THE RESPIRATION OF STREPTOCOCCUS PYOGENES

I. Optimal Conditions of Respiration[1]

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Studies on the respiration of washed suspensions of various bacteria are quite numerous. However, only a few attempts have been made with *Streptococcus pyogenes*, possibly because the washed suspensions of this group of organisms respire very little. A systematic study of the respiratory enzyme systems of various strains of hemolytic and non-hemolytic streptococci, *Streptococcus viridans*, *S. liquefaciens*, *S. lacticus*, *S. fecalis*, and *S. bovis* was carried out by Farrell (1935). He reported that the oxygen uptake of the washed streptococci suspended in saline as measured in the Warburg apparatus was negligible after a period of eight hours. In the presence of glucose these microörganisms consumed approximately 15 cmm. of oxygen within the eight hour period (personal communication). Chu and Hastings (1938) stated that their experiments with β-hemolytic streptococci (*Streptococcus pyogenes*), were unsuccessful because in the presence of glucose the oxygen uptake by the washed suspensions of these bacteria was negligible. Barron and Jacobs (1938) on the other hand, experimenting with erysipelas, scarlet fever, and septicemia strains, found that, out of seven strains, three consumed oxygen in the presence of glucose. They further stated that different strains of hemolytic streptococci varied greatly in this respect.

In the present study washed suspensions of *Streptococcus pyogenes*, strains C203M, C203S, C203R, 1685M, and 1685S were used.[2] The volume of oxygen consumed in the presence of glu-

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[2] The first three are mucoid, smooth and rough variants of a strain which is classified by Lancefield (1940) as belonging to both types I and III. Strains 1685M and S are mucoid and smooth variants of a strain belonging to Griffith type I.

TABLE 1

Aerobic and anaerobic respiration of streptococci

NUMBER	AEROBIC RESPIRATION OF STREPTOCOCCUS PYOGENES cmm. O_2/hr./mgm. streptococci					ANAEROBIC GLYCOLYSIS BY STREPTOCOCCUS PYOGENES cmm. CO_2/hr./mgm. streptococci				
	Aerobic system	C203M	C203S	C203R	1685M	Anareboic system (95% N_2 + 5% CO_2)	C203M	C203S	C203R	1685M
	ml.					*ml.*				
1	Streptococci......0.4 Saline............2.4 No glucose	4 4	6	3 0		Streptococci......0.4 Saline............2.2 $NaHCO_3$ 1M......0.2 No glucose	0 0		2 2	4 0
2	Streptococci......0.4 Saline............2.0 Glucose 0.1 M....0.4	24 20 17	25 42 30	46 8 13 39		Streptococci......0.4 Saline............2.0 $NaHCO_3$ 1M......0.2 Glucose 0.2 M....0.2	44 13 36 50 204	35 5 4	27 23 35	34 103 65 136
3	Streptococci......0.4 Yeast extract.....1.0 Saline............1.4 No glucose	11 25	26	6 5		Streptococci......0.4 Yeast extract.....1.0 Saline............1.2 $NaHCO_3$ 1M......0.2 No glucose	3 1	34	2	0 0
4	Streptococci......0.4 Yeast extract.....1.0 Saline............1.0 Glucose 0.1M.....0.4	67 57 132 163	109 113 99	55 41 36 67	91 87 97	Streptococci......0.4 Yeast extract.....1.0 Saline............1.0 $NaHCO_3$ 1M......0.2 Glucose 0.2M.....0.2	262 266 220	495 373 148	236 242 252	364 463 402
5	Streptococci......0.4 Rabbit serum.....1.0 Saline............1.4 No glucose	13 15	11	52 44		Streptococci......0.4 Rabbit serum.....1.0 Saline............1.2 $NaHCO_3$ 1M......0.2 No glucose	4 3		2 0	
6	Streptococci......0.4 Rabbit serum.....1.0 Saline............1.0 Glucose 0.1M.....0.4	77 51 101 109	65 87 71	60 47 68	97 97	Streptococci......0.4 Rabbit serum.....1.0 Saline............1.0 $NaHCO_3$ 1M......0.2 Glucose 0.2M.....0.2	244 241 302 291 336 408 182	144 167 117 65	265 270 293 204	324 313 317
7	Streptococci......0.4 Yeast extract.....0.5 Rabbit serum.....0.5 Saline............1.4 No glucose	20 19	26	29 24		Streptococci......0.4 Yeast extract.....0.5 Rabbit serum.....0.5 Saline............1.2 $NaHCO_3$ 1M......0.2 No glucose	3 1		21 21 3	

TABLE 1—*Concluded*

NUMBER	AEROBIC RESPIRATION OF STREPTOCOCCUS PYOGENES CMM. O_2/HR./MGM. STREPTOCOCCI					ANAEROBIC GLYCOLYSIS BY STREPTOCOCCUS PYOGENES CMM. CO_2/HR./MGM. STREPTOCOCCI				
	Aerobic system	C203M	C203S	C203R	1685M	Anaerobic system (95% N_2 + 5% CO_2)	C203M	C203S	C203R	1685M
	ml.					*ml.*				
8	Streptococci......0.4	208	81	75	88	Streptococci......0.4	351	345	549	552
	Yeast extract.....0.5	109	148	107	121	Yeast extract.....0.5	345	375	556	620
	Rabbit serum.....0.5	176	120	104	115	Rabbit serum.....0.5	326	150	262	385
	Saline.............1.0	122	98		88	Saline.............1.0	398			448
	Glucose 0.1M.....0.4	141				$NaHCO_3$ 1M......0.2	338			
		197				Glucose 0.2M.....0.2	655			
		270					558			
							487			

cose and phosphate buffer of pH 7.4 was of the order of 15–40 cmm. per milligram dry weight of organisms per hour. The anaerobic glycolysis of glucose by these strains was likewise low (see table 1, systems 1 and 2). Either the suspensions of the organisms lacked the necessary respiratory factors (co-enzymes, etc.), or by washing the organisms they were deprived of these factors. By the addition of yeast extract, normal serum, or both, or of whole defibrinated blood to the system, the oxygen consumption and anaerobic glycolysis in the presence of glucose were readily accelerated several fold (table 1). Figure 1 shows that the amount of respiration under these conditions is nearly proportional to the number of organisms.

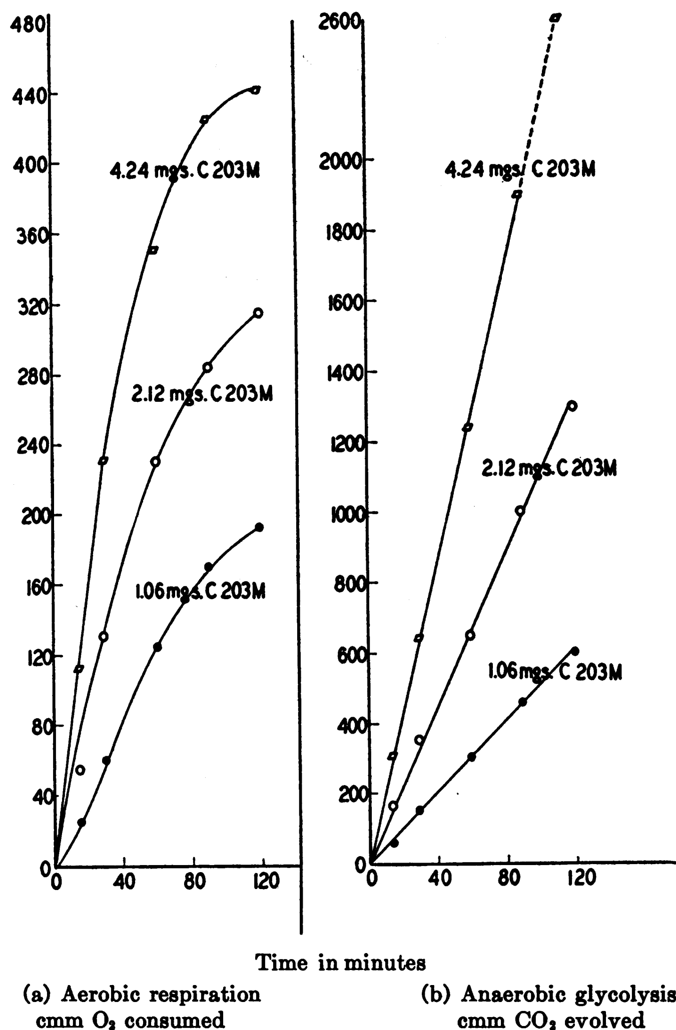
Experiments to determine the optimal conditions for the respiration of these organisms were carried out since further work was planned to determine the effect of various drugs on bacterial respiration.

EXPERIMENTAL

Method of culture

The stock cultures are kept in the refrigerator. The M and S strains in blood broth are transferred to fresh blood broth every three days. The R strains are kept in plain broth. From these 0.2 ml. are planted in 100 ml. of 1 per cent peptone broth contain-

ing 5 per cent normal horse serum and 0.5 per cent glucose. At the end of 16 hours growth the culture is centrifuged, most of the



Time in minutes

(a) Aerobic respiration cmm O_2 consumed

(b) Anaerobic glycolysis cmm CO_2 evolved

FIG. 1. AMOUNT OF RESPIRATION OF STREPTOCOCCI AS A FUNCTION OF NUMBER OF ORGANISMS

supernatant discarded, leaving about 20–25 ml.; the bacterial sediment is mixed in this and transferred to 600 ml. of broth containing 5 per cent serum and 0.5 per cent glucose and allowed to

grow for four hours. By this process we obtain young actively growing cultures of M, S, and R strains. From several preliminary experiments with cultures of varying ages we found the four-hour culture the most active and therefore more suitable for this study. At the end of the four-hour period the culture is centrifuged in an angle centrifuge at the speed of 3500–5000 r.p.m. to spin down the slimy organisms. The sediment is then washed twice; with physiological saline for anaerobic glycolysis of glucose, with m/15 phosphate buffer of pH 7.4 for the aerobic respiration. The culture is plated on blood agar to test for purity and for colony characteristics.

Measurement of respiration. Aerobic and anaerobic respiration were measured by means of the usual Barcroft-Warburg apparatus calibrated with mercury and checked by a chemical method. The flasks were of two different sizes, one set suitable for systems of small volumes (2–2.8 ml.) and another set for systems of larger volumes (5–5.8 ml.). The temperature of the water bath was held constant at 37.5 ($\pm$0.1) degrees C. In measuring the oxygen consumption glucose was used as a substrate. The evolving carbon dioxide was absorbed by a roll of No. 40 filter paper which had been wetted with 0.3 ml. of 20 per cent KOH. The absolute volume of oxygen consumed was thus measured. In the anaerobic glycolysis of glucose the carbon dioxide measured was the result of a reaction between the sodium bicarbonate added to the system and the lactic acid and other acids or both formed from the breakdown of glucose. The essential reactants were kept separate in the side arms of the flasks until after the necessary preparatory steps such as the equilibration of the temperature, etc. were taken; they were then mixed.

The weight of streptococci used for each test. The weight of washed streptococci used in each test was determined by the total nitrogen content of the samples used. The total nitrogen $\times$ 7.51 = weight of streptococcal sample. The factor 7.51 is derived from analysis of streptococci for total nitrogen which averaged 13.2 per cent (Sevag, Smolens, and Lackman, 1940). For the nitrogen determinations carried out during this study we are indebted to Mr. J. Smolens.

Number of cocci in 1 mgm. of streptococci. The number of cocci

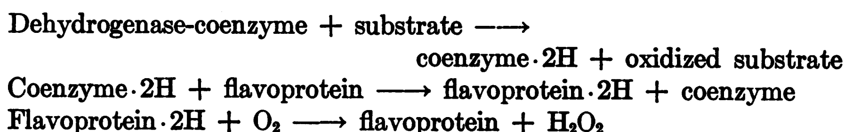
in several of the samples used for respiration was counted by Mr. Joseph Snyder using the methods of Breed (1911) and Hanks and James (1940). In seven determinations the number of individual cocci in 1 mgm. of streptococci (C203M) ranged from 2.27×10^9 to 3.05×10^9 and gave an average of 2.75×10^9 cocci per milligram of streptococci, and from a similar counting the average number was 3.74×10^9 cocci per milligram of C203S strain.

Preparation of yeast extract. Extracts were prepared from baker's yeast cakes or beer yeast cultures. Both extracts were found to be equally active in accelerating the respiration of streptococci. The extract from beer yeast was made as follows:

One liter of a thick yeast culture was filtered through a Buchner funnel and washed repeatedly with distilled water until the filtrate was colorless and the smell of beer was completely eliminated. The caked yeast suspended in 1 liter of distilled water was boiled for 10–15 minutes, cooled and centrifuged. The supernatant was filtered through a Seitz E.-K. filter. The filtrate was then concentrated *in vacuo* and after filtering through a Seitz filter was dried *in vacuo* from the frozen state (Flosdorf and Mudd, 1938; Flosdorf, Stokes, and Mudd, 1940). Seven and three-tenths grams of dry material were thus obtained. Three to 6 mgm. of the dry extract was used in each respiration system.

Experiments regarding hydrogen peroxide formation. The fundamental findings of M'Leod and Gordon (1923) have shown that hemolytic streptococci do not contain catalase and are hydrogen-peroxide formers. They classified streptococcal strains into non-, mild-, and moderate hydrogen-peroxide-forming groups. Fuller and Maxted (1939) investigated 75 strains of streptococci grown in various media for their hydrogen-peroxide-forming property, using a sensitive method which detected 1 in 3 million parts of hydrogen peroxide. Ten type 3 strains failed to show peroxide. Of the other 65 strains only 2 (one type 5 and one type 1), failed to form peroxide. Farrell (1935) reported that streptococci do not contain catalase, cytochrome, or indophenol-oxidase, showing the absence of the cytochrome-oxidase respiratory system in streptococci. It is also known that pneumococci (Sevag, 1933a, 1933b; Sevag and Maiweg, 1934), *Lactobacillus acidophilus*

(Fromageot and Roux, 1934), and *Lactobacillus bulgaricus* (Bertho and Gluck, 1932) are hydrogen peroxide formers. It is believed that these organisms respire according to the following system:



Dehydrogenase-coenzyme + substrate ⟶ coenzyme·2H + oxidized substrate
Coenzyme·2H + flavoprotein ⟶ flavoprotein·2H + coenzyme
Flavoprotein·2H + O_2 ⟶ flavoprotein + H_2O_2

which accounts for the hydrogen-peroxide-forming property of haemin-free bacterial cells.

We also studied this aspect of the problem so that the necessary precautions could be taken in our experiments and in evaluating

TABLE 2

Aerobic respiration of Streptococcus pyogenes in the presence and absence of catalase

AEROBIC SYSTEM (CMM. O_2/HR./MGM. STREPTOCOCCI)		STRAIN C203M	
		Without catalase	With 0.2 ml. catalase
	ml.		
Streptococci in phosphate pH 7.4	0.4	104	109
Saline	1.0	88	88
Yeast extract	0.5	54	83
Normal serum*	0.5	56	49
Glucose 0.1M	0.4	67	72
		91	93

* Serum inactivated at 56°C. for one hour is free of catalase activity.

the results. At the conclusion of the respiration experiments with C203M strain we analyzed the reaction systems for hydrogen peroxide. In none of the aerobic systems were we able to demonstrate the presence of hydrogen peroxide. The volume of oxygen in 8 experiments ranged from 110–270 cmm. O_2 per hour per milligram of streptococci. This volume of oxygen in the form of hydrogen peroxide is capable of liberating iodine from potassium iodide, equivalent to 2 to 4.83 ml. of 0.005 N $Na_2S_2O_3$ solution. By this method an amount of hydrogen peroxide corresponding to 12 cmm. oxygen (= 0.2 ml. of 0.005 N $Na_2S_2O_3$ solution) was measurable.

It has previously been shown in numerous instances (Fuller and

Maxted, 1939; Sevag, 1933a and b; Sevag and Maiweg, 1934; Dixon, 1925) that the respiration and growth of H_2O_2-forming bacteria, as well as the H_2O_2-forming enzyme systems, are accelerated many fold in the presence of catalase. In our experiments the absence of the toxic effect or the accumulation of measurable amounts of hydrogen peroxide in the system oxidizing glucose by strain C203M was also confirmed by the lack of accelerative response to catalase in the presence of yeast extract and serum and absence of significant acceleration in the presence of rabbit whole-blood (see tables 2 and 3). These indicate that hydrogen peroxide which theoretically is believed to be formed (Wieland) from the oxidation of glucose by streptococci is, perhaps, utilized in the nascent state by a secondary reaction such as that between pyruvic acid and H_2O_2 (Sevag, 1933a and b; Sevag and Maiweg, 1934).

TABLE 3

Aerobic and anaerobic respiration of Streptococcus pyogenes in the presence of rabbit whole blood

NUMBER	AEROBIC RESPIRATION IN WHOLE BLOOD (CMM. O_2/HR./MGM. STREPTOCOCCI C203M)		ANAEROBIC RESPIRATION IN WHOLE BLOOD (CMM. CO_2/HR./MGM. STREPTOCOCCI C203M)	
	Aerobic systems	O_2	Anaerobic systems	CO_2
	ml.	*cmm.*	*ml.*	*cmm.*
1	Rabbit blood*............1.0	15	Rabbit blood*............1.0	90
	Saline....................4.0	23	Saline....................4.4	28
	Glucose 0.1M............0.4	17	Glucose 0.2M............0.2	69
	Phosphate pH 7.4........0.4	14	$NaHCO_3$ 1M.............0.2	
2	Rabbit blood............1.0	164†	Rabbit blood............1.0	215†
	Saline....................4.0	138	Saline....................4.0	328
	Glucose 0.1M............0.4	129	Streptococci (in saline)..0.4	165
	Streptococci (in phosphate pH 7.4)..........0.4	91	Glucose 0.2M............0.2	
			$NaHCO_3$ 1M.............0.2	

* Rabbit blood defibrinated by beads, citrate or heparin gave similar results. Experiments with 1 ml. rabbit blood in a respiration system of 2.8 ml. also gave results similar to those given in the table.

† These figures are corrected for the control figures in system 1.

SUMMARY AND CONCLUSION

Washed suspensions of β-hemolytic streptococci oxidize glucose aerobically only to a negligible degree. The amount of anaerobic

glycolysis of glucose by these organisms is likewise very low. This negligible respiration is found to be due to the absence of respiratory factors (coenzymes, etc.) in the washed suspensions of streptococci. When yeast extract, serum, yeast extract plus serum, or whole blood are added to the washed suspension the aerobic and anaerobic respiration are several fold accelerated. As will be shown in a following paper, under certain of these conditions of respiration the organisms do not appear to multiply.

Attempts to demonstrate the accumulation of detectable amounts of hydrogen peroxide in aerobic systems after 2- to 3-hour periods of respiration failed. The oxygen consumption was not accelerated by the addition of catalase or whole blood to the system, showing that hydrogen peroxide does not accumulate to slow down the respiration. This is in contrast to the accelerating effect of catalase on pneumococcal respiration which shows measurable amounts of hydrogen peroxide. It may possibly be that hydrogen peroxide, which is theoretically expected to be formed by streptococci, is used up in a nascent state in a secondary reaction, or as soon as it forms. We are investigating this question further.

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