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Studies on the ultrastructure of the avian embryonic nervous system : with comprehensive reviews of fundamental ultrastructive, fine structure of the adult nervous system and cytoarchitecture of embryonic tissue

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STUDIES ON THE ULTRASTRUCTURE OF THE AVIAN EMBRYONIC NERVOUS SYSTEM - WITH COMPREHENSIVE REVIEWS OF FUNDAMENTAL ULTRASTRUCTIVE, FINE STRUCTURE OF THE ADULT NERVOUS SYSTEM AND CYTOARCHITECTURE OF EMBRYONIC TISSUE

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INTRODUCTION

Since this research has occupied so much time during my medical education, it seems proper to recapitulate its evolution as a preface to this senior thesis. While studying the ontogeny of branchial arch musculature as a freshman, I felt suspicious of the use of cranial nerves as criteria of homologous muscle structures in the embryo and adult vertebrate. This suspicion stimulated literature research which subsequently became my freshman paper in Anatomy. Cursory as it was, this literature review seemed to substantiate my suspicions, for the validity of this theory of muscle homology was based solely upon the assumption of the specificity of nerve fibers emerging from the embryonic neural tube for their presumptive end organs. Since this specificity had never been demonstrated conclusively, and indeed was the subject of great controversy, the use of innervation as index of muscle homologies appeared to be unproven.

During the following summer a review of the literature concerning factors influencing peripheralization of nerve fibers revealed a paucity of data especially with respect to the time of emergence of the first nerve fibers. It appeared that the first step in investigating the factors influencing the early course of 'pioneer' nerve fibers after emergence from the neural tube, was to find the earliest emergence of cervical 'pioneer' fibers. (See 239) I first studied this with the light microscope, utilizing Lyon's blue as a specific stain for nerve fibers. Due to a multitude of factors, essentially no valid data was

gained during this summer.

During the sophomore year I continued working on this project on a part-time basis hoping that improved technique with the previously unsuccessful method would solve the problems. Throughout this year and the early portion of the following summer, the results were disappointingly poor due to the inability of Lyon's blue dye to stain embryonic nerve fibers and the low resolution of light microscopy. At this time I decided that further studies with light microscopy were futile and commenced pursuit of the problem with the electron microscope. Initial results were exasperating; gradually as technic improved and standard procedures for adult tissues were adapted to embryonic use, valid but unpublishable data was acquired. Enthused by this, I decided to continue the research on a full-time basis through the following academic year.

During this year I read voraciously in order to understand cellular ultra structure and find new techniques and procedures which could improve the quality of results. This research yielded sufficient understanding of the ultrastructure of the embryonic neural tube and pioneer nerve fibers to provide a thesis for my Master of Science Degree. Although the research was completed by the beginning of my junior year, my thesis was written on a part-time basis throughout the course of my junior year in medicine.

The extensive literature review done for the Masters Degree covered almost all of the papers published up to March of 1960 on nervous system,

and all papers dealing with electron microscopy of embryonic tissue up through January, 1961. Investigation of the neural tube was incomplete for only those stages up to Hamilton and Hamburger (98) Stage 19+ had been investigated. During one month of senior elective, I chose to continue this research by investigating older stages of embryonic development and fill in certain other gaps, as well as bring my bibliography on fine structure of the nervous system and embryonic tissue up to date.

The literature review of this paper will emphasize specific aspects of ultrastructure, rather than present a chronological history. In order that the reader, who perchance may be unfamiliar with the terminology of ultracytology may easily understand this paper, the literature review begins with a section on basic fine structure of tissue. Subsequent sections will describe adult nervous system cytology and the fine structure of the embryonic cells.

The section on materials and methods covers all techniques used on previous and recent work, emphasizing modifications necessary to adequately preserve fine structure of older specimens.

The results will deal solely with data attained in recent investigation of both developing nervous system and developing somite.

The conclusion will be limited to discussion of recent findings and alterations in concepts of cellular differentiation in the embryonic nervous system wrought by these recent findings and the recent literature.

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REVIEW OF LITERATURE

PART I. FUNDAMENTAL FINE STRUCTURE OF TISSUE

The following three sections concerning the general ultrastructure of tissue, submicroscopic morphology of the nervous system, and the specific characteristics of embryonic tissue are presented to avail the reader with a rudimentary but essential understanding of the submicroscopic structure of tissues. The light microscope reveals three discontinuous phases of tissues: The extracellular space, cytoplasm and nucleus, delimited by two barriers; the plasma or cell and nuclear membrane. Therefore, this section is divided into three units concerning the nucleus and nuclear membrane; cytoplasm, plasma membrane and their organelles, and the extracellular space.

Section 1. Nucleus and Nuclear Membrane.

The nucleus is the area of cell bounded by a nuclear membrane composed of two trilaminate (214) membranes, the outer and inner nuclear membranes, separated by an electron lucent space, the perinuclear cistern. At certain points along the nuclear membrane, the outer and inner nuclear membranes are contiguous; in profile it appears that the inner nuclear membrane is reflected back upon itself to become the outer nuclear membrane. When two such reflections appear adjacent to one another, a gap is formed between them; this gap is known as the nuclear pore (48, 100, 129, 173, 174, 180, 184-186, 202, 203, 233, 236, 256 and 259). In tangentially sectioned nuclear membranes these nuclear pores have the appearance of circular "holes"; attached to the outer nuclear

membrane at the margin of these pores are Palade granules (small particulate component, ribosomes) (256, 259). Some authors (48) believe that the "pores" represent a defect in the nuclear membrane, through which there is direct continuity of the nucleoplasm and cytoplasm, which permits nuclear granules to pass into the cytoplasm to become ribosomes. Most recent evidence indicates that the gap in the nuclear membrane is filled with dense material which gives a higher radiodensometric reading than the adjacent imperforate nuclear membrane (129, 256). Further research has demonstrated that a dense ground-glass appearing matrix fills the pores; through this matrix pass fine filamentous profiles oriented perpendicular to the nuclear membrane pass (256, 259). The outer nuclear membrane of most cells is enclothed in ribosomes in the same spiraling, circular or rosette pattern found on membranes of the granular endoplasmic reticulum, discussed subsequently (172-174, 202, 203, and 256). Nevertheless, ribosomes are absent from the outer nuclear membrane of neurones (184-186). The outer nuclear membranes and the granular endoplasmic reticulum are continuous parts of the same membrane system; thus the perinuclear cistern is in direct continuity with the "lumen" of the endoplasmic reticulum (173, 174, 180, 184-186, 202, 203, 233, 235, 256 and 259). The inner nuclear membrane is similarly part of the same system, by virtue of confluence with the outer nuclear membrane and is in direct apposition to the nucleoplasm. Though ribosomes probably do not pass through the nuclear pores, morphological continuity between the nucleoplasm and cytoplasm through the pores, provides a route

for functional continuity, yet no direct evidence supports it. During mitosis, the intrapore matrix undergoes dissolution, the nuclear pore enlarges and the adjacent pores coalesce; it appears that the granular endoplasmic reticulum retracts its portion which forms the nuclear membrane (202).

Recent improvement in tissue preparation has produced data that invalidates previous concepts of nucleoplasm morphology. Previously, two phases were distinguished; an electron lucent ground-glass continuous phase is confluent with the cytoplasmic matrix through the nuclear pores (174, 186, 202, 203, 234, 235, 256 and 259). This phase was presumed to consist of a hydrous solution of electrolytes, small organic molecules (sugars, nucleic acids and amino acids) and their larger polymers (carbohydrates, nucleotides and proteins) (123). Embedded in this continuous phase are granules morphologically indistinguishable from ribosomes but composed of desoxyribonucleoprotein (171, 172, 178, 198, 202, and 227) rather than ribonucleoprotein predominating in the ribosomes (123, 178, 227, 228, and 231). The recent findings of Palay (184) and others not cited here indicate that the nucleus is composed of one finely granular electron dense phase and that the biphasic appearance is due to suboptimal preparation.

Within the nucleoplasm are embedded one or more aggregations of extremely electron dense particles arranged in reticular patterns identified as nucleoli (180, 203 and 235) and composed of ribonucleoproteins (123). In embryonic cells no nucleoli are present (122, 123), and

aggregations of electron dense granules, often erroniously interpreted as nucleoli, probably represent the desoxynucleoprotein rich chromocenter familiar to histochemists and microspectrophotometrists (122 and 123).

Section 2. Cytoplasm.

That portion of the tissue which lies between the plasma and nuclear membranes of each cell is the cytoplasm, the seat os cellular characteristics and site of all cell function except replication and ribonucleic protein anabolism. The cytoplasm is composed of an amorphous continuous phase, the cytoplasmic matrix and formed cyto-organelles of various composition and morphology including cytofilaments, lipid inclusions, granules, granular and agranular membranes, vesicles, vacuoles, mitochondria, liposomes, centrioles and ciliary basal bodies. This section presents the basic concepts of cytoplasmic structure and function.

Subsection 1. Cytoplasmic Matrix.

The cytoplasmic matrix, is noted for its marked lack of characteristic structure; close observation reveals that this amorphous nature varies with cell type and tissue preparation techniques. Thus is found a great span in the appearance of the ground substance from the electron lucence of "leached" cells to the electron density of overstained cells yet all appear to have a faintly 'ground-glass' texture.

At the nuclear pores the cytoplasmic matrix appears continuous with its nuclear counterpart, as previously mentioned. However, factual existence of a direct morphologic or biochemical continuity remains a

moot point.

The cytoplasmic matrix is composed of water, electrolytes, carbohydrates, sugars and polysaccharides, amino acids, intermediate peptides and dissolved proteins, ribonucleic acids (RNA), ribonucleotides and ribonucleoproteins (RNP). This homogenous mixture is in a colloid state varying from sol to gel; this dynamic equilibrium between sol to get produces cellular motion with concomittant mechanical mixing which promotes diffusion and enhances intracellular transport of nutrients, excretia, waste products and intermediate organic compounds. A state of chemical equilibrium exists between the intrinsic equilibrium system of the cytoplasmic matrix and the intrinsic equilibrium of various formed structures, for example the ribonucleoproteins of the ribosomes and the dissolved ribonucleoproteins of the amorphous ground substance (178, 227-231) and protein cytofilaments and dissolved protein (223).

In addition to the dynamic physical and chemical kinetic equilibrium systems mentioned above, there exists osmotic equilibrium systems between the cytoplasmic matrix and a host of dissimilar phases, such as the extracellular compartment across the plasma membrane, the mitochondrial matrix across the mitochondrial membrane, the nuclear matrix across the nuclear membrane, contents of vesicles, vacuoles and tubules of the various cytomembrane systems (thus indirectly with the extracellular compartment) and secretion vacuoles across the membranes of the agranular reticulum (122, 123, and 234).

Subsection 2. Formed Structures of the Cytoplasm.

A. Cytofilaments. Within the cytoplasm appear long hairlike profiles known as cytofilaments. These profiles vary in diameter, between 90 - 350 Angstroms, and in length. Their character is that of a fibrillar protein composed of various α-amino acids. The periodicity, function and contractility of these cytofilaments vary with cell type; yet cytofilaments, regardless of individual cellular character and composition, are ubiquitous among cells. Thus, they are found in the neuronal perikarya (141, 152, 180, 184, 186, 188 and 226), and axons (65, 97, and 112), astrocytes (28-30, 143, 152, 153, 225 and 226), and oligodendroglia (28-30, 140, 141, 144, 150, 152, 153, 187 and 226), as the contractile elements of muscle myofilaments, (both smooth and striated) (113, 118, 176, 238 and 255), (in striated muscle they present a distinct periodicity). In epithelial cells they are associated with desmosomes as structural elements (62 and 75) or cilia as the contractile elements (75 and 223). They are the prime structural elements of the osteoblasts (99), in the notochord of Amphioxus (63) and in fibroblasts. Though the protein composition of filaments varies with cell type and species the morphology is essentially the same. The morphology and behavior, physical and chemical, of one representative cytofilament, the neurofilament of neurofibrillae, has been revealed after isolation from squid giant axons as follows (223):

1) Per cent of axon equals 0.07.

2) Intrinsic birefringence equals 0.005.

3) X-ray diffraction shows it to have the alpha pattern of fibrous proteins of the KMEF class.

4) Filament diameter equals 100 to 200 Angstroms.

5) Nodose appearance due to associated granules suggest actual periodicity or approximately 250 Angstroms.

6) Reversible depolymerization by increase of the ionic strength of the medium.

7) Protein equals 70 per cent of total macromolecular content of axoplasm or 0.35 per cent of wet weight and 2.6 per cent of dry weight.

8) Iso-electric point is at pH of 6.0.

9) Axial ratio equals 100 and decreases as pH increases.

10) Flexible rod rather than globulin at iso-electric point, but as pH rises to 8.5 depolymerization occurs into globular macromolecules of molecular weight approximating 70,000.

11) The fibrous structure seems to split into two fibers as the pH is increased from 6.0 to 7.7.

B. Ribosomes. The ribosomes, variously known as Palade granules, the small particular component and cytoplasmic granules, are ubiquitously embedded in the cytoplasmic matrix in those areas not occupied by other organelles. They appear as small round, square or polygonal electron dense profiles approximately 150 Angstrom units in diameter and may be associated with alpha cytomembranes or 'free'. Whether 'free' or associated with membranes they may assume circular, spiral or rosette patterns (171,

172, 174, 178, 180, 185, 186, 198, 199, 227, 235 and 236). These granules are primarily composed of ribonucleic acid and proteins; the proteins are either free or combined with the ribonucleic acids to form ribonucleic protein. Thus an intrinsic equilibrium between free and RNP protein exists. These proteins are simple proteins without phospholipid or mucopolysaccharide prosthetic groups (178 and 227).

A morphologic equilibrium between free and associated ribosomes appears to exist. Chemical equilibria also exist between the soluble ribonucleic acids and proteins and soluble ribonucleoproteins in the cytoplasmic matrix, the soluble matrical ribonucleoprotein and its counterpart in the ribosomes, both free and associated with alpha cyto-membranes and the alpha amino acids and ribonucleic acids and their ribosome polymers (122-127, 178, 182, 227-231).

As the prime locus of cellular ribonucleoproteins, the ribosomes are the primary sites of cytoplasmic protein production.

The role of ribosomes in protein anabolism has been extensively studied in pancreatic acinar cells by Palade and Siekevitz (178, 182, 227-231) who found that C14 tagged alpha amino acids were rapidly incorporated into the ribosomes initially but later were found in the protein fraction of the ribosomes and intracysternal granules; even later they attained high concentrations in the zymogen granules as trypsin-activatable-proteolytic activity (more commonly known as chymotrypsin) and RNAse (228 and 230). In the liver as in the pancreas tagged amino acids were found initially concentrated in the ribosomes,

and rapidly incorporated into ribosomal protein, but remained in granules gradually decreasing over the ensuing three to four weeks (230). Experimental starvation yielded a parallel decrease in the numbers of intracisternal granules and the amount of chymotrypsin and RNAse, indicative of decreased protein production by the ribosomes, thus illustrating the relationship of the ribosomes in cellular protein production (229). These workers have demonstrated three microsomal fractions:

1) Ribosomes attached to membranes (alphacytomembranes).
2) Ribosomes suspended freely in the cytoplasmic matrix.
3) Soluble ribonucleic proteins.

These fractions are listed above in descending order with respect to the RNA/protein ratio and the ascending order with respect to the speed of incorporation of adenine-8-C^{14}, which is an index of protein and RNA turnover; moreover, the greatest concentrations of alpha-amino acid activating enzymes are located in the soluble RNP fraction (231). It appears that alpha-amino acids are activated by the ribonucleic acids of the soluble RNP fraction, this RNA activated alpha amino acid conjugate becomes associated with free ribosomes to form the ribonucleoproteins which act as protein templates (as theorized by Pauling) and finally these ribosomes, containing the RNP templates upon which the finished protein molecules have been formed, may become associated with the alpha-cytomembranes into whose cysternae the finished protein is deposited.

Hyden, et al., has shown that the shift of RNP from the oligodendroglial cells to the Deiter's cells of the vestibular nuclei during

increased function involves the soluble RNP fraction since cytoplasmic basophilism which depends upon ribosomal concentration, is not ostensibly increased (64, 123-127). They show that during neuronal development in the chick embryo the rate of protein and RNP production remain parallel and constant until the 7th to 8th day of incubation when rapid acceleration of both RNA and protein production takes place coinciding with the formation of the Nissl substance. The first stage of protein production (up to the 8th day) in which there is a gradual increase in total cellular protein, is characteristic of all embryonal cells, but the late accelerated phase of protein production is noted only in neurons (122, 123 and 239). Electron microscopy shows that this Nissl substance, whose appearance initiates the phase of accelerated protein production, is composed of ribosomes and alpha-cytomembranes (177, 180, 183-186), which invites correlation with the findings of Palade and Siekevitz (noted above).

While most Nissl bodies are composed of alpha-cytomembranes with affixed ribosomes, some species also have Nissl bodies which are exclusively composed of ribosomes and are more metabolically active than the membranous Nissl bodies found in the same cells (240, 241). Degranulation, demembranization and subsequent dissolution of ribosomes in reticular Nissl bodies is observed with the use of some chemicals, malononitrile (253) and when perikaryal chromatolysis occurs, which is a process of degranulization and demembranization. Under all these conditions RNP content of the perikaryon increases enormously with a parallel increment in protein metabolism (102, 119, 120).

The origin of the ribosomes is uncertain. Three theories are postulated. The first presumes that ribosomes produced by the nuclear DNP migrate through the nuclear pores directly into the cytoplasm (48). Another assumes that ribosomes formed as above attach to the inner nuclear membrane to be "towed" to the cytoplasm as this membrane is everted due to membrane motion in which portions of the inner nuclear membrane become part of the outer nuclear membrane; subsequently the granules dissociate becoming free ribosomes (198, 199). These two hypotheses appear unlikely in view of subsequent research on the structure of nuclear pores (see above). Present conceptions are that RNP shed from their nuclear DNP templates pass through the nuclear pores and subsequently become aggregated into ribosomes or remain soluble cytoplasmic RNP.

In summary, the ribosomes are small electron dense granules which may or may not be associated with alpha-cytomembranes, are composed of RNP produced in the nucleus, whose primary function is the production of cellular structural and enzyme proteins. The initial phase of protein production is association of alpha-amino acids with soluble RNP (alpha-amino acid activator enzymes) to form a conjugate which subsequently associates with free ribosomes which in turn associate with the alpha-cytomembranes, at which point the activated alpha amino acids are joined to form proteins which dissociate from their template RNP into the lumen of the endoplasmic reticulum where they form ill-defined granules which may be passed to the Golgi apparatus and on to the extracellular space via the secretion granules (182) or directly across the plasma membrane

as in induction (121). Physiological states of increased protein production are characterized by numerical decrease in membrane associated ribosomes and a shift of RNP from the ribosomes into the soluble RNP fraction of cytoplasm; with re-establishment of protein balance, reversal of these phenomena is noted. Thus, stressed neurons losing great quantities of protein react by chromatolysis, a shift of RNP from ribosomes to a soluble form. Similarly, an increased RNP production occurs within the nucleus of such injured cells. These two factors account for the enormous increment in soluble RNP and subsequent increased protein anabolism.

C. Cytomembranes. The cytomembranes are lipoprotein membranes which though mutually interconnecting, may be differentiated into several classes: the plasma membrane, the granular endoplasmic reticulum and the agranular reticulum. The only membranes not included are those associated with the mitochondria and lysosomes. Sjostrand (235-237) defines three membrane systems within the cell: the alpha, the beta and the gamma cytomembranes.

The alpha-cytomembranes, the granular endoplasmic reticulum, is differentiated from the other two systems in that it consists of paired membranes approximately 40 A. thick and separated by a variable interval associated with these membranes are ribosomes (see previous section). The beta-cytomembranes include the plasma membrane and its invaginations such as those commonly found in the basal portion of pancreatic acinar cells. The plasma membrane averages 105 A. in total thickness, is

trilaminate, and consists of two electron dense 40 A. thick leaflets, separated by an electron lucent 25 A. thick space. The beta-cytomembrane itself consists of two trilaminate membranes, invaginated from the plasma membrane, separated by a regular 40 A. electron lucent space, which is contiguous with the amorphous basement membrane. Beta-cytomembranes are confluent with the alpha-cytomembranes. The gamma cytomembranes, the agranular reticulum, are composed of paired lipoprotein membranes 60 A. in diameter and devoid of ribosomes; the Golgi apparatus is the prominent part of this system.

While the plasma and beta-cytomembranes are morphologically stereotyped, both the alpha- and gamma-cytomembranes assume various cytoarchitectural forms such as tubules and vesicles of varying diameters, expansive interconnected cisternae whose aggregates form Golgi bodies or ergastoplasm. Each one of these cytomembrane systems appears to be directly connected with the other; thus, the perinuclear cistern, part of the granular endoplasmic reticulum, directly connects to the plasma membrane and extracellular space, via both the granular and agranular reticuli. Whether these connections are permanent or transitory is uncertain, yet most cytologists believe that continuous cytoplasmic motion brings membranes of all systems into apposition and that transitory connections between systems continuously are formed and destroyed.

Granular Endoplasmic Reticulum.

The granular endoplasmic reticulum characterized above, includes the nuclear membrane (174, 185, 186, 198, 202, 203, 235-237, 256, 259, and 263) which has previously been discussed will not be reconsidered

here, and the ergastoplasm. The granular endoplasmic reticulum enjoys ubiquitious distribution within the cytoplasm with frequent connections to the plasma membrane (173, 235, 237), nuclear membrane and the Golgi apparatus (38-42, 116, 174, 198, 202, 203, 235-237). The paired, 40 A. thick, electron dense membranes form on serial reconstruction tubules, vesicles and cysternae which are flat, broad, pancake-shaped cavities bounded by cytomembranes (174, 198, 202, 203, 235-237). When such cisternae become stacked upon each other with their planes of orientation parallel, a lamellar pattern is formed; it is called the ergastoplasm. (263). The plane of orientation of these lamellae may be flat, concave, cup-shaped, or biconcave. The ergastoplasm is the basophilic component of cells seen with the light microscope; this basophilia is due to the associated ribosomes, not the membranes. The ergastoplasm is most prominent in epithelial cells such as exocrine glands, intestinal mucosa and neurons, and also mesodermal elements especially the plasma cells of the reticuloendothelial system and renal tubule cells (174, 179, 182, 196, 198, 197, 199, 201, 202, 235-237 and 263).

In the nervous system the ergastoplasm is encountered in the neuronal Nissl body. Yet, all that is 'Nissl' is not ergastoplasm. The Nissl body was originally described as a basophilic component or body found in neurons using the light microscope; when investigated with both light and electron microscopy on adjacent sections of nervous tissue, the basophilic masses most often appear as ergastoplasm, that is, parallel lamellae of granular endoplasmic reticulum but in a less compressed pattern than found in exocrine cells (180, 183, 186, 217-219, and 227). However,

the degree of organization of the endoplasmic reticulum in basophilic areas varies widely, depending on neuron type, (the highest degree of orientation in the anterior horn cells and least in the internuncial cells of the spinal cord), and physiologic state. Furthermore, Nissl bodies of some lizard neurons are normally composed entirely of ribosomes (the 'areticular' Nissl body) (240 and 241) and the dissolution of the membranous component of ergastoplasmic Nissl bodies occurs after malononitrile treatment of live frogs. In summary, the Nissl substance has two components, a regular high concentration of ribosomes and an irregular membranous component which appears to be part of the granular endoplasmic reticulum; the specific architecture varies with cell type and the physiologic state. Nevertheless, we shall consider the Nissl substance to be the neuroergastoplasm.

The membranes of the granular endoplasmic reticulum are composed primarily of lipoprotein molecules. Biochemically, the endoplasmic reticulum is known as the microsomal fraction. The microsomes possess large quantities of protein nitrogen, RNA and phospholipid phosphate. Upon treatment of the microsomal fraction with Versene the ribosomes dissolve leaving only the bare membranes; desoxycholate treatment degranulates the membranes, permitting differential centrifugation and separation of a pure membrane fraction; both the Versene insoluble fraction and post-deoxycholate membrane fractions of the microsomes possess high concentrations of protein nitrogen and phospholipid phosphate, and also variable amounts of DPNH-cytochrome reductase activity, alcohol soluble hemochromogen and other cytochromes (178,227),

The amount of endoplasmic reticulum is an index of the amount of protein production in a cell (175, 182, 227-231 and 263) especially where ergastoplasm is found. Often this is due to enzyme synthesis as in pancreatic exocrine cells (227-231), however, ergastoplasm is associated with many types of protein production other than enzymes such as monoamines (176), posterior pituitary hormones,(183), acetylcholinesterase, and production of immune bodies by plasma cells of the reticuloendothelial system (173). Direct evidence that localization of protein hormone and enzyme synthesis in the granular endoplasmic reticulum is found in starvation during which decreased production of proteolytic enzymes by the pancreas is associated with quantitative decrease in ergastoplasm (229) and in the rapid incorporation of leucine-1-C_{14} into the microsomal fraction and thence into the secretion granules in both pancreas and liver (230).

In summary, the granular endoplasmic reticulum is a system of membranes and associated ribosomes which are found with a high degree of orientation in ergastoplasm or Nissl bodies and are the primary locus of protein synthesis, including various enzymes, protein hormones, monoamines, acetylcholesterase and immune globulins. Substantiation of its role in protein synthesis is supported not only by its association with cells and metabolic phases of high protein production but also by tracing radioactive alpha-amino acids.

Agranular Reticulum

The agranular reticulum or gamma-cytomembranes are ubiquitously distributed throughout the cytoplasm, taking the form of the Golgi

apparatus, synaptic vesicles, axonal tubules and the vesicles and vacuoles of secretion. It is contiguous with the nuclear membrane, plasma membrane directly and via secretion granules, and the granular endoplasmic reticulum (38-42, 174, 198, 199, 203) from which it is distinguished by the greater average thickness of its membranes and absence of associated ribosomes. In the early days of electron microscopic cytology much debate between Palade and co-workers and Dalton and Felix occurred concerning the synonymity of the agranular reticulum and the Golgi apparatus. Subsequently, Palade admitted that the Golgi apparatus of light microscopy was part of the membrane system he has described as the agranular reticulum. However, subsequent studies have revealed that the agranular reticulum is a more extensive system than even Palade had considered it to be, while the Golgi apparatus remains as a highly oriented aggregate of the gamma-cytomembrane system.

The agranular reticulum is found in the diffuse form primarily in the form of tubules (axonal tubules), vesicles (synaptic vesicles, Golgi vesicles) and multibranched tubules associated with steroid secretion (182). The diffuse agranular reticulum may form cisternae or lamellae with perforations called annulate lamellae; they may be found in aggregate or solitary form. The highly organized form of agranular reticulum known as Golgi apparatus (38-42, 132, 133, 175 and 243) consists of closely apposed flattened cysternae or lamellae composed of parallel tubules; the interposed cytoplasmic matrix has a dense ground glass appearance with slightly higher electron density than the surrounding matrix. The

lamellae may assume flat, curvilinear, biconcave or concave-convex contours. The flattened cysternae split into rows or parallel tubules which terminate in chains of vesicles at the periphery of the Golgi body. These terminal vesicles increase in size at the extreme periphery of the Golgi body to form Golgi vacuoles, the precursors of secretion vacuoles. The Golgi apparatus may consist entirely of Golgi lamellae, entirely of Golgi vesicles and vacuoles, or any intermediate state depending on cellular activity. The characteristic architecture of the Golgi apparatus described above has been found in a variety of species and organ systems (38-42, 132, 133, 174, 175, 198, 199, 203, and 243).

The chemical composition of the agranular reticulum is unknown because this element fails to separate out as a specific fraction upon differential centrifugation and is assumed to be included in the microsomal fraction.

The agranular reticulum may be understood as a collecting system for cell products and pathway of transport to the plasma membrane for disposal. The Golgi apparatus functions as a collecting center into which all cell products are transported; these cell products are incorporated into vesicles and then vacuoles which 'migrate' from the Golgi apparatus' usual central location to the apex or periphery of the cell where they empty by a mechanism of approximation, confluence and disintegration of the plasma and vacuolar membranes. This has been demonstrated in a variety of conditions such as proteolytic enzyme secretion in the exocrine pancreas (228, 229, 235, 236), neurosecretion of the supraoptic nucleus (31, 183), mucin and enzyme formation in the

gland cells of duodenum, formation of cytoplasmic vesicles in the testis (34, 38, 39) and the contractile vacuoles of protozoans and parazoans (90).

Particular cell areas containing an abundance of multibranched agranular reticulum have been implicated as sites of steroid synthesis. (182). Nevertheless, the major function of the Golgi apparatus and agranular reticulum seems to be the collection and disposition of cell products (182). This hypothesis applied to neurons presumes that acetylcholine or acetylcholinesterase (the 'transmitter' substance of the synapse) synthesized in the perikaryon is transported to the synapse via the gamma-cytomembranes of the Golgi apparatus, axonal tubules to the synaptic vesicles. Temptingly plausible as this seems, neither Ach or Achase has been demonstrated in the agranular reticulum by present electron microscopy histochemical techniques. Another independent system of cytomembranes related to the agranular reticulum has been suggested; agranular in nature, but with more delicate membranes than the gamma-cytomembranes, it was dubbed the irregular tubulomembranous component of cytoplasm (10); Subsequent study of steroid producing cells reveals similar systems which are indisputably gamma-cytomembranes. Rosenbluth reports subsurface cisterns and other cisternal aggregates in neuronal cytoplasm different than granular or agranular reticulum (218). Nevertheless, the status of these membrane systems is not clearly understood and they along with the annulate lamellae are presently considered as variants of the agranular reticulum.

Plasma membrane.

The plasma membrane, separating the cytoplasm and extracellular space, is a trilaminate lipoprotein membrane approximately 105 A. thick and is connected with the granular endoplasmic reticulum and agranular reticulum (236). So simple is the plasma membrane's ultrastructure that it hardly presents a challenge; however, the morphological simplicity belies the complexity of its molecular and physical nature. Far more interesting from a morphologist's standpoint are the modifications of the plasma membranes which are discussed presently. The plasma membrane usually presents a straight or undulant linear profile, in electron micrographs, which may be altered by interdigitation with adjacent cell membranes, by invagination to form beta-cytomembranes, by simple thickening to form a terminal bar, or by the appearance of additional laminae between adjacent cells and convergence of cytofilaments upon the area to form a desmosome, by the projection of microvilli and by such organelles as flagella and cilia.

Interdigitation of plasma membranes of adjacent cells, are interlocking projections of one cell into another; they may be branch or simple, multiple or solitary. Such interdigitations enhance stability of adjacent cells by locking them together. They are found in epithelial cells in the pancreatic exocrine cells (235-237), testicular and epididymal cells (38-42), intestinal epithelium and at the neuron's axon hillock (152, 153).

The electron microscope reveals the "brush border" of light microscopy to be composed of individual microvilli; these are minute

cylindrical evaginations of the apical cytoplasm which vary in diameter from 500 A. to 2000 A., but maintain a uniform diameter and length for each type of cell. Some are straight; others are spiraling, but all are covered by a normal plasma membrane. In the crypts between the microvilli, the plasma membrane appears to form vesicles under the microvillar apical border of the cell a layer of very fine cytofilaments known as the terminal web is found (5).

Desmosomes are adaptations of the plasma membranes of adjacent cells to enhance stability and intracellular adhesion, and are of two types, the terminal bar and complex desmosome. The terminal bar is an area of increased electron density and depth of adjacent plasma membranes (105 A. to 150 A.). They are often found at apices of exocrine cells as in the pancreas (235-237), the testicular and epididymal epithelia (38, 42), the intestine medulloblasts of the neural plate (239) and between neurons and adjacent glia (152, 153), between layers of loose myelin (217-219) and between ciliated ependymal cells (27, 75). The complex desmosome consists of increase in the thickness of adjacent plasma membranes to 300 - 400 A., an increased electron density, interposition of five extra linear profiles (3 electron opaque and 2 electron lucent leaflets) between adjacent plasma membranes, and termination of many cytofilaments in the thickened plasma membrane. Complex desmosomes appear in almost every stratified squamous epithelium investigated (75, 217-219) and in the areaopaca of the chick embryo vitelline membranes (12-16 and J. H. Smith, unpublished data). The cilium is an organelle derived from the centrioles and plasma membrane conjointly. Explanation

of ciliary origin begins with the description of the centriole which is composed of nine parallel, electron dense, finely granular rods arranged in a cylindrical plane. Two centrioles usually approximate each other with their axes perpendicular. The first stage of cilium formation is the approximation of the paired centrioles and the plasma membrane; then the centriolar axial angle becomes more obtuse until the centriolar axial angle is obliterated. The tanden centrioles lie adjacent and perpendicular to the plasma membrane. The plasma membrane becomes slightly invaginated into the cytoplasm at the point of centriolar contact. Shortly a cytoplasmic process is evaginated from the center of the depression; this process lengthens and tubules are elaborated from the terminae of the centriolar rods into the evaginated process. These tubules split longitudinally and increase in electron density forming the nine paired filaments of the adult cilium. Meanwhile, the area of plasma membrane-centriolar contact forms a plate into which are bound the proximal ends of the ciliar fibrils, and the peripheral centriole forms the basal body by proliferation of electron dense material between the centriolar rods to form an intact cylinder. In non-motile cilia the excess cytoplasm in the ciliar process is sloughed and the second centriole disengages from the basal body(242).

The structure of non-motile and motile cilia though basically similar are not identical. The non-motile cilium consists of the shaft with nine pairs of tubular filaments radially arranged in an amorphous granular cytoplasmic matrix, a neck piece consisting of a flat plate of

dense amorphous material into which the proximal ends of the ciliar filaments are embedded. Under the neck piece and continuous with it is a cylinder of amorphous dense material in which are embedded the nine centriolar filaments known as the basal body. From the basal body may extend one or more "rootlets" which appear, in electron micrographs as striated fibrillar bands and are collectively known as the peribasal apparatus. The motile cilia in addition to the components of the stereocilium possesses a paired central filament connected with the neck piece, but not extending into the center of the basal body, and a peribasilar apparatus which consists of one or more striated fibrillar bodies known as 'rootlets' (222).

Cilia are found within the central nervous system, (183), in the ciliated ependyma (27) and in the developing neural tube (56, 239, 242).

Though flagellae are cellular organelles intimately associated with plasma membrane, they are not discussed here because they were not encountered in the investigations of this paper.

D. Mitochondria, Lysosomes. The electron microscope reveals the acidophilic granules known to the light microscopist as mitochondria to be an organelle of unique structure and vital function. The cyto-architecture of the mitochondrion is constant regardless of its shape which may be round, oblong, filamentous, simple or branched.

The mitochondrion consists of a central mitochondrial matrix surrounded by two trilaminate membranes, the inner and outer mitochondrial membrane. The mitochondrial matrix has a homogeneous ground-glass appearance similar to the cytoplasmic matrix but of great electron

density. Occasionally minute spherules of extreme density are found in the mitochondrial matrix; they are probably associated with degenerative processes. The outer mitochondrial membrane is 70 - 120 A. thick with thick, electron dense outer and inner leaflets and central electron lucent, hydrophilic layer. The outer mitochondrial membrane completely circumscribes the mitochondrion separating it from the cytoplasmic matrix, and is not continuous with any other cytomembrane system. The trilaminate inner mitochondrial membrane is identical to the outer mitochondrial membrane which it closely approximates along most of its course. The inner mitochondrial membrane is invaginated into the mitochondrial matrix to form shelf-like structures, the cristae mitochondiales. These cristae mitochondiales may assume the form of simple or branched shelves, tongues or fimbria. The material interposed between inner and outer membranes is identical to the mitochondrial matrix. From early investigation of mitochondrial ultrastructure it was unclear whether the cristae mitochondriales were continuous with the inner mitochondrial membrane or separate structures embedded in the mitochondrial matrix. The continuity of the cristal and inner mitochondrial membranes was eventually substantiated by a number of workers (46, 69, 137, 170).

Differential centrifugation of tissue homogenates separates out a fraction composed entirely of mitochondria, which has permitted extensive biochemical investigation which has revealed that the mitochondrion is the prime locus of energy transformation within the cell. This energy transformation produces ATP which is the common denominator of intracellular energy transportation and storage. The mitochondrial

fraction contains higher concentrations of the enzymes of the citric acid or tricarboxylic acid cycle than any other tissue homogenate fraction and is also rich in enzymes of amino acid metabolism, gluconeogenesis, ketone formation and fatty acid metabolism. The enzymes of the electron transport chain which couple the tricarboxylic acid cycle to ATP are concentrated in the mitochondrial fraction (96, 136). Lysis of the mitochondrial fraction with detergents into membrane and matrix fractions which may be separated by differential centrifugation. Analyzes of these fractions localizes the electron transport chain in the mitochondrial membranes, while the oxidative enzymes of the tricarboxylic acid are confined to the mitochondrial matrix (232). The mitochondria are the power houses of the cell.

One would then anticipate that the character and quantity of the mitochondria would vary directly with cellular oxidative rate. Such correlation is found between the number of mitochondria and the characteristic metabolic rate of each cell type and between the number of mitochondria and the metabolic rate of each cell. For example, in Wallerian regeneration, perikaryal mitochondria numerically increase (102, 105, 119 and 120). As metabolic rate increases the complexity of mitochondrial internal structure manifests as a numerical increase in cristae mitochondriales, increases; tissues exhibiting a high metabolic rate have a more complex cristae and more branched cristae than tissues of a low metabolic rate (17, 18, 46, 118, 206 and 233).

Explanation of mitochondrial origin or fate are conflicting; the

most plausible present theory is that they replicate by fission of previously existing mitochondria. Nevertheless, the studies of mitochondrial origin and fate brings us to the problem of identifying several organelles of diverse structure associated by the literature with mitochondrial formation and degeneration.

The multivesicular body is similar in size to mitochondria, possesses a single or double limiting membrane enclosing a matrix of amorphous ground-glass appearance in which many tiny vesicles, 100 - 300 A. in diameter are embedded (12, 13, 183-186). No exhaustive study has been done on these organelles and though they are frequently noted, neither their morphology or function have been defined.

The next organelle considered is the liposome, lipochrome granule, lysosome, lipoid body, gliosome or senile mitochondrion; the array of names reflects the variety of cytoarchitectural entities ranging from mitochondria with slightly increased electron density (possibly due to the vagaries of technique) to amorphous highly electron dense masses without well-defined membranous limits included; several of these are described. Some mitochondria display great electron density in their matrices without loss of internal structure, while others show some deterioration of internal structure. A seemingly 'further' stage appears as an amorphous black oval mass circumscribed by two trilaminate limiting membranes; similar profiles possessing more limiting membranes are found as well as those possessing myelin figures in their matrices. Further along the spectrum are found membrane bounded electron dense granules in which the myelin fragments are oriented at angles with each other. Finally, electron dense ground-glass oval profiles are

known which have no limiting membrane. The highly osmophilic property of these profiles indicates that they probably contain ample lipid material, probably as phospholipid since myelin forms so readily, yet this group is so poorly defined that it may include several discrete entities, quite different from one another. These bodies appear more frequently in older cells such as neurons which have a long life span and are not replaced. Because of this, many presume that these forms are derived from mitochondrial degeneration. Other workers infer that these are primordial stages in formation of mitochondria. Their classification as cellular organelles is still unclear (18, 20, 36, 58-60, 70, 71, 120 and 197).

Recently published research revealed a discrete organelle previously included in this spectrum, the cytolysosomes, electron dense membrane bounded profiles containing high concentrations of acid phosphatase and formed from the Golgi apparatus; it is postulated that these organelles have a role in cellular catabolism, especially RNP catabolism (166).

E. Lipid and Yolk Granules. Lipid and yolk granules are distinctly classified entities not to be confused with the liposomes, etc. of the preceding section. Lipid such as sphingomyelin and the cerebrosides, often found in the lipoprotein membrane systems, may under certain conditions separate and aggregate to form the myelinoid inclusions found in bladder epithelial cells and nerve cells (185-186); they appear as concentrically wrapped lipoprotein membranes similar to those described in pure lipoprotein systems by Satorius (see 218).

Pure triglycerides and waxes appear as multilobate, electron dense profiles with prominent compression waves and, therefore, have a pied appearance; they are the beta yolk granules. Alpha yolk granules are membrane bounded systems enclosing concentric layers of lipoprotein granules of various electron densities. Some appear to form multivesicular bodies; others seemingly grow smaller as digested (13, 14, 15).

Section 3. The Extracellular Space.

The last discontinuous phase of tissue considered is the extracellular space that lies outside the plasma membrane. Its continuous phase has a ground-glass appearance and varies widely from usual electron lucency to the high densities found in colloid and bone. It is composed primarily of mucopolysaccharides, mucoproteins, water and electrolytes; the electrolytes may crystallize to form bone (hydroxyapatite), or the mucopolysaccharide may become concentrated so that cartilage is formed (91, 106). The discontinuous phase of the extracellular space consists of secreted cell products, collagen and elastin fibrils, necrotic material, and lipid in transport. The basement membrane is a zone of increased electron density of 200 - 700 A. thickness, composed of a dense ground-glass continuous phase and small filaments of collagen. Collagen is distinguished by its prominent 640 A. periodicity. Collagen fibers contribute the majority of the formed phase of the extracellular space. Recently, Pease has demonstrated that elastin appears as broad fibers and filaments (191).

PART II. ULTRASTRUCTURE OF THE NERVOUS SYSTEM

This section, dealing with the specific cytoarchitecture of the nervous system, will attempt to present the rudimentary concepts of ultrastructure of the nervous system and review the pertinent past and recent literature. Therefore, this part is divided into seven sections; the neuron and the general organization of the nervous system, the nerve fiber, the synapse, the glial cells, the myelin sheath and nodes of Ranvier, degeneration and regeneration and the ependyma.

Section 1. The Neuron and General Organization of the Nervous System.

The nucleus and nucleolus of the neuron are similar to those of other cells, as previously described (152, 180, 183-186, and 226). The only variation described is the agranular character of the outer nuclear membrane (185, 186) which has not been specifically substantiated. The nucleus has a homogenous ground-glass electron dense appearance with adequate fixation and the nucleolus has a coarse reticular appearance (184). Frequent nuclear pores are described and the nuclear membrane consists of an inner and outer nuclear membrane contiguous at the nuclear pores and separated elsewhere by the perinuclear cistern which is continuous with the lumina of endoplasmic reticulum (186).

The cytoplasmic matrix of the neuron is not remarkable (152, 153, 186, 226). The mitochondria are smaller than those of most other cells and tend to be filamentous (104 and 105). Later in Wallerian degeneration perikaryal mitochondria distinctly increase (102). Better technique, biochemical studies, and quantitation of mitochondria volume show an absolute and relative increase in number, size and activity of

perikaryal mitochondria following axonal section (119). Reports of lysosomes in the nervous system have increased in the last year (57, 58, 120, 197). Several investigators have reported an unquantitated impression that these lysosomes increase with neuronal age. The prevalence and distribution of ribosomes varies with neuronal type and the activity of neurons (122-127, 152, 183-186), are more prominent within the perikaryon than in dendrites and are excluded from the axons. The RNP-rich ribosomes decrease as soluble RNP increases, concomittant with RNA and protein synthesis during hyperactivity, growth and axonal section (122-127). Furtermore, in tricyanoaminopropene stimulus of RNA synthesis reversal of the pyrimidine-pyridine ratio of Deiter's nucleus neurons occurs. Neuronal endoplasmic reticulum, though existing diffusely, forms highly oriented aggregates of ergastoplasmic character, recognized as the Nissl bodies (185, 186). These Nissl bodies are composed of a regular granular component and an irregular membranous component. The Golgi apparatus usually occurs perinuclearly with an extensive system of diffuse agranular reticulum pervading the entire neuron (152, 185, 186, 226). Reported recently is the subsurface cistern, an agranular membranous cistern closely approximating (200 - 400 A.) the plasma membrane (218). It is hypothesized that such membranes lie near enough to alter the plasma membrane's electromagnetic character, subsequently modifying conductivity and excitability. The agranular reticulum

continues into the axon as the axonal tubules which terminate in the synaptic vesicles (253). Neurofilaments, fibrous proteins, characterized by Schmidt (223) have been described in both perikaryon and axon (152, 183-186, 226). Neurofilaments are numerically increased in embryonal dorsal root neurons made hypertrophic by augmenting their periphery (188).

The neuronal plasma membrane appears to be a typical trilaminate lipoprotein membrane of perfect uniformity except at the synapses where foci of increased depth and electron density appear (152, 183-186, 226) and at areas adjacent the subsurface cisterns (218)/ at the axon hillock where terminal bar desmosomes are found in some cells (152, 226). Quantitation of the contact of perikaryal plasma membranes with other central nervous system components reveals that synaptic endings contribute only 15 per cent while glial cells cover 35 per cent of the perikaryal surface and dendrites, neighboring perikarya, myelin and unidentified material occupy the rest (unidentified material enveloping 17 - 21 per cent of total surface) (23). That synaptic endings comprise such a small portion of parikaryal surface is not surprising since perikaryal synapse are probably non-functional due to the paucity of acetylcholinesterase present (45).

Recently several studies utilizing new and apparently quite successful methods of fixation have confirmed many aspects of neuronal cytology mentioned above; these papers have dealt with the rat cerebral cortex (184), the acoustic nerve ganglion (217-219), the rat spinal cord (264) and sympathetic ganglia sheath cells of the frog (266). Intact

centrifuged spinal ganglion cells fixed to preserve the resultant layering of cellular components revealed four layers, as follows:

1) A layer of membranes and granules, subdivided into three continuous layers, free ribosomes having the greatest density, ribosomes and infrequent membranes having lesser density, and ribosome associated membranes, the least density.
2) The next discrete layer contained only mitochondria.
3) Above this latter layer appeared a layer of empty vesicles impossible to correlate with any naturally occuring neuronal component.
4) The topmost layer held lipid vacuoles, and lysosomes (11).

Section 2. Nerve Fibers.

Two types of nerve fibers exist, axons and dendrites; yet such division is equivocal. Axons and dendrites have different ultrastructure as will be shown below; yet the dendrites, at least functionally, of peripheral sensory nerves have axonal ultrastructure.

Subsection 1. Dendrites.

Dendrites are perikaryal processes which serve as the primary receptors of impulses from other neurons (45), and emerge from the peritaryon in a main trunk from which originate many fine branches (56, 57, 184, 264), each of which protrudes small processes, the dendritic spines, which are the postsynaptic components of CNS synapses (93-95). The dendrites possess an abundance of ribosomes, frequent profiles of granular endoplasmic reticulum, scant agranular reticulum, and many ovoid

mitochondria especially in post-synaptic areas on the dendrite spines (93-95). Neurofilaments are abundant, though not as prominent as in the perikaryon and axon. In summary, the cytoarchitecture of dendrite cytoplasm and perikaryal cytoplasm are identical except that less agranular reticulum and no ergastoplasmic Nissl bodies are found (57, 184, 264).

The axon and the peripheral sensory dendrite are identical and considered together here. The earliest valid study of axons by Hess and Lansing (112) described the very pale axoplasm due to the virtual absence of ribosomes and the undulant anastomosing filaments, 70 A. in diameter, whose long axes roughly parallelled the axonal axis, the neurofilaments. Existence of neurofilaments was further confirmed using Epon 812 and various stains by Elfvin (65). Longitudinally oriented membranous profiles of the agranular reticulum are found. Thin filamentous mitochondria with normal internal structure are frequently found throughout the axoplasm. The axolemma, axonal plasma membrane is thin and trilaminate. These findings were quickly confirmed by Hama (97), Palay and Palade (185, 186) and recently have been reaffirmed using better technique (184, 264). Several investigations have shown that the axonal agranular reticulum which is associated with its counterpart in the perikaryon, including the Golgi apparatus, is the source of synaptic vesicles whose exact function in the synapse is as yet uncertain (93, 94, 253). The relationship of the axonal protein and neurofilaments have been adequately described and chemically characterized by Schmidt (223) previously mentioned. These basic concepts

of axonal ultrastructure have been affirmed by the recent introduction of vastly improved fixation and embedding techniques (57, 184 and 264).

Section 3. The Synapse.

Broadly speaking a synapse is a contact between two cells across which passes an impulse, the nature of which is unsettled and may be electromagnetic, chemical or electronic. Some synapses excite while others inhibit the effected cell, and a variety of substances have been postulated as mediator of the impulse passage across the synapse. Synapses may be classified as exictory or inhibitory, intraneuronal or neuromuscular; the intraneuronal synapses may further be classed as axodendritic, axosomatic, or axonal, while the excitory neuromuscular synapses are divided into rapid-reacting and slow-reacting synapses.

There are five essential morphological elements in every synapse: the presynaptic cytoplasm, the presynaptic plasma membrane, the synaptic cleft, the post-synaptic membrane and the postsynaptic cytoplasm. Each one of these has distinct characteristics regardless of the nature of theccell contributing it and these characteristics will be considered first. The presynaptic cytoplasm is always the axonal bouton termineaux. It is enveloped in a normal plasma membrane and surrounded by the sheath or Schwann element except at the contact point with the effector organ. There are no neurofilaments, ample spherical eliptical mitochondria with many simple cristae. In the cytoplasmic matrix are embedded scattered profiles of endoplasmic reticulum and great numbers of synaptic vesicles whose diameter varies from 100 - 300 A. (35, 43,

49-51, 53, 55, 177, 179, 181). As previously noted, these vesicles originate at the termina of the axonal tubules (253). It has been postulated that these synaptic vesicles contain acetylcholine and that the content of these vesicles is poured into the synaptic cleft to act as the mediator substance (49, 55).

The presynaptic membrane which is a continuation of the axolemma around the bouton termineaux at the synapse is closely applied to the effector organ plasma membrane from which it is separated by an interval of only 200 A. The thickness of the presynaptic membrane is 70 - 120 A. except at certain foci of increased thickness and electron density (177, 179, 181); these have been postulated to be the sites of liberation of acetylcholine from the synaptic vesicles (49, 55). The synaptic bars may correspond to foci shown by electron microscope histochemical techniques to possess acetylcholinesterase activity (9, 45).

The synaptic cleft is usually 200 A. across but may be expanded under a variety of conditions. The cleft is filled with an amorphous ground-glass appearing material which is PAS positive, stains with Alcian blue and, therefore, is probably a mucopolysaccharide; this material is contiguous with the basement membrane surrounding the periaxonal sheath cell and the effector organ (35, 43, 205 and 267). It is known as the amorphous synaptic material (ASM) and contains ample acetylcholinesterase as demonstrated by light (35) and electron microscopic histochemical techniques (9 and 45), as are foci along the presynaptic membrane (9, 45).

The postsynaptic membrane is the trilaminate plasma membrane of the effector cell, either a muscle cell or a neuron. It may show synaptic (terminal) bars adjacent those of the presynaptic membrane (177, 179, 181) which have been postulated as recipient areas of synaptic vesicle contents (49-53, 55). The postsynaptic membrane possesses the highest concentration of acetylcholinesterase and cholinesterase found with light or electron microscopic techniques (9, 35, 45).

The postsynaptic cytoplasm contains all of the characteristics of the effector cell's cytoplasm plus aggregation of mitochondria at the post-synaptic area.

This simple fundamental pattern of synapses is exactly duplicated by central nervous system synapses. Approximately 15 per cent of the perikaryon surface is involved in such synapses (23); however, these axosomatic synapses possess a paucity of acetylcholinesterase and the only areas of acetylcholinesterase activity in the cerebral cortex are at the axodendritic synapses (45). These axodendritic synapses assume the form of dendritic spines (94).

In the peripheral nervous system three types of synapses have been described. The autonomic ganglionic synapse resembles the central nervous system synapses except that the bouton termineaux is flattened around the perikaryon of the effector neuron like a pancake (35). Slow-reacting neuromuscular synapses are identical to central nervous system synapses, as are those of unstriated muscle (205; inhibitory

synapses differ only by the presence of annulate lamellae and ergastoplasmic profiles on the presynaptic side (196). The most highly developed synapse is the rapid-acting neuromuscular synapse or myoneural junction. All the fundamental characteristics of the CNS synapse are present except that the postsynaptic membrane and cytoplasm is furrowed by numerous deep narrow parallel troughs called secondary synaptic clefts (9, 35, 43 and 267). Both primary and secondary synaptic clefts are filled with amorphous synaptic material described above (267), and as previously noted, the postsynaptic membrane, amorphous synaptic material and foci along presynaptic membrane have high acetylcholinesterase activity (9). No acetylcholinesterase activity is evident within the synaptic vesicles (9). Though the synaptic vesicles may contain high concentrations of acetylcholine, they are devoid of acetylcholinesterase or cholinesterase; the significance of this data is not immediately apparent.

Section 4. The Glia.

The supporting elements of the nervous system have presented considerably greater problems than the neurons. First, nomenclature of glia and, second, the derivation of myelin have been sources of controversy; the history of these conflicts has adequately been reviewed previously (239).

Glial nomenclature has been a subject of much dispute since the earliest investigations of the nervous system; at the time of a previous paper (239) two rival systems had arisen, yet recent developments have markedly altered this situation. Two major divisions of central nervous

system gliocytes, the microglia and the macroglia, exist. The microglia have evoked no dispute and appear as small dendritic cells with large round or ovoid nuclei and cytoplasm compactly filled with granular endoplasmic reticulum and ribosomes. The osmophilia of these cells is so pronounced that they appear the same regardless of preparation technique.

The nomenclature of macroglia has been the subject of much dispute. As previously reviewed (239), two rival schools existed; Luse, et al., (140-150) proposed that certain electron lucent cells of the central nervous system were the oligocytes of the light microscopist, while other electron lucent cells filled with bundles of cytofilaments were fibrous astrocytes, and other cells with cytoplasm of electron density intermediate between the oligocyte and the neuron were protoplasmic astrocytes. The other scheme of nomenclature supported by Pease, et al., (152, 153, 225 and 226), Farquahr (74) and Torack, et al. (247, 248) believed that the electron lucent cells were protoplasmic astrocytes while cells containing a multitude of cytofilaments were fibrous astrocytes, and the cells with cytoplasm of intermediate electron density were oligodendroglia. Each system ignored great variations in nuclear size and shape, cell size and shape and position with respect to neurons or capillaries extant within each cellular class. It was soon apparent that both systems agreed that cells possessing bundles of cytofilaments were fibrous astrocytes. Most interesting are recent works establishing the location of edema within the nervous system. Originally, Torack (247) had indicated that cerebral edema was entirely intracelluhr lying

within the protoplasmic astrocytes, confirmed by Luse and co-workers with the anticipated difference in nomenclature (150); yet, edema in myelinated white matter lies within the extracellular space (150, 247, and 248). While the validity of these works is in question in view of the results of recent technical improvements, the consistency produced by these workers certainly represents a functional, if not anatomic, difference. The concept of the blood-brain barrier recently has been philosophized and reviewed (89).

Because of several new prefixation techniques, including aortic perfusion, injection into the ventricular system, especially the fourth ventricle, and direct immersion of spinal cord in the cold fixative solution our understanding of macroglial types has been markedly altered (28-30, 184, 217, 219, 264, and 265). Furthermore, studies on pretreatment of unmyelinated neurite sheath cells have shown that hypertonic solutions produce extremely electron lucent cells appearing like protoplasmic astrocytes while isotonic solutions produce cells similar to oligodendroglia and hypertonic solutions appear to crenate the cells to appear like microglia (68).

The results of these new studies vary, yet show only two types of glia, the fibrous astrocytes and the oligodendroglia. Only one type of glial cells were found in the area postrema (264, 265) and cerebellum (220) the oligodendroglia; while the reverse was found in the rat spinal cord where oligodendroglia were found associated with myelinated and unmyelinated nerve fibers while fibrous astrocytes were found interspersed between these neurite-oligoglial bundles, especially in subpial

layers (264). Palay found two macroglial types of cells (184), the first of which was a "relatively small cell with rounded nucleus and a narrow rim of cytoplasm, which is crowded with ribonucleoprotein particles. The endoplasmic reticulum is usually disposed in the form of one or two broad cisternae concentric with the nuclear envelope. Processes are not usually encountered but when they are included in the section, they often display fine long canaliculi similar to those characteristically found in the dendrites and axons. The nuclear envelope and endoplasmic reticulum exhibit a much greater tendency to swell and distend than do those of any other cell in our experience. The boundary or plasmalemma of this cell is difficult to follow as it seems always to lie tangentally to the plane of section over most of the perimeter of the cell. The mitochondria are sparse, usually elongate with numerous ill-defined, transversely oriented cristae. This cell is here identified as the oligodendrogliocyte. It is commonly encountered both in the gray and white matter, often in rays between myelinated fibers and as a satellite to the large neurons." (184)

"The second neuroglial cell is usually larger than the first and has either a rounded or irregularity elongated nucleus. Its cytoplasm is much more voluminous and is occupied by prominent bundles of very fine long filaments, sometimes oriented in swirls about the nucleus and nearly always extending into the several processes. The endoplasmic reticulum consists of dispersed small vesicles and shortened tubules. The ribonucleoprotein particles although present are not conspicious. Consequently, the cytoplasm appears lighter than that of the first cell.

The mitochondria are few but larger than those in the neurons. The limiting membrane of this cell is more readily followed than that of the first cell. It extends over numerous processes or arms which insinuate themselves between myelinated nerve fibers or along the basement membranes of capillaries. Consequently the cell usually has a stellate shape even in thin sections. This cell is here identified as the fibrous astrocyte." (184)

It may be seen from this reference that the cell formerly known as the protoplasmic astrocytes may be no more than a fixation artifact; that apparently two macroglial cell types normally exist, the oligodendroglia and the fibrous astrocytes. However, Bunge, et al. (28-30), have demonstrated a cell occurring during the reparative phase of central nervous system lesions which they call the 'reactive macroglia.' It is larger than an oligodendroglia with an oval though often irregular nucleus; the cytoplasm is characterized by dense endoplasmic reticulum and ribosomes, normal mitochondria, and the cytofilaments though numerically greater than in oligocytes are less frequent than in the astrocytes, and not aggregated into bundles as in fibrous astrocytes. The oligodendroglial cells have often been associated with the formation of myelin within the central nervous system and are considered part of a greater family of cells, the sheath cells, including ganglion sheath cells and Schwann cells of peripheral nerve fibers. Recently it was shown that cells of oligodendrogliomas apparently possess the ability to form whorls which after expression of its cytoplasm appears

as loose myelin, but without association with nerve fibers (216). This would indicate the intrinsic ability of the oligodendrogliocyte to form whorls independent of other stimuli. Sheath cells in Schwann cells of regenerating dorsal root ganglia have the capacity to form collagen (163) and similar perivascular cells of the central nervous system, cells clearly of neurogenic origin are capable of becoming phagocytes where they are (154). Thus phagocytosis appears to be accomplished in the nervous system by microglia, as previously thought, perivascular cells (154) and oligodendroglial cells (28-30), as well as fibrocytic infiltration from the arachnoid and pial membranes.

Section 5. Myelin.

Beta-cytomembranes have previously been discussed, and in the nervous system its representative is myelin. The subject of myelin has initiated much dispute since the early days of electron microscopic investigation. The first dispute concerned the source of the birefingence of myelinated nerve fibers. DeRobertis (47 and 52) and Bennett (19) held that the source of this birefingence was neural tubules which they had described, while Fernandez-Moran and Finean believed that myelin laminae caused the birefingence (77-80).

Another source of contention concerns the myelin formation. In 1954, Schmidt and Geren made a remarkable discovery concerning the myelinization of sciatic nerves in frogs (224, 249, 251); they described first the formation of the mesaxon by invagination of the axon into the

sheath cell, then formation of loose myelin by spiral wrapping of the Schwann cell cytoplasm around the axon carrying the mesaxon with it in a spiral manner and finally condensation of the loose myelin into compact myelin by expression of cytoplasm from between adjacent spirals of mesaxon. Subsequent to this cytoplasmic expulsion, apposition of the inner electron dense lamina of the trilaminate plasma membrane of adjacent mesaxon spirals produced the major dense line of myelin, while adjacent external electron dense leaflets of the two trilaminate plasma membranes of the mesaxon form the minor dense line (224, 249, 251). Maintenance without further alteration was demonstrated by investigating the ultrastructure of myelin subjected to hypotonic and hypertonic solutions in vivo revealing the cleavage of myelin by splitting of the mesaxon between the external leaflets comprising the minor dense line (212). The morphology of compact myelin has been extensively investigated using both electron microscopic and x-ray diffraction techniques; these studies (65-68, 77-83, 158, 193-195, 208-215) reveal a series of membranes consisting of a 75 A. major dense line, a 30 A. electron lucent leaflet, two 25 A. thick electron dense lines comprising the minor dense line / a 30 A. electron lucent line and a 75 A. major dense line, forming the serial repeating unit of compact myelin. The dimensions of the lamina vary greatly with the preparation technique. A radial component of compact myelin was also described (195). The Schmidt-Lanterman clefts are ascribed to shearing defects (211). Subsequently Luse ascertained a similar mechanism of myelinization in the central nervous system (142). Palade and Gasser showed that the relationship of olfactory and

peripheral unmyelinated nerve fibers to their sheath cell mesaxons was the same as the primordial mesaxon formed in myelinization, but that bundles of olfactory neurites utilized a single mesaxon (85, 86). Hess affirmed that in the peripheral unmyelinated nerve a mesaxon bears a single neurite, and further corroborated findings of Schmidt and Geren, and demonstrated that myelinated and unmyelinated nerve fibers might use the same sheath cell (108). DeLorenzo confirmed Gasser and Palade's findings concerning the olfactory nerve structure and sheath cell relationships (44).

In 1954, DeRobertis, et al., proposed a new theory of myelinization in the central nervous system by formation within the sheath cells of myelin laminae which were subsequently applied to the axons and reconstituted to form compact myelin (54). This theory though never generally accepted, stimulated much study. The sheath cell relationship of unmyelinated peripheral sensory ganglion nerves were subsequently redescribed as similar to those originally described by Gasser and Palade (88, 89) as were unmyelinated sympathic splenic autonomic nerve fibers (65-67). Optic nerve investigation by Maturana revealed desmosomes on the mesaxon, and several fascicles of unmyelinated nerve fibers as well as solitary myelinated nerve fibers enveloped on secondary mesaxons springing from a primary one (151). Peters concluded that central nervous system myelin is similar to that of the peripheral nervous system except that the inner spirals of the mesaxon are separated with cytoplasm extrusion was incomplete. The outer lamina of myelin was not covered by an envelope of sheath cell cytoplasm (193, 194).

DeRobertis' theory was further invalidated by Robertson's studies on a variety of central and peripheral nervous system sources in a variety of species; included the expansion of myelin in hypertonic and hypotonic solutions (208-215).

Bunge, et al., (28-30), while studying remyelinization in the spinal cord ascertained the process of central nervous system myelinization in greater detail than previously provided and explained the differences between the central and peripheral myelin noted by Peters. Initially, an oligodendroglial process coils about an adjacent axon like a pancake being rolled into a suzette with subsequent expression of the cytoplasm and condensation into normal compact myelin (28-30, 193, 194, 212 and 249). Recently a perikaryal meylin sheath has been described which is composed of loose myelin, that is, myelin in which the mesaxon has been formed but expulsion of intervening cytoplasm and condensation into compact myelin has not occurred. This perikaryal myelin appears to be derived from several sheath cells, branching appears to be common and condensation of the myelin varies greatly. The occurrence of the perikaryal myelin has so far been reported only for acoustic nerve ganglion (217, 219). The nodes of Ranvier are located at the junctures of adjacent sheath cells along an axon. At the terminae of myelin sheaths, the major dense line cleaves to encompass a narrow rim of sheath cell cytoplasm; the minor dense line is split to form the outer electron dense leaflet of an apparently normal plasma membrane surrounding the rim of sheath cell cytoplasm (67, 209, 213, 250, and 252). The major difference between central peripheral nodes of Ranvier is that in the

central nervous system the innermost myelin lamina open out to form cytoplasmic bleb proximally while the most external lamellae open up distal (to the sheath cell nucleus), while in the peripheral nervous system the reverse is true; the external lamellae opening out proximally and the internal lamellae opening distally.

The composition of myelin biochemically has been extensively investigated by means of x-ray diffraction studies in which a regular arrangement of lipoprotein molecules has been found. It appears that the major dense line is composed of a lipid bileaflet with each leaflet containing a repeating unit of lipid moieties in the following order: phosphatidyl serine, cholesterol, cerebroside, cholesterol and sphingomyelin; the protein or hydrophilic component has yet to be characterized despite vigorous efforts (77-83, 158).

Section 6. Degeneration and Regeneration in the Nervous System.

Understanding of nervous system morphogenesis is greatly aided by studies of degeneration and regeneration of adult nervous systems; degeneration occurs quite frequently in the embryonic nervous system (239) and regeneration of severed axons entails chromatolysis and increased acidophilia processes that return neurons to a state of growth. Axon regeneration affords an opportunity to view growing adult neurites and to correlate it with initial neurite protrusion in the embryo. In the first 48 hours after neurotomy the highly organized alpha-cytomembranes of the Nissl bodies are disrupted by dissolution of membranes and solution of

both attached and free ribosomes; by the fifth day a numerical increase in perikaryal mitochondria herald inception of Wallerian regeneration (102, 119, and 167). Ten to eleven days after hypoglossal neurotomy there occurs a numerical increase in perikaryal mitochondrial profiles per unit area, and an increase in the mitochondrial/cytoplasmic volume ratio to approximately 70 per cent; 3/5ths of this is due to mitochondrial swelling and 2/5th due to absolute numerical increase of mitochondria (119).

The first evidence of degeneration in axons is the accumulation of axonal mitochondria at the nodes of Ranvier in the first 12 hours (260); almost simultaneously vesiculation of agranular reticulum tubules occurs (167, 254). During the following 24 to 48 hours the protoneurofilaments dissolve, fragment, and subsequently agglomerate into granules which during the third day agglutinate forming irregular electron dense masses. Simultaneously mitochondria enlarge; their matrices assume an increased electron density and by the third to fifth day there is disintegration of the cristae mitochondriales (111, 167, 254). During the third to fifth day the entire axoplasm agglomerates to form highly electron dense profiles which decrease in size to disappear by the fifteenth day leaving no remnant of the axon (149).

The character of myelin degeneration varies with the locus in the nervous system and the type of trauma exerted. Three types of injury have been studied: neurotomy, experimental diphtheritic neuritis and sudden compression-decompression by rapid withdrawal and reinjection of spinal fluid. Normal enfoldings and indentations occur in the myelin

sheath; at the apices of such enfoldings in either the Schwann cell or axonal cytoplasm are ellipsoids of degenerating myelin (261). In areas of post-neurotomy degeneration similar but more expansive myelin figures indicate the internal collapse of the myelin; this internal collapse is secondary to the collapse of the axon by physical stress and produces the myelinoid profiles known as ellipsoids (83, 167), which vary from myelin-like figures to granulation of the myelin lamellae and ultimate disaggregation and digestion of these granules (167). This ellipsoid formation is ubiquitous by the third day after peripheral nerve neurotomy (92) and the fourth day is followed by phagocytosis by Schwann cells (92); however this phenomenon takes place more slowly in the central nervous system such as in the optic nerve where myelin phagocytosis is not seen until 100 - 200 days after neurotomy. These findings correlate with the paucity of lipid loss in the central nervous system until 100 days after damage while in the peripheral nervous system significant loss occurs by the 16th day and scant myelin or lipid remains at 100 days (92). In experimental diphtheritic neuritis, the first sign of ellipsoid formation is at the ends of the myelin sheaths, the nodes of Ranvier, and this ellipsoid formation correlates with the onset of clinical weakness; subsequent ellipsoid formation along the greater length of the myelin sheath commences at infoldings and loci of previously existing Schmidt-Lantermann clefts (62). A different sequence of events has been demonstrated in the rat spinal cord after rapid withdrawal and reinjection of spinal fluid. In this type of regeneration axons remain intact while Schwann cells and

myelin degenerate. By 29 hours the myelin of the subpial areas has formed ellipsoids. Oligodendrioglia have degenerated and phagocytes known as "Gitter" cells appear; the origin of these cells is obscure, and though microglia have previously been implicated this author has demonstrated that macrophages from the blood are one assured source of Gitter cells. Cells which have the appearance of lymphocytes differentiate from the perivascular areas to form Gitter cells. By the third day the axons remain intact while myelinolysis continues; by the sixth day demyelinization is complete with no evidence of lipid remaining. By the 10th to 14th day cells called the 'reactive' macroglia previously described proliferate from an unknown source and appear to remyelinize the naked axons; these reactive macroglial processes envelope the naked axons in the same manner that the foot plate of a squid tentacle wraps around a man's arm (28-30).

Helpful in understanding structure and behavior of regenerating neurites and their growth cones is a paper describing in vitro mechanisms of nerve fiber growth. Nakai and Kawasaki had described very fine processes extending from the amoboid processes of the growth cone, called phylopodia. They notice a difference in the manner of adhesion to different substances, such as cell debris, glass, metal, starch, polyethylene paraffin and cholesterol, indicating that the growth cone follows the pathway pre-established by the phylopodia which indiscriminately palpate the objects in its path and react to each of them in a manner intrinsic in the phylopodia; such substances as cholesterol repel the phylopodia

while phylopodia have a distinct affinity for mesodermal fragments which presents an adequate substrate upon which the growth cone may grow. These workers demonstrated that the phylopodia exerted a definite force of approximately 3×10^{-10} dynes and are capable of clot lysis; active adhesiveness, tensile strength and retractility; they have a definite affinity for neurites which facilitates the formation of nerve trunks (161, 162). Also essential to understanding the peripheralization of nerve fibers is the concept, proposed by Weiss, of the existence of an intracellular lattice of colloidal micelles whichpprovides a substrate upon which 'pioneer' nerve fibers travel to the periphery (239).

The regenerating axon bears many similarities to adult axons. Its ground substance is pale; mitochondria are infrequent; protoneurofilaments are finer and thinner than in adult axons; the granular endoplasmic reticulum is scant, and the agranular reticulum is implicated in filament proliferation, and presents vesicles correlated with pinocytosis (167). These axons can penetrate the basement membrane surrounding the Bungner bands which are composed of Schwann cells remaining after degeneration of the previous axons and myelin sheaths (168). The growth cone possesses many microvesicles, 200 - 700 A. in diameter, frequent elongated and often degenerate mitochondria and abundant multivesicular bodies. Protoneurofilaments are found in the neck of the growth cone but not in the growth cone itself (72). Chains of large vesicles passing from the growth cone into the axon may correlate with pinocytosis (13).

Section 7. Ependyma.

The ependyma is composed of cuboidal and columnar cells, each with a round regularly margined nucleus bounded by outer and inner nuclear membranes enclosing a perinuclear cistern perforated by the nuclear "pores". The cytoplasmic matrix of moderate electron lucency contains numerous normal mitochondria, loose ergastoplasmic profiles similar to loose Nissl substance, scant numbers of ribosomes and stereocilia, typical non-motile cilia with nine paired peripheral filaments and without central filaments (27). Similar cytoarchitecture has been described in the developing neural tube in young rats and fetal rabbits (244, 245); formation of stereocilia in the ependyma was originally portrayed by Sotelo and Trujillo-Cenoz (242). Ciliogenesis occurs at the time of closure of the neural tube in the chick embryo (239).

PART III. ELECTRON MICROSCOPY OF EMBRYONIC TISSUES

This section will be divided into two parts. The first emphasizes cytoarchitectural characteristics specific to embryonic cells, while the second deals with various individual embryonic tissues.

Section 1. General.

Two phases of embryonic cell adaptation, proliferation, and differentiation predetermine the cytoarchitectural character which is quite different in these two adaptive states; all stages of a gradient exist between the two extremes.

The embryonic cell adapted to mitotic division has a nucleus identical to that in the resting phase of other embryonic and adult cells; however, during mitosis the nuclear pores increase in size and coalesce as retraction of the perinuclear cistern and nuclear membrane occurs. This abolishes the nucleocytoplasmic barrier permitting an undetermined amount of admixture; soon after, the chromosomes become visible; the extent of chromosome demonstrability varies with the preparation technique used (20). The cytoplasm of mitotic cells possess prominent centrioles from which very fine protein filaments radiate toward the chromosomes; these are the spindle fibers. The plasma membrane is extremely thin, often being invisible in tangential sections. The demand imposed by the extensive increase in the total surface area of plasma membrane during growth and division apparently exceeds the production of lipoprotein for membrane, thus necessitating a thinner plasmalemma. (75) The structure of the plasma membrane has never been specifically demonstrated

as trilaminate in embryonic cells. The granular endoplasmic reticulum of mitotic cells is usually scanty; its role in the proliferation and retraction of nuclear membranes has been mentioned above. The diffuse agranular reticulum rarely presents discrete Golgi bodies. Despite the great metabolic demands imposed by proliferation on mitotic cells, the mitochondria remain extremely simple but proliferate numerically assuming an ovoid or circular form with few simple cristae mitochondriales. The mode of mitochondria proliferation is unknown. Most remarkable is the abundance of ribosomes; biochemical studies show that embryonic cells are rich in both ribosomes and soluble RNP (122) which accounts for the rapid incorporation of labelled amino acids into cellular protein (122 and 123).

As cells begin to differentiate mitoses with their alteration of nuclear membranes and pores occur less frequently terminating in a mixture of the nucleoplasm and cytoplasm. Centrioles previously occupied in spindle formation initiate cilia formation (242). The thickened plasma membrane assumes a trilaminate character and forms terminal bar type desmosomes (75). The extensive hypertrophy of these differentiating cells explains the failure of the plasma membrane to attain the thickness of adult cells. In early differentiating cells the endoplasmic reticulum becomes more extensive, yet this hyperplasia is less than that of later stages of differentiation when ergastoplasm or Nissl substance is formed. The ribosomes and their associated soluble RNP remain most prominent. Among the various forms of protein produced by the RNP of the cytoplasm is that which is ultimately

incorporated into cytoplasmic filaments; these are most numerous in ectodermal cells. The probably site of cytofilament origin is the agranular reticulum (72, and 255); the Golgi apparatus hypertrophies quantitatively approaching that of most adult cells and forms discrete Golgi bodies. Some mitochondrial hyperplasia occurs with a predilection for filamentous forms without increase in complexity of the internal structure. Various forms of alpha yolk granules and yolk degradation products, the beta lipoid granules which assume an irregular multilobular profile and show artifactual compression waves, numerically decrease as maturation progresses (12, 15). Glycogen within the cytoplasm is seen as distinctly electron lucent circular profiles which may be single, multiple, or confluent giving a scalloped lacy appearance to the cytoplasm; the electron lucence is due to extraction of the glycogen which usually occurs with processing of the specimen (128). Dead cells are more frequent than in adult tissues. Upon the stage of degeneration depends the appearance of such profiles from the initial decomposition of membranes, and lipoid degeneration of mitochondria, subsequent formation of irregular lipoid bodies and agglomeration of ribosomes into electron dense bodies to the terminal myelinoid profiles or large lipoid masses. The nuclei suffer the same fate as the ribosomes. Such figures may be enclosed within the degenerating cell's plasma membrane, separated by electron lucent areas, or within the cytoplasm of phagocyte (16).

Section 2. Ultrastructure of specific embryonic tissues.

This section is subdivided into three sections, each dealing with a primordial germ layer; however, the first section includes yolk of extraembryonic origin with ectodermal structures because of the close functional relationship with the blastodisc membrane.

Subsection 1. Ectoderm and yolk.

The earliest study of embryonic tissue was done in an effort to demonstrate the ultrastructure of viruses known to infect embryonic membranes of the chick (5-7 and 129); however, these works preceded the advent of Palade's fixative, and contributed little knowledge of virus morphology or embryonic membrane structure. Nevertheless, subsequent works have demonstrated a variety of viruses in infected membranes as well as virus particles in normal embryonic cells (8 and 86) which are similar to particles found in cells of the Rous sarcoma of chickens.

The earliest valid electron microscope study of embryonic tissues concerned the areas opaca and pellucida of the chick embryo blastodisc (12) and the chorioallantoic membrane (24); it was shown that the areas opaca and pellucida are composed of cuboidal cells with relatively undifferentiated cytoplasm which contains much yolk. The area pellucida possesses two cell layers, endoderm and ectoderm, which are cytoarchitecturally similar; the endodermal layer ingests yolk particles and passes them on to the ectodermal layer which transports them to the embryo. In the area opaca only one ectodermal layer is found; these

cells are more differentiated than ectodermal cells of the pellucida possessing stereocilia on the dorsal surface, interdigitated cell membranes, and terminal bar and complex desmosomes (12; J. H. Smith, unpublished data). A theory of yolk digestion based on morphologically differentiable yolk granules was proposed by Bellairs. She presently feels that this work is equivocal, yet her classification of yolk granules into alpha 1, 2, 3, 4, 5 and beta types remain extremely useful (12). She has recently demonstrated the ultrastructure of unincubated yellow and white yolk and is presently preparing a paper on the yolk of incubated eggs (15). Cell death and phagocytosis in the embryo has been most able characterized in the blastodisc membranes (14 and 16).

The only paper specifically dealing with embryonic ectoderm itself preceded Palade's fixative and yielded equivocal results (61). Previou researches of the author have demonstrated that embryonic ectoderm is composed of relatively undifferentiated cuboidal and low columnar cells.

Most remarkable of the ectodermal derivitives is the nervous system. At the earliest stages the neural plate possesses a basement membrane, the external limiting membrane, which arises at the two somite stage in the chick embryo (56). This membrane persists, surrounding the entire neural tube as it differentiates from the ectoderm (239) remaining until the emergence of 'pioneer' nerve fibers at Hamilton-Hamburger (98) stage 14 when it is broken (239) and disappears by Hamilton-Hamburger stage 17 to 19 (239, 244). The plasma membrane of the

medulloblasts is even thinner than that of other embryonic cells (13, 156, and 239). Terminal bar desmosomes and terminal webs are found in the juxtaluminal zone at very early stages (13, 56, 239, 244); often these terminal bars lock several medulloblasts together, as if they radiated from one point on the ependymal lumen, forming the ependymal 'cones' (239). Cytoplasmic processes and cilia were reported at the juxtaluminal surface of medulloblasts (56) and in the foot processes of spongioblasts (244); these developed during closure of the neural tube (239) in a manner amply demonstrated in the chick embryo (242). Bellairs (13) has shown that cells of the neural tube contain beta type yolk droplets which gradually decrease and disappear by Hamilton-Hamburger stages 16-17. She demonstrated proliferation of mitochondria until Hamilton-Hamburger stages 10, followed by a decrease in mitochondria inversely proportional to proliferation of the endoplasmic reticulum; this reversal is accelerated at Hamilton-Hamburger stages 23 through 35 which corresponds to the appearance of Nissl substance in perinuclear zones at stages 23-26 and peripherally in stages 26-35. This is comparable to similar phenomenon in Wallerian degeneration and regeneration after neurotomy (100 and 119). Focal dilatation of the endoplasmic reticulum of neuroblast cell processes is correlated with pinocytosis. Neurofilaments appear at Hamilton-Hamburger stages 16 (13) or earlier by the author at Hamilton-Hamburger stage 18 (239). Tennyson (244) has described the architecture of ependymal spongioblasts and germinal cells, undifferentiated cells of the mantle layer,

neuroblasts and the invasion of the ependymal layer by capillaries penetrating the poorly developed basement membrane in chick embryos of an unidentified age.

Smith (239) attempted to trace differentiation of the neural plate cells in early stages (Hamilton-Hamburger stages 7-19) emphasizing evolution of neurons and gliocytes from the germinal cell of His. Though the present work renders equivocal much of this previous paper, he characterized 3 cell types in the neural plate, the germinal cells, columnar medulloblasts and intermediate forms. The columnar medulloblasts of this stage typically display an apically-polarized Golgi apparatus and basally polarized ribosomes and often form ependymal cones. This basic pattern is altered only by increased longitudinal and transverse axis growth of the basal-part of columnar cells until the closure of the neural folds when cilia appear in medulloblast foot processes. The development of an affinity for phosphotungstic acid stain by the neural tube at Hamilton-Hamburger stages 12-14 was demonstrated to precede dynamic cytoarchitectural alterations commencing in Hamilton-Hamburger stages 12 and 13; these changes include a numerical increase in ependymal mitoses, formation of bipolar neuroblasts with protoneurofilaments. The marginal layer is formed during Hamilton-Hamburger stages 13 - 17 by interposition of axons of the neuroblasts between the external limiting membrane and columnar elements. Rupture of the basement membrane by emerging pioneer nerve fibers occurs at Hamilton-Hamburger Stages 14 in the cervical segments by which time the lateral mesoderm has already differentiated, losing its segmental

pattern. The circumferential cleavage of the ependymal palisade composed of columnar cells into an internal palisade (presumptive ependyma) and outer palisade (presumptive mantle layer) precedes differentiation of the outer palisade into the bipolar and unipolar neuroblasts of the definitive mantle layer. Theoretical sequences of neurono- and gliogenesis were proposed (239).

Induction, an embryonic process, has been studied in the lens placode where certain (PAS-positive) electron dense masses were shown in the extracellular space between the presumptive lens and ribosome-rich areas of the inductor optic cup (121). As previously mentioned in the section on myelin, formation of myelin sheaths and nodes of Ranvier were first demonstrated in the larval frogs (224, 249-251). Definitive maturation of cortical cells in the rat does not occur until the first 16 days postpartum when typical cell position are assumed, definitive synapses formed and glial and neuronal cell processes elaborated (204).

Subsection 2. Mesoderm.

Mesodermal structures have been less thoroughly investigated. The notochord is formed of relatively undifferentiated polygonal cells surrounded by a basement membrane (13 and 56) which apparently is distinctly separate from the basement membrane of the neural tube (56). Certain extracellular mesenchymal fibrils originate at the notochord margins and are found in greatest concentration near the notochord, spreading as development proceeds (56). Recent work on the notochord

of Amphioxus (61) reveals a markedly different structure than in the embryo (13, 56, 239, 244) with longitudinal tubulofibrillar component apparently passing through a series of notochordal cells (61). While early studies on the chick embryo heart indicated that myofibrils were first found to appear on the third day and that intercalated discs developed by the fifth day (113, 114), recent work reveals myofibrils spontaneously forming at 29 hours; composed of sarcoplasmic reticulum, of larger bundles and by 48 hours the sarcoplasmic reticulum of the Z-bands of these older bundles of myofibrils appear to act as proliferatory centers for genesis of new myofibrils (255). The ultrastructure of embryonic avian osteoclasts has been correlated with function as revealed by cinephotomicrography, showing that the osteoclast engulf collagen fibrils embedded in bone and then 'shakes' them, thus fragmenting the bone salts into small granules which are phagocytosed by the osteoclast as are fragments of collagen (99).

Subsection 3. Endoderm.

The endodermal derivatives of the chick embryo have been neglected. A recent study of the rat fetal thyroid between the 17th to 20th day of gestation during which the first increase in I131 uptake and first chemically detectable thyroglobulin is noted, shows that all cytoplasmic components and organelles are present before differentiation of function and that functional activation correlates with an increase in the organization of the endoplasmic reticulum and increase in the extent of the Golgi apparatus (76). Early differentiation of the liver has been studied

with special emphasis on the fate of an ultrastructure of glycogen, certain dense inclusions, the formation of bile canaliculi and hematopoiesis (128). Decreasing nuclear-mitochondrial proximity as differentiation occurs in the chick embryo liver is correlated with the increase in cellular metabolic control and differentiation of cell function (165). At the end of this section on embryonic researches is a recent investigation of the bursa of Fabricius, a lymphoepithelial organ found in avian cloacae, which presents convincing ultrastructural evidence of the formation of lymphocytes from the relatively undifferentiated epithelial cells.

MATERIALS AND METHODS

All fertile eggs used in these experiments originated from the Poultry Husbandry Department of the University of Nebraska College of Agriculture. These eggs possessed a low viability, approximately 42 per cent, but no other source of fertile eggs could be found which consistently delivered the small number of eggs needed throughout the course of this research. The eggs used were collected at intervals of one to eight hours; thus an initial error in embryo age of up to eight hours was introduced. After collection, these eggs were cooled, stored, and then transported from Lincoln to Omaha by an unrefrigerated truck which during warm weather permitted reincubation for periods of up to four hours. The eggs were again cooled for storage and later incubated at approximately 39.7° C. Supposedly, the abundance of inviable blastodiscs was effected by incubation beyond the stage of early primitive streak preceding cooling on our freezer, since embryos do not withstand cooling once the primitive streak is formed. Furthermore, since an unknown period of previous incubation had occurred, a wide variation in the age of embryos incubated for a given period occurred. Nevertheless, embryos of a given age were obtained by summating the desired age and 12 hours allowed for warming time of the egg (Duval, as in 239). In order to eliminate abnormal embryos examination of each embryo used was performed with a dissecting microscope at the time of "staging" (see below).

In all 148 embryos were collected; 25 were prepared for light microscopy, while 123 were examined with the electron microscope, these

ranging in age from Hamilton-Hamburger (98) stages 8 through 41. During the last six hours, eggs were placed with the air space superiorly, the blastodisc rotating to lie underneath it.

Three methods were used for collection of embryos. Early stages (Hamilton-Hamburger stages 8 through 11) were first candled and a circle drawn on the shell locating the blastodisc; then another line was drawn about 7 to 10 mm. below the first. The egg was submerged to the latter circle in an absolute ethyl alcohol-dry ice mixture for 2 to 3 minutes producing rapid cooling of the egg without formation of intracellular ice crystals in the embryo. The shell and outer shell membrane over the blastodisc were quickly removed and the inner shell membrane carefully stripped from the underlying embryo. A filter paper ring was placed to surround the blastodisc without touching the embryo. The egg then was put into a container with a ground glass lid to prevent exposure to the toxic Osmium tetroxide fumes and cold fixative (see below); squirted on it until the overlying albumin was washed away and direct contact of embryo and fixative was attained. This whole process takes 1 to 1½ minutes after removal of the egg from the cooling bath. Upon completion of fixation the embryo attached to the filter paper ring is removed from the egg, washed in 50% ethyl alcohol and placed in a watch glass of 50% ethyl alcohol for "staging". All procedures in which the fixative was not enclosed in a container with a ground glass lid, were completed under an exhaust hood to prevent inhalation and air-tight goggles were worn to prevent corneal fixation by fumes of Osmium tetroxide.

The second method of collection was used for older embryos (stages 12 thought 29) in which the first procedure failed, probably due to penetration of the embryo by fixative from only one surface. The egg is opened under Chick Ringers solution; the blastodisc is cut away from the yolk and floated to a watch glass while submerged in Chick Ringers solution, then spread and observed under a dissecting microscope at which time it was "staged". If the embryo was of stages 12 - 16, it was placed on a filter paper ring, transferred to a weighing jar (with ground glass lid) filled previously with cold fixative. If the specimen was of stages 17 to 19, multiple transection of the cervical region was effected to facilitate penetration of fixative; the embryo was then placed on a filter paper ring and transferred to a weighing bottle of cold fixative. In embryos of stages 19-23 the cervical region was dissected out, the developing heart and ventral portion of the cervical region removed and the remaining dorsal cervical region, containing the neural tube, was transferred to a weighing bottle of cold fixative; in older stages of this latter series, the cervical region was occasionally transected in 3 or 4 places while in Chick Ringers solution to permit more complete fixation.

The composition of Chick Ringers solution is as follows:

Sodium chloride ($NaCl$)	0.9 grams
Potassium chloride (KCl)	0.042 grams
Calcium chloride ($CaCl_2$)	0.024 grams
Sodium Bicarbonate ($NaHCO_3$)	0.02 grams
Distilled water	100.0 ml.

In the third method, adapted for Hamilton-Hamburger stages 33 through 41, the egg was opened while submerged in Chick Ringers

solution; the chorioallantoic membrane was incised carefully avoiding any major blood vessels; the embryo and amnionic covering was then evulsed through the incision, and the amnion was similarly opened. The yolk stalk was then tied off with cotton thread near the embryo and cut distal to the embryo which was then transferred to a watch glass for staging and dissection under a dissecting microscope. The dorsal cervical ectoderm was then incised in the midline and reflected laterally, as was the underlying gelatinous dorsal cervical musculature. This exposed the vertebral column which was transected with a scissors at the level of the sixth cervical vertebra (approximately 7th cervical neural segment). The supraoccipital portion of the occipital bone was removed, revealing the underlying cerebellum. Without disturbing this, a scissors blade was directed caudally through the dorsal foramen magnum which was cut, along with each neural arch. The cerebellum was then reflected cephalad and the brain stem transected at the level of the medullopontine junction. The entire skull was then removed, the medulla protruding from the cervical stump. A small dissecting forceps was slid into the vertebral canal, allowed to expand (successfully retracting the neural canal along its entire course); and the medulla and cervical neural tube was then removed en bloc and plunged into a waiting weighing jar of cold fixative.

Early attempts to fix embryonic neural tube with Palade's fixative (169) at a pH of 7.2 to 7.4 and Palade's fixative as modified by Caulfield (33) failed. Thus, Zetterquist's solution (see 190) was

modified as below. ( ). This utilizes an acetate-veronal (169) buffered solution of Osmium tetroxide (3, 4, 155, 201), brought to isotonicity with Sodium, Potassium and Calcium chloride (190) and sucrose (33), and to a pH between 8.3 and 8.8 with Hydrochloric acid (the higher pH's nedded for younger embryose) (190).

This fixative is prepared as follows:

I. Stock Buffer Solution

a.	Sodium veronal	2.94 grams
b.	Sodium acetate	1.94 grams
c.	Distilled water	100.0 cc.

II. Stock Salt Solution

a.	Sodium chloride	8.0 grams
b.	Potassium chloride	0.40 grams
c.	Calcium chloride	0.20 grams
d.	Distilled water	100.0 cc

A one-half gram vial of Osmium tetroxide is broken in a jar (with ground glass lid) into which has previously been pipetted (25.0 cc. of distilled water, and the crystals allowed to completely dissolve. While waiting for this to dissolve, a small flask of Stock Buffer Solution is placed on a Beckman pH meter (previously warmed up for 10 minutes and freshly standardized with standard solution (pH, 7.0 at 20° C.)). The stock Buffer has a pH of 10.2 to 10.5 which is lowered to the pH previously mentioned by the addition of 0.1 N HCl. About 3 cc. of acid for every 5 cc. of Stock Buffer yields the pH 7.2 - 7.4 of Zetterquist's fixative; however, only 1 - 2 cc bring the pH to 8.3 - 8.8. Exactly 9.0 cc of acidified buffer in which has been dissolved 250 mg of sucrose (to replace the osmolar deficit of the deleted HCl) and 1.7 cc. of Stock Salt Solution are added to the Osmium tetroxide solution to give

35.7 ml. of fixative with a pH of 8.3 to 8.8, osmolarity of 0.3411 osmoles and 1.44 per cent Osmium tetroxide. This is cooled in ice water to a final temperature of 2° C. (239). The embryo remains at this temperature during dehydration until the change from 85 to 95 per cent ethyl alcohol.

The optimal duration of fixation varied with the age of embryo and the manner in which the tissue was collected. In the first and second methods of specimen collection (stages 8 - 19) the optimal fixation time was 20 to 30 minutes; however, in older embryos, a slightly longer fixation period of one hour produced excellent material. In embryos older than stage 19 in which the cervical spinal cord and medulla were obtained by microdissection the optimal period was three hours. Underfixed tissue displayed indistinct, blurred and low electron density plasma and cytoplasmic membranes. Over-fixed specimens presented distinct membranes, but the cytoplasm was leached.

Pre-embedding staining was attempted with saturated solutions of Uranyl acetate in 50% ethyl alcohol for durations up to 1½ hours without major improvement over staining of sections (see below). Pre-embedding staining with Phosphotungstic acid (5% solution in absolute ethyl alcohol) (138) produced rather surprising results when durations of 22 hours, as recommended in the above reference (24 to 48 hours) were used; specimens thus handled were impossible to section because of the brittle quality of the methacrylate embedded tissue. Either the neural tube or the glass knife were shattered. Eight hour staining produced similar results; one hour staining permitted sectioning, but these

sections were too poor to photograph. Forty-five minute staining permitted sectioning with difficulty and electron micrographs were obtained which are passible. In summary, at stages 12 to 14 the neural tube of the chick embryo acquires a preferential affinity for Phosphotungstic acid, while adjacent structures, the notochord, endoderm, and somites show no excess staining. This property disappears after stage 18.

Immediately after fixation, the embryo is transferred to a series of increasing concentrations of ethyl alcohol (50%, 70%, 85%, 95% and absolute for dehydration); the specimen remains for 4 to 15 minutes in each solution (190) except absolute alcohol in which it is subjected to three fresh baths of 20 minutes each. In embryos of stages 33-41, a 10 minute bath of 25% of ethyl alcohol was also used. The specimens remain at 2^{o} C. until they reach the 95% ethyl alcohol. Some embryos are "staged" during the 50% alcohol bath. It is during dehydration that pre-staining was done.

"Staging" of embryos is a procedure in which the embryo is examined under a dissecting microscope and compared with standard pictures and criteria previously determined by Hamilton and Hamburger (98); in embryos which reach the dehydrating bath intact staging is delayed to that point; however, in embryos altered previously to dehydration staging is done while the embryo is immersed in Chick Ringers solution, before any dissection is started.

Two embedding media were used; methacrylate and Epon 812, an epoxy resin. Methacrylate (25, 26 and 164) was handled as by Pease (190) and Farquahr (73). Inhibitor was removed from both methyl and butyl

monomers by washing with 40% Sodium hydroxide in distilled water and contaminating ions were subsequently removed with six washings of distilled water. Water was removed by filtering through indicator Drierite (anhydrous Calcium sulfate) or Calcium chloride 3-5 times. The final mixture was composed of 15% methyl monomer, 85% butyl monomer and 2% Luperco CBD (polymerization accelerator) whose active ingredient is Benzoyl peroxide. After three changes of absolute alcohol the tissue was washed with methyl methacrylate monomer with inhibitor for one-half hour, a half hour bath in "15-85" with inhibitor removed, and two half-hour baths of "15-85" with inhibitor removed and 2% Luperco CBD added. The embryo was then embedded in prepolymerized (syrupy) "15-85" without inhibitor and with 2% Luperco CBD added in 00 gelatin capsules; care was taken to orient the neural tubes along the long axis of the capsule so that sections would be transverse. This, however, was not always possible. Capsules were then incubated at 58° C. (25). It has been noted that capsules cured for two or more weeks at room temperature after polymerization are more easily sectioned.

Epon 812, an epoxy resin (84), has been handled according to the techniques advised by Luft (139). Two mixtures are used. The first mixture (A) is composed of Epon 812 (Shell Chemical Company) and Dodecenyl succinic anhydride (DDSA) (National Aniline Division of Allied Chemical Corporation) in a ratio of 62 to 100; mixture B is composed of Epon 812 and methyl nadic anhydride MNA (National Analine Division of Allied Chemical Corporation) in a ratio of 100 to

89. These two mixtures may be stored indefinitely, but before use must be mixed together thoroughly. The hardness of the mixture is dependent on the relative proportion of parts A and B; mixture A apparently being the harder of the two. These mixtures may be expected to remain uneffected in the refrigerator for approximately six months, but must be warmed before opening after removal from the refrigerator to prevent absorption of condensed water. Immediately before use an accelerator which is 2,4,6-tri(dimethylaminomethyl) phenol (DMP-30) must be added in a concentration of 1.5 to 2% by volume and mixed thoroughly. Care must be taken to avoid direct contact with DMP-30 since severe contact dermatitis may result. Though mixtures A and B were used in a 5 to 5 ratio for all the micrographs shown in this paper, recent experience indicates that a 6 to 7 ratio sections more easily with less compression artifact. While many tissue blocks were polymerized in a 60° C. oven over night, it was discovered that these blocks were extremely hard to cut and that polymerization occurring overnight at 35° C., 45° C. the following day, and 60° C. that night, offered better results with less compression artifact.

The face of the block of embedded tissue was trimmed to include the whole embryo, thick sections (1 to 5 microns) and cut, placed on glass slides to be stained with alkaline methylene blue (methacrylate sections only) and viewed by light microscopy. From observation of the tissue's position in the block, the face of the block was trimmed to include only neural tube and immediately adjacent structures (26).

Thick sections of Epon embedded tissue were suspended in water and viewed with a phase contrast microscope to determine position of the tissue.c

Sections were cut on a Porter-Blum (200) microtome with glass knives (134) scored by the method of Cameron (32) at angles which most consistently produce straight check-free edges (246). Sections were collected in "boats" containing 20 to 40% acetone in distilled water (190) and spread with xylene fumes from either a bottle or an impregnated applicator stick (221), and sections displaying light gold, silver or blue gray interferences colors (189) were picked up upon Phillips "slot" grids or RCA mesh grids. Methacrylate sections were picked up on grids previously coated with 0.075% Formvar in Ethylene dichloride (190). Care was taken throughout the entire procedure to avoid contamination (117) or sectioning artifact (159). The sections were placed upon a filter paper in a Petri dish for at least two hours to permit thorough drying.

After drying the grid, sections could be "post-stained" anytime before exposure to the electron beam. Several stains were tried: Phosphotungstic acid (138), Potassium permanganate (135), Ferric chloride (21), Barium hydroxide, Phosphomolybdic acid and Uranyl acetate (257, 258). Of these Uranyl acetate most consistently produced effective staining of embryonic tissue with the least amount of artifact and was used for staining embryos of stages 8 - 14, after which cytoplasmic components were so distinct in unstained material that no

stain was used. Embryos of stages 33 - 41 needed staining; Uranyl acetate again was found to be optimal. A saturated solution of Uranyl acetate in distilled water was prepared in a stock bottle; small amounts were pipetted into plastic bottle caps upon which dried grids were floated, face down; the optimal staining time for younger stages was 20 to 30 minutes, however, overnight staining gave optimal results in the older embryos. After staining the grids were washed in 4 to 6 changes of distilled water and dried as above.

Sections were viewed with a Phillips EM-100B electron microscope and interpreted with care so as not to mistake artifact derived from polymerization damage, poor fixation, sectioning, effects of electron beam or contamination as normal structure (25, 26, 33, 73, 117, 159, 164, and 190). Previous research by others adequately demonstrates that this technique does not grossly alter the structure of the cell as seen by phase contrast microscopy (3, 4, 25, 26, and 160). Selecte areas of grids were photographed with the roll camera on Eastman Kodak Fine Grain Positive 35 mm. film and developed in D-19 developer.

It has been noted that practice is the prime factor in increasing quality of results. Several factors have recently improved micrographs, such as focusing on granular rather than membranous profiles, under focusing, use of standard exposure times, magnifications, illumination, and personal supervision of micrograph printing..

Since part time work does not permit one to attain the fine touch at sectioning required to obtain adequate uncompressed thin sections of Epon embedded material, a full time technician did the sectioning on the Epon material.

RESULTS

The purpose of this study was to fill certain gaps remaining at the time of completion of my Master's Thesis and to expand the studies to stages older than those already investigated. Therefore, the results obtained are fragmentary and would be completely disjointed if a minimum of information obtained during previous research was not included.

The stem cell of the nervous system is the germinal cell of His; the nature of which has been adequately covered in a previous paper (239) and is not immediately pertinent to this research. The cell differentiating from this germinal cell is the medulloblast (Fig. 1), the main component of the medullary plate. Its important characteristics are the basally polarized ribosomes, abundant alpha and beta yolk granules and apically polarized Golgi apparati which are essentially the same as in adult cells (Fig. 2). A further more detailed description (239) is the source of these figures repeated here solely to impart continuity to this paper.

The medullary plate displays several characteristics, the formation of intercellular lacunae, ependymal cones, and growth in depth and diameter of the basal pole of the medulloblasts to the neural tube (239). Recent investigation of Epon 812 embedded ependymal velum (Fig. 2) reveals more distinct cell membranes than previously demonstrated, endoplasmic reticulum which is more extensive, cell processes into the lumen of the neural tube, frequent pinocytotic vacuoles, and prominent terminal bars. As was mentioned in an earlier section of this paper,

cilia formation commences in the ependymal velum as the neural tube closes.

Subsequent to neural tube formation, the basal plate deepens due to longitudinal growth of the medulloblasts; these cells do not appear altered until Hamilton-Hamburger (98) stages 13 and 14, at which time neuroblasts are formed. Previously chemical alteration of the cytoplasm of medulloblasts resulting in increased affinity of the cytoplasmic matrix and the mitochondrial membranes for Phosphotungstic acid occurs. This phenomena, previously not demonstrable morphologically, is shown in Figure 3, attained by shortening the recommended duration of Phosphotungstic acid staining from 22 hours to 45 minutes. The extreme electron density of the cytoplasm is apparent upon comparison with the beta yolk granule (yb) which is usually absolutely electron opaque. It appears that an alteration in cell metabolism precedes morphological alterations by about one stage or four to six hours of incubation.

The morphological alteration of the basal plate during stages 13 and 14 is illustrated in Figure 2 which shows the entire depth of the basal plate from ependyma (ep) with its still mitotically active germinal cells of His (mc) to the external limiting membrane (elm) which surrounds the entire neural tube. In the interval are found two kinds of cells: the typical medulloblasts with their apically polarized Golgi apparatus and basally polarized ribosomes and the bipolar neuroblasts (bnb) (Figs. 4-7) with densely packed perikaryal ribosomes, oblate eccentric nucleus and striking external process, the axon (a). The bipolar neuroblast

four definitive parts: the perikaryon or cell body, the axon, the growth cone and the internal processes. The previously mentioned perikaryon has a high concentration of ribosomes or small particulate component (spc) (Figures 7); remarkable is the virtual absence of endoplasmic reticulum and Golgi apparatus. In the axon ribosomes are rarely found in the watery appearing axoplasm which displays a few scattered profiles of agranular reticulum and myriads of fine cytofilaments, the protoneurofilaments. This peculiar type of cytoplasm called axoplasm extends into the neck of the growth cone, the expanded axonal terminus possessing frequent mitochondria, many protoneurofilaments and abundent ribosomes (Figures 4, 5, and 8). The internal process (Fig. 4, ip) has a watery appearance similar to axons, but with noprotoneurofilaments; in it are found rare ribosomes, few profiles of endoplasmic reticulum and the prominent Golgi apparatus. (239)

A third cell, rarely seen in the neural tube at this stage, lies immediately under the external limiting membrane (elm), has a large round or oblong central nucleus with prominent nucleolus and the cytoplasm possessing a moderate abundance of ribosomes and frequent simple intact mitochondria. It has no internal process, and its external process or axon is not prominent. Its cell membrane is frequently invaginated giving it a lobate profile, but most remarkable is the presence of numerous cytofilaments and electron lucent areas similar to glycogen granules. This cell is the unipolar neuroblast (Figures 4 and 7, unb). Its cytoarchitesture is easily distinguishable from that of the bipolar

neuroblast (Figure 7), and is differentiated from the medulloblasts by the presence of the cytofilaments, protoneurofilaments.

The external limiting membrane (Figures 4, 5 and 8, elm) is a typical basement membrane composed of small collagen fibrils and an amorphous electron dense ground-glass appearing matrix. The collagen fibrils often extend into the surrounding extracellular space; though previous concepts hold that collagen does not exist in embryos of these early stages (see 239), Figure 8 quite clearly demonstrates their presence. During stage 14 the growth cones of 'pioneer' nerve fibers invade the mesenchyme; it may be seen in Figures 9 and 10 that the external limiting membrane is broken and reflected upon itself by the 'pioneer' emerging nerve fiber. The emerged pioneer nerve fiber appears to drift or float in the surrounding extracellular space (Figure 9); if such pertains, the mechanical theory of neurotaxis proposed by Paul Weiss (see 239) has no validity. However, Figure 11 shows that the extracellular space is not a watery void but that proper staining reveals an extensive network of nodose reticular fibers in the mesenchymal ECS. Figure 12 shows a later stage in which three axons were found far out in the mesenchyme pursuing an undulant course, demonstrating mutual adhesion and affinity for mesenchymal cells. Interspersed throughout the mesenchymal extracellular space in Figures 9 and 12 are small granular profiles, tiny membrane bound fragments of cytoplasm, and profiles of transversely cut fibrils but no reticular network as seen with Phosphotungstic acid pre- or post-staining (Figure 11).

During stages 13-15 the axons of the unipolar neuroblasts and their precursor bipolar neuroblasts radiate toward the periphery until reaching the external limiting membrane which deflects their course circumferentially; thus, the marginal layer is created. Eventually the growth cones of presumptive primary neuroblasts break through the external limiting membrane to become the pioneer nerve fibers of the ventral root. Meanwhile, the ependymal palisade medulloblasts and bipolar neuroblasts elongate forming a pseudostratified epithelium which subsequently splits circumferentially to form the inner and outer palisades which differentiate into the mantle and ependymal layers. The outer palisade cells become neuroblasts to yield the definitive mantle layer and the inner palisade cells remain similar to the primordial medulloblasts of the earlier stages. By stages 17 through 19 the mantle layer neuroblasts have matured and their axons follow the pioneer fibers into the mesenchyme to form a definitive nerve root. The basement membrane has been obliterated; the only barrier that separates nervous system from the surrounding mesenchyme is a few thin, flat cells of the mesenchyme. By stage 19 mesenchymal capillaries begin to invade the embryonal spinal cord via the nerve roots. Simultaneously small pleomorphic cells whose cytoplasm is filled with ribosomes and occasional simple mitochondria (Figure 13), identical to adult microglia but not actively phagocytic, enter the nervous system.

At the termination of previous research, no typical Nissl substance had been found. Several light microscopists had described them in the six day chick (239); microspectrophotometrists demonstrated no Nissl

substance until the seventh to eighth day of incubation (122, 123). Therefore, chick embryos of Hamilton-Hamburger stages 38- (eight days) and 39+ (nine days) were examined. The anterior horn cells of the cervical spinal cord (Figures 14-16) characteristically display shrinkage artefact, and their prominent large eccentric nuclei show extensive leaching, but the cytoplasm remains intact. The cytoplasm has two distinct regions, the endoplasm and ectoplasm. The endoplasm contains abundant agranular reticulum and mitochondria while the ectoplasm is primarily composed of protoneurofilaments, few scattered mitochondria and ribosomes. A few embryonal neurons of stage 39+ present small ergastoplasmic profiles, the Nissl bodies in the perinuclear endoplasm. These are primitive as shown in Figure 16 and composed of only two to five parallel cisternae. Elsewhere, the cytoplasm displays a mixture of ribosomes, endoplasmic reticulum, agranular reticulum and mitochondria, occasional cytolysosomes and abundent protoneurofilaments. The mitochondrial cristae are bizarrely shaped and oriented. Except for the absence of elaborate Nissl bodies and the complexity of the mitochondria, these embryonal neurons resemble their adult counterparts.

During the study of these later stages, attention was drawn from the neurons to the macroglial cells which were usually one of two types. One type was scattered throughout the neuropile (Figure 17); its cytoplasm appears to have a scalloped margin, but in reality engulfs the surrounding neurites (z). This cytoplasm possesses many ribosomes, a prominent Golgi apparatus and

an elongate, multilobulate or grooved nucleus (Figures 17-19). High magnifications reveal a profusion of cytofilaments, abundant endoplasmic reticulum, mitochondria, agranular reticulum, cytolysosomes and ribosomes. The most constant feature of these cells was the prominent Golgi apparatus (Figure 17-21). The other macroglial cell type (Figures 18, 20 and 21) is most frequent in the juxtaependymal regions where its well developed ergastoplasmic profiles identify it. Upon first investigation, these were mistaken for neuronal Nissl substance; however, they are easily distinguished by their juxtaependymal location, paucity of ribosomes, and cytofilaments, ample agranular reticulum, infrequent small mitochondria and the prominent ergastroplasm (Figures 18, 20 and 21, e). The nuclei are often indented or lobulated. These cells are distinctly columnar and though often located near the ependyma never abut upon the lumen from which they are always separated by ependymal cells. These two macroglial types predominate, yet less common intermediate forms are scattered through the neuropile (Figure 22b); these cells have cytoplasm similar to the juxtaependymal cells, possessing ergastoplasmic profiles, yet they evidently act as supporting cells of neurites; they also display a distinct proclivity to form whorls (Figure 22b).

Two kinds of neurites may be differentiated in the central nervous system of the later stages, the axons and dendrites (Figures 22 and 22B). Figure 22 represents sagittally sectioned axons with their prominent bizarre filamentous mitochondria, the axonal tubules of agranular

reticulum and the cytofilaments. They differ from axons of earlier stages only in the prominence of the agranular reticulum. Enveloping the neurites is the cytoplasm of their sheath cells. The marked difference in axons and dendrites is seen in Figure 22b; the electron lucent round profiles of transversely sectioned axons contrast remarkably with the smaller ribosome filled profiles of transversely sectioned dendrites. Neither are completely invested with macroglial cytoplasm.

Recently, pursuit of emergent pioneer nerve fibers has extended into the mesenchyme adjacent the neural tubes, where the early differentiation of the myoblasts have been observed. The early differentiation of the somite has been noted, yet micrographs obtained were of too poor technical quality to be included here; the three regions of the somite are quite distinct. The dermatome possesses a watery cytoplasm with few ribosomes; the myotome has an intermediately dense cytoplasm with a profusion of mitochondria and agranular reticulum, and the sclerotome is composed of moderately dense ribosome-laden cells. The myotomic cells elongate with a fusiform nucleus and retain the abundance of sarcoplasmic reticulum and mitochondria. Subsequent differentiation of lateral myotome myoblasts is seen in Figures 23-26. In the first stages (figure 23), multiple and solitary unoriented cytofilaments appear in the cytoplasm. Soon dark areas, associated with the sarcoplasmic reticulum, appear from which many filaments radiate. The undulant myofilaments then become loosely aggregated with their long

axes parallel and the Z-band with its sarcoplasmic reticulum become prominent (Figure 24). The mitochondria, typical of differentiating cells are bizarre, possessing broad cristae which often extend the entire width of the mitochondrion as if to partition it. Subsequently, the sarcoplasmic reticulum of the Z-band beoomes more intricate; the myoblast cytoplasm displays many cytofilaments not associated with the myofibrils; ribosomes are sparse, and myofilaments have formed a compact myofibril by expression of the intervening sarcoplasm; occasionally a faint periodicity is noted within the sarcomere (Figure 25). Finally the diameter of the myofibril increases by the addition of myofilaments at its periphery; the bundle becomes more compact and electron dense (Figure 26).

CONCLUSIONS

Recently acquired information forces me to alter certain earlier conclusions and to add new parameters to study of the embryo.

Demonstration of the affinity of medulloblasts of stages 12-14 for Phosphotungstic acid raises the question of the nature of this pre-morphological alteration in cellular composition. It is known that Phosphotungstic acid has a definite affinity for RNA, protein, glutathione and uric acid. If this increased staining of medulloblasts is an increase in RNA, it may represent a process of induction, or perhaps an increase in soluble RNA preceding formation of the profusion of ribosomes seen in the bipolar neuroblasts. If it is an increase in protein, it may be understood as preparation for rapid growth. Nevertheless, microspectrophotometric studies by Hyden, et al., (122, 123) on growing neurons has demonstrated no remarkable increase in RNA or protein during these stages. Therefore, the source of the increased Phosphotungstic acid affinity remains a mystery; its appearance one or two stages preceding neuroblast differentiation may or may not be correlated with neurogenesis.

Interestingly, this research has yielded evidence supporting Weiss's theories of neurite peripheralization (see 239). The axons of primary anterior horn cells emerge from the neural tube at approximately stage 14 long after obliteration of the segmental pattern (239). Weiss, et al., (See 239) propose, based on <u>in vitro</u> studies, that these 'pioneer' neurites proceed along a ultramicroscopic network of colloidal micelles in the extracellular space. Earlier studies failed

to indicate the existence of any such reticulum; however, the present study uncovers the existence of collagen fibrils, fibrils stainable with Phosphotungstic acid and fine mesenchymal cell processes which could indeed provide such a pathway through the mesenchyme. Certainly collagen fibrils are apparent in the mesenchymal extracellular space as early as stage 13+ (Figure 8). Again Figure 11 shows an extensive fibrillar reticulum, which is not demonstrable except by Phosphotungstic acid staining, and which appear to have the same distribution as extracellular fibrils found by Duncan (56). Neither the source or composition of these reticular fibers is known. Figure 12 shows several neurofibrils which are mutually adherent and have an affinity for a mesodermal cell; both phenomena hypothesized by Weiss and demonstrated in vitro by Nakai (161, 162). These findings substantiate in vivo many concepts acquired by study of behavior of nerve fibers in vitro. The presence of two easily distinguishable types of neurites, the axons and dendrites, by stages 38- and 39+ (8 and 9 days of incubation) (Figures 22 and 22b) reflects the maturity of fetal neurons at this time and the similarity to adult spinal cord demonstrated by Duncan and Williams (57 and 264).

Duncan (57) suggests that improved technique may yet demonstrate a basement membrane completely enveloping the emerging neurite, yet present studies fail to reveal any such membrane. He bases his assumption upon evidence that adult neurites are always separated from the extracellular space by intervention of a basement membrane.

The invasion of the central nervous system by microglia at stage 19 indicates that these cells achieve an adult form before the macroglia differentiation occurs. Whether these cells are the actual precursors of adult microglia is uncertain, yet the cytoarchitectural similarity is evident.

Without question the medulloblasts (Figure 1) are precursors of the spongioblasts, and thereby may be considered as a stage of gliogenesis. From the present studies, no dogmatic statements may be made concerning the immediate precursor of the bipolar neuroblasts; either the germinal cell directly differentiate into bipolar neuroblasts ormedulloblasts act as intermediate cell types. The bipolar neuroblasts presumably eventually withdraw their internal process, the xoplasm invades the perikaryon as previously shown (239) to become the unipolar neuroblast. Later, the unipolar neuroblasts develop an endoplasm crowded with abundant endoplasmic reticulum and ribosomes, and an ectoplasm, characterized by complex branching mitochondria and a greatly convoluted plasma membrane. By stages 38 and 39, however, the neuron has vastly increased its size; its nucleus is eccentric with an entire margin, its endoplasm is crowded with agranular reticulum and mitochondria and are found parallel cisternae of endoplasmic reticulum, the Nissl substance in perinuclear region. The ectoplasm contains scattered protoneurofilaments and ribosomes. The transition between the unipolar neuroblast and the fetal neuron are yet to be investigated. The correlation of Nissl substance formation in the 8 and 9 day old chick embryo and the microspectro-

photometric studies of Hyden, et al., revealing increased neuronal protein production are interesting matters for speculation (122, 123).

Still unsettled is the problem of macrogliogenesis and differentiation. Recent research by others (28-30, 184, 217-219, 264 and 265) indicate that only two macroglial types occur in adult tissue, the oligodendrogliocyte and the fibrous astrocyte. The macroglia found in the embryo resemble neither adult types; however, recently Bunge, et al., (28-30) have demonstrated the reactive macroglia in tissue experimentally demyelinated without destruction of the axons; these cells appearing during the reparative phase engulf axons and subsequently producing myelin around them. Comparison of these cells with those found enveloping neurites in the fetal nervous system (Figures 17-20) show that the two cells are nearly identical. Two differences are that the embryonal cells have nuclear lobulation and more prominent Golgi apparati. Bunge, et al., (28-30) believe that these reactive macroglia are the precursors of oligodendrogliocytes associated with the myelin of the adult central nervous system; if so, perhaps the similar embryonal macroglia are the precursors of the adult oligodendroglia. It seems rational that these cells appearing identical to precursors of adult oligodendroglia and supporting neurites, as do the adult oligodendroglia, are the embryonal precursors of oligodendroglia. However, intervening stages have not been demonstrated to establish a continuity between these cells and adult oligodendroglia.

In view of the number of intermediate forms (Figure 22b), it

appears that there is continuity between the cells described above and the other type of embryonal macroglia characterized by the well developed ergastoplasm. Such a continuum must indicate either a transition from the latter cell to the former or both must have a common origin.

It is presently presumed that this latter cell is the spongioblast because of its cylindrical form and its location in proximity to the ependyma. If this is so, it is the precursor of the previously described macroglial type. Yet, its pale cytoplasm is similar to an astrocyte. It must be recalled that the spongioblast gives rise to both adult macroglia. The precise function of the ergastoplasm characteristic of these cells is unknown.

Another interesting facet of the macrogliogenesis is noted in Figure 22b; where tendency of the intermediate cells (O) to form whorls is noted. The recent demonstration by Robertson, et al., (216) that oligodendroglioma cells show a similar proclivity to whorling and similar cytoplasm, invites the presumption that oligodendrogliomas are a form of dedifferentiation to a more primitive stage represented by the cells in Figure 22b.

Formation of striated muscle in the chick starts with the differentiation of elongated myoblasts with fusiform nuclei and abundant mitochondria and sarcoplasmic reticulum. Myofibril formation commences in these cells with formation of unoriented cytofilaments which appear to originate from the sarcoplasmic reticulum as in embryonic cardiac

muscle (255). Subsequently, aggregates of sarcoplasmic reticulum form the Z-bands. The next stage is sarcomere formation in which the myofilaments align themselves along a parallel axis; their superimposed attachments to the sarcoplasmic reticulum automatically form the Z-bands which delineate the sarcomere. Thus, the first stage is filamentogenesis, and the second formation of the sarcomere. Nevertheless, at the onset of sarcomere formation the myofilaments remain undulant and separated by sarcoplasm. In the third stage, this intervening sarcoplasm is expressed rendering a narrow but compact sarcomere. At this stage a faint periodicity may become apparent in the cytofilaments. Finally, myofibril diameter is increased by the peripheral addition of myofilaments. Typically adult periodicity was not observed in these specimens.

SUMMARY

Provided here is a fundamental text on the ultrastructure of tissue, in general, and the adult nervous system; furthermore, there is a compilation of the present concepts of fine structure of the embryonal nervous system. The materials and methods used since the beginning of this research are reviewed and the pertinent results of studies of the developing ultrastructure of the embryonal nervous system and lateral mesoderm are reported.

The locus of Phosphotungstic acid affinity of medulloblasts of chick embryos of stages 12-19 is demonstrated and discussed.

Certain facets of gliogenesis are shown; specifically, these are the invasion of the neural tube by capillaries and electron dense phagocytic cells, possible precursors of the microglia, during stage 19. Two macroglial cell types whose position in macrogliogenesis is uncertain, but which appear to be the spongioblast and primitive oligodendroglia (reactive macrocyte) were found in eight and nine day old embryos.

The lower motor neurons of the eight and nine day chick embryos were shown to possess primitive Nissl bodies.

Peripheralization of pioneer fibers was further investigated with the demonstration of a fibrillar mesenchymal extracellular reticulum which perhaps serves as the "colloidal mecellar latticework" of Weiss' theories of mechanical taxis of neurites and the demonstration of *in vivo* affinity of pioneer nerve fibers for mesodermal cells.

Finally, preliminary studies on the differantiation of the lateral mesoderm and especially skeletal muscle were presented.

Again, the research was closed with a partial understanding of the structure and genesis of the nervous system. Additional research is necessary to completely understand neurono- and gliogenesis. It is hoped that in the future the pathways of cell differentiation may be mastered in order that we may better appreciate the processes involved and the stages of dedifferentiation found in tumors.

Figure 1a - Neural Plate

This portion of the neural plate shows two medulloblasts with their polarized Golgi apparatus (ar), alpha (yb) and beta (ya) yolk granules, basally concentrated ribosomes, intercellular lacunae (il), mitochondria (m) and basement membrane (bm).

Stage: 9, posterior cervical neural tube.

Fixation: Osmium tetroxide, pH of 8.4; duration of 22 minutes.

Magnification: 5870 X

Figure 1b - Golgi Apparatus

This shows the typical form of the agranular reticulum when it is concentrated in the form of the Golgi apparatus (ar). Golgi lamellae, vesicles and vacuoles are noted.

Stage 9, middle cervical neural tube.

Fixation: Oxmium tetroxide, pH of 8.4, time of 22 minutes.

Uranyl acetate staining of section.

Magnification: 121,980 X


1a
yb
ar
er
ar
m
ya
il
bm

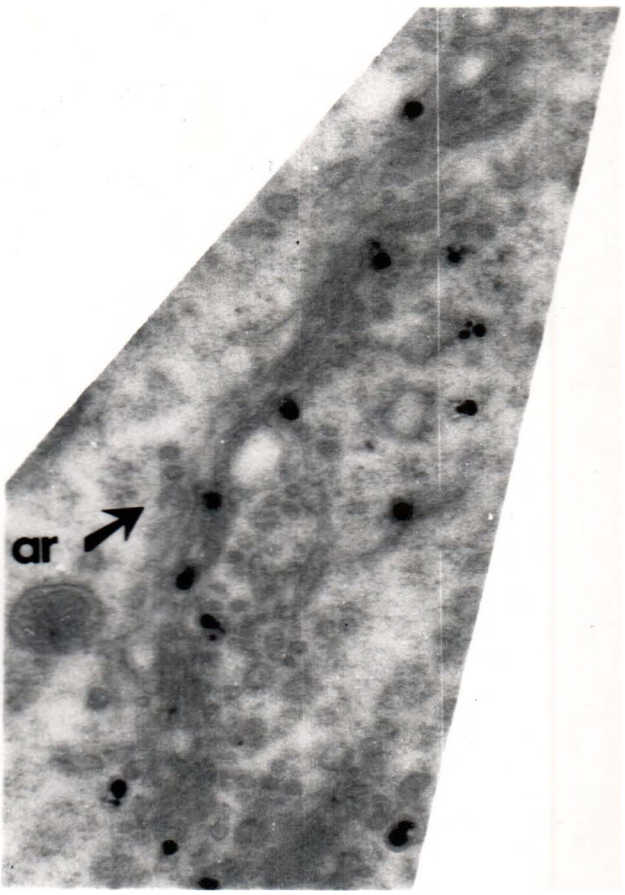

1b
ar

Figure 2 - Ependymal Layer of Embryonic Spinal Chord.

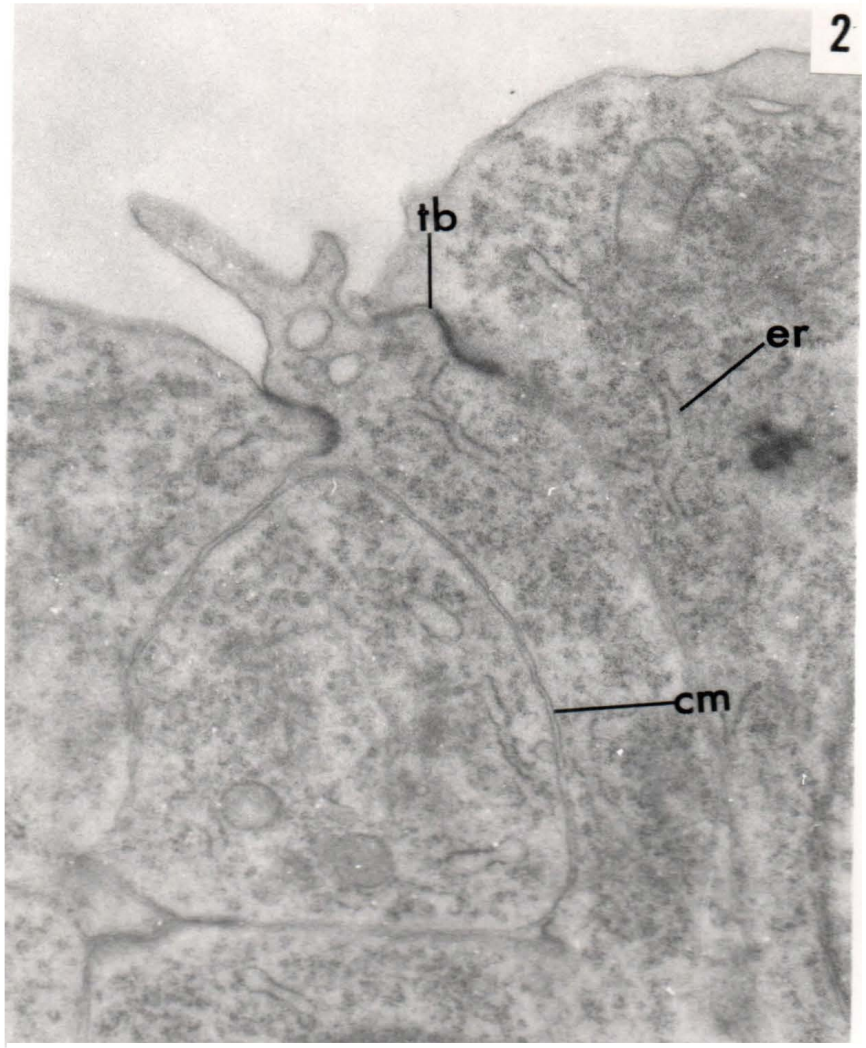
Here is seen an area of the luminar surface of the ependyma which demonstrates terminal bars (tb), the fine cell membranes (cm), the endoplasmic reticulum (er), a microvillus-like process extending into the lumen, pinocytotic vacuoles between the terminal bars and an abundance of ribosomes.

Stage 22
Fixation: Osmium tetroxide, pH of 8.0, time of 83 minutes.
Uranyl acetate strain.
Epon 812
Magnification: 16,900 X

Figure 3 - Neural Tube, Prestained with Phosphotungstic Acid.

This shows the great affinity of neural tube of stage 12+ for Phosphotungstic acid. The density of the cytoplasm is noted by comparing it with the highly osmophilic beta lipid granule (yb) and usually dense nuclei (n). Obviously some of this is artifactual leaching of the nucleus, but this micrograph was taken at 100 KV at an exposure that would normally over-expose the film. The great affinity appears to be localized in the mitochondrial membrane and the cytoplasmic matrix.

Stage: 12+
Fixation: Osmium tetroxide, pH of 8.2, time of 28 minutes.
Pre-embedding stain with Phosphotungstic acid for 45 minutes.
Methacrylate
Magnification: 10,250 X


2
tb
er
cm

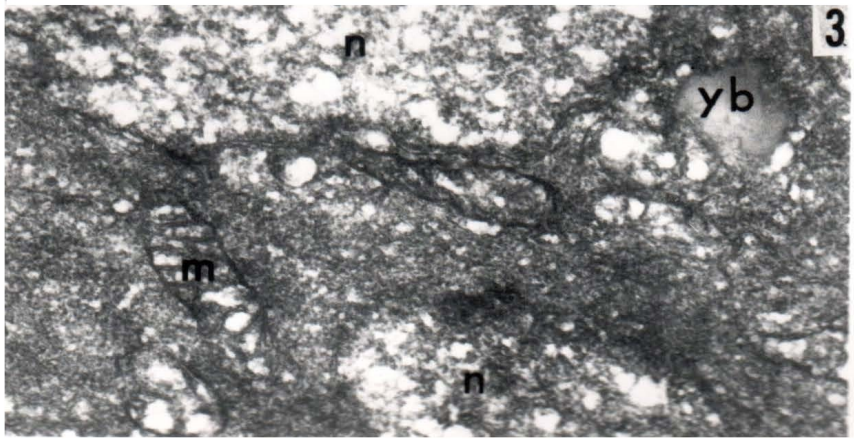

3
n
yb
m
n

Figure 4 - Basal Plate at Stage 13 plus.

At the bottom is the ependymal velum (ep) where a germinal cell is observed in mitosis (mc) and the internal (ip) processes of the early bipolar neuroblasts (bnb) are noted. Toward the external limiting membrane is noted the perikarya of bipolar neuroblasts with their axons (a) terminated in the growth cones (gc), and a single early unipolar neuroblast (unb). Inset 1 contains the axon and growth cone of a bipolar neuroblast (early stage) which is Figure 5; inset 2 shows the unipolar neuroblast, while inset 3 (within inset 2) contrasts the cytoplasm of an early bipolar and an early unipolar neuroblast, see Figure 7.

Stage 13 plus.
Fixation: Osmium tetroxide, pH of 8.3, time of 32 minutes.
No stain.
Magnification: 4300 X

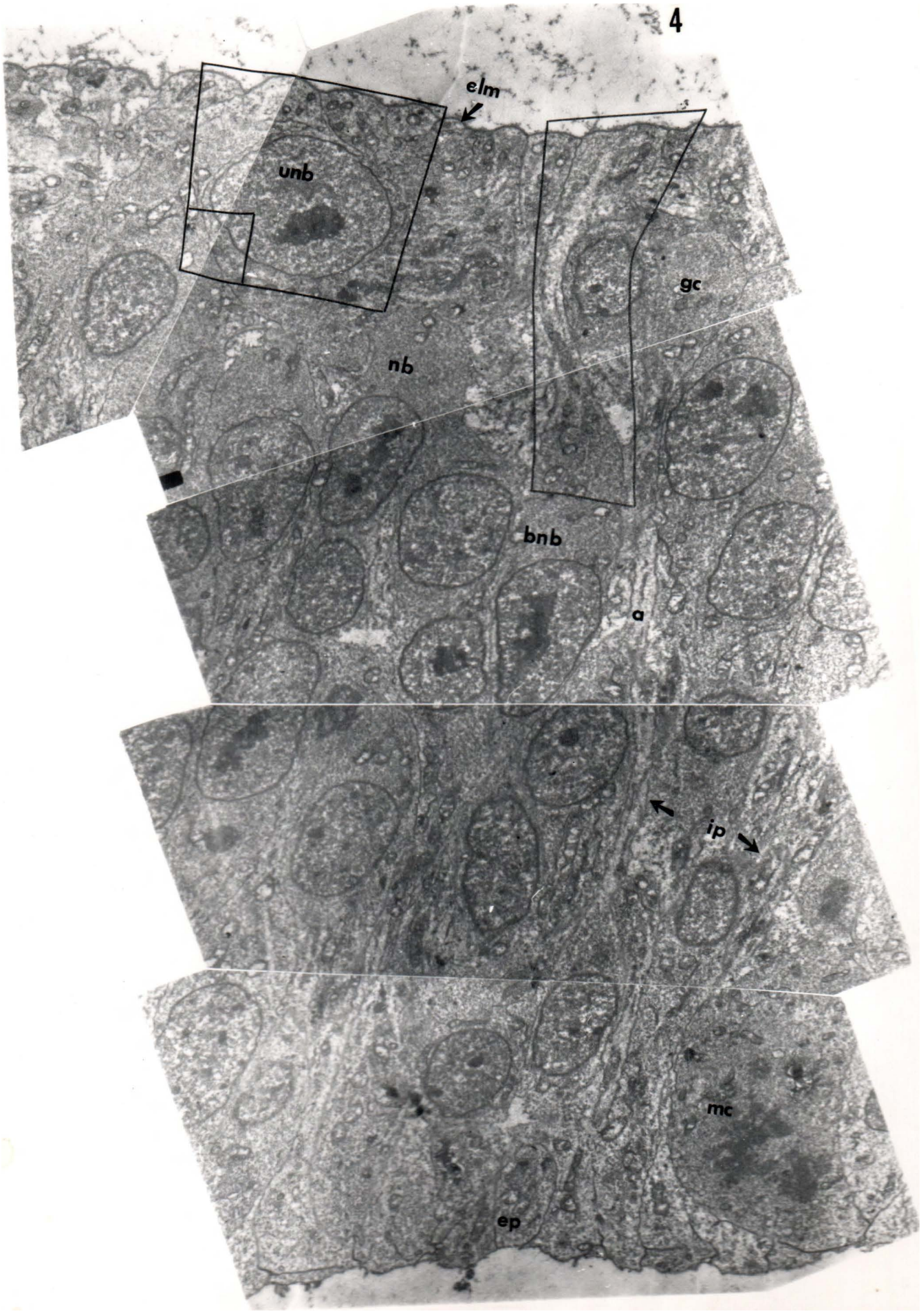

4
elm
unb
gc
nb
bnb
a
ip
mc
ep

Figure 5 - Early Bipolar Neuroblast.

This shows early bipolar neuroblast from its perikaryon (p) with mitochondria (m) and highly concentrated small particulate component (spc) to the growth cone (gc) which lies immediately under the external limiting membrane (elm). Interposed is the axon (a) with its neurofibrillae (nf). Inset shown in Figure 6.

Stage 13 plus.

Fixation: Osmium tetroxide, pH of 8.3, time of 32 minutes.

No stain.

Magnification: 23,400 X


elm
5
m
gc
nf
a
m
spc
p

Figure 6 - Contrast of Axoplasm and Cytoplasm of Early Bipolar Neuroblast.

To the lower right is the neuroblast cytoplasm, characterized by the high concentration of Palade granules, with electron lucent spaces possibly filled with glycogen, and rare profiles of endoplasmic reticulum. In the upper left is the axon with its dense concentration of neurofibrillae (nf).

Stage 13 plus, inset of Figure 5.
Fixation: Osmium tetroxide, pH of 8.3, time of 32 minutes.
No stain.
Magnification: 84,000 X

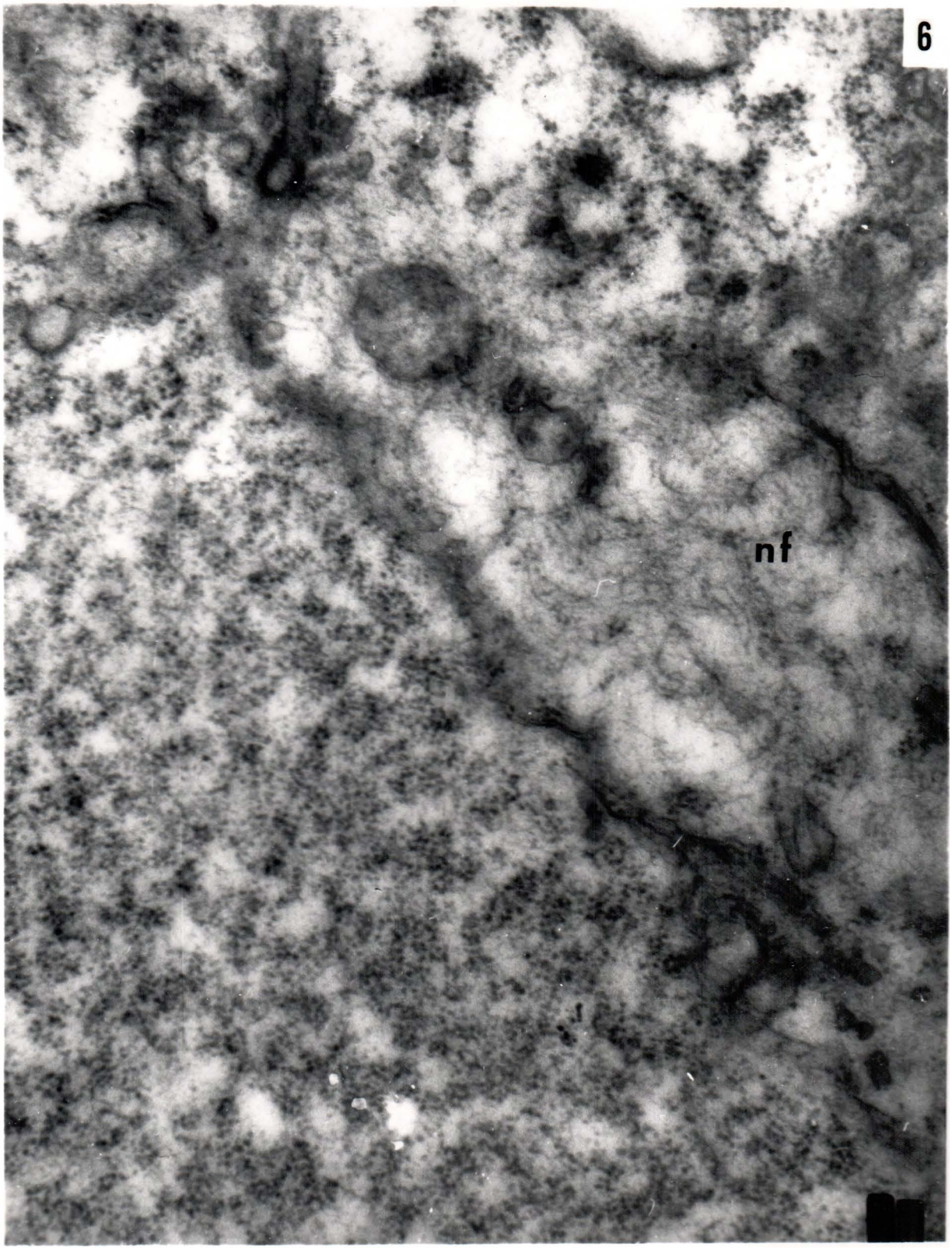

6
nf

Figure 7 - Contrast in Cytoplasm of Early Bipolar Neuroblast and Unipolar Neuroblast.

Below is seen the early bipolar neuroblast cytoplasm (bnb) with its dense small particulate component, while the upper cell is an early unipolar neuroblast (unb). The latter has less dense concentration of Palade granules and close examination reveals neurofibrillae. Also demonstrated is the perinuclear cistern with the outer and inner nuclear membranes, and numerous mitochondria with scant cristae.

Stage 13 plus.
Fixation: Osmium tetroxide, pH of 8.3, time of 32 minutes.
No stain.
Magnification: 45,000 X

7
unb
bnb

Figure 8 - Growth Cone under Basement Membrane.

This shows the essential characteristics of the growth cone (gc) cytoplasm and external limiting membrane (elm). The growth cone is noted to contain mitochondria, rosettes of Palade granules and some neurofibrillae. The basement membrane is composed of collagen fibers and matrix. Notice the discrete collagen fiber of the mesenchymal extracellular space at the upper right.

Stage 13 plus.
Fixation: Osmium tetroxide, pH of 8.3, time of 32 minutes.
No stain.
Magnification: 47,500 X

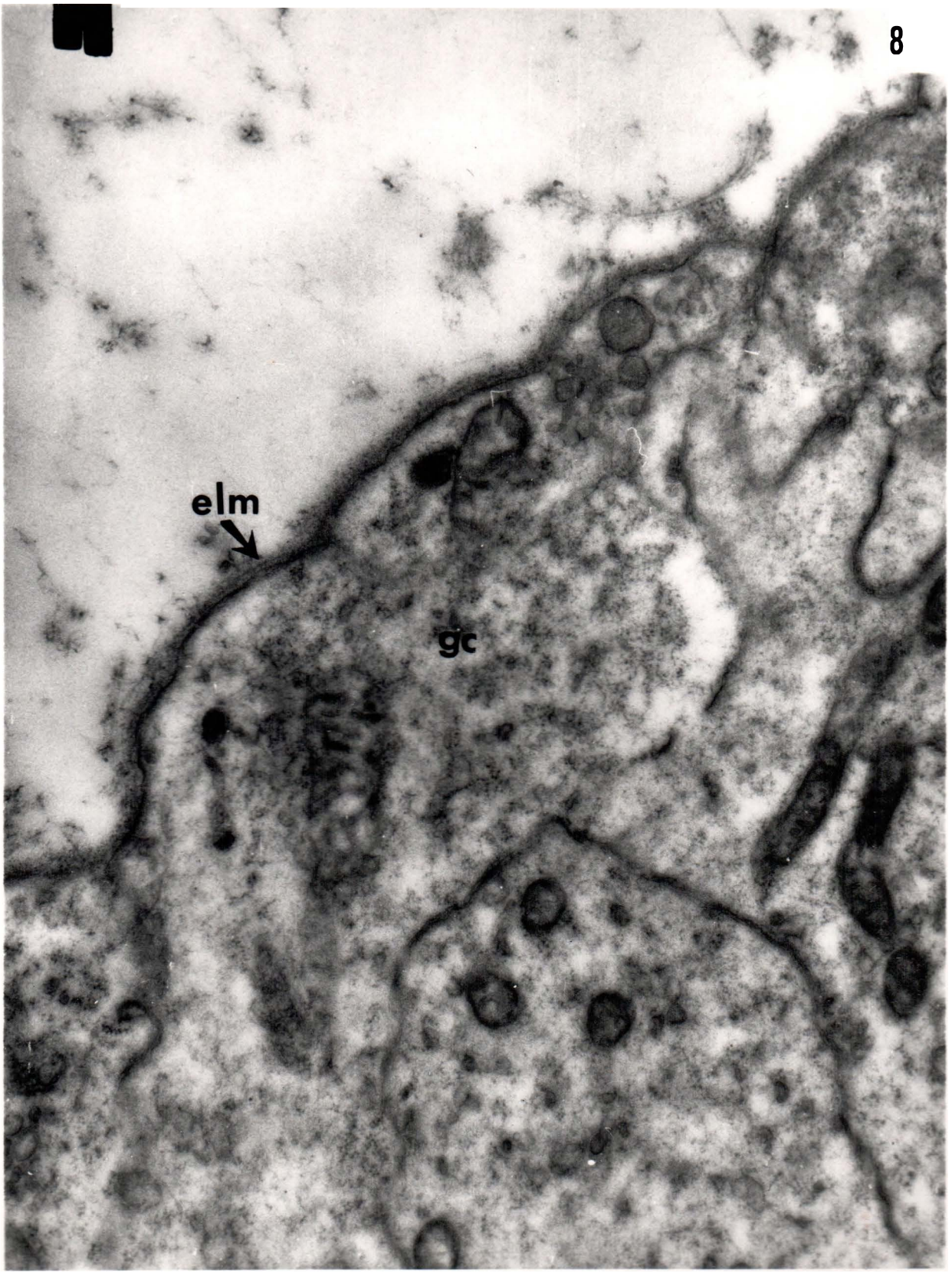

elm
gc

Figure 9 - 'Pioneer' Nerve Fiber.

These micrographs show the 'pioneer' nerve fiber with its root in the neural tube and its growth cone apparently loose in the mesodermal extracellular space. The neurite is composed of typical axoplasm; its growth cone appears to be surrounded by a matrix in which are embedded chains of vesicular profiles, possibly representing fine, undulant villous processes.

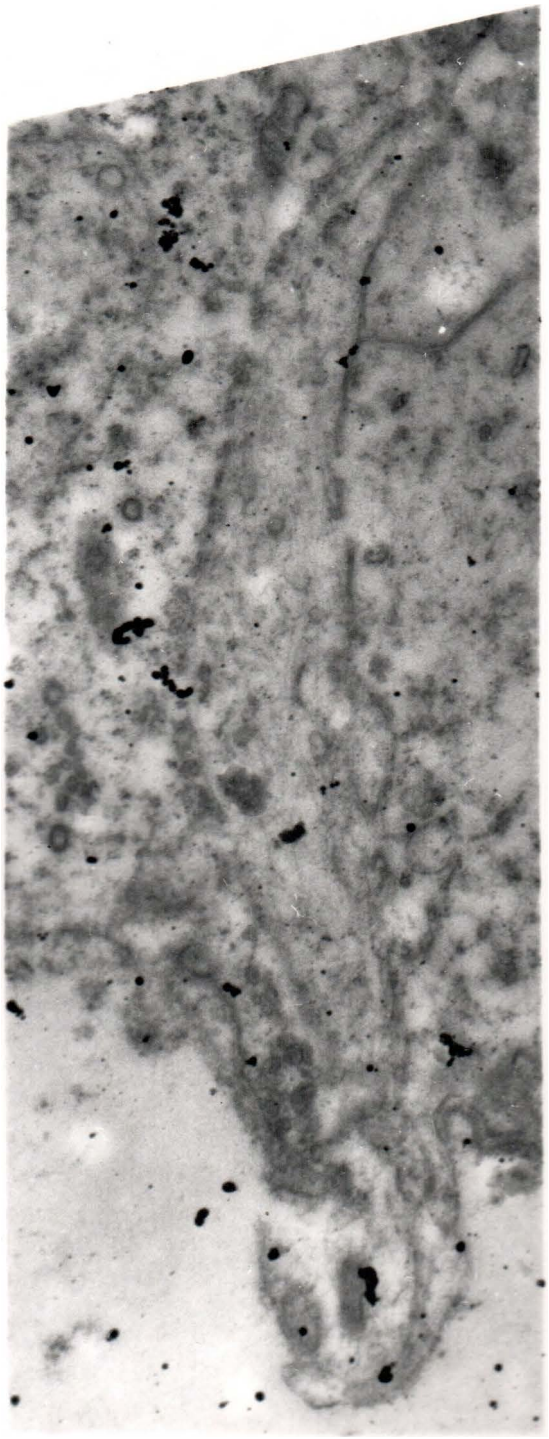
Stage 14 plus.
Fixation: Osmium tetroxide, pH of 8.32, time of 30 minutes.
No stain.
Magnification: 8000 X

Figure 10 - Early Stage in Process of the Emergence of a Neurite.

Here is noted the emergence of a growth cone by what is apparently simply rupture of the basement membrane. The portion of the neurite remaining in the neural rube is shown in Figure 20. The basement membrane seems to be rolled back upon itself on the right side of the emerging 'pioneer' nerve fiber.

Stage 17 minus.
Fixation: Osmium tetroxide, pH of 8.32, time of 30 minutes.
No stain.
Magnification: 33,000 X.

10



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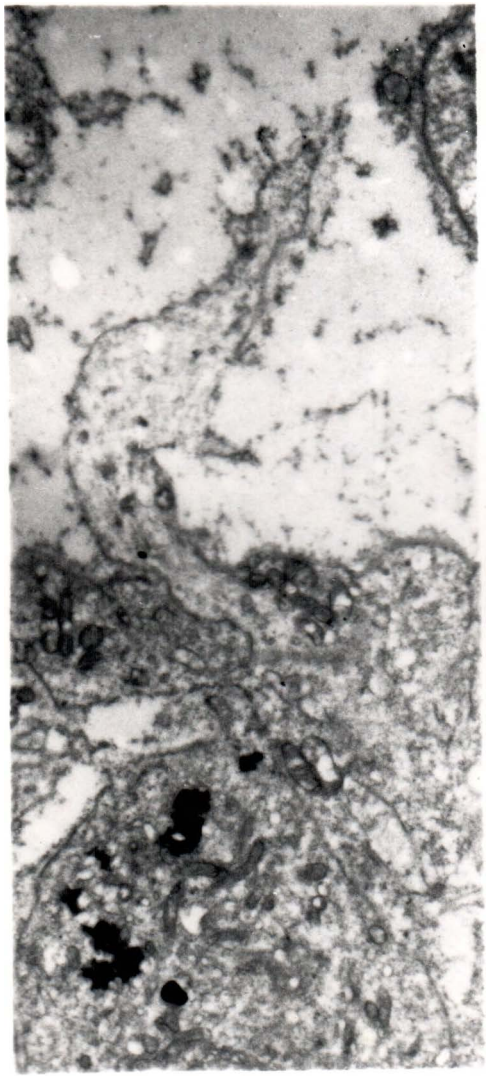


Figure 11 - Mesenchymal Extracellular Space.

This micrograph shows the complexity of the mesenchymal interstitial space; the branching nodular fibrils seen are found only by staining sections with Phosphotungstic acid. These fibers appear to be most numerous near the notochord.

Stage 13 minus.
Fixation: Osmium tetroxide, pH of 8.25, duration of 26 minutes.
Phosphotungstic acid post-stain.
Magnification: 67,200 X.

Figure 12 - 'Pioneer' Neurites in Mesenchyme.

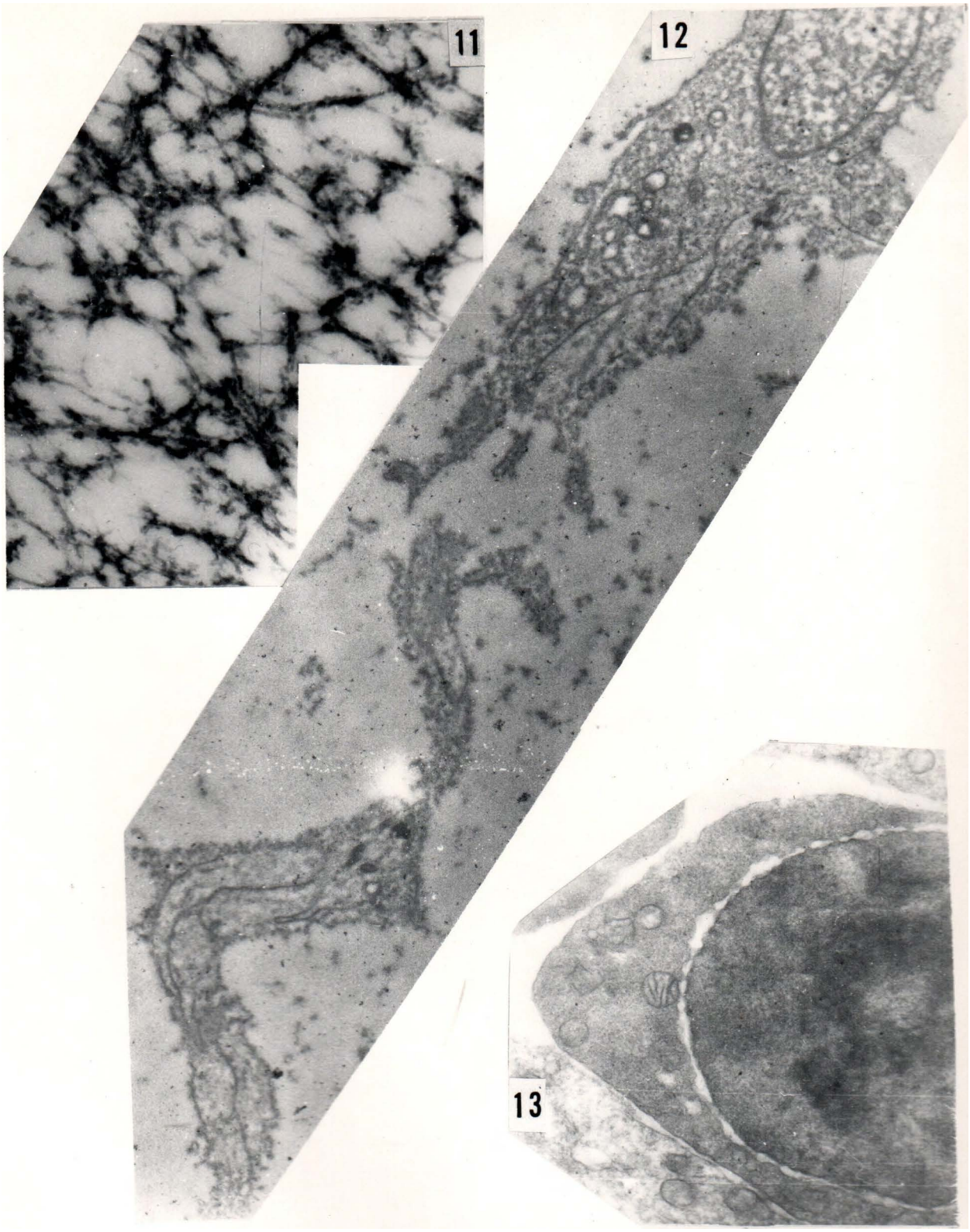
These 'pioneer' neurites pursue an undulant course through the mesenchymal ECS and adhere to a mesodermal cell (upper right). Notice the amorphous granular material concentrated around the neurites but also diffusely scattered through the ECS; similar material is associated with the mesodermal cell.

Stage 15
Fixation: Osmium tetroxide, pH of 8.3, duration 22 minutes.
No stain.
Magnification: 50,000 X.

Figure 13 - Primordial Microglia

This cell may be found invading the embryonal spinal chord with the capillaries during Stage 19. The point of invasion is the nerve root (only the ventral is present at this time). The dense accumulation of ribosomes in the cytoplasm of these cells is identical to the adult nervous system microglia.

Stage 19.
Fixation: Osmium tetroxide, pH of 7.9, duration of 39 minutes.
Unstained.
Magnification: 4,100 X.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Figures 14 and 15 - Anterior Horn Cells of the Fetal (8 day) Spinal Chord.

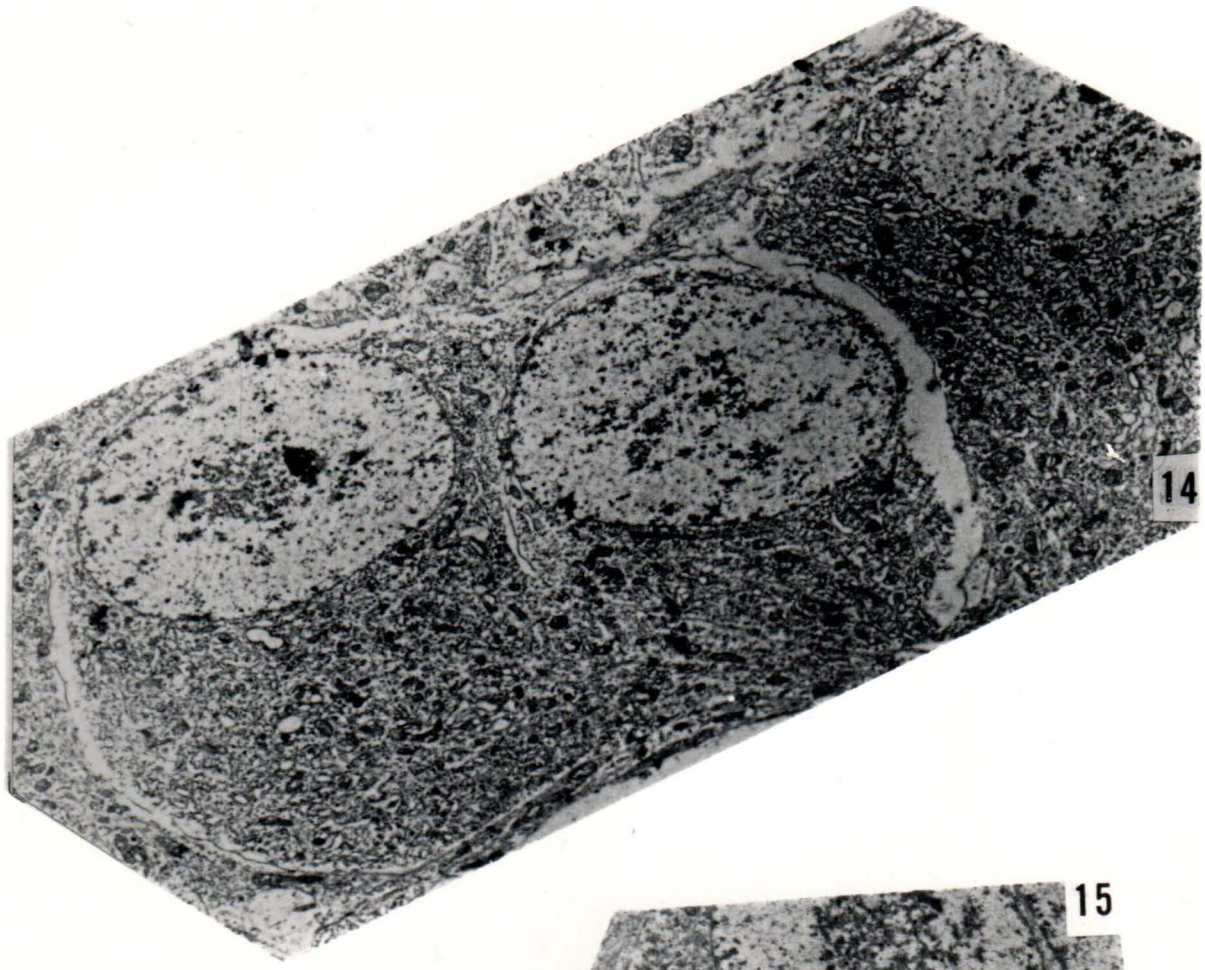
These micrographs show the large nucleus and expansive cytoplasm of the anterior horn cells of the fetal chick. The endoplasm displays prominent Golgi bodies and mitochondria (especially in Figure 15); the upper medial border of the cell on the extreme right in Figure 14 presents a small cluster of Nissl substance, while the middle cell shows an even earlier stage in the inferior perinuclear region (see figure 16). The artifactual electron lucent area around these neurons arises from shrinkage during dehydration and was a constant attribute of these cells.

Stage 39 plus

Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.

Uranyl acetate stain.

Magnification: 4,850 X.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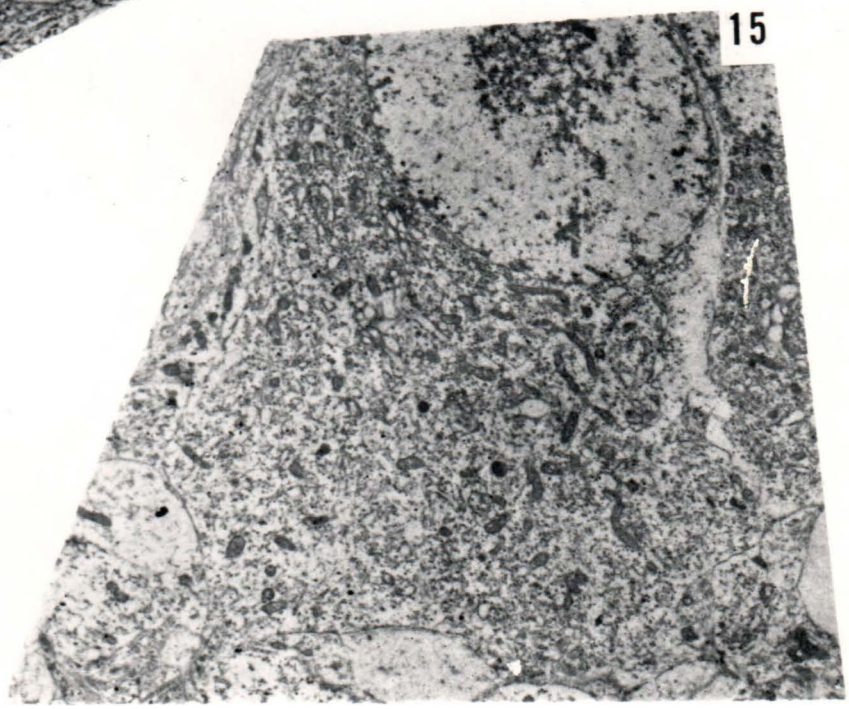
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Figure 16 - The Cytoplasm of the Primary Anterior Horn Cell.

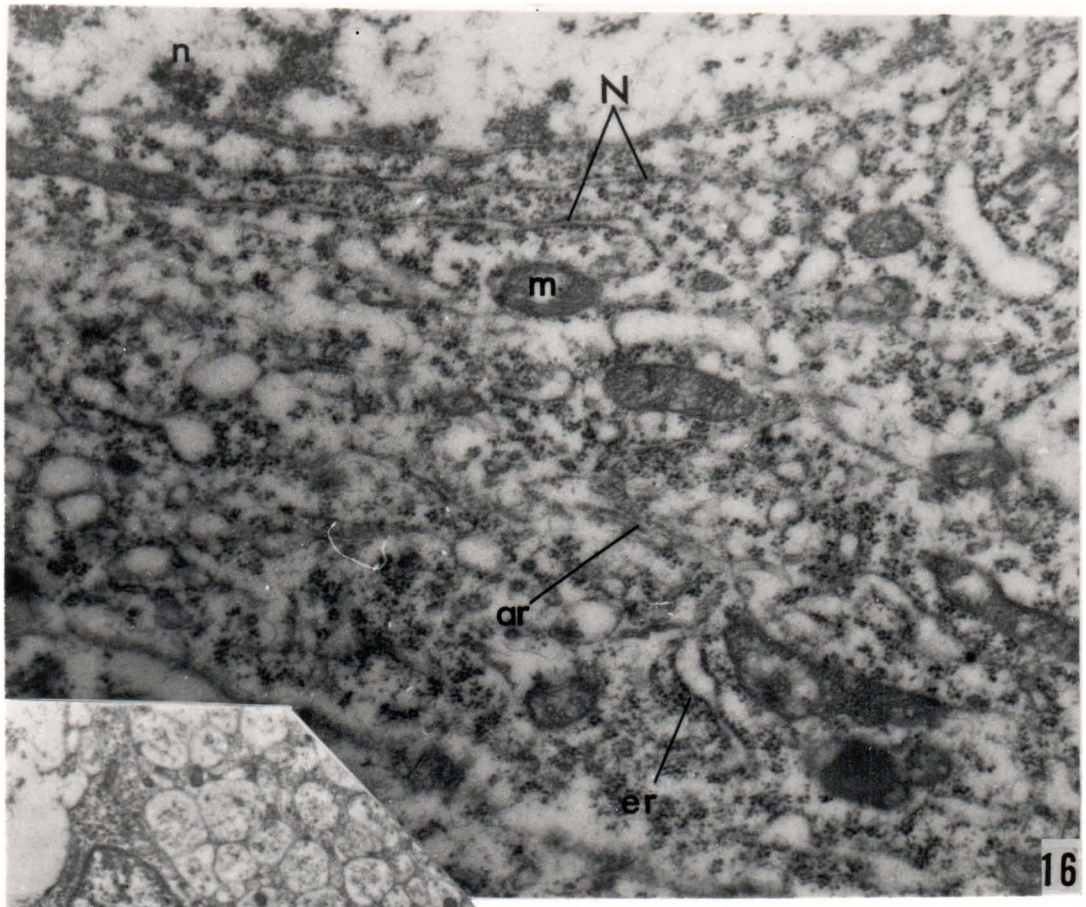
Parallel to the nuclear membrane and closely approximating the nucleus (n) is found the first vestige of Nissl substance (N); frequent dilated profiles of endoplasmic reticulum are seen. The agranular reticulum (ar) is forming a few primitive Golgi apparati, and the mitochondria (m) display a bizarrely complex cristae. Close inspection reveals many fine neurofilaments.

Stage 39 plus.
Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.
Uranyl acetate stain.
Magnification: 32,250 X

Figure 17 - Primitive Macroglia

The elongate nucleus is eccentrically placed in the cytoplasm which is seen peripherally to extend between the neighboring neurites (z). The cytoplasm itself possesses abundant ribosomes and a prominent Golgi apparatus.

Stage 38 minus.
Fixative: Osmium tetroxide, pH of 8.1, duration of three hours.
Uranyl acetate stain.
Magnification: 7,300 X.



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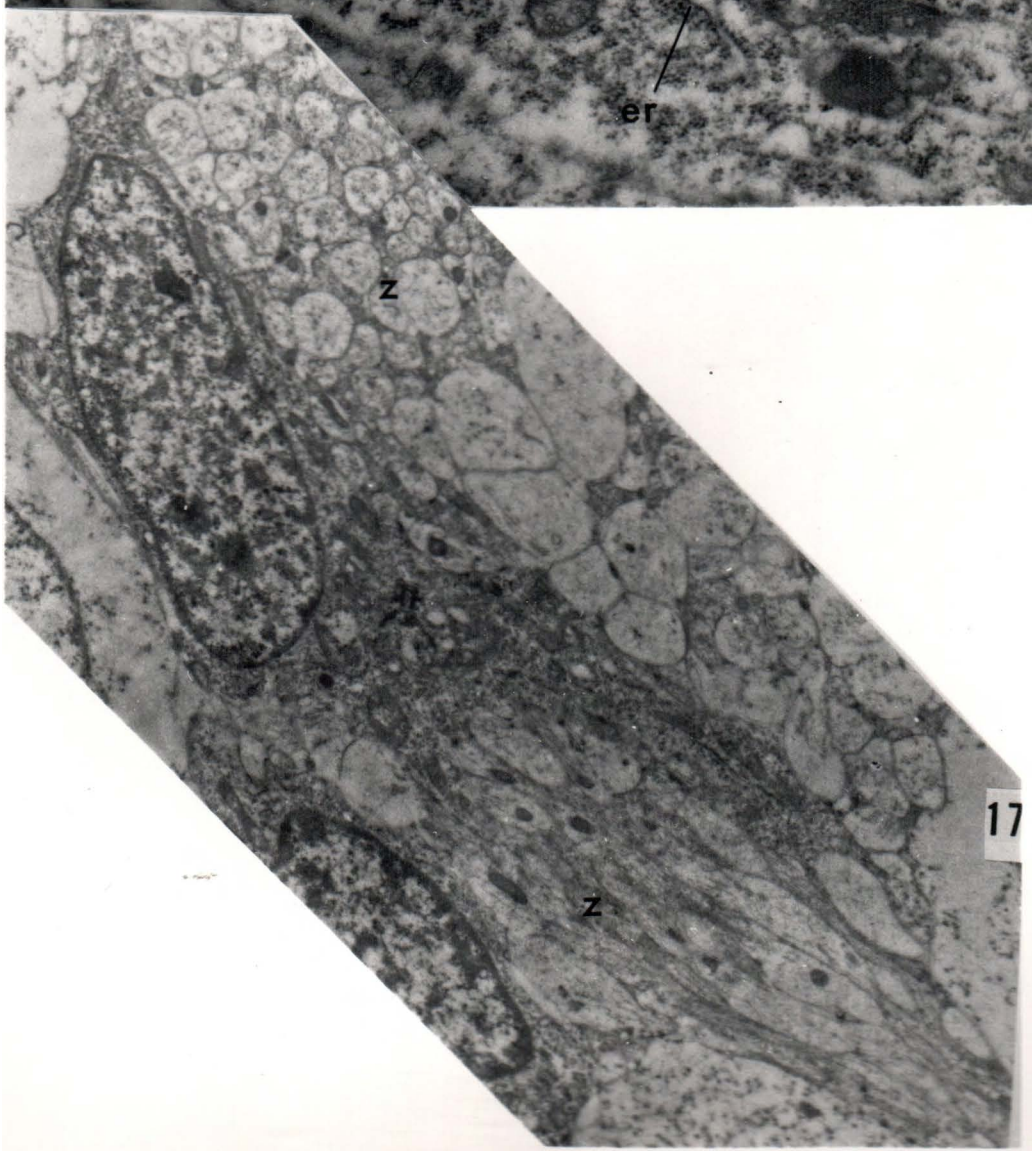

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Figure 18 - Primitive Macroglia and Spongioblast.

Here is shown a spongioblast (s) characterized by the ergastoplasmic profile (e), grooved nucleus and ribosome-poor cytoplasm and the primitive macroglia (o) identified by its enclosed axons (a). The macroglia also has a lobulated nucleus; its agranular reticulum is disposed in a dilated Golgi apparatus.

Stage 39 plus.

Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.

Uranyl acetate stain.

Magnification: 10,000 X.

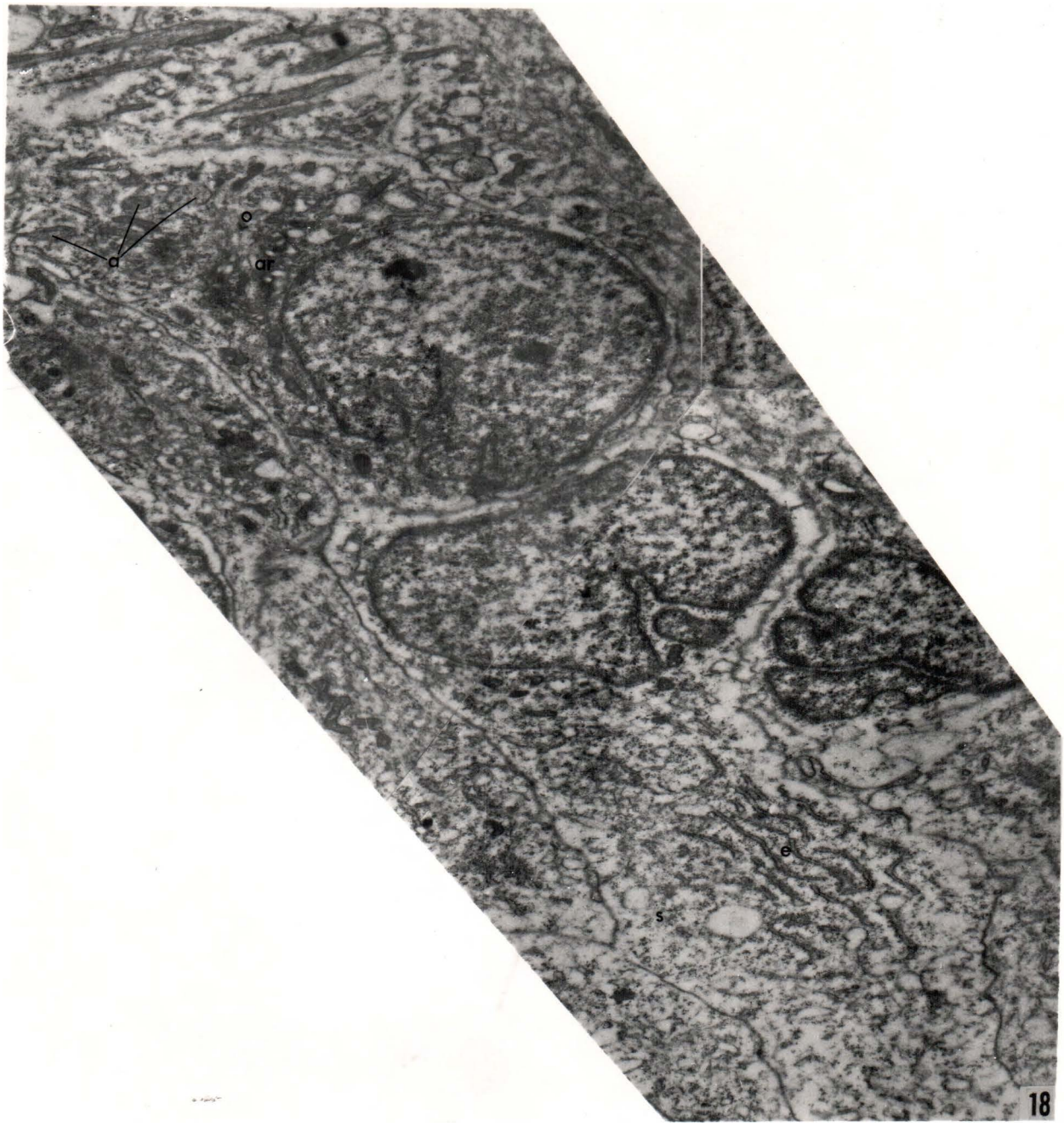

o
d
ar
e
s
18

Figure 19 - High Magnification of Primitive Macroglia.

Here may the axons (a) be seen more clearly; the axon at the extreme left has a short mesaxon. The mitochondria are small, dark and possess few bizarre cristae; the Golgi apparatus (ar) is prominent; the endoplasmic reticulum is scant and the ribosomes are not abundant.

Stage 39 plus.

Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.

Uranyl acetate stain.

Magnification: 14,400 X.

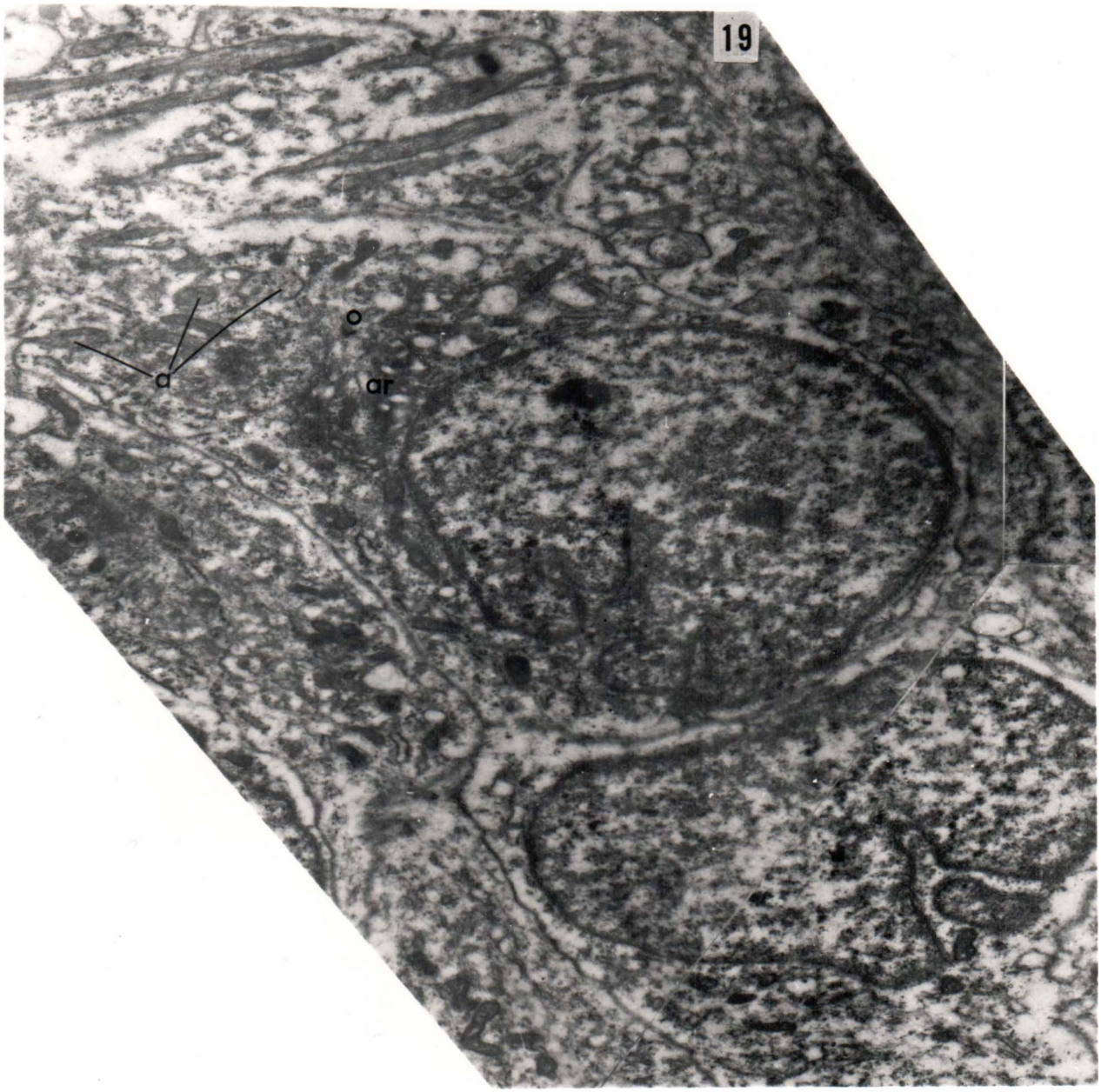

19
o
ar
d

Figure 20 - Spongioblast and Primitive Macroglia.

This spongioblasts (s) possesses a prominent ergastoplasm (e) as well as scattered profiles of agranular reticulum, but no cytofilaments and rare ribosomes. The macroglia (o) inferiorly shows many cytofilaments, frequent profiles of endoplasmic reticulum of an unorganized sort, agranular reticulum, few mitochondria and several lysosomes.

State 39 minus.

Fixation: Osmium tetroxide, pH of 811, duration of three hours.

Uranyl acetate stain.

Magnification: 33,800 X.

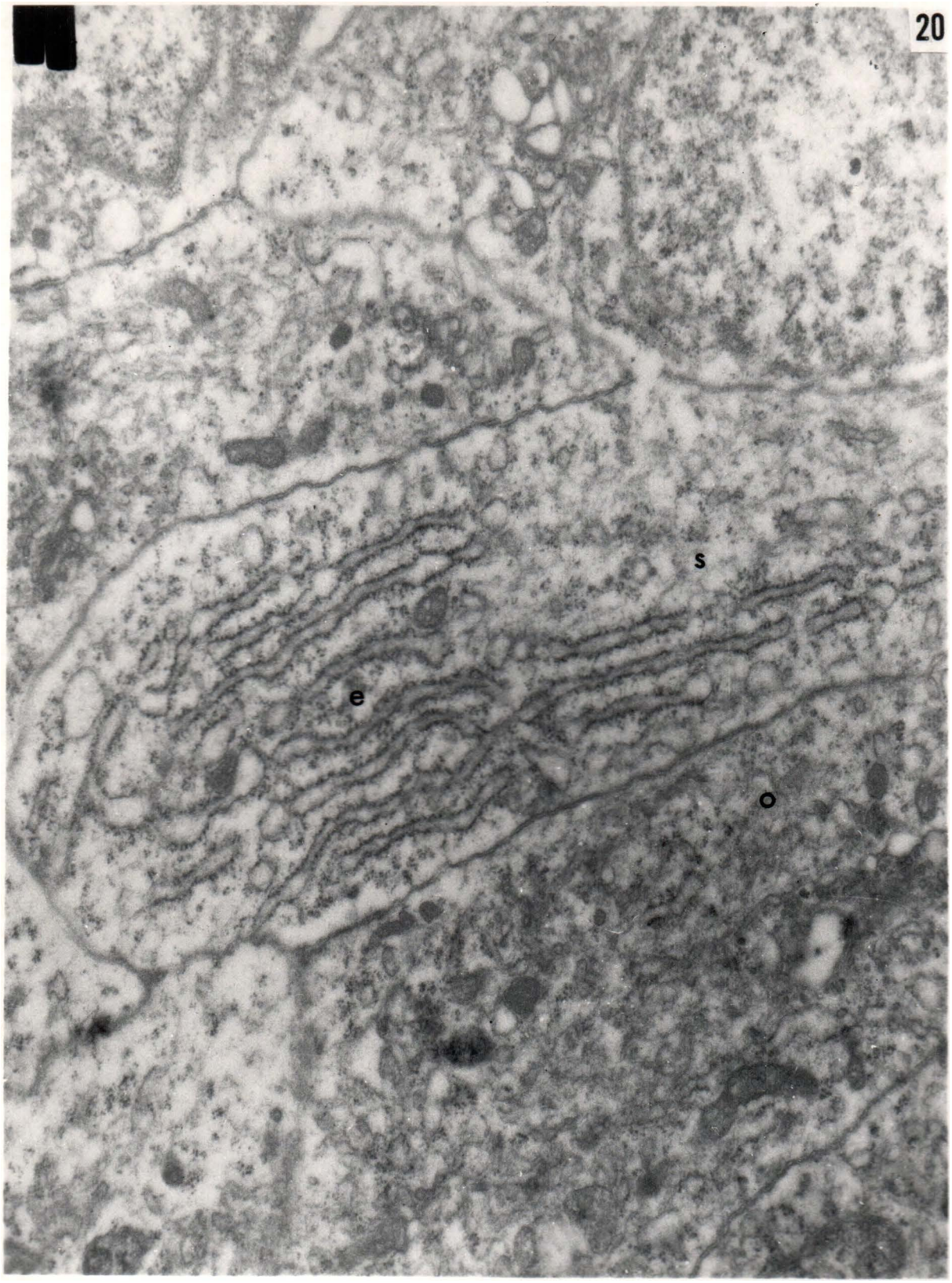

s
e
o

Figure 21 - Higher Magnification of Area in Figure 20.

The fine structure of the ergastoplasmic profile is shown to be composed of parallel cisternae of endoplasmic reticulum (er). In the lower cell, the macroglia, can be seen the numerous cytofilaments (f), mitochondria (lower right corner) and several dark membrane-bound profiles, the lysosomes.

Stage 38 minus.

Fixation: Osmium tetroxide, pH of 8.1, duration of three hours.

Uranyl acetate stain.

Magnification: 68,250 X.

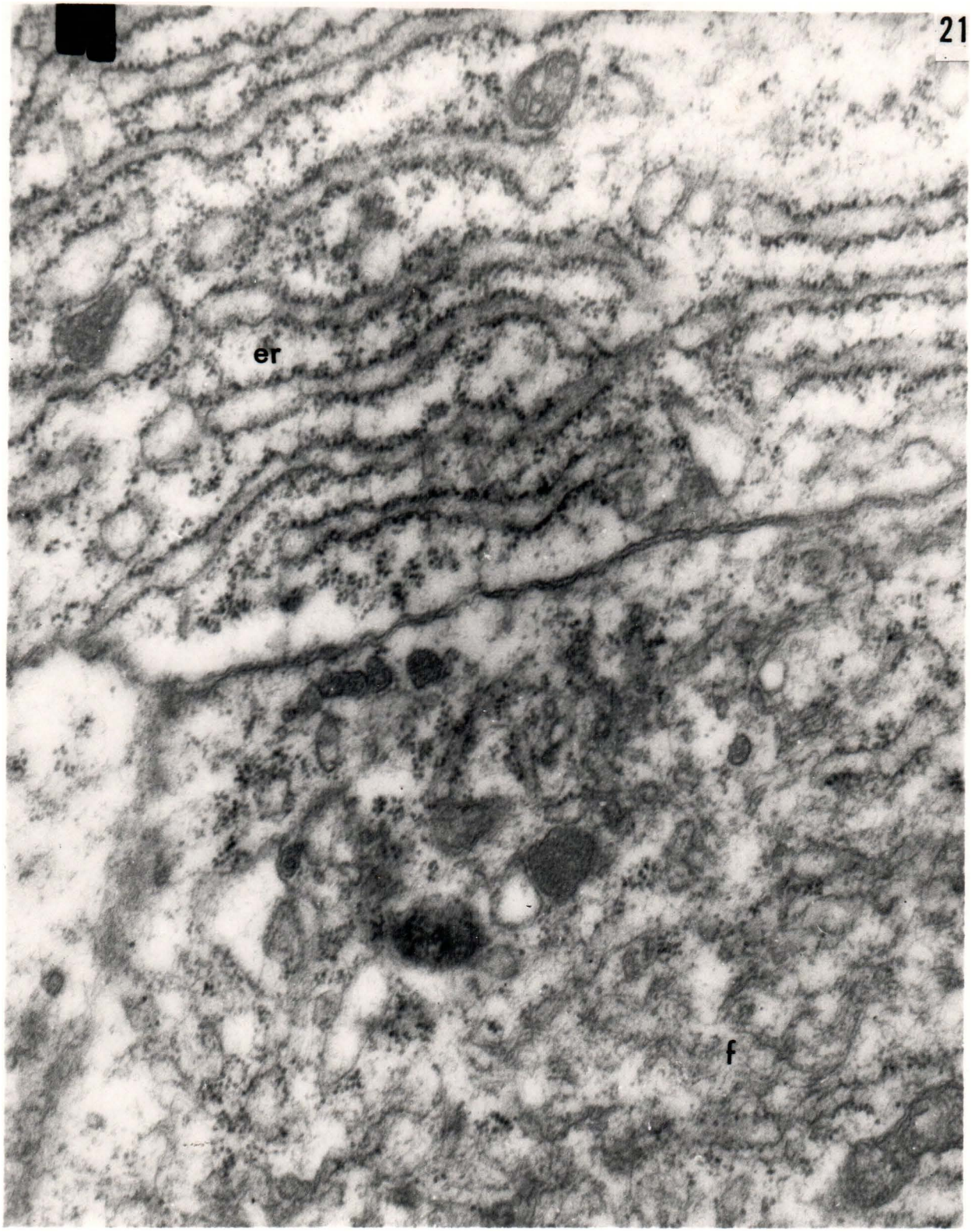

21
er
f

Figure 22 - Neuropile.

Neurites such as the axons (a) in this micrograph possess filamentous mitochondria (m) with many bizarre cristae, frequent neurofilaments and profiles or agranular reticulum (ar) are prominent. The macroglial cytoplasm with its endoplasmic reticulum and ribosomes is seen to the right and also interspersed between the neurites.

Stage 39 plus.

Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.

Uranyl acetate stain.

Magnification: 67,000 X

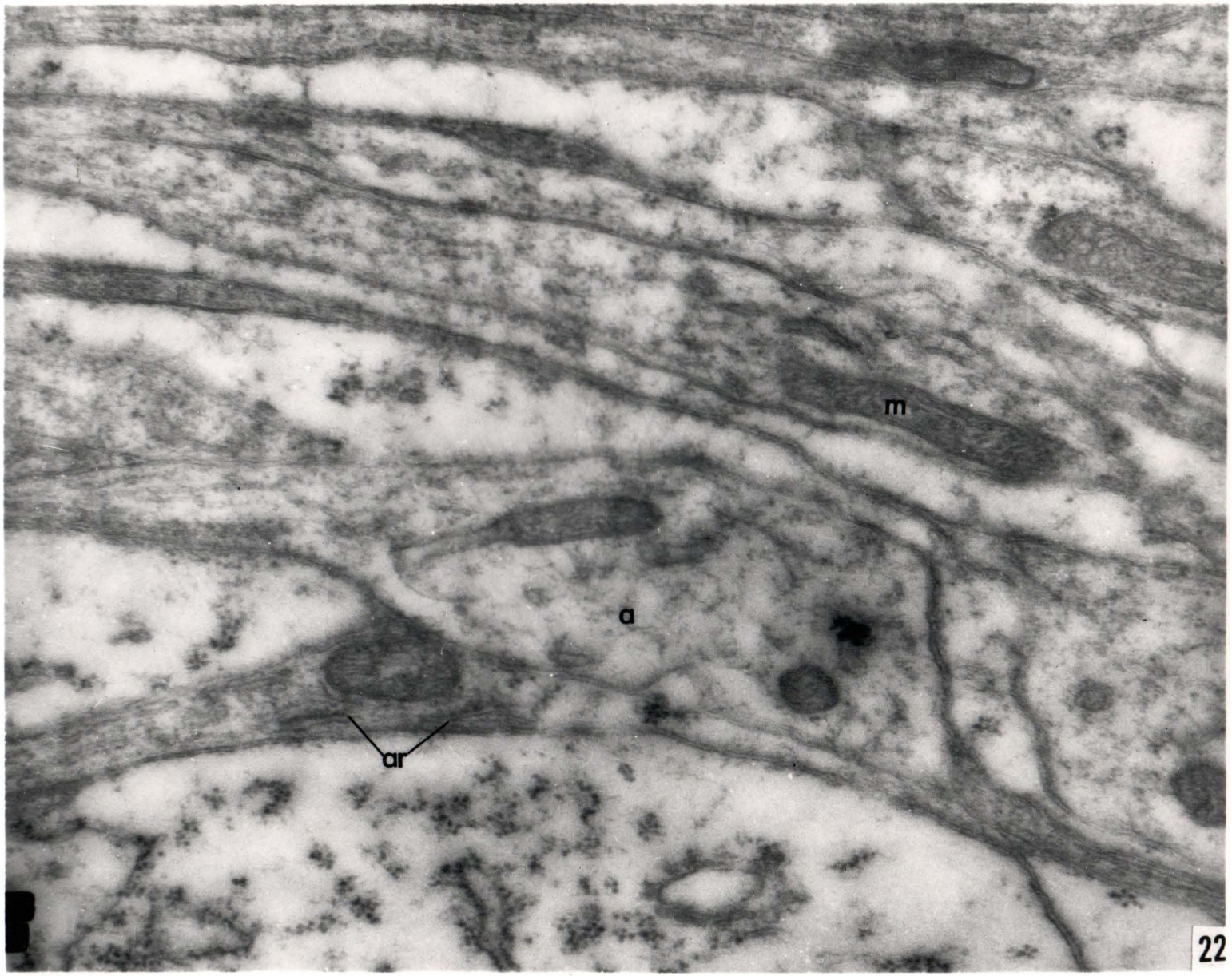

m
a
ar

Figure 22b - Intermediate Cells

These cells found among the neurites (z) of the neuropile show distinct whorling tendencies of oligoglia, their cytoplasm extends into the neuropile to intertwine among the axons (a) and dendrites (d) but contains no cytofilaments and few ribosomes and displays a distinctly ergastoplasmic profile (e).

Stage 39 plus

Fixation: Osmium tetroxide, pH of 7.8, duration of five hours.

Uranyl acetate stain.

Magnification: 16,100 X.

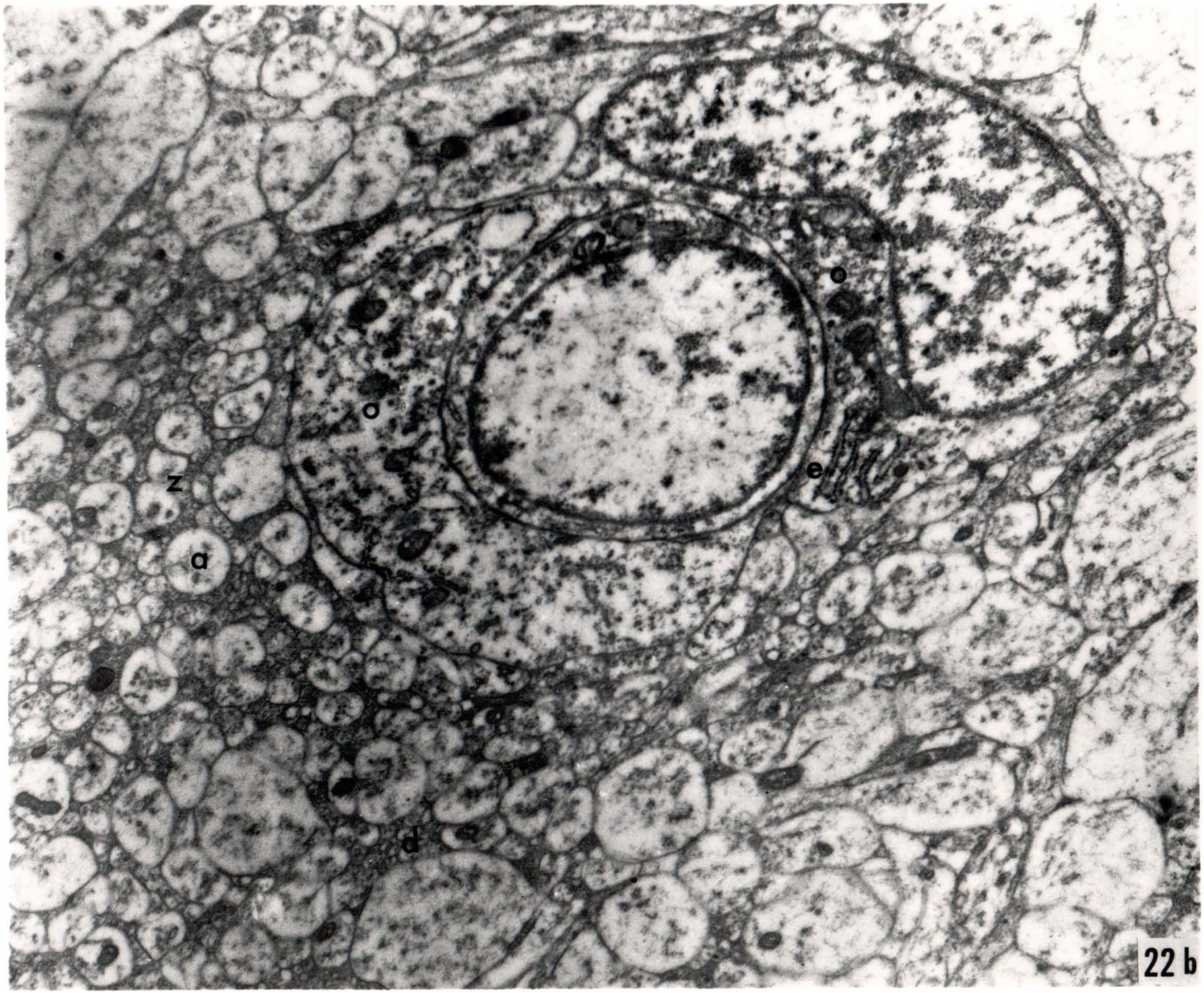

o
o
z
e
a
d
22 b

Figure 23 - Early Myofilament Formation.

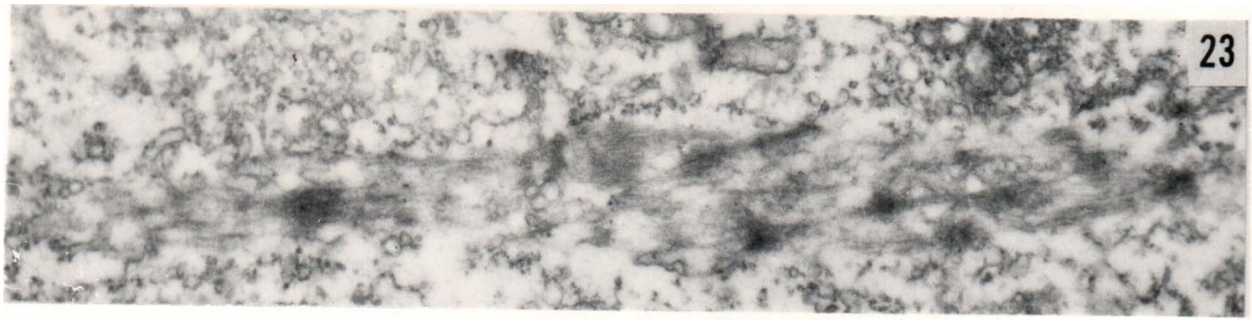
This earliest stage of myofibril differentiation consists of randomly oriented single and multiple cytofilaments which display no periodicity. Close inspection reveals certain areas along the long axis of multiple filaments from which other filaments appear to radiate. Prominent are the profiles of reticulum.

Stage 23 plus.
Fixation: Osmium tetroxide, pH of 8.2, duration of 33 minutes.
Uranyl acetate stain.
Magnification: 23,000 X.

Figure 24 - Myofilament Formation.

At this older stage the myofilaments are parallelly oriented and separated by sarcoplasm but undulant. The Z-bands (arrow) are distinct and close inspection reveals the profiles of sarcoplasmic reticulum so characteristic. No real evidence of periodicity is noted. Remarkable are the mitochondria (m) whose bizarre cristae are typical of differentiating tissue.

Stage 23 plus.
Fixation: Osmium tetroxide, pH of 8.2, duration of 33 minutes.
Uranyl acetate stain.
Magnification: 23,000 X.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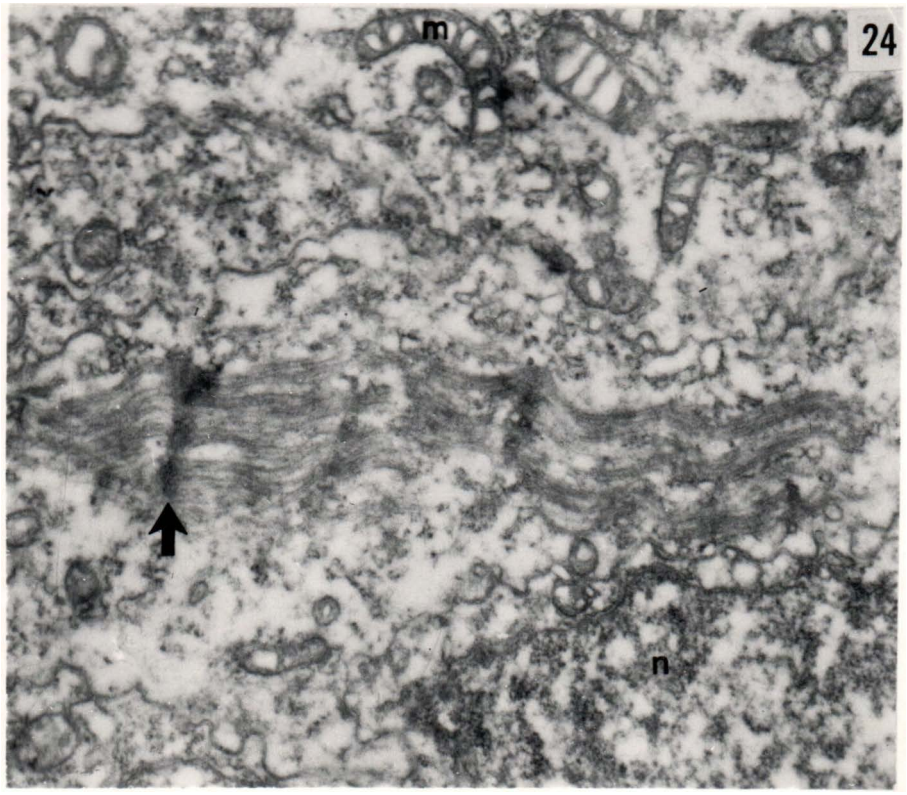

24
m
n

Figure 25 - Myofilament Development.

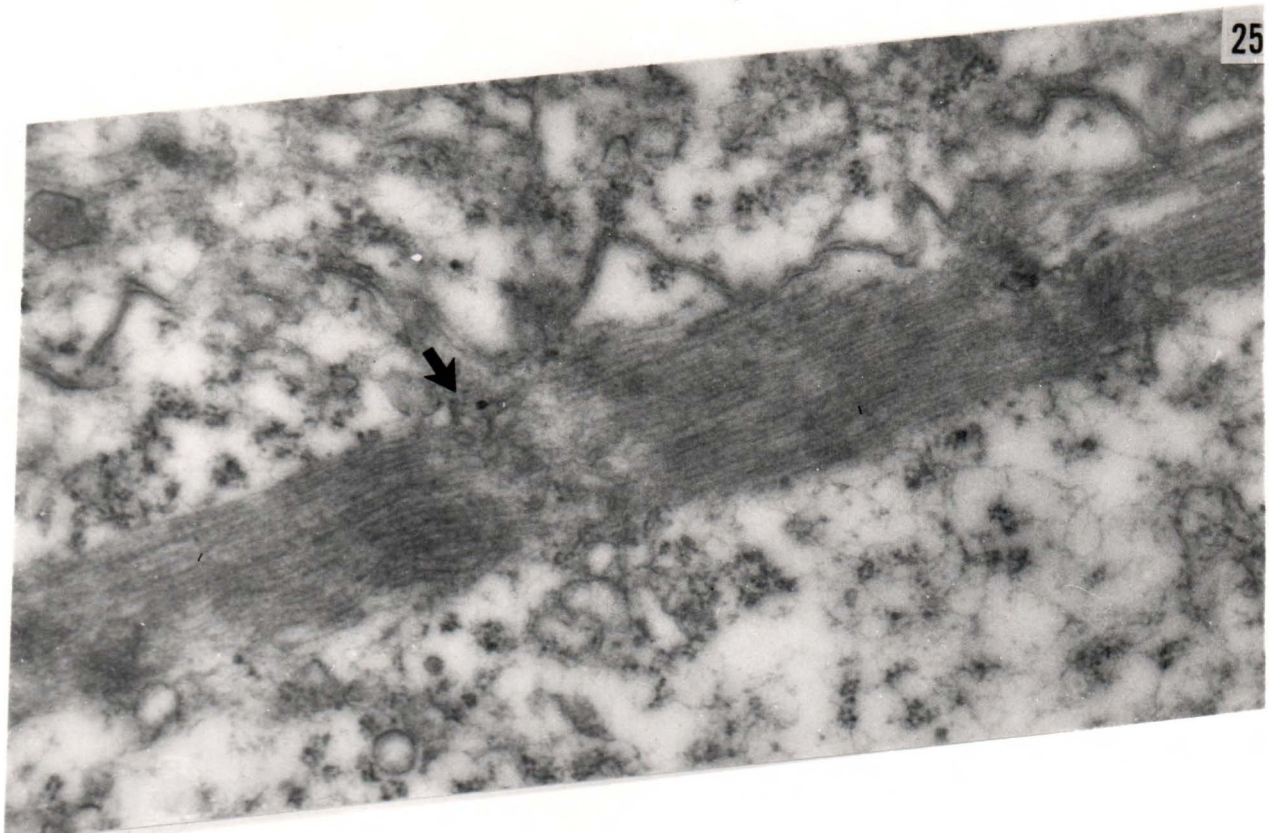
This even later stage shows the loss of myofilament undulance, the expression of intervening sarcoplasm from between the myofilaments and the increase in prominence of the sarcoplasmic reticulum of the Z-band (arrow). Note also the trace of periodicity within the sarcomere.

Stage 23 plus
Fixation: Osmium tetroxide, pH of 8.2, duration of 33 minutes.
Uranyl acetate stain.
Magnification: 63,000 X.

Figure 26 - Myofilament Maturation.

The sarcoplasmic reticulum of the Z-band (arrow) becomes more electron dense; there is some periodicity within the sarcomere and myofibril increases in diameter due to the addition of myofilaments peripherally.

Stage 23 plus.
Fixation: Osmium tetroxide, pH of 8.2, duration of 33 minutes.
Uranyl acetate stain.
Magnification: 63,000 X.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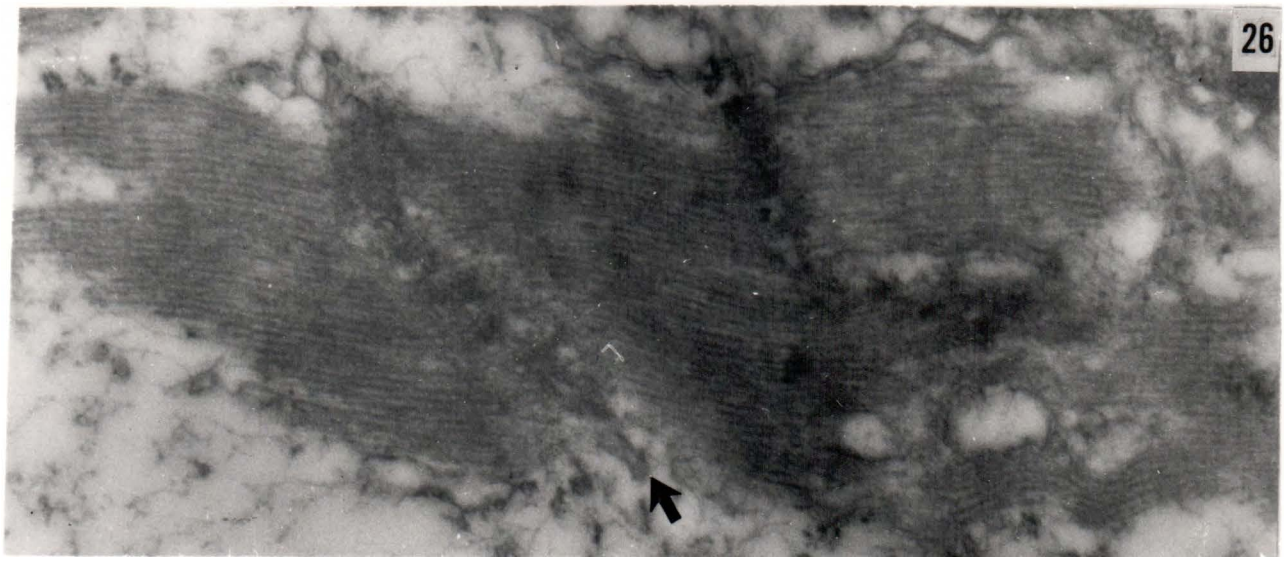
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