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Formation and Structure of Mesosomes in Myxococcus xanthus

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With 9 Figures in the Text

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"Chondrioids" (RYTER and KELLENBERGER 1958), "mesosomes" (FITZ-JAMES 1960) or "plasmalemmosomes" (EDWARDS and STEVENS 1963) seem to be ubiquitous in bacterial species. In Gram-negative bacteria mesosomes (this term will be used throughout this paper) have been demonstrated in *Spirillum serpens* (MURRAY 1960/61), *Escherichia coli* (KAYE and CHAPMAN 1963; VANDERWINKEL and MURRAY 1962), *Neisseria gonorrhoeae* (FITZ-JAMES 1964), *Moraxella* (RYTER and PIÉCHAUD 1963), *Treponema* (RYTER and PILLOT 1963), *Fusobacterium* (TAKAGI et al. 1963) and *Caulobacter* (STOVE POINDEXTER 1964). They seem to be formed under unfavorable growth conditions (MURRAY 1962), though no pertinent data have been reported as to the physiological factors leading to the formation of mesosomes. In contrast to Gram-positive bacteria, mesosomes in Gram-negative species do not seem to occur regularly near the division plane (FITZ-JAMES 1964). The function of mesosomes has been related to cell wall resynthesis in L-forms (RYTER and LANDMAN 1964), to septa formation during sporogenesis (FITZ-JAMES 1960; OYHE and MURRELL 1962), to cell division (CHAPMAN and HILLIER 1953) and to partition of nucleoids (RYTER and JACOB 1964). Large mesosomes and "lamellar bodies" have been reported for germinating *Azotobacter* cysts (TCHAN et al. 1962). The function of the "lamellar bodies" has been ascribed to the production of enzymes for the dissolution of the cyst coat and exosporium during cyst germination. It is worth noting that the authors made a distinction between the mesosome as an intrusion of the cytoplasmic membrane and the "lamellar bodies", both located in the apical part of the germinating cyst. No explanation was given as to the origin of the "lamellar bodies", nor was a typical mesosomal structure discernible for these organelles.

Mesosomes have not been previously observed in the myxobacteria; however, it has been shown (VOELZ and DWORKIN 1962) that during microcyst formation by the fruiting strain FB of *Myxococcus xanthus*

invaginations of the cytoplasmic membrane appear to form membrane-bound vacuoles. These invaginations were denoted as peripheral bodies though being functionally different from those described by CHAPMAN and HILLIER (1953) for *Bacillus cereus*. They were tentatively correlated to the process of rounding up of the vegetative cells prior to completion of the formation of the microcysts. This report concerns the formation of mesosomes in *M. xanthus* and its physiological basis.

Material and Methods

Organism and media. The fruiting strain FB of *M. xanthus* was used in all experiments. The strain has been maintained for more than 3 years on casitone agar. The casitone medium, exclusively used in the experiments, as well as the medium and method for spheroplast formation was described by DWORKIN and VOELZ (1962). Casitone as a liquid medium is referred to as CT-broth; casitone plus 2% agar is referred to as CT-agar. Dispersed growth in CT-broth was measured with a Klett-Summerson colorimeter using a No. 54 filter. Cell counts were made on CT-agar and generation time was determined in the conventional manner.

Electron microscopy. Specimens for thin sectioning were prepared by the method of RYTER and KELLENBERGER (1958) using Epon 812 for embedding. For glutaraldehyde fixation, cells were suspended in 2% glutaraldehyde in Ryter-Kellenberger buffer for 10 min at room temperature, washed 5 times in buffer, treated with buffered 1% OsO_4 for 30 min and processed for electron microscopy by the method mentioned above. Thin sections were observed under a RCA EMU-3G electron microscope at 100 KV.

Physiological experiments. For dispersed growth cells were grown in 1000-ml Erlenmeyer flasks containing 500 ml of CT-broth medium. The cultures were aerated by bubbling with various concentrations of O_2 in mixtures of N_2 plus 5% CO_2 using Berkefeld filter candles as described by Moss (1956). The O_2-concentration in the medium was determined with a Beckman oxygen sensor attached to a Beckman pH meter with extended scale. Aeration pressure was maintained at 50 mm. The oxygen concentration in the medium was regulated by adjustment of the oxygen cylinder valve according to control measurements with the oxygen sensor. Anions and cations to be tested were added to casitone broth in concentrations of 0.02 and 0.2 M.

Results and Discussion

1. Formation and regression of mesosomes

When *M. xanthus* was maintained on CT-agar as vegetative cells, i.e., without passing through a resting stage (microcysts), numerous mesosomes were found to be widely distributed throughout the whole cell. They were relatively small and their sites were not confined to the division plane (Fig. 1). When transferred into CT-broth, the cells began to divide more rapidly than on agar with a generation time of 3.5 hrs (DWORKIN 1962). After transfer of mesosomes-containing cells from colonies into CT-broth and incubating at 28°C with aeration the first cell division occurred in about 10 hrs. During this time the mesosomes decreased in number and size. During the first or second cell division after transfer into CT-broth, mesosomes were formed at the division plane (Fig. 2). These

membranous structures were large and more complex than those seen in cells continuously grown on CT-agar. After a second transfer into fresh CT-broth, no mesosomes were observed after six generations and division

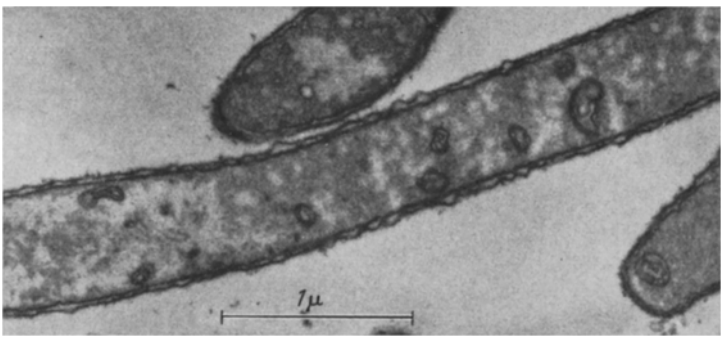

Fig. 1. Thin section of vegetative cells of *Myxococcus xanthus* grown on CT-agar containing mesosomes dispersed throughout the cells. ×24600

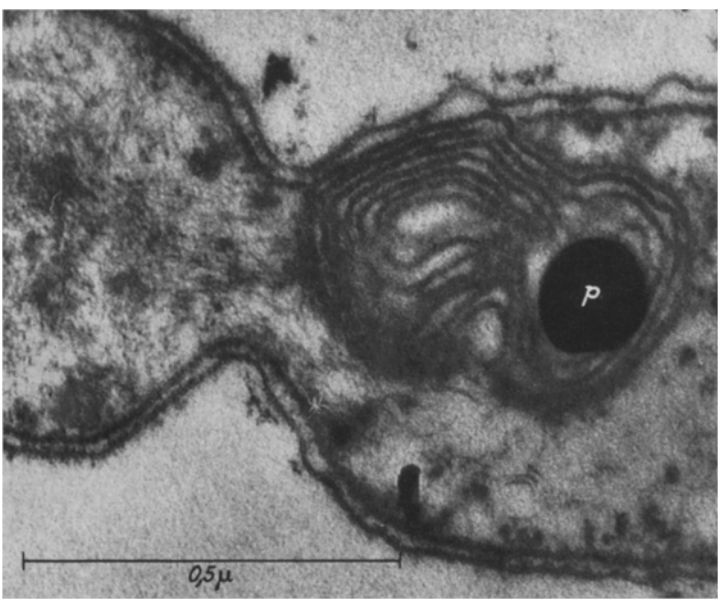

Fig. 2. Large mesosome formed at the division plane after transfer of cells into CT liquid. Poly-phosphate granule (*p*) enclosed by the mesosome. × 100000

was by constriction without forming any detectable membranous folds, particulate cytoplasmic organelles or inclusions (Fig. 3). Upon transfer of cells, lacking mesosomes, onto CT-agar small mesosomes and short infoldings of the cytoplasmic membrane occurred at the division plane during the first three or four cell divisions (Fig. 5). The cells pile up and

produce considerable amounts of slime while moving over the agar surface by a gliding motion. Sections through these cells revealed numerous small mesosomes which were at no particular area but were dispersed within the cytoplasm as seen in Fig. 1. The exact number of cell generations necessary for the acquisition of mesosomes which appeared after growth on CT-agar could not be determined.

2. Physiological conditions for the formation of mesosomes

From the observations on the formation of new mesosomes described in the previous section, one may presume that invaginations of the cytoplasmic membrane and consequently the formation of large mesosomes in *M. xanthus* grown in CT-broth were a reaction of the cell to a change in growth conditions. Assuming that the bulk of respiratory enzymes are located in the cell envelopes (literature reviewed by SALTON 1964), a rapid increase of oxygen in the medium may indirectly influence growth and subsequently infolding of the cytoplasmic membrane, thereby enlarging the respiratory surface. Thus, in case of formation of mesosomes by depletion or rapid increase of oxygen in the medium, the formation of this cytoplasmic organelle may rather be a transient requisite of a functional unit than a congenital structure comparable with the mitochondria of higher cells as was suggested by MUDD et al. (1951) and others; see also MURRAY (1962). This would not exclude the possibility that mesosomes

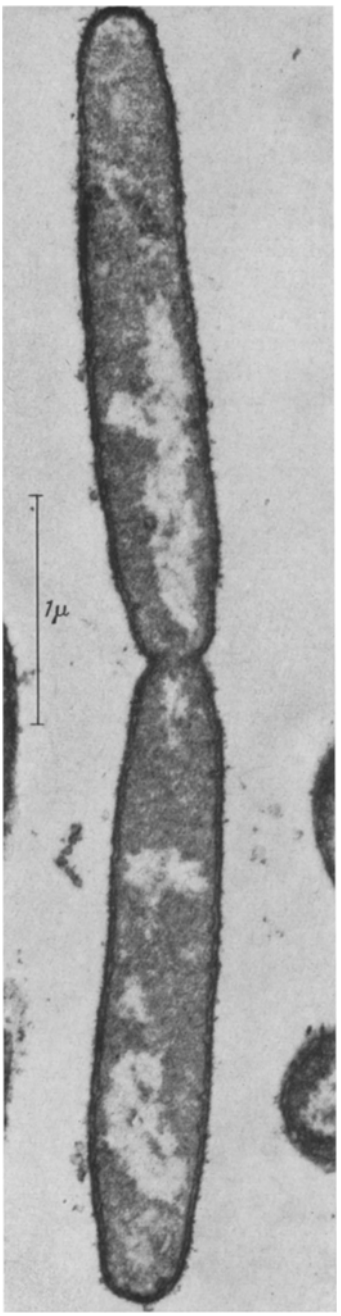

Fig. 3. Vegetative cells during cell division after growth in CT-liquid for more than 6 generations. × 30000

under certain conditions may assume a specific function, e.g., harboring the majority of the redox-system of the cell (VANDERWINKEL and MURRAY 1962; TAKAGI et al. 1963; VAN ITERSON and LEENE 1964a, b). Apart from the valuable observations on *S. serpens* by VANDERWINKEL and MURRAY (1962), no pertinent data are available as to the factors controlling the formation of mesosomes. We have, therefore, attempted to define the physiological conditions which initiate the formation of mesosomes or infoldings of the cytoplasmic membrane in *M. xanthus*.

In preliminary experiments it was noticed that mesosomes were formed when cells, containing no mesosomes at the beginning of the experiment, were grown in CT-broth without aeration. These cells, however, settled to the bottom of the culture flasks and died very quickly. An estimate of their growth rate was, therefore, impossible. In experiments with dispersed growth, cells were maintained in CT-broth with aeration at 28° C until mesosomes were no longer detectable in thin sections. A mixture of O_2 and N_2 plus 5% CO_2 at various O_2-concentrations was bubbled through cultures with a cell density of 350—400 Klett units. The cell density was measured hourly. Samples were taken every 3 hrs and processed for thin sectioning. When the O_2-concentration in the medium was lowered to $5 \cdot 10^{-5}$ M the cell generation time increased to 15 hrs compared with 3.5 hrs under optimal growth conditions. Small mesosomes and invaginations of the cytoplasmic membrane appeared in thin sections of most of the cells after the cells were subjected to a low O_2-concentration as of 10^{-4} M at an approximate cell density of 450 Klett units for 6—9 hrs. Growth came to a complete stop when the O_2-concentration was lowered below 10^{-5} M. A gradual increase of the O_2-concentration in these cultures caused linear increase of the growth rate proportional to the increase of O_2-concentration in the medium. Mesosomes were, however, still present and located at no particular area within the cell. An increase of O_2 did not result in a formation of new mesosomes near the division plane nor did continued aeration eliminate the mesosomes. After transfer of these cells into a fresh medium with optimal aeration, mesosomes gradually disappeared.

These results as well as the observation that mesosomes were temporarily formed at the division plane after transfer of cells, harboring mesosomes, from CT-agar into CT-broth (section 1, p. 61) suggest that a low oxygen concentration in the medium may have induced the formation of a factor responsible for the formation of mesosomes. This postulated factor, or chain of factors, once formed by the cell could presumably not be eliminated by an increase of oxygen in the medium. Only a transfer of cells into fresh medium with optimal aeration established growth conditions under which mesosomes disappeared. It follows that this factor was secreted into the medium when oxygen concentration was low. In

order to see whether altered physiological conditions would enhance or suppress the effect of this factor, growth conditions were varied in cultures of cells with or without mesosomes.

Cations such as Na^+, K^+, Mg^{++}, as chlorides or sulfates, or anions such as Cl^-, SO_4^{--}, PO_4^{---} added to the medium caused no stimulatory or inhibitory effect on mesosome formation at any oxygen level. Temperatures above 32°C resulted in autolysis of cells at an O_2-concentration of $5 \cdot 10^{-5}$ M but caused chain formation of cells with or without mesosomes at optimal aeration. Temperatures below 23° C further lowered the growth rate without any detectable influence on mesosome formation. Within the pH range of 6.0—8.0, only the growth rate varied. It decreased at pH lower than 7.0 but no particular change was noticed above pH 7.0. Neither formation nor regression of mesosomes occurred under these conditions. Further studies will be concerned with the identification of the factor which stimulates ingrowth of the cytoplasmic membrane.

3. Structure of mesosomes

The mesosomes of *M. xanthus* are multi-layered and intrude into the cytoplasm or nucleoids. No portion of either one, however, seems to become engulfed by the folds (Fig. 4 a and b). The membranes constituting a mesosome were found to vary in their fine structure. Fig. 6 shows a mesosome in a cell taken from one of the first five or six generations after transfer from CT-broth onto CT-agar. In contrast to the triple-layered mesosomes shown in Figs. 2, 4 a, 4 b, 5 and 7, the single membranes of this mesosome were composed of five layers. Three dense lines of the same width were found to alternate with two translucent layers (Fig. 6, arrows). Two triple-layered membranes seem to have merged during infolding, though one could expect a central dense line twice as wide as the outer ones. The width of the membranes which constitute the mesosomes was approximately 120 A. This is, however, not in accord with our assumption that two portions of the cytoplasmic membrane have merged, since one could then expect a width of the mesosomal membrane of 160 A [80 A as being the width of the cytoplasmic membrane of *M. xanthus* VOELZ and DWORKIN (1962)]. High metabolic activity near the mesosome in Fig. 2 may be responsible for the formation of a large poly-phosphate granule found to be enclosed by the mesosome. (The formation of poly-phosphate granules in *M. xanthus* and their identification will be reported elsewhere). Mesosomes are not known to function in the accumulation of reserve material, however, there exists a striking similarity between the formation of this granule in a mesosome and the occurrence of calcium phosphate granules in mitochondria (LEHNINGER 1964). It would be of interest to see whether the mesosomes have a specific function in this regard comparable to that of mitochondria.

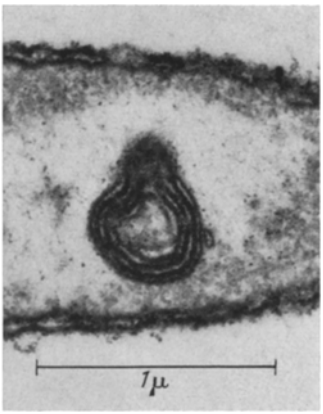

Fig. 4a

Fig. 4a. Oblique section through a spherical mesosome located in the nucleoid. × 64000

Fig. 4b. Cross section through a mesosome located in the cytoplasm. × 50000

Fig. 5. Vegetative cell after retransfer onto CT agar. Short infoldings of the cytoplasmic membrane and small mesosomes at the division plane. × 60000

Fig. 6. Mesosome consisting of 5-layered membranes (arrows). × 170000

Fig. 7. Cytoplasm invaded by mesosomes. Notice shape of mesosomes at border between cytoplasm and nucleoids. × 70000

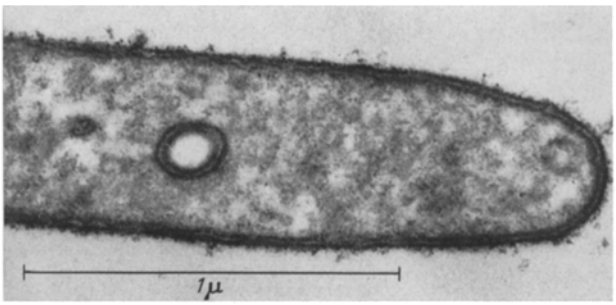

Fig. 4b

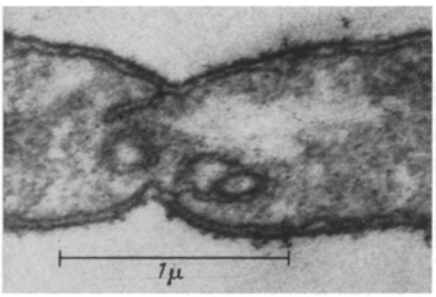

Fig. 5

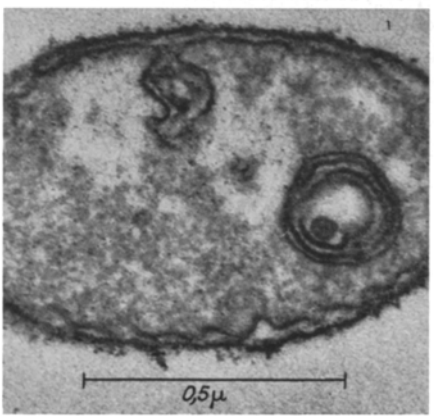

Fig. 7

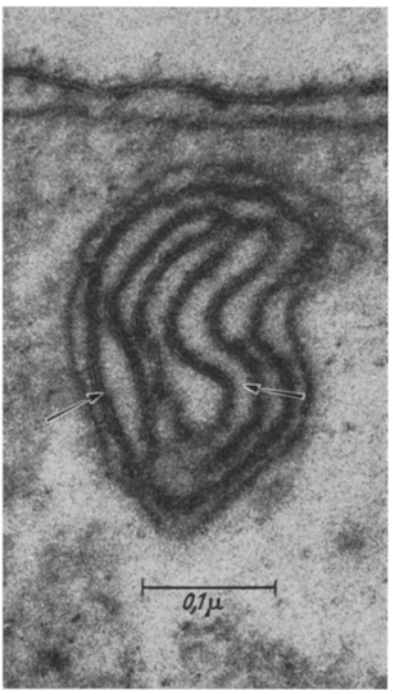

Fig. 6

One may discern two types of organelles in *M. xanthus* both resembling mesosomes but structurally, physiologically and possibly functionally different. There is evidence that the mesosomes of cells from colonies and those of anaerobically-grown-liquid cells differ in: a) the appearance of granules in one type, but not the other, though grown in the same medium; b) different number of layers in the two types. Structure and function of mesosomes apparently change with the physiological state of the cell.

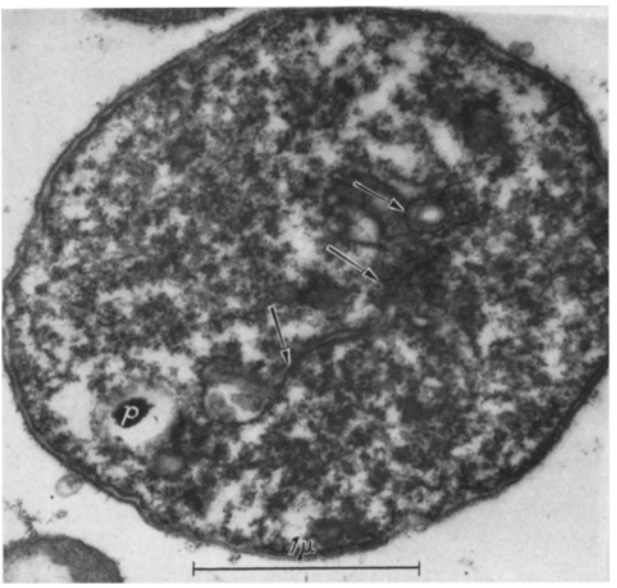

Fig. 8. Spheroplast of *M. xanthus* with unrolled mesosome (arrows) and poly-phosphate granule (*p*). × 30000

The infoldings of the cytoplasmic membrane, forming a mesosomal structure, constitute a more or less spherical figure provided the cytoplasm or nucleoid into which they intrude is homogeneous. Homogeneity of the nuclear material is presumably responsible for the shape of the spherical mesosome in Fig. 4a and the homogeneous cytoplasm for the spherical mesosome in Fig. 4b. Heterogeneity between cytoplasm and nuclear material is demonstrated in Fig. 7. It shows a spherical mesosome located in the cytoplasm with one edge bordering the nuclear region and thereby slightly compressed at the bordering side. A second mesosome located in the cytoplasm is entrenched between two nucleoids which account for its shape. One may assume that the shape of a mesosome is affected by the physical state of the cyto- and nucleoplasm rather than by autonomous growth of the mesosome forming a predetermined structure. Further proof for the plasticity of mesosomes could be derived from

deformed mesosomes in spheroplasts. Fig. 8 shows a section through a spheroplast with a large, partially unrolled mesosome stretching almost throughout the whole cell.

The question whether the formation of mesosomes is restricted to a certain area of the cell, e.g., the division plane, cannot easily be answered. Based on the studies of COTA-ROBLES (1963), one may assume that an area of a less intimate association between cell wall and cytoplasmic membrane may be prerequisite for an ingrowth of the cytoplasmic

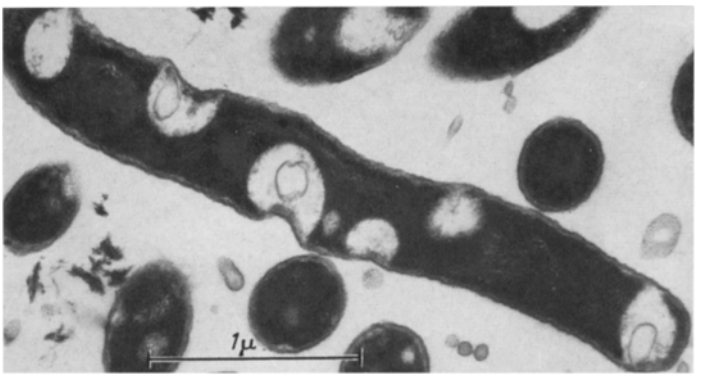

Fig. 9. Plasmolyzed cells of *M. xanthus*. Glutaraldehyde fixation. Indented cell wall at plasmolyzed zones points to a lack of a rigid layer. × 28200

membrane. In order to define these areas, cells not containing any mesosomes or infoldings of the cytoplasmic membrane were plasmolysed during their log phase. Cells were grown in CT-broth with optimal aeration for more than six generations. Samples were briefly washed in distilled water and subjected to 0.5 M sucrose in 0.01 M phosphate buffer, pH 7.2 for 10 min. The cells then were fixed in glutaraldehyde followed by osmium tetroxide treatment and processed for electron microscopy. The time for plasmolysis was critical. A prolonged exposure of cells to sucrose resulted in an undesired complete plasmolysis. In order to achieve plasmolysis it was necessary to wash the cells in distilled water prior to treatment with sucrose, and fixation in an aldehyde. The electron micrograph (Fig. 9) of a longitudinal section through a partially plasmolyzed cell demonstrates the location of plasmolyzed zones typical of the majority of the population under the same conditions. The plasmolyzable zones appeared not to be confined to certain areas in the cell, though less resistant zones could be found at the tips of the cells, and in the zone predetermined for cell division. Provided that this assumption is correct, it then appears likely that under certain growth conditions, the ingrowth of the cytoplasmic membrane may take place at any area in the cell preferably at the division plane.

The process of ingrowth of the cytoplasmic membrane leading to the formation of mesosomes is certainly of a very complex nature. These investigations may thus be considered only preliminary attempts to analyze morphogenetical events in the bacterial cell induced by physiological factors. Moreover, the data presented pertain only to one species. They cannot be generalized nor applied to other species, particularly not to Gram-positive bacteria. However, the results presented strongly support the suggestion by MURRAY (1962), that mesosomes occur in cells subjected to underoptimal growth conditions.

Summary

Vegetative cells of *Myxococcus xanthus*, strain *FB*, were found to contain numerous small mesosomes distributed throughout the cell. They persisted in the cell as long as the cells were maintained on casitone-agar. When these cells were transferred into casitone-broth and grown under aeration large mesosomes were newly formed at the division plane during the first and second cell division after transfer. After four to six more generations when transferred a second time into fresh casitone broth mesosomes were no longer detectable in the cells but reappeared when the cells were retransferred onto casitone-agar.

A low oxygen concentration in the medium caused the formation of an unidentified factor found to be responsible for the formation of mesosomes in cells of colonies or in a liquid medium.

The shape of the mesosomes seems not to be predetermined but depends upon the inhomogeneity of cytoplasm and nucleoids into which they intrude. In some large mesosomes the infolded membrane consisted of five layers, one dense layer alternating with a translucent one with dense layers limiting the membrane. The width of these membranes was 120 A instead of 160 A as could be expected for two merged triple-layered cytoplasmic membranes each measuring about 80 A. A large poly-phosphate granule was found to be enclosed by a mesosome.

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References

CHAPMAN, G. B., and J. HILLIER: Electron microscopy of ultra-thin sections of bacteria. I. Cellular division in *Bacillus cereus*. J. Bact. **66**, 362—373 (1953).

COTA-ROBLES, E. H.: Electron microscopy of plasmolysis in *Escherichia coli*. J. Bact. **85**, 499—503 (1963).

DWORKIN, M.: Nutritional requirements for vegetative growth of *Myxococcus xanthus*. J. Bact. **84**, 250—257 (1962).

—, and H. VOELZ: The formation and germination of microcysts in *Myxococcus xanthus*. J. gen. Microbiol. **28**, 81—85 (1962).

EDWARDS, M. R., and R. W. STEVENS: Fine structure of *Listeria monocytogenes*. J. Bact. **86**, 414—428 (1963).

FITZ-JAMES, P. C.: Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. biophys. biochem. Cytol. **8**, 507—528 (1960).

— Thin sections of dividing *Neisseria gonorrhoeae*. J. Bact. **87**, 1477—1482 (1964).

KAYE, J. J., and G. B. CHAPMAN: Cytological aspects of anti-microbial antibiosis. III. Cytologically distinguishable stages in antibiotic action of colistin sulfate on *Escherichia coli*. J. Bact. **86**, 536—543 (1963).

LEHNINGER, A. L.: The Mitochondrion. New York: W. A. Benjamin, Inc. 1964.

MOSS, F.: Adaption of the cytochromes of *Aerobacter aerogenes* in response to environmental oxygen tension. Aust. J. exp. Biol. **34**, 395—406 (1956).

MUDD, S., L. C. WINTERSCHEID, E. D. DELAMATER, and H. J. HENDERSON: Evidence suggesting that the granules of *Mycobacteria* are mitochondria. J. Bact. **62**, 459—475 (1951).

MURRAY, R. G. E.: Fine structure and taxonomy of bacteria. 12. Symp. Soc. Gen. Microbiol., p. 119—124 (1962).

— The structure of microbiological cytoplasm and the development of membrane systems. Lectures on theoretical and applied aspects of modern microbiology. Sponsored by the American Cyanamid Co., Chas. Pfizer Co. and Merck and Co., University of Maryland (1960/61).

RYTER, A., et F. JACOB: Étude au microscope électronique de la liaison entre noyau et mesosome chex *Bacillus subtilis*. Ann. Inst. Pasteur **107**, 385—400 (1964).

—, et E. KELLENBERGER: Étude au microscope électronique de plasmas contenant de l'acid desoxyribonucléique. I. Les nucleoides des bactéries en croissance active. Z. Naturforsch. **13 b**, 597—605 (1958).

—, and O. E. LANDMAN: Electron microscope study of the relationship between mesosome loss and the stable L State (or protoplast state) in *Bacillus subtilis*. J. Bact. **88**, 457—467 (1964).

—, et M. PIÉCHAUD: Étude au microscope électronique de quelques souches de *Moraxella*. Ann. Inst. Pasteur. **105**, 1071—1079 (1963).

—, et J. PILLOT: Étude au microscope électronique de la structure externe et interne du Tréponème Reiter. Ann. Inst. Pasteur **104**, 496—501 (1963).

SALTON, M. R. J.: The bacterial cell wall. New York: Elsevier Publ. Co. 1964.

STOVE POINDEXTER, J.: Biological properties and classification of the *Caulobacter* group. Bact. Rev. **28**, 231—295 (1964).

TAKAGI, A., K. UEYAMA, and M. UEDA: Reduction of tetrazolium salts to formazans in *Fusobacterium polymorphum*. J. gen. appl. Microbiol. (Tokyo) **9**, 287—298 (1963).

TCHAN, Y. T., A. BIRCH-ANDERSEN, and H. L. JENSEN: The ultra-structure of vegetative cells and cysts of *Azotobacter chroococcum*. Arch. Mikrobiol. **43**, 50—66 (1962).

VANDERWINKEL, E., et R. G. E. MURRAY: Organelles intracytoplasmiques bactériens et site d'activité oxydo-reductrice. J. Ultrastruct. Res. **7**, 185—199 (1962).

VAN ITERSON, W., and W. LEENE: A cytochemical localization of reductive sites in a Gram-positive bacterium. Tellurite reduction in *Bacillus subtilis*. J. Cell Biol. **20**, 361—376 (1964a).

— — A cytochemical localization of reductive sites in a Gram-negative bacteria. Tellurite reduction in *Proteus vulgaris*. J. Cell Biol. **20**, 377—388 (1964b).

VOELZ, H., and M. DWORKIN: Fine structure of *Myxococcus xanthus* during morphogenesis. J. Bact. **84**, 943—952 (1962).

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