

Fig. 1. Comparison of multiple exposures (M) and single shots (S) in the cleaving egg.

Films, with a time-lapse between frames of 1 sec, were made of the cleaving eggs, and as cleavage took about 13 min, approximately 780 frames were exposed for each cleavage. Sequential 1 sec exposures of the completed negative film at normal running speed (24 frames/sec) were projected on to Agfa rapidoprint FPS1 paper. To provide a standard of reference, single frame exposures from each of the 24 frame groupings were also made.

By comparison of multiple exposures (M) and single shots (S), it was obvious that non-random movement in the cleaving egg could readily be detected (Figure 1) by the extended streaks made by moving sub-cellular par-

ticles, whereas areas of random movement were blurred. Areas exhibiting these streaks were represented on a line diagram (Figure 2), which was prepared by close examination of large prints of the multiple exposures and comparing these with the single shots, the areas of supposed movement being drawn on the diagram. The technique, therefore, provides a useful means for detecting sub-cellular movements that are discernable with the naked eye but difficult to record and quantify.

It would seem that the method is potentially versatile and capable of application to a variety of problems involving cell movements. A more detailed analysis of movement in cleaving eggs will be published in due course⁴.

Zusammenfassung. Eine Methode zur einfachen Quantifizierung von gerichteten Bewegungen subzellularer Teile, zum Beispiel in Furchungszellen, wurde entwickelt.

I. AP GWYNN and P. C. T. JONES

Department of Zoology, University College of Wales, Aberystwyth (Wales, UK), 4 May 1970.

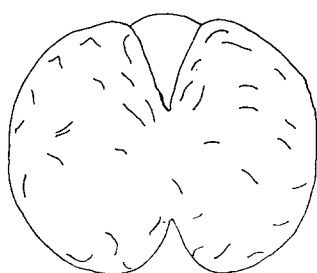


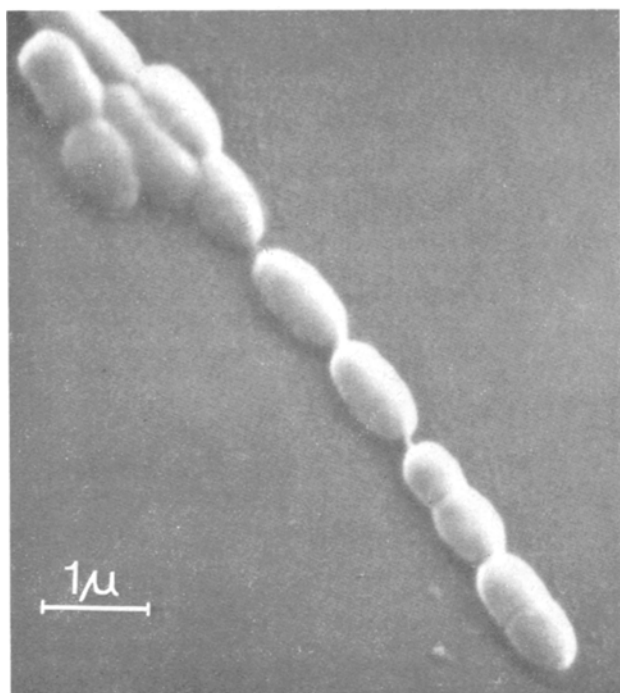
Fig. 2. Areas of supposed movement.

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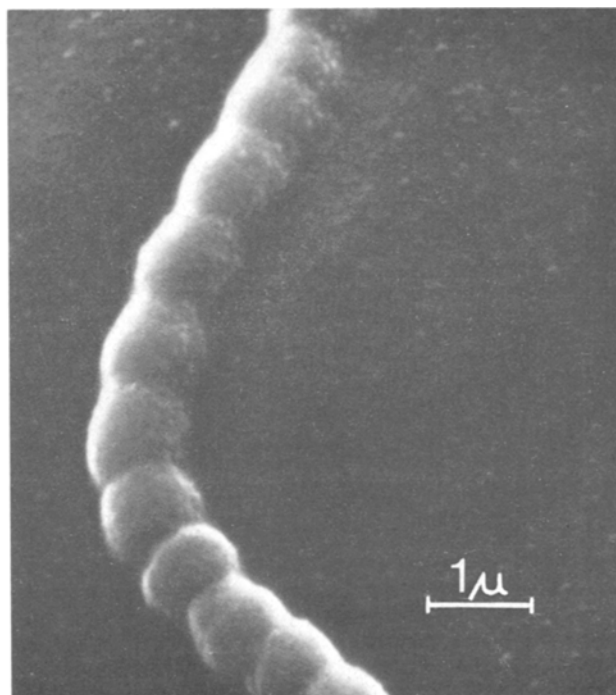
Scanning Electron Microscopy of the Antigen-Antibody Complex

The Scanning Electron Microscope (SEM) has had limited use in microbiology. The instrument has been commercially available since 1965, and its application to studies of bacterial topography have been few¹⁻⁵. The purpose of this study was to examine, using SEM, the antigen-antibody complex formed on the surface of a bacterial cell.

Streptococcus mutans K-1R was used as the antigen. The organism was obtained from the National Institutes of Health, Bethesda, Maryland. This oral streptococcus is of human origin, and has been shown to cause dental caries in experimental animals⁶. Antiserum to this organism was prepared in rabbits in our laboratory according to the method of KALONAROS and BAHN⁷. The K-1R



A. Unaltered cells of *Streptococcus mutans* K-1R. $\times 10,000$.



B. Antibody-complexed cells of *Streptococcus mutans* K-1R. $\times 10,000$.

organisms were grown at 37°C in Todd-Hewitt Broth (Difco), supplemented with 0.5% glucose, for 18 h in an atmosphere of 95% nitrogen and 5% carbon dioxide. The cells were harvested and washed 3 times in sterile 0.85% sodium chloride, and were then divided into 2 groups. One group was fixed in 1% glutaraldehyde and 5% sucrose for 16–18 h⁴. The remaining cells were mixed with the K-1R antiserum. A strong agglutination reaction occurred between the cells and antiserum. The agglutinated particles were then sedimented by centrifugation and fixed in glutaraldehyde and sucrose, as described above. Both groups of cells were washed twice in sterile deionized water to remove excess fixative. The samples were resuspended in water to a concentration of 1.0×10^5 cells per ml of water and transferred onto circular glass coverslips. The specimens were allowed to air dry and the coverslips were mounted on standard aluminium SEM specimen holders. The specimens were coated with a vaporized gold-palladium alloy prior to examination. A Cambridge, Mark II Stereoscan Electron Microscope (Cambridge Instruments Company Ltd., London, England) was used to view the cells. The specimens were examined at 15 to 18 kV at a magnification of $\times 10,000$ and a specimen angle of 45°. Polaroid photomicrographs were obtained to record the results.

The untreated K-1R organism is shown in Figure A. A chain of cells, typical of streptococci was noted. Their surface morphology appeared smooth with the exception of a transverse striation, or band, at the equator of some cells. Such equatorial bands are considered as active sites of cell wall synthesis^{8,9}. A thin intercellular connection was observed between 2 of the cells.

The antiserum agglutinated cells are shown in Figure B. The chain arrangement of cells was again noted. The cells appeared more closely positioned than the untreated K-1R cells. Also, the surface morphology was noticeably altered when compared to that of untreated cells. A flocculent, rough coating was observed adhering to the cell surface,

in contrast to the relatively smooth surface of the unreacted cells. This flocculent coating was considered to represent the specific immunoglobulin to the K-1R organism.

Although the immunological information reported here is not new, the application of high magnification and extended depth of field, as offered by the SEM, has improved optical viewing and resolution of the antigen-antibody complex^{10–12}.

Zusammenfassung. Elektronenmikroskopischer Beitrag für die Verwendung des Scanning-Mikroskops zur Darstellung von Antigen-Antikörper-Bindungen.

L. G. SIMONSON and I. L. SHKLAIR

US Naval Dental Research Institute, Microbiology Division, Great Lakes (Illinois 60088, USA), 27 April 1970.

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¹² The opinions or assertions contained herein are those of the authors and not to be construed as official or reflecting the views of the Department of the Navy or the Naval Service at large.