A STUDY OF THE RELATIONSHIP BETWEEN THE SURFACE CHARGE AND THE ADSORPTION OF ACID DYES BY BACTERIAL CELLS[1]

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Studies of the adsorption of acid dyes of the fluorane series have shown that more of the dye is adsorbed by bacteria under acid conditions than under basic or neutral conditions (Harris, 1949). The amount of dye adsorbed was also found to be related to the ionization of the dye molecule. Since both the bacterial cell and the dye anion carry negative charges, these findings suggest that as the H^+ of the medium is increased the charge on the cell becomes less, thus allowing greater combination between the cell and the dye anion. It is known that a number of environmental factors influence the electrical charge of the bacterial cell (Abramson, 1934). This investigation was undertaken to study the adsorption of acid dyes in relation to the charge of the bacterial cell as determined by electrophoretic measurements.

EXPERIMENTAL RESULTS

The procedures used in growing large numbers of bacterial cells and the measurement of dye adsorption were the same as described previously (Harris, 1949). Electrophoretic measurements were made following the general methods suggested by Moyer (1936). Sodium acetate, acetic acid, and hydrochloric acid were combined to give buffers of the desired pH. Bacterial cells were washed and suspended in M/50 buffer for dye adsorption and electrophoretic measurements. Since fluorane dyes are unstable in very acid solutions, sodium salts of certain sulfonaphthalein derivatives commonly used as acid-base indicators were studied instead. Quantitative determinations of these dyes were made spectrophotometrically in phosphate buffer, pH 8.0. The uptake of dyes is reported as milligram equivalents (m.e.) of the dye combining with 100 grams of dry cells.

Typical S-shaped curves, resembling adsorption isotherms, were obtained when the amounts of bromthymol blue, bromthymol purple, and bromcresol green combining with the cells in acid solution were plotted against the concentrations of the dye. The results obtained were similar to those reported for other acid and basic dyes by McCalla (1940). Quantitative removal of the adsorbed dye was easily accomplished by changing the reaction to slightly alkaline. The results of a typical experiment are shown in table 1, in which are recorded both the adsorption of bromthymol blue by *Bacillus subtilis* at different reactions and the subsequent desorption of the dye when the dye-coated cells were adjusted to pH 8.5.

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Adsorption of these dyes by *Bacillus subtilis*, *Bacillus megatherium*, *Rhodospirillum rubrum*, *Escherichia coli*, *Sarcina lutea*, *Azotobacter chroococcum*, and *Rhizobium meliloti* and the electrophoretic mobility were compared at different pH levels. The negative charge on the cell decreased and the adsorption of the acid dye increased as the pH of the suspending medium was lowered. The

TABLE 1

Adsorption of bromthymol blue by Bacillus subtilis at various pH levels and the subsequent desorption of these dyes when the cells were resuspended at pH 8.5

	pH								
	8.33	7.50	6.80	5.94	5.15	4.50	4.13	3.12	2.50
M.E. bromthymol blue adsorbed per 100 g cells at each pH level	1.00	17.0	30.0	40.0	43.0	82.0	90.0	102	102
M.E. bromthymol blue desorbed per 100 g cells at pH 8.5	0.60	9.50	21.0	30.0	36.0	66.0	71.0	77.0	77.0

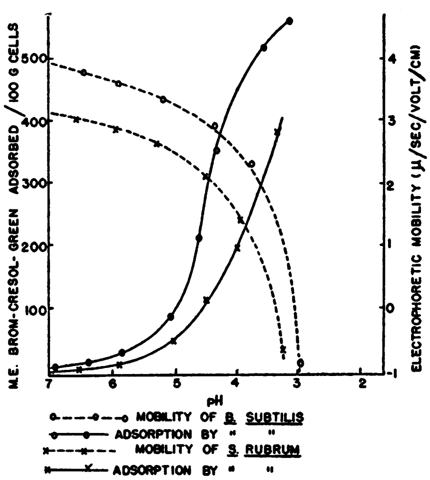

Figure 1.

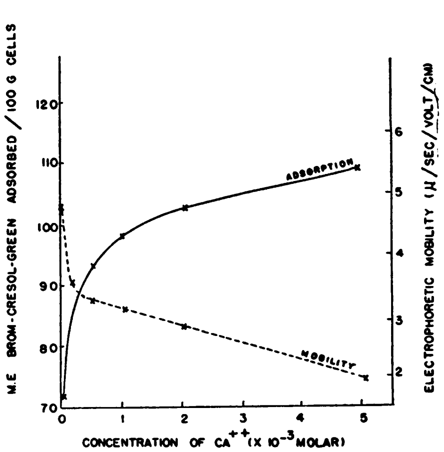

Figure 2.

Figure 1. A comparison of electrophoretic mobility and the absorption of bromcresol green by *Bacillus subtilis* and *Spirillum rubrum* at different pH values.

Figure 2. The effect of the addition of varying concentrations of calcium upon the electrophoretic mobility of *Bacillus subtilis* and the adsorption of bromcresol green, pH 5.0.

mobility and the uptake of bromcresol green by *Bacillus subtilis* and *Spirillum rubrum* in various hydrogen ion concentrations are shown in figure 1. A definite relationship is evident between the uptake of the acid dye and the electrophoretic mobility (electrical charge) of the cell.

The effect of various cations on the adsorption of the indicator dyes and the electrophoretic mobility was studied in an acid solution by varying the concentration of various cations. The data obtained with *B. subtilis* and varying calcium concentrations are shown in figure 2. As the concentration of Ca^{++} was increased, the uptake of bromcresol green increased and the mobility of the cells decreased, indicating a decrease in the negative charge carried by the cell.

Certain cations of the heavy metals are known to be adsorbed readily by bacterial cells (McCalla, 1940). The results of an experiment using a number of metallic cations (0.005 M) are shown in table 2. Mercuric and cupric ions decreased the mobility of the cells only slightly and the former had very little influence on the adsorption of bromthymol blue. In contrast, zinc, barium, strontium, and aluminum ions all resulted in significant increases in dye adsorption and decreases in the mobility of the cells. These results indicate that factors other than electrical charge influence the uptake of the acid dyes.

Since Dyar and Ordal (1946) have shown that cationic surface-active agents are particularly effective in reducing the electrical charge of bacterial cells, it

TABLE 2

The effect of addition of certain cations (0.005 M) upon adsorption of bromthymol blue by Bacillus subtilis and upon the electrical charge of the bacterial cell as indicated by electrophoretic mobility, pH 4.2

CATIONS ADDED	BUFFERS ONLY	Hg^{++}	Ca^{++}	Cu^{++}	Zn^{++}	Ba^{++}	Sr^{++}	Al^{+++}
M.E. bromthymol blue adsorbed per 100 g cells	91.0	91.2	162	146	235	298	257	264
% decrease in mobility		29.0	37.0	29.0	44.6	45.3	68.0	157*

* The charge was reversed.

might be expected that such compounds would lead also to greater adsorption of acid dyes. A number of experiments with members of the quaternary ammonium series have indicated that this occurs. This is in agreement with Dyar (1947), who observed staining of the cell wall by acid dyes in the presence of a cationic agent. However, quantitative experiments with surface-active agents are complicated by the fact that above certain critical concentrations micelle formation occurs, resulting in a change in the color of many indicators (Corrin and Harkins, 1946). In the present study trimethylbenzylammonium chloride and cetyldimethylbenzylammonium chloride were found to increase the adsorption of acid dyes and decrease the mobility of the cells. In all instances, the data obtained with these surface-active agents agreed with those obtained with cations of lower molecular weight and already recorded.

Since the electrical charge of the bacterial cell is increased when the cells are suspended in dilute buffer solutions, it would be expected that the concentration of the buffer might influence the adsorption capacities of the bacterial suspensions. A number of experiments have shown this to be the case. For example, *Azotobacter* cells were washed in pH 4.0 buffers of varying ionic concentrations and the dye uptake was measured in these buffers. Adsorption of bromthymol blue in such an experiment follows:

M/10	buffer	.... 84.2	m.e. per 100 g cells
M/50	"	.... 75.0	" " " " "
M/500	"	.... 72.3	" " " " "
M/1,000	"	.... 70.0	" " " " "
M/5,000	"	.... 37.0	" " " " "

These results indicate that reducing the charge on the cell by increasing the concentration of the buffer solutions is of more importance in influencing the uptake of the dye than is the competition between the acetate and dye anions for adsorptive positions on the cell surfaces. In the case of competitive adsorption the uptake of the dye would decrease as the concentration of the buffer was increased.

In three types of experiments, the uptake of the dye anions has been shown to increase when the conditions were such that the negative charge on the cell was decreased. This constitutes experimental evidence supporting the theory that the relatively poor staining of bacteria by acid dyes under ordinary conditions is due to a repulsion between the like (negative) charges of the dye anion and the bacterial cell. It is likely that other factors such as the ionization of the dye molecule (Harris, 1949), the chemical nature of the cell, the ionic composition of the suspending medium, and the solubility of dye components in the cell surfaces also have a marked influence upon the uptake of dyes by the cells. Certain phases of this problem are being investigated.

SUMMARY

Three members of sulfonaphthalein type dyes, bromthymol blue, bromcresol purple, and bromcresol green, were shown to combine with bacterial cells under acid conditions. Quantitative desorption of these dyes is observed at pH 8.5. The negative charge of common bacterial cells was reduced by lowering the pH of the suspending medium, by the addition of readily adsorbed cations, and by increasing the ionic concentration of the suspending medium. Greatest adsorption of the acid dyes occurred when the conditions were such that the electrical charge on the cells was least negative. These results are discussed in relation to the staining of bacteria by acid dyes.

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