STUDIES ON CELL MEMBRANE ULTRASTRUCTURE, AND THE USE OF THE FREEZE-FRACTURING TECHNIQUE IN ELECTRON MICROSCOPY

by

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ABSTRACT

1) The Ultrastructural appearance of frozen-fractured mouse liver cell membranes, and consistent variations in the distribution of membrane-associated particles caused by various pretreatments as described by previous investigators, were confirmed in this study.

2) Freeze fracturing, was evaluated as a method for studying the ultrastructure of single celled microorganisms. Large prokaryotes with previously undescribed morphologies were investigated using freeze fracturing, thin sectioning, and negative staining techniques.

3) Structures thought to represent flagellar attachment sites were demonstrated in frozen fractured bacterial preparations for the first time.

4) A previously undescribed level of cell organization was discovered in a rumen organism classified morphologically as Selenomonas.

5) A technique was devised to retrieve replicas from both sides of the fracture of a single frozen-fradured specimen. The results obtained by its use support the theory that frozen cell membranes fracture along some interior plane rather than at the membrane-cytoplasm boundary. The technique also showed that the particles seen on frozen fractured membranes lie within the thickness of the membrane.

6) For determination of the third dimension in freeze fracture replicas, i.e. the heights of various features, an alternative method to stereoscopy was devised. This method, which can be made semiautomatic in execution, involves microdensitometry of electron micrograph negatives and certain mathematical manipulation of the optical density data.

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LIST OF FIGURES

amplification of one thousand the output E_o is read on the ten volt scale of a volt-ohm meter. The circuit includes an integrated circuit operational amplifier, represented by the triangle on the diagram. The circuit contained within the operational amplifier is shown in the lower diagram.

Fig 1.4 pg. 19

Basic steps in the freeze-fracturing procedure - I In liquid nitrogen at atmospheric pressure.

A) Specimen holder with specimen is placed into the hole of the bottom brass block.

B) Specimen is fractured with a cold blade.

C) The parts of the cold block are assembled under the liquid nitrogen.

Fig 1.5 pg. 19

Basic steps in the freeze-fracturing procedure - II In the vacuum evaporator.

A) Cold-block in pot is correctly oriented with respect to the two evaporant sources. A crane is attached to the lid.

B) When high vacuum is achieved, the lid is lifted, and platinum/carbon, then carbon, is evaporated on to the fracture face of the specimen via the shadowing tunnels.

Fig 1.6 pg. 28

An integrating circuit, with adjustable leak, as used in preliminary experiments on recovering profile heights from electron micrographs of freeze-fracture replicas. The dashed line connected to one potentiometer represents a mechanical attachment to the recording pen of the microdensitometer used. The output from the circuit, E_o, was fed to a variable speed chart recorder.

Fig 2.1 pg. 36

Two diagrams of liver cell histology and cytology taken from Bloom & Fawcett (1968).

A) The histological structure of liver tissue.

B) A liver cell with typical ultrastructure. The plasma membrane specializations considered here, occur in the vicinity of the bile canaliculus.

Fig 2.2 pg. 65

a) An interpretation of the structure of the tight junction, based on the theory that freeze-fracturing reveals true membrane surfaces. (Adapted from Staehelin et al. 1969).

b) An interpretation of the structure of the gap junction, based on the same theory as a). m_1, m_2 represent apposed plasma membranes. c_1, c_2 represent cytoplasm.

Fig 2.3 pg. 47

Four graphs relating bell-jar pressure and cold-block temperature with time, for four different freeze fracture runs.

Fig 3.1 pg. 99

Interpretation of the fracturing behaviour of Quin's Oval cell membranes on the assumption that true membrane surfaces are revealed by fracturing.

Fig 3.2 pg. 99

Alternative interpretation of the Q.O. cell membrane fracture planes on the assumption that the membrane is split internally by a unique fracture plane. The two, distinct (+) and (-) fracture faces are thus seen to be mating faces.

Fig 3.3 pg. 107

An interpretation of the ultrastructure of the peripheral layers of a Q.O. cell. A portion of the specialized flagella region and flanking "polar" membrane is shown.

Fig 3.4 pg. 107

An interpretation of the ultrastructure of the peripheral layers of a Selenomonas cell. The flagellar sac (with attached flagella) is shown.

Fig 4.1 pg. 119

The modified freeze-fracture specimen holder. Normal holders were split lengthwise, and semicircular "wings" were added to simplify handling.

Fig. 4.2 pg. 119

Modified holder in the end-to-end configuration. The specimen to be fractured lies across the joint between the two halves. After freezing, the specimen and surrounding ice cement the halves together.

Fig. 4.3 pg. 119

Modified specimen holder shown assembled in the bottom cold block immediately after fracturing. Previously mating halves of the fractured specimen are both oriented uppermost and lie side by side.

Fig 4.4 pg. 121

a) Folding line of a book.

b) Folding line for two fracture faces in the modified holder.

Fig 4.5 pg. 121

a) Relationship of the modified holder to the shadowing source - side view.

b) and c) Relationship of the modified holder to the shadowing source - plan view.

In b) the specimen holder is in orientation "A", whereas in c) the holder is in orientation "B".

Fig 4.6 pg. 121

Profile view of shadowed fracture faces along the direction of shadowing. (The same point on both faces is represented by X). In orientation "A", (above), matching features receive the same quantity of shadowing material, however, this is not normally true when orientation "B" is used.

Fig 4.7 pg. 137

Model illustrating the hypothetical collapse of membrane lipid tails during freeze fracturing explaining the usual lack of particle depressions.

a) The possible structure of an unfractured membrane.

b) Two "matching" membrane faces after fracturing.

Fig 4.8 pg. 139

a) Interpretation of the liver cell plasma membrane gap-junction, based on evidence from matched fracture faces. The model represents an imaginary thin section across the membrane region corresponding to lines L - L' in plate 49.

b) An interpretation of the tight junction based on evidence from matched fracture faces. The model represents an imaginary thin section across the membrane at right-angles to the tight junction ridges. It can be compared with line L - L' on plate 8.

Fig 5.1 pg. 148

Profiles reconstructed from the densitometer trace of plate 52 and fig 5.4, using the analogue circuit shown in fig 1.6

Fig 5.2 pg 149

Curve A is the computed profile using the erroneous theory described on page 149 and the density data from plate 52 and fig 5.4. Curves (1) (2) and (3) are computed from the same density data using the program on page 164. Because an incorrect value was used for D_{min} in curve (1), it appears unnaturally steep in some regions.

Fig 5.3 pg. 159

A hypothetical region of the platinum/carbon replica of a fracture face together with the corresponding optical density profile of the electron microscope negative for corresponding points along the X-axis.

Fig 5.4 pg. 163

Optical density trace across the E.M. negative along the line marked in Plate 52. The vertical O.D. axis here shows the values used in computing curves 2) and 3) of fig 5.2, (D_{min} = 0, D_{max} = 230, D_{hor} was taken to be 125, 110, for curve 1) D_{min} = 45 D_{max} = 230, D_{hor} = 100).

Fig 5.5 pg. 168

Microdensitometer traces taken across coverslips positioned at the level of a fractured specimen in the brass block and coated with carbon or platinum/carbon, as in a freeze-fracture run. Curves 1) and 2) are of the carbon coating in two traces taken at right angles to each other. Curves 3) and 4) are of the platinum/carbon coating, at right angles, and parallel to the shadowing direction respectively. Variation in coating thickness would be virtually negligible across a normal replica.

Magnification x 10

Fig 5.6

a) Calculated graph showing variation of optical density D, on the negative with θ, the inclination of the fracture face for a range of values for m_1

b) Data as in 5.6a, but the graph axes have been rearranged to show the dependence of D on m_1 for several values of θ. pg. 177

The graphs are calculated from the relationship

$$D = e^{-m_1} \cdot D_{max} \text{ , where } m_1 = \frac{-at_1}{\sqrt{2}} \cdot (\tan\theta + 1).$$