Nuclear and Cytoplasmic Nucleic Acid Synthesis in *Acetabularia*

By

ILSE MARIE JOHANNE ORTABASI

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1971

This dissertation is dedicated to my husband

Uğur

as an expression of my gratitude for his exceptional friendship during this long expedition of learning.

ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. J. H. Gregg, the chairman of her supervisory committee for his guidance and generous support in every sense during the course of this work. Thanks are due to Drs. J. W. Brookbank, T. W. O'Brien and H. M. Wallbrunn for their participation on the author's committee.

The author would like to thank Dr. G. Ceron and Dr. E. M. Johnson whose help and suggestions have contributed so significantly to this research. The author would particularly like to thank her sister Doris Knatz and Mrs. Donna Gillis for typing the manuscript and her friend Dr. W. Sue Badman for reading the manuscript and for constructive criticism.

The valuable discussions with Dr. L. Racey, Dr. A. R. Larkin and Dr. P. Byvoet are appreciated. The author's thanks are also extended to Miss Jill Gomery and Miss Pamela High for helping with the algae cultures. Finally the author wishes to thank her husband, Uğur, to whom this dissertation is dedicated.

This work was performed with support from the Department of Zoology to whom the author wants to express her gratitude.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS iii

LIST OF FIGURES vi

ABSTRACT vii

INTRODUCTION 1

Nucleic Acids as Genetic Material 1

Replication of DNA and RNA 2

The Genetic Code and Messenger RNA 4

Protein Biosynthesis 6

RNA in Eukaryotic Cells 8

The Nucleolus and Its Role in RNA Synthesis 8

Control of r-RNA Synthesis 10

Ribosomal Subunits, Monosomes, and Polyribosomes . . 11

Messenger RNA 12

Control of Protein Biosynthesis 13

Regulation at the Transcriptional Level 13

Regulation at the Translational Level 15

Cytoplasmic RNA Synthesis 16

Enucleated Cells 17

Differentiation in Acetabularia 18

Life Cycle 19

Morphogenetic Substances 24

Synthesis of Macromolecules in Nucleate and
Anucleate Fragments of Acetabularia 30

Protein biosynthesis 30
The role of the chloroplast. 32
RNA synthesis. 34
Purpose of the Work for This Dissertation 38
MATERIALS AND METHODS 39
Culture of *Acetabularia* Cells 39
Sterility Tests 41
Nucleic Acid Extraction 45
Enzyme Treatment. 47
Electrophoresis 48
Enucleation of *Acetabularia* Cells 53
Labeling of *Acetabularia* Cells. 53
Determination of Radioactivity in Polyacrylamide
Gel Fractions After Electrophoresis. 53
Autoradiography of *Acetabularia* RNA 54
RESULTS 55
DISCUSSION. 71
SUMMARY 77
APPENDIX. 68
LIST OF REFERENCES. 99
BIOGRAPHICAL SKETCH 107

LIST OF FIGURES

		Page
Figure 1	Adult *Acetabularia* plants.	21
Figure 2	The nucleus of *Acetabularia*.	23
Figure 3	Life cycle of *Acetabularia*	26
Figure 4	Gametogenesis in *Acetabularia crenulata*.	28
Figure 5	Cultures of *Acetabularia*	43
Figure 6	UV absorption spectrum of the nucleic acids extracted from *Acetabularia*.	57
Figure 7	Polyacrylamide electrophoresis of the nucleic acids from *Acetabularia*. Staining patterns.	59
Figure 8	RNA synthesis in *Acetabularia*. Autoradiographs of polyacrylamide electrophoresis patterns.	61
Figure 9	Comparison of electrophoretically separated nucleic acids from *Escherichia coli* B/r and *Acetabularia mediterranea*	64
Figure 10	Calibration curve for high molecular weight RNA.	66
Figure 11	RNA synthesis in *Acetabularia*. Radioactivity pattern of the polyacrylamide electrophoresis.	69

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of
the Requirements for the Degree of Doctor of Philosophy

NUCLEAR AND CYTOPLASMIC NUCLEIC ACID SYNTHESIS IN *ACETABULARIA*

By

Ilse Marie Johanne Ortabasi

March, 1971

Chairman: Dr. J. H. Gregg
Major Department: Zoology

Nucleic acids from *Acetabularia crenulata* and *Acetabularia mediterranea* were fractionated into 12 bands by polyacrylamide gel electrophoresis. The sedimentation coefficients of these nucleic acid molecules were estimated by comparison with a standard. The banding pattern obtained from the two species was identical. The two r-RNA species consist of two components each. These RNA components possess approximate sedimentation coefficients of 25 S, 23 S, 17 S and 15 S. A 13 S RNA molecule was also observed which is possibly identical with the stable 15 S RNA described in the literature. Enucleated *A. crenulata* cells no longer synthesized all of the RNA species synthesized in whole plants. Enucleated *A. mediterranea* cells no longer synthesized the 25 S and 17 S RNA species, which appear to be nuclear in origin. The 23 S and 15 S RNA species which are synthesized in the enucleated plants are believed to be synthesized by the chloroplasts. Enucleated *A. mediterranea* cells were able to synthesize the 13 S RNA component, a finding which is in agreement with the literature. Polyacrylamide gel electrophoresis as a means

to analyze RNA metabolism specifically in relation to the "morpho-
genetic substances" is discussed.

INTRODUCTION

Nucleic Acids as Genetic Material

Over the last 30 years, evidence has accumulated showing that the genetic information of living organisms is laid down in specific nucleic acid molecules. The first clue supporting the notion that genes are composed of DNA was that DNA is largely restricted to the chromosomes in the nuclei of cells. Correlation of the behavior of chromosomes during meiosis with genetic studies had established the chromosomes as the vehicles of inheritance. Quantitative measurements of the amount of DNA in cells show that somatic cells of a given species contain a constant amount of DNA, regardless of metabolic state, and haploid cells contain half the amount. In 1944, Avery, MacLeod and McCarty demonstrated that transformation in Diplococcus pneumoniae was mediated by DNA and thereby showed that nucleic acids can carry genetic specificity. Hershey and Chase (1952) showed that only the DNA of a bacteriophage enters the bacterium and directs the synthesis of new viral particles in the infected cell. The genetic role of nucleic acids was also demonstrated very elegantly in tobacco mosaic virus (TMV) by Fraenkel-Conrat and Singer (1957). Hybrid viruses can be produced by combining the RNA from one strain of tobacco mosaic virus with the protein from another. When tobacco plants were infected with these hybrid viruses it was found that the new virus produced in the plant is identical to the virus of the strain that provided the RNA of the synthetic hybrid.

The Beadle and Tatum one gene-one enzyme hypothesis (1941) was later modified to the one gene-one polypeptide or one triplet-one amino acid hypothesis. Cytochemical research by Caspersson (1950) and Brachet (1950) established the importance of RNA in growing tissues as a concomitant of protein synthesis. Cytological autoradiography studies by Prescott (1959, 1962) showed that RNA was synthesized in the nucleus, the site of the genetic material, and transferred to the cytoplasm, the site of protein synthesis. Finally, the Watson and Crick (1953) complementary strand model of the DNA molecule, made it seem likely that DNA must somehow serve as a template for RNA synthesis. This central dogma of modern molecular biology has been confirmed by many *in vivo* experiments but largely through successful studies of systems for cell-free nucleic acid and protein synthesis.

Replication of DNA and RNA

Evidence for the semiconservative nature of DNA replication was obtained through experiments performed with bacterial DNA by Meselson and Stahl (1958) by bouyant density gradient centrifugation and by Cairns (1963) through autoradiography. Analogous experiments with chromosomes of higher organisms were performed by Taylor, Woods and Hughes (1957) giving results consistent with the hypothesis. The simplest model consistent with the isotope incorporation studies by Taylor *et al*. (1957) and kinetic studies of deoxyribonuclease (DNAase) action on lampbrush chromosomes (Gall, 1963), is a chromatid containing one very long DNA double helix.

DNA polymerases have been isolated from a variety of bacterial and animal cells. The DNA polymerase isolated from *E. coli* which has been worked on most extensively by Kornberg (1969) and his associates.

catalyzes the addition of mononucleotide units to the 3' hydroxyl-terminus of a primer DNA chain which serves also as template. However, it is not yet clear how this *in vitro* system is related to the normal processes of synthesis *in vivo*, since the enzyme is almost inactive with native, double-stranded primers. It was also shown by electron-microscopy that a large percentage of the product DNA molecules are branched.

Weiss (1960) reported the first RNA polymerase in nuclei of rat liver, calf thymus and ascites tumor cells. A similar enzyme was discovered in pea seedlings by Huang *et al*. (1960). Both of these enzymes are tightly bound to the chromatin fraction and could be separated from the DNA-nucleoprotein complex by Huang and Bonner (1962).

By contrast, bacterial RNA polymerase is a readily soluble enzyme and has been used for the analysis of DNA dependent RNA synthesis. It was shown by Furth *et al*. (1961) that the base ratio of RNA synthesized by *E. coli* RNA polymerase *in vitro* is complementary to the base ratio of the primer DNA and furthermore it was demonstrated by Hurwitz *et al*. (1962) that the nearest-neighbor nucleotide frequencies are similar for the primer DNA and the product RNA.

It was shown by DNA-RNA hybridization experiments that the base sequence of the product of the RNA polymerase reaction is complementary to the DNA template (Spiegelman *et al*., 1961). Hayashi *et al*. (1963) discovered that living cells infected with phage ØX174 contain RNA, which is complementary to only one of the two replicative strands. This evidence indicated that RNA polymerase *in vivo* transcribes only one strand of the DNA double helix.

The Genetic Code and Messenger RNA

In visualizing a precise mechanism by which nucleic acid information might be related to protein structure, a special coding system was proposed in which three nucleotides code for one amino acid and the message is read in threes from a fixed point. The code is degenerate and appears to be universal. An overlapping code could be eliminated as a possibility, since single amino acid substitutions are found in hemoglobin and TMV mutants. The mutations in bacteriophage T4 obtained by Crick et al. (1961) suggest that the loss of a single base pair disrupts reading of the entire message rather than of a single amino acid and this implies that the reading mechanism operates sequentially. Crick also confirmed that the minimum coding unit consists of three nucleotides. He hypothesized that a missing nucleotide might be corrected by deleting two more nucleotides. Even though a few wrong amino acids might be built into the chain between mutation sites, most of the message could be read correctly. When Crick combined mutant phages in this way he observed in fact that phenotypically wild-type phages were regenerated.

The greatest impetus to the study of RNA metabolism was provided by the prediction of messenger RNA (m-RNA) by Jacob and Monod (1961) followed shortly by the demonstration of an unstable RNA intermediate carrying information from genes to ribosomes for protein synthesis in E. coli by Brenner et al. (1961) and Gros et al. (1961).

When Nirenberg and Matthaei (1961) introduced their template dependent system for cell-free protein synthesis from E. coli it was found that an artificial poly U strand specifies the synthesis of a polyphenyl alanine chain, suggesting that the coding triplet for phenyl alanine must be UUU. Other artificial templates with different

base compositions and ratios were used to incorporate amino acids. These techniques provided sufficient evidence that a specific coding system was operating even though these methods provided no way to distinguish between triplets of identical base composition but different base sequence. In 1964, Nirenberg and Leder discovered that the binding of specific amino acyl-soluble RNA's (amino acyl s-RNA) to the ribosome is dictated by messenger RNA and the minimum messenger length is the trinucleotide. Each of the 64 trinucleotides was tested for the ability to specify the binding of one or another of the 20 amino acyl s-RNA's. Nishimura et al. (1965) have analyzed polypeptides synthesized in the E. coli cell-free system with template RNA's of regularly alternating base sequence. They have found that polypeptides were formed which contain two amino acids in regular alternation. This finding implies that the coding unit consists of an odd number of bases. Since the code for the amino acids was already known, this experiment established the fact that the messenger RNA is read three nucleotides at a time.

The triplets UAA and UAG are believed to be terminator triplets, signaling the end of a given polypeptide (Takanami and Yan, 1965; and Brenner et al., 1965). The triplets AUG and GUG code for the initiator amino acid N-formylmethionine.

Evidence suggesting that the genetic code is universal comes from the experiments in cell-free systems derived from E. coli and rabbit reticulocytes both of which induce synthesis of polyphenyl alanine from a poly U template. TMV when used as a template in the E. coli system directs the synthesis of proteins that are very similar to native TMV protein. Single base changes in the E. coli

glutamic acid triplet can give rise to six possible amino acid substitutions, five of these but no unexpected substitutions occur in human hemoglobin, thus indicating that the code is universal.

Protein Biosynthesis

The mechanism of protein biosynthesis has recently been reviewed by Lengyel and Söll (1969) and in the 1969 Cold Spring Harbor Symposium on Quantitative Biology. For specific references regarding the information in the following paragraphs it is referred to these two references.

Biosynthesis of proteins depends on the presence of several enzymes, ATP, GTP, amino acids, transfer RNA (t-RNA or s-RNA), messenger or template RNA (m-RNA) and a complex multimolecular structure: the ribosome.

The t-RNA's serve as adapter molecules to assemble the amino acids on the surface of the m-RNA template. In every organism in which this was tested there exist at least 20 amino acid-t-RNA synthetases which link the correct amino acid to the 3' hydroxyl of the terminal adenosine of a specific t-RNA molecule. The specific enzyme activates the corresponding free amino acid forming an adenyl-amino acid enzyme complex. This step requires ATP and Mg^{++}; pyrophosphate is released. In the transfer step, the activated amino acid is transferred to its specific t-RNA molecule with the release of AMP and the amino acid t-RNA synthetase. When cysteine was converted to alanine while complexed with the specific cysteine t-RNA, polypeptide chains were synthesized *in vitro* which contained alanine at specific sites where cysteine should have been. This shows that the specificity of the amino-acyl-t-RNA for the codon on the m-RNA resides with the t-RNA moiety.

It was demonstrated that after infection of *E. coli* with the bacteriophage T4 a phage specific m-RNA is formed which attaches to the preexisting *E. coli* ribosomes. This and other experiments underlie the concept that ribosomes provide a machinery for assembling polypeptide chains which lacks species specificity. Ribosomes are large ribonucleoprotein particles. There are two functionally different subunits of *E. coli* ribosomes, the 30 S and the 50 S subunits. The 30 S subunit consists of about 20 different proteins and one species of RNA (16 S), whereas the 50 S subunit contains about 30 different proteins and two species of RNA (5 S and 23 S). The biosynthesis of ribosomes in prokaryotic and eukaryotic organisms will be discussed later.

The components known to be involved in peptide chain initiation are formyl methionine-t-RNA (f-Met-t-RNA) responding to initiator codons on the m-RNA, several initiation factors, the 30 S ribosomal subunit and GTP. The complex containing a 30 S subunit, m-RNA and f-Met-t-RNA is a necessary intermediate for initiation. The f-Met-t-RNA is believed to be translocated from the AA-t-RNA receptor site to the peptidyl donor t-RNA binding site on the 70 S ribosome m-RNA complex. Peptide elongation can now proceed from the N-terminal to the C-terminal end. The attachment of AA-t-RNA to the donor site requires GTP and elongation factors. Subsequently, the carboxyl group of the formyl methionyl residue of the f-Met-t-RNA is released from its linkage to t-RNA and is linked in a peptide bond with the α-amino group of the AA-t-RNA peptidyl transferase; the enzyme catalyzing this reaction apparently is part of the 50 S subunit. The following events are presumed to occur: the discharged t-RNA is released from the ribosome, the f-Met-AA-t-RNA is shifted to the peptidyl donor site

and the ribosome moves the length of one codon along the m-RNA in the 5'-to-3' direction. This involves another elongation factor and GTP. The stage is now set for the addition of another amino-acyl residue. The cycles are repeated and the peptide chain grows until a terminator codon in the m-RNA is reached. Two release factors were found to be involved in the peptide chain termination process.

At the present time the study of the synthesis, structure and function of ribonucleic acid molecules in functioning cells, differentiated cells and especially in differentiating cells is one of the important branches of molecular biology.

RNA in Eukaryotic Cells

In eukaryotic cells the RNA comprises a mixture of many different molecules of defined lengths; some are larger molecules which are precursors to ribosomal RNA (45 S; 32 S), the ribosomal RNA's (28 S, 5 S, 7 S and 16 S-18 S), transfer RNA (4 S), as well as heterogeneous nuclear (giant) and cytoplasmic RNA. For reviews see Prescott (1964), Penman (1966), Perry (1967), Darnell (1968), Osawa (1968).

The Nucleolus and its Role in RNA Synthesis

One of the most conspicuous structures in nuclei of cells of higher organisms is the nucleolus. Sufficient evidence has accumulated to attribute a key role in the biosynthesis of ribosomes to the nucleolus.

Embryos of the mutant of *Xenopus laevis* that lacked the ability to form nucleoli were also incapable of synthesizing r-RNA (Brown and Gurdon, 1964). Ritossa and Spiegelman (1965) determined the number of DNA complements of r-RNA in stocks of *Drosophila melanogaster* containing X chromosomes either deficient or duplicated for a short piece of heterochromatin containing the nucleolar

organizer, by using the technique of DNA-RNA hybridization. Birnstiel et al. (1966) did a similar study on Xenopus embryos. The isolation of the r-RNA cistrons was achieved with the Xenopus system. It is believed that visualization of nucleolar genes from amphibian oocytes was accomplished by Miller and Beatty (1969).

A satellite DNA band was found in CsCl density gradients which was not found in the DNA of the anucleolate mutant. The r-RNA cistrons exist in extended clusters. It is believed that the cistrons for the two components of r-RNA are arranged in an alternating sequence. This is a likely possibility, considering the nature of the 45 S precursor and the coordinated synthesis of the two r-RNA components. Edström (1960) demonstrated a close similarity in base composition between nucleolar and cytoplasmic RNA, the bulk of which is ribosomal. Low doses of actinomycin D, which selectively inhibit nucleolar RNA synthesis, also selectively inhibit the synthesis of the precursor to r-RNA. (Perry, 1962, 1963). That the nucleolus contains the necessary enzymatic apparatus for the polymerization of RNA and its subsequent methylation was shown by Siebert et al. (1966), Liau and Hurlbert (1966), and Birnstiel et al. (1963). Several lines of evidence combine to indicate that the 45 S RNA component is the early precursor of r-RNA. Perry (1962), Sherrer et al. (1963) and others observed that in experiments in which cells were pulse-labeled with RNA precursor and then chased in the presence of sufficient actinomycin D to inhibit subsequent r-RNA synthesis completely, the 45 S component disappeared and labeled 28 S and 18 S r-RNA components appeared. Base composition analyses showed that the 45 S RNA component resembles a composite of the 28 S and 18 S components; the guanine + cytosine content is high. Diminished synthesis of 45 S RNA in the

anucleolate mutants of Xenopus, which are incapable of r-RNA synthesis, was observed by Brown and Gurdon (1964). Nemer (1963) found a proportionately reduced synthesis of 45 S RNA in early stages of embryogenesis that are also characterized by a lack of r-RNA synthesis.

Synthesis, methylation and finally cleavage of the 45 S precursor molecule to form the 28 and 18 S components of the cytoplasmic ribosomes appears to occur in the nucleolus. In pulse-chase experiments one observes a sequential labeling of 45 S, then 32-35 S and 18 S, and finally 28 and 18 S components. All the incorporated methyl groups in the 45 S RNA are conserved during processing; nonmethylated portions of the RNA chain are lost during cleavage. 5 S RNA, one molecule of which is found in every larger ribosomal subunit, has a separate origin from the 45 S pre-ribosomal RNA.

Control of r-RNA Synthesis

Control of the rate at which new ribosomes are formed is important, because of the central role occupied by ribosomes in protein synthesis and, ultimately therefore, in cellular metabolism.

Regulation of the transcription of r-RNA genes is found in systems with variable amounts of nucleolar organizer. Activation and suppression of r-RNA genes is apparent during development. In unfertilize eggs the r-RNA genes are quiescent. After fertilization they are reactivated during an early cleavage stage in mammalian embryos (Mintz, 1964). In amphibia (Brown and Littna, 1964), echinoderms (Nemer, 1963) and other forms r-RNA synthesis occurs at the onset of gastrulation. Among these systems reserve r-RNA is synthesized during maturation of the oocyte.

It has been noted morphologically that multiple large nucleoli exist in oocytes of frogs and salamanders and that the amount of DNA

complementary to r-RNA was increased perhaps as much as 100-fold compared to DNA from other tissues or whole animals (Brown, 1966; Brown, 1968; Gall, 1966). Variations of r-RNA synthesis are also observed during the cell cycle.

Ribosomal Subunits, Monosomes and Polyribosomes

Within the cell, r-RNA is always found in combination with protein. It was found that the first appearance of both 18 S and 28 S RNA after labeling was in the form of 40 S and 60 S ribosomal subunits (Girard *et al.*, 1965; Joklik and Becker, 1965), which were apparently assembled in the nucleolus (Liau and Perry, 1969). Subsequently the newly formed particles appear in polyribosomes and only later as part of the mono-ribosome pool. Polyribosomes are groups of ribosomes which are attached to the same m-RNA molecule actively synthesizing protein. The obligatory initiation of protein synthesis by subunits has been demonstrated in bacteria by Nomura *et al.* (1967). In 1962, Marks *et al.* discovered that most of the amino acid incorporation during hemoglobin synthesis is associated with a ribosome fraction sedimenting faster than 100 S. Warner *et al.* (1962) could show by electronmicroscopy that this fraction contained primarily pentamers of the 80 S ribosomes. After brief digestion with RNAase, the pentamer peak disappeared while the monomer peak increased, showing that the aggregate is held together by a single-stranded RNA. On high resolution electron micrographs a nucleic acid fiber can actually be seen to connect the ribosomes (Slayter *et al.*, 1963). Evidence that the RNA connecting strand is in fact m-RNA has been presented by Gierer (1963), who noted that the addition of artificial poly U to a cell free reticulocyte system stimulates both phenylalanine incorporation and the formation of aggregates.

Messenger RNA

Although much effort has been expended in attempting to study m-RNA in eukaryotic cells, very little concrete knowledge about this RNA species has been uncovered. In bacteria m-RNA has several defined properties, it represents only a small proportion of the total cellular RNA, it is rapidly synthesized and degraded and its base composition reflects the average DNA base composition.

The species of rapidly labeled cytoplasmic RNA considered to be m-RNA is that which is associated with polyribosomes and can be discharged by EDTA or puromycin treatment.

Kinetic studies and annealing studies in cultured cells and differentiated cells indicate that the DNA-like, high molecular weight heterogeneous nuclear RNA may not serve a cytoplasmic m-RNA function (Attardi et al., 1966; Penman et al., 1963; Harris, 1962).

Scott and Bell (1964) have studied the survival times of m-RNA in various tissues of the chick embryo by using actinomycin D to inhibit the RNA polymerase reaction and observing the decline in protein synthesis. They found that many cell types have short-lived messengers, but that the cells of the ocular lens and of the down feather primordia are able to continue protein synthesis for over 24 hours without renewing their messengers. It has also been estimated that the half-life of m-RNA in HeLa tumor cells is about 3 hours (Penman et al., 1963). In rabbit reticulocytes and rat liver the m-RNA's appear to be stable, with half-lives extending over many days. From work on the sea urchin it was concluded that one of the limiting factors of protein synthesis in unfertilized eggs is the presence of stable, "masked" m-RNA (Tyler, 1967). Sidebottom and

Harris (1969) and Harris *et al*. (1969) believe that the nucleolus not only governs the transport of "structural" r-RNA but also of the RNA which is made on other parts of the chromosome, *e.g.*, m-RNA. These workers propose that the informational polydisperse 16 S nuclear RNA is shielded from intranuclear degradation through association with protein in the nucleolus. The ribosome in the cytoplasm is envisaged by Harris (1968) as consisting of a subunit containing 28 S RNA and a subunit containing 16 S RNA, which carries the information for protein synthesis. Nucleoproteins containing m-RNA or informosomes have been proposed by Spirin (1969) and his group. These particles exhibit a great heterogeneity in size (20 S-110 S) which is believed to be due to the heterogeneity in the sizes of the m-RNA contained in them. They are found in the nucleus as well as in the cytoplasm of various species of animals. The most distinctive feature of these RNA-protein complexes is their relatively low buoyant density as determined by CsCl density gradient centrifugation which evidences the significant predominance of protein over the RNA moiety. The value of their buoyant density does not depend on the size of the particles in a wide range of sedimentation coefficients.

Control of Protein Biosynthesis

Regulation at the Transcriptional Level

Many events of interest in cells are expressions of changing patterns of protein synthesis. Therefore the control of protein synthesis in cellular metabolism, differentiation, cell division, regeneration, etc. represents a significant problem. In bacteria, the expression of genetic information is regulated largely by homeostatic mechanisms. Jacob and Monod (1961) proposed their operon

theory as a model for control of gene expression on the transcriptional level. The activities of genes that determine the structures of enzymes concerned with a particular metabolic pathway are coordinately affected by certain metabolites of the pathway. These effector metabolites function by interacting with the product of a regulatory gene whose role is specifically to repress the activity of the structural genes. The metabolites may activate (co-repressors) or inactivate (inducers) the repressor substance. The site of action of the repressor is the operator or the product of the operator. The gene or group of genes controlled by a given operator is termed an operon. The operon theory holds for bacterial systems but is not applicable in its simplest form to all enzyme and structural protein systems of higher organisms, namely because it fails to account for biochemical changes that tend to persist after the stimuli that initiated them have disappeared. The genetic information in cells of a complex metazoan organism is believed to be identical to that of every other cell of that organism. Within a given organism the tremendous diversity of cell phenotypes must therefore derive from the fact that each cell expresses only a limited amount of its full genetic potential and that different cell types express different portions of their genome. The development of an adult organism from a single cell or the differentiation of a single cell is a process which requires an orderly progression of gene activities until the differentiated stage is reached.

In nucleated cells where basic histone proteins are complexed with DNA a possible role for histones as selective inhibitors of RNA transcription was proposed. Supporting evidence for such a role comes from the fact that addition of histones to cell-free DNA polymerase

systems leads to reduction in the reaction rates (Huang and Bonner, 1962; Huang *et al*, 1964). When total histone or specific histone fractions are added to a cell free preparation of thymus nuclei, nuclear RNA synthesis is inhibited (Allfrey *et al*., 1963). It was discovered that histone acetylases are present in the nuclei. Histones which have been chemically acetylated lose much of their inhibitory effects on RNA synthesis *in vitro* (Allfrey *et al*., 1964). Since the primary structure of histone molecules is very homogeneous from tissue to tissue and organism to organism, specific chemical modification of histones after the molecule has been synthesized and/or interaction of histones with specific RNA molecules has been suggested to provide an answer to the problem of the very selective control of gene expression. It was also determined that the histones of the diffuse, active chromatin fraction are acetylated to a much greater extent than those of the repressed fraction. In insects only certain chromosomal loci synthesize RNA at a given time during development in cells of a given tissue (Beermarn, 1960). The roles of acidic proteins associated with chromatin in transcriptional control is largely unknown. An increase in acidic proteins at dipteran chromsome puffs may be correlated with an increase in gene activity. Possibly the acidic proteins function as antagonists to the DNA histone interaction.

Regulation at the Translational Level

In addition to the transcriptional control of gene expression in eukaryotes, nuclear activity is influenced by the state of the cytoplasm and factors in the cytoplasm regulate the translation of genetic messages of nuclear origin.

The existence of translational control mechanisms of both protein-specific and nonspecific nature, has been postulated for a number of

systems. Liver polysomes isolated from rats that had been fed a protein-free diet were found to sustain much lower levels of amino acid incorporation *in vitro* than polysomes from protein-fed animals (Von der Decken, 1967). Similar effects on the activity of *in vitro* ribosomal preparations have been found by Kerr *et al.* (1966) who were working with mouse ascites tumor cells. Subcellular factors have been described which influence *in vitro* protein synthesis (Beard and Armentrout, 1967; Hoagland *et al.*, 1964). Examples of protein-specific regulation at the translational level are also known. Adrenal steroids induce the synthesis of the enzyme tyrosine amino transferase in rat hepatoma cells. Tomkins *et al.* (1969) proposed that the steroid hormones antagonize a posttranscriptional repressor which both inhibits messenger translation and promotes messenger degradation.

The fact that protein is synthesized in the cytoplasm of enucleated cells makes it obvious that the synthesis of protein in these cells does not require the continued synthesis of short-lived m-RNA molecules as in bacteria. It has been shown that the synthesis of a specific phosphatase can be regulated in the enucleated *Acetabularia* cell (Spencer and Harris, 1964). One possibility for translational control could be that the rate of release of polypeptides from the templates on which they are formed can be regulated.

Cytoplasmic RNA Synthesis

In organisms which contain chloroplasts and/or mitochondria the processes of gene expression are further complicated by the presence of these organelles and the role they play in cellular differentiation.

It is generally accepted that chloroplasts contain DNA (Brawerman, 1966) and since chloroplasts also contain a DNA-dependent RNA polymerase,

it appears that specific RNA's are synthesized from chloroplast DNA acting as a template (Kirk, 1966). The discovery of protein synthesis in chloroplasts by Stephenson and Zamecnik (1956) was followed by Lyttleton's (1962) isolation of chloroplastic ribosomes. These ribosomes are smaller than cytoplasmic ribosomes from the same cells and the ribosomal RNA extracted from them has lower sedimentation constants than the corresponding cytoplasmic r-RNA's (Loening and Ingle, 1967; and Ingle et al., 1970). The exact mechanism of coordination between nuclear DNA and chloroplastic DNA is almost unknown, however, nuclear DNA clearly plays a determining role in the synthesis of chlorophyll, carotenoids, and the photosynthetic electron carriers (Kirk, 1966).

One of the earliest reports of mitochondrial DNA was that of Nass and Nass (1963) in thin-sectioned chick mitochondria. In 1964, Luck and Reich isolated mitochondrial DNA from Neurospora. Protein synthesis by mitochondrial preparations has been reported and ribosome like particles that incorporate amino acids into proteins have been isolated from mitochondria (Roodyn, 1962; Kroon, 1963). 55 S ribosomes have been isolated from rat liver mitochondria by O'Brien and Kalf (1967). Penman et al. (1969) have observed the presence of a unique RNA synthesized in association with mitochondria which has sedimentation constants of 12 S and 21 S with two more slowly migrating shoulders associated with them.

Mitochondria like chloroplasts appear to multiply by fragmentation or division. It has been suggested that chloroplasts as well as mitochondria may be descended from symbiotic micro-organisms.

Enucleated Cells

Studies on the metabolism of enucleated cells have been performed to reach an understanding of the interactions between the nucleus and

cytoplasm. Only very few cells are amenable to microsurgical enucleation. In most other cells the anucleate condition must be initiated chemically by inhibiting nuclear RNA synthesis. A few highly specialized cells show naturally occurring nuclear degeneration. One example is the mammalian red blood cell. In a wider sense, every differentiated cell may be considered anucleate with respect to a number of repressed genes. Studies on the anucleate state of cells consist of characterizing the metabolic changes occurring in cells which result in exhausting their supply of genetic information of nuclear origin. A breakdown of the cellular organization sooner or later occurs. The anucleate cellular system can, however, provide valuable information concerning regulation of metabolism. In some instances information on cellular differentiation and the contribution of DNA-containing organelles to the cellular metabolism and differentiation can be obtained.

In general, it can be concluded from studies on anucleate cells that both in pro- and eukaryotic cells, various protein-specific messengers differ greatly in their mean functional life. Concurrent existence of short- and long-lived messengers in the same cell type supports the concept of a genetically determined messenger life (Keck, 1969).

Differentiation in *Acetabularia*

In the preceding discussion, mechanisms related to RNA metabolism which appear most likely to be true, considering the experimental evidence, have been selected. Results from various organisms have been generalized. In the following section results pertaining to these problems will be reviewed which have been obtained in experiments with the green alga *Acetabularia*.

Life Cycle

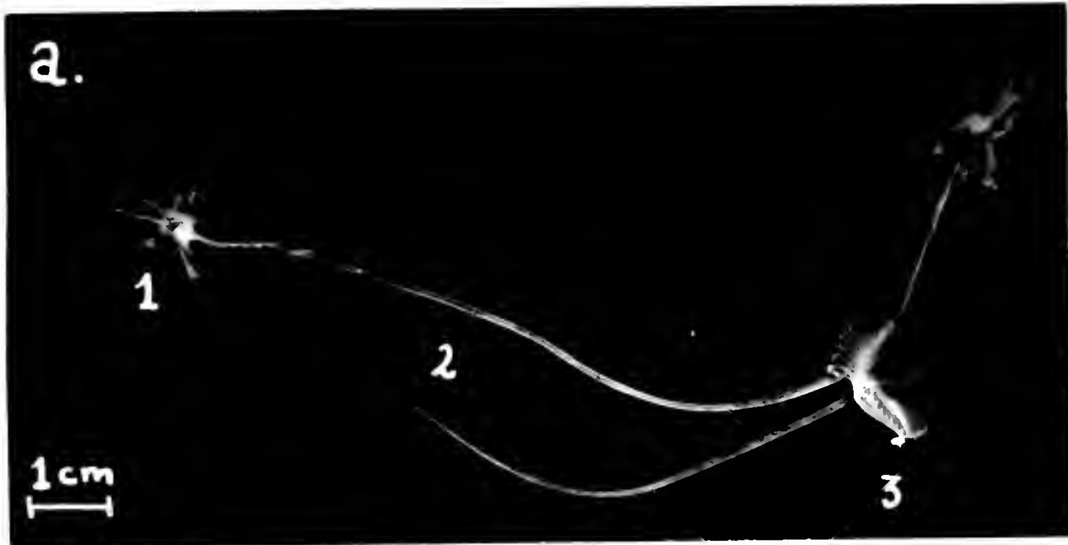
Acetabularia and its relatives combine a number of unusual features that make them desirable experimental organisms for the study of nucleo-cytoplasmic relationships during morphogenesis. The plants are giant, uninucleate single cells (Figure 1). The size facilitates enucleation and grafting experiments by surgical means. The details of development have been described by Hämmerling (1931) and Schulze (1939). The diploid zygote arises from the fusion of the two haploid isogametes and germinates to form a rhizoid and a stalk. At its apical end the stalk forms many deciduous whorls and finally a cap or umbrella. The rhizoid contains the single nucleus. In the zygote nucleus, nucleolar material is almost completely lacking; after germination a small round nucleolus appears. During the subsequent growth of the nucleus, which contains almost undetectable amounts of DNA, many big, rod-shaped branched nucleoli are formed, which are rich in RNA (Figure 2). In cells with mature fully differentiated caps, the primary giant nucleus in the rhizoid undergoes mitotic divisions and gives rise to many secondary nuclei which are transported to the mature cap by cytoplasmic streaming in the stalk. Hämmerling (1939, 1953) showed that the division of the rhizoid nucleus in *A. mediterranea* depends upon the presence of a mature cap. If the mature cap is removed just before the nucleus enters mitosis, division is postponed until a new cap is formed. By repeating the operation, nuclear division can be delayed indefinitely. Conversely, if a young nucleate rhizoid is transplanted to a plant containing a large mature cap, mitosis may begin as early as 2 weeks after grafting instead of at the normal time of about 2 months. These experiments indicate that information of an unknown nature is conveyed from the cap to the nucleus and is responsible for initiating nuclear

Figure 1. Adult _Acetabularia_ plants.

a. _Acetabularia crenulata_.

b. _Acetabularia mediterranea_.

1 : rhizoid
2 : stalk
3 : cap


a.
1
2
3
1cm

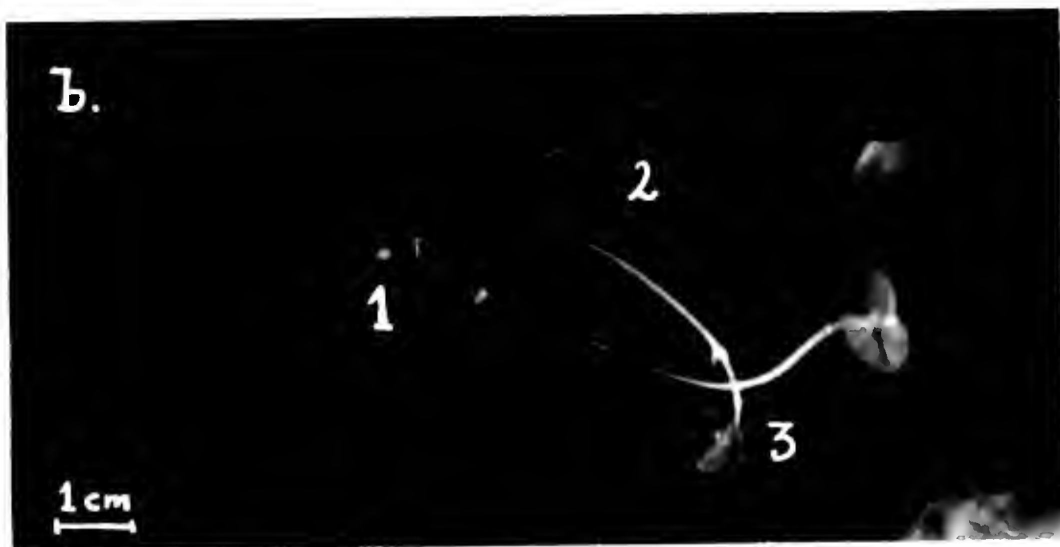
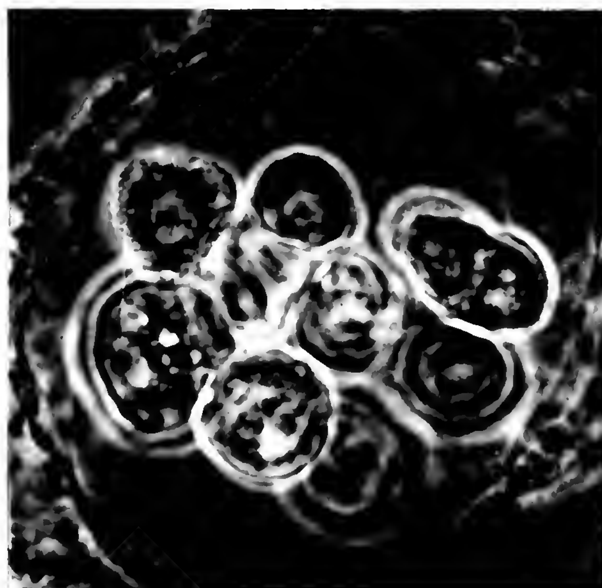

b.
1
2
3
1cm

Figure 2. The nucleus of *Acetabularia*.

a. Young rhizoid of *A. crenulata* stained with acridine orange. Nucleus: green. Small round nucleolus: red. Diameter of nucleus approximately 2 μ.

b. Nucleus of an adult *A. crenulata* plant, shortly before nuclear division, with many nucleoli. Diameter of nucleus approximately 60 μ. Live phase contrast.

a.



b.

division. In the rays of the cap, a cyst containing cytoplasm forms around each nucleus which undergoes further mitotic divisions. Finally meiosis occurs and haploid gametes are produced. Subsequently the cap chambers disrupt and the cysts extrude flagellated gametes, thus closing the life cycle of the organism (Figures 3 and 4).

Morphogenetic Substances

Nucleate segments are capable of complete regeneration and anucleate segments are capable of prolonged growth and can even regenerate a very complex structure, the cap, which is normally the reproductive organ of the alga (Hämmerling, 1934). Intra- and interspecific grafts can be made readily and have shown that the nucleus of the alga produces species-specific "morphogenetic substances" (MS) which migrate toward the tip of the stalk and are distributed according to a decreasing apico-basal concentration gradient (Hämmerling, 1934, 1953). The apico-basal concentration gradient of the MS is established in light as well as in darkness, but in darkness only if the nucleus is present. In anucleate parts kept in the dark, the concentration gradient soon disappears, resulting in an equal distribution of the MS over the whole stalk. If light is given, the gradient is soon re-established. The direction of the light may cause the MS to accumulate at the end of the stalk piece which was formerly proximal to the rhizoid. In nucleate parts, however, the direction of light is without influence on the distribution of the MS; accumulation always occurs at the distal stalk end (Hämmerling, 1934, 1959).

It has been postulated that the MS are stable m-RNA molecules. The evidence for the identity of MS and m-RNA molecules comes from several kinds of experiments. Autoradiography shows that nuclear

Figure 3. Life cycle of *Acetabularia*.

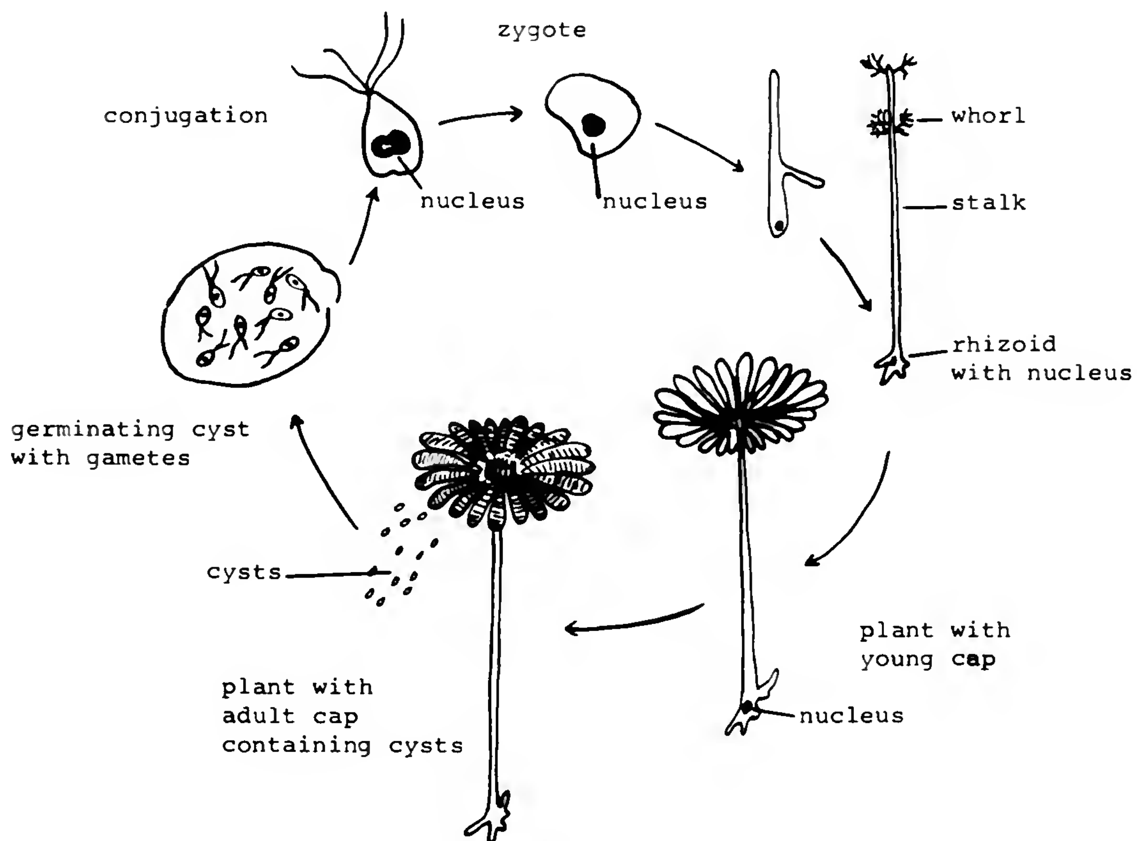
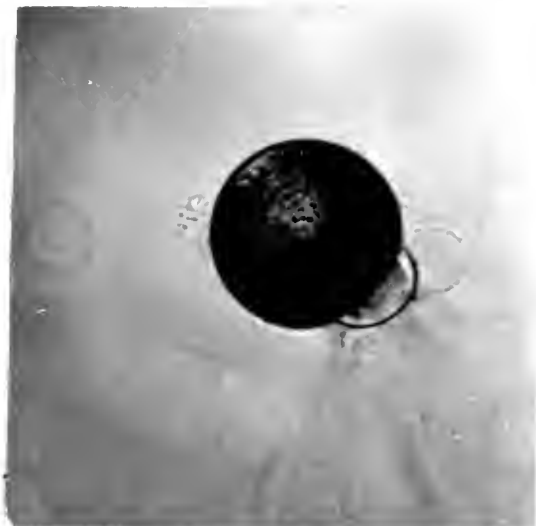

Life cycle of Acetabularia
(after Brachet)
zygote
conjugation
nucleus
nucleus
whorl
stalk
rhizoid
with nucleus
germinating cyst
with gametes
cysts
plant with
young cap
nucleus
plant with
adult cap
containing cysts

Figure 4. Gametogenesis in <u>Acetabularia</u> <u>crenulata</u>.

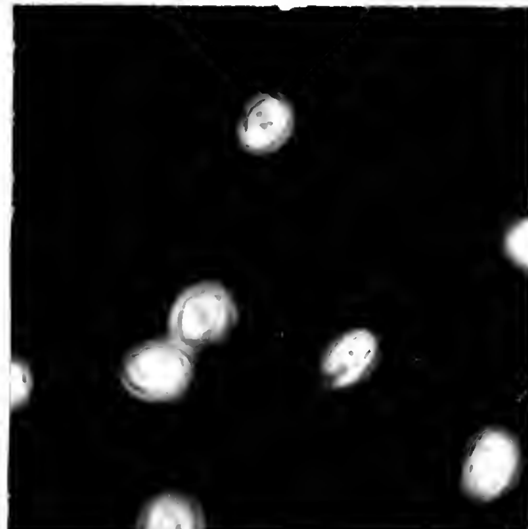
a. Cap chamber containing cysts.
b. Cyst releasing isogametes.
c. Empty cyst. Diameter: approximately 100 μ.
d. Isogametes with two flagellae.
Length: approximately 5 μ.

a.



b.

c.



d.

and nucleolar RNA is labeled first and moves out of the nucleus and migrates toward the apex of the stalk (Olszewska and Brachet, 1961; Olszewska _et al_., 1961; de Vitry, 1965). Cytochemical observations carried out by Werz (1961) have demonstrated that high-molecular weight RNA and certain proteins accumulate at the apex of the stalk. The effects of ultraviolet irradiation on regeneration indicate that RNA is one of the factors controlling morphogenesis (Olszewska _et al_., 1961). _In vivo_ treatment with ribonuclease completely inhibits regeneration of nucleate or anucleate segments as long as the enzyme is present in the medium. The inhibition is reversible in the case of the nucleate fragments but remains irreversible in the case of the anucleate ones. The effects of actinomycin D have been studied in _Acetabularia_ by Brachet _et al_. (1964); by de Vitry (1965) and by Zetsche (1964, 1966a). It was found that actinomycin D will not inhibit regeneration in anucleate halves; on the other hand, regeneration of the nucleate fragments is prevented as long as actinomycin is present in the culture medium. Since actinomycin D inhibits RNA synthesis presumably without destroying preexisting RNA molecules, these results are consistent with the idea that the MS and m-RNA are identical. Zetsche (1966b) further concludes from actinomycin D experiments that the genes responsible for the formation of the stalk and the cap must be active simultaneously for m-RNA synthesis. The information for stalk formation is expressed immediately in the cytoplasm, while that for cap formation is stored and remains inactive, thus the control of the passage from one information to the other would occur at the level of translation in the case of cap formation.

Synthesis of Macromolecules in Nucleate and Anucleate Fragments of Acetabularia

In the following the results of biochemical work carried out with nucleate and anucleate cell fragments of Acetabularia as well as with subcellular fractions of the algae shall be reviewed and summarized as far as they appear relevant to the problem of characterizing the morphogenetic behavior of the cell in molecular terms. Significant difficulties have been encountered in attempts to isolate and characterize macromolecules from Acetabularia, concomitant with the demonstration of their role during morphogenesis in anucleate and nucleate cells. These difficulties include the problem of consistently preparing undegraded RNA, the reaction of Acetabularia to the trauma associated with the enucleation procedure and the complications introduced by the simultaneous transcription of RNA from several different classes of DNA from sub-cellular organelles. The effects of light and darkness on growth, on cap formation, on the accumulation of MS and on the establishment of the apico-basal concentration gradient of these MS, on macromolecular synthesis, further point to the complexity of the process of differentiation in this "simple" organism.

Protein biosynthesis

Brachet et al. (1955) showed that net synthesis of proteins occurs under conditions of good illumination in anucleate fragments of the algae. According to Clauss (1958), both chloroplastic and cytoplasmic proteins increase in the absence of the nucleus. The enzymes invertase (Keck and Clauss, 1958), aldolase (Baltus, 1959), phosophorylase (Clauss, 1959), UDPG-4-epimerase (Zetsche, 1966c), and ribonuclease (Schweiger, 1966) are synthesized by anucleate fragments. Special attention has been concentrated on acid phosphatase.. Spencer and

Harris (1964) and Triplett et al. (1965) found that acid phosphatase in Acetabularia is a complex of many isozymes. There appears to be independent regulation of the synthesis or activity of these isozymes during morphogenesis even in the absence of the nucleus. The enzymatic activity of two of the isozymes of acid phosphatase which are associated with the chloroplast fraction increases when caps are formed by anucleate fragments. UDPG-4-epimerase activity also increases at the time of cap formation, even in enucleated cells. One can conclude from these studies that enzyme synthesis is controlled on the translational level, unless it is only the activity of these enzymes that changes, or the enzymes are synthesized by the chloroplasts. Special attention should perhaps be given to the enzymes involved in the synthesis of the polysaccarides forming the cell wall, since they may be the most important for morphogenesis. Zetsche (1966c) for instance found that the cell wall of the cap is richer in galactose than that of the stalk. Experiments which reveal the contribution of information by the nucleus have been performed. The enzyme acid phosphatase is one example (Keck, 1961; Keck and Choules, 1963). Several species-specific molecular forms of acid phosphatase are known to exist in Acetabularia. In graft combinations between cells of A. mediterranea and Acicularia Schenckii (acic) the expression of the A. mediterranea phosphatase is dominant. There is evidence that in such grafts the preexisting acid phosphatase is converted via an intermediate form to the A. mediterranea phosphatase. The isozyme patterns of malic dehydrogenase and lactic dehydrogenase (Schweiger et al., 1967b; Reuter and Schweiger, 1969) are species-specific. If nuclei of two different species of Acetabularia are exchanged, the isozyme pattern is changed within

4 weeks so that the old isozyme pattern disappears and a new one which corresponds to the species of the implanted nucleus is formed. Ceron (1969) found that the protein spectrum from the soluble fraction remains constant throughout all stages of development. The protein pattern from the membrane fraction changes at the time of cap morphogenesis. Enucleated cells are capable of synthesizing all of the proteins of the soluble and membrane fractions up to 6 weeks after enucleation. The changes in the protein spectrum that occur in the membrane fraction during cap morphogenesis also take place in enucleated cells. When purified chloroplasts were pulsed *in vitro* with radioactive precursors, only some of the chloroplastic proteins became labeled.

Several general principles for nuclear and/or cytoplasmic control over cell differentiation in *Acetabularia* emerged from these various studies. Synthesis of proteins continues after enucleation at almost normal rates for a certain period of time, the length of which is characteristic for each protein. It was thus concluded that this synthesis is controlled by extranuclear mechanisms and is directed by genetic messages, which, in the case where they are nuclear in origin, possess a relatively long functional half-life. In *Acetabularia* the sequential expression of the genome which is phenotypically expressed in the sequential character of the morphogenetic events would depend upon a regulated sequential translation of the gene products.

The role of the chloroplast

The presence of chloroplasts in *Acetabularia* is a complicating factor and it appears to be very important to establish the degree of autonomy of these organelles in relation to the nucleus and their relative role in the morphogenetic events of the cell.

Plastids of Acetabularia have been shown to contain DNA (Gibor and Izawa, 1963; Baltus and Brachet, 1963; Green et al., 1967, 1970; Berger and Schweiger, 1969) and to be capable of synthesizing DNA (Gibor, 1967; Berger and Schweiger, 1969). In addition they have been shown to contain RNA (Naora et al., 1960; Janowski, 1965; Schweiger, 1967) and to be capable of synthesizing RNA (Schweiger and Bremer, 1961; Janowski et al., 1969) as well as protein (Clauss, 1958; Goffeau and Brachet, 1965; Bonotto et al., 1969; Ceron, 1969). Chloroplasts can multiply in anucleate fragments (Shephard, 1965a) and synthesis of chloroplastic enzymes and other proteins takes place in anucleate cells (Shephard, 1965b; Zetsche, 1966d; Ceron, 1969). Growth and morphogenesis in Acetabularia require light, which produces the energy needed for the various syntheses through photophosphorylation. Removal of the nucleus exerts no measurable effect on the photosynthetic capacity, even after several weeks (Brachet et al., 1955). Werz (1966) has shown that young chloroplasts and mitochondria of nucleate and anucleate fragments can differentiate in the dark. At least some of the information which codes for the structural and functional components of the plastids has to be supplied by the nuclear DNA, because the amount of DNA per plastid which has been estimated to be on the order of 10^{-16}g is not sufficient for the synthesis of all proteins required for the construction and function of the chloroplast (Gibor and Izawa, 1963; Baltus and Brachet, 1963). 10^{-16}g of DNA corresponds to approximately 1.88×10^5 nucleotides. According to Berger (1967), chloroplastic DNA must code for 23 S, 16 S, 9 S and 4 S RNA and for ribosomal proteins. This leaves only a maximum of 8.6×10^4 nucleotides available for the coding of approximately 100 non-ribosomal proteins having an average molecular weight of 40,000. More recently Green et al. (1970) have

estimated the DNA content of Acetabularia chloroplasts to be on the order of 150×10^{6} daltons. This brings the DNA content up to a quantity which would be sufficient for a free-living organism.

RNA synthesis

It is well established that net RNA synthesis occurs in anucleate fragments of Acetabularia if they are given adequate illumination (Brachet et al., 1955; Richter, 1959; Naora et al., 1959). Naora et al. (1960) attributed the greater part of RNA synthesized in the enucleated cytoplasm to the action of the chloroplasts. The base composition of chloroplast RNA is similar to the ratio of incorporation of P^{32}-orthophosphate into the four nucleotides of RNA in enucleated cells (Schweiger, 1962). Schweiger and Bremer (1960, 1961) demonstrated that the amount of light given to the cells is an essential factor in these experiments. The RNA content of the anucleate and nucleate fragments decreases 30-40 percent if the fragments are kept in darkness for 10 days. But if nucleate fragments kept in the dark for 10 days are amputated from their nucleus and if the resulting anucleate fragments are illuminated, their RNA content increases. It is concluded from these experiments that substances promoting cytoplasmic RNA synthesis are probably delivered by the nucleus; these substances are accumulated in the cytoplasm of nucleate plants during darkness and consumed in light. RNA synthesis is sensitive to actinomycin D in anucleate fragments of Acetabularia (Brachet and Six, 1966). Janowski (1965) who fractionated the P^{32}-labeled RNA of the algae by column chromatography was able to demonstrate the presence of three different kinds of RNA labeled in the absence of the nucleus: a small molecular weight RNA, a ribosomal type RNA associated with the chloroplasts and a chloroplastic RNA that is closely associated with chloroplastic DNA.

Richter's (1966) findings are in agreement with those reported by Janowski (1965) in that they provide evidence for the existence of rapidly labeled RNA fractions in normal and anucleate cells of Acetabularia. Incorporation of radioactive precursors of RNA into the nucleated and enucleated cells of A. mediterranea and Polyphysa cliftonii and isolation of the labeled RNA produces a sucrose density gradient profile of radioactivity which is similar to that of RNA isolated from E. coli ribosomes (Schweiger et al., 1967a). Woodcock and Bogorad (1970) separated two major species of r-RNA (25 S and 18 S) in A. mediterranea by polyacrylamide electrophoresis. Two minor species in the 16 S and 12-14 S position were attributed to degradation products. A comparison of the width of the A. mediterranea bands with those of E. coli indicates considerable size heterogeneity in the former. The heterogeneity of the two r-RNA fractions was demonstrated directly by polyacrylamide gel electrophoresis (Schweiger, 1970). By this method each of the two r-RNA peaks of Acetabularia was separated into two components corresponding to 25 S and 23 S for the faster sedimenting peak and to 18 S and 16 S for the more slowly sedimenting peak. Schweiger and Berger (1964) and Berger (1967) have demonstrated that isolated chloroplasts retain their ability to synthesize 23, 16, 9 and 4 S RNA. Incorporation of RNA precursors is inhibited by darkness, actinomycin D, and DNAase. Schweiger and Dillard (1968) have proposed the occurrence of two components of ribosomal RNA to account for the sigmoid form of the short term synthesis kinetics curves. According to Janowski and Bonotto (197 the 25 S RNA disappears at a higher rate than the 16 S RNA after

enucleation or cap formation. They also found that the pulse labeled RNS's (17 and 23 S) do not sediment at the same rate as the unlabeled RNA's (16 and 25 S). Furthermore, chase experiments indicate the existence of a stable 15 S RNA, which is synthesized in the anucleate fragments as well as in whole plants. This last result was confirmed by Farber (1969a and 1969b). The 15 S RNA appears to be synthesized in the chloroplasts; the label is retained after a 35 day chase. The sedimentation profiles of labeled RNA become polydisperse after a 58 day chase, when the plants have formed caps. However, when plants at this stage were labeled, the 15 S component is still the most predominantly labeled one. Farber's results indicate that the 24 S absorbance peak disappears in both nucleate and anucleate fragments and that there is a diminution of the 15 S fraction in the nucleate halves one month after the surgery. Nucleate halves had formed caps by this time. The absorbance profiles of whole plants display the same changes upon cap formation. Several workers have developed methods for the detection of ribosomes and polysomes in whole algae and in their fragments and have attempted to characterize the RNA species present in these particles. Incorporation of tritiated uridine into whole _A. mediterranea_ cells and anucleate fragments allowed the detection of polysomes, 82 S, 65 S and 48 S particles (Janofski, 1966, 1967; Baltus _et al._, 1968). Ribonuclease converts the heavier radioactive components to 82 S particles, which are probably 82 S monomers. Increasing the Mg^{++} concentration does not result in a lowering of the level of the 65 S and 48 S radioactivity; it seemed therefore unlikely that the 65 S and 48 S components consist of subunits of dissociated monosomes. After removal of the nucleus the radioactivity transferred from the polysomal region to the 82 S ribosomes

by ribonuclease treatment is very small. However the incorporation of uridine into the polysomes is stimulated by a two day dark period prior to the incubation. It was suggested that chloroplastic m-RNA might combine with the ribosomes to form active polysomes. Janowski et al. (1969) report that anucleate fragments of Acetabularia as well as whole plants are able to incorporate H^3-uridine into the ribosomes and polyribosomes of both the chloroplastic and non-chloroplastic fractions. Janowski et al. (1969) corrected the sedimentation coefficients of the various particles from 82, 65 and 48 S to 70, 50 and 30 S. There appears to be no detectable difference in size between the chloroplast and non-chloroplast labeled ribosomes. These workers were unable to successfully isolate non-chloroplastic unlabeled ribosomes. It has been demonstrated that the 50 S particles contain 23 S uridine labeled RNA while the 30 S particles contain 16 S RNA. Baltus et al. (1968) made extensive studies on the base composition of the various RNA species of Acetabularia. The nucleolar RNA of A. mediterranea is rich in adenine (A) and uridine (U), it also shows a tendency towards symmetry between A and U and between guanine (G) and cytosine (C). The A content of the nucleolar RNA is the same as that of the nuclear DNA. The base composition of the nucleoplasm is characterized by a very high content in U. The RNA from chloroplastic ribosomes has a base composition of the GC type (G+C=51.1 percent), the G+C content of the cytoplasmic r-RNA is 41.3 percent and the r-RNA from whole chloroplasts is different from that of chloroplastic r-RNA: its G+C content is only 42 percent. This difference may be explained, since whole chloroplasts probably contain many different RNA species enabling them to perform independent protein synthesis.

Purpose of the Work for This Dissertation

It becomes obvious from the preceding review that an understanding in molecular terms of differentiation in Acetabularia has not been achieved. The purpose of the present study was to gain further insight into RNA metabolism of this organism. Before one may establish the presence in Acetabularia of the postulated stable cytoplasmic m-RNA's, the elucidation of all other RNA molecules must also be achieved. A study of the available data showed that the RNA's extracted from Acetabularia display many puzzling properties. In order to gather further information about the various RNA species, it appeared to be necessary to develop a new method of investigation, since fractionation of these RNA's in sucrose gradients will not demonstrate differences in sufficient detail. Therefore it was attempted to develop polyacrylamide electrophoresis for RNA extracted from Acetabularia. Since the work reported in this dissertation was completed Woodcock and Bogorad (1970) and Schweiger (1970) have also used gel electrophoresis analysis of Acetabularia r-RNA. The research reported in this dissertation has beed directed towards answering the following specific question. Is it possible to observe qualitative differences between the behavior of whole plants and anucleate fragments as far as RNA synthesis is concerned?

MATERIALS AND METHODS

Culture of *Acetabularia* Cells

Stock solutions and reagents.

A. Detergent solution:

1 g Alconox in 100 ml sterile sea water

B. Antibiotic solution:

100 ml sterile sea water

200,000 units Penicillin-G (Pfizer)

200 mg Streptomycin Sulfate (Squibb)

100,000 units Mycostatin (Squibb)

200 mg Neomycin Sulfate (Upjohn)

C. Soil extract:

200 g dark, humus-rich dried soil in 1000 ml sea water.

D. Sea water

Filtered sea water was kindly provided by Marineland of Florida, Florida, U. S. A.

E. Salt concentrate

4 g Na_2HPO_4

20 g $NaNO_3$

in 1000 ml of distilled water.

F. Erdschreiber medium

2500 ml of filtered sea water

100 ml of distilled water

35 ml soil extract

15 ml salt concentrate

A. crenulata cyst-bearing caps were obtained through the generosity of Dr. G. Ceron from his stock cultures; these caps originated from plants that were collected at Key Largo, Florida U.S.A. The culture methods were adopted from Keck (1964), Gibor and Izawa (1963), Lateur (1963), and Hämmerling (1931). The caps were stored in sterile sea water for several weeks in the dark, prior to the initiation of a new culture. About 10 caps were then transferred to the solution containing 1 g of Alconox detergent in 100 ml of sterile sea water and kept in the dark for 24 hours. Then the caps were placed in an antibiotic mixture devised by Keck (1964) and Gibor and Izawa (1963). This mixture was passed through a millipore filter prior to use.

Following the antibiotic treatment, the caps were disrupted with iredectomy scissors to free the cysts. Induction of gametogenesis was accomplished by suspending the spores in about 1 ml of sterile distilled water for 2 minutes and then resuspending them in 200 ml of sterile sea water contained in cotton stoppered Erlenmeyer flasks. The cysts were illuminated for periods of 12 hours by means of fluorescent lights of approximately 2500 lux. Free-swimming flagellated isogametes usually appeared 5 days after induction. The gametes are positively phototactic and were readily recognized by the appearance of a large green spot on the side wall of the Erlenmeyer flask which was facing the light source. They were collected under sterile conditions by drawing them up into a Pasteur pipette and seeding them into 1000 ml Erlenmeyer flasks containing approximately 600 ml of sterile Erdschreiber medium. Erschreiber medium was prepared as follows: 2500 ml of filtered sea water, 100 ml of distilled water and 35 ml of soil extract were combined and autoclaved for 35 minutes. After cooling, the medium was

completed by adding 15 ml of cold, sterile salt concentrate. The soil extract was prepared by suspending 200 g dark, humus-rich dried soil in 1000 ml sea water and autoclaving this mixture 25 minutes for extraction. The suspension was filtered through Pyrex glass wool, followed by a filtration through fine filter paper (Whatman No. 1) until a clear brown solution was obtained. This solution was re-autoclaved and stored at $4^{o}C$.

The cultures were kept in an alternating 12 hour light-dark cycle in a walk-in Forma environmental room. The light intensity was about 2500 lux and the temperature was kept between $22^{o}C$ and $25^{o}C$. The medium was changed every two weeks under sterile conditions. Several cultures of all developmental stages were maintained simultaneously (Figure 5).

A. mediterranea plants were cultured in similar fashion. Cyst-bearing caps were kindly supplied by A. Gibor.

Sterility Tests

Stock solutions and reagents:

A. Nutrient agar

2 percent Bactoagar (Difco)

1 percent Bactopeptone (Difco)

1 percent Bactodextrose (Difco)

in Erdschreiber medium

B. Erdschreiber medium

containing 1 mg/ml Ampicillin

C. Nutrient agar

containing 1 mg/ml Ampicillin

Acetabularia cultures appeared clean but they were not sterile, as shown by bacterial colonies formed on nutrient agar. It is unlikely

Figure 5. Cultures of *Acetabularia*.

that the contribution of foreign RNA would appear in the stained bands after electrophoresis in the polyacrylamide gel considering the large numbers of algae utilized in comparison to the possible quantity of contaminating organisms. However, in labeling studies, bacterial intervention cannot easily be discounted. The cultures used for these studies contained approximately 5000 bacteria per 1 cm long plant. This number was obtained from "colony counts." Twenty algae were homogenized in 10 ml of sterile sea water. Samples of this homogenate were spread out on nutrient agar plates in serial dilution. The contaminating organisms were grown at 23°C and colonies were counted after 48 hours of incubation. If one assumes that one bacterium weighs approximately 10^{12} daltons and that six percent of this is RNA (Watson, 1965), one obtains 0.15 μg bacterial RNA per 300 labeled Acetabularia plants. For each electrophoretic fractionation 10 μg of Acetabularia RNA were used, of these approximately 1 μg was labeled. Assuming that all of the bacterial RNA is labeled, only one-seventh of the label can be contributed by the incorporation of uridine into the bacterial RNA.

Farber (1969) has shown that autoradiograms show some contamination in the region of the rhizoid membrane of A. mediterranea, but it was concluded that the quantity of foreign radioactivity present, compared to the total amount of radioactivity situated in Acetabularia itself should not influence the labeling pattern. Moreover, Janowski et al. (1969) have shown that the radioactivity incorporated in Acetabularia and in the contaminating micro-organisms differs greatly. Under the same conditions of incorporation, 10 whole algae incorporate as much radioactivity as 15-20 ml of highly infected culture medium. In my studies, I found that 1 mg/ml of Ampicillin completely inhibited

contaminating microbial growth on nutrient agar. However, it was apparent that a 12 hour incubation of *Acetabularia* cells in Erdschreiber medium containing 1 mg/ml Ampicillin did not decrease the viable bacterial population as compared to the controls when samples were taken for colony counts on agar plates. This suggests that the metabolic activity of contaminating organisms was negligible under routine experimental conditions. In the experiment in which nucleated cells were compared to enucleated cells, the rhizoids of the nucleated cells were removed before the RNA extraction, so that any differences observed in the labeling pattern were not due to a difference in the microbial contamination. Nucleic acids were extracted from microorganisms that had been cultivated from *Acetabularia* cultures. Four to 5 bands were obtained on the polyacrylamide gel after electrophoresis which were identified as DNA, 23 S RNA, 16 S RNA, 5 S RNA and 4 S RNA (Figure 7, Column 1). The 5 S RNA and 4 S RNA are not seen in Figure 7, Column 1. The electrophoresis banding pattern obtained from *Acetabularia* is clearly more complex.

Since the work for this dissertation was completed a paper by Shephard (1970) has appeared describing the axenic culture of *Acetabularia* cells.

Nucleic Acid Extraction

Stock solutions and reagents:

A. Homogenizing buffer pH 5.0

0.01 M sodium acetate

0.54 M glucose

0.001 M ethylenediamine tetraacetic acid (EDTA)

1 mg/ml naphtalenedisulfonate

B. Homogenizing medium pH 5.0

0.01 M sodium acetate

0.54 M glucose

0.001 M EDTA

1 mg/ml naphtalenedisulfonate

2 M lithium chloride (LiCl)

C. Phenol reagent

85 ml redistilled phenol (Baker)

15 ml H_2O

1 percent sodiumdodecylsulfate

This reagent was always freshly prepared before each RNA extraction.

D. Chloroform isoamyl alcohol reagent

35 ml chloroform (Mallinkrodt)

1 ml isoamyl alcohol

E. Sodium chloride solution

20 percent sodium chloride (NaCl) in distilled water

F. 100 percent Ethyl alcohol

Approximately 3000 algae 1-2 cm long were washed several times in sterile sea water and one time in the homogenizing buffer. The homogenizing buffer was described by Baltus and Quertier (1965). The plants were homogenized foroneminute in 20 ml of homogenizing medium, in a high-speed Virtis 45S homogenizer at top speed, surrounded by an ice bath. All operations were carried out in the cold room between 0-4°C. The RNA was precipitated with 2 M LiCl after Barlow et al. (1963) and Farber (1969). Precipitation occurred for 16 hours in the cold, after which the suspension was centrifuged for 20 minutes in a clinical centrifuge at about 3000 g. The supernate was decanted and the sediment was washed twice with homogenizing medium. Following the washes, the sediment was resuspended in 5 ml of homogenizing

buffer to which an equal volume of the phenol reagent was added. The aqueous phase was extracted twice with the phenol reagent for 3 minutes each time. This was followed by one 2 minute extraction with an equal volume of the chloroform isoamyl alcohol reagent. After a 5 minute centrifugation in the clinical centrifuge, to separate the phases, the aqueous phase was removed with a Pasteur pipette, it was made 1 percent in NaCl and 2 volumes of 100 percent ethyl alcohol were added. The precipitation of the nucleic acids required at least 2 hours at -20°C.

Plants which were radioactively labeled were washed several times with sterile sea water and one time with the homogenizing buffer before they were added to the carrier plants. These plants were not labeled and were added in order to increase the amount of RNA to work with. RNA extraction was carried out in the same way as for unlabeled plants.

In order to determine the purity and concentration of the RNA preparation, the precipitate was dissolved in water or a suitable buffer and extracted several times with ether after Byvoet (1961) to remove trace amounts of phenol. The remaining ether was removed by bubbling air through the preparation. The absorption spectrum was then measured with a Zeiss spectrophotometer and the concentration was estimated from the optical density reading at 260 mμ. This estimation was based upon the assumption that a solution of 10 μg RNA/ml had an absorbance of 0.24 at 260 mμ. The A260:A230 ratio of the RNA preparations was 1.73.

Enzyme Treatment

Reagents:

A. DNAase buffer pH 7.0

0.02 M NaCl

0.00 M potassium phosphate

0.001 M magnesium sulfate

B. DNAase (Worthington, RNAase free)

C. 0.01 M sodium acetate buffer pH 5.0

D. RNAase, beef pancreas (Worthington)

The final nucleic acid precipitates were dissolved and treated with either RNAase or DNAase.

For the RNAase treatment, the precipitate was dissolved in 2 ml of 0.1 M sodium acetate buffer pH 5.0 before 160 μg RNAase were added. For the DNAase treatment the precipitate was dissolved in 2 ml of the DNAase buffer and 100 μg DNAase were added. Both samples were incubated for ten minutes at 4°C, at the end of which they were extracted once with an equal volume of chloroform isoamyl alcohol reagent. The nucleic acids were precipitated from the aqueous phase.

Electrophoresis

Reagents:

A. 10 percent Monomer

19 g recrystallized acrylamide (BioRad)

1 g recrystallized methylene bisacrylamide (BioRad)

in 200 ml of distilled water

This solution was stored in a brown bottle in the refrigerator.

B. Tris-EDTA-Borate buffer pH 8.3

108 g Tris-(hydroxymethyl)-aminomethane

55 g boric acid

9.3 g EDTA

in 1000 ml of distilled water

The buffer was filtered and stored in the refrigerator. The concentration of this buffer was ten times the working concentration.

C. DMAPN, 6.5 percent (catalyst)

6.4 ml 3-dimethylaminopropionnitrile (DMAPN)

in 100 ml distilled water.

This solution was stored in a brown bottle in the refrigerator.

D. Ammonium persulfate 1.6 percent

0.08 g ammonium persulfate

in 5 ml of distilled water.

This solution was prepared fresh for each electrophoretic run.

E. Agarose (Seakem, Bausch and Lomb)

F. 20 percent Sucrose-EDTA-urea-bromphenol blue dye solution

20 g sucrose (Baker Analyzed Reagent)

approximately 0.1 g bromphenol blue

in 100 ml 0.001 M EDTA.

G. 1 M Acetic acid

100 ml glacial acetic acid

1640 ml distilled water

H. Methylene blue staining solution pH 4.7

2 g methylene blue

500 ml 0.4 M sodium acetate

500 ml 0.4 M acetic acid

The stain was reused several times.

I. "Stains-all" stock staining solution after Bunting.

0.1 percent "Stains-all" = 1-Ethyl-2- 3-(1-ethyl-natptho-[1,2d] thiazolin-2-ylidine)-2-methylpropenyl -naphtho [1,2d] thiazolium bromide. (Eastman Organic Chemicals) in 100 percent formamide (Reagent grade from Fisher)

0.1 g stain were dissolved in 100 percent formamide on a magnetic stirrer for 3-4 hours and stored in the dark.

J. "Stains-all" working stain solution

10 ml stock staining solution

90 ml 100 percent formamide

100 ml water

The electrophoresis was performed following the procedures of Peacock and Dingman (1967, 1968), Dingman and Peacock (1968) and Bunting.

The electrophoretic runs were carried out in the vertical analytical cell of Raymond, E-C470 (E-C Apparatus Corp., Philadelphia, Pa.).

The polyacrylamide slab technique was preferred over the cylindrical gel, because a more accurate comparison of samples is possible, since conditions are identical across all slots. Other advantages included that water layering was not necessary; it took less time to prepare one gel instead of several cylindrical gels and since the slab is flat and had a thickness of only 3 mm it destained more rapidly. The gel slab is also suitable for two-dimensional electrophoresis and when dried to a thin membrane, can be used for autoradiography.

The cleaned and dried E-C cell was assembled after placing a sponge strip in the bottom of the cell and all hoses to the cooler as well as to the buffer pump were connected. The cooling water to which an antifreeze solution had been added was kept at -2°C and circulated through the coils for a few minutes before pouring the gel. Composite 2 percent acrylamide - 0.5 percent agarose gels were used routinely.

160 ml mixed gel solution are needed for a gel slab of 3 mm thickness in the E-C cell. To prepare one gel, 0.8 g agarose were placed in a 250 ml cotton stoppered Erlenmeyer which contained a

magnetic stirring bar, 97 ml distilled water were added and the suspension was autoclaved for 10 minutes. During this time the slot former was cooled in the freezer and the monomer solution was prepared. 32 ml of the 10 percent monomer solution, 16 ml of the 10 x concentrated run buffer and 10 ml of the 6.4 percent DMAPN solution were mixed in a 250 ml Erlenmeyer flask and warmed to 40°C. After cooling the agarose solution to 40°C, the monomer catalyst solution was added under stirring, finally 5 ml of a 1.6 percent ammonium persulfate solution were added and the solution was mixed gently by swirling. The solution was then poured immediately into the tilted precooled electrophoresis cell, the cooled slot former was inserted and a tightly stoppered tube of crushed ice was placed in front of the slot former. During this procedure care had to be taken to avoid trapping air bubbles. In low percent polyacrylamide gels it is important that the agarose gels first, and this was insured by precooling the cell, the slot maker and the test tube, since polymerization of the acrylamide is retarded at lower temperature. Gelation was allowed to occur for one hour with the cell in a horizontal position.

After gelation, while the cell was still horizontal, the gel block in front of the slot former and the test tube were carefully removed. The cell was then placed in the vertical running position, and the diluted Tris-EDTA-Borate buffer (about 2000 ml) was poured into the reservoirs until the electrodes and the slot area were well covered. Now the slot former was carefully removed, as gels tore very easily.

The coolant was now started to circulate. Next 25 µl samples of 20 percent sucrose-EDTA-urea-bromphenol blue dye solution were applied to each of the 8 slots to test whether they had been damaged during the removal of the slot former. The lid was placed on the upper

reservoir and the gel was pre-run for about 60 minutes at 200 V prior to the application of the RNA sample. The buffer was circulated during the pre-run. After completion of the pre-run the buffer pump was turned off, but the coolant continued to be pumped through the coils. Approximately 10 μg of RNA in 25 μl of the 20 percent sucrose-EDTA-urea-bromphenol blue dye solution were added to each slot for analysis. The RNA precipitate was dissolved in the cold room and applied to the gel with a 0.5 ml syringe which had a flexible Teflon tubing tip instead of a needle. The 25 μl drop was removed from the test tube by means of a 25 μl pipette and placed on a piece of "Parafilm" from which the drop was easily drawn into the syringe.

The length of the electrophoretic run was approximately 90 minutes at 200 V, the current read 30-40 ma using the Tris-EDTA-Borate buffer. The run was stopped when the bromphenol blue tracking dye had reached a certain mark on the electrophoresis cell, which ensured constant migration distances among experiments.

Ribonucleic acids carry a net negative charge over a wide pH range and therefore will migrate from the negative to the positive pole in an electric field. The cathode was therefore set at the electrode of the top reservoir and the anode at the electrode of the bottom reservoir. After completion of the run, the buffer was removed from the reservoirs and the cell was carefully dismantled. The gel slab was removed from the cell and placed in a tray filled with 1 M acetic acid for 10 minutes. The gel was then transferred to another tray, containing the methylene blue staining solution and stained for 30 minutes. Destaining was carried out in running water until good contrast for photographic purposes between the bands and the gel background was attained.

Occasionally gels were stained following the "Stains-all" staining method of Bunting. "Stains-all" stands for 1-ethyl-2- 3-(1-ethyl-naphtho-[1,2d]thiazolin-2-ylidine)-2-methylpropenyl -naphtho[1,2d] thiazolium bromide. This chemical stains RNA bluish-purple, DNA blue and protein pink or red, when used as described. The working stain solution was mixed well and poured over the gel in a tray. The tray was covered with aluminum foil and left overnight. The gel was then destained in running water out of direct light, since the stain is photosensitive.

Enucleation of *Acetabularia* Cells

Acetabularia cells which had attained an average length of 3 cm were enucleated after a dark period of ten days, by cutting the rhizoid off at the basal portion of the stalk using iridectomy scissors (Schweiger and Bremer, 1960, 1961). The fragments were allowed to recuperate from the surgical procedure for 48 hours in subdued light.

Labeling of *Acetabularia* Cells

Whole cells or algal fragments were incubated for 96 hours in Erdschreiber medium containing 10 μc/ml of uniformly labeled (H^3) uridine (specific activity 2C/mMole, New England Nuclear Corp.) or C^{14} uridine (specific activity 500 mC/mMole, Nuclear Chicago).

The cells were washed with sea water several times and homogenizing buffer once, and then immediately processed.

Determination of Radioactivity in Polyacrylamide Gel Fractions After Electrophoresis

The gel fractions containing the stained bands and appropriate blanks were cut out with a thin wire and collected in glass counting vials containing 5 ml of acetone. After 15 minutes, the acetone was

removed by aspiration and each gel piece was weighed. Dried gel fractions were solubilized by incubation overnight in capped vials at 60°C in the presence of 0.2 ml of 30 percent hydrogen peroxide (Young and Fulhorst, 1965; Tischler and Epstein, 1968). The resulting solutions were rendered miscible with scintillation fluid (15 ml of toluene scintillator containing 5 g of PPO and 0.1 g of POPOP/l of toluene) prior to counting, by addition to each vial of 1 ml NCB solubilizer. The samples were counted in a Packard liquid scintillation counter and the counts expressed as counts/minute/mg gel.

Autoradiography of *Acetabularia* RNA

After staining, destaining and photography of the RNA pattern, the acrylamide gel slice was dried onto a glass plate.

The dried slices were placed in contact with Kodak no-screen x-ray film and kept in the dark during the exposure period. The film was developed after an exposure of three to fourteen days.

RESULTS

Before experiments of biological significance could be performed, a method had to be worked out to extract undegraded RNA from *Acetabularia*. After attempting to extract nucleic acids from *Acetabularia* in many ways, the 2 M LiCl method was finally successful. The material obtained by biochemical extraction had to be identified and analyzed for chemical purity, before the technique of one-dimensional polyacrylamide electrophoresis could be developed.

The bands obtained by polyacrylamide electrophoresis of material extracted from *Acetabularia* represent nucleic acids for the following reasons: (1) the material was isolated by phenol extraction and precipitated with alcohol; (2) the material was susceptible to RNAase or DNAase treatment; (3) the migration distances of the bands after electrophoresis in the polyacrylamide gel were similar to those of nucleic acids from other sources; (4) the staining properties with methylene blue and "Stains-all" of the bands were characteristic for nucleic acids; (5) H^3 uridine and C^{14} uridine were incorporated into the material; (6) the UV absorption spectrum of the extracted material was typical for nucleic acids. The purity of the material was assured by determining the UV absorption spectrum. Figure 6 illustrates the UV absorption spectrum of purified nucleic acids from *A. crenulata*. The A 260/A 230 ratio = 1.73. This value was obtained repeatedly from different samples.

The staining patterns represented in Figure 7 and the autoradiographs in Figure 8 show the resolution that can be obtained by poly-

Figure 6. UV absorption spectrum of the nucleic acids extracted from _Acetabularia_.

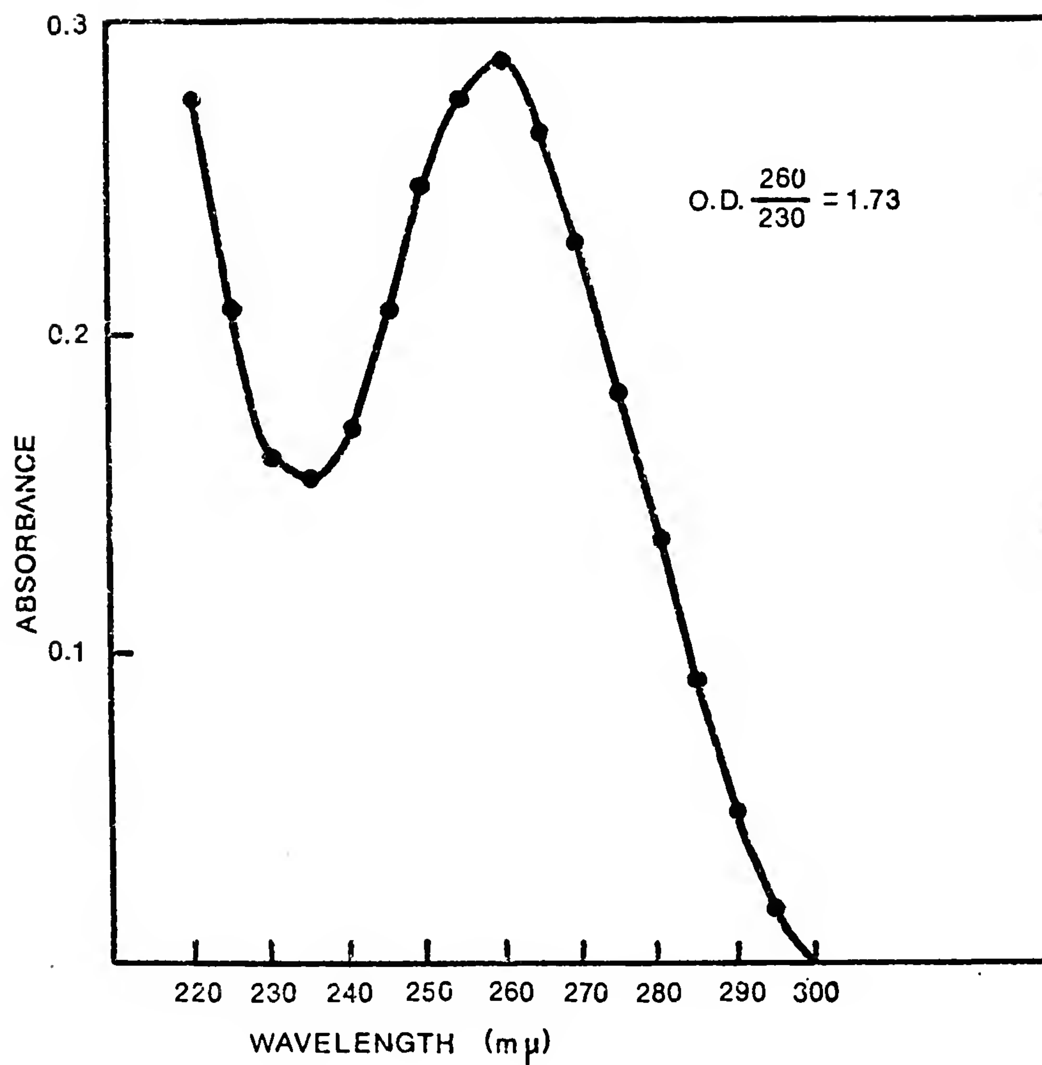

0.3
0.2
0.1
ABSORBANCE
O.D. 260/230 = 1.73
220
230
240
250
260
270
280
290
300
WAVELENGTH (mμ)

Figure 7. Polyacrylamide electrophoresis of the nucleic acids from *Acetabularia*. Staining patterns.

Column 1:	Nucleic acids from contaminating micro-organisms 1.9 cm = DNA 3.0 cm = 23 S RNA 3.8 cm = 16 S RNA
Column 2:	4 S yeast RNA
Columns 3-7:	*A. crenulata* nucleic acids, different electrophoretic runs
Columns 8 and 9:	*A. mediterranea* nucleic acids, different electrophoretic runs

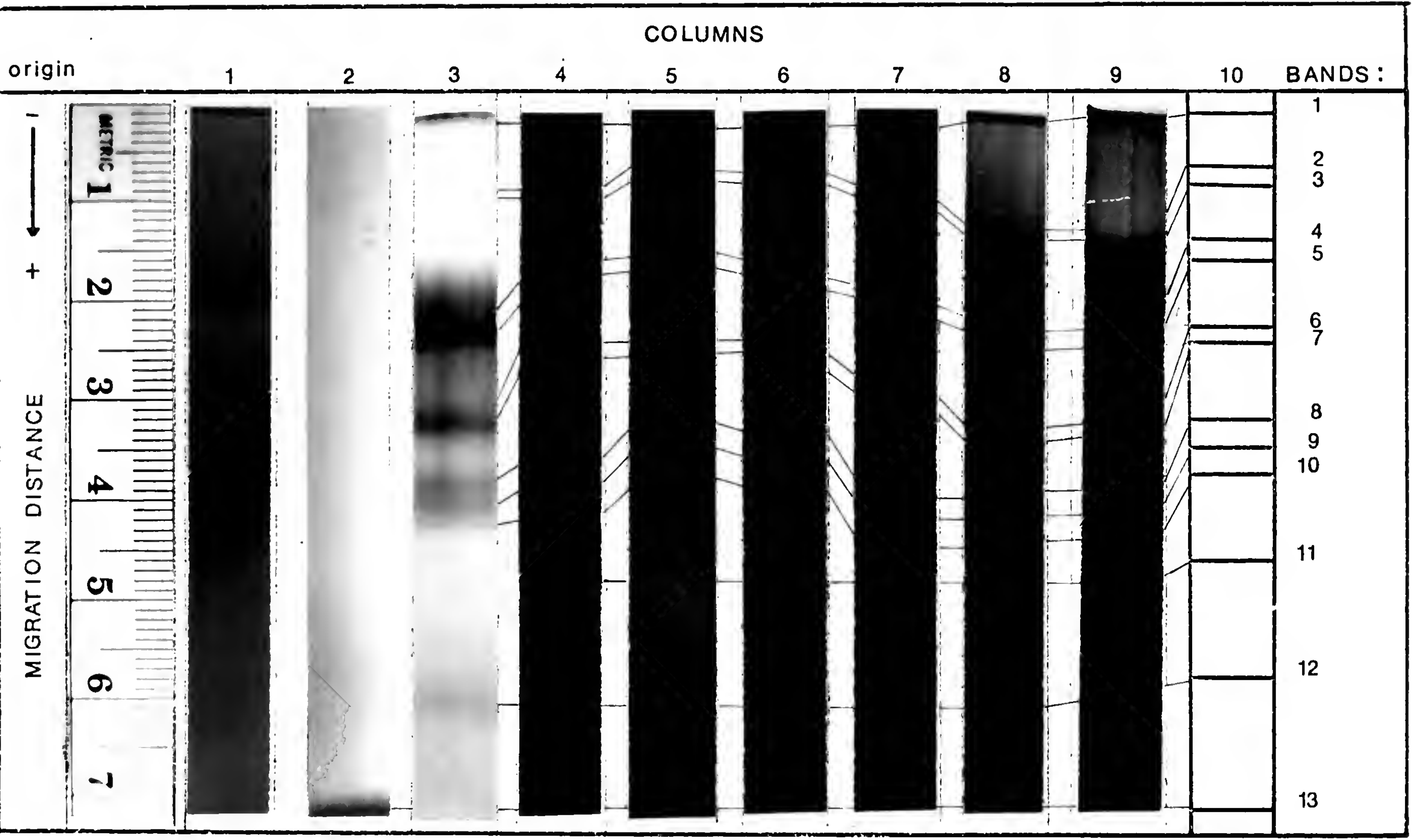

COLUMNS
origin
1 2 3 4 5 6 7 8 9 10
BANDS:
1 2 3 4 5 6 7 8 9 10 11 12 13
METRIC 1 2 3 4 5 6 7
MIGRATION DISTANCE
- +

Figure 8. RNA synthesis in *Acetabularia*. Autoradiographs of polyacrylamide electrophoresis patterns.

Column 1: *A. crenulata*: whole plants (autoradiograph)

Column 2: *A. crenulata*: whole plants (staining pattern)

Column 3: *A. crenulata*: whole plants (autoradiograph, corresponding to Column 2)

Column 4: *A. crenulata*: anucleate plants (autoradiograph)

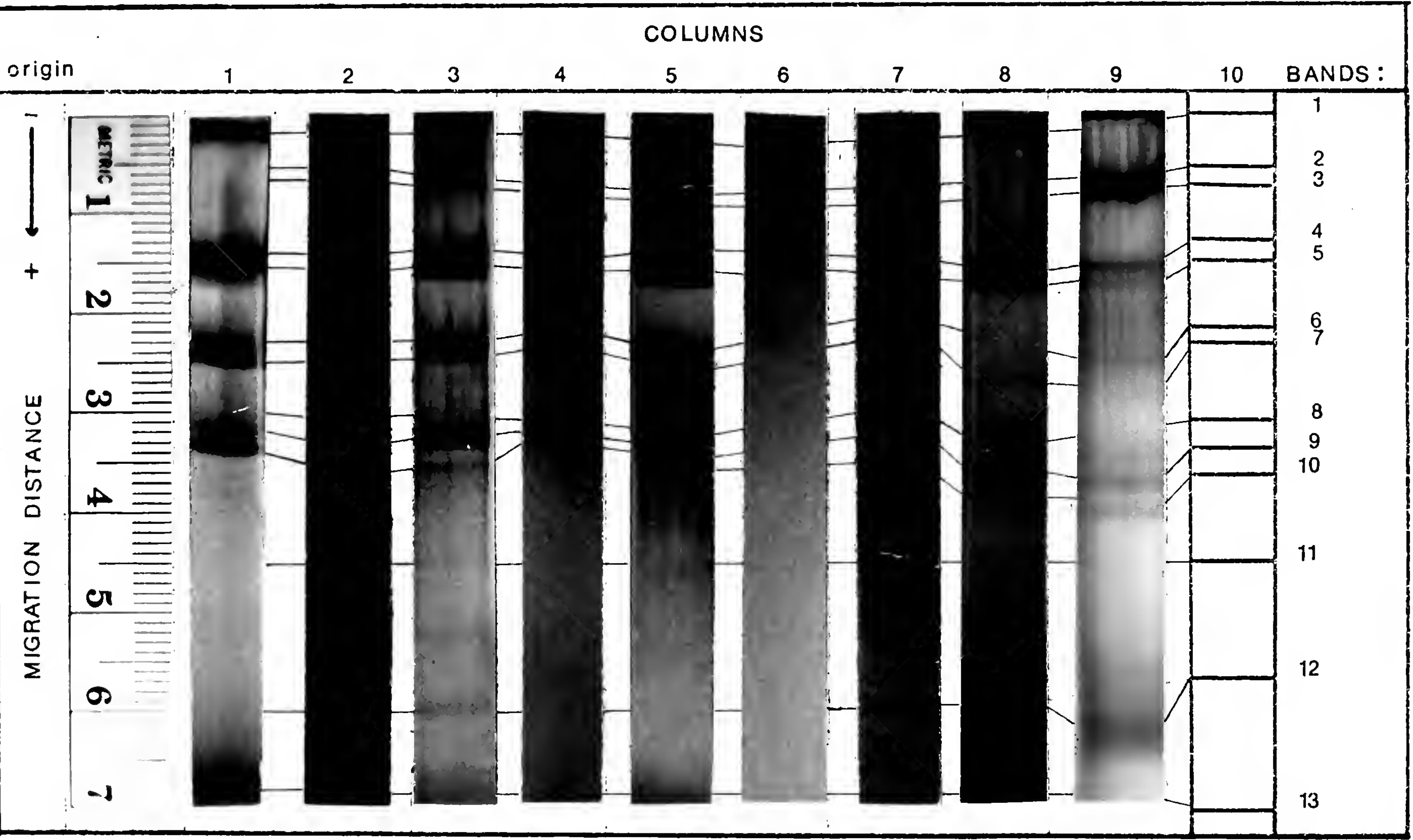
Column 5: *A. crenulata*: whole plants (autoradiograph)

Column 6: *A. crenulata*: whole plants (autoradiograph; RNAase treated; corresponding to Column 5)

Column 7: *A. mediterranea*: whole plants (autoradiograph)

Column 8: *A. mediterranea*: anucleate plants (autoradiograph)

Column 9: *A. mediterranea*: whole plants (carrier) (staining pattern corresponding to Column 8)


COLUMNS
origin
1 2 3 4 5 6 7 8 9 10
BANDS:
1 2 3 4 5 6 7 8 9 10 11 12 13
- +
MIGRATION DISTANCE
METRIC 1 2 3 4 5 6 7

acrylamide gel electrophoresis of nucleic acids from Acetabularia. A maximum of 13 bands could be resolved with this method under optimal conditions. Because of the uncertain identity of some of these bands, not all of them will be discussed here.

The bands 1, 2 and 3 (Figure 7, Figure 8) are unidentified high molecular weight macromolecules, which incorporate C^{14} uridine and do not completely disappear with RNAase treatment. Band 2 stains pink with "Stains-all", indicating that it is a protein.

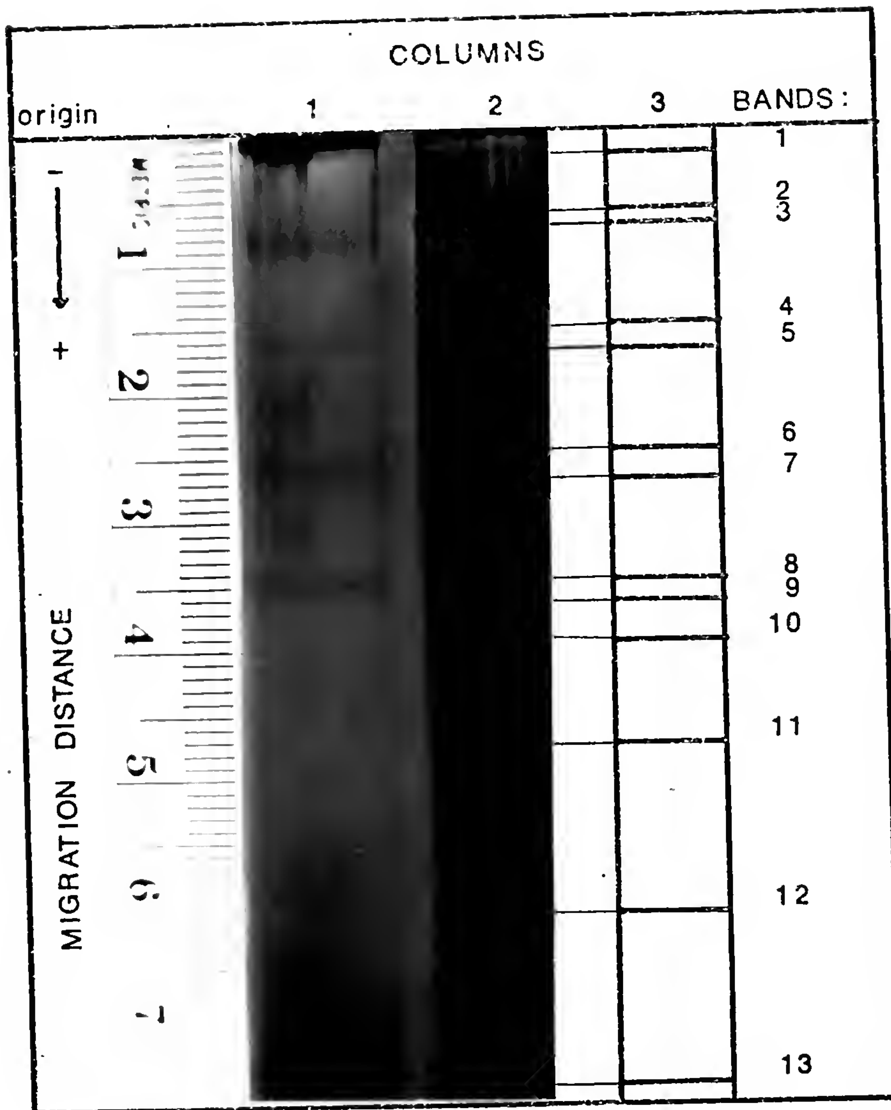
There are two DNA bands in Acetabularia (4 and 5). These bands were identified as DNA according to their disappearance after DNAase treatment and persistence after RNAase treatment. The two DNA bands may represent the nuclear and the chloroplastic DNA. In Acetabularia the isotope from uridine which carries a general label of H^3 or C^{14} becomes incorporated into DNA (Figure 8).

According to Loening (1968) polyacrylamide electrophoresis can be used for the comparative estimation of molecular weights or sedimentation constants of r-RNA of very different base composition. At the salt concentrations used for electrophoresis, changes in the secondary structure of RNA which affect the mobility of the molecule are very small. According to Loening (1968), Bishop et al. (1967), and Peacock and Dingman (1968) the migration distances of high molecular weight RNA in polyacrylamide gels after electrophoresis are linearly related to the sedimentation coefficient or the reciprocal of the log of the molecular weights. Therefore, E. coli RNA can be used as a standard for determining the S values of RNA from other sources. Figure 9 shows an electrophoretic run of nucleic acids extracted from E. coli B/r and nucleic acids from A. mediterranea. The graph in Figure 10 was obtained by plotting the migration distances of the

Figure 9. Comparison of electrophoretically separated nucleic acids from *Escherichia coli* B/r and *Acetabularia mediterranea*.

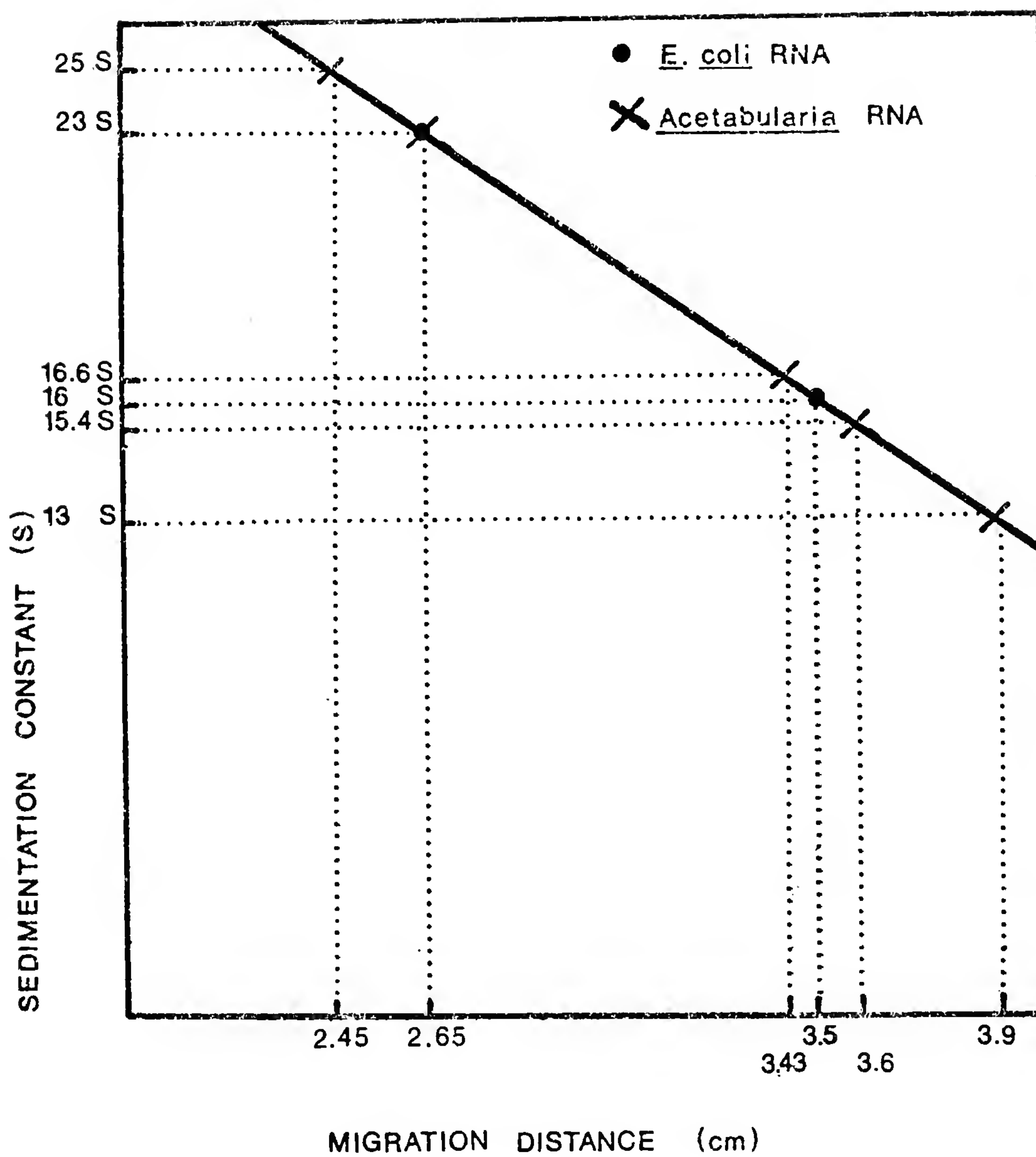
Column 1: *E. coli* nucleic acids
2.65 cm = 23 S
3.5 cm = 16 S

Column 2: *Acetabularia* nucleic acids



COLUMNS
origin
1
2
3
BANDS:
1
2
3
4
5
6
7
8
9
10
11
12
13
- +
MIGRATION DISTANCE

Figure 10. Calibration curve for high molecular weight RNA. This graph was obtained by plotting the migration distances of the *E. coli* RNA bands from Figure 9 versus the sedimentation coefficients corresponding to these RNA species.


E. coli RNA
Acetabularia RNA
25 S
23 S
16.6 S
16 S
15.4 S
13 S
SEDIMENTATION CONSTANT (S)
2.45
2.65
3.43
3.5
3.6
3.9
MIGRATION DISTANCE (cm)

E. coli RNA bands from Figure 9 versus the sedimentation coefficients corresponding to these RNA species. From this graph the sedimentation coefficients of the nucleic acid bands from Acetabularia could be estimated. The bands correspond to the following S values: Band 6 = 25 S = 2.45 cm, Band 7 = 23 S = 2.65 cm, Band 8 = 17 S = 3.43 cm, Band 9 = 15 S = 3.6 cm, Band 10 = 13 S = 3.9 cm. Therefore, according to their migration distances when compared to RNA from bacteria and in agreement with the literature the bands 6, 7, 8, 9 and 10 appear to represent the ribosomal RNA species of Acetabularia and possibly the stable 15 S RNA obtained on sucrose gradients. This fact that there are at least 5 RNA bands in the ribosomal RNA size range in Acetabularia whereas there are only two bands (23 S and 16 S) in bacteria will be discussed later. Usually several low molecular weight RNA species were found in polyacrylamide gels after electrophoresis (Bands 11, 12 and 13). The fastest moving one (13) probably represents the s-RNA or 5 S r-RNA fraction of the plants; the others may be degradation products. Band 12 is obtained quite consistently, it may correspond to the 9 S species found by other workers.

No significant difference is noted in the nucleic acid banding pattern from the two species. In general it was found that material extracted from A. mediterranea gave more consistent results.

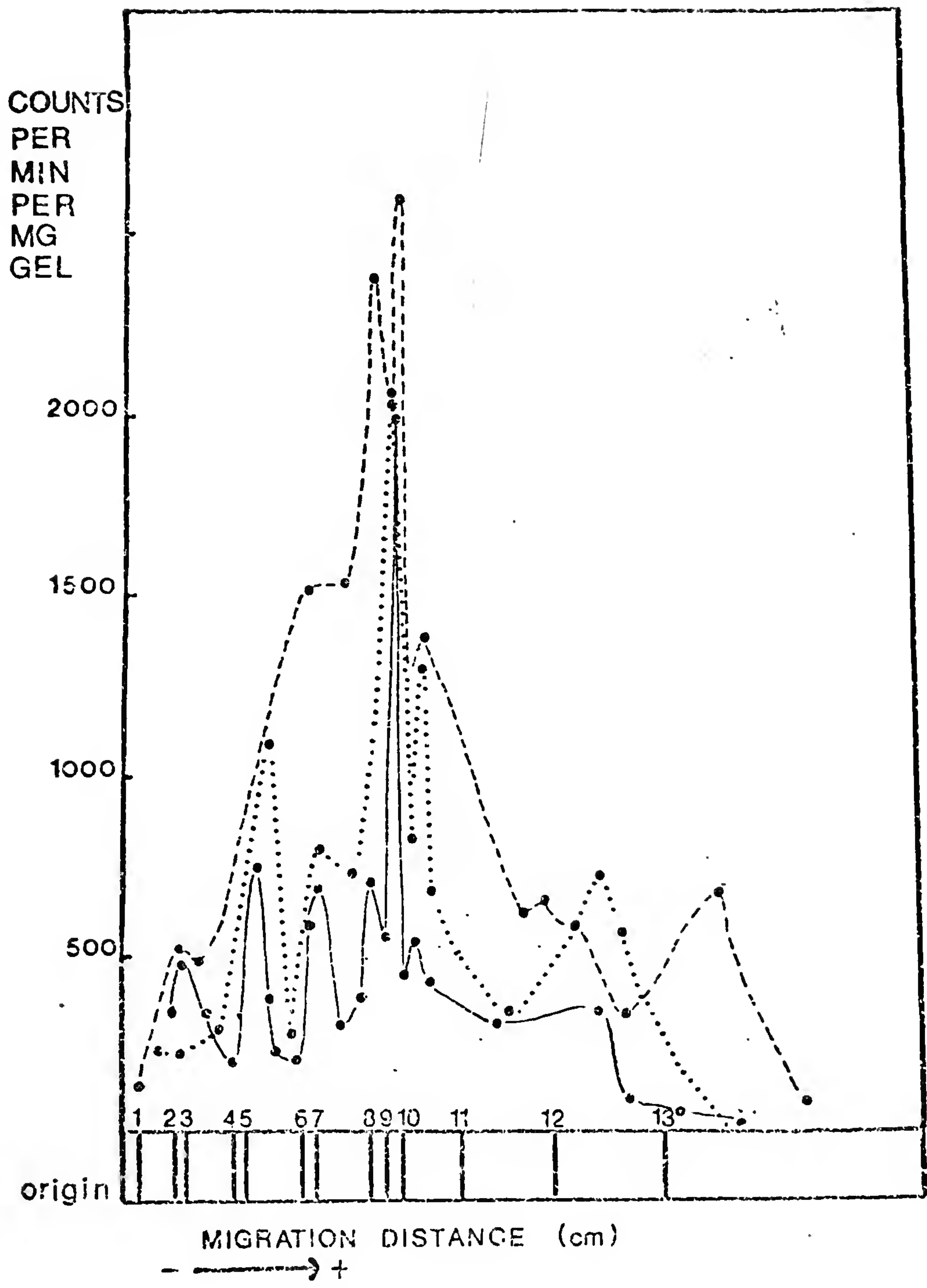
Part of the work was concentrated on comparing RNA synthesis in whole plants with enucleated plants in the two species. Figure 11 shows typical results obtained from such experiments. The data represent the radioactivities of gel slices after electrophoresis of the nucleic acids. It is difficult to interpret these results as to qualitative differences in RNA synthesis between whole cells and enucleated cells. Possibly

Figure 11. RNA synthesis in *Acetabularia*. Radioactivity pattern of the polyacrylamide electrophoresis.

——— *A. crenulata* whole cells

.... *A. crenulata* enucleated cells

----- *A. mediterranea* whole cells


COUNTS
PER
MIN
PER
MG
GEL
2000
1500
1000
500
1
2
3
4
5
6
7
8
9
10
11
12
13°
origin
MIGRATION DISTANCE (cm)
- → +

the slicing method is not sensitive enough to resolve the bands adequately.

Figure 8 shows the results obtained by autoradiographing the dried polyacrylamide gel slabs after electrophoresis. It can be noticed that the labeling pattern of whole cells in both species is similar to the corresponding staining pattern (Columns 2, 3, 7, 9, Figure 8). The staining pattern corresponding to Column 5 did not show separation of the bands 6, 7, 8, 9, 10. It can also be seen that RNAase treatment (Column 6) completely removes the uridine-labeled bands 6 through 10 as well as the low molecular weight RNA species.

The labeling pattern obtained by incubating enucleated A. crenulata plants with C^{14} uridine and electrophoresing the nucleic acids extracted from them is shown in the 4th column of Figure 8. The staining pattern from this experiment displayed bands 6, 7, 8, 9 and 10 clearly, therefore the display of only 2 RNA bands on the autoradiograph cannot be explained by an unsatisfactory electrophoretic resolution of the RNA species. The significance of this result will be discussed in a subsequent section of this dissertation. The same experiment was also performed with A. mediterranea plants, the results of which are shown in Column 8 and Column 9 of Figure 8. Column 8 represents the autoradiograph of the labeling pattern, and Column 9 represents the corresponding staining pattern. The labeled bands are the DNA band 5 and the RNA bands 7, 9, and 10. Thus, these are the faster moving or lower molecular weight species of each set of bands. This phenomenon will be discussed below. Also an attempt will be made to explain the differences between the results obtained with A. crenulata and those obtained with A. mediterranea

DISCUSSION

If the ability of enucleated *Acetabularia* cells to synthesize RNA is to be evaluated, precautions have to be taken to assure that the enucleated cells are free from nuclei or nuclear fragments. The procedure of enucleation in *Acetabularia* satisfies this requirement. Another point of importance is that the cells and especially the enucleated ones are in good condition (Schweiger and Bremer, 1960, 1961). If this is not the case, misleading results may be observed. A third requirement is that the cells are free from contamination with micro-organisms. Therefore, sterility tests had to be performed as described under "Materials and Methods".

The results of this work demonstrate that polyacrylamide gel electrophoresis is the method of choice if the synthesis of several RNA species of similar molecular weights is to be studied. From the literature review on metabolic studies on nucleic acids in *Acetabularia*, it was obvious that density gradient centrifugation could not resolve all of the nucleic acid species present in this plant in sufficient detail. Other workers have fractionated 3 (Schweiger *et al.*, 1967b; Schweiger and Berger, 1964; Berger, 1967; Janowski and Bonotto, 1970; and Baltus *et al.*, 1968) and at the most 4 (Berger, 1967) species of RNA from *Acetabularia* by sucrose density gradient sedimentation. These are believed to represent the two ribosomal RNA species with S values of 23 and 16 and a soluble 4 S RNA species. The fourth RNA molecule has an S value of 9. Polyacrylamide gel electrophoresis,

on the other hand, clearly fractionates the nucleic acids from Acetabularia into 13 bands, 10 of which appear to be RNA, the other two being DNA and the remaining one being a high molecular weight protein. The S values obtained for the bands 6, 7, 8, 9, and 10 are comparable to the S values of the RNA species obtained by sucrose density gradient sedimentation. It would appear that the bands 6 and 7 are the ones that sediment together as one 25 S or 23 S peak and that the bands 8, 9 and 10 sediment as the 16 S or 15 S peak on the sucrose gradient.

Work on the molecular weights of the ribosomal RNA of a wide range of species suggests that there are at least four classes of ribosomes in nature. The bacterial or chloroplast ribosomes sediment at 70 S and contain 23 S and 16 S ribosomal RNA. The ribosomes of plants and animals sediment at 80 S, those of the plants contain 25 S and 16 S or 18 S RNA and those of the animals 28 S and 18 S RNA. Ribosomes from rat liver mitochondria sediment at 55 S. The presence of several components for each ribosomal RNA species in Acetabularia was suspected from studies on the enucleated cells and Schweiger and Dillard (1968) have proposed the occurrence of two components for each ribosomal RNA species from kinetic studies. Loening and Ingle (1967) and Ingle et al. (1970) observed the presence of additional RNA components in green compared to non-green plant tissues and suggested that they were associated with the chloroplasts. Since the work reported in this dissertation was completed, Woodcock and Bogorad (1970) and Schweiger (1970) have also used polyacrylamide gel electrophoresis to fractionate the RNA from A. mediterranea cells. Woodcock and Bogorad (1970) observed a 25 S and an 18 S RNA band. In addition, two minor bands were found

in the 16 S and 12-14 S regions. These were interpreted as being degradation products of the higher molecular weight RNA's.

Schweiger (1970) however, separated each of the two r-RNA peaks into two peaks corresponding to 25 S and 23 S for the faster sedimenting peak and to 18 S and 16 S for the more slowly sedimenting peak. These workers concluded that the slower moving RNA component of each of the two ribosomal RNA species, represents the ribosomal RNA coded for by the nucleus whereas the faster moving ones appear to be synthesized by the chloroplasts. The results presented in this dissertation are therefore interpreted as follows: The Acetabularia RNA bands 6 and 8 with approximate sedimentation constants of 25 S and 17 S represent the two ribosomal RNA species coded for by nuclear genes. The bands 7, 9 and 10 with approximate sedimentation constants of 23 S, 15 S and 13 S represent the ribosomal RNA species coded for by chloroplastic genes or possibly mitochondrial genes.

If this interpretation is correct, the plants should not be capable of synthesizing the "nuclear" ribosomal RNA species in the absence of the nucleus.

One cannot, however, exclude the possibility that the additional bands are specific degradation products of higher molecular weight RNA. Hartman et al. (1970) for example observed that hydrolysis of E. coli r-RNA with pancreatic ribonuclease under controlled conditions leads to specific fragments. Since hydrolysis of intact ribosomes leads to fewer fragments these workers conclude that there are several specific sites available for ribonuclease attack or degradation, which are protected by protein in the intact ribosome.

Enucleated A. crenulata plants are no longer capable of synthesizing some of the RNA species that are synthesized by intact algae.

(Figure 8, Column 4). It is, however, impossible to decide from this experiment which of the bands designated as RNA are missing. One possible conclusion from this experiment is that enucleated Acetabularia cells can no longer synthesize any RNA. The labeled bands would then have to be accounted for by incorporation of C^{14} uridine into the RNA of the contaminating micro-organisms. It is also possible that the enucleated cells used for this experiment were not in good condition and therefore only the two diffuse RNA bands could be obtained. Possibly the optimal conditions for enucleated A. crenulata cells to synthesize RNA differ from those determined for enucleated A. mediterranea cells by Schweiger and Bremer (1960, 1961). As stated before, the corresponding staining pattern of this experiment displayed a very satisfactory electrophoretic resolution of the RNA species. The bands obtained with the stain were of course due to the RNA extracted from the intact carrier A. crenulata plants which were added to the labeled anucleate fragments after incubation in C^{14} uridine. Lastly it is also possible that the two diffuse RNA bands represent the r-RNA species synthesized by the chloroplasts in A. crenulata in the absence of the nucleus.

Enucleated A. mediterranea plants are no longer capable of synthesizing the bands 6 and 8 (Figure 8, Column 8). These bands are believed to be the "nuclear" r-RNA bands, since they are the higher molecular weight components of each r-RNA species. This experiment thus proves that enucleated A. mediterranea fragments do synthesize RNA and this RNA is most likely coded for by the chloroplastic DNA since the bands represent the lower molecular weight RNA components of the two r-RNA species (23 S, 15 S and 13 S).

It also appears likely from this experiment, that the enucleated A. mediterranea cells do not synthesize the slower moving DNA band (band 4). Possibly this band is the nuclear DNA of the plant, whereas the other DNA band is the chloroplastic DNA.

Band 10 with an approximate sedimentation coefficient of 13 is not unique in Acetabularia. It was also observed in green plant tissue by Loening and Ingle (1967) and Ingle et al. (1970). This band may be identical with the 15 S stable RNA found by other workers on sucrose gradients (Janowski and Bonotto, 1970; and Farber, 1969), even though its sedimentation constant is lower (13 S), as was estimated from the electrophoresis experiment. It is unlikely that it is the proposed stable m-RNA carrying the species-specific information for cap formation since Janofski and Bonotto (1969) and Farber (1969) found that this stable 15 S RNA is synthesized in anucleate fragments as well as in whole plants. Band 10 is also synthesized in A. mediterranea anucleate fragments as can be seen on the autoradiograph Figure 8, Column 8. Information that is dependent on the nucleus cannot be synthesized in its absence.

However, since Farber (1969) observed that the 15 S RNA fraction appears to be used up during cap formation the 15 S RNA may be species-unspecific information for cap formation. In this case one would have to assume that the chloroplasts supply this information since Farber (1969) also found that this RNA fraction is synthesized in the chloroplasts.

It can be concluded from the results presented in this dissertation that much additional work will be required before we can understand the function of all of these molecules in Acetabularia. Polyacrylamide

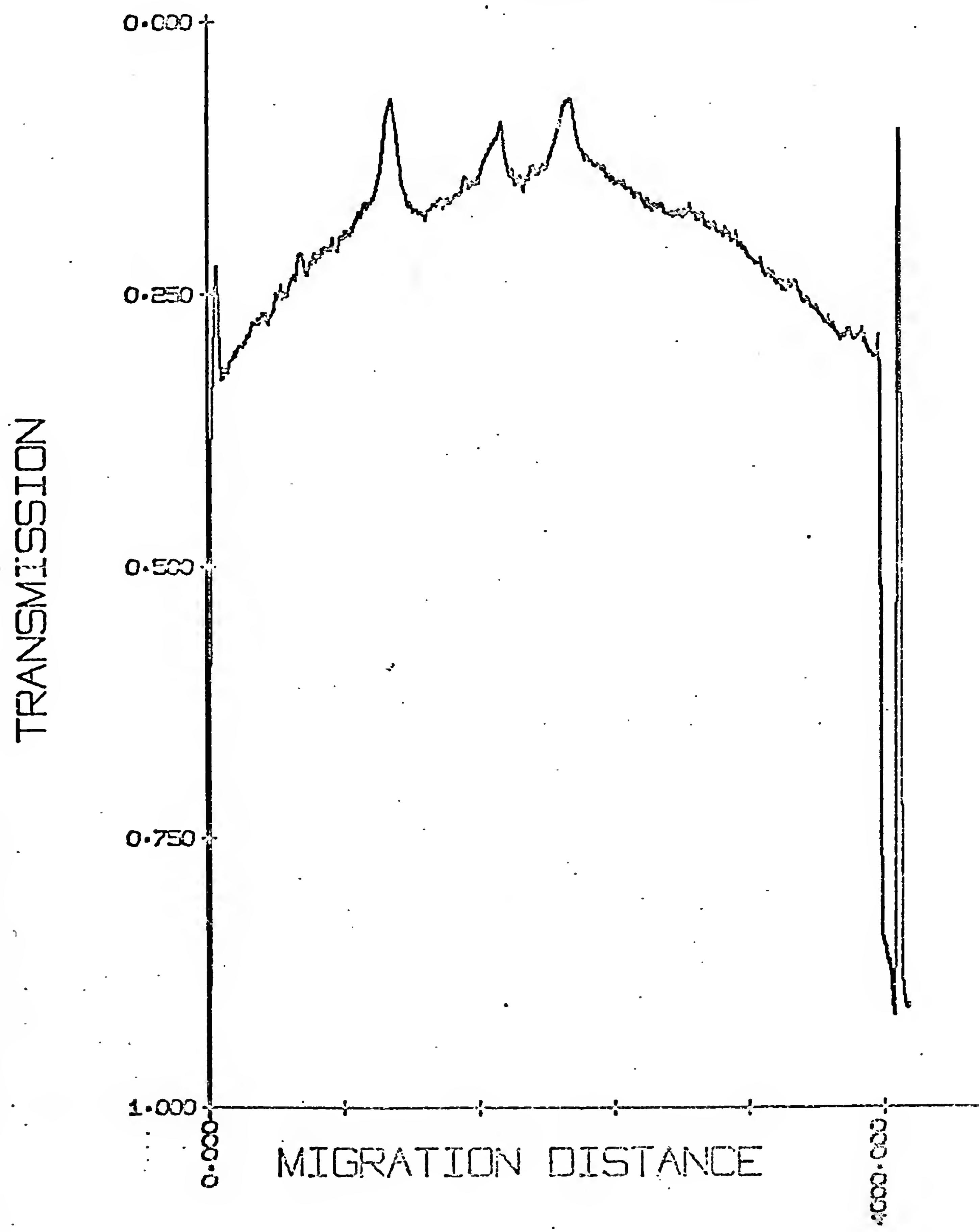
gel electrophoresis appears to be a promising approach for the analysis of RNA molecules in this plant. Eventually it might be possible to find the "mysterious" "morphogenetic substances." It has been suggested that the mature cap supplies the nucleus in the rhizoid with substances that will induce mitotic nuclear division (Hämmerling, 1939). It is possible that this feed back mechanism is in some way related to the production of m-RNA or "morphogenetic substances."

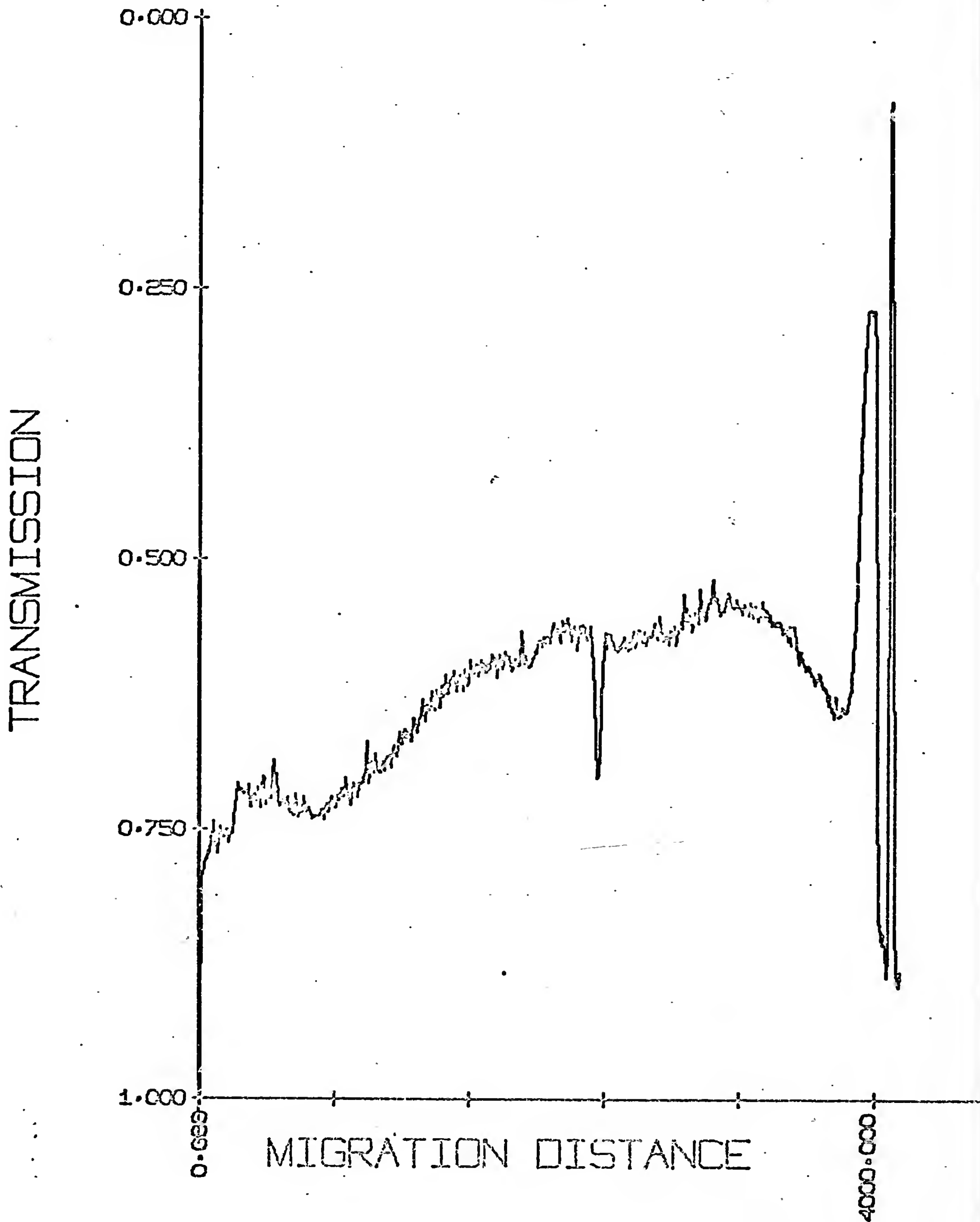
It might be worthwhile to analyze the nucleolar RNA of this organism, since the nucleolus is such a prominent structure in the nucleus of the older plants. Aside from being responsible for r-RNA synthesis the nucleolus may have other functions related to the production of the "morphogenetic substances."

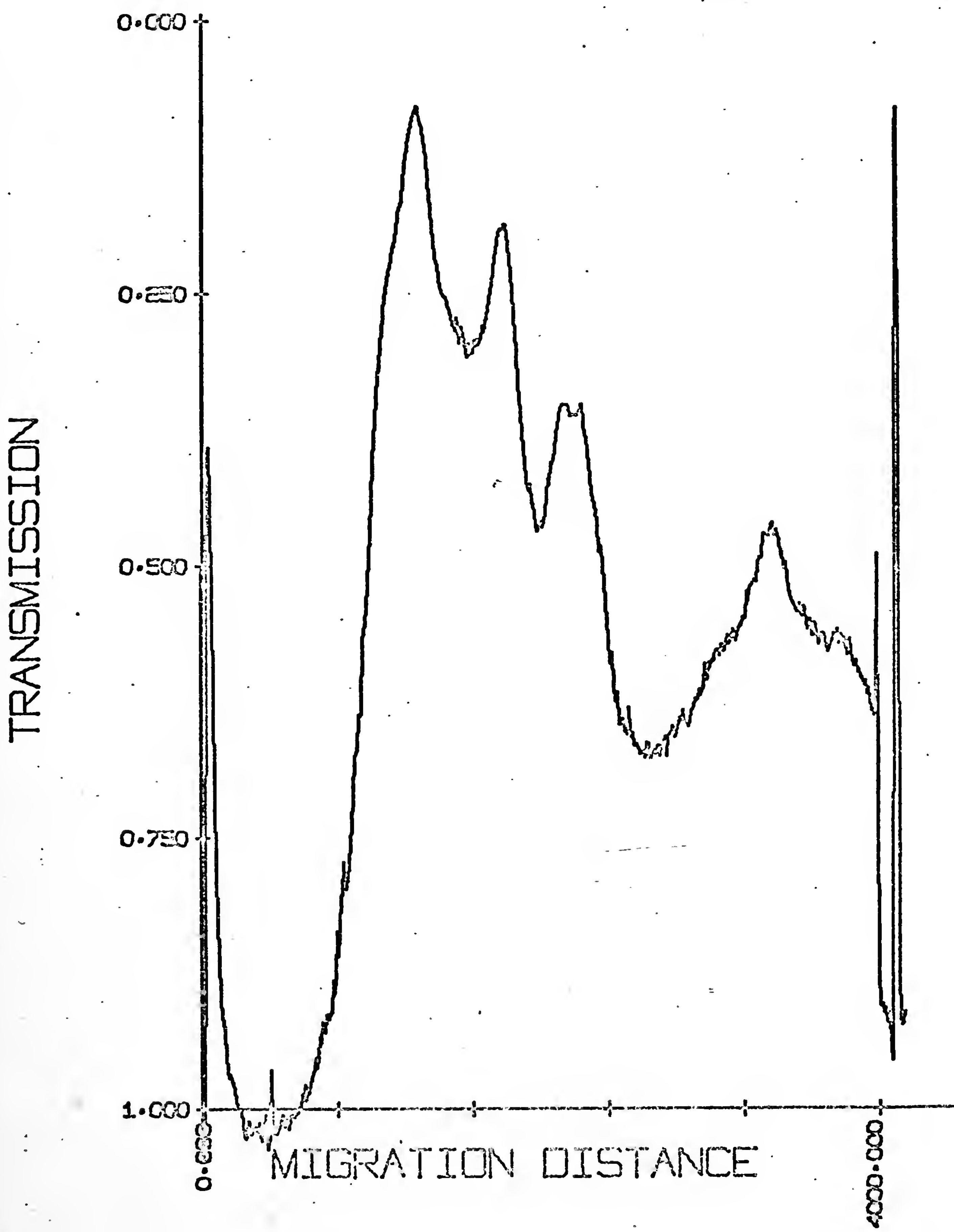
SUMMARY

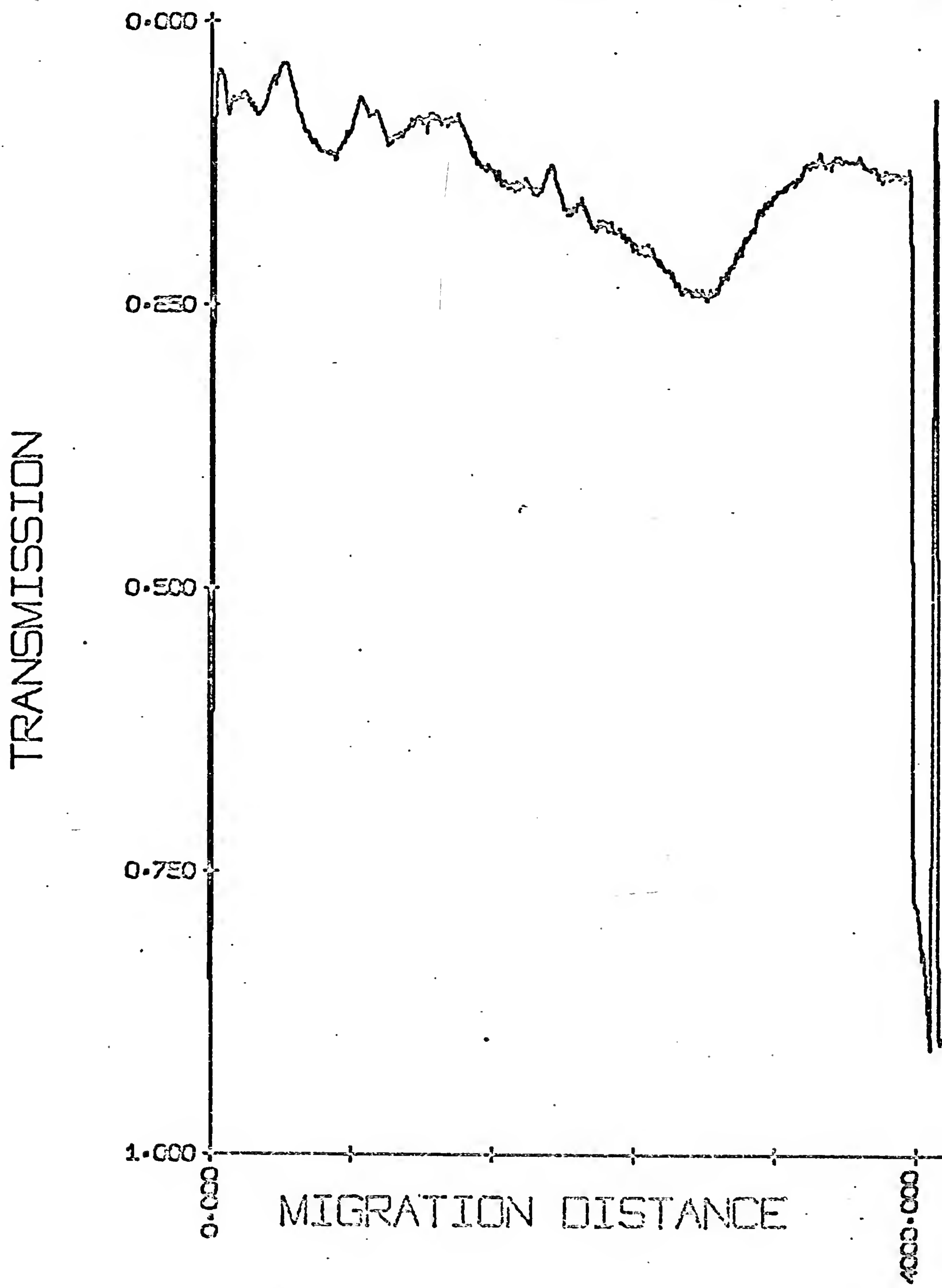
1. Nucleic acids were isolated from Acetabularia homogenates by precipitation with 2 M LiCl.
2. Polyacrylamide gel electrophoresis was used to fractionate the nucleic acid species present in the plant. This method yielded 13 bands of which the sedimentation coefficients could be esimated by comparison with a standard.
3. The two r-RNA species could be fractionated into two components each. These RNA components possess approximate sedimentation coefficients of 25 S, 23 S, 17 S and 15 S.
4. A 13 S RNA molecule was also observed.
5. There was no significant difference in the RNA species present in A. crenulata and A. mediterranea.
6. Enucleated A. crenulata cells no longer synthesized all of the RNA species synthesized in whole plants.
7. Enucleated A. mediterranea cells no longer synthesized the 25 S and 17 S RNA species, which appear to be nuclear in origin. The 23 S and 15 S RNA species are believed to be synthesized by chloroplasts.
8. Enucleated A. mediterranea cells were able to synthesize the 13 S RNA components.

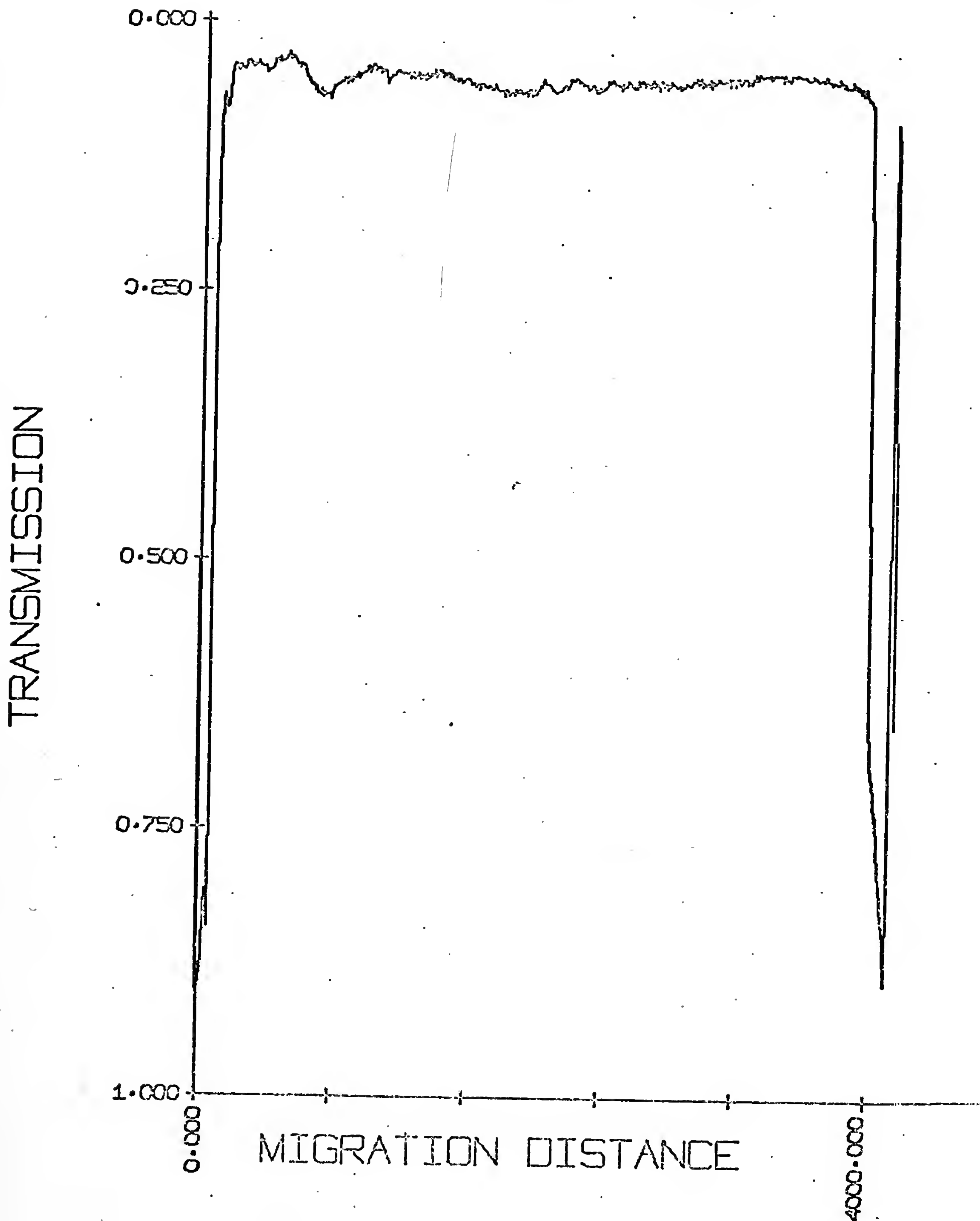
APPENDIX

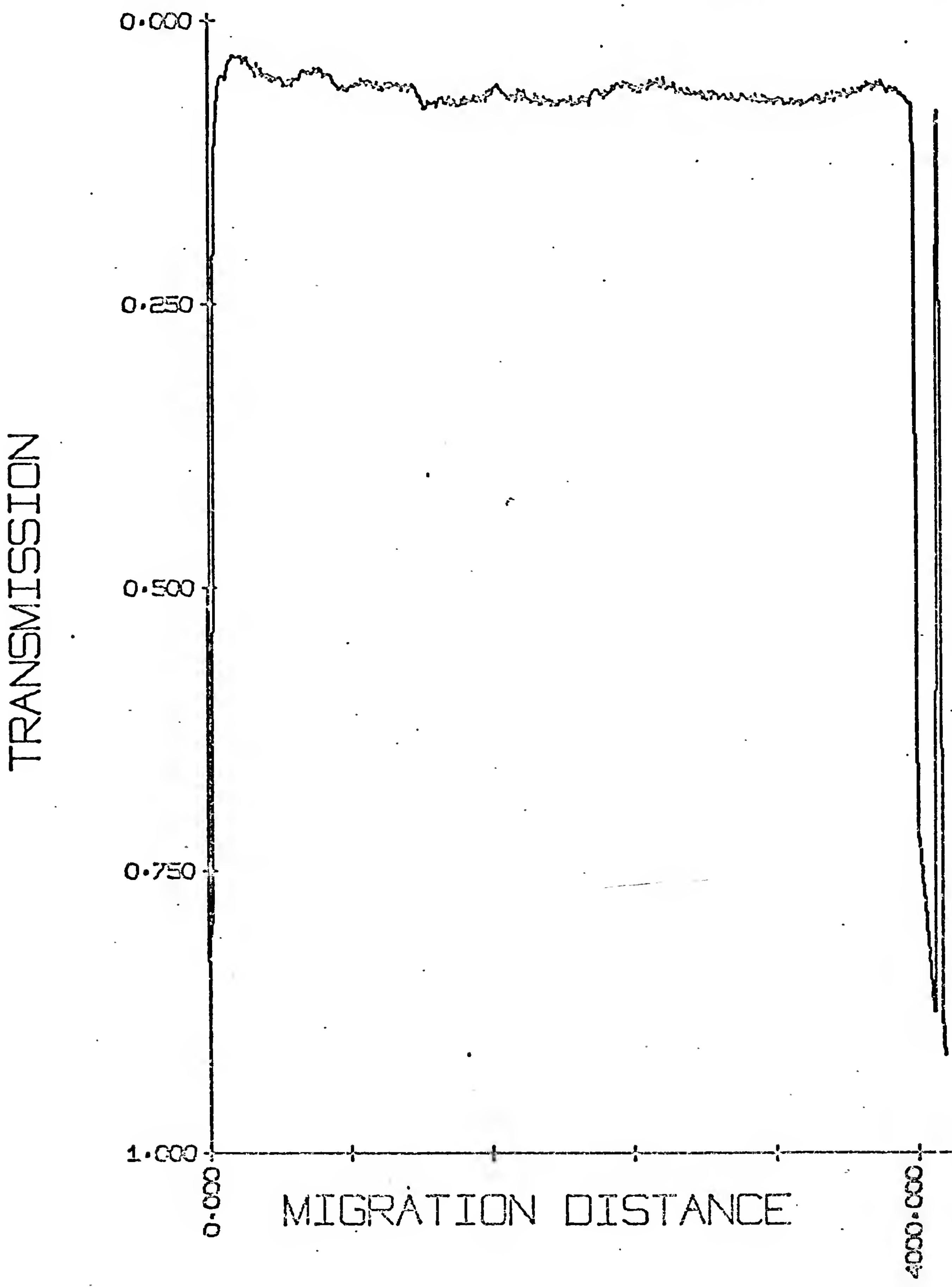

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7; COLUMN 1.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

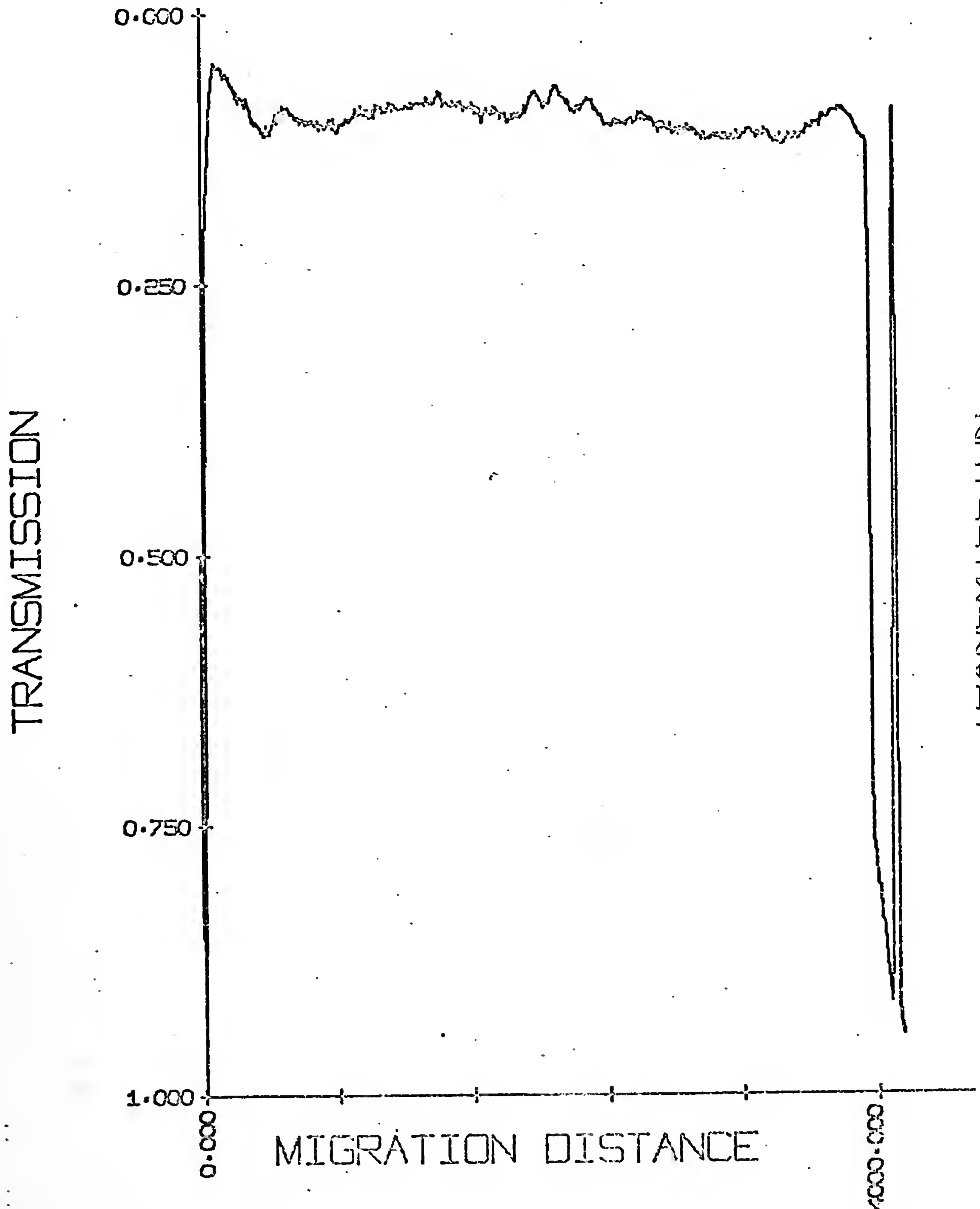

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 2.
TRANSMISSION
0.000
0.250
0.500
0.750
1.000
MIGRATION DISTANCE
0.000
4000.000

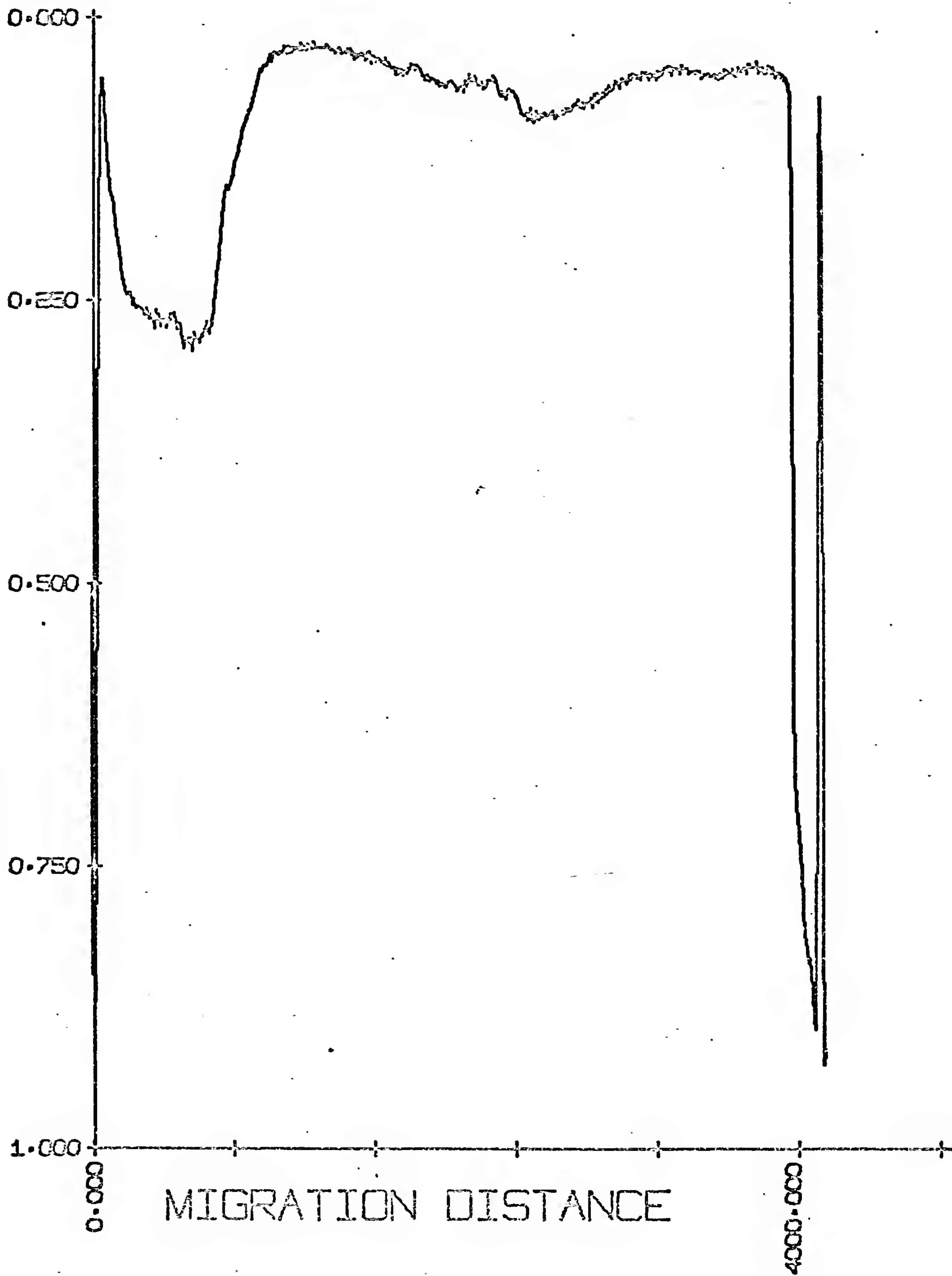

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 3.
TRANSMISSION
0.000
0.250
0.500
0.750
1.000
MIGRATION DISTANCE
0.000
4000.000

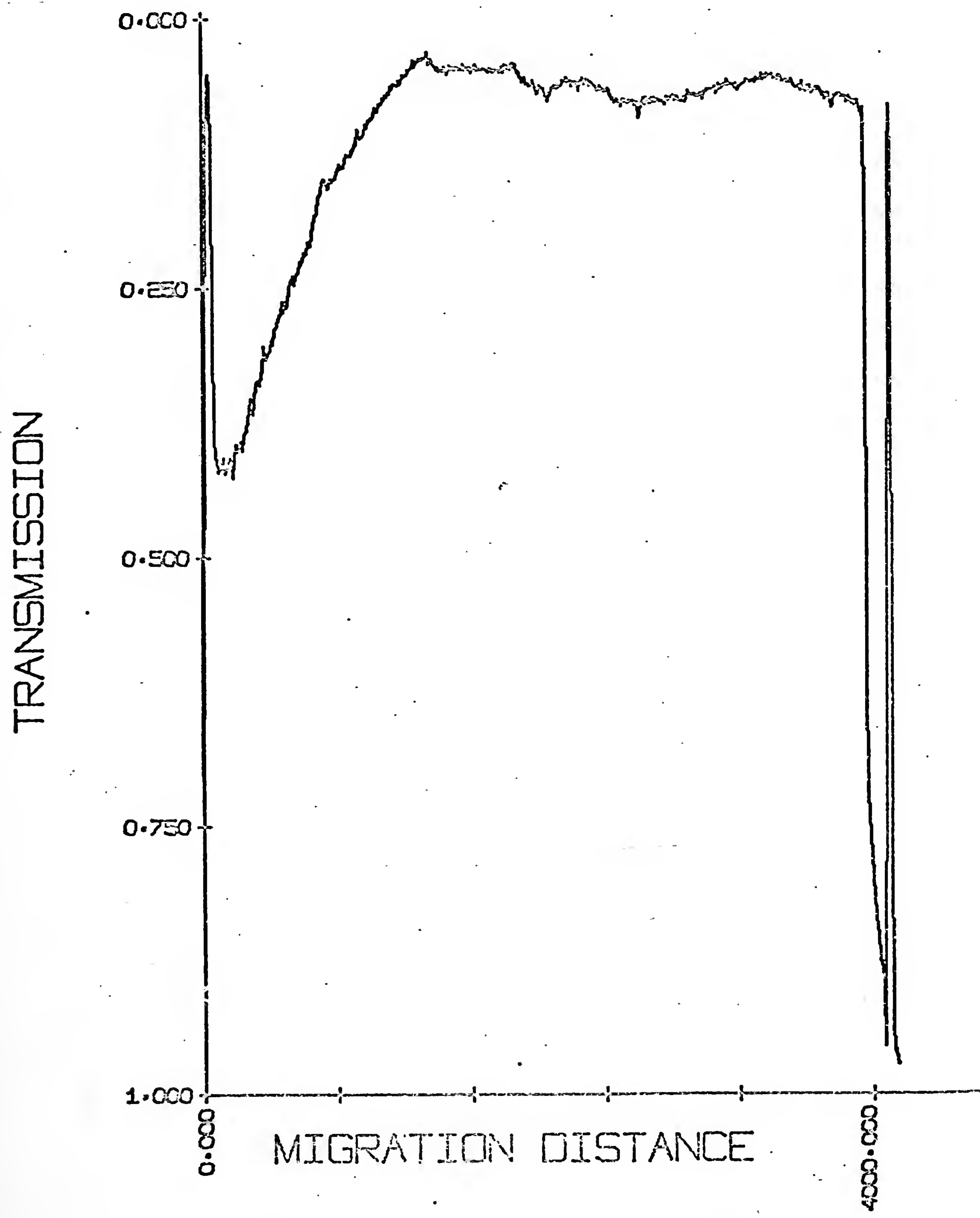

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 4.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

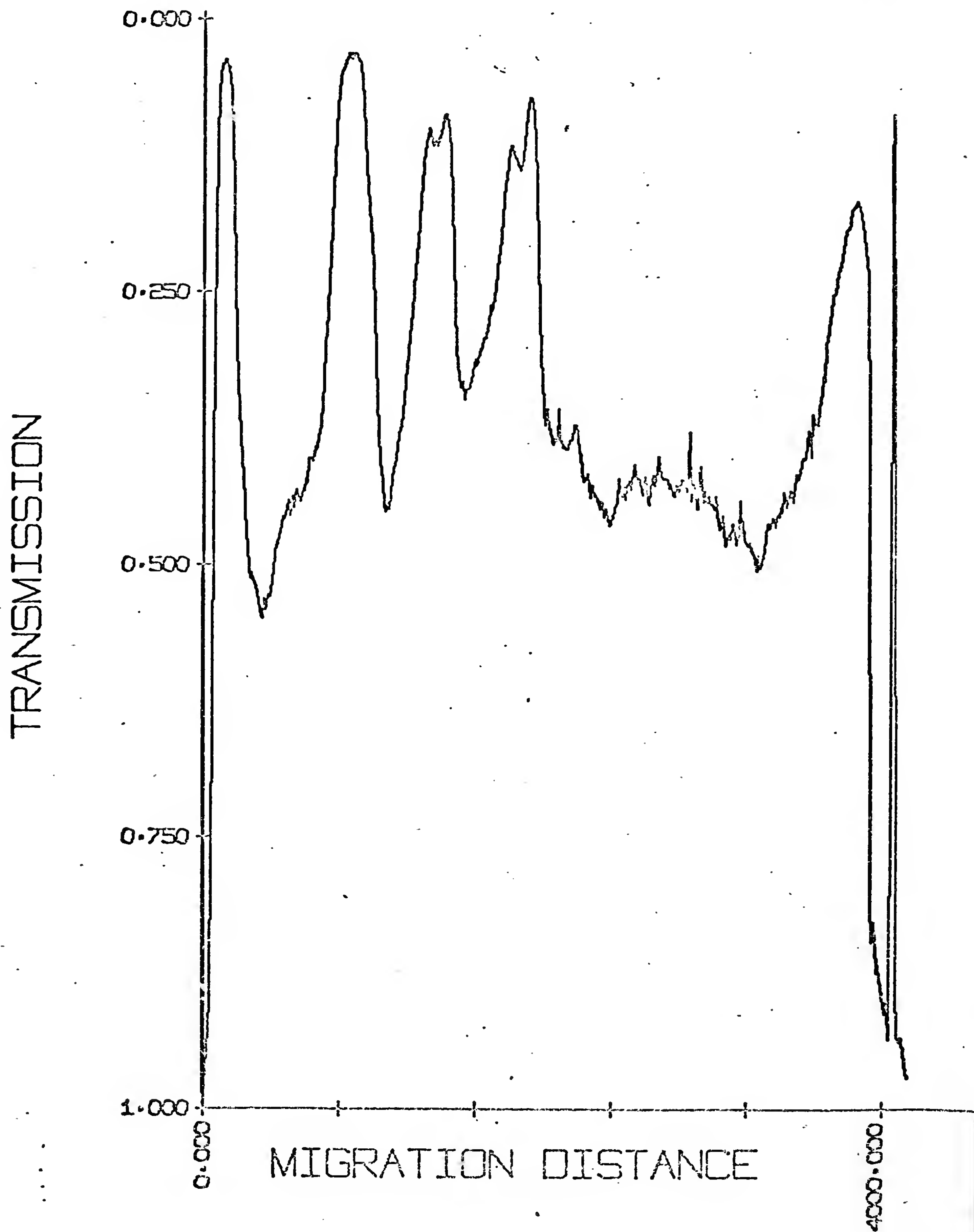

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 5.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
MIGRATION DISTANCE
4000.000

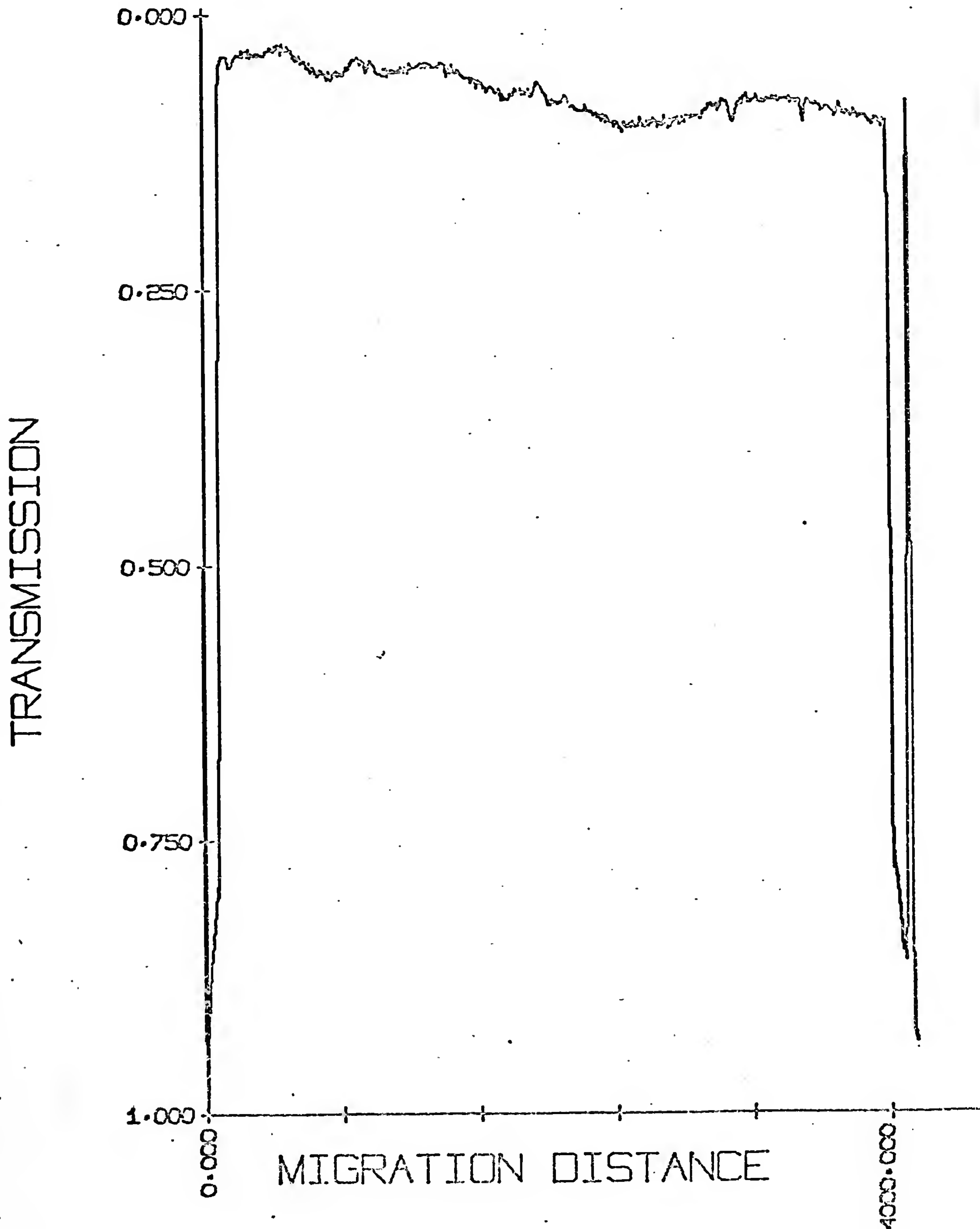

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 6.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

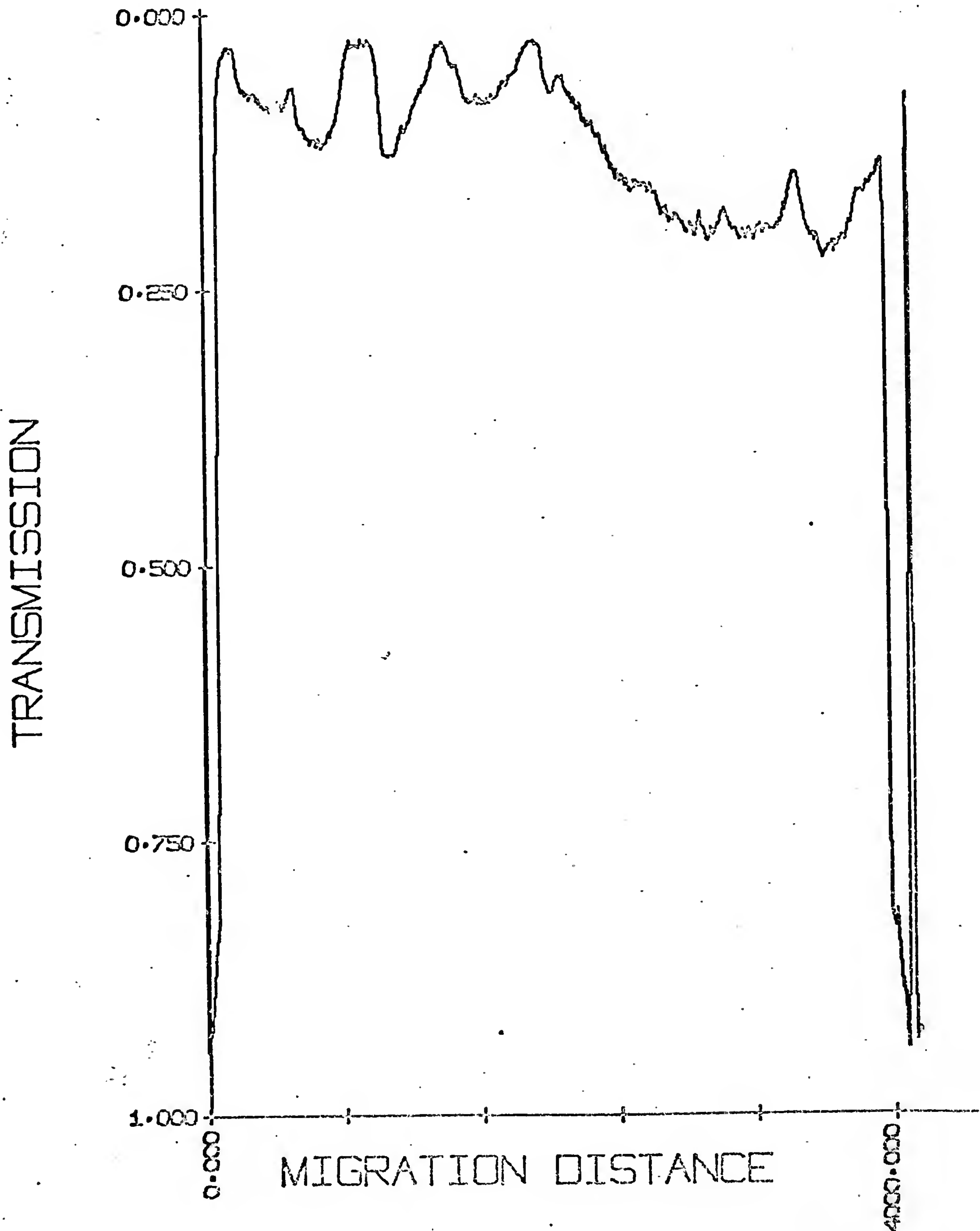

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN 7.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

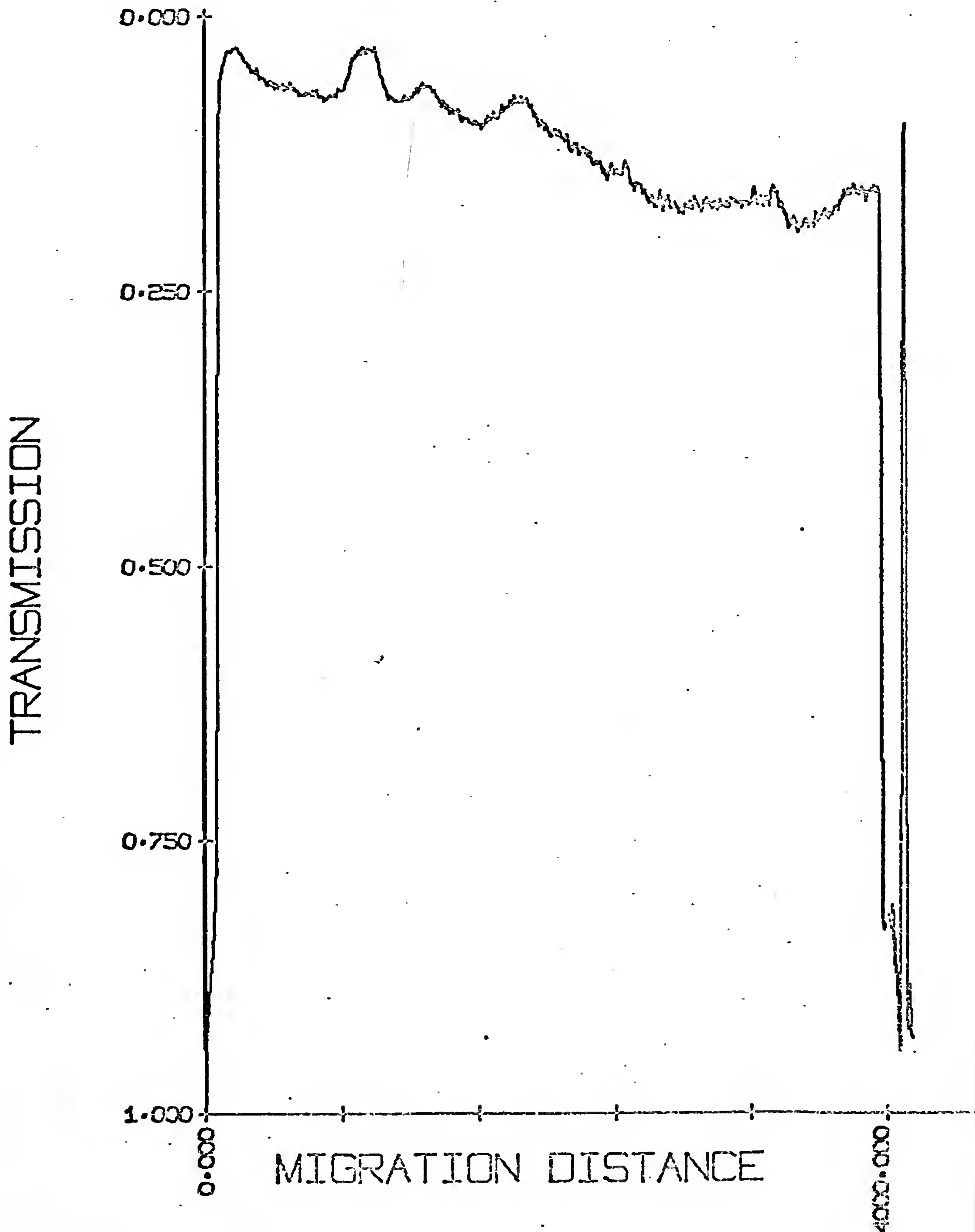

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 7, COLUMN B.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

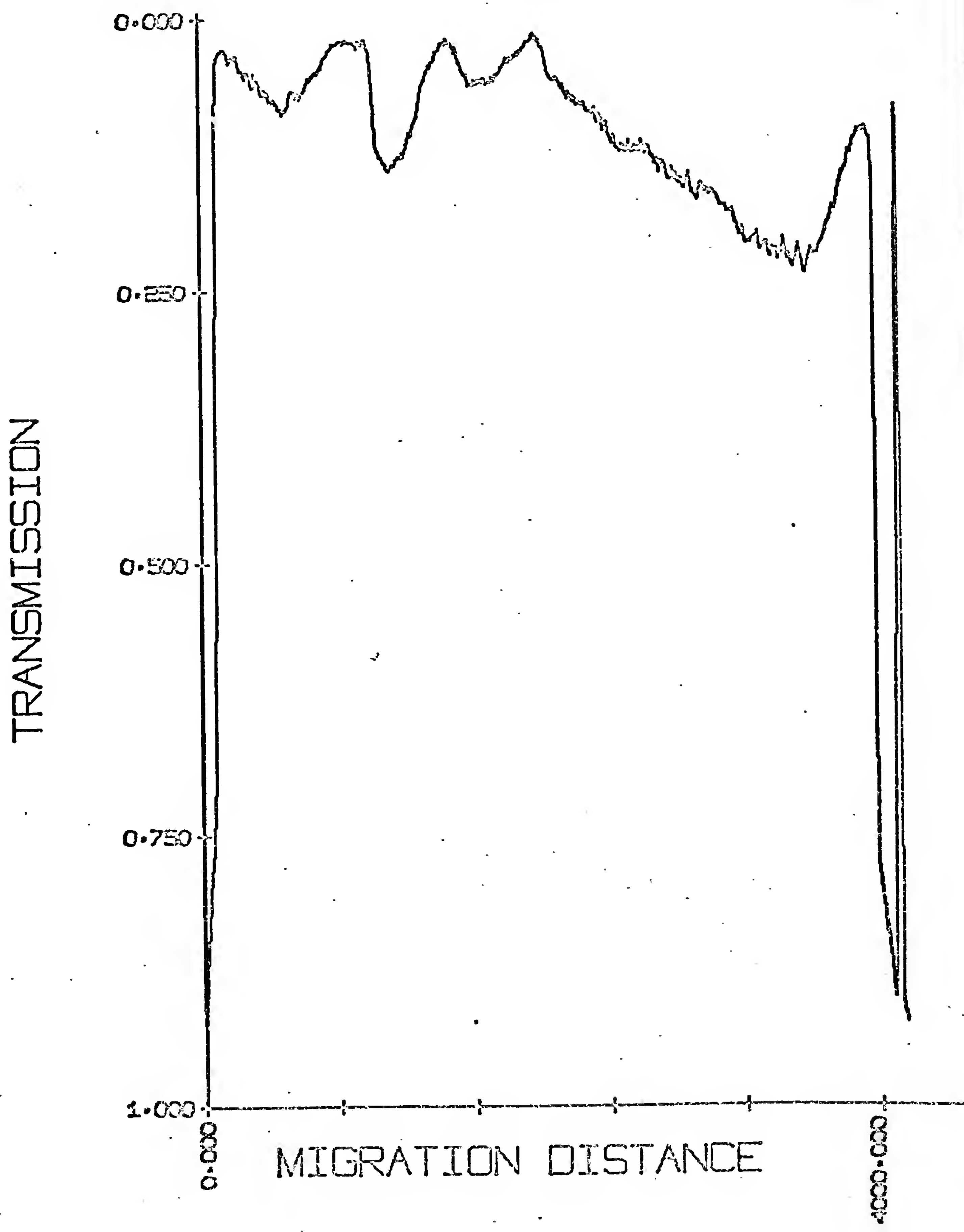

DENSITOMETER TRACING CORRESPONDING TO FIGURE 7, COLUMN 9.
TRANSMISSION
0.000
0.250
0.500
0.750
1.000
MIGRATION DISTANCE
0.000
4000.000


DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 1.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

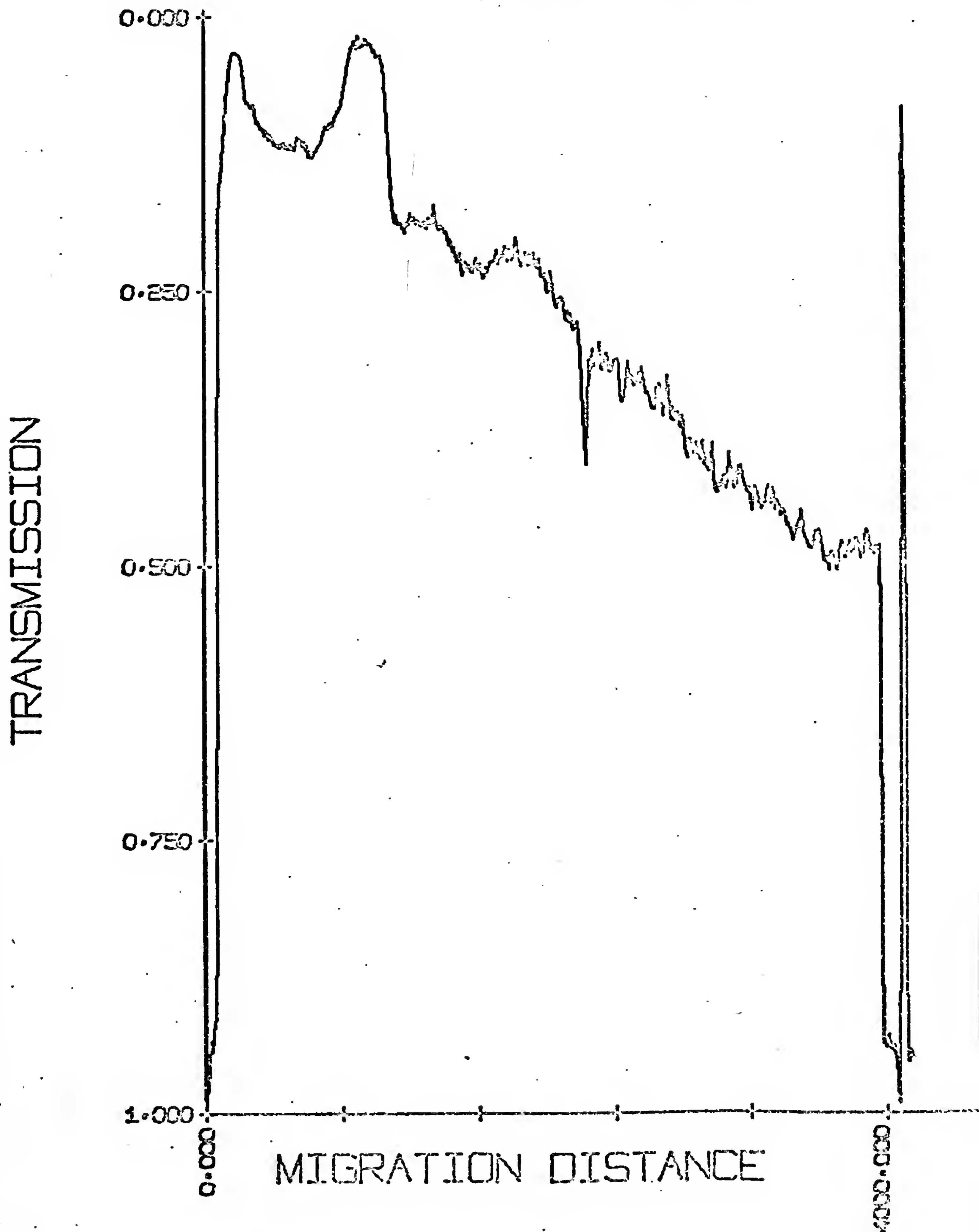

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 2.
TRANSMISSION
0.000
0.250
0.500
0.750
1.000
MIGRATION DISTANCE
0.000
4000.000


DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 3.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
MIGRATION DISTANCE
4000.000

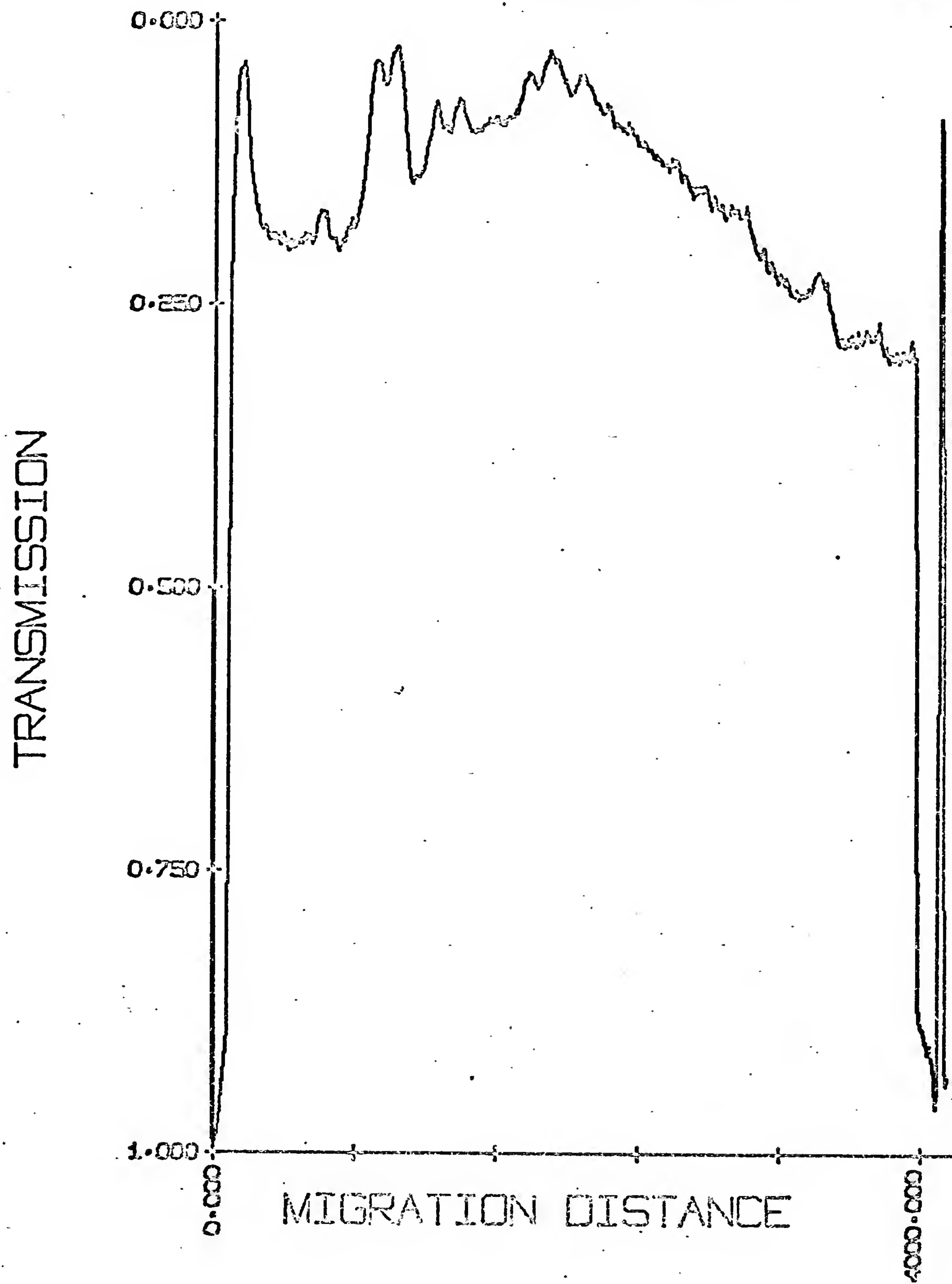

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8; COLUMN 4.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
400.000
MIGRATION DISTANCE

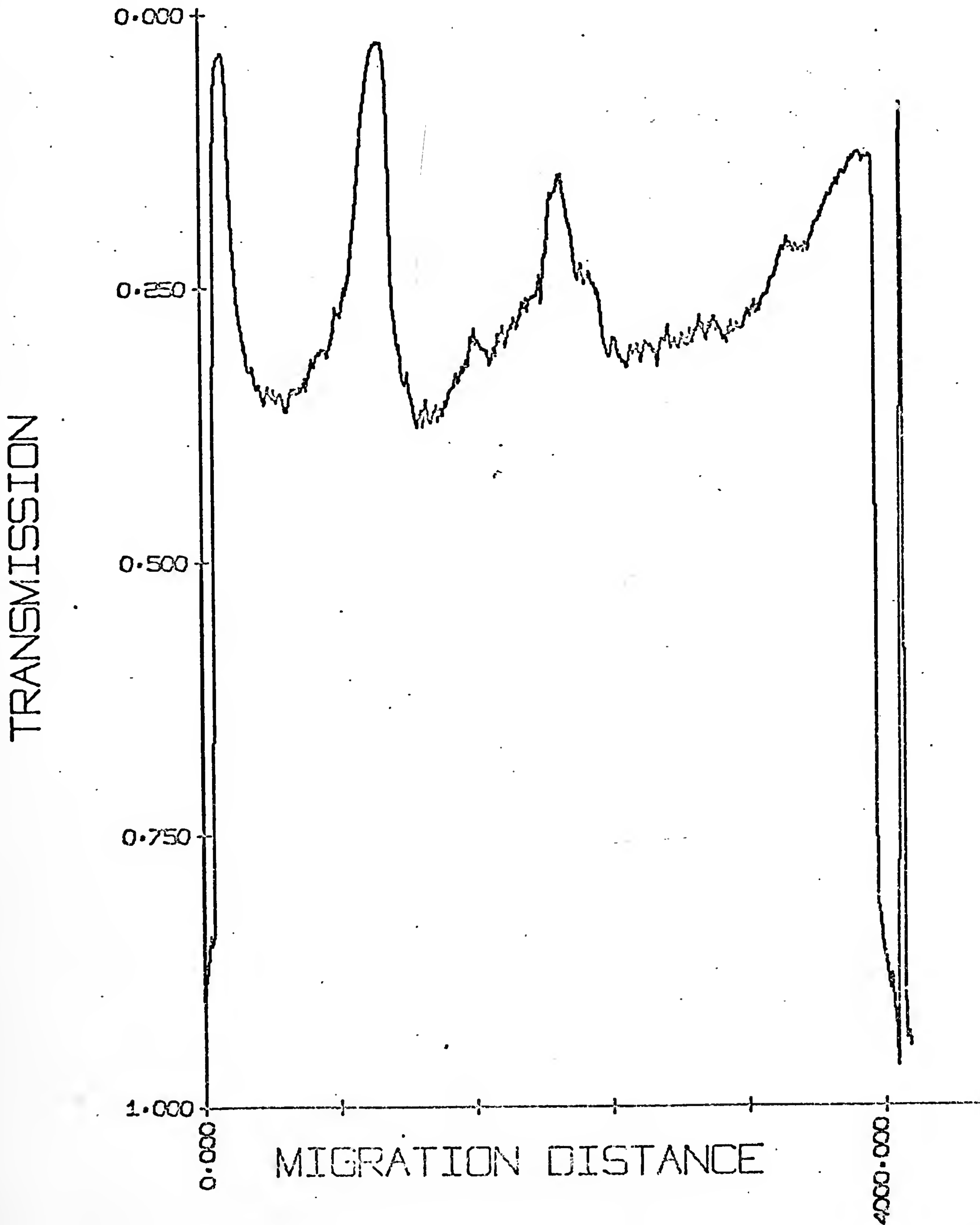


DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 5.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
MIGRATION DISTANCE
1000.000

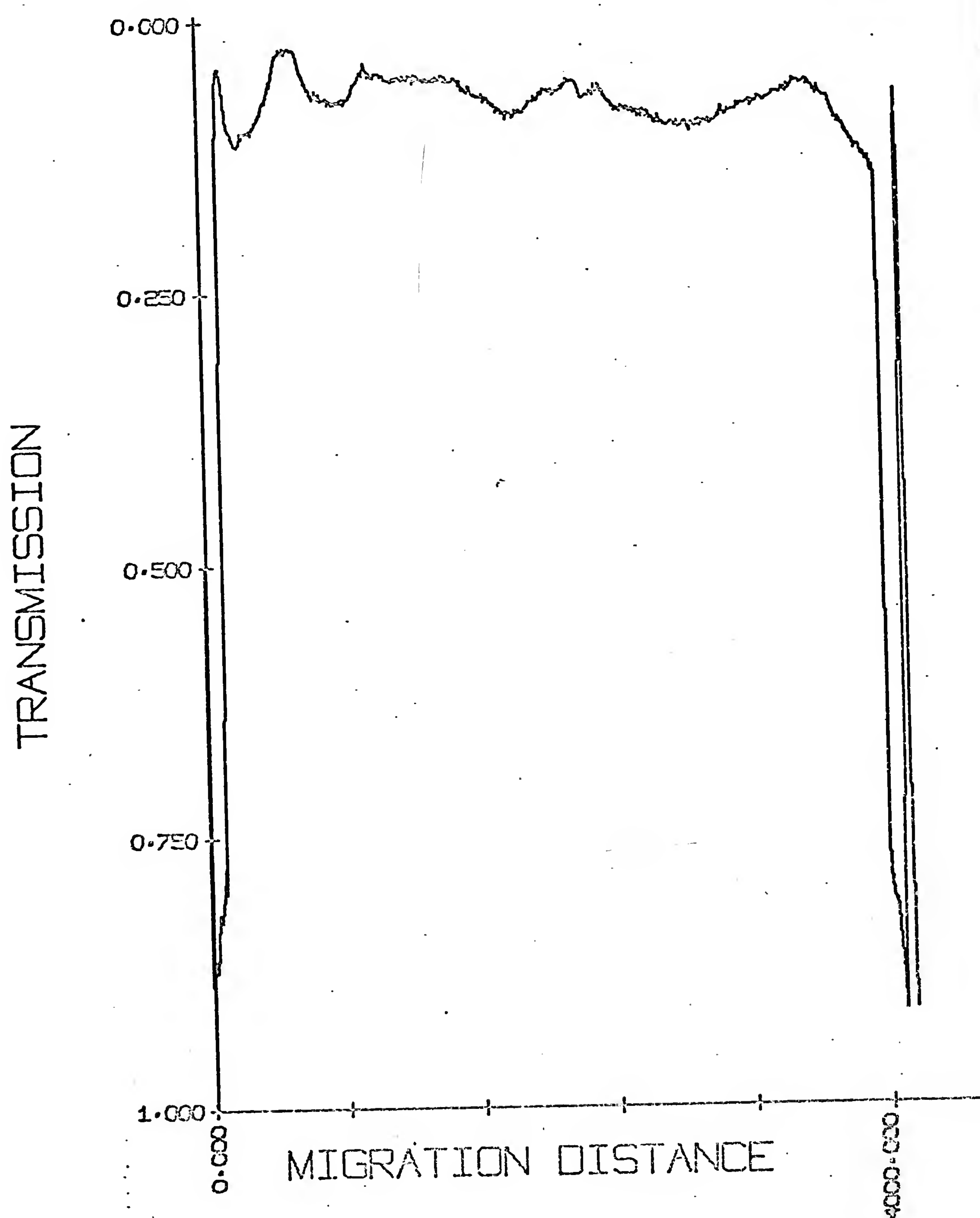

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 6.
TRANSMISSION
0.000
0.250
0.500
0.750
1.000
0.000
4000.000
MIGRATION DISTANCE

DENSITOMETER TRACING CORRESPONDING TO FIGURE 8; COLUMN 7.

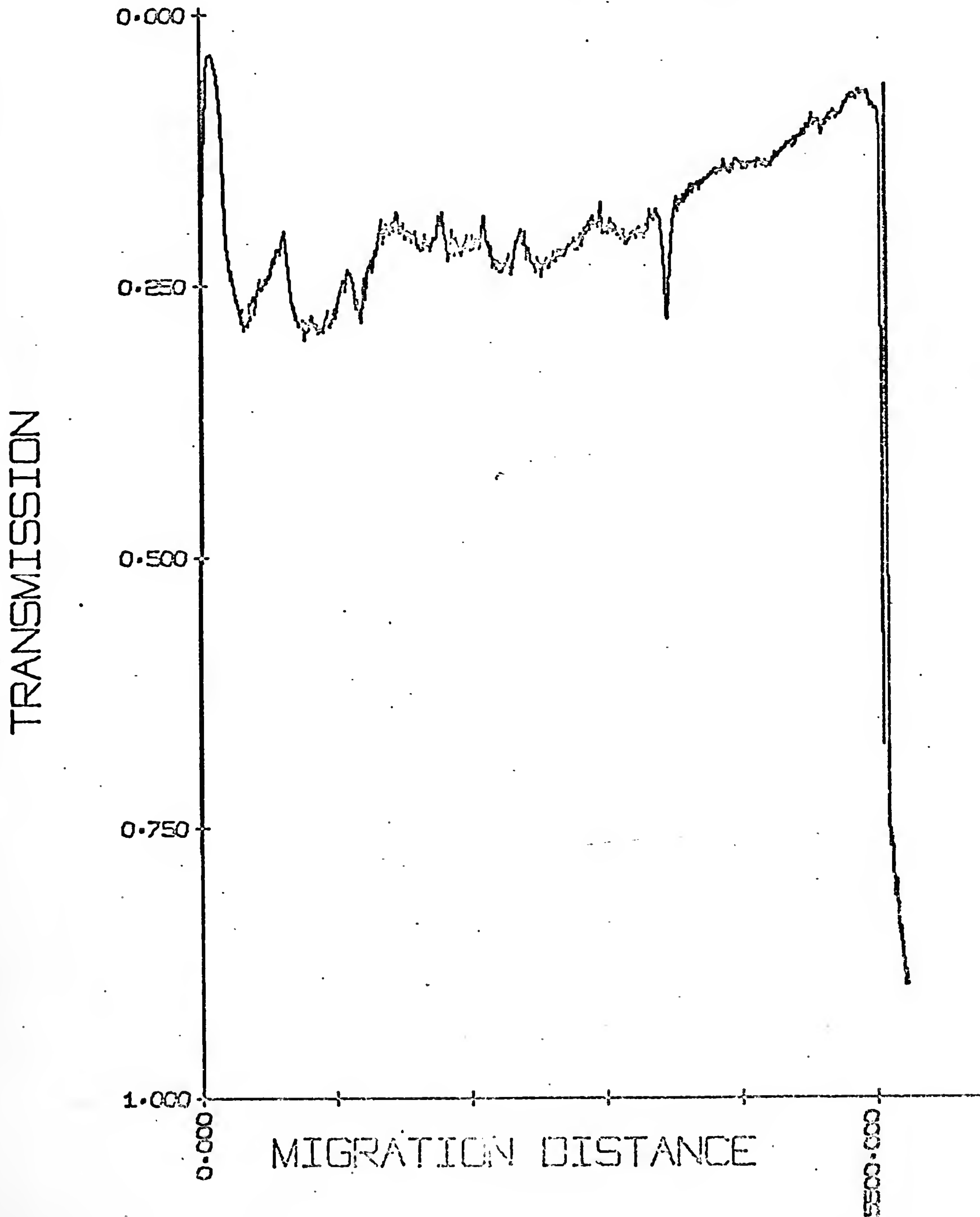


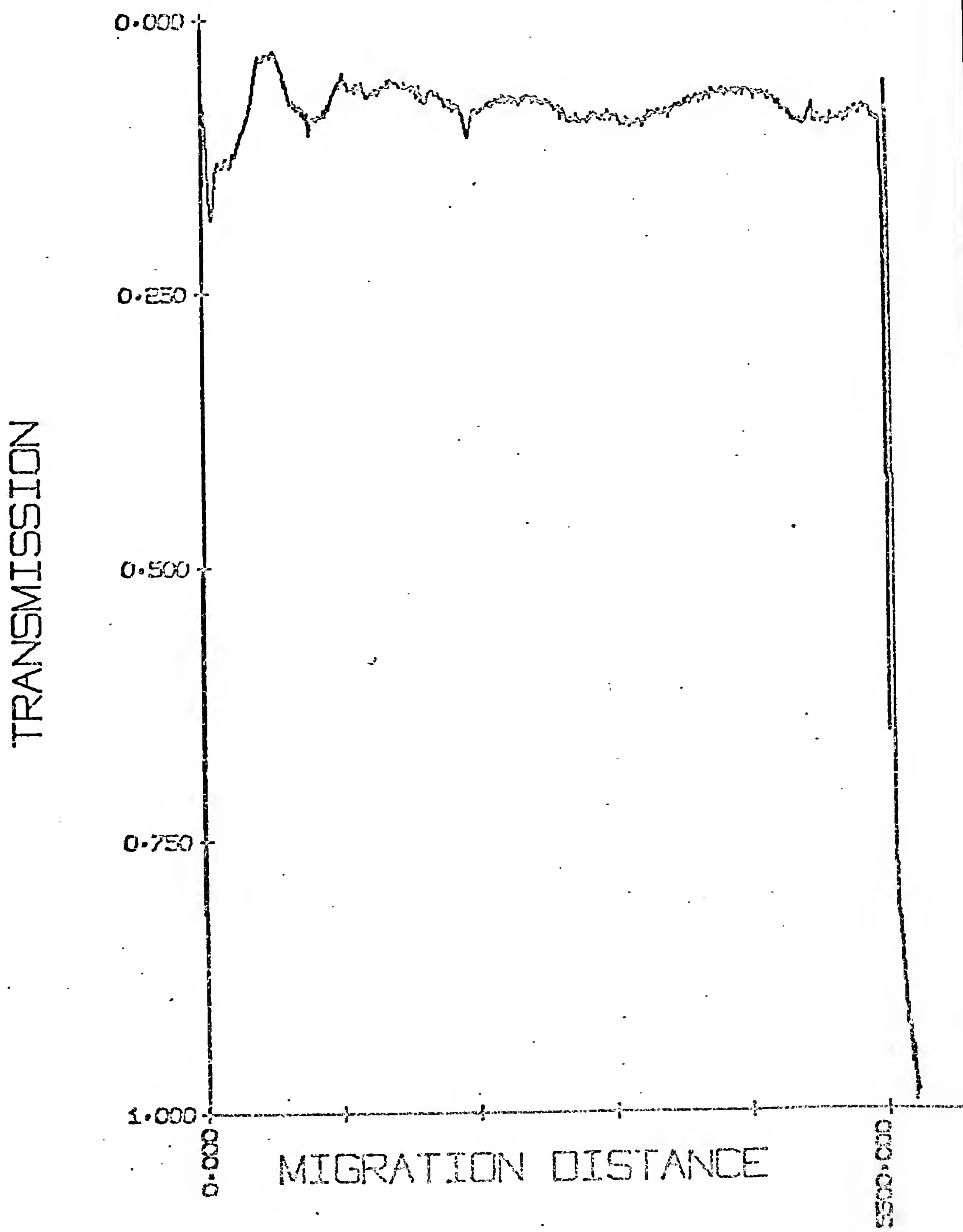

DENSITOMETER TRACING CORRESPONDING TO
FIGURE 8, COLUMN 8.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
4000.000
MIGRATION DISTANCE

DENSITOMETER TRACING CORRESPONDING TO FIGURE 8, COLUMN 9.



DENSITOMETER TRACING CORRESPONDING TO FIGURE 9, COLUMN 1.




DENSITOMETER TRACING CORRESPONDING TO
FIGURE 9, COLUMN 2.
0.000
0.250
0.500
0.750
1.000
TRANSMISSION
0.000
MIGRATION DISTANCE
5500.000

LIST OF REFERENCES

Allfrey, V. C., Faulkner, R., and Mirsky, A. E. (1964). Proc. Natl. Acad. Sci. U.S. 51: 786.

Allfrey, V. G., Littau, V. C., and Mirsky, A. E. (1963). Proc. Natl. Acad. Sci. U.S. 49: 414.

Attardi, G., Parnas, H., Hwang, M., and Attardi, B. (1966). J. Molec. Biol. 20: 145.

Avery, O. T., MacLeod, C. M., and McCarty, M. (1944). J. Exptl. Med. 79: 137.

Baltus, E. (1959). Biochem. Biophys. Acta 33: 337.

Baltus, E., and Brachet, J. (1963). Biochem. Biophys. Acta 76: 490.

Baltus, E., and Quertier, J. (1966). Biochem. Biophys. Acta 119: 192.

Baltus, E., Edstrom, J. E., Janowski, M., Quertier, J., Tencer, R., and Brachet, J. (1968). Proc. Natl. Acad. Sci. U.S. 59: 401.

Barlow, J. T., Mathias, A. P., Williamson, R., and Gammack, D. B. (1963). Biochem. Biophys. Res. Commun. 13: 61.

Beadle, G. W., and Tatum, E. L. (1941). Proc. Natl. Acad. Sci. U.S. 27: 499.

Beard, N. W., Jr., and Armentrout, S. A. (1967). Proc. Natl. Acad. Sci. U.S. 58: 750.

Beermann, W. (1960). Chromosoma 11: 263.

Berger, S. (1967). Protoplasma 64: 13.

Berger, S., and Schweiger, H. G. (1969). Physiological Chemistry and Physics 1: 280.

Birnstiel, M. L., Fleissner, E., and Borek, E. (1963). Science 142: 1577.

Birnstiel, M. L., Wallace, H., Sirlin, J. L., and Fischberg, M. (1966). Natl. Cancer Inst. Monograph 23: 431.

Bishop, D. H. L., Claybrook, J. R., and Spiegelman, S. (1967). J. Mol. Biol. 26: 373.

Bonotto, S., Goffeau, A., Janowski, M., Van den Driessche, T., and Brachet, J. (1969). Biochem. Biophys. Acta 174: 704.

Brachet, J. (1950). In "Chemical Embryology." Wiley (Interscience), New York.

Brachet, J., Chantrenne, H., and Vanderhaeghe, F. (1955). Biochem. Biophys. Acta 18: 544.

Brachet, J., Denis, H. and de Vitry, F. (1964). Develop. Biol. 9: 398.

Brachet, J., and Six, N. (1966). Planta 68: 225.

Brawerman, G. (1966). In "Biochemistry of Chloroplasts" (T. W. Goodwin, ed.). Academic Press, New York 1: 301.

Brenner, S., Jacob, F., and Meselson, M. (1961). Nature 190: 576.

Brenner, S., Stretton, A., and Kaplan, S. (1965). Nature 206: 994.

Brown, D. D., and Gurdon, J. B. (1964). Proc. Natl. Acad. Sci. U.S. 51: 139.

Brown, D. D. (1966). Natl. Cancer Inst. Monograph 23: 297.

Brown, D. D. (1968). Current Develop. Biol. 2: 47.

Brown, D. D., and Littna, E. (1964). J. Mol. Biol. 8: 669.

Bunting, S. L. "Methods of preparing acrylamide gels and agarose-acrylamide gels for vertical slab electrophoresis in the E-C 470 apparatus of Raymond." Personal communication.

Byvoet, P. (1961). Biochem. Pharmacology 6: 161.

Cairns, J. (1963). J. Mole. Biol. 6: 208.

Caspersson, T. O. (1950). In "Cell Growth and Cell Function." Norton, New York.

Ceron, G. (1969). Dissertation, University of Florida.

Clauss, H. (1958). Planta 52: 334.

Clauss, H. (1959). Planta 52: 534.

Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. (1961). Nature 192: 1227.

Darnell, J. E. (1968). Bacteriological Reviews 32: 262.

de Vitry, F. (1965). Bull. Soc. Chem. Biol. 47: 1325.

Dingman, C. W., and Peacock, A. C. (1968). Bull. Soc. Chem. Biol. 7: 656.

Edström, J. E. (1960). J. Biophys. Biochem. Cytol. 8: 47.

Farber, F. (1969a). Biochem. Biophys. Acta 174: 1.

Farber, F. (1969b). Biochem. Biophys. Acta 174: 12.

Fraenkel-Conrat, H., and Singer, B. (1957). Biochem. Biophys. Acta 24: 540.

Furth, J., Hurwitz, J., and Goldman, M. (1961). Biochem. Biophys. Res. Commun. 4: 362.

Gall, J. G. (1963). Nature 198: 36.

Gall, J. G. (1966). Natl. Cancer Inst. Monograph 23: 475.

Gibor, A. (1967). In "Biochemistry of Chloroplasts." (T. W. Goodwin, ed.) 2: 321.

Gibor, A., and Izawa, M. (1963). Proc. Natl. Acad. Sci. U.S. 50: 1164.

Gierer, A. (1963). J. Mol. Biol. 6: 148.

Girard, M., Latham, H., Penman, S., and Darnell, J. E. (1965). J. Mol. Biol. 11: 187.

Goffeau, A., and Brachet, J. (1965). Biochem. Biophys. Acta 95: 302.

Green, B. R., Heilporn, V., Limbosch, S., Bouloukhère, M. and Brachet, J. (1967). Proc. Natl. Acad. Sci. U.S. 58: 1351.

Green, B. R., Burton, H., Heilporn, V., and Limbosch, S. (1970). In "Biology of Acetabularia." (J. Brachet, S. Bonotto, eds.) Academic Press, New York. 35.

Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W., and Watson, J. D. (1961). Nature 190: 581.

Hämmerling, J. (1931). Biol. Zentr. 51: 633.

Hämmerling, J. (1939). Biol. Zentr. 59: 158.

Hämmerling, J. (1953). Int. Rev. Cytol. 2: 475.

Hämmerling, J. (1934). Wilhelm Roux Arch. Entwickl. Mech. Org. 131: 1.

Hämmerling, J. (1959). Biol. Zentr. 78: 703.

Harris, H. (1968). In "Nucleus and cytoplasms." Clarendon Press, Oxford.

Harris, H. (1962). Biochem. J. 84: 60.

Harris, H., Sidebottom, E., Grace, D. M., and Bramwell, M. E. (1969). J. Cell Sci. 4: 499.

Hartman, K. A., Amaya, J., and Schacter, E. M. (1970). Science 170: 171.

Hayashi, T., Holtzman, E., and Lamont, H. C. (1963). Arch. Biochem. Biophys. 97: 551.

Hershey, A. D., and Chase, M. J. (1952). J. Gen. Physiol. 36: 39.

Hoagland, M. B., Scornik, O. A., and Pfefferkorn, L. C. (1964). Proc. Natl. Acad. Sci. U.S. 51: 1184.

Huang, R. C., and Bonner, J. (1962). Proc. Natl. Acad. Sci. U.S. 48: 1216.

Huang, R. C., Bonner, J., and Murray, K. (1964). J. Mol. Biol. 8: 54.

Huang, R. C., Maheshwari, N., and Bonner, J. (1960). Biochem. Biophys. Res. Commun. 3: 689.

Hurwitz, J., Furth, J., Anders, M., and Evans, A. (1962). J. Biol. Chem. 237: 3752.

Ingle, J., Possingham, J. V., Wells, R., Leaver, C. J., and Leoning, U. E. (1970). Lymp. Soc. Exp. Biol. 23: 303.

Jacob, F., and Monod, J. (1961). J. Mol. Biol. 3: 318.

Janofski, M. (1966). Life Sci. 5: 2113.

Janofski, M. (1967). Arch. Intern. Physiol. Biochem. 75: 172.

Janowski, M., and Bonotto, S. (1970). In "Biology of Acetabularia." (J. Brachet and S. Bonotto, eds.) Academic Press, New York.

Janowski, M., Bonotto, S., and Bolouk̀here, M. (1969). Biochem. Biophys. Acta 174: 525.

Janowski, M. (1965). Biochem. Biophys. Acta 103: 399.

Joklik, W. K., and Becker, Y. (1965). J. Mol. Biol. 13: 511.

Keck, K. (1961). Aim N.Y. Acad. Sci. 94: 741.

Keck, K. (1964). In "Methods in Cell Physiology." (D. M. Prescott, ed.) Academic Press, New York 1: 189.

Keck, K. (1969). Int. Rev. Cyt. 26: 191.

Keck, K., and Choules, E. A. (1963). J. Cell Biol. 18: 459.

Keck, K., and Clauss, H. (1958). Botan. Gaz. 120: 43.

Kerr, I. M., Cohen, N., and Work, T. S. (1966). Biochem. J. 98: 826.

Kirk, J. T. O. (1966). In "Biochemistry of Chloroplasts." (T. W. Goodwin, ed.), 1: 319. Academic Press, New York.

Kornberg, A. (1969). Science 163: 1410.

Kroon, A. M. (1963). Biochem. Biophys. Acta 69: 184.

Lateur, L. (1963). Rev. Alg. 7: 26.

Lengyel, P., and Söll, D. (1969). Bacteriological Reviews 33: 264.

Liau, M. C., and Hurlbert, R. B. (1966). Proc. Am. Assoc. Cancer Res. 7: 42.

Liau, M. C., and Perry, R. P. (1969). J. of Cell Biol. 42: 272.

Loening, U. E. (1968). J. Mol. Biol. 38: 355.

Loening, U. E., and Ingle, J. (1967). Nature 215: 363.

Luck, D. J. L., and Reich, E. (1964). Proc. Natl. Acad. Sci. U.S. 52: 931.

Lyttleton, J. W. (1962). Exptl. Cell Res. 26: 312.

Marks, P., Burka, E., and Schlessinger, D. (1962). Proc. Natl. Acad. Sci. U.S. 48: 2163.

Meselson, M., and Stahl, F. W. (1958). Proc. Natl. Acad. Sci. U.S. 44: 671.

Miller, O. J., Jr. and Beatty, B. R. (1969). Science 164: 955.

Mintz, B. (1964). J. Exptl. Zool. 157: 85.

Naora, H., Naora, H., and Brachet, J. (1960). J. Gen. Physiol. 43: 1083.

Naora, H., Richter, G., and Naora, H. (1959). Exp. Cell Res. 16: 434.

Nass, M. M. K., and Nass, S. (1963). J. Cell Biol. 19: 593.

Nemer, M. (1963). Proc. Natl. Acad. Sci. U.S. 50: 230.

Nirenberg, M., and Leder, P. (1964). Science 145: 1399.

Nirenberg, M., and Matthaei, J. (1961). Proc. Natl. Acad. Sci. U.S. 47: 1588.

Nishimura. S., Jones, D., Ohtsuka, E., Hayatsu, H., Jacob, T., and Khorana, H. (1965). J. Mol. Biol. 13: 283.

Nomura, M., Lowry, C. V., and Guthrie, C. (1967). Proc. Natl. Acad. Sci. U.S. 58; 1487.

O'Brien, T. W., and Kalf, G. F. (1967). J. Biol. Chem. 242: 2172, 2180.

Olszewska, M., and Brachet, J. (1961). Exptl. Cell Res. 22: 370.

Olszewska, M., de Vitry, F., and Brachet, J. (1961). Exptl. Cell Res. 24: 58.

Osawa, S. (1968). Ann. Rev. Biochem. 37. 109.

Peacock, A. C., and Dingman, C. W. (1968). Biochemistry 7: 668.

Peacock, A. C., and Dingman, C. W. (1967). Biochemistry 6: 1818.

Penman, S. (1966). J. Mol. Biol. 17: 117.

Penman, S., Scherrer, K., Becker, Y., and Darnell, J. E. (1963). Proc. Natl. Acad. Sci. U.S. 49: 654.

Penman, S., Vesco, C., Weinberg, R., and Zylber, E. (1969). Cold Spring Harbor Symposium on Quantitative Biology 24: 535.

Perry, R. P. (1962). Proc. Natl. Acad. Sci. U.S. 48: 2179.

Perry, R. P. (1963). Expt. Cell Res. 29: 400.

Perry, R. P. (1967). Progress in Nucleic Acid Research and Molecular Biology 6: 219.

Prescott, D. M. (1959). J. Biophys. Biochem. Cytol. 6: 203.

Prescott, D. M. (1962). J. Histochem. Cytochem. 10: 145.

Prescott, D. M. (1964). In "Progress in Nucleic Acid Research." (J. N. Davidson, W. E. Cohn, eds,) 3: 35. Academic Press, New York.

Reuter, W., and Schweiger, H. G. (1969). Protoplasm 68: 357.

Richter, G. (1959). Biochem. Biophys. Acta 34: 401.

Richter, G. (1966). Nature 212: 1363.

Ritossa, F. M., and Spiegelman, S. (1965). Proc. Natl. Acad. Sci. U.S. 53: 737.

Roodyn, D. B. (1962). Biochem. J. 85: 177.

Schweiger, H. G. (1962). Coll. Intern. Centre Nat. Resch. Scient. 106: 356.

Schweiger, H. G. (1966). Planta 68: 247.

Schweiger, H. G. (1967). Arzneimittel Jorsch. 17: 1433.

Schweiger, H. G. (1970). Symposia of the Society for Experimental Biology 24: 327.

Schweiger, H. G., and Berger, S. (1964). Biochem. Biophys. Acta 87: 533.

Schweiger, H. G., and Bremer, H. J. (1960). Exp. Cell Res. 20: 617.

Schweiger, H. G., and Bremer, H. J. (1961). Biochem. Biophys. Acta 51: 50.

Schweiger, H. G., and Dillard, W. L. (1968). Biochem. Biophys. Acta 169: 561.

Schweiger, H. G., Dillard, W. L., Gibor, A., and Berger, S. (1967a). Protoplasma 64: 1.

Schweiger, H. G., Master, R. W. P., and Werz, G. (1967b). Nature 216: 554.

Scott, R. B., and Bell, E. (1964). Science 145: 711.

Schulze, K. L. (1939). Arch. Protistenk. 92: 179.

Shephard, D. C. (1965a). Exptl. Cell Res. 37: 93.

Shephard, D. C. (1965b). Biochem. Biophys. Acta 108: 635.

Shephard, D. C. (1970). In "Methods in Cell Physiology." (D. M. Prescott, ed.) Academic Press, New York 4: 49.

Sherrer, K., Latham, H., and Darnell, J. E. (1963). Proc. Natl. Acad. Sci. U.S. 49: 240.

Sidebottom, E., and Harris, H. (1969). J. Cell Sci. 5: 351.

Siebert, G., Villalobos, J., Ro, T. S., Steele, W. J., Lindenmayer, G., Adams, H., and Busch, H. (1966). J. Biol. Chem. 241: 71.

Slayter, H. W., Warner, J. R., Rich, A., and Hall, C. E. (1963). J. Mol. Biol. 7: 652.

Spencer, T., and Harris, H. (1964). Biochem. J. 91: 282.

Spiegelman, S., Hall, B., and Storek, R. (1961). Proc. Natl. Acad. Sci. U.S. 47: 1135.

Spirin, A. S. (1969). Eur. J. Biochem. 10: 20.

Stephenson, M. L., and Zamecnik, P. C. (1956). Federation Proc. 15: 362.

Takanami, M., and Yan, Y. (1965). Procl Natl. Acad. Sci. U.S. 54: 1450.

Taylor, J. H., Woods, P., and Hughes, W. (1957). Proc. Natl. Acad. Sci. U.S. 43: 122.

Tischler, P. V., and Epstein, C. J. (1968). Anal. Biochem. 22: 89.

Tomkins, G. M., Gelehrter, T. D., Granner, D., Martin, D., Samuels, H. H., Tompson, E. B. (1969). Science 166: 1474.

Triplett, E. L., Steers-Lievens, A., and Baltus, E. (1965). Exptl. Cell Res. 38: 366.

Tyler, A. (1967). Devel. Biol. Suppl. 1: 170.

Von der Decken, A. (1967). J. Cell Biol. 33: 657.

Warner, J. R., Rich, A., and Hall, C. E. (1962). Science 138: 1399.

Watson, J. D. (1965). In "Molecular Biology of the Gene." W. A. Benjamin, Inc. New York.

Watson, J. D. and Crick, F. H. C. (1953). Nature 171: 737, 964.

Weiss, S. B. (1960). Proc. Natl. Acad. Sci. U.S. 46: 1020.

Werz, G. (1961). Z. Naturforsch. 16b: 126.

Werz, G. (1966). Planta 68: 256.

Woodcock, C. E. F. and Bogorad, L. (1970). Biochem. Biophys. Acta 224: 639.

Young, R. W., and Fulhorst, H. W. (1965). Anal. Biochem. 11: 389.

Zetsche, K. (1964). Z. Naturforsch. 19b: 751.

Zetsche, K. (1966a). Z. Naturforsch. 21b: 375.

Zetsche, K. (1966b). Planta 68: 360.

Zetsche, K. (1966c). Biochem. Biophys. Acta 124: 332.

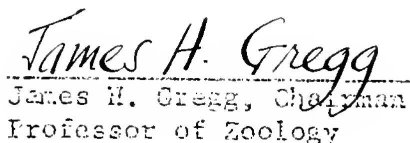
Zetsche, K. (1966d). Z. Naturforsch. 21b: 88.

BIOGRAPHICAL SKETCH

Ilse Marie Johanne Ortabasi was born November 28, 1941, at Hamburg, Germany. In March, 1961, she was graduated from the Schule Schloss Salem in Baden, Germany. In February, 1965, she passed the "Vordiplom" examination in Biology at the University of Hamburg. In 1966, she enrolled in the Graduate School of the University of Florida. She worked as a teaching assistant in the Department of Zoology until August, 1967, and held a fellowship from the University of Florida from September, 1967, until June, 1969, after which she worked as a teaching assistant in the Department of Zoology and as a research assistant for Dr. J. H. Gregg, Professor of Zoology. From September, 1966, until the present time she has pursued her work toward the degree of Doctor of Philosophy.

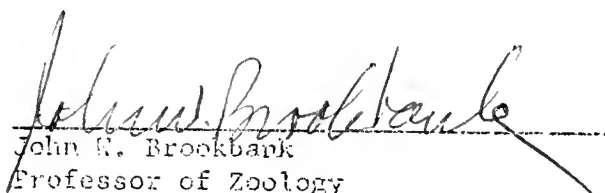
Ilse Marie Johanne Ortabasi is married to Uğur Ortabasi.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



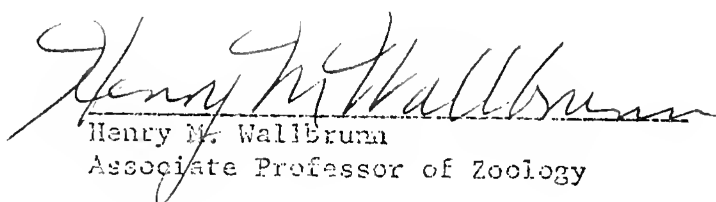
James H. Gregg, Chairman
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



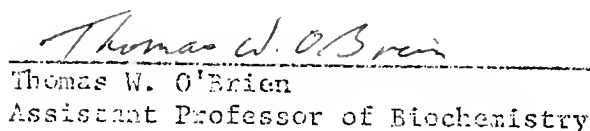
John W. Brookbank
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Henry M. WallBrunn
Associate Professor of Zoology

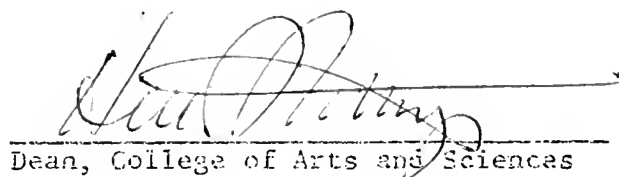
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Thomas W. O'Brien
Assistant Professor of Biochemistry

This dissertation was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1971



Dean, College of Arts and Sciences

Dean, Graduate School