THE CHARACTERIZATION OF THE RIBONUCLEIC ACID FRACTION OF POLYRIBOSOMES OF ULTRAVIOLET LIGHT IRRADIATED *ESCHERICHIA COLI* KEPES ML3 BY CELLULOSE ACETATE ELECTROPHORESIS

A DISSERTATION

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COLLEGE OF

ARTS AND SCIENCES

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INTRODUCTION

The development of new experimental techniques and instrumentation has provided numerous fundamental insights into living systems, especially at the molecular level. Many important discoveries have redefined and broadened our concepts of biological phenomena.

The investigations now being reported in this dissertation were concerned with the development and perfection of methods for the extraction and spectrophotometric identification of polyribosomes from ultraviolet irradiated bacteria. The ribosomal ribonucleic acid fraction of the polysomes was subsequently separated, and the studies were conducted to identify macro-molecular changes which had occurred in that fraction as a result of radiation exposure in a living system. To facilitate the characterization, electrophoretic methods were developed which would allow for the use of cellulose acetate, a medium that offers rapid separations and high resolution of the separated cellular fractions.

The first recorded results of the effects of radiation on microorganisms was reported by Downes and Blount (1877), who found that organisms which had been exposed to bright sunlight from five to seven hours became inactive. Almost simultaneously, two investigators working independently concluded that ultraviolet wave lengths in the sunlight were responsible for the bactericidal effect (Arsonval 1894;

Ward 1894). Strebel (1901), using isolated regions of the spectrum showed that the strong killing action for bacteria was confined to the ultraviolet wave lengths. Bie (1900) computed the lethal effects of ultraviolet radiations to be in the 295 nm to 200 nm range. While a number of investigations showed the bactericidal effectiveness of ultraviolet radiations, the lack of uniformity in light sources, inadequacy in measurement of intensity of radiation, fluctuations in the duration of exposure, and variations in the physiological state of the organisms tested, did not allow for an understanding of the basic mechanisms involved (Duclaux 1885; Bayne-Jones and Van der Lingen 1923).

The sources of the ultraviolet radiations had ranged from such artificial sources as lamps burning fluids in oxygen and high tension disruptive electric spark discharge between metal electrodes, to iron or carbon arc sources with mixed spectra ranging from 360 nm to 200 nm. With the development of the quartz mercury vapor lamp, and its subsequent refinement, monochromatic sources were made available for biological research (Kuch and Stark 1905). Investigations by Coblentz, Long and Kahler (1919) resulted in the introduction of a method for determining quantitatively the intensity of the emission of the quartz mercury vapor lamp.

While working with Staphylococcus aureus, Gates (1928) observed the relative bactericidal effectiveness of

different wave lengths. These were chiefly 237 nm to 312 nm of monochromatic ultraviolet radiation. He noted that the incident energy required to kill S. aureus at 260 nm to 270 nm was less than for any other region of the spectrum examined and was the same as that of the relative absorption of ultraviolet radiation by deoxyribonucleic acid derivatives. The action spectrum for Escherichia coli showed a maximum of bactericidal effectiveness and absorption at 260 nm and a minimum at 238 nm (Gates 1930). Ehrismann and Noethling (1932) reported a maximum sensitivity for E. coli at 251 nm in bactericidal action studies, while Wyckoff (1932), Hollaender and Claus (1936), and Hollaender and Duggar (1936) found the maximum sensitivity of E. coli to be 265 nm in similar bactericidal action spectra analyses. These observations of maximum efficiency of bactericidal effects at ~260 nm focused attention on purine and pyrimidine constituents of nucleic acids.

Studies using ultraviolet radiations as a mutagenic agent showed a correlation between mutation rates and the wave lengths of incident light (Knapp et al 1939; Hollaender and Emmons 1941). The action spectrum for ultraviolet mutagenesis was shown to follow a nucleic acid absorption spectrum.

Beukers and Berends (1960) observed the formation of thymine dimers, apparently preceded by radical formation

(Eisinger and Schulman 1963), after ultraviolet irradiation of thymine in frozen solutions. The same type of dimerization was observed in the hydrolysis products of DNA from ultraviolet light irradiated bacteria (Wacker, Dellweg and Lodemann 1961). Although DNA is the target most likely to be responsible for the permanent loss of viability in cells, various classes of RNA may be similarly sensitive when damaged by photons.

Sinsheimer and Hastings (1949) found that uracil, both its nucleoside and its nucleotide, form a uracil photoproduct which is reversible by the use of heat or acid. The principal photoproduct obtained from uracil solutions irradiated with ultraviolet light ~230 nm was a hydrate. In experiments with ultraviolet irradiation of uracil and uridine, dimer formation has been observed (Wacker et al 1961; Swenson and Setlow 1963; Setlow et al 1965; Wang 1965). Uracil dimers have been isolated from UV irradiated RNA (Schuster 1964).

Cytosine nucleosides exposed to ultraviolet radiations add water (Shugar 1960) and subsequently undergo deamination (Wacker 1963). Dimers of cytosine have been obtained in ultraviolet irradiated photoproducts (Wacker 1963; Setlow, Carrier and Bollum 1965). Ultraviolet radiations can lead to a cytosine uracil transition (Johns, LeBlanc and Freeman 1965).

Polyribosomes represent a complex of mRNA-ribosomes-tRNA

and forming peptide chains (Warner et al 1962). Luria et al (1943) described particles obtained from differential centrifugation of bacterial spheroplasts with a high RNA content. In 1958, Dintzis suggested the term "ribosome" to distinguish the particulate material. Warner et al (1962) found that ribosomes existed not only singly, but also as aggregates, and introduced the terms polyribosomes or polysomes. In vitro studies have shown that synthetic m-poly-UA formed aggregates with single ribosomes (Spyrides and Lipmann 1962; Barondes and Nirenberg 1962).

The first intensive study of the physio-chemical properties of bacterial ribosomes was conducted by Tissieres and Watson (1958) and Tissieres et al (1959). These workers noted that 80-90% of the RNA of exponentially growing E. coli was in the form of ribonucleoprotein particles. The RNA/protein ratio of these ribosomes was about 65:35 and their structured integrity was apparently dependent upon magnesium ions. Britten and McCarthy (1959) found that in E. coli, most ribosomes are 70S, but that they can be dissociated into smaller particles of 30S and 50S.

Electron microscopic studies of E. coli ribosomes supported the occurrence of the two unit structure of monosomes (Hall and Slayter 1959; Huxley and Zubay 1960). Electron micrographs showed that polysomes consist of variable numbers of ribosomes, seemingly linked together by a

fiber 10-15A in diameter (Slayter et al 1963). The strand joining the ribosomes consisted of mRNA, according to Gilbert (1963). He suggested that a 70S ribosome became attached to the end or rather the beginning of a mRNA strand and progressed along its length as the corresponding polypeptide was synthesized. A second, third and other 70S ribosomes became successively attached to the mRNA strand so that at any time several forming polypeptides may be at different stages in the progression of the 70S ribosome along the mRNA strand. Gilbert (1963) found that ribosomes detached themselves from the mRNA when they reached the end of the mRNA strand, released the polypeptide chain at the same time and then were free to repeat the process.

The role of ribosomes as centers of protein synthesis was established with bacteria, using exponentially growing cultures labeled for short periods with ^{35}S-sodium sulfate (McQuillen, Roberts and Britten 1959). The cellular radioactivity appeared as amino acid sulfur in association with the 70S ribosomes. In experiments with a 15 sec labeling period followed by a 2 min chase at 37 C using a large excess of ^{35}S-sulfate and cysteine, analysis of extracts from the cells showed most of the labeled, newly formed protein had reached the soluble fraction. From the time course of the reaction, it was calculated that the amount of the labelled ^{35}S-sulfate that passed through the ribosomes

was sufficient to account for the entire protein synthesis in the cells. Thus, for the first time it was demonstrated that ribosomes were involved as the major site of protein synthesis. The non-specificity of the ribosomes was first shown in investigations where it was found that the net synthesis of ribosomal RNA stops immediately after phage infection, and yet several new protein species, not produced in uninfected bacteria, are formed in large quantities (Cohen 1948; Hershey 1953). In vivo studies by Brenner, Jacob and Meselson (1961), as well as in vitro studies by Tsugita et al (1962), Loeb and Zinder (1961) and Nathans et al (1962), further substantiate the fact that ribosomes are nonspecific with respect to the kind of protein being synthesized.

Since polyribosomes are sensitive to shearing forces, a variety of methods for gentle lysis of bacteria have been developed to prevent extensive breakage. Polysomes have been rapidly extracted from E. coli by sequential passage of cells through solutions of a chelating agent and lysozyme in a centrifugal field, followed by sucrose density-gradient sedimentation (Dresden and Hoagland 1965; Mangiarotti and Schlessinger 1966; and Flessel, Ralph and Rich 1967). The studies reported in this disseration employed the use of a refrigerated unit which allowed for disruption of the bacterial cell wall without appreciably altering the protoplasm.

The sequence of events involved in protein synthesis make it apparent that errors can occur at each step (i.e. in the replication of DNA, in the transcription of DNA into mRNA and in the translation of the RNA into protein). The importance of the protein fraction in the functional activity of the ribosome has been shown by the sequential analysis of the amino acids found in the nucleoprotein structure of the E. coli ribosome (Spahr 1962); the synthesis of ribosomal units (Nomura, Traub and Guthrie 1968); in addition to amino acid starvation studies (Mandelstam 1960).

From a personal communication, Rupert (1969), made available information concerning action spectrum studies of monochromatic ultraviolet irradiation of ribosomes of E. coli, which was subsequently published by Ekert, Muel, and Latarjet (1970). The irradiated ribosomes were tested for relative activity for carrying out polyphenylalanine synthesis, using polyuridylic acid as a messenger. The action spectrum showed differences in the magnitudes of the peaks at 260 nm and 280 nm, indicating that both RNA and protein were inactivated. Ultraviolet induced conformational alterations in the ribonucleic acid fraction of the ribosome was revealed. The research now being reported in this dissertation was concerned with the phenomenon and the use of ultraviolet radiation damage as a probe to find macromolecular changes in the ribonucleic acid fraction.

MATERIALS AND METHODS

ORGANISM

The bacterial strain Escherichia coli Kepes ML3 was utilized as the source of the polyribosomes studied in this investigation. The ATCC #15233 culture was obtained from the American Type Culture Collection (Rockville, Maryland). The strain was inducible for β-galactoside permease and for galactoside transacetylase ($i^+ z^+ y^-$).

MEDIA

The medium used for culturing the experimental organisms was a basal medium containing the following:

Potassium phosphate, monobasic (KH_2PO_4)	5.4 g
Potassium phosphate, dibasic (K_2HPO_4)	36.6 g
Ammonium sulfate, $(NH_4)_2SO_4$	4.0 g
Water distilled	1,000.0 ml

The solution was sterilized in 2,000 ml Erlenmeyer flasks for 15 min at 15 lb pressure at 121 C. The medium was supplemented by the addition of 1% sterile glycerol as an energy source and 10 ml of a sterile solution of trace elements.

The solution of trace elements was prepared by dissolving the following substances in 1,000 ml of distilled water:

Magnesium sulfate, ($MgSO_4$ 7 H_2O)	40.0 g
Calcium chloride, ($CaCl_2$)	1.0 g
Ferrous sulfate, ($FeSO_4$ 7 H_2O)	0.5 g
Sodium citrate ($Na_3C_6H_5O_7$ 2 H_2O)	100.0 g

Stock cultures were maintained on Difco Bacto Nutrient Agar Slants and in the basal medium previously described. New stock cultures were transferred every seven days. The inoculated agar slants were incubated 12 hr at 37 C prior to being stored at 5 C. The bacteria were grown on basal medium at 37 C using constant agitation in a New Brunswick Gyrotory Incubator-Shaker Model G-25 for 12-14 hr and then refrigerated.

In addition, periodic checks of the organism were made by use of Levine Eosin Methylene Blue Agar (Baltimore Biological Laboratory), a differential medium for the detection of coliform and other enteric bacilli (Levine 1918).

CULTURING OF ORGANISM

In order to assure a relatively uniform cell population throughout the investigation, harvested bacterial cells were brought to a reading of 200 units on the Klett-Summerson Photoelectric Colorimeter using a #42 blue filter, with a spectral range of 400-465 nm. Erlenmeyer flasks (125 ml capacity each containing 50 ml of basal medium, were ino-

culated with 0.5 ml of the bacterial stock solution and incubated 12 hr at 37 C with constant shaking.

The bacterial cells were then collected by 10 min centrifugation at 0 C in a refrigerated Sorvall RC 2-B centrifuge at an average force of 12,000 x g, using an SS-34 rotor. The bacteria were washed twice by resuspending them in incomplete basal medium (ie. without the supplements), and centrifuging for 10 min. The washed cells were then suspended in a sufficient amount of incomplete basal medium to bring them to a Klett reading of 200, using a #42 blue filter, with a spectral range of 400-465 nm.

ULTRAVIOLET IRRADIATION

Thirty ml quantities of the log phase bacterial cell suspension were placed in 150 mm quartz tubes with an I.D. of 20 mm and aerated by a gentle bubbling of air. Irradiation was done with four germicidal lamps, General Electric - G 15TB, 15W, resulting predominantly in 253.7 nm radiation. The low mercury vapor pressure fluorescent lamps were housed in sets of two in two desk lamp holders with light shields. The lamps were placed at a distance of 30 cm. The suspension was irradiated in 5 sec intervals up to 25 sec for preliminary studies to be used to determine the variation of dose-effect as indicated by cell survival numbers.

The intensity measurements were made with a Blak-Ray UV

Intensity Meter, J-225. The dose delivered at the 40 ml capacity quartz tube was 28.0 ergs/mm^2/sec. A control suspension, protected from irradiation by use of a drop cloth was run simultaneously. All irradiation was done in the absence of visible light in a constant temperature room at 30 C. The irradiated cells and the control suspensions were placed immediately into sterile 125 ml actinide flasks to prevent photoreactivation.

GROWTH STUDIES OF ULTRAVIOLET IRRADIATED BACTERIAL CELLS

Bacterial cells in the log phase were exposed to ultraviolet radiation in 5 sec intervals up to 25 sec and utilized to study dose-effects. A 1 ml aliquot was placed in 9 ml of sterile distilled water to give a 1:10 dilution. Serial tenfold dilutions were subsequently made to the 1:10^6 dilution. The 1:10 dilution was discarded. From the remaining dilutions a 0.1 ml aliquot was inoculated on nutrient agar by the spread plate technique. The plates prepared under yellow light to prevent photoreactivation were then incubated for 48 hr at 37 C. Counts of the colonies produced by surviving cells were made, and UV survival curves plotted.

β-GALACTOSIDASE INDUCTION

The strain of E. coli ATCC 15223 used in this investigation was inducible for β-galactoside permease and for

galactoside transacetylase ($i^+ z^+ y^-$). Preliminary studies in the development of techniques to be utilized has been done in the laboratory of Dr. Kenneth A. Fry using E. coli, Kaja, obtained from Dr. Howard Adler, Oak Ridge Institute of Nuclear Studies.

Cells were incubated 9 hr at 37 C, with shaking and then centrifuged at 0 C for 10 min at 12,000 x g. The cells were washed twice in distilled water, using the same centrifugation time and settings. The pellet was then suspended in cold incomplete basal medium to bring the sample to a Klett Reading of 200 units, using a #42 blue filter, with a spectral range of 400-465 nm.

The cells were transferred to 40 ml capacity quartz test tubes for ultraviolet irradiation. The cell suspension was irradiated for 15 sec using 4 germicidal lamps, General Electric-G15TB, 15 w, giving predominantly 253.7 nm, at 28 ergs/mm^2/sec. The control cell suspension was protected from UV exposure by use of a drop cloth. Both control and the irradiated suspensions were transferred to sterile 125 ml actinide flasks and equilibrated for 15 min at 37 C in the New Brunswick Gyrotory Shaker, Model G-25.

The cells were induced with Methyl-Beta-D-thiogalactopyranoside (TMG), using 0.1 ml/ml of the cell suspension. For an energy source, glycerol citrate was added (0.005 ml/ml

of the cell suspension). Cultures were incubated for 1 hr with shaking at 37 C. The enzyme assay was made using σ-nitrophenyl-β-galactoside (ONPG). One ml aliquots of cells were removed from the irradiated and control cultures and layered with 0.06 ml of toluene prior to being placed in the Gyrotory Shaker for 10 min at 37 C. The tubes were then placed in a Blue-M Microtrol Magni-Whirl Constant Temperature Water Bath at 37 C. For the enzyme assay both the irradiated and non-irradiated cell suspensions were tested in 14 x 150 mm pyrex tubes. Each assay tube contained:

NaH_2PO_4-Na_2HPO_4 Buffer, pH 7.0	0.1 ml
Water, distilled	0.7 ml
ONPG	0.1 ml

To each tube, 0.1 ml of the cell suspension was added at 30 sec intervals. At the end of 5 min, a 2 ml addition of 1M Na_2CO_3 was made and the solution in the tubes was allowed to stand at 37 C for a 10 min period. The mixture was then diluted with 3 ml of distilled water. The tube contents were read in the Klett-Summerson Photoelectric Colorimeter Model 800-3 and recorded as optical density in Klett units, using a #42 blue filter with a spectral range of 400-465 nm.

RIBI-REFRIGERATED CELL FRACTIONATION

Log phase harvested cells that had been washed twice with distilled water were suspended in Nirenberg—Matthaei buffer and brought to a Klett reading of 200 units using a #42 blue filter, with a spectral range of 400-465 nm. The cell suspension was then irradiated for 15 sec under conditions previously described. The control and irradiated cell suspensions were transferred to sterile 250 ml actinide flasks and refrigerated at 4 C. The cell suspensions were allowed to equilibrate for a period of 2 hr.

The suspension was then processed in a Sorvall-Ribi Refrigerated Cell Fractionator, RF-1 at 12,500-20,000 psi. As a modified French pressure cell, the Sorvall Ribi-RF-1 is a self-contained unit. Cells were broken under the force of the nitrogen cooled Ribi Valve assembly. Both the cell suspension flask and the lysate receiving flask were kept in an ice bath to maintain the temperature below 4 C. The opalescent lysate was collected in a sterile 250 ml actinide flask. The lysate was transferred to sterile tubes and centrifuged in the HB-4 Swinging Bucket Rotor in the Sorvall RC 2-B Refrigerated Centrifuge at 27,000 x g for 10 min at 0 C. The supernatant was quickly frozen in liquid nitrogen and stored in a Revco Ultra Low Temperature Cabinet, Model ULT-657 at -70 C.

SUCROSE DENSITY-GRADIENTS

The techniques employed in this investigation were a modification of the enzyme method of polyribosome isolation by Dresden and Hoagland (1965). The sucrose density gradients used were as described by Dresden and Hoagland (1965). The sucrose solutions were made up in the buffer of Nirenberg and Matthaei (1961), which contained 0.06 M KCl; 0.01 M magnesium acetate; 0.006 M 2-mercapto-ethanol; 0.01 M Tris (hydroxymethyl)-aminomethane, pH 7.8.

Linear sucrose gradients, 15 to 30%, were prepared in the cold room. The gradients were made up in Beckman cellulose tubes, #302235, with a 13.5 ml capacity.

The quantities of solution were layered from the bottom of the tube as follows:

Sucrose in Nirenberg-Matthaei Buffer, pH 7.8	3.0 ml	30%
Sucrose in Nirenberg-Matthaei Buffer, pH 7.8	2.5 ml	25%
Sucrose in Nirenberg-Matthaei Buffer, pH 7.8	2.0 ml	20%
Sucrose in Nirenberg-Matthaei Buffer, pH 7.8	1.5 ml	15%

The contents were left to chill and to allow for linear gradient equilibration.

SEDIMENTATION OF BACTERIAL POLYSOMES IN SUCROSE DENSITY-GRADIENTS

The cell lysate was layered in 0.2 to 0.5 ml quantities on the surface of a chilled linear sucrose gradient in a 13.5 ml capacity. The Beckman cellular centrifuge tube #302235 was sealed with an aluminum assembly cap, Beckman Spinco type #330860 and placed in a Spinco Type 50 Ti, Highest Force Fixed Angle Rotor and centrifuged in a Spinco Model L 2-65 Preparative Ultracentrifuge at 4 C, with a relative centrifugal force of 200,000 x g for 1-1/4 hr.

COLLECTION OF POLYSOME FROM SUCROSE DENSITY-GRADIENTS

The tube containing the sedimented lysate fraction was carefully punctured at the bottom with a 20 gauge syring needle. The fractions were collected in 5 drop aliquots. The fractions were diluted with Nirenberg-Matthaei Buffer, pH 7.8.

SPECTROPHOTOMETRIC CHARACTERIZATION

The contents of the linear sucrose density-gradient sedimentation tubes were collected from the bottom of the tube in fractions of 5 drops each. To each fraction, Nirenberg-Matthaei buffer was added to bring the volume to 3 ml. The diluted fraction was placed in a 3 ml capacity quartz cuvette and the optical measurements of the ultraviolet

absorbing material at 260 nm were made on the Model 139 Hitachi-Perkin-Elmer Spectrophotometer. The instrument was set at zero using Nirenberg-Matthaei buffer as a blank.

HYDROLYSIS OF POLYSOMES

The method of hydrolysis chosen was one that would yield both purines and pyrimidines (Markham and Smith 1951). The purine and the pyrimidine bases were liberated as nucleotides. One ml 1 N HCl acid was added to 2 ml of the first five fractions of the polysome suspension and sealed in a glass tube. The tube was then placed in a water bath at 100 C for 1 hr and then cooled.

CELLULOSE ACETATE ELECTROPHORESIS OF THE HYDROLYSATE

Electrophoretic separations were made of the hydrolysate along with reference standards. The standards used were 10 mM solutions of adenine, quanine, uracil, cytosine, uridine-3' (2') phosphoric acid 4 H_2O and cytidine sodium monophosphate·2 H_2O. The cellulose acetate support medium used was the Sepraphore squares, 17 cm x 17 cm. The support medium was prepared by soaking in 0.05 M sodium citrate buffer, pH 3.0.

The separations were performed at low voltage using 0.05 M sodium citrate buffer in a Gelman Deluxe Electrophoresis Chamber. The Gelman Power Supply Amperage was set at

4.5 ma and the voltage at 300 v for the 2-1/2 to 4 hr electrophoretic run. All electrophoresis was done in the cold room at 4 C. Protective covers were placed over the power supply and power lines to prevent excessive condensation of moisture on the surfaces.

The wet electrophoretograms were observed immediately after removal from the chamber using ultraviolet light in the Chromato-Vue Model C-5 with both long wave 320 nm and short wave 254 nm lamps. The spots were marked with a pencil.

The hydrolysate spots were identified in accordance with their migration as compared to the locations of the reference standard solutions areas.

EXPERIMENTAL RESULTS

The survival curve shown in Figure 1 plots the surviving fractions of E. coli Kepes ML3 as the log ($N/N_o \times 10^6$) versus the dose (ergs/mm^2).

In the fractional survival, N/N_o:

N_o = the initial number of cells

N = the number of surviving cells at a given dose

D = dose of radiation delivered

K = constant value of dose delivery

When applied,

$$\frac{dN}{N} = kdD$$

This may be expressed as:

$$\log N/N_o = -kD$$

The statistical means of data from five experiments are presented in Table 1. The graphical presentation in Figure 2, indicated that an exponential loss of active cells occurred with increasing ultraviolet radiation exposure dose. These results are in agreement with the concept that when photons impinge on a surface, random energy absorption occurs and results in the inactivation of a proportion of the cells at each dose level. The experimental data being reported in this dissertation, indicate that the organism used, E. coli Kepes ML3, was sensitive to ultraviolet

Figure 1. Dose effect curve expressed as fractional surv of ultraviolet irradiated Escherichia coli ML3, after exposure to ultraviolet light of inantly monochromatic wavelength, 253.7 nm at 28 ergs/mm^2/sec. The bacteria plated on agar, were incubated 48 hr at 37 C in the dark. The survival data represents the means of five experiments.

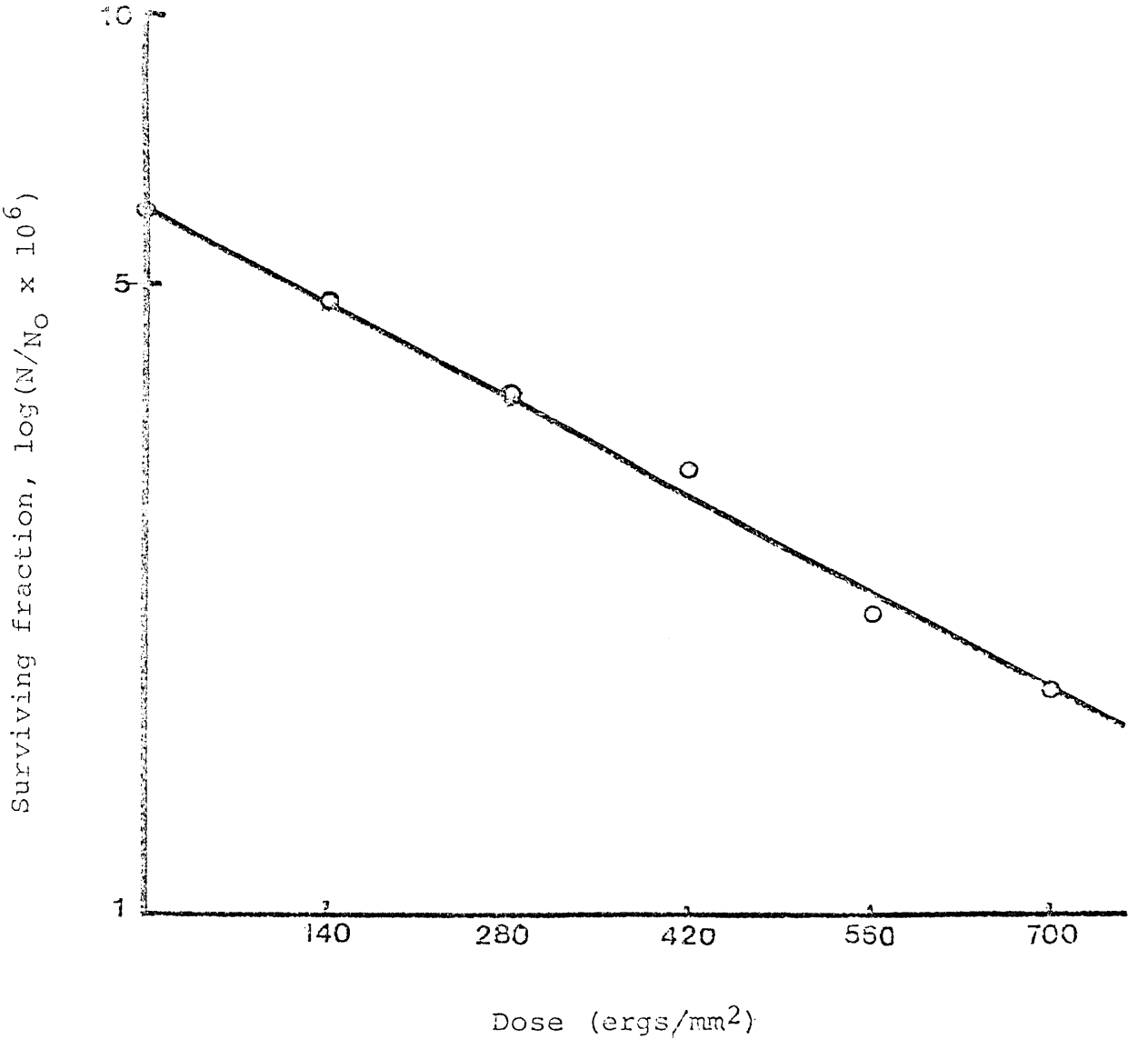
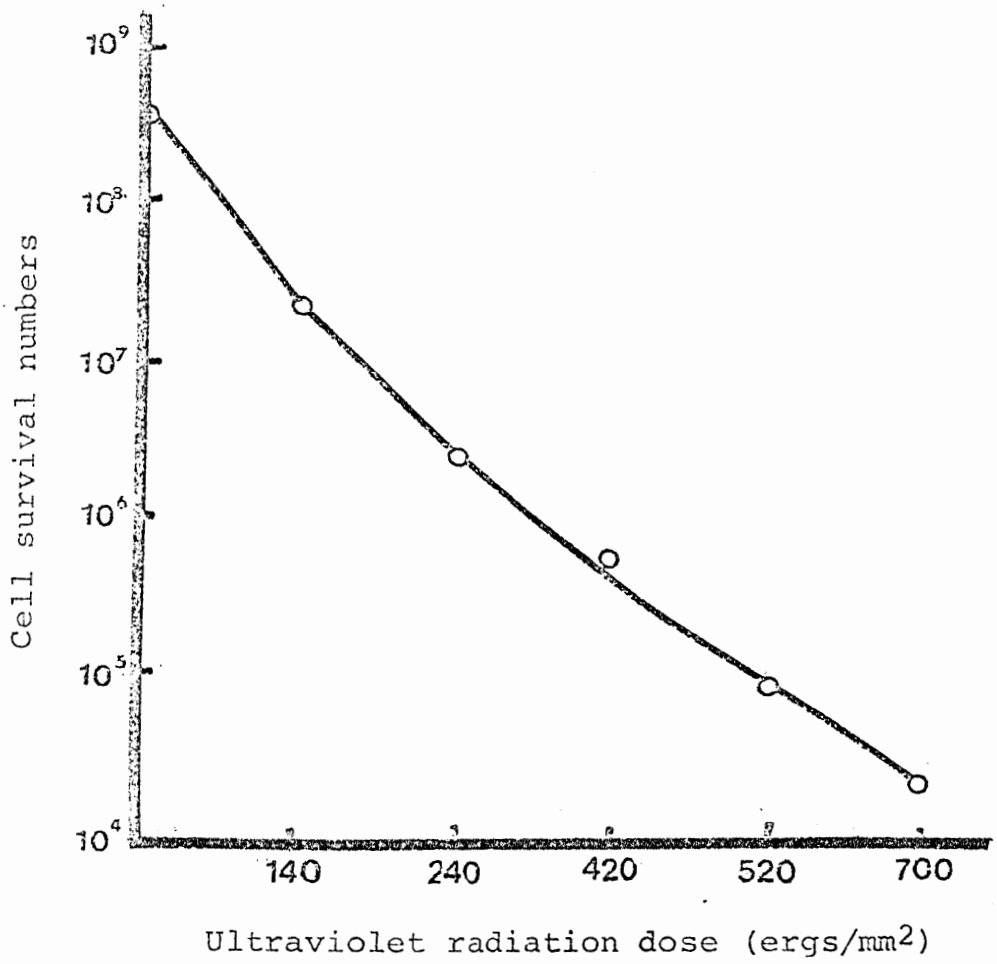

10
5
1
140
280
420
560
700
Surviving fraction, log(N/N$_o$ x 10^6)
Dose (ergs/mm^2)

Table 1. The survival of Escherichia coli Kepes ML3 after exposure to ultraviolet radiation, 253.7 nm, at 28 ergs/mm^2/sec.*

Ultraviolet Exposure Time in sec	Ultraviolet Exposure Dose in ergs/mm^2	Cell Survival Number	Fractional Survival		
			N/N_o	$(N/N_o \times 10^6)$	$\log(N/N_o \times 10^6)$
0	0	5.8×10^8	1.0000000	1,000,000.0	6.00000
5	140	3.9×10^7	0.0678000	67,800.0	4.83123
10	280	4.3×10^6	0.0073700	7,370.0	3.86747
15	420	7.9×10^5	0.0013550	1,355.0	3.13194
20	560	9.1×10^4	0.0001570	157.0	2.19309
25	700	3.6×10^4	0.0000621	62.1	1.79309

*The bacteria were plated on nutrient agar and incubated 48 hr, at 37 C in the dark. The data represent the means from five experiments.

Figure 2. Cell Survival of Escherichia coli Kepes ML3 exposure to ultraviolet radiation at 28 sec, grown on nutrient agar 48 hr at 37 C in dark. The data represents the means of five experiments, and are plotted as the total of cells surviving the irradiation versus


Cell survival numbers
10⁹
10⁸
10⁷
10⁶
10⁵
10⁴
140
240
420
520
700
Ultraviolet radiation dose (ergs/mm²)

radiation at a wavelength of 253.7 nm. This substantiates the results of other scientists.

Escherichia coli Kepes ML3 was tested for β-galactoside permease and galactoside transacetylase ($i^+ z^+ y^-$) induction. This data shown in Table 2 and Figure 3 show a marked drop in ONPG hydrolysis by irradiated bacteria compared to that of the non-irradiated control organisms, indicating inhibition of induction due to irradiation. The intial cell sample assayed showed a difference in the levels of enzymatic synthesis between the irradiated bacteria and the non-irradiated microorganisms.

Pardee (1957) found that irradiation of Escherichia coli with ultraviolet light stopped induced β-galactosidase synthesis within a few minutes. He attributed the repression of the enzyme to accumulation of catabolites by the damaged bacteria. From the data of Table 2 and Figure 3 it is evident that ultraviolet radiation will affect the rate of the synthesis of the protein, β-galactosidase, in Escherichia soli Kepes ML3.

Five drop fractions of the effluent were collected from the sucrose density gradients. The number of fractions collected was usually 30 $\pm$ 2. From the results shown in Table 3 it is apparent that there was a difference in the quantity of the polyribosomal content of the irradiated

Table 2. Ultraviolet radiation inactivation of induced β-galactosidase enzyme synthesis in Escherichia coli Kepes ML3 as measured by the o-nitrophenyl-β-D-galactosidase assay procedure.*

Sampling Time min	Optical Density in Klett-Summerson units**		Enzyme units*** x 10^{-2}	
	Control	Irradiated	Control	Irradiated
0	70	50	3.5	2.5
30	110	70	5.5	3.5
60	180	90	9.0	4.5
90	220	120	11.0	6.0
120	270	150	13.5	7.5

*Data are the means from seven experiments.

**The optical density values were read and recorded as Klett-Summerson units, using a #42 blue filter with a spectral range of 400-465 nm.

***Enzyme units $= \frac{\text{Klett unit x Dilution factor x Time}}{720}$

Figure 3. Inactivation of induced β-galactosidase en
synthesis in ultraviolet irradiated
coli Kepes ML3. Enzyme assays were per
after the exposure of the cells to 420
of predominantly monochromatic ultraviolet
radiation at a wavelength of 253.7 nm. (
time represents sampling time immediately
irradiation).

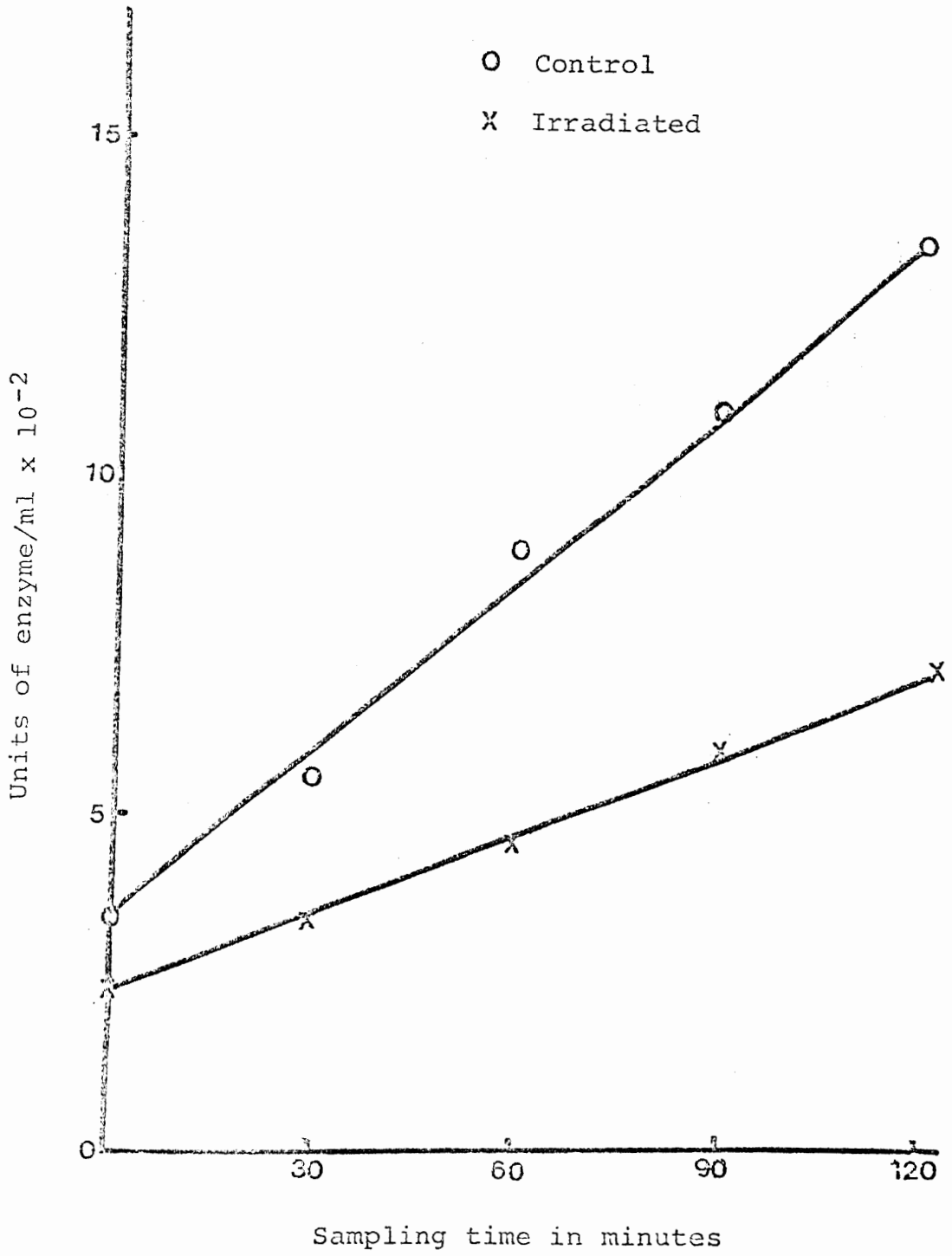

O Control
X Irradiated
Units of enzyme/ml x 10⁻²
15
10
5
0
30
60
90
120
Sampling time in minutes

Table 3. Ultraviolet absorption of effluent fractions* from sucrose density gradient analyses of cell lysates from ultraviolet irradiated and non-irradiated E. coli Kepes ML3.** Centrifugation of the sucrose gradients was done in a Spinco Type 50 Ti fixed angle rotor, at 200,000 x g for 1¼ hr.

Fraction	Absorbance of Effluent Fraction at a wavelength of 260 nm	
	Control	Irradiated
1	0.435	0.115
2	0.495	0.130
3	0.470	0.115
4	0.485	0.123
5	0.490	0.135
6	0.485	0.155
7	0.485	0.147
8	0.492	0.145
9	0.498	0.140
10	0.502	0.135
11	0.495	0.125
12	0.480	0.128
13	0.505	0.130
14	0.545	0.140
15	0.485	0.145
16	0.475	0.155
17	0.460	0.152
18	0.495	0.152
19	0.495	0.148
20	0.515	0.148
21	0.515	0.135
22	0.550	0.175
23	0.595	0.178
24	0.595	0.191
25	0.596	0.190
26	0.598	0.192
27	0.607	0.192
28	0.735	0.197
29	0.750	
30	0.784	
↑ Top		

* Five drop fractions were collected from the bottom of the linear sucrose gradient tube.

**These determinations were made on the Hitachi-Perkin-Elmer Spectrophotometer, Model 139, using quartz cuvettes.

and non-irradiated fractions. There also appears to be a fluctuation in the reading that might be accounted for by the use of the angle rotor in the sedimentation analysis. The fractions collected could have had a mixture of sedimentation layers. This is suggested by the angulation of the sediments. Figure 4 shows the possible pattern of sedimentation in a fixed-angle rotor. This could be corrected by use of the Swinging Bucket Rotor for the preparative ultracentrifugation. The sedimentation pattern of the lysate shows predominantly polysomes with an almost neglible 70S peak forming at the end of the profile (Figure 5).

The first five fractions collected from the sucrose density-gradient analysis were used for hydrolysis. During the period of hydrolysis the solution turned from a light straw color to a deep amber color.

The reproduction of the electrophoretograms of the irradiated hydrolysate showed that there was an apparent difference in the nucleotides of the irradiated and non-irradiated samples. There was an overlap of what appears to be two components in terms of the outline of the migrated material in position two from the point of origin (Figure 6). The intensity of the observed spot under 254 nm was uniform throughout. While this may possibly be two components, it may also be the result of dimerization

Figure 4. Sedimentation process in a fixed-angle rotor. Sedimentation behavior is expressed in terms of a sedimentation coefficient (S), which is the velocity of the sedimenting molecules per unit field of force:

$$S = \frac{1}{\omega^2 r} \frac{dr}{dt}$$

where:
- S= sedimentation coefficient
- ω= angular velocity, in radians per second
- r= distance of the boundary from the axis of rotation, in centimeters
- t= time, in seconds

(Beckman-Spinco Instruction Manual, 1250-1M-2)

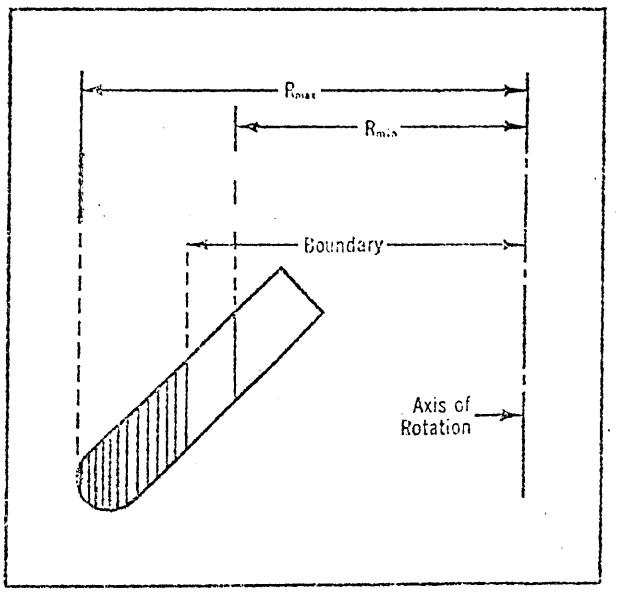

R_{max}
R_{min}
Boundary
Axis of
Rotation

Figure 5. Ultraviolet absorption of effluent fractions from sucrose density gradient sedimentation analysis of cell lysates from ultraviolet irradiated and non-irradiated Escherichia coli Kepes ML3, with absorbance determined on the Model 139, Hitachi-Perkin-Elmer Spectrophotometer at 260 nm.

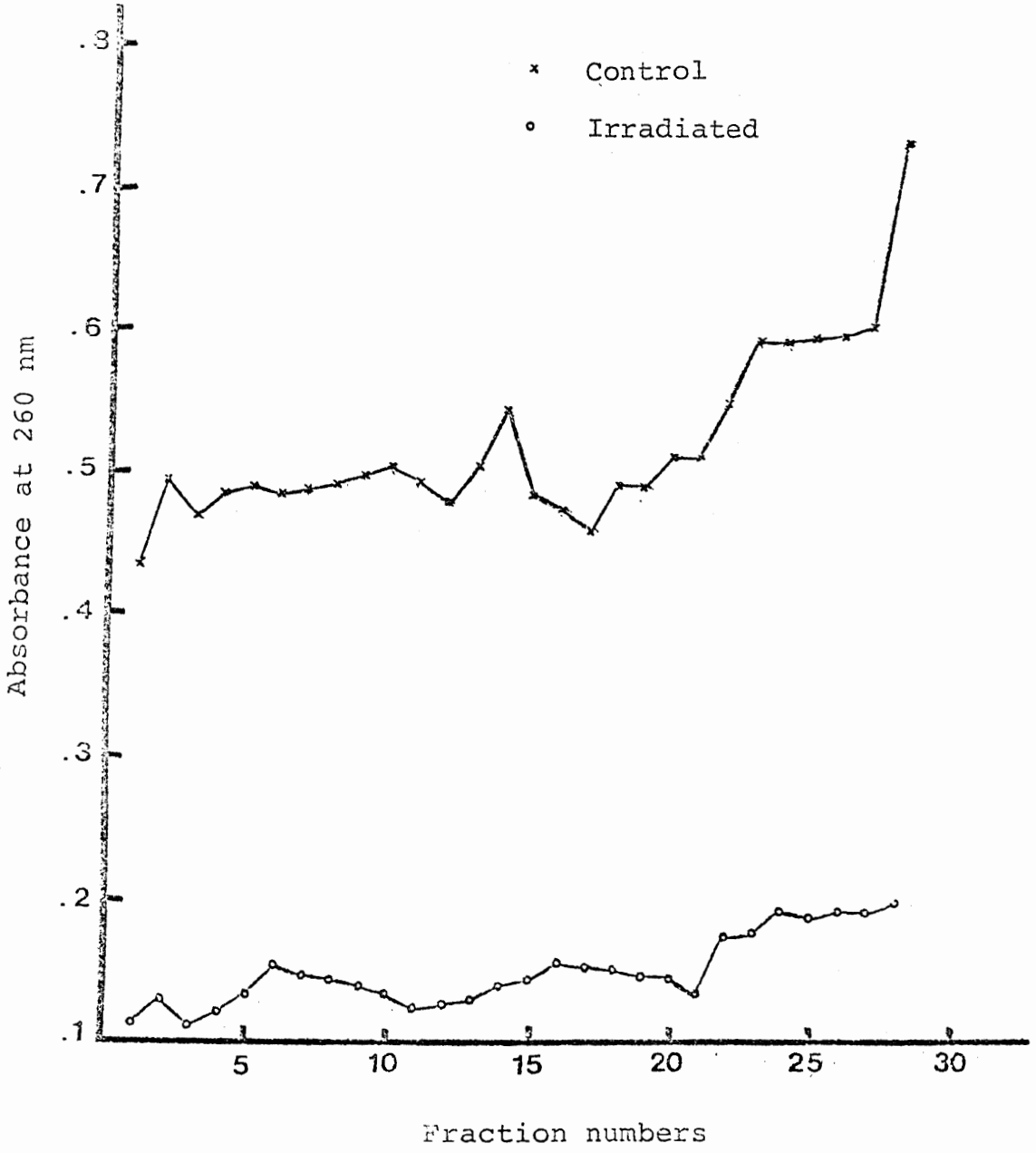
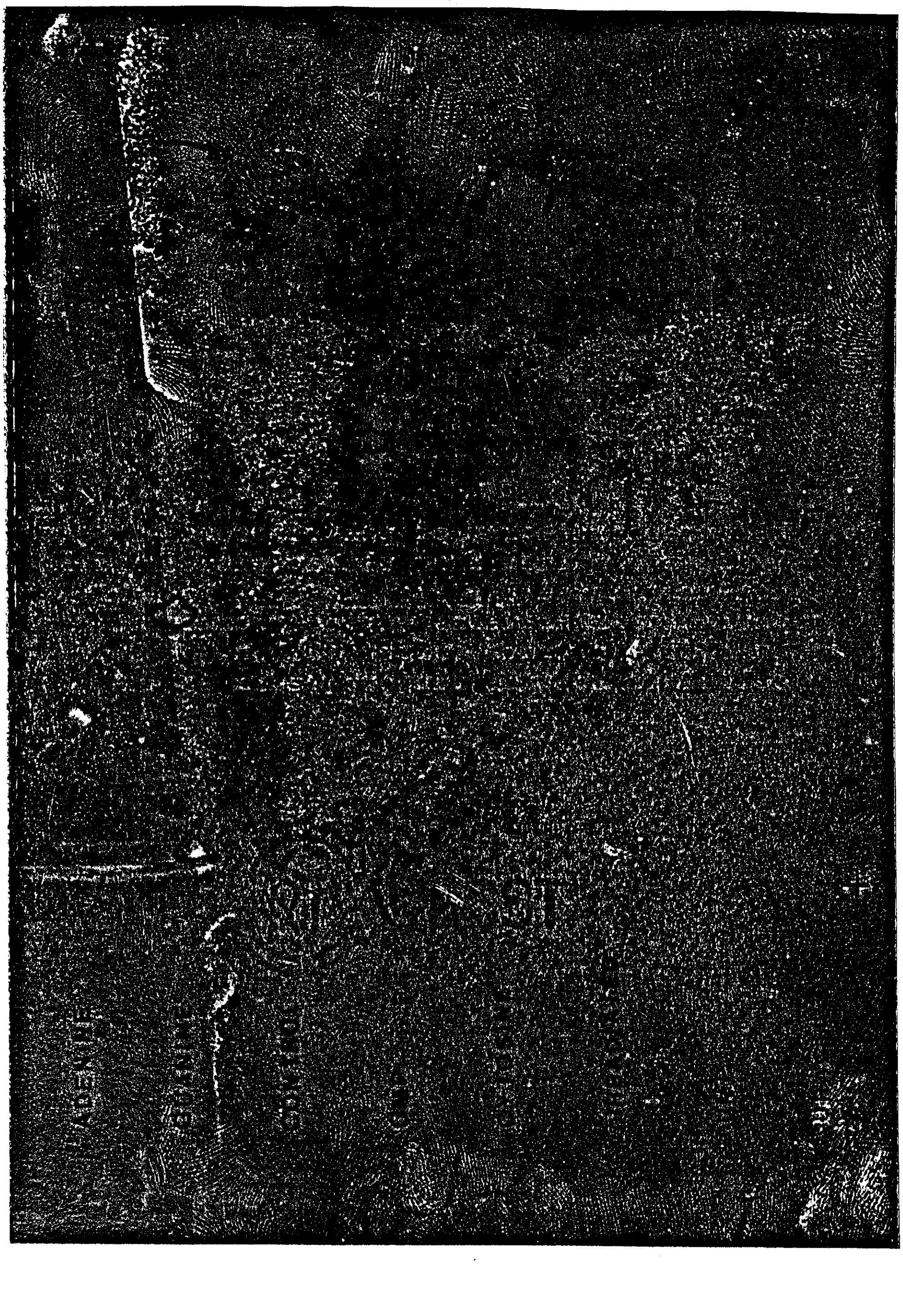

Control
Irradiated
Absorbance at 260 nm
.8
.7
.6
.5
.4
.3
.2
.1
5
10
15
20
25
30
Fraction numbers

Figure 6. Electrophoretogram of polyribosome extract lysates obtained from ultraviolet irradiated non-irradiated Escherichia coli Kepes ML3. E phoretic separations were performed at low vol in sodium citrate buffer (0.05M, pH 3.0), using cellulose acetate support media at 4 C.



and hydration. Sinsheimer (1954) postulated that the reversible photoproduct of uridylic acid is a substance with a water addition at the 5-6 position of the ring. Such a product has been shown to allow growth in Neurospora crassa (Rapport, Canzanelli and Sossen, 1955).

DISCUSSION

This investigation was concerned with the characterization of the ribosomal-ribonucleic acid fraction of polyribosomes which were extracted from ultraviolet irradiated Escherichia coli Kepes ML3. According to Smith and Hanawalt (1969), ultraviolet radiation can be used as a very sensitive and specialized probe into the function of the intracellular machinery. Low doses of ultraviolet irradiation result in inhibitory effects on the synthesis of DNA, RNA and protein (Swenson and Setlow 1966).

Photons emitted by ultraviolet radiations, which are absorbed by biological molecules are more often destructive and degradative than useful to the necessary functions of these molecules (Smith and Hanawalt 1969). The damaging events vary in terms of molecular size, sensitivity, charge, quantity, and biological importance. In addition, certain conditions may decidedly effect the severity or degree of damage, including the presence and the amount of water and molecular oxygen.

The bactericidal effectiveness (Zelle and Hollaender 1955), the mutagenicity (Knapp et al 1939; Hollaender and Emmons 1941) and inhibitory activity against the rates of synthesis of both protein and RNA of ultraviolet radiations have been well established (Rushizky et al 1960; Hanawalt and Setlow 1960).

The response of Escherichia coli to ultraviolet radiation has also been shown to be affected by several genetic loci (Witkin 1946; Hill 1958; Howard-Flanders and Theriot 1962; Rorsch et al 1966).

Ultraviolet radiation is absorbed at random. Although a large number of photons usually have to be absorbed by a cell before it becomes inactivated, the actual inactivation is usually caused by only one photon. The action of the absorbed ultraviolet photons can cause photochemical reactions in many constituents of the living cell. The most harmful events are the result of the absorption of the photons by the nucleic acids, as shown in the correlation of action spectra for inactivation of bacteria and the absorption spectra of nucleic acids or nucleoproteins (Zelle and Hollaender 1954).

The genetic material, DNA or RNA, is functionally involved in reproductive growth, concerned with both genetic information obtained from the parent by the offspring and the protein-synthesizing machinery of the organism. Rupert and Harm (1966), in a review of the actions of ultraviolet radiation, have shown through a compilation of studies that ultraviolet inactivation can be the result of interference with either the copy or translation of the genetic information. The micro-organisms that retain the ability to form colonies after ultraviolet irradiation are termed "survivors" and the effect

of the irradiation can be characterized by a "survival curve" (Rupert and Harm 1966).

The killing of bacteria described by Lea (1962) was similar to that reported here (Table 1 and Figure 2), in that the numbers of cells killed by successive dose increments were not equal, but were in the same proportion to the number of organisms which had survived until then. These results were attributed to a single direct hit of a vital target. The inference "single" vital target does not imply that this is only one substance, but the interruption of any one site essential to the synthetic machinery of the cell. The survival curve expressed as fractional survival (Figure 1) as well as the cell survival (Figure 2) are both exponential. Similar studies by Wyckoff (1932) and Lea and Haines (1940) using monochromatic ultraviolet light, have shown that the survival curves are exponential and that the effect of a given dose is independent of the intensity.

The sugar lactose (glucose-4-β-D-galactoside) can be used by E. coli as its sole carbon source. There are two proteins which are essential specifically for the metabolism of lactose (Pardee, Jacob and Monod 1959). One is a galactoside-permease found in the bacterial membrane, which directs the transport and accumulation of lactose within the cell. The second protein is intracellular β-galactosidase, an enzyme, which catalyzes the hydrolysis of β-galactosides into its two com-

ponent monosaccharides, glucose and galactose. Both the permease and the hydrolase are inducible in wild type Escherichia coli (Perrin 1963; Ullmann, Perrin, Jacob and Monod 1965). The structure of the permease is determined by the y gene, the enzyme structure by the z gene, and an i gene affects both proteins allowing for the conversion from the inducible to the constitutive state (Ullmann, Jacob and Monod 1968). These genes are clustered in the "Lac" region of the Escherichia coli chromosome (Lederberg 1947; Wollman and Jacob 1955; Pardee et al 1959). Included in the "Lac" region also are the lac (lactose) gene, the p (promoter) gene and the a gene for a third protein called thiogalactoside transacetylase which increases in amount when the cell is "induced". The group of genes transcribed into a single polygenic messenger RNA species is termed an operon (Jacob and Monod 1961). The inhibition of β-galactosidase induction by irradiation reported here is in agreement with Pardee (1957) who found that irradiation of Escherichia coli with ultraviolet radiation stopped induced β-galactosidase synthesis.

The addition of isopropyl β-D-thiogalactoside (IPTG) to a growing culture of Escherichia coli causes immediate onset of messenger RNA synthesis on the lac operon (Kepes 1963), with the steady-state rate achieved about three minutes later (Pardee and Prestidge 1961). Ultraviolet radiation could

apparently affect any of the parameters defined in the mode of utilization of mRNA as a template for protein synthesis, such as: messenger initiation rate, transcription time, inactivation rate and translation time (Jacquet and Kepes 1971).

In polyribosome extraction procedures utilized by Godson and Sinsheimer (1967), Flessel et al (1967), and Hotham-Iglewski and Franklin (1967), the sedimentation of the polyribosomes was 80-90% of the sedimented ribosomal material. Similiar results were obtained in the investigations being reported in this dissertation. A review of the polysome extraction methods used by various investigators, shown in Table 4, indicated that the method of cell rupture was extremely important in polysome recovery. In all of the investigations the workers used sucrose density gradient sedimentation analysis for the ribosomal recovery. Other variations also make it apparent that a number of factors affect the percentage of recovery of polysomes from cell lysates, including the age of the cell (Li and Umbreit 1966), growth medium (Mangiarotti et al 1966), concentration of magnesium ions (Ron, Kohler and Davis 1968), and temperature (Flessel, Ralph and Rich 1967).

The most effective parameters for extraction were selected for use in the studies reported here. The Sorvall-Ribi Refrigerated Cell Fractionator used for these investigations

Table 4. Polyribosome extraction yields using various methods of cell rupture. All of the procedures terminated in sucrose density gradient centrifugation analysis.

Percentage of Polyribosomes in the Total Ribosomal Distribution	Method of Cell Rupture	Reference
30	Grinding with alumina powder	Tissieres et al. 1960
34	French pressure cell	Schaecter 1963
50	Homogenized with sodium deoxycholate	Wettstein et al. 1963
70-80	0.5 % BRIJ 58	Kiho and Rich 1964
42-52	EDTA-Lysozyme	Dresden and Hoagland 1965
50-65	0.5 % sodium deoxycholate	Mangiarotti and Schlessinger 1966
50-75	Freezing and thawing in Lysozyme	Ron et al. 1966
74	Triton X100	Cundliffe and McQuillen 1967
80	0.6 % BRIJ 58	Hotham-Iglewski and Franklin 1967
85-90	0.5 % BRIJ and deoxyribonuclease	Flessel et al. 1967
83	Lubrol W	Godson and Sinsheimer 1967
75-80	0.05 % sodium deoxycholate	Kelley and Schaecter 1969

had an autoclave type needle valve, that can be maintained at O C, and which allowed the controlled pressurized microbial suspension to be released at the orifice. The efficiency of the instrument as a method for the isolation of subcellular substances has been shown in studies of the purification of bacterial endotoxins (Fukushi et al 1964); activity of purified enzymes from E. coli (Duerre and Ribi 1963) and E. coli ribosomal proteins (Duerre 1964).

Phillips and Franklin (1969) in an analysis of data presented by various investigators on E. coli ribosomal distribution, stated that there was a difference in recovery of polysomes based upon monovalent cations in the lysis media and/or gradients. Two types of distribution patterns were described: Type I consisted of polysomes with little or no 70S monomers, 50S or 30S ribosomal subunits; Type II consisted of polysomes, with a prominent peak of 70S monomers, 50S, and 30S ribosomal subunits. Results reported here fit the Type I pattern.

The number of ribosomes on the polysome has been described in E. coli to vary from five or six ribosomes to as many as forty (Guttman and Novick 1963; Kiho and Rich 1964; Davidson 1969). The sedimentation pattern of polysomes reflected the number of ribosomes associated with mRNA strand.

The structural complexity of the ribosome first became apparent as a result of the work of Waller (1964), who

demonstrated that there are many different proteins in the bacterial ribosome. Waller fractionated the ribosomal proteins by starch gel electrophoresis, as well as by carboxymethyl cellulose chromotography. He was able to demonstrate that there were at least 24 separable components which are not artifacts caused by the aggregation of smaller number of proteins. In addition, Waller showed that the proteins of the 30S and 50S subunits were characteristic of each subunit. The structural complexity of the ribosome established by Waller implied that the function of the ribosome was complex.

Extensive purifications of ribosomal proteins were reported by Wittman and his co-workers (Kaltschmidt et al 1967). Twenty-two ribosomal proteins from E. coli were purified by polyacrylamide electrophoresis. The amino acid compositions, tryptic peptides, and molecular weights of 16 of these proteins were shown to be different. Simultaneously, Traut et al (1967) reported the purification of some of the 30S ribosomal proteins. Some of the protein of the 30S ribosomal subunit of E. coli was isolated and partially characterized by Fogel and Sypherd (1968), as well as by Moore et al (1968). Twenty proteins were purified from the 30S ribosomal subunit and shown to have unique amino acid compositions, tryptic peptides, and molecular weights (Kurland et al 1969). In a study of the peptide patterns from isolated 30S and 50S ribosomal

proteins of E. coli, Rombauts et al. (1971) described 43 pure ribosomal proteins and the separation of the tryptic peptides by column chromatography. Kurland (1970) estimated that there are roughly 50 different proteins in the ribosomes of E. coli, some of which are acidic or neutral proteins, but most are basic proteins. Their molecular weights range from 10,000 to 60,000 daltons; the average is in the neighborhood of 20,000 (Kurland 1970). Since the amino acid compositions of some of these are not very different from one another, the possibility existed that some of the proteins are structural homologs (Wittmann and Wittmann-Liebold 1967). However, a comparison of the tryptic peptides of the different proteins reveals ver little homology except in a few limited cases that are probably fortuitous (Kurland 1966; Hardy and Kurland 1966; Kurland et al 1970). Recent immunochemical studies by Stöffler and Wittmann (1972) have failed to demonstrate significant cross reactions when the antibodies prepared against individual ribosomal proteins were reacted with heterologous proteins. Therefore, all of the proteins seem to be quite unique. The overwhelming impression is that the ribosome is far from being a primitive organelle. Instead, it seems to be a highly evolved and complicated entity, containing a much larger number of components that can be accounted for by present views of protein synthesis.

The functional analysis of individual ribosomal proteins was first performed with the proteins of the 30S fraction (Traub et al 1967). These were fractionated by column chromatography. Then the 30S subunits were reconstituted in the absence of individual proteins so that the separate contribution of each component might be assessed. A class of essential and a class of dispensable proteins were discovered. The activities of the ribosomes lacking a single protein do not permit the assignment of specific functions to each individual protein. When the ribosome lacks a single dispensable or essential protein, all its activities are depressed in a coordinate fashion and the absence of any one protein must reflect a more subtle disruption of cooperative interactions between the proteins that are necessary to maintain the ribosome in a unique optimum configuration. Since the diagrams of 50S particle (Figure 7) show the ribosome to be a relatively compact object, this aspect of ribosome structure is not surprising (Bruskov and Kiselvev, 1968).

Observations by Hart (1965) on the surface features of separate 50S subparticles of E. coli by shadow casting, showed fine ridges and grooves with spacings of about 35 A. Bruskov and Kiselvev (1968), using negative staining with uranyl acetate, have made studies of E. coli ribosomes. They found a large number of ribosomes which, on the border between subparticles, exhibit something like a hollow or an

Figure 7. Schematic representation of the 50S subparticle (a) to (d) The subparticle is rotated in the plane which separates it from the other subparticle through various angles with respect to the observer (10°, 20°, 60°, 80°, and 90°).

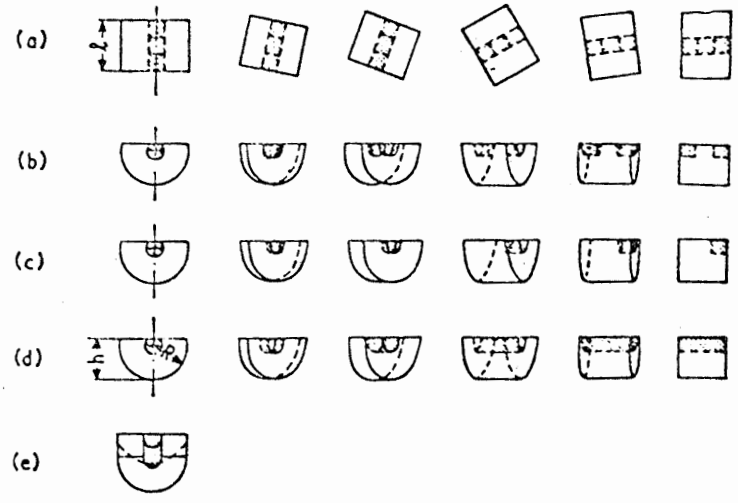
(a) The subparticle viewed from above. The channel is shown fully penetrated by stain. Dotted line shows boundary of penetration by 1/3.

(b) The subparticle viewed in a side-on projection. The channel is penetrated by stain, to 1/3 from both sides.

(c) The same, but with the channel penetrated to 1/3 from one side.

(d) The same, but with the channel fully penetrated.

(e) The subparticle viewed obliquely (Bruskov and Kiselvev, 1968).


(a)
(b)
(c)
(d)
(e)

opening, which was penetrated by stain and seen in the micrographs as black spots that seem rounded, with a diameter of 30 to 40 A. In some cases they gave an impression of either a through channel viewed from the side or a deep lateral depression. When complete ribosomes are viewed along the channel, the 50S subparticles have roundish configurations and resemble an arc. In some instances 2 to 3 concentric half-rings arranged about the rounded "opening" of the channel were seen. The small subparticles were characterized in this projection by a trapezoidal to rectangular form. Both the rounded and the rectangular-like form are the same subparticle with different orientations on the EM-grid.

The completely spontaneous nature of the assembly of ribosomes was demonstrated through total reconstitution of 30S subunits from purified ribosomal RNA and the separate protein (Traub and Nomura, 1968). As a consequence, each of the 30S proteins could be subjected to functional analysis and it became possible to identify ribosomal proteins leading to functional classification, as well as a definitive enumeration of the minimum number of different 30S proteins (Nomura et al 1969).

Maruta et al (1971) reassembled functionally active 50S subunits with the addition of ribosomal RNA's 23, 16 and 5S to 50S ribosomal protein digests. They found that 5S RNA is

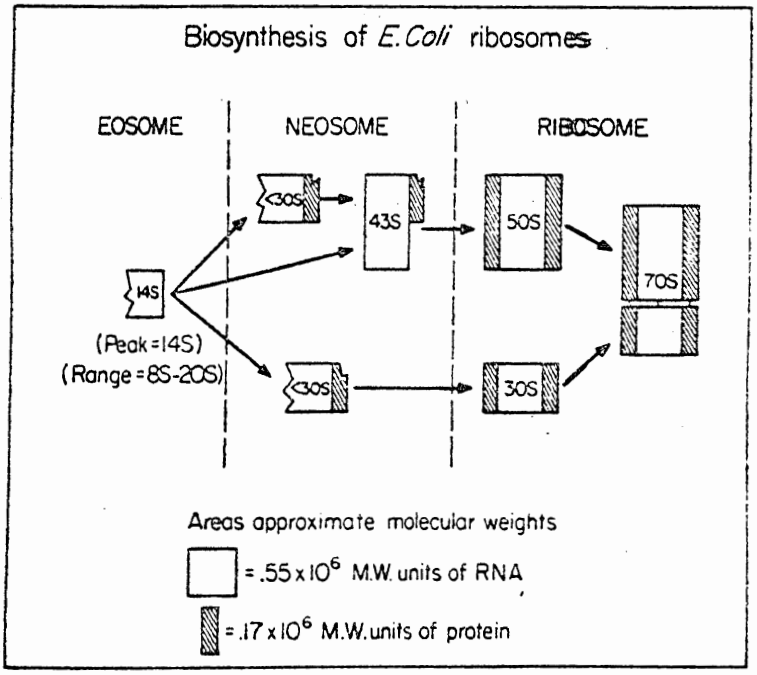
required for the reformation and that the reassembly of the 50S subunit depends on either the presence of 30S particles or simultaneous reassembly of 30S subunits from 16S-RNA and proteins.

In the course of ribosome synthesis, protein is attached to the rRNA in a stepwise fashion according to Lerman et al (1966). Gierer and Gierer (1968) studied the formation of the protein fraction produced by functional ribosomes and the subsequent attachment of the protein to ribonucleoprotein particles or cores. The kinetics of incorporation of nucleotides into ribosomes has been shown by Roberts, Britten and McCarthy (1962) to involve a time lag of several minutes during which various intermediates appear in a definite time sequence. A scheme of the biosynthesis of ribosomes proposed by the investigators is shown in Figure 8.

McCarthy et al (1962) analyzed the process of ribosome formation by labeling E. coli cells in a steady growth for various periods with ^{14}C-uracil. Their findings support the stepwise formation of ribosomes. The radioactivity appeared first in the 14S fraction, then shifted to the fraction of 30S and 43S, and finally incorporated into 30S and 50S ribosomes. They concluded that the sequence of processes leading to ribosome formation may be summarized by a scheme:

Pool ⟶ Eosome ⟶ Neosome ⟶ Ribosome

Figure 8. The biosynthesis of ribosomes in Escherichia The open and shaded areas are proportional to weights of RNA and protein, respectively (Roberts, Britten and McCarthy, 1962).


Biosynthesis of E.Coli ribosomes
EOSOME
NEOSOME
RIBOSOME
30S
43S
50S
70S
14S
(Peak=14S)
(Range=8S-20S)
30S
30S
Areas approximate molecular weights
= .55 x 10^6 M.W. units of RNA
= .17 x 10^6 M.W. units of protein

In a review of the ribosome structure and function, Kelley and Schaecter (1968) postulated the life cycle of the ribosome (Figure 9).

The 70S functional ribosome of protein synthesis associates with mRNA to form polysomes. Kelley and Schaecter, (1968), suggest four possible hypothetical models of the turnover of the polysome complex based on current research (Figure 10).

The primary structure of ribosomal ribonucleic acid is a polynucleotide chain with 3', 5' linkages (Carter and Cohn, 1949; Cohn and Volkin, 1953). The secondary structure is one of the internal hydrogen bonding types, and the helical content of rRNA has been reported as 78%. (Spirin 1963; Boedtker, 1968; Hartman and Thomas, 1970). Examination of hydrolysis products of protein-free rRNA makes it apparent that a definite tertiary structure exists for rRNA in solution (Gould 1966).

Both in vivo and in vitro studies have confirmed that rRNA is produced by the same mechanism as other cellular RNA, which is by the action of RNA polymerase on ribonucleotide triphosphates directed by a DNA template. In vitro studies established that rRNA may be synthesized by the action of RNA polymerase and DNA primer (Ochoa et al 1961). These

Figure 9. The "life cycle" of the bacterial ribosome (Kelley and Schaecter, 1968).

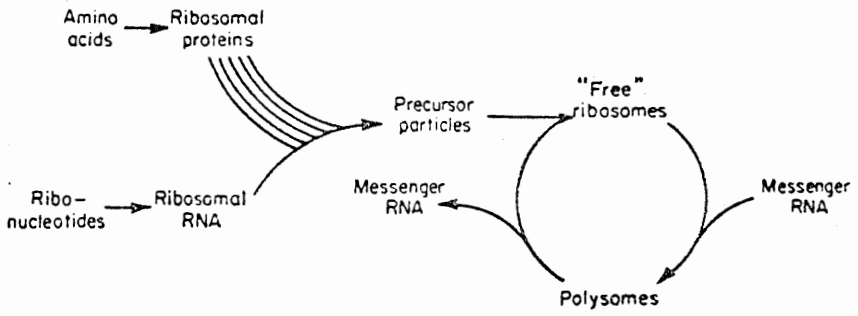
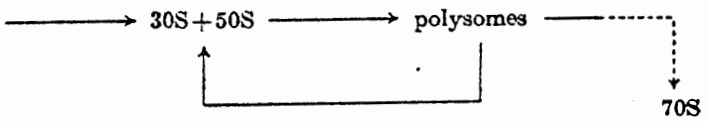
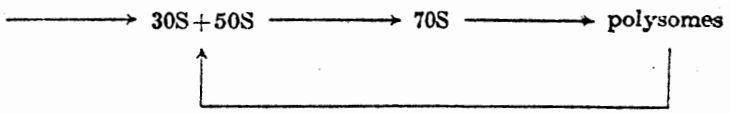

Amino acids
Ribosomal proteins
Precursor particles
"Free" ribosomes
Messenger RNA
Messenger RNA
Polysomes
Ribo- nucleotides
Ribosomal RNA

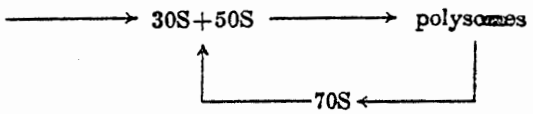
Figure 10. Hypothetical models of the turnover of the mRNA complex (Kelley and Schaecter, 1968).



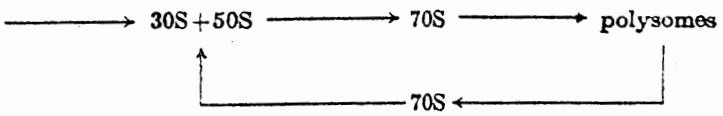
SCHEME A



SCHEME B

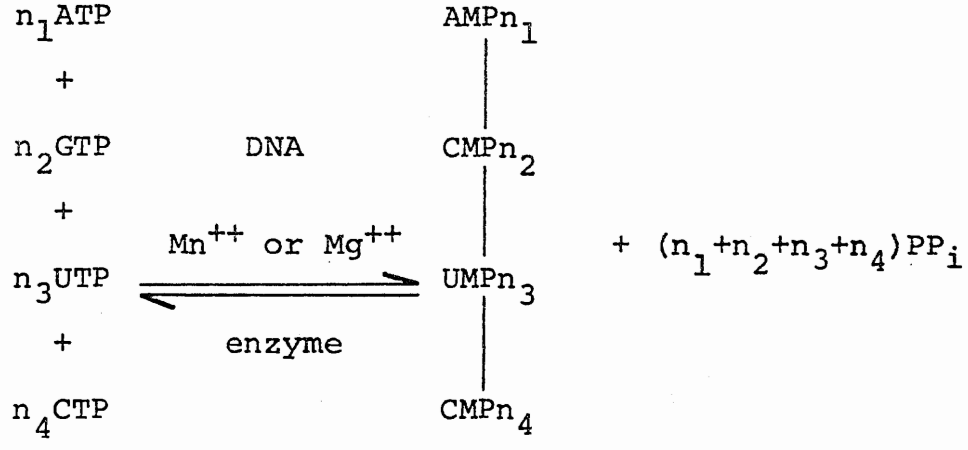


SCHEME C



SCHEME D

investigators summarized the stepwise synthesis as follows:



Ribosomal RNA of E. coli was found to hybridize at specific points with DNA (Spielgelman 1965). Through tracer experiments using ^{14}C-labelled 16S and 23S ribosomal fractions, Avery and Midgley (1968), established that the total ribosomal RNA of E. coli was synthesized coordinately from 0.45 $\pm$ 0.02% of the DNA, which would be 45 cistrons with a total molecular weight of 5×10^5. Therefore, it was postulated that the rRNA species from individual cistrons are present in the same frequencies as the DNA cistrons themselves. Pigott and Midgley (1968) previously reported that in an analysis of hybridization of rapidly-labelled and randomly labelled RNA's and DNA of E. coli, the rapidly-labelled RNA consists of 30-33% mRNA and 67-70% of precursors to ribosomal RNA. The rRNA precursors in rapidly-labelled RNA were present in the same relative amounts as in the RNA of 70S ribosomes. Gorelic (1970), employing the hybridization technique, localized rRNA to specific cistrons on the E. coli chromosome.

The primary concern of the investigations being reported in this dissertation was to identify ultraviolet radiation damage in the ribosomal ribonucleic acid fraction of the ribonucleoprotein structure. Since it has been shown that purine and pyrimidine bases, as well as their nucleotides and nucleosides, are UV sensitive, particular interest was placed on these fractions in the UV irradiated material. After subjecting the polysome fraction to hydrochloric acid hydrolysis, the golden brown hydrolysate was separated by cellulose acetate electrophoesis.

The initial electrophoretic separations were started in buffer which had been chilled to 4 C, and the electrophoretic run made at room temperature. The subsequent heating which occurred did not allow for distinct separations. The procedure was modified to stabilize the temperature to 4 C by performing the electrophoresis in the cold room.

According to Loening (1961), electrophoretograms were allowed to air dry at room temperature prior to visualization with ultraviolet light at 254 nm. However, this procedure was not successful, since no spots were visible when viewed in this manner. It was, therefore, necessary to alter the procedure. The electrophoretograms were examined immediately after removal from the electrophoresis chamber at the end of the run. All of the migrated components, including reference standards, were readily observed and outlined on

the moist electrophoretogram.

The components identified in the investigations reported in this dissertation included the purine and pyrimidine bases common to ribosomal ribonucleic acid and two pyrimidine mononucleotides. The position and conformational appearance of the nitrogenous bases from the ultraviolet irradiated bacteria made it obvious that a difference existed between the structure of irradiated and non-irradiated material.

The cellulose acetate electrophoretic medium used in the research being presented in this dissertation offers an excellent tool for the isolation of ultraviolet damaged material. Since the role of ribosomal ribonucleic acid in the structure and function of ribosomes has not yet been resolved, cellulose acetate electrophoresis offers a method for the isolation and subsequent identification of individual components. This makes possible a comparative study of the conformational structure of ribosomal ribonucleic acids from ultraviolet irradiated and non-irradiated sources. The metabolic aberrations induced by ultraviolet damage might well be the method by which the adaptation of structure to function of the ribosomal ribonucleic acid can be established.

Thus, the results reported here confirm the alteration at the molecular level by UV. The mechanism of such alterations is not determined since hybridizations were not run. Hybridization experiments with isolated polysomal fractions

are necessary to elucidate the site of alteration on the genes.

Since the completion of the work for this manuscript Normura (1973), in a review of the assembly of bacterial ribosomes, described evidence that supports the functional role of ribosomal RNA. The inactivity of 30S subunits isolated from colicn E3-treated E. coli in polypeptide synthesis in vitro suggests both a functional and a structural role of rRNA. A second indication comes from the analysis of kasugamycin-resistant mutants of E. coli (Helser et al. 1972). The ribosomes from these mutants are resistant to kasugamycin during polypeptide synthesis in vitro. Chemical analysis and reconstitution studies showed that an alteration in 16S RNA was responsible for the mutant behavior. With the current progress in technology, complete elucidation of the primary structure of all the ribosomal components is probably just a matter of time.

SUMMARY AND CONCLUSIONS

1. Investigations were conducted to develop and perfect methods for the extraction and spectrophotometric identification of polyribosomes from ultraviolet light irradiated Escherichia coli Kepes ML3 cells. The ribosomal ribonucleic acid fraction was separated from the polysomes and analyzed by cellulose acetate electrophoresis.

2. The results of the investigations being presented in this dissertation provide evidence that Escherichia coli Kepes ML3 was sensitive to predominantly monochromatic ultraviolet radiation at a wavelength of 253.7 nm, delivered at an exposure dose of 28 ergs/mm^2/sec. The data presented showed that both lethality of the bacteria and inactivation of enzyme synthesis occurred when the organisms were exposed to the radiant energy source.

3. Results of the studies on the bactericidal effectiveness of ultraviolet irradiation indicated that an exponential loss of active cells occurred with increasing ultraviolet radiation exposure dosage. The values obtained are in agreement with the concept that when photons impinge on a substance random energy absorption occurs, resulting in the inactivation of a proportion of the cells at each dose level.

4. It was determined that ultraviolet radiation reduced the rate of induced synthesis of β-galactosidase by E. coli Kepes ML3. The evidence was obtained by σ-nitrophenol-β-galactosidase (ONPG) assay of cell samples taken from cultures that had been exposed to ultraviolet radiation and non-irradiated cell cultures.

5. The Sorvall Ribi RF-1 Refrigerated Cell Fractionator was found to be an efficient tool for disrupting bacterial cell walls. The instrument allowed for a controlled application of pressure at 0 C, which is essential in the extraction of subcellular components.

6. Spectrophotometric analyses were made of the effluents of the irradiated and non-irradiated cell lysates sedimented on 15 to 30% linear sucrose density gradients. The data showed that 80-95% of the ribosomal material was of polysomes, with the remaining fraction being single monomers. From the sedimentation profiles it was apparent that there were quantitative differences between the polysomal fractions of the irradiated and the non-irradiated bacteria.

7. The use of cellulose acetate electrophoresis proved to be a rapid, reproducible technique for the separation of subcellular components. The separations made at pH 3.34 occurred best at 4 C. The migrated materials could easily be visualized under ultraviolet light at 254 nm and outlined on the wet electrophoretogram.

8. Purine and pyrimidine bases were identified as the components of the ribosomal ribonucleic acid fraction of the isolated polyribosomes. This was accomplished by the comparison of the migration patterns of the rRNA isolated from E. coli Kepes ML3 and the reference standards.

9. The results of the investigations presented in this dissertation suggest that ultraviolet radiation can be used as a very sensitive and specialized probe into the function of intracellular components. The inhibitory effectiveness of UV light at the low doses utilized in this study make this apparent.

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VITA

Versia Lindsay Lacy, the youngest of three daughters, was born to Mr. and Mrs. Oscar Lindsay on March 15, 1929 in Houston, Texas. In May 1944, she graduated with honors from Booker T. Washington High School, Houston, Texas. She received the B.S. degree from Tillotson, Austin, Texas with a major in biology and an minor in chemistry in May, 1948. She continued her studies at Atlanta University, Atlanta, Georgia, and was awarded the M.S. degree in Biology, August 26, 1950. During the summer, 1952, and both regular and summer sessions 1955-56 she studied at the University of Texas, Austin, Texas. In 1958 she was a participant in a NSF Summer Institute in Marine Biology at the University of Oregon School of Marine Biology, Charleston, Oregon. As an AEC-NSF participant, she attended a Summer Institute in Radiation Biology for college teachers at Argonne National Laboratory in 1965. In September, 1966 she enrolled in the doctoral program, at Texas Woman's University where she was a Graduate Research Assistant in the laboratory of Dr. Kenneth A. Fry from 1966 to 1969.

From 1950 to 1955, she was an instructor of biology and chemistry at Paul Quinn College, Waco, Texas, in addition to serving as Dean of Women, 1952-54. She taught

at Tyler District Junior College, Tyler Texas from 1956 to 1966. Since September, 1969, she has been an Associate Professor of Biology at Bishop College at Dallas, Texas.

On June 7, 1958, she was married to J. W. Lacy. They are the parents of two children, Lindsay Keith, 12, and Elizabeth Juliene, 9. She has one stepdaughter, Brenda Joyce, a teacher in the Aldine Public Schools, Houston, Texas.

The recipient of two national scholarships from Zeta Phi Beta Sorority, Inc., Mrs. Lacy was the Third National Anti-Basileus of this organization from 1952-1954. The founder and organizer of the Tyler Club of the National Association of Negro Business and Professional Women's Clubs, Inc., she is currently serving as the Vice Governor of the South Central District. Mrs. Lacy is also the former president of the Tyler Chapter of the Top Ladies of Distinction, Inc. and the National Editor of the Crown.

Mrs. Lacy is a member of the Radiation Research Society, The American Association for the Advancement of Science, American Institute of Biological Sciences, National Institute of Science, Southwest Photobiology Group, Texas Association for Radiation Research, Texas Academy of Science, North Texas Biological Society, Beta Beta Beta Biological Society, Beta Kappa Chi Scientific Honor Society and the Texas Woman's University Club of Sigma Xi Society.