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*J DENT RES* 1937; 16; 151

DOI: 10.1177/00220345370160020501

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## SALIVARY CHOLESTEROL<sup>1</sup>

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Cholesterol has been considered an active participant in the mechanism of various pathological conditions. Increased amounts in blood have been reported for kidney disorders, arteriosclerosis, hypertension, obesity, diabetes, and obstructive jaundice. Acute infections such as typhoid fever, scarlet fever, pneumonia, erysipelas, and syphilis frequently give rise to a marked decrease in blood cholesterol—the higher the fever, the lower the cholesterol content. Cholesterol analysis applied to other body fluids remains practically unexplored. A few investigators have studied spinal fluid (3, 4, 5, 6), but there seems to be no previous record of any attempt to ascertain its presence in saliva. The present paper gives a method for the quantitative determination of cholesterol in saliva, analyses showing the distribution of values generally as well as that obtaining for dental caries and erosion.

*Method of Analysis.* Preliminary tests proved that the technique of Myers and Wardell, with little modification, could be employed for the estimation of even the minute amounts of cholesterol in saliva (1, 2). As finally adopted, 4.0 cc. of fresh saliva in a 75 cc. casserole, is evaporated over a water bath to approximately 1 cc. and treated as described in the procedure for blood cholesterol (1). After extraction, the flask containing the extract is removed from the hot plate when the level of  $\text{CHCl}_3$  in the extraction chamber is one-half the height of the siphon tube. The extract is evaporated to about 5 cc., filtered through fat-free Whatman #50 filter paper to a 10 cc. graduate, capped with filter paper and allowed to evaporate spontaneously at room temperature to 1.0 cc. To this is added 0.02 cc. of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) and 0.4 cc. of acetic anhydride,

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<sup>1</sup> Preliminary report of some of the data was published in the *J. Den. Res.* 14: 226, 1934. Cholesterol in dental caries and erosion was presented as part of Clinical and Biochemical Studies of Dental Erosion and Abrasion by Isadore Hirschfeld, Frances Krasnow and Edith B. Oblatt at the meeting of the New York Section of the International Association for Dental Research, June 1936.

the mixture shaken, and placed in the dark for ten minutes. Micro-cups and plungers are used, and the color developed matched against naphthol green B which has been standardized against cholesterol. Because a brown coloration frequently develops when the extract is mixed with  $H_2SO_4$  and acetic anhydride, a red disc is employed for matching. Such discs frequently necessitate correction factors, which always obtained in our earlier work. Recently, however, an adjustment was arranged which obviates the necessity of any corrections.

*Results.* Duplicate determinations checked within limits to be expected for the concentrations existing in saliva, as follows (values are mgm. for 100 cc.): case I-21, 2.6, 2.8; case H.T., 5.7, 6.1; case O-7, 9.0, 9.0. The analyses thus far carried out<sup>2</sup> are detailed in Table I.

There is a fairly wide range of variation—the lowest content being 2.3 mgm. per 100 cc. and the highest 50.0 mgm. Clinically normal individuals (identified in Table I by "I") who have had no dental disease, have salivary cholesterol contents falling between 2.3 and 9.4 mgm., per cent. Very few cases have yet been relegated to this group and so the general average 5.4 mgm. per cent (or 7.9 mgm. for postabsorptive samples) may be used as the normal figure only tentatively.

Table I includes also determinations obtained in a comparative series of salivas from subjects suffering with active caries and erosion (abrasion). The figures deduced from the analytical data are summarized in Table II.

In tabulating the results, our attention was drawn to differences between cases with alkaline saliva and those with acid saliva. Treated as separate groups the two types gave averages which placed the alkaline set lower and nearer to the normal level, but raised and removed the acid group farther from that value. Thus witness  $5.3 \pm 2.1$  and  $9.2 \pm 0.7$  mgm. cholesterol per 100 cc. of alkaline saliva in contrast to  $15.7 \pm 2.9$  and  $13.5 \pm 2.0$  in acid saliva from erosion and caries patients, respectively. If not subdivided, the average of each type is above the normal by an amount which

<sup>2</sup> We acknowledge with appreciation the kindness of Dr. Isadore Rosen in offering the facilities of his laboratory at the New York Post Graduate School and Hospital and that of Dr. A. S. Rosen for aid in the analyses.

TABLE I  
General distribution of salivary cholesterol values

CASE	SEX	AGE	DATE	COLLECTION				MG. PER 100 CC.	DISTRIBUTION OF VALUES <sup>1</sup>	
				Stimulation	Time	Period	cc.		Range	Per cent
I-28	M	22	1-15-32	Paraffin	5:20*	30	25	2.3		
39 E	F	17	10-15-35	Unstimulated	Postab <sup>5</sup>	30	25	2.5		
I-21	M	21	1-13-32	Paraffin	5:00	—	25	2.7		
S-15	M	21	1-12-32	Paraffin	5:00	—	25	2.9		
S-31	M	22	1-21-32	Paraffin	5:50	10	23	3.1		
S-11	M	21	1-11-32	Paraffin	5:30	15	20	3.2		
I-13	M	20	1-11-32	Paraffin	5:20	10	20	3.2		
34	F	30?	12-28-34	Unstimulated	Postab	40	28	3.3		
I-16	M	21	1-12-32	Paraffin	5:00	—	20	3.6		
S-30	M	19	1-11-32	Paraffin	5:00	—	25	3.6		
31 E	F	20?	12-18-34	Unstimulated	Postab	30	19	3.7		
40 E	F	33	10-15-35	Unstimulated	Postab	75	25	3.8		
S 26	M	21	1-22-32	Paraffin	5:00	—	25	3.9		
S 14	M	19	1-13-32	Paraffin	5:00	—	25	4.0		
I 25	M	20	1-14-32	Paraffin	5:00	15	25	4.0		
S 18	M	19	1-13-32	Paraffin	5:00	—	25	4.1		
32 E	F	47	12-18-34	Unstimulated	Postab	30	26	4.4		
11 I	F	10	12- 3-35	Unstimulated	Postab	45	45	5.1		
41 E	M	43	10-15-35	Unstimulated	Postab	50	25	5.6		
H T-I	F	5 mos.	12-15-33	Unstimulated	Postab	30+30*	4.5+3.5	6.1		
S-23	M	21	1-22-32	Paraffin	4:30	90	33	6.1		
19 I	F	5	12- 3-35	Unstimulated	Postab	130	36	6.2		
35 E	M	41	5-14-35	Unstimulated	Postab	60	24	6.3		
9 C	F	9	10-29-35	Unstimulated	Postab	45	50	6.5		
17 C	F	11	11-19-35	Unstimulated	Postab	60	46	6.5		
21 C	F	7	12-10-35	Unstimulated	Postab	140	45	6.5		
10 E	F	25	10-29-35	Unstimulated	Postab	30	50	6.6		
S 22	M	20	1-14-32	Paraffin	5:45	25	35	6.8		
I 24	M	20	1-14-32	Paraffin	5:15	30	25	7.0		
I 27	M	21	1-15-32	Paraffin	5:00	—	25	7.0		
S 29	M	23	1-15-32	Paraffin	6:10	5	25	7.1		
45 E	F	32	2-11-36	Unstimulated	Postab	150	45	7.2		
15 CE	F	24	11-19-35	Unstimulated	Postab	30	49	7.3		
46 E	M	34	2-11-36	Unstimulated	Postab	135	45	7.7		
48 I	F	20	2-11-36	Unstimulated	Postab	95	45	7.9		
30 C	F	21	1-21-36	Unstimulated	Postab	130	45	8.3		
47 C	F	30	2-11-36	Unstimulated	Postab	90	45	8.6	2.3- 8.6	52.9
O-7	F	35	7-19-34	Unstimulated	Postab	265	18	9.0		
26 CE	M	34	1-14-36	Unstimulated	Postab	65	45	9.1		
28 C	M	11	1-21-36	Unstimulated	Postab	105	45	9.2		
6 I	F	7	3-17-36	Unstimulated	Postab	115	62	9.4		
23 C	M	8	12-10-35	Unstimulated	Postab	60	45	9.6		
24 C	F	8	12-10-35	Unstimulated	Postab	50	35	10.0		
3 C	F	9	3-17-36	Unstimulated	Postab	75	65	10.0		
27 C	F	22	1-28-36	Unstimulated	Postab	78	47	10.3		
54 E	M	54	5-12-36	Unstimulated	Postab	115	60	10.7		
8	F	8	10-29-35	Unstimulated	Postab	105	50	10.9		
16 C	F	15	11-19-35	Unstimulated	Postab	55	48	10.9		
50 C	M	24	2-18-36	Unstimulated	Postab	130	50	10.9		
18 E	M	25	12- 3-35	Unstimulated	Postab	70	45	11.0		
51 E	F	16	5-12-36	Unstimulated	Postab	—	60	11.1		
22 C	F	7	12-10-35	Unstimulated	Postab	150	20	11.5		
37 E	F	12	6-25-35	Unstimulated	Postab	75	25	11.6		
44 E	F	35	7- 1-35	Unstimulated	Postab	155	25	11.6		
29 C	F	13	1-21-36	Unstimulated	Postab	135	45	12.6		

TABLE I—*Concluded*

CASE	SEX	AGE	DATE	COLLECTION				MG. PER 100 CC.	DISTRIBUTION OF VALUES <sup>3</sup>	
				Stimulation	Time	Period	cc.		Range	Per cent
53 C	F	20	3-17-36	Unstimulated	Postab	135	50	13.1	8.7-15.0	27.1
51 E	F	41	5-12-36	Unstimulated	Postab	130	40	15.3		
14 C	F	13	11-12-35	Unstimulated	Postab	125	30	15.4		
25 EC	F	42	1-14-36	Unstimulated	Postab	160	45	15.6		
42 E	F	26	6-25-35	Unstimulated	Postab	75	25	15.8		
57 E	F	47	6- 2-36	Unstimulated	Postab	90	60	15.9		
60 P	F	49	6- 2-36	Unstimulated	Postab	110	23	16.7		
43 E	F	26	6-25-35	Unstimulated	Postab	130	25	16.8		
13 C	M	12	11-12-35	Unstimulated	Postab	140	34	18.6		
59 E	M	14	6- 2-36	Unstimulated	Postab	105	29	19.4		
20 C	M	22	12-10-35	Unstimulated	Postab	140	45	19.6	15.1-21.4	14.3
49 E	F	21	2-11-36	Unstimulated	Postab	90	13	27.3	21.5-27.7	1.4
55 C	F	6	6-2-36	Unstimulated	Postab	110	25	33.0		
36 E	M	53	5-21-35	Unstimulated	Postab	120	24	33.3		
56 P	F	32	6- 2-36	Unstimulated	Postab	162	48	34.5	27.8-34.5	4.3
58 E	F	38	6- 2-36	Unstimulated	Postab	210	19	50.0		

<sup>3</sup> The difference between the lowest value 2.3 and the highest value (not including 50.0) was divided arbitrarily into five equal intervals varying from each other by 6.4.

<sup>4</sup> Evening collections were made approximately four hours after the noon meal.

<sup>5</sup> Saliva from physically normal subjects in postabsorptive state was collected in the morning after a good night's rest, before washing mouth, brushing teeth, smoking, or breakfast, a 1½ to 2-hour period having elapsed between rising and collection, this interval including a fifteen-minute rest at laboratory (40-50 cc. collected).

<sup>6</sup> To obtain enough saliva for this analysis, two collections of two consecutive mornings were mixed. That secured the first day was stored in the ice chest. Previous experimentation indicated no deterioration of the cholesterol when thus treated.

TABLE II

*Comparative levels of salivary cholesterol for dental caries and erosion  
Analyses on total unstimulated saliva*

TYPE OF CASE	NUMBER OF CASES	TOTAL CHOLESTEROL AVERAGE, MG. PER CENT	PERCENTAGE DISTRIBUTION OF VALUES WITH REFERENCE TO THE NORMAL AVERAGE RANGE		ODDS AGAINST RANDOM OCCURRENCE OF DEVIATIONS CALCULATIONS BASED ON D/P.E. <sup>7</sup>
			Below	Above	
Normal.....	6	7.9 ±1.0 <sup>8</sup>			
Erosion.....	21	13.7 ±2.5	33	57	5.4
Alkaline saliva.....	4	5.3 ±2.1	75	25	1.0
Acid saliva.....	17	15.7 ±2.9	24	65	9.9
Caries.....	22	11.8 ±1.3	14	73	8.5
Alkaline saliva.....	9	9.2 ±0.7	22	67	1.2
Acid saliva.....	13	13.5 ±2.0	8	77	9.9

<sup>7</sup> D/P.E. as suggested by Pearl (7) is used to indicate the odds against random occurrence of a difference between two series. D = difference between the two classes under consideration, P.E. = probable error.

<sup>8</sup> This value is the standard deviation,  $\sigma = \sqrt{\frac{\sum d^2}{N(N-1)}}$ .  $\Sigma$  = sum, d = difference of an individual value from the mean, N = total number of analyses in any series.

does not give overlapping. When, instead of considering the averages, the percentage distribution below and above the average normal range is tabulated, a high percentage of cases falls beyond the high normal value and this percentage is increased for the subgroups with acid saliva. To test further whether these differences are significant, computations were made for the odds against their random occurrence. Values for caries-acid saliva and for erosion-acid saliva deduced by employing the formula, D/P.E. (7) approach significance. There is apparently a very close agreement of the three methods for the evaluation of analytical results differentiating the salivary composition accompanying abnormal dental conditions from the normal. It is interesting to note that simultaneous quantitative studies of other salivary constituents indicate general relationship of cholesterol to pH, protein and lipid phosphorus.

#### CONCLUSIONS

1. The method proposed for analysis of salivary cholesterol yields good reproducibility. Duplicate determinations check within 10 per cent.

2. The series of cases thus far studied present marked variation in salivary cholesterol—the limits being 2.3 to 50.0 mgm. per 100 cc.

3. There is apparently a distinct tendency to increased cholesterol in saliva (especially when acid) of caries and erosion susceptible individuals.

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