MEMBRANE ASSEMBLY IN *ESCHERICHIA COLI* I. SEGREGATION OF PREFORMED AND NEWLY FORMED MEMBRANE INTO DAUGHTER CELLS

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SUMMARY. Density labeling of the lipid phase of membranes has been used to test the possibilities that the cytoplasmic membrane of *Escherichia coli* is assembled at one or two fixed foci, either at the equitorial perimeter or at the poles of the organism. Synchronized cultures of *E. coli* were switched from light to heavy medium, and samples were removed after either one or two cell division cycles of growth in heavy medium and processed by equilibrium density gradient centrifugation. No separation of definitively light or heavy membrane bands was observed indicating that preformed and newly formed membranes are not assembled at one or two fixed foci and then segregated with conservation of structure.

An attractive model proposed to explain the mechanism of chromosome segregation in bacteria is based on the assumption that DNA is bound to membrane, and that the membrane grows at a fixed focus (1). The binding of DNA at specific sites on the chromosomes of *E. coli* and *Bacillus subtilis* to the bacterial membrane has been reviewed (2). Two recent articles conclude with statements to the effect that the membranes of *E. coli* (3) and *Bacillus megaterium* KM (4) grow by extension at the poles of the cells with concomitant conservation of preformed and newly formed membrane structure. Experiments with an unsaturated fatty acid auxotroph of *E. coli* indicate that certain proteins are incorporated into membranes preferentially with newly synthesized lipids and remain associated with the lipids with which they were initially incorporated into the membrane matrix (5). Models of mem-

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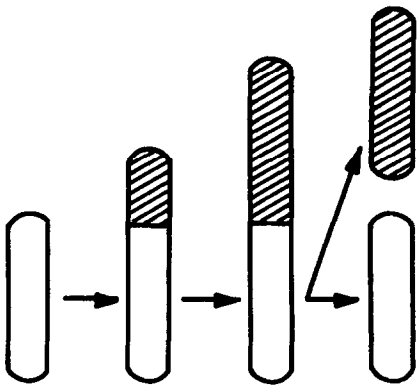
brane growth at one or a few fixed foci (1-3) are consistent with these experimental data. The development of a density labeled precursor for lipids has enabled us to test directly models for membrane growth at one or two distinct foci using synchronized cultures of an unsaturated fatty acid auxotroph of *E. coli* (6).

METHODS. Strain 30E, an unsaturated fatty acid auxotroph of *E. coli* K 12 (5-6) was grown in medium A (7) supplemented with 0.5% Difco casamino acids, 0.25% of the detergent Triton X-100 (Rohm and Haas), 5 µg/ml of thiamine•HCl, and 0.02% of the indicated fatty acid. Oleate medium contained oleic acid (Hormel Institute, Austin, Minn.) and bromostearate medium, bromostearic acid which was synthesized as described previously (6). A synchronized population of newly divided daughter cells was obtained by a modification (8) of the Mitchison-Vincent procedure (9). The bacteria growing in oleate medium were labeled with 0.5 µCi/ml of ^{3}H-isoleucine (New England Nuclear) for 3.5 generations of growth in the experiment described in Figure 1, and with 1.0 µCi/ml for the experiment described in Figure 2. After switching to growth in bromostearate medium, the cells were labeled with ^{14}C-isoleucine (New England Nuclear) at 0.5 µCi/ml in both experiments.

RESULTS. Model I considers growth from a single pole with conservation of old and new membrane structure giving rise to two daughter cells, one



MODEL I. Unidirectional growth of membrane from a single pole of a newly divided daughter cell with conservation of preformed and newly formed membrane, detectable after one generation of growth following a density shift.

with a membrane composed of material made prior to the last generation of growth, the other with a membrane composed of material made entirely during the last generation of growth. To test Model I, a synchronized culture of cells grown in oleate (light) medium containing a ^{3}H-amino acid was switched to bromostearate (heavy) medium containing a ^{14}C-amino acid. After one round of cell division, growth was arrested, and the cytoplasmic membrane fraction was prepared and subjected to isopycnic banding in a linear sucrose density gradient (Figure 1). No distinct separation of light (^{3}H-labeled) and heavy (^{14}C-labeled) membranes was observed. In contrast, when two batches of cells, one light and ^{3}H-labeled and the other heavy and ^{14}C-labeled are mixed and treated in an identical fashion, a distinct ^{3}H-labeled

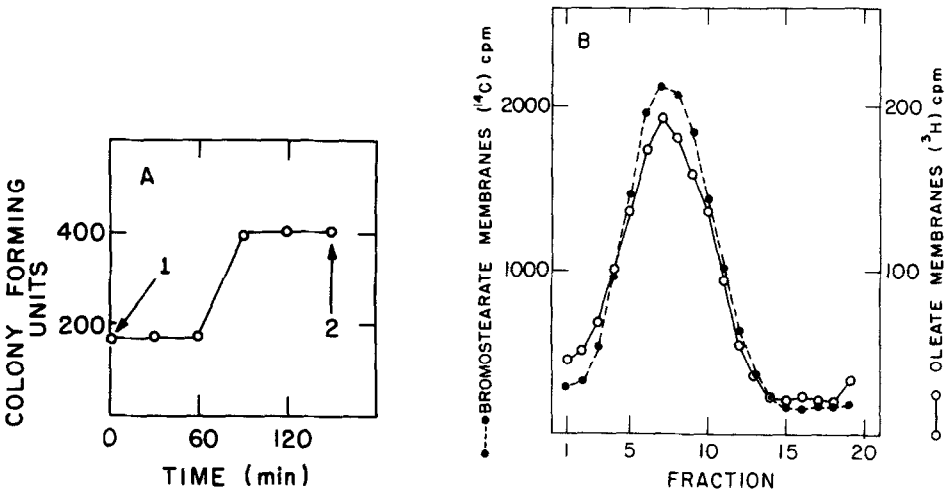
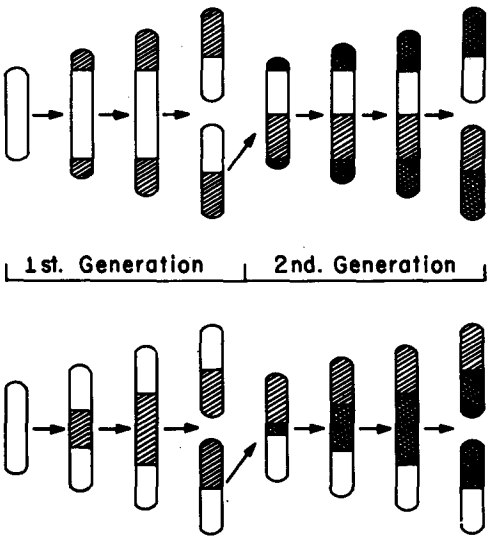


FIGURE 1. *TEST OF MODEL I.* A synchronized population of newly divided daughter cells labeled with ^{3}H-isoleucine during growth in oleate medium was suspended at a cell density of approximately 1.5×10^{8} cells per ml in bromostearate medium containing ^{14}C-isoleucine (Figure 1A, arrow 1). An aliquot was removed at this point and at 30 minute intervals, and 0.1 ml of a 10^{5} dilution was plated to determine the cell population density. After the first cell division, but before the second (arrow 2), growth was arrested by the addition of chloramphenicol and by chilling to ice bath temperature, and the cytoplasmic membranes were prepared (5). The cytoplasmic membranes were banded by equilibrium centrifugation in a 60% to 35% sucrose density gradient for 20 hours at 45,000 rpm in the Spinco SW50.1 rotor (Figure 1B). Fractions of the gradient were collected from the bottom of the tube after piercing with a needle. The most dense fraction is fraction 1. Radioactivity in the fractions was determined by liquid scintillation counting employing the solution described by Patterson and Green (10) containing 3:1 toluene-Triton X-100.

light peak is observed at about fraction 10 of the gradient, and a distinct ^{14}C-labeled heavy peak at fraction 5. These data make it possible to reject Model I.

Model II considers growth from the equitorial perimeter or from both poles with conservation of old and new membrane structures. In contrast to



MODEL II. Bidirectional growth of membrane from the equitorial perimeter or growth at both poles of newly divided daughter cells with conservation of preformed and newly formed membrane, detectable after two generations of growth following a density shift.

Model I, it is necessary to allow at least two rounds of cell division before a density difference can be expressed. The experimental test of Model II, however, gives no evidence of any density separation between membrane formed before and after the density shift (Figure 2).

DISCUSSION. The experiments described here indicate that the models of membrane growth proposed by others and considered here can be used to explain chromosome segregation only with modification. Our data contrast sharply with the autoradiographic studies of Morrison and Morowitz which suggest that the newly synthesized lipids incorporated into membrane during a pulse-label experiment are localized at the poles of *B. megaterium* KM (4).

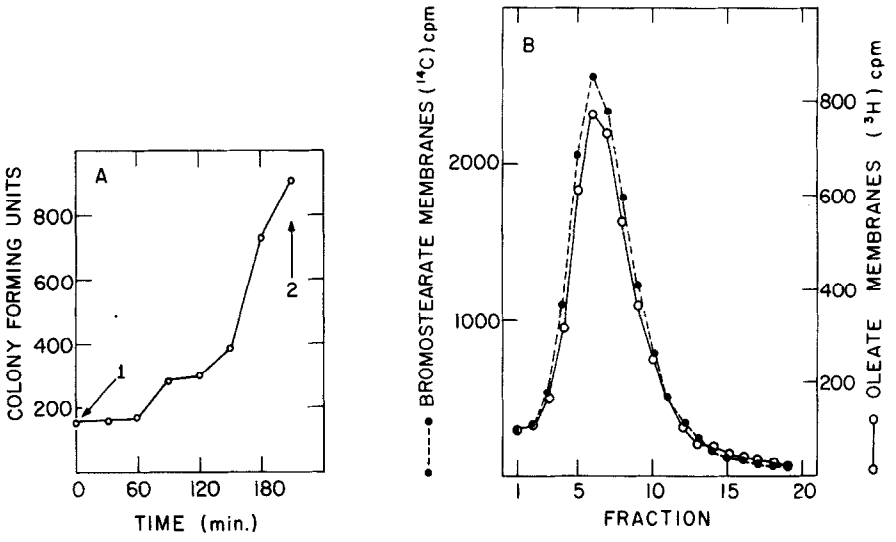


FIGURE 2. *TEST OF MODEL II.* The experimental conditions are essentially those of Figure 1, except that the cells used for the preparation of membranes were processed after two generations of growth (Figure 2A, arrow 2) following the shift (Figure 2A, arrow 1) from oleate medium containing ^{3}H-isoleucine to bromostearate medium containing ^{14}C-isoleucine. The conditions for isopycnic banding of membranes (Figure 2B) are identical to those given for Figure 1B.

Mindich, however, has shown by autoradiography that the pulse-label incorporation of a radioactively labeled lipid precursor into the membrane lipids of *Bacillus subtilis* does not result in any distinct topographical localization of the isotope (11). Certainly, the incorporation of lipids and proteins into the *E. coli* membrane does not occur at one or two fixed foci with concomitant conservation of structure of old and newly synthesized membrane. It is conceivable, of course, that lipids and proteins of membranes might be assembled at fixed points, and that the lipids might then diffuse laterally throughout that monolayer of the bilayer structure into which they were initially incorporated. Diffusion of this sort has recently been observed with magnetic resonance techniques in small (300 Å in diameter) lipid vesicles, but has not yet been detected in membranes of biological origin (12). Since lipids of membranes might diffuse without the accompanying diffusion of membrane proteins, we have exploited the properties of a

minicell producing strain of *E. coli* to test the described membrane assembly models (Models I and II), modified so that only the membrane proteins need be accounted for. These experiments are described in the accompanying paper by Wilson and Fox.

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