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MOIRÉ PATTERNS IN ELECTRON MICROGRAPHS OF A BACTERIAL MEMBRANE

AUDREY M. GLAUERT
Strangeways Research Laboratory, Cambridge

SUMMARY

A regular hexagonal array of morphological units is observed in negatively stained preparations of a membrane from the surface of the spherical bacterium *Micrococcus radiodurans*. Fragments of this membrane are frequently folded so that structural details from two or more layers are superimposed. The appearance of the resulting moiré patterns is illustrated and the need to be aware of the possibility of the formation of these patterns in similar biological specimens is stressed.

INTRODUCTION

Recent studies on the fine structure of the radiation-resistant bacterium *Micrococcus radiodurans* by electron microscopy of negatively stained preparations (Glauert, 1962; Murray, 1962; Thornley, Horne & Glauert, 1965) have shown that a well-defined pattern of hexagonally arranged units is present in one of the surface layers. Work & Griffiths (private communication) have successfully separated this patterned layer from the other layers of the surface and have found it to consist of carotenoids, lipids, carbohydrates and proteins. About 25% of the layer is extracted by lipid solvents, suggesting that it contains sufficient lipid to act as a permeability barrier and to be classified as a membrane. The exact position of the membrane in intact cells is still in doubt since the hexagonal pattern has been observed only in preparations of isolated 'cell walls', but it is generally agreed that it is outside the rigid, mucopeptide-containing layer of the wall (Thornley *et al.*, 1965; Work & Griffiths, private communication).

A layer with a similar hexagonal pattern is also found in isolated cell walls of the Gram-negative bacterium *Spirillum serpens* and has been described in detail by Murray (1963). This description was simplified by the fact that the fragments of patterned material tended to be flat and unfolded so that no confusion was introduced due to the superposition of patterns from two or more layers. In contrast, the fragments of membrane from *M. radiodurans* are frequently folded so that moiré patterns are often observed.

METHODS

Details of the isolation and growth of *M. radiodurans* are described by Thornley *et al.* (1965). These authors studied the fine structure of the surface layers of the bacterium in negatively stained preparations of disrupted cells. After breakage with a

Mickle disintegrator the four different structured layers of the bacterial surface ('grape-skin', 'compartment', 'holey' and 'hexagonal' layers) were all visible. Smaller fragments were obtained by subsequent treatment with ultrasonic vibrations. Attempts were then made to separate the four structured layers by centrifugation, sucrose density and electrophoresis without success. One of the preparations of small cell-wall fragments in 0·1 M TRIS (tris(hydroxymethyl)-aminomethane-HCl) buffer containing 0·001 M EDTA (ethylene-diamine-tetraacetic acid, disodium salt) was stored at 5 °C. After 2½ years it was noticed (Thornley & Glauert, unpublished observations) that a greatly increased proportion of the preparation consisted of the 'hexagonal layer', and that the 'holey' and 'compartment' layers had disappeared.

A few millilitres of this suspension were mixed with an equal volume of the negative-staining solution (2 % potassium phosphotungstate or 2 % ammonium molybdate) and a small drop of the mixture was placed on the collodion-carbon support film on an electron microscope grid. Excess fluid was removed with filter paper and the specimen allowed to dry.

Electron micrographs were taken with a Siemens Elmiskop I electron microscope operating at 60 kV with a 50-μ objective aperture.

The diagrams to illustrate the formation of the moiré patterns were made photographically. A simplified version of the basic, one-layer pattern observed in the electron micrographs was first made by drawing a hexagonal array of circles on a piece of white card and then inking in the background. This pattern can be seen at the corners of the diagram in Fig. 2 B. The ratio of the diameter of a white circle to the centre-to-centre distance between adjacent circles was chosen to be the same as in the micrographs. The diagram of the basic pattern was then copied on to a number of quarter-plate lantern slides so that a series of identical negatives was obtained. The moiré patterns were formed by placing two of these negatives one on top of the other in the enlarger, with emulsion touching emulsion. The angle and position of the plates relative to each other were adjusted until the moiré patterns produced in the enlarger were similar to those observed in the electron micrographs. Prints were then made of these moiré patterns.

OBSERVATIONS

The basic, one-layer pattern of the membrane is visible in the upper, left-hand region of Fig. 1; it appears as a hexagonal array of 'pegs' with a centre-to-centre spacing of about 175 Å. The membranous sheet is folded back on itself so that it is double in the remainder of the picture. The fold is irregular and consequently the angle between the two superposed layers changes from one area to another with an accompanying alteration in the resulting moiré pattern. Two of these moiré patterns are shown at higher magnification in Figs. 2 A and 3 A, and their formation is illustrated diagrammatically in Figs. 2 B and 3 B.

In some fragments of the membrane little of the basic pattern is visible (Fig. 4) and most of the surface is covered with moiré patterns (Figs. 5 A, 6 A) formed by the superposition of detail from two or more layers. This tendency of the layer to fold has caused some confusion in the interpretation of the patterns and led Murray (1962), who was the first to recognize the existence of superposed patterns in this membrane, to suggest that a double layer of hexagonally arranged pegs is always present in the cell wall. It seems more likely, however, that the basic pattern is a simple hexagonal array and that moiré patterns are observed in isolated fragments as a result of the tendency of the layer to fold.

DISCUSSION

The negative-staining technique is being used increasingly in the study of the fine structure of biological membranes, and regular arrays of subunits have already been observed in various membranes, including the outer envelopes of a spirochete (Listgarten & Socransky, 1964), the exosporium of *Bacillus* spores (Gerhardt & Ribi, 1964), the membranes of bacterial chromatophores (Holt & Marr, 1965) and the membranes of outer segments of retinal receptors (Blasie, Dewey, Blaurock & Worthington, 1965). Moiré patterns may easily arise in such preparations when fragments of membranes are isolated and, as the diagrams in this paper illustrate, may give a very misleading impression of the morphology of the structural components. It is particularly fortunate that the membrane from *M. radiodurans* displays both one- and two-layer patterns in the same fragments, so that the interpretation of the basic pattern is clear.

In other biological specimens, such as the polyheads of bacteriophage T4, two superposed patterns are almost always seen since both upper and lower surfaces of the particle are contrasted by the negative stain (Finch, Klug & Stretton, 1964). A study of the observed moiré patterns enabled Kellenberger & Boy de la Tour (1965) to deduce information about the geometry of the lattice cell and to confirm the conclusions reached earlier by Finch *et al.* (1964) using an optical diffraction technique (Klug & Berger, 1964) There have been some difficulties, however, in deciding upon the exact distribution of density in the surface lattice (Klug, Finch, Leberman & Longley, 1966).

In the interpretation of electron micrographs of negatively stained membranes the possibility that moiré patterns may be formed should always be borne in mind.

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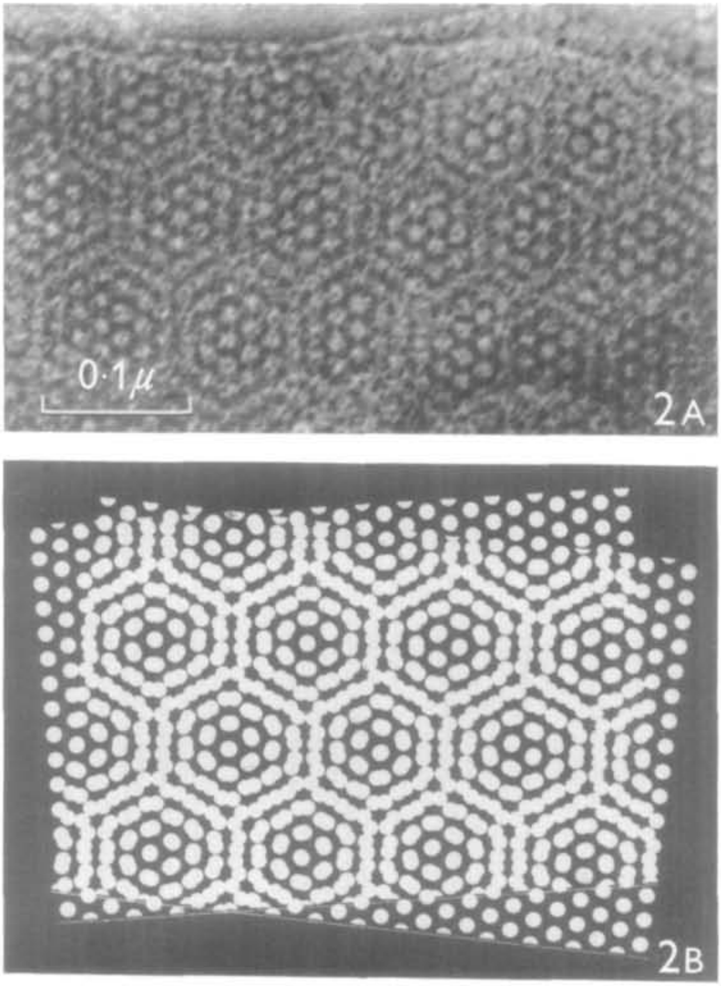
Fig. 1. Part of a membrane from *M. radiodurans* negatively stained with ammonium molybdate. The basic, hexagonal pattern is visible in the upper left-hand region. The opposite edge of the membrane is folded and moiré patterns are seen. × 150000.


0·1μ
1

Fig. 2A. Enlargement of a moiré pattern from Fig. 1. × 200000. Fig. 2B. Diagram of the moiré pattern in Fig. 2A. This diagram was formed by overlapping two identical hexagonal arrays of white 'dots'. The one-layer pattern is visible at the corners of the diagram.

Fig. 3A. Enlargement of a moiré pattern from Fig. 1; scale = 0·1 μ. × 200000. Fig. 3B. Diagram of the moiré pattern in Fig. 3A.


0·1μ
2A
2B

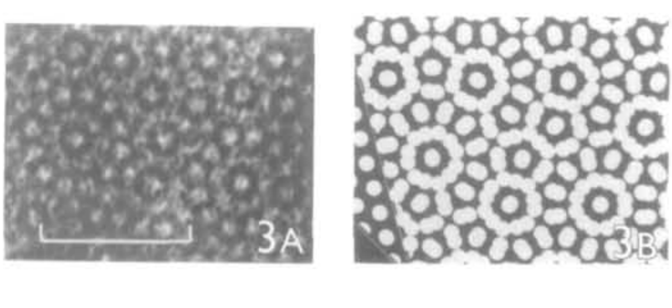
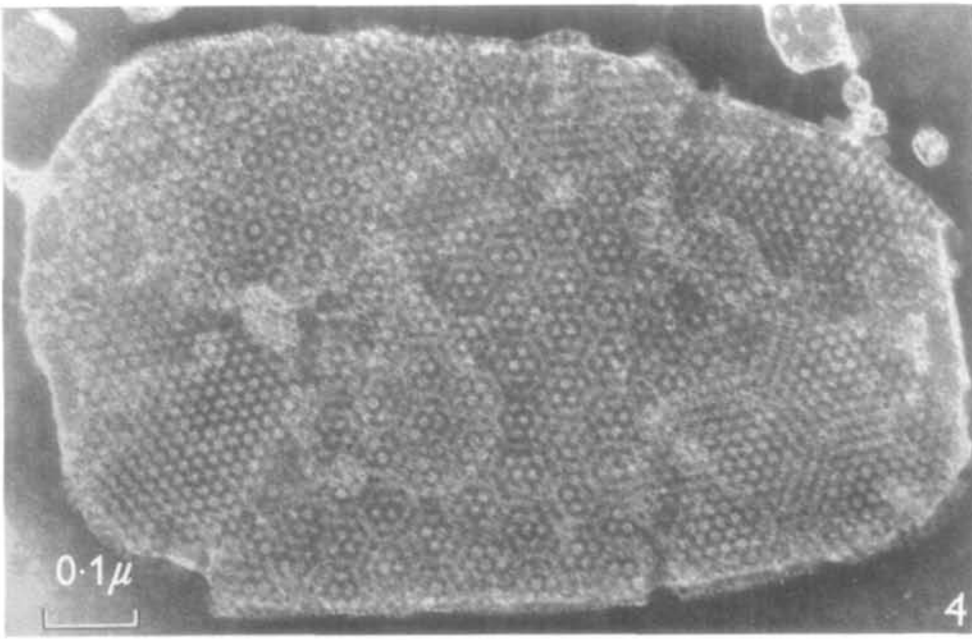
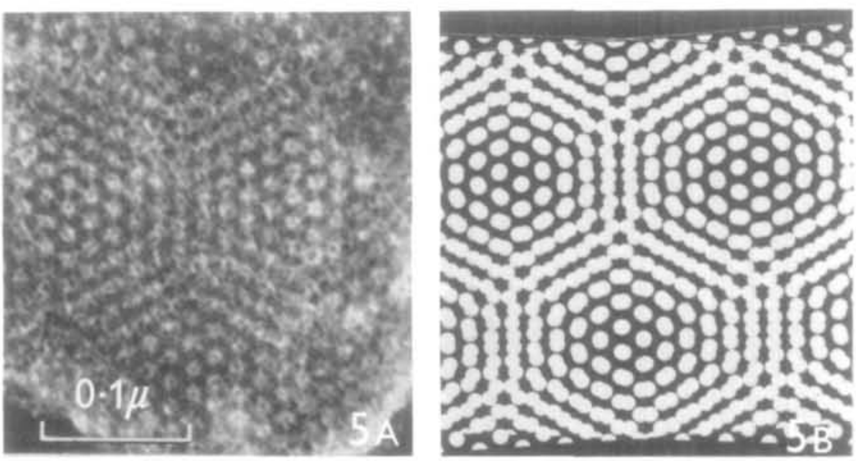

3A
3B

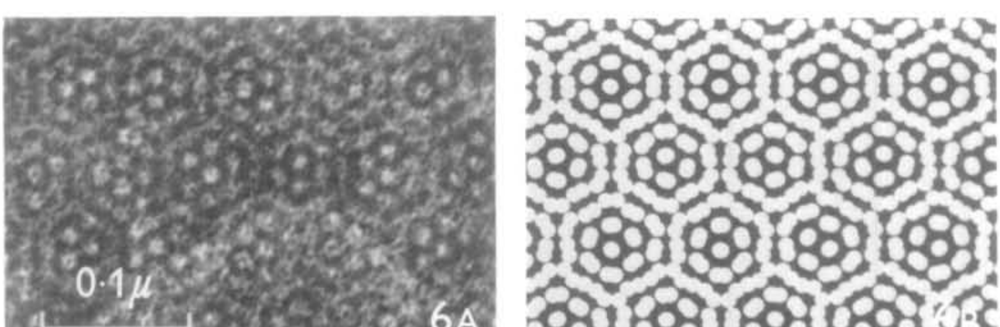
Fig. 4. Fragment of membrane from *M. radiodurans* negatively stained with potassium phosphotungstate. Most of the surface is covered with moiré patterns. × 120000.

Fig. 5A. Enlargement of a moiré pattern from Fig. 4. × 200000. Fig. 5B. Diagram of the moiré pattern in Fig. 5A.

Fig. 6A. Enlargement of a moiré pattern from Fig. 4. × 200000. Fig. 6B. Diagram of the moiré pattern in Fig. 6A.


0·1 μ
4


0·1 μ
5A
5B


0·1 μ
6A
6B