BBA 75262

SYNTHESIS AND TURNOVER OF MEMBRANE PROTEIN AND LIPID IN *MYCOPLASMA LAIDLAWII*

ITZHAK KAHANE AND SHMUEL RAZIN

Department of Clinical Microbiology, the Hebrew University–Hadassah Medical School, Jerusalem (Israel)

(Received November 19th, 1968)

SUMMARY

The relationship of lipid to protein synthesis in *Mycoplasma laidlawii* membranes was studied in heavy suspensions of the organisms in complex or partially defined media. Under the test conditions used the organisms did not multiply, and the amount of total cell protein remained constant. Lipid synthesis was measured by the incorporation of [^{3}H]oleic acid into membrane lipids, while protein synthesis was measured by the incorporation of L-[^{14}C]phenylalanine into membrane proteins. Though the ability of the cells to synthesize both membrane proteins and lipids declined steeply with age their protein-synthesizing ability was impaired at a much earlier stage. When membrane protein synthesis was arrested by treating the cells with chloramphenicol, the membrane lipid synthesis was not affected, so that the cell membranes formed had a density of 1.158 g/cm^3 as against 1.170 g/cm^3 for untreated cell membranes. The total membrane proteins labeled with L-[^{14}C]phenylalanine turned over at a relatively high rate, having a half life of approx. 3 h. Turnover of the total membrane lipids labeled with [^{3}H]oleic acid became apparent only after a lag period of several hours after the beginning of the chase. It was thus found that in *M. laidlawii* membrane lipid synthesis can be uncoupled from the synthesis of the membrane protein, so that the two processes are not necessarily synchronized. The possibility of varying the lipid to protein ratio in biological membranes without affecting their function is discussed.

INTRODUCTION

Studies on the biosynthesis of biological membranes are still in their infancy, and the information available is fragmentary and restricted to a few membrane types. So far attention has been focused mainly on the mitochondrial membranes, but the recent finding that the inner and outer mitochondrial membranes are synthesized by different systems[1,2] complicates the study of this model. The synthesis of the chloroplast membranes (*e.g.*, ref. 3) may also involve complex mechanisms owing to the presence of at least two membrane types in this highly developed organelle.

Very little has so far been done to elucidate the synthesis of the plasma membrane[4]. One of the major obstacles is that in the eucaryotic cell it is difficult to separate this membrane from the intracellular membranes. This problem does not

arise with the procaryotic Mycoplasma organisms which have only a plasma membrane that can be readily separated from the cytoplasmic constituents by the gentle procedure of osmotic lysis[5]. The molecular organization of the protein and lipid in the plasma membrane of *Mycoplasma laidlawii* has been extensively investigated[6–12], and though the highly disputed problem of the biological membrane structure has not been solved, valuable information has been obtained on the type of bonds responsible for the assembly of membrane protein and lipid to a membranous structure. In view of the considerable information available on the structure and function of the *M. laidlawii* membrane (reviewed in ref. 13), it was decided to embark on the investigation of its biosynthesis. In the present study it was intended to elucidate some aspects of membrane protein and lipid synthesis, and principally to answer the question whether membrane lipid synthesis is necessarily synchronized with membrane protein synthesis, or whether it can proceed without it. The results summarized in the present paper indicate that the synthesis of membrane lipid may be uncoupled from that of membrane protein when membranes with a different lipid to protein ratio are formed which are still able to perform their physiological functions.

MATERIALS AND METHODS

Organism and growth conditions

Mycoplasma laidlawii (oral strain) was grown statically in a modified Edward medium[14]. The organisms were harvested after 14–16 h of incubation at 37°. Cells were washed once or twice in the cold with 0.25 M NaCl containing 0.01 M $MgCl_2$.

Isolation of cell membranes

Cell membranes were isolated by osmotic lysis of the organisms[10,14].

Incorporation of L-[^{14}C]*phenylalanine and* [^{3}H]*oleic acid into the cell membrane*

Cells washed twice in the NaCl *plus* $MgCl_2$ solution were suspended in Edward medium to about 0.8 mg cell protein/ml. The suspension was agitated in a 37° water bath. A portion of the suspension was kept in crushed ice and served as a control for nonspecific adsorption of the amino acid or fatty acid. After 15 min incubation 0.1 μC of L-[^{14}C]phenylalanine (485 mC/mmole) and/or 0.15 μC of [9,10-^{3}H]oleic acid (3.02 C/mmole) were added to each ml of the suspensions. (The radioactive materials were the products of the Radiochemical Centre, Amersham, England.) Incubation continued for another 30 min, when the cells were separated by centrifugation and washed 3 times in 0.25 M NaCl without magnesium. A period of 30 min was chosen because at this time incorporation of the labeled compounds was still linear even with the most active cell preparations. The cell membranes were isolated and washed twice in deionized water and once with 0.05 M NaCl in 0.01 M phosphate buffer (pH 7.5). To determine their radioactivity samples of the membranes (containing 0.1–0.2 mg protein) were transferred to scintillation vials and were solubilized by sodium dodecyl sulfate (2 mg detergent/mg protein). Each vial received 10 ml of a scintillation mixture composed of 850 ml of dioxane liquor (50 g naphthalene, 7 g 2,5-diphenyloxazole, 50 mg 1,4-bis-(5-phenyloxazolyl-2)benzene in 1 l dioxane) and 150 ml of toluene scintillation liquor (4 g 2,5-diphenyloxazole, 100 mg 1,4-bis-(5-phenyloxazolyl-2)benzene in 1 l of toluene). Radioactivity was determined in a

Packard Tri-Carb liquid scintillation spectrometer model 3375. Isotope spectrum discrimination and efficiencies were determined by the use of Packard [^{14}C]- or [^{3}H]-toluene standards.

Turnover of membrane protein and lipid

Washed cells were suspended in the partially defined medium of RAZIN AND COHEN[15] from which L-phenylalanine was omitted. The suspension (7 mg cell protein per ml) was agitated in a 37° water bath for 15 min and 0.2 μC of L-[^{14}C]phenylalanine with or without 0.5 μC of [^{3}H]oleic acid were added per ml of reaction mixture. Incubation continued for another 90 min, and the cells were separated by centrifugation and washed once in the cold with Edward medium. For turnover determination part of the cells were resuspended (to give 2 mg cell protein/ml) in Edward medium prewarmed to 37°, while the other part was resuspended in this medium kept at 4°. Samples were withdrawn at various time intervals up to 3 h. The cells were sedimented, washed twice with 0.25 M NaCl, and cell membranes were isolated and their radioactivity determined. In some experiments radioactivity was also determined in the "soluble" cytoplasmic fraction obtained after sedimenting the membranes. To ensure the removal of small membrane particles this soluble fraction was centrifuged for 1 h at 37 000 $\times$ *g* and the supernatant fluid was collected. Radioactivity in proteins of the "soluble" fraction was determined according to KAHANE AND POLJAKOFF-MAYBER[16].

Lipid extraction

Membrane lipids were extracted with chloroform–methanol (2:1, v/v). The extract was washed according to FOLCH *et al.*[17], and the solvents were evaporated under N_2. Polar lipids were separated from the nonpolar lipids by chromatography on silicic acid columns, as described by RAZIN *et al.*[18].

Thin-layer chromatography of lipids

Chromatography was carried out on Silica Gel G (0.25 mm thick) chromatoplates. The developing solvent systems were chloroform–methanol–water (65:25:4, by vol.) for the separation of polar lipids, and benzene–diethyl ether–acetic acid–ethanol (50:40:0.2:2, by vol.) for the separation of nonpolar lipids. The lipid spots were detected with iodine vapor, phospholipid spots with molybdate spray reagent[19], glycolipids with $AgNO_3$ reagent[20], cholesterol with the Liebermann–Burchard reagent[21]. The lipid spots were scraped and their radioactivity was determined.

Polyacrylamide-gel electrophoresis of membrane proteins

Freeze-dried lipid-extracted membranes were solubilized in a phenol–acetic acid–water mixture (2:1:0.5, w/v/v). The solubilized material was centrifuged at 30 000 $\times$ *g* for 20 min. Samples of the supernatant fluid were run in polyacrylamide gels containing 7.5% acrylamide, 35% acetic acid and 5 M urea. The gels were prepared according to the method described by RAZIN[22], with some modification. *N*,*N*'-Methylenebisacrylamide was replaced by ethylene diacrylate, 0.25% (K and K Laboratories N.Y.), so that the gel could be solubilized for protein and radioactivity measurements[23]. Only 50 μl of the phenol–acetic acid–water-solubilized membranes (containing about 200–500 μg protein) with no added sucrose were layered on top of the gel. The electrophoretic run was about 3 h. Each of the samples was run in

triplicate; one of the gels was stained with 1% Amido Black 10B in 7% acetic acid, while the other two were not stained. All three gels were then sliced into 2-mm thick slices, using an instrument built by E. Yechezkely (Mechanical Workshop of the Hebrew University-Hadassah Medical School). The gel slices were solubilized in 1 ml of 25% NH_4OH solution[23]. Protein was estimated by measuring the absorbance of the solubilized stained gel slices at 600 mμ. Radioactivity in the solubilized slices was determined with the Bray scintillation mixture[24]. Appropriate quenching corrections were included when counting the stained-gel solubilized slices.

Density gradient centrifugation

Samples (0.15 ml, 100–300 μg protein) of labeled membranes (washed 10 times, as described in ref. 10) were layered on 4.2 ml linear 31–45% sucrose gradients. The gradients were centrifuged at 39 000 rev./min for 2 h at 10° in an SW-50 rotor and a Beckman model L-2 ultracentrifuge. Fractions (0.16 ml) were collected by puncturing the bottom of the centrifuge tube, and assayed for radioactivity.

Assay procedures

Colony-forming units in suspensions were estimated according to BUTLER AND KNIGHT[25], using Edward medium containing 1.2% agar. Protein was determined by the Folin phenol method of LOWRY *et al.*[26] with crystalline bovine plasma albumin as standard. ATPase (EC 3.6.1.3) activity was assessed as described by ROTTEM AND RAZIN[27].

RESULTS

Incorporation of phenylalanine and oleic acid into cell membranes

Table I shows that [^{3}H]oleic acid is selectively incorporated into membrane lipids, while L-[^{14}C]phenylalanine is found almost exclusively in the lipid-extracted membrane fraction, which is almost entirely composed of protein. Thus the incorporation of L-[^{14}C]phenylalanine can serve as a marker for protein synthesis, while the incorporation of [^{3}H]oleic acid can serve as a marker for lipid synthesis. Nonspecific binding to cells of the fatty and amino acid was estimated by including cold controls, *i.e.*, reaction mixtures incubated at 4°. The values obtained for [^{3}H]oleic acid binding

TABLE I

SPECIFICITY OF THE INCORPORATION OF LABEL FROM L-[^{14}C]PHENYLALANINE INTO PROTEIN, AND [^{3}H]OLEIC ACID INTO LIPID OF *M. laidlawii*

Incorporation was tested in the partially defined medium of RAZIN AND COHEN[15]. Membranes were isolated and their lipid extracted as described under MATERIALS AND METHODS.

Fraction	*Radioactivity (counts/min per mg membrane protein)*	
	^{14}C	^3H
Original membranes	8100	37 100
Lipid-extracted membranes	8100	400
Membrane lipids	110	44 500

at 4° were about 5–10% of those obtained at 37°, while the values of L-[^{14}C]phenylalanine binding at 4° were about 1–5% of those obtained at 37°. In all experiments cold control values were subtracted from the values obtained at 37°. The higher ^{3}H radioactivity observed with extracted membrane lipids than with whole membranes (Table I) may be due to the high self-absorption of ^{3}H irradiation by membrane components other than lipids.

The distribution of the label from incorporated [^{3}H]oleic acid in membrane lipids is shown in Table II. Over 90% of the radioactivity was found in phospholipids and glycolipids, while less than 4% of the radioactivity was found in the free fatty acid fraction. In the cold controls over 50% of the radioactivity was found in the free fatty acid fraction.

TABLE II

INCORPORATION OF [^{3}H]OLEIC ACID INTO LIPIDS OF *M. laidlawii* MEMBRANES

Incorporation of [^{3}H]oleic acid was tested in the partially defined medium of RAZIN AND COHEN[15]. Lipids were extracted and separated by thin-layer chromatography using two different solvent systems. The spots were identified and their radioactivity determined as described under MATERIALS AND METHODS.

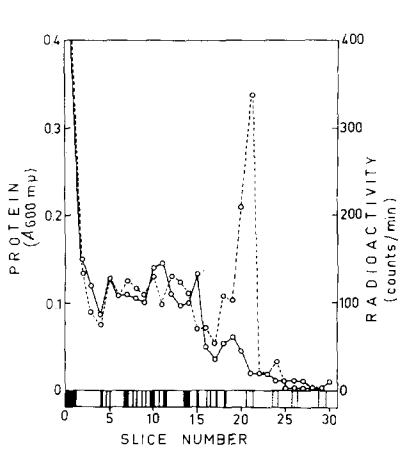
Developing solvent system	*Spot No.*	R_F	*Tentative identification*	*Percent of total radioactivity*
Chloroform–methanol–water (65:25:4, by vol.)	1	Origin	Not identified	1.10
	2	0.55	Phospholipid	2.80
	3	0.75	Phospholipid	59.40
	4	0.85	Glycolipid	31.90
	5	0.95	Free fatty acids	3.30
	6	Front	Carotenoids, cholesterol	1.50
Benzene–diethyl ether–acetic acid–ethanol (50:40:0.2:2, by vol.)	1	Origin	Phospho- and glycolipids	90.40
	2	0.08	Phospho- and glycolipids	4.60
	3	0.35	Free fatty acids	3.60
	4	0.75	Cholesterol	0.03
	5	0.90	Not identified	0.50
	6	Front	Carotenoids	0.90

The distribution of the label from L-[^{14}C]phenylalanine in membrane proteins is shown in Fig. 1. This label is seen to be incorporated into most, if not all, membrane proteins. Although the electrophoretic and gel-slicing techniques employed are most probably unable to separate all the membrane proteins, the results clearly indicate an uneven distribution of radioactivity in the protein bands. Thus slices No. 20–22 had a low protein content but high radioactivity values, indicating the presence of highly labeled protein(s) in these fractions.

Membrane protein and lipid synthesis by organisms harvested at various growth phases

Fig. 2 shows that the ability to synthesize membrane protein, as measured by L-[^{14}C]phenylalanine incorporation, is highest at a very early phase of growth, after which it declines steeply. The ability to synthesize membrane lipid, as measured by [^{3}H]oleic acid incorporation, reached its maximum about 10 h later. The initial

increase in the ability of the cells to synthesize both protein and lipid at a very early age of the culture is most probably due to the replacement of the relatively old and inactive inoculum cells by more metabolically active organisms.



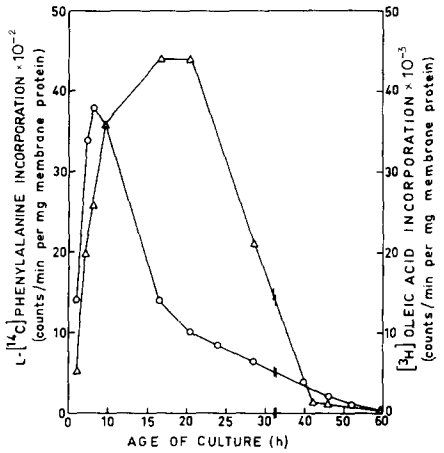


Fig. 1. Electrophoretic separation of *M. laidlawii* membrane proteins in polycrylamide gels. Highly labeled membranes were obtained from cells incubated for 90 min at 37° in the partially defined medium of RAZIN AND COHEN[15] containing L-[^{14}C]phenylalanine. Solubilization and electrophoretic separation of membrane proteins and the determination of protein and radioactivity in the gel slices are described under MATERIALS AND METHODS. A schematic representation of the electrophoretic pattern is attached at the bottom of the figure. O—O, protein; O---O, radioactivity.

Fig. 2. Incorporation of L-[^{14}C]phenylalanine (O—O) and [^{3}H]oleic acid (△—△) into membranes of *M. laidlawii* cells harvested at different ages of culture. The harvested cells were tested for the incorporation of the labeled protein and lipid precursors by incubation for 30 min at 37° in Edward medium containing the radioactive compounds as described in detail under MATERIALS AND METHODS. Unlabeled oleic acid (10 μg/ml) was added to the incorporation medium to prevent exhaustion of the fatty acid supply.

The effect of chloramphenicol on membrane protein and lipid synthesis

Fig. 3 shows the results of phenylalanine and oleic acid incorporation into membranes of cells incubated in Edward medium for various periods of time at 37°. Colony counts showed no significant change in the number of viable cells throughout the experimental period and the amount of total cell protein also remained constant. As may be seen from Fig. 3, the ability of the cells to incorporate radioactive phenylalanine dropped after 1 h of incubation, while their ability to incorporate radioactive oleic acid remained constant for about 4 h, declining steeply afterwards. Chloramphenicol did not affect oleic acid incorporation into membrane lipids but completely inhibited phenylalanine incorporation (Fig. 3). Colony counts performed on the chloramphenicol-treated suspensions did not show any significant decrease in the number of viable cells. Membranes isolated from the chloramphenicol-treated cells exhibited the same ATPase activity as membranes from untreatedcells, and a similar electrophoretic protein pattern in polyacrylamide gels.

From the continued incorporation of [^{3}H]oleic acid into membrane lipids in the absence of L-[^{14}C]phenylalanine incorporation it could be conjectured that the membranes formed in the presence of chloramphenicol have a higher lipid to protein ratio, which should be reflected in their density. To achieve a finer resolution in the

density-gradient analyses, the chloramphenicol-treated cells were labeled with [^{3}H]oleic acid, while the untreated cells were labeled with L-[^{14}C]phenylalanine.

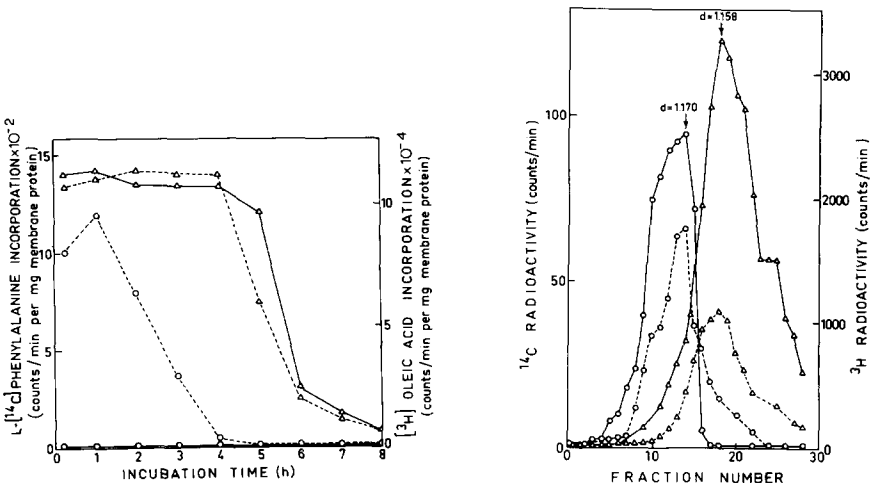


Fig. 3. The effect of chloramphenicol on the incorporation of L-[^{14}C]phenylalanine and [^{3}H]oleic acid into membranes of *M. laidlawii* cells. A suspension of washed *M. laidlawii* cells (0.8 mg cell protein/ml) was prepared in Edward medium with 200 μg/ml chloramphenicol (———) or without this drug (– – – –). The suspension was incubated at 37°, samples were withdrawn at various time intervals, and the ability of the cells to incorporate L-[^{14}C]phenylalanine (○) and [^{3}H]oleic acid (△) was tested in Edward medium as described under MATERIALS AND METHODS.

Fig. 4. The effect of chloramphenicol treatment on the density of *M. laidlawii* membranes. Washed cells were incubated at 37° for 7 h in Edward medium containing 0.15 μC/ml of [^{3}H]oleic acid and 200 μg/ml chloramphenicol (△), or with 0.1 μC/ml of L-[^{14}C]phenylalanine in the absence of chloramphenicol (○). The membranes derived from these cells were analyzed on linear sucrose density gradients (31–45%). The top of the gradient is on the right. – – – –, results of the density analysis of a mixture consisting of two parts of ^{14}C-labeled membranes and one part of ^{3}H-labeled membranes.

Fig. 4 shows that the peak density of the membranes of the chloramphenicol-treated cells was 1.158 g/cm^3 as against 1.170 g/cm^3 of the membranes isolated from untreated cells.

Turnover of membrane proteins and lipids

Incorporation of L-[^{14}C]phenylalanine into membrane proteins of washed *M. laidlawii* cells incubated in the partially defined, phenylalanine-poor, medium of RAZIN AND COHEN[15] was about 10-fold higher than in the phenylalanine-rich Edward medium. Thus, in order to obtain cells with highly labeled membranes for turnover studies the labeling was done in the partially defined medium. Turnover of membrane proteins and lipids in these highly labeled membranes was tested in Edward medium. This complex medium enables the rapid synthesis of membrane components while providing an excess of unlabeled L-phenylalanine and oleic acid, making it most suitable for turnover studies. Fig. 5 shows that the total Mycoplasma membrane proteins turned over at a relatively high rate, having a half life of approx. 3 h. The soluble cytoplasmic proteins, on the other hand, did not show any apparent turnover during the experiment. Chloramphenicol decreased the rate of membrane protein turnover apparently by inhibiting new membrane protein synthesis. No turnover

could be detected at 4°. A variable but slight increase in radioactivity in both protein and lipid was noted in all experiments done at 4°. This finding remains unexplained at the present. The total cell protein values remained constant throughout the turnover experiments. Fig. 6 shows that the distribution of radioactivity in the major

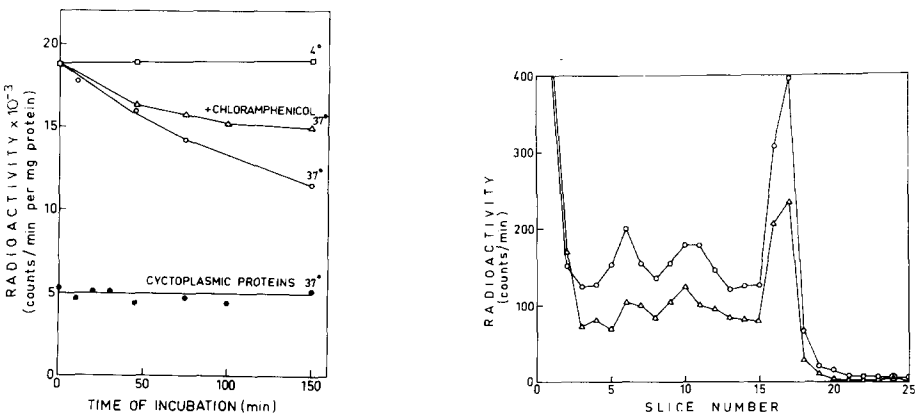


Fig. 5. Turnover of membrane and cytoplasmic proteins of *M. laidlawii*. Cell proteins were labeled by incubating washed cells for 90 min at 37° in the partially defined medium containing L-[^{14}C]-phenylalanine. The cells were washed once, transferred to Edward medium and incubated at 37° or at 4°. Samples were withdrawn at various time intervals and the cells were sedimented, washed three times, lysed and the membranes were separated from the cytoplasmic fraction. Radioactivity was determined in both the membrane and cytoplasmic fractions. □—□, membranes of cells incubated at 4°; △—△, membranes of cells incubated at 37° in the presence of 200 μg/ml chloramphenicol; ○—○, membranes of cells incubated at 37° without chloramphenicol; ●—●, cytoplasmic protein fraction of cells incubated at 37°.

Fig. 6. The effect of turnover on the distribution of radioactivity in *M. laidlawii* membrane proteins. Cells labeled with L-[^{14}C]phenylalanine were incubated in Edward medium for 2.5 h at 37° and their membranes were isolated. Membrane proteins were separated electrophoretically in polyacrylamide gels. The gels were sliced and their radioactivity was determined as described under MATERIALS AND METHODS. ○—○, membrane proteins of cells before incubation; △—△, membrane proteins of cells after incubation.

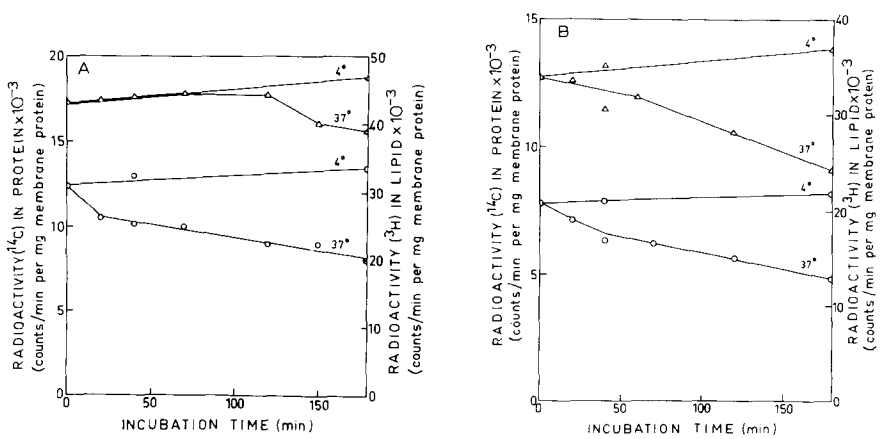


Fig. 7. Turnover of protein (○—○) and lipid (△—△) in *M. laidlawii* membranes at 37° and at 4°. The procedure was as described under Fig. 5, but the cells were labeled with both L-[^{14}C]phenylalanine and [^{3}H]oleic acid. (A) Cells harvested from a 16-h culture. (B) Cells harvested from a 24-h culture.

groups of membrane proteins did not change after a chase period of 2.5 h, though the radioactivity in all protein peaks markedly decreased. As the amount of protein determined colorimetrically in corresponding gel slices before and after the chase was about the same, the decrease in the radioactivity values shown in Fig. 6 reflects a decrease in the specific radioactivity of all major groups of membrane proteins.

To compare the turnover rates of membrane proteins and lipids, the cells were labeled with both L-[^{14}C]phenylalanine and [^{3}H]oleic acid. Fig. 7A shows that the turnover of membrane lipids became apparent only after a lag period, while that of membrane protein commenced immediately at the beginning of the chase. The lag in lipid turnover was not seen in cells harvested at a later phase of growth. In these cells (from a 24-h culture) the turnover rates of total membrane proteins and lipids were similar (Fig. 7B).

DISCUSSION

The experimental systems used for the assessment of membrane protein and lipid synthesis are complex, and several objections can be raised regarding the validity of some of our assumptions. Thus our conclusions are based on the synthesis of membrane proteins incorporating L-phenylalanine, which probably do not represent all the membrane proteins. However, the electrophoretic analysis of *M. laidlawii* membrane proteins showed that all protein bands were labeled with L-[^{14}C]phenylalanine, though to a different degree. On the other hand the electrophoretic technique apparently does not separate all membrane proteins, so that the electrophoretic pattern frequently represents groups of biophysically related proteins rather than single protein species. Furthermore, proteins which comprise less than 1% of the total membrane protein and migrate singly might not be detected. The high purity of the *M. laidlawii* membrane preparations as indicated by their low nucleic acid content (less than 0.4% by weight), speaks against part of the proteins measured being cytoplasmic or belonging to ribosomes that stick to the plasma membrane.

Another possible reservation concerns the physiological state of the cells tested. It is difficult to include them in the ill-defined category of "resting" cells, since they were suspended in complex or semi-defined media capable of supporting the growth of *M. laidlawii*. At the same time the high concentration of the organisms in the suspensions (about 4–10 times that found in a good growing culture) seems to have prevented their growth, as evidenced by viable counts and by total cell protein determinations, though enabling some membrane protein and lipid synthesis to occur.

Lipid synthesis was measured by the incorporation of [^{3}H]oleic acid into membrane lipids. Over 90% of the label incorporated was found in membrane glycolipids and phospholipids, which constitute over 90% of the total membrane lipids of *M. laidlawii*[5]. The incorporation of label from oleic acid can thus serve as a marker for the biosynthesis of almost all *M. laidlawii* membrane lipids.

In spite of the reservations, the experimental system used seems adequate for answering the question of whether membrane lipid synthesis is necessarily synchronized with membrane protein synthesis. The answer seems to be in the negative, as membrane lipid synthesis measured by oleic acid incorporation continued for an appreciable time after the complete cessation of protein synthesis. Evidence that oleic acid incorporation is a measure of net lipid synthesis and not just the result of fatty

acid exchange is provided by the significantly lower density of membranes obtained from cells treated with chloramphenicol; 1.158 g/cm^3 as against 1.170 g/cm^3. Nevertheless, this lipid-rich membrane was still functional, as the organisms remained viable and started multiplying upon the removal of the drug. The lipid-rich membranes also exhibited the same electrophoretic pattern of proteins and ATPase activity as did the normal membranes.

It seems that the biological membrane can withstand most pronounced qualitative and quantitative changes in its lipid component without losing its vital physiological functions. Thus the fatty acid composition of *M. laidlawii* membrane lipids could be radically changed by varying the fatty acid supply in the growth medium[18]. An extreme example recently found is the introduction of elaidic acid as the only fatty acid in membrane lipids of a growing goat Mycoplasma[28]. Marked changes in the lipid composition of *Staphylococcus aureus* membranes accompanying transfer of cells from anaerobic to aerobic conditions were recorded by FRERMAN AND WHITE[29]. Changes in the fatty acid composition of the endoplasmic reticulum membranes of rat hepatocytes had no effect on their enzymic content[30]. Other interesting observations are the pronounced changes described by GOLDHOR[31] in the density and lipid to protein ratio of liver mitochondria of chicken during development. These changes probably reflect a variation in the lipid to protein ratio in the mitochondrial membranes, although no experimental proof has been furnished to support this suggestion.

All the experiments testing membrane protein and lipid synthesis in cells of different ages of culture or under chloramphenicol treatment basically show the same result, *i.e.*, that lipid synthesis continues almost without interruption long after protein synthesis has ceased. From the complete inhibition of protein synthesis by chloramphenicol the decay time of the enzymatic system responsible for fatty acid activation and incorporation into membrane lipids could be estimated at 4 h. This system is apparently more stable than the protein-synthesizing system, perhaps because it is less complex.

Our turnover experiments failed to demonstrate different turnover rates among the various membrane proteins, though they probably exist. The data presented must therefore be regarded as the mean turnover rate of the total membrane proteins. The same conclusion also applies to membrane lipids. The turnover rates of different membrane phospholipids and glycolipids are known to vary considerably[32–34]. Since the amount of total cell protein was constant throughout the experiment, the fall in radioactivity noted in the membranes after the chase cannot be attributed to isotope dilution by new membrane synthesis. A possible interpretation of our results is that the rate of synthesis of new membrane proteins is roughly equivalent to the rate of membrane protein breakdown, in line with the observation of WARREN AND GLICK[4] on the turnover in the plasma membrane of nongrowing animal cells in culture. However, the turnover rate of total membrane proteins in the nongrowing *M. laidlawii* cells was much faster than that of the plasma membrane proteins of the nongrowing animal cells.

Turnover of membrane lipids in young *M. laidlawii* cells became apparent only after a lag period. This might indicate different rates of turnover for membrane proteins and lipids. However, this lag was not recorded in cells harvested at a later phase of growth. In cells from a 24-h culture the turnover rates of total membrane proteins and lipids were similar. The lag in the turnover of *M. laidlawii* lipids may

only be apparent and not real. The labeled oleic acid released during lipid breakdown might have been immediately utilized for lipid synthesis, owing to the low water solubility of the free acid and its tendency to adsorb to the membrane, where lipid synthesis is presumably taking place. In aged cells when the rate of lipid synthesis is low (Fig. 2), the fatty acid liberated might not be utilized so fast, and the turnover becomes noticeable from the beginning of the chase. OMURA *et al.*[35] reported different rates of turnover for total proteins and lipids in endoplasmic reticulum membranes of rat hepatocytes, while WARREN AND GLICK[4], working with the surface membranes of mouse fibroblasts in tissue culture, claim that there was no significant difference in the turnover rates of total membrane proteins and lipids. It is possible that conclusions drawn from the study of one type of membrane may not apply to another.

ACKNOWLEDGMENT

This work was supported by grant FG-Is-174 from the U.S. Department of Agriculture under Public Law 480.

REFERENCES

1 D. S. BEATTIE, *J. Biol. Chem.*, 243 (1968) 4027.
2 A. J. LAMB, G. D. CLARK-WALKER AND A. W. LINNANE, *Biochim. Biophys. Acta*, 161 (1968) 415.
3 I. OHAD, P. SIEKEVITZ AND G. E. PALADE, *J. Cell Biol.*, 35 (1967) 521.
4 L. WARREN AND M. C. GLICK, *J. Cell Biol.*, 37 (1968) 729.
5 S. RAZIN, *Ann. N.Y. Acad. Sci.*, 143 (1967) 115.
6 S. RAZIN, H. J. MOROWITZ AND T. M. TERRY, *Proc. Natl. Acad. Sci. U.S.*, 54 (1965) 219.
7 D. M. ENGELMAN, T. M. TERRY AND H. J. MOROWITZ, *Biochim. Biophys. Acta*, 135 (1967) 381.
8 T. M. TERRY, D. M. ENGELMAN AND H. J. MOROWITZ, *Biochim. Biophys. Acta*, 135 (1967) 391.
9 A. W. RODWELL, S. RAZIN, S. ROTTEM AND M. ARGAMAN, *Arch. Biochem. Biophys.*, 122 (1967) 621.
10 S. ROTTEM AND S. RAZIN, *J. Bacteriol.*, 94 (1967) 359.
11 D. M. ENGELMAN AND H. J. MOROWITZ, *Biochim. Biophys. Acta*, 150 (1968) 376.
12 S. ROTTEM, O. STEIN AND S. RAZIN, *Arch. Biochem. Biophys.*, 125 (1968) 46.
13 S. RAZIN, in L. HAYFLICK, *The Mycoplasmatales and the L-Phase of Bacteria*, Appelton Century Crofts Inc., New York, 1969, p. 134.
14 S. RAZIN, *J. Gen. Microbiol.*, 33 (1963) 41.
15 S. RAZIN AND A. COHEN, *J. Gen. Microbiol.*, 30 (1963) 141.
16 I. KAHANE AND A. POLJAKOFF-MAYBER, *Plant Physiol.*, 43 (1968) 1115.
17 J. FOLCH, M. LEES AND G. H. SLOANE-STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
18 S. RAZIN, M. E. TOURTELLOTTE, R. N. MCELHANEY AND J. D. POLLACK, *J. Bacteriol.*, 91 (1966) 609.
19 J. C. DITTMER AND R. L. LESTER, *J. Lipid Res.*, 5 (1964) 126.
20 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.
21 D. WALDI, in E. STAHL, *Thin Layer Chromatography*, Academic Press, New York, 1965, p. 483.
22 S. RAZIN, *J. Bacteriol.*, 96 (1968) 687.
23 D. F. CAIN AND R. E. PITNEY, *Anal. Biochem.*, 22 (1968) 11.
24 G. A. BRAY, *Anal. Biochem.*, 1 (1960) 279.
25 M. BUTLER AND B. C. J. G. KNIGHT, *J. Gen. Microbiol.*, 22 (1960) 470.
26 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
27 S. ROTTEM AND S. RAZIN, *J. Bacteriol.*, 92 (1966) 714.
28 A. RODWELL, *Science*, 160 (1968) 1350.
29 F. E. FRERMAN AND D. C. WHITE, *J. Bacteriol.*, 94 (1967) 1868.
30 G. DALLNER, P. SIEKEVITZ AND G. E. PALADE, *J. Cell Biol.*, 30 (1966) 97.
31 S. GOLDHOR, *J. Cell Biol.*, 37 (1968) 823.
32 P. F. SMITH, *Ann. N.Y. Acad. Sci.*, 143 (1967) 139.
33 E. F. GALE AND J. P. FOLKES, *Biochim. Biophys. Acta*, 144 (1967) 452.
34 Y. KANEMASA, Y. AKAMATSU AND S. NOJIMA, *Biochim. Biophys. Acta*, 144 (1967) 382.
35 T. OMURA, P. SIEKEVITZ AND G. E. PALADE, *J. Biol. Chem.*, 242 (1967) 2389.