THE UNIVERSITY OF MANITOBA

INFLUENCE OF AUTOTROPHIC AND HETEROTROPHIC GROWTH ON RIBOSOME STRUCTURE OF PSEUDOMONAS OXALATICUS OX1.

BY

RICHARD TERRENCE MARTIN

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"INFLUENCE OF AUTOTROPHIC AND HETEROTROPHIC GROWTH ON RIBOSOME STRUCTURE OF PSEUDOMONAS OXALATICUS OX1"

by

RICHARD TERRENCE MARTIN

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TO MY WIFE, PARENTS AND FAMILY

ABSTRACT

by RICHARD TERRENCE MARTIN

under the supervision of Dr. P. Y. Maeba

The ribosomes of Ps. oxalaticus OX 1 grown under different cultural conditions were studied. Analysis of ribosomal protein complements of formate - and casamino acid-grown cells by pH 4.5 polyacrylamide disc gel electrophoresis indicated 3 protein band differences could not be shown by two dimensional gels. pH 8.7 disc gel electrophoresis showed 3 additional proteins present in the heterotrophic but not the autotrophic ribosomes. From two dimensional polyacrylamide gel estimates of 53-58 heterotrophic and 50-56 autotrophic ribosomal proteins were obtained. Analysis of the ribosomal subunits also failed to locate and identify the specific alterations involved in adaptation of the ribosome. However, a minimum of twenty-six 50 S and fifteen 30 S heterotrophic and twenty-five 50 S and eighteen 30 S autotrophic ribosomal proteins were identified. Analysis of r RNA indicated that the 23 S

r RNA of intact 70 S ribosomes contained "hidden scission". Dissociation of ribosomes into their respective subunits was accompanied by further breakdown of both 16 S and 23 S r RNA of both autotrophic and heterotrophic ribosomes.

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ABBREVIATIONS

Although growth on formate as sole carbon source can not be classified as true autotrophy, it will be referred to as autotrophy in this thesis. The pathway of incorporation of formate carbon into cell material is analogous to that in autotrophs.

Throughout this thesis, ribosomes and subunits isolated from cells grown autotrophically or heterotrophically will be referred to as "autotrophic ribosomes or subunits" and "heterotrophic ribosomes or subunits", respectively.

The ribosomal proteins are numbered horizontally from left to right according to the vertical distance of migration from the origin of the two dimensional electropherogram with the letters S and L representing proteins of the small, or 30 S, and the large, or 50 S, subunits respectively. This nomenclature has been used to describe ribosomal proteins of E. coli in the "Historical". In this thesis, this terminology has been applied to the Ps. oxalaticus OX1 ribosomal proteins.

A_{260}	-	Absorbance at 260 mμ
A_{260} equivalents of protein	-	Amount of protein extracted from 1 A_{260} unit of ribosome or subunit
ATCC	-	American Type Culture Collection
DNAase	-	Deoxyribonuclease
DTT	-	Dithiothreitol (Cleland's Reagent)
Mg acetate	-	Magnesium acetate
RNAase	-	Ribonuclease
rRNA	-	Ribosomal ribonucleic acid
SDS	-	Sodium dodecyl sulphate
TCA	-	Trichloroacetic acid
TK	-	0.01 M tris-HCl + 0.05 M KCl, pH 7.8
TKM	-	0.01 M tris-HCl + 0.05 M KCl + 0.01 M Mg acetate, pH 7.8
TM	-	0.01 M tris-HCl + 0.01 M Mg acetate, pH 7.8
TM-Urea-DTT	-	0.1 M tris-HCl + 0.01 M Mg acetate + 7.0 M urea + 1.0 mM dithiothreitol, pH 8.0

I INTRODUCTION

The heterogeneity of bacterial ribosomal proteins is well documented (Historical). Measurement of protein content showed that some 30 S proteins exist in less than one copy per ribosome (Kurland et al., 1969; Traut et al., 1969; Voynow and Kurland, 1971; Weber, 1972). On the other hand, some proteins of the 50 S subunit were present in more than 1 copy per ribosome while others were in less than one copy per ribosome (Weber, 1972). These facts suggested that different classes of ribosomes exist in a given cell such that the number of copies of a particular protein reflects an average of the ribosome population (Kurland et al., 1969; Kurland, 1970; Voynow and Kurland, 1971).

Two models based upon this "time-average" hypothesis were suggested, the steady state and static models. In the steady state model the ribosomal protein complement changes as a given ribosome proceeds through initiation, propagation, and termination of protein synthesis. The static model requires distinct classes of ribosomes, each with a specific function and specific protein complement.

A functional basis for heterogeneity of ribosomes was suggested by Deusser (Deusser and Wittmann, 1972; Deusser, 1972) who detected a variation in the protein composition of E. coli ribosomes grown under different conditions, i.e., in rich and minimal medium. Park (1973) demonstrated gross changes in protein composition of ribosomes of Thiobacillus novellus grown under autotrophic and heterotrophic conditions. One explanation for these observations was that under different growth conditions different mRNA's would be produced and translated by specific ribosomes.

However, the T. novellus system was difficult to investigate due to the technical problems encountered in growing the organism. In this thesis, investigation into the influence of growth conditions on ribosomal protein composition was studied employing the organism Pseudomonas oxalaticus OX1 (Khambata and Bhat, 1953). This organism is capable of utilizing sodium formate as its sole source of carbon and energy. Conversion of formate to CO_2 which is fixed via the Calvin cycle closely resembles autotrophic growth (Quayle and Keech, 1959 a, b, c). Growth on formate and adaptation to a more heterotrophic growth, and vice versa, are rapid making this system more suitable for this investigation.

II HISTORICAL

Protein synthesis is a very complex cellular process involving over 100 macromolecules. Such components as the transfer RNA (tRNA) molecules; the amino acid - activating enzymes; various supernatant factors involved in chain initiation, propagation and termination; messenger RNA (mRNA); and the 55-60 components of the ribosome are required for protein synthesis. The ribosome plays a central role in this process serving to bind tRNA and mRNA and interacting with various supernatant factors. It contains peptidyl transferase activity which is required for peptide bond formation and is involved in translocation of mRNA during translation as well as the translocation of nascent peptide chains from the "A" to "P" sites. Obviously, an understanding of the ribosome and its various components is necessary for elucidation of the mechanism of protein synthesis.

The Escherichia coli ribosome is characterized by its sedimentation coefficient of approximately 70 S and molecular weight of 2.6×10^6 daltons of which 65%

is RNA (Tissières et al., 1959). It consists of 2 unique subunits of unequal size that function together to mediate protein synthesis. The small, or 30S, subunit has a molecular weight of 0.8×10^6 daltons (Tissières et al., 1959; Hall and Slayter, 1959; Huxley and Zubay, 1960) consisting of a 16S ribosomal RNA molecule of molecular weight $0.53 - 0.56 \times 10^6$ (Kurland, 1960; Midgely, 1965; Stanley and Bock, 1965) and a protein mass of about $0.23 - 0.28 \times 10^6$ daltons, i.e., 30-33% protein (Craven et al., 1969). The other subunit, the 50 S, is roughly twice the size of the 30 S with a molecular weight of 1.8×10^6 daltons (Tissières et al., 1959; Hall and Slayter, 1959). The 50 S particle consists of a 23 S rRNA with molecular weight $1.0 - 1.12 \times 10^6$ (Kurland, 1960; Midgely, 1965; Stanley and Bock, 1965), a 5S rRNA of molecular weight 0.04×10^6 daltons (Brownlee and Sanger, 1967; Brownlee et al., 1967), and an aggregate protein mass of $0.52 - 0.56 \times 10^6$ daltons (Dzionara et al., 1970; Mora et al., 1970).

The ribosome was pictured as a simple entity, much like a virus, until Waller and Harris (1961) demonstrated its structural complexity. Using starch gel electrophoresis in 6 M urea approximately 20 constituent ribosomal proteins, with an average molecular weight of 25,000 daltons, were identified. In further

work, Waller (1964) resolved over 24 proteins and, as well, ruled out the possibility that this number might be due to artifacts arising through aggregation of proteins. Furthermore the electrophoretic patterns of proteins extracted from 30 S and 50 S subunits were markedly different. Due to his research, the ribosome was pictured as a particle with a heterogeneous population of proteins.

Further work on purification of ribosomal proteins carried out in a number of labs indicated that the complexity of the ribosome was underestimated even by Waller's work. The proteins of the 30 S subunit of E. coli have been fractionated into 21 distinct species (Moore et al., 1968; Fogel and Sypherd, 1968; Craven et al., 1969; Hardy et al., 1969; Kurland et al., 1969; Nomura et al., 1969; Kaltschmidt and Wittmann, 1970 a; Wittmann et al., 1971; Hindennach et al., 1971 a) and that of the 50 S subunit into 34 different proteins (Craven et al., 1969; Dzionara et al., 1970; Kaltschmidt et al., 1970; Kaltschmidt and Wittmann, 1970 b; Mora et al., 1971; Hindennach et al., 1971 b). Aggregation, deamidation, carbamylation, disulphide interactions, nucleotide binding, and partial proteolysis have been ruled out as causes for the large numbers of

observed components (Waller, 1964; Traut, 1966; Möller and Chrambach, 1967).

Using polyacrylamide gel electrophoresis with sodium dodecyl sulphate the molecular weights of the 30 S and 50 S E. coli proteins were determined. The values ranged from 10,900 to 65,000 for the 30 S proteins and from 9,600 to 31,500 for those of the 50 S subunit (Dzionara et al., 1970). These values agreed with molecular weights determined by equilibrium sedimentation (Traut et al., 1969; Craven et al., 1969).

The 21 proteins of the E. coli 30 S subunit have been extensively characterized. All proteins are different with no sequence similarities (Kaltschmidt et al., 1967; Traub et al., 1967; Moore et al., 1968; Fogel and Sypherd, 1968; Craven et al., 1969; Hardy et al., 1969; Hindennach et al., 1971 a). Also, each of the 21 proteins reacts with only its homologous antiserum indicating no sequence similarities exist between the proteins (Kaltschmidt et al., 1967; Traut et al., 1969; Stöffler and Wittmann, 1971).

The 50 S proteins have also been characterized although not as extensively. Although two proteins, L7 and L12, were identical except for the acetylation of the N-terminal serine residue in protein L7 (Möller

et al., 1972; Terhorst et al., 1972) the rest of the proteins were unique (Fogel and Sypherd, 1968; Dzionara et al., 1970; Kaltschmidt et al., 1970 b; Mora et al., 1971; Stöffler and Wittmann, 1971; Hindennach et al., 1971 b).

Comparison of 30 S proteins with those of the 50 S subunit by polyacrylamide gel electrophoresis (Strnad and Sypherd, 1969) and by immunological activity (Stöffler and Wittmann, 1971) indicated that no common structure existed between 30 S and 50 S subunits. However, recently, the proteins S 20 (30 S) and L 26 (50 S) have been shown to share some common sequences.

The first indications that ribosomes are functionally, as well as structurally, heterogeneous came from the work of Staehelin (Staehelin and Meselson, 1965; Raskas and Staehelin, 1967) and Nomura (Hosokawa et al., 1966; Traub et al., 1967). Fractionation of subunit in cesium chloride resulted in the removal of some proteins, the "split proteins", from the subunit and the production of a protein-deficient particle, the "core". These workers produced functional ribosomes by the addition of "split proteins" to the protein-deficient "cores". It was apparent that a number of different proteins were bound to the rRNA to form specific sites with catalytic and binding activities.

Once total reconstitution of the 30 S subunit from 16 S rRNA and 30 S proteins was obtained by Traub and Nomura (1968), investigation into the function of each protein was undertaken. For example, S1 is believed to be responsible for the association between mRNA and the 30 S subunit (van Duin and Kurland, 1971); S 2, S 3 and S 14 are involved in the aminoacyl site of the ribosome (Randall-Hazelbauer and Kurland, 1972); S 21 inhibited the binding of N-formyl methionyl -tRNA (van Duin et al., 1972); L 7 and L 12 are involved in translocation (Highland et al., 1973); and L 11 is believed to be the peptidyltransferase enzyme (Nierhaus and Montejo, 1973). The assignment of specific functions to specific ribosomal proteins confirmed the functional heterogeneity of the ribosomal proteins.

The number-average molecular weight for the 30 S proteins was approximately 20,000 daltons (Waller, 1964; Möller and Chrambach, 1967; Craven et al., 1969; Dzionara et al., 1970). Since the 30 S particle contained only 0.23 - 0.26 x 10^6 daltons of protein, 12 different proteins present as one copy per ribosome would be expected. Consistent with this assumption, 13 of the 30 S proteins were purified and found to exist as one copy per subunit (Moore et al, 1968; Sypherd et al., 1969). However, the identification

of 21 unique 30 S proteins was incompatible with this assumption (Hardy et al., 1969; Kaltschmidt and Wittmann, 1970 a). The total mass of these 21 proteins was approximately 0.44×10^6 daltons or 0.2×10^6 daltons greater than the expected value (Craven et al., 1969; Kurland et al., 1969; Traut et al., 1969; Hardy et al., 1969). Thus there was an excess of protein which could not be accomodated by the 30 S subunit.

Several possible artifacts which could account for this discrepancy have been investigated. Such artifacts as overestimation of molecular weights (Craven et al., 1969), contamination of ribosomes by reversibly bound supernatant proteins or enzymes (Kurland, 1966; Hardy and Kurland, 1966) and partial stripping of proteins during ammonium sulphate purification (Hardy et al., 1969) have been ruled out.

On the basis of stoichiometric analysis, the 30 S proteins were divided into 3 classes: "unit proteins" which were present in 0.8 to 1.0 copies per 30 S particle; "fractional proteins", 0.6 or less copies per subunit; and "marginal proteins" which could be either "unit" or "fractional" (Kurland et al., 1969; Traut et al., 1969; Voynow and Kurland, 1971; Weber, 1972). This data strongly supported the

conclusion that purified ribosomes are structural heterogeneous *in vitro* and that no 30 S protein is present in more than one copy per ribosome (Voynow and Kurland, 1971). The discrepancy between total protein mass per subunit and the aggregate mass of 21 proteins could be compensated for by several proteins being present in less than 1 copy per ribosome.

This structural and functional heterogeneity of 30 S subunits has been interpreted by Kurland (Kurland *et al.*, 1969; Kurland, 1970; Voynow and Kurland, 1971) as a "time-average" of the different states that the ribosome occupied during protein synthesis. Two models have been formulated based upon this interpretation - the steady state and the static models. The steady state model required only one "ribosome" made up of "unit proteins" to form a basic particle. Its protein complement changes during initiation, elongation and termination of protein synthesis. The alteration of functional capacities of the "ribosome" is mediated by the displacement of one set of "fractional proteins" by a second set required for the next function of the ribosome cycle. A basic ribosomal particle consisting of "unit proteins" was also postulated for the static model. In this

case, however, different "fractional proteins" were permanently bound to the basic particle to produce different types of 30 S subunits. Each type was active in all phases of protein synthesis of a particular kind, e.g., translation of a specific mRNA.

When stoichiometric information was compared with 30 S assembly data obtained from reconstitution studies, an interesting correlation arose. "Unit proteins" were found to be proteins that bind to RNA to give particles of higher S value, i.e., those in whose absence particles with S - values of 30 could not be formed. Almost all of the "fractional proteins" were required for restoration of full activity of the ribosome in various functional assay systems (Traub et al., 1967). The absence of fractional proteins did not interfere with the assembly of the 30 S subunit but were required for formation of functional particles (Nomura et al., 1969; Kurland, 1970).

Supporting the steady state hypothesis was the following observation. When 30 S subunits were isolated only a fraction were functionally active. By allowing the proteins of the subunits to exchange with specific 30 S proteins, that fraction of active subunits could be increased (Kurland et al., 1969) suggesting that some proteins were able to exchange

with exogenous proteins. Further support came from experiments in which "fractional proteins" were added to ribosomes participating in specific functions. S 21 addition inhibited (van Duin et al., 1972) whereas proteins S 2, S 3 and S 14 enhanced (Randall-Hazelbauer and Kurland, 1972) binding of N-formyl methionyl -tRNA to ribosomes. It was concluded that S 21 was present in "propagating" ribosomes, i.e., ribosomes involved in peptide chain elongation, and absent in an "initiating" ribosome, i.e., ribosomes involved in the initiation of protein synthesis, whereas S 2, S 3 and S 14 were present in "initiating" ribosomes but not in "propagating" ribosomes. It was suggested that the presence of S 21 prevents ambiquity in translation of the A U G codon during chain propagation by altering the ribosomal configuration of an "initiating" ribosome (van Duin et al., 1972)

Further examination of this concept of different "initiating" and "propagating" ribosomes was undertaken by Bollen et al.,(1972) using matrix-bound mRNA to isolate specific 30 S complexes engaged in initiation. Bollen was able to demonstrate that 3 proteins (S 1, S 2 and one unidentified protein) were drastically decreased in "initiating" ribosomes. Both S 1 and S 2 are

"fractional proteins". In contrast, no variation in the stoichiometry of "marginal" and "unit" proteins were observed. Sedimentation analysis in sucrose gradients demonstrated that S 1 and S 2 were released from ribosomes in the "initiation" complexes whereas they were present in free-state (not bound to mRNA) ribosomes. Thus the protein complement of the 30 S subunit is altered during various stages of the ribosome cycle.

The structure of the 50 S subunit initially appeared simpler although it contained more proteins. The aggregate mass of the 35 50 S proteins was approximately equal to the protein mass of 50 S subunits. Consequently, it was suggested that the 50 S subunit was structurally homogeneous containing one copy of each 50 S protein (Traut et al., 1969; Dzionara et al., 1970; Mora et al., 1971). Weber (1972) studied the stoichiometry of 50 S proteins and observed four classes of proteins. They were: "fractional" (0.1 - 0.6 copies per 50 S subunit); "unit" (0.8 - 1.2 copies); "fractional repeated" (1.4 - 1.7 copies); and "repeated" (1.8 - 2.2 copies). These results indicated a greater degree of heterogeneity than for the 30 S subunit.

Although isolated 30 S and 50 S subunits were heterogeneous, no direct evidence supported the concept that heterogeneity occurred in living cells. Deusser

(Deusser and Wittmann, 1972; Deusser, 1972) investigated in vivo heterogeneity of ribosomes isolated from cells grown in enriched and minimal medium containing either ^{14}C - or ^{3}H - labelled amino acids. Cells were combined and ribosomes were extracted. Proteins extracted from the mixed ribosomes were resolved by 2- dimensional gel electrophoresis and the $^{14}C/^{3}H$ ratio determined for each protein. Deviation from the medium ratio (normalized to 1) would indicate certain proteins may be in greater or lesser quantity when grown under different conditions. The proteins were classified into 4 groups:

a) Proteins with normalized ratios between 0.90 to 1.10. This group was made up of 2/3 of the 50 S and 30 S proteins whose concentration in ribosomes was not influenced by growth conditions.

b) S 1, S 10, S 11, S 19, S 20, L 8 + L 9, L 17, L 27, L 32, L 33. These proteins appeared in greater concentration when cells were grown in enriched or minimal medium. The normalized ratios for these deviated 10-25% from the median and the values fluctuated in identical experiments.

c) L 7 and L 16. These proteins are similar to (b) except the 10-25% deviation from the median normalized ratio was repeatable in all experiments.

d) S 6, S 21 and L 12. These 3 proteins appeared in greater concentrations when cells were grown in enriched medium. Their normalized ratios were 2.46, 2.36 and 3.10 respectively.
These findings indicated that structural heterogeneity of ribosomal population can be induced *in vivo* in response to an alteration of growth conditions.

Additional support for *in vivo* heterogeneity was supplied by Foster and Parish (1973). In *Myxococcus xanthus*, they demonstrated that the only difference between ribosomes of myxospores and vegetative cells was in the quantities of 30 S proteins S 4, S 6 and S 7. This indicated that differentiation may have been responsible for the heterogeneity.

Many investigators have studied the ribosomes of eucaryotic organisms in an attempt to determine whether there are structural differences in ribosomes isolated from different tissues and organs of the same organism. The results to date are somewhat questionable due to the lack of good extraction and purification procedures for ribosomal proteins.

MacInnes (1971) observed a difference in the Mg^{++} concentration required for dissociation of brain and liver ribosomes of mice. Analysis of these ribosomes showed no major difference in protein composition of

brain and liver ribosomes, although the gel patterns suggested a quantitative difference in the amounts of some proteins. However, extensive fractionation of these proteins into separate, identifiable bands was not obtained. van Tan et al., (1971) reported intraspecific differences between ribosomal proteins from rabbit reticulocytes and liver. Also Lambertsson (Lambertsson et al., 1970; Lambertsson, 1972) resolved 53 larval, 50 pupal and 52 adult ribosomal proteins in Drosophila melanogaster suggesting heterogeneity of ribosomes induced by differentiation.

In this thesis, the heterogeneity of bacterial ribosomes is further investigated in the organism Pseudomonas oxalaticus strain OX1. This bacterium was chosen since it can utilize formate, oxalate or more reduced organic compounds as a sole source of carbon.

Using oxalate enrichment procedures, Khambata and Bhat (1953) were able to isolate the bacterium Pseudomonas oxalaticus from the intestinal tract of the Indian earthworm. Six strains of this oxalate utilizer were isolated: OX1, OX2, OX3, OX4, OX6 and OX23. These strains were characterized as gram-negative motile rods (0.3 - 0.4 μ X 0.9 - 1.5 μ) with 1 to 3 polar flagella. Strains OX1, OX2, OX3 and OX23 were also capable of growth on formate as the sole source of carbon and energy.

The ability of an organism to utilize formic acid as a sole source of carbon is restricted to procaryotes. Such aerobic organisms as Bacterium formicium, Bacterium formoxidans, Hyphomicrobium spp., Nitrobacter winogradskyi, Protaminobacter alboflavas, Protaminobacter ruber, Pseudomonas AM1, Pseudomonas M 27 and Vibrio extorquens are capable of formate utilization. Photosynthetic organisms such as Chloropseudomonas spp., Rhodopseudomonas palustris, and some non-photosynthetic anaerobic bacteria like Methanobacterium formicicum and Methanococcus vanniellii also utilize formate (Quayle, 1972).

The ability of Ps. oxalaticus OX1 to grow on formate depends upon its capacity to synthesize 3 carbon compounds from this 1 carbon molecule. The pathway was elucidated by following the fate of ^{14}C isotopes incorporated by the organism as formate or bicarbonate. Around 80-90% of the total recovered radioactivity appeared initially in phosphorylated compounds, phosphoglyceric acid being the major constituent (Quayle and Keech, 1959 a, b, c). Combined with the requirement for the addition of ribulose -1, 5-diphosphate or ribose - 5 - phosphate and ATP for fixation of the label by cell-free extracts, the

results also suggested that carbon assimilation into cellular constituents occurred via the carboxylation of ribulose -1, 5-diphosphate and the Calvin cycle. The suggestion was substantiated by the identification in cell-free extracts of phosphoriboisomerase, phosphoribulokinase, and carboxydismutase which are key enzymes of this cycle. (Quayle and Keech, 1959 b, c).

The assimilation of formate carbon into cellular constituents by this pathway required carbon dioxide as an intermediate metabolite, and when bicarbonate replaced formate identical labelling patterns were obtained. Furthermore, identification of a NAD^+-linked formic dehydrogenase combined with the fact that 94% of the carbon assimilated was exchangeable with carbon dioxide proved that CO_2 was an intermediate metabolite in formate utilization (Quayle and Keech, 1959 b).

The energy requirement for the biosynthesis of one molecule of triose phosphate (e.g.: phospho-glyceric acid) from formate demands a large quantity of energy as shown by equation (1):

$$3CO_2 + 9\ ATP + 6NADH + H^+ + H_2O \longrightarrow TRIOSE\ PHOSPHATE + 9ADP + 8Pi + 6NAD^+ \text{ ------ (1)}$$

This requirement was primarily met by the oxidation reaction carried out by the NAD^+ - linked formic dehydrogenase (equation (2)):

$$\text{FORMATE} + NAD^+ \longrightarrow CO_2 + NADH + H^+ \text{ ------ (2)}$$

The mode of growth of Ps. oxalaticus 0X1 on formate is, therefore, basically autotrophic in that CO_2 was fixed into organic material.

As well, this organism was capable of heterotrophic growth on oxalate, acetate, succinate, lactate, tartrate, citrate, ethyl alcohol, glucose, dextrin and starch (Khambata and Bhat, 1953). Yet, none of these substrates were capable of inducing the enzymes necessary for formate utilization. Studies on growth on mixed substrates (formate + another organic substrate) suggested that formate induction of these key enzymes was under some sort of metabolic repression (Blackmore and Quayle, 1968; Blackmore et al., 1968). The exact mechanism has not yet been determined.

The organism is capable of growth either as an autotroph or heterotroph. In adapting from one pattern of metabolism to another, various adaptive responses must be made by the organism. In this thesis, an attempt is made to determine whether such responses involve alterations of ribosome structure.

III MATERIALS AND METHODS

Organism

The organism used in this investigation was Pseudomonas oxalaticus strain OX 1 (ATCC 11883). This organism was maintained at 4¾C on trypticase soy agar slants (Difco) covered with sterile paraffin oil and transferred to fresh medium at 1 1-1½ month intervals. Batch cultures were grown in a liquid medium described by Jayasuriya (1955). The composition of the medium was slightly modified and consisted of the following components (expressed as grams/litre): K_2HPO_4, 8.7; $NaH_2PO_4.H_2O$, 6.9; $(NH_4)_2SO_4$, 2.5; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.001; $FeSO_4.7H_2O$, 0.005; $MnSO_4.5H_2O$, 0.0025; $Na_2MoO_4.2H_2O$, 0.0025; plus a carbon and energy source. For autotrophic growth, 6.8 grams/litre sodium formate served as sole source of carbon and energy and for heterotrophic growth, 2.0 grams/litre vitamin-free casamino acids (Difco) replaced the formate salt. The pH of the medium was approximately 6.8.

Autotrophic growth was carried out in 15 litre carboys aerated by forcing air through porous glass tubing. At pH 7.5 to 8.0, optimal growth took place at 28°C although the maximum temperature for growth was 34°C. During logarithmic growth, the pH increased rapidly to 8.5 - 9.0 and had to be continually adjusted to pH 7.5 - 8.0 with 1.43 M sterile phosphoricaacid. Failure to adjust pH resulted in lysis of cells. pH determinations were made on 5 ml samples aseptically removed from the culture vessel.

Two litres of culture grown as shake cultures in the same medium for 12-14 hr was used to inoculate the carboys. Cell growth was stopped in late logarithmic phase by addition of ice. Cells were harvested in a Sharples centrifuge, packaged in 25 grams (wet-weight) lots, and stored at - 76°C.

Identical procedures were used for heterotrophic growth of Pseudomonas oxalaticus except that casamino acids were used as the carbon and energy source. With this substrate, no pH adjustments were necessary.

Isolation of Ribosomes

Ribosomes were isolated from 25 grams wet-weight of cells following the method of Kurland (1966) modified in the following way. Cells were suspended

in 35 ml TM buffer (0.01 M tris-HCl, pH 7.8 + 0.01 M Mg acetate) containing 100 µg of DNA ase I (RNA ase-free, Worthington Biochemical Corporation). The cells were disrupted by 2 passages through an Aminco french pressure cell at 20,000 psi. The lysate was centrifuged at 27,000 x g for 30 min in a Sorval RC-2B centrifuge to remove cell debris. The supernatant was then subjected to three successive ammonium sulphate fractionations. The final pellet was resuspended in TM buffer containing 76 grams/litre ammonium sulphate (Schwarz/Mann) and pelleted by centrifugation at 40,000 rpm for 3 hr or 15 hr at 20,000 rpm in a Beckman 60 Ti rotor. This washing procedure was repeated twice. The final pellet was dissolved in TM buffer + 1.0 mM dithiothreitol (Cleland's Reagent, Calbiochem) and dialyzed against the same buffer for 6 hr. Ribosomes were stored at - 76°C in 1 ml quantities at a concentration of 400-800 A_{260} units/ml.

Isolation of Ribosomal Subunits

50 S and 30 S subunits were obtained from purified 70 S ribosomes. Ribosomes were dialyzed in TK buffer (0.01 M tris- HCl, pH 7.8 + 0.05 M KCl) + 1.0 mM Mg acetate for at least 6nhr then subjected

to sucrose gradient centrifugation.

a) Small Quantities

Dialyzed ribosomes (200-250 A_{260} units) were applied to 55 ml of 5-20% linear sucrose density gradient (RNA ase-free, Schwarz/Mann) made in TK + 1.0 mM Mg acetate buffer. After centrifugation in a Beckman SW 25.2 rotor at 24,000 rpm for 12 hr, the gradients were fractionated into approximately 1.5 ml fractions and assayed at 260 mμ in a Gilford Model 2000 spectrophotometer. The peak fractions of 30 S and 50 S subunits were pooled, made to 0.01 M Mg acetate and precipitated with 0.67 volumes ethanol (Staehelin et al., 1969). The precipitated fractions were allowed to stand overnight at - 20°C and then collected by centrifugation at 27,000 x g for 20 min. The pellet was dissolved in TKM buffer (0.01 M tris-HCl, pH77.8 + 0.05 M KCl + 0.01 M Mg acetate) and dialyzed against the same buffer for at least 6 hr. The subunits were stored at - 76°C in 1 ml quantities at a concentration of 100 - 200 A_{260} units/ml.

b) Large Quantities

When larger quantities of subunits were required, 1200 A_{260} units of dialyzed 70 S ribosomes were centrifuged in 55 ml of a 5-20% RNA ase-free linear sucrose gradient made in TK + 1.0 mM Mg acetate buffer for 12 hr at 24,000 rpm (60 Ti rotor).

The gradient was fractionated and assayed as previously described. Fractions were pooled and precipitated with ethanol at - 20°C for 1-2 hr. The precipitate was collected by centrifugation, dissolved and dialyzed for 6 hr in TK + 1.0 mM Mg acetate buffer. The material was subjected to a second sucrose gradient centrifugation and fractionated as described above. The purified subunits were stored at - 76°C in 1 ml quantities at a concentration of 100-200 A_{260} units/ml.

Sedimentation Analysis of Purified Subunits

2-3 A_{260} units of isolated subunits (30 S or 50 S) were applied to 5 ml of 5-20% sucrose gradient made in TK + 1.0 mM Mg acetate buffer and centrifuged in a Beckman SW 50.1 rotor for 105 min at 49,000rrpm. Gradients were analyzed at 260 mμ with a flow-through cell in a Gilford Model 2000 recording spectrophotometer. The recorded peaks were cut out from tracings and weighed in order to determine the percentage cross-contamination.

Isolation of Ribosomal Protein

Proteins were extracted from ribosomes or subunits following the method of Waller and Harris (1961) as modified by Hardy et al. (1969). The Mg acetate concentration of samples of ribosomes in TM buffer + 1.0 mM DTT was raised to 0.1 M prior to

add

addition of 2 volumes of glacial acetic acid. This mixture was kept on ice for 1-2 hr and mixed on a vortex mixer every 10-15 min. The precipitated RNA was pelleted by centrifugation at 12,000 x g for 15 min and the supernatant containing the extracted ribosomal proteins was lyophilized.

Analysis of Ribosomal Proteins

Three procedures were used during this investigation to analyze Ps. oxalaticus ribosomal proteins.

a) Polyacrylamide Disc Gel Electrophoresis, pH 4.5

The lyophilized protein was dissolved in TM-Urea-DTT buffer (0.1 M tris-HCl, pH 8.0 + 0.01 M Mg acetate + 7.0 M Urea + 1.0 mM DTT) and dialyzed against the same for 6 hr. Electrophoresis was carried out at pH 4.5 as described by Leboy et al. (1964) in 10% polyacrylamide - 0.15% bisacrylamide (Eastman) gels in 8.0 M urea (Fisher). The volumes of the separation, spacer and sample gels were 2.4, 0.15 and 0.15 ml respectively. The sample contained 4.0 to 8.0 A_{260} equivalents of protein (65 to 130 µg). Electrophoresis was conducted at 10°C in a β-alanine-acetic acid discontinuous buffer system (pH 4.5) at 3 ma/gel. A small amount (10 µl) of pyronine red

(0.1%) served as tracking dye during electrophoresis. Gels were fixed in 12.5% TCA (trichloroacetic acid) at 37°C for 2 hr and stained overnight at 37°C with 0.05% Coomassie Brilliant Blue (Sigma) in 12.5% TCA. Gels were destained in 10.0% TCA at room temperature.

b) Polyacrylamide Disc Gel Electrophoresis, pH 8.7

Proteins were also analyzed by electrophoresis in 7.5% polyacrylamide - 0.1% bisacrylamide gels at pH 8.7. Electrophoresis was carried out in a tris-glycine discontinuous buffer system (pH 8.7) at 10°C using bromophenol blue (Sigma) as the tracking dye. Sample amounts and staining procedure are the same as described above.

c) Two-dimensional Polyacrylamide Gel Electrophoresis.

Ribosomal proteins were analyzed, as well, by the two-dimensional electrophoresis method of Kaltschmidt and Wittmann (1970 a). Glass tubing (180 mm x 6 mm inside diameter) was filled to a depth of 100 mm with 4% acrylamide gel and allowed to polymerize using ammonium persulphate. Lyophilized protein sample (30-100 A_{260} equivalents or 0.48 to 1.6 mg) was dissolved in 0.15 ml of sample gel and layered on top of the separation gel and polymerized with riboflavin. The remainder of the glass tubing was filled with separation gel such that the sample was sandwiched between two columns of separation gel.

Electrophoresis was conducted at 3 ma/gel for 24 hr at pH 8.6 after which the gel was removed from the tubing and stirred in "starting buffer" (pH 4.6) for 2 hr. In other analysis the gel was dialyzed against cold 0.3 M HCl for 5 min followed by 3 successive washings with cold distilled water (Avital and Elson, 1974). The advantages of the latter procedure for lowering gel pH is that it is quick and prevents the loss of proteins that occurred during the long dialysis against the "starting buffer". The disc gel was polymerized to the second dimension slab gel (18% polyacrylamide, pH 4.6) and electrophoresis was conducted at 105 volts for 44 hr at 10°C in alglycine-glacial acetic acid buffer. The Model 490 vertical gel electrophoresis cell (E-C Apparatus Corporation) used for electrophoresis yields gels of dimensions 250 mm x 208 mm x 4 mm. After electrophoresis the gel was stained with 0.55% Napthol Blue Black (Sigma) in 5.0% glacial acetic acid for 15 min, rinsed with cold running water for 1 hr and destained with 1.0% glacial acetic acid for 2 to 4 days.

Analysis of Ribosomal RNA

RNA was analyzed by electrophoresis in 2.65% polyacrylamide - 0.15% bisacrylamide gel containing 0.2% sodium dodecyl sulphate (SDS) in a tris-EDTA-

borate-SDS (TEBS) continuous buffer (pH 8.0) system (Peacock and Dingman, 1967). The gel was pre-run at 3 ma/tube for at least 1 hr. Approximately 0.6 - 1.2 A_{260} units of ribosomes or subunits in TEB + 0.1% SDS buffer mixed with sucrose (5.0%) and bromophenol blue (tracking dye) was layered on top of the gels and electrophoresis was carried out at 3 ma/tube for 2 hr. With SDS incorporated into the system there is no need for extraction of RNA from ribosomes (Bishop et al., 1967). After fixing gels in 1.0% glacial acetic acid, the gels were scanned at 260 mμ in a Shimadzu MPS 50 L spectrophotometer equipped with linear transport accessory or in a Chromoscan (Joyce, Loebl). After scanning, gels were fixed in 40.0% methanol and 1.0% glacial acetic acid for 1 hr at 37°C. Fixed gels were stained in 0.1% Toluidine Blue (Sigma) in 30.0% methanol and 1.0% glacial acetic acid at room temperature and destained in 30.0% methanol and 1.0% glacial acetic acid at room temperature.

IV RESULTS

Autotrophic and Heterotrophic Growth of *Ps. oxalaticus* OX1

Growth studies were conducted in 15 litre of medium in carboys so that growth would take place under identical conditions to those under which batch cultures were grown. Maximum growth took place at 28°C in minimal salts medium, pH 6.8, (Methods) with either 6.8 grams/litre sodium formate (autotrophic growth) or 2 grams/litre vitamin-free casamino acids (heterotrophic growth) fulfilling the carbon and energy requirements. The carboys were inoculated with 12-14 hr old cultures grown on the same medium as in the carboy. Approximately 0.13 volumes of inoculum was added to initiate growth. Growth was followed by withdrawing 5 ml samples from the culture vessel and determining its optical density at 660 mμ in a Klett-Summerson colorimeter. pH of the sample was determined by a Black-Simpson Radiometer pH meter.

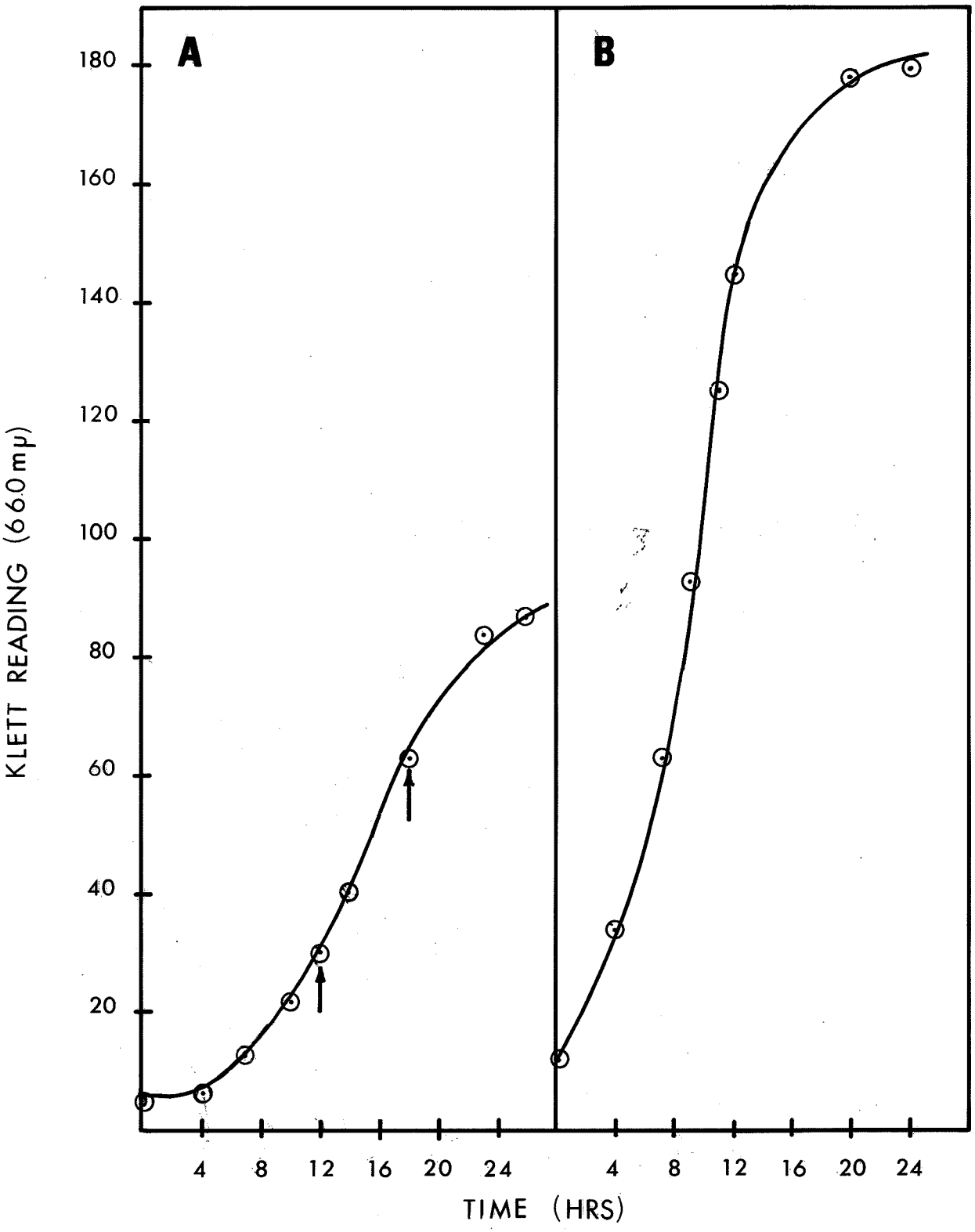
Figure 1 shows the growth curves for *Ps. oxalaticus* OX1 growing under autotrophic and heterotrophic conditions.

FIGURE 1. Growth of Pseudomonas oxalaticus OX1. Cells were grown in 15 litre carboys at 28°C and aerated by forcing air through porous glass tubing.

A. Autotrophic growth with 6.8 grams/litre sodium formate.

B. Heterotrophic growth with 2.0 grams/litre vitamin-free casamino acids.

For details see "Methods".


A
B
180
160
140
120
100
80
60
40
20
KLETT READING (660 mμ)
4
8
12
16
20
24
4
8
12
16
20
24
TIME (HRS)

When grown autotrophically (Figure 1 A), the culture entered logarithmic growth phase within 8-10 hr after inoculation. Logarithmic growth continued for 10-12 hr before the cells entered the phase of negative growth acceleration (late logarithmic) at which time the cells were harvested (approximately 20 hr after inoculation. Growth onssodium formate was accompanied by clumping of some cells. The pH of the medium was lowered to 7.5 twice prior to harvesting as indicated by the arrows in Figure 1 A. Autotrophic growth on formate yielded 0.6-0.8 grams wet weight of cells per litre of medium.

Heterotrophic growth on casamino acids was more rapid as shown in Figure 1 B. Within 4-5 hr after inoculation logarithmic growth commenced and continued for 8-10 hr before entering the late logarithmic growth phase. No pH change was detected during growth although some clumping of cells was observed. Cells were harvested at 14 hr after inoculation - the yield being 1.8 to 2.2 grams wet weight of cells per litre of medium.

As can be seen by comparison of the two growth curves (Figures 1 A and B), growth was faster, with a reduced lag period of proceeded to a greater extent with casamino acids as the carbon and energy source.

Isolation of Ribosomes

From 25 grams wet weight of cells, 1,250 - 2,000 and 5,500 - 6,400 A_{260} units of purified 70 S ribosomes from autotrophic and heterotrophic cells, respectively, were isolated (Methods). Poor breakage of autotrophic cells (observed visually) may have been responsible for the lower yield of ribosomes from autotrophic cells. Assays of extracts at 260 mμ showed that after breakage of 25 grams of autotrophic and heterotrophic cells 10,000 and 23,000 A_{260} units of material, respectively, were present. After fractionation, 19.6% and 25.6% of the A_{260} absorbance in extracts of autotrophic and heterotrophic cells, respectively, were recovered as purified 70 S ribosomes. The results suggest that the lower yield of ribosomes may be due to inefficient breakage of autotrophic cells or lower internal concentrations of ribosomes in autotrophic cells rather than to loss during the fractionation process.

Dissociation of 70 S Ribosomes

Ribosomes were dissociated into subunits to facilitate the study of structural alterations that may have been induced by different growth conditions. The optimum Mg acetate concentration required for

dissociation was first determined. Purified ribosomes isolated from organisms grown under different cultural conditions were dialyzed in TK buffer with varying Mg acetate concentrations and analyzed in 5 ml sucrose gradients made up in the same buffer. Gradients were centrifuged in a Beckman SW 50.1 rotor at 49,000 rpm for 105 min.

As shown in Figures 2 and 3, dissociation of both heterotrophic and autotrophic ribosomes was effected by 3 mM Mg acetate, and reducing the Mg acetate concentration to 0.5 mM did not induce greater dissociation. For routine preparation of subunits, ribosomes were dialyzed and centrifuged in buffers containing 1 mM Mg acetate. This concentration was sufficiently high to maintain intact subunits (as judged by their sedimentation rate) yet prevented aggregation of particles that normally occurs at higher Mg^{++} concentrations.

Since the mass of the 30 S subunit is one-half that of the 50 S subunit (Historical), one would expect 33.3% of the 70 S ribosome to be 30 S. However, determination of area under the curves of sedimentation profiles (Figure 2 and 3 B) showed that the 30 S autotrophic subunit represented 30.1% of the 70 S ribosome whereas the 30 S heterotrophic subunit represented 33.9% of the ribosome. One explanation for this dis-

FIGURE 2. Sedimentation profile of heterotrophic Ps. oxalaticus 0X1 ribosomal subunits after centrifugation inaa Beckman SW 50.1 rotor at 49,000 rpm for 105 min. A 5-20% linear sucrose gradient prepared in TK buffer with varying Mg acetate concentrations was used.

A. TK + 3 mM Mg acetate

B. TK + 1 mM Mg acetate

C. TK + 0.5 mM Mg acetate

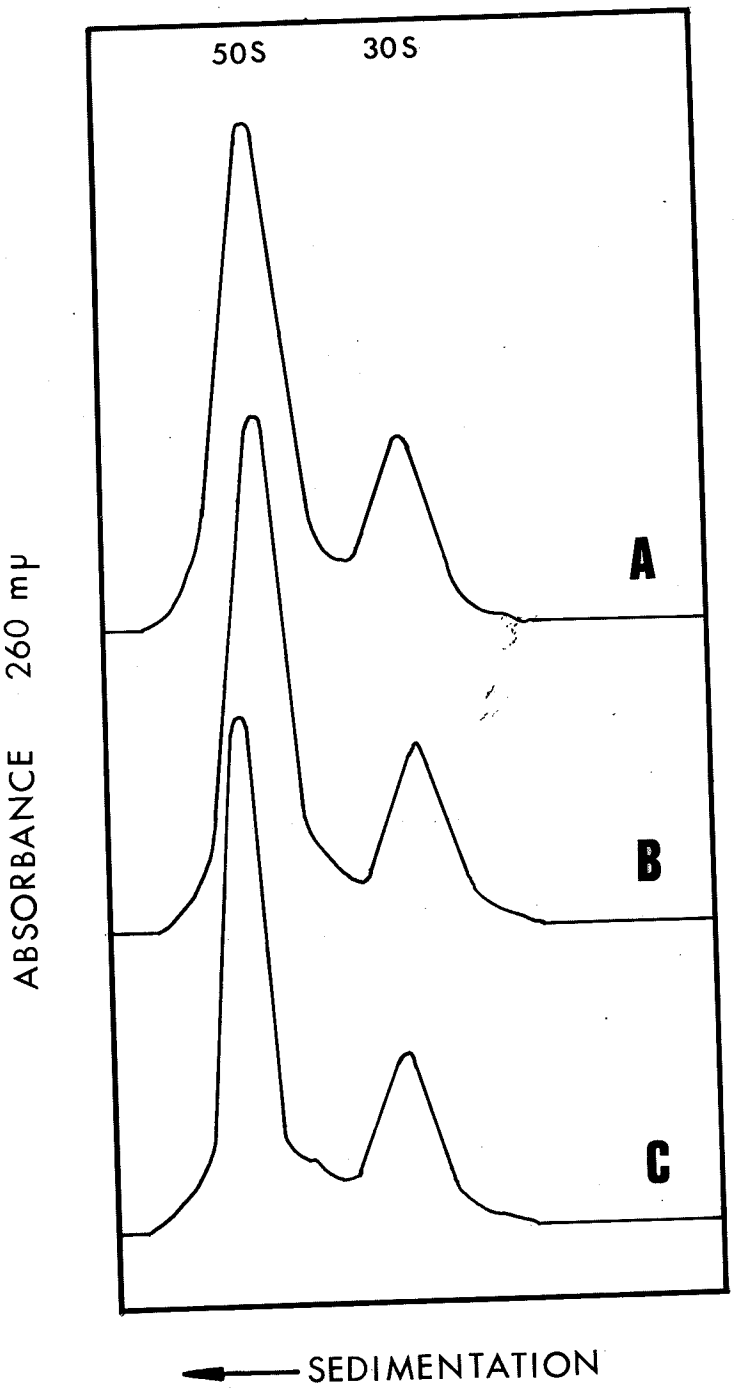
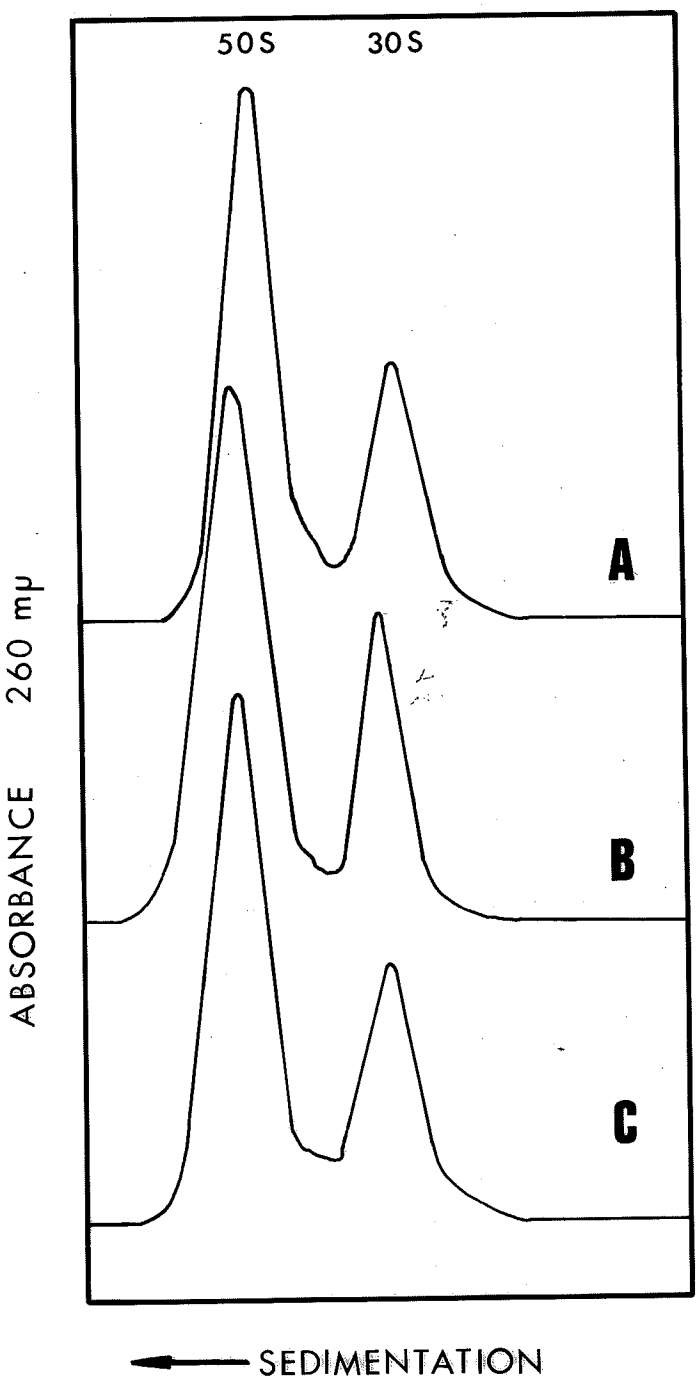

50S
30S
A
B
C
ABSORBANCE 260 mμ
SEDIMENTATION

FIGURE 3. Sedimentation profile of autotrophic Ps. oxalaticus 0X1 ribosomal subunits analyzed in linear 5-20% sucrose gradients prepared in TK buffer with varying Mg acetate concentrations. Gradients were centrifuged in a Beckman SW 50.1 rotor at 49,000 rpm for 105 min.

A. TK + 3 mM Mg acetate

B. TK + 1 mM Mg acetate

C. TK + 0.5 mM Mg acetate


50S
30S
A
B
C
ABSORBANCE 260 mμ
SEDIMENTATION

crepancy is that the 30 S subunit from autotrophs may have formed dimers which sediment with the 50 S peak. However this observation was not investigated further.

Isolation of Ribosomal Subunits

The procedure used for subunit isolation was dependent upon the quantity required (Methods). For small quantities, a single centrifugation in a 55 ml 5-20% linear sucrose gradient was sufficient for preparing subunits from 200-250 A_{260} units of ribosomes. Figure 4 represents a typical sedimentation profile of ribosomes after centrifugation for 12 hr in a SW 25.2 Beckman rotor. The 30 S and 50 S fractions were pooled and precipitated as indicated in Figure 4. The subunit preparations showed less than 10% cross contamination.

For larger quantities, 1,200 A_{260} units of ribosomes were separated on gradients in a procedure that consisted of two successive centrifugations. The subunits pooled from the first gradient (Figure 5) were heavily cross contaminated. After precipitation of the subunits with alcohol, they were dialyzed and applied to a second 55 ml 5-20% sucrose density gradient as described in "Methods". The profiles obtained after the second centrifugation is shown in Figure 6 A and B. The subunits obtained from either isolation procedure were collected and subjected to analytical centrifugation to determine the extent of cross contamination.

FIGURE 4. Sedimentation profile of 50 S and 30 S ribosomal subunits after preparative centrifugation of 200-250 A_{260} units 70 S ribosomes. Subunits were isolated on 55 ml 5-20% sucrose gradients made in TK + 1 mM Mg acetate buffer centrifuged for 12 hr in a SW 25.2 rotor at 24,000 rpm. Fractions were pooled as indicated and precipitated with 0.67 volumes ethanol. For further details see "Methods".

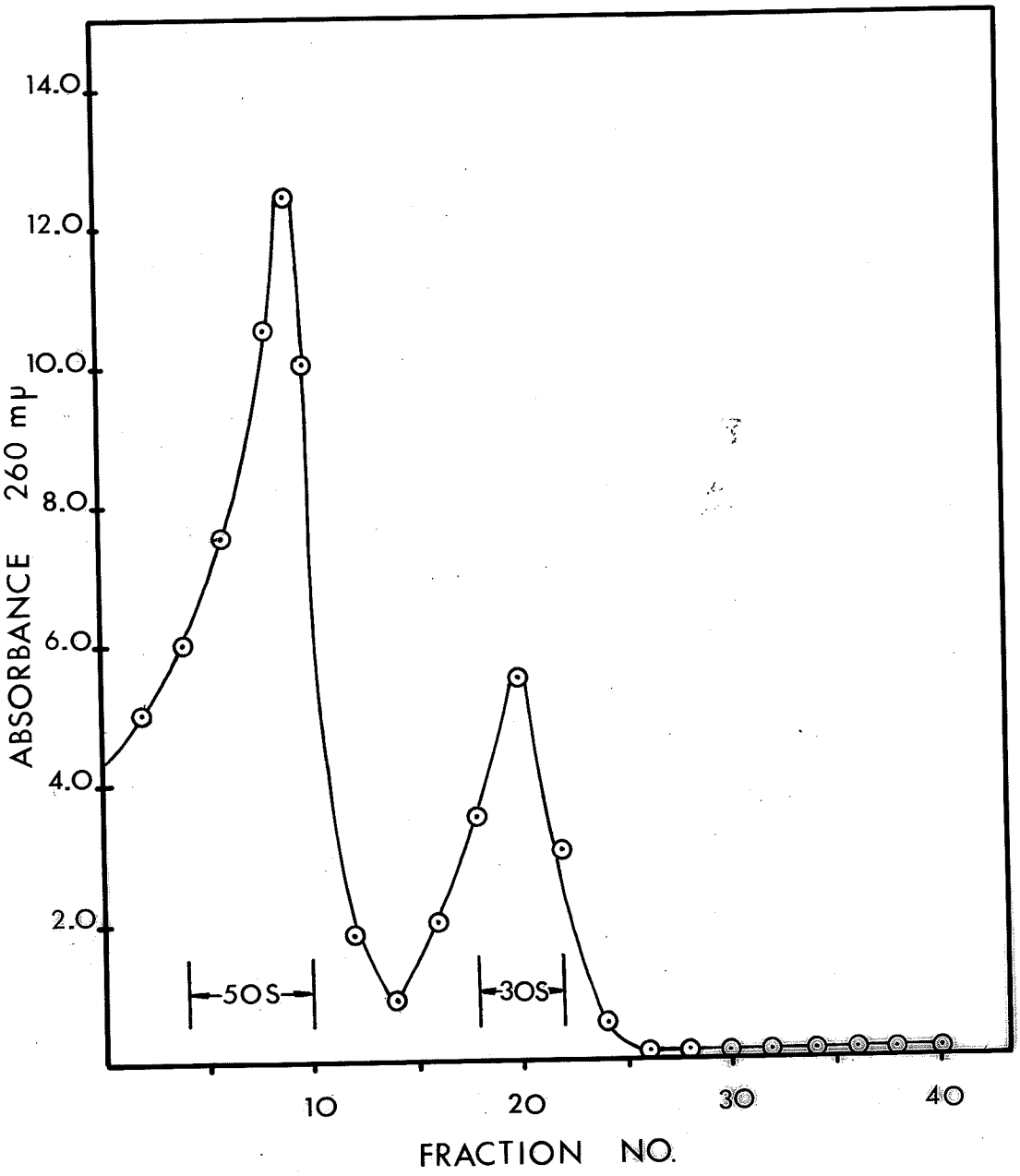

14.0
12.0
10.0
8.0
6.0
4.0
2.0
ABSORBANCE 260 mμ
50S
30S
10
20
30
40
FRACTION NO.

FIGURE 5. Sedimentation profile of ribosomal subunits after centrifugation of 1,200 A_{260} units of dissociated ribosomes. Fractions were collected as indicated and subunits precipitated with 0.67 volumes of ethanol. The pooled fractions were dialyzed against TK + 1 mM Mg acetate and applied to a second gradient for separation of contaminating subunits (Figure 6).

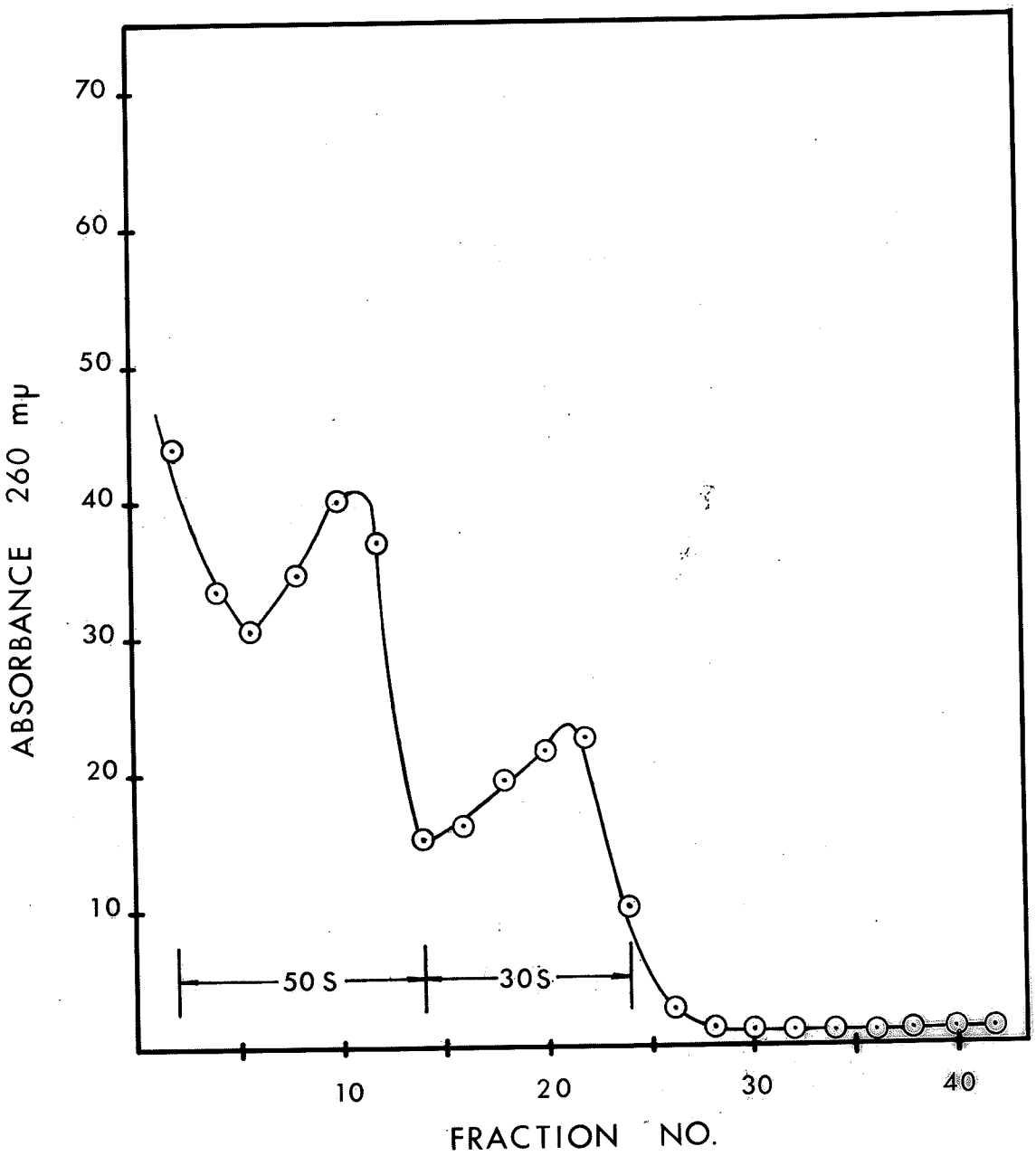
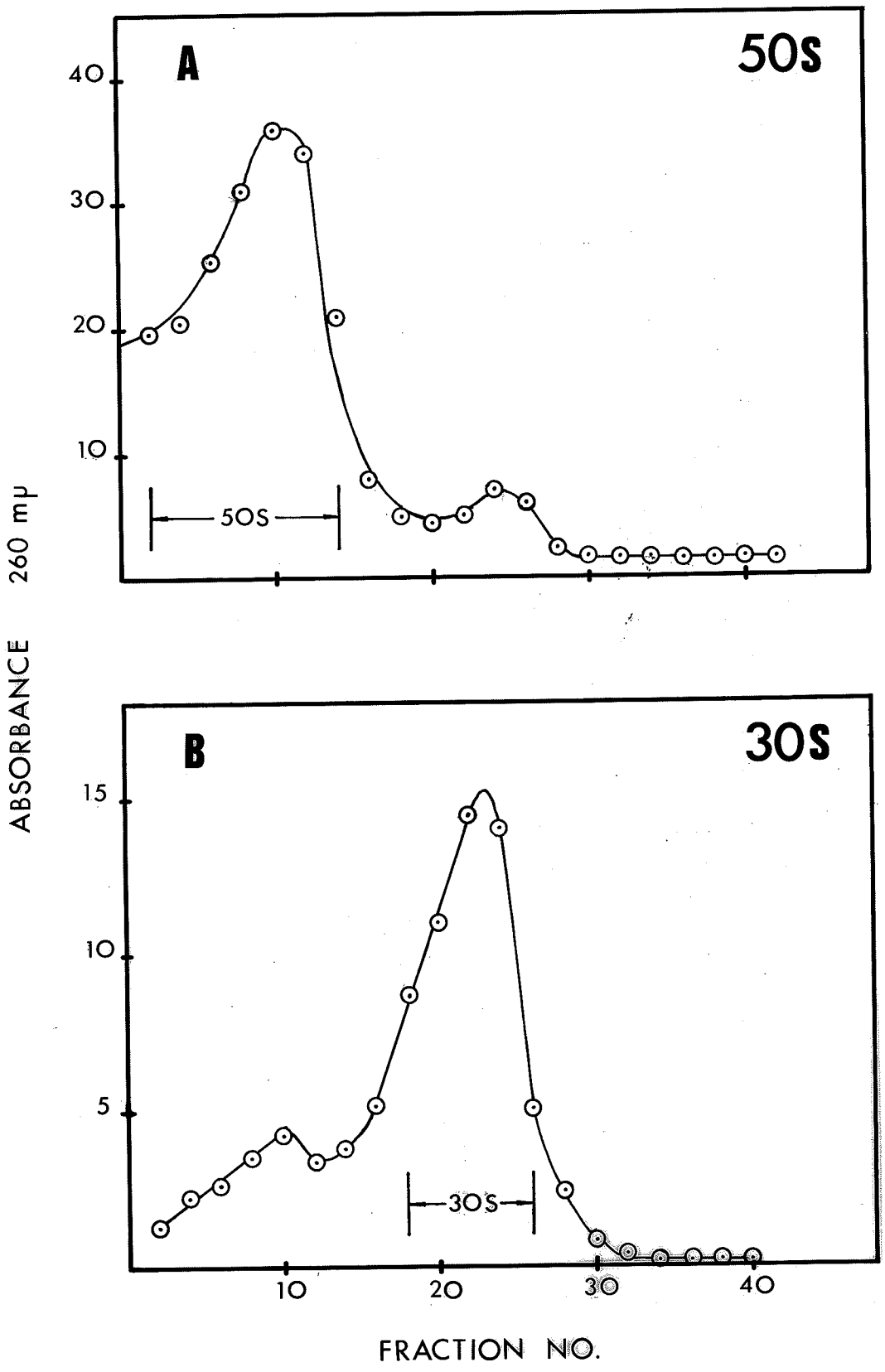

70
60
50
40
30
20
10
ABSORBANCE 260 mμ
50 S
30S
10
20
30
40
FRACTION NO.

FIGURE 6. Sedimentation profiles of 50 S (A) and 30 S (B) ribosomal subunits following a second centrifugation on 55 ml 5-20% sucrose gradient. Samples were obtained after rough separation by a previous gradient centrifugation step (Figure 5). Fractions were pooled as indicated.


A
50S
40
30
20
10
50S
B
30S
15
10
5
30S
10
20
30
40
ABSORBANCE 260 mμ
FRACTION NO.

Cross Contamination

Purified subunits were subjected to analytical centrifugation on a 5 ml 5-20% linear sucrose gradients prepared in TK + 1 mM Mg acetate buffer and tracings of the sedimentation profiles were obtained (Methods). Figure 7 shows a typical tracing. The area under each peak was calculated by cutting out and weighing the peaks. These estimates of peak sizes showed that the extent of cross contamination of autotrophic 30 and 50 S subunits was 9.7% and 8.2%, respectively, whereas 9.4% of the 30 S and 7.3% of the 50 S subunit preparations from heterotrophic ribosomes were cross contaminated. Generally subunit preparations were cross contaminated to the extent of 6 to 10%.

Analysis of Ribosomal RNA from 70 S Ribosomes

The possibility that growth by 2 widely different means, i.e., autotrophic and heterotrophic, might result in detectable differences in ribosomal RNA was studied. The electrophoretic patterns of rRNA in 2.65% polyacrylamide (pH 8.4) of autotrophic and heterotrophic ribosomes were compared (Methods).

Electrophoresis of autotrophic and heterotrophic ribosomes together (Figure 8 A) demonstrated that 16 S

FIGURE 7. Sedimentation profiles of purified 50 S and 30 S subunits centrifuged for 105 min at 49,000 rpm in a SW 50.1 rotor in 5 ml 5-20% sucrose gradients prepared in TK buffer + 1 mM Mg acetate.

A. Heterotrophic 30 S subunit

B. Heterotrophic 50 S subunit

C. Autotrophic 30 S subunit

D. Autotrophic 50 S subunit

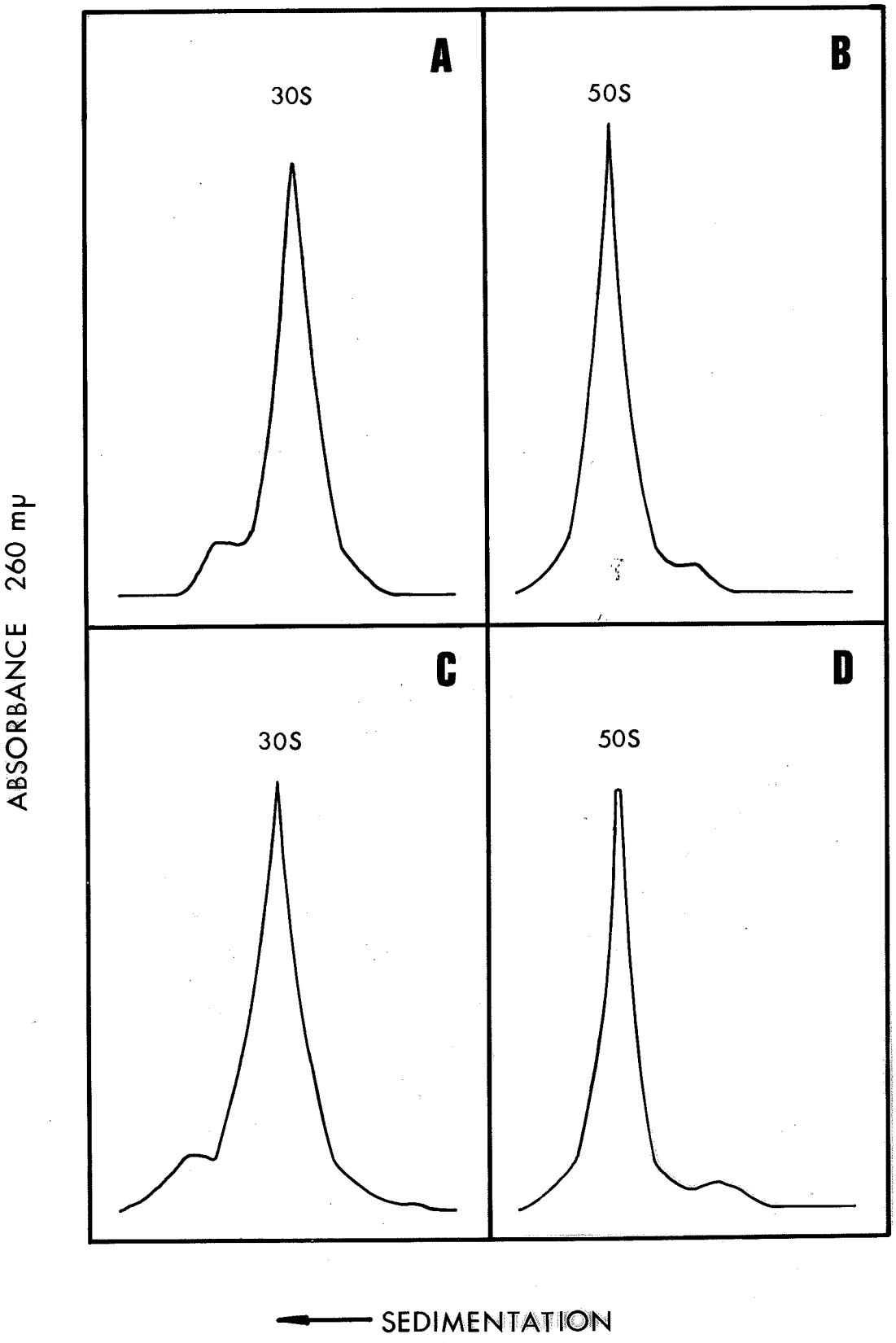
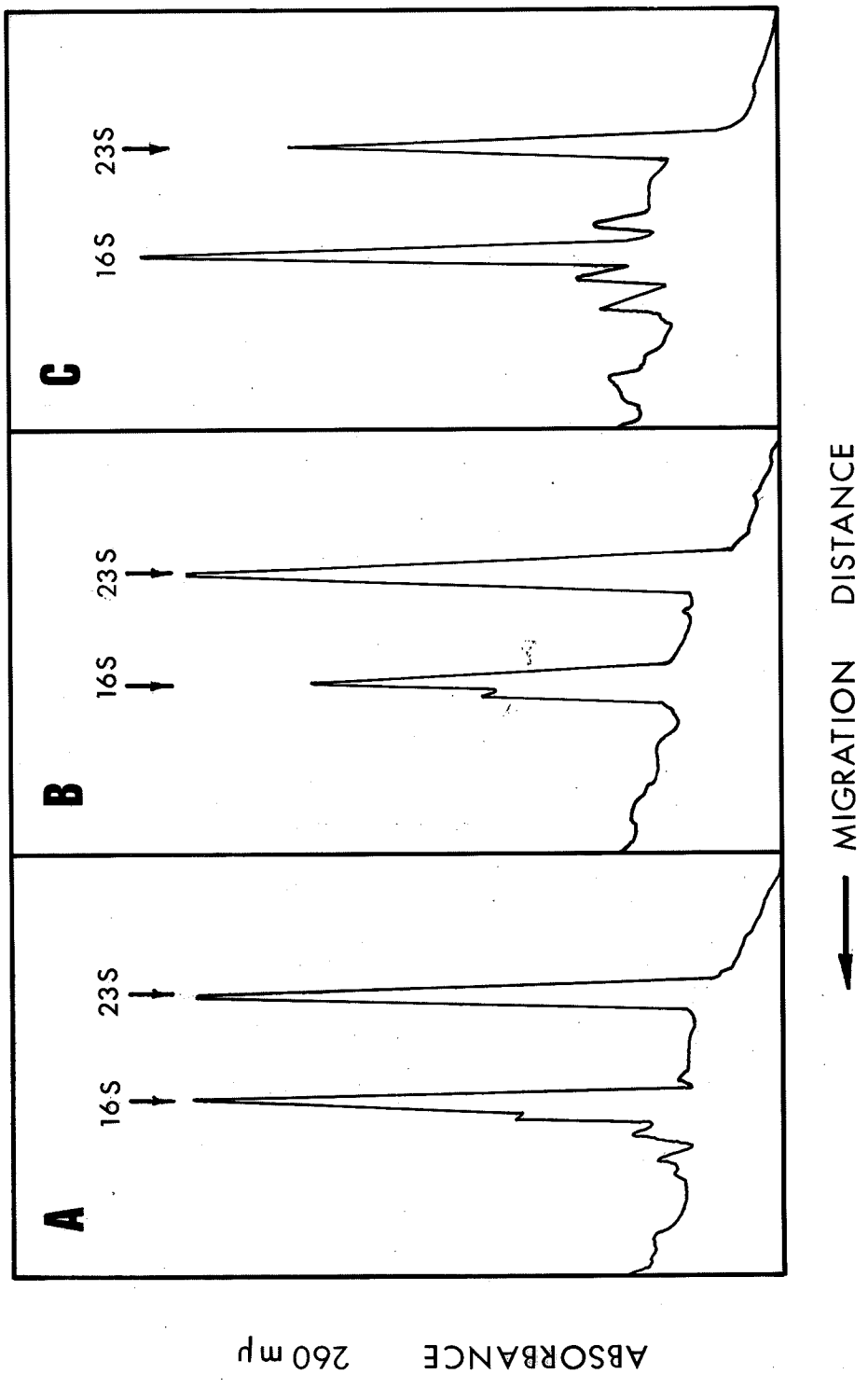

A
30S
B
50S
C
30S
D
50S
ABSORBANCE 260 mμ
SEDIMENTATION

FIGURE 8. Electrophoretic analysis of ribosomal RNA in intact 70 S ribosomes. Analysis carried out in 2.65% polyacrylamide - 0.15% bisacrylamide gels containing 0.2% SDS as described in "Methods". Approximately 1.2 A_{260} units of ribosomes were applied to each gel and after electrophoresis, these were scanned at 260 mμ. RNA patterns are from a mixture of 70 S autotrophic and heterotrophic ribosomes, (A), 70 S heterotrophic, (B), and 70 S autotrophic, (C), ribosomes of Ps. oxalaticus OX1.


A
16S
23S
B
16S
23S
C
16S
23S
MIGRATION DISTANCE
ABSORBANCE 260 mμ

and 23 S RNA comigrated, suggesting that gross differences in the structure of these species were not induced by the two cultural conditions. Comparison of the two molecules of 5 S RNA was not undertaken. The appearance of several smaller RNA peaks and the size of the 23 S peak (23 S RNA peak should enclose twice the area of the 16 S peak because of their mass differences) suggested a breakdown of 23 S RNA. Electrophoresis of heterotrophic (Figure 8 B) and autotrophic (Figure 8 C) ribosomes, separately, showed breakdown of 23 S RNA in both cases.

To test the integrity of rRNA, ribosomes were heated to 75°C for 1 min in the presence of 0.2% SDS prior to application to gels. Heating in the presence of SDS disrupts the secondary structure of rRNA and reveals any "hidden scissions" that might be present (Breuning and Bock, 1967) (Figure 9). Comparison of electrophoretic profiles of heat-treated (Figure 9) and untreated ribosomes (Figure 8) shows that a significant proportion of the RNA molecules contains a "nicks" and that it is primarily the 23 S species that harbor these scissions.

Analysis of Subunit Ribosomal RNA

The RNA of purified subunits was studied to precisely determine which ribosomal RNA species con-

FIGURE 9. Electrophoretic analysis of ribosomal RNA of 70 S ribosomes (1.2 A_{260} units) after heating to 75°C for 1 min. Analyzed in 2.65% polyacrylamide - 0.15% bisacrylamide gels containing 0.2% SDS as described in "Methods". Patterns, obtained by scanning gels at 260 mμ, of rRNA from 70 S heterotrophic, (A), and autotrophic, (B), ribosomes.

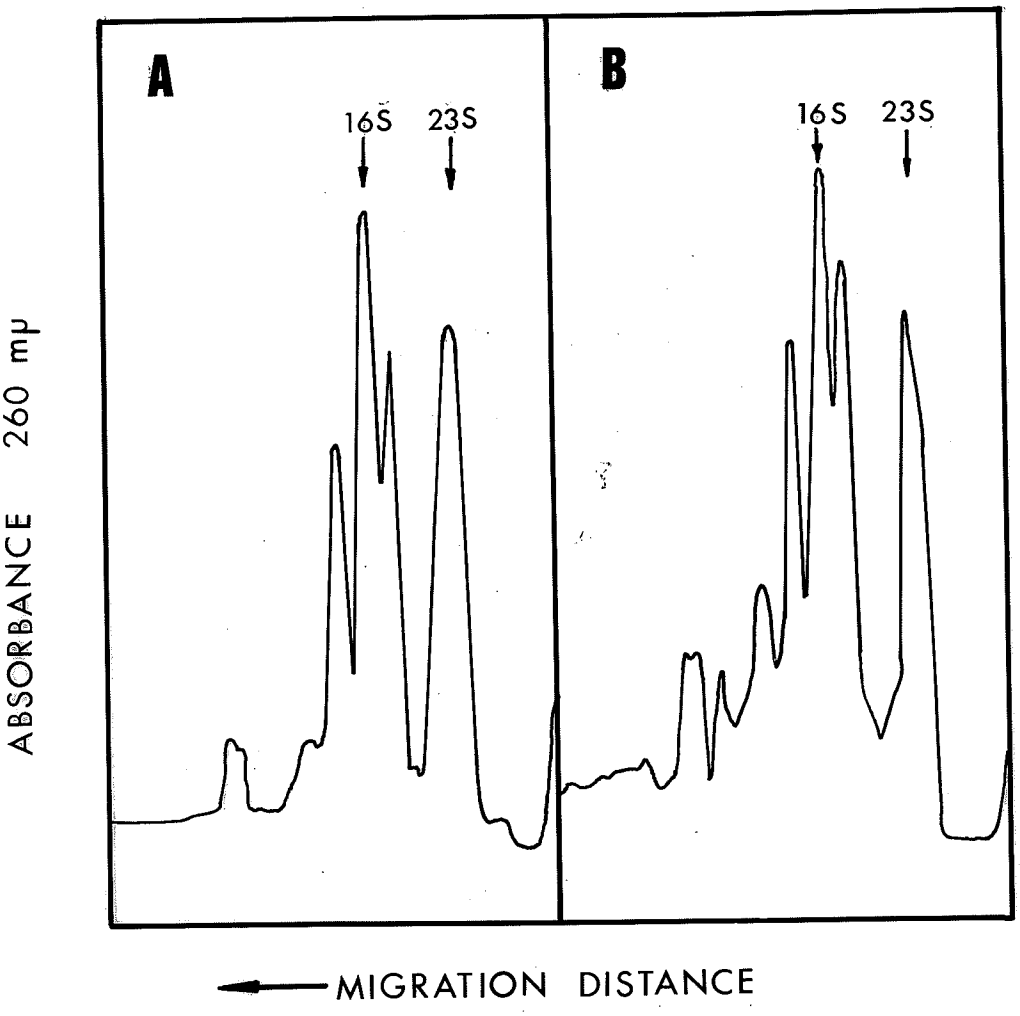

A
16S
23S
B
16S
23S
ABSORBANCE 260 mμ
MIGRATION DISTANCE

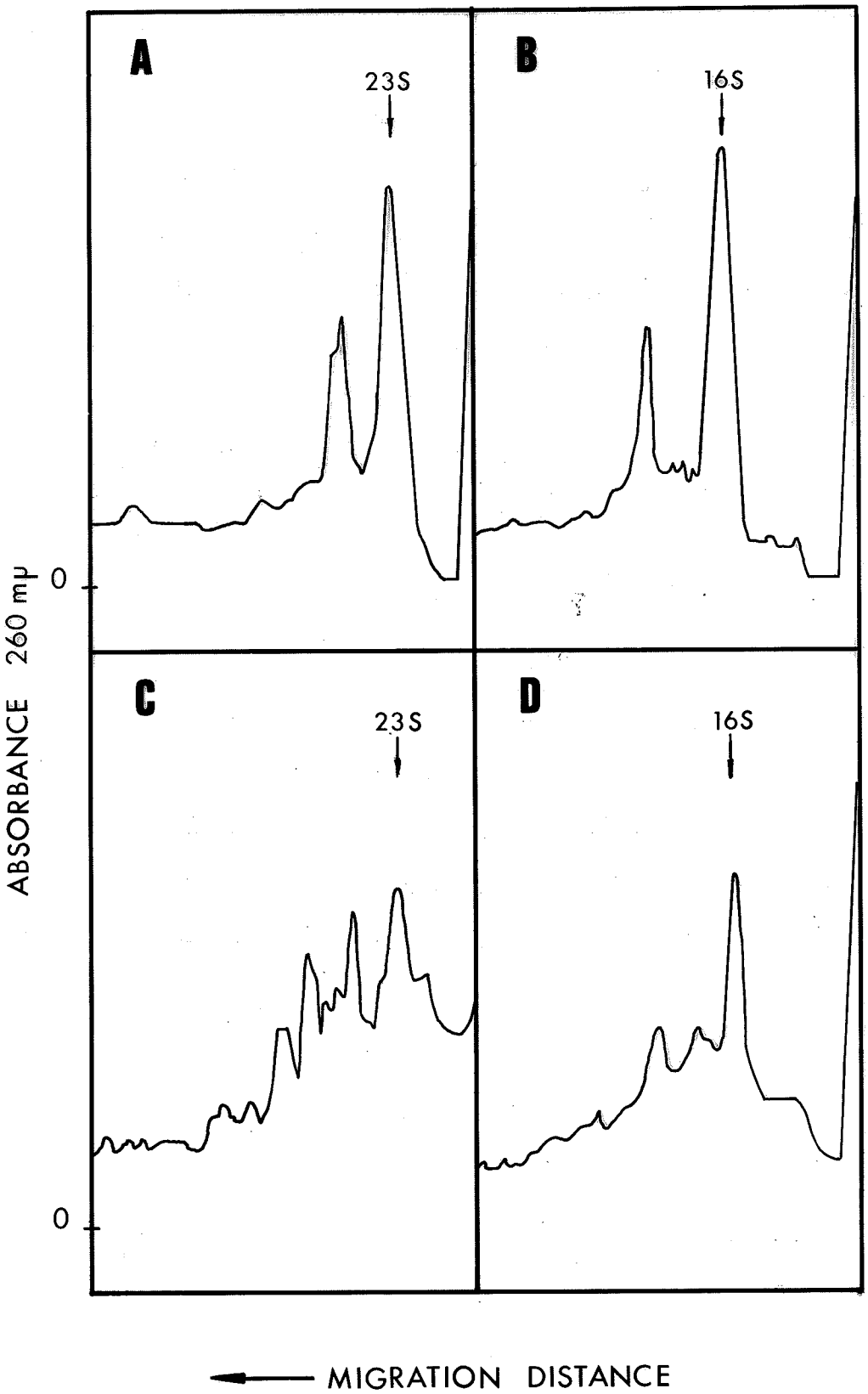
FIGURE 10. Electrophoretic profiles of autotrophic 16 S and 23 S RNA. Approximately 1.2 A_{260} units of purified subunits were analyzed at pH 8.4 in 2.65% polyacrylamide - 0.15% bisacrylamide gels containing 0.2% SDS. After electrophoresis, these gels were scanned at 260 mμ. The samples electrophoresed are as follows:

A. 50 S subunit, no heating

B. 30 S subunit, no heating

C. 50 S subunit, heated to 75°C for 1 min

D. 30 S subunit, heated to 75°C for 1 min


A
23S
B
16S
C
23S
D
16S
ABSORBANCE 260 mμ
0
0
MIGRATION DISTANCE

tained scissions. Surprisingly the RNA from both autotrophic 30 S and 50 S subunits migrated as more than 1 major peak in gel electrophoresis indicating degradation of both types of RNA (Figure 10 A and B). Thus dissociation of autotrophic ribosomes into its respective subunits is accompanied by scissions in both the 16 S and 23 S RNA's. Heating the subunit to 75°C for 1 min resulted in extensive breakdown of both rRNA's (Figure 10 C and D). However, 16 S and 23 S RNA's are the predominant species in the gels.

Analysis of 30 S and 50 S heterotrophic subunits again revealed breakdown of 16 S and 23 S RNA as shown in Figure 11 A and B. However, subjecting the subunit to heat (75°C for 1 min) resulted in surprisingly different results than was seen for the autotrophic subunits. Disruption of the secondary structure of 23 S RNA revealed numerous "hidden scissions" as indicated by the 23 S RNA electrophoretic profile (Figure 11 C). On the other hand, heating the 30 S heterotrophic subunit did not produce further breakdown of 16 S RNA (Figure 11 D). The reason(s) for this result is not apparent.

Analysis of Ribosomal Proteins

To investigate the possible influence different cultural conditions had on ribosomes, the proteins of 70 S ribosomes of autotrophic and heterotrophic cells

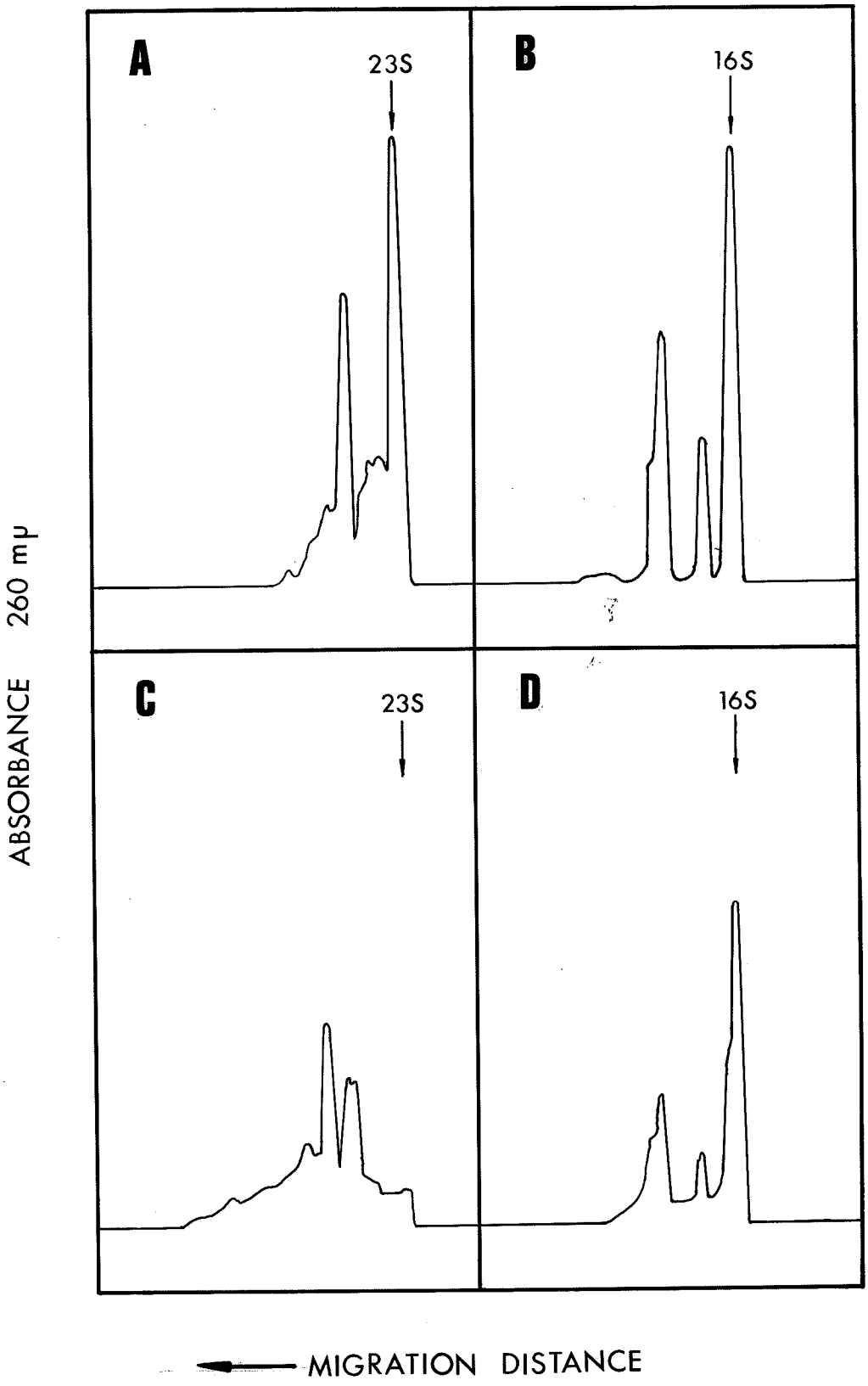
FIGURE 11. Electrophoretic profiles of heterotrophic 16 S and 23 S RNA. Approximately 1.2 A_{260} units of purified subunits were analyzed at pH 8.4 in 2.65% polyacrylamide - 0.15% bisacrylamide gels containing 0.2% SDS. After electrophoresis, these gels were scanned at 260 mμ. The samples electrophoresed are as follows:

A. 50 S subunit, no heating

B. 30 S subunit, no heating

C. 50 S subunit, heated to 75°C for 1 min

D. 30 S subunit, heated to 75°C for 1 min


A
23S
B
16S
C
23S
D
16S
ABSORBANCE 260 mμ
MIGRATION DISTANCE

were analyzed by one dimensional polyacrylamide disc gel electrophoresis at pH 4.5 (Methods). Plate 1 compares the electrophoretic protein patterns of autotrophic (A and B) and heterotrophic (C and D) ribosomes at two protein concentrations and indicates 3 possible differences between them. At positions 2 and 3 (Plate 1), the autotrophic ribosomal protein pattern appears to lack protein bands that are present in the heterotrophic ribosomal protein pattern. This can be most clearly visualized by comparing gels B and C at position 2, and gel D with either A or B at position 3. If the slowest moving band of each protein pattern is studied (position 1), it can be seen that this band migrates as a doublet in electropherograms of proteins from autotrophic ribosomes, but as a singlet in proteins from heterotrophic ribosomes.

The exact number of components which differ can not be evaluated by disc gel electrophoresis since the method can neither differentiate between proteins of the same electrophoretic mobility nor resolve closely neighbouring proteins (Kaltschmidt and Wittmann, 1970 a). Although, the lack of resolution may result in undetectable differences, the electropherogram indicated discrete differences, involving at least 3 proteins, between ribosomes of autotrophically - and heterotrophically-grown cells.

PLATE 1. Electropherograms of 70 S ribosomal proteins of Ps. oxalaticus 0X1 in polyacrylamide gels at pH 4.5 (Methods). Ribosomes were extracted from either autotrophically - (A and B) or heterotrophically - grown (C and D) cells.

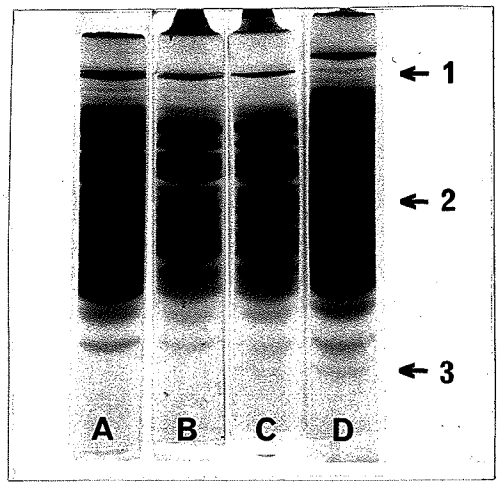
A. 130 µg protein sample

B. 100 µg protein sample

C. 100 µg protein sample

D. 130 µg protein sample

Arrows indicate position of differences in protein pattern of ribosomes of autotrophic and heterotrophic cells.



Two Dimensional Analysis of 70 S Ribosomal Proteins

Since disc gel electrophoresis cannot resolve all proteins in a complex mixture two dimensional polyacrylamide gel electrophoresis was employed (Methods). In this procedure proteins are first electrophoresed in 4% polyacrylamide disc gels at pH 8.6. In the second dimension proteins are electrophoresed out of the disc gel into an 18% polyacrylamide slab gel at pH 4.5.

The conditions used in this study were optimal for resolution of E. coli ribosomal proteins (Kaltschmidt and Wittmann, 1970 a) though not necessarily for Ps. oxalaticus OX1 ribosomal proteins. Plate 2 shows a typical separation of proteins from 100 A_{260} units of 70 S heterotrophic ribosomes. It appeared that some larger spots were composed of several proteins. In order to resolve these spots, the polyacrylamide concentration was decreased to 16% (from 18%) in the second dimension slab gel with the results shown in Plate 3. Combining the results of electrophoresis under different conditions, a composite electrophoretic pattern (Figure 12) was obtained which shows the maximum resolvable number of proteins.

The exact number of heterotrophic ribosomal proteins could not be estimated from Figure 12 since one large spot representing 3 to 6 proteins (designated Z in Figure 12) could not be resolved despite various

PLATES 2 and 3. Electropherograms of 70 S heterotrophic ribosomal proteins as resolved by two dimensional gel electrophoresis of a 100 A_{260} equivalent sample (1.6 mg) as described in "Methods". Dimensions of thessecond dimension slab gel are 250 mm x 208 x 4 mm and of the disc gel, 180 mm x 6 mm.

Plate 2. Standard conditions with 18% polyacrylamide gel

Plate 3. Standard conditions with 16% polyacrylamide gel

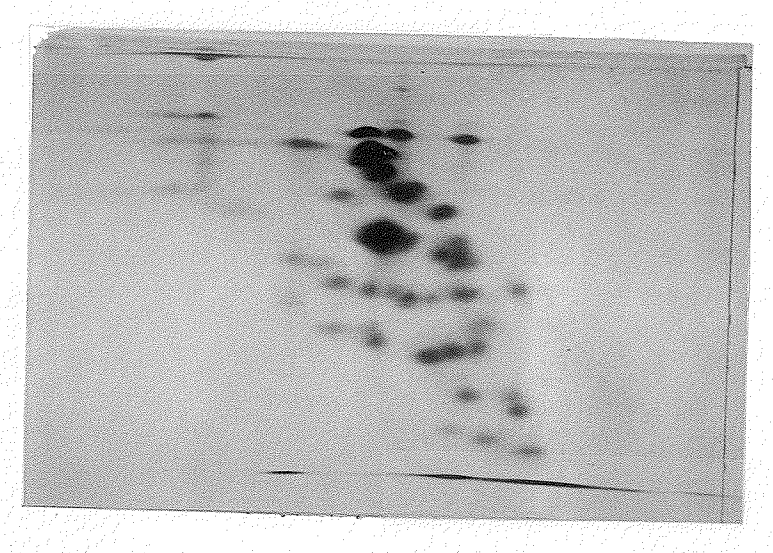


PLATE 2

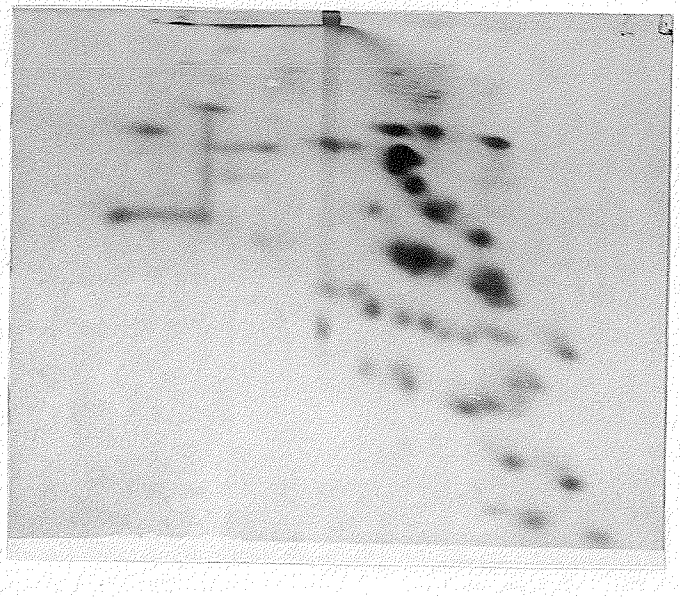
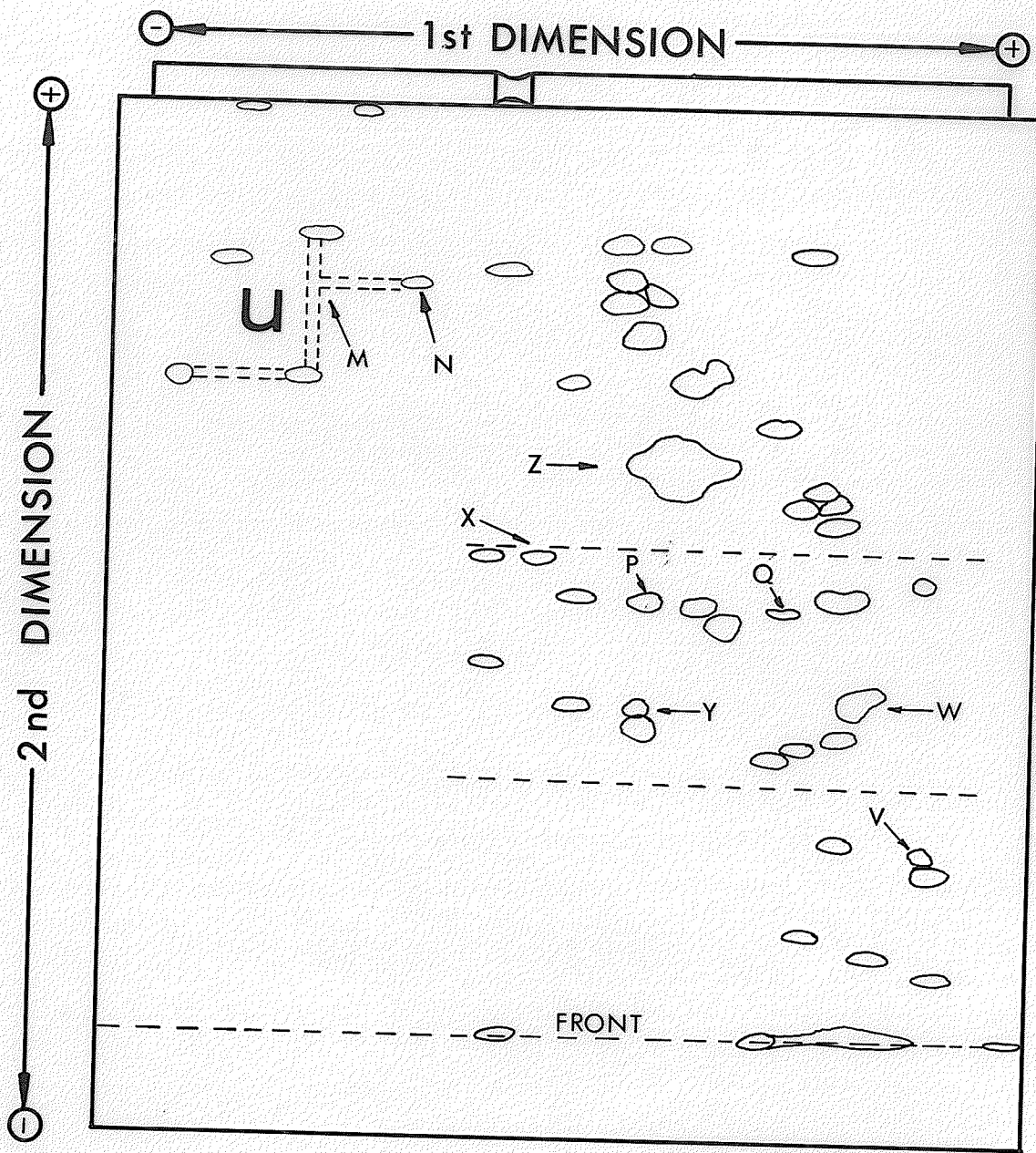


PLATE 3

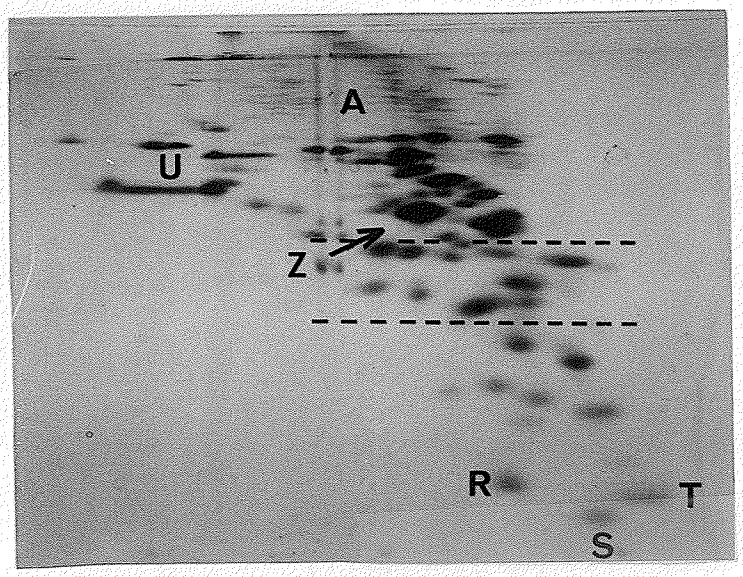
FIGURE 12. Composite electrophoretic pattern of 70 S heterotrophic ribosomal proteins. The diagram was prepared by combining the results from Plates 2 and 3 and several other electropherograms. Proteins of uncertainty are designated V, W, X and Y.


1st DIMENSION
2nd DIMENSION
u
M
N
Z
X
P
Q
Y
W
V
FRONT

conditions used. Also almost every electropherogram was plagued by the formation of a "front" which decreased resolution by impeding several of the faster migrating proteins. The distance from the origin at which the "front" was formed varied with each separation. Determination of ribosomal protein numbers was further hindered by the appearance of faint spots even with samples containing 2.0 - 2.25 mg protein. The faint spots may be due to: 1) contamination by cellular proteins, 2) ribosomal proteins in low quantities, 3) an aggregate, or 4) fragments of larger proteins. Thus faint spots like V, W, X and Y may or may not be ribosomal proteins. The final problem encountered in enumeration of ribosomal proteins resulted from trailing of proteins which have an isoelectric point of greater than 8.6 (left hand side of the gel) represented by U. The reason for trailing is not known but it may have obscured some protein spots. The approximate number of heterotrophic ribosomal proteins resolved by this method consequently ranges between 50 and 58.

In order to resolve whether the faint spots were due to oxidation induced aggregation, protein samples were electrophoresed with and without reduction by DTT. In the absence of DTT protein aggregation through disulphide bridge formation would be favored and faint spots should become more distinct. Plate 4 shows a typical electropherogram of an unreduced sample. A

PLATE 4. Electropherogram of 70 S heterotrophic proteins which were not reduced in DTT. Resolution of three additional proteins R, S and T was obtained. Conditions under which electrophoresis was carried out is described in "Methods".



large number of additional spots are observed and comparison with Figure 12 shows that these additional spots appeared mainly around the large spot Z and in the regions designated U and A. Furthermore, the area between the dotted lines showed a different protein pattern from that of the reduced samples. As a result of this alteration in protein patterns, the status of protein spots W, X and Y (Figure 12) remained unclear. In the electropherograms of unreduced samples, the front was close to the gel bottom allowing the resolution of 3 additional spots R, S and T. This increased the estimated number of heterotrophic ribosomal proteins to between 53 and 58.

Similar studies with autotrophic 70 S ribosomal proteins produced protein patterns quite similar to that of the heterotroph. Plate 5 represents a typical electropherogram obtained with 1.6 mg (100 A_{260} equivalents) of protein. Resolution of several large composite spots could not be obtained with a 16% gel but were resolved by decreasing the protein sample to 1 mg (60 A_{260} equivalents) as shown in Plate 6. A composite diagram made up of Plates 5 and 6, i.e., Figure 13, facilitated the identification of 49 to 56 ribosomal proteins. As previously mentioned the exact number of ribosomal proteins could not be determined.

PLATES 5 and 6. Electropherograms of 70 S autotrophic ribosomal proteins as resolved by two dimensional gel electrophoresis (Methods). Two protein concentrations were used:

Plate 5. 1.6 mg or 100 A_{260} equivalents of protein

Plate 6. 1.0 mg or 60 A_{260} equivalents of protein

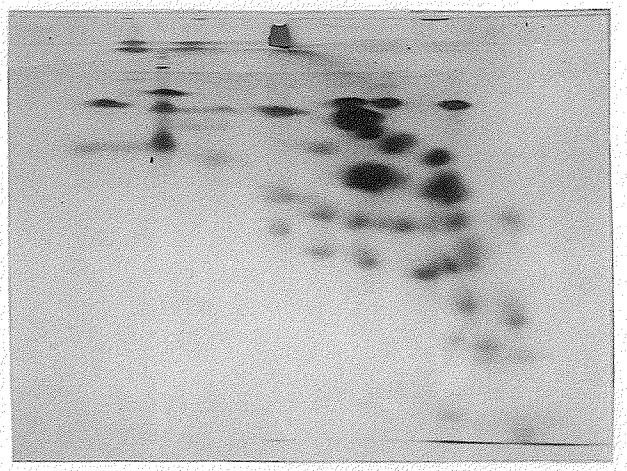


PLATE 5

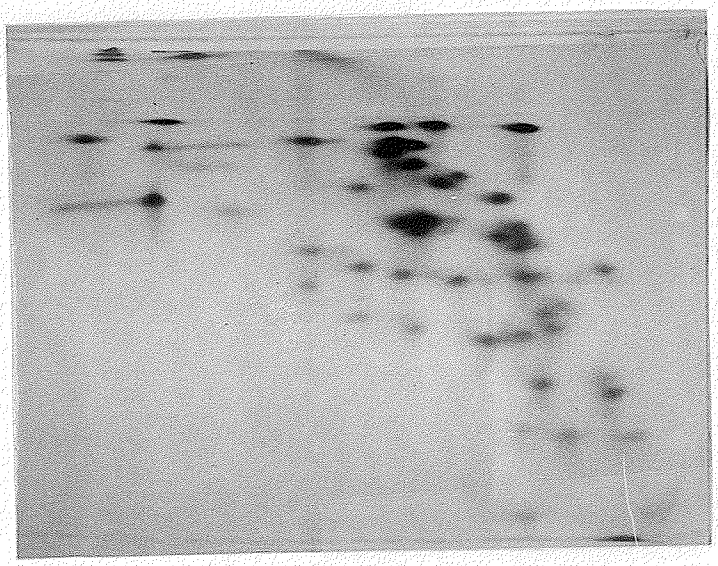


PLATE 6

The electropherogram of autotrophic ribosomes were plagued by trailing (U), composite spot (Z), formation of a "front" and several faint spots (V, W and Y). In addition, two spots designated P and Q were present in some 1.6 mg samples (Plate 5) but not in 1.0 mg samples (Plate 6). When unreduced samples (1.6 mg) were electrophoresed, P and Q were not present (Plate 7) but three additional proteins R, S and T could be identified. Two of these proteins, R and T, were present in the composite diagram, Figure 13. This raised the number of ribosomal proteins to between 50 and 56. However, Plate 7 does not contain the additional protein spots that the unreduced sample of the heterotroph (Plate 4) had around spot Z and region U.

Comparison of the two composite diagrams, Figures 12 and 13, revealed very little difference between the autotrophic and heterotrophic 70 S ribosomal protein patterns. The differences which were apparent in the disc gels (Plate 1) were not observed. Possible differences between protein patterns were observed in the area between the dotted lines of the composite diagrams (Figures 12 and 13). The proteins P,Q and X were present in Figure 12 were absent from autotrophic ribosomes (Figure 13). In region U, proteins M and N could reflect the influence that the mode of growth has upon the ribo-

PLATE 7. Electropherogram of 70 S autotrophic ribosomal proteins which were not reduced in DTT. Conditions under which electrophoresis was carried out are described in "Methods". Additional ribosomal proteins R, S and T could be resolved with this method.

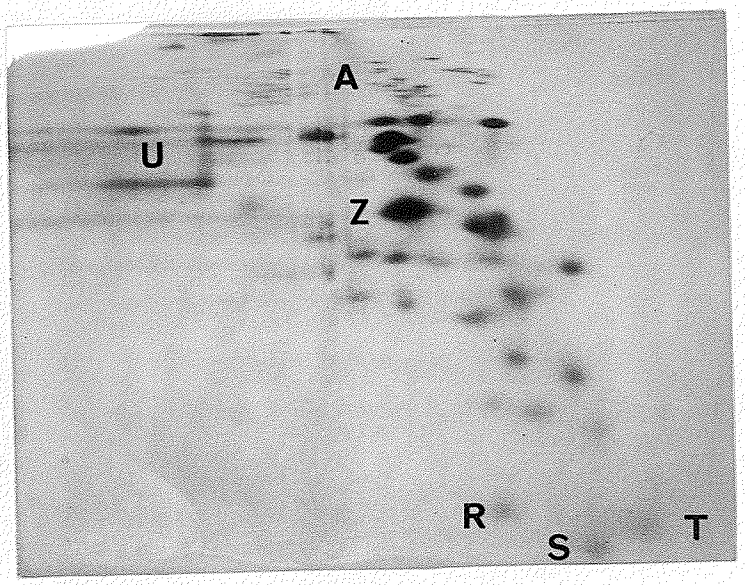
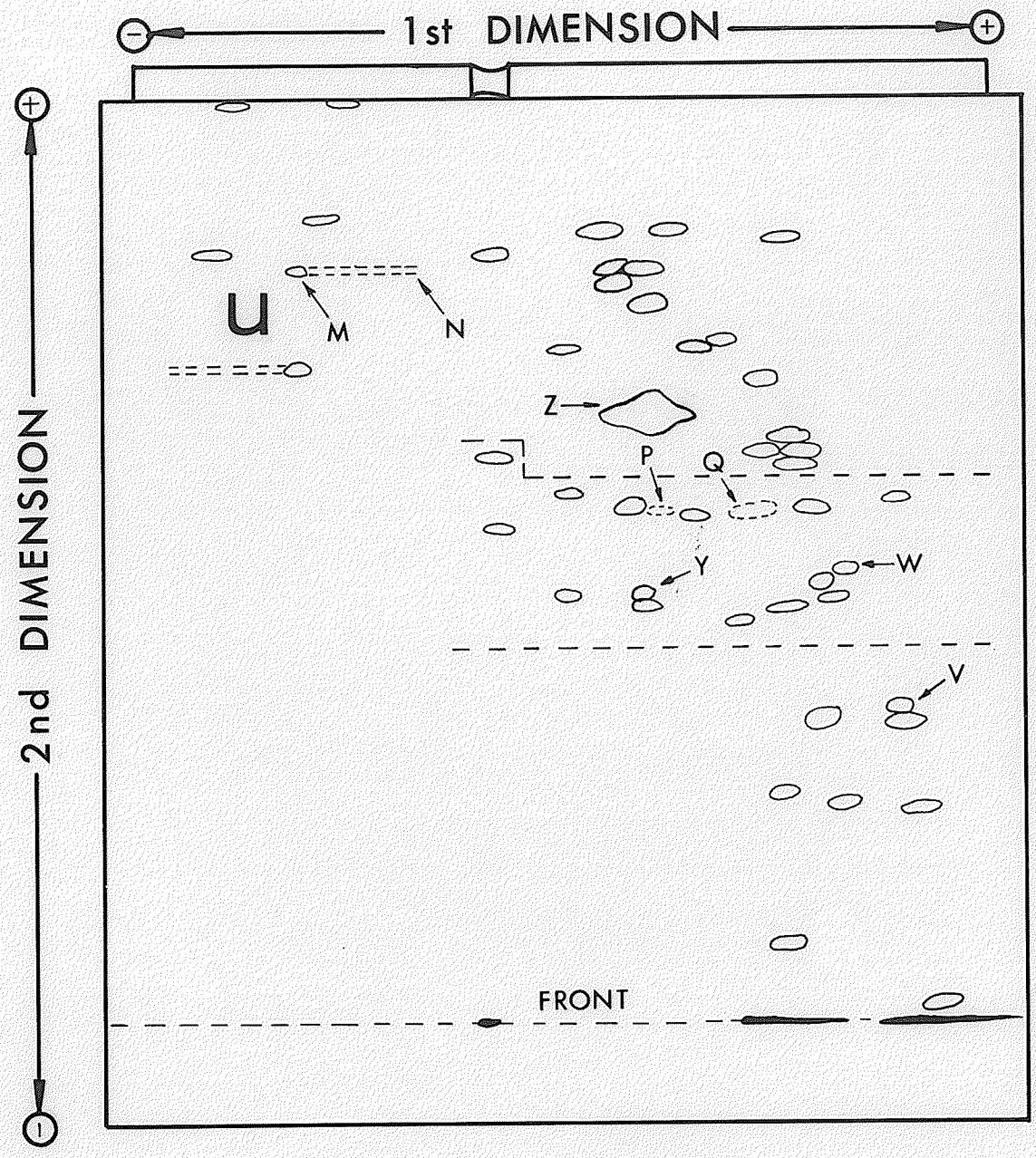


FIGURE 13. Composite electrophoretic pattern of 70 S autotrophic ribosomal proteins. The diagram was prepared from Plates 5 and 6 and other electropherograms.


1st DIMENSION
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W
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somal proteins. The heterotroph possessed protein N but not M (Figure 12) with the reverse true for the autotroph (Figure 13).

In order to resolve the protein spots in region U, polyacrylamide disc gels, pH 8.7 (Methods), were used. This procedure allowed the separation of proteins with an isoelectric point of greater than pH 8.6. Plate 8 confirmed that the protein patterns of ribosomes of formate - and casamino acid-grown cells differed when acidic proteins were compared. Definite differences were shown in proteins 1, 2 and possibly 3 of the heterotroph (C + D) and the autotroph (A + B). The heterotroph contained all these proteins while the autotroph had none. Thus the different growth conditions influenced the acidic proteins of the ribosomes.

In an attempt to demonstrate ribosomal protein differences of autotrophic and heterotrophic ribosomes the two dimensional polyacrylamide gel electrophoresis procedure was again modified slightly. After the first dimension run the alkaline gel must be acidified in preparation for the second dimension run. This was done originally by immersing the gel in a second dimension "starting buffer" (Kaltschmidt and Wittmann, 1970 a) for two 1 hr periods. This step was suspected of causing a loss of proteins by diffusion out of the gel which may explain why no discrete differences were observed in

autotrophic and heterotrophic ribosomes. To reduce the loss of proteins the alkaline disc gel was shaken in 100 ml of cold 0.3 M HCl for 5 min and then quickly rinsed 3 times in cold distilled water (Avital and Elson, 1974) prior to the second dimensional run. Studies by Avital and Elson (1974) showed the rapid lowering of pH with HCl significantly increased the amount of radioactivity labelled proteins recovered from the second dimension gel. Treating Ps. oxalaticus 0X1 ribosomal protein samples in this way gave protein patterns equivalent to those obtained with the original procedure. No additional differences in the ribosomal protein patterns from the 2 cultural conditions could be demonstrated. However, this procedure was retained because of the speed with which the gel could be acidified while retaining most of its protein.

Two Dimensional Analysis of Subunit Proteins

The differences observed in two dimensional ribosomal protein patterns were investigated by electrophoresis of subunit proteins. This analysis allowed a better comparison of the autotrophic and heterotrophic ribosomes as well as locating where differences occurred, i.e., in the 30 S or 50 S subunits.

Appropriate quantities (30 S - 0.55 mg; 50 S - 1.0 mg) of acetic acid extracted ribosomal proteins were electrophoresed for 24 hr in the first dimension and 44 hr in the second dimension. In all electropherograms the subunit preparation was at least 90% free of cross contaminating material. The stained gels were photographed and compared to each other.

A) The 50 S Subunit

Plate 9 is the gel obtained from electrophoresis of DTT reduced heterotrophic 50 S preparation. From this and other electropherograms of DTT reduced samples the composite electrophoretic pattern, Figure 14, was produced. Figure 14 revealed a minimum of 23 50 S ribosomal protein spots labelled as proteins L1-L23 where L represents the large, or 50 S, subunit. Plate 10, the separation of an unreduced sample of the same subunit, resolved 3 additional proteins L24, L25 and L26 raising the number of 50 S proteins to 26. However, L11 and L12 appear to be made of several unresolvable proteins so that the total number of 50 S heterotrophic ribosomal protein may be between 26 and 36.

Plate 11 and Figure 15, a composite diagram, are results obtained from two dimensional electrophoresis of DTT reduced 50 S autotrophic ribosomes. Twenty-two distinct protein spots were detected (Plate 11). With

PLATE 8. Electropherograms of ribosomes of formate - (A and B) and casamino acid - grown (C and D) cells as analyzed in polyacrylamide disc gel at pH 8.7 (Methods).

A. 100 µg of autotrophic 70 S proteins

B. 130 µg of autotrophic 70 S proteins

C. 140 µg of heterotrophic 70 S proteins

D. 110 µg of heterotrophic 70 S

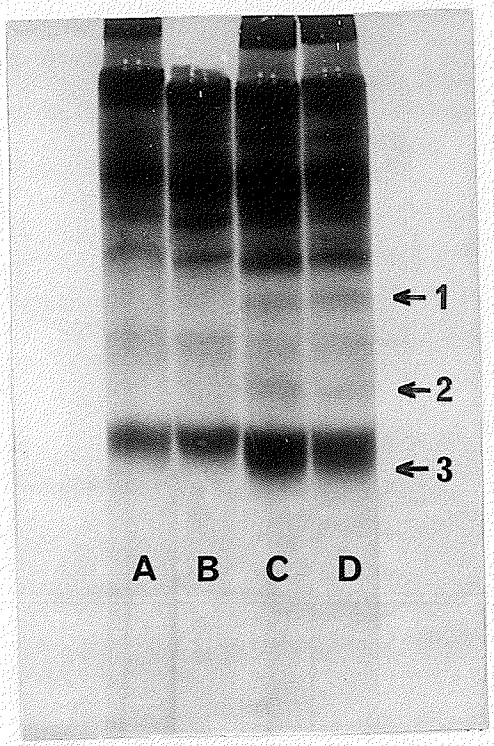
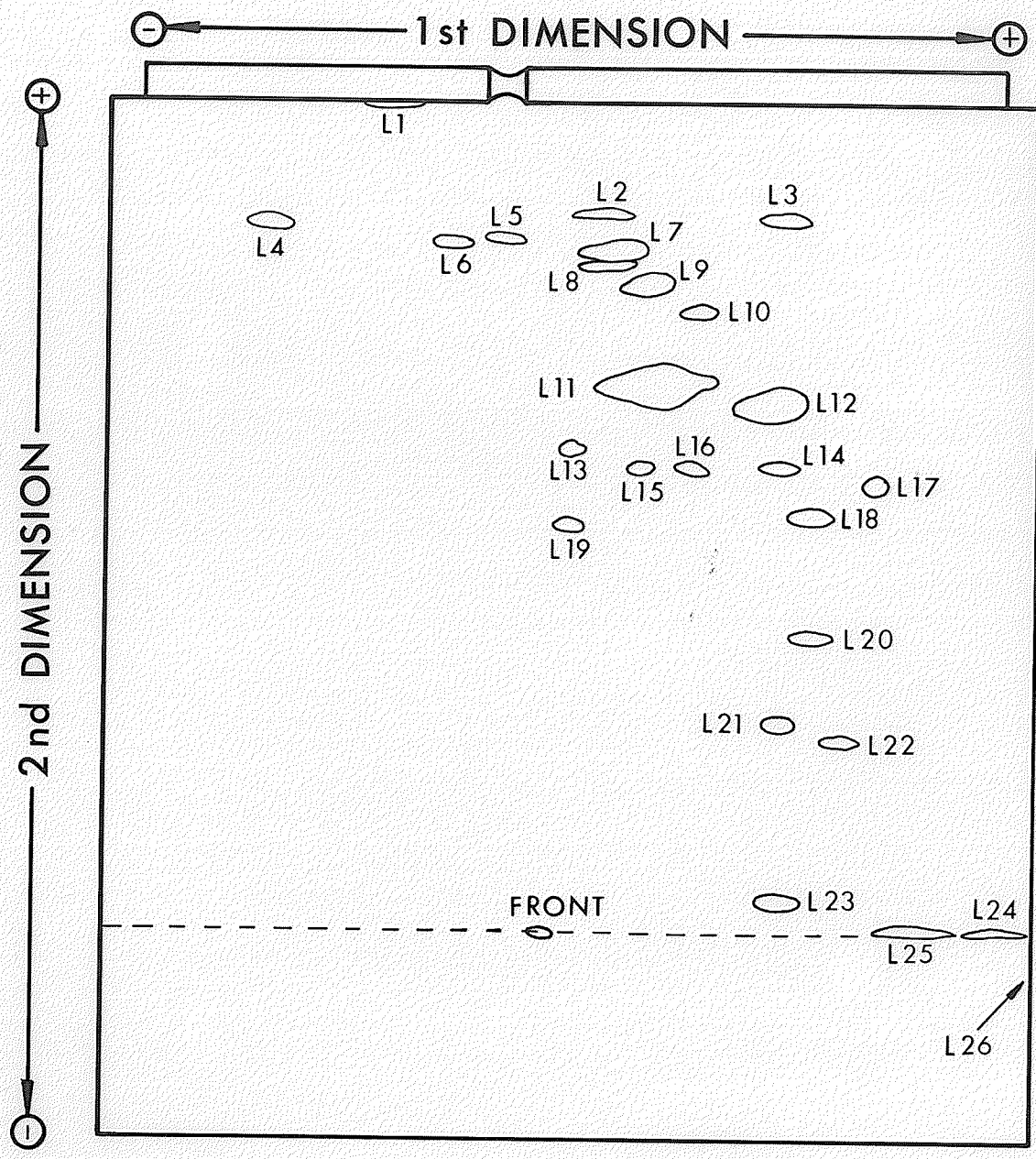


FIGURE 14. Composite diagram prepared from Plates 9 and 10 and other electropherograms showing a minimum of 26 ribosomal proteins from heterotrophic 50 S subunits.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L23
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L25
L26

PLATES 9 and 10. Electrophoretic separation of the heterotrophic 50 S subunit proteins using two dimensional polyacrylamide gels (Methods). Samples were:

Plate 9. 1.0 mg (60 A_{260} equivalents) of DTT reduced 50 S proteins

Plate 10. 1.0 mg of unreduced 50 S proteins

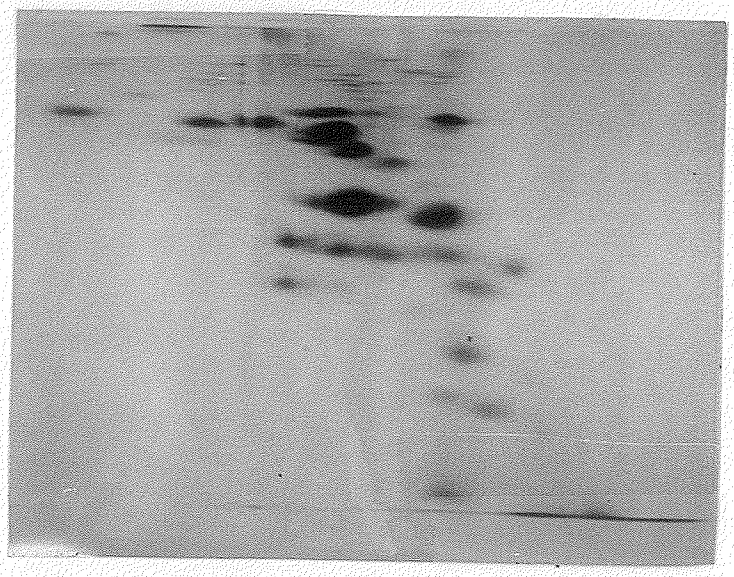


PLATE 9

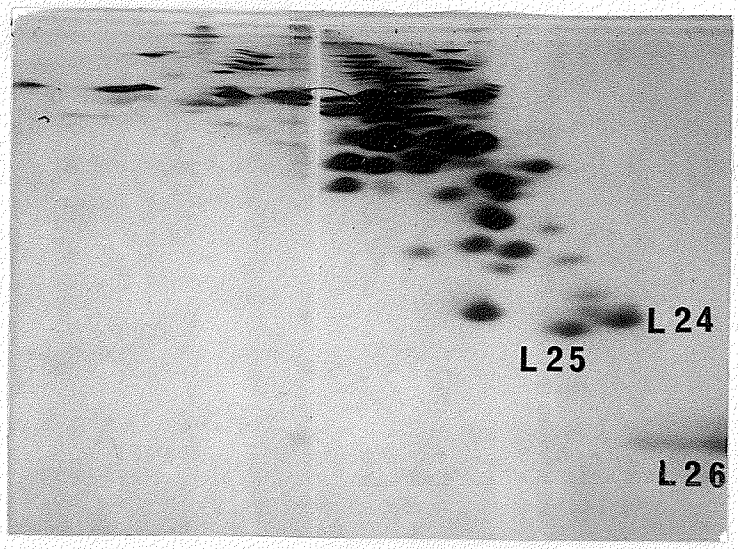
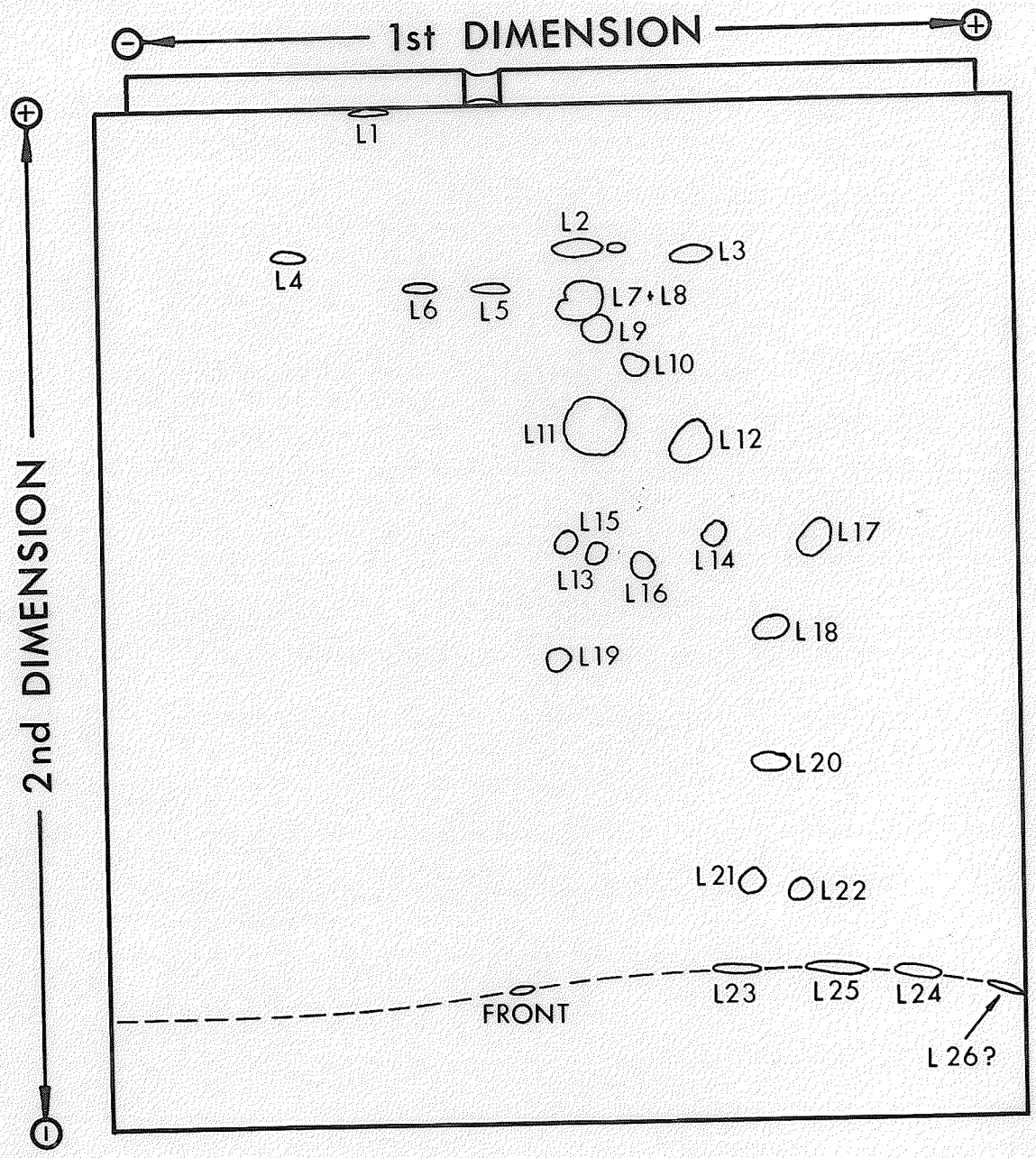


PLATE 10

FIGURE 15. Composite electrophoretic pattern of the 50 S autotrophic ribosomal proteins prepared from Plates 11 and 12 and other electropherograms. A minimum of 25 ribosomal protein spots can be detected.


1st DIMENSION
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L6
L7+L8
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L14
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L16
L17
L18
L19
L20
L21
L22
L23
L24
L25
FRONT
L 26?

unreduced samples three additional proteins L23, L24 and L25 (Plate 12) were detected, therefore raising the minimum number of ribosomal proteins on the 50 S autotrophic subunit to 25. The protein spots L11 and L12 again appeared to be composed of more than 1 protein so that the estimate of 50 S proteins lies between 25 and 35. For comparison, the proteins of the autotrophic subunit were labelled as described for the heterotroph.

Comparison of Figure 14 and 15 revealed only one difference between the electrophoretic patterns of the autotrophic and heterotrophic 50 S subunit. Protein L26 was present in heterotrophic ribosomes and absent in the autotrophic ribosomes. However, trapping of protein L26 in the "front" of the electropherogram (Figure 15) could account for this difference.

The protein differences observed in the two dimensional 70 S protein patterns were not confirmed by electrophoresis of 50 S autotrophic and heterotrophic subunits. Proteins P, Q and X were present in the 70 S heterotrophic ribosomes (Figure 12) but absent from autotrophic ribosomes (Figure 13). Comparison of composite electrophoretic patterns of 50 S autotrophic and heterotrophic proteins (Figure 14 and 15) in this region (L13-L19) failed to show these differences.

PLATES 11 and 12. Electrophoretic separation of proteins of the autotrophic 50 S subunit using two dimensional polyacrylamide gels (Methods). Samples which underwent analysis were:

Plate 11. 1.0 mg (60 A_{260} equivalents) of DTT reduced 50 S proteins

Plate 12. 1.0 mg of unreduced 50 S proteins

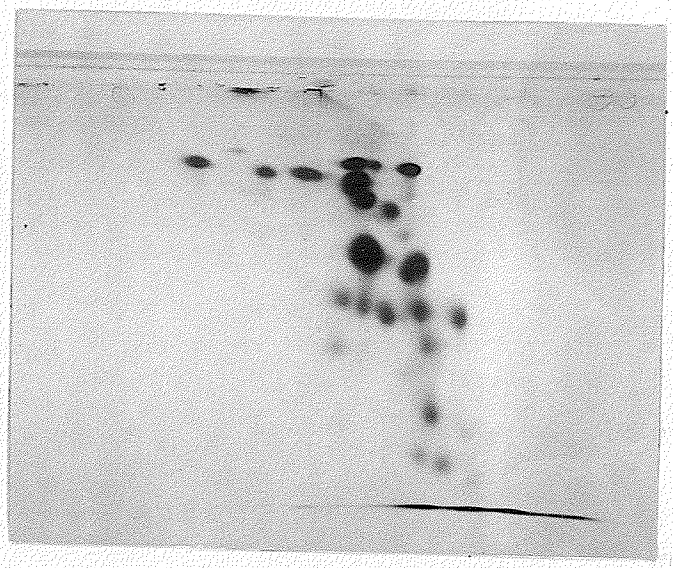


PLATE 11

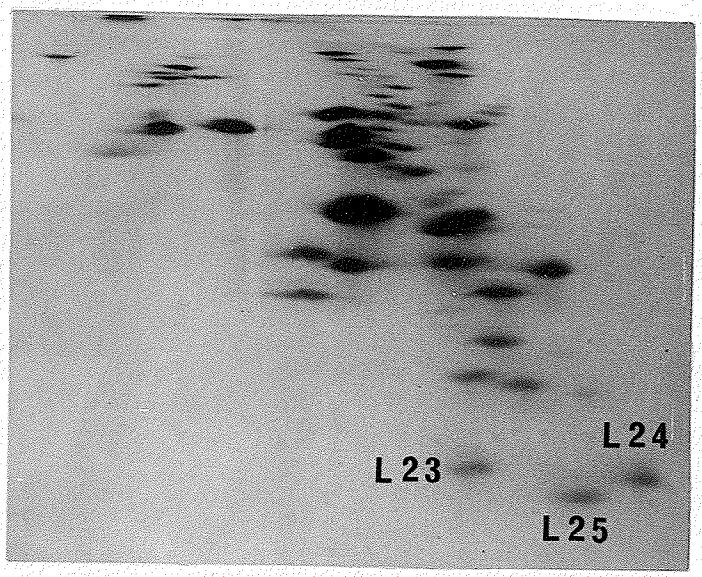


PLATE 12

Furthermore, comparison of 70 S proteins with isoelectric points greater than pH 8.6 (region U, Figures 12 and 13) had shown that the heterotroph possessed protein N but not M with the reverse true for the autotroph. This difference was not confirmed upon comparison of 50 S protein patterns (Figure 14 and 15). Both type of 50 S subunits possessed proteins L1, L4, L5 and L6. Thus the only possible alteration induced by cultural conditions as shown by two dimensional gels occurs in the 30 S subunits.

B) The 30 S Subunit

Electrophoresis of 0.55 mg (35 A_{260} equivalents) of 30 S heterotrophic ribosomal proteins resulted in the separation of 15-18 proteins (Plate 13). From this electropherogram and others of the 30 S heterotrophic subunit the composite electrophoretic pattern, Figure 16, was formed. Approximately 15 protein spots labelled S1 - S15 could be detected where S designates the small, or 30 S, subunit. In several electropherograms the "front" was very close to the bottom of the second dimension gel allowing the detection of protein S18 (Figure 16). Although spots S5, S8, S9 and S11 appeared as faint spots in Plate 13, in other electropherograms with higher concentrations of protein these spots were much more distinctive and thus included in

FIGURE 16. Composite electrophoretic pattern of 30 S heterotrophic proteins from Ps. oxalaticus 0X1 showing a minimum of 16 protein spots. Additional proteins may be present and their location is indicated by S?.

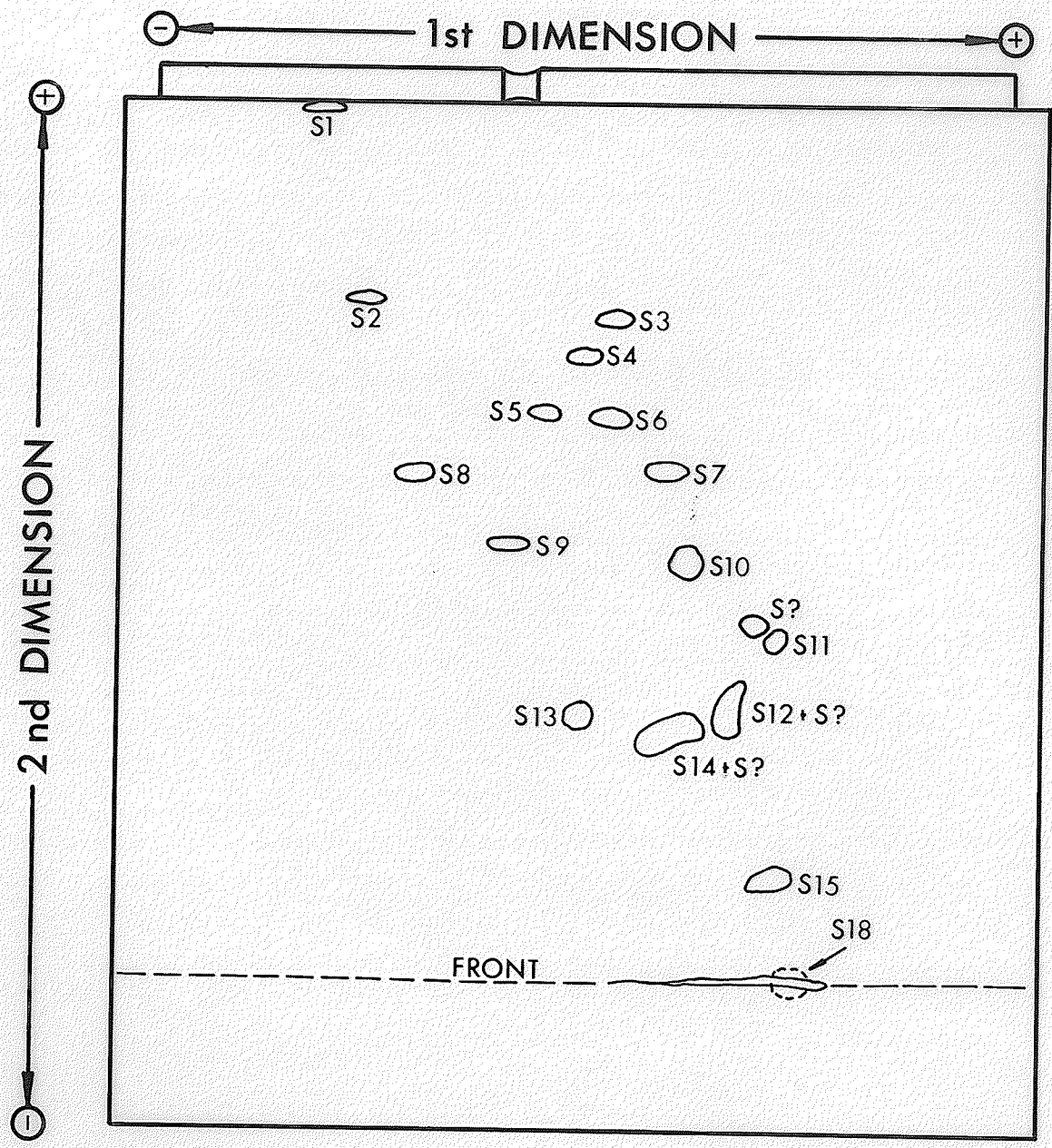

1st DIMENSION
2nd DIMENSION
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S?
S11
S13
S12+S?
S14+S?
S15
S18
FRONT

PLATE 13. Electropherogram of a 0.55 mg protein sample of 30 S heterotrophic subunits after two dimensional analysis on polyacrylamide gels.

PLATE 14. Electropherogram of a 0.55 mg protein sample of 30 S autotrophic subunits after two dimensional polyacrylamide gel analysis.

PLATE 13

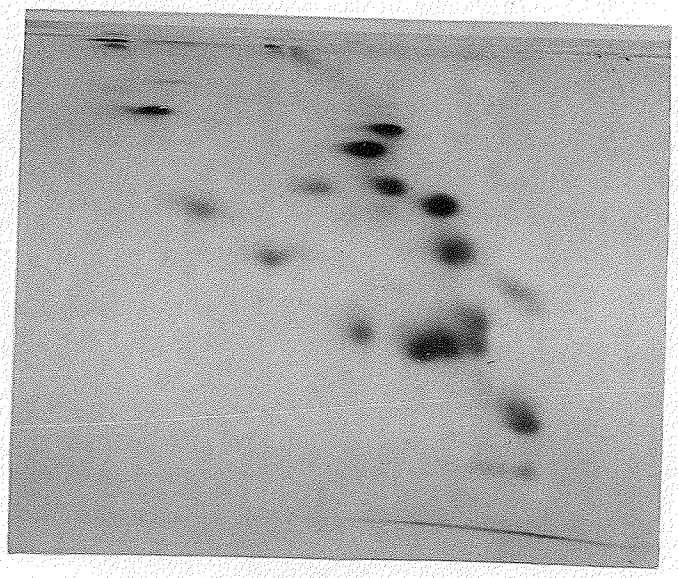
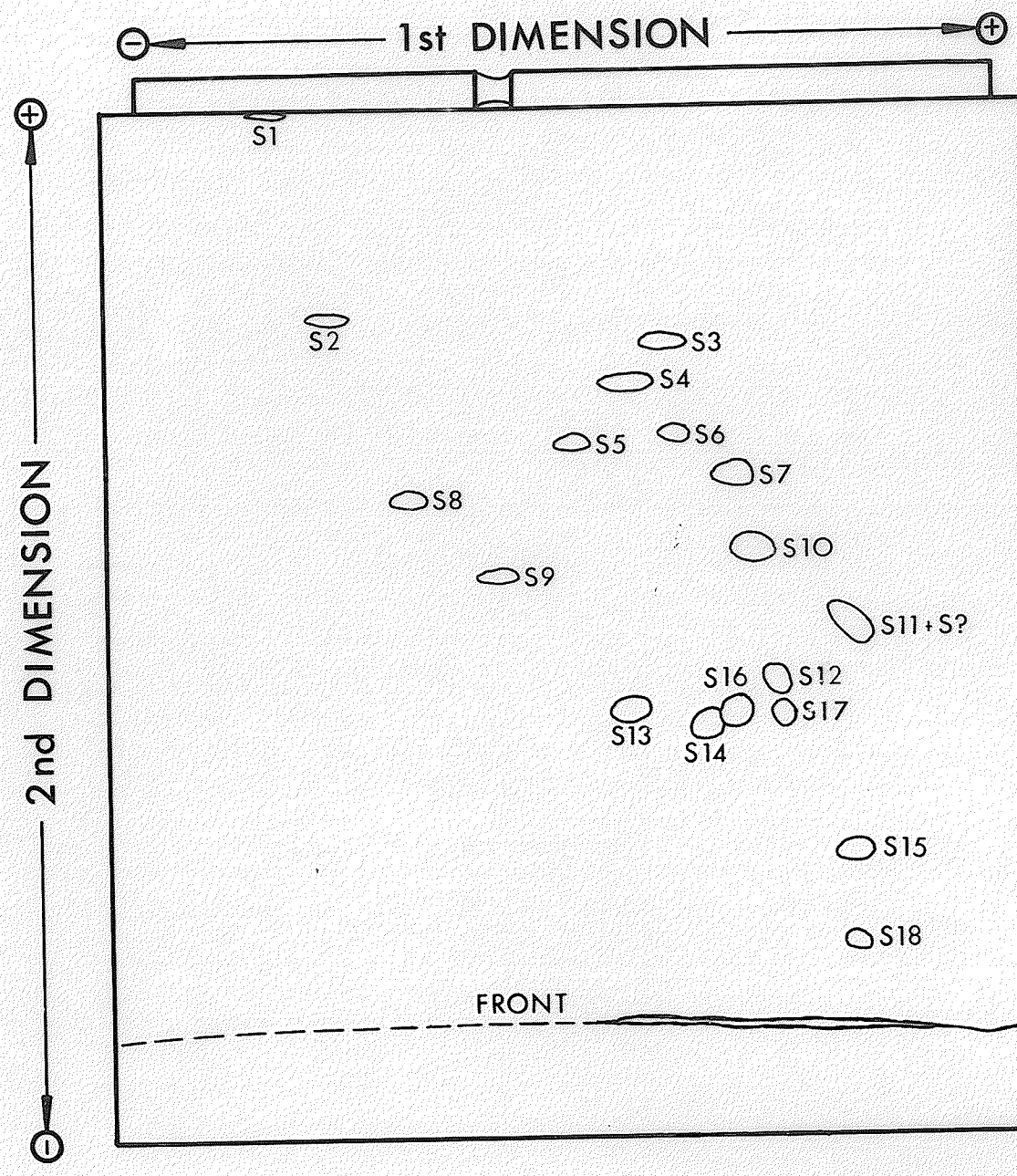


PLATE 14

FIGURE 17. Composite electrophoretic pattern of 30 S autotrophic proteins of Ps. oxalaticus 0X1 showing a minimum of 18 protein spots. An additional protein S? may be located with protein S11.


1st DIMENSION
2nd DIMENSION
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11+S?
S16
S12
S17
S13
S14
S15
S18
FRONT

the catalogue of 30 S heterotrophic proteins.

Plate 14 shows the 30 S autotrophic ribosomal protein pattern. Here, better resolution has allowed the identification of a minimum of 18 spots, S1-S18, with still additional protein(s) trapped in the "front". Proteins S16 and S17 were not detected in heterotrophic electropherograms probably as the result of poor resolution of the 30 S heterotrophic proteins.

In all electropherograms analyzed, it was difficult to determine if some proteins [S11, S12 and S14 of the 30 S heterotrophic subunit (Figure 16) and S11 of the 30 S autotrophic subunit (Figure 17)] consisted of one protein that trailed or two proteins. However, in both composite diagrams these proteins are depicted as one protein and the label S? was added to indicate a second possible protein located in this spot.

Comparison of the two composite diagrams (Figures 16 and 17) failed to show any difference in the electrophoretic protein pattern of the 30 S autotrophic and heterotrophic ribosomes.

V DISCUSSION

Adaptation to different cultural conditions requires cells to undergo physiological and structural changes such as the synthesis of new enzymes and alterations to the structure of cell membranes and organelles. One organelle which undergoes structural alterations in response to different cultural conditions is the ribosome. Support for this belief came from the work of Deusser (Deusser and Wittmann, 1972; Deusser, 1972). She grew E. coli in rich and minimal media, labelling the cells with different isotopes (^{14}C or ^{3}H - amino acids) to mark their origin. Cells were combined, ribosomes were extracted and their proteins identified on two dimensional polyacrylamide gels. The ratio of $^{14}C/^{3}H$ radioactivity associated with each protein spot was calculated. Comparison of these ratios showed variations in the amounts of some ribosomal proteins as a result of growth under different cultural conditions. Park (1973) was able to demonstrate gross changes in the ribosomal protein composition of Thiobacillus novellus cells grown under autotrophic and heterotrophic

conditions. In addition, Park was able to demonstrate an alteration in the Mg^{++} requirement for dissociation of autotrophic and heterotrophic ribosomes. The heterotrophic ribosome dissociated at 3 mM Mg^{++} whereas autotrophic ribosomes failed to show dissociation at even 0.5 mM. Alterations in amounts of protein on the ribosome as well as alterations in the protein composition were induced by different cultural conditions.

Ps. oxalaticus 0X1 was the organism chosen for this investigation. It was capable of growth on formate or other organic substrates as its sole source of carbon and energy. Growth on formate required the conversion of formate to CO_2 which is assimilated into cell constituents via the carboxylation of ribulose-1, 5-diphosphate and the Calvin cycle (Quayle and Keech, 1959 a, b, c). Energy for this process was derived from the oxidation of formate to CO_2. Thus the growth of Ps. oxalaticus 0X1 on formate is basically autotrophic. Growth of this organism on oxalate and other more reduced organic compounds was shown to be heterotrophic. Growth on organic substrates (other than formate) failed to induce the enzymes necessary for formate utilization (Blackmore and Quayle, 1968; Blackmore et al., 1968). This point combined with the rapid rate of growth, particularly for the autotroph (Figure 1), made this

organism ideal for the investigation of the influence of cultural conditions on protein composition and properties of ribosomes.

Initial analysis of the protein complements from ribosomes of formate and casamino acid-grown cells by electrophoresis in pH 4.5 polyacrylamide disc gels (Plate 1) indicated that ribosomes from the 2 types of ribosomes differed in 3 protein bands. Subjecting the ribosomes to further analysis on two dimensional gels failed to confirm these alterations. Difficulties in separation such as trailing of proteins, failure to resolve conglomerate spots, observation of faintly visible spots and the formation of a "front" were given as the reasons for the failure to confirm protein composition alterations.

One particular difficulty, trailing of proteins, was investigated further. Trailing occurred with proteins that have an isoelectric point of greater than pH 8.6. These proteins appeared in the left hand side of the two dimensional gels. No reason for trailing can be given but it is not confined to Ps. oxalaticus 0X1 having been observed in the separation of E. coli ribosomal proteins. In an attempt to resolve any protein differences hidden by trailing, pH 8.7 polyacrylamide disc gel electrophoresis was

carried out on these proteins. The heterotroph was found to contain 3 additional proteins not found in the autotroph (Plate 8). However, analysis of the proteins of ribosomal subunits by 2 dimensional gel electrophoresis failed to show these differences.

An attempt was made to resolve this disparity by increasing the sensitivity of the two dimensional electrophoresis procedure to that used in the E. coli system. Attempts to increase resolution by altering voltage (90 - 180 volts), time of electrophoresis, and temperature at which electrophoresis was carried out (6^{o}C to room temperature) failed. Alteration of second dimension polyacrylamide gel (from 18% to 16%) increased resolution but it was still insufficient for resolving some larger conglomerate spots (eg. spot Z, Figures 12 and 13).

Also, the possibility existed that single protein spots on two dimensional gels represented more than one protein. Such hidden proteins may represent some of the differences seen in the disc gels. With E. coli ribosomes, proteins L8 and L9 were resolved from a single protein spot by varying the first dimension gel concentration (4-12%) and the pH of the buffer from 4.9 to 12.0 (Kaltschmidt and Wittmann, 1970 b). Verification that single protein spots do represent a single protein in Ps. oxalaticus would require similar studies.

An alternative explanation is that proteins were lost during lowering of the first dimension gel pH from 8.7 to 4.6. This could account for not seeing alterations in ribosomal protein compositions by the two dimensional gels. The original acidification procedure involved dialysis of the gel against a "starting buffer" for two 1 hr periods ("Methods"). In the E. coli system, the recovery of radioactive proteins in the second dimension gel was only 51 to 58% of the original sample applied to the first dimension (Avital and Elson, 1974). However, when HCl was used to lower the pH ("Methods") recoveries of 78 to 92% were obtained. Applying this procedure to the Pseudomonas system failed to reveal any additional differences in the protein patterns of autotrophic and heterotrophic ribosomes. However, a protein loss of 8-22% still remains to be accounted for and although it may be assumed that this loss is split evenly between all ribosomal proteins, the possibility can not be excluded that the loss was caused by only a few proteins. If for example this loss represented only 5 of the approximately 53 to 58 ribosomal proteins, the protein differences seen in disc gels can be accounted for. Verification of this suggestion requires further manipulation of the two dimensional electro-

phoretic procedure.

Growth under different cultural conditions may influence the ribosome in ways other than protein composition. Disruption of autotrophic cells resulted in much lower yields of ribosomes per gram of cells than for the heterotroph ("Results"). Poor breakage of cells was interpreted as the reason for this phenomenon. Park (1973) encountered a similar situation when disrupting autotrophic and heterotrophic T. novellus cells. Despite using various disruption and extraction procedures, the yield of autotrophic ribosomes remained low. Park (1973) suggested that this difference in ribosome yields may reflect a difference in the ribosome content of the two types of cells as dictated by the differences in their protein - synthesizing capacity. The results obtained with Ps. oxalaticus makes this suggestion a distinct possibility. Attempts to increase ribosome yield from cells should be undertaken to verify the possibility that cultural conditions influence the ribosome content of the cell.

Analysis of rRNA on 2.65% polyacrylamide - 0.15% bisacrylamide gels containing 0.2% SDS indicated that the 23 S rRNA of intact 70 S ribosomes (Figure 8 and 9) contained hidden nicks. Dissociation of ribosomes into their respective subunits was accompanied by further

breakdown in both the 16 S and 23 S rRNA of both autotrophic and heterotrophic ribosomes (Figures 10 and 11). Similar results were obtained by Szer (1969) working with E. coli strain Q 13. Szer found that intact 70 S ribosomes kept at 0°C for 15-30 days resulted in almost complete degradation of 23 S rRNA with a concomitant formation of 16 S rRNA in the larger subunit. Dissociation of intact ribosomes into subunits and incubation at 0°C resulted in degradation of both 23 S and 16 S rRNA. Analysis of these rRNA's showed that they were "halved" first and then underwent further degradation. Endonucleolytic activity (RNA ase IV type) associated with the "split protein" fraction from either subunit was demonstrated to be responsible for the instability of the rRNA's (Szer, 1969). With the similarities between the E. coli Q 13 and Ps. oxalaticus 0X1 systems, it is tempting to speculate that there is a similar activation of a nuclease activity upon dissociation of ribosomes from Ps. oxalaticus and that the nuclease could be associated with the "split protein" fraction. This nuclease could be part of the ribosomal protein complement as shown for E. coli MRE 600 (Ceri, 1973) or a contaminating protein.

Degradation of 23 S rRNA of intact 70 S ribosomes and that of the 16 S and 23 S rRNA of the subunits was not reflected in the sedimentation profiles of the ribosomal subunit except perhaps for the 30 S autotrophic subunit (Figures 2 and 3). The question arises as to the activity of these subunits in protein synthesis. Would the activity of these subunits be affected to an extent corresponding to its RNA degradation? If no loss of activity resulted, would the ribosomes from the 2 types of cells show functional differences in the protein synthesis assay as a result of the differences in their ribosomal protein composition? These questions warrant investigation in order to determine the extent of ribosomal changes induced by the different cultural conditions.

An investigation, similar to that of Deusser (Deusser and Wittmann, 1972; Deusser, 1972), into the possibility that the amounts of ribosomal proteins were altered in response to different cultural conditions was undertaken. Ps. oxalaticus was grown in the presence of 2.5 μc/ml of ^{14}C- labelled sodium formate. Cells were collected, mixed with cold cells and ribosomes were extracted. However, small amounts of ribosomes with a specific activity of only 4400 cpm/A_{260} unit made

a two dimensional study unfeasible. The problem with this labelling procedure was that 58% of the total radioactivity in the vessel was caught in a CO_2 trap. To overcome the technical difficulty, ^{14}C-labelled bicarbonate could be used so that the radioactivity remains in the medium. Possibly then ribosomes with a high specific activity could be isolated and combined with ^{3}H-amino acids to investigate the variation in the amount of ribosomal proteins between the two cultural conditions.

Growth of Ps. oxalaticus 0X1 on formate and casamino acids resulted in alterations to the protein complements of the ribosomes as shown by polyacrylamide disc gels. However confirmation of these differences could not be shown by two dimensional gels and thus failed to locate and identify the specific alterations involved. Despite this handicap, this investigation does suggest a possible functional basis for heterogeneity of ribosomal proteins. The static model ("Historical") suggests that the functional specializations of subclasses of ribosomes are permanently fixed by stable association of specific "fractional proteins" with a common core of "unit proteins". An organism grown under different conditions such as autotrophy and heterotrophy would have different mRNA complements and consequently the function specializations of

ribosomes would be different. This suggests that the protein complement of the ribosome would be different as a result of the stable association of different "fractional proteins".

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