THE GRAM REACTION AND THE ELECTRIC CHARGE OF BACTERIA

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A number of investigators, particularly Winslow and his associates, have shown that bacteria carry a negative electric charge. The charge and consequently the rate of migration under the influence of an electric current can be modified by changes in pH. Stearns and Stearns (1924; 1925) have attempted to explain the Gram reaction of bacteria as related to or determined by the isoelectric point and the pH. Winslow and Upton (1926) failed to see any relation between the electric charge and the Gram reaction. The experiments described here were made to determine whether there exists any relation of this sort.

EXPERIMENTAL

A Gram-positive yeast and a Gram-negative bacillus, *Escherichia communior*, were used as the test organisms. These were grown on potato extract agar for 24 hours, scraped off with a loop and transferred directly to the desired pH solution. After shaking for 15 minutes to break up the clumps the suspension was centrifuged and the supernatant fluid poured off. More of the suspension fluid was added, shaking was continued for five minutes and the pH adjusted to the desired point.

The suspension fluid was made of distilled water adjusted to the approximate pH. After the organisms were suspended in it the pH was adjusted with the aid of the hydrogen electrode. The organisms were made up in suspensions ranging in pH from 1.0 to 14.0.

The cell used for measuring the migration of the organisms was a modification of the apparatus described by Northrop (1922).

Care was taken to prevent the interior of the cell from becoming coated with the organism and thus interfering with the test. The apparatus was kept level to prevent drifting. A D.C. current of 112 volts was used.

The suspension of the organisms was placed in the cell and the migration rates determined. The electric charge of the organism is proportional to the migration velocity. The migration rate is given as micra per second. The figures given in the table represent an average of 20 readings. These readings were obtained by determining the rate of migration of 5 organisms in the upper half and 5 in the lower half of the cell. This was repeated after reversing the current. The organisms selected were at mid points of the upper and lower halves of the cell.

Smears were made from the various suspensions and Gram stained by the Burke technique (1922) except that no sodium bicarbonate was added to the dye on the slide.

The results are given in table 1. Upon exposure to a pH of 1.5 and 13.5 the electric charge of *Esch. communior* became neutral and in a pH of 1.0 and 14.0 reversed or positive. The cells were Gram-negative in all reactions. The migration toward the positive pole was greatest at about neutrality and gradually decreased as the solution became either more acid or alkaline. There is nothing to indicate a relation between the electric charge and the Gram reaction since the charge was greatly modified and reversed without affecting the Gram reaction.

With the Gram-positive yeast cells the migration velocity curve differs somewhat from that of *Esch. coli.* The greatest rate of migration was at about pH 10.0. The charge was reversed at pH 13.0 to 13.5. The charge became neutral at pH 2.0 and did not become reversed at pH 1.0, i.e., the cells were still motionless. The Gram-positive character of the cells began to be lost at a pH of 12 and 3.5. At these pH values the cells still carried a negative though reduced charge.

It is seen from this experiment that it is possible to reverse the charge of a Gram-negative cell without affecting the Gram reaction. And that with a Gram-positive cell it is possible to reverse the Gram reaction without reversing the charge. Whether it is possible to reverse the Gram reaction without affecting the negative charge was not determined by this experiment. Burke and Barnes (1929) however, have shown that by breaking the cell

TABLE 1

ESCH. COMMUNIOR				YEAST			
pH		Velocity	Gram reaction	pH		Velocity	Gram reaction
1.0	=	+1.0	−	1.0	=	0.0	−
1.5	=	0.0	−	1.5	=	0.0	−
2.0	=	−0.9	−	2.0	=	0.0	−
2.5	=	−2.4	−	2.5	=	−2.5	±
3.0	=	−4.0	−	3.0	=	−6.0	±
3.5	=	−6.0	−	3.5	=	−8.0	±
4.0	=	−8.6	−	4.0	=	−10.2	+
4.5	=	−12.3	−	4.5	=	−12.2	+
5.0	=	−12.0	−	5.0	=	−12.5	+
5.5	=	−11.6	−	5.5	=	−12.0	+
6.0	=	−14.2	−	6.0	=	−12.6	+
6.5	=	−15.0	−	6.5	=	−13.4	+
7.0	=	−16.8	−	7.0	=	−15.0	+
7.5	=	−16.8	−	7.5	=	−15.2	+
8.0	=	−15.0	−	8.0	=	−15.8	+
8.5	=	−14.0	−	8.5	=	−16.5	+
9.0	=	−13.0	−	9.0	=	−18.0	+
9.5	=	−11.5	−	9.5	=	−18.4	+
10.0	=	−10.5	−	10.0	=	−19.0	+
10.5	=	−9.5	−	10.5	=	−18.6	+
11.0	=	−8.0	−	11.0	=	−18.0	+
11.5	=	−7.1	−	11.5	=	−14.0	+
12.0	=	−6.5	−	12.0	=	−10.2	±
12.5	=	−5.5	−	12.5	=	−5.0	±
13.0	=	−2.0	−	13.0	=	−4.0	−
13.5	=	0.0	−	13.5	=	+5.0	−
14.0	=	+26.0	−	14.0	=	———	−

Velocity expressed as average velocity in micra per second at various pH values. − indicates migration toward anode, + migration toward cathode. In reference to the Gram reaction + indicates Gram positive, − Gram negative, and ± Gram amphophile.

wall the Gram reaction of a Gram-positive cell can be reversed. The pH of the protoplasm appears to be the same as that of the unbroken cells which remain Gram-positive.

CONCLUSIONS

Gram-positive and Gram-negative cells may show a similar migration curve under the influence of an electric current. Both carry a negative electric charge and move toward the positive pole. The electric charge on the cells can be altered by the addition of acid and alkali. The charge on the Gram-negative cells may be reversed without reversing the Gram reaction. The Gram reaction of the Gram-positive cells may be reversed without reversing the electric charge.

The Gram reaction is apparently not determined by, and may not be correlated with, the electric charge on the cell. This experiment adds support to the conclusion of Winslow and Upton that there is no apparent relation between the electric charge and the Gram reaction such as was postulated by Stearns and Stearns (1924; 1925).

REFERENCES

Burke, Victor 1922 Jour. Bacteriol., **7**, 159.

Burke, Victor, and Barnes, Mildred Winchester. 1929. Jour. Bacteriol., **18**, 69.

Northrop, J. H. 1922 Jour. Gen. Physiol., **4**, 629.

Stearn, E. W., and Stearn, A. E. 1924 Jour. Bacteriol., **9**, 479.

Stearn, E. W., and Stearn, A. E. 1925 Jour. Bacteriol., **10**, 13.

Winslow, C.-E. A., and Upton, M. F. 1926 Jour. Bacteriol., **11**, 367.