RESPIRATORY STUDIES OF THE MICROCOCCI

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by

Tom Duvall Nunheimer

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Introduction

The respiration of bacteria is a subject of primary importance in understanding the mechanism by which energy is furnished to the cell. These energy mechanisms in turn form an hypothesis which enables a better understanding of the methods by which foods are made available for life processes.

Respiratory studies likewise assist in an understanding of many biochemical reactions such as one finds in the oxidation-reduction of many indicators and dyes used in bacteriology such as methylene blue, litmus, dyes used in Endo's medium, indicator in tellurite medium, eosin-methylene blue and many others.

The sensitivity of gram-positive and gram-negative organisms to certain dyes such as brilliant green and crystal violet might indicate a fundamental difference in the enzymatic makeup of the organism.

A study of the respiratory enzymes found in bacteria leads to a better understanding of the fundamental differences between aerobiosis and anaerobiosis, and has helped to explain the relation of oxidation to fermentation.

In view of the fact that respiratory enzymes play an important part in many of the fundamental reactions of the cell and since there has been only a very limited amount of work done upon the respiratory enzymes of the Micrococci, the present study was undertaken.

Literature Survey of the Dehydrogenases

It is generally accepted that biological oxidation-reduction manifests itself as a transfer of hydrogen (electrons) from a donator to an acceptor, the transfer yielding energy to the organism. The role of oxygen is that of a hydrogen acceptor, while under anaerobic conditions the oxygen is replaced by some other suitable acceptor. Metabolites such as glucose, succinic acid, ethyl alcohol, etc., are so changed under the influence of certain specific cellular agents that hydrogen atoms become transferred to reducible substances. An activation of the metabolite by the cell initiates the hydrogen transfer; the specific enzymes activating this hydrogen transfer being known as dehydrogenases.

The presence of dehydrogenases is generally demonstrated by means of the Thunberg (1918) methylene blue technique.

P revious to 1937 studies of the dehydrogenases produced by bacteria were limited almost entirely to the colon-typhoid group. (Braun and Wordehoff 1933, Cook 1930, Green and Stickland 1934a,1934b, Quastel and Wooldridge 1928, Wooldridge, Knox and Glass 1936, and Yudkin 1933,1934 and 1937)

In 1932 Braun and Vasarhelyi studied the dehydrogenases of Staphylococcus aureus and found that relatively few substrates were dehydrogenated. Among the carbohydrates and related compounds fructose, maltose, sucrose and glycerol were oxidized while arabinose,galactose,lactose,mannitol and dulcitol were not activated.

Of thirteen organic acid salts used only formate, lactate, pyruvate and fumarate were actively dehydrogenated while such salts as succinate, tartrate, acetate, malate and citrate were inactive. Most of the amino acids were activated with the exception of glycine, valine, leucine and phenylalanine.

Fabre (1935) studied the dehydrogenases produced by Staphylococcus aureus. Of the 67 substrates tested only 27 were found to be active. The most actively dehydrogenated were glucose, mannose, galactose, sucrose, lactose, xylose, maltose, lactate and formate. Substrates weakly activated were succinate, fumarate, ethyl alcohol and glutamate. The hydrogen donors which were inactive or doubtful were dulcitol, rhamnose, acetate, citrate, oxalate, alanine, phenylalanine, asparagine and aspartate.

Ehrismann (1937) made a study of the dehydrogenases produced by micrococci and streptococci. The dehydrogenase activity was determined for Staphylococcus aureus and albus, Streptococcus pyogenes, Micrococcus tetragenus and candicans.

Most of the substrates were found to be activated by all the organisms, the difference being quantitative rather than qualitative. The main exceptions were the inability of M. candicans to activate malate, ethyl alcohol and arabinose; the inability of M. tetragenus to activate dulcitol; and the ability of Staphylococcus albus to dehydrogenate asparagine.

Experimental

Study of the Dehydrogenase Activity of the Micrococci

The various strains of micrococci were tested by the Thunberg (1918) technique to determine the dehydrogenase activity.

Method

The organisms were grown in Roux bottles at room temperature for 30 to 34 hours on a medium of the following composition:

0.5 per cent peptone
0.2 per cent yeast extract
0.2 per cent peptonized milk
0.2 per cent meat extract
3.0 per cent agar
pH 7.0-7.1

After incubation the growth was removed and washed three times (by centrifugation) with saline and suspended in saline in a bottle fitted with a tube for sterile aeration. The cell suspension was made up to a volume of ten milliliters for each Roux bottle in the harvest. The cell suspension was stored at five degrees and aerated at that temperature before use.

In the Thunberg tube was placed 1 ml. substrate (M/10 unless otherwise indicated), 1 ml. saline, 1 ml. methylene blue 1-5000, and 1 ml. of phosphate buffer. In the hollow stopper was placed 1 ml. of aerated cells.

The buffer used was a mixture of M/30 K_2HPO_4 and M/30 KH_2PO_4 with a pH of 6.85.

The tubes were evacuated for 2.5 minutes with constant shaking and incubated in a water bath at 40°C. After allowing for equilibrium the cells were tipped into the reaction mixture and the time required to bring about complete reduction of the methylene blue was noted.

The cell suspension was diluted with saline so that the reduction time, in the absence of added substrate, was about one hour. In this connection, Yudkin (1937) found that with Escherichia coli dilution of the cells resulted in a disproportionate fall in the dehydrogenase activity. This was found to be due to the presence of a coenzyme which became the limiting factor at higher dilutions. In view of this fact, boiled cells were added to the diluted cell suspension to reduce the reduction time to the value calculable from the degree of dilution.

The substrates were made up to M/10 unless otherwise indicated and adjusted to pH 7.1. The time required for complete reduction of the methylene blue was noted and the dehydrogenase values were calculated and reported in the following manner:

$$\frac{\text{reduction time of control}}{\text{reduction time with substrate}} \times 100 = \text{Dehydrogenase Value}$$

It can be seen that the reduction time of the control in which there was no added substrate would in each case result in a Dehydrogenase Value of 100, while a Dehydrogenase Value of 200 in the presence of a substrate would indicate that the reduction time was half that of the comtrol.

Preliminary tests showed the dehydrogenase activity to be stable for three or four days if the cell suspensions were stored at five degrees centigrade.

Table 1. The Rate of Dehydrogenation of Sugars and Alcohols by Micrococci. The Figures represent Dehydrogenase Values.

	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebareus
d-Arabinose	144	113	145	170	155
l-Arabinose	122	149	127	213	298
d-Xylose	127	130	133	516	425
d-Glucose	250	145	171	1260	2533
d-Fructose	329	176	133	971	1900
d-Mannose	210	183	124	744	1100
d-Galactose	123	159	297	919	316
Lactose	171	147	212	669	62
Maltose	1000	283	225	821	1100
Sucrose	367	579	136	956	1090
Raffinose	835	213	428	1075	1950
l-Rhamnose	143	169	111	1016	459
Ethyl Alcohol	350	758	6400	300	2655
Glycerol	14o	166	628	300	810
Dulcitol	161	200	102	225	2400
d-Mannitol	264	172	229	225	3900

Table 1. Continued. The Rate of Dehydrogenation of Organic and Amino Acids by Micrococci.

Substrate	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebareus
Formate	126	117	2500	168	2509
Acetate	343	180	277	299	1750
Lactate	360	200	1091	261	1520
Citrate	400	240	159	421	156
Oxalate	100	100	118	117	117
Malate	325	211	151	110	224
Succinate	442	261	261	922	228
Fumarate	303	185	142	117	101
Maleate	520	249	341	143	1017
Tartrate	292	122	103	108	208
Asparagine	447	200	351	389	320
a-Alanine	490	183	261	389	688
b-Alanine	282	175	154	130	233
Glycine	262	141	141	187	158
dl-Leucine °	182	137	129	331	177
dl-Isoleucine °	158	105	140	432	61
dl-b-Phenylalanine °	135	123	113	187	1500
d-Glutamate °	313	131	392	714	1052
Aspartate °	129	102	200	302	1157

° Substrates used in M/30 concentration

Table 2. The Relative Rates of Dehydrogenation, with Glucose taken as the Standard, equal to 100.

Substrate	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebareus
d-Glucose	100	100	100	100	100
d-Arabinose	58	78	85	13	6
l-Arabinose	49	103	74	17	12
d-Xylose	51	90	78	41	17
d-Fructose	132	121	78	77	75
d-Mannose	84	126	73	59	43
d-Galactose	49	110	174	73	13
Lactose	68	101	124	53	3
Maltose	400	195	132	65	43
Sucrose	147	399	80	76	43
Raffinose	334	147	250	85	77
l-Rhamnose	57	117	65	81	18
Ethyl Alcohol	140	523	3743	24	105
Glycerol	56	114	367	24	32
Dulcitol	64	138	60	18	95
d-Mannitol	106	119	134	18	154

Table 2. Continued. The Relative Rate of Dehydrogenation, with Glucose taken as the Standard, equal to 100.

Substrate	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebaraus
Formate	50	81	1462	13	99
Acetate	137	124	162	24	69
Lactate	144	138	638	21	60
Citrate	160	166	93	33	6
Oxalate	40	69	69	9	5
Malate	130	146	88	9	10
Succinate	177	180	155	73	9
Fumarate	121	128	83	9	4
Maleate	208	172	199	11	40
Tartrate	117	84	60	9	82
Asparagine	179	138	205	31	13
a-Alanine	196	126	153	31	27
b-Alanine	113	121	90	10	9
Glycine	105	86	82	15	6
dl-Leucine °	73	94	75	26	7
dl-Isoleucine °	63	72	82	34	3
dl-b-Phenylalanine °	54	85	65	15	59
d-Glutamate°	125	90	228	57	41
Aspartate °	52	70	117	24	46
Endogenous respiration	40	69	51	8	4

° Substrates used in M/30 concentration

Discussion

The dehydrogenase activities of the organisms are shown on Table 1 and Table 2. It was found that the order of activity varied greatly among the organisms. In the case of the carbohydrates it was found that raffinose, maltose, sucrose, glucose and fructose were readily activated. Certain exceptions were evident since variations among strains and species occur. Among these the following instances might be mentioned. M. flavus was more active against carbohydrates than were any of the other organisms since all of the sugars except d-arabinose and d-xylose were dehydrogenated at a rate superior to that of glucose which was taken as the standard for comparison. M. aurantiacus dehydrogenated lactose and galactose at a greater rate than did the other organisms. M. freundenreichii did not activate strongly any of the sugars except glucose, raffinose and rhamnose.

Ethyl alcohol and d-mannitol were readily activated by all the organisms except M. freundenreichii which did not activate any of the alcohols to an appreciable extent. Dulcitol would be classed as 'fair to good' as a substrate for dehydrogenation by M. flavus and M. cinnebareus, but only 'poor' to the other organisms; while glycerol would be classed as 'good to excellent' with M. flavus and M. aurantiacus, but 'poor to inert' in the case of the other organisms.

The most actively dehydrogenated of the organic acids were the four carbon dicarboxylic acids succinate and maleate. Citrate, lactate, acetate and malate were activated at an appreciable rate, while fumarate, tartrate and formate served as poor donators to methylene blue. Oxalate was not activated by M. luteus and M. flavus and served as a very poor substrate for the other organisms. With M. aurantiacus and M. cinnebareus formate was found to serve as an excellent hydrogen donator, superior even to succinate and maleate. M. cinnebareus activated only formate, tartrate, acetate and lactate to an appreciable extent while such acids as succinate, citrate and malate were practically inert.

The amino acids used were not activated to an appreciable expent with the exception of glutamate and a-alanine which, together with asparagine, served as 'good to excellent' substrates. Beta alanine was not activated as rapidly as was alpha alanine but was superior to the majority of amino acids tested.

M. flavus was more active against the carbohydrates as a group than were any of the other organisms while M. cinnebareus actively dehydrogenated glucose, fructose and raffinose. M. flavus was also most active against the alcohols and amino acids.

Study of the Complete Respiratory System of the Micrococci

The various strains of micrococci were tested by the Warburg apparatus to determine the rate of oxygen uptake in the presence of the different substrates.

Method

The organisms were cultivated as for the dehydrogenase studies and were stored at five degrees centigrade and aerated each time before use.

Before determining the oxygen uptake in the presence of the different substrates, the optimum conditions for respiration were determined. It was found that the oxygen uptake was practically independent of the concentration of phosphate buffer (K_2HPO_4-KH_2PO_4) between M/10 and M/90 concentrations. Temperature and pH exerted a greater effect; the optimum temperature was between 35° and 39° centigrade. It was found, however, that at 37° and above, the rate of oxygen uptake decreased with time, so respiration studies were conducted at 35°C. which showed about the same initial rate as the higher temperatures yet decreased the tendency for heat inactivation. Using glucose as a representative substrate it was found that the respiratory system of the cells was quite stable since the oxygen uptake remained constant for four days if the cells were stored and aerated at low temperatures. The optimum pH was between 6.3 -6.8.

In light of the above facts, respiration studies were conducted at 35°C. in the presence of M/30 phosphate buffer (mixture of M/30 K_2HPO_4 and M/30 KH_2PO_4 , pH 6.7). The substrates were made up M/30 and adjusted to 7.05. The washed cell suspension was stored at 5°C. and aerated at this temperature for thirty minutes before use. The cell suspensions were considered as being stable for three days.

In the Warburg vessel were placed 1 ml. aerated cells, 1 ml. buffer pH 6.7, 0.6 ml. saline, 1 ml. substrate M/30, and in the center cup was placed 0.2 ml. of twenty per cent NaOH to take up the carbon dioxide evolved. The cells were added last, and immediately after their addition the manometers were placed in the bath and the shaking was started. After allowing ten minutes for equilibrium the cocks were closed and measurements were taken for the next 60 minutes.

Nitrogen determinations were made on each cell suspension by the Kjeldahl-Gunning method. The indicator used was a mixture of methylene blue and methyl red as suggested by Johnson and Green (1930). The oxygen uptake was reported as qO_2 which represents the cubic mm. oxygen uptake per hour per milligram of nitrogen.

The rates of oxygen uptake with time are shown in Table 4, and the results are summarized in Table 3.

Table 3. The Rate of Oxygen Uptake by Micrococci. The figures represent the qO_2 or the c.mm. oxygen uptake per hour per milligram nitrogen.

Substrate	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebareus
d-Arabinose	36.5	27.7	31.3	63.0	56.5
l-Arabinose	39.1	40.4	30.3	64.1	55.9
d-Xylose	56.5	130.3	32.5	74.3	65.4
d-Glucose	145.7	79.5	141.7	318.7	204.0
d-Fructose	67.9	40.7	99.4	465.8	74.1
d-Mannose	62.9	40.3	51.9	108.4	134.6
d-Galactose	98.7	138.3	35.8	70.2	60.8
Lactose	81.3	139.2	7.3	54.6	57.4
Maltose	145.8	193.4	41.1	371.5	180.0
Sucrose	201.8	143.2	340.3	421.2	164.1
Raffinose	58.9	163.0	35.6	79.4	66.5
l-Rhamnose	53.3	147.5	25.1	51.2	46.8
Ethyl Alcohol	150.0	194.8	333.1	406.7	552.5
Glycerol	46.3	151.4	279.6	378.5	230.1
Dulcitol	95.0	112.3	18.7	43.5	46.7
d-Mannitol	62.6	43.5	71.3	415.7	37.6

Table 3. Continued. The Rate of Oxygen Uptake by Micrococci.

Substrate	M. luteus	M. flavus	M. aurantiacus	M. freundenreichii	M. cinnebareus
Formate	43.0	55.0	127.8	207.9	33.1
Acetate	141.6	170.7	57.0	511.7	32.3
Lactate	63.5	60.1	242.4	537.6	333.8
Citrate	43.9	137.9	19.0	41.6	51.9
Oxalate	79.5	122.1	18.6	39.8	49.9
Malate	131.8	127.8	22.9	343.7	57.8
Succinate	189.7	429.0	51.9	408.5	66.9
Fumarate	174.2	313.0	33.1	292.5	63.7
Maleate	43.4	34.5	19.8	58.2	36.9
Tartrate	67.6	106.6	24.4	28.1	41.7
Asparagine	137.0	126.0	77.2	477.1	151.5
a-Alanine	102.8	161.7	33.5	338.9	465.6
b-Alanine	93.9	118.8	33.9	94.9	49.2
Glycine	116.3	134.3	154.6	237.8	60.0
dl-Leucine	56.9	37.3	32.6	57.5	41.4
dl-Isoleucine	79.4	82.0	39.6	129.5	173.5
dl-b-Phenylalanine	179.7	177.8	24.0	439.6	80.0
d-Glutamate	141.2	306.1	288.4	613.5	477.6
Aspartate	47.2	162.8	61.9	168.9	84.1
Endogenous respiration	41.8	23.1	16.2	38.1	31.5

Discussion

Table 3 indicates that sucrose, maltose and glucose were excellent hydrogen donators when oxygen was used as the ultimate hydrogen acceptor. M. flavus was more active against the sugars than were the other organisms since xylose, galactose, raffinose and rhamnose were oxidized at a more rapid rate than glucose which was taken as the standard for comparison. M. flavus alone was very active against rhamnose and M. cinnebareus was very active against fructose while these sugars were not oxidized very rapidly by any of the other organisms.

Ethyl alcohol was rapidly oxidized by all the organisms as was glycerol except in the case of M. luteus. Dulcitol was activated at an appreciable rate by M. flavus, while mannitol served as an excellent substrate for M. cinnebareus, although both were poor substrates for the other organisms.

Succinate and lactate were generally oxidized at a very rapid rate while fumarate, acetate and malate were readily activated only by M. luteus, M. flavus and M. cinnebareus. M. aurantiacus and M. freundenreichii oxidized lactate readily but were not very active against the other acid salts.

Glutamate and asparagine were oxidized rapidly by all the organisms. Alpha alanine was activated at a rate greater than that of beta alanine by all the organisms except M. aurantiacus which oxidized both at the same rate. Dl-isoleucine was oxidized at an appreciable rate by M. freundenreichii and M. cinnebareus and with all the organisms the rate was greater than for dl-leucine.

The oxygen uptake with most of the substrates was constant or decreased slightly with time, although with a few of the substrates the initial rate of respiration increased with time during the 60 minute test. This is shown in Figure 1. Glutamate showed a slight increasing oxidation rate with all the organisms except M. flavus. Acetate and dl-b-phenylalanine showed an increasing rate with M. flavus, M. cinnebareus and M. freundenreichii as did glycine and aspartate in the presence of M. aurantiacus. The initial lag noticed in the case of these substrates suggests the formation of some intermediate compound which provides a better substrate for respiration.

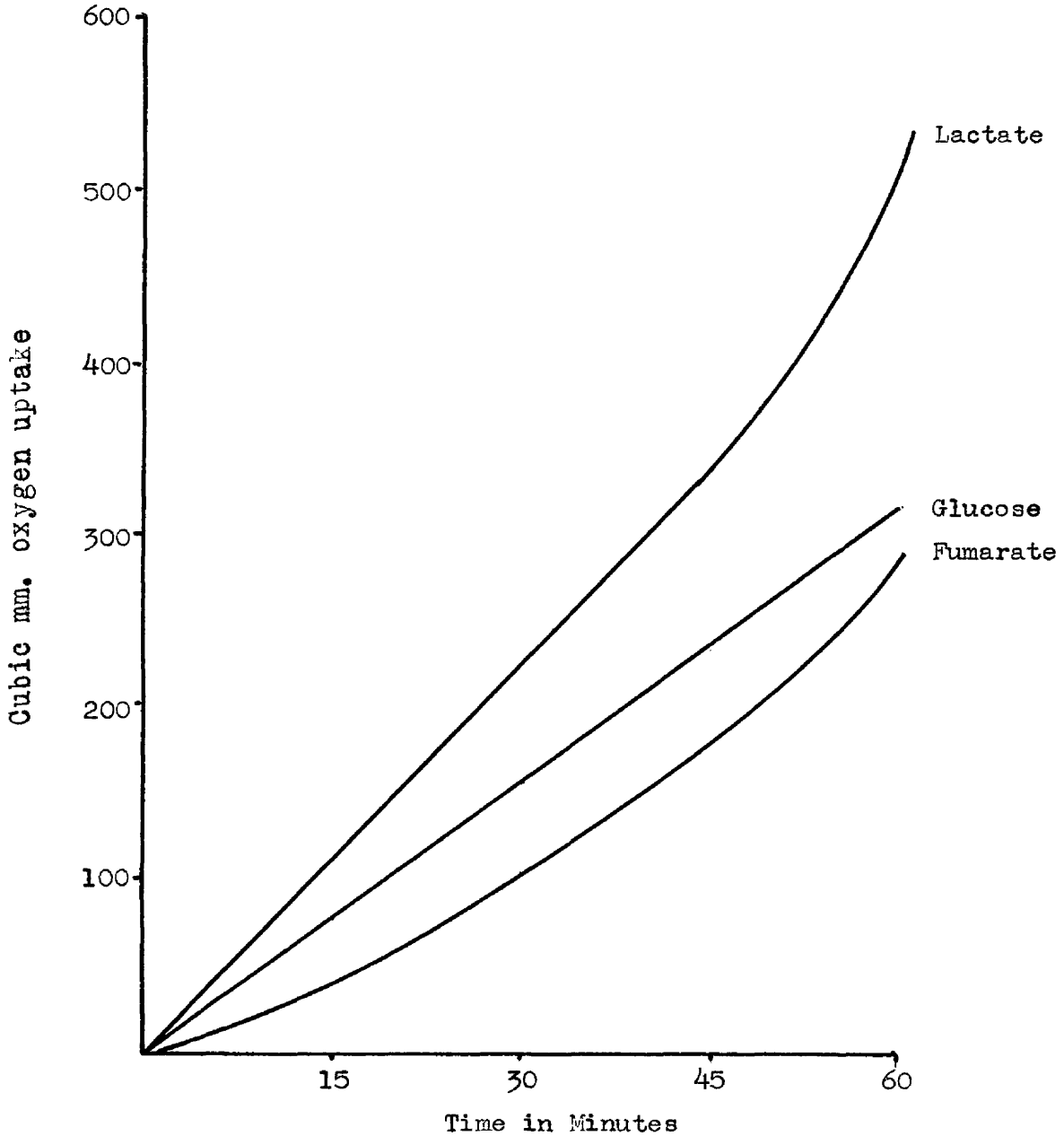

Figure 1. The Rate of Oxygen Uptake by M. cinnebareus

Table 4. The Oxygen Uptake of M. luteus . The figures represent the c. mm. oxygen per mgm. nitrogen.

Substrate	Time in minutes			
	15	30	45	60
d-Arabinose	5.3	16.6	28.3	36.5
l-Arabinose	6.6	18.2	29.6	39.1
d-Xylose	18.2	31.2	44.9	56.5
d-Glucose	43.6	80.4	114.3	145.7
d-Fructose	21.4	38.9	54.3	67.9
d-Mannose	23.5	38.9	51.4	62.9
d-Galactose	44.7	67.6	85.4	98.7
Lactose	36.5	54.3	79.2	81.3
Maltose	43.8	79.9	113.5	145.8
Sucrose	55.9	107.7	146.6	201.8
Raffinose	22.1	36.1	49.2	58.9
l-Rhamnose	18.9	31.4	43.8	53.3
Ethyl Alcohol	39.9	77.4	116.0	150.4
Glycerol	21.2	31.3	38.3	46.3
Dulcitol	48.8	71.3	85.3	95.0
d-Mannitol	27.4	41.7	52.3	62.6

Table 4. Continued. The Oxygen Uptake of M. luteus. The figures represent the c. mm. oxygen uptake per mgm. nitrogen.

Substrate	Time in minutes			
	15	30	45	60
Formate	15.6	26.2	34.9	43.0
Acetate	46.8	90.7	128.5	141.6
Lactate	19.7	33.7	52.5	63.1
Citrate	16.1	27.2	37.6	43.9
Oxalate	38.2	58.6	70.5	79.5
Malate	33.5	67.1	100.2	131.8
Succinate	51.4	101.1	147.2	189.7
Fumarate	50.1	95.6	140.7	174.2
Maleate	15.8	26.7	35.7	43.4
Tartrate	38.5	48.4	59.0	67.6
Asparagine	32.2	65.3	104.0	137.0
a-Alanine	27.8	52.4	76.6	102.8
b-Alanine	22.2	43.7	67.0	93.9
Glycine	33.1	63.1	91.1	116.3
dl-Leucine	18.9	32.8	45.5	56.9
dl-Isoleucine	25.6	45.4	63.5	79.4
dl-b-Phenylalanine	48.3	90.2	137.7	179.7
d-Glutamate	37.6	64.5	107.0	141.2
Aspartate	15.8	28.3	38.4	47.2
Endogenous respiration	14.5	22.2	30.9	41.8

Table 4. Continued. The Oxygen Uptake of M. flavus. The figures represent the c. mm. oxygen uptake per mgm. nitrogen.

Substrate	Time in minutes			
	15	30	45	60
d-Arabinose	9.1	16.9	21.1	27.7
l-Arabinose	12.1	22.4	29.3	40.4
d-Xylose	36.2	70.9	101.6	130.3
d-Glucose	24.8	45.0	63.7	79.5
d-Fructose	15.1	25.4	33.3	40.7
d-Mannose	16.9	26.4	34.2	40.3
d-Galactose	40.4	74.7	107.9	138.3
Lactose	37.1	81.6	111.5	139.2
Maltose	45.5	85.4	137.8	193.4
Sucrose	36.7	74.0	107.5	143.2
Raffinose	42.9	83.1	121.7	163.0
l-Rhamnose	39.3	75.2	113.2	147.5
Ethyl Alcohol	45.8	92.7	136.1	194.8
Glycerol	38.9	76.7	100.3	154.0
Dulcitol	32.0	63.0	92.3	112.3
d-Mannitol	17.4	31.7	38.4	43.5

Table 4. Continued. The Oxygen Uptake of M. flavus. The figures represent the c. mm. oxygen uptake per mgm. nitrogen.

Substrate	Time in minutes			
	15	30	45	60
Formate	18.1	34.9	46.1	55.0
Acetate	39.2	77.1	106.7	170.7
Lactate	19.0	34.4	47.4	60.1
Citrate	38.9	78.7	112.3	137.9
Oxalate	34.8	66.5	98.2	122.1
Malate	32.2	63.9	93.6	127.8
Succinate	114.2	229.3	333.2	429.0
Fumarate	99.8	219.3	287.6	313.0
Maleate	12.6	21.8	28.1	34.5
Tartrate	31.8	60.0	83.5	106.6
Asparagine	39.7	64.9	103.1	126.0
a-Alanine	39.2	77.4	132.8	161.7
b-Alanine	23.9	54.5	85.6	118.8
Glycine	30.0	63.7	96.5	134.3
dl-Leucine	13.8	23.4	31.4	37.3
dl-Isoleucine	20.9	41.2	61.8	82.0
dl-b-Phenylalanine	44.0	86.5	131.1	177.8
d-Glutamate	76.1	153.2	232.8	306.1
Aspartate	37.6	77.7	120.1	162.8
Endogenous respiration	9.7	15.8	20.3	23.1

Table 4. Continued. The Oxygen Uptake of M. aurantiacus. The figures represent the c.mm. oxygen uptake per mgm.nitrogen.

	Time in minutes			
Subatrate	15	30	45	60
d-Arabinose	9.5	18.6	24.8	31.3
l-Arabinose	9.2	18.0	24.8	30.3
d-Xylose	12.0	19.4	25.6	32.5
d-Glucose	33.9	68.2	103.9	141.7
d-Fructose	23.4	50.7	72.0	99.4
d-Mannose	15.1	27.8	40.2	51.9
d-Galactose	11.7	20.1	29.0	35.8
Lactose	2.4	3.4	5.8	7.3
Maltose	9.3	19.4	40.0	41.1
Sucrose	117.7	213.5	327.8	390.4
Raffinose	14.2	24.5	30.3	35.6
l-Rhamnose	8.4	15.0	21.1	25.1
Ethyl Alcohol	82.1	164.6	249.6	333.1
Glycerol	64.2	134.8	205.1	279.6
Dulcitol	6.2	11.4	15.6	18.7
d-Mannitol	14.8	29.6	49.4	71.3

Table 4. Continued. The Oxygen Uptake of M. aurantiacus. The figures represent the c.mm. oxygen uptake per mgm, nitrogen.

Substrate	Time in minutes 15	30	45	60
Formate	40.2	74.8	104.7	127.8
Acetate	15.4	32.6	41.9	57.0
Lactate	83.6	160.5	228.7	242.4
Citrate	6.0	12.0	14.8	19.0
Oxalate	6.6	12.5	14.8	18.5
Malate	5.8	10.6	16.8	22.9
Succinate	12.1	23.0	39.0	51.9
Fumarate	7.9	16.0	24.0	33.0
Maleate	7.3	12.1	16.0	19.8
Tartrate	12.2	17.4	18.1	24.4
Asparagine	17.1	36.8	56.7	77.2
a-Alanine	14.7	20.3	28.5	35.3
b-Alanine	10.1	18.4	24.6	33.9
Glycine	33.8	71.7	100.2	154.6
dl-Leucine	12.1	18.3	26.1	32.6
dl-Isoleucine	8.9	19.9	26.7	39.6
dl-b-Phenylalanine	4.0	8.6	14.3	24.0
d-Glutamate	55.3	124.9	201.2	288.4
Aspartate	11.2	24.8	40.3	61.9
Endogenous respiration	5.5	10.1	12.6	16.2

Table 4. Continued. The Oxygen Uptake of M. cinnebareus. The figures represent the c.mm. oxygen uptake per mgm.nitrogen.

	Time in minutes			
Substrate	15	30	45	60
d-Arabinose	20.0	39.0	50.7	63.0
l-Arabinose	20.3	38.4	52.0	64.1
d-Xylose	24.3	43.5	60.7	74.3
d-Glucose	81.5	151.2	230.1	318.7
d-Fructose	91.6	204.9	326.7	465.8
d-Mannose	30.8	58.9	83.7	108.4
d-Galactose	23.6	41.2	56.2	70.2
Lactose	18.5	32.0	44.2	54.6
Maltose	97.2	193.9	281.8	371.5
Sucrose	110.9	224.1	320.6	421.2
Raffinose	31.1	49.3	65.9	79.4
l-Rhamnose	14.9	28.6	42.6	51.2
Ethyl Alcohol	113.3	219.9	317.8	406.7
Glycerol	97.6	180.4	280.3	378.5
Dulcitol	17.7	29.5	38.1	43.5
d-Mannitol	99.3	209.0	311.9	415.7

Table 4. Continued. The Oxygen Uptake of M. cinnebareus. The figures represent the c.mm. oxygen uptake per mgm. nitrogen.

Substrate	Time in minutes 15	30	45	60
Formate	60.3	120.1	166.8	207.9
Acetate	117.3	250.8	391.3	511.7
Lactate	119.2	234.7	337.2	537.6
Citrate	16.4	25.7	34.1	41.6
Oxalate	13.5	24.8	32.7	39.8
Malate	47.5	117.7	224.1	343.7
Succinate	79.4	174.6	284.4	408.5
Fumarate	43.0	104.2	184.7	292.5
Maleate	17.6	32.9	45.2	58.2
Tartrate	10.9	18.6	24.0	28.1
Asparagine	107.4	239.3	358.5	477.1
a-Alanine	79.4	186.3	246.1	338.9
b-Alanine	29.5	58.5	80.1	94.7
Glycine	53.7	117.3	177.4	237.8
dl-Leucine	19.2	36.4	47.6	57.5
dl-Isoleucine	32.6	66.1	98.7	129.5
dl-b-Phenylalanine	93.9	202.6	320.5	439.6
d-Glutamate	149.6	297.2	457.1	613.5
Aspartate	46.2	88.3	133.1	168.9
Endogenous respiration	13.1	25.3	32.0	38.1

Table 4. Continued. The Oxygen Uptake of M. freundenreichii. The figures represent the c.mm. oxygen uptake per mgm. nitrogen.

	Time in minutes			
Substrate	15	30	45	60
d-Arabinose	18.7	33.3	45.4	56.5
l-Arabinose	18.5	32.2	44.1	55.9
d-Xylose	21.5	39.6	62.3	65.4
d-Glucose	56.4	110.5	158.4	204.0
d-Fructose	20.6	37.7	51.3	74.1
d-Mannose	34.9	70.0	101.9	134.6
d-Galactose	22.8	37.4	50.0	60.8
d-Lactose	20.9	34.9	45.9	57.4
Maltose	62.4	107.4	143.5	180.0
Sucrose	47.5	90.0	127.3	164.1
Raffinose	26.3	42.8	56.1	66.5
l-Rhamnose	16.1	27.8	37.9	46.8
Ethyl Alcohol	148.0	277.6	409.5	552.5
Glycerol	64.1	123.9	178.0	230.1
Dulcitol	17.1	28.1	37.3	46.7
d-Mannitol	11.0	20.2	29.7	37.6

Table 4. Continued. The Oxygen Uptake of M. freundenreichii. The figures represent the c.mm. oxygen per mgm. nitrogen.

Substrate	Time in minutes			
	15	30	45	60
Formate	12.0	19.6	27.2	34.1
Acetate	74.3	147.9	228.2	325.0
Lactate	110.5	199.6	269.3	333.8
Citrate	19.3	33.2	42.7	51.9
Oxalate	20.6	32.3	41.7	49.9
Malate	15.4	31.3	44.9	57.8
Succinate	17.0	36.6	51.5	66.9
Fumarate	16.7	33.8	49.5	63.7
Maleate	8.9	18.7	25.9	36.9
Tartrate	13.8	25.6	33.8	41.7
Asparagine	33.4	76.1	113.6	151.5
a-Alanine	146.2	284.1	382.6	465.6
b-Alanine	16.1	29.1	39.1	49.2
Glycine	20.9	36.3	49.0	60.0
dl-Leucine	12.9	22.8	33.8	41.4
dl-Isoleucine	36.7	79.3	128.9	174.5
dl-b-Phenylalanine	18.0	37.3	58.8	80.0
d-Glutamate	101.7	214.3	313.2	447.6
Aspartate	21.8	43.9	62.9	84.1
Endogenous respiration	10.7	19.0	23.8	31.5

Study of Respiratory Inhibitors

Several respiratory inhibitors have been used in an attempt to reveal certain properties and modes of action of enzymes. Narcotics have been found to inhibit the dehydrogenases and are used to identify such systems. Cyanide has been found to inhibit iron catalyzed reactions, hence reacting with cytochrome oxidase, catalase and peroxidase, while it is considered to have little effect upon the dehydrogenases, Elvehjem, Wilson et al (1939), Green and Brosteaux (1936), and Hawthorne and Harrison (1939). It has been found, however, that the l-malic dehydrogenase of E. coli is sensitive to higher concentrations of cyanide (Gale and Stephenson,1939), as are many dehydrogenase reactions performed with the intact cells (Green and Brosteaux,1936, Ehrismann,1937).

There is little information on the literature concerning the action of sodium azide on bacterial dehydrogenases though it has been found to inhibit catalase, peroxidase and cytochrome oxidase. The mono-halogen derivatives of acetic acid have been reported as inhibiting the reactions occurring during fermentation, Das (1937).

Ehrismann (1937) studied the effect of cyanide on the dehydrogenase activity of Staphylococcus aureus in the presence of lactate, glucose, fructose, glycine and alanine. It was found that M/100 cyanide decreased the reduction time of methylene blue

(increased the dehydrogenase activity) in the presence of lactate, glucose and fructose but inhibited the oxidation of glycine and alanine. Lactate and amino acid dehydrogenation were inhibited about 50 per cent by M/10 cyanide. Lower concentrations exerted correspondingly less action on lactate while a concentration as low as M/1000 still inhibited the dehydrogenation of the amino acids.

The effect of different concentrations of brom-acetic acid on Staphylococcus aureus was determined in the presence of the above substrates. The activation of glucose and fructose was inhibited over 50 per cent by M/100 brom-acetate, while the oxidation of glycine and alanine was only slightly inhibited. However, in the presence of M/10 brom-acetate the dehydrogenation of lactate was stimulated while the activation of glucose and fructose were completely inhibited.

The influence on oxido-reductions of the halogenated acids appeared to be directed upon the primary point of attack of these compounds in respiration. Of special importance is the indicated action upon sulfhydryl groups since these are concerned with oxido-reductions (as the nitrate and tellurite reactions of Corynebacterium, Ehrismann,1935).

Influence of Inhibitors upon Dehydrogenases

Method

In studying the effect of inhibitors on the dehydrogenases, the inhibitors sodium azide, sodium cyanide and sodium monochlor-acetate were used.

In the Thunberg tube were placed 1 ml. M/30 phosphate buffer pH 7.05, 0.3 ml. saline, 0.7 ml. inhibitor, 1 ml. methylene blue 1-20,000, and 1 ml. cells, and in the hollow stopper was placed 1 ml. of M/10 substrate.

When cyanide was used as the inhibitor the tubes were set up as above and allowed to stand for 20 minutes (after the addition of the cells) before the substrate was tipped into the tube. The substrate was placed in the stopper and after twenty minutes the tube was evacuated with shaking for 2.5 minutes, the substrate tipped into the reaction mixture and the tube incubated at 40°C. The time required for complete reduction of the methylene blue was determined. When sodium azide or sodium monochlor-acetate was used as the inhibitor the cells, methylene blue, saline and inhibitor were allowed to stand ten minutes before the substrate was added.

The effect of the inhibitors upon the dehydrogenases is shown in Table 5.

Table 5. The Per Cent Increase or Decrease in Dehydrogenase Activity in the Presence of Inhibitors.

		Potassium cyanide			
Initial concentration		M/10	M/100	M/1000	M/10,000
Final concentration		1.4×10^{-2}	1.4×10^{-3}	1.4×10^{-4}	1.4×10^{-5}
M. luteus	Glucose	+ 64	+ 71		
	Sucrose	+104	+ 29		
	Lactate	+ 48	+115	+ 47	
	Succinate	+ 14	+ 18		
	Glycine				- 37
	dl-b-Phenyl-alanine				- 52
M. flavus	Glucose	+225	+105		
	Sucrose	+246	+217		
	Lactate	+133	+156	+184	
	Succinate	+129	+107		
	Glycine				- 65
	dl-b-Phenyl-alanine				- 80
M. aurantiacus	Glucose	- 31	- 36		
	Sucrose	- 56	- 48	- 37	
	Lactate	- 57		- 5	
	Succinate	- 60	- 57		
	Glycine			- 80	
	dl-b-Phenyl-alanine				- 67
M. cinnebareus	Glucose	- 22	+ 52		
	Sucrose	- 36	+ 44		
	Lactate	- 34		+116	
	Succinate	- 15		+ 4	
	Glycine				- 50
	dl-b-Phenyl-alanine				- 5
M. freunden-reichii	Glucose	- 25	+324		
	Sucrose	- 14	- 6		
	Lactate	+ 2.4	± 0		
	Succinate	+157	+500		
	Glycine				- 74
	dl-b-Phenyl-alanine				- 68

Table 5. Continued. The Per Cent Increase of Decrease in Dehydrogenase Activity in the Presence of Inhibitors.

		Sodium monochlor-acetate			
Initial concentration		M/10	M/100	M/1000	M/10,000
Final concentration		1.4×10^{-2}	1.4×10^{-3}	1.4×10^{-4}	1.4×10^{-5}
M. luteus	Glucose	- 31	- 30		
	Sucrose	- 28	- 22		
	Lactate	- 10	- 16		
	Succinate	- 3	+ 13		
	Glycine				- 31
	dl-b-Phenyl-alanine				- 58
M. flavus	Glucose	+ 18	+ 7.1		
	Sucrose	- 7.2	- 11		
	Lactate	- 8.8	+ 6.4		
	Succinate	+ 25	+ 28		
	Glycine				- 59
	dl-b-Phenyl-alanine				- 72
M. aurantiacus	Glucose	+ 59			
	Sucrose	- 46	+ 23		
	Lactate	- 26	- 5		
	Succinate	- 56	- 6.9		
	Glycine				- 81
	dl-b-Phenyl-alanine				- 66
M. cinnebareus	Glucose	- 35	- 24		
	Sucrose	+ 24	+ 33		
	Lactate	+ 11			
	Succinate	- 11	+ 51		
	Glycine				- 50
	dl-b-Phenyl-alanine				- 5
M. freunden-reichii	Glucose	- 18	- 5.4		
	Sucrose	- 18	- 7.8		
	Lactate	- 24	- 38		
	Succinate	- 31			
	Glycine				- 39
	dl-b-Phenyl-alanine				- 80

Table 5. Continued. The Per Cent Increase or Decrease in Dehydrogenase Activity in the Presence of Inhibitors.

		Sodium azide		
Initial concentration		M/100	M/1000	M/10,000
Final concentration		1.4×10^{-3}	1.4×10^{-4}	1.4×10^{-5}
M. luteus	Glucose	- 12	- 4	
	Sucrose	- 29		
	Lactate	+ 6	- 20	
	Succinate	- 40	- 16	
	Glycine			- 27
	dl-b-Phenyl-alanine			- 62
M. flavus	Glucose	+ 22		
	Sucrose	+ 15	+ 36	
	Lactate	+ 17	+ 16	
	Succinate	- 3.3		
	Glycine			- 47
	dl-b-Phenyl-alanine			- 71
M. aurantiacus	Glucose	+ 64		
	Sucrose	- 32	+ 3.5	
	Lactate	- 29		
	Succinate		- 6.4	
	Glycine		- 16	
	dl-b-Phenyl-alanine			- 77
M. cinnebareus	Glucose	- 39	- 8	
	Sucrose	+ 26		
	Lactate	+160		
	Succinate	- 32	+ 50	
	Glycine			- 61
	dl-b-Phenyl-alanine			- 40
M. freunden-reichii	Glucose	+ 3.7		
	Sucrose		- 55	
	Lactate		- 38	
	Succinate		- 60	
	Glycine			- 60
	dl-b-Phenyl-alanine			- 60

Discussion

A great difference was found among the organisms in regard to their sensitivity toward inhibitors (Table 5). The dehydrogenases of M. luteus and M. flavus were stimulated by a cyanide concentration as high as 1.4×10^{-2} molar while the other organisms were generally inhibited by this concentration. M. aurantiacus showed the greatest sensitivity toward cyanide, being inhibited by a concentration of 1.4×10^{-4} molar.

The specific dehydrogenases were found to vary in their sensitivity to inhibitors since dehydrogenation of the amino acids was greatly inhibited by a cyanide concentration of 1.4×10^{-5} molar while this concentration did not affect the other dehydrogenases.

M. cinnebareus and M. flavus were the least sensitive to monochlor-acetate since they were generally stimulated by a concentration of 1.4×10^{-2} molar, while M. aurantiacus appeared to be the most sensitive toward sodium monochlor-acetate in this concentration. The amino acid dehydrogenases were more sensitive toward sodium monochlor-acetate than were the sugar and fatty acid dehydrogenases.

Sodium azide appeared to be a stronger inhibitor than sodium cyanide since a concentration of $1.4 \times LO^{-3}$ molar sodium azide inhibited the dehydrogenases to a greater extent than did this concentration of sodium cyanide.

M. flavus, however, was generally stimulated by both sodium cyanide and sodium azide, and in this case the stimulation produced by azide was much less than that produced by cyanide.

The glucose dehydrogenase of M. aurantiacus appeared to be excpetionally resistant to both sodium monochlor-acetate and sodium azide while the other dehydrogenases of this organism appeared to be very sensitive to all the inhibitors. The amino acid dehydrogenases were found to be uniformily more susceptible to all the inhibitors than were the other dehydrogenases produced by the organisms.

Influence of Inhibitors upon Oxygen Uptake by Micrococci

In 1924 Callow studied the oxygen uptake of a number of bacteria and found very little oxygen uptake for Staphylococcus aureus and Sarcina aurantiaca.

Gerard (1931) found that the QO_2 (c.mm.oxygen uptake per hour per mgm. dry weight of cells) for Sarcina lutea to be between four and six, while the QO_2 in glucose was about 20.

Methylene blue increased the oxygen uptake of endogenous respiration about 50 per cent while the oxidation of lactate was inhibited between 10 and 30 per cent.

Cyanide in a concentration of M/100 did not decrease the oxygen uptake of the cells in the presence of any of the substrates, while a concentration of M/10 inhibited respiration about 50 per cent. Working with the same strain one year later, however,

Barron (Gerard,1931) found that M/100 cyanide inhibited a respiration about 50 per cent.

Cook, Haldane and Mapson (1931) found that cyanide concentrations below $2x10^{-3}$ molar did not bring about serious inactivation of the dehydrogenases of E. coli. With formate as the substrate it was found that cyanide strongly inhibited oxygen uptake, but that the addition of methylene blue together with cyanide resulted in an oxygen uptake nearly that of the formate alone in the absence of inhibitor. With lactate as the substrate methylene blue alone (M/250) inhibited respiration about 74 per cent, and when cyanide and methylene blue were added together only 67 per cent of the original uptake (lactate alone) was obtained.

After studying the effect of the various inhibitors upon the dehydrogenases, the effect of the inhibitors upon the complete respiratory mechanism of the cell was determined.

Method

In the Warburg vessel were placed 1 ml.cells, 1 ml. buffer pH 7.05, 0.1 ml. saline, and 0.5 ml. inhibitor which was adjusted to pH 7.1. In the center cup was placed 0.2 ml. of twenty per cent NaOH to take up the carbon dioxide evolved. The buffer was a mixture of M/30 K_2HPO_4 and M/30 KH_2PO_4.

When sodium monochlor-acetate and sodium azide were used as the inhibitors the cells, buffer, saline and inhibitor were allowed to stand, with frequent agitation, for ten minutes

before the addition of M/30 substrate. In those cases where cyanide was used as the inhibitor, the cyanide was allowed to remain in contact with the cells, buffer, and saline for twenty minutes before the addition of the substrate, and the carbon dioxide absorbant used was the KOH-KCN mixture suggested by Krebs (1935).

After the addition of the substrate the vessels were placed in the water bath at 35°C. and ten minutes allowed for equilibrium. The oxygen uptake was followed for the next 60 minutes.

Using glucose as a representative substrate it was found that M. luteus was resistant to cyanide. Figure 2 indicates that a cyanide concentration of 1.4×10^{-2} molar inhibited respiration about 69 per cent. A concentration of 1.4×10^{-3} molar inhibited respiration slightly during the first 45 minutes, but this period was followed by an increase in rate so that the total oxygen uptake at the end of the 60 minutes was identical with the uptake in the absence of inhibitor. M.aurantiacus likewise showed such an increase with glucose as the substrate.

Cyanide concentrations less than 1.4×10^{-3} molar brought about a stimulation. This amounted to 43 per cent in a cyanide concentration of 1.4×10^{-4} molar while half this concentration of cyanide stimulated 21 per cent.

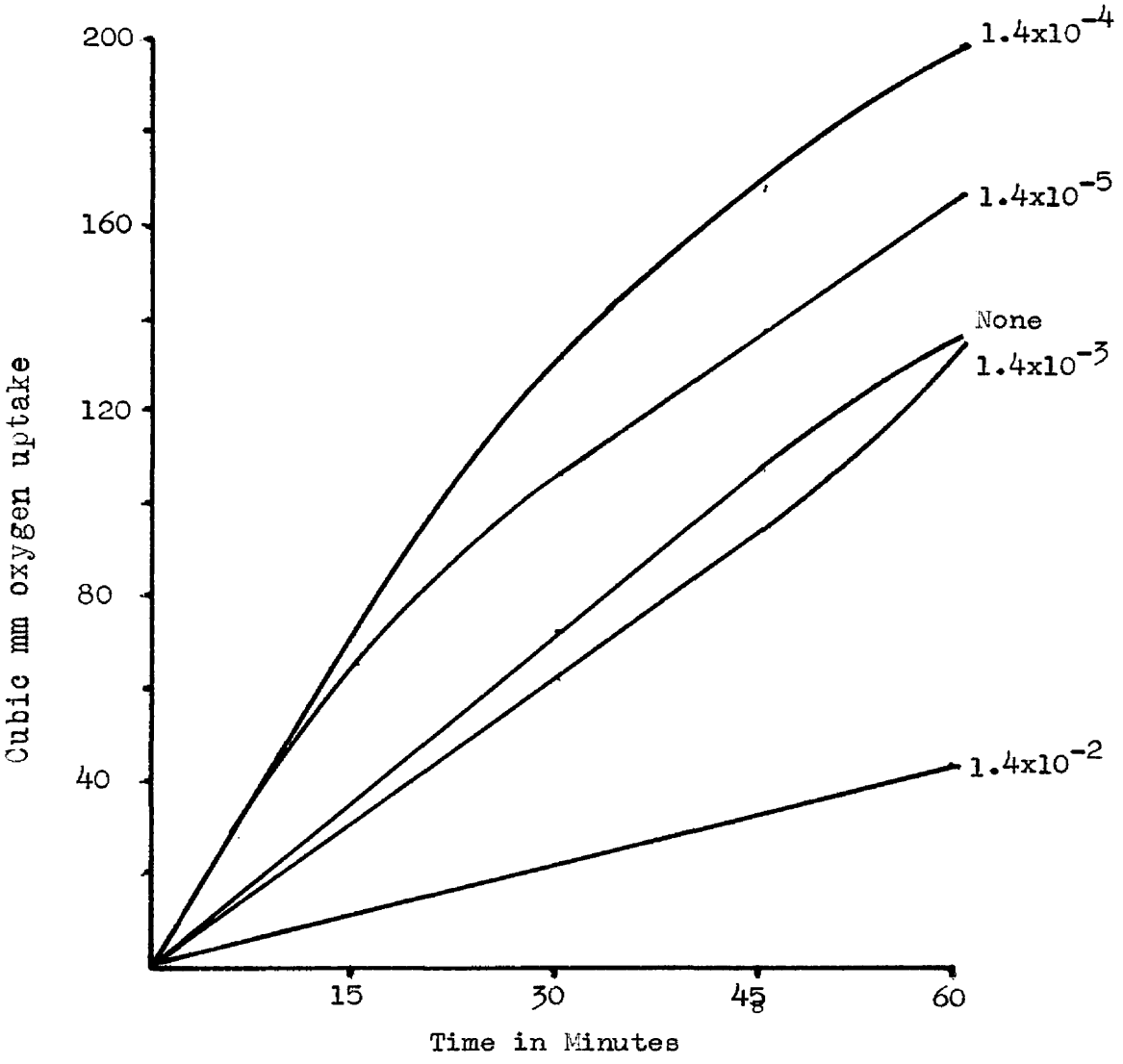

Figure 2. The Effect of Potassium Cyanide (in molar concentration) upon the Oxygen Uptake of M. luteus.

The influence of inhibitors upon the oxygen uptake of the organisms was determined using as substrates glucose, sucrose, lactate, succinate, glycine and dl-b-phenylalanine. A concentration of inhibitor was chosen which did not exert too pronounced an effect upon the dehydrogenase and the effect of this concentration on the oxygen uptake of the cells was determined.

The results are shown in Table 9 and are summarized in Tables 6,7 and 8.

Table 6. The Per Cent Increase or Decrease in Respiration in the Presence of Potassium Cyanide and Methylene Blue.

		Potassium cyanide				
Final molar concentration		1.4×10^{-2}	1.4×10^{-3}	1.4×10^{-4}	KCN + ° M.B.	M. B. alone
M. luteus						1 mgm.
	Glucose	-.70			- 45	- 3.5
	Sucrose	- 77			- 13	+157
	Lactate	- 79			- 74	+ 45
	Succinate	- 95			- 94	- 6.5
M. flavus						1 mgm.
	Glucose	- 38			- 38	+101
	Sucrose	- 78			+ 33	+ 44
	Lactate	- 76			- 71	+ 5.1
	Succinate	- 93			- 90	- 3.3
M. aurantiacus						0.1 mgm.
	Glucose			+ 14	+ 42	- 33
	Sucrose			- 6.4	- 91	- 83
	Lactate			- 12	- 47	- 15
	Succinate		- 17		- 76	- 63
M. cinnebareus						0.1 mgm.
	Glucose	- 88			- 80	- 51
	Sucrose	- 92			- 82	- 35
	Lactate	- 93			- 71	+ 57
	Succinate			- 26	- 87	- 73
M. freundenreichii						0.5 mgm.
	Glucose	- 77			- 73	+ 25
	Sucrose		+ 9.6		+ 0.4	+ 38
	Lactate		- 25		- 63	+ 39
	Succinate	- 54			- 51	- 59

° The cyanide concentration used was that recorded in the column to the left, and the methylene blue concentration was that amount in mgm. indicated in the column to right.

Table 7. The Per Cent Increase or Decrease in Respiration in the Presence of Sodium monochlor-acetate.

		Sodium monochlor-acetate		
Final molar concentration		1.4×10^{-2}	1.4×10^{-3}	1.4×10^{-5}
M. luteus	Glucose	+ 97		
	Sucrose	+ 3.6		
	Lactate	- 26		
	Succinate	- 19		
	Glycine			- 6.5
M. flavus	Glucose	+ 6		
	Sucrose	+ 9.1		
	Lactate	- 27		
	Succinate	- 7.6		
M. aurantiacus	Glucose	+ 16		
	Sucrose		- 1.8	
	Lactate		- 7.2	
	Succinate		+ 8.9	
M. cinnebareus	Glucose		+ 9.2	
	Sucrose	- 50		
	Lactate	- 44		
	Succinate	- 54		
	dl-b-Phenyl-alanine			+ 4.7
M.freundenreichii	Glucose	+ 3.2		
	Sucrose		- 0.4	
	Lactate	- 28		
	Succinate	+ 8.5		

Table 8. The Per Cent Increase or Decrease in Respiration in the Presence of Sodium Azide.

		Sodium azide		
Final molar concentration		1.4×10^{-3}	1.4×10^{-4}	1.4×10^{-5}
M. luteus	Glucose	+161		
	Sucrose	+ 16		
	Lactate	- 26		
	Succinate	- 15		
	Glycine			- 42
M. flavus	Glucose	- 4.6		
	Sucrose	+ 16		
	Lactate	- 9		
	Succinate	- 7.4		
M. aurantiacus	Glucose	+ 0.2		
	Sucrose		- 3.8	
	Lactate			- 0.4
	Succinate		+ 2.1	
	Glycine		+ 8.8	
M. cinnebareus	Glucose		+ 2.6	
	Sucrose	- 39		
	Lactate	- 35		
	Succinate		- 14	
M.freundenreichii	Glucose	- 1.3		
	Sucrose	+ 4.1		
	Lactate		- 2.8	
	Succinate		- 34	

Discussion

It was found (Table 6) that cyanide in a concentration of 1.4×10^{-2} molar greatly inhibited the oxygen uptake of the cells. In general the inhibition of glucose was less than for the other substrates.

Since most of the amino acid dehydrogenases were so strongly inhibited by a cyanide concentration as low as 1.4×10^{-5} molar, the effect of cyanide upon the oxygen uptake was not determined. However, the dl-b-phenylalanine dehydrogenase of M. cinnebareus was an exception since it was inhibited only five per cent and the oxygen uptake only nine per cent by a 1.4×10^{-5} molar concentration of cyanide.

The decrease in respiration in the presence of cyanide indicated that the cell carriers operative in normal respiration were inhibited. Therefore the ability of methylene blue to replace these natural carriers was determined.

Influence of Methylene Blue upon Cyanide Inhibition

The methods were the same as for the study of the inhibitors alone except that for this work the methylene blue was made up with the cyanide and the pH of the mixture was adjusted to 7.05. This mixture of cyanide and methylene blue was added to the cells, buffer and saline and allowed to stand for 20 minutes before the addition of the substrate. The oxygen uptake was compared

with that in the presence of methylene blue alone which was likewise allowed to remain in contact with the cells for 20 minutes before the addition of the substrate.

The organisms varied in their sensitivity toward methylene blue (Table 6). M. luteus and M. flavus were stimulated by the presence of 1 milligram of methylene blue in the presence of all substrates except succinate. The other organisms were found to be inhibited by this dye concentration, although M. freundenreichii was stimulated by 0.5 milligram in the presence of all substrates except succinate. M. cinnebareus was still more sensitive to methylene blue since even 0.1 milligram exerted strong inhibitive action in the presence of all substrates except lactate with which there was stimulation.

Methylene blue was not capable of replacing the natural cell carriers to any great extent. For most of the organisms the cyanide inhibition was lessened slightly by methylene blue in the presence of glucose, sucrose and lactate (Table 6). The cyanide inhibition was completely removed by methylene blue only in the case of M. flavus with sucrose as the substrate and M. aurantiacus with glucose as the substrate.

The difference shown in the susceptibility toward cyanide and in the ability of methylene blue to act as a carrier indicates that one single mechanism is not operative in the oxidation of the different substrates.

Sodium monochlor-acetate in a concentration of 1.4×10^{-2} molar inhibited the oxidation of all the substrates with the exception of glucose with which there was stimulation (Table 7). In two cases sucrose and succinate were also stimulated.

Although sodium azide (Table 8) was found to be a stronger inhibitor than sodium monochlor-acetate, the stimulation and inhibition paralleled, indicating the point of attack of both inhibitors to be the same.

The action of the inhibitors indicates that only one mechanism is probably operative for the oxidation of glucose. It is stimulated by sodium monochlor-acetate and sodium azide and inhibited by sodium cyanide. The cyanide inhibition, however, is partially removed by the presence of methylene blue.

Two mechanisms exist for the oxidation of sucrose. With M. luteus, M. flavus and M. freundenreichii the oxidative mechanism is not susceptible to sodium monochlor-acetate or sodium azide, is inhibited by sodium cyanide but is not to any great extent reactivated by methylene blue. This mechanism may be identical with the oxidative mechanism operative in the presence of glucose. M. aurantiacus and M. cinnebareus differ in that the oxidation of sucrose is susceptible to all inhibitors, but reactivation does not take place in the presence of methylene blue.

The oxidation of lactate is sensitive to all inhibitors. The cyanide sensitivity is removed by methylene blue only in the case of M. luteus, M. flavus and M. cinnebareus.

The amino acid dehydrogenases were very susceptible to the inhibitors, being strongly inhibited by a concentration as low as 1.4×10^{-5} molar, therefore the inhibition of oxygen uptake was not determined. The dl-b-phenylalanine dehydrogenase of M. cinnebareus, however, was inhibited by only five per cent in this concentration of cyanide and sodium monochlor-acetate. The oxygen uptake in the presence of this amount of sodium cyanide was found to be nine per cent, while the uptake in the presence of sodium monochlor-acetate was stimulated 4.7 per cent.

The response of a dehydrogenase to an inhibitor, and the inhibition or stimulation of oxygen uptake in the presence of that inhibitor frequently did not parallel. This can be explained by the fact that respiration is the sum total of individual reactions. If the dehydrogenase activity limits the respiratory rate, any inhibition of the dehydrogenase will decrease the oxygen uptake (respiratory rate) to the same extent. If the dehydrogenase activity does not limit the respiratory rate, the dehydrogenase may be inhibited to a certain extent and still not decrease the rate of oxygen uptake. However, if the dehydrogenase and another enzyme is inhibited, the oxyegn uptake may or may not parallel the dehydrogenase activity depending upon which enzyme or reaction becomes the limiting factor.

Table 9. The Influence of Inhibitors upon Oxygen Uptake. The figures represent the c.mm. O_2 uptake per mgm. nitrogen.

Micrococcus luteus						
Substrate	Inhibitor	Final molar Concentration	Time in minutes 15	30	45	60
Glucose	None		22.2	40.7	56.0	72.6
	CH_2ClCO_2Na	$1.4x10^{-2}$	41.2	81.1	115.6	143.2
	NaN_3	$1.4x10^{-3}$	46.6	94.0	139.9	189.5
	KCN	$1.4x10^{-2}$	5.0	10.3	15.6	21.4
	° KCN + M.B.	1 mgm. MB.	16.2	25.9	34.2	40.1
	M. B.	1 mgm.	39.8	61.2	66.8	74.2
Sucrose	None		55.2	102.8	146.2	185.0
	CH_2ClCO_2Na	$1.4x10^{-2}$	51.4	102.8	146.6	192.1
	NaN_3	$1.4x10^{-3}$	58.7	110.3	157.6	214.4
	KCN	$1.4x10^{-2}$	10.1	20.2	30.2	41.8
	° KCN + MB.	1 mgm. MB.	46.9	88.2	124.0	160.3
	M. B.	1 mgm.	137.8	223.8	269.5	475. 4
Lactate	None		47.9	98.7	148.7	191.5
	CH_2ClCO_2Na	$1.4x10^{-2}$	37.5	75.8	110.1	142.2
	NaN_3	$1.4x10^{-3}$	35.0	71.4	105.7	139.3
	KCN	$1.4x10^{-2}$	12.6	21.3	31.7	40.9
	° KCN + MB.	1 mgm. MB.	17.1	30.0	41.9	50.1
	M. B.	1 mgm.	105.1	174.2	235.7	278.2
Succinate	None		131.9	374.8	463.7	620.9
	CH_2ClCO_2Na	$1.4x10^{-2}$	113.1	239.3	375.5	501.9
	NaN_3	$1.4x10^{-3}$	115.0	244.5	383.7	526.7
	KCN	$1.4x10^{-2}$	6.7	14.8	22.1	33.7
	° KCN + M.B.	1 mgm. MB.	12.9	22.3	28.5	35.9
	M. B.	1 mgm.	126.6	349.8	426.9	580.8
Glycine	None		78.6	163.2	220.7	351.3
	CH_2ClCO_2Na	$1.4x10^{-5}$	80.6	164.8	252.0	328.6
	NaN_3	$1.4x10^{-5}$	50.9	99.0	148.9	203.1
	KCN	$1.4x10^{-5}$	50.4	98.3	147.2	204.1
	° KCN + M.B.	1 mgm. MB.	30.7	46.9	58.0	67.0
	M. B.	1 mgm.	95.8	149.7	180.4	196.1

° 1 mgm. of methylene blue + that concentration of cyanide given immediately above.

Table 9. Continued. The Influence of Inhibitors upon Oxygen Uptake.

The figures represent the c. mm. O_2 uptake per mgm. nitrogen.

Micrococcus flavus						
Substrate	Inhibitor	Final molar concentration	Time in minutes 15	30	45	60
Glucose	None		16.2	32.3	47.2	61.0
	CH_2ClCO_2Na	$1.4x10^{-2}$	24.7	39.3	53.6	64.8
	NaN_3	$1.4x10^{-3}$	17.1	32.1	36.1	58.2
	KCN	$1.4x10^{-2}$	8.7	17.7	27.8	37.6
	° KCN + M.B.	1 mgm. MB.	13.9	24.0	31.2	37.6
	M. B.	1 mgm.	47.1	81.0	106.7	122.5
Sucrose	None		43.9	85.5	122.2	157.6
	CH_2ClCO_2Na	$1.4x10^{-2}$	40.6	74.2	144.2	171.9
	NaN_3	$1.4x10^{-3}$	49.0	98.4	145.9	182.5
	KCN	$1.4x10^{-2}$	7.5	16.2	24.5	35.2
	° KCN + M.B.	1 mgm. MB.	49.5	103.4	155.8	209.2
	M. B.	1 mgm.	55.2	113.6	156.0	227.1
Lactate	None		66.6	99.0	141.8	177.8
	CH_2ClCO_2Na	$1.4x10^{-2}$	34.4	68.6	100.8	130.4
	NaN_3	$1.4x10^{-3}$	42.4	82.2	123.7	161.6
	KCN	$1.4x10^{-2}$	11.1	19.4	31.0	42.4
	° KCN + M.B.	1 mgm. M.B.	18.1	29.0	43.2	51.6
	M. B.	1 mgm.	84.0	113.9	154.6	186.8
Succinate	None		108.6	265.0	453.3	468.2
	CH_2ClCO_2Na	$1.4x10^{-2}$	104.2	215.8	329.5	432.8
	NaN3	$1.4x10^{-3}$	103.7	215.5	321.8	432.7
	KCN	$1.4x10^{-2}$	8.9	19.6	28.2	37.2
	° KCN + M.B.	1 mgm. MB.	14.9	28.3	38.0	47.7
	M. B.	1 mgm.	67.8	179.9	311.5	452.9

° 1 mgm. of methylene blue + that concentration of cyanide given immediately above.

Table 9. Continued. The Influence of Inhibitors upon Oxygen Uptake.

The figures represent the c.mm. O_2 uptake per mgm. nitrogen.

Micrococcus aurantiacus						
Substrate	Inhibitor	Final molar concentration	Time in minutes 15	30	45	60
Glucose	None		39.1	81.7	121.7	163.7
	CH_2ClCO_2Na	$1.4x10^{-2}$	44.8	93.9	141.7	190.3
	NaN_3	$1.4x10^{-3}$	41.5	83.1	122.0	164.0
	KCN	$1.4x10^{-4}$	32.9	73.3	112.1	187.0
	° KCN + MB.	0.1 mgm.MB.	54.1	113.9	171.7	232.9
	M. B.	0.1 mgm.	29.7	54.5	84.6	110.2
Sucrose	None		117.6	234.5	325.2	390.7
	CH_2ClCO_2Na	$1.4x10^{-3}$	121.8	237.1	318.7	383.6
	NaN_3	$1.4x10^{-4}$	113.3	227.9	312.6	376.0
	KCN	$1.4x10^{-4}$	104.6	201.1	270.7	365.6
	° KCN + MB.	0.1 mgm.MB.	9.9	19.0	26.9	36.2
	M. B.	0.1 mgm.	29.1	47.4	59.5	65.8
Lactate	None		68.5	131.4	187.3	242.7
	CH_2ClCO_2Na	$1.4x10^{-3}$	60.2	117.0	169.2	225.2
	NaN_3	$1.4x10^{-5}$	65.8	128.7	183.4	241.7
	KCN	$1.4x10^{-4}$	55.8	111.1	160.6	212.9
	° KCN + M.B.	0.1 mgm.MB.	23.8	53.4	89.4	127.8
	M. B.	0.1 mgm.	44.4	93.6	149.7	205.2
Succinate	None		64.0	126.5	191.1	248.6
	CH_2ClCO_2Na	$1.4x10^{-3}$	68.1	139.6	209.1	270.8
	NaN_3	$1.4x10^{-4}$	64.1	128.2	194.1	253.9
	KCN	$1.4x10^{-3}$	43.8	96.8	148.4	206.2
	° KCN + M.B.	0.1 mgm.MB.	14.4	29.3	44.2	60.1
	M. B.	0.1 mgm.	26.9	44.8	51.6	92.1
Glycine	None		39.0	82.6	126.8	176.4
	NaN_3	$1.4x10^{-4}$	43.0	88.4	136.6	192.0

° 0.1 mgm. of methylene blue + that concentration of cyanide given immediately above.

Table 9. Continued. The Influence of Inhibitors upon Oxygen Uptake.

The figures represent the c.mm. O_2 uptake per mgm. nitrogen.

Micrococcus cinnebareus						
Substrate	Inhibitor	Final molar concentration	Time in minutes 15	30	45	60
Glucose	None		64.6	139.0	204.0	277.1
	CH_2ClCO_2Na	$1.4x10^{-3}$	72.1	155.8	229.o	302.6
	NaN_3	$1.4x10^{-4}$	65.1	139.8	211.4	284.4
	KCN	$1.4x10^{-2}$	11.5	17.0	24.1	32.1
	° KCN + M.B.	0.1 mgm.MB.	18.5	30.1	42.6	54.6
	M. B.	0.1 mgm.	38.6	73.1	106.2	136.3
Sucrose	None		77.1	159.8	240.0	315.6
	CH_2ClCO_2Na	$1.4x10^{-2}$	53.5	96.7	130.8	157.2
	NaN_3	$1.4x10^{-3}$	72.6	130.8	166.3	192.4
	KCN	$1.4x10^{-2}$	4.6	16.1	16.6	24.6
	° KCN + M.B.	0.1 mgm.MB.	12.2	29.6	40.2	56.7
	M. B.	0.1 mgm.	43.7	94.9	147.1	204.3
Lactate	None		85.9	180.9	266.8	355.8
	CH_2ClCO_2Na	$1.4x10^{-2}$	50.7	107.6	154.8	200.0
	NaN_3	$1.4x10^{-3}$	70.8	137.2	189.9	231.1
	KCN	$1.4x10^{-2}$	6.9	16.1	21.1	26.2
	° KCN + M.B.	0.1 mgm. MB.	26.3	52.3	79.0	104.6
	M. B.	0.1 mgm.	153.9	292.7	430.6	557.3
Succinate	None		63.9	142.3	233.4	342.5
	CH_2ClCO_2Na	$1.4x10^{-2}$	45.3	89.5	123.7	157.9
	NaN_3	$1.4x10^{-4}$	60.4	128.8	207.2	292.7
	KCN	$1.4x10^{-4}$	53.5	111.1	180.9	252.8
	° KCN + M.B.	0.1 mgm.MB.	18.1	28.6	37.7	44.2
	M. B.	0.1 mgm.	28.6	52.3	72.9	92.5
dl-b-phenyl-alanine	None		104.9	222.5	341.8	464.5
	CH_2ClCO_2Na	$1.4x10^{-5}$	106.0	228.8	354.4	486.3
	KCN	$1.4x10^{-5}$	98.5	207.8	297.9	421.3
	° KCN + M.B.	0.1 mgm.MB.	13.8	25.8	33.3	40.2
	M. B.	0.1 mgm.	14.3	23.0	28.7	34.4

° 0.1 mgm. of methylene blue + that concentration of cyanide given immediately above.

Table 9. Continued. The Influence of Inhibitors upon Oxygen Uptake.

The figures represent the c. mm. O_2 uptake per mgm. nitrogen.

Micrococcus freundenreichii						
Substrate	Inhibitor	Final molar concentration	Time in minutes 15	30	45	60
Glucose	None		63.7	133.3	173.3	213.7
	CH_2ClCO_2Na	$1.4x10^{-2}$	74.5	135.2	183.2	220.5
	NaN_3	$1.4x10^{-3}$	59.6	117.8	168.4	216.5
	KCN	$1.4x10^{-2}$	15.5	26.6	36.9	48.4
	° KCN + M. B.	0.5 mgm.MB.	18.1	34.7	49.4	58.5
	M. B.	0.5 mgm.	83.1	168.4	221.0	268.4
Sucrose	None		62.5	121.8	174.6	226.2
	CH_2ClCO_2Na	$1.4x10^{-3}$	65.1	124.0	173.7	225.3
	NaN_3	$1.4x10^{-4}$	62.4	125.2	180.0	235.5
	KCN	$1.4x10^{-3}$	51.4	116.8	182.6	248.0
	° KCN + M. B.	0.5 mgm.MB.	53.4	112.0	174.4	227.1
	M. B.	0.5 mgm.	89.5	172.9	231.9	312.5
Lactate	None		100.1	188.1	265.4	333.5
	CH_2ClCO_2Na	$1.4x10^{-2}$	62.3	146.7	194.1	238.7
	NaN_3	$1.4x10^{-4}$	94.6	179.7	257.5	324.0
	KCN	$1.4x10^{-3}$	54.9	117.7	186.7	249.2
	° KCN + M. B.	0.5 mgm.MB.	39.5	72.8	100.8	122.8
	M. B.	0.5 mgm.	136.1	265.6	383.1	463.8
Succinate	None		19.2	36.1	50.3	63.7
	CH_2ClCO_2Na	$1.4x10^{-2}$	21.8	38.9	54.7	69.1
	NaN_3	$1.4x10^{-4}$	12.3	24.0	33.6	41.8
	KCN	$1.4x10^{-2}$	8.3	15.1	21.5	29.3
	° KCN + M. B.	0.5 mgm.MB.	12.2	21.6	27.5	31.3
	M. B.	0.5 mgm.	12.8	17.4	22.9	25.8

° 0.5 mgm, of methylene blue + that concentration of cyanide given immediately above.

Examination of Micrococci for the Presence of Other Respiratory Enzymes

Examination for the Presence of Polyphenol Oxidase

Polyphenol oxidase is specific in that it will oxidize the ortho di-hydroxy phenols such as catechol and pyrogallol, yet is practically inert against the meta- and para-hydroxy phenols. The oxidation of the former is used as a basis for the determination of polyphenol oxidase. The oxygen uptake by the cells was determined at 35°C in the presence of 5 milligrams of catechol.

In the Warburg vessel were placed 1 ml. cells, 1 ml. M/30 phosphate buffer pH 7.05, 1.1 ml. saline and in the center cup was placed 0.2 ml. of twenty per cent NaOH to take up the carbon dioxide evolved. Five milligrams of catechol in 0.5 ml. of water was placed in the side arm and added at equilibrium. The oxygen uptake was followed for 60 minutes and compared with the oxygen uptake of the cells in the absence of catechol.

The results are shown in the following table.

Table 10. The Polyphenol Oxidase Activity of Micrococci

Organism	qO_2 in absence of catechol	qO_2 in presence of catechol
M. luteus	30.4	59.9
M. flavus	25.5	116.8
M. aurantiacus	26.6	47.6
M. cinnebareus	38.1	185.4
M. freundenreichii	33.9	38.0

Examination for the Presence of Catalase

The manometric method similar to that introduced by Blaschko (1935) was used. This method makes use of the fact that catalase breaks down hydrogen peroxide to water and oxygen. Catalase can be determined, then, by measuring the oxygen given off from peroxide. A blank must be run to determine the oxygen uptake by the cells which must be added to the oxygen given off from the peroxide to give the total ogygen evolved.

In the Warburg vessel were placed 1 ml. cells, 1 ml. phosphate buffer pH 7.0, 1.1 ml. saline and in the center cup was placed 0.2 ml. of twenty per cent NaOH to take up the carbon dioxide evolved. One-half milliliter of diluted hydrogen peroxide was placed in the side arm and added at equilibrium. For the control, to determine the oxygen uptake by the cells in the absence of peroxide, the vessels were set up as above except that no peroxide was present and 1.6 ml. of saline was added to keep the volume of liquid in the vessel at 3.6 ml. Preliminary trial showed the amount of peroxide used to be in excess. Respiration measurements were conducted at 35°C. for 30 minutes.

The results are shown on the following table.

Table 11. The Catalytic Activity of Micrococci

Organism	C.mm. O_2 uptake in absence of H_2O_2	C.mm. O_2 liberated in 30 Min,/Mgm. N
M. luteus	17.9	46.9
M. flavus	17.6	46.8
M. aurantiacus	20.1	80.2
M. cinnebareus	31.1	291.9
M. freundenreichii	18.8	42.1

Examination for the Presence of Cytochrome Oxidase

The cytochromes, or at least cytochrome-c, is oxidized by the enzyme cytochrome oxidase. This oxidase is a hemin compound and is inhibited by carbon monoxide and light and by cyanide.

Stotz, Sidwell and Hogness (1938) found that cytochrome-c and the oxidase were involved in the oxidation of both hydroquinone and para-phenylenediamine, and that cytochrome-b, which does not oxidize hydroquinone, could function quite independently of this system in the oxidation of p-phenylenediamine.

The ability of the Micrococci to oxidize hydroquinone and p-phenylenediamine was determined as follows: In the Warburg vessel were placed 1 ml. cells, 1 ml of phosphate buffer pH 7.05, 1.1 ml. saline and in the center cup was placed 0.2 ml. of twenty per cent NaOH to take up the carbon dioxide evolved. In the side arm was placed 0.5 ml. of hydroquinone or p-phenylenediamine 0.15 molar.

The p-phenylenediamine used was the hydrochloride which was adjusted to pH 6.95 immediately before use.

It was found that hydroquinone showed a considerable rate of autoxidation at pH 7.05 and therefore this rate was determined as a blank in each experiment. Para-phenylenediamine, on the other hand, showed very little autoxidation.

Hydroquinone was not oxidized by any of the organisms with the possible exception of M. freundenreichii which showed an endogenous respiration qO_2 of 33.9 while in the presence of hydroquinone the qO_2 was 34.3.

In the presence of p-phenylenediamine an increased oxygen uptake was observed. This is shown in the following table.

Table 12. The Oxygen uptake of Micrococci in the Presence of Para-phenylenediamine.

Organism	qO_2 in absence of p-phenylenediamine	qO_2 in presence of p-phenylenediamine
M. luteus	27.2	102.5
M. flavus	23.5	93.1
M. aurantiacus	14.5	181.4
M. cinnebareus	49.0	68.2
M. freundenreichii	30.7	261.3

M. auranticaus and M. freundenreichii were found to be most active in the oxidation of p-phenylenediamine while M. cinnebareus oxidized the compound very slowly. Since hydroquinone was not oxidized by any of the organisms, cytochrome-c

may be lacking in the cells. The rapid oxidation of p-phenylene-diamine might indicate that cytochrome-b is very active since Stotz et al. (1938) found that cytochrome-b could act independently of cytochrome-c and cytochrome oxidase in the oxidation of this compound. The absence of cytochrome-c would class these organisms with Staphylococcus aureus, albus and citreus and Sarcina aurantiaca which have been found by Frei at al (1934) to contain the cytochrome components a,b and d. In this connection Krampitz and Werkman (1941), however, found that Micrococcus lysodeikticus oxidized p-phenylenediamine but not hydroquinone. It was suggested that the cytochrome-c of this organism had a protein bearer differing from that of beef heart (which oxidized both) which caused the potential of the former to be negative with respect to hydroquinone and hence inactive.

Study of the Bacterial Pigment

No principal differences have been found between the carotenoids of higher and lower plants but those of the higher plants anly have been studied. For the algae only fucoxanthin is well characterized while with regard to the fungi and bacteria still less is known (Zechmeister, 1934).

Aside from the brief observations of Schrötter (1895) that the pigments of Serratia aurantiaca and Staphylococcus aureus show the solubility and lipocyan reaction of the carotenoids,

our knowledge regarding carotenoid producing species of bacteria is due to Zopf (1899a,b; 1891, 1892) who has described the chromolipids in eight species of bacteria. The descriptions of Zopf point with certainty to carotenoids in the case of four species only, namely, Bacillus egregrium, Bacillus chrysoglia, Staphylococcus aureus and Sphaerotilus roseus.

According to Zopf B. egregrium and B. chrysoglia and Staphylococcus aureus produce a yellow carotenoid pigment characterized as follows: The cells produce an intense blue color with concentrated H_2SO_4 (lipocyan reaction). The pigment was slowly extracted by warm absolute alcohol and after extraction was soluble in alcohol, ether, chloroform, methyl alcohol, benzene and petroleum ether. The alcoholic solution showed two absorption bands, one covering the F line, the other between F and G.

Sphaerotilus roseus, a red organism, gave a yellow-red alcoholic extract. The yellow poiment was water soluble while the red pigment was soluble in the fat solvents. In alcohol the latter pigment showed absorption bands at 492-474 mu and 456-442 mu.

In 1925 Reader studied the carotenoid pigment of Sarcina aurantiaca . The cell suspension was saponified and the unsaponifiable fraction was extracted with petroleum ether, evaporated to dryness and taken up in light petroleum (B.P. 40-60°). This solution was passed through a chalk column and divided into fractions and examined spectroscopically. Two principal fractions were found, one of which appeared to be lycopin with absorption

bands between 509-490 mu, 477-458 mu, and 447-435 mu, while the other fraction possessed bands at 491-476mu and 456-440 mu and was believed to be carotene.

Experimental

Studies were conducted to determine whether the yellow pigment of M. luteus, the red pigment of M. cinnebareus, and the orange pigment of M.aurantiacus were carotenoid in nature.

M. luteus, M. flavus and M. aurantiacus reacted positively to the lipocyan test with concentrated H_2SO_4.

The yellow pigment of M. luteus was found to be soluble only in butyric acid, normal and secondary butyl alcohol. After extraction and reduction to dryness under reduced pressure the material was soluble only in butyric acid, normal and secondary butyl alcohol and glacial acetic acid. This material, after extraction and evaporation to dryness, no longer reacted positively to the lipocyan test.

The absorption spectrum of the butyl alcohol extracted material was determined by means of the Cenco-Spectrophotometer. Before each reading the galvanometer was set at 100 with the secondary butyl alcohol alone and the reading of the solvent containing the pigment was determined.

The butyl alcohol extract of M. luteus was found to possess absorption bands at 420 mu. and at 450 mu.

After treatment of the cells with acetic acid a yellow extract was obtained with butyl alcohol, acetone or a mixture of

acetone and alcohol. This yellow extract obtained after treatmant with acetic acid, however, possessed no absorption bands between 350 mu. and 680 mu.

The orange pigment of M. aurantiacus possessed the same characteristics as the yellow pigment of M. luteus except that the absorption bands were located at 420 mu. and 490 mu.

M. cinnebareus and M. freundenreichii did not react positively to the lipocyan test. Their pigments were found to be soluble in secondary butyl alcohol. The butyl alcohol extracted material of M. cinnebareus had a red color and possessed rather weak absorption bands at 510 mu. and 480 mu., while the butyl alcohol extracted material of M. freundenreichii was light yellow in color and possessed rather strong absorption bands at 465 mu., 450 mu. and at 420 mu.

Summary

The respiratory activities of Micrococcus luteus, Micrococcus flavus, Micrococcus aurantiacus, Micrococcus cinnebareus and Micrococcus freundenreichii were studied.

With methylene blue as the hydrogen acceptor the compounds found to be most readily activated were raffinose, maltose, sucrose, glucose, ethyl alcohol, succinate, maleate and glutamate.

When molecular oxygen was used as the hydrogen acceptor the compounds most readily activated were sucrose, maltose, glucose, ethyl alcohol, succinate, lactate, glutamate and asparagine.

The oxygen uptake of most of the substrates was constant or decreased slightly with time, while some few of the substrates, mainly glutamate and dl-b-phenylalanine, showed an increasing oxidation rate.

The influence on the cells of several respiratory inhibitors was determined. The dehydrogenases of Micrococcus luteus and Micrococcus flavus were stimulated by a cyanide concentration of 1.4×10^{-2} molar, while the other organisms were inhibited by this concentration. The specific dehydrogenases were found to vary in their sensitivity toward inhibitors, those active against the amino acids being most susceptible in that they were strongly inhibited by sodium cyanide, sodium monochlor-acetate and sodium azide in a concentration of 1.4×10^{-5} molar.

Using glucose as the representative substrate it was found that Micrococcus luteus was inhibited about 70 per cent by a cyanide concentration of 1.4×10^{-2} molar, while a 1.4×10^{-3} molar concentration inhibited respiration for a short time only after which an increasing rate was observed so that the total oxygen uptake at the end of one hour was identical with the uptake in the absence of inhibitor. A cyanide concentration of 1.4×10^{-4} molar stimulated respiration about 40 per cent.

Since the cell carriers operative in normal respiration were inhibited by the higher concentrations of cyanide, the ability of methylene blue to supplant these carriers was determined. Cyanide inhibition was generally decreased by methylene blue but was completely removed only in the case of Micrococcus flavus with sucrose as the substrate and Micrococcus aurantiacus with glucose as the substrate.

Methylene blue when used alone was found to exert an influence upon the cells. Micrococcus luteus and Micrococcus flavus were stimulated by the presence of one milligram of methylene blue in the presence of all substrates except succinate, while the other organisms were inhibited by this amount. Micrococcus freundenreichii was stimulated by 0.5 milligram in the presence of all substrates except succinate while Micrococcus aurantiacus and Micrococcus cinnebareus were inhibited by a concentration as low as 0.1 milligram.

Sodium monochlor-acetate in a concentration of 1.4×10^{-2} molar inhibited the oxidation of all substrates with the exception of glucose with which there was stimulation.

Sodium azide was found to be a stronger inhibitor than sodium monochlor-acetate but the stimulation and inhibition paralleled, indicating the point of attack of both inhibitors to be the same.

With the exception of Micrococcus freundenreichii, all the organisms possessed moderate polyphenol oxidase and catalase activity.

Hydroquinone was not oxidized by any of the micrococci, while p-phenylenediamine was oxidized by all the organisms. This might indicate that cytochrome-b is abundant in the organisms or that cytochrome-c has a protein bearer which causes its potential to be negative with respect to hydroquinone and hence inactive.

Micrococcus luteus, Micrococcus flavus and Micrococcus aurantiacus reacted positively to the lipocyan test with concentrated H_2SO_4. After extraction with secondary butyl alcohol the absorption spectrum of the pigments was determined. The pigment of Micrococcus luteus possessed absorption bands at 420 mu and at 450 mu. The orange pigment of Micrococcus aurantiacus possessed absorption bands at 420 mu. and at 490 mu.

Micrococcus cinnebareus and Micrococcus freundenreichii did not react positively to the lipocyan test. Their pigments were extracted with secondary butyl alcohol and possessed absorption bandsa at 510mu and 480 mu; and at 465mu, 450 mu and 420 mu, respectively.

Bibliography

Blaschko,H. (1935) The machanism of catalase inhibitions. Biochem. J. 29: 2303-2312.

Braun,H. and Vasarhelyi,J.v. (1932) Ueber die oxydrierenden und reduzierenden Fahigkeiten der Proteusbazillen und uber die Beeinflussung dieser Fermentwirkungen durch Antikorper und Antiseptika. Zentr. Bakt. Parasitenk.I Abt. Orig. 127: 105-111.

Braun,H. and Wordehoff, Ph. (1933) Ueber die Oxydativen und reduzierenden Fermentwirkungen des Ruhrbazillus Flexner. Zbl. Bakteriol. Orig. 128: 50-81.

Callow, A.B. (1924) The oxygen uptake of bacteria. Biochem. J. 18: 507-

Cook, R.P. (1930) A comparison of the dehydrogenases produced by B. coli Communis in the presence of oxygen and methylene blue. Biochem. J. 24: 1538-1550.

Cook,R.P., Haldane,J.B.S. and Mapson,L.W. (1931) The relationship between the respiratory catalysts of B. coli. Biochem. J. 25: 534-550.

Das, N.B. (1937) Identity of lactic and malic cehydrogenases. Biochem.J. 31: 1116-1123.

Ehrismann,O. (1935) Ueber die reduzierenden Wirkungem der Diphtherienbakterien. Zentr. Bakt. Parasitenk. I Abt. Orig. 135: 56-60.

Ehrismann, O. (1937) UberDehydrierenden Leistungen von Mikrokken und Streptokokken. z.f. Hyg. und Infektsk. 119: 572-612.

Elvehjem,C.A., Wilson,P.W. et al (1939) Respiratory enzymes. Burgess Publishing Co., Minneapolis.

Fabre,R. (1935) The hydrogen donors for the Staphylococci. Compt. rend. 120: 608-610.

Frei,W., Riedmüller,L. and Almasy,F. (1934) Über Cytochrom und das Atmungssystem der Bakterien. Bio. Z. 274: 253-267.

Gale, E.F. and Stephenson,M. (1939) L-Malic dehydrogenase and codehydrogenases of Bact. coli. Biochem. J. 33: 1245-1256.

Gerard,R.W. (1931) Observations on the metabolism of Sarcina lutea. Biol. Bull. 60: 213-241.

Green,D.E. and Stickland,L.H. (1934) Studies on reversible dehydrogenase systems. I. The reversibility of the dehydrogenase system of Bacterium coli. Biochem. J. 28: 898-900.

Green,D.E. and Stickland,L.H. and Tarr,H.L.A. (1934) Studies on reversible dehydrogenase systems. III Carrier-linked reactions between isolated dehydrogenases. Biochem. J. 28: 1812-1824.

Green,D.E. and Brosteaux,J. (1936) The lactic dehydrogenase of animal tissues. Biochem. J. 33: 1489-1508.

Hawthorne,J.R. and Harrison,D.C. (1939) Cytochrome-C as a carrier with the glucose dehydrogenase system. Biochem. J. 33: 1573-79.

Johnson,A.A. and Green,J.R. (1930) Modified methyl red and sodium alizarin sulfonate indicators. J.Ind. and Eng. Chem. (Analytical Edition) 2: 2-5.

Krampitz,L.O. and Werkman,C.H. (1941) Some properties of a bacterial cytochrome system (Abstract) J. Bact. 41: 21-22.

Krebs,H.A. (1935) Metabolism of amino-acids. III. Deamination of amino-acids. Biochem. J. 29: 1620-1644.

Quastel, J.H. and Wooldridge,W.R. (1928) Some properties of the dehydrogenating enzymes of bacteria. Biochem.J. 22: 689-702.

Reader,V. (1925) A note on the lipochromes present in certain bacteria. Biochem. J. 19: 1039-1046.

Schrötter,vH. (1895) Vorlaufige Mittheilung über das Pigment von Sarcina aurantica und Staphylococcus pyogenes aureus. Centr.Bakt.Parasitenk. (1) 18: 781-782. from Palmer,L.S., Carotenoids and related pigments, Chemical Catalog Co. N.Y.,1922.

Stotz,E., Sidwell,A.E. and Hogness,T.R. (1938) Thr role of the cytochromes in the action of "Indophenol Oxidase." J. Biol. Chem. 124: 733-744.

Thunberg,T. (1918) Zur Kenntnis der Einwirkung tierischer Gewebe auf Methylenblau. Skand. Arch. Physiol. 35: 163-195.

Wooldridge, W.R.,Knox,R. and Glass,V. (1936) Variability in the activity of bacterial enzymes.I.The effect of the age of the culture. Biochem. J. 30: 926-931.

Yudkin,J. (1933) The dehydrogenase of Bact. coli. I.The effect of dilution: with a note on the existence of a coenzyme of glucose dehydrogenase. Biochem. J. 27: 1849-1858.

Yudkin,J. (1937) The dehydrogenases of Bact. coli. III.The coenzyme of glucose dehydrogenase. Biochem. J. 31: 865-868.

Zechmeister,L. (1934) Carotenoide, Julius Springer, Berlin, 1934.

Zopf,W. (1889a) Ueber Pilzfarbstoffe. Botan. Ztg. 49: 53-61, 69-81, 85-91. From Palmer,L.S. Carotenoids and related pigments , Chemical Catalog Co., New York, 1922.

Zopf,W. (1889b) Ueber das microchemische Verhalten von Fettfarbstoffen und Fettfarbstoff-haltigen Organen. Z.wiss. Micros.6: 172-177., from Palmer,L.S., Carotenoids and related pigments, Chemical Catalog Co., New York, 1922.

Zopf,W. (1891) Ueber Ausscheidung von Fettfarbstoffen (Lipochromen) seiten gewisser Spaltpilze. Ber. botan. Ges. 9: 22-28., from Palmer, L.S., Carotenoids and related pigments, Chemical Catalog Co., New York, 1922.

Zopf,W. (1892) Zur Kenntniss der Farbungsursachen niederer Organismen (Zweite Mittheilung) Beitr. Physiol. Morphol. niederer Organismen 2: 3-48. , from Palmer,L.S., Carotenoids and related pigments, Chemical Catalog Co., New York, 1922.