From: The Laboratory of the County Hospital, Holbaek, Denmark

OXYGEN UPTAKE OF THE SALIVARY MICROFLORA AND I TSRELATION TO CARIES ACTIVITY

I. The oxygen consumption without added substrates

by

H. Eggers Lura

INTRODUCTION

The oxygen uptake of saliva was first demonstrated by the author in 1944 (13). *Hartles* and *McDonald,* 1953 (8), *Burnett,* 1952 (2), 1954 (3), *Bramstedt* and *Vonderlinn,* 1952 (1), *Naujoks* and *Vonderlinn,* 1953 (15), *Naujoks* and *Kröncke,* 1954 (14) and *Eggers Lura,* 1954 (12) have also reported on salivary oxygen uptake. The latter five authors reported that caries resistance was accompanied by a high respiration rate. *Kröncke* and *Naujoks,* 1954 (10) used the oxygen uptake of saliva as a new caries test and demonstrated its clinical usefulness. The negative results of the former authors with respect to correlation with caries may be explained by several factors: *Hartles* and *McDonald* used paraffin stimulated saliva, while the latter authors used unstimulated, fasting saliva which seems to have a more concentrated and caries-related bacterial content. Moreover, *Hartles* and *McDonald* as well as *Burnett* used either the DMF index or the Snyder test both of which are rather uncertain criteria of caries activity. As several factors connected with salivary oxygen uptake still seem to be insufficiently elucidated and in view of the importance of this problem not only in caries pathogenesis, but also in general oral hygiene, the author has deemed it appropriate to make further investigations.

EXPERIMENTAL

Material and Technique

Special efforts were made to obtain salivary samples from persons who might be designated as physiologically "caries-active" or "caries-resistant". To procure uniform material persons of almost the same age group (5—16 years) were chosen. Most of the children were from the author's own practice. Some were from a state reformatory where the prevalence of caries is very low and where the children are living under very healthy and regular dietary conditions. Only salivas from persons who had been observed through a period of more than one year were used. Those designed as *C-R* (caries-resistant) did not show any new caries during the experimental period of one year. Most of these were completely caries-free, especially those from the reformatory, and the others only had very few old fillings.

Those in the *C-A* (caries-active) group showed the well-known picture of acute, rampant caries and both the number and the size of cavities increased during the experimental period. Due to this strict classification the formation of two further groups appeared necessary. Those grouped as *C-R ?* (questionably caries-resistant) developed certain caries-like alterations e.g. fissure pigmentation during the experimental period. The individuals classified as *C-A ?* (questionably caries-active) had offered the impression of caries activity at the first examination, but later inspection showed no alterations with respect to caries.

A clear distinction was made between artificially *stimulated* and *unstimulated* (or rather minimally stimulated) saliva. Saliva collected in quantities greater than 3 ml during a collecting period of 10 minutes were called maximally stimulated and those of quantities less than 3 ml in 10 minutes unstimulated (or minimally stimulated). As far as possible any storage of saliva was avoided.

For measuring the salivary oxygen uptake a Warburg-manometer, Model P, from B. Braun, Melsungen, Germany was used. The apparatus has 8 double capillary manometers with thermoregulation $\pm$ 0.03° C. A description of the Warburg technique is unnecessary, very good instructions being given in the books by *Dixon* (6) and *Umbreit, Burris* and *Stauffer* (16).

DATA

Effect of storage upon the salivary oxygen uptake

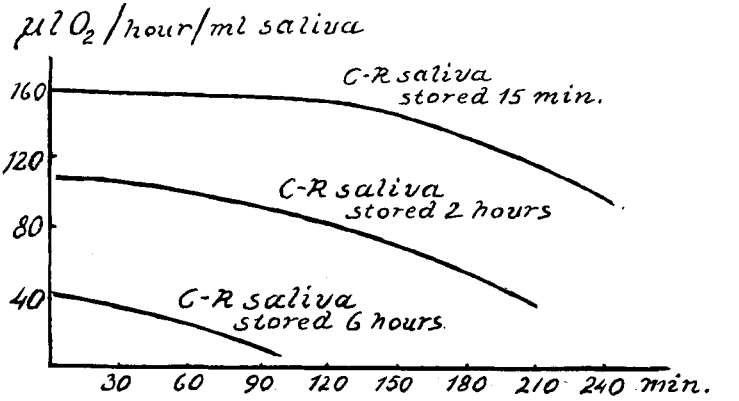

Fig. 1. Used: 1 ml saliva + 0.5 ml phosphate buffer pH 7.2. Temp. 37° C.

Since the air is used as the gas phase in the manometer the saliva must be exposed to air for sufficient time for the CO_2 to equilibrate between saliva and air. In the experiment this equilibration was made at room temperature for 15—30 minutes. The storage of the saliva was made in a thermostate at 37° C. During the first two hours the salivary respiration rate (measured as cmm O_2 uptake per ml saliva per hour) is practically unchanged. After a two hour storage the activity is decreased with 50—100 per cent and after four hours the respiration power has almost disappeared. Consequently, for all the following experiments only salivary samples were used which had not been stored for more than 30 minutes and the experimental time did not exceed one hour.

Diurnal variations of the salivary oxygen uptake

The line graphs (Fig. 2) represent 16 different samples with the following variations:

C-R (stimulated) Mean = 142 ± 16 C-R (unstimulated) Mean = 175 ± 18
C-A (stimulated) Mean = 36 ± 2.2 C-A (unstimulated) Mean = 68 ± 4.5

The highest oxidizing activity was found in the unstimulated (minimally stimulated) morning and fasting saliva.

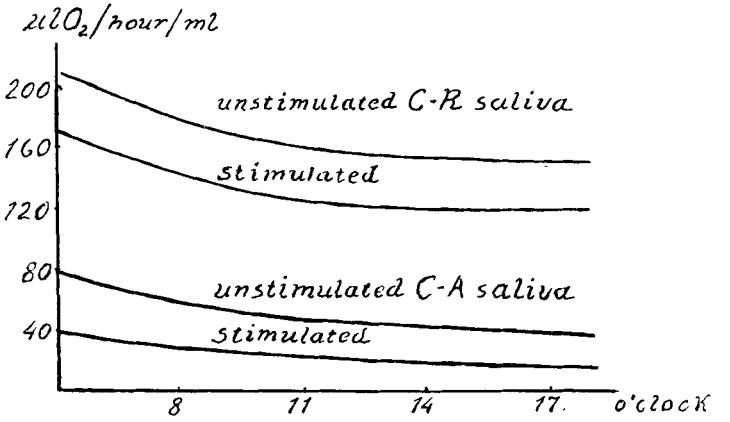

Fig. 2. Used: 1 ml saliva + 0.5 ml phosphate buffer pH 7.2. Temp. 37° C.

The respiration of parotid saliva

Since it is likely that the viable microorganisms of the whole saliva or their enzyme systems are chiefly responsible for the oxygen consumption, the uncontaminated saliva of the parotid glands was obtained by using a device proposed by *Curby*, 1953

Fig. 3. The device of Curby for collecting parotid saliva.

(5). The oxygen consumption of the uncontaminated saliva was then determined for comparison with that of whole saliva obtained from the same person.

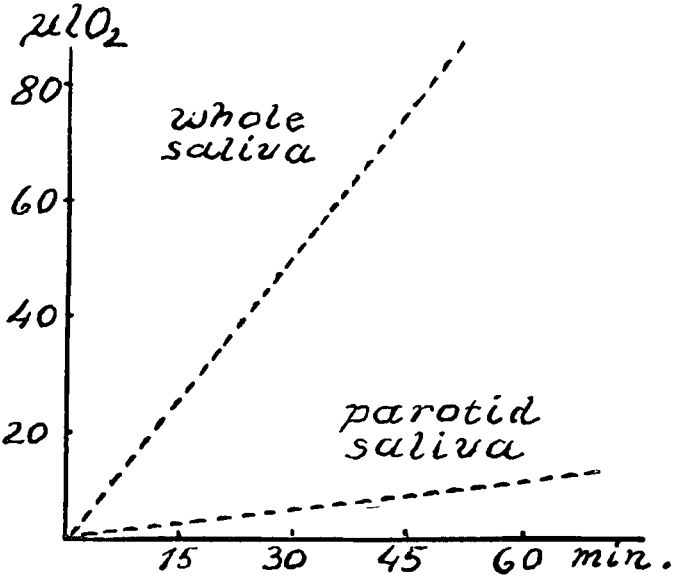

Fig. 4. Used: 0.5 ml parotid saliva + 0.25 ml phosphate buffer pH 7.2.

The uncontaminated parotid saliva only utilizes 5—10 per cent oxygen of that utilized by the microbial flora of the whole saliva. Removal of bacteria by means of filter or centrifuge gives similar results. *Burnett* (3). The faint respiration of pure parotid saliva probably is due to the presence of easily oxidizable substances such as ascorbic acid and citric acid.

The salivary oxygen consumption by inflammations of the oral tissues

The non-specific gingivitis or stomatitis generally shows the characteristics of a serous inflammation with the following main alterations of the biochemical environment: 1) Change of the inorganic ion-milieu, 2) pH changes toward the acid side, 3) Increase of the carbohydrate-metabolism, 4) Alterations of the normal flora. Investigations of the different germ-quotients of the salivary flora from normal persons and persons with oral diseases have been made by *Lammers,* 1953 (11) who was able to demonstrate a surprising uniformity in the ratio between

staphylococci, streptococci, Neisseria, b. acidophilus and the coli-aerogenes group both in healthy and non-healthy mouths, as seen in Table I. It may be noted that the number of the definite aerobes such as Neisseria and staphylococci is considerably increased in the group with oral inflammations in comparison with the caries-active group where the anaerobes and facultative anaerobes such as b. acidophilus and the streptococci are dominating.

Table I, from Lammers (11)

Ratio: Staphyl./streptoc.	*Ratio:* b. acidoph./total bact. number	Neisseria (Gram neg. diplococci)	Coli-aerogenes
Germ quotient of normal saliva (calculated means)			
1 : 0.7	1 : 8000	9.2 %	0.2 %
Germ quotient of caries-active saliva			
1 : 5	1 : 65	2 %	0 %
Germ quotient of saliva from persons with oral inflammations (gingivitis, stomatitis, pharyngitis)			
1 : 2	1 : 4000	12 %	15 %

It has also been demonstrated by *Glickman, Turesky* and *Hill,* 1949 (7) that chronic inflammation of human gingiva will increase oxygen consumption of that tissue. The same could be demonstrated by *Naujoks* and *Kröncke,* 1954 (14).

In the following experiment salivary samples were collected exclusively from persons with oral inflammations such as gingivitis, stomatitis and pharyngitis from random age groups. The material was divided into the four above mentioned groups with respect to caries and the oxygen uptake was measured.

According to the results in Table II it appears that the oxygen consumption of the microflora of saliva from persons with oral inflammations and from different age groups cannot be correlated with caries activity. The bacterial content of saliva in

Table II

No.	Age	Sex	Caries-group	cmm O_2/per ml saliva per hour	Standard deviation	Standard error of mean
1	45	♂	C-R	402		
2	27	♂	C-R	165	109	48
3	8	♀	C-R?	96	of total	of total
4	10	♂	C-R?	212	C-R group	C-R group
5	55	♂	C-R	120		
6	12	♂	C-R	310		
7	18	♀	C-A?	325		
8	27	♂	C-A	110	94.6	42
9	38	♀	C-A?	208	of total	of total
10	14	♂	C-A	68	C-A group	C-A group
11	25	♂	C-A	242		
12	10	♂	C-A	78		

these cases seems to be dominated by the inflammatory microflora rather than by the caries-disposing one. Also the varying age groups may influence the flora as shown by *Lammers* (11).

The salivary oxygen uptake of two caries-resistant and two caries-active groups from a standardized material

The following saliva samples (Table III, A-D) were collected as unstimulated fasting and morning saliva from children aged 5 to 16 years. All the samples were taken from children with healthy oral tissues.

The differences between the means of the two groups may be regarded as significant with 99 % probability, because:

$$M_1 - M_2 > 3 \cdot \sqrt{\delta^2_{M_1} + \delta^2_{M_2}};\ 165 - 54 > 3 \cdot \sqrt{213.4 + 34.8};\ 111 > 48.$$

The normal O_2 consumption and CO_2 output of C—R and C—A saliva

Some of the above experiments were performed with the use of two manometer vessels, one with added NaOH and another without NaOH, in order to measure both the O_2 consumption and the CO_2 output from the same saliva.

Table III

The oxygen uptake of 50 caries-resistant saliva samples

A				B			
C-R (definitely caries-resistant)				*C-R?* (questionably caries-resistant)			
no.	sex	age	O_2/ml saliva/hour	no.	sex	age	O_2/ml saliva/hour
1	M	14	210	1	M	5	74
2	M	11	312	2	F	12	60
3	M	8	140	3	F	6	174
4	F	8	96	4	M	12	87
5	M	12	412	5	M	10	260
6	F	14	182	6	M	15	157
7	M	6	122	7	F	17	160
8	F	10	280	8	M	12	198
9	F	14	160	9	F	8	219
10	M	12	202	10	M	15	80
11	M	10	105	11	M	14	214
12	M	9	216	12	M	11	82
13	F	8	115	13	F	8	220
14	M	16	88	14	M	7	136
15	F	12	138	15	M	16	88
16	M	17	86	16	F	17	118
17	M	15	310	17	M	12	176
18	M	9	210	18	M	10	158
19	F	10	164	19	F	14	316
20	M	16	186	20	M	7	204
21	F	14	101	21	M	13	114
22	M	16	212	22	F	13	148
23	M	14	171	23	M	12	120
24	M	12	144	24	M	8	90
25	M	16	90	25	M	6	152
Mean (M) = 178 cmm O_2/ml/hour				Mean (M) = 152 cmm O_2/ml/hour			
Standard deviation (δ) = 79.7				Standard deviation (δ) = 67			
Standard error of mean (δ_M) = 15.9				Standard error of mean (δ_M) = 13.4			

(cont.)

Table III

The oxygen uptake of 50 caries-active saliva samples

C D

C-A (definitely caries-active)				*C-A*? (questionably caries-active)			
no.	sex	age	cmm O_2/ml/hour	no.	sex	age	cmm O_2/ml/hour
1	M	8	8	1	M	10	36
2	M	16	82	2	F	11	70
3	F	10	42	3	M	8	150
4	M	7	64	4	M	9	45
5	F	15	16	5	F	10	124
6	M	8	26	6	M	12	19
7	M	12	33	7	M	8	105
8	F	15	18	8	M	8	88
9	M	13	72	9	F	12	30
10	F	8	10	10	M	10	24
11	M	9	68	11	M	11	64
12	F	7	24	12	F	8	42
13	M	12	66	13	M	16	88
14	M	11	82	14	M	15	62
15	M	14	28	15	M	16	40
16	M	14	44	16	F	12	68
17	F	12	18	17	M	8	86
18	M	9	6	18	M	6	104
19	M	12	18	19	M	12	58
20	F	9	38	20	M	8	60
21	M	13	74	21	F	12	72
22	M	8	40	22	M	10	30
23	M	10	16	23	M	7	18
24	M	10	50	24	M	11	48
25	M	14	74	25	F	15	51
Mean (M) = 45 cmm O_2/ml/hour				Mean (M) = 63 cmm O_2/ml/hour			
Standard deviation (δ) = 25				Standard deviation (δ) = 34			
Standard error of the mean (δ_M) = 5				Standard error of the mean (δ_M) = 6.8			

Mean of the total caries-resistant group (A)+(B) M = 165 ± 14.6
Mean of the total caries-active group (C)+(D) M = 54 ± 5.9

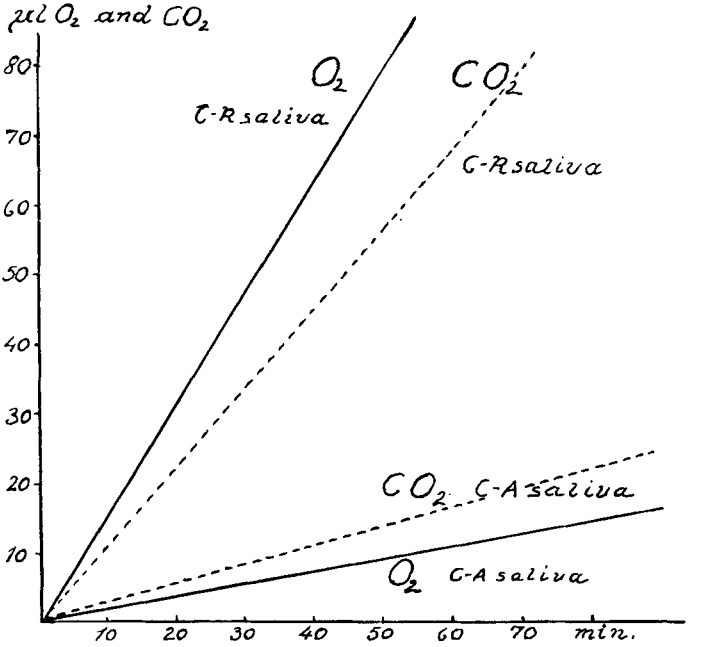

Fig. 5. Used: 1.5 ml saliva + 0.5 ml phosphate buffer pH 7.2. Temp. 37° C. $k_{O_2} = 1.17$; $k_{CO_2} = 1.28$ (Mean values of 15 samples).

Mean $O_2 = 168$	Standard deviation (ϑ) = 71.2	$\vartheta_M = 18.2$
Mean $CO_2 = 135$	Standard deviation (ϑ) = 48.8	$\vartheta_M = 12.5$

Both the O_2 consumption and the CO_2 output occur *proportionally* with time. In normal saliva samples from caries-free persons the respiration quotient $\left(RQ = \frac{CO_2}{O_2}\right)$ is about 0.73. In the saliva samples from caries-active persons the CO_2 output rate is faster than in the caries-resistant types, probably due to slower oxidation of and accumulation of acid which will expel some of the bound carbon dioxide from such saliva.

DISCUSSION

The above results may be interpreted to indicate that a mainly *aerobic* microflora has adapted itself to healthy caries-free mouths, while the caries-active saliva seems to be dominated by an *anaerobic* or facultative anaerobic microflora. [*Clapper* and *Heatherman*, 1955 (4)]. The struggle for life between the dif-

ferent kinds of microorganisms is carried out with metabolic products and in the course of time the interaction between the environment-determining factors will result in the predominance of a special flora in healthy mouths which mainly uses the aerobic pathways for the degradation of the substrates. As long as we do not know all details of caries pathogenesis, it is difficult to classify the bacteria with regard to their caries pathogenicity, and at any rate no single type of bacteria seems to be responsible for the initial attack upon the enamel. If we compare the above results with those of numerous bacteriologic investigations over the past years, it seems likely that the saprophytic microflora of caries-resistant people mainly consists of strains of aerobic staphylococci, gram-negative micrococci (Neisseria), Sarcinae and some aerobic proteolytic bacteria, while the caries-disposing flora mainly consists of anaerobic or facultative anaerobic strains of the lactobacilli and the enterobacteria. [*Lammers,* 1953 (11), *Krasse,* 1954 (9)]. When using the salivary oxygen uptake as a criterion of the presence of a caries-resistant or caries-active microflora, however, we have to standardize the material very carefully. As may be seen from the results of the experiment, Table II, small groups of saliva samples are not valid for correlation with caries, especially as long as we do not pay attention to the general state of health of the oral tissues. The negative results with respect to correlation wih caries obtained by some authors [*Hartles* and *McDonald* (8), *Burnett* (3)] may be explained by the fact, that *Burnett* used pooled saliva and that one single sample from a person with acute gingivitis, stomatitis or pharyngitis may spoil the results. The DMF caries index used by *Hartles* and *McDonald* is a very bad criterion of caries activity. Furthermore, their material originates from an older age group and the age factor seems to play an important role in the salivary microflora. [*Lammers* (11)].

SUMMARY

The oxygen uptake of the salivary microflora of 50 caries-resistant and 50 caries-active children has been measured in Warburg manometer under standardized experimental conditions. Caries resistance was associated with a high salivary respiration rate, the differences between the two groups being clearly signi-

ficant. Inflammations of the oral tissues, however, may influence the salivary oxygen uptake and unless the collection of the salivary samples is postponed until the inflammation has been eliminated, no statistical correlation with caries-activity can be obtained.

RÉSUMÉ

LA CONSOMMATION D'OXYGÈNE DE LA FLORE MICROBIENNE SALIVAIRE ET SA RELATION AVEC L'ACTIVITÉ DE LA CARIE

La consommation d'oxygène de la flore microbienne salivaire a été mesurée par le manomètre Warburg dans des conditions expérimentales standardisées sur 50 échantillons salivaires d'enfants resistants à la carie, et sur 50 autres d'enfants avec une grande activité de carie. La résistance à la carie était associée à une intensité eleveé de respiration salivaire, les differences entre les deux groupes se montrant significatives. Cependant des inflammations des tissus buccaux peuvent influencer la consommation d'oxygène et tant que ces inflammations ne sont pas guéries nulle correlation statistique à la carie ne peut être établie.

ZUSAMMENFASSUNG

DIE SAUERSTOFFAUFNAHME DER SPEICHELMIKROFLORA UND IHRE BEZIEHUNG ZUR KARIESAKTIVITÄT

Die Sauerstoffaufnahme der Speichelmikroflora wurde im Warburg-Manometer unter standardisierten Versuchsbedingungen an 50 Speichelproben von kariesresistenten und 50 Proben von kariesaktiven Kindern gemessen. Die Kariesresistenz war mit einer grösseren Respiration der Speichelmikroflora verbunden, und die Unterschiede der zwei Gruppen waren deutlich signifikant. Entzündungen der oralen Gewebe können jedoch die Speichelsauerstoffaufnahme beeinflussen, und solange die Entzündungen nicht zu Ende gebracht worden sind, können keine sichere statistischen Werte zwischen den zwei Versuchsgruppen erreicht werden.

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Address: *18, Smedelundsgade*
Holbaek
Denmark