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AN INVESTIGATION OF THE RESPIRATORY ACTIVITIES OF ORAL BACTERIA[1]

by

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The anaerobic breakdown of carbohydrates to organic acids has been the part of the metabolism of oral bacteria that has attracted most attention in dental research. The reason for this has been the obvious importance of this activity in the process of dental caries. The uptake of oxygen, the output of carbon dioxide and the processes involved in these respiratory exchanges have, on the other hand, received very little attention. The aim of the present work is to estimate quantitatively the aerobic respiration of oral bacteria. Experiments have also been performed to determine the anaerobic acid production by measuring the amount of carbon dioxide driven out from a bicarbonate solution by the lactic acid formed.

Since the aerobic oxidation of carbohydrates to water and carbon dioxide does not give any products injurious for the enamel, this phase of the chemical activities of mouth bacteria is interesting from the point of view of dental caries. When these bacteria have access to oxygen aerobic oxidation and anaerobic acid production take place at the same time. Under strictly anaerobic conditions there is, of course, only acid production. If some factor or factors which would change most of the metabolism to aerobic oxidation could be introduced, this would have a caries inhibiting effect. In an earlier investigation (*Strålfors*, 1950, page 72) it was found, that the acid production by oral staphylococci was very much decreased, if oxygen was bubbled vigorously through the fermentation liquid containing

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the bacteria, sugar and buffer. This decrease did not take place when lactobacilli were treated in the same way.

PRINCIPLES OF RESPIRATION MEASUREMENTS

The Warburg respirometer is the standard apparatus for measuring the respiration of cells or tissues. It is based on the principle that, if the volume of a gas is kept constant at a constant temperature, every exchange of gas can be determined from the change in pressure (*Warburg*, 1926; *Dixon*, 1943; *Umbreit et al.*, 1947).

If the following symbols are used

V_g = volume of gas in the Warburg flask
V_f = volume of liquid in the Warburg flask
T = the absolute temperature
α = the Bunsen coefficient — milliliters of gas dissolved per milliliter of liquid when the pressure of the gas is one atmosphere
P_0 = a pressure of 760 mm Hg expressed in milliliters of manometer liquid
h = the change of pressure in the manometer reading in mm
x = the exchange of gas measured in microliters at 0° C and 760 mm Hg

the following equation applies

$$x = h \cdot \frac{V_g \cdot \frac{273}{T} + V_f \cdot \alpha}{P_0}$$

$$k = \frac{V_g \cdot \frac{273}{T} + V_f \cdot \alpha}{P_0}$$

The quantity k is called the "flask constant" and it is constant for a given Warburg flask with a given volume of liquid. Because of the difference in solubility between oxygen and carbon dioxide, ($\alpha_{O_2} = 0.0239$ and $\alpha_{CO_2} = 0.567$ at 37° C) the "flask constant" is different for these two gases.

In order to determine the uptake of oxygen, 10 % KOH is used in the central well of the Warburg flask. The carbon di-

oxide is absorbed completely by the alkali as soon as it is formed, and the oxygen will then be the only gas that is exchanged. If we know the dry weight of the tissue or the cells and the time during which oxygen absorption is observed the respiratory activity can be calculated. The quantity Q_{O_2} is the amount of oxygen in microliters absorbed per milligram dry weight of the living tissue per hour.

To measure the output of carbon dioxide one can use either the direct method of Warburg or the indirect method. In this investigation the direct method has been applied. An equal volume of the same bacterial suspension is added to each of three Warburg flasks. The center well of the first flask is supplied with KOH and thus shows the uptake of oxygen. In the two other flasks the center well is supplied with water. In flask no. 2 acid is tipped in from a side arm at the termination of the experiment to determine the carbon dioxide taken up by the buffer solution. In flask no. 3 acid is tipped in at the start of the respiration experiment to determine the amount of carbon dioxide bound in the solution at the beginning.

The amount of carbon dioxide, X_{CO_2}, produced by the bacteria is obtained from the equation:

$$X_{CO_2} = \left(h_2 - h_3 \cdot \frac{k_{3\,CO_2}}{k_{2\,CO_2}} - \frac{X_{O_2}}{k_{2O_2}} \right) \cdot k_{2CO_2}$$

h_2 is the change in mm on the manometer of flask no. 2
h_3 is the change in mm on the manometer of flask no. 3
X_{O_2} is the oxygen taken up, shown by flask no. 1
k_{2O_2} is the "flask constant" for oxygen for flask no. 2
k_{2CO_2} is the "flask constant" for carbon dioxide for flask no. 2
k_{3CO_2} is the "flask constant" for carbon dioxide for flask no. 3

The respiratory quotient, R. Q., is obtained by dividing the volume of carbon dioxide produced, by the volume of oxygen taken up. Since X_{O_2} has a negative value the equation will be:

$$R.\,Q. = -\frac{X_{CO_2}}{X_{O_2}}$$

EXPERIMENTAL METHODS

A large bacterial mass was grown from the bacterial strain originating from the human mouth. This was accomplished for lactobacilli and streptococci in a liquid substrate, viz. beef peptone infusion broth with glucose, which was shaken during the whole growth period. Staphylococci, gaffkya and sarcina cocci, on the other hand, were grown on the surface of beef infusion peptone agar. After an appropriate time, the bacteria were harvested from the glucose broth by centrifuging and from the agar surface by suspending in sterile Ringer solution with a sterile platinum triangle. In each case, the bacteria were then washed twice in the centrifuge with Ringer solution. Gram stained streak preparations were made to check that no contamination had occurred during the growth.

Then the wet weight of the bacteria was determined and they were suspended in a buffer solution. For the experiments on aerobic respiration, a phosphate buffer with the following composition was used:

$Na_2HPO_42H_2O$	concentration 50 mC
KH_2PO_4	concentration 50 mC

Accordingly, the total concentration of phosphate was 100 mC.

It is to be remarked that experiments with these acid producing bacteria must be made in a well-buffered medium, as otherwise there would be a rapid pH decrease which would reduce the respiration to a very low level.

For the experiments on anaerobic acid production, a bicarbonate buffer with the following composition was used:

$NaHCO_3$	concentration 50 mC
NaCl	concentration 100 mC

To determine the dry weight of the bacteria, 5.0 ml of the bacterial suspension was first pipetted into a weighing vessel. Into the main compartment of each Warburg flask, 2.0 ml of the bacterial suspension was added.

The additions to the center well and the side arm as well as the tipping in of the contents of the side arm varied in the different types of experiment as will be described later in this paper.

The dry weight of 5.0 ml of buffer solution without any bacteria was determined. This figure was then subtracted from the weight of the dried 5.0 ml of buffer-bacteria suspension to obtain the dry weight of the bacteria alone. A correction was made for the space taken up by the bacteria in the suspension. The specific gravity of the bacteria was *estimated at* 1.1. The wet weight divided by 1.1 thus gave the volume of the bacteria.

After the proper liquids had been added to the compartments of the Warburg flasks, these were immersed in a water-bath kept at a constant temperature (37° C). The flasks were shaken for approximately 20 minutes to obtain equilibrium between the liquids and gases. Then the respiration experiment proper was started by tipping in the glucose solution. The manometers were read every ten minutes. A thermo-barometer was also placed in the bath so that changes in air pressure and bath temperature could be observed.

At the end of the experiment, 0.2 ml of 5-normal H_2SO_4 was tipped into the main compartment. It had been previously found that this gave a pH of about 1.5. Thus the bacterial metabolism was effectively stopped.

After the Warburg flasks had been removed from the water bath, the liquid in the main compartment was transferred to tubes and the bacteria separated by centrifuging. The amount of lactic acid in the liquid was determined by the method of *Barker & Summerson* (1941) and the glucose content according to *Folin & Wu* (1920). By means of these determinations, comparisons could then be made between the aerobic respiration and the anaerobic respiration (i.e. the acid production) in the same experiment. It was also possible to compare the reduction in the quantity of glucose with the amount of glucose that ought to have been used up by respiration and acid production.

EXPERIMENTAL DETAILS

A. Experiments on aerobic respiration

1. *Endogenous respiration compared with respiration in glucose.*

Experiments were performed in duplicate (a. and b.) with the same amount of bacteria in each. In a. the respiration took place in phosphate buffer alone while in b. glucose was added to the buffer solution. The experiments were numbered from 1a and 1b to 11a and 11b.

	Vol. ml	Flask a	Flask b
Main chamber, bacteria in phosphate	2.0	+	+
Center well, KOH, 10 %	0.2	+	+
Side arm 1, glucose 280 mC in phosphate	0.3		+
Side arm 1, phosphate	0.3	+	
Side arm 2, H_2SO_4, 5-normal	0.2	+	+
At the start the contents of side arm 1 were tipped in		+	+
At the end the contents of side arm 2 were tipped in		+	+

2. *Experiments on uptake of oxygen in glucose. Determination of the respiratory activity.*

Experiments a) and b) were performed with equal volumes from the same bacterial suspension. The medium was phosphate buffer with glucose. The two experiments were identical. These experiments were numbered 12a, 12b, 13a ..... 44b.

	Vol. ml	Flask a	Flask b
Main chamber, bacteria in phosphate	2.0	+	+
Center well, KOH, 10 %	0.2	+	+
Side arm 1, glucose 280 mC in phosphate	0.3	+	+
Side arm 2, H_2SO_4, 5-normal	0.2	+	+
Gas: air		+	+
At the start side arm 1 is tipped in		+	+
At the end side arm 2 is tipped in		+	+

3. *Determination of the oxygen uptake as well as the carbon dioxide output for determining the respiratory quotient.*

Each experiment was carried out with three Warburg flasks. Experiment numbers: 45a, 45b, 45c ..... 88c.

	Vol. ml	Flask a	b	c
Main chamber, bacteria in phosphate ...	2.0	+	+	+
Center well, KOH, 10 %	0.2	+		
Center well, H_2O	0.2		+	+
Side arm 1, glucose 280 mC in phosphate	0.3	+	+	+
Side arm 2, H_2SO_4, 5-normal	0.2	+	+	+
Gas: air				
At the start side arm 1 is tipped in		+	+	+
At the start side arm 2 is tipped in				+
At the end side arm 2 is tipped in		+	+	

B. Experiments on the anaerobic acid production

In these experiments, the air in the Warburg flasks was replaced by a mixture of 50 % nitrogen and 50 % carbon dioxide. These commercial gases were run through a tube with copper wire-netting heated to glowing to free them from any remaining oxygen. The Warburg flasks were filled by the evacuation method of Burris *(Umbreit et al.* 1947, page 43).

Experiment numbers: 89 to 128.

	Volume ml
Main chamber, bacteria in bicarbonate ...	2.0
Side arm 1, glucose 280 mC in bicarbonate	0.3
Side arm 2, H_2SO_4, 5-normal	0.2
Gas: N_2 50 % and CO_2 50 %	+
At the start side arm 1 is tipped in	+
At the end side arm 2 is tipped in	+

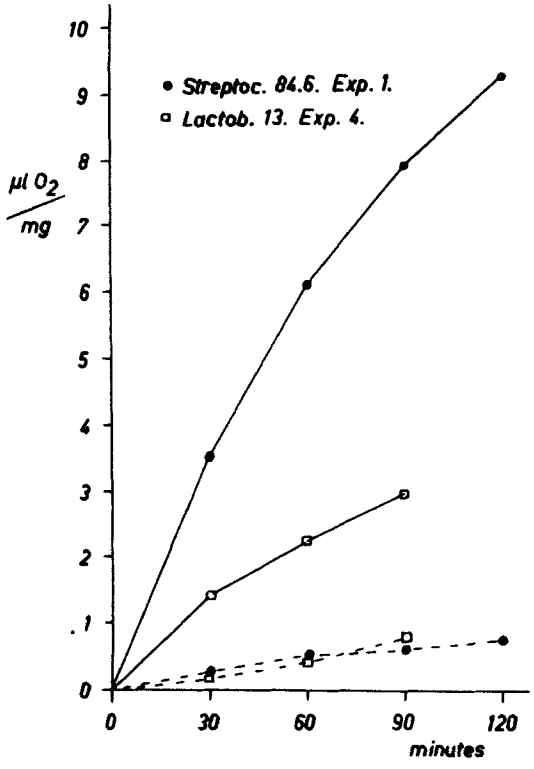


Fig. 1. The course of respiration. The continuous curve represents the respiration in glucose and the dotted curve the endogenous respiration.

RESULTS

Endogenous respiration compared with respiration in glucose

The results are given in Table 1. The endogenous respiration varied from 3 to 27 % of the respiration in glucose. This type of respiration occurs without any substrate added, i.e. the cell uses its own supply of nutritive substances.

In determining the amount of oxygen taken up per unit of substrate, it is a problem to decide whether one should subtract from the oxygen uptake observed in the presence of substrate, the oxygen taken up over the same interval in the absence of substrate. That is, when a substrate is being rapidly oxidized does the endogenous respiration continue at its constant rate, is it suppressed, or does it increase? These questions have not yet been answered. *Van Niel* (1943) gives a thorough discussion.

Figures 1 and 2 show diagrammatically the course of endo-

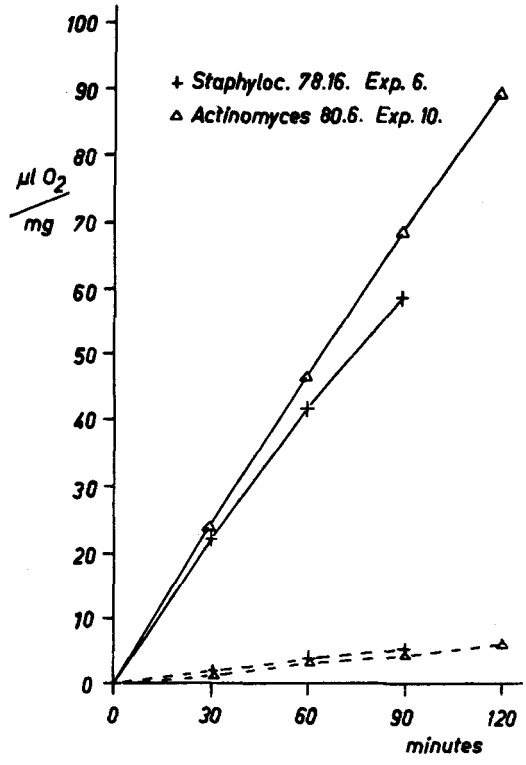


Fig 2. The course of respiration. The continuous curve represents the respiration in glucose and the dotted curve the endogenous respiration.

genous and glucose respiration. The continuous curves represent the glucose and the dotted curves the endogenous respiration.

Determination of the respiratory activity, Q_{O_2}

The rate of oxygen uptake, Q_{O_2}, expressed as microliters of oxygen taken up per milligram dry weight of bacteria per hour, is demonstrated in three experimental series. First there is that part of the study on endogenous respiration which was performed with glucose, then there are the duplicate experiments for determining Q_{O_2} and finally there is part a) of the experiments on the respiratory quotient. All these results are grouped together in Table 2.

The following types of bacteria were examined: streptococci, lactobacilli of dental origin, one strain of lactobacilli from the plant world, staphylococci, gaffkya, sarcina and actinomyces. The

strains used are the same as in an earlier work of the author (*Strålfors,* 1950) and the designations of the strains are the same. Columns 3, 4 and 5 show the experimental numbers of the three experimental series. The next four columns show the values of Q_{O_2} during four different periods of time in each experiment, i.e. 0—30, 30—60, 60—90 and 90—120 minutes. The last column gives the mean value of Q_{O_2} during the whole experiment.

The Q_{O_2} values at the different intervals have a characteristic trend. For streptococci and lactobacilli the activity falls during the course of the experiment but this is not the case, at least not to the same degre, for the other groups of bacteria.

Figure 3 shows some typical curves of respiration. The ordinate represents microliters of O_2 per milligram of dry weight of bacteria and the abscissa the time in minutes. The slope of the curve or, mathematically expressed, the derivate of the curve, represents Q_{O_2}. It can be seen from Fig. 3 how the respiration follows a straight line for the staphylococci, gaffkya cocci and sarcina cocci. In the case of streptococci and lactobacilli, on the other hand, the slope of the curve and hence the respiratory activity is falling.

Several explanations for the cause of this decreasing respiratory activity are possible. The pH might be of importance. To check this, the pH of the liquid was measured in a number of experiments instead of tipping in sulphuric acid. Table 5 shows the results. Experiments no. 18b, 19a, 19b and 27b have, according to Table 2, a decreasing value of Q_{O_2} but the pH value was relatively high, from 6.62 to 6.82, at the end of the experiment. Consequently, it does not seem likely that the pH value has been a deciding factor in the change in Q_{O_2}.

Another possibility is that the aerobic oxidation diminishes because of the formation of hydrogen peroxide. The oral streptococci and lactobacilli, at least the strains used in these experiments, are devoid of the enzyme catalase (*Strålfors,* 1950, pages 30—32). Thus any hydrogen peroxide which is formed cannot be decomposed.

MacLeod & Gordon (1924, 1925) and *Avery & Morgan* (1924) have shown that pneumococci and streptococci form H_2O_2 in the presence of oxygen. *Bertho & Glück* (1932) found that two species of lactobacilli, B. acidificans longissimus and B. acido-

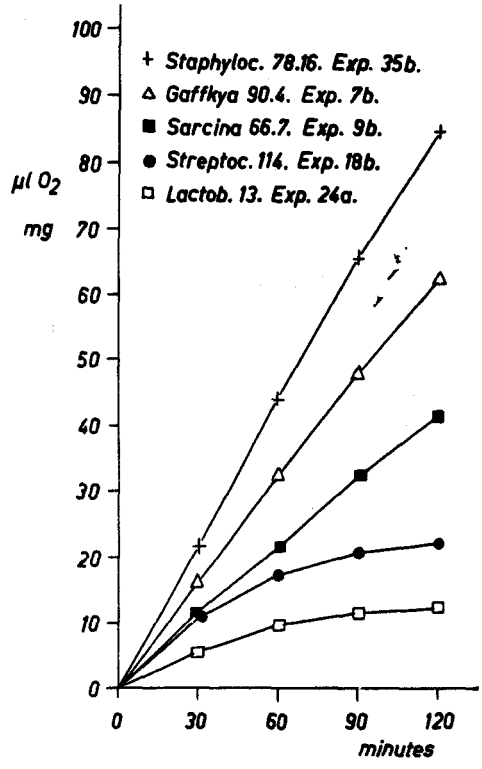


Fig. 3. The course of respiration. Typical curves for different groups of bacteria. A uniform activity for staphylococci, gaffkya and sarcina. A decreasing activity for the streptococci and lactobacilli.

philus, formed hydrogen peroxide. We may assume that, in the experiments in the present investigation, hydrogen peroxide had accumulated and inhibited the respiration.

It is to be presumed, however, that the conditions are quite different in the oral plaques with their composite flora of bacteria. The most numerous bacteria in the plaque after streptococci are the neisseria cocci which contain catalase *(Strålfors,* 1950, page 36). It therefore seems very probable that the hydrogen peroxide, as soon as it is formed by the lactic bacteria, will be decomposed by the catalase of the neisseria.

In Table 3 the mean values are given for each strain as well as the mean values for each group of bacteria. It appears that staphylococci, gaffkya, sarcina and actinomyces have higher Q_{O_2} values than streptococci and lactobacilli. Since the mean values in this table represent periods of time up to two hours, the difference between bacterial groups depends partly upon the

falling activity of streptococci and lactobacilli. In Table 4 mean values are given for the interval 0—30 minutes only, and we see that also here the lactic bacteria are at a lower level of oxygen uptake.

It is of special interest to compare the activity of aerobic respiration with the anaerobic glycolysis investigated in the earlier study *(Strålfors,* 1950, pages 52—68). We find that there is a reversed relation. The lactic bacteria have a higher rate of acid production than staphylococci and gaffkya. Thus the streptococci and lactobacilli have a tendency to let their carbohydrate metabolism proceed with a relatively small uptake of oxygen but with a rapid acid production, while the staphylococci, gaffkya and sarcina decompose the carbohydrate, mostly by means of aerobic oxidation. As was mentioned above, a lactic acid analysis of the contents of the Warburg flasks was made after the respiration experiment. Therefore we are able to compare, in the same experiment, the oxygen uptake with the acid production when there is access to air. This will be dealt with later.

Table 1

Endogenous respiration compared with respiration in glucose

Bacteria	Strain no.	Exp. no.	a. Q_{O_2} in phosphate	b. Q_{O_2} in glucose + phosphate	Ratio a/b
Streptococci	84.6	1	0.37	4.62	0.08
	84.6	2	1.03	5.13	0.20
	84.6	3	0.60	4.52	0.13
Lactobacilli	13	4	0.54	1.97	0.27
Staphylococci	78.16	5	2.89	27.5	0.11
	78.16	6	3.39	38.8	0.09
Gaffkya	90.4	7	2.68	31.2	0.09
Sarcina	60.5	8	4.79	19.5	0.25
	66.7	9	4.21	20.7	0.20
Actinomyces	80.6	10	2.96	44.4	0.07
	86.14	11	1.13	40.0	0.03

Table 2

Respiratory activity, Q_{O_2}, in oral bacteria

(in phosphate buffer with glucose)

Bacteria	Strain no.	Experiment no.			Q_{O_2} in interval				Q_{O_2} mean value
		Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	0—30 min.	30—60 min.	60—90 min.	90-120 min.	
Streptococci	84.6	1b			7.03	5.14	3.56	2.73	4.62
		2b			4.83	5.88	4.66	—	5.13
		3b			6.02	6.42	4.01	1.61	4.52
			12a		13.1	5.61	2.80	2.16	5.92
			12b		13.4	6.06	2.81	2.59	6.22
			13a		6.97	5.97	3.78	2.49	4.80
			13b		7.30	5.90	3.50	2.50	4.80
				45a	13.6	9.47	5.19	3.53	7.95
	111		14a		6.87	5.29	3.34	1.86	4.34
			14b		6.40	5.19	3.25	1.95	4.20
			15a		2.12	2.97	1.45	0.63	1.64
			15b		2.21	2.46	1.45	0.57	1.67
				46a	3.96	2.64	0.84	—	2.48
	113			47a	10.6	9.84	—	—	10.2
				48a	10.3	5.13	—	—	7.70
	114		16a		2.33	1.73	1.56	1.27	1.72
			16b		2.57	1.80	1.20	0.88	1.61
			17a		11.5	4.66	2.47	1.10	4.92
			17b		9.27	5.42	2.96	0.07	4.43
			18a		13.5	9.00	6.08	4.02	8.15
			18b		21.8	12.8	6.38	3.33	11.1
			19a		24.1	14.7	8.81	5.28	13.2
			19b		14.0	15.2	11.7	5.85	11.7
				49a	24.1	17.3	7.91	5.73	13.8
				50a	21.1	7.83	—	—	14.5
	115		20a		21.1	13.3	8.28	5.26	12.0
			20b		19.9	9.28	4.21	2.54	8.98
			21a		22.7	14.0	7.42	4.28	12.1
			21b		21.3	14.5	7.18	4.47	11.9
				51a	8.57	3.72	1.21	0.78	3.57
				52a	24.9	14.9	—	—	19.9
	116			53a	11.1	4.44	—	—	7.77
				54a	17.4	12.8	—	—	15.1
	117		22a		10.0	1.98	0.72	0.63	3.33
			22b		9.38	1.99	0.72	0.63	3.18
				55a	17.1	5.49	—	—	11.3
	118			56a	13.1	8.25	—	—	10.7
				57a	42.0	29.7	—	—	35.8
	119			58a	23.3	14.7	—	—	19.0
				59a	20.8	10.4	—	—	15.6
	120			60a	9.39	3.36	—	—	6.38

(Cont.)

Table 2 (continued)

Bacteria	Experiment no.				Q_{O_2} in interval				Q_{O_2} mean value
	Strain no.	Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	0—30 min.	30—60 min.	60—90 min.	90-120 min.	
Lactobacilli of dental origin	13	4b			2.76	1.71	1.43	—	1.97
			23a		9.67	6.93	3.39	1.45	5.36
			23b		11.9	6.93	3.23	1.78	5.96
			24a		4.21	3.36	1.79	0.84	2.55
			24b		4.73	3.36	1.68	0.84	2.65
				61a	8.03	4.44	2.10	1.60	4.04
				62a	5.10	1.53	1.28	—	2.64
	14		25a		0.78	2.09	2.88	2.09	1.96
			25b		5.76	4.45	3.92	2.88	4.25
			26a		5.80	4.89	2.99	1.72	3.85
			26b		7.60	5.97	5.43	4.17	5.79
			27a		6.33	5.43	7.23	4.97	5.99
			27b		14.5	2.72	1.81	2.72	5.44
			28a		5.48	5.01	3.46	2.38	4.08
			28b		6.20	5.02	3.46	2.50	4.29
				63a	6.01	7.79	5.52	—	6.44
	19		29a		6.65	4.32	4.51	2.52	4.50
			29b		6.51	4.51	4.14	2.17	4.33
				64a	8.25	6.03	—	—	7.14
	20			65a	11.2	10.7	—	—	10.9
				66a	9.17	3.77	—	—	6.47
	22		30a		5.98	4.93	3.58	2.09	4.15
			30b		6.28	4.93	3.59	2.10	4.23
				67a	8.33	4.97	—	—	6.65
				68a	7.10	5.63	—	—	6.37
Lactobacilli from the plant world	26		31a		47.5	52.1	46.4	42.4	47.1
			31b		42.9	48.8	44.6	40.8	44.3
			32a		35·9	30.6	27.6	19.8	28.5
			32b		32.9	27.6	25.9	22.8	27.3
				69a	54.2	59.9	49.5	45.4	52.2
Staphylococci	78.16	5b			30.9	33.1	30.3	15.8	27.5
		6b			43.8	38.6	34.1	—	38.8
			33a		46.0	51.9	47.4	44.6	47.5
			33b		42.1	48.0	43.1	41.1	43.6
			34a		33 5	30.5	29.3	26.0	29.8
			34b		43.0	44.6	43.0	37.8	42.1
				70a	57.0	58.8	52.7	46.6	53.8
	79.5		35a		36.0	38.0	32.6	21.9	32.1
			35b		33.2	36.9	34.9	22.4	31.9
			36a		13.4	26.4	42.0	42.7	31.1
			36b		36.3	39.5	36.0	30.9	35.7
			37a		33.1	32.7	33.4	30.5	32.4
			37b		31.7	29.7	31.1	28.6	30.3
			38a		23.6	23.6	24.4	18.9	22.6
			38b		36.3	23.6	20.5	11.0	22.9

(Cont.)

Table 2 (continued)

Bacteria	Strain no.	Experiment no.			Q_{O_2} in interval				Q_{O_2} mean value
		Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	0—30 min.	30—60 min.	60—90 min.	90-120 min.	
Staphylococci	80.3		39a		39.3	40.2	36.9	32.2	37.1
			39b		33.6	35.8	32.5	29.2	32.8
				71a	33.8	41.3	34.7	—	38.3
	89.2		40a		27.4	28.4	26.6	20.9	25.8
			40b		26.4	27.9	27.4	25.2	26.7
				72a	36.2	39.8	—	—	38.0
	67.7		41a		44.7	45.3	42.4	40.4	43.2
			41b		53.0	51.6	42.5	45.7	48.2
			42a		24.8	24.8	26.4	24.8	25.2
			42b		25.0	26.1	27.5	24.5	25.8
				73a	62.7	64.3	59.4	59.4	61.4
	82.9		43a		34.5	31.1	36.6	17.5	29.9
			43b		34.1	33.6	32.1	23.1	30.7
			44a		70.5	28.2	11.8	2.33	28.2
			44b		16 5	18.8	18.8	11.8	16.5
Gaffkya	88T3			74a	53.6	54.9	52.0	—	53.5
				75a	72.8	85.3	—	—	79.1
	88T9			76a	40.8	43.8	—	—	42.3
				77a	37.1	55.7	—	—	46.4
	88T10			78a	51.2	55.9	—	—	53.5
				79a	63.7	61.3	—	—	62.5
	90.4	7b			32.4	32.7	30.4	29.3	31.2
				80a	33.7	34.0	28.9	27.3	31.0
Sarcina	60.5	8b			25.0	17.1	19.7	16.1	19.5
				81a	26.1	27.0	24.7	—	25.9
	64.1			82a	19.6	20.2	—	—	19.9
				83a	41.3	46.9	—	—	44.1
	66.7	9b			21.1	21.8	21.8	18.2	20.7
				84a	19.0	20.5	20.5	—	20.0
	73.3			85a	23.6	24.0	—	—	23.8
				86a	41.4	45.7	—	—	43.5
Actinomyces	76a			87a	43.1	75.9	—	—	59.5
	76c			88a	68.0	85.7	—	—	76.8
	80.6	10b			47.3	45.2	43.7	41.2	44.4
	86.14	11b			52.2	41.9	38.4	27.8	40.0

Table 3

Respiratory activity in oral bacteria
Mean values

Bacteria	Strain no.	Mean value	Bacteria	Strain no.	Mean value
Streptococci	84.6	5.50	*Staphylococci*	78.16	40.4
	111	2.87		79.5	29.9
	113	8.95		80.3	36.1
	114	8.51		89.2	30.2
	115	11.4		67.7	40.8
	116	11.4		82.9	26.3
	117	5.94		Mean value	34.0
	118	23.3			
	119	17 3	*Gaffkya*	88T3	66.3
	120	6.38		88T9	44.4
	Mean value	10.2		88T10	58.0
				90.4	31.1
Lactobacilli	13	3.60		Mean value	50.0
of dental	14	4.68			
origin	19	5.32	*Sarcina*	60.5	22.7
	20	8.69		64.1	32.0
	22	5.35		66.7	20.4
	Mean value	5.53		73.3	33.7
				Mean value	27.2
Lactobacilli	26	39.9	*Actinomyces*	76a, 76c,	55.2
from the				80.6 and	
plant world				86.14	

Table 4

Respiratory activity in oral bacteria
Mean values for the interval 0—30 minutes

Bacteria	Strain no.	Mean value	Bacteria	Strain no.	Mean value
Streptococci	84.6	9.03	*Staphylococci*	78.16	42.3
	111	4.31		79.5	30.5
	113	10.5		80.3	35.6
	114	14.4		89.2	30.0
	115	19.7		67.7	42.0
	116	14.3		82.9	38.9
	117	12.2		Mean value	36.6
	118	27.6			
	119	22.1	*Gaffkya*	88T3	63.2
	120	9.39		88T9	39.0
	Mean value	14.4		88T10	57.5
				90.4	33.1
Lactobacilli	13	6.63		Mean value	48.2
of dental	14	6.50			
orgin	19	7.14	*Sarcina*	60.5	25.1
	20	10.2		64.1	30.5
	22	6.92		66.7	20.1
	Mean value	7.48		73.3	32.5
				Mean value	27.2
Lactobacilli	26	42.7	*Actinomyces*	76a, 76c,	52.7
from the				80.6 and	
plant world				86.14	

Table 5

pH values in respiration experiments

Bacteria	Strain no.	Experiment no.		pH at the end of the exp.	Falling respiratory activity
		Q_{O_2} in dupl. exps.	R. Q. exps.		
Streptococci	84.6		45a	5.83	+
	114	18a		5.98	+
		18b		6.62	+
		19a		6.74	+
		19b		6 83	+
Lactobacilli	13		61a	4.12	+
	14	26a		4.85	+
		26b		4.85	+
		27a		6.03	+
		27b		6.73	+
Staphylococci	78.16		70a	6.73	
	79.5	37a		6.43	
		~~37b~~		~~6.68~~	
		38a		6.82	
		38b		6.87	
	67.7		73a	6.72	

Table 6
Respiratory quotient, R.Q.

Bacteria	Strain no.	Exp. no.	Respiratory quotient	Mean value for bacterial group
Streptococci	84.6	45	1.06	0.89 ± 0.04
	111	46	0.78	
	113	47	0.79	
		48	0.78	
	114	49	0.92	
		50	0.92	
	115	51	0.87	
		52	0.90	
	116	53	0.94	
		54	1.19	
	117	55	0.76	
	118	56	0.82	
		57	0.95	
	119	58	0.79	
		59	0.66	
	120	60	1.14	
Lactobacilli	13	61	1.36	1.45
		62	1.37	
	14	63	1.05	
	19	64	1.55	
	20	65	1.50	
		66	1.42	
	22	67	1.65	
		68	1.86	
	26	69	1.30	
Staphylococci	78.16	70	0.90	0.93
	80.3	71	0.88	
	89.2	72	1.01	
	67.7	73	0.94	
Gaffkya	88T3	74	1.01	0.99
		75	0.95	
	88T9	76	0.98	
		77	0.89	
	88T10	78	1.00	
		79	1.03	
	90.4	80	1.06	
Sarcina	60.5	81	1.03	1.02
	64.1	82	1.15	
		83	1.03	
	66.7	84	0.93	
	73.3	85	0.91	
		86	1.04	
Actinomyces	76a	87	1.01	1.12
	76c	88	1.22	

Table 7

Ratio between lactic acid production and aerobic oxidation at access to air

Bacteria	Strain no.	Experiment no.			Ratio		
		Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	for separate exper.	mean value for bacterial strain	mean value for bacterial group
Streptococci	84.6	2b			19.4	14.1	19.6
		3b			14.0		
			12a		3.79		
			12b		3.61		
			13a		18.7		
			13b		24.8		
	111		14a		0.279	3.38	
			14b		0.548		
			15a		10.3		
			15b		5.49		
				46a	0.283		
	113			47a	45.3	77.7	
				48a	110		
	114		16a		3.13	7.06	
			16b		6.25		
			17a		13.6		
			17b		14.6		
				49a	3.23		
				50a	1.56		
	115		20a		3.83	7.72	
			20b		8.43		
			21a		9.62		
			21b		11.2		
				51a	0.133		
				52a	13.1		
	116			53a	0.66	2.09	
				54a	3.52		
	117		22a		28.2	23.4	
			22b		28.6		
				55a	13.5		
	118			56a	0.60	2.52	
				57a	4.44		
	119			58a	12.6	6.87	
				59a	1.13		
	120			60a	50.7	50.7	

(Cont.)

Table 7 (continued)

Bacteria	Experiment no.				Ratio		
	Strain no.	Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	for separate exper.	mean value for bacterial strain	mean value for bacterial group
Lactobacilli of dental origin	13	4b			49.3	83.4	61.2
			23a		58.5		
			23b		48.5		
			24a		172		
			24b		96.2		
				62a	75.8		
	14		25a		115	65.9	
			25b		65.9		
			28a		41.5		
			28b		45.2		
				63a	62.0		
	19		29a		55.6	67.6	
			29b		68.0		
				64a	79.2		
	20			65a	30.7	33.8	
				66a	36.9		
	22		30a		60.2	55.3	
			30b		55.7		
				67a	46.2		
				68a	59.0		
Lactobacilli from the plant world	26		31a		3.39	26.2	26.2
			31b		3.47		
			32a		26.4		
			32b		94.8		
				68a	2.76		
Staphylococci	78.16	6b			0.0884	0.113	0.42
			33a		0.0479		
			33b		0.0602		
			34a		0.172		
			34b		0.195		
	79.5		35a		0.433	0.857	
			35b		0.866		
			36a		1.06		
			36b		1.07		
	80.3		39a		0.0410	0.111	
			39b		0.280		
				71a	0.0110		
	89.2		40a		0.830	0.591	
			40b		0.828		
				72a	0.114		

(Cont.)

Table 7 (continued)

Bacteria	Strain no.	Experiment no.			Ratio		
		Endogenous respir.	Q_{O_2} in dupl. exps.	R. Q. exps.	for separate exper.	mean value for bacterial strain	mean value for bacterial group
Staphylococci	67.7		41a		1.59	3.26	2.49
			41b		2.43		
			42b		5.75		
	82.9		43a		1.34	1.72	
			43b		1.54		
			44a		1.36		
			44b		2.62		
Gaffkya	88T3			74a	0.253	0.231	0.20
				75a	0.208		
	88T9			76a	0.438	0.438	
	88T10			78a	0.0906	0.0906	
	90.4	7b			0.0198	0.0293	
				80a	0.0388		
Sarcina	60.5	8b			0.155	0.154	0.10
				81a	0.153		
	64.1			82a	0.0687	0.0448	
				83a	0.0209		
	66.7	9b			0.0158	0.0223	
				84a	0.0288		
	73.3			85a	0.283	0.162	
				86a	0.0409		
Actinomyces	76a			87a	0.0637	0.0637	0.07
	76c			88a	0.0329	0.0329	
	80.6	10b			0.126	0.126	

Table 8

Sum of glucose remaining, glucose converted to lactic acid, and glucose aerobically oxidated

Mean values for strains and for groups of bacteria

Bacteria	Strain no.	Sum of glucose micro mols
Streptococci	84.6	56.2
	111	77.9
	113	66.3
	114	74.5
	115	65.4
	116	89.3
	117	54.0
	118	77.7
	119	80.5
	120	72.9
	Mean value	71.5
Lactobacilli of dental origin	13	70.6
	14	66.0
	19	79.3
	20	72.0
	22	61.1
	Mean value	69.8
Lactobacilli from the plant world	26	48.3
Staphylococci white	78.16	33.7
	79.5	49.4
	80.3	31.0
	89.2	26.0
	Mean value	35.0
Staphylococci yellow	67.7	48.6
	82.9	62.3
	Mean value	55.5
Gaffkya	88T3	66.9
	88T9	83.9
	88T10	84.1
	90.4	67.5
	Mean value	75.6
Sarcina	60.5	76.1
	64.1	79.2
	66.7	86.3
	73.3	71.6
	Mean value	78.3
Actinomyces	76a	77.6
	76c	79.5
	80.6	79.2
	Mean value	78.8

Table 9

Rate of acid production measured in the Warburg apparatus

Bacteria	Strain no.	Exper. no.	10^{-9} mol of acid (mg dry weight) × (minute)
Streptococci	114	89	62.3
		90	182
		91	184
	117	92	205
		93	189
	120	94	91.7
		95	84.9
		96	204
Lactobacilli	13	97	131
		98	137
		99	153
		100	142
		101	168
		102	181
	14	103	72.2
		104	73.7
		105	131
	19	106	175
		107	164
		108	162
	22	109	70.4
		110	129
	26	111	35.9
		112	38.4
		113	27.4
		114	23.0
Staphylococci	78.16	115	11.8
		116	7.27
		117	8.34
	79.5	118	14.3
		119	14.1
		120	14.2
		121	9.60
	67.7	122	20.6
Gaffkya	88T3	123	12.7
		124	11.7
Sarcina	60.5	125	3.09
		126	4.87
		127	4.28
	66.7	128	0

Table 10

Cytochrome in some groups of bacteria

Bacteria	Species	Form of cytochrome		
Sarcina	S. aurantiaca	a	b	—
	S. lutea	a	b	c
Staphylococcus	S. albus	a	b	—
	S. aureus	a	b	—
	S. citreus	a	b	—
Pneumococcus	P. I	a	b	—
Streptococcus	S. acidi lactici	—	—	—
	S. pyogenes	—	—	—
	S. faecalis	—	—	—
	S. agalactiae	—	—	—
Lactobacillus	L. delbrückii	—	—	—
	L. acidophilus	—	—	—

CALCULATION OF THE POSSIBILITY OF ANAEROBIC CONDITIONS BEING PRESENT IN THE INNER PART OF THE DENTAL PLAQUES

As is well known, a number of strictly anaerobic kinds of bacteria have been isolated from dental plaques. Among these are the fusiforms and the spirochaetes. The fact that these bacteria are able to live and multiply in plaques is to be ascribed to the coexistence of other bacteria taking up the oxygen. It should be possible to calculate approximately how thick a layer of plaque material is required to remove the oxygen completely. The pressure of oxygen at the surface of the plaque is the same as in the air, i.e. about 21 % of 760 mm Hg equal to 160 mm Hg. Further, in the plaque the pressure falls because of bacterial oxygen uptake. At what depth is the pressure zero?

The distance is expressed by the equation (*Strålfors*, 1950, page 23)

$$H = \sqrt{2c \cdot \frac{D}{Q}}$$

c = conc. of oxygen in the air

D = the diffusion coefficient for oxygen

Q = the rate of aerobic respiration

D is, according to *Krogh* (1919), 1.4×10^{-5} ml O_2 (0°, 760

mm Hg) through 1 cm^2 per minute if the concentration gradient is 1 atmosphere per cm.

c will then be expressed in atmospheres

Q will then be expressed in ml oxygen/ (ml plaque volume) $\times$ (minute).

If we limit the calculations to the viridans streptococci, since they are the most numerous bacteria in the plaque, we find the following:

Mean value (period 0—30 minutes) is 15.2 microliters O_2/(mg dry weight) $\times$ (hour). In the plaque there are about 23×10^9 cells per ml plaque, corresponding to 23/2.03 mg dry weight of streptococci (*Strålfors,* 1950, page 81). Consequently, the oxygen uptake in the plaque should be $15.2 \times 23/2.03$ microliters O_2/(ml plaque) $\times$ (hour) $= 2.87 \times 10^{-3}$ ml O_2/ (ml plaque) $\times$ (minute).

According to the equation given above, the oxygen value should be zero at a depth of 0.45 mm. Plaques on the teeth often exceed this thickness especially in the interproximal spaces, where the distance has to be measured in a bucco-lingual direction.

We have not, however, considered the other bacteria in the plaque. If we estimate the respiration of the neisseria, cocci and other bacteria to be of the same magnitude as that of the streptococci, the depth given above should be divided by $\sqrt{2}$ resulting in the figure 0.32 mm.

Determination of the respiratory quotient

The results of the experiments to determine R.Q. are shown in Table 6.

A total of 44 experiments have been performed and two experiments have been made with each of 14 bacterial strains. As the bacteria in the two paired experiments have come from two separate cultures the total error of the method includes the variance in the growth, the pipetting, the reading of the manometer and so on.

The mean value of the deviations in the duplicate experiments is 0.09 with a standard deviation of $\pm$ 0.02.

If complete conversion of glucose to carbon dioxide and water took place without any divergent processes the total reaction would be:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Then the respiratory quotient would be exactly 1. The most marked deviation from this figure is found with the lactobacilli with values from 1.05 to 1.86 (mean 1.45). The reasons for this relatively high R.Q. cannot be explained without thorough complementary investigations. It should be pointed out, however, that the glycolysis may not have been purely homofermentative. Carbon dioxide, ethyl alcohol and organic acids other than lactic acid (e.g. acetic acid) may have been formed. Since the lactobacilli have a much more intense acid production than aerobic oxidation (the ratio is 61: 1, as will be reported later in this paper) even a very small part of the glycolysis taking place heterofermentatively with production of CO_2 would give a substantially higher respiratory quotient.

The streptococci have a lower R.Q. than 1, varying from 0.66 to 1.19 with a mean value of 0.89. A total of 13 values are below 1 and only three are above. The standard deviation of the mean is $\pm$ 0.04. Thus the mean value is significantly lower than 1. The cause of this low R.Q. could not be conclusively demonstrated from this investigation. However, it seems likely that the production of H_2O_2 may provide the explantion. If all the oxidation occurred to H_2O_2 the total reaction would be:

$$C_6H_{12}O_6 + 6H_2O + 12O_2 \rightarrow 6CO_2 + 12H_2O_2.$$

The R.Q. would then be $6/12 = 0.5$.

When the hydrogen peroxide is partly decomposed to oxygen and water the result is an R.Q. between 0.5 and 1.

The H_2O_2 production of lactobacilli should also lower the respiratory quotient. Evidently, however, this effect is counteracted by the CO_2 production coupled to the anaerobic glycolysis resulting in a R.Q. higher than 1. For the other micro-organisms, staphylococci, gaffkya and sarcina the quotient is quite near to 1.

The quantitative ratio of lactic acid production to aerobic oxidation in the presence of the atmospheric oxygen

A lactic acid analysis was made after the termination of most of the respiration experiments. Thus the ratio between lactic

acid production and aerobic oxidation could be calculated in each experiment.

In these calculations it has been supposed that the oxidation had followed the formula

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O.$$

One molecule of glucose corresponds to six molecules of oxygen. One micromol occupies 22.4 microliters at 0° and 760 mm Hg. Thus, by dividing the figure for oxygen by 6×22.4, the number of micromols of glucose oxidized is obtained.

According to the equation

$$C_6H_{12}O_6 \rightarrow 2\,C_3H_6O_3$$

two mols of lactic acid are formed from one mol of glucose. The number of micromols of lactic acid has therefore to be divided by two to obtain the number of micromols of glucose metabolized.

Table 7 shows the ratio of glucose converted to lactic acid to glucose oxidized aerobically for each separate experiment as well as the mean value for each bacterial strain and the mean value for each group of bacteria.

There is obviously a marked difference between the lactic bacteria and the other groups. The lactobacilli have the highest ratio 61.2 and then the streptococci follow with 19.6. An interesting fact is that strain no. 113, which is a Streptococcus salivarius (*Strålfors*, 1950, page 30), has the high value of 77.7, thereby deviating from the other strains of streptococci, which belong to the species Streptococcus mitis.

The white and yellow staphylococci have different values; the white form lower (0.42) than the yellow form (2.49).

Still lower values are characteristic of the other bacteria: gaffkya 0.20, sarcina 0.10 and actinomyces 0.07.

The sum of glucose remaining after the experiment, glucose changed to lactic acid, and glucose broken down by aerobic oxidation.

In addition to the lactic acid analysis, a determination of the glucose content was also made in most of the experiments. If the only reactions that took place were the breakdown of glucose

to lactic acid and glucose plus oxygen to carbon dioxide and water, the sum of the glucose used up in these two processes plus the glucose remaining should be exactly equal to the glucose originally supplied.

Deviations from this scheme are, however, to be expected. Other fermentation products than lactic acid will result. It is known that staphylococci produce valeric acid (*Fosdick & Rapp,* 1943) as well as lactic acid. Sarcinae produce acetic acid (*Fosdick & Calandra,* 1945).

Furthermore, part of the glucose may be assimilated, i.e. synthesised to more complex substances, which will be incorporated in the cell contents. That this is the case not only for proliferating cells but also in a washed suspension was shown by *Giesberger* (1936) for Spirillum serpens and by *Clifton* (1937) for Pseudomonas calco-acetica.

The figures for "summed up glucose" are shown in Table 8. Only the mean value of the experiments is given for each strain. The table also gives the mean value for each bacterial group. All the values are smaller than the amount of glucose added originally which was 0.30 ml of a 280 mC glucose solution equal to 84 micromols of glucose. The staphylococci have remarkably low values indicating that they metabolize to a great extent along other pathways.

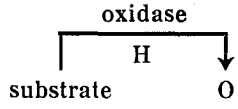
Anaerobic acid production measured in the Warburg apparatus

The results from this experimental series appear in Table 9. Compared with the results of the earlier work by the author with a pH measuring method (*Strålfors,* 1950, pages 55—58) they are of approximately the same magnitude although somewhat lower. Since the pH value is not under control in the Warburg respirometer an exact comparison cannot be made. The technique is quite laborious in this type of experiment, and it does not seem to have any advantages over the pH measuring method.

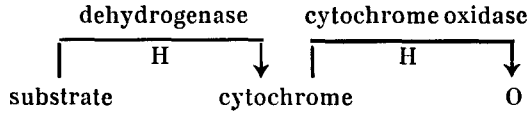
DISCUSSION

In biologic oxidations, the hydrogen is only rarely transferred directly from the substrate to the oxygen. This does take place, however, with amino acid oxidases found in animal tissues and

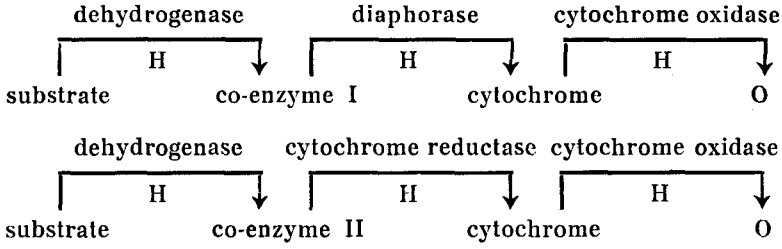
in the bacterial species Proteus vulgaris. Oxidation then follows the scheme:



Generally, the hydrogen is transferred from the substrate to an intermediate carrier, which then gives it off again. There are different oxidation systems of this kind. The hydrogen may be transferred to cytochrome and then from cytochrome to oxygen by the action of cytochrome oxidase.



A still more complicated system is that in which the hydrogen is first transferred to co-enzymes I or II.



There are three forms of cytochrome, called a, b and c, distinguished by their different absorption spectra. A bacterial species may have all three forms of cytochrome, or two, or one or none at all.

The bacteria investigated in this work and the species closely related to them are characterized by their cytochrome content as shown in Table 10 (*Gate,* 1947, page 40; *Porter,* 1947, page 553; *Stephenson,* 1949, page 25).

As far as the author could ascertain, no investigation has been made of the cytochrome in viridans streptococci from the mouth. They are very closely related, however, to the pneumococci and the question is then if they contain cytochrome a and b or if they, like the other streptococci, are completely devoid of cytochrome.

Concerning the bacteria used in this investigation it may thus be supposed, that in the staphylococci and sarcina the respiration is mediated by a cytochrome system.

The process of respiration in bacteria lacking cytochrome is not known, but it probably occurs by the direct transfer of hydrogen to oxygen by means of an oxidase (*Stephenson,* 1949, page 27). As yet, however, no such oxidase has been found in any bacteria.

When hydrogen combines with oxygen, either water or hydrogen peroxide may be formed. As described above, hydrogen peroxide was most probably formed by the streptococci and lactobacilli.

SUMMARY

The Warburg constant volume respirometer has been used to investigate the aerobic and anaerobic breakdown of carbohydrates. Chemical determinations of the amounts of lactic acid and glucose were made simultaneously with the gasometric measurements.

The groups of bacteria studied were: streptococci, lactobacilli, staphylococci, gaffkyacocci, sarcinacocci and actinomyces. The characteristics of the bacterial strains used were given in an earlier publication (*Strålfors,* 1950).

The following results were obtained

1) Endogenous respiration accounted for only a relatively small fraction of the respiration of the bacteria in glucose.

2) Streptococci and lactobacilli showed decreasing respiratory activity, in terms of oxygen taken up, as the experiment proceeded. The other bacterial groups had a more uniform activity curve.

3) The absorption of oxygen was on a lower level for the streptococci and lactobacilli than for the other bacteria.

4) It was possible to make an approximate calculation of the depth in the dental plaque, under which strictly anaerobic conditions would prevail.

5) The respiratory quotient was especially high with lactobacilli, the mean value being 1.45. For streptococci it was significantly lower than 1, with a mean value of 0.89 ± 0.04. For the other bacterial species, the quotient was very near to 1. Various possible explanations of the different values are discussed.

6) The relationship between the amount of glucose which was changed to lactic acid in the presence of air, and the amount that was, at the same time, aerobically oxidated, was variable. Mean values for this relationship were: for lactobacilli 61, for streptococci 20, for yellow staphylococci 2.5, for white staphylococci 0.42, for gaffkya 0.20, for sarcina 0.10 and for actinomyces 0.07. Thus it appears that lactobacilli and streptococci predominantly break down carbohydrates anaerobically to acid even when they have good access to air.

7) The sum of the amount of glucose remaining after the experiment, the glucose changed to lactic acid and the glucose metabolized by aerobic oxidation was constantly lower than the total amount originally supplied. Staphylococci showed the lowest value. Various other possible ways of glucose consumption are discussed.

8) The acid production, measured by the gasometric method under strictly anaerobic conditions, was of approximately the same magnitude as was reported earlier by the present author using a method with pH determination (*Strålfors,* 1950). The gasometric method of determining acid production does not seem to be particularly advantageous.

RÉSUMÉ

UNE ÉTUDE SUR L'ACTIVITÉ RESPIRATOIRE DES BACTÉRIES BUCCALES

Des expériences sur la respiration des microbes de la cavité buccale ont été faites selon la méthode de Warburg.

Les groupes de microbes examinés ont été les suivants: streptocoques, lactobacilles, staphylocoques, gaffkyas, sarcines et actinomycètes. Les caractéristiques de ces microbes sont précisées dans l'ouvrage précédent par l'auteur (*Strålfors,* 1950).

Les résultats des recherches présentes sont:

1) La respiration endogène constituait seulement un part peu considérable de la respiration dans du glucose.

2) Les streptocoques et les lactobacilles montraient une activité décroissante de l'absorption d'oxygène. Les autres microbes montraient une activité de l'absorption d'oxygène plus uniforme.

3) Les streptocoques et les lactobacilles montraient une activité de l'absorption d'oxygène inférieure à celle des microbes restants.

4) Un calcul approximatif a été fait de la profondeur des matériaux de plaques à laquelle des conditions anaérobies pourraient se produire.

5) Le quotient respiratoire était spécialement élevé chez les lactobacilles, en moyenne 1.45. Pour les streptocoques il était notablement inférieur à 1, en moyenne 0.89 ± 0.04. Pour les autres sortes de bactéries le quotient etait très proche de 1. On a discuté différentes possibilités pour expliquer les diverses valeurs de quotient.

6) Le rapport entre la quantité de glucose transformé en acide lactique en présence d'air — et la quantité qui a subi une oxydation aérobie etait très variable pour les groupes différents de microbes. Les moyennes etaient: pour les lactobacilles 61, pour les streptocoques 20, pour les staphylocoques jaunes 2.5, pour les staphylocoques blancs 0.42, pour les gaffkyas 0.20, pour les sarcines 0.10 et pour les actinomycètes 0.07.

7) La somme de glucose restant après l'expérience, de glucose transformé en acide lactique et de glucose transformé par l'oxydation aérobie, était partout inférieure à la quantité ajoutée au début. La valeur la plus basse a été trouvée chez les staphylocoques. On a mis en discussion les possibilités différentes d'une autre dépense de glucose.

8) La production d'acide mesurée pendant des conditions anaérobies dans l'appareil de Warburg a donné des résultats presque les mêmes que les résultats obtenus par l'auteur par la mesure du pH et publiés dans l'ouvrage précédent (*Strålfors*, 1950). L'emploi de la méthode de Warburg ne parait pas offrir d'avantages pour la determination de la production d'acide.

ZUSAMMENFASSUNG

EINE UNTERSUCHUNG ÜBER DIE RESPIRATORISCHE AKTIVITÄT DER MUNDBAKTERIEN

Mit Warburgs Respirometer wurden Bestimmungen der aeroben und anaeroben Spaltung der Kohlenhydrate ausgeführt. Auch wurden Milchsäure und Glukose mit chemischen Methoden bestimmt.

Die untersuchten Bakterien waren: Streptokokken, Laktobazillen, Staphylokokken, Gaffkyakokken, Sarcinakokken und Aktinomyces. Die karakteristischen Eigenschaften der angewandten

Stämme sind in einer früheren Arbeit näher beschrieben (*Strålfors*, 1950).

Folgende Resultate wurden erzielt:

1) Endogene Respiration machte nur einen geringen Teil der Respiration in Glukose aus.

2) Streptokokken und Laktobazillen zeigten eine fallende Aktivität der Sauerstoffabsorption während des Experimentes. Die übrigen Bakterienarten zeigten eine mehr gleichmässige Aktivität.

3) Streptokokken und Laktobazillen hatten eine niedrigere Sauerstoffabsorption als die übrigen Bakterien.

4) Es war möglich approximativ zu berechnen, in welcher Tiefe des Plaquematerials streng anaerobe Verhältnisse herrschen.

5) Die respiratorische Quote war besonders hoch für Laktobazillen, Mittelwert 1.45. Für Streptokokken war die Quote niedriger als 1, Mittelwert 0.89 ± 0.04. Für die anderen Bakterienarten war die Quote sehr nahe 1. Mehrere Möglichkeiten, diese Verschiedenheiten zu erklären, werden diskutiert.

6) Das Verhältnis zwischen der Glukosemenge, die zu Milchsäure verwandelt wurde, und der, die in derselben Zeit aerob oxydiert wurde, variierte. Mittlere Werte für diese Quote waren: für Laktobazillen 61, für Streptokokken 20, für gelbe Staphylokokken 2.5, für weisse Staphylokokken 0.42, für Gaffkya 0.20, für Sarcina 0.10 und für Aktinomyces 0.07. Es ergibt sich also, dass Laktobazillen und Streptokokken auch bei guter Zufuhr von Luft die Kohlenhydrate grösstenteils anaerob zu Milchsäure spalten.

7) Bei Zufuhr von Luft wird die Glukose teils zu Milchsäure verwandelt, teils zu Kohlendioxyd und Wasser oxydiert, und ausserdem bleibt ein Teil der Glukose unverändert in der Lösung. Die Summe dieser drei Glukosemengen war in allen Experimenten geringer als die ursprünglich zugeführte Quantität. Die Umsetzungsprodukte anderer Art, die also entstehen müssen, wurden diskutiert.

8) Die anaerobe Säureproduktion gasometrisch bestimmt war von ungefähr derselben Grösse wie die in der früheren Arbeit des Verfassers mittels pH-Messungen gefundene. (*Strålfors*, 1950). Die gasometrische Methode der Säurebestimmung scheint gegenüber der pH-Messungsmethode keinen Vorteil zu haben.

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