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Evidence for the Involvement of Membranous Bodies in the Processes Leading to Genetic Transformation in *Bacillus subtilis*

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ABSTRACT

WOLSTENHOLME, DAVID R. (Max-Planck-Institut für Biologie, Tübingen, Germany), CORNELIUS A. VERMEULEN, AND GERHARDUS VENEMA. Evidence for the involvement of membranous bodies in the processes leading to genetic transformation in *Bacillus subtilis*. J. Bacteriol. **92**:1111–1121. 1966.—Data obtained from electron microscopic autoradiographs of profiles of cells of a *Bacillus subtilis* population exposed to H^3-thymidine-labeled donor deoxyribonucleic acid (DNA) during the phase of maximal competence indicated that molecules originating from absorbed DNA are closely associated with membranous bodies, particularly with those situated in the cytoplasm, but that most if not all of the radioactive molecules are outside the bodies. It is suggested that membranous bodies produce enzymes essential to the eventual incorporation of transforming DNA into the bacterial genome, or to the breakdown and utilization or expulsion of absorbed DNA not incorporated as transformant (or to both processes). During the phase of maximal competence, the total number of membranous bodies seen in profiles increased continuously to as much as 2.3 times the numbers found during earlier stages of culture. This increase was not accounted for by a decrease in bacterial cell volume, but resulted from an actual increase in total volume of membranous bodies. The number of membranous bodies visibly connecting plasma membrane and nuclear region increased during maximal competence to as much as 30 times the numbers found in earlier stages. As both increases were found in the absence of donor DNA and only began after maximal competence was attained, it seemed most probable that they were an expression of a physiological state influenced by the continuing deficiency of nutrients in the growth medium during this phase of culture.

In electron micrographs of two readily transformable species of bacteria, *Bacillus subtilis* (5, 15, 21) and *Diplococcus pneumoniae* (19), membranous bodies [which have also been referred to as chondroids (15), peripheral bodies (5, 21), and mesosomes (3)] are often found which are frequently attached to the plasma membrane of developing cross walls. Such bodies are sometimes seen to be in direct contact with the nuclear region of the cell. These observations, together with the suggestion of a correlation between the state of division of the cell and competence in *D. pneumoniae* (6), have led to a consideration of the possibility that the membranous bodies play a part in the entry of donor deoxyribonucleic acid (DNA) into a recipient cell (19, 20).

This report deals with the results of experiments involving electron microscopic autoradiography of *B. subtilis* cells exposed to H^3-thymidine-labeled donor DNA during an extended period of maximal competence (18, 23), made in an attempt to determine whether the membranous bodies are involved in any of the cell processes leading to transformation.

MATERIALS AND METHODS

The *B. subtilis* cells used as recipient in all experiments were of a strain auxotrophic for indole and autotrophic for tyrosine (*ind tyr*$^+$). Competent cultures of these cells were made by the method described by Venema, Pritchard, and Venema-Schröder (23). This involved growing the bacteria at 37 C with constant aeration in Spizizen's minimal salt medium with 0.5% glucose, 0.02% casein hydrolysate, 10^{-7} M Mn^{+2}, and 20 μg of indole per ml added. The

growth of the cultures was followed by measuring the extinction at a wavelength of 450 mμ in a Zeiss PMQ II spectrophotometer. At the end of the logarithmic phase of growth, when the rate of increase was approximately one-half maximal, the cells were diluted twofold with growth medium without indole and casein hydrolysate. Maximal competence was reached 90 min later, and was maintained for at least 1 hr (23).

The DNA used as donor was extracted by a modification (23) of the method described by Kirby (8), and added to the recipient population at a concentration of 5 μg/ml of recipient cells. In the first experiment, H^3-thymidine-labeled DNA was prepared from cells of a strain autotrophic for indole and auxotrophic for tyrosine and thymidine (*ind*$^+$ *tyr thy*), which had been grown in a medium supplemented with 10 μg/ml of H^3-thymidine (specific activity, 240.8 μc/mmole). This DNA was added to an *ind tyr*$^+$ culture 90 min after dilution, and the culture was incubated an additional 60 min.

Samples (3 ml) for electron microscopy were taken at each of the times indicated in Fig. 6. Each sample was centrifuged at 2,000 × *g* for 5 min at room temperature (≃21 C). The supernatant fluid was poured off, and the bacteria were mixed with 5 ml of Kellenberger's 1% OsO_4 at 0 C. (In Fig. 6, the times shown are those at which the cells were removed from the cultures. In all cases, the actual time of fixation was approximately 5 min later.) After fixation at room temperature for 24 hr, the cells were treated with uranyl acetate (15), dehydrated in a graded series of ethyl alcohol, and embedded in methacrylate-divinyl benzene (9). Thin sections (≃600 A) were cut by use of an LKB Ultrotome microtome with a diamond knife, and mounted on copper grids covered with a carbon-coated Formvar film.

The presence or absence of membranous bodies and the number and position of these bodies, when present, were noted in each of 1,000 profiles of cells from each sample at 20,000 × in a Siemens Elmiskop I electron microscope. (When a membranous body was found lying partly in the cytoplasm and partly in the nuclear region, it was awarded to the region containing the most of it. To minimize the inclusion of more than one profile from the same bacterium, not more than one section in ten was used.) Also recorded at 20,000 × were the maximal and minimal dimensions of 25 membranous bodies situated in the nuclear region and 25 membranous bodies situated in the cytoplasm of profiles selected at random from each of the nine samples. The maximal and minimal diameters of 100 profiles of cells from each of samples 1–3 and 7–9 (see Fig. 6) were noted from negatives taken at 8,000 ×.

Electron microscopic autoradiographs were made of sections of cells of samples 7, 8, and 9, and also of cells of a sample taken near the end of the logarithmic-growth phase from the free H^3-thymidine-supplemented culture of *ind*$^+$ *tyr thy* cells, from which the radioactive donor DNA was prepared. The autoradiographs were made by use of the method of Caro, Van Tubergen, and Kolb (2), which involves direct application of a gelled loop of Ilford L_4 nuclear emulsion to mounted sections, and development for 5 min in Microdol-X. Exposure was for about 90 days. Negative photographs of 50 grains associated with bacterial profiles from each of the four samples were made with a Zeiss EM 9 electron microscope at 20,000 ×. A grain was considered to be associated with a bacterial profile if its center lay within one-half its maximal diameter from the edge of the profile. From a consideration of the number of grains not associated with cell profiles over the area searched for the 50 grains in each sample, and from estimates of the total area occupied by bacteria in the sections examined, it was determined that in any of the three samples no more than 4.6% of the grains can be attributed to background. Measurements of the distances of the centers of grains (i.e., the point judged to be equidistant from the extremities of the grain viewed as a two-dimensional object in the micrograph) to the various parts of the cell profile were made on the negatives.

In the second experiment, approximately 90 min after a population of *ind tyr*$^+$ cells was diluted at the end of the logarithmic phase of growth, the culture was divided into two equal parts. Nonradioactive donor DNA (*ind*$^+$ *tyr*) was immediately added to one of the subcultures, and the two subcultures were incubated for an additional 70 min. One sample for electron microscopy was taken during the logarithmic phase of growth and another at the beginning of the phase of maximal competence, just before the culture was divided. Further samples were taken from each of the subcultures at 18, 38, and 70 min. The presence or absence of membranous bodies in profiles was determined as in the first experiment.

In previous experiments under the present conditions of culture and with the same recipient strain and either of the donor strains, the transformation rate for indole was constantly about 0.01% at the end of the logarithmic-growth phase, and about 0.1% during the phase of maximal competence.

RESULTS

Many of the membranous bodies of *B. subtilis* were composed of a spiral of membranes (Fig. 1–5), with the outer three-layered membrane having the same characteristics of width and density as the unit membrane forming the plasma membrane of the cell. The inner membranes appeared to be formed by the infolding of the outer membranes. Some of the membranous bodies appeared vesiculated (Fig. 5), each vesicle being bounded by either a unit membrane or what appeared to be a single-layered membrane about 30 A thick. Such bodies were sometimes found attached to the more frequent spiral membranous bodies (Fig. 5). As well as being found attached to regions of the plasma membrane involved in cross-wall formation (Fig. 3 and 5), in *B. subtilis* membranous bodies were also found attached to the plasma membrane at places where it was clearly not involved in new cross-wall formation

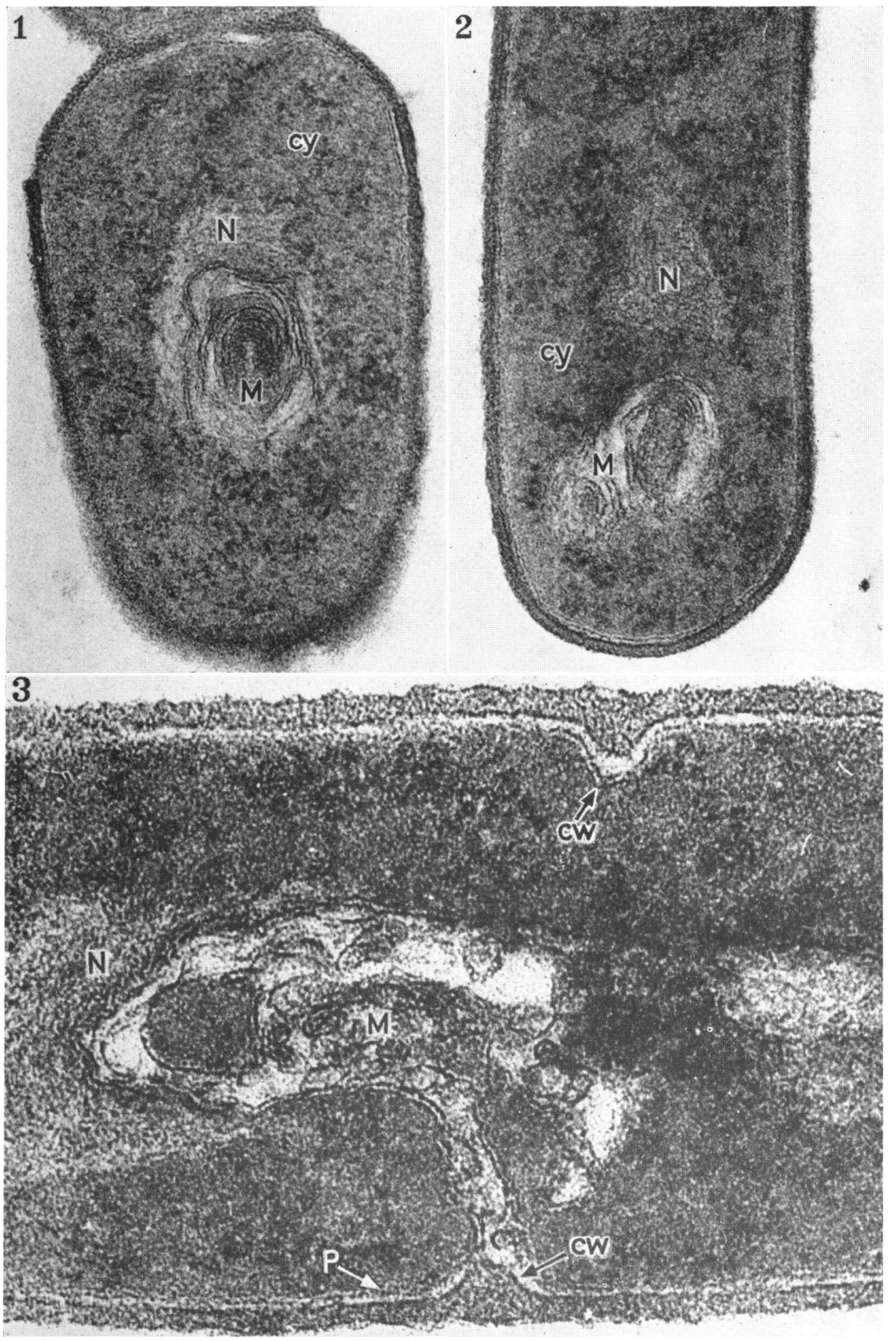

FIG. 1. *Electron micrograph of a thin section of a cell of Bacillus subtilis showing a membranous body (M) in the nuclear region (N); cy, cytoplasm. × 120,000.*

FIG. 2. *Electron micrograph of a thin section of a cell of Bacillus subtilis showing a membranous body (M) in the cytoplasm (cy); N, nuclear region. × 120,000.*

FIG. 3. *Electron micrograph showing a membranous body (M) which lies in the nuclear region (N), and is also continuous with the plasma membrane (P), where the latter is involved in the formation of a new cross-wall (cw). × 170,000.*

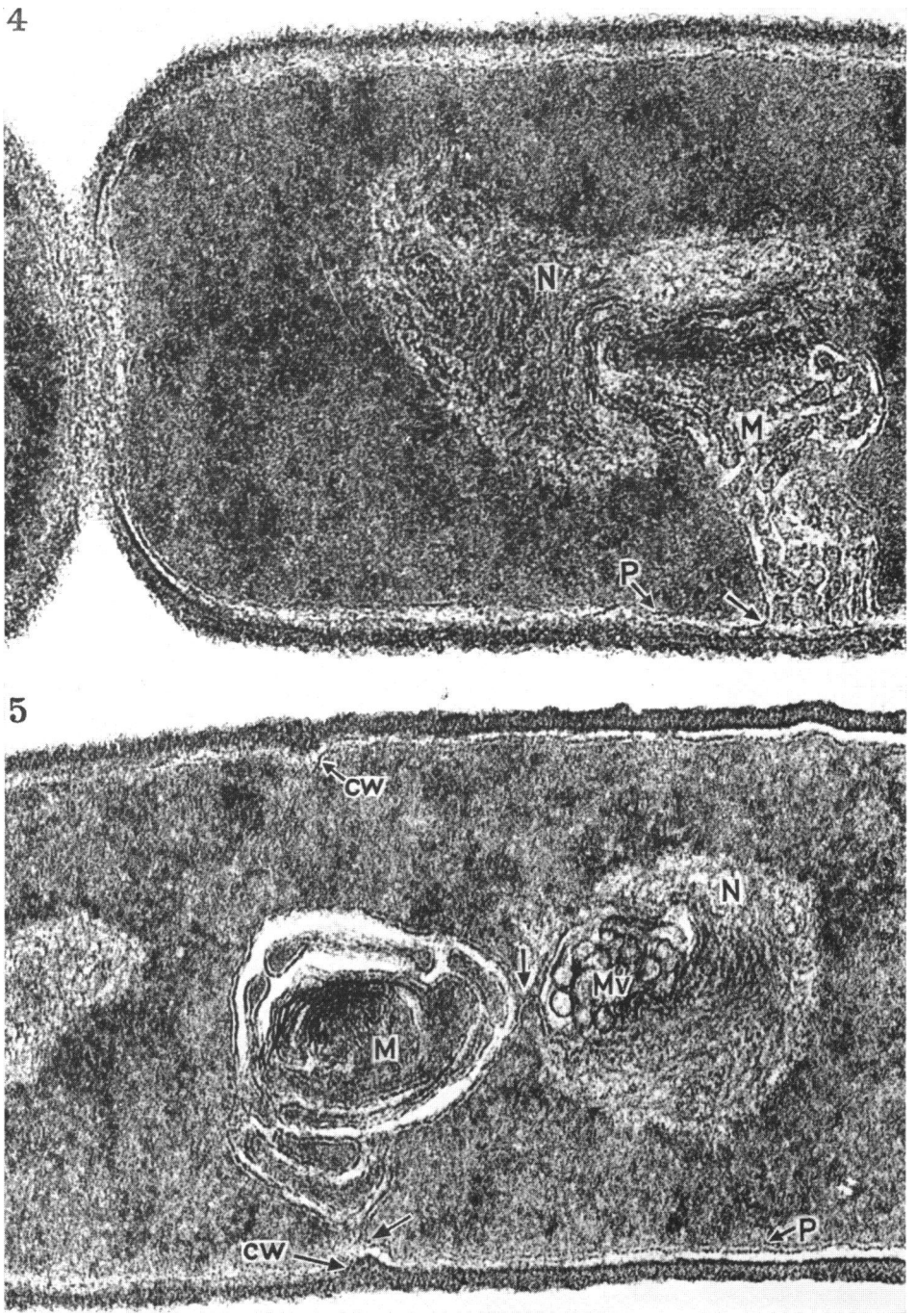

FIG. 4. *Electron micrograph showing a membranous body (M) which lies in the nuclear region (N), and is also continuous (arrow) with the plasma membrane (P), where the latter is clearly not involved in the formation of a new cross-wall.* × *170,000.*

FIG. 5. *Electron micrograph showing a rare case of a membranous body (M) which is attached (arrows) both to the plasma membrane (P) at the place where the latter is involved in the formation of a new cross-wall (cw), and to a second, vesiculated membranous body (Mv) embedded in the nuclear region (N).* × *170,000.*

(Fig. 4). Membranous bodies at both points of attachment to the plasma membrane were sometimes found to penetrate the nuclear region of the cell (Fig. 3, 4, and 5).

After the addition of donor DNA in the first experiment, there was a continuous increase in the number of membranous bodies observed in both the cytoplasm and the nuclear region of cell profiles during the phase of maximal competence examined (Fig. 7). Since the volume of bacterial cells did not decrease during maximal competence [the mean maximal and minimal dimensions of cells taken during maximal competence were 1.101 μ ($\pm$0.025) and 0.585 μ ($\pm$0.004), respectively, compared with the similar dimensions of 0.952 μ ($\pm$0.023) and 0.559 μ ($\pm$0.004) for cells taken during the logarithmic phase of growth],

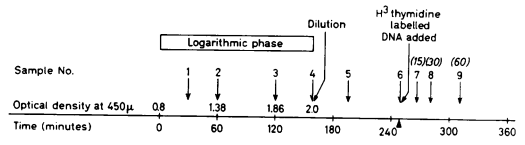

FIG. 6. *Times at which the samples of cells were taken for electron microscopy during the different stages of culture of a population of Bacillus subtilis. The solid arrow head beneath the abscissa indicates the beginning of the phase of maximal competence. The time in minutes at which each sample was taken after addition of the labeled DNA is shown in parentheses above each sample number.*

these increases represented an increase in the total volume of membranous bodies. The results of measuring membranous bodies in each of the samples (Fig. 8) indicated that an increase in volume of the individual membranous bodies contributed to at least the initial rise in the observed body numbers. Towards the end of maximal competence, an actual increase in the number of individual bodies in the nuclear region must, however, occur.

After addition of donor DNA, there was also a striking continuous increase in the number of membranous bodies found connected to the plasma membrane and also penetrating the nuclear region (Fig. 9). This increase was greatest for connections to the plasma membrane where the latter was not visibly involved in cross-wall formation.

In autoradiographs (Fig. 10), there was a successive significant increase in the distance of the mean positions of grains from the outer limit of the cell wall in samples 7–9 (t_{98} = 3.17, P = < 0.01, > 0.001 for the difference between the means of samples 7 and 9) (Fig. 11). This finding was consistent with there being a continuous increase in the mean number of radioactive molecules in the nuclear region relative to those found either at the cell wall or in the cytoplasm of cells of the population with time after the addition of the labeled donor DNA. The distribution

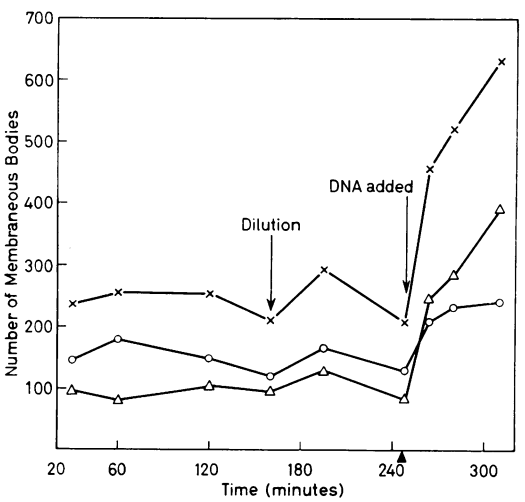

FIG. 7. *Number of membranous bodies observed in 1,000 profiles (×, total; ○, cytoplasm; △, nuclear region) of cells from each of the samples taken during the different stages of culture of a population of Bacillus subtilis. The solid arrow beneath the abscissa indicates the beginning of the phase of maximal competence.*

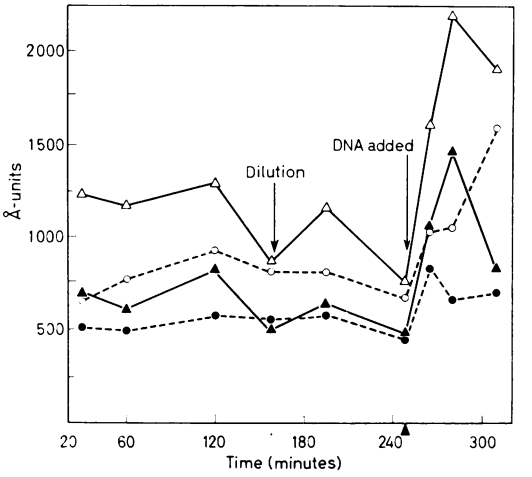

FIG. 8. *Mean maximal (○) and minimal (●) diameters of 25 membranous bodies found in the cytoplasm, and the mean maximal (△) and minimal (▲) diameters of 25 membranous bodies found in the nuclear region in profiles of cells of each sample taken during the different stages of culture of a population of Bacillus subtilis. The solid arrow head beneath the abscissa indicates the beginning of the phase of maximal competence.*

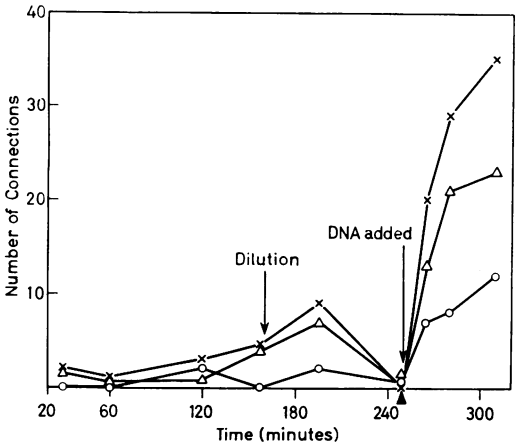

FIG. 9. *Number of connections formed by membranous bodies between the nuclear region and the plasma membrane (×, total; ○, plasma membrane at new cross-wall formation; △, plasma membrane at place other than new cross-wall formation) observed in 1,000 profiles of cells from each of the samples taken during the different stages of culture of a population of Bacillus subtilis. The solid arrow beneath the abscissa indicates the beginning of the phase of maximal competence.*

of grains for the *ind*$^+$ *tyr thy* sample showed what would be expected if all of the radioactive source were in the nuclear region.

In profiles of bacteria with which a grain was associated, from each of samples 7, 8, and 9, membranous bodies occurred with a frequency significantly higher than that found in nonautoradiographed profiles (Table 1). This suggested that membranous bodies are found in the vicinity of the source of radioactivity responsible for activation of the silver grains more often than would be expected owing to chance. In all three samples of autoradiographs (Table 1), the percentage of profiles in which the nearest membranous body to the grain was in the cytoplasm was significantly in excess of what would be expected from a consideration of the percentage of nonautoradiographed profiles which contain cytoplasmically situated membranous bodies. Similarly, in autoradiographs, the percentage of profiles in which the nearest membranous body to the grain was in the nuclear region was less than expected.

In all three of the competent samples, there appeared to be a concentration of grains lying outside the membranous bodies and within a rather constant distance (mean positions, 1,300–1,600 A) from their boundaries (Fig. 12), suggesting again that positions of the sources of radioactivity and the membranous bodies are closely related. In comparison with these latter distributions, however, the greatest concentration of grains in the *ind*$^+$ *tyr thy* sample lay over the edge of the nuclear region (which was assumed to be the only source of radioactivity in this sample). Of these grains, 84% lay within 1,000 A of the nuclear edge, and their mean position was at 350 A outside this boundary.

These latter findings are consistent with similar measurements of the positions of grains over H^3-thymidine-labeled profiles of *B. subtilis* made by Caro (1). Of the grains, 89% lay within 1,000 A of the nuclear margin, and the mean position of these grains [calculated from Fig. 8 of Caro (1)] was 180 A from the limit of the nuclear region. A comparison of this grain distribution with that resulting from a much smaller source offered by H^3-thymidine-labeled bacteriophage (roughly spherical, 600 A in diameter), shown by Caro (1), revealed that the number of grains over the smaller source was less, and that the mean and mode of the grain distribution lay further away from the edge of the smaller source. The mean lay at 1,000 A from the edge of the bacteriophage [calculated from Fig. 6 of Caro (1)].

In the present experiment, the mean sizes of the membranous bodies in each of the competent samples were smaller than the mean size of the nuclear region of the *ind*$^+$ *tyr thy* profiles (see Fig. 12). From the above consideration of Caro's data, therefore, the differences in positions of the means and modes of the grain distributions of the competent and *ind*$^+$ *tyr thy* samples did not in itself rule out the possibility that, in cells of the competent samples the sources of radioactivity lay completely within the limits of the membranous bodies. However, the mean sizes of the extended sources offered by the membranous bodies were larger than the bacteriophages studied by Caro (1), but the mean positions of the grains were further away from the edge of the membranous bodies than they were from the edge of the bacteriophages. This suggests that the grain distributions of samples 7, 8, and 9 might result from sources of radioactivity most of which, if not all, lie outside the limit of the membranous bodies, rather than from points distributed at random throughout them.

That the grains associated with membranous body-containing profiles in each sample were actually closer to the membranous bodies than would be expected if the radioactive source and membranous bodies were distributed in the profiles independent of each other was confirmed in the following way. From the mean maximal and minimal measurements of cell profiles with which a grain was associated and which contained a membranous body, a diagram of a cell profile was constructed on graph paper for each of sam-

ples 7, 8, and 9 (Fig. 13). The mean curvature of each of the two narrow ends was considered to be exactly one-half the arc of a circle. [That the areas of the figures constructed in this way were at least as small as those of the actual mean areas of the samples of profiles was ascertained by comparing the mean area of 30 labeled membranous body-containing profiles (10 of which were taken at random from each of samples 7, 8, and 9), calculated as described above, with the mean area of the same profiles calculated from graph-paper outlines. The values obtained by the two methods were 0.5023 and 0.5027 μ^2, respectively.] Before each figure was constructed, the

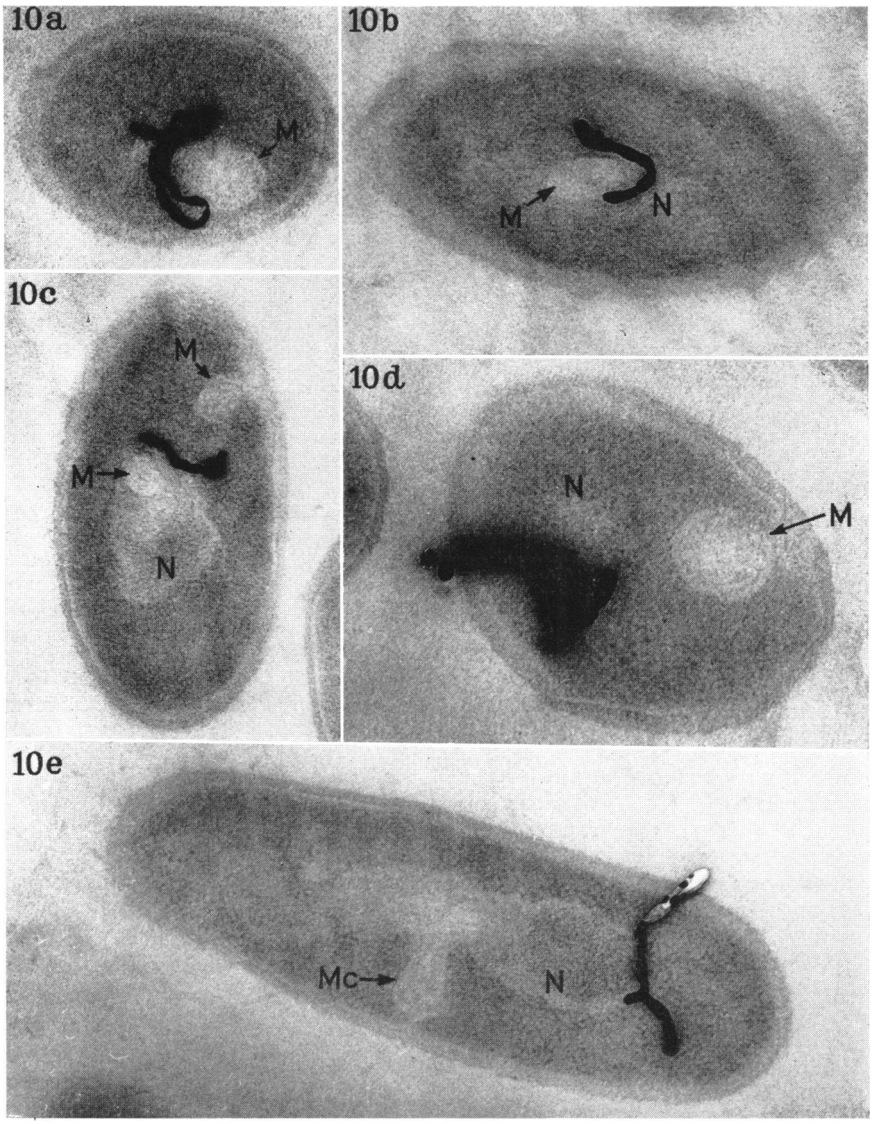

FIG. 10. *Electron microscopic autoradiographs of membranous body containing profiles of cells of a population of Bacillus subtilis exposed to H^3-thymidine-labeled donor DNA during the phase of maximal competence. The membranous bodies, M, are clearly distinguishable from small sections of nuclear region, N, by their more definite outline resulting from their membrane boundary. (a and b) A membranous body lying in the cytoplasm (samples 8 and 7, respectively). (b) A membranous body lying in the nuclear region (sample 9). (c) This profile contains two membranous bodies, one lying in the nuclear region and one in the cytoplasm (sample 8). (e) A membranous body, Mc, connecting the plasma membrane and the nuclear region (sample 7). All micrographs, × 60,000.*

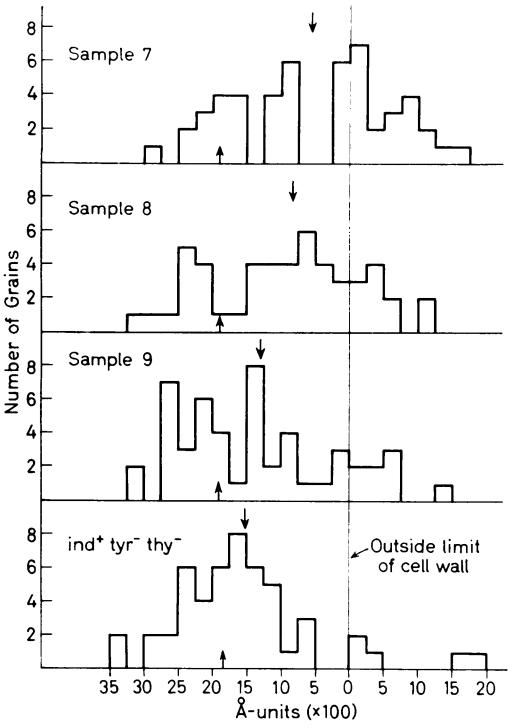

FIG. 11. *Comparative frequency distributions, relative to the outer limit of the cell wall, of 50 grains over profiles of cells of each of the three samples taken during the phase of maximal competence after addition of H^3-thymidine-labeled donor DNA, and of ind+ tyr thy cells grown in a medium supplemented with free H^3-thymidine. The arrow above each histogram indicates the sample mean, and the arrow on each abscissa, the mean position of the edge of the nuclear region.*

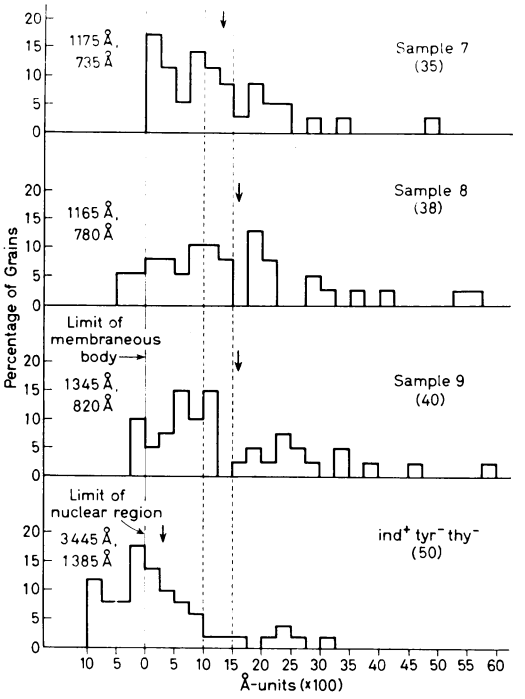

FIG. 12. *Comparative percentage frequency distributions of grains over membranous body-containing profiles of cells of each of the three samples taken during the phase of maximal competence after addition of H^3-thymidine-labeled donor DNA, relative to the nearest edge of the nearest membranous body, and of grains over profiles of ind+ tyr thy cells grown in a medium supplemented with free H^3-thymidine, relative to the edge of the nuclear region. The arrow above each histogram indicates the sample mean. The numbers to the left of each histogram represent the mean maximal and minimal dimensions of the nearest membranous bodies to the grains in each sample. The number of observations for each sample is shown in parentheses.*

mean dimensions were corrected for the presence of more than one body in some of the profiles by halving the maximal dimension of each profile containing two bodies, and reducing to one-third the maximal dimension of each profile containing three bodies, etc. The areas of the constructed figures were corrected for the thickness of the cell wall (about 250 A). The mean position of the grains relative to the outer limit of the cell wall was marked at the mid-position of the greater diameter of the figure. A 2,500-A arc was then drawn, taking the mean position of the grains as its center. The radius of 2,500 A was made up of 1,500 A, the distance from the edge of the membranous bodies within which the greatest concentration of grains occurred on the histograms in Fig. 12, plus 1,000 A, the mean diameter of the membranous bodies, taken in a line from the center of the grain to the nearest edge of the body, for all three cell samples. The area within the 2,500-A arc and the area outside the arc, but within the cell wall of the bacterial profile, was calculated from each figure. The number of membranous bodies lying completely within a 2,500-A arc was then compared with the number of membranous bodies lying outside this arc. In each sample (Table 2), more membranous bodies were found within the 2,500-A radius of the mean position of the grains than would be expected if the radioactive source and membranous bodies were distributed over the area of the profiles independent of each other. The chi-square values were progressively less from sample 7 through sample 9, indicating a mean decrease in association of membranous bodies and radioactivity with time.

The mean distance from the center of grains associated with profiles of sample 7, in which either a developing cross-wall or a newly formed cross-wall was evident, to the nearest point of the

TABLE 1. *Comparison of the occurrence and positions of membranous bodies in grain-associated profiles and in normal, nonautoradiographed profiles of each of the three samples taken during the phase of maximal competence*[a]

Determination	Sample 7		Sample 8		Sample 9	
	Grain-associated profiles	Normal profiles	Grain-associated profiles	Normal profiles	Grain-associated profiles	Normal profiles
Membranous bodies are found	70***	35.1	76***	40.6	80***	50.7
Two membranous bodies are found	22***	6	6†	7.9	16†	10.5
Three or more membranous bodies are found	8***	1.8	4†	1.6	2†	1
The nearest membranous body to the grain is in the cytoplasm	56***		68***		56***	
A membranous body is found in the cytoplasm		21.4		23.7		24.0
The nearest membranous body to the grain plasm	14§		8**		24*	
A membranous body is found in the nuclear region		24.3		28.1		39.2

[a] Levels of significance for chi-square are: †, >0.2; §<0.1, >0.05; *, <0.05, >0.01; **, <0.01, >0.001; ***, <0.001. Results expressed as percentage of profiles in which the various determinations were made.

new cross-wall formation was found to be 0.506 μ ($\pm$0.091); the distance to the furthest point at the opposite end of the profile was 0.435 μ ($\pm$0.54) (t_{24} = 0.678, P = <0.6, >0.5). This result does not support the view that donor DNA entered the cell preferentially at new cross-wall formations.

In the second experiment, the total number of membranous bodies observed in profiles of cells of the subculture from which DNA was omitted was found to increase continuously during maximal competence to 1.7 times the number found in any previous stage of culture, compared with a similar increase of up to 1.3 times in profiles of cells of the subculture supplemented with donor DNA. Also, during maximal competence, the number of membranous-body connections between the plasma membrane and the nuclear region in cells of the subculture lacking DNA was found to increase to 30 times the number found at earlier stages of culture, compared with 15 times in cells of the culture to which DNA was added. The presence of donor DNA in the culture medium was not, therefore, a prerequisite for the increases in membranous bodies to take place, but appeared, in fact, to partially inhibit body increase.

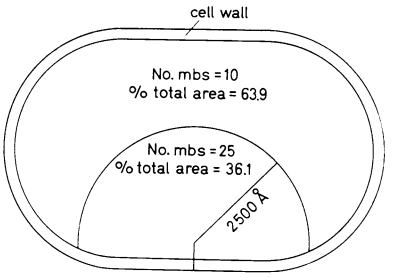

FIG. 13. *Bacterial profile constructed from the mean maximal and minimal dimensions, corrected for the presence of more than one membranous body (mbs), of membranous body-containing profiles of sample 7. The arc of radius 2,500 A is drawn from a point representing the mean position of the center of the grains relative to the outer limit of the cell wall.*

DISCUSSION

In *B. subtilis* and *D. pneumoniae*, only one strand of a donor DNA molecule taken into a cell appears to be incorporated into the recipient genome (10, 23). There is little information at the present time concerning either the fate of the unincorporated strand or the percentage of DNA absorbed which is physically integrated as an intact single strand in *B. subtilis*. For *D. pneumoniae*, however, estimates of absorbed DNA which bring about transformation vary from 20 to 50% (4, 10, 12), and evidence has been presented (10) that the remainder is degraded, and that at least some of the products are incorporated into the nuclear DNA by normal synthetic pathways. It is possible, therefore, that in the present experiment the activation of grains may have resulted from radioactivity resident in a variety of molecular species originating from absorbed donor DNA. The finding that the mean position of the grains in the autoradiographs is nearer to the nuclear edge with time indicates that, in *B. subtilis*, the H^3-labeled molecules which result from degradation of absorbed donor DNA not in-

TABLE 2. *Data concerning the relative distribution of membranous bodies and silver grains in profiles of each of the samples taken during the phase of maximal competence*

Determination	Sample 7 (15 min after DNA addition)	Sample 8 (30 min after DNA addition)	Sample 9 (60 min after DNA addition)
Number of membranous body-containing profiles	35	38	40
Mean diameter of profiles (corrected for presence of more than one body)			
Maximal	8,750 A	9,200 A	9,880 A
Minimal	5,090 A	5,490 A	5,540 A
Mean distance of the center of the grains from the outer edge of the cell wall	575 A	657 A	1,255 A
Percentage area of profile contained within a radius of 2,500 A from mean position of center of grains	36.1	31.5	35.2
Percentage membranous bodies found completely within a radius of 2,500 A from mean position of center of grains	71.4	63.2	57.5
Chi-squared for deviation in expected and observed numbers membranous bodies lying within and outside 2,500-A arc	19.0 (P, <0.001)	17.7 (P, <0.001)	8.7 (P, <0.01, >0.001)

corporated as an intact single strand must eventually be either incorporated into the nuclear material or lost from the cell.

The close association of molecules originating from H^3-thymidine-labeled donor DNA and the membranous bodies, indicated by the autoradiography data, suggests that the membranous bodies are essential to some process concerning the donor DNA after its entry into the bacterial cell. This process seems to involve primarily cytoplasmically situated membranous bodies, since the nearest membranous body to the grain is more often in the cytoplasm and less often in the nuclear region than expected. The finding of grain-associated profiles which do not contain membranous bodies in each of samples 7, 8, and 9 indicates that at least some of the products of absorbed donor DNA are not confined within the membranous bodies; a consideration of the distribution of grains in membranous body-containing profiles, relative to the nearest membranous body, leads to the conclusion that most if not all of the radioactive molecules are either on the edge of, or outside, the bodies, rather than being distributed at random within them [c.f. the postulation of Miller and Landman that "DNA enters the cell interior via the mesosome" (Mendel Memorial Symp. Prague II, p. 36, 1965)]. The noted decrease in chi-square with time for the measure of a close association of radioactive source and membranous bodies indicates this association to be transient. The fact that after 1 hr of incubation the association can still be demonstrated suggests, however, that it is maintained for some time.

There is evidence that the membranous bodies are the mitochondrial equivalents of *B. subtilis* (11, 17, 22). It seems plausible, therefore, that their association with the molecules resulting from donor DNA reflects their involvement in producing enzymes necessary for one or more of the processes essential for the eventual incorporation of donor DNA into the bacterial genome, and the further utilization or expulsion of breakdown products of DNA not incorporated as an intact single strand.

Since the noted increase in volume of membranous bodies takes place in the absence of donor DNA, and since such increases are not found to accompany the continuous increase in competence of the population which takes place during the 90-min period after dilution (Venema, *unpublished data*), it must be an expression of the physiological state of the cells during the phase when the population is maximally competent. The increase in membranous bodies may be a response to the low concentration of nutrients present in the growth medium by the beginning of maximal competence and the continuing decrease in nutrients during this phase. This would imply that an increase in body volume is necessary for normal enzyme production to continue and,

plausibly, for the maximal level of competence to be maintained, after the point when specific exogenous nutrients reach a certain minimal concentration. Evidence has been presented (14) that the membranous bodies forming connections between the nuclear region and the plasma membrane are instrumental in division of the bacterial genome. Because the rate of division of cells of the strain examined does not increase during the phase of maximal competence (Vermeulen, *unpublished data*), the noted increase in membranous-body connections (of which the total increase in membranous bodies must be, at least in part, a function) suggests that, at least at this time, they have an additional function. Their formation may be a general means of facilitating rapid exchange of materials throughout the cytoplasm, and also a means of ensuring close association between the membranous bodies and the molecules resulting from absorbed donor DNA during the intracellular passage of the latter. The initial association of membranous body and donor DNA could be ensured by the membranous bodies determining the point of entry of the donor DNA into the cell.

The apparent inhibitory effect of donor DNA on membranous-body increase may be a reflection of an overall decrease in enzyme-mediated activity after absorption of the DNA.

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