of secretion from these papillae).

All subjects were instructed to spit into beakers containing 95 per cent ethanol. The secretion which appeared during the first hour following meals was discarded.

From the ethanol the mucous fraction of the saliva was collected on glass rods by gentle stirring. A small amount of amorphous residue (containing minute amounts of carbohydrate) was usually left at the bottom after this procedure. The material thus collected was treated three times with 40 per cent ethanol (to get rid of the main part of dialysable, low-molecular constituents), and afterwards thoroughly washed with 99 per cent ethanol and ether. The preparations were then dried in desiccators and analysed.

The analytical procedures have been reported in detail earlier (31).

Hexosamine was determined by the *Morgan-Elson* method as modified by *Blix* (3). The "total" *hexose* content (including fucose) was determined by the orcinol-sulphuric acid method described by *Vasseur* (30), using 20 min. heating and the Beckman Model B spectrophotometer at 490 mμ. *Fucose* was determined by the method of *Dische* (7). The "true" hexose content was deduced by subtracting the fucose figure from the "total" hexose value. *Sialic acid* determinations were done by the *Bial* method (32), and in some cases the *Ehrlich* method (32) was also used. There was close agreement between the findings of the two methods. *Nitrogen* was determined by the micro-Kjeldahl method (9).

The monosaccharide constituents were also assayed qualitatively by paper partition chromatography. Usually butanol-acetic acid was used as solvent and aniline-phthalic acid as spray-reagent (21, 22).

RESULTS

The analytical figures for the pooled specimens for each individual are given in Table 1. In the case of submaxillary-sublingual saliva (GB) the figures from two separate samplings are given (I and II).

Table I

Specimens of saliva	Contents in per cent of dry weight of					Sugars (except hexosamine) demonstrated by chromatography after hydrolysis[1]
	Nitrogen	Hexosamine	Hexose	Fucose	Sialic acid	
Mixed Saliva						
I. M.	11.4	6.4	6.8	4.4	1.9	Galactose, fucose, traces of mannose
L. O.	11.8	5.3	5.8	3.2	2.1	Galactose, fucose
A. P.	10.4	6.9	11.1	4.9	1.9	Galactose, fucose, traces of mannose
E. L.	11.0	6.7	9.4	2.9	2.7	Galactose, fucose
I. A.	10.0	4.2	5.9	2.1	1.8	" "
L. W.	—	7.3	8.2	3.2	1.8	" "
U. L.	—	5.3	9.1	2.3	—	" "
Mean values	10.9	6.0	8.0	3.3	2.1	
Submaxillary—sublingual secretion						
G. B. I.	—	6.6	[2] —	—	5.3	Galactose, fucose
G. B. II.	—	6.0	—	—	3.9	" "

[1] In a pooled specimen traces of glucose were observed.

[2] The figure for hexose+fucose was 10.0 (single determination).

The figures reported are the means of duplicate determinations, except for the figures for G.B., the hexosamine figures for E.L and U.L., and the fucose figures for E.L., U.L., I.A., and L.W. Duplicate determinations were not performed in these cases because of lack of material, and ash and moisture determinations were omitted for the same reason. The figures in the table are thus uncorrected. In our experience similar preparations usually contain 10—15 per cent of moisture and 3—5 per cent of ash.

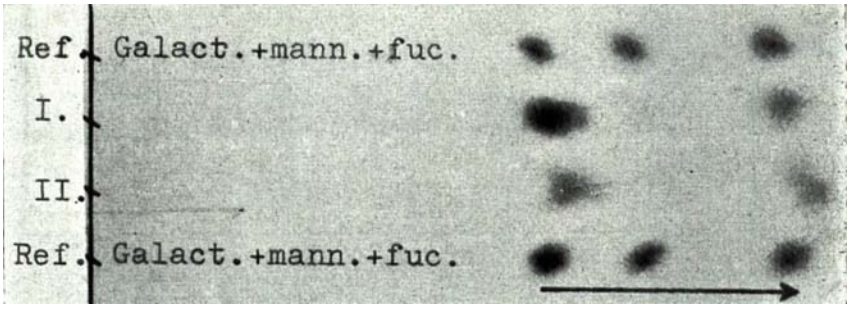

Fig. 1. Chromatogram showing sugar components of two hydrolysed specimens of saliva (I, II). Solvent: Butanol-acetic acid-water. Sprayed with aniline-phthalic acid. Descending chromatography.

The chromatograms all showed the presence of hexosamine, galactose, and fucose. In two cases faint traces of mannose were also present. The results are summarized in Table 1. A typical chromatogram is shown in Fig. 1.

DISCUSSION

A detailed historical survey of the chemical composition of salivary mucin was recently published by *Clement* (6). Only the main data which have led to the present, somewhat contradictory concepts of the carbohydrate composition of salivary mucin will therefore be mentioned here.

First to demonstrate the presence of reducing substance in hydrolysates of salivary gland extracts was *Eichwald,* in 1865 (8). The first monosaccharide identified was hexosamine, isolated from submaxillary secretion as the hydrochloride by *F. Müller* (19, 20), and supposed by him to be glucosamine.

In his monograph on *Hexosamines and Mucoproteins* (13) *Levene* claimed that the carbohydrate complex of submaxillary mucin was mucoitin sulphuric acid, i.e. a polysaccharide composed of glucuronic acid and sulphuric acid (23, 24, 25, 26).

The question of the composition of submaxillary mucin was later more closely studied by *Blix* (2). He demonstrated that bovine submaxillary mucin contains two different kinds of carbohydrate groups. One, constituting the minor part of the carbohydrate moiety, was composed of hexosamine and hexose. *Blix*

and collaborators later (5) showed that the hexose fraction not only contained galactose and some mannose but also a methylpentose, fucose, thereby establishing it as the same type of substance as the blood-group antigens. The main carbohydrate constituent was isolated in crystalline form, and was found to be an acid with an elementary composition approximately corresponding to $C_{14}H_{25}NO_{11}$. The acid was later shown to be accompanied by an equimolecular amount of chondrosamine (2-aminogalactose) (5). No hexuronic acid and only traces of sulphate-sulphur were found in the mucin. The presence of mucoitin-sulphuric acid could thus be excluded.

A similar composition for sublingual mucin was reported by *Tanabe* (21, 28, 29). The polyhydroxy acid, now named *sialic acid,* has been extensively studied in latter years. A detailed report on the chemical and physical properties of sialic acids from different animal species has recently been published (4).

In summary, it may be stated that the mucin from extracts of submaxillary glands contains two main types of glycoprotein. One is an acid glycoprotein containing hexosamine and sialic acid; this constitutes the major part of the mucin.* The other, present in much smaller amounts, is a neutral glycoprotein of the same type as the blood-group substances, and thus contains hexosamine, galactose, and fucose. In addition to these two main components, there are probably also small amounts of mannose-galactose-N-acetylglucosamine containing glycoprotein of the type common in blood and tissue.

Our examination of the submaxillary-sublingual secretion (Table 1) shows that the same two types of glycoprotein are secreted in the saliva. There is a difference, however, with regard to the relative amounts. The relative amount of the blood-group type of substance, the fucomucin, is considerably higher in the secretion than in the glandular extract. This might possibly be explained by the fact that the glycoprotein of the glandular extract had been precipitated by acidification in water solution, whereas the material from the secretion was obtained by pre-

* In this connexion it may be mentioned that *Meyer* (18) reported the isolation of glucosamine and gluconic acid from a similar preparation. Detailed analytical data were not given, however, and no confirming report has yet appeared.

cipitation with alcohol. In the former case, part of the fucomucin might have remained in solution. The specimens of mixed saliva also contained the same carbohydrate constituents, indicating the presence of both types of glycoprotein. Here the difference in the relative amounts is still more pronounced, fucomucin forming the major part of the secretion (Table 1). It may be concluded that the combined secretion of the buccal, palatal, and lingual glands is richer in fucomucin than in sialomucin, and is thus similar to mucous secretion found elsewhere (31).

Regarding the possible relation of mucous secretion to dental caries, it can be safely stated that none of the monosaccharide constituents present in appreciable amounts in the saliva belongs to the type of sugar that is easily degraded by natural or bacterial enzymes normally present in the oral cavity. Furthermore, with the exception of sialic acid, they are all firmly bound to protein residues, and partial fermentation thus seems still more improbable.

Sialic acid might possibly be of some importance. The acid is easily split off from the mucin. It has a pK_a of about 2.6 and is thus a considerably stronger acid than, for example, lactic acid or acetic acid. The marked acidic properties of the mucin are no doubt due to the carboxyl groups of the sialic acid component. Most of these carboxyl groups are probably free, and should form salts at the pH of the saliva. Some of the sialic acid, however, may be bound in such a way that the carboxyl group is released when the acid is split off from the mucin. The mucin may be assumed to have a higher concentration on the surface of the teeth than in the saliva, and liberated sialic acid may possibly influence and accelerate the decalcification of carious areas. To some extent, then, the old theories of *Lohmann* (15, 16) and of *Lothrop and Gies* (17) on the decalcifying action of salivary mucin would be vindicated.

SUMMARY

Normal human saliva contains two main kinds of glycoproteins. One is a neutral glycoprotein, a fucomucin, containing hexosamine, galactose, and fucose, and is thus of the same type as the blood-group substances found in all epithelial mucous secretions of the body. The other is an acid glycoprotein, a sialo-

mucin, containing hexosamine and sialic acid. This type is also found in most epithelial mucous secretions.

In mixed salivary secretion the fucomucin is predominant. The secretion from the submaxillary and sublingual glands is richer in sialoprotein than the mixed salivary secretion.

As sialic acid is a relatively strong acid it might, if liberated in the oral cavity, possibly influence the development of dental caries.

RÉSUMÉ

LES CONSTITUANTS HYDROCARBONÉS DE LA SALIVE HUMAINE

La salive normale humaine contient deux espèces des glycoprotéides. L'une est une glycoprotéide neutre, une fucomucine, contenant: hexosamine, galactose et fucose, étant ainsi du type des antigènes des groupes sanguins, trouvés dans toutes les secrétions muqueuses épithéliales du corps. L'autre est une glycoprotéide acide, une sialomucine, qui contient: hexosamine et acide sialique. Ce type est aussi trouvé dans la plupart des secrétions muqueuses épithéliales.

Dans la secrétion salivaire mixte la fucomucine prédomine. La secrétion des glandes submaxillaires et sublinguales est plus riche en sialoprotéide que la secrétion salivaire mixte.

Puisque l'acide sialique est un acide relativement fort, il est possible, s'il est libéré dans la cavité buccale, qu'il ait une influence sur le développement des caries dentaires.

ZUSAMMENFASSUNG

DIE KOHLENHYDRATKOMPONENTE DES MENSCHLICHEN SPEICHELS

Im normalen Speichel des Menschen treten zwei Formen von Glykoproteiden auf. Die eine ist ein neutrales Glykoproteid, ein Fucomucin, das Hexosamin, Galactose und Fucose enthält. Es ist somit von demselben Typus als die Blutgruppensubstanzen, die in allen mucösen Sekreten des Körpers vorkommen. Die andere ist ein saures Glykoproteid, ein Sialomucin, das Hexosamin und Sialinsäure enthält.

Im gemischten Mundspeichel überwiegt das Fucomucin. Das Sekret der Submaxillaris- und Sublingualisdrüsen ist reicher an Sialoprotein als der gemischte Speichel.

Da Sialinsäure eine verhältnismässig starke Säure ist, könnte sie möglicherweise, falls sie in der Mundhöhle abgespalten wird, die Entwicklung von Zahnkaries beeinflussen.

LITERATURE

1. *Baer, H., E.A.Kabat and V.Knaub:* J.Exptl.Med. *91:* 105, 1950.
2. *Blix, G.:* Z.physiol.Chem. *240:* 43, 1936.
3. —»— Acta Chem. Scand. *2:* 467, 1948.
4. *Blix, G., E.Lindberg, L.Odin, I.Werner:* Acta Soc. Med. Upsal. *61:* 1, 1956.
5. *Blix, G., L.Svennerholm, I.Werner:* Acta Chem. Scand. *6:* 358, 1952.
6. *Clement, A.J.,* J.D.A. South Africa *6:* 277, 1951.
7. *Dische, Z., and L.B.Shettles:* J.Biol.Chem. *175:* 595, 1948.
8. *Eichwald, E.:* Ann.d.Chem.u.Pharm. *134:* 177, 1865.
9. *Friedrich, A.:* Microanalyse, F.Deuticke, Leipzig, 1933.
10. *Harte, R.A.,* J.Biol.Chem. *167:* 873, 1947.
11. *Knox, K.W., and J.L.Still:* J.D.Res. *32:* 379, 1953
12. *Landsteiner, K., and R.A.Harte,* J.Biol.Chem. *140:* 673, 1941.
13. *Levene, P.A.:* Hexosamines and Mucoproteins, New York, Longmans, Green & Co., London, 1925.
14. *Leskowitz, S., and E.A.Kabat:* J.Am.Chem.Soc. *76:* 4887, 1954.
15. *Lohmann:* Arch. für Zahnheilkunde, June 1904.
16. —»— D.Cosmos *46:* 981, 1904.
17. *Lothrop, A.P., and W.J.Gies:* Jour. Allied Dent. Societies *5:* 262, 1910.
18. *Meyer, K.:* Advances in Protein Chemistry, ed. M.Anson and J.T.Edsall. Vol. II, New York, Academic Press Inc. 268, 1945.
19. *Müller, F.:* Zeit.f.Biol. *42:* 466, 1901.
20. —»— Ibid. *42:* 561, 1901.
21. *Partridge, S.M.,* Biochem.J. *42:* 238, 1948.
22. —»— Nature *164:* 443, 1949.
23. *Schmiedeberg, O.:* Arch.f.Exp.Path.u.Pharm. *87:* 1, 1920.
24. —»— Ibid. *87:* 44, 1920.
25. —»— Ibid. *87:* 47, 1920.
26. —»— Ibid. *87:* 31, 1920.
27. *Tanabe, Y.,* J.Biochem. (Tokyo) *28:* 227, 1938.
28. —»— Ibid. *29:* 387, 439, 1939.
29. —»— Ibid. *30:* 181, 1939.
30. *Vasseur, E.:* Acta Chem. Scand. *2:* 693, 1948.
31. *Werner, I.,* Acta Soc. Med. Upsal. *58:* 1, 1953.
32. *Werner, I., and L.Odin:* Acta Soc. Med. Upsal. *57:* 230, 1952.

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