JOURNAL OF BACTERIOLOGY, May 1969, p. 784-796

Vol. 98, No. 2
Printed in U.S.A.

Effects of Oleate Starvation in a Fatty Acid Auxotroph of *Escherichia coli* K-12

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Received for publication 27 February 1969

The effects of oleate starvation on an oleate auxotroph of *Escherichia coli* K-12 were investigated. Following removal of oleate from the mutant growing in a minimal glycerol-peptone medium, the cells stopped making deoxyribonucleic acid, ribonucleic acid, protein, and phospholipids; they began to die exponentially and finally lysed. During oleate starvation in minimal medium minus peptone, inhibition of macromolecular syntheses and death occurred; however, lysis did not follow. When growth ceased, no further dying was observed. It is shown that none of the early effects (inhibition of macromolecular syntheses and death) can be due to leakiness of the cells, induction of a prophage or a colicin, or lack of energy sources. The cause of inhibition of macromolecular syntheses remained unknown. Since the rate of death was the same as the generation time under different conditions, it appears that death is due to the defective synthesis of some cellular structure (quite possibly, cytoplasmic membrane) during phospholipid deficiency. Lysis was found to require protein synthesis; electron microscopy revealed a peculiar type of "lysis from within"; i.e., the shape of the cells did not change but fragmentation of the inner layer of the cell envelope occurred. The murein was found to be unaltered. Most likely, lysis was a consequence of the cell's attempt to synthesize cytoplasmic membrane with altered phospholipid composition or during phospholipid deficiency. Several membrane functions (respiration, adenosine triphosphate formation, permeability) existing before oleate removal were not lost during starvation. Therefore, general damage to the membrane did not occur, and it could be that most, if not all, described effects were due to defective de novo membrane synthesis.

In the course of studies on the synthesis of *Escherichia coli* succinate dehydrogenase, which is bound to the cytoplasmic membrane (22), we obtained some indirect evidence that control of the synthesis of the enzyme may be coupled with control of membrane growth. To test this possibility, we wished to differentiate between membrane synthesis and synthesis of soluble proteins. It was thought that one approach could consist in the use of mutants which would be unable to grow under certain conditions but would, at least for some time after arrest of growth, exhibit unaltered inducible enzyme synthesis. A mutant of the desired type was envisioned in an oleate auxotroph, as isolated recently by Silbert and Vagelos (24) and by Overath et al. (14). One might assume that upon removal of the fatty acid from the medium such a mutant must stop growth because of the lack of one class of building blocks for the cell envelope. Possibly, the synthesis of a number of different components of the cell envelope is regulated in such a way that the amount of each component available meets the requirements of the assembly process. Deficiency of one component may then cause inhibition of the synthesis of other components, and growth inhibition should follow. Synthesis of soluble proteins could still be possible for a while after arrest of growth, as is the case with spheroplasts (19).

We found, however, that these considerations do not apply if the missing component is an unsaturated fatty acid. In an oleate auxotroph, protein synthesis decreased fairly rapidly—and *before* growth stopped—after deprival for oleate. This somewhat unexpected phenomenon prompted a more detailed study on the effects of oleate starvation in the mutant.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The *E. coli* K-12 parental strain was a thiamine-requiring HfrH. The oleate-requiring auxotroph was obtained

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from this strain after treatment with diethylsulfate followed by penicillin selection in a glucose-minimal medium. As will be seen from the properties of the mutant described here, recovery of this mutant would not have been expected with the penicillin selection procedure. [We have not determined the enzymatic defect in the mutant; most likely it is the same as is present in the oleate auxotroph reported by Silbert and Vagelos (24); they found that their mutant lacked β-hydroxy-decanoyl thioester dehydrase activity. This mutant (donated by D. F. Silbert) was compared with ours concerning the properties described in Fig. 1 and 2. They behaved indentically. Another oleate auxotroph (isolated from *E. coli* K-12, strain YMel, donated by P. Overath) also showed the characteristics depicted in Fig. 1 and 2.]

Minimal medium was the tris(hydroxymethyl)-aminomethane medium of Weigert and Garen (27; with potassium phosphate concentration changed to 6.4×10^{-4} M) supplemented with glycerol (0.5%), potassium oleate (100 μg/ml), peptone (Difco; 0.25%), and thiamine (1 μg/ml). Complete medium was a glucose-containing broth (Antibiotic Medium no. 3, Difco). In all experiments, cells were grown at 37 C with vigorous aeration. Turbidity was measured at 578 nm (1-cm light path, Eppendorf photometer).

Analytical procedures: deoxyribonucleic acid (DNA) synthesis. From cultures growing in glycerol medium (cf. Fig. 4), 6.5-ml samples were removed and further aerated after addition of ^{3}H-thymidine (1.9 c/mmole; Schwarz BioResearch, Orangeburg, N.Y.) at 2 μc/ml. At 2, 5, and 10 min after addition of thymidine, 2-ml samples of the labeled culture were processed as described by Carr and Rosenkranz (2). The rate of incorporation was found to be linear with time over the interval measured. The amounts of ^{3}H-thymidine incorporated after 2, 5, and 10 min were used to calculate the rate of DNA synthesis per 1 ml of culture per 0.1 optical density (OD) (578 nm; turbidity of the culture).

Ribonucleic acid (RNA) synthesis. For RNA synthesis, 6.5-ml samples were further aerated after addition of 2-^{14}*C*-uracil (7 mc/mmole; New England Nuclear Corp., Boston, Mass.) at 0.5 μc/ml. Samples (2 ml) of the labeled culture were removed 5, 10, and 20 min after uracil addition, mixed with 2 ml of cold 10% trichloroacetic acid and filtered through membrane filters (Millipore Corp., Bedford, Mass., HAWP 02500; pore size 0.45 μm). The filters were washed two times with 10 ml of cold 5% trichloroacetic acid and twice with 10 ml of water-saturated ether. The filters were dried and counted. Rate of incorporation was also found to be linear with time up to 20 min of labeling. The amounts of ^{14}C-uracil incorporated after 5, 10, and 20 min were used to calculate the rate of RNA synthesis the same way as described for DNA synthesis.

Protein synthesis. For protein synthesis, 1-ml samples were further aerated after addition of ^{14}C-L-leucine (uniformly labeled, 22 mc/mmole; New England Nuclear Corp.) at 0.1 μc/ml; 0.1-ml samples of the labeled culture were removed 5, 10, and 15 min after leucine addition and mixed with 2 ml of cold, 10% trichloroacetic acid. The suspension was further treated as described for RNA synthesis, except that 10% trichloroacetic acid was used.

In all cases, the dry membrane filters were placed in glass vials with a 0.4% solution of Omnifluor (New England Nuclear Corp.) in toluene, and radioactivity was measured with a Tri-Carb liquid scintillation spectrometer-3002 (Packard Instrument Co., Inc., Downers Grove, Ill.).

Pool size of adenosine triphosphate (ATP). A 20-ml amount of the growing culture was removed and further aerated after addition of carrier-free ^{32}P-orthophosphate (Buchler, Braunschweig, Germany) at 10 μc/ml, yielding a specific radioactivity in the medium of 16.7 mc/mmole. Radioactivity of the ATP, normalized to an OD (578 nm, turbidity of the culture) of 0.1, did not increase after 10 min of labeling; therefore, all pool size determinations were performed after growth for 15 min in the presence of radioactive phosphate. Labeled cells were spun, without cooling, as rapidly as possible (about 2 min). The pellet was suspended with 2 ml of cold 0.1 M HCl containing 2 μmoles of carrier ATP. After 5 min in an ice bath, the cells were removed by centrifugation and again extracted with 2 ml of 0.1 M HCl. (No more ATP could be recovered from the cells by additional washings with HCl.) The combined HCl extracts were dried in vacuo. From 10 to 20% of the residue dissolved in water was chromatographed on plastic sheets coated with polyethyleneimine-treated cellulose according to the method of Neuhard et al. (13). (Solvents I-2, I-3a, and I-3b were used.) Adsorption of the HCl extracts to charcoal was found to be unnecessary for satisfactory resolution of ATP. ATP was located with ultraviolet light, cut out, and counted in a gas flow Packard Radiochromatogram Scanner-7200. The area under the ATP peak was measured planimetrically, converted to nc, and normalized to an OD (578 nm) of 0.1, as mentioned above. [It has been shown by Cole et al. (3) that ATP can be lost from the cells quite easily. We have not determined the dry weight of our cultures; however, assuming one cell weighs 10^{-12} g, it can be calculated that the ATP concentrations found fall in the same order of magnitude as those reported by these authors.]

Phospholipid synthesis. Samples (20 ml) were removed from growing cultures and further aerated after the addition of carrier-free ^{32}P-orthophosphate at 2 μc/ml (specific radioactivity in the medium, 3.4 mc/mmole).

The rates of phospholipid synthesis were found to be linear with time up to 20 min of labeling and became exponential at approximately 30 min. Therefore, labeling was performed in all cases (cf. Fig. 1 and 2) for 5, 10, and 20 min.

^{32}P-Labeled phospholipids were extracted by the method of Kanfer and Kennedy (6). Extractable phospholipids were chromatographed on thin-layer plates, as described by Rouser and Fleischer (16). Solvents used were chloroform-methanol-water (65:25:4, first dimension) and *n*-butanol–acetic acid–water (60:20:20, second dimension). Autoradiography revealed that chromatography in the second dimension was not necessary; cardiolipin, phosphatidylethanolamine, phosphatidylglycerol, and (usually only traces

of) phosphatidylserine were well separated in the first dimension, and no additional radioactive compounds were found after chromatography in the second direction. We did not observe that phosphatidylglycerol would give rise to two spots, one of which might have moved together with phosphatidylethanolamine as described by Kanemasa et al. (5). Individual phospholipids were identified as follows. Phosphatidylethanolamine and cardiolipin were identified by comparison with pure substances (phosphatidylethanolamine from the California Corporation for Biochemical Research; cardiolipin from *E. coli*, a generous gift from W. Stoffel). Phosphatidylglycerol was identified by its R_F values and absence of a positive ninhydrin reaction. Phosphatidylserine was present in all cases in such low amounts that its contribution to total phospholipid was negligible. It has not been measured quantitatively.

Electron microscopy. Bacteria were treated according to the method of Ryter et al. (18) of fixation, dehydration, and embedding in Vestopal 310 (a gift from the Chemische Werke, Hüls). Ultrathin sections were performed with an LKB Ultrotome, using glass knives with a knife angle of 35°. Sections were placed onto grids coated with carbonized Formvar. The thickness of specimens, determined by measuring the width of wrinkles in the sections (M. K. Reedy, *personal communication*), was found to be in the range of 40 to 80 nm. Sections were stained with lead citrate (15; Fig. 8, 9, 11, 17, 18) or with 5% uranyl acetate in 50% methanol (Figs. 10, 12–14). For shadowing, specimens were prepared with the agar filtration technique (8) and shadow-cast with platinum alloy 1010 (Usine Genevoise de Dégrossissage d'Or, Genève). Preparations were examined with a Siemens Elmiskop I at 80 kv with double condensor illumination using a 50-μm objective aperture. Micrographs were taken at instrumental magnifications of 3,500, 8,000, 20,000, and 40,000. Magnification was checked with a diffraction grating replica (Fullam, 2160 lines/mm).

RESULTS

Growth, survival, and macromolecular syntheses in the mutant during oleate starvation. After removal of oleate, the size of the population, growing in peptone-glycerol medium, increases about fourfold; subsequently, the cells begin to lyse. The rates of ^{14}C-leucine incorporation and of ^{32}P-phospholipid synthesis decrease earlier than visible growth (Fig. 1).

The decrease of phospholipid synthesis is not the same for all individual phospholipids (Fig. 2). Cardiolipin synthesis, barely detectable before oleate removal, has increased about 10-fold 2.5 hr after oleate removal and constitutes almost one-half of the total extractable phospholipid. This situation is specific for the mutant. In an identical experiment with wild-type cells, the ratios of the individual phospholipids did not change much upon removal of oleate.

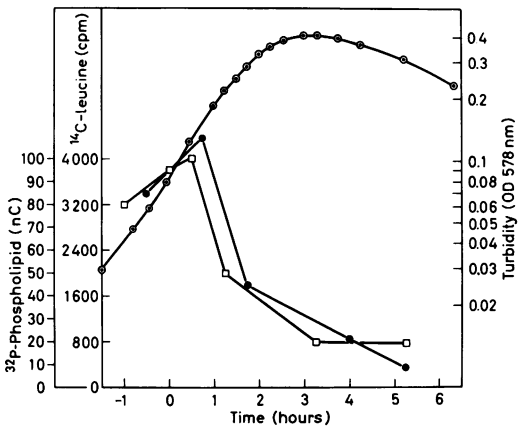

FIG. 1. *Effects of oleate starvation on the mutant in glycerol-peptone medium. The syntheses measured are expressed as rates of isotope incorporation. ^{14}C-Leucine incorporation(□)is given as counts/min per ml of cells, normalized to an OD of 0.1 (578 nm, turbidity of culture; ◉); ^{32}P incorporation (●) is given as nc per ml of cells, normalized to an OD of 0.1. Oleate was removed at time zero.*

Since the cells eventually lyse, the explanation for the behavior shown in Fig. 1 might be trivial; leakiness for intracellular substances as ions or energy sources may begin soon after oleate deprival and cause the observed decrease in the rate of protein synthesis. However, lysis is not an obligatory consequence of oleate starvation, at least within a certain time range. Whereas the cells lyse in complete glycerol-peptone and glucose media shortly after the growth rate decreases, they do not do so in the glycerol medium for at least 8 hr after removal of oleate. It was found later, by accident, that this property can be lost. The mutant had often been reisolated after growth on glycerol medium, and the behavior depicted in Fig. 3 A1 could not be reproduced. The population lysed shortly after cessation of growth. The "old" mutant grew with a mean doubling time of about 3 hr, the "new" mutant with one of about 2 hr. Thus, selection of a variant with faster growth on the glycerol medium had occurred. It appears, therefore, that whether lysis occurs sooner or later (or more likely, not at all) after cessation of growth in the absence of oleate is a function of the generation time rather than of certain constituents of the medium. All experiments described have been performed with the "old," slow-growing mutant.

Figure 3 demonstrates the growth physiology of the mutant during oleate starvation in broth and in the glycerol medium. Two facts of interest may be noted. First, the increase of turbidity is

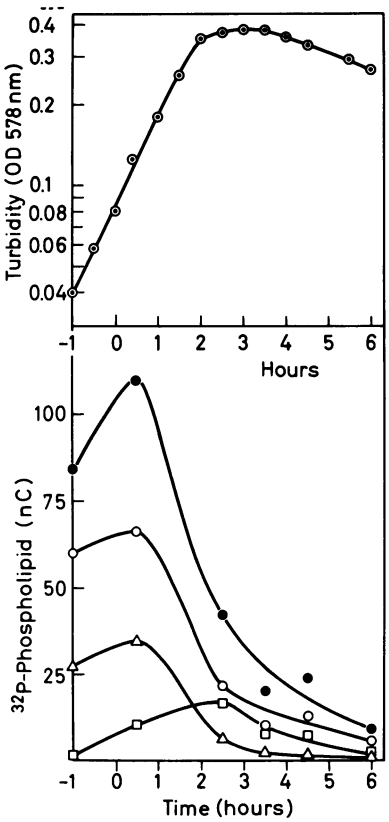

FIG. 2. *Rates of synthesis of individual phospholipids. From the mutant cells, growing in glycerol-peptone medium, oleate was removed at time zero. Symbols:* ●, *total extractable phospholipid;* ○, *phosphatidylethanolamine;* △, *phosphatidylglycerol;* □, *cardiolipin.* ^{32}P *incorporation was calculated as indicated in Fig. 1.*

paralleled by the increase of the cell count but not by the increase of cells able to form colonies. The concentration of colony formers in the minimal medium remains fairly constant up to 8 hr after oleate removal. Second, the cell count does not decrease, although the culture lyses; i.e., the cells remain easily visible in the phase contrast microscope (but become transparent). Since lysis does not interfere with observation in the glycerol medium, macromolecular syntheses were followed before and after oleate removal under this growth condition. (Fig. 4). DNA and RNA syntheses, after an initial period of acceleration, decrease more rapidly than protein synthesis.

Thus, we can distinguish three different effects of oleate starvation: (i) inhibition of DNA, RNA, protein, and phospholipid syntheses; (ii) death; and (iii) lysis.

One may conceive of four entirely different mechanisms causing the early effects (inhibition of macromolecular syntheses). (i) As mentioned already, the cells may become leaky. (ii) Oleate removal may lead to induction of a defective prophage or a colicin. (No plaque-forming particles were found in the supernatant fluid of a lysed culture.) Such a process could be less effective in minimal medium than in broth. (iii) Some direct effect on the cytoplasmic membrane may be followed by inhibition of macromolecular syntheses and death. (iv) The early effects may be due to a control mechanism, such as one observes upon removal of a required amino acid (cessation of DNA and, in the stringently controlled state, of net RNA synthesis). Specifically, it has been shown (25) that total lipid synthesis is also subject to this stringent control in *E. coli*.

Evidence against leakiness of cells starved for oleate. A general leakiness for intracellular substances does not follow deprival for oleate. Cells uniformly labeled with ^{32}P or ^{14}C-leucine before oleate removal do not, on any medium, release radioactivity upon oleate removal as long as no lysis occurs. Appearance of label in the medium occurs only and exactly at the time the turbidity begins to decrease. This fact does not exclude, of course, a specific leakiness, e.g., for K^+ ions. Such a possibility will be considered again below.

Evidence against prophage or colicin induction. Induction of a defective prophage or a colicin would have to be specific for oleate starvation. Neither the parent HfrH nor a revertant of the mutant could be induced to lyse by ultraviolet irradiation, treatment with mitomycin, or growth at 42 C. Such an induction, therefore, is extremely unlikely.

Evidence against a control mechanism causing all oleate starvation effects. Not only inhibition of macromolecular syntheses but also cell death may be caused by a control mechanism. If, for example, decreasing phospholipid synthesis were followed by inhibition of some other reaction, one (or a number of) toxic substance(s) might accumulate. The fraction of survivors in such a case would be expected to decrease exponentially with time. This is true (Fig. 3A), but only so long as growth continues. Therefore, a "toxic accumulation" appears to be very unlikely. Since growth is required for the cells to die (*see* following section), it appears much more reasonable to assume that the same type of mechanism kills the cells as the one which causes lysis.

Experiments concerning effects on the cytoplasmic membrane. In the mutant starved for oleate, we can envisage two kinds of effects on the cytoplasmic membrane (and, of course, other cell wall layers). It is known (5, 6) for *E. coli* that rapid turnover of phosphatidylglycerol occurs. Lack of substrates for such a turnover may lead

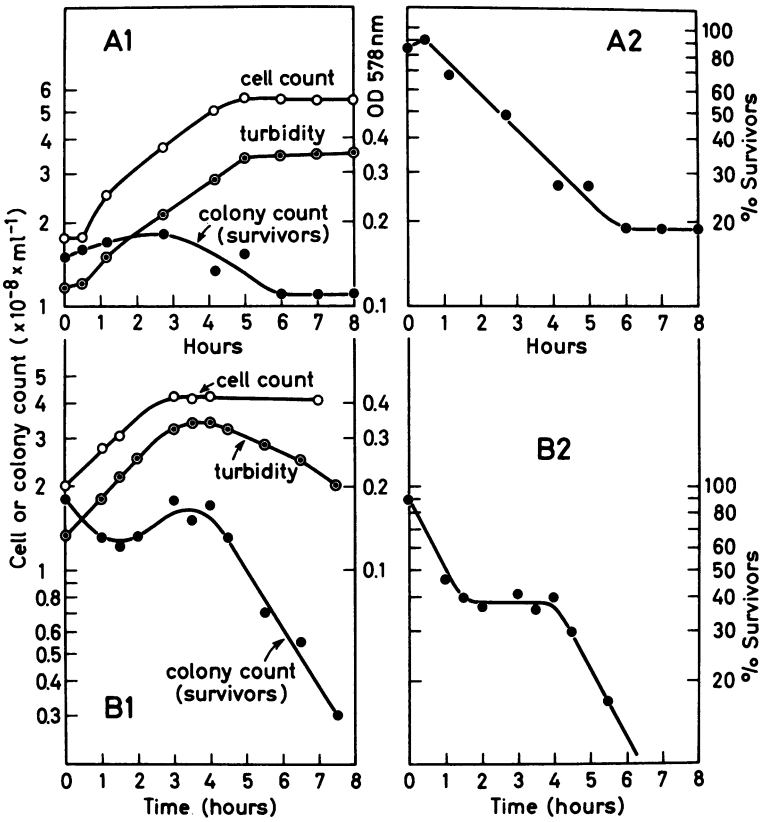

FIG. 3. *Physiology of growth of the mutant in different media in the absence of oleate. Oleate removal was at time zero. A1, glycerol medium; B1, glycerol-peptone medium; A2, death rate expressed as fraction of survivors of the whole population (cell count) in the glycerol medium; B2, same plot as A2 but for cells in the glycerol-peptone medium.*

to loss of some vital function of the membrane. Secondly, assembly of the membrane should be affected unless control mechanisms would lead to a cessation of this assembly as soon as one class of building blocks (e.g., phospholipids in this case) is no longer available.

To obtain some information on the first possibility, we asked whether preexisting membrane functions are lost during oleate starvation under conditions where no lysis occurs.

For cells growing aerobically on glycerol as carbon source, the main source of ATP production must be oxidative phosphorylation, a process which certainly is associated with the cytoplasmic membrane (7). Therefore, formation of ATP and respiration were measured in the mutant before and after removal of oleate during growth in glycerol medium. Data obtained for the pool size of ATP are shown in Fig. 5.

There is a rather drastic increase of intracellular ATP concentration after oleate removal. The ATP concentration reaches prestarvation values again after only about 3 hr of oleate deprival. (This result, of course, also proves that there is no loss of permeability for the carbon source, glycerol.) The same increase of ATP pool size was found in wild-type cells, pregrown in the same medium, upon removal of oleate. Whether oleate removal, or merely resuspension in the fresh medium, is responsible for the increase of ATP concentration, we do not know; it is obvious, however, that the decrease of macromolecular syntheses is not due to lack of ATP. The rate of succinate-dependent respiration was also found to be unaltered up to 6 hr of oleate starvation (measured manometrically; data not shown). We can conclude that a number of preexisting membrane functions such as respiration, phosphorylation and permeability—at least for glycerol and succinate—are not grossly altered when growth begins to cease. These results also argue rather strongly against a possibility mentioned before,

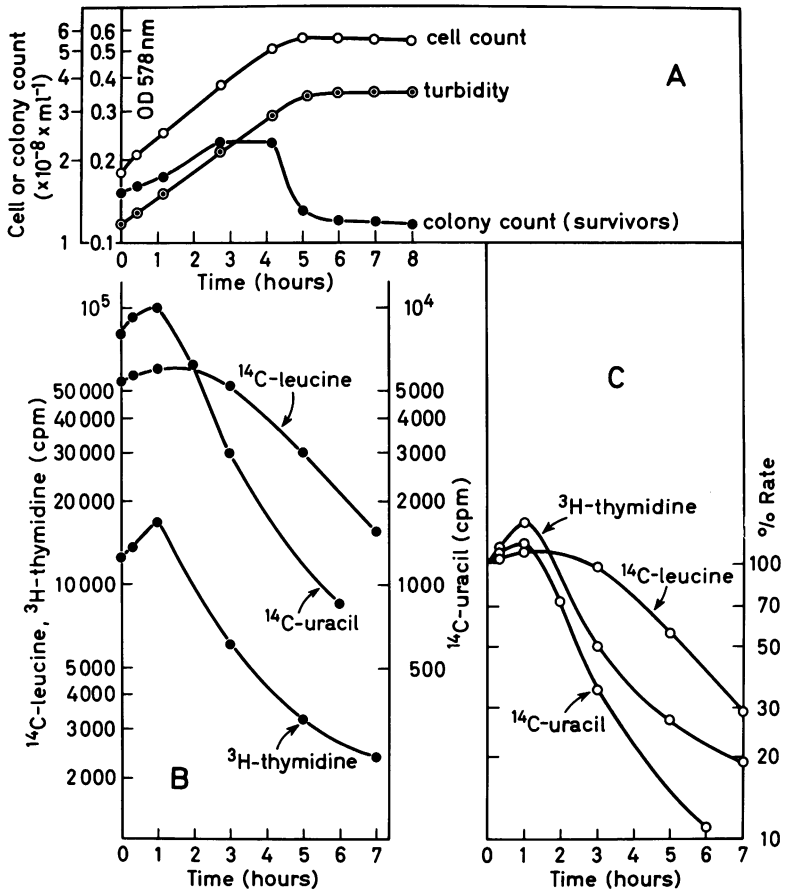

FIG. 4. *DNA, RNA, and protein syntheses in the mutant upon oleate removal. Oleate was removed from the cells, growing in glycerol medium, at time zero. (A) Physiology of growth. (B) Rates of incorporation of ³H-thymidine, ¹⁴C-uracil, and ¹⁴C-leucine. The values are counts/min incorporated per ml of cells, normalized to an OD of 0.1 (turbidity). (C) The same rates are expressed as per cent of the rates measured at time-zero.*

namely, selective leakiness for an intracellular substance (not containing phosphorus or leucine) as, e.g., certain ions. It seems very unlikely that a process such as oxidative phosphorylation should not respond to a significant change of ionic environment in the cell.

At a time when the growth rate begins to decrease, 50 to 80% of the population does not survive. At the same time, respiration or phosphorylation had not decreased significantly below prestarvation values. Thus, it appears quite safe to assume that all cells remained metabolically active when macromolecular syntheses were already strongly inhibited.

Technical difficulties are limiting for experiments aiming to answer the second question; namely, are effects on the growing membrane responsible for the consequences of oleate starvation? If so, one would expect protein synthesis to be required for the expression of these consequences. We found, however, that oleate present in cells pregrown in oleate-supplemented media could not be removed completely by any other means than starvation. Starvation causes cell death and inhibition of macromolecular syntheses; requirement of protein synthesis for these effects to be expressed, therefore, cannot be tested.

Lysis. Lysis requires protein synthesis. The mutant was grown in glycerol-oleate and starved in the same medium for oleate for 8 hr. If peptone was then added, lysis occurred (although only after a lag period of 4 hr). Lysis was completely prevented when chloramphenicol was added 1 hr before peptone. It should be stressed, however, that the demonstration of this complete effect of

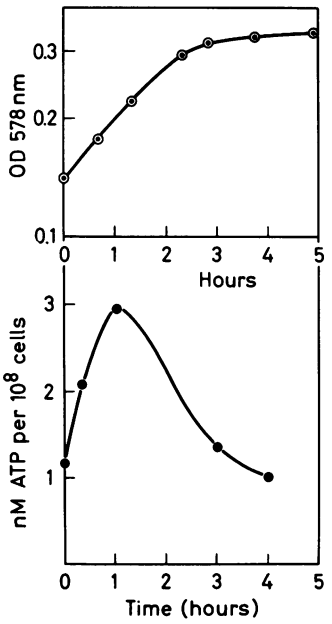

FIG. 5. *ATP pool sizes in the mutant during oleate starvation. Oleate was removed at time zero; the medium was glycerol. The calculation of nm ATP is described in the Materials and Methods.* ◉ = *turbidity of the culture.*

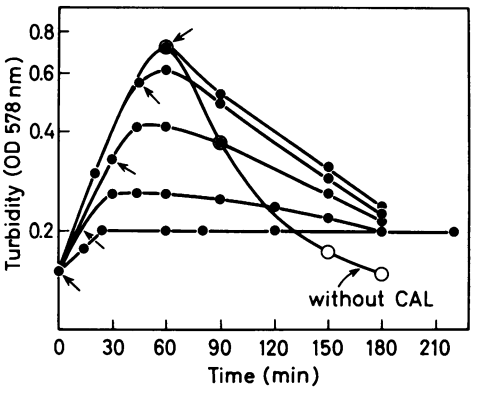

FIG. 6. *Effect of chloramphenicol on lysis. Oleate was removed from the nutrient broth at time zero. Chloramphenicol was added (200 μg/ml) to five cultures at the times indicated by arrows.*

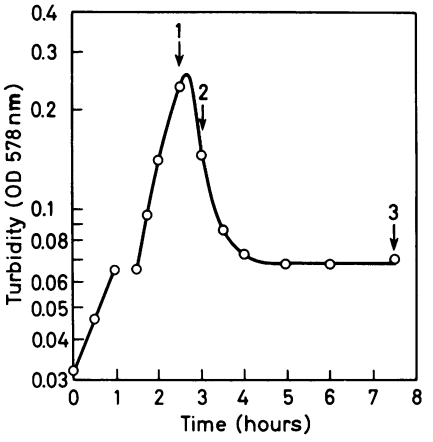

FIG. 7. *Growth of the mutant population used for the electron micrographs. Oleate was removed from the culture, growing in nutrient broth, at 1.5 hr. The arrows indicate removal of samples for electron microscopy; the numbers will be referred to in Fig. 8–16.*

chloramphenicol was not possible under all conditions of growth. When the drug was added to cells growing in glycerol-peptone, subsequent removal of oleate caused lysis in the presence of chloramphenicol, although the rate of lysis in this case was about 10-fold slower than in the absence of chloramphenicol.

Figure 6 demonstrates that lysis is an active process. Chloramphenicol, even when added to a culture already beginning to lyse in broth, considerably reduces the rate of lysis. Microscopic examination of the cultures of Fig. 6 showed that faster rates of decrease of turbidity were accompanied by the appearance of more lysed cells. Thus, a slow decrease of turbidity does not represent partial lysis of all cells but complete lysis of only a few cells.

Electron microscopy revealed that alterations in cells starved for oleate in broth concern the nuclear material and the cytoplasmic membrane. The distribution of nuclear material and the appearance of this membrane do not change visibly after oleate deprival before the beginning of lysis (Fig. 8), although at the time the sample of Fig. 8 was taken about 80% of the cells did not survive. In samples taken shortly before lysis, however, frequently mesosome-like structures or rather multiply reduplicated membrane aggregates were found (Fig. 8; cf. Fig. 13 and 14). Whether the formation of these aggregates is a consequence of oleate starvation could not be clarified. Similar structures, although much less clearly distinguishable from the background, were also found occasionally in mutant cells from cultures growing in oleate-supplemented media. They were never observed, however, in the parent HfrH strain.

Cells during and after completion of lysis are shown in Fig. 11 and 12, respectively. The predominant features are fragmentation of the cytoplasmic membrane and condensation of the nuclear material. Condensation does not appear

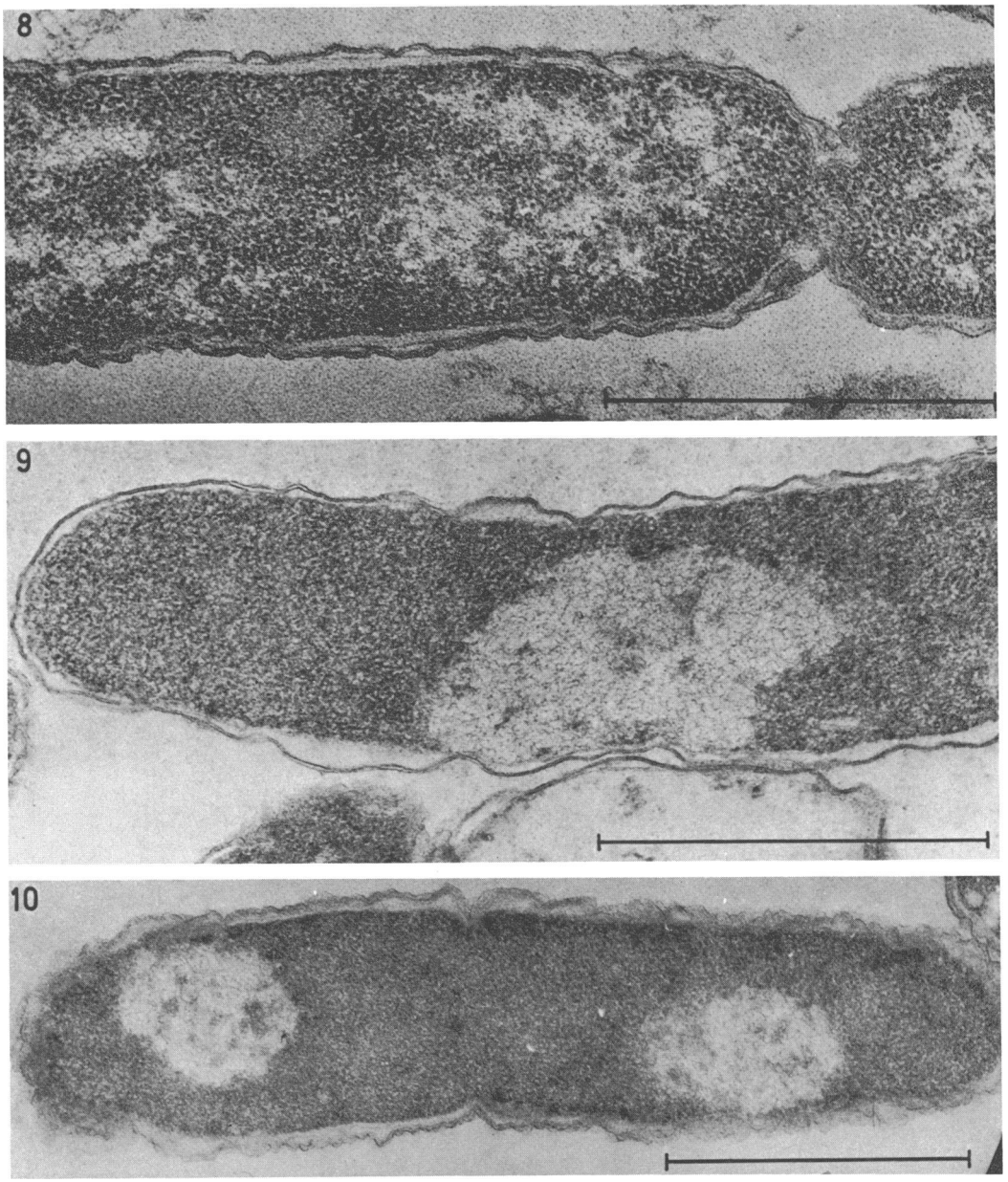

FIG. 8. *Single cell from a sample removed shortly before the onset of lysis (arrow 1 of Fig. 7). M represents a mesosome-like structure. The line in all micrographs, with the exception of Fig. 14, is 1 μm.*

FIG. 9. *Single cell from the lysing culture (arrow 2 of Fig. 7).*

FIG. 10. *Cell from lysing culture (arrow 2 of Fig. 7) with two condensed nuclear bodies.*

to necessarily precede lysis; the finely distributed state of nuclear material seen in two cells on the left margin of Fig. 11 can pertain. It is of interest that in final stages the condensed nuclear body is almost always found in close association with the cytoplasmic membrane (Fig. 9 and 10). It also appears that this association occurs at the site where cell division may begin; Fig. 10 seems to indicate that two nuclear bodies are attached to sites determined not for the next but for the next

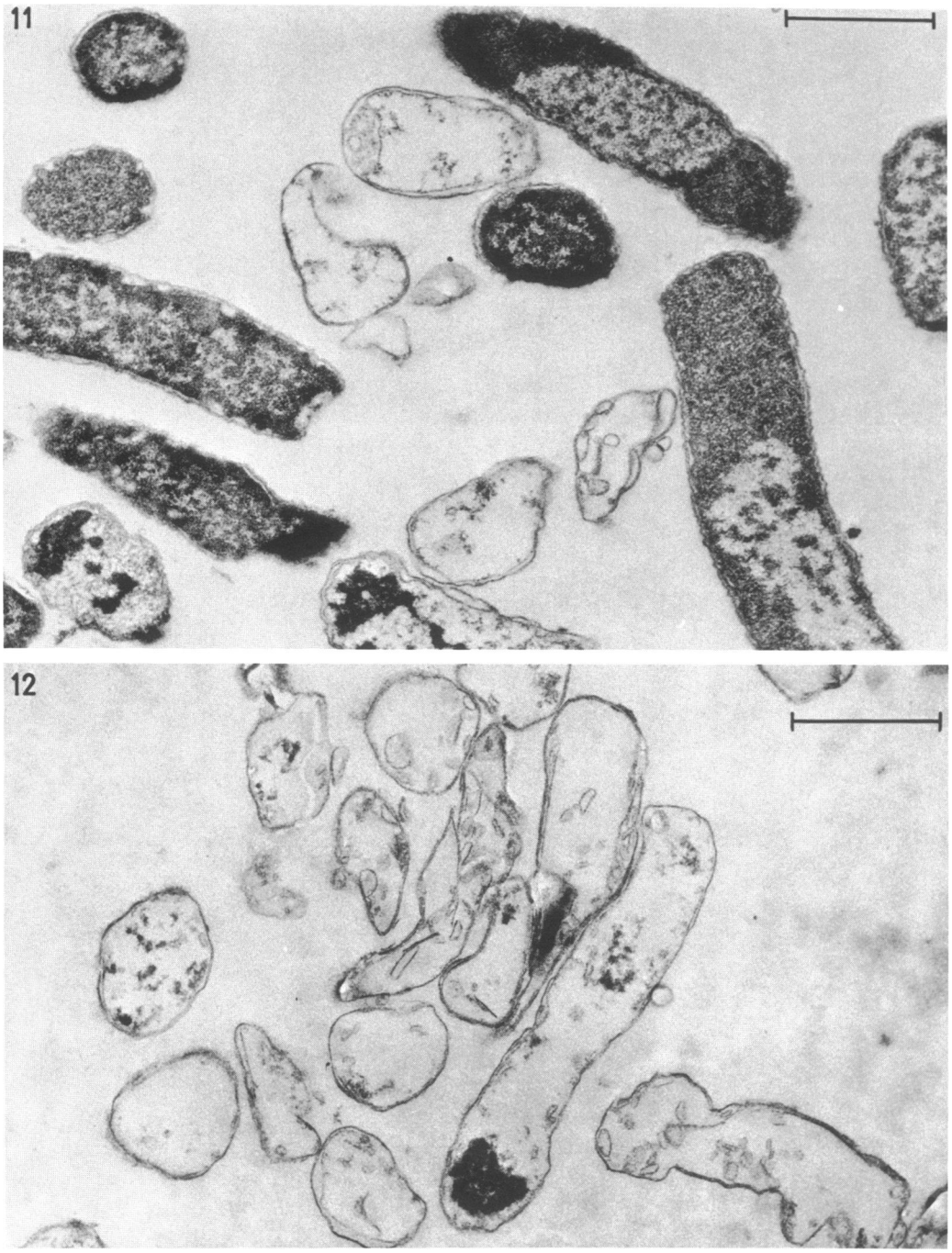

FIG. 11. *Lysing cells; sample was taken at arrow 2 of Fig. 7.*
FIG. 12. *Cells 4 to 5 hr after beginning of lysis; sample was taken at arrow 3 of Fig. 7.*

succeeding septum formation. During lysis the reduplicated membrane aggregates become more distinct (Fig. 13 and 14).

Fragmentation of the cytoplasmic membrane during lysis (Fig. 15) is also clearly seen from shadowed preparations. Since the shape of the cells after "lysis from within" does not change, it was suspected that the so-called rigid layer of the

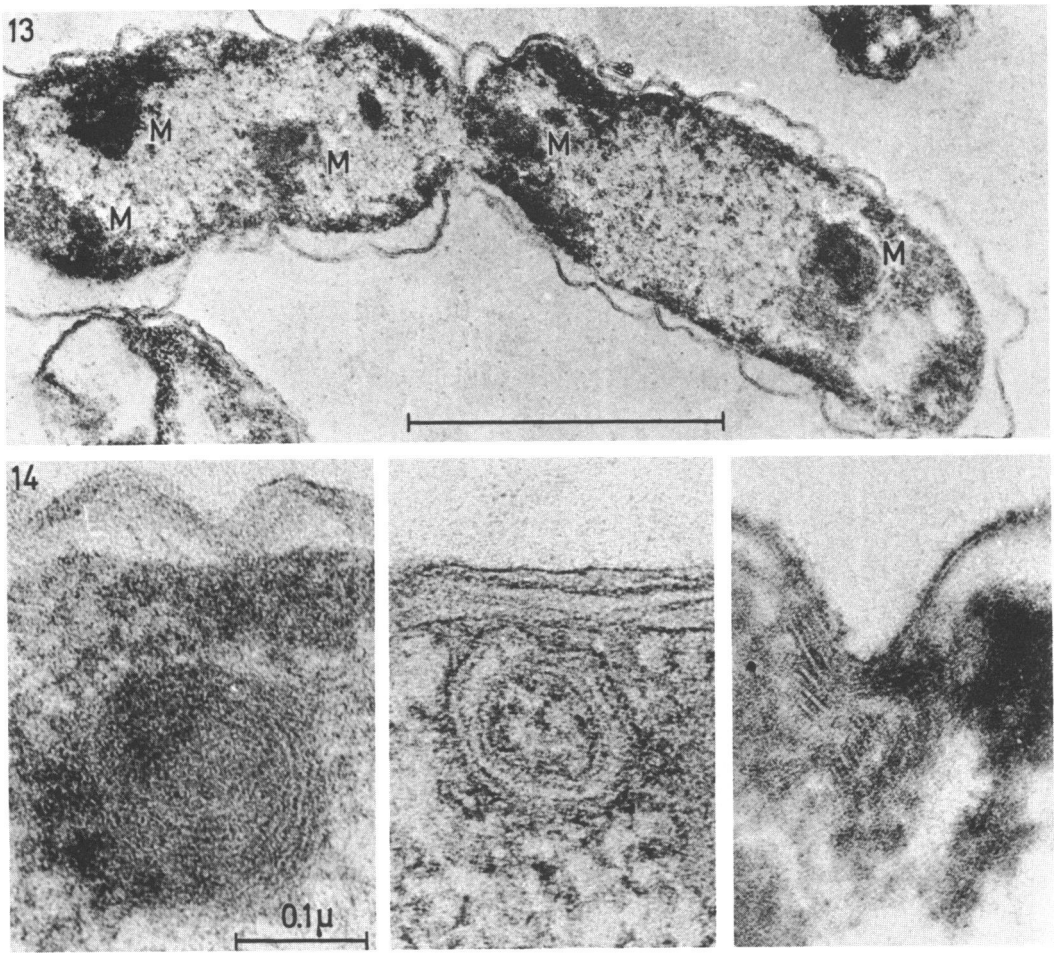

FIG. 13. *Mesosome-like structures in a cell from the lysing culture (arrow 2 of Fig. 7).*
FIG. 14. *Mesosome-like, multiply reduplicated membrane structures at higher magnification.*

cell envelope, the murein (26), remained intact. Preparation of the murein by treatment of lysed cells with hot dodecylsulfate (9) revealed unaltered murein structures (26; Fig. 16).

Oleate starvation in glycerol medium is not followed by lysis. The only visible alteration in cells starved for oleate in this medium for 8 hr (Fig. 17 and 18) concerns the nuclear material, which attains a more electron-dense appearance. In starved cultures containing more than 10% of survivors (Fig. 18), it was not possible to distinguish two classes of cells by electron microscopy.

DISCUSSION

Three distinct effects were observed in the oleate auxotroph upon oleate starvation: inhibition of macromolecular syntheses, death, and lysis.

Cell death appears to be related to growth and may, just as lysis, require a certain rate of protein synthesis. This assumption would also explain why the mutant with faster generation time lyses in glycerol, whereas the same mutant with slower generation time does not. An interesting feature may be noted regarding the event of cell death. The concentration of survivors remains fairly constant so long as the cells do not lyse. In other words, the rate at which the cells die roughly equals the mean doubling time. Since this is true for two different growth rates, it certainly does not appear to be fortuitous.

If death is closely related to growth, then it seems likely that this type of death is connected with the synthesis of some essential cellular structure(s). For instance, there is a variety of evidence for the existence of specific DNA-attachment sites on the cytoplasmic membrane (17). Lack of or altered composition of phospho-

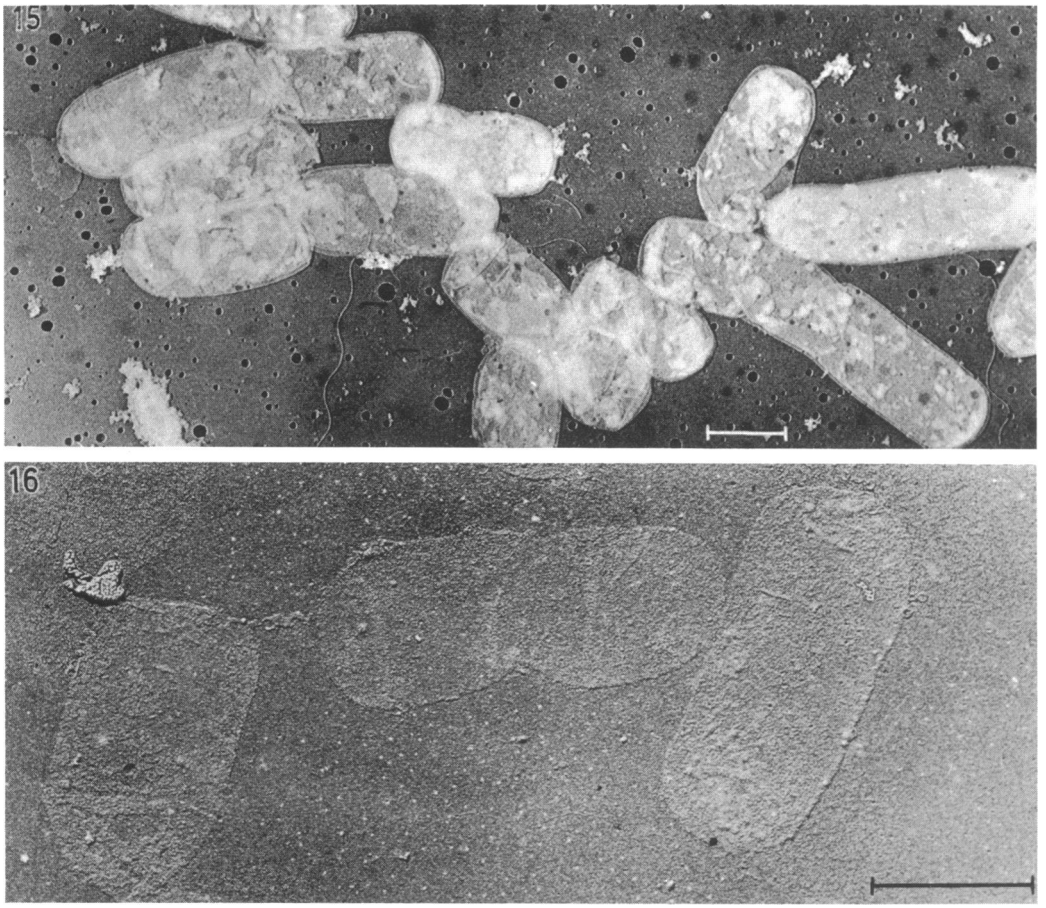

FIG. 15. *Shadowed preparation of lysed cells (arrow 3 of Fig. 7).*
FIG. 16. *Shadowed preparation of murein of lysed cells (arrow 3 of Fig. 7).*

lipids during the formation of a new attachment site might prevent survival.

We have already discussed that the inhibition of DNA, RNA, and protein syntheses may be due to a control mechanism. Using strain W 945 (*thi, thr, leu, trp*), we found that starvation for tryptophan or leucine practically abolished phospholipid synthesis; similar results were obtained by Sokawa et al. (25). Chloramphenicol also reduced the rate of phospholipid synthesis to about 20% of the control value. [The latter finding appears to disagree with the data obtained by Sokawa et al. (25); however, we did not measure phospholipid synthesis immediately upon chloramphenicol addition but only when growth had stopped 30 to 50 min after this addition.] It appears that normal phospholipid synthesis requires protein synthesis. As yet incomplete data favor the view that phospholipid synthesis behaves much as the synthesis of a coenzyme (Rehn, *unpublished data*). That is, inhibition of enzyme synthesis will also inhibit coenzyme synthesis, but not vice versa. If this situation were true for phospholipid synthesis, we would not expect the inhibition of macromolecular syntheses upon oleate starvation to be due to a control mechanism. Beyond this, it is obvious that the situation is much too complex to allow a reasonable speculation concerning a mechanism causing the inhibition under discussion.

Concerning the fact that cardiolipin synthesis does not follow the same pattern of decreasing rates of syntheses as the other phospholipids during oleate starvation, it should be mentioned that it has been shown by Buller and Astrachan (1) and by Furrow and Pizer (4) that T4 infection causes certain changes in the rates of syntheses of individual phospholipids. Furthermore, the first authors mentioned that T4rII infection, as opposed to r^+ [of *E. coli* (λ)], greatly stimulates

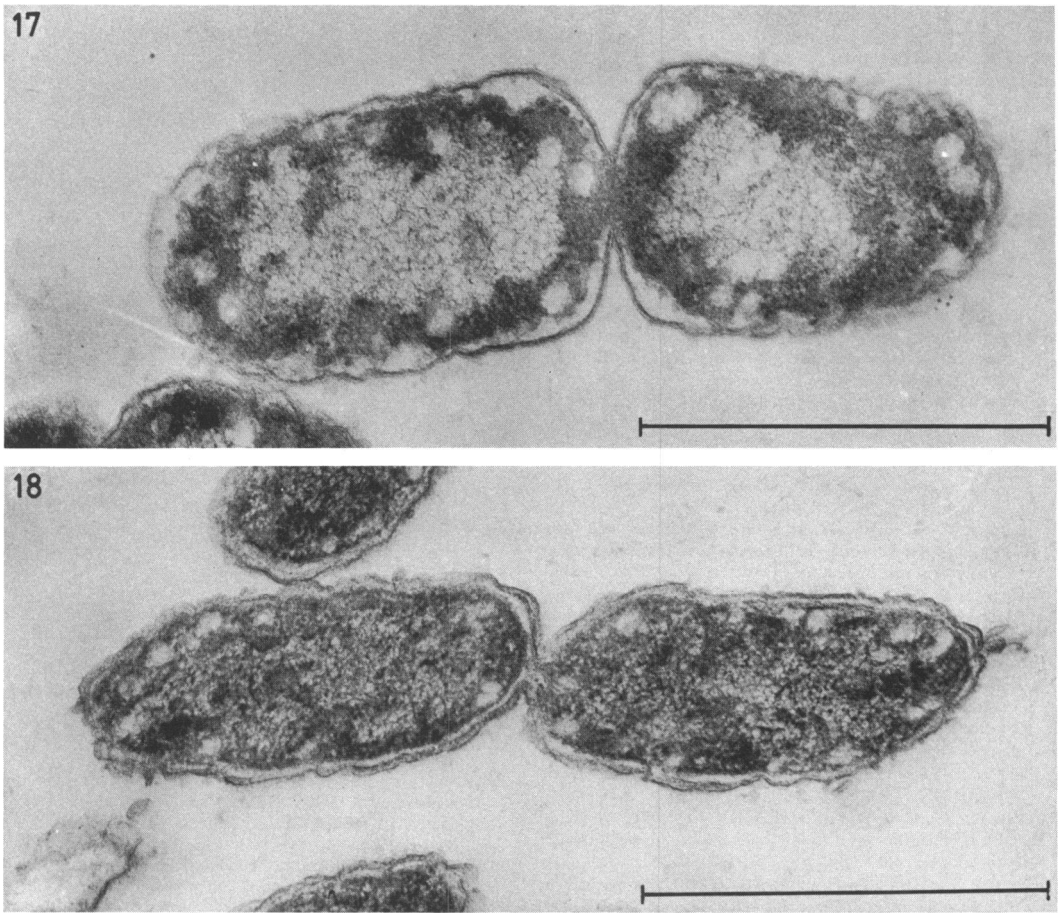

FIG. 17. *Cells from a culture growing logarithmically in the glycerol medium supplemented with oleate.*
FIG. 18. *Cells from the same medium 8 hr after removal of oleate.*

cardiolipin synthesis. T4rII infection of *E. coli* (λ) may lead to defective membrane repair or synthesis (1, 23). Increased cardiolipin synthesis upon oleate starvation and T4rII infection may thus point to a common alteration of regulation of phospholipid synthesis; it could be of interest to know whether still other types of defective membrane synthesis [e.g., the abnormal membrane formation following f1 amber mutant infection of *E. coli* (20)] have the same effect.

Lysis is "lysis from within" and as such is the same as was found upon infection of *E. coli* B with T4r phages unable to produce active lysozyme (11, 21). The requirement, at least under certain conditions, of protein synthesis for lysis and the type of lysis observed by electron microscopy—ultimately leading to an almost complete fragmentation of the cytoplasmic membrane—appears to indicate that lysis results from the cell's attempt to synthesize cytoplasmic membrane during phospholipid deficiency or with altered phospholipid composition. (No such "degradation" of the outer layers of the cell envelope occurs.)

The condensation of the nuclear material certainly is not specific for oleate removal. It has been observed under a large variety of conditions, including changes in the ionic environment (28), phage infection (12), and chloramphenicol treatment (10). It may be that the attachment to the membrane of the condensed nuclear bodies is specific. It has been observed without exception in each ultrathin section examined; such an attachment appears to be absent or less frequent in cells treated with chloramphenicol (10).

ACKNOWLEDGMENTS

We thank Helga Gnirke for excellent technical assistance in electron microscopy. We appreciate the help of L. Bockstahler in the preparation of the manuscript. We thank M. K. Reedy for communication of results prior publication, H. Schaller for advice

concerning the measurements of the ATP pool sizes, and U. Schwarz for advice regarding the preparation of the murein. We also thank P. Overath (who independently found cell death and lysis as consequences of oleate starvation) for communicating unpublished results.

LITERATURE CITED

1. Buller, C. S., and L. Astrachan. 1968. Replication of T4rII bacteriophage in *Escherichia coli* K-12 (λ). J. Virol. 2: 289–307.
2. Carr, H. S., and H. S. Rosenkranz. 1966. H^3-thymidine and the conservation of deoxyribonucleic acid. J. Bacteriol. 92:1840–1841.
3. Cole, H. A., J. W. T. Wimpenny, and D. E. Hughes. 1967. The ATP pool in *Escherichia coli*. I. Measurement of the pool using a modified luciferase assay. Biochim. Biophys. Acta 143:445–453.
4. Furrow, M. H., and L. I. Pizer. 1968. Phospholipid synthesis in *Escherichia coli* infected with T4 bacteriophages. J. Virol. 2:594–605.
5. Kanemasa, Y., Y. Akamatsu, and S. Nojima. 1967. Composition and turnover of the phospholipids in *Escherichia coli*. Biochim. Biophys. Acta 144:382–390.
6. Kanfer, J., and E. P. Kennedy. 1963. Metabolism and function of bacterial lipids. I. Metabolism of phospholipids in *Escherichia coli B*. J. Biol. Chem. 238:2919–2922.
7. Kashket, E. R., and A. F. Brodie. 1963. Oxidative phosphorylation in fractionated bacterial systems. VIII. Role of particulate and soluble fractions from *Escherichia coli*. Biochim. Biophys. Acta 78:52–65.
8. Kellenberger, E., and W. Arber. 1957. Electron microscopical studies of phage multiplication. I. A method for the quantitative analysis of particle suspensions. Virology 3:245–255.
9. Mardarowicz, C. 1966. Isolierung und Charakterisierung des Murein-Sacculus von Brucella. Z. Naturforsch. 21b: 1006–1007.
10. Morgan, C., H. S. Rosenkranz, H. S. Carr, and H. M. Rose. 1967. Electron microscopy of chloramphenicol-treated *Escherichia coli*. J. Bacteriol. 93:1987–2002.
11. Mukai, F., G. Streisinger, and B. Miller. 1967. The mechanism of lysis in phage T4-infected cells. Virology 33:398–404.
12. Murray, R. G. E., and J. F. Whitfield. 1953. Cytological effects of infection with T5 and some related phages. J. Bacteriol. 65:715–726.
13. Neuhard, J., E. Randerath, and K. Randerath. 1965. Ion-exchange thin-layer chromatography. XIII. Resolution of complex nucleoside triphosphate mixtures. Anal. Biochem. 13:211–222.
14. Overath, P., E.-M. Raufuss, W. Stoffel, and W. Ecker. 1967. The induction of the enzymes of fatty acid degradation in *Escherichia coli*. Biochem. Biophys. Res. Commun. 29: 28–33.
15. Reynolds, E. S. 1963. The use of lead citrate at high *p*H as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17:208–212.
16. Rouser, G., and S. Fleischer. 1967. Isolation, characterization, and determination of polar lipids of mitochondria, p. 385–433. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol X. Academic Press Inc., New York.
17. Ryter, A. 1968. Association of the nucleus and the membrane of bacteria: a morphological study. Bacteriol. Rev. 32: 39–54.
18. Ryter, A., E. Kellenberger, A. Birch-Anderson, and O. Maaløe. 1958. Etude au microscope électronique des plasmas contenant de l'acide désoxyribonucleique. Z. Naturforsch. 13b:597–605.
19. Schlesinger, M. J. 1968. Secretion of alkaline phosphatase subunits by spheroplasts of *Escherichia coli*. J. Bacteriol. 96:727–733.
20. Schwartz, F. M., and N. D. Zinder. 1968. Morphological changes in *Escherichia coli* infected with the DNA of bacteriophage f1. Virology 34:352–355.
21. Séchaud, J., E. Kellenberger, and G. Streisinger. 1967. The permeability of cells infected with T4r and r^+ phages. Virology 33:402–404.
22. Sedar, A. W., and R. M. Burde. 1965. Localization of the succinic dehydrogenase system in *Escherichia coli* using combined techniques of cytochemistry and electron microscopy. J. Cell Biol. 24:285–295.
23. Sekiguchi, M. 1966. Studies on the physiological defect in rII mutants of bacteriophage T4. J. Mol. Biol. 16:503–522.
24. Silbert, D. F., and P. R. Vagelos. 1967. Fatty acid mutant of *E. coli* lacking a β-hydroxydecanoyl thioester dehydrase. Proc. Nat. Acad. Sci. U.S.A. 58:1579–1586.
25. Sokawa, Y., E. Nakao, and Y. Kaziro. 1968. On the nature of the control by RC gene in *E. coli*: amino acid-dependent control of lipid synthesis. Biochem. Biophys. Res. Commun. 33:108–112.
26. Weidel, W., and H. Pelzer. 1964. Bagshaped macromolecules—a new outlook on bacterial cell walls. Adv. Enzymol. 26: 193–232.
27. Weigert, M. G., and A. Garen. 1965. Amino acid substitutions resulting from suppression of nonsense mutations. I. Serine insertion by the Su-1 suppressor gene. J. Mol. Biol. 12:448–455.
28. Whitfield, J. F., and R. G. E. Murray, 1956. The effects of the ionic environment on the chromatin structures of bacteria. Can. J. Microbiol. 2:245–260.