

## THE SIALIC ACID CONTENT OF HUMAN SALIVA

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The occurrence of sialic acids in many mucoproteins of animal tissues (1,2) coupled with the presence of "mucoids" in human saliva prompted the present study. To our knowledge no published information has demonstrated the presence of sialic acid in either whole or parotid human saliva despite the well-documented presence of this class of compounds in the salivary glands (1,2). Using the direct Ehrlich reaction, Schneyer (3) could not detect sialic acid in parotid saliva.

Samples of parotid saliva (P.S.) using the Lashley cup (4) and mixed whole saliva (W.S.) were obtained consecutively with paraffin-gum stimulation from 18 individuals. For the determination of total sialic acid, 1.0 ml of saliva was hydrolyzed with 0.1 ml 3N  $\text{H}_2\text{SO}_4$  at 80° C for 1 hour, and 0.2 ml were taken for analysis according to the thiobarbituric acid procedure of Warren (5) using N-acetylneuraminic acid (NANA) as standard. The results are shown in Table 1. The paucity of samples, however, does not permit any correlation between the disease entities and sialic acid content.

The presence of sialic acid in parotid and mixed whole saliva was substantiated as described below. Seventy-five ml of parotid and mixed whole saliva were each hydrolyzed with 1/10 volume of 3N  $\text{H}_2\text{SO}_4$  at 80° C for 1 hour. The hydrolyzates were adjusted to pH 6.0 with saturated  $\text{Ba}(\text{OH})_2$ , centrifuged, lyophilized and made up to 10.0 ml with distilled water. Aliquots removed at this juncture gave a faint Bial and faint direct Ehrlich test (6). In addition, absorption spectra were taken of the chromophores developed on an additional aliquot according to Warren (5). The remaining 9.0 ml were placed on a Dowex 2-X8, 200-400 column in the acetate form, eluted with 1.0 M

Table 1

Total Sialic Acid Content of Human Saliva

mg %									
<u>Normal Young Adults</u>			<u>Periodontal Disease</u>			<u>Rheumatoid Arthritis</u>			
P.S.*	W.S.**	P.S./W.S.	P.S.	W.S.	P.S./W.S.	P.S.	W.S.	P.S./W.S.	
2.0	3.2	0.63	1.4	2.7	0.52	6.0	7.1	0.85	
3.7	5.8	0.64	2.2	5.6	0.39	2.9	6.0	0.48	
0.9	2.6	0.35	2.6	4.2	0.62	3.6	4.8	0.75	
3.6	8.6	0.41	0.8	2.9	0.28	2.6	3.4	0.77	
3.9	5.1	0.77	2.6	4.8	0.54	2.4	5.1	0.47	
1.8	2.4	0.75							
1.5	7.3	0.21							
1.6	2.7	0.59							
Mean	2.4	4.7	0.54	1.9	4.0	0.47	3.5	5.3	0.66

\* Parotid saliva  
\*\* Whole saliva

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acetate buffer, pH 4.6 (6), passed through a Dowex 50-X12, 50-100 mesh column in the hydrogen form, lyophilized, and taken up in 2.0 ml water. Aliquots chromatographed on Whatman #1 with ethanol-water-ammonium hydroxide (8:2:1) and sprayed with the reagents of Warren (7) revealed a spot in both the mixed and parotid saliva preparations which migrated with the NANA standard. An additional aliquot was used for the development of the chromophores (5) and the absorption spectra were again taken. It was apparent that the absorption spectra of thiobarbituric acid chromophores of the hydrolyzed saliva samples before and after column elution and standard NANA were identical and indicated the absence of materials producing a peak at 532 mμ (5). Calculations based on Equations 1 and 2 of Warren (5) to correct for optical density which did not derive from N-acetylneuraminic acid, indicated that over 95% of the chromophore derived from sialic acid. Evans (8) has reported the presence of 7 mg% fucose in whole saliva, while Warren (5) has indicated that L-fucose may cause as much as a 35% decrease in the optical density due to N-acetylneuraminic acid. In the present study, quantitative recoveries of NANA added to both parotid and whole saliva were obtained indicating the absence of fucose interference in saliva. According to the criterion of Warren (5), 5 - 10% of the total sialic acid in parotid and mixed whole saliva was free.

The concentration of sialic acids was appreciably greater in mixed whole saliva than in parotid saliva from the same individual as shown in Table 1. This may be attributed to its presence in oral bacteria (9), in secretions of the other salivary glands, and in oral epithelial debris.

The absence of sialic acid in parotid saliva as reported by Schneyer (3) may be due to the relative insensitivity of the direct Ehrlich reaction which has only 1/28 the extinction coefficient of the thiobarbituric acid procedure of Warren (5).

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