CELL DIVISION IN A SPECIES OF ERWINIA.

ENVELOPE FATTY ACIDS AND TRANSPORT

By

THOMAS W. RICE

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1966

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1969

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
December, 1973

CELL DIVISION IN A SPECIES OF ERWINIA.

ENVELOPE FATTY ACIDS AND TRANSPORT

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. E. A. Grula for his guidance, counsel, understanding and patience throughout the last seven years.

Appreciation is extended to Dr. Mary M. Grula without whose help most of the studies concerning division inhibition would not have been possible.

Appreciation is extended also to Drs. Lynn L. Gee, Norman N. Durham and Billy G. Hudson for their service as committee members.

Special thanks are extended to Dr. George V. Odell, Dr. H. Olin Spivey, Lois Hwang and Paul Buck for their guidance in the areas of gas-liquid chromatography and spectrophotofluorimetry.

I wish to express appreciation to my fellow graduate students (Roy Hopfer, Clifton Savoy, Bill Taylor and special thanks to Wick Johnson and Richard Staerkel) for their continual friendship and help during some special aspects of these studies.

Financial assistance furnished by NDEA Title IV Fellowship, the National Institutes of Health through a grant award (AI-02530) to Dr. E. A. Grula, Oklahoma State University Microbiology Department (teaching assistantship), and U.S. Bureau of Mines contract to Dr. Lynn L. Gee is deeply appreciated.

Finally, special appreciation is extended to my wife, Sue (N. t. F.), for continual encouragement, understanding, and lists, which assisted in the preparation of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Since the initial discovery that Erwinia species, when grown on glucose supplemented agar, formed filamentous cells (Grula, 1960a), the study of cell division inhibition has been the major area of investigation in this laboratory.

The D-forms of serine, methionine, phenylalanine, threonine, tryptophan, and histidine have been shown to bring about filament formation in this organism (Grula, 1960b). Other compounds or treatments shown to cause significant inhibition of division are penicillin, ultraviolet light, vancomycin, 5-fluorouracil, D-cycloserine, and mitomycin c. The division inhibiting agents most extensively investigated have been D-serine and certain antibiotics.

One unifying theme in the study of division inhibition from this laboratory has been the involvement of the cell wall mucopeptide. A good deal of evidence has been accumulated indicating that inhibition of mucopeptide synthesis is associated with filament formation. Spheroplast formation and lysis were reported to occur during division inhibition caused by the D-amino acids (Grula, 1960b). Grula and Grula (1964) reported that division inhibited cells generated by D-serine, vancomycin, penicillin, mitomycin c, D-cycloserine, or ultraviolet irradiation had a decrease in mucopeptide of from 30-40 %. Weinbaum (1966) reported that nutritionally induced filaments of Escherichia coli also possess a

reduced mucopeptide content.

Another approach in the study of division is the prevention or reversal of filament formation. Although a large number of unrelated compounds or treatments can cause division inhibition, it is apparent that an equally varied group of compounds can prevent or reverse its occurrence. Filament formation caused by D-serine can be prevented by incorporation of ammonium salts, D-alanine, L-alanine, p-aminobenzoic acid, calcium, manganese, zinc, or altering the pH of the growth medium (Grula, 1960b). In addition to preventing division inhibition, caused by D-serine or penicillin, pantoyl lactone (PL) and ω-methyl pantoyl lactone can induce preformed filaments to revert to normal sized cells (Grula and Grula, 1962a). Hypertonic levels of NaCl, propylene glycol, and α-methyl-D-glucoside can also prevent division inhibition in many cases.

Grula and Grula (1964) suggested that the observed decrease in mucopeptide content in filamentous cells may allow secondary membrane damage. This proposed lesion may have more to do with division inhibition than the diminished mucopeptide content because, as they also reported, reversal agents such as PL and hypertonic conditions allow cell division to occur without the restoration of normal mucopeptide levels. Further evidence that division inhibition may be a membrane problem has been reported by Grula and King (1971). Changes in membrane protein composition and conformation as well as alterations in transport activity and cell wall content were reported for non-dividing cells of Micrococcus lysodeikticus disIIp$^+$. Reduced aspartic acid transport ability in non-dividing cells of Erwinia sp. has also been reported (Grula, et al., 1968). There has been sufficient indirect evidence gathered to indicate that cell division inhibition as well as its prevention is closely

related to cell membrane stability. This study of the fatty acid composition of lipids isolated from Erwinia sp. was undertaken because cell division inhibition appears to be a membrane-related problem and lipids are major structural and functional components of biological membranes.

There have been numerous reports that the fatty acid (FA) composition of microorganisms undergoes alterations in response to changes in environmental conditions. An increase in the proportion of unsaturated fatty acids (uFA) with the lowering of the growth temperature is one of the most well known alterations (Marr and Ingraham, 1962; Cullen, Phillips, and Shipley, 1971; Shen, et al., 1970; and Shaw and Ingraham, 1965). Marr and Ingraham (1962) also reported that in E. coli there is an increase in cyclopropane FA as cultures enter the stationary growth phase. These investigators also reported that when ammonium salts are the limiting growth substance, cells contain an increased proportion of saturated FA. When the growth rate is limited by glucose concentration, a slight increase in uFA was observed. Kito, et al. (1972) reported stationary cultures of E. coli contain decreased amounts of uFA compared to cultures in exponential growth. Knivett and Cullen (1967) also noted an increase in cyclopropane FA in stationary phase cultures of E. coli when ammonium salts or phosphate were limiting, but not when sulfate or magnesium were limiting. Aeration and pH are other factors that can alter FA composition (O'Leary, 1967).

The role of uFA in microbial membranes has been the subject of much recent research. The importance of uFA in E. coli has been dramatically demonstrated by the isolation of uFA auxotrophs (Silbert and Vagelos, 1967). Fox (1969) has reported that uFA are necessary for the induction of the functional lactose transport system. The relationship between

transport rate and temperature is altered depending on the uFA supplement in uFA auxotrophs (Wilson and Fox, 1971). Similar alterations in activity of the membrane enzyme 3-phosphate acyltransferase have been reported with changes in uFA supplement (Mavis and Vagelos, 1972). Using osmotically shocked cells of Staphylococcus aureus, Gale and Llewellin (1971) reported that uFA stimulated aspartic acid uptake. More closely related to the present study, Weinbaum and Panos (1966) reported a deficiency in cyclopropane FA in filamentous cells of E. coli. Research in our laboratory has shown that there is a change in the FA composition of M. lysodeikticus when cells are grown in the presence of D-cycloserine (a division inhibition agent) or PL (Staerkel, 1973).

One of the goals of this project was to determine if alterations in FA composition could be detected in division inhibited cells of Erwinia sp. (induced by a variety of agents) or cells grown in the presence of PL (a reversal agent). Pantoyl lactone was the reversal agent of choice in this study because it has proven to be effective against division inhibition induced by all agents thus far investigated.

The other phase of this project concerned the effect of PL on various transport properties in Erwinia sp. These studies were undertaken in the hope that any information gained might help elucidate the effect of PL on the cell membrane.

Pantoyl lactone effects can be categorized as immediate and long term. The most pronounced immediate effect is on transport. The inhibitory effect on transport of many compounds in both M. lysodeikticus and Erwinia sp. has been documented (Grula and King, 1971; Hopfer, 1972; and Staerkel, 1973). Inhibition can be readily reversed by simple washing of the cells in buffer. Inhibition is not limited to any one class

of compounds (amino acids, carbohydrates, organic acids, or nucleic acid bases) and both active and passive transport processes appear to be impaired. It has also been demonstrated that PL inhibits the efflux of 2-deoxy-D-glucose and D-alanine. Pantoyl lactone does not alter the activity of lysozyme, lactic dehydrogenase, NADH dehydrogenase, catalase, or the aspartic acid binding protein from Erwinia sp. These results plus the fact that passive transport is inhibited renders it doubtful that the mode of action of PL is by interaction with the various transport proteins. Surface tension or osmotic pressure alterations have been ruled out as the reason for uptake inhibition. Other membrane actions noted are the crenation or lysis of horse erythrocytes (Hopfer, 1972). Long term effects requiring growth in the presence of PL for one hour or longer include division inhibition reversal, alteration of the FA composition in M. lysodeikticus, and the potentiation of glucose and acetate transport after PL has been removed. Investigations concerning these long term effects are a part of this study.

CHAPTER II

MATERIALS AND METHODS

Test Organism

The organism employed in this study was a species of Erwinia belonging to the soft-rot group. Biochemical tests indicate it most closely resembles Erwinia carotovora (Grula, 1960a).

Media and Growth Conditions

The basal salts medium contained the following compounds per 100 ml: K_2HPO_4, 174 mg; KH_2PO_4, 136 mg; $MgSO_4 \cdot 7H_2O$, 3 mg; and trace minerals (Grula, 1960b). The pH of this salts solution was 6.8.

The aspartate medium consisted of the basal salts (100 ml) supplemented with 280 mg DL-aspartic acid (adjusted to pH 6.8 prior to addition). The glucose-aspartate medium consisted of the basal salts supplemented with 280 mg of aspartic acid and 250 mg of D-glucose per 100 ml (autoclaved separately at 12 psi for 10 min and added aseptically). All other sterilizations were at 15 psi for 15 min. The ammonium-glucose medium consisted of basal salts supplemented with 250 mg of D-glucose and 80 mg of NH_4Cl (low ammonium) or 400 mg of NH_4Cl (high ammonium) per 100 ml. At times, this medium was also supplemented with 530 µg of sodium pantothenate.

To initiate cell division inhibition, any of the following compounds were added to 100 ml of the above media: D-cycloserine 124 mg,

D-serine 88 mg, vancomycin 1 mg, mitomycin c 1.2 μg, or penicillin 100 units. These agents were sterilized by filtration through a 0.45 μm pore size Millipore filter pad. When PL was incorporated into the growth medium it was also filter sterilized prior to addition.

Media were inoculated with cells grown on nutrient agar slants (12-24 hr). Inoculum cells were washed once with 0.85 % NaCl and adjusted to an optical density of about 0.1 (540 nm). An inoculum of 0.5 ml was used for 100 ml of culture medium contained in a 250 ml Erlenmeyer flask. Cultures were incubated on a rotary shaker at 25 C for 10 to 16 hr. Growth was monitored by optical density (15 mm tube at 540 nm).

Transport Measurements

Cells were harvested by centrifugation, washed twice with basal salts solution, and resuspended in that solution to an optical density of 0.16 (540 nm). This cell suspension was shaken on a Burrell Wrist-action shaker at a setting of 1.0 at room temperature for 20 min prior to the initiation of uptake measurements. The standard uptake mixture was composed of 2.5 ml of cell suspension, 0.3 ml of basal salts or test solution, and 0.15 ml of the labeled compound (0.5 μCi/ml). Test solutions were added 30 sec prior to the addition of the labeled compound. At appropriate time intervals 0.5 ml samples were withdrawn and passed through a 0.45 μm Millipore filter pad using vacuum suction after which the filter pad was washed three times with 1 ml portions of basal salts solution (25 C). Filter pads were allowed to dry overnight at room temperature before addition of 10 ml Aquasol. Samples were shaken at 37 C for a minimum of 4 hr before counting.

The following is a list of the stock solutions used in uptake

measurements. Aspartic acid: 5 μCi of L-aspartic-U-^{14}C (154 mCi/mM) and 10 mg of non-radioactive aspartic acid per 10 ml.
Acetic acid: 8 X 10^{-6} M acetic acid-2-^{14}C (6 mCi/mM) and 8 X 10^{-6} M non-radioactive acetic acid. Both stock solutions were made up using basal salts solution (pH 6.8).

Measurement of Transport at Various Temperatures

The general procedure for transport measurement was the same as previously described. Because these experiments took up to 6 hr to perform, the washed cell suspensions were held in an ice bath and portions removed and brought to the desired temperature for 10-15 min prior to the determination of transport rate. Temperature was regulated to ± 0.5 C by mixing hot and cold running water which circulated around the sample.

Measurement of Phase Transitions

An Amicon-Bowman Spectrophotofluorimeter, located in the Department of Biochemistry, Oklahoma State University, was used for monitoring fluorescence. The procedures followed were essentially those described by Overath and Trauble (1973). Recrystallized 8-anilino-naphthalene-1-sulfonate (ANS) at a final concentration of 5 X 10^{-3} M was used as the fluorescent probe. Recrystallized ANS was obtained by forming a saturated solution of ANS in the presence of a molar excess of $MgCl_2$ in water at about 70 C. After heating, a small amount of decolorizing carbon (Norit-A) was added and the sample boiled for 5 min. Non-dissolved material was removed by filtration. Crystallization was accomplished by allowing the sample to cool to room temperature. Crystals were collected by filtration. Excitation wavelength was 360 nm and the emission

wavelength was 480 nm. Continuous recording of light intensity vs. temperature was accomplished by using an X-Y recorder. A thermophile was positioned directly in the cuvette and connected to the X axis of the recorder, while changes in light intensity were recorded on the Y axis. Phospholipids, isolated from 100 ml of log phase Erwinia sp. were suspended in basal salts (5 ml) and sonicated for 15 sec at room temperature to obtain a stable dispersion. This dispersion was diluted ten fold in basal salts solution for fluorescence measurements. When intact cells were used they were suspended to an optical density of 0.16 (540 nm) in basal salts solution. Pantoyl lactone, when incorporated in the assay mixture, was at a final concentration of 7.7×10^{-2} M.

Lipid Extraction

Cells from 100 ml of growth medium were harvested by centrifugation and resuspended in 5 ml of methanol. The suspension was flushed with nitrogen, sealed in a screw cap tube (Teflon insert), and heated at 55 C for 30 min. After cooling to room temperature 10 ml of chloroform was added, the suspension again flushed with nitrogen, and the extraction allowed to continue overnight in the sealed tubes. The insoluble material was removed by filtration using a 0.45 µm Millipore filter pad and vacuum suction. The filtrate was washed twice by inversion in glass stoppered test tubes with equal volumes of 2 M KCl followed by a single wash using distilled water. The chloroform extract was concentrated to about 2 ml at 55 C under a stream of nitrogen and passed through a sodium sulfate column (52 mm X 80 mm) to remove any residual water. The extract was taken to dryness under nitrogen (the last 1 ml at room temperature).

Preparation of Methyl Esters

Fatty acids were cleaved from the lipids and the methyl esters formed using 2 % H_2SO_4 (v/v) in anhydrous methanol. Lipids were dissolved in 4.5 ml of the methanolic H_2SO_4, flushed with nitrogen and then allowed to stand at room temperature overnight in screw cap tubes. Distilled water (4.5 ml) was added to each tube and the sample transferred to glass stoppered tubes. The sample was extracted 3 times with hexane (3 ml) and the pooled hexane extracts washed once with distilled water (3 ml). The hexane extract (containing the methyl esters of the fatty acids) was passed through a column containing sodium sulfate and the hexane evaporated under a stream of nitrogen at 55 C (the last 1 ml at room temperature). Methyl esters were stored at -20 C under nitrogen and dissolved in 0.05 to 0.07 ml of iso-octane just prior to gas-liquid chromatography.

Gas-Liquid Chromatography

Analyses were done using a Perkin Elmer model 990 Gas Chromatographic unit equipped with a hydrogen flame detector.

Chromatographic conditions:

Column	6' X 1/4" glass
Liquid Phase	Diethylene Glycol Succinate (5 %)
Column Temperature	165 C
Injection Port Temperature	250 C
Detector Temperature	250 C
Carrier Gas	Nitrogen
Flow Rate	40 liter/hr
Hydrogen Pressure	15 psi

Air Pressure	40 psi
Chart Speed	10 mm per min
Attenuation	8X - 128X

Identification of Methyl Esters of Fatty Acids

The primary mode of identification was by comparison of retention times (relative to palmitic methyl ester) of methyl esters to a series of standard methyl ester mixtures obtained from Sigma.

Standard 189-2: Arachidic (C 20:0), Linolenic (C 18:3), Oleic (C 18:1), Stearic (C 18:0) and Palmitic (C 16:0).

Standard 189-3: Capric (C 10:0), Caprylic (C 8:0), Lauric (C 12:0), Myristic (C 14:0), and Palmitic (C 16:0).

Standard 189-4: 11-Eicosenoic (C 20:1), Erucic (C 22:1), Oleic (C 18:1), and Palmitoleic (C 16:1).

Standard 189-6: Heneicosanoic (C 21:0), Heptadeconic (C 17:0), Nonadeconic (C 19:0), Pentadeconic (C 15:0), and Trideconic (C 13:0).

Branch chain fatty acids for which we had no standard compounds were tentatively identified by comparison with published relative retention times (Thorne and Kodicek, 1962) and comparison with the known branched chain fatty acids that can be isolated from lipid extracts of M. lysodeikticus. Use was also made of the relationship that the log of the relative retention time is proportional to the length of the carbon chain for a homologous series of fatty acids (i.e., saturated, one double bound, etc.). Cyclopropane fatty acids were tentatively identified by comparison with cyclopropane fatty acids present in lipid extracts of E. coli B. We were able to substantiate the identification of the major

fatty acid components (C 14:0, C 16:0, C 16:1, C 18:0, and C 18:1) isolated from Erwinia sp. by use of a coupled mass spectrometer-gas chromatography unit (Barber-Coleman model 5000 GLC unit and a LKB-9000 low resolution mass spectrometer) located in the Department of Biochemistry.

Determination of Percentage Composition of Fatty Acids

The percentage composition was obtained by determining the area of each peak and dividing by the total area of all peaks. The method used to determine the area was to multiply the height by the width at one half of the height. The height was measured in mm to the nearest 1 mm. The width was measured in mm to the nearest 0.1 mm with the aid of a magnifying lens having an inscribed scale. Width measurements were made from the inside of the pen line. This method was less biased than using the outside or the center of the line especially with very narrow peaks. When more than one attenuation setting was used in a single run the peak areas were normalized by multiplying the peak area by the attenuation. A sample calculation is given below.

Height X Width (at 1/2 Height) = Area

Area X Attenuation = Corrected Area

Cell Size Measurements

Cell sizes were determined using stained (crystal violet), heat-fixed preparations. Measurements were done using a Vicker's Instruments Image Splitting Eyepiece and Adjustable Gauging Unit. Cells were selected that appeared to be single cells, rather than a chain of cells

or cells in the process of division. Measurements of this type were used for experiments concerning the effect of growth in the presence of PL on cell size, but not for division inhibition experiments.

CHAPTER III

RESULTS AND DISCUSSION

Fatty Acid Studies

Fatty Acid Composition of Erwinia Species

Cho and Salton (1966) reported the FA composition for 5 Gram-positive organisms and 16 Gram-negative organisms. Judging by this report it would seem that in Gram-positive bacteria branched-chain FA predominate, while in Gram-negative bacteria straight-chain, cyclopropane, and unsaturated FA are the most common. Although many FA were reported for each organism tested, only a few (3-5) were present in large amounts (each more than 10 % of the total FA composition).

Data are given in Table I comparing the FA composition of Erwinia sp. to the composition of E. coli B (Mavis and Vagelos, 1972) and Pseudomonas fluorescens (Cullen, Phillips, and Shipley, 1971). The major FA detected were the same in all three organisms, however the distribution was different. The FA composition of Erwinia sp. seems similar to that reported for other Gram-negative bacteria (Cho and Salton, 1966) and quite similar in distribution to the FA from P. fluorescens. Three FA (C 16:0, C 16:1, and C 18:1) make up 97 % of the total FA detected and about 76 % of the total FA are unsaturated. The relative amounts of C 12:0, C 14:0, and C 18:0 isolated from Erwinia sp. are much closer to 1 % than any of the others reported in trace amounts and under different

TABLE I

COMPARISON OF THE FATTY ACID COMPOSITIONS OF *ERWINIA* SPECIES, *ESCHERICHIA COLI* AND *PSEUDOMONAS FLUORESCENS*

Fatty Acid*	Percentage Composition[a]		
	Erwinia sp.[b]	*E. coli*[c]	*P. fluorescens*[d]
C 12:0	trace	-	-
C 13:0	trace	-	-
C 14:b	trace	-	-
C 14:0	trace	3	trace
C 15:b	trace	-	-
C 15:0	trace	-	-
C 16:b	trace	-	-
C 16:0	24	44	31
C 16:1	46	4	40
C 17:b	trace	-	-
C 17:0	trace	-	-
C 17:Δ	trace	21	trace
C 18:0	trace	1	trace
C 18:1	27	20	27
C 19:b	trace	-	-
C 19:Δ	trace	trace	-

*Number on the left refers to the carbon chain length. The symbol on the right refers to the degree of unsaturation (0, saturated; 1, one double bond), chain branching (b, branched), or a cyclopropane fatty acid (Δ).

[a]Fatty acids present in less than 1% of the total are referred to as trace; (-) indicates values not reported.

[b]Cells grown in the aspartate medium to an O. D. of 0.39 (540 nm).

[c]After Mavis and Vagelos (1972). Cells grown in glycerol minimal medium and harvested in late log phase.

[d]After Cullen, Phillips, and Shipley (1971). Cells grown in nutrient broth and harvested after 20 hr of growth.

growth conditions may exceed 1 %.

Because the phase of growth has been reported to affect the FA composition of bacteria (see Introduction) the effect of duration of incubation in the aspartate medium on the FA composition was investigated (Table II). The time zero values were obtained from cells grown on nutrient agar and appear significantly different from the values for cells grown in the aspartate medium. Nutrient agar grown cells appear to have more cyclopropane FA and a lesser proportion of C 16:0. Once growth is established in the aspartate medium, the FA composition is relatively constant over the time periods tested. Cells in late logarithmic-early stationary phase (15 hr of incubation) appear to have increased relative amounts of C 16:1. Cells well into stationary phase (36 hr) appear to have slightly reduced relative amounts of C 16:1 and slightly increased amounts of C 18:1.

Effect of Division Inhibition on Fatty Acid Composition

The simplest system for establishment of division inhibition employs the aspartate medium, where aspartic acid serves as the sole source of carbon, nitrogen, and energy, and selected compounds are used to bring about division inhibition. Comparative FA compositions for normal and division inhibited cells (penicillin and mitomycin c grown) are given in Table III. These data indicate that even though division is profoundly inhibited, the FA composition is not significantly altered. The previously reported decrease in cyclopropane FA for filamentous cells of E. coli was not observed (Weinbaum and Panos, 1966). It must be pointed out, however, that E. coli contains a much greater proportion of

TABLE II

EFFECT OF INCUBATION TIME ON THE FATTY ACID COMPOSITION OF ERWINIA SPECIES

Fatty Acid	Percentage Composition				
	Hours of Incubation*				
	0[a]	11[b]	13[c]	15[d]	36[e]
C 12:0	trace	1	trace	trace	trace
C 14:0	trace	2	trace	trace	2
C 15:b	trace	1	trace	trace	trace
C 15:0	1	trace	trace	trace	trace
C 16:0	12	21	24	22	23
C 16:1	41	47	46	53	39
C 17:Δ	2	trace	trace	trace	1
C 18:0	trace	1	trace	trace	1
C 18:1	25	23	27	23	30
C 19:b	1	trace	trace	trace	2
C 19:Δ	13	trace	trace	1	trace

*All cells grown in the aspartate medium unless otherwise stated.

[a]Harvested after 24 hr growth on nutrient agar.

[b]Harvested at an O. D. of 0.15 (early logarithmic phase).

[c]Harvested at an O. D. of 0.39 (logarithmic phase).

[d]Harvested at an O. D. of 0.69 (early stationary phase).

[e]Harvested at an O. D. of 0.72 (late stationary phase).

TABLE III

EFFECT OF MITOMYCIN C AND PENICILLIN ON THE FATTY ACID COMPOSITION OF ERWINIA SPECIES

Fatty Acid	Percentage Composition				
	Growth Condition*				
	Control[a]	Pen-1[b]	Pen-2[c]	Mito-1[d]	Mito-2[e]
C 12:0	1	trace	trace	1	trace
C 14:0	2	1	2	3	1
C 15:b	1	2	trace	2	2
C 16:0	21	23	22	18	19
C 16:1	47	46	50	50	48
C 18:0	1	trace	1	3	2
C 18:1	23	24	19	18	23
C 19:b	trace	trace	trace	1	trace
C 19:Δ	trace	trace	trace	trace	1

*All cell grown in the aspartate medium.

[a]Cells harvested after 10.5 hr of growth (O. D. = 0.15).

[b]Medium contained 10 units per ml of penicillin G. Cells harvested after 10.5 hr of growth (O. D. = 0.12). Cell length, > 40 μm.

[c]Medium contained 15 units per ml of penicillin G. Cells harvested after 10.5 hr of growth (O. D. = 0.11). Cell length, > 40 μm.

[d]Medium contained 12 ng per ml of mitomycin c. Cells harvested after 11 hr of growth (O. D. = 0.07). Cell length, > 40 μm.

[e]Medium contained 14 ng per ml of mitomycin c. Cells harvested after 11 hr of growth (O. D. = 0.06). Cell length, > 40 μm.

cyclopropane FA (Table I). The effects of division inhibition caused by vancomycin and D-cycloserine (DCS) were also investigated (Table IV). The DCS induced division inhibition was of particular interest because of the effect on FA composition observed in M. lysodeikticus disIIp$^+$ grown in the presence of DCS (Staerkel, 1973). Although the relative distribution is not greatly altered, a slight increase in the C 19:b component was noted. This must be considered a DCS effect rather than a general division inhibition effect, because it was not observed in the other division inhibition situations (penicillin, mitomycin c, or vancomycin). Filament formation induced by vancomycin (extremely long cells) was not accompanied by any change in FA composition. In the "prevention" situation (no filaments produced), where vancomycin and Mn^{++} were added at the same time, cells had a FA composition almost identical to control cells and vancomycin-induced filaments. When division inhibition was prevented by incorporation of PL into the growth medium, a significant reduction in the major FA, C 16:1, was observed. This drop from about 40 % to 30 % was outside the normal range of variation. Also a slight increase in the relative amounts of all of the minor components was seen. More in depth investigations into the effect of PL on the FA composition are reported in a later section.

Reduced pantothenate levels have been reported in D-serine induced filaments of Erwinia sp. (Grula and Grula, 1962b). It was also noted that when pantothenate was added to the culture medium, the growth inhibitory effect of D-serine was partially overcome (Grula and Grula, 1962a). Data given in Table V show the FA composition of cells grown in the low-ammonium-glucose medium with added D-serine, pantothenate, or D-serine plus pantothenate. The addition of pantothenate has no effect on the FA

TABLE IV

EFFECT OF VANCOMYCIN AND D-CYCLOSERINE ON THE FATTY ACID COMPOSITION OF ERWINIA SPECIES

Fatty Acid	Percentage Composition				
	Growth Condition*				
	Control[a]	Vm[b]	Vm + Mn^{++} [c]	Vm + PL[d]	DCS[e]
C 12:0	1	1	1	1	-
C 14:0	1	1	1	3	3
C 15:0	trace	trace	trace	1	trace
C 16:0	28	29	26	24	19
C 16:1	42	40	40	31	41
C 18:0	trace	1	1	4	2
C 18:1	26	28	28	29	29
C 19:b	trace	trace	trace	2	6
C 19:Δ	trace	trace	trace	4	trace

*All cells grown in the aspartate medium.

[a]Cells harvested after 15 hr of growth (O. D. = 0.28). Cell length, 2-5 μm.

[b]Medium contained vancomycin (20 μg/ml). Cells harvested after 15 hr of growth (O. D. = 0.034). Cell length, 40 % 50-100 μm or greater.

[c]Medium contained vancomycin (20 μg/ml) and $MnCl_2$ (1×10^{-5} M). Cells harvested after 15 hr of growth (O. D. = 0.13). Cell length, 2-3 μm.

[d]Medium contained vancomycin (20 μg/ml) and PL (4.6×10^{-2} M). Cells harvested after 14.5 hr of growth (O. D. = 0.054). Cell length, 80 % 3-5 μm and 20 % 5-10 μm.

[e]Medium contained D-cycloserine (1.2 μg/ml). Cells harvested after 14.5 hr of growth (O. D. = 0.01). Cell length, 30-50 μm.

TABLE V

FATTY ACID COMPOSITION OF CELLS GROWN IN THE LOW-AMMONIUM-GLUCOSE MEDIUM IN THE PRESENCE AND ABSENCE OF D-SERINE AND/OR PANTOTHENATE*

Fatty Acid	Percentage Composition			
	Control[a]	Pantothenate[b]	Pantothenate + D-Serine[c]	D-Serine[d]
C 12:0	trace	1	1	1
C 14:0	1	2	1	2
C 16:b	1	trace	trace	trace
C 16:0	21	23	20	21
C 16:1	44	44	56	50
C 18:0	1	1	trace	trace
C 18:1	25	23	18	17
C 19:b	2	2	1	2
C 19:Δ	trace	2	1	1

*All cells grown in the low-ammonium-glucose medium (NH_4Cl 80 mg per 100 ml).

[a]Harvested after 14.5 hr of incubation (O. D. = 0.048). Cell length, 3-5 μm.

[b]Harvested after 16 hr of incubation (O. D. = 0.04). Cell length, 3-5 μm.

[c]Medium contained 530 μg pantothenate and 88 mg D-serine per 100 ml added after 12 hr of growth. Harvested after 16 hr of incubation (O. D. = 0.025). Cell length, 75 % 5-20 μm, 25 % greater than 20 μm.

[d]Medium contained 88 mg of D-serine per 100 ml added after 12 hr of growth. Harvested after 16 hr of incubation (O. D. = 0.02). Cell length, 90 % 2-10 μm and 10 % greater than 10 μm.

composition of cells grown in the presence or absence of D-serine. Growth in the presence of D-serine (with or without added pantothenate) appeared to cause an increase in the relative amount of C 16:1 and a corresponding decrease in C 18:1 when compared to control values. It is tenuous to associate this alteration with filament formation because a similar change was not seen in filaments initiated by other agents. It is difficult to attach much significance to these values for C 16:1 and C 18:1 because they are within the range of other normal values (see Tables II and VI). Growth in the presence of D-serine and DCS in the high-ammonium-glucose medium with added pantothenate were also investigated. Cells grown in the presence of D-serine or DCS do not have FA compositions significantly different from control cultures (Table VI).

The D-serine data given in Tables V and VI are for cultures to which D-serine was added after 12 hr of incubation. Division inhibition may also be initiated by incorporating D-serine into the medium at the time of inoculation. The FA compositions of cells grown in the high-ammonium-glucose medium with added D-serine or DCS are given in Table VII. No DCS-induced effect was evident. This is in accord with all other experiments conducted with DCS. D-Serine, when added at the time of inoculation rather than after 12 hr of incubation, did have a distinct effect on the FA composition. The relative amounts of many minor components (C 12:0, C 14:0, C 15:0, C 15:b, C 18:0, C 19:b, and C 19:Δ) increased while the major FA component (C 16:1) showed a marked decrease. These changes are quite definite and the trend is reproducible. This type profile was in no way similar to the FA profile for any control culture tested. This alteration is not likely to be linked to division inhibition; however, because filament formation is more pronounced when D-serine is added

TABLE VI

FATTY ACID COMPOSITION OF CELLS GROWN IN THE HIGH-AMMONIUM-GLUCOSE MEDIUM PLUS PANTOTHENATE IN THE PRESENCE AND ABSENCE OF D-SERINE OR D-CYCLOSERINE*

Fatty Acid	Percentage Composition		
	Control[a]	D-Serine[b]	DCS[c]
C 12:0	trace-trace	trace	trace
C 14:0	trace- 1	1	1
C 15:b	trace- 1	trace	1
C 16:0	19 - 24	22	20
C 16:1	57 - 47	52	54
C 17:b	trace-trace	trace	trace
C 17:0	trace-trace	trace	trace
C 18:0	trace- 1	trace	trace
C 18:1	19 - 23	20	17
C 19:b	trace-trace	trace	trace
C 19:Δ	2 -trace	trace	5

*All cells grown in the high-glucose-ammonium medium for 15-16 hr.

[a]O. D. = 0.062 and 0.082. Cell length, 2-5 μm.

[b]O .D. = 0.060. Cell length, 50 % 5-10 μm, 50 % greater than 10 μm. D-Serine (440 μg/ml) added after 12 hr growth.

[c]O. D. = 0.052. Cell length, 50 % 3-5 μm, 49 % 5-10 μm and 1 % greater than 10 μm. D-Cycloserine (1.2 μg/ml) added at the time of inoculation.

TABLE VII

FATTY ACID COMPOSITION OF CELLS GROWN IN THE HIGH-AMMONIUM-GLUCOSE MEDIUM IN THE PRESENCE OF D-SERINE OR D-CYCLOSERINE

Fatty Acid	Percentage Composition		
	Growth Situation		
	Control[a]	DCS[b]	D-Serine[c]
C 12:0	trace	trace	5- 6
C 14:0	1	1	7- 6
C 15:b	1	trace	2- 4
C 15:0	trace	trace	trace- 2
C 16:b	trace	trace	trace- 2
C 16:0	25	24	23-20
C 16:1	47	45	26-14
C 18:0	1	2	3- 5
C 18:1	19	23	29-26
C 19:b	trace	1	5-11
C 19:Δ	2	3	trace- 6

[a]Cells harvested after 14.5 hr of incubation (O. D. = 0.048). Cell length, 3-5 μm.

[b]Cells harvested after 15 hr of incubation (O. D. = 0.025). Cell length, 80 % 1-5 μm and 20 % > 5 μm. D-Cycloserine (1.2 μg/ml).

[c]Cells harvested after 16 hr of incubation (O. D. = 0.022, 0.008). Cell length, 2-5 μm, 5 % > 5 μm; 80 % 2-5 μm, 20 % > 5 μm. D-Serine (440 μg/ml and 1.1 mg/ml).

after 12 hr of incubation, a situation not associated with a similar FA alteration.

The major points established from this rather extensive survey, employing four growth media and five agents which induce filament formation, are quite simple. Although two previous reports (Weinbaum and Panos, 1966; and Staerkel, 1973) have linked an alteration in FA composition to division inhibition, this is definitely not an obligatory association. Growth inhibition caused by the various agents with the possible exceptions of D-serine and PL is not associated with an alteration in FA composition. Also, prevention of vancomycin-induced filament formation by Mn^{++} salts is not accompanied by an alteration in FA composition. Prevention of division inhibition by PL might be associated with an alteration in the relative distribution of the various FA.

Effect of PL on Cell Length, Growth, and Fatty Acid Composition

From the data given in Table IV it can be seen that growth in the presence of PL is one of the few conditions investigated which brings about a significant alteration in FA composition. It has been reported (Staerkel, 1973) that *M. lysodeikticus* disIIp^{+} grown in the presence of PL exhibited a marked shift in FA distribution. On this basis further investigations were undertaken to elucidate some of the parameters associated with this change. An attempt was made to correlate the effects of PL on cell length, growth and FA composition.

The data in Table VIII show that concentrations of PL up to 3.8×10^{-2} M do not drastically inhibit growth, but at a PL concentration of 7.7×10^{-2} M growth is significantly inhibited. Also presented in

TABLE VIII

EFFECT OF PANTOYL LACTONE ON GROWTH AND CELL SIZE

PL Concentration (M)	O. D.*	Cell Size** (μm)
0	0.18	2.0
5.3×10^{-3}	0.14	1.5
9.6×10^{-3}	0.15	1.7
1.9×10^{-2}	0.16	1.3
3.8×10^{-2}	0.14	1.3
7.7×10^{-2}	0.05	1.1

*Optical density (540 nm) after 13 hr of incubation in the aspartate medium.

**Method of measurement given in Materials and Methods.

Table VIII are data which indicate that PL not only can cause a reduction in the length of filamentous cells, but has a similar effect on non-division inhibited cells. Reduction in cell length occurs at concentrations of PL that are not extremely inhibitory to growth. Significant alterations in the FA profile (Table IX), reduction in the relative amounts of the uFA (C 16:1 and C 18:1) and a relative increase in a number of the minor components, did not occur unless the concentration of PL was at least 7.7×10^{-2} M. Significant growth inhibition and alteration in FA profile were observed only at the highest concentration of PL tested, while cell length reduction (Table VIII) was seen at a ten-fold lower concentration. This strongly suggests that the action of PL on cell size does not occur because of an alteration in FA composition.

To more closely define the concentration of PL necessary to effect an alteration in FA profile the above titration was repeated using a different concentration range of PL in both the aspartate and glucose-aspartate media. Pantoyl lactone had a similar effect on growth and cell length in both media. Increasing the concentration of PL caused greater growth inhibition and progressively smaller cells (Table X). With increasing concentrations of PL in the growth medium a progressive decrease in C 16:1 and an increase in C 14:0, C 18:0, and C 19:b was observed in the glucose-aspartate medium (Table XI). This same pattern was observed when the experiment was performed in the aspartate medium, but the degree of alteration was more pronounced (Table XII). Quite significant alterations in FA composition were seen only under conditions of extreme growth inhibition. Growth inhibition as such was not sufficient to bring about an alteration in FA composition, because pronounced growth inhibition was observed in a number of previous experiments involving antibiotics

TABLE IX

EFFECT OF PANTOYL LACTONE CONCENTRATION ON FATTY ACID COMPOSITION

Fatty Acid	Percentage Composition					
	PL Concentration ($M \times 10^3$)*					
	0	5.7	9.6	19	38	77
C 12:0	trace	trace	trace	trace	trace	2
C 14:0	1	1	1	1	2	5
C 16:b	trace	trace	trace	trace	trace	13
C 16:0	19	19	18	19	17	18
C 16:1	52	49	50	50	53	34
C 18:0	trace	1	1	trace	1	3
C 18:1	23	27	26	27	23	18
C 19:b	trace	trace	trace	trace	trace	1
C 19:Δ	trace	1	trace	trace	trace	4

*All cultures harvested after 13 hr incubation in the aspartate medium. PL present from time of inoculation.

TABLE X

EFFECT OF PANTOYL LACTONE ON GROWTH AND CELL SIZE IN THE ASPARTATE AND GLUCOSE-ASPARTATE MEDIA

PL Concentration (M)	O. D. (540 nm)		Cell Size* (μm)	
	Aspartate[a] Medium	Glucose-Aspartate[b] Medium	Aspartate Medium	Glucose-Aspartate Medium
0	0.39	0.48	3.2	6.3
3.1×10^{-2}	0.22	0.34	1.9	4.3
4.6×10^{-2}	0.15	0.19	2.0	2.8
6.2×10^{-2}	0.08	0.16	1.6	2.3
7.7×10^{-2}	0.03	0.07	1.4	1.9
9.2×10^{-2}	0.03	0.05	1.2	1.6

*Method given in Materials and Methods.

[a]Harvested after 13.5 hr of incubation.

[b]Harvested after 12 hr of incubation.

TABLE XI

EFFECT OF PANTOYL LACTONE CONCENTRATION ON FATTY ACID COMPOSITION OF CELLS GROWN IN THE GLUCOSE-ASPARTATE MEDIUM

Fatty Acid	Percentage Composition				
	PL Concentration ($M \times 10^2$)*				
	0	3.1	4.6	6.2	9.2
C 14:0	trace	trace	1	1	3
C 15:0	trace	trace	trace	trace	1
C 16:0	13	19	19	23	24
C 16:1	68	56	55	45	32
C 18:0	trace	trace	trace	trace	5
C 18:1	16	21	20	24	23
C 19:b	trace	3	trace	4	9

*Cultures harvested after 12 hr of incubation. PL present from the time of inoculation.

TABLE XII

EFFECT OF PANTOYL LACTONE CONCENTRATION ON FATTY ACID COMPOSITION OF CELLS GROWN IN THE ASPARTATE MEDIUM

Fatty Acid	Percentage Composition					
	PL Concentration ($M \times 10^2$)*					
	0	3.1	4.6	6.2	7.7	9.2
C 14:0	trace	1	1	4	6	5
C 15:b	trace	trace	trace	trace	2	7
C 15:0	trace	trace	trace	trace	2	5
C 16:0	27	20	20	20	23	24
C 16:1	54	41	48	42	26	10
C 18:0	trace	2	1	2	5	7
C 18:1	18	23	26	24	18	12
C 19:b	trace	9	trace	7	17	30
C 19:Δ	trace	trace	2	trace	trace	trace

*Cultures harvested after 13.5 hr of incubation. PL present from the time of inoculation.

without an alteration in FA composition.

In an attempt to dissociate the action of PL on growth and cell length from its effect on FA composition, PL (9.2×10^{-2} M) was added to growing cultures of Erwinia sp. after 12 hr of incubation (O. D. = 0.15) and incubation was continued for an additional 6 hr. Growth inhibition and reduction in cell length were observed 2 hr after the addition of PL (Fig. 1). Although growth was inhibited and cell length was reduced, no change in FA composition occurred even after 6 hr of growth in the presence of PL (Table XIII). This experiment reveals two important points: a) The ratio of the amount of PL to the number of cells present at the time of the addition of PL to the culture may be important in determining whether or not PL has an effect on the FA composition of the cell; b) Because growth inhibition and reduction in cell length occur under conditions that do not alter the FA profile, one cannot attribute either of these two effects of PL to an alteration in FA composition.

The above experiment suggests that prevention of division inhibition by PL could be accomplished without any alteration in FA composition. Data given in Table XIV indicate that this is indeed the case. D-Serine was added to growing cultures after 12 hr of incubation and division inhibition was observed about 4 hr later. If PL was added at the time of inoculation or at the same time as D-serine, no filament formation was observed at the time of harvest. In none of the situations tested, either division inhibition by D-serine or prevention by PL, was there a significant (greater than 5 %) alteration in FA composition.

The following experiment was done to determine the period of time necessary for FA composition to return to normal values after the removal of PL from the medium. Data given in Table XV indicate that cells grown

Figure 1. Influence of Pantoyl Lactone on Growth and Cell Length of Erwinia Species. ●, optical density of control cells; ▲, optical density of cells grown in the presence of PL (9.2×10^{-2} M); o, length of control cells; Δ, length of cells grown in the presence of PL (9.2×10^{-2} M).

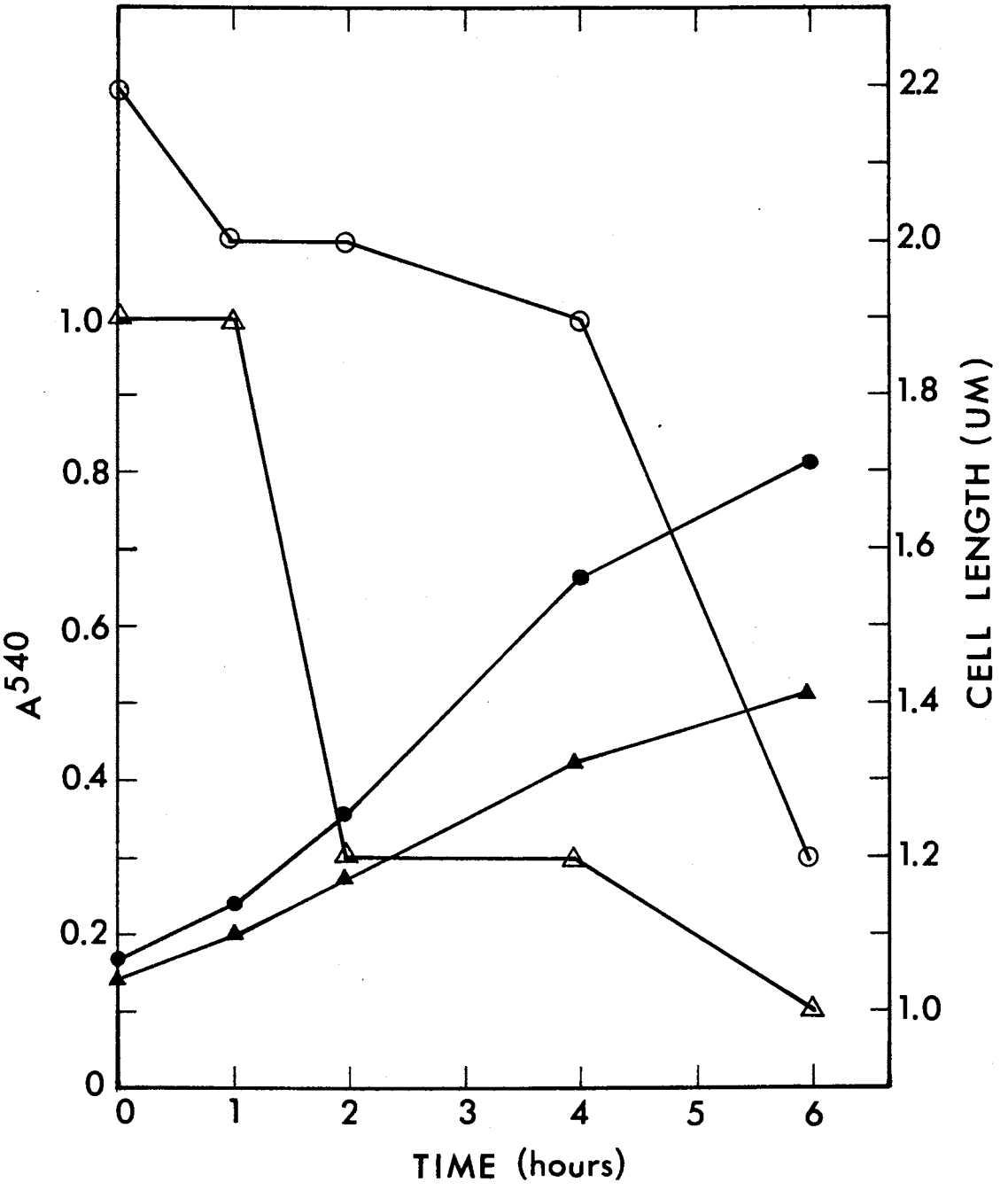

A540
CELL LENGTH (UM)
TIME (hours)
0
0.2
0.4
0.6
0.8
1.0
1.0
1.2
1.4
1.6
1.8
2.0
2.2
0
1
2
3
4
5
6

TABLE XIII

EFFECT OF THE ADDITION OF PANTOYL LACTONE AFTER TWELVE HOURS OF GROWTH ON FATTY ACID COMPOSITION

Fatty Acid	Percentage Composition					
	Time (hr) After the Addition of PL*					
	0[a]	1[b]	2[c]	4[d]	6[e]	Control[f]
C 14:0	1	1	trace	trace	1	trace
C 15:0	trace	trace	trace	trace	trace	1
C 16:0	17	20	17	19	14	19
C 16:1	59	57	60	58	61	54
C 18:1	21	21	20	22	22	23

*All cells grown in the aspartate medium. PL concentration (9.2×10^{-2} M).

[a]Harvested after 12 hr of incubation.

[b]Harvested after 1 hr of growth in the presence of PL.

[c]Harvested after 2 hr of growth in the presence of PL.

[d]Harvested after 4 hr of growth in the presence of PL.

[e]Harvested after 6 hr of growth in the presence of PL.

[f]Harvested after 18 hr of growth (no PL added to the culture).

TABLE XIV

EFFECT OF GROWTH IN THE PRESENCE OF D-SERINE AND PANTOYL LACTONE ON FATTY ACID COMPOSITION

Fatty Acid	Percentage Composition			
	Growth Condition*			
	Control[a]	DS (12)[b]	DS+PL (0)[c]	DS+PL (12)[d]
C 14:0	trace	1	trace	trace
C 16:0	24	22	20	20
C 16:1	47	52	53	56
C 18:0	trace	3	3	1
C 18:1	23	20	20	19
C 19:b	trace	trace	trace	trace

*All cells grown in the high-ammonium-glucose medium with added pantothenate (530 µg/100 ml).

[a]Harvested after 15.8 hr of incubation (O. D. = 0.062). Cell length was 2-5 µm.

[b]Harvested after 15.8 hr of incubation (O. D. = 0.06). D-Serine (88 mg/100 ml) added after 12 hr. Cell length was 50 % 5-10 µm and 50 % > 10 µm.

[c]Harvested after 15.8 hr of incubation (O. D. = 0.076). D-Serine (88 mg/100 ml) and PL (4.6×10^{-2} M) added after 12 hr. Cell length was 2-5 µm.

[d]Harvested after 15.8 hr of incubation (O. D. = 0.035). PL (4.6×10^{-2} M) added at the time of inoculation and D-serine (88 mg/100 ml) added after 12 hr incubation. Cell length was 2-3 µm.

TABLE XV

EFFECT OF REMOVAL OF PANTOYL LACTONE ON FATTY ACID COMPOSITION

Fatty Acid	Percentage Composition			
	Time After Removal of PL (hr)*			
	0[a]	2[b]	3[c]	5[d]
C 14:0	1	trace	trace	trace
C 15:0	1	trace	trace	trace
C 16:0	21	21	22	18
C 16:1	41	43	46	56
C 18:0	1	trace	trace	trace
C 18:1	21	27	28	20
C 19:Δ	10	6	3	5

*All cells grown in the aspartate medium containing PL (9.2×10^{-2} M) for 17 hr (O. D. = 0.08), washed twice in basal salts solution, and resuspended in fresh aspartate medium without PL and incubated for the times indicated.

[a]Harvested after washing (O. D. = 0.05).

[b]Harvested after 2 hr in fresh medium (O. D. = 0.09).

[c]Harvested after 3 hr in fresh medium (O. D. = 0.18).

[d]Harvested after 5 hr in fresh medium (O. D. = 0.33).

in the presence of PL for 17 hr regained a more normal FA profile within 3-5 hr after the cells were placed in fresh medium without PL. Some recovery is noticeable after about the time necessary for the cell mass to double (2 hr).

While it has been demonstrated that growth in the presence of PL can induce an alteration in FA distribution of Erwinia sp. and that this alteration is reversible, this alteration can not be directly related to the effect PL has on prevention or reversal of division inhibition.

Temperature Dependence of Aspartate Transport

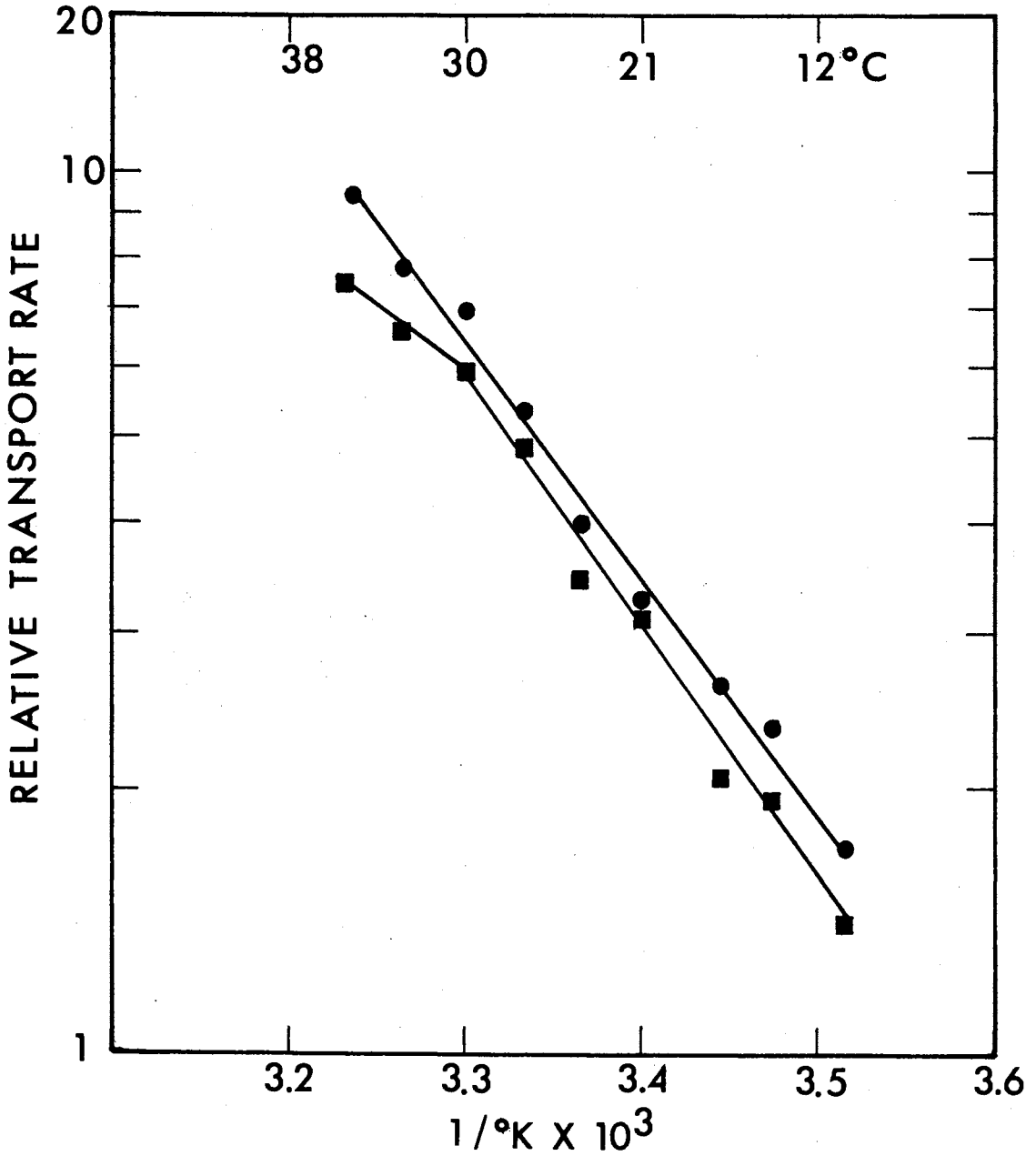
It has been well documented that PL has an immediate inhibitory effect on the transport of many substances in both Erwinia sp. and M. lysodeikticus disIIp$^+$ (Grula and King, 1971; Hopfer, 1972; and Staerkel, 1973). Hopfer (1972) suggested that PL exerts its effect by interaction with the lipid portion, in particular the uFA, of the bacterial membrane. A number of observations are consistent with this suggestion. Pantoyl lactone is appreciably soluble in a number of relatively apolar solvents (Hopfer, 1972). Pantoyl lactone has a partition coefficient (n-octanol/water) of 0.5 (M. Grula, unpublished data). The ability of a compound to partition into n-octanol is closely related to its ability to penetrate into biological membranes (Hansch and Glave, 1971). In general, membrane-active organic compounds stabilize membranes at low concentrations and are disruptive at higher concentrations (Seeman, 1966). Pantoyl lactone has been shown to have these latter properties in experiments using horse erythrocytes (Hopfer, 1972). Based on data of this type, it was postulated that PL inhibits aspartate transport by partitioning into the lipids of the cell membrane.

The physical state of membrane lipids has been shown to exert an effect on the transport properties of β-glucoside and lactose (Fox and Tsukagoshi, 1972). Plots of the logarithm of transport rate versus temperature in 1/°K (Arrhenius plots) show inflection points at a temperature that corresponds to the phase transition temperature (melt-point) of the lipid bilayer. Using a strain of E. coli, which requires uFA, it has been shown that the transition temperature and the inflection temperature of transport plots can be altered by supplementation of different uFA during growth (Fox and Tsukagoshi, 1972). It was thought if PL does partition into the lipids in Erwinia sp., this could change the physical state of the lipid bilayer which might, in turn, alter the inflection point seen in Arrhenius plots of aspartate uptake.

Arrhenius plots for aspartate transport were linear over the temperature range tested (Fig. 2). When PL (7.7×10^{-2} M) was present during transport measurements, a break in the line at about 28 C was evident (Fig. 2). It can be seen that PL inhibits aspartate transport at all temperatures, but inhibition is much greater above 28 C.

The fact that aspartate transport plots obtained in the absence of PL show no break may be explained in a number of ways. Aspartate uptake may not be sensitive to phase transitions of the lipids. Certain membrane enzymes have been shown to lack this property (Mavis and Vagelos, 1972). Alternately, the break temperature may be outside the temperature range tested. Overath and Trauble (1973) reported the transition temperature for E. coli supplemented with naturally occurring uFA to be quite low (10-15 C). Baldassare, McAfee, and Ho (1973) reported two transition temperatures for membrane vesicles prepared from a different strain of E. coli; one at about 20 C and another at about 30 C. Because lipids

Figure 2. Arrhenius Plot for Aspartate Transport in the Presence and Absence of Pantoyl Lactone. Cells grown in the aspartate medium. ●, control cells; ■, cells assayed in the presence of PL (7.7×10^{-2} M). Transport rate was determined as described in the Materials and Methods section. Each rate value is the average of three rate determinations (1 min, 2 min, and 4 min) normalized to 4 min. The units are counts per 10 min $\times 10^{-3}$ per 0.5 ml sample.


20
10
1
38
30
21
12°C
RELATIVE TRANSPORT RATE
3.2
3.3
3.4
3.5
3.6
1/°K X 10³

isolated from Erwinia sp. contain about 76 % uFA at least one transition temperature would be expected to be rather low. The break point in the aspartate uptake plot might be above 36 C. This could not be investigated because the transport rate drops rapidly above 36 C, presumably due to thermal denaturation of some protein important to aspartate transport. It seems unlikely that the lipid phase transition would occur at such a high temperature in view of the large amounts of uFA present.

The reason a break in the Arrhenius plot was observed when PL was present is not clear. If indeed there is a phase transition at about 28 C, and even though it may not affect aspartate uptake, the more fluid state might allow more PL to enter the membrane and in some manner cause greater inhibition of transport. Alternately the phase transition may be normally out of the range of the assay (above or below) and PL may alter the physical state of the lipids such that the transition temperature is shifted to 28 C. Overath and Trauble (1973) have reported that incorporation of exogenous molecules tends to lower the transition temperature. This would predict that the normal transition temperature is above 36 C. As mentioned before this is unlikely in view of the large amounts of uFA present in Erwinia species. Ultimately the interpretation of these data depends on obtaining a physical measurement of the lipid transition temperature in Erwinia sp. in the presence and absence of PL.

Phase Transitions

Overath and Trauble (1973) have recently described methods for using the fluorescent probe, 8-anilinonaphthalene-1-sulfonate (ANS), to measure lipid phase transitions in whole cells, isolated membranes, and phospholipid dispersions.

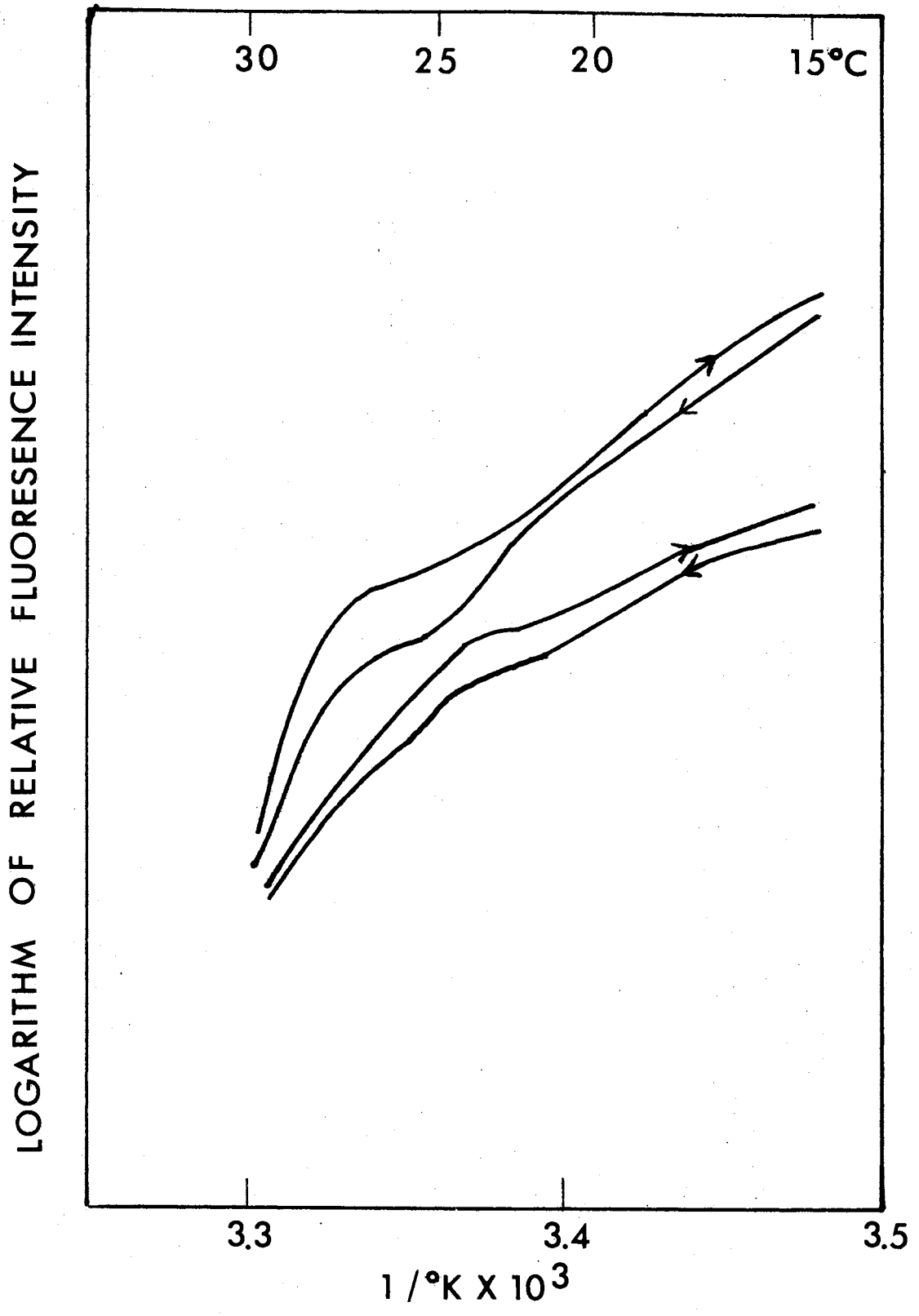
The objective of these investigations was to determine if a lipid phase transition existed at about 28 C, the temperature at which the break in the Arrhenius plot of aspartate transport measured in the presence of PL was detected. Because ANS will interact with proteins as well as lipids (Augustin and Hasselbach, 1973), it was decided that these experiments be performed using both isolated phospholipids and whole cells.

Although the data are not shown, ANS in basal salts solution fluoresces only very weakly and the relative fluorescence is independent of temperature between 15 and 30 C. When PL (7.7×10^{-2} M) was added a ten fold increase in fluorescence was observed. This increase in fluorescence probably reflects the relatively apolar nature of the PL molecule. The relative fluorescence of ANS in the presence of PL was also independent of temperature.

When ANS was added to a phospholipid dispersion and measurements made from 15 C to 30 C, it was found that the relative fluorescence increased as the temperature was lowered (Fig. 3). An apparent phase transition was detected between 22 C and 28 C with a midpoint of about 25 C. The shape of the curves obtained was very similar to results reported for isolated membranes (Overath and Trauble, 1973). The shape of the curves are not, however, like those obtained using isolated phospholipids by these investigators. When these measurements were repeated with PL present, the curves were somewhat flatter than the control curves and the transition temperature was apparently shifted to a lower (23 C) temperature. This lowering of the transition temperature could be expected if PL mixes with lipids (Overath and Trauble, 1973).

A similar experiment was performed using whole cells instead of

Figure 3. Phase Transition Temperature of Isolated Phospholipids From Erwinia Species Using ANS as the Fluorescent Probe. Arrows indicate increasing or decreasing temperature during the scan. ANS (5×10^{-3} M); PL (7.7×10^{-2} M). Top curves are controls; lower curves were made with PL present.


30
25
20
15°C
LOGARITHM OF RELATIVE FLUORESCENCE INTENSITY
3.3
3.4
3.5
1 /°K X 10³

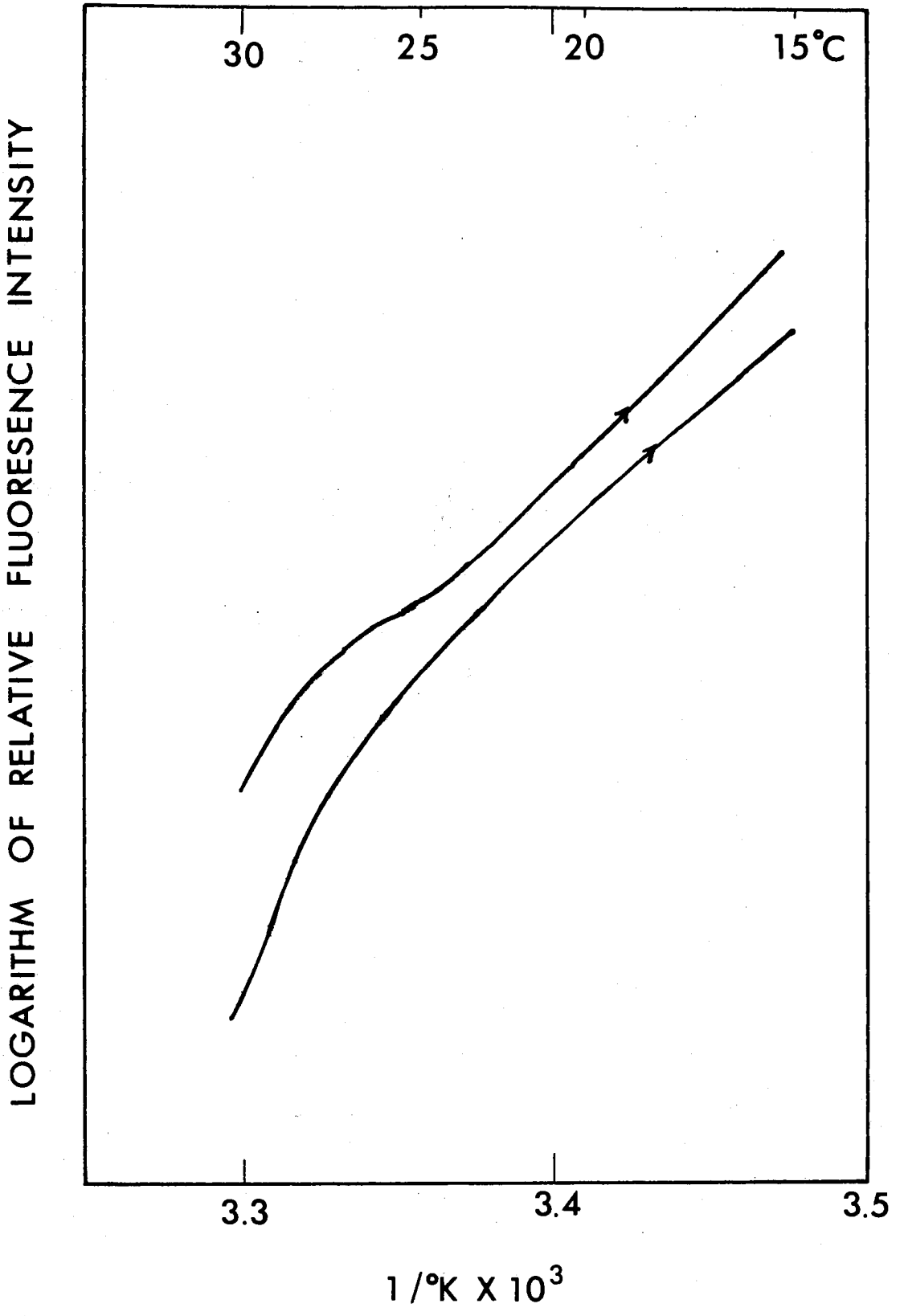
isolated phospholipids. An indication of a phase transition was observed at about 26 C for control cells (Fig. 4). When PL was added, this transition appeared much broader or may have been absent.

The transition temperature measured by this method, using either whole cells or isolated phospholipids, agrees rather closely with the data obtained from Arrhenius plots of aspartate transport in the presence of PL. They do not agree with data gathered in the absence of PL since no break in the Arrhenius plot was observed. These physical measurements support the contention that the break in the Arrhenius plot for aspartate transport in the presence of PL is probably due to more PL entering the membrane above the transition temperature and thereby somehow interfering with the uptake system.

These fluorescence studies give direct physical evidence that PL can interact with phospholipids isolated from Erwinia species. This property may well be associated with the biological activity of PL. Unfortunately, we have no way to yet measure possible direct effects of PL on transport proteins.

As discussed previously, the major phase transition for lipids containing such large amounts of uFA would be expected to be much lower than 25 C. Unfortunately, measurements could not be made below 15 C. Baldassare, McAfee, and Ho (1973) have reported the measurement of two separate lipid phase transition temperatures, one at approximately 20 C and another at approximately 30 C, for membrane vesicles isolated from E. coli. It is possible that there are also two transition temperatures for the lipids from Erwinia sp., one at approximately 25 C and one at a temperature below 15 C.

Figure 4. Phase Transition Temperature Using Whole Cells With ASN as the Probe. Arrow indicates decreasing temperature during the scan. ANS (5×10^{-3} M); PL (7.7×10^{-2} M). Cells suspended to an optical density of 0.16 (540 nm). Top curve is the control; lower curve made with PL present.


30
25
20
15°C
LOGARITHM OF RELATIVE FLUORESENCE INTENSITY
3.3
3.4
3.5
1/°K X 10³

Influence of PL on the Transport of Acetic Acid-2-^{14}C

Acetate transport is the only system so far investigated in our laboratory that is not inhibited by PL (Staerkel, 1973). This particular finding has warranted further investigation.

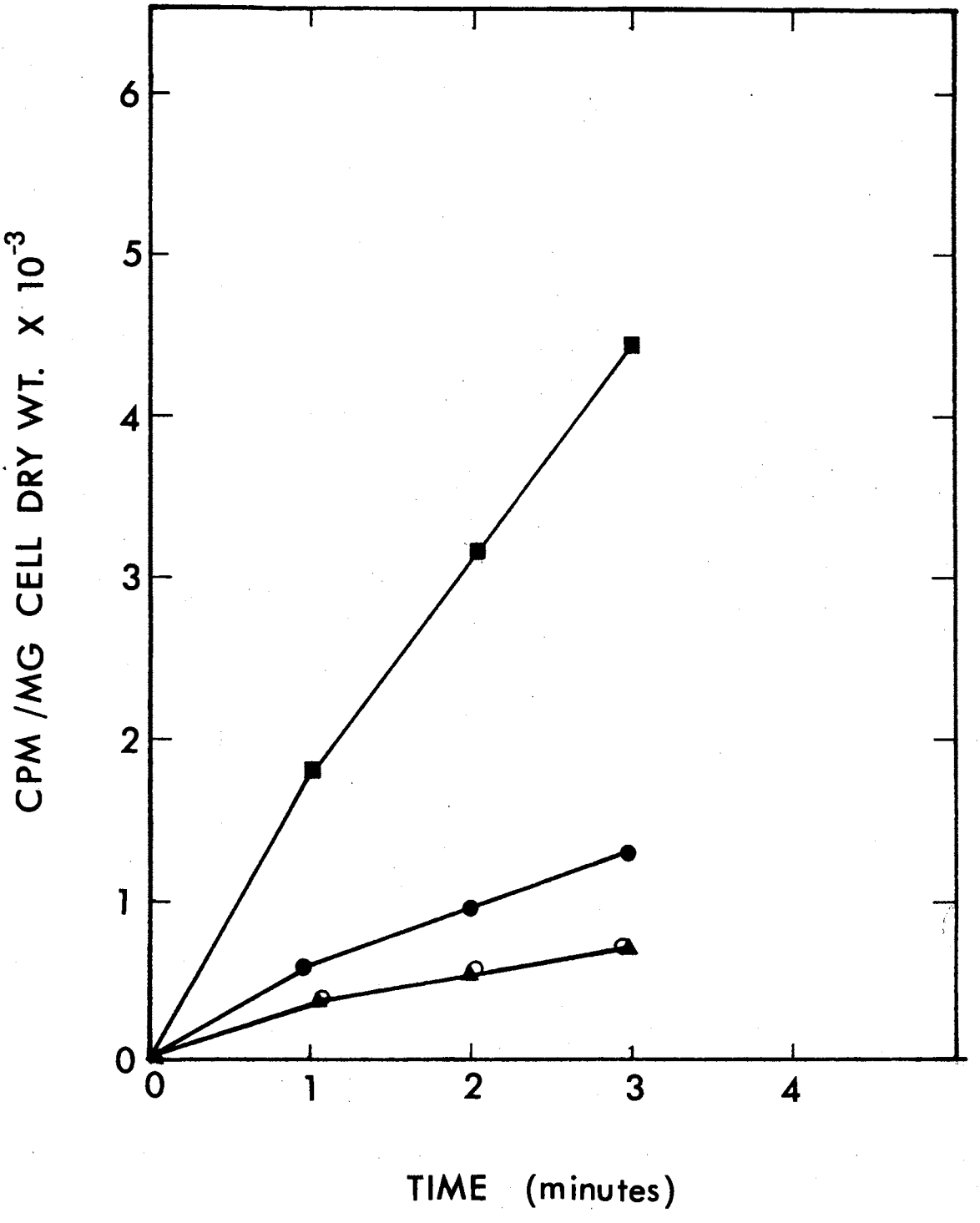
When acetate transport was measured both in the presence and absence of PL, unexpected results were obtained. Unlike the situation in M. lysodeikticus disIIp$^+$, PL inhibited acetate transport about 45 % (Fig. 5).

Another interesting property of acetate transport was observed by Staerkel using cultures of M. lysodeikticus disIIp$^+$. Cells grown in the presence of PL, which were subsequently washed to remove the PL, exhibited a four-fold higher uptake ability compared to control cells (Staerkel, 1973). Data shown in Fig. 5 indicate a three-fold increase was observed under similar circumstances using Erwinia sp. It was also noted that acetate transport for cells grown in the presence of PL was more sensitive (60 % reduction, compared to a 45 % reduction) to PL inhibition than control cells (Fig. 5). This is contrary to the findings of Staerkel (1973).

Enhanced transport ability for glucose using cells grown in the presence of PL was reported by Hopfer (1972). This is not always the effect seen in cells grown in the presence of PL; Staerkel (1973) observed that D-alanine transport is not increased. Hopfer (1972) suggested that enhancement of glucose transport was a "repression-derepression" process. The following series of experiments were done in an attempt to gain more information about the PL-induced stimulation of acetate transport ability.

When PL (7.7×10^{-2} M) was incorporated into the growth medium,

Figure 5. Transport of Acetic Acid-2-^{14}C by Cells Grown in the Presence and Absence of Pantoyl Lactone. ●, cells grown in the aspartate medium for 10 hr; ■, cells grown in the presence of PL (7.7×10^{-2} M) for 14 hr; ▲, control cells assayed with PL present; ○, PL grown cells assayed with PL present.


CPM /MG CELL DRY WT. X 10^{-3}
0
1
2
3
4
5
6
0
1
2
3
4
TIME (minutes)

growth was quite slow compared to control cultures; therefore, it was thought that such cells might retain some properties of inoculum cells. Acetate transport for inoculum cells (nutrient agar grown) was compared to that in control cells (grown in the aspartate medium for 10 and 12 hr). The transport ability was 5 times higher in the cells grown on nutrient agar (Fig. 6). Cells grown to an O. D. of only 0.1 (aspartate medium) did not retain an elevated acetate transport rate. This was approximately the amount of growth obtained when cells were grown in the presence of PL in previous experiments.

To determine whether PL actually causes an increase in acetate transport ability, aspartate-grown cells were used as a source of inoculum for fresh aspartate media with and without PL. Cells from the PL-containing medium, when assayed after the removal of PL, showed a 2-3 fold enhancement of acetate transport compared to control cells (Fig. 7). It can be concluded that growth in the presence of PL causes cells to acquire an increased acetate transport ability rather than just retaining it.

It is possible that the PL-induced stimulation of acetate uptake might occur because the cells retain some diffusible compound that functions to stimulate acetate uptake. This concept was considered a possibility because PL is known to cause a reduction in the efflux of 2-deoxyglucose and D-alanine in M. lysodeikticus disIIp$^+$ (Staerkel, 1973). Cells were incubated in the presence of PL (7.7×10^{-2} M) for 13.5 hr, harvested by centrifugation, washed twice in basal salts solution, and shaken in basal salts solution for 2, 4, and 6 hr at 25 C. Data given in Fig. 8 indicate that acetate transport remained constant for 6 hr after the removal of PL. Therefore, the accumulation of a diffusible

Figure 6. Comparison of the Transport of Acetic Acid-2-^{14}C for Cells Grown on Nutrient Agar and in the Aspartate Medium. ●, control cells grown to an O. D. of 0.1; ▲, control cells grown to an O. D. of 0.34; ■, cells grown on nutrient agar.

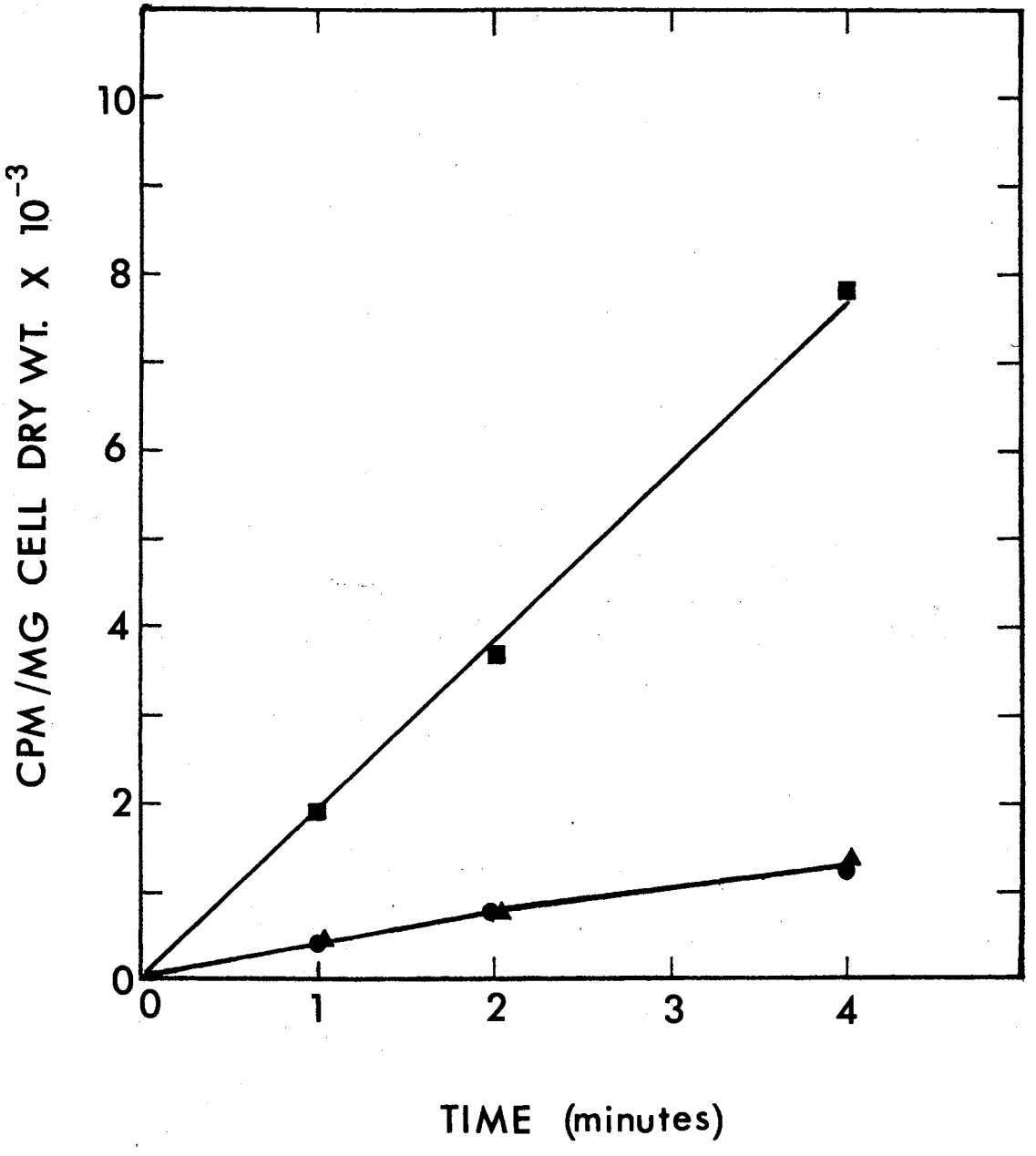

CPM/MG CELL DRY WT. X 10^{-3}
10
8
6
4
2
0
0
1
2
3
4
TIME (minutes)

Figure 7. Influence of Pantoyl Lactone in the Growth Medium on the Transport of Acetic Acid-2-^{14}C. Cells grown in the aspartate medium were used as a source of inoculum. ●, control; ■, cells grown in the presence of PL (7.7×10^{-2} M).

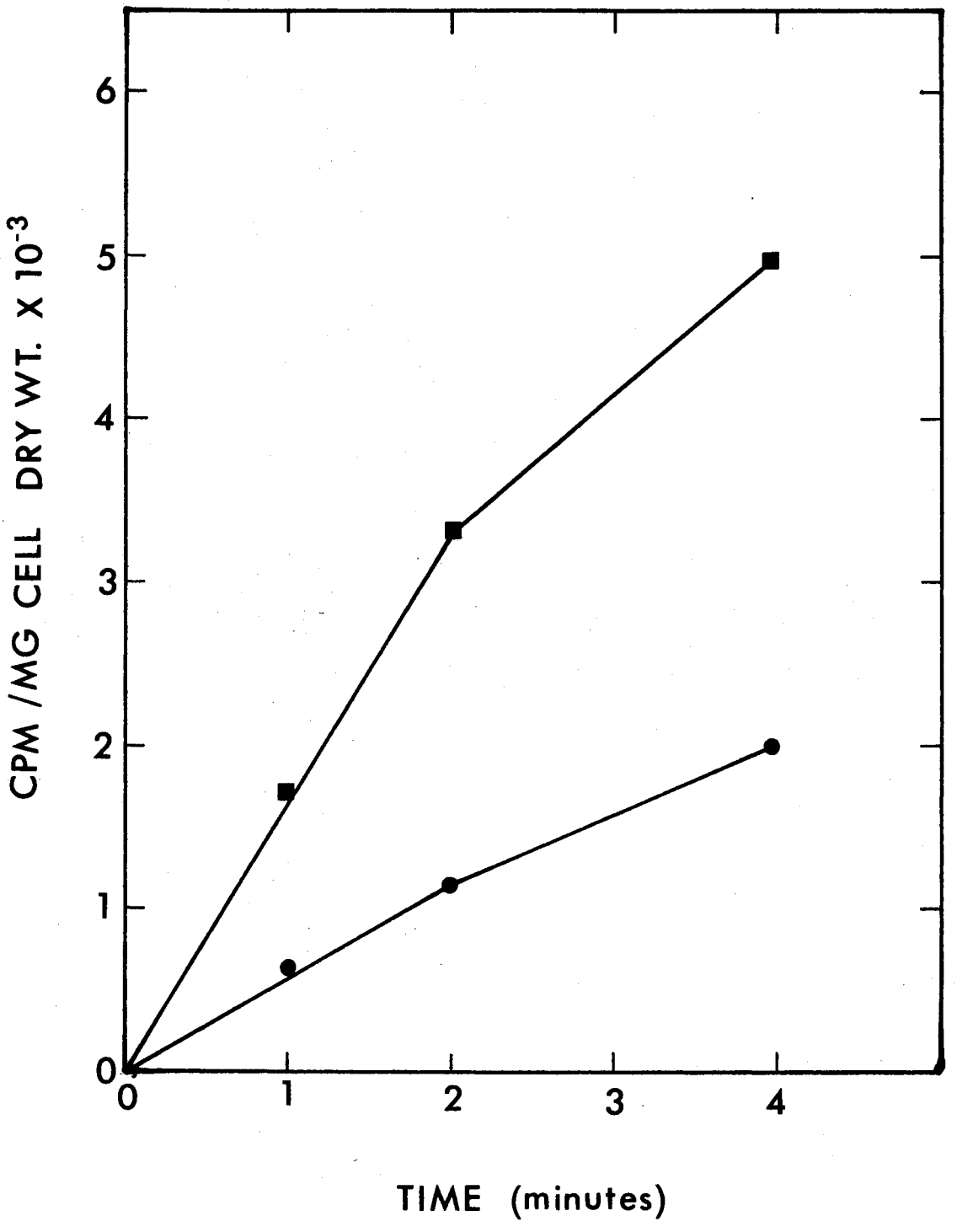
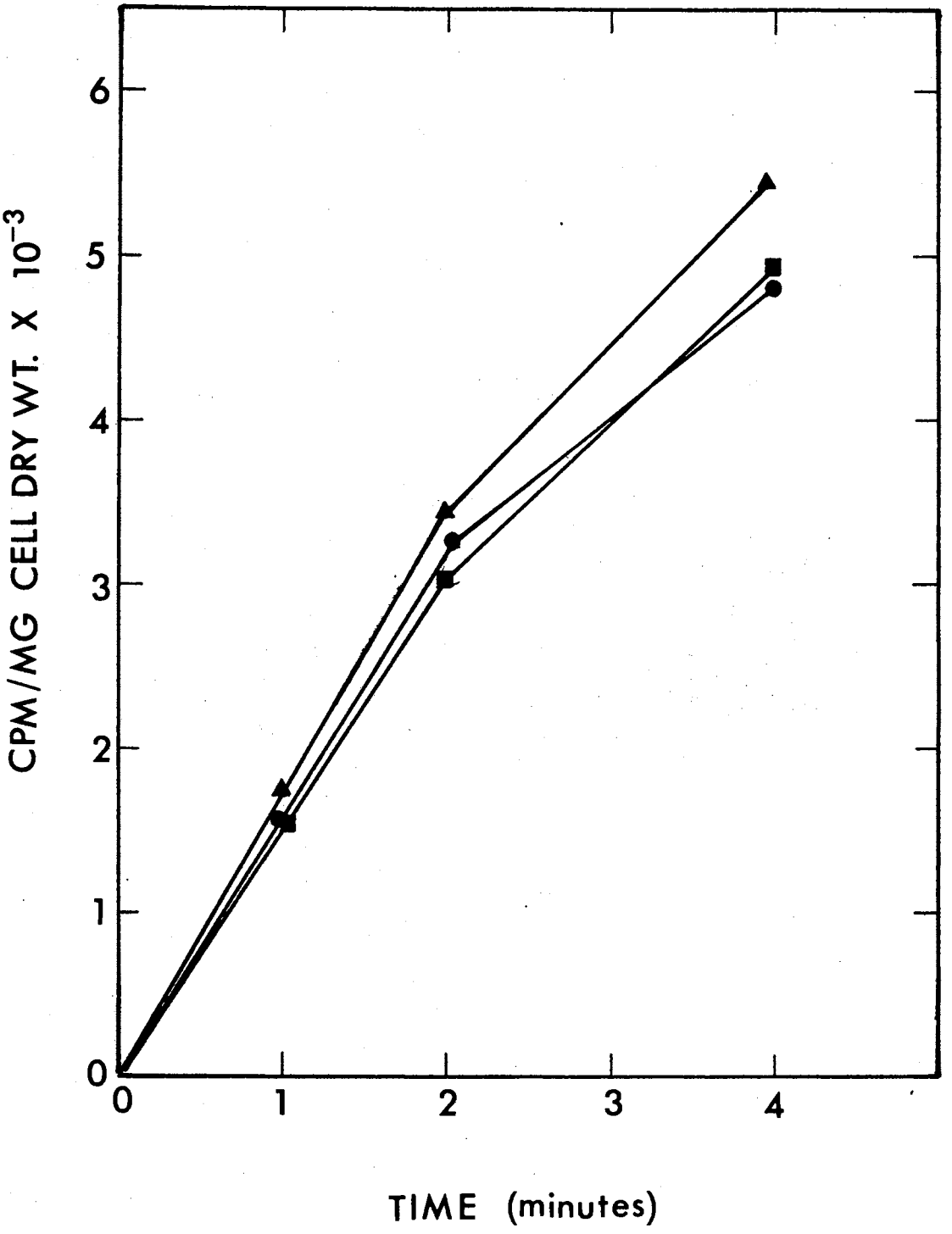

CPM /MG CELL DRY WT. X 10^{-3}
0
1
2
3
4
5
6
0
1
2
3
4
TIME (minutes)

Figure 8. Transport of Acetic Acid-2-^{14}C by Cells Grown in the Presence of Pantoyl Lactone and Held in Basal Salts Solution for 6 Hours. Cells were grown in the presence of PL (7.7×10^{-2} M) for 14 hr, washed twice, and shaken at room temperature for 6 additional hr. ■, 2 hr after removal from the growth medium; ●, 4 hr after removal from the growth medium; ▲, 6 hr after removal from the growth medium.


CPM/MG CELL DRY WT. X 10^{-3}
0
1
2
3
4
5
6
0
1
2
3
4
TIME (minutes)

molecule which stimulates acetate transport is probably not the mechanism by which PL enhances acetate uptake. Some permanent alteration to the cell has probably occurred.

Another possible explanation is that some portion of the acetate transport system is inducible; the system is induced when cells are grown on nutrient agar, but not in the aspartate medium. Cells grown in the presence of PL could accumulate the compound(s) necessary for induction, which would normally be released from the cell at a greater rate. In an attempt to test such a hypothesis, cells were grown in the presence of 3'-5' cyclic adenosine monophosphate (cAMP) as well as acetate, because cAMP and its leakage from the cell have been implicated in "glucose-repression" (Pastan and Perlman, 1970). Cells grown in the aspartate medium (non-induced) were used as inoculum. Inclusion of cAMP into the medium proved to be toxic to growth (as was acetate to a lesser extent). The presence of cAMP or cAMP plus acetate during growth caused a slight stimulation of acetate uptake, whereas presence of acetate alone caused a slight reduction (Fig. 9). Stimulation by the inclusion of cAMP in the growth medium was not nearly as great as that produced in the presence of PL. The results of this experiment do not yield convincing evidence that the PL stimulation of acetate transport is due to induction of the acetate transport system.

If the PL enhancement of acetate uptake is due to the induction of a protein necessary for acetate transport, then chloramphenicol (CAP) should to some extent prevent this induction. Data given in Table XVI indicate that, even though the inclusion of CAP in the medium will cause growth inhibition, the PL-induced stimulation of acetate uptake is not prevented. In fact, when both PL and the higher concentrations of CAP

Figure 9. Effect of Growth in the Presence of cAMP and Acetate on Acetic Acid-2-^{14}C Transport. ●, control; ■, acetate (10^{-3} M) present during growth; ▲, cAMP (2×10^{-3} M) present during growth; o, acetate (10^{-3} M) and cAMP (2×10^{-3} M) present during growth.

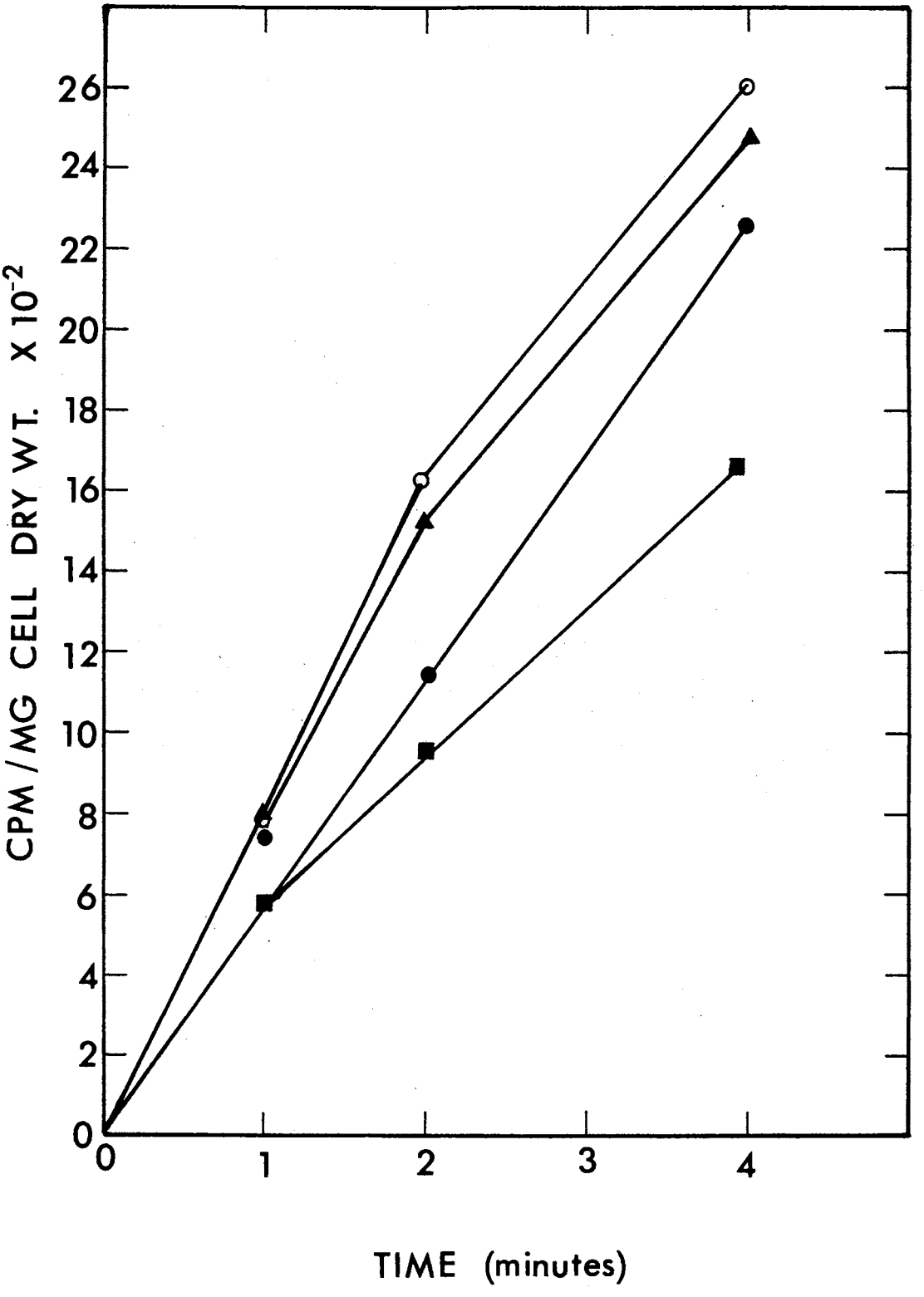

CPM / MG CELL DRY WT. X 10^{-2}
0
2
4
6
8
10
12
14
16
18
20
22
24
26
0
1
2
3
4
TIME (minutes)

TABLE XVI

EFFECT OF GROWTH IN THE PRESENCE OF PANTOYL LACTONE AND CHLORAMPHENICOL (CAP) ON THE TRANSPORT OF ACETIC ACID-2-^{14}C

Additions to Medium[a]	Initial O. D.	Final O. D.[b]	CPM/mg Cell Dry Wt.[c]
None	0.038	0.090	2,706
PL[d]	0.042	0.080	3,398
PL + CAP (1 µg/ml)	0.035	0.070	2,644
PL + CAP (5 µg/ml)	0.032	0.047	3,946
PL + CAP (10 µg/ml)	0.030	0.039	4,055

[a]Inoculum cells were obtained from a 12 hr culture grown in the aspartate medium.

[b]Incubation was for a period of 2 hr in the aspartate medium with the indicated additions.

[c]Values were taken after 4 minutes in the presence of the labeled compound.

[d]PL when present was always at the final concentration of 7.7×10^{-2} M.

were present during growth, acetate uptake was stimulated to a greater extent than when PL alone was present. On the basis of these results it would seem unlikely the stimulation in acetate transport observed, when cells are grown in the presence of PL, is due to the induction of a protein in the acetate transport system. Growth in the presence of PL must in some yet unknown manner alter the permeability properties of the cell, at least with respect to acetate transport.

There is some indication that this stimulation of acetate transport may be associated with an alteration in FA composition. When cells were grown in the presence of PL for 17 hr and resuspended in fresh medium, which did not contain PL, the ability to transport acetate decreased with continued incubation (Table XVII). In addition, FA composition returned to a more normal profile, as mentioned in a previous section (Table XV). Cells grown on nutrient agar also exhibit greater acetate uptake than do cells grown in the aspartate medium (Fig. 6). There appear to be certain similarities in the FA composition of cells grown on nutrient agar and for cells grown in the presence of PL. Values for C 16:1 are in general lower than for cells grown in the aspartate medium. Also, a number of minor FA are present in greater proportions. It should be noted that cells grown in the presence of PL from the time of inoculation (Fig. 5) show a greater stimulation of acetate transport than do cells grown in the presence of PL for only 2 hr (Table XVI). Fatty acid composition is altered when PL is present from the time of inoculation (Table IX, Table XI, and Table XII), while no such alteration was observed when PL was added after significant growth was established (Table XIII). Further investigation will be needed to establish if the increase in acetate uptake for cells grown in the presence of PL is due to an altered FA composition.

TABLE XVII

EFFECT OF REMOVAL OF PANTOYL LACTONE ON THE TRANSPORT OF ACETIC ACID-2-^{14}C

Time After Removal of PL (hr)[a]	CPM/mg Cell Dry Wt.[b]
0	2,285
2	2,025
3	1,340
5	1,338

[a]All data on the cultural conditions are given in Table XV.

[b]Values were taken after 3 minutes in the presence of the labeled compound.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The major objective of this project was to determine if either growth conditions, which bring about division inhibition or prevention of division inhibition, cause an alteration in the FA composition of the lipids isolated from Erwinia sp. Grula and Grula (1964) suggested that division inhibition may be the result of secondary membrane damage, which occurs because of a decrease in mucopeptide synthesis. For this study it was proposed that this secondary membrane damage might be reflected in an altered FA composition. This hypothesis was investigated by inducing filament formation by a variety of agents. Five different inhibitors of division and four different growth media were used. In only one instance was division inhibition associated with a significant alteration in FA composition. D-Serine, when added at the time of inoculation, causes a reduction in the major FA (C 16:1) and an increase in a number of the minor components. This change, however, cannot be directly correlated with D-serine-induced division inhibition. When filament formation was induced by the addition of D-serine after 12 hr of growth, no alteration in FA profile was observed.

Prevention of division inhibition might be considered a more likely situation to be associated with an alteration in FA composition. Grula and Grula (1964) reported that agents that prevent or reverse division inhibition do not restore mucopeptide synthesis to control levels. It

was therefore suggested that these agents may act by stabilizing the cell membrane. Manganese salts effectively prevent filament formation induced by vancomycin without any alteration in FA composition. Pantoyl lactone was considered the compound of greatest interest because it has proven to be effective in the prevention of division inhibition in all situations so far investigated. Prevention of vancomycin-induced filament formation by PL was associated with a significant change in FA distribution; however, it was demonstrated that a change in FA composition was not necessary for PL to prevent division inhibition. The inclusion of PL in the growth medium along with D-serine effectively prevented filament formation, while the FA composition remained unaltered (Table XIV).

It can be concluded that neither division inhibition nor the prevention of filament formation are associated with an altered FA profile. The two previous reports (Weinbaum and Panos, 1966; and Staerkel, 1973) which have associated changes in FA composition with division inhibition should be considered as describing parallel events rather than causative ones.

Cells grown in the presence of PL exhibit a concentration dependent reduction in cell length, growth inhibition, and alteration in FA composition. The effects on growth and cell size are evident at concentrations of PL not sufficient to alter FA composition. Consequently one cannot attribute the effect PL has on cell size and on growth to a change in FA distribution. When sufficient PL is present to bring about a change in the FA composition growth is also severely inhibited. Growth in the presence of mitomycin c also proved to be extremely inhibitory to growth, but no change in FA distribution was observed. Therefore growth inhibition as such cannot be the reason PL causes a change in the FA profile.

The mechanism by which PL causes an alteration in FA distribution may be determined when possible effects of PL on the enzymes involved in FA synthesis and desaturation are investigated.

Evidence was obtained that is consistent with the hypothesis that PL inhibits transport by interacting with the lipids in the cell membrane. Arrhenius plots for aspartate transport showed a break point, but only when PL was present. Alterations in transport Arrhenius plots have been demonstrated to be directly reflective of alterations in the lipid character of the membrane (Fox and Tsukagoshi, 1972). The break temperature in the plot of aspartate transport measured in the presence of PL corresponds well with the temperature for a lipid phase transition obtained by spectrophotofluorimetric means. It is suggested that the break in the Arrhenius plot for aspartate transport, when measured in the presence of PL, is due to more PL entering the membrane lipids at temperatures above the phase transition temperature. It was also found that PL influenced the phase transition temperature when measured using whole cells or isolated phospholipids. The phase transition temperature was lowered and the general shape of the curves altered when PL was present. These findings were taken as evidence that PL can interact with the lipids of Erwinia species.

Uptake of acetate is the only transport system so far investigated which is not inhibited by PL in M. lysodeikticus disIIp^{+} (Staerkel, 1973). When acetate transport was measured in Erwinia sp. it was found that PL was inhibitory to acetate uptake. This may indicate that acetate is taken up by a different mechanism in the two organisms or that the transport system may be in a different environment, such that PL has a different effect on acetate transport in the two organisms.

Although it seemed likely that the increase in acetate transport might be due to the induction of a protein(s) involved in the transport of acetate, the results of two experimental approaches indicate that this is not the case. Growth in the presence of acetate and/or cAMP did not cause an increase in acetate transport to nearly the extent that growth in the presence of PL did. Also cells grown in the presence of CAP did not show any decrease in the PL-induced stimulation of acetate transport. On this basis it is suggested that growth in the presence of PL may in some manner alter the permeability properties of the cell membrane such as to alter acetate transport. There is some indication that the stimulated acetate uptake may be associated with the observed changes in FA composition produced when cells are grown in the presence of PL.

LITERATURE CITED

Augustin, J., and W. Hasselbach. 1973. Studies on the fluorescence of 1-anilino-8-naphthalenesulfonate by the membranes of the sarcoplasmic reticulum. Eur. J. Biochem. 35:114-121.

Baldassare, J., Alice Gerke McAfee, and C. Ho. 1973. A spin label study of E. coli membrane vesicles. Biochem. Biophys. Res. Commun. 53:617-623.

Cho, K. Y., and M. R. J. Salton. 1966. Fatty acid composition of bacterial membrane and wall lipids. Biochim. Biophys. Acta 116:73-79.

Cullen, J., M. C. Phillips, and G. G. Shipley. 1971. The effects of temperature on the composition and physical properties of the lipids of Pseudomonas fluorescens. Biochem. J. 125:733-742.

Fox, C. F. 1969. A lipid requirement for induction of lactose transport in Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. 63:850-855.

Fox, C. F. (ed.), and N. Tsukagoshi. 1972. The influence of lipid phase transitions on membrane function and assembly, pp. 145-153. In Membrane Research. Academic Press, New York.

Gale, E. F., and J. M. Llewellin. 1971. Effect of unsaturated fatty acids on aspartate transport in Staphlococcus aureus and on staphlococcal lipid monolayers. Biochim. Biophys. Acta 233:237-242.

Grula, E. A. 1960a. Cell division in a species of Erwinia. I. Initial observations relating to nutritional dependency. J. Bacteriol. 80:369-374.

Grula, E. A. 1960b. Cell division in a species of Erwinia. II. Inhibition of division by D-amino acids. J. Bacteriol. 80:375-385.

Grula, E. A., and Mary M. Grula. 1962a. Cell division in a species of Erwinia. III. Reversal of inhibition of cell division caused by D-amino acids, penicillin, and ultraviolet light. J. Bacteriol. 83:981-988.

Grula, E. A., and Mary M. Grula. 1962b. Cell division in a species of Erwinia. V. Effect of metabolic inhibitors on terminal division and composition of a "division" medium. J. Bacteriol. 84:492-499.

Grula, E. A., and Mary M. Grula. 1964. Cell division in a species of Erwinia. VII. Amino sugar content of dividing and non-dividing cells. Biochem. Biophys. Res. Commun. 17:341-346.

Grula, E. A., and R. D. King. 1971. Changes in the cell membranes of dividing and non-dividing cells of Micrococcus lysodeikticus disIIp$^+$. Biochem. Biophys. Res. Commun. 44:1356-1363.

Grula, Mary M., R. W. Smith, C. F. Parham, and E. A. Grula. 1968. Cell division in a species of Erwinia. XII. A study of nutritional influences in D-serine inhibition of growth and division of Erwinia sp., and of certain specific sites of D-serine action. Can. J. Microbiol. 14:1225-1238.

Hansch, C., and W. R. Glave. 1971. Structure-activity relationships in membrane-perturbing agents. Mol. Pharmacol. 7:337-354.

Hopfer, R. L. 1972. Pantoyl lactone-induced alterations in biological systems. Ph.D. thesis. Oklahoma State University.

Kito, M., S. Aibara, M. Kato, and T. Hata. 1972. Differences in fatty acid composition among phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin of Escherichia coli. Biochim. Biophys. Acta 260:475-478.

Knivett, V. A., and J. Cullen. 1967. Fatty acid synthesis in Escherichia coli. Biochem. J. 103:299-306.

Marr, A. G., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in Escherichia coli. J. Bacteriol. 84:1260-1267.

Mavis, R. D., and P. R. Vagelos. 1972. The effect of phospholipid fatty acid composition on membranous enzymes in Escherichia coli. J. Biol. Chem. 247:652-659.

O'Leary, W. 1967. The Chemistry and Metabolism of Microbial Lipids. p. 9. The World Publishing Co., Cleveland and New York.

Overath, P., and H. Trauble. 1973. Phase transitions in cells, membranes, and lipids of Escherichia coli. Detection by fluorescent probes, light scattering, and dilatometry. Biochemistry 12:2625-2634.

Pastan, I., and R. Perlman. 1970. Cyclic adenosine monophosphate in bacteria. Science 169:339-344.

Seeman, P. M. 1966. Membrane stabilization by drugs: Tranquilizers and anesthetics. Int. Rev. Neurobiol. 9:145-221.

Shaw, M. K., and J. L. Ingraham. 1965. Fatty acid composition of Escherichia coli as a possible controlling factor of the minimal growth temperature. J. Bacteriol. 90:141-146.

Shen, P. Y., E. Coles, J. L. Foote, and J. Stenesh. 1970. Fatty acid distribution in mesophilic and thermophilic strains of genus Bacillus. J. Bacteriol. 103:497-499.

Silbert, D. F., and P. R. Vagelos. 1967. Fatty acid mutant of E. coli lacking β-hydroxydecanoyl thioester dehydrase. Proc. Nat. Acad. Sci. U.S.A. 58:1579-1586.

Staerkel, R. E. 1973. Effects of pantoyl lactone on transport and fatty acids composition in Micrococcus lysodeikticus. M. S. thesis. Oklahoma State University.

Thorne, Kareen J. I., and E. Kodicek. 1962. The metabolism of acetate and mevalonic acid by Lactobacilli IV. Biochim. Biophys. Acta 59:306-312.

Weinbaum, G. 1966. Characteristics of cell walls from morphological variants of Escherichia coli. J. Gen. Microbiol. 42:83-92.

Weinbaum, G., and C. Panos. 1966. Fatty acid distribution in normal and filamentous Escherichia coli. J. Bacteriol. 92:1576-1577.

Wilson, G., and C. F. Fox. 1971. Biogenesis of microbial transport systems: Evidence for coupled incorporation of newly synthesized lipids and proteins into membrane. J. Mol. Biol. 55:49-60.

VITA

Thomas W. Rice

Candidate for the Degree of

Doctor of Philosophy

Thesis: CELL DIVISION IN A SPECIES OF ERWINIA. ENVELOPE FATTY ACIDS AND TRANSPORT

Major Field: Microbiology

Biographical:

Personal Data: Born in Tulsa, Oklahoma, March 14, 1944, the son of Earl L. and Amy M. Rice; married to Susan N. Stokely on February 18, 1972.

Education: Graduated from Daniel Webster High School, Tulsa, Oklahoma, in May, 1962; received Bachelor of Science degree from Oklahoma State University, with a major in Microbiology, in May, 1966; received Master of Science degree from Oklahoma State University, with a major in Microbiology, in August, 1969; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in December, 1973.

Professional Experience: NDEA Fellow and Graduate Teaching Assistant, Department of Microbiology, Oklahoma State University, 1966-69; active duty with U.S. Army, Departments of Experimental Nephrology and Neuroendocrinology, Walter Reed Army Institute of Research, 1969-71; Graduate Research Assistant, Department of Microbiology, Oklahoma State University, 1971-72; Research Technician, U.S. Bureau of Mines contract on Microbial Flora of Deep Geological Formations, Department of Microbiology, Oklahoma State University, 1973.

Professional Organizations: Member of the American Society for Microbiology and Missouri Valley Branch - American Society for Microbiology.