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THE INFRARED SPECTRAL CHARACTERISTICS OF HUMAN WHOLE STIMULATED SALIVA COLLECTED FROM INDIVIDUALS CLASSIFIED AS TO DENTAL CARIES EXPERIENCE

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Infrared spectral curves (2-16 μ) were accomplished on the saliva of 398 United States Air Force enlistees, who were classified according to dental caries status. Slight variations in the characteristic absorption spectra of human saliva appeared correlated with the carious surface score. Infrared scores, based on spectral curve characteristics, were inverse to 24-hour bacterial turbidity readings and directly correlated to flow rate. Infrared analysis presents a potential method for evaluating the immediate caries susceptibility status of an individual; however, this hypothesis needs confirmation in a time-function study.

Infrared spectrophotometry has been utilized extensively in analytical chemistry for the detection, quantitation, and description of chemical compounds (9, 16, 23). Although its use has been extended to the field of biochemistry, there are no data currently available in the literature on the infrared absorption characteristics of saliva. This information is desirable because of the ability of the infrared spectrum to identify molecular structure. Also, such a study offers the inherent possibility of discerning some characteristic of the envelope of the infrared absorption curve that might be correlated with caries resistance or susceptibility. This possibility is increased by the fact that Green (17) has recently reported the finding of a protein substance in the saliva of caries-immune individuals that is not present in the caries-susceptible individual.

The current study concerns the infrared absorption spectra of saliva collected from 398 airmen who were recently inducted into the United States Air Force and who were classified according to dental caries experience. Concurrently evaluated were the volume and the 24-hour bacterial turbidity of a culture inoculated with an aliquot of the saliva.

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TECHNIC

The young men (17 to 22 years of age) utilized in this study were recent enlistees into the U.S. Air Force. Only individuals with no restorations were included. All had successfully passed a physical examination, and all were subject to a routine of eating and sleeping characteristic of the life of an enlistee. All geographic locations of the United States were represented in the sampled group.

A thorough dental examination was accomplished with the use of a mouth mirror and explorer. No x-rays were taken. Saliva was collected in calibrated centrifuge tubes by having the subject chew on a pure gum rubber band, size 32, 1/8 inch wide, for a period of five minutes, after which time the specimen was refrigerated until removed for analysis. Samples were centrifuged five minutes at 1,500 r.p.m. at 5° C., after which time the volume was recorded. The resultant supernatant fluid was used as follows: 1 ml. was utilized to inoculate 9 ml. of Rogosa's broth (28) (from which the potassium monobasic phosphate and agar had been omitted) and allowed to incubate (37° C.) for 24 hours, at which time the turbidity was evaluated using a Klett-Summerson colorimeter (No. 66 red filter).

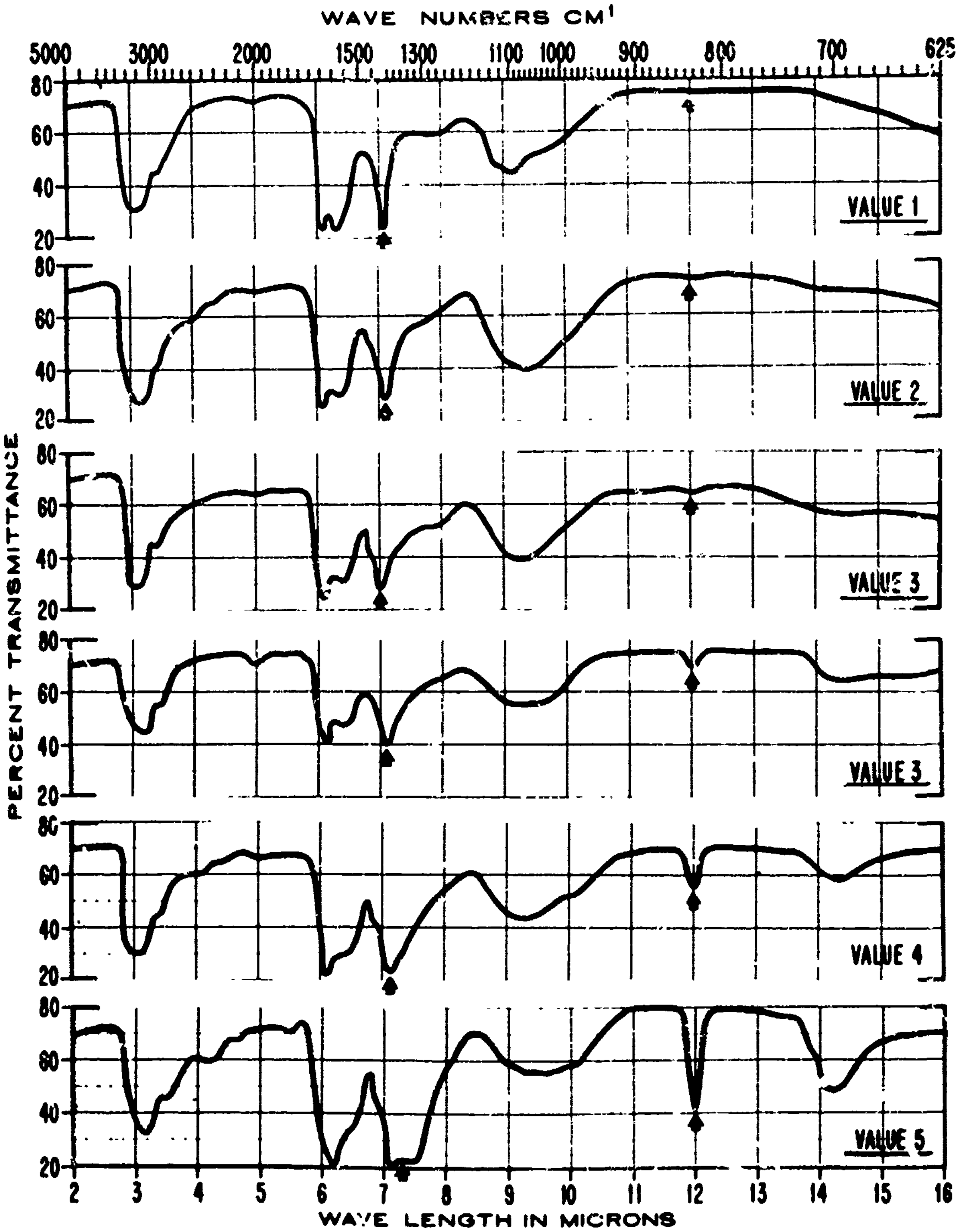
WAVE NUMBERS CM⁻¹
5000
3000
2000
1500
1300
1100
1000
900
800
700
625
PERCENT TRANSMITTANCE
VALUE 1
VALUE 2
VALUE 3
VALUE 3
VALUE 4
VALUE 5
WAVE LENGTH IN MICRONS

FIGURE 1

A second milliliter was placed in 4 ml. of ion exchange water to which 1 gm. of potassium bromide was added. This was immediately shell-frozen in acetone and dry ice, and lyophilized; 150 mg. of the homogeneously dispersed lyophilyzate was used to make a KBr pellet. Details of pellet fabrication include the following: evacuation of the die chamber for one minute with a dual-seal pump and then increasing hydraulic press pressure at the rate of 2,000 p.s.i. per stroke up to 30,000 lb.; this requires approximately 45 seconds. The procedure produced a clear transparent window. Infrared spectroscopy (2 to 16 μ) was accomplished by use of a modified Baird 400-B automatic recording, double-beam instrument operating at "normal speed" (15 min./scan) and with the starting absorption arbitrarily established at 65 percent.

RESULTS

Human saliva possesses a characteristic absorption curve, with slight variations (fig. 1). There are strong absorption peaks at 3.1, 6.1, 7.2 μ, and a broader absorption peak at 9 μ. Other less marked absorption peaks are sometimes seen at 5, 12, and 14 μ (with the latter two paralleling each other in direction and extent of change).

In a cursory examination of preliminary absorption curves, only those independent peaks at 7.2 and 12 μ appeared sufficiently correlated with a zero DMF[1] index for possible association with the caries-resistant state. An arbitrary value of 5 was assigned to the broad absorption peak at 7.2 as well as to the sharp peaking at 12 μ. Conversely, a value of 1 was separately assigned to the sharp peak at 7.2 and to the absence of a peak at 12 μ as these conditions appeared related to the group correlated with a high carious surface score. Intermediate values between 1 and 5 for each peak were possible (fig. 1). Thus, in considering each individual curve, a total infrared (IR) score of 2 to 10 was possible. To test the hypothesis that a low IR score would be correlated with a high carious surface score, and vice versa, both scores were calculated for the 398 individuals included in the study. The IR scores of 2 to 4, inclusive, were found least often in the zero DMF group and became more numerous as the carious surface involvement increased (table I). Similarly, it was typical that those with the highest IR scores (8 to 10 inclusive) decreased in prevalence as surface involvement increased.

Table II indicates that total turbidity readings of a 24-hour bacterial culture decreases significantly ($P > .01$) with increasing IR scores. However, there is a considerable overlap within each surface category, for horizontal values in table II show no consistent fall-off with increasing score, except for the > 40 group. The vertical picture in the table is more consistent; there is an increase in each column with each increase of carious surface score.

[1] Decayed, missing, and filled teeth.

TABLE I

Number of persons in categories classified by caries experience and by infrared scores

	Infrared scores			Total
Decayed surfaces	2 - 4	5 - 7	8 - 10	
0	19 (20.0)*	22 (22.9)	55 (57.3)	96
1-10	15 (20.5)	18 (24.6)	40 (54.8)	73
11-20	21 (34.4)	19 (31.1)	21 (34.4)	61
21-40	42 (37.5)	42 (37.5)	28 (25.0)	112
> 40	28 (50.0)	10 (17.8)	18 (32.1)	56
Total	125 (31.4)	111 (27.9)	162 (40.7)	398

*Percent.

TABLE II

Means and standard deviations of bacterial turbidometric measurements of individuals classified according to dental caries experience and by infrared score

Surfaces	Scores									Totals		
	2 - 4			5 - 7			8 - 10					
	N	$\bar{X}$	σ	N	$\bar{X}$	σ	N	$\bar{X}$	σ	N	$\bar{X}$	σ
0	19	213.8	180.5	22	120.3	173.7	55	135.9	156.9	96	147.8	1[illegible]2
1-10	15	218.7	197.3	18	180.8	192.1	40	264.4	178.2	73	231.4	186.4
11-20	21	309.8	169.3	19	35[illegible].9	123.7	20	295.4	160.0	60	318.7	152.4
21-40	42	414.4	124.3	42	316.3	128.1	28	390.9	81.1	112	383.0	119.4
> 40	28	448.0	91.2	10	421.5	80.2	18	395.0	116.3	56	426.2	100.8
	125	350.4	171.9	111	282.6	178.1	161	261.0	179.3			

TABLE III

Means, standard deviations, and sample sizes of saliva volume of individuals classified according to caries experience and infrared scores

Surfaces	Scores									Totals		
	2 - 4			5 - 7			8 - 10					
	N	$\bar{X}$	σ	N	$\bar{X}$	σ	N	$\bar{X}$	σ	N	$\bar{X}$	σ
0	19	5.36	1.92	22	5.50	2.09	55	8.56	2.47	96	7.22	2.71
1-10	15	5.56	1.28	18	6.98	2.47	40	8.14	2.42	73	7.32	2.4[illegible]
11-20	21	5.17	1.57	19	6.87	2.10	21	9.40	2.25	61	7.16	2.65
21-40	42	5.16	1.71	42	6.58	2.35	28	8.38	2.96	112	6.50	2.61
> 40	28	5.15	1.60	10	7.09	2.51	18	9.05	2.33	56	6.75	2.65
	125	5.24	1.63	111	6.53	2.32	162	8.59	2.51			

Observing the mean at the bottom of each vertical IR score column in table III, one finds that the volume increases significantly with increasing IR scores ($P > .001$). Yet, the volume means are not significantly different from the means of the total carious surface scores. There is possibly a slight decline (7.22 7.32, 7.16, 6.50, 6.75) with increasing surfaces affected, but this is not striking.

DISCUSSION

In any study attempting to relate the chemical composition of saliva to caries status, there is the extremely complicating fact that DMF indices represent *past* carious episodes of unknown duration, whereas the chemical data are related to the *present* resistant-susceptible status. This fact, together with the probability that individual caries status possibly reflects a dynamic shifting biochemical equilibrium, makes it extremely difficult to assess the effect of recently altered food and living habits (3, 27), changing psychologic stresses (5, 22, 24), and different geographic (20, 26, 30) and climatic conditions (13, 25) occasioned by the recent enlistment of these individuals.

Since the IR score was originally based on the actual carious surfaces involved, it would be expected that individuals with zero surfaces affected would tend to have a higher score. Such a relationship is seen in table I. Despite the high statistical correlation, the results show far from perfect individual association. Thus, one cannot, at this time, conclude from these results that the IR score is more indicative of true susceptibility-resistance status than the surfaces-affected classification. Conversely, the IR score may more truly measure the immediate caries susceptibility status, but this still has to be proved.

The IR score agrees with accepted concepts that a higher bacterial count should be expected with higher caries scores (1, 10). However, since *Lactobacillus acidophilus* counts have not been found to be *individually* correlated with caries development over a specified period (18), it is again not possible to determine whether the IR score is better than the turbidity index in establishing present susceptibility status.

The component appearing in the IR spectrum at 7.2 and 12 μ appears to be specifically associated with higher flow rates. The fact that there is a marked increase of flow rate with increasing IR score that is not paralleled by an inverse carious surface score and volume relationship, indicates that *if* flow rate is a factor in caries development, then the IR score (and the quantitative differences in the components going to make up that score) might be used to indicate caries status. Thus, a review of the possible role of flow rate in the caries process is indicated.

Flow rates in a considerable number of studies have resulted in nonsignificant correlations between carious surfaces and flow rates (1, 23). Balancing these investigations have been as many, or more, in which a trend for a higher flow rate has been found in a caries-resistant group (2, 15, 2: 34). Possibly this lack of agreement among investigators is due to the heretofore-mentioned insensitivity of the DMF-type indices to indicate *present* carious status, since it is improbable that technical errors in volume measurement are responsible. In the few studies where individual volume measurements were evaluated before and after a period of time in which the intervening caries attack rate was measured, volume has been higher in those developing fewer carious lesions (11, 12, 32).

It appears logical that a higher flow rate should be related to a more resistant state. Xerostomia, whether as a result of animal experimentation (6, 7, 8, 29) or as seen in humans as a result of salivary gland dysfunction (19, 31, 35), has been accompanied by higher caries scores. Also, in human subjects where the tooth surface has been isolated from the saliva following a glucose mouthwash, the pH has remained lower for a longer period of time when saliva is diverted from the plaque area (14). Thus, a general extrapolation appears in order that the greater the flow rate, the more dilution that occurs to the bacterial end-products responsible for caries development.

Despite the fact the IR scores correlate favorably with the carious surface scores, the 24-hour bacterial turbidity reading, and the flow rate, the true resistance-susceptibility status of the mouth at the time of chemical examination remains to be demonstrated. This is partially due to the fact that there is *no* reliable method at present to determine the immediate susceptibility status of an individual. Therefore, these IR data should be regarded as preliminary in nature, with final conclusions as to their ultimate value as a prognostic index awaiting the outcome of a time-function study, where both the chemistry and the caries attack rate are measured over a given period of time.

SUMMARY

A method for completing infrared spectroscopy of saliva has been presented. The envelope of the infrared absorption curve of human saliva from 2 to 16 μ is characteristic. Variations in the curve occur at 7.2 and 12 μ and appear correlated with caries susceptibility status. The components represented by the absorption peaks at 7.2 and 12 μ are associated with a lower 24-hour bacterial turbidity reading and with a higher flow rate than seen in a caries susceptible group.

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