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Effects of Surface-Active Agents on Carbohydrate Metabolism in Yeast.* (24610)

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Although inhibitory effects of surface-active agents on microbial metabolism are well known(1,2,3,4), several reports note that very small quantities of certain agents increase utilization of glucose, consumption of oxygen and production of CO_2(1,5,6,7). As far as is known, no detailed investigation of these stimulatory effects has been made.

Methods and materials. All experiments were performed on nonproliferating suspensions of commercial baker's yeast (Standard Brands) washed 4 times and aerated 4 hours prior to experiment. Gas exchange measurements were performed by standard Warburg technics. All suspensions were buffered with 0.03 M sodium succinate-tartrate adjusted to appropriate pH by addition of sodium hydroxide. With aid of commercially available glucose oxidase preparation· (Glucostat—Worthington Biochemical Corp.) glucose utilization was determined by measuring disappearance of glucose from the medium. The highest concentration of benzalkonium chloride in yeast suspensions had no significant effect on glucose determinations. Most studies were made with surfactant, benzalkonium chloride, but in some experiments cetylpyridinium chloride, dodecylammonium chloride or sodium dioctyl sulfosuccinate were used. All surfactant concentrations are recorded as mg of surfactant salt/mg yeast. All Figures and Tables represent averages of 2 experiments.

Results. Effects of benzalkonium ion on anaerobic metabolism of glucose. The effects of cationic surfactant, benzalkonium chloride, on anaerobic CO_2 production from glucose at pH 5.5 are shown in Fig. 1. At benzalkonium ion concentration below 0.003 (mg benzalkonium chloride/mg yeast) anaerobic CO_2 production was only slightly inhibited. Inhibition became more marked with higher surfactant concentrations until at 0.006 surfactant concentration anaerobic CO_2 production was inhibited approximately 70%. For comparison with later experiments it should be noted that, in the range of 0.003 to 0.004 of surfactant concentration, inhibition increased from 14 to 35%. It is concluded (Fig. 1) that benzalkonium chloride was either without effect or was inhibitory to anaerobic metabolism of glucose in the range of surfactant concentrations used.

Effects of benzalkonium ion on aerobic metabolism. Effects on metabolism of glucose. In Fig. 2 are plotted data for aerobic CO_2 production and oxygen consumption as functions of benzalkonium chloride concentration. Both CO_2 production and oxygen consumption at first rose and then declined as surfactant concentration was increased. In the range 0.003-0.004 rate of CO_2 production rose about 1 μM/mg yeast/hr to peak value approximately twice the control value. Rate of oxygen consumption in 0.003-0.004 range showed a smaller rise of about 0.24 μM/mg yeast/hr, a 35% increase over the control. If it is assumed that CO_2 produced by process involv-

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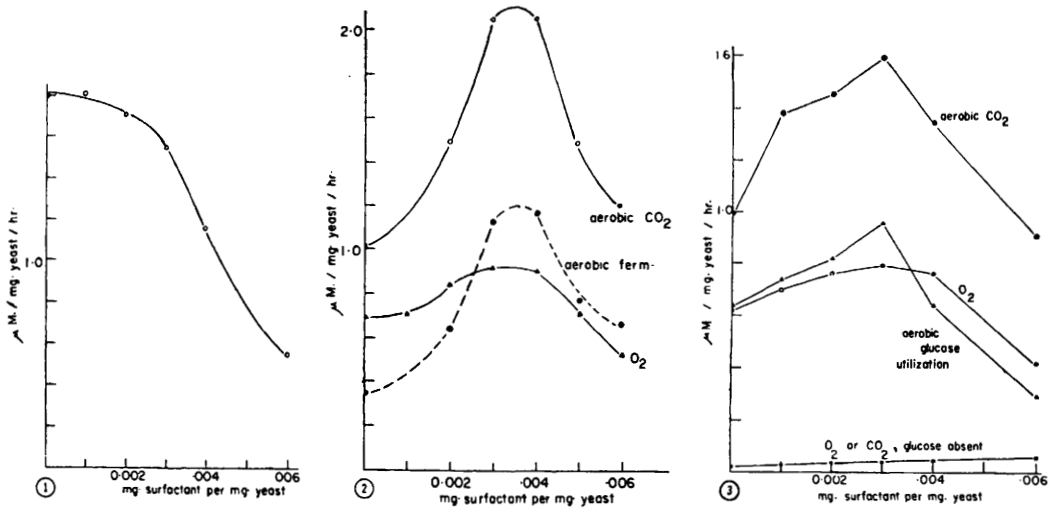


FIG. 1. Effect of benzalkonium chloride concentration on anaerobic CO_2 production by baker's yeast. Suspension contained 5 mg yeast/ml, 0.1 M glucose, 0.03 M sodium succinate-tartrate at pH 5.5, varying concentrations of benzalkonium chloride. Total volume was 3 ml; surfactant concentration expressed in terms of mg benzalkonium chloride/mg yeast. Warburg vessels gassed with helium prior to experiment.

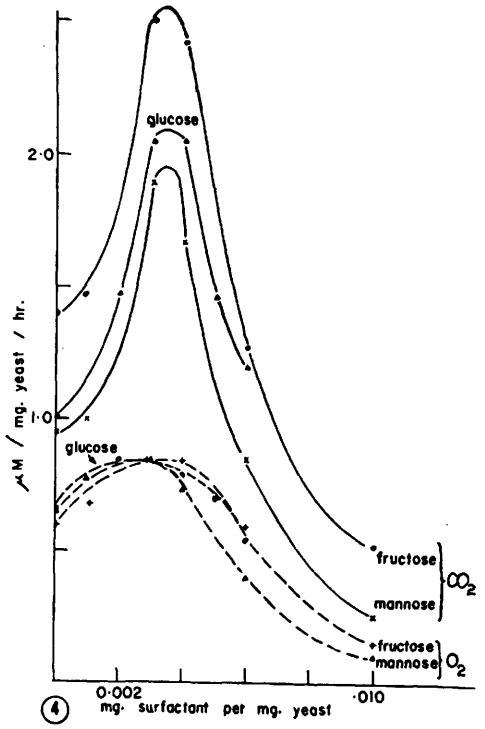
FIG. 2. Effect of benzalkonium chloride concentration on O_2 consumption and aerobic CO_2 production. Conditions same as for Fig. 1, except that air replaced helium in gaseous phase.

FIG. 3. Effect of benzalkonium chloride on oxygen consumption, aerobic CO_2 production and aerobic glucose utilization. Conditions same as in Fig. 2, except that initial glucose concentration was .03 M.

ing O_2 uptake (respiration) is in a one-to-one molar ratio with oxygen consumed, then the difference between rate of total aerobic CO_2 production and rate of oxygen consumption can be considered to be rate of aerobic fermentation, *i.e.*, rate at which anaerobic process (fermentation) is proceeding in presence of air. Thus, in the surfactant range 0.003-0.004 calculated aerobic fermentation rises to a maximum which is approximately 350% of control (Fig. 2). Increased aerobic CO_2 production in presence of benzalkonium ion therefore would be due primarily to an increase in, or a "release" of, fermentation by benzalkonium chloride.

Breakdown of carbohydrate stores with concomitant rise in CO_2 production and oxygen consumption was unlikely, since relatively negligible rates of CO_2 production and oxygen consumption (Fig. 3) were obtained at all surfactant concentrations when suspensions contained buffer but no substrate. Furthermore, comparison of rates of aerobic fermentation of Fig. 2 with rates of fermentation of Fig. 1 shows that aerobic fermentation never exceeded anaerobic fermentation at corresponding values of surfactant concentration; *i.e.* increased CO_2 production occurring in air when benzalkonium was added was limited by fermentation values found in anaerobic curve of Fig. 1.

Finally, a "release" of fermentation as the result of added surfactant would require that glucose utilization increase, to account for increased aerobic CO_2 production. Fig. 3 shows average results for 2 aerobic experiments in which rates of CO_2 production, O_2 consumption, and glucose utilization are plotted against surfactant concentration. It will be noted that rate of glucose utilization at peak value rose 0.3 μM/mg yeast/hr above control. The increase in rate of CO_2 production at peak value amounted to approximately 0.6 μM/mg yeast/hr above CO_2 control. Thus, for each extra mole of glucose utilized it appears that approximately 2 extra moles of CO_2 are produced, a ratio in agreement with stoichiometry of fermentation process. Since 6 extra moles of oxygen would need to be consumed for each extra mole of glucose utilized by respiration, it is evident that increase in oxygen consump-



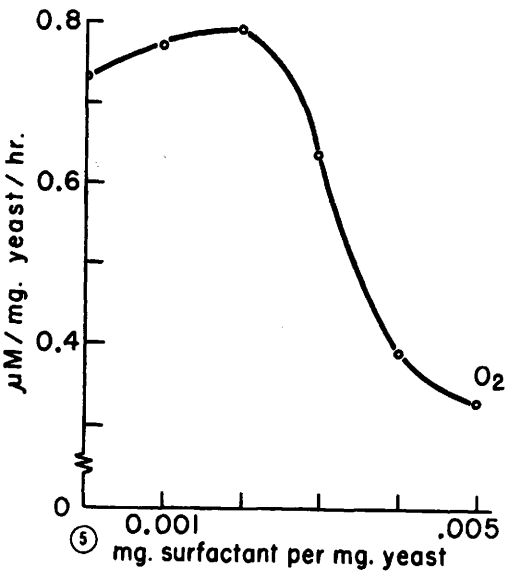


FIG. 4. Comparison of effects of benzalkonium chloride on aerobic metabolism of glucose, mannose, and fructose. Conditions same as in Fig. 2, except that in studies on mannose or fructose metabolism, appropriate hexose was substituted in equal concentration for glucose.

FIG. 5. Effect of benzalkonium chloride on oxidation of ethyl alcohol by baker's yeast. Conditions same as in Fig. 2, except that glucose was replaced by 0.02 M ethanol.

tion played a negligible role in increased utilization of glucose.

On the basis of experiments represented by Figs. 1, 2, and 3, therefore, it is concluded that appropriate concentrations of benzalkonium chloride bring about increased fermentation in presence of air.

Effects of benzalkonium chloride on the metabolism of other substrates. In Fig. 4 are shown comparative effects of benzalkonium ion on metabolism of glucose, mannose, and fructose. It is interesting that the same effect of surfactant was found with all 3 hexoses, both with regard to percentage stimulation of rates of CO_2 production and oxygen consumption, and with regard to surfactant concentration at which the peaks occur. In Fig. 5 results are shown for effects of benzalkonium chloride on oxidation of alcohol. In contrast to experiments with hexose substrates, oxygen consumption sharply declined in the surfactant range 0.002-0.004.

Comparison of effects of benzalkonium chloride and other surfactants on metabolism and effect of low pH. The effects of several surface-active agents on metabolism are summarized in Table I. At pH 5.5 all 3 cationic surfactants produced a maximum rate of aerobic CO_2 production. The peak occurred at different concentrations of surfactant in each case, however, and these differences in peak position do not correlate with molecular weights of the agents. At pH 5.5 the anionic surfactant, sodium dioctylsulfosuccinate, at 10 times the highest concentration of any cationics, produced no alteration of either rate of CO_2 production or rate of O_2 consumption. Cetyl pyridinium chloride at pH 5.5 and sodium dioctylsulfosuccinate at pH 2.5 inhibited oxygen consumption at all concentrations of surfactant tested (to 0.008 mg/mg yeast). This inhibition contrasted with effects of quaternary ammonium cationic agents which stimulated O_2 consumption.

At pH 2.5 benzalkonium chloride and sodium dioctylsulfosuccinate brought about a rise in CO_2 production, and in the case of benzalkonium chloride, a slight rise in O_2 consumption (see Table) was also noted.

When compared to curve of Fig. 2, the stimulatory effect of benzalkonium chloride at

TABLE I. Comparison of Effects of Surfactants on Aerobic Metabolism in Yeast. In addition to varying amounts of appropriate surfactant, suspensions contained 0.1 M glucose, 5 mg/ml yeast, 0.03 M sodium succinate-tartrate buffer.

Surfactant	Type	pH 5.5 Relative surfactant conc. at peak stimulation and % stimulation at peak				pH 2.5 Relative surfactant conc. at peak stimulation and % stimulation at peak			
		CO_2	% above control	O_2	% above control	CO_2	% above control	O_2	% above control
Benzalkonium chloride	Quat. ammonium cationic	.003–.004	105	.003–.004	31	.0005	35	.0005	10
Dodecylammonium chloride	*Idem*	.001	16	.001–.002	10				
Cetyl pyridinium chloride	Pyridinium cationic	.002–.003	60	No peak	Continuous decline				
Sodium dioctylsulfosuccinate	Anionic	*		———	* ———	.0005	41		Progressive inhibition

* No effect.

pH 2.5 occurred at lower surfactant concentration, but degree of stimulation at the peak was smaller than at pH 5.5.

Discussion. Figs. 1 and 2 indicate that 3 main factors in metabolism of hexoses are affected by benzalkonium ion: the controlling mechanism which is responsible for the Pasteur Effect, the fermentative process, and respiration.

Very low concentrations of benzalkonium ion inhibit the Pasteur Effect when the latter is measured either by glucose utilization or CO_2 production. Thus, there occurs an increased fermentation which is countered at higher benzalkonium concentrations by the inhibitory effect of the agent on the fermentation process itself. The presence of certain concentrations (0.003-0.004) of surfactant produces the expected peak in rate of CO_2 production.

The less pronounced but definite peak value reached in oxygen consumption when glucose was metabolized in presence of benzalkonium chloride is not explained. The fact that oxidation of alcohol (Fig. 5) was inhibited at surfactant concentration 0.003-0.004 makes it unlikely that increase in oxygen consumption is due to oxidation of the extra alcohol produced by "release" of fermentation. It is possible that benzalkonium ion produces stimulation in oxygen consumption by the fact that surface-active agents have a potent effect in disrupting the organization and permeability of the cell membrane(2,4,6). Increased permeability might then allow hexose substrate or an intermediate metabolite greater access to a reactive site. However, low concentrations of benzalkonium chloride were used in this study; whether these low concentrations have a great effect on membrane permeability has not been determined. In addition, increased oxygen consumption occurred only when the 2 quaternary-ammonium compounds were used, the other 2 compounds being strictly inhibitory to oxygen consumption. Perhaps, therefore, increased oxygen consumption is a specific effect of quaternary ammonium compounds rather than a nonspecific breakdown of a barrier to substrate penetration.

Since metabolism of mannose, glucose and fructose showed such similar responses to benzalkonium ion, the suggestion is made that there is a common "point of attack" by benzalkonium ion in metabolism of all 3 substrates. For example, benzalkonium ion might affect one or more enzymes in the pathway common to metabolism of all 3 substrates. Our findings are in agreement with those of Aisenberg and Potter(8) that the Pasteur Effect is mediated through inhibition of hexokinase or phosphohexokinase or both. Yeast hexokinase is known to phosphorylate all 3 hexoses(9,10). Since the release of fermentation by benzalkonium ion necessitates inhibition of the control factor or factors in the Pasteur Effect, the possibility arises that the normal "inhibition" of these enzymes is itself affected by the agent. Or again, the similarity of effect among various substrates could be a membrane or barrier phenomenon involving loss of structural integrity in the cell.

Within the range studied, cationic agents become increasingly potent as external pH is raised, wheras an opposite effect is seen for anionic type surfactants(1). As reported by us, the effects of the anionic agent, sodium dioctylsulfosuccinate, is in agreement with the literature. However, we found that the cationic agents tested produce their effects at lower surfactant concentrations when pH is lowered to 2.5. It may be conjectured that the pH tested is an extreme pH and that the cell is being subjected to abnormal stress at such low pH. It is well established that normal hexose metabolism by yeast is definitely inhibited at low pH(11). Although the effect of benzalkonium ion on metabolism occurs at lower surfactant concentration at pH 2.5 than at pH 5.5, the *extent* of the effect is much reduced at lower pH. The latter finding could suggest a directly inhibitory effect of hydrogen ion on metabolism of the injured cell.

Summary. 1. The effects of several surface-active agents on carbohydrate metabolism have been studied. Fermentation, oxygen consumption, and the Pasteur Effect are affected by these agents. 2. With the exception that oxygen consumption is affected differently by various surfactants tested, all 4 agents show qualitative similarities in their effects on carbohydrate metabolism. Benzalkonium chloride showed greatest effect on aerobic CO_2 production and the Pasteur Effect. 3. Several hexoses used as substrates showed the effect of benzalkonium ion to be non-specific in its action on metabolism of 3 hexose substrates. 4. Effects of the agents on oxygen consumption and effects of pH were discussed.

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