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BACTERIAL MESOSOMES
REAL STRUCTURES OR ARTIFACTS?

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SUMMARY

The ultrastructural study of membrane organization in gram-positive bacteria related to the OsO_4 fixation conditions revealed that large, complex mesosomes are observed only when the bacteria are subjected to an initial fixation with 0.1 % OsO_4 in the culture broth, as in the prefixation step of the Ryter-Kellenberger procedure. Evidence was obtained suggesting that the large mesosomes are produced by this prefixation. The kinetic study of the membrane morphological alterations occurring during the prefixation of *Bacillus cereus* with 0.1 % OsO_4 in the culture broth showed that the amount of mesosome material increases linearly from zero to a maximum observed at 1.7 min of prefixation and that at about this time a maximum is reached for the number of mesosomes per unity of cell area and for the average individual mesosome area. The large mesosomes observed in gram-positives fixed by the complete Ryter-Kellenberger procedure would be the result of the membrane-damaging action of 0.1 % OsO_4. Such damaging action was deduced from the observation that 0.1 % OsO_4 quickly lyses protoplasts and induces a quick and extensive leakage of intracellular K^+ from *B. cereus* and *Streptococcus faecalis*. In support of that interpretation is the observation that in bacteria subjected to several membrane-damaging treatments, mesosome-like structures are seen after three different fixation procedures. In bacteria initially fixed with 1 % OsO_4, 4 % OsO_4 or 2.5 % glutaraldehyde, no large, complex mesosomes are observed, small and simple invaginations of the cytoplasmic membrane being present. The size of these minute mesosomes is inversely proportional that causes of fixation. Uranyl acetate was found among the studied fixatives the one to the rate the least damage to bacterial membranes. This fixative satisfactorily preserves protoplasts. In bacteria initially fixed with uranyl acetate no mesosomes were found. The results of the present work throw serious doubts on the existence of mesosomes, both large and small, as real structures of bacterial cells. It is proposed that a continuous cytoplasmic membrane without infoldings (mesosomes) would be the real pattern of membrane organization in gram-positives.

INTRODUCTION

Mesosomes [1] are membranous structures which have been observed by electron microscopy of thin sections in several bacteria mainly after the introduction of the Ryter-Kellenberger OsO_4 fixation procedure [2]. The typical mesosomes are prominent and complex and consist of a pocket formed by the invagination of the cytoplasmic membrane filled with vesicules, tubules or lamellae [3]. On the basis of morphological data, we arrived at the conclusion that the conventional picture of the mesosome [3] can not be accepted with confidence [4, 5], and, later, we considered the possibility of mesosomes being artifacts [6, 7]. In the present paper we report additional results which favour the interpretation that mesosomes may well be artifacts produced by membrane-damaging fixation procedures. The production of mesosomes by several other membrane-damaging treatments is also described. Part of this work has been previously presented in abstract form and in a review article [7].

MATERIALS AND METHODS

Microrganisms, culturing conditions and membrane-damaging treatments

Bacillus cereus (strain NCTC 7587) was grown aerobically in 0.1 % Bacto Tryptone (Difco)+0.5 % NaCl, pH 7.2, at 30 °C, with shaking, to $3–5 \cdot 10^8$ cells/ml. *Streptococcus faecalis* (strain ATCC 9790) was grown aerobically in 1.0 % Bacto Tryptone (Difco)+0.5 % Yeast Extract (Difco) +1.0 % glucose, pH 7.2, at 37 °C, with shaking, to $3–5 \cdot 10^8$ cells/ml. Samples of the cultures were taken to serve as controls. Other samples from the same cultures were subjected to the following treatments. (1) Bacteria in the broth were heated at 60 °C for 2 min (*B. cereus*) or 100 °C for 5 min (*S. faecalis*) and then quickly cooled down to 20 °C by immersion in cold water, as previously reported [8]. (2) Pellets obtained by centrifugation of the cultures were resuspended in 50 mM Tris · HCl buffer/50 mM Na succinate/0.61 mM Nitroblue Tetrazolium chloride (Sigma), pH 7.0, and incubated at the culture temperatures. Under these conditions the Nitroblue Tetrazolium is reduced in the membranes of *B. cereus* to diformazan through the activity of succinic dehydrogenase [9], which is a membrane bound enzyme [10]. No significant reduction occurs with *S. faecalis*. (3) Phenethyl alcohol (Sigma) was added to samples of the cultures to final concentration of 40 mM; incubation was continued for 15–60 min at growth temperatures.

Protoplasts from *B. megaterium* (strain KM, CCM 2037, kindly supplied by Dr. M. Kocur) were grown as indicated above for *B. cereus* and were prepared according to the procedure described by Fitz-James [11]. Protoplasts from *S. faecalis* were produced as previously described [12].

Fixation procedures

Control and treated bacteria were fixed by: (1) OsO_4 followed by uranyl acetate, according to the Ryter-Kellenberger procedure [2], with and without [5] the prefixation step. In both cases the fixative was quickly mixed with the broth or the pelleted bacteria, respectively, by vortexing. (2) By glutaraldehyde (TAAB, London) at 2.5 % in 0.1 M cacodylate buffer, pH 7.0, for 2 h, followed by procedure 1. (3) By uranyl acetate (Merck) at 0.1–0.2 % in Ryter-Kellenberger veronal acetate buffer

(final pH 5.0) or in a veronal acetate-bicarbonate buffer (final pH 6.5) [7], for 30 min, followed by the Ryter-Kellenberger OsO_4 fixation. Fixations 2 and 3 were also applied to control, untreated *B. cereus* subjected to the prefixation step of the Ryter-Kellenberger procedure. Control, untreated *B. cereus* were fixed by the complete Ryter-Kellenberger procedure with the following modification: the prefixation step was carried out by adding 2.5 ml of a 7.0 % solution of OsO_4 in water to 15 ml of the culture (final OsO_4 concentration: 1.0 %). Control, untreated *B. cereus* cells were fixed by the Ryter-Kellenberger procedure without prefixation and with the main fixation carried out with 4 % OsO_4 instead of the standard 1.0 %.

To the suspensions of protoplasts in the stabilizing media, OsO_4, glutaraldehyde or uranyl acetate were added to final concentrations of 0.1, 2.5 and 0.1 %, respectively. After 30 min, the suspensions of protoplasts were treated in two ways. (1) They were centrifuged and the pellets postfixed with OsO_4 and uranyl acetate according to the Ryter-Kellenberger procedure. (2) They were mixed with glutaraldehyde (stock 25 % solution) to a final concentration of 2.5 %; after 2 h the suspensions were centrifuged and postfixed by the Ryter-Kellenberger procedure. No significant differences in the integrity of the protoplasts were observed with these two procedures. Fixation with 1.0 % OsO_4 was also applied to pelleted, unfixed, protoplasts.

All fixations were carried out at 20 °C. The fixed specimens were processed for ultramicrotomy and electron microscopy as described in ref. 13.

Kinetic study of membrane morphological alterations during prefixation with 0.1 % OsO_4

B. cereus cells were subjected to the prefixation step of the Ryter-Kellenberger procedure by adding 50 ml OsO_4 fixative to 500 ml of culture. After 0.5, 1, 1.5, 2, 3, 4 and 5 min of prefixation samples were taken and quickly mixed with glutaraldehyde (stock 25 % solution) to a final concentration of 2.5 %. Samples with less than 0.5 min of prefixation were not studied for technical reasons. The fixed samples were processed for electron microscopy as described [13] and micrographs of sections of similar thickness were taken at magnification 8000. Photographs of 300–500 individual sections were taken in preparations corresponding to each of the seven samples. In prints enlarged 24 000 × the following values were determined: total cell area, total mesosome area, total number of mesosomes. In preparations corresponding to samples taken at times 0.5 through 3 min the number of complex and simple mesosomes was counted. From these values the following parameters were calculated: ratio total mesosome area/total cell area, ratio number of mesosomes/total cell area, average individual mesosome area.

Calculation of the average individual mesosome area as related to the fixation conditions

From thin sections of cells fixed by several procedures prints were obtained at magnifications of 24 000–96 000 ×. The average individual mesosome area was determined in the prints by dividing the total mesosome area by the number of mesosomes. The values given in Table II correspond to mesosomes enlarged to 24 000 × so that these values can be compared with those in column 7 of Table I.

K^+ efflux experiments

B. cereus and *S. faecalis* were grown in the media indicated above supplement-

TABLE I

VALUES OBTAINED WITH THE KINETIC STUDY OF THE MEMBRANE MORPHOLOGICAL ALTERATIONS OCCURRING DURING THE RYTER-KELLENBERGER PREFIXATION IN *B. CEREUS*

All parameters were determined in prints enlarged 24000 ×.

Time of prefix (min)	Total cell area	Total mesosome area	No of mesosome	Mesosome/cell ratio (area)	No. of mesosome/unity of cell area			Average individual mesosome area
					Total	Simple	Complex	
0.5	59.03	0.300	147	0.00508	2.49	1.44	1.05	0.00204
1.0	68.32	0.680	350	0.00995	5.12	2.24	2.88	0.00194
1.5	60.67	0.905	351	0.01492	5.78	0.93	4.85	0.00258
2.0	65.33	1.130	375	0.01729	5.74	0.53	5.21	0.00301
3.0	67.06	1.120	373	0.01670	5.56	0.22	5.34	0.00300
4.0	58.78	1.030	312	0.01752	5.31	–	–	0.00330
5.0	83.76	1.400	521	0.01671	6.22	–	–	0.00268

TABLE II

SPEED OF FIXATION AND MESOSOME SIZE (*B. CEREUS*) AS RELATED TO THE FIXATION CONDITIONS

Fixation	Time of gellification of albumin*	No. of mesosome counted	Average individual mesosome area**
0.1 % OsO_4 in broth	> 2 h	201	0.00285
1.0 % OsO_4	15.5 min	426	0.00052
3.5 % OsO_4	1.9 min	287	0.00025
2.5 % glutaraldehyde	3.5 min	200	0.00032

* 15 % bovine serum albumin.

** In prints enlarged 24 000 ×.

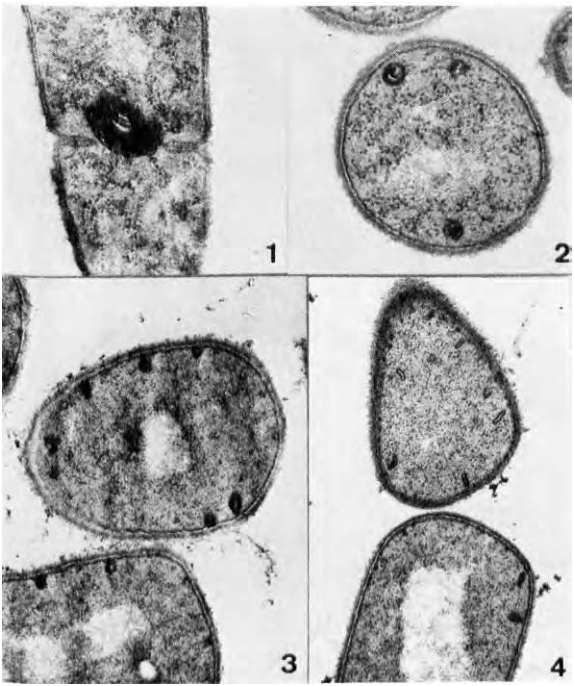
ed with 0.5 % dipotassium phosphate. The cells were washed twice with 0.1 % peptone to remove extracellular K^+. The final suspensions of washed bacteria were made in 0.1 % peptone. The leakage of K^+ from *B. cereus* and *S. faecalis* subjected to the fixations and treatments described above was determined by measuring the K^+ concentration in filtrates obtained at intervals by passing the samples through Millipore filters (type HA, pore size 0.45 μm). K^+ was assayed with an EEL flame photometer, model 150. The 100 % leakage was taken as the value of K^+ leaked from cells boiled for 30 min (*B. cereus*) or treated with 10 % HNO_3 (*S. faecalis*).

Study of the speed of fixation of different fixatives

This was carried out using the procedure described by Millonig and Marinozzi [14]. Bovine serum albumin (Sigma) was dissolved in water at 30 % concentration and mixed with OsO_4 and glutaraldehyde. Final concentrations were: albumin, 15 %; OsO_4, 0.1, 1 and 3.5 %; glutaraldehyde, 2.5 %.

RESULTS

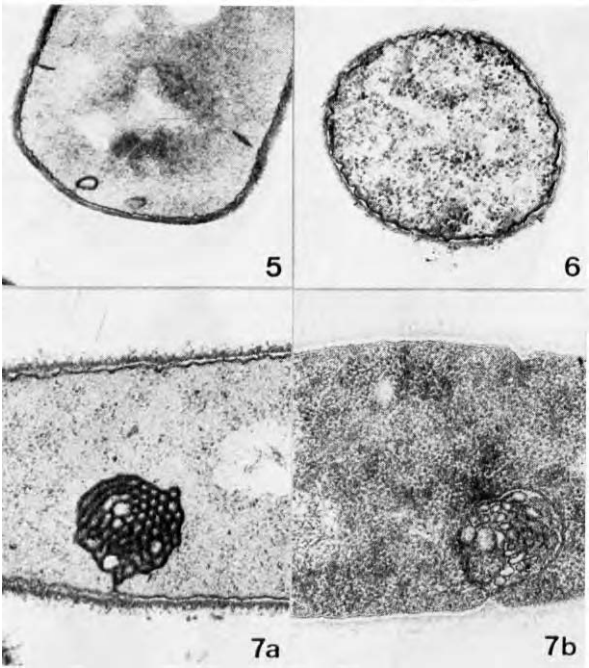
Control, untreated *B. cereus* and *S. faecalis* fixed by the complete Ryter-Kellenberger procedure exhibit prominent, complex mesosomes (Fig. 1), henceforth called large mesosomes. They consist of large invaginations of the cytoplasmic membrane filled with vesicules. When the prefixation step of the Ryter-Kellenberger procedure is omitted, or when this step is carried out with 1 % OsO_4 instead of the standard 0.1 %, the large mesosomes are not present, simple and small invaginations of the cytoplasmic membrane being observed (Fig. 2 and 3), henceforth called small mesosomes. The study of serial sections showed that these small mesosomes frequently consist of a single vesicule. Results similar to those reported above were obtained with other bacteria belonging to the genera *Bacillus* [5], *Nocardia* [15], *Micrococcus* [16] and *Sporosarcina* [17]. The intrusions of the cytoplasmic membrane are even smaller when the cells are fixed by the Ryter-Kellenberger procedure without prefixation and with 4 % OsO_4 in place of the standard 1 % (Fig. 4 and Table II). Fixation of control, untreated bacteria with glutaraldehyde-OsO_4-uranyl acetate (procedure 2) results in the presence of small mesosomes (Fig. 5 and Table II). Control, untreated bacteria fixed with uranyl acetate-OsO_4 (procedure 3) do not show mesosomes, a continuous



Figs. 1–4. All electron micrographs are of *B. cereus* sections contrasted with lead citrate for 5 min. Growing cells fixed by: Fig. 1, OsO_4 according to the complete Ryter-Kellenberger procedure (prefixation included), × 48 000. Fig. 2, Ryter-Kellenberger procedure without the prefixation step. × 48 000. Fig. 3, as in Fig. 1 but prefixation with 1 % OsO_4, × 40 000. Fig. 4, as in Fig. 2 but OsO_4 at 4 %, × 45 000.

cytoplasmic membrane being observed (Fig. 6). Large mesosomes are also observed in samples prefixed with 0.1 % OsO_4 in broth (Ryter-Kellenberger prefixation) and postfixed by procedures 2 or 3 (Fig. 7).

Protoplasts from *B. megaterium* and *S. faecalis* are quickly lysed when prefixed with 0.1 % OsO_4, as deduced from macroscopic and light microscopic observations, as well as from the study of thin sections by electron microscopy (Fig. 8). When initially fixed with glutaraldehyde or uranyl acetate the protoplasts do not lyse (Fig.



Figs. 5–7. All electron micrographs are of *B. cereus* sections contrasted with lead citrate for 5 min. Growing cells fixed by: Fig. 5, glutaraldehyde-OsO_4-uranyl acetate (procedure 2), × 51 000. Fig. 6, uranyl acetate-OsO_4 (procedure 3), × 48 000. Fig. 7, prefixation according to the Ryter-Kellenberger method followed by procedure 2 (Fig. 7a) or by procedure 3 (Fig. 7b), × 62 000.

9). Satisfactory preservation of protoplasts is also achieved by fixation with 1 % OsO_4.

Table I shows the values obtained with the kinetic study of the membrane morphological alterations occurring during the prefixation of *B. cereus* in broth with 0.1 % OsO_4. With these values the graphs presented in Fig. 10 were drawn. These graphs show: (1) the ratio total mesosome area/total cell area increases linearly from 0.5 min to about 1.7 min (correlation coefficient: 0.999); (2) after about 1.7 min no significant change in the total mesosome area occurs; (3) the number of mesosomes per unity of cell area increases until a maximum is reached at about 1.5 min; (4) the number of small mesosomes (consisting of a single vesicule) drops quickly after the first minute of prefixation; (5) the average individual mesosome area increases to a

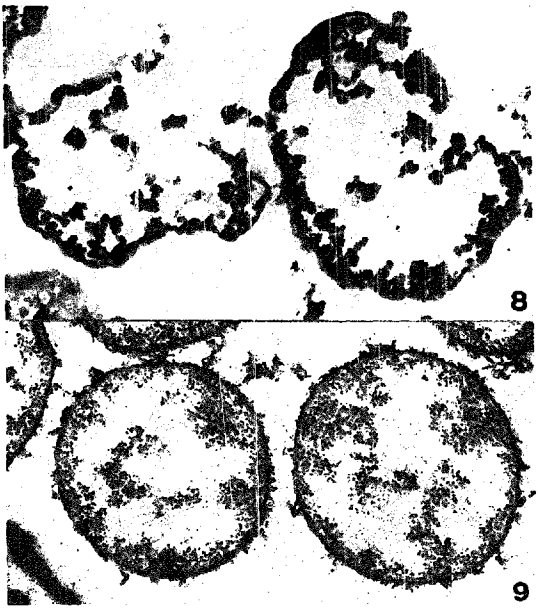


Fig. 8. Protoplasts from *S. faecalis* prefixed with 0.1 % OsO_4 and postfixed with glutaraldehyde-OsO_4-uranyl acetate. × 45 000.

Fig. 9. Protoplasts from *S. faecalis* prefixed with 0.1 % uranyl acetate followed by Ryter-Kellenberger 1 % OsO_4. × 40 000.

maximum observed at 2 min. Assuming that the process of membrane alteration which occurs during the first 0.5 min of prefixation has kinetics identical to that observed for the period 0.5–2 min., total mesosome area at time 0 (in unfixed, native cells) would be statistically not different from zero (giving the value 0 to total mesosome area at time 0, the correlation coefficient for the part of the graph A corresponding to time 0–1.5 min is 0.999).

B. cereus subjected to the described three membrane-damaging treatments and fixed by procedure 1 without prefixation and by procedures 2 and 3, exhibit complex, prominent membranous structures (Figs. 11-16) similar to the large mesosomes found in the control, untreated cells when the fixation is by the complete Ryter-Kellenberger

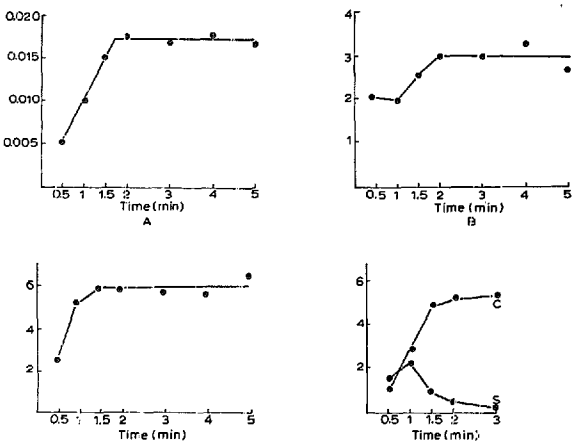
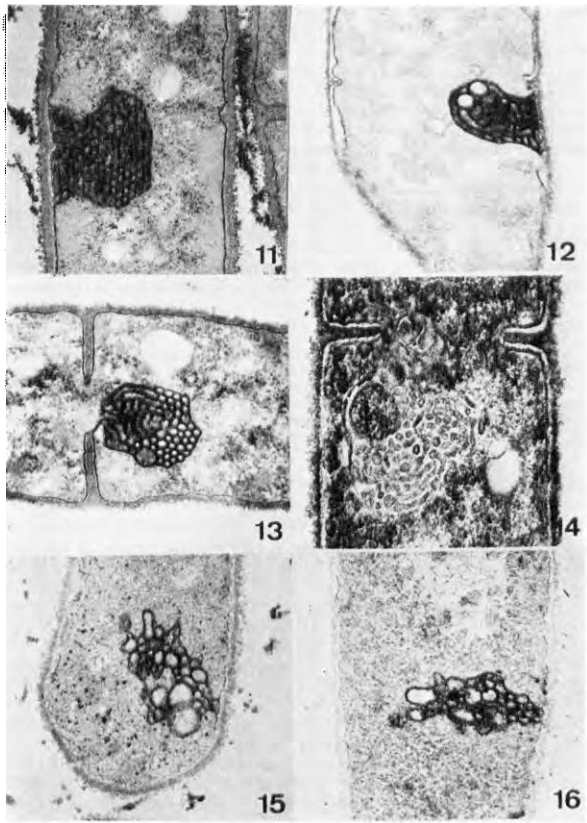


Fig. 10. Graphs drawn with the results of the kinetic study of membrane morphological alterations occurring during the prefixation step of the Ryter-Kellenberger procedure (*B. cereus*). A. Variation of the ratio total mesosome area/total cell area. B. Variation of the average individual mesosome area. C. Variation of the number of mesosomes per unity of cell area. D. As in C but discriminating between small, simple mesosomes (S) and complex, prominent mesosomes (C).

procedure. In *S. faecalis* heated at 100 °C for 5 min large mesosomes are found (see Figs. 2–4 in ref. 18). Membrane ultrastructure of *S. faecalis* was found to be unaffected by the treatment with phenethyl alcohol under the conditions described above. The influence of the treatment with Nitroblue Tetrazolium on the ultrastructure of *S. faecalis* was not studied.

The treatment with 0.1 % OsO_4, moist heat, phenethyl alcohol, and Nitroblue Tetrazolium inflict a serious damage to the cytoplasmic membrane of *B. cereus* as deduced from the quick and extensive K^+ leakage observed in bacteria subjected to these treatments (Fig. 17A). OsO_4 and moist heat were also found to produce an important breakdown of the permeability barrier in *S. faecalis* (Fig. 17B). Phenethyl alcohol exhibited a reduced action on the permeability to K^+ in *S. faecalis* (Fig. 17B); the action of Nitroblue Tetrazolium was not studied. Glutaraldehyde and uranyl acetate induce a slight leakage of K^+ from *B. cereus* and *S. faecalis* (Fig. 17A and B).

Table II shows the results of the study of the speed of fixation of albumin by OsO_4 and glutaraldehyde (uranyl acetate was found to precipitate the albumin but not to gellify it). There is a good correlation between speed of fixation of albumin and mesosome size, this becoming smaller as speed of fixation increases.



Figs. 11–16. All electron micrographs are of *B. cereus sections* contrasted with lead citrate for 5 min. Cells subjected to membrane-damaging treatments. Fig. 11, cell heated at 60 °C for 2 min and fixed by procedure 2, × 50 000. Fig. 12, as in Fig. 11 but fixation by procedure 3, × 48 000. Fig. 13, cell treated with 40 mM phenethyl alcohol for 30 min at 30 °C and fixed by procedure 2, × 48 000. Fig. 14, as in Fig. 13 but fixation by procedure 3, × 55 000. Fig. 15, cell incubated in 50 mM succinate/0.61 mM nitroblue tetrazolium for 60 min at 30 °C and fixed by procedure 2, × 48 000. Fig. 16, as in Fig. 15 but fixation by procedure 3, × 48 000.

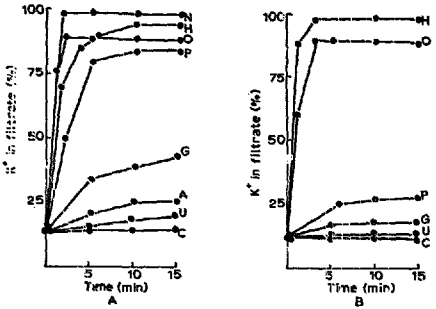


Fig. 17. K^+ efflux from *B. cereus* (A) and *S. faecalis* (B). Symbols: C, control, untreated cells. U, cells treated with 0.1 % uranyl acetate. G, cells treated with 2.5 % glutaraldehyde. P, cells treated with 40 mM phenethyl alcohol. O, cells treated with 0.1 % OsO_4+0.01 % $CaCl_2$ (as in the prefixation step of the Ryter-Kellenberger procedure). H, cells heated at 60 °C (*B. cereus*) or 100 °C (*S. faecalis*). N, cells treated with 0.61 mM nitroblue tetrazolium. A, cells treated with 10 mM sodium azide. All chemical treatments were carried out at 20 °C. When necessary, pH was kept at the appropriate values (see Materials and Methods) with 50 mM s-Collidine/HCl.

DISCUSSION

Present and previous results [5, 7, 13] clearly show that large mesosomes are found in thin sections of several gram-positives only when the bacteria are subjected to an initial fixation with 0.1 % OsO_4 in the culture broth (prefixation step of the Ryter-Kellenberger procedure). The use of 1 % OsO_4, 2.5 % glutaraldehyde or 0.1–0.2 % uranyl acetate after this prefixation, showed no significant influence on the size and complexity of the mesosomes. This indicates that the presence of the large mesosomes is determined by the initial Ryter-Kellenberger prefixation.

On the other hand, OsO_4 at 0.1 % concentration was found to damage bacterial membranes, since it quickly lyses protoplasts and induces a rapid and extensive efflux of K^+ from *B. cereus* and *S. faecalis*. The observed rates of K^+ efflux indicate that OsO_4 is acting directly on the cytoplasmic membrane of the studied bacteria, causing a primary breakdown of its permeability. As discussed below, this indicates that important disturbances in membrane molecular organization occur. Indeed, if OsO_4 would be acting as a mere metabolic inhibitor or as an inhibitor of the (Na^++K^+)-ATPase, the rate of K^+ efflux would be much smaller than the observed one, as in the case with sodium azide-treated *B. cereus*. This potent inhibitor of *Bacillus* (Na^++K^+)-ATPase [19] induces a rather small rate of K^+ efflux from *B. cereus* at 10 mM concentration (Fig. 17A).

Taking together the results discussed above, we propose that the large mesosomes would be produced by the Ryter-Kellenberger prefixation as a result of the membrane-damaging action of OsO_4. In support of this interpretation is the observa-

tion that *B. cereus* subjected to other membrane-damaging treatments reported above or to treatment with the local anesthetics tetracain and nupercain [7], consistently exhibits large mesosome-like structures when fixed by any of the three procedures described. Those treatments, like treatment with 0.1 % OsO_4, induce K^+ effluxes that are indicative of a direct damage of bacterial membranes, with a primary breakdown of their permeability. It seems significant that no large mesosomes are observed in *S. faecalis* treated with 40 mM phenethyl alcohol, a treatment that induces a slight K^+ leakage in that bacterium. These observations are in accordance with the well known resistance of *S. faecalis* to phenethyl alcohol [20].

Mesosome production would be a common effect of the treatment of bacterial cells with 0.1 % OsO_4 in broth and with the other membrane-damaging treatments reported above. The difference between the action of OsO_4 and of the other treatments resides in the fact that OsO_4 has a membrane-stabilizing capacity. As a result of this capacity, the membranes initially modified by the primary membrane-damaging action of OsO_4 are later stabilized. In proposing this interpretation we assume that the membrane alterations which would result in the formation of mesosomes should occur before membrane stabilization. Such an assumption is likely since OsO_4 is known to be a slow fixative [14] and we found that membrane damage induced by 0.1 % OsO_4 occurs rather quickly. The results of the study of the kinetics of the membrane morphological alterations that occur during the Ryter-Kellenberger prefixation of *B. cereus* are in agreement with this interpretation. In fact, such results show that the large mesosomes are progressively formed during the initial part of the prefixation, indicating that the process is self-limiting and is finished before the usual time of the Ryter-Kellenberger prefixation.

Our interpretation that the large mesosomes are artifacts produced by some fixation procedures is in accordance with the results from other laboratories. Nanninga [21] and very recently Fooke-Achterrath et al. [22], on the basis of morphological studies involving freeze-etch electron microscopy, proposed that the large mesosomes do not correspond to real structures present in the living bacterial cell, the real mesosomes being small intrusions of the cytoplasmic membrane. An alternative for this hypothesis is suggested by our results obtained with uranyl acetate fixation. Such results show that no mesosomes are found in *B. cereus* and *S. faecalis* when the fixation procedure uses uranyl acetate as first fixative. This holds true for *B. subtilis* and *N. asteroides* [7]. Although we have no definite proof indicating that this alternative is the correct one, we favour it on the following basis. (1) The results of the kinetic study show that the amount of mesosome material of unfixed cells is statistically not different from zero. (2) There is evidence indicating that the small mesosomes may well be artifacts, too. The time-related dynamics of the evolution of the number of the two types of mesosomes during the initial period of the Ryter-Kellenberger prefixation suggests that the small mesosomes may well be the initial step in the formation of the large ones. The small vesicules could either be transformed in, or aggregated to form the large, vesicular mesosomes typical of *B. cereus* fixed by the complete Ryter-Kellenberger procedure. Small mesosomes are observed not only in cells prefixed with 0.1 % OsO_4 in broth for short times, but also after fixation with 1 % OsO_4 and 2.5 % glutaraldehyde. These two fixatives are more efficient than 0.1 % OsO_4, as shown by the observation that, contrary to 0.1 % OsO_4, they are able to fix protoplasts and by the results of the study of the speed of fixation of albumin. It seems

conceivable, therefore, to admit that in the case of these more rapid fixations, the artifact production is stopped early, not proceeding beyond the initial step corresponding to the formation of the small mesosomes. The results of the fixation with 4 % OsO_4 are in accordance with this interpretation, showing that even smaller mesosomes are present in cells subjected to this faster fixation. (3) There are good reasons to accept uranyl acetate as an efficient fixative for membranes. Uranyl ions have been shown to have a stabilizing action on bacterial membranes and on other biomembranes [13, 23, 24]. Low concentrations of uranyl acetate were found to fix protoplasts. Uranyl ions are known to interact with both proteins and phospholipids (see ref. 25), the main components of biomembranes. The binding of UO_2^{2+} to phospholipids is very strong for acidic phospholipids [26] which are largely represented in the membranes of the bacteria we studied [12, 27]. Such binding results in a condensation and stabilization of phospholipid bilayers, with a decrease in their fluidity [26]. The results of the study of the K^+ efflux from bacteria treated with uranyl acetate are in accordance with these biophysical observations. Conversely, the permeability changes induced by the membrane-damaging treatments we used in the present study, including treatment with 0.1 % OsO_4, indicate that opposite effects, that is, increase in membrane fluidity and membrane expansion, are likely to be produced by such treatments. Moreover, in the case of treatment with local anesthetics, which also results in the presence of mesosomes as discussed above, the occurrence of increased membrane fluidity and membrane expansion have been demonstrated [28–30]. Mesosome production may well be related to these alterations.

The conclusion emerging from the results here described, namely that a continuous cytoplasmic membrane without infoldings (mesosomes) would be the real image of the membrane organization in gram-positive Eubacteria, extend to these bacteria the membrane pattern accepted for most gram-negatives and for L-forms and Mycoplasmae. Moreover, that conclusion is in accordance with accumulating evidence indicating that the functions ascribed in the past to mesosomes are not associated with these structures [3, 31]. The cytoplasmic membrane of gram-positives appears, therefore, as the structure with the task of performing the functions attributed to mesosomes, as has been accepted for those bacteria in which mesosomes have not been found.

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