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Effect of Surface Active Agents on Oxidations of Lactate by Bacteria.

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Baker, Harrison and Miller[1] have studied the effect of synthetic surface active agents on the metabolism of bacteria as measured in the Warburg manometric apparatus. Cationic surface active agents were found to inhibit equally the metabolism of both Gram positive and Gram negative bacteria. Anionic agents, in general, inhibit the metabolism of Gram positive bacteria only. In a subsequent paper[2] studies on bactericidal effects were reported. Dubos[3] discussed the differential susceptibility of Gram positive and Gram negative bacteria to different injurious agents, and suggested the use of anionic and cationic surface active agents as tools for the study of bacterial structure.

In this paper we report the effects of an anionic surface active agent, the dioctyl ester of sodium sulfosuccinate, and of a cationic agent, cetyl pyridinium chloride, on a Gram positive organism and a Gram negative organism. As an index of the action of these agents we used the oxidation of lactate by molecular oxygen as compared with anaerobic oxidation by methylene blue.

Methods. Two organisms were used, the Insecticide Board strain of *Staphylococcus aureus*, a Gram positive organism, and *Escherichia coli*, University of Washington No. 406, a Gram negative organism. These two cultures were selected because they possessed relatively active lactic dehydrogenases as determined by the Thunberg technic.

Staphylococcus aureus was grown on the broth prescribed for the Food and Drug Administration procedure for the phenol coefficient.[4] After 24 hours' incubation, the organisms were collected by means of a continuous flow Sharples centrifuge and washed 3 times with M/100 phosphate buffer at pH 7.0. *Escherichia coli* was grown on Blake bottles containing F. D. A. broth to which 2% agar had been added. The organisms were collected and then washed as described above for *Staphylococcus aureus*.

1 Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **73**, 249.

2 Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **74**, 611.

3 Marshall, E. K., Jr., Lockwood, J. S., and Dubos, R. J., *Chemotherapy*, Philadelphia, University of Pennsylvania Press, 1941.

4 Circular No. 198, 1931, U. S. D. A.

TABLE I.
Effect of Surface Active Agents on Lactic Dehydrogenases in *Staphylococcus aureus* and *Escherichia coli*.

	Staphylococcus aureus				*Escherichia coli*				
Conc. of OT, moles per l	0	1.6x 10^{-4}	3.2x 10^{-4}	6.4x 10^{-4}	0	5.3x 10^{-4}	2.1x 10^{-3}	2.5x 10^{-2}	5.1x 10^{-2}
Reduction time of methylene blue in min.	3	6	33	60	4	5	11	33	60
Conc. of CPR in moles per l	0	4.3x 10^{-6}	8.6x 10^{-6}	1.7x 10^{-5}	0	3.4x 10^{-5}	1.4x 10^{-4}	5.5x 10^{-4}	11.1x 10^{-4}
Reduction time of methylene blue in min.	3	3	11	60	4	5	13	38	60

OT—dioctyl ester of sodium sulfosuccinate.
CPR—cetyl pyridinium chloride.

The activity of lactic dehydrogenases in the cell suspensions was determined by the modified Thunberg method of Ordal and Halvorson[5] with methylene blue as indicator. Each Thunberg tube contained 5 ml M/15 phosphate buffer at pH 7.56, 0.25 ml 0.2 M sodium lactate, 1 ml 1-5000 methylene blue, 0.25 ml cell suspension and 2 ml H_2O or surface active agent. Tests for dehydrogenase activity were terminated at 60 minutes, and in general, controls without substrate showed no signs of decolorization at this time. All experiments were performed at 37°C.

The effect of the presence of varying concentrations of the dioctyl ester of sodium sulfosuccinate and of cetyl pyridinium chloride on the activity of the lactic dehydrogenases of *Staphylococcus aureus* and of *Escherichia coli* is shown in Table I.

The oxidation of lactate by oxygen was determined in conventional Warburg manometers. The final concentrations of phosphate, lactate and cell suspensions were identical with those used in the

TABLE II.
Oxygen Uptake from Lactate Solutions by Suspensions of *Staphylococcus aureus* in the Presence of Surface Active Agents.

Surface active agent	Conc. in moles/l	Oxygen uptake μl at 15 min	30 min	60 min	120 min
None		8	17	34	60
OT	8.0×10^{-5}	7	17	33	57
"	1.6×10^{-4}	6	11	24	43
"	3.2×10^{-4}	2	5	10	22
"	6.4×10^{-4}	—1	3	6	10
CPR	2.2×10^{-6}	7	14	27	42
"	4.3×10^{-6}	6	12	17	21
"	8.6×10^{-6}	1	3	6	7

OT—dioctyl ester of sodium sulfosuccinate.
CPR—cetyl pyridinium chloride.

[5] Ordal, E. J., and Halvorson, H. O., *J. Bact.*, 1939, **38**, 199.

TABLE III.
Oxygen Uptake from Lactate Solutions by Suspensions of *Escherichia coli* in the Presence of Surface Active Agents.

Surface active agent	Conc. in moles/l	Oxygen uptake in μl at				
		15 min	30 min	45 min	60 min	75 min
None		35	71	103	132	160
OT	2.5×10^{-2}	33	67	94	118	142
"	5.1×10^{-2}	31	65	90	111	130
CPR	1.1×10^{-5}	28	56	84	103	122
"	2.2×10^{-5}	12	26	36	40	42
"	4.5×10^{-5}	2	7	9	11	11

OT—dioctyl ester of sodium sulfosuccinate.
CPR—cetyl pyridinium chloride.

Thunberg tubes. The results of tests with varying concentrations of the surface active agents are shown in Tables II and III.

Results. It is apparent from the data shown in Table I that the lactic dehydrogenase of *Staphylococcus aureus* is far more susceptible to the action of both surface active agents than that of *Escherichia coli.* However, the inhibitory effect of the surface active agents disappears quickly with dilution with *Staphylococcus aureus* while with *Escherichia coli* there is a considerable range of concentrations in which there is a relatively slow change in inhibition. In particular, the lactic dehydrogenase of *S. aureus* is inhibited by 1.7×10^{-5} M cetyl pyridinium chloride. On dilution to one-fourth this concentration, the inhibitory effect disappears. On the other hand, a concentration of 1.1×10^{-3} M cetyl pyridinium chloride is required to inhibit the lactic dehydrogenase of *E. coli*, and a dilution in excess of 33 times is necessary before all traces of inhibition disappear.

Examination of the data in Tables II and III, shows that both surface active agents inhibit the oxidation of lactate by *S. aureus,* but that only the cationic agent inhibits the oxidation of lactate by *E. coli.*

For convenience in comparing the susceptibility of the two organisms, we have designated as "critical" concentrations those concentrations of surface active agents inhibiting the reduction by methylene blue for 60 minutes in the tests for dehydrogenases, and the concentrations giving nearly complete inhibition after 60 minutes in the tests for oxygen uptake. These data are collected in Table IV.

In the case of *S. aureus,* there is little difference between the concentrations of surface active agents required to inhibit oxidation of lactate by oxygen, as compared to oxidation by methylene blue. Presumably, therefore, the cytochrome systems normally concerned in the oxidation of lactate by oxygen in *S. aureus* possess no special

TABLE IV.
"Critical" Concentrations of Surface Active Agents Inhibiting Oxidation of Lactate by Oxygen and by Methylene Blue.

Surface active agent	Oxidation by	Organism	
		Staph. aureus	*E. coli*
Dioctyl ester of sodium sulfosuccinate	methylene blue	6.4×10^{-4}M	5.1×10^{-2}M
	oxygen	6.4×10^{-4}M	—*
Cetyl pyridinium chloride	methylene blue	1.7×10^{-5}M	1.1×10^{-3}M
	oxygen	8.6×10^{-6}M	4.5×10^{-5}M

*A concentration of 5.1×10^{-2}M only slightly slowed down the oxidation of lactate by oxygen (See Table III).

sensitivity to these surface active agents. Anson[6] has shown that synthetic surface active agents are extremely active in the denaturation of proteins. It seems likely that the proteins of *S. aureus* concerned in the oxidation of lactate by methylene blue are relatively accessible to the surface active agents.

There are striking differences in the effects of the surface active agents on the oxidations of lactate by *E. coli*. Since the oxidation of lactate by molecular oxygen is inhibited by a lower concentration of cetyl pyridinium chloride than oxidation by methylene blue, it would appear either that the cytochrome systems peculiar to *E. coli* are more sensitive to the surface active agent than the other proteins concerned in the oxidation of lactate, or are more available to the action of the agent.

The anomalous result with the dioctyl ester of sodium sulfosuccinate, inhibition of methylene blue reduction by a 5.1×10^{-2} M concentration, a concentration which affects only slightly the oxidation of lactate by oxygen, is most logically due to the reduced availability to the cells of the methylene blue due to the high concentration of the surface active agent which is present in this concentration largely in the form of micelles.

The enzymes of the two organisms which are concerned in the oxidation of lactate, differ profoundly in sensitivity to the anionic surface active agent. This is in accord with the results of Baker, Harrison and Miller.[1, 2]

Dubos[3] has pointed out that Gram negative bacteria contain so-called lipo-polysaccharide antigens which appear to form an outer coating on the cells, and Baker, Harrison and Miller[7] have shown that certain phospholipids protect bacterial cells against the action of both cationic and anionic surface active agents. It is possible,

[6] Anson, M. L., *J. Gen. Physiol.*, 1939, **23**, 239.

[7] Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **74**, 621.

therefore, that a cell membrane containing substances of this character in the Gram negative bacteria may account for the lack of sensitivity to the anionic agent. We are, therefore, attempting to prepare cell-free enzymes from *S. aureus* and *E. coli* in order to determine the rôle played by the cell membranes in the results shown above.

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Sulfonamide-Resistant Strains of Staphylococci: Clinical Significance.*

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Strains of pneumococci, gonococci, *E. coli* and *S. aureus* have been rendered resistant *in vitro* to the bacteriostatic action of sulfonamide drugs.[1-6] Rammelkamp[7] has also observed that *S. aureus* may become "fast" to tyrothricin, both *in vitro* and *in vivo*. The present report is concerned with the *in vitro* and *in vivo* production of sulfonamide resistant strains of staphylococci.

The *in vitro* observations were made with 2 strains of *S. aureus* isolated from patients with severe staphylococcal infections. A chemically-defined medium as described by Gladstone was used throughout.[8] These strains were made resistant by exposure to increasing concentrations of sodium sulfathiazole in this medium. Initial growth was established with a minute inoculum from an agar slant. Subsequent transfers of the cultures in the synthetic medium were made with a 5 mm wire loop. The two strains showed an initial difference in susceptibility to the action of sulfathiazole. Strain 14

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1 MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet*, 1939, **1**, 562.

2 MacLeod, C. M., and Doddi, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

3 Lowell, F. C., Strauss, E., and Finland, M., *Ann. Int. Med.*, 1940, **14**, 1001.

4 Westphal, L., Charles, R. L., and Carpenter, C. M., *Ven. Dis. Inform.*, 1940, **21**, 183.

5 Strauss, E., Dingle, J. H., and Finland, M., *J. Immunol.*, 1941, **42**, 313.

6 *Ibid.*, 1941, **42**, 331.

7 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 346.

8 Gladstone, G. P., *Brit. J. Exp. Path.*, 1939, **20**, 189.