THE COMPOSITION OF SALIVA IN RELATION TO THE INCIDENCE OF DENTAL CARIES

A THESIS SUBMITTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
AT THE UNIVERSITY OF CALIFORNIA

BY

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MAY, 1917

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REPRINTED FROM THE AMERICAN JOURNAL OF PHYSIOLOGY,
Vol. XLIII, No. 2, May, 1917

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 43, No. 2, May, 1917

THE COMPOSITION OF SALIVA IN RELATION TO THE INCIDENCE OF DENTAL CARIES[1]

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Received for publication March 5, 1917

INTRODUCTION

It has been previously reported by the writer (4) that the ratio of the neutralizing powers, or the power to maintain neutrality, of normal resting saliva and of the activated saliva, obtained by the chewing of paraffine, bears a definite relationship to the incidence of dental caries. In persons with carious teeth this ratio, expressed as a percentage, exceeds 80 while in persons whose teeth are temporarily immune from (or, more correctly, resistant to) caries the ratio falls below 80. In other words, as the difference between normal resting saliva and activated diminishes so does the liability to the incidence of caries increase. Shepard and Gies, in discussing this relationship or "salivary factor" have maintained (5) that it is inconstant. They based their conclusions, however, upon data which included all the different types of stimuli indiscriminately without regard to the nervous impulses and reflexes produced by the sense of taste. In their experiments they used paraffine, sucrose, sodium chlorid, alcohol and certain combinations of these. It was subsequently stated by the writer (8) that comparisons of saliva, the samples of which have been collected under different conditions, are inadmissible since such procedure ignores entirely psychic influences. Recalculation of their figures confirms the findings originally reported (4).

In a second communication (6) the writer presented data which both substantiated and developed the above thesis. Reports were made of investigations conducted in some of the state institutions for the in-

[1] Submitted in partial satisfaction of the requirement for the Degree of Doctor of Philosophy in the University of California and accomplished in part under the auspices of the Research Institute of the National Dental Association.

sane, and consisted of analyses of saliva from certain cases of dementia praecox and epilepsy. This work was likewise criticised by Gies (7) and answered in turn by the writer (9). No data have been presented which disprove any of the conclusions drawn in either paper (4 and 6) and it is in the further development of the consequences arising out of these conclusions that the following experiments were undertaken.

There are two main questions which fall under consideration in this connection, namely, first, the origin of the change of the neutralizing power of saliva which occurs in response to certain stimuli, and second, the significance of this change in relation to the incidence of dental caries. In other words, whether the alteration of the salivary factor is a contributing cause of dental caries, or conversely, an effect produced by dental caries, and lastly, whether there is a cause common to both altered factor and dental caries in which case the factor would become merely an incidental symptom.

In connection with the first of these problems, I have sought to throw light on the origin of the differences between the neutralizing powers of different samples of saliva: (a) By dialysis experiments in which the attempt has been made to determine the relative magnitudes of the parts played by the inorganic, or at least the diffusible substances, and the non-diffusible, and presumably organic, constituents of saliva. (b) By the determination of the amino nitrogen content of various samples of saliva after hydrolysis with a view to estimating more exactly the part played by protein in contributing to the difference in properties and composition between normal resting and activated salivas. In connection with the second problem I have sought to extend the observations of Pickerill upon the relationship of diet and habit to the incidence of dental caries and furthermore to determine the influence of the locality of the stimulus upon the neutralizing power of the secretion which is evoked.

Part 1

THE ORIGIN OF THE DIFFERENCES IN NEUTRALIZING POWERS DISPLAYED BY NORMAL RESTING AND PARAFFINE ACTIVATED SALIVA

a. The relative magnitude of the parts played by diffusible and non-diffusible substances in determining the neutralizing power of saliva

The experiments were carried out as follows: Saliva was titrated and a second set of samples was dialyzed for a period of time; then the liquids both outside and inside the membrane were separately titrated.

The dialysis of the samples was made in these cases where a sufficient quantity of saliva could be obtained without conscious exertion on the part of the patient. Following the method of Clausen (10) and Porter (11) a solution of gun cotton in an ether alcohol mixture was made. From this solution collodion thimbles were fabricated and then placed in recently boiled distilled water until ready for use. From 2 to 5 cc. of the sample were measured directly into the thimble and then placed in a small prescription vial. Boiled distilled water was pipetted into the bottle until the level of the sample exactly coincided with that of the water. Decomposition of the sample was prevented by adding

TABLE 1

Comparison of undialyzed and dialyzed saliva

NUMBER	RESTING SALIVA								ACTIVATED SALIVA							
	Resting saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Activated saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed
	Cubic centimeters of HCl				Cubic centimeters of NaOH				Cubic centimeters of HCl				Cubic centimeters of NaOH			
E 4	6.60	3.00	2.00	5.00	13.75	9.20	4.20	13.40	24.15	16.00	2.65	18.65	4.75	2.85	1.65	4.50
E 1	9.00	6.20	5.40	11.60	9.50	8.25	0.20	8.45	17.75	14.00	0.95	14.95	5.50	4.40	0.69	5.09
E 6	19.50	12.30	1.28	13.58	6.50	6.00		6.00	48.30	46.50	3.40	49.90	1.20			
E 7	8.75	6.50	0.00	6.50	9.80	10.70		10.70	25.20	21.40	2.40	23.80	4.55	3.40	1.00	4.40
E 9	10.70	8.10	1.00	9.10	7.30	4.85	0.85	5.70	15.80	14.45	1.15	15.60	3.10	3.00		3.00
E10	14.00	9.25	3.15	12.40	5.00	4.90	0.75	5.65	16.35	13.70	2.00	15.70	4.80	2.10	2.25	4.35
E11	17.45	10.25	7.00	17.25	7.40	3.30	0.90	4.20	19.10	18.30	3.00	21.30	2.90	1.75	1.00	2.75
E12	7.25	4.00	3.15	7.15	11.75	8.10	2.70	10.80	19.90	16.70	2.20	18.90	4.85	3.10	0.95	4.05
E13	8.50	4.85	3.20	8.05	12.40	9.60	3.00	12.60	22.70	20.10	1.50	21.60	5.40	4.00	0.90	4.90
E14	16.40	9.70	5.80	15.50	8.90	4.80	3.90	8.70	35.40	23.10	3.40	26.50	1.70	1.00		1.00

one drop of chloroform and one drop of xylol. The bottle was tightly corked and placed in an air tight cabinet for twenty-four hours, at a temperature between 20° and 24°C. At the end of this time the liquid outside the membrane was titrated separately from that inside the membrane. Theoretically, if there were no loss of CO_2, from the sample, the sum of the two titration figures for either alkalinity or acidity should equal the original titration value. But a slight precipitation of phosphates, which is always evident, demonstrates that some loss of CO_2 has occurred. Data based on twelve, thirty-six and forty-eight hour dialyses showed a wider variation than those based on the twenty-

four hour limit and this later time, consequently, was chosen as a standard.

The results of this work are reported in tables 1 and 2. The analyses, although of questionable quantitative value demonstrate, qualitatively, that the greater percentage of alkalinity and acidity is found in that portion of the sample which has dialyzed through the membrane and is due, therefore, to inorganic constituents. With subject No. E4 the alkalinity of 10 cc. of the resting saliva was 6.60 cc. N/200 HCl. After dialysis the alkalinity outside of the membrane was 3.00 cc. and inside the membrane, 2.00 cc. The activated sample exhibited a

TABLE 2

Dialysable proportion of neutralizing power

NUMBER	CONDITION OF MOUTH	NORMAL RESTING SALIVA			PARAFFINE ACTIVATED SALIVA			
		Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Salivary factor
E 4	Immune	20.35	18.40	90.01	28.90	23.15	80.10	70.41
E 1	Immune	18.50	20.05	108.38	23 25	20.04	86.19	79.57
E 6	Immune	26.00	18.58	75.31	47 10	49.90	105.94	55.20
E 7	Immune	18.45	17.20	93.22	29.75	28.20	94.79	62.02
E 9	Carious	18.00	14.80	82.03	18.90	18.60	98.41	95.23
E10	Carious	19.00	18.05	95.00	20.15	20.05	99.50	94.29
E11	Carious	24.85	21.45	86.11	22.00	24.05	109.32	113.00
E12	Immune	19.00	17.95	94.47	24.75	22.95	92.77	76.76
E13	Immune	20.90	20.65	98.80	28.10	26.50	94.31	74.37
E14	Immune	25.30	23.20	91.70	37 10	27.50	74.12	68.20

marked difference for the titration figure of the undialyzed sample was 24.15 and for the dialyzed, 16.00 outside the membrane and only 2.65 inside the membrane. The acidities likewise show the same phenomena, the undialyzed normal resting saliva having a reading of 13.75 cc. N/200 NaOH and the dialyzed 9.20 ouside the thimble and 4.20 inside.

b. The amino-nitrogen yielded by hydrolysis of normal resting and activated saliva

In the utilization of the Van Slyke apparatus for the determination of the amino-nitrogen in the saliva the author has employed a method

which combines accuracy with simplicity. The wide application which this apparatus has found in blood analysis recommends it favorably to the problem at hand. The procedure outlined in Hawk (14) was followed with a few modifications. The technique of the analytical work was performed by Mr. S. A. Waksman and I take pleasure in acknowledging his service.

It was at first thought best to analyze the samples as soon as they were obtained from the patient but this procedure is open to objection on account of the fact that there is so small an amount of gas evolved in the reaction that accurate readings of the gas volume are exceedingly difficult. Since it has been the custom in the salivary work to secure the material between eight and eleven in the morning it was found inconvenient to make the determinations at the same time. To overcome these objections all the samples were hydrolyzed. Ten cubic centimeters of well mixed saliva were measured into a special digestion tube and 4 cc. of concentrated HCl were added. The tube employed was of soft glass about nine inches long and one inch wide. One end was sealed off and the other drawn out until the diameter was reduced to nearly $\frac{1}{4}$ inch. After the introduction of the sample and the addition of the acid the small end was sealed. These tubes closed at both ends were placed in a water bath and digested at 100°C. for four hours. Attempts to digest the samples by boiling over a flame and using a reflux condenser were unsatisfactory as bumping and loss of the material could not be controlled.

Having prepared the Van Slyke micro-apparatus in the usual manner 2 cc. of the well mixed hydrolyzed sample were carefully transferred to the measuring tube and run into the decomposing bulb. The bulb was shaken for ten minutes and the NO absorbed by the permanganate solution. The volume of nitrogen was read and the room temperature and barometer noted. Duplicate determinations were made and, in many instances, triplicates.

The results of the analyses are reported in tables 3 and 4 and represent the cubic centimeters of amino nitrogen at standard pressure and temperature yielded by 10 cc. of sample. This calculation was made so that the data would be comparable to those of the titration experiments. Inspection of the table shows that, in the greater percentage of those cases which were classed as immune from dental caries, the nitrogen content of the normal resting saliva is appreciably higher than that of the paraffine activated saliva. Those samples, however, taken

from the mouths in which caries existed did not exhibit the same difference. These facts substantiate the work done on the dialysis experiments. For it was shown that the increase of total neutralizing power in paraffine saliva from immune cases was due to a larger amount of inorganic constituents. These later tests demonstrate that a smaller percentage of organic bodies, represented by the amino nitrogen values, is contained in this same type of saliva and that therefore the difference in titration equivalents must be due to inorganic material.

TABLE 3

Caries

DATE	SERIAL NUMBER	NORMAL RESTING, CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE
September 18, 1916	G 2	2.20	3.00	
September 21, 1916	G 2	2.50	3.00	
September 25, 1916	G 8	3.70	3.80	
October 27, 1916	G28	3.70	3.60	
October 27, 1916	G29	3.70	3.60	
October 27, 1916	G31	4.30	4.30	
November 2, 1916	G36	4.10	3.60	
November 2, 1916	G37	4.20	3.80	
September 25, 1916	G 9	3.10	3.30	
October 5, 1916	G 9	3.00	3.00	
October 30, 1916	G 9	3.40	2.80	
September 29, 1916	G10	3.30	3.40	3.80
November 8, 1916	G10	4.15	4.27	
November 10, 1916	G10	3.77	3.63	
November 10, 1916	G18	2.80	2.95	
November 7, 1916	G18	3.10	3.20	
November 9, 1916	G18	4.10	4.00	
November 4, 1916	G38	3.90	3.80	
November 5, 1916	G42	4.20	4.10	
November 8, 1916	G44	4.40	4.50	
November 15, 1916	G 9	4.27	3.70	
November 20, 1916	G 9	5.80	3.57	
November 23, 1916	G10	4.02	4.02	
November 2, 1916	G35	4.10	4.00	
November 4, 1916	G43	5.90	4.87	3.90
November 21, 1916	G52	4.48	4.84	
November 21, 1916	G53	3.80	4.10	
November 23, 1916	G58	3.12	3.39	

TABLE 4

Immunity

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
September 16, 1916	G 1	2.40	2.30	2.20
September 19, 1916	G 1	3.20	2.90	
September 19, 1916	G 3	4.60	3.80	3.50
September 26, 1916	G 3	5.70	3.30	
September 21, 1916	G 4	3.00	2.60	
September 28, 1916	G 4	3.50	4.00	3.20
October 9, 1916	G 4	3.80	3.20	3.40
September 21, 1916	G 5	2.90	2.70	
September 21, 1916	G 5	3.20	3.00	
September 28, 1916	G 5	3.70	3.90	4.60
October 9, 1916	G 5	3.60	3.00	2.90
October 3, 1916	G 6	3.40	2.60	
October 3, 1916	G11	3.40	3.10	
October 9, 1916	G11	4.90	4.10	
October 3, 1916	G12	5.00	4.10	
October 9, 1916	G12	4.20	3.80	
October 9, 1916	G14	4.00	3.40	
October 9, 1916	G15	4.20	3.20	
October 13, 1916	G16	3.90	3.70	
October 13, 1916	G17	4.60	4.00	
October 20, 1916	G19	2.90	2.30	
October 20, 1916	G20	3.60	3.40	
October 20, 1916	G21	3.50	2.70	
October 20, 1916	G22	4.20	3.40	
October 20, 1916	G23	4.50	3.40	
October 23, 1916	G24	4.10	3.70	
October 25, 1916	G24	4.20	3.90	
October 27, 1916	G24	4.10	4.00	
October 25, 1916	G25	5.60	2.90	
October 25, 1916	G26	5.60	3.00	
October 28, 1916	G26	4.30	3.60	
October 28, 1916	G32	5.10	4.30	
October 28, 1916	G33	5.60	4.50	
October 28, 1916	G34	4.30	3.80	
November 2, 1916	G34	4.20	3.90	
November 18, 1916	G26	5.80	4.10	
November 11, 1916	G26	5.75	4.00	
November 4, 1916	G39	4.80	4.10	
November 4, 1916	G40	4.60	4.15	
November 4, 1916	G41	5.70	4.05	
November 11, 1916	G45	4.87	4.63	

TABLE 4—Continued

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
November 11, 1916	G46	4.80	4.00	
November 11, 1916	G47	6.24	4.37	
November 11, 1916	G48	4.97	3.97	
November 13, 1916	G48	4.63	4.05	
November 14, 1916	G48	4.70	4.13	
November 15, 1916	G48	4.90	4.06	
November 16, 1916	G48	5.04	4.20	
November 17, 1916	G48	3.51	3.30	
November 23, 1916	G25	5.25	3.10	
November 24, 1916	G25	5.44	3.19	
November 24, 1916	G26	3.80	3.20	
November 13, 1916	G26	5.80	4.10	
November 15, 1916	G26	5.75	4.00	
November 6, 1916	G49	4.97	4.14	
November 13, 1916	G49	4.87	4.14	
November 14, 1916	G50	5.50	4.06	
November 17, 1917	G50	5.21	3.99	
December 4, 1916	G55	5.93	3.67	
December 11, 1916	G55	5.70	3.67	

On averaging these tables, the following figures are obtained:

Immunity normal resting saliva	4.47 cc. amino N
Immunity paraffine activated saliva	3.63 cc. amino N
Difference	0.84
Equals	20.7 per cent of the mean
Caries normal resting saliva	3.89 cc. amino N
Caries paraffine activated saliva	3.72 cc. amino N
Difference	0.17
Equals	4.5 per cent of the mean

The difference in nitrogen content between the normal resting saliva in cases of immunity and that in caries is strikingly brought out by the above tabulation. In the determination of averages however there is always an error which must be measured before the data may be considered conclusive.

The determination of the standard deviation of the mean demonstrates, however, the reliability of the results presented. These calculations were evaluated from the formula given by Davenport (15) as follows:

$$\text{Standard deviation} = \sqrt{\frac{\text{sum of the squares of the deviation from the mean}}{\text{number of measurements}}},$$

$$\text{Probable error of the mean} = 0.6745 \times \frac{\text{standard deviation}}{\sqrt{\text{number of measurements}}}.$$

The appended table thus summarizes the data:

	CARIES	IMMUNITY
Standard deviation normal resting saliva	0.645	0.913
Probable error	0.08	0.07
Equals	2.1 per cent of the mean	1.8 per cent
Standard deviation paraffine saliva	0.5564	0.569
Probable error	0.0735	0.0496
Equals	2.0 per cent of the mean	1.4 per cent

It is evident from these figures that the difference between the yields of amino nitrogen by normal resting and activated saliva in persons afflicted with dental caries is only twice the probable error of the mean; in other words, that there is either no difference, or at the most only a slight one between the average protein content of normal resting saliva and activated saliva secreted by such persons. In normal individuals, on the contrary, the difference between the amino nitrogen yields is no less than ten times the probable error of the determination and it is evident that in such persons there is a very definite divergence of composition between normal resting and activated saliva. The normal resting saliva of a person with caries approaches, in protein content, the activated saliva of a normal person, and stimulation by chewing paraffine results in little change either in protein content or in neutralizing power of the saliva secreted.

These relations are illustrated in the appended table showing the average neutralizing powers (that is the number of cubic centimeters of N 200 acid and alkali required to change 10 cc. of saliva from neutrality to phenolphthalein to neutrality to paranitrophenol) of normal resting and activated saliva in normal persons and in persons afflicted with caries. These averages are compiled from salivary analyses completed within the last two years and comprise data obtained from over one hundred individuals. It is evident that saliva from subjects with

carious teeth presents two distinct differences from saliva of immune subjects, namely, that the neutralizing power of the resting saliva is supernormal while that of the paraffine activated saliva is subnormal.

Average total neutralizing power

	NORMAL RESTING SALIVA	PARAFFINE ACTIVATED SALIVA	AVERAGE SALIVARY FACTOR
Immunity	23.693	38.324	61.82
Caries	30.096	30.952	97.24

The normal resting saliva of persons with carious teeth is, therefore, characterized by (1) a relatively high neutralizing power and therefore, presumably, (2) a high content of diffusible substances; (3) a low content of proteins.

TABLE 5

Comparison of values of amino nitrogen with the total neutralizing power

NUMBER	NORMAL RESTING SALIVA				PARAFFINE ACTIVATED SALIVA				
	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	Salivary factor
	cc.	cc.		cc.	cc.	cc.		cc.	
G24	17.10	5.00	22.10	4.10	38.50	1.00	39.50	3.70	55.95
G26	27.40	4.70	32.10	5.60	58.45	−1.50	56.95	3.15	56.40
G26	20.00	4.50	24.50	4.30	49.00	0.50	49.50	3.60	49.50
G27	15.00	12.90	27.90	8.00	41.10	1.75	42.85	4.90	65.10
G 9	27.75	2.55	30.30	3.40	36.55	1.50	38.05	2.80	79.60
G45	28.50	−1.60	26.90	4.87	57.60	−4.15	53.45	4.63	50.30
G48	34.75	2.15	36.90	4.63	47.55	2.50	45.05	4.05	81.90
G48	24.00	3.30	37.30	4.90	35.00	0.60	35.60	4.06	76.70
G49	16.10	5.70	21.80	4.97	35.50	1.25	36.75	4.14	59.30
G40	18.40	3.70	22.10	5.50	31.60	2.30	33.90	4.06	65.20
G52	33.00	8.00	41.00	4.48	40.65	1.00	41.65	4.84	98.45
G53	24.15	3.95	28.10	3.80	30.80	2.80	33.60	4.10	83.60
G58	7.70	8.00	15.70	3.12	32.45	1.30	33.75	3.39	46.50
G58	11.90	6.00	17.90	4.10	33.45	1.25	34.70	3.70	51.60
G59	24.55	2.75	27.30	3.19	26.70	0.90	27.60	3.00	98.90

The inter-relation between the amino nitrogen and the salivary factor is shown in table 5. Subjects 52 and 59 were classed as carious, the others as immune. This connection of the one to the other is rendered all the more striking when the results of the dialysis experiments

are kept in mind, for the data evaluated in table 1 pointed to the fact that the increase of the total neutralizing power is due primarily to an increased amount of inorganic substances. Conversely, the lowered amount of organic bodies in activated salivas coupled with their greater neutralizing power brings further evidence to substantiate this conclusion.

From these results it is evident that immune persons secrete in response to stimulation by chewing tasteless substances a saliva which has a greater neutralizing power than normal resting saliva and is furthermore differentiated from normal resting saliva by a considerably lower content of protein and higher content of inorganic salts. The alteration in the character of the saliva is not merely due to dilution, consequent upon more rapid secretion, but involves a marked change in the relative proportion of the constituents. Persons with carious teeth differ from normal persons in that their normal resting saliva approximates in composition and neutralizing power to the composition of the activated saliva, in other words the salivary glands of such persons behave as though they were constantly receiving stimulus analogous to that constituted by the act of chewing a tasteless substance. Such a stimulus might conceivably be provided by carious teeth themselves, or on the other hand, both conditions may be attributable to a common underlying cause.

Part 2

Relationship of dental caries and the composition of saliva to dietary conditions and the locality and nature of stimuli promoting secretion

a. The relation of diet to the incidence of caries

The alteration in the salivary factor may be due to either a direct or an indirect cause. If the former, then the presence of dental caries in an otherwise healthy mouth would initiate the change. If the latter, then the change is either incidental or comprises a portion of a vicious circle.

Among the indirect factors which may initiate an acute disturbance may be mentioned diet, a lesion or an infection, a chronic peripheral nervous affection, a central nervous affection or lastly a defect in the processes of repair and growth correlated with a defect in salivary function.

Diet comprises per se two factors, namely composition and taste.

The former has been the subject of much discussion and research and the conclusions reached by the many authorities appear to be rather negative in character. Data hereinafter reported will deal more particularly with this phase.

It has been shown (6) that the salivary factor is constant in certain types of insanity to the same extent as in the normal individual and from this fact the deduction may be made that differences in neutralizing power are not related to the central nervous system.

In reviewing the literature concerned with the problems of dental caries and its possible relation to habits of cleanliness, climatic conditions, diet and general health of an individual or of a race, one notes a lack of uniformity in the recorded observations. This may be attributed to the fact that the many different writers were influenced in their judgment by different standards of observation, so that teeth which superficial examination would designate as non-carious might disclose, upon a more thorough examination, exactly the opposite condition.

Pickerill (1) states that certain food investigations point to the fact that the modern dietary of the civilized world differs from the diet of the uncivilized world in that the former is less hard but more tough and requires, therefore, more triturating but less crushing. From this conclusion the idea is advanced that, since different sets of muscles are used in triturating than in crushing, the over-development of these triturating muscles (buccinator and pterygoids) is responsible for both the abnormally shaped as well as the undeveloped dental arch. This, in turn, accounts for the increasing number of malposed teeth which accompany the under-developed arch. Malposed teeth are very susceptible to dental caries and therefore the increase of this disease among the modern civilized nations is correlated to our changed habits in masticating. Pickerill notes further that of the races of the world, the meat eaters, or at least those whose food is largely protein in character, were quite as susceptible to caries as those whose diet was mainly vegetarian. This is contrary to the views expressed by both Mummery and Patrick. The argument advanced by Pickerill is that the immune races, which include according to some authorities the Asiatics, Africans, Polynesians, Australians, et cetera, owe their comparative freedom from caries to the fact that their diets were both varied and sapid. Their universal use of masticatories resulted in the prevention of stagnation within the oral cavity (p. 314) a fact which other investigations appears to support.

Dr. R. Thurnwald, in speaking to the author of his anthropological researches in New Guinea, mentioned that the inhabitants in that section of the world seem to be comparatively free from dental caries. Their diet is mainly vegetable consisting of yams, sago, rice and sugar cane, etc.; meat is rather an accessory and, with the exception of the rather scarce mango, there are no acid fruits. The custom of chewing the betel nut plays, unintentionally, an important part, no doubt, in their oral prophylaxis. For at the age of puberty this custom, often connected with one of the initiatory ceremonies, is commenced and continued throughout the life of the individual.

Contrary to the usual belief, this betel nut habit does not blacken the teeth. For that purpose there is employed a mixture of cocoanut oil and soot which is vigorously rubbed on the teeth.

The betel nut is not used alone but is combined with seeds or leaves of a peppery nature together with pulverized lime from calcined shells. These three substances are taken into the mouth one after the other and are masticated between meals. The old men are edentulous and prepare a paste by mixing the material before chewing it. The natives expectorate profusely after the use of this mixture and the saliva is colored blood red. This coloration might be ascribed to the bleeding or to a compound formed by the action of the lime on the betel nut. The teeth are lost between the ages of forty and fifty and are exfoliated comparatively rapidly once the process has commenced. This exfoliation is accompanied by swollen and bleeding gums.

Calculus is deposited upon the teeth in almost unbelievable quantity, and it is often the case with the adults to see the size of the lower incisors increased by these concretions to 300 or 400 per cent.

Underwood (2) after examinations of skulls from different collections makes the following comment:

> In the hot belt of the earth including India, Africa and Southern China, bathing and washing are natural habits because of the heat; rinsing the mouth after meals and the use of sticks, toothpower, ashes and salt for cleansing the mouth is almost universal among the natives; while the food is largely rice and no alcohol is used. In all of them caries is so rare that to all intents and purposes the natives may be regarded as immune.

He states further that the people of the arctic regions whose personal habits, at least in regard to the care of the mouth, leave much to be desired, and whose diet is quite different, likewise enjoy immunity. He considers the Australian native equally immune. These observations lead him to conclude that the use of artificial foods and the re-

placing of breast feeding exerts a direct influence in the "weakening of the tooth defences." Just what constitutes "tooth defence" is not mentioned.

The effect of certain drugs upon the teeth has been dealt with by Austen (3). The systemic conditions which are supposed to favor the development of caries are anemia, dyspepsia, pregnancy, acute rheumatism, enteric and other continued fevers. Various salts and compounds of mercury, lead, bismuth, silver and copper were used in the experiments. Although it was found that the drug was partly excreted into the oral mucosa, yet it is rather an open question whether this excretion at one time may be so long continued as to accelerate or even cause any deleterious effect upon the erupted teeth. Another point however which may well be considered, is that the frequent drugging of growing children promotes a disturbance in the nutrition of the ameloblast and of the odontoblast thereby bringing about structural changes in the enamel and dentin respectively. Histological examinations conducted along this line of experimentation would undoubtedly throw light upon certain phases of present therapeutic methods.

In table 6 are presented certain abstracts and notations upon the teeth and diet of a few races from different parts of the world. Definite information on the subject appears to be rather scattered for in many instances an author may detail the foods at great length, the manner of cooking and habits of eating but will overlook entirely the conditions of the masticatory apparatus. In so far as the relative amounts of protein to carbohydrate in the diet are concerned, the data appear to confirm Pickerill's (1) conclusion in this regard, namely, that the protein eating races are as susceptible to dental caries as those whose food is mainly carbohydrate. On the other hand they appear to negative the popular impression that the teeth of primitive races are relatively immune to caries.

b. Relation of type and locality of stimulus to the neutralizing power of the secretion of saliva evoked by the stimulus

If the chronic disturbance of the neutralizing power be due to a chronic peripheral nervous affection or to a lesion or an infection remote from the salivary glands or teeth, such a disturbance would probably act through nervous reflexes. If the locality of the lesion is important, then by applying a definite stimulus to a circumscribed area in the oral mucosa such nervous irritation so produced might be expected to

TABLE 6

RACE	TEETH	DIET AND HABITS	AUTHORITY
Australian	"Toothache and indigestion common"	Large proteid diet. Flesh sometimes eaten raw. Only vegetable. "Nardu" grass, fern roots, yams	Eagle Bank and Crow, J. Mathieu. David Nutt, London
Australian	Teeth very tender	Chewing fiber from making of twine wears down teeth and makes them tender	The Australian Race, E. M. Curr. Tribunder & Co., London
Australian		Eat almost anything in the shape of animal food	Customs, Rites, etc., of the Aboriginal Tribe of the Gulf of Carpentaria, W. G. Stretton and E. C. Stirling. Trans. Roy. Soc. So. Australia, vol. 17, part 2, p. 240
Australian		Eat all the mammalian fauna	Report of the Horn Expedition, E. C. Stirling. Part IV, p. 51
Northern Australians		Natives eat anything that is edible	Native Tribes of the Northern Territory of Australia, Spencer. MacMillan & Co., 1914, New York
Brazilian	Unusually liable to decay	Milk, cheese, farina, sun dried meat. Food is bolted and all expectorate rather excessively	Highlands of Brazil, R. F. Burton. Tinsley Bros., London
Eesa or Bedoin branch of Somal	Teeth do not project, are poor and stained from surat tobacco	Mutton, milk, butter, rice, millet bread, dates	First Footsteps in East Africa, R. F. Burton. Dent & Co., London

Egyptians,* Ancient and Modern	Toothache common	Diet mainly vegetables, bread of millet or maize; milk, eggs, melons, beans, lupins. The cleaning of teeth a religious rite.	Account of Manners and Customs of Modern Egyptians, E. W. Lane. John Murray, London
Filipinos	Caries present in 20.90 per cent of cases examined	Diet includes great quantities of sugar	Ottofy Lancet, 176, 1909, 263
Friendly Isles	In general good teeth, but poorer than those of other nations	(See natives of Island of Otaheite)	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Gallois Tribe	(See Somal)	Gourds the only vegetable. Habitual drunkards. Habitually use the tooth stick	First Footsteps in East Africa, R. F. Burton. Dent, London
Igorots	Caries present in only 2.05 per cent of a large number of cases examined	Carnotes, a sweet potato, 30 per cent of diet. Rice, 30 per cent of diet. Other vegetables, maize, etc., 30 per cent of diet. Fish, meat and eggs, 10 per cent. Sugar is distasteful and is not used	Ottofy Lancet, 176, 1909. 253
Otaheite	White and regular	Mainly carbohydrate in character. Teeth cleansed six times a day	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Natives of Sarawak	"Toothache and decayed teeth are almost unknown"	Rice, yams, cucumbers, fowls, and fish	Natives of Sarawak and British North Borneo, H. L. Roth. Truslone & Hansone, London, p. 77.
Somal	Scattered teeth considered a sign of warm temperament	(See Eesa)	First Footsteps in E. Africa, R. F. Burton. Dent, London

* Note that Patrick quoted by Pickerell cites the Egyptians as one of the immune races, the ratio of caries in 3,306 teeth examined being only 3.418 per cent.

TABLE 6—Continued

RACE	TEETH	DIET AND HABITS	AUTHORITY
Somal	Teeth do not project, are poor and stained by tobacco	The teeth are cleaned by tooth stick. Flesh, holcus, few vegetables. Millet beer	First Footsteps in East Africa, R. F. Burton. Dent, London
Tasmanians	Among forty-two natives examined all had "very good teeth"	Refuse European food; eat rats, many vegetables of various kinds. Have enormous appetites. Meals are eaten with great greediness and great quantities are eaten at one time. Live chiefly on animal food	Aborigines of Tasmania, H. E. Roth. King & Son, Halifax, England, pp. 8 and 87
Tasmanians	Are reported by Cook, La Billardiere and Widowson as having good teeth		
Tasmanians	Teeth much worn; decay uncommon	Soundness of teeth due to chewing hard and tough material	Daily Life of the Tasmanians, Bonwick
Makololo	Beautiful teeth	General mixed diet, including: – porridge, milk, butter, corn, honey, beer, wild fruit, elephant meat	Narratives of an Expedition to the Zambesi, etc., David and Charles Livingstone. John Murray, London, 1865
Kebrabasa		Fowls, eggs, sugar cane, sweet potatoes, tomatoes, rice, maize; Kaffir corn	Ibid.
Manganja		Millet, maize, beans, nuts, yams, rice, pumpkins, cassava, sweet potatoes, etc.	Ibid.
Matumboka	"Teeth here are more solid and often wear down to the gums in old persons without decay. Cases of toothache not at all uncommon nevertheless"	Mixed diet	Ibid.

influence the neutralizing power. One of the easiest methods of stimulation is the use of an electric current which has been passed through an inductorium, and experiments along these lines were projected.

In this series of experiments it was desired to determine what comparative differences would result in titratable acidity and alkalinity by the use of the electric current at different parts of the oral mucosa. It has been demonstrated that the mechanical stimulus obtained by the chewing of paraffine excites a flow of saliva which is markedly different from that found normally in the mouth. With the employment of the electric current, obtained from an inductorium, a third sample was secured which differed in titration value from either the normal resting or paraffine saliva. The amount of current used and the locality at which it was applied did not appear to produce any marked deviation from the general result. Although the strength of current varied with different individuals, only that strength was used which at the end of two minutes produced a tingling sensation at the point of contact. Whenever this amount was appreciably increased it was found to be prejudicial to salivary activity, as an unnecessary nervous tension was thus produced.

The apparatus consisted of two Edison Lelande cells, type Z connected in series with an inductorium and a key. The electrodes consisted of two platinum points mounted on a vulcanite handle. The electrode was applied to the mucous membrane at some predetermined point and the saliva thus obtained titrated in the usual manner. The different localities at which the electrode was applied were, first, the opening of Stenson's duct opposite the upper second molar in the buccal mucosa; second, the openings of Wharton's ducts and the ducts of Bartholin on either side of the frenum linguae; third, on the dorsum of the tongue at the juncture of the posterior with the middle third near the apex of the V formed by the convergence of the two lines of the circumvallate papillae; fourth, at the gingivae. In applying the current at the bilateral structures one side was stimulated for two minutes and then the opposite side. No inflammation of the mucosa at the point of contact was developed at any time. The results of these experiments are reported in table 7. In the first column is noted the serial number of the patient, in each column "A" the alkalinity of 10 cc. of sample expressed in cubic centimeters of N/200 HCl.; in "B" the acidity of 10 cc. sample expressed in cubic centimeter of N/200 NaOH., and in "C" the total neutralizing power. The salivary factor appears whenever the paraffine saliva was taken; the distance of the

TABLE 7

"Electrical stimulus"

NUMBER	NORMAL RESTING SALIVA			PARAFFINE STIMULATED SALIVA			ELECTRICALLY STIMULATED SALIVA			FACTOR	COIL	DATE
	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.			
	A	B	C	A	B	C	A	B	C			
					Stenson's duct							
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*			*cm.*	
E 1	14.05	5.25	19.30	25.50	2.35	27.85	4.80	16.50	21.30	69.30	27.50	December 28, 1915
E 2	15.30	5.80	21.10	34.40	3.25	37.65	18.20	5.50	23.70	56.50	25.50	January 5, 1916
E 2	4.10	15.30	19.40	24.50	3.40	27.90	9.45	11.00	20.45	69.50	27.00	December 28, 1915
E 4	6.60	13.75	20.35	24.15	4.75	28.90	7.55	17.75	25.30	70.40	30.00	January 7, 1916
E 5	17.70	24.75	42.45	32.75	3.35	36.10	47.80	8.50	26.30	117.60	27.00	January 10, 1916
E 6	19.50	6.50	26.00	48.30	−1.20	47.10	25.45	2.50	27.95	55.20	27.00	January 8, 1916
E 7	8.75	9.80	18.55	25.20	4.55	29.75	10.65	7.00	17.65	62.40	27.00	January 12, 1916
G 8	20.40	3.30	23.70	39.80	0.50	40.30	27.10	11.30	38.40	58.80	27.00	September 1, 1916
					Wharton's and Bartholin's ducts							
E 1	9.00	9.50	18.50	17.75	5.50	23.25	10.75	5.30	16.05	79.60	30.00	January 5, 1916
E 2	16.95	1.30	18.25	34.70	−3.75	30.95	20.15	−1.50	18.65	58.90	32.00	January 12, 1916
E 4	17.80	4.60	22.40	19.40	8.25	27.65	15.90	7.10	23.00	81.00	29.50	January 8, 1916
F 1	16.25	6.95	23.20	29.50	2.70	32.20	19.05	6.25	25.30	72.09	30.00	January 15, 1916
F 2	21.10	3.40	24.50	34.20	1.90	36.10	19.30	3.10	22.40	67.90	29.75	January 16, 1916
F 3	20.10	3.10	23.20	24.15	2.75	26.90	15.00	2.30	17.30	86.20	29.50	January 17, 1916
F 3	16.10	6.25	22.35	22.00	1.85	23.85	14.40	2.50	16.90	93.70	29.50	January 18, 1916
F 5	17.70	4.50	22.20	37.40	2.50	39.90	14.25	3.95	18.20	55.70	29.00	January 18, 1916
G 7	22.85	2.50	25.35				19.90	2.70	22.60		29.50	February 22, 1916
G 8	15.15	6.50	21.65	44.85	1.50	46.35	27.55	11.00	38.55	46.71	27.80	July 22, 1916
G 8	26.05	7.75	34.80	44.55	3.10	47.65	26.40	11.80	38.20	74.70	26.00	August 16, 1916

Tongue												
E 1	8.70	10.50	19.20	15.75	4.85	20.60	11.35	4.45	15.80	93.20	28.00	January 22, 1916
E 4	11.00	14.70	25.70				12.50	8.40	20.90		28.50	November 11, 1915
Gingivae												
E 6	18.25	7.60	25.85				22.95	3.30	26.25		31.00	January 10, 1916
E 7	10.50	8.15	18.65				12.25	5.75	18.00		29.50	January 13, 1916
G 43	17.25	8.00	25.25	21.60	2.75	24.35	16.55	5.35	21.90	103.70	25.50	November 5, 1916
E 5	18.40	23.55	41.95				25.55	6.90	32.45		31.00	January 11, 1916

secondary coil from the primary shows the comparative strength of the current and is expressed in centimeters.

The data submitted demonstrate first, that the alkalinity of the electrically excited saliva is lower than that of the paraffine sample; second, that the acidity of the former is relatively higher than that of the latter; and third, that the neutralizing power of the saliva obtained by electric stimulus is lower in every instance than that collected by the paraffine method. This fact indicates that the use of the inductorium does not promote salivary activity to the same extent as the paraffine.

It was hoped that differential areas of irritation in the mouth could be demonstrated by means of this electrical stimulation for it was desired to determine whether one part of the oral mucosa is more sensitive to extraneous influences than another, so that a local irritation in one circumscribed locality would tend to produce a greater change in the salivary neutralizing power than in another locality. The experiments, however, so far reported, have failed to supply any conclusive evidence.

The repeated use of the inductorium on one individual produces a marked effect upon the relationship of the normal resting saliva to the activated paraffine saliva. This electric irritation alters the factor in a few days from one which is comparatively low to one with a much higher valuation. As examples of this condition may be cited subjects E1, E4 and G8. In the first instance the experiment was commenced on December 28, 1915. The salivary factor at this time was 69.3. Electrical stimulation was applied and after eight days a second test was made. The normal resting saliva and the paraffine saliva were first collected and the application of the current repeated. It was found that the factor rose steadily from 69.3 to 79.6 and finally to 93.2. Similarly with E4 the factor at the start was 69.5 but rose in eleven days to 81.0. For subject G8 the factor evaluated on July 23, 1916 was 46.7; on August 1 it had risen to 74.7. One month later it had returned to nearly the same ratio as at the start and duplicate samples on successive days gave a factor of 58.8. The results reported are based on duplicate analyses and on duplicate samples obtained on successive days.

The determination of the reaction of the taste impulses upon salivary secretion was attempted by comparing the results obtained with a normal resting sample with those secured by the use of different substances of marked taste. Howell (12) and other investigators describe the taste sense as being composed of four fundamental sensations, namely, bitter, sweet, acid and salty. Tastes other than these are combinations of any two or more of the primary sensations and produce, therefore, a

mixed stimulation of the sense organ. There was used for this experiment quinine on one day and sucrose on the second or third day following.

In securing the samples there was collected first a resting saliva and then the saliva secreted from the use of the taste stimulant. The results are reported in table 8. In the first column appears the subject number. Figures in columns A, B and C represent respectively the alkalinity, acidity and total neutralizing power of each sample. It may be stated in general that the action of sucrose, the sweet stimulant, tends to lower the total neutralizing power of the saliva. Quinine, the bitter stimulant, appears to produce the opposite condition yielding saliva resembling that secreted in response to the stimulus of chewing

TABLE 8

Comparison of effects of different types of stimulation upon salivary secretion

NUMBER	SALIVA NORMAL RESTING			SALIVA FROM QUININE STIMULUS			SALIVA FROM SUCROSE STIMULUS		
	A	B	C	A	B	C	A	B	C
F1	16.25	6.95	23.20	20.65	4.55	25.20	13.80	10.70	24.50
F2	19.55	4.20	23.75	33.95	2.00	35.95			
F2	25.50	5.00	30.50	25.80	3.75	29.55	17.15	8.00	19.15
F2	21.15	7.50	28.65				18.45	5.50	23.95
F3	20.70	3.70	24.40				13.25	8.00	21.25
F3	18.40	5.40	23.80				9.95	8.25	18.20
F4	19.25	4.75	24.00				15.00	6.20	21.20
F5	19.10	5.75	24.85				10.65	8.00	18.65
G7	22.85	2.50	25.35				12.60	3.60	16.20
F1	18.05	4.70	22.75	19.60	13.40	33.00	13.85	7.00	20.85

paraffine. The well known work of Miller (13) demonstrates that fermenting carbohydrates such as would be found on the teeth tend to promote caries. From the above experiments it would be logical to infer that saliva favors the condition as well, since the neutralizing power of the sucrose-stimulated saliva approaches that of the normal resting saliva, and a factor evaluated on this basis would be of a magnitude corresponding to that which under the paraffine stimulus would indicate the presence of caries.

SUMMARY

Dialysis of saliva shows that the total neutralizing power is chiefly due to inorganic constituents.

The use of the Van Slyke apparatus in the determination of amino-nitrogen in saliva is a new application of this method. The results so

obtained show that there is a definite correlation between the concentration of inorganic constituents, the amino-nitrogen content, and the neutralizing power of saliva; namely, that a high neutralizing power is associated with a correspondingly high percentage of inorganic constituents and with a low percentage of protein.

Data are presented which confirm Pickerill's observations concerning the effect of different constituents of the diet upon dental caries.

The use of the electric current as a salivary stimulant excites a secretion markedly low in alkalinity and correspondingly high in acidity when compared to the saliva resulting from the paraffine stimulus. The neutralizing power of saliva secreted in response to electrical stimuli is less than that secreted in response to the chewing of paraffine.

Differential areas of stimulation in the oral mucosa cannot be demonstrated.

The comparison of analyses of saliva obtained by a sweet stimulus (sucrose) with that obtained by a bitter stimulus (quinine) proves that the former yields a saliva low in neutralizing power and that the latter produces the opposite result.

In conclusion I wish to express my deep appreciation to Dr. T. Brailsford Robertson for his interest evinced throughout the work as well as for the valuable suggestions offered. My thanks are also due to those students and clinic patients who have provided the necessary material for the experimental purposes.

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BALTIMORE, U. S. A.