CORRELATION BETWEEN PHYSICOCHEMICAL PROPERTIES OF THE SURFACE OF BACTERIA AND THEIR SUSCEPTIBILITY TO PHAGOCYTOSIS BY POLYMORPHS

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The electrophoretic velocities (electrokinetic potentials) and the degree of hydration (hydrophilicity) of the surface of bacteria and the intensity of their ingestion by rabbit blood phagocytes were determined for 12 strains of Escherichia coli. A close connection was found between the degree of hydration of the bacterial surface and the intensity of their phagocytosis by leukocytes: the higher the degree of hydration the more active the phagocytosis. No correlation could be found between the electrophoretic mobility of the bacteria and the intensity of their phagocytosis.

Many investigators have demonstrated the role of physicochemical mechanisms in the initial phases of phagocytosis [4, 6-9, 11] but information in the literature on this question is contradictory [2].

The object of this investigation was to make a quantitative study of correlation between the electrophoretic velocities (electrokinetic potentials) and the degree of surface hydration of bacterial cells with their susceptibility to phagocytosis by the polymorphs of the blood. Sensitive methods of colloid chemistry, giving quantitative information on the size of the hydration layers on the surface of bacterial cells or on the degree of hydrophilicity of their surfaces were used for the first time in this investigation.

EXPERIMENTAL METHOD

Experiments were carried out with 12 strains of Escherichia coli in the s-form. The cells grown on meat-peptone agar (20 h) were washed off with distilled water and divided into two portions, one of which was washed three times with distilled water and the other with 0.102 M (for the electrophoresis experiments 203×10^{-4} iM) citrate-phosphate buffer, pH 7.0.

The electrophoretic velocities of the bacteria were determined with the apparatus of Pešak and Kostka [10].

The degree of hydration of the cell surfaces was measured in two ways: 1) by the method of determining the free water in the dispersion medium of the suspensions, as developed by Dumanskii [3]. It is based on the fact that free water, unlike the water of the hydration membranes, is a solvent. By adding a known quantity of sucrose to the suspension and then measuring its concentration with a saccharimeter, the quantity of solvent water in the dispersion medium can easily be calculated. To reduce the entrance of the sugar into the cells to a minimum, the cells were saturated with the sugar before the experiment, for which purpose the sucrose was added to the water used to wash the bacteria off the agar; 2) by the increase in viscosity of bacterial suspensions on an increase in their concentration from 4.15 to 14% dry weight. This method is based on the well-known rule: the higher the degree of hydration of the particles, the greater the rise in viscosity of the suspension accompanying an increase in their concentration. The viscosity was judged from the time taken for a metal ball to drop in a cylinder filled with the bacterial suspension. Each value was calculated as the mean of 11 measurements.

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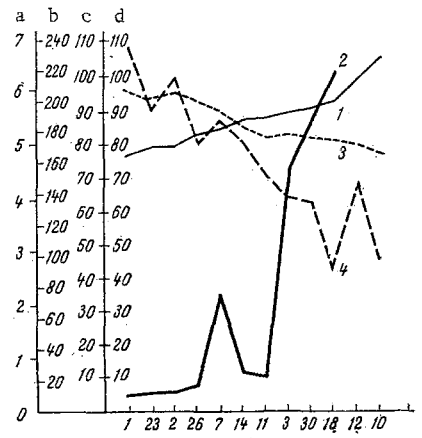

Fig. 1. Correlation between intensity of phagocytosis of E. coli cells by leukocytes and various indices reflecting degree of hydrophilicity of the bacterial surface: 1) phagocytic number; 2) increase in time taken for ball to drop*; 3) content of free water; 4) contact angles. Abscissa, no. of culture; ordinate: a) phagocytic number, b) increase in time for ball to drop (in sec), c) content of free water (in percent), d) contact angles of drops (in deg.).

*The ball did not drop in 14% suspensions of E. coli nos. 10 and 12.

The degree of hydrophilicity of the bacterial surface was measured by the method developed in colloid chemistry by Rebinder [5]. It consists of measuring the contact angles of drops of water and buffer solution on the surface studied – in this case on dry films of bacteria. Drops on films immersed in kerosine to remove hysteresis were photographed and their contact angles measured on highly enlarged projections of the photographic images. The mean values of 11-12 measurements of the angles were recorded. Statistical analysis showed high significance in all cases.

The phagocytic number (the number of bacteria per polymorph) was determined for each culture from the blood of ten rabbits, and the arithmetic mean was then calculated. This was necessary to smooth out the effect of individual differences.

EXPERIMENTAL RESULTS

No correlation was found between the intensity of phagocytosis of the bacteria and their electrophoretic mobility and, consequently, their electrokinetic potentials. By contrast a strict correlation was observed between phagocytosis and the surface hydration of the bacteria. In Fig. 1 the cultures studied are arranged in order of increasing susceptibility to phagocytosis by leukocytes. The increase in the phagocytic number clearly went parallel with the increase in hydrophilicity of the bacterial surface. Viscosity of the bacterial suspensions was increased with an increase in their concentration from 4.15 to 14%, the content of free water in the intercellular space of the suspensions was reduced, and the contact angles of the drops on dry bacterial films were reduced. The results of the experiments using water and buffer solution as dispersion media were approximately the same, so that only the former are shown in Fig. 1. The coefficient of correlation between the phagocytic number and indices such as the increase in time for the ball to drop, the percentage of free water, and the size of the contact angles was 0.83 ± 0.09, -0.92 ± 0.04, and -0.97 ± 0.02 for determination of the degree of hydration in water, and 0.83 ± 0.13, -0.84 ± 0.08, and -0.97 ± 0.02 respectively, when the experiments were carried out in buffer. In all cases $P < 0.05$. Cultures in 14% suspensions of which the ball did not fall were excluded from calculations of the corresponding coefficients of correlation.

The results described above show a well-defined correlation between the degree of hydration (or hydrophilicity) and the susceptibility of bacteria to phagocytosis. The higher the degree of hydration the more readily the cells are ingested by polymorphs. It was shown conclusively that this rule applies only if the phagocytic number for each strain is determined in the blood of several (ten) rabbits, and the mean value is then calculated. Phagocytic numbers obtained from the blood of each rabbit individually correlated much less closely with the surface properties, indicating the need for repetition of the experiments.

Without a series of additional experiments it is impossible to say whether the increased hydration favors phagocytosis directly or indirectly, through selective adsorption of certain blood proteins on the bacteria. However, the fact that this correlation exists provides a good basis for the further study of the physicochemical mechanisms of the phagocytic response, and the methods used are clearly suitable for this purpose.

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