A LIGHT AND ELECTRON MICROSCOPIC STUDY OF DEVELOPING MAUTHNER'S CELLS IN *XENOPUS LAEVIS* (DAUDIN)

by

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I. INTRODUCTION

A. History, structure, and function of Mauthner's cell

The history of Mauthner's neurons began with the discovery of two giant fibers in the spinal cord of Esox lucius (common pike) by Mauthner in 1859. The earliest investigators believed that these so called Mauthner's fibers were the result of a fusion of fibers originating from several cells in the brain, and were therefore "multiaxial" (Sanders, 1879). This concept was so well accepted that the discovery and description of two giant neurons in the medulla of the carp by Mayser (1881) did not result in the immediate announcement of the possible relationship between Mauthner's fibers in the spinal cord and these huge nerve cells. The situation remained this way until Goronowitsch, in 1888, succeeded in tracing Mauthner's fiber to its emergence from one of the giant cells.

Since that time Mauthner's neurons and their fibers have been identified in selachians, teleosts, urodeles and anurans (Kingsbury, 1895, 1896; Tagliani, 1905; Beccari, 1907; Tello, 1909; Herrick, 1914; Bartelmez, 1915; Coghill, 1934; Szepsenwol, 1935, 1936; Stefanelli and Osti, 1942; Stefanelli, 1945a,b,c, 1949, 1950, 1951; Willis, 1947; De Angelis, 1950; Wilson, 1959).

Morphological studies, particularly those of Tagliani

(1905), Beccari (1907), Bartelmez (1915) in fish, and Larsell (1934), Szepsenwol (1935, 1936), Stefanelli (1942, 1946), Leghissa (1941) in amphibia, demonstrated the close relationship of Mauthner's cell (M cell) to fibers of the VIIth, VIIIth, and IXth nerves and the fibers of the motorium tegmentum of the medulla. These observations instigated a series of experiments designed to elucidate the behavioral and physiological relationships of the M cell to these areas and to the motor neurons of the trunk and tail regions of the spinal cord.

It was strongly suspected from the histological findings that the M cell behaved as a miniature associative center. It was assumed to correlate information received from the lateral line branches of the VIIth and IXth nerves, from the acoustico-vestibular organs via the VIIIth nerve, and from the motor areas of the brain, and to initiate the correct response in the trunk and tail musculature via synapses of the huge M axon on the motor neurons in the spinal cord.

Evidence supporting these assumptions and indicating the role of the M cell as mediator of the "startle" reflex or avoidance response came from a series of extirpation experiments in fish and amphibians (Detwiler, 1927, 1933,a,b, 1947; Retzlaff, 1957; Sims, 1962; Rodgers and Melzak, 1963), and electrophysiological experiments with fish (Retzlaff, 1955, 1957; Retzlaff and Fontaine, 1960a,b; Furshpan and Furukawa, 1962; Fukami, Furukawa and Asada, 1965; Yasargil and Diamond,

1968; Diamond and Yasargil, 1969).

Further studies on teleost M cells with both light (Bartelmez, 1915; Bartelmez and Hoerr, 1933; Bodian, 1937b, 1942, 1952; Barbey, 1942; Stefanelli and Caravita, 1964), and electron microscopy (Robertson, 1963; Robertson, Bodenheimer and Stage, 1963; Kohno, 1970; Nakajima and Wang, 1971) reveled an abundance of morphologically distinguishable synaptic contacts localized on the cell body, on the massive dendrites and on the axon hillock regions of the M cell. These synapses were then experimentally investigated and found to demonstrate a variety of electrophysiological phenomena. For example, the huge club endings located on the lateral part of the lateral dendrite were supposedly the source of both electrical and chemical excitatory potentials (Furshpan, 1964), while the axon hillock region was the target for both electrical and chamical inhibition (Furukawa and Furshpan, 1963; Eccles, 1964). The inhibitory nature of several prospective transmitters was subsequently tested in some of these areas with electrophoretic application of various agents to specific synaptic sites (Diamond, 1968; Roper, 1970).

Mauthner's cells in amphibians have been found to be less complex than those known in fish. They have no recognizable axon cap, nor do they have any recognizable club endings (Stefanelli, 1951; Moulton, Jurand and Fox, 1968). They are also somewhat smaller and therefore not as desirable for neurophysiological studies.

For developmental studies, however, M cells in amphibian larva have tremendous potential. The amphibian system provides an outstanding tool because (a) there are only two cells in each animal, (b) they are obviously larger than any other neuron in their vicinity and are easily recognizable, and (c) they regress and atrophy while other nerve cells adjacent to them remain normal. These characteristics proved to be most valuable because they allowed for the study of a complete life cycle of a normal cell in a normal environment, and in addition would permit the manipulation an analysis of factors that would elicit a specific response in an identifiable cell.

The M cell in Xenopus is determined in early neural plate stage (Stefanelli, 1951), and in either gastrula (Holtfreter, 1931), neural plate (Piatt, 1945; Stefanelli, 1951) or somewhere in between (Detwiler, 1944) in Ambystoma. In any case the event occurs before any accessory fibers or structures, eg, the otic vesicles or fibers of the VIIIth nerve, with which the M cell was closely associated in the mature state, are present. The question as to the extent to which these factors are involved in the maturation and ultimate degeneration of the M cell was approached from several points of view. Stefanelli (1951) using an ecological argument noted that those anurans whose tadpoles were entirely aquatic, with well developed tail musculature and lateral line were also the ones having the largest and best developed M cell (Rana, Hyla, Xenopus). Those animals that were almost

completely terrestrial (Bufo) had almost vestigial M cells or none at all. He therefore attributed the growth and maintainance of M cells to these accessory structures, particularly the lateral line and tail, and its atrophy to their loss during metamorphosis (Stefanelli and Osti, 1942; Stefanelli, 1949, 1951). Larsell (1934) and Baffoni and Catte (1951) agreed with his analysis. However, Oppenheimer (1941), Piatt (1943, 1947, 1950, 1969), Weiss and Rossetti (1951) and Pesetsky (1960) demonstrated that M cells were able to develop to varying extents without the lateral line, acoustico-vestibular systems, or tail. However a larger number of completely normal M cells developed with fully differentiated dendrites when the accessory structures were present.

Baffoni and Catte (1950,1951), and Pesetsky (1962) found that the presence of thyroid hormone was absolutely essential for the maintainance of the M cell regardless of the presence or absence of lateral line, vestibular, or spinal cord connections. Its normal degeneration was attributed to a drop in thyroxin concentration at the completion of metamorphosis to a level insufficient to sustain a fully differentiated M cell resulting in subsequent cell atrophy (Pesetsky, 1962, 1966; Fox and Turner, 1967; Fox and Moulton, 1968).

Mauthner's cells have also been the center of considerable attention as a model for axonal growth and specification (Piatt, 1944; Stefanelli, 1950, 1951; Jacobson,

C.O., 1964; Hibbard, 1965; Swisher and Hibbard, 1967).

The most neglected aspect of M cell differentiation has been cytological. A few general studies have been done using light microscopy (Baffoni and Catte, 1951; Stefanelli and Osti, 1942), but only one paper is available on the ultrastructure of developing M cells (Moulton, Jurand and Fox, 1968). These investigators looked only at stages in which the M cell was already functionally active, is indicated by the presence of the "startle" reflex. They dealt primarily with the development of the granular endoplasmic reticulum in relation to metamorphosis, and only incidentally with other cytoplasmic constituents.

B. General aspects of neuronal differentiation

The cytology of neurogenesis has been studied much more extensively in other types of nerve cells. Investigations have been carried out primarily on the mammalian (Windle, 1935; Windle and Baxter, 1936a,b; Herman and Kauffman, 1966; LaVelle and LaVelle, 1969; Tennyson, 1969; Hinds and Ruffet, 1971), avian (Cowdry, 1914; Barron, 1943, 1946; Duncan, 1957; Bellairs, 1959; Blechschmidt, 1960; Eschner and Glees, 1963; Lyser, 1964; Wechsler, 1966), and to a much lesser extent on amphibian nervous systems (Fisher and Jacobson, 1970).

Earlier studies employing silver impregnation revealed a close parallelism between nerve cell differentiation and neurofibrillar elaboration (Ramon y Cajal, 1909; Cowdry, 1914; Windle, 1935; Windle and Baxter, 1936a; Barron, 1943,

1946). These investigators agreed to some extent on a sequence of changes usually observed in newly differentiating neurons, definable by the number of processes emanating from the cell body and by the pattern of the neurofibrillar distribution. Germinal cells (Ramon y Cajal, 1929) or prefibrillar cells (Cowdry, 1914; Windle and Baxter, 1936a) contained some faint granular accumulations adjacent to the nucleus which were subsequently replaced by neurofibrils in the anaxonic or apolar neuroblast. The fibrils were concentrated around the nucleus and were oriented toward those parts of the cell still attaced to the inner and outer borders of the neural tube. The inner processes contained no evidence of fibrillar material and remained attaced to the border of the neurocoel. The distal process, showing a strong argentophilia, detached itself and elongated into the axon. At this stage the cell was designated a primitive bipolar neuroblast. Once the axon began to grow, the inner transitory process was retracted resulting in a unipolar neuroblast. Neurofibrils were then accumulated at points from which the future dendrites emerged, transforming the unipolar neuroblast into a multipolar neuroblast. In each case the neurofibrils were distinctly associated with the formation of functional processes.

With the advent of electron microscopy, several attempts were made to correlate the presence of argentophilic neurofilaments with a subcellular organelle. Many workers concluded that the neurofibrils of light microscopy most

closely paralleled the distribution of 100 A (diameter) neurofilaments (Peters, 1956; Gray and Guillery, 1961, 1966; Coggeshall and Fawcett, 1963; Guillery, 1965; Lund and Westrum, 1966; Sechrist and LaVelle, 1966; Sechrist, 1968, 1969; LaVelle and LaVelle, 1969). However, some discrepency as to the universality of these findings has been pointed out. Walberg (1964) examined some nerve endings in the inferior olive that reacted quite strongly with silver, but found the area devoid of neurofilaments. Lyser (1968) found the early distribution of microtubules more closely related to the neurofibrls of the light microscope studies than did the neurofilaments, although neither was ound in large enough quantity to account for the intensity of the silver stain seen in her embryos. To complicate matters further, Tennyson (1965) observed that silver particles attaced to a variety of organelles, including the granular endoplasmic reticulum, depending on which silver technique was emplotyed.

Nissl bodies, are found in all nerve cells and their pattern of distribution and organization has been found to be characteristic for a given type of neuron (Einarson, 1935). It has been proposed (LaVelle, 1951, 1956) that the appearance of this structure represented one of the earliest signs of nerve cell differentiation. However, other workers do not agree with this contention.

LaVelle and LaVelle (1958), and Eschner and Glees (1963) observed the appearance of some fine cytoplasmic particles at the base of the neurite in bipolar and unipolar

neuroblasts which subsequently accumulated and differentiated as the Nissl bodies. This occurred close to the nuclear membrane opposing the base of the process. The amount of Nissl progressively increased with maturity of the young neuron. Upon electron microscopic examination of mature neurons, granular endoplasmic reticulum was found to be the counterpart of the Nissl bodies of light microscopy (Palade, 1955; Palay and Palade, 1955; Porter, 1961) and the elaboration of granular endoplasmic reticulum was also related to early nerve cell development (Bellairs, 1959; Tennyson and Pappas, 1964; Eschner and Glees, 1963; Meller, Eschner and Glees, 1966; Tennyson, 1965; Fujita, 1966; Pannesse, 1968). Lyser (1964) and Fisher and Jacobsen (1970), however, argued that the first definitive sign of neuronal differentiation was the appearance of a recognizable axon, and at that time only a few scattered profiles of granular endoplasmic reticulum were present and in such small amounts that the early differentiating neurons were indistingishable from the ventricular cells (i.e. those cells lining the neurocoel and still presumably capable of mitosis). Therefore, the beginning of Nissl body formation was clearly separable in their systems from the onset of nerve cell differentiation.

Any proposals that involved granular endoplasmic reticulum with developing processes automatically implicated the nucleolus. Extensive work on a variety of cell types, including neurons, has shown that the nucleolus is the source of the ribosomal RNA component of the granular endoplasmic

reticulum (Edstrom and Eichner, 1958; Leblond and Amano, 1962; Birnsteil, Chipchase and Hyse, 1963; Esper and Barr, 1964; Perry, 1965, 1966; Sidebottom and Harris, 1969).

Not only has the nucleolus been related to ribosome formation but it has also been proposed to play a role in the actual differentiation of granular endoplasmic reticulum or Nissl substance (Barr and Bertram, 1951; LaVelle, 1951, 1956; Parvis, 1954; Eschner and Glees, 1963; Olszowski, 1954; Watson, 1968).

The Golgi apparatus is another cytoplasmic constituent recognizable both light and electron microscopically, which has been associated with the growth of processes. Ramon y Cajal (1929) described a "reticular apparatus" composed of a neurofibrillar zone and a Golgi complex. He then went on to correlate its appearance with regions "most capable of generating outgrowths" (Ramon y Cajal, 1929). More recently Dvorak (1968, 1970), employing histochemical techniques, observed Golgi complexes at points of emergence of the Purkinje cell dendrites and postulated that they might be involved in the origin, or more likely in the growth and maintainance of the processes.

The factors which determine the site of origin of axons and dendrites from the soma have not been elucidated. Experimentally it has been demonstrated that intrinsic properties of the neurons must contribute significantly if not completely to this phenomenon (Van der Loos, 1965; Globus and Scheibel, 1967).

The growing tip of the axon was first identified as a growth cone by Ramon y Cajal (see Ramon y Cajal, 1917). It has since been demonstrated in the fin of a living tadpole (speidel, 1933, 1941), in tissue culture (harrison, 1907 1910, 1912, 1914; Lewis and Lewis, 1912; Hughes, 1953; Nakai, 1956; Nakai and Kawasaki, 1959; Pomerat, Hendelman, Raiborn and Massey, 1967; Bray, 1970), an in regenerating nerves (Harrison, 1910; Estable, Acosta-Ferreira and Sotelo, 1963; Speidel, 1964; Lampert, 1967; Lentz, 1967). The growth cone usually appears slightly enlarged and gives off filopodia which measure approximately 3u in diameter and 10u-20u in length.

Morest (1968, 1969a,b) investigated dendritic development in Golgi preparations and found growth cones that resembled those described at the ends of elongating axons. Filopodia extended from the growing portion of dendrites and also from the cell body. The latter, which he termed somatic filopodia, were indistiguishable from those found on the axons and dendrites. Somatic filopodia were transitory structures and decreased in number as the cells differentiated more fully. They were most numerous at times when the cell body and its denrites were growing the most.

Two different ultrastructural configurations have been proposed for growth cones. Bodian (1966) and Del Cerro and Snider (1968) found accumulations of large agranular vesicles at the growing tips of axons and called them growth cones. Other workers (Candiollo and Filogamo, 1966;

Larramendi and Lemkey, 1966) have commented on the presence of such vesicles either directly under the surface of the cell body or in the distal portions of processes that extended for varying distances from the soma. They did not assign any functional significance to them.

Tennyson (1970) argued that the structures observed by Del Cerro and Snider (1968) were not followed far enough to be properly identified as growth cones. She examined serial sections of growing dorsal root ganglion cell processes and described growth cones as consisting of large varicositites containing mitochondria, smooth endoplasmic reticulum, neurofilaments and meurotubules with smaller filopodia containing find fibrils emerging from them. This view corresponds to the observations of Yamada, Spooner and Wessels (1971) on cultured dorsal root ganglion cells.

Once the dendrite begins to differentiate, its growth and maintainance is believed to be profoundly influenced by two factors. The first is the termination of the axon (Ramon y Cajal, 1909; Barron, 1943; Morst, 1969a). Evidence supporting the contention that a dendrite would not develop beyond a certain point (if at all) until the axon completed its terminal connections is largely circumstantial (Weiss, 1936, 1942, 1947; Kollors, 1943; Miner, 1956; Jacobson and Baker, 1968, 1969). The second factor believed to be important in the development of dendritic structure is the formation of functional synapses on the developing dendrite itself (larsell, 1931; Levi-Montalcini, 1949; Filogamo, 1950).

Without synapses the dendrites remained stunted and abnormal.

The development of synapses has been found to follow a certain pattern within each cell. Synapses appear first on the dendrites and later on the soma (Pappas and Purpura, 1961; Vowller, Pappas and Purpura, 1963; Purpura, Shofer, Housepian and Noback, 1964). The factors which determine the temporal sequence and spatial arrangement of the various synaptic connections have not been elucidated. Equally confusing has been the question of which synaptic elements appear first. Although some workers on rat brain and neuromuscular junction reported an increase in synaptic vesicles as being the first indication of synapse formation (Hirano, 1967; Teravainen, 1968), other studies claimed that thickened membranes were the earliest characteristics (Eschner and Glees, 1963) or finally, that both structures appeared simultaneously (Bodian, 1968; Kornguth, Anderson and Scott, 1968; Kanemitsu, Yohro and Nakai, 1970).

C. Mauthner's cell as a model for neuronal development

It is obvious from this brief review of nerve cell development that a considerable amount of literature has accumulated on various aspects of differentiation of vertebrate nerve cells. In all but one or two cases, the gathering of information about various morphological events occurring within developing nerve cells has relied on the use of populations of cells, since identification of specific individual cells has been impossible in the spinal cord or in most parts of the brain. In these *in vitro* or *in situ*

systems, a reasonably accurate sequence of events could be mapped out with respect to the appearance and arragements of various structures. The ideal situation would be to use one cell and trace its development from the time it was first determined as a neuron until it matured. In this respect, the Mauthnerian apparatus offered a unique opportunity, and was therefore used in this study to investigate morphological changes occurring within a developing nerve cell. Identification could be made at a relatively early age, with a certain amount of accuracy, and its development could be traced from that point without confusing it with any other cell (Figs. 1 and 74). One difficulty encountered was that in the very early stages, the M cell, was found to be indistinguishable from other cells in the medulla. It could be recognized only at the point when it already had its axon and began to differentiate as a neuron. However very early nerve cell development has been dealt with to a certain extent in other cells of the nervous system (Lyser, 1964, 1968; Fisher and Jacobson, 1970). It was still possible to study relatively early stages and to follow the morphological manifestations of dendritic origin as well as the growth and maturation of the cell body and all of its processes in one specific cell.

II. MATERIALS AND METHODS

A. Breeding and maintainance of embryos

Adult Xenopus laevis (Daudin) were obtained from the Lemberger Co., Oshkosh, Wisconsin. The males and females were kept in separate tanks maintained at room temperature in either spring water (Crystal Spring Water Co.), or tap water treated with Long-life Aqua Age tablets (Long-life Fish Food Products) to remove the chlorine. The containers were fitted with wire mesh to prevent the toads from jumping out and at the same time to allow for adequate ventilation.

The adults were fed beef liver cut into 2 mm cubes once or twice a week. The water was changed the next day and/or when the water became turbid due to accumulating waste products. The toads appeared to do well on this regimen as evidenced by their pear shape (Bles, 1905), by the dorsal protrusion of their lungs, and by the fact that they continued to breed and produce healthy embryos for two years.

Since all the toads were kept together in only two tanks, a method was necessary for distinguishing the individuals. Xenopus laevis, or the African clawed toad, has, as its name implies, a horny claw on each of the outer three toes of each hind limb. These were clipped in various

combinations, so that the toads could be distinguished by the number and position of clipped claws. The procedure was painless to the animals and although regeneration did occur within a few months, the claws could be easily re-clipped at that time.

Adult toads were induced to spawn in the laboratory by the injection of human chorionic gonadotropins (Brown and Littna, 1964). Variations in number and quality of gametes produced throughout the year were common, with September and March being the most productive, and the summer months, the least. The first dose appeared to act as a priming dose (Henriquez, 1964) for the subsequent week's injection. As many as one to two thousand fertilized eggs were harvested during the second and third weeks in the fall months, and so the fourth injection was rarely required. When it was attempted, primarily during the summer, the results were poor. This could have been due to inadequate environmental controls resulting in unduly elevated laboratory temperatures, or in a shifting of the dose response curve of the hormone to a higher threshold level. In some cases, gametes were obtained during these periods by increasing the dosage and injecting a priming dose the day before the toads were mated.

Mature females could be recognized by enlarged cloacal valves, while the mature males showed black "nuptial pads" on the inner aspects of their forelimbs (Nieuwkoop and Faber, 1967). In some cases the "nuptial pads" appeared

after the first hormonal injection. In no case were zygotes produced when the male did not show these markings.

When eggs were required, the animal was grasped by the legs, usually with an absorbant towel or gauze to prevent it from sliding away, and the hormone was injected into the dorsal lymph sac by passing the needle through the dorsal femoral muscle and piercing the septum between the trunk and hind limb (Ochse, 1948). This prevented seepage from the sac by contraction of the muscle upon withdrawal of the needle. If the animal was held with its head downward, and the injection accurately administered, the fluid could be seen to collect over the head region. The male was injected with 1 cc of Antuitrin S (Parke-Davis) containing 280 I.U. dissolved in distilled water. The female was injected with 2 cc of the same hormone solution containing 580 I.U.

The pair was then put into a 2 liter tank with a wire mesh placed 2 cm above the bottom. The grid spaces of 1 cm squares were large enough for the eggs to drop through as they were spawned, and, at the same time, small enough to prevent the adults from trampling on them. A mesh lid was placed on top to keep the breeding toads confined. No attempt was made to regulate either temperature, light or noise levels since such controls did not improve the quality or the quantity of fertilized eggs.

Spawning began a few hours later and lasted 10 to 14 hours. The following morning, the toads, who were

usually found still clasped if a successful mating had occurred, were removed from the breeding tank and replaced into their original containers. The feces were removed from the water and the embryos were then very gnetly removed from the tank and grid, to which many of them had adhered, and put into 0.5 liter autoclaved glass dishes or large petri dishes, kept at room temperature. They were provided with gentle aeration through plastic tubing connected to a simple aquarium aerator. Viability was further enhanced by separating the zygotes from each other and by removing the unfertilized and abnormal eggs as quickly as possible. Almost all the embryos developed well in those dishes where the population density was low, allowing at least 10 ml space for each embryo. Larger volumes were required as the embryos grew older. The survival rate declined dramatically as the degree of crowding increased.

Healthy fertilized eggs were light brown with a slightly darker brown region on top corresponding to the animal pole.

The larvae were fed beginning on the fifth day with a fine suspension of nettle powder (Herba urticae, provided through the courtesy of S.B. Penick and Co., N.Y.) prepared by squeezing a small amount of the powder through a double thickness of bandage gauze. The larvae were fed every day, and the water changed often.

A few of the tadpoles were reared through metamorphosis which began at about stage 57 (according to Nieuwkoop

and Faber, 1967) and lasted about 17 days. At stage 58, when the tails began to atrophy and the skin began to take on adult features, the tadpoles were put into shallow water. They became sluggish, stopped eating and sank to the bottom. They began to eat again towards the end of metamorphosis. At this time they were fed Tubifex worms and were gradually shifted to a diet of beef liver.

The larvae and tadpoles used in the following studies were removed from their tanks at regular developmental intervals. They were examined under a Bausch and Lomb dissecting microscope and staged according to the external characteristics outlined by Nieuwkoop and Faber (1967). They were then dropped whole into fixatives for both light and electron microscopy.

B. Light microscopy

Two hundred seventy two larvae and tadpoles, stages 22 through 54 were studied by light microscopy. They were fixed and stained as in Table 1.

All the procedures carried out from fixation to infiltration were done while the specimens remained in cork stoppered fixing vials. The solutions were withdrawn and replaced by means of disposable glass pipettes thus eliminating direct manipulation of the extremely fragile specimens.

The embryos and tadpoles were fixed at room temperature in either Allen's modification PFA 3 of Bouin's original fixative (McClung and Allen, 1929), in Lavdowsky's solution (as given in Guyer, 1953), in Bodian's number 1,2,4,

5 and 9 (Bodian, 1937a) in phosphate buffered glutaraldehyde (Sabatini, Bensch and Barnett, 1963), or in Karnovskys fixative (Karnovsky, 1965) for a period of one to ten days. All the samples were dehydrated in a graded series of ethanol, cleared in xylol, and embedded in Paraplast. Each block was trimmed and cut in serial sections 7μ, 10μ, or 20μ thick with a rotary microtome (Spencer "820", American Optical Co.), and mounted on glass slides. Twenty-four specimens were sectioned frontally, and 250 were sectioned transversely.

Thirty-one larvae and tadpoles were stained with Harris" hematoxylin and eosin for familiarization with the overall histology of the animals. The remaining embryos and tadpoles were impregnated with silver.

Holmes' silver nitrate (Holmes, 1942), and Bodian's protargol (Bodian, 1936) were tried in combination with the above fixatives for each developmental stage studied. Both procedures involved the deposition and subsequent reduction of ionic silver, followed by a re-impregnation and reduction of ionic gold to further enhance contrast. The overall effect was a purple to black appearance of the nervous tissue (depending on methods and reagents emplobed within each technique) with a lighter backtround in the same tones. Using colloidal suspensions of silver protargol as the impregnating media, generally gave more consistently good results than using the silver nitrate solutions. However, all the methods used depended to a marked extent on the

fixative used and even more so on the inherent properties of the tissue itself.

Bodian's original method required an extended impregnation period, ranging from one to three days, in a 1% colloidal suspension of Protargol-S (Winthrop Stearns), at 37 C, followed by reductions with hydroquinone and subsequent gold toning. The basic method was successful only with older specimens, eg, stage 46, and was progressively worse as the age of the larvae declined. The following two methods were modifications of Bodian's method, and each worked better for some stages.

The first method (Davenport, 1960) incorporated a pretreatment period of one hour with 10% aqueous silver nitrate at 60 C. This enhanced the affinity of the sections for the silver proteinate to such an extent that the impregnation could be carried out in a reduced concentration of 0.2% Protargol for a period of one hour at room temperature. The results obtained were similar to good examples of the original method with a slight improvement in staining intensity, particularly of individual fibrils. The major advantage was that the staining procedure could be standardized. The youngest larvae still stained very poorly.

The second modification (Davenport, McArthur and Bruesch, 1939), substituted amidol which is a more powerful reducer, for hydroquinone, to reduce both the silver and the gold. The combination of this staining procedure with a short fixation time (one hour) in Lavdowsky's mixture considerably improved

the differential staining of the nerve cells in all but the very early larvae, stages 22-27, where the over abundance of yolk further complicated matters.

The sections were examined with a Bausch and Lomb or Zeiss Standard Universal microscope and photomicrographs were taken with a Nikkormat FTN or Nikon Microflex camera on Panatomic X film (Eastman Kodak).

C. Electron microscopy

Tadpoles were selected and staged according to Nieuwkoop and Faber (1967) and fixed by immersion in the following fixatives maintained at pH 7.2:

a) 1% osmium tetroxide in 0.2 M phosphate buffer at 25 C (Millonig, 1961)
b) 3% glutaraldehyde, post fixed in 1% osmium tetroxide, buffered with 0.05-0.5 M phosphate at 4 C and at 25 C (Sabatini, Bensch and Barnett, 1963)
c) 4% paraformaldehyde, 5% glutaraldehyde, post fixed in 1% osmium tetroxide, buffered with 0.2 M phosphate at 25 C (Karnovsky, 1965)
d) 4% paraformaldehyde, 5% glutaraldehyde, 3% acrolein, post fixed in 1% osmium tetroxide, buffered with 0.1M or 0.2 M phospate or 0.1 M or 0.2 M S-collidine at 4 C or 25 C (Hayat, 1970)
e) simultaneous fixation in 3% glutaraldehyde and 1% osmium tetroxide buffered with 0.1 M or 0.2 M phosphate or 0.1 M or 0.2 M S-collidine at 4 C or 25 C.

Contrast was considerably enhanced by staining the

tissue en bloc for five minutes with a 2% solution of uranyl acetate in either 70% ethanol or 70% acetone during dehydration (Hayat and Giaquinta, 1970; Locke, Krishan, McMahan, 1971). The tissues were dehydrated in a graded series of ethanol and put through propylene oxide before embedding, or dehydrated in acetone and embedded directly in Epon 812 (Luft, 1961). The final mixtures of Epon A and B were in ratios of 3:2 or 1:1. For embedding, the specimens were placed in Beam capsule tops (Beam) or on Epon platforms in 00 gelatin capsules and polymerized at 60 C.

Come larvae and tadpoles were fixed and embedded by a rapid method requiring only four hours from fixation to cutting (Hayat and Giaquinta, 1970) with good results as long as the tissues were trimmed to pieces not exceeding 1 mm^3. Rapid polymerization was obtained at 100 C.

Blocks were rough trimmed transverse to the long axis of the embryo, beginning at the anterior end, up to the level of the ear vesicle. From there thick sections of 1-2u were cut on a Sorvall Porter Blum MT-1, or MT-2 microtome and stained at 60 C with a 2:1 mixture of 1% pyronine GS and 1% toluidine blue dissolved in 1% borax (Modified from Ito and Winchester, 1963). The block was trimmed for thin sectioning as soon as a recognizable portion of the M cell appeared in the thick sections.

Thin sections were cut using glass knives or a diamond knife (Dupont) and sections showing silver or light gold interference colors corresponding to approximate thicknesses

of 600 -900 A and 900-1500 A respectively were picked up on uncoated copper grids of 100, 150, or 200 mesh or on grids with a center hole of 0.8 mm in diameter coated with formvar. The formvar film was prepared from a 0.5% or 0.25% solution of Formvar 15/95 E (Shawinigan Resins Corp.) dissolved in either chloroform (Bradley, 1965) or in 1,2, dichloroethane.

Sections stained en bloc in most cases needed no further treatment. The others were first stained with 2% aqueous or alcholholic uranyl acetate (Watson, 1958; Locke and Krishan, 1971), and secondarily stained with lead citrate (Reynolds, 1963; Venables and Coggeshall, 1965). Those stained with hot (60 C) alcoholic (95%) uranyl acetate (2%) did not require double staining with lead. Vanadium molybdate and vanadium sulfate were also tried (Callahan and Horner, 1964), but with poor results.

The sections were examined with an RCA EMU-3H, Siemens Elmiskop IA or Phillips 200 electron microscope. Micrographs were taken at original magnifications of 2,000 x to 40,000 x on Kodak medium or contrast projector slide plates, and developed in D-19 or Dektol (Eastman Kodak). Prints were made with an Omega (Simmons) enlarger on Kodabromide papers ranging from F 1 to F 5 and developed in Dektol.

Table I. Compilation of embryos by stage, and methods of fixation and staining

Stage		22	23	24	25	26	27	28	29/30	31	32	33/34	35/36	37/38	39	40	41	42	43	44	45	46	47	48	49	50	54
*	**																										
B	HE	1		1				2	2	1	1	2	2	3	2	2	2	2	2		1	4					
B	H			2		1		2	8	1		1		1							1			1		1	
L	H			1	1		1	1	1	1	2	2	2	1	1	1	1	1		1	1	1					
L	Ph																				1	1	2	1			
L	Pa	6	3	5	2	2	4	13	12	14	10	6	5	3	1												
1	Ph									1	1	1		1	1			1			1	2	2				
2	Ph							1	2		1	2		2	1	1	1	1				2	2				
4	Ph							4	2	3	6	1	2	1	1	1	1		1	1	1	2	2		4		
4	Pa							3	9	4	1								3					3		2	1
5	Ph									1		1		1		1	1	1	1	1	1	2	2	2			
9	Ph								1	1		1			1			1	1		1	2	2				
G	Ph								2	2										2							
K	Ph																			2							

* Fixatives
B, Bouins's
L, Lavdowsky's
1,2,4,5,9 Bodian's # 1,2,4,5,9
G, Glutaraldehyse
K, Karnovsky's

** Stains
HE, Hematoxylin and eosin
H, Holmes'
Ph, Bodian's protargol with hydroquinone
Pa, Bodian's protargol with amidol

III. RESULTS

A. Light microscopy

Mauthner's cells in Xenopus laevis were located in the medulla oblongata at the level of entry of the VIIIth nerve. In the mature state (Stage 54, the cell (Fig.1), could be seen to contain a large dendrite directed dorsolaterally, apparently terminating in the vicinity of the VIIIth nerve and lateral line fibers. The two ventral dendrites, also quite large, projected into the tegmentum where they probably entered into synaptic contacts with fibers of the brain's motor centers. The large axons emerged medially, ran along the border of the intermediate and marginal zones (terminology according to the Boulder Committee, 1970)[1], decussated in the midline, and traveled down the spinal cord giving off collaterals to motor neurons of the trunk and tail regions of the spinal cord.

1. Stage 22

The rhombencephalic portion of the neural tube at the level of the ear placodes was composed mainly of pseudo-

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[1]Ventricular zone - proliferating cells which ultimately give rise to the neurons and macroglia of the central nervous system. Intermediate zone - in the early stages contained immature neurons, later invaded by axons and macroglia. Marginal zone - the outermost processes of the ventricular cells but gradually replaced by ingrowing axons and dendrites from other zones.

stratified columnar cells oriented perpendicular to the neurocoel (Fig. 2). In favorable sections these ventricular cells could be traced the entire width of the neural tube. The elongated nuclei, measuring approximately 15μ in length, stood out from the less well impregnated cytoplasm. Large droplets of yolk and lipid (up to 12μ in diameter) were the most prominent components of the neural tube cells, effectively obscuring any cytoplasmic detail that might be present at this stage (Fig. 3).

One or two cells with rounded nuclei were found in each section, located at the periphery of the neural tube. The long axis of these cells ran perpendicular to the ventricular cells in the plane of section. None of the cells on either side of the 10 embryos examined (with various silver techniques in sections of 7μ, 10μ or 20μ) were conspicuous enough with respect to size, shape, number or position of processes, or location of the cell body to be positively identified as a Mauthner's cell.

2. Stage 23 to stage 26

From stage 23 to stage 26 the rhombencephalon became broader and its roof simultaneously became thinner so that this portion of the neural tube resembled an inverted triangle. The thickened ear placodes, which delimited the area within which the future M cell would be located, began to invaginate at stage 24; a process that was completed by stage 27. A slow but progressive increase in the number of cells located at the periphery of the neural tube and

oriented parallel to the neurocoel were noted. These peripheral cells containing rounded nuclei had presumably begun to differentiate, although no axonal or dendritic processes could be seen. No single cell of the group was morphologically distinguishable from the others (Fig. 4).

3. Stage 27

The rhombencephalon at stage 27 did not undergo any major modifications compared to the previous stages. Three to five differentiating cells were found on each side of the neural tube in each section. In some embryos a cell was noticed on the extreme dorso-lateral edge of the neural tube and adjacent to the otic vesicle. It resembled an early M cell with respect to its location and general appearance, although it did not have any specific distinguishing characteristics (Fig. 5), and only appeared larger than neighboring cells in some cases. The cell was spindle shaped with a lightly stained dorsal area and a dense ventral region that appeared to contain some fibrillar material. The dorsal part seemed to be made up of some sort of poorly stained globular material, possibly lipid, or remnants of lipid extracted during alcohol fixation. The cell's nucleus was approximately 9u in diameter which was only slightly larger than the nuclear diameters of the neighboring cells.

After stage 27 the M cell was more easily recognizable in all specimens examined.

4. Stage 28

At stage 28, the closed otic vesicle became detached from the overlying ectoderm. A few early acoustic ganglion cells were observed extending from the medial side of the otic vesicle to the dorso-lateral border of the neural tube (Fig. 6). The amount of yolk found in the neural tube cells had diminished to such an extent that it no longer formed a major barrier to visualization of the individual cells. Very fine axons could be seen here for the first time although they were probably present but not detectible earlier. The first axons of the rhombencephalon were seen emerging from cells with round nuclei whose long axes were oriented either perpendicular to or parallel to the neurocoel in the plane of section. The M cell was found at the level of the acoustic ganglion cells at the lateral border of the neural tube.

From stage 28 to stage 35/36 the nucleolus of the M cell enlarged at a much faster rate than its nucleus. As a result the nuclear nucleolar diameter ratio decreased from 7.5 to 3.5 during this period, and remained relatively constant after stage 35/36. The presence of this prominent nucleolus was one of the most reliable indications of an M cell.

The dorsal part of the cell contained an abundance of yolk and other amorphous poorly stained material. There was no clearly defined dorsal dendrite. The M cell seemed to have only one process, which extended ventrally and was probably the axon (Fig. 7).

5. Stage 29/30

A few fibers were seen extending from the acoustic ganglion cells into the neural tube. The cytoplasm of the M cell, which had become much more fibrillar than at the preceding stages, contained a number of distinct fibrils lateral to the nucleus. The fibrils extended dorsally and terminated in close proximity to the entering fibers of the VIIIth nerve (Fig. 8). There was no clearly defined dorsal dendrite at this stage. The ventral portion of the M cell contained fibrils that converged and then extended ventro-medially and ventro-laterally (Figs. 8, 9). After careful comparisons of serial sections of M cells from stage 29/30 and older stages, the medial fibrillar extension was identified as the axon, while the lateral group of fibrils were most probably early dendritic.

6. Stage 31

At stage 31, the M cell and its surroundings were essentially in the same condition as at stage 29/30. The M cell itself was somewhat larger than the other cells in its vicinity and its axon could be followed for a longer distance from its origin (Fig. 10).

Four M cells out of 184 examined from stages 28 to 31 contained two nucleoli instead of one (Fig. 11). Since no other cell even vaguely resembling the M cell could be found in any of the serial sections ipsilateral to the double nucleolate one, it was assumed that this cell was a true M cell. The two nucleoli were usually morphologically equivilent. The contralateral M cell consistently displayed only one

nucleolus (Fig. 12).

In one case, a normal appearing stage 31 tadpole, the double-nucleolate M cell and also the contralateral M cell were morphologically abnormal (Compare Figs. 11, 12 to Fig 10). Both had very elongated nuclei and large amounts of globular material in their cytoplasm. The nucleolus of the right cell (Fig. 12) was much smaller and seemed to be almost fragmented as compared to a normal M cell (Fig. 10). It was interesting to note that of all the M cells examined subsequent to stage 31 (approximately 300) with particular attention to the number of nucleoli in each nucleus, no M cell was ever found with more than one nucleolus.

7. Stage 32

The M cell was spindle shaped. Dorsally the cell body tapered to a large now clearly distinguishable dendrite that appeared to bifurcate a short distance from its source. Two ventral dendrites were also visible for the first time at this stage, both emerging lateral to the axon.

The nucleus was oval, and its long axis was 1.45 x as great as that of the next largest nuclei found at this level of the neural tube. Its round nucleolus was dense and measured 4.5μ in diameter as compared to adjacent nucleoli whose diameters were approximately 1.6μ. The M cell cytoplasm appeared densely fibrillar except for a narrow band of lesser density around the ventral part of the nucleus and a larger light area lateral and dorso-lateral to the nucleus. The perikaryal fibrils continued dorsally into the dendrite

and diverged into two bundles outlining the course of the dentritic branches (Fig. 14). The ventro-medial dendrite was quite large while the ventro-lateral dendrite was small and relatively inconspicuous in relation to the other processes. The two dendrites were identified by comparing their relative sizes and positions in larva and tadpoles of subsequent stages.

A few fine fibers crossed the M cell at the base of the ventral processes. Some appeared to be extensions of the cells in the intermediate layer, while others seemed to be coming from outside the medulla. The relationships of these fibers to the M cell were not apparent.

8. Stage 33/34

The neural tube at stage 33/34 was divided into three distinct developmental zones. The ventricular zone was restricted to a narrow band lining the neurocoel while the intermediate and marginal zones became progressively wider with the acquisition of immature neurons and fibers. The M cell was situated in such a way as to form a boundary between the intermediate and marginal zones (Fig. 15) and appeared to be the target for a number of converging fiber groups (Fig. 16).

The first set of fibers, terminating in the vicinity of the dorsal dendrite of the M cell, seemed to originate from the cells of the VIIIth ganglion (Fig. 16, F1). The second group of fibers, located in the vestibular region, were directed along the lateral side of the cell from the

dorsal dendrite to the base of the ventral dendrite (Fig. 16, R 2). A third group of fibers projecting longitudinally in the neural tube seen in cross section were closely applied to the base of the axon (Fig. 16, circle). Lastly, a few fibers encircled the ventral part of the cell body at the point of exit of the two ventral processes (Fig. 16, F 3). Although most of these fibers have not been identified specifically as to their origin or function, some authors have suggested that they were part of the acoustico-vestibular system and tegmental apparatus (Bartelmez, 1915, Larsell, 1934, Stefanelli, 1951, Sims, 1962).

The size and distribution of the major constituents of the cell body did not change significantly from stage 32.

9. Stage 35/36 to stage 54

The M cell by stage 35/36 contained all of the major cell constituents detectible by Bodian's Protargol method. From this point to stage 54, the developmental changes occurring within the cell body and its extensions were primarily quantitative, manifested by a increase in size, and by ramification of the cell's processes.

The nucleus of the Mauthner's cell grew quite large, increasing in diameter from approximately 13.6 μ at stage 35/36 to about 18μ-20μ at stage 46. The nucleus was lightly stained and contained some typical vesiculations. The ratio of nuclear-mucleolar diameter remained relatively constant at 3.5. The nucleolus appeared either densely homogeneous (Figs. 25, 31), lobulated (Fig. 17), ringlike (Fig. 19),

speckled (Fig. 32), or any combination of the above. By slowly varying the focus on transverse and longitudinally cut 10u and 20u silver stained sections, the presence of the ringlike form, with a hollow central area was always seen in addition to the other forms. The structure that would best fit in with these observations would be that of a roughly speherical organelle with a heterogeneous distribution of materials and containing one major and/or several minor lightly stained central areas. The large less dense central region was found to be peculiar to the M cell, at least on the light microscopic level. It was not found in any other cells of the rhombencephalon nor even in any of the giant primary motor neurons of the spinal cord whose nucleoli were also rather conspicuous (3.3μ diameter in a nucleus measuring 23μ in diameter (Fig. 33) as compared to the M cell nucleolus which measured 5.8μ with a nucleus of 20μ, at the same stage (Fig. 1).

The perikaryal cytoplasm became progressively more fibrillar from stage 37/38 to stage 54, with a definite increase in the density and thickness of each fibril. The fibrils were arranged parallel to the long axis of the cell and were dispersed around the dorsal, ventral and lateral parts of the nucleus. The remaining medial region was conspicuously devoid of fibrils except for a very narrow band seemingly contiguous with the nuclear envelope.

The two major branches of the dorsal dendrite grew and ramified during these stages, thereby increasing their

potential for interaction with other neuronal, or possibly even extra-neuronal, components of the medulla (Figs. 14, 16, 22, 31, 32). The number of fibers which terminated in the vicinity of the M cell processes increased with the age of the embryos and the growth of the M cell.

The ventral dendrites (Figs. 13, 16, 17, 18, 21, 23, 27, 30) of the M cell underwent dramatic elongations. It was very difficult to capture the two dendrites and the axon in one focal plane as in Figs. 21 and 23, therefore, most of the micrographs show either one or the other dendrite in relation to the axon. The axon by stage 39 achieved a width comparable to that of the ventral-medial dendrite (in silver impregnated preparations) so that in some sections which transect both processes close to the cell body, they were distinguishible solely on the basis of relative positions (Fig. 18).

The M axon was seen to emerge near to but not from the same point as the medial dendrite, or as a direct extension of the latter. The axon was quite prominent at most levels of the cord and could be traced with increasing ease as the tadpole matured. By stage 54 it could be followed down the medial longitudinal fasciculus to the level of the lumbar spinal ganglion 9 (hind limb bud) where it became indistinguishible from the giant fibers of the huge primary motor neurons, consequently the M axon collaterals could not be specifically identified in transverse or longitudinal sections.

Of the two ventral dendrites, the medial one was the longer and showed some branching among the fibers of the tegmental region. The shorter ventro-lateral dendrite could be seen as early as stage 32 but was much more obvious at stage 39, leaving the cell body at an acute angle to the medial dendrite. Figures 21 and 23 show the dendritic and axonal relationships in an embryo of stage 46.

One embryo of stage 48, seemingly indistinguishable from other embryos at that stage with respect to gross morphology and behavior, cntained two Mauthner's cells on one side. One was approximately 20μ posterior to the other (Figs. 25, 27). Each of the cells bore the characteristic features of the M cell and each was approximately the same size as the one M cell found on the opposite side (Fig. 26), and not one-half the size as previously reported by Stefanelli, (1951). Each of the two duplicated cells gave off an axon that traversed the medulla, decussated normally, and coursed down the opposite median longitudinal fasciculus side by side. The contralateral axon also followed its normal course (Fig. 28).

A large clear area in the medial and dorso-medial portions of the cell apparently devoid of any cytoplasmic components was first noted at stage 33/34 and showed up with increasing regularity in all the older stages. In order to define the nature of this space, a series of stages 44-50 (stages at which the space is always present) were examined using a variety of fixatives and staining procedures.

The space was present to some extent in all specimens stained with Bodian's Protargol after fixation in Bodian's fixatives #1 (Fig. 30), #4 (Fig. 32), and #5 (Fig. 20), #9 (Fig. 24), or Lavdowsky's (Fig. 17), which was used primarily for early stages. There was virtually no difference with respect to the clear area in material stained with protargol using hydroquinone or amidol as the reducer, or in material stained by Holmes' silver method (Fig. 34).

B. Electron microscopy

1. Stage 27

The neural tubes of three embryos were examined at the level of the otic vesicle. The cells within the neural tube were primarily ventricular and oriented with their long axes perpendicular to the neurocoel. As observed by light microscopy, a few of the cells towards the periphery of the neural tube appeared to be in early stages of differentiation, and occasionally one cell could be singled out as a probable M cell (Fig. 5). With electron microscopy, however (Fig. 35), it was not possible to distinguish between a ventricular cell, an early differentiating neuron, or an M cell. All the cells at this level of the neural tube, at this embryonic stage were similar with respect to their content and distribution of organelles.

Nuclei in the outer cell layer were less elongated than those of the ventricular layer, but not as clearly rounded in the electron micrographs as in the silver stained paraffin sections (Figs. 4, 6).

The nuclei, bounded by two unit membranes, were composed of a fairly evenly dispersed nucleoplasm. Some of the nuclei contained one (Cell 2, Fig. 35) or more (Cell 1, a,b,c, Fig. 35) condensations similar to those described as chromocenters (Hyden, 1943) or as pre-nucleolar bodies (Hay, 1968). When a few such elements were present in one nucleus, they were scattered throughout the nucleoplasm, and one was always much larger than the others.

Yolk, lipid, mitochondria and ribosomes were dispersed throughout the cytoplasm. In addition there were other particles similar to ribosomes as well as to the beta glycogen particles described by Perry (1967). Neurotubules, filaments, and granular endoplasmic reticulum were also present to a lesser extent. A few small Golgi complexes were located in the lateral perinuclear region.

The extracellular space appeared to be larger in these young embryos of stage 27 (Fig. 35 *), than in mature tadpoles of stage 47.

2. Stage 28

At stage 28 a group of cells at the border of the neural tube could be distinguished from the ventricular cells on the basis of fine structure. They contained a somewhat rounder nucleus with a clearly defined nucleolus, and proportionately more membranous components in the cytoplasm. Among this latter group, at least one of the pair of M cells could usually be recognized because of its slightly larger size and more prominent nucleolus. In all other respects, the M cell was indistinguishable from the other early differentiating neurons. It was very difficult to obtain a thin section parallel to the long axis of the M cell, because the M cell was slightly oblique to the cross section of the neural tube. Therefore, the information concerning its overall morphology was pieced together from a series of sections.

The spindle shaped M cell was relatively large with a slightly eccentric nucleus. Ventrally the perikaryon tapered to a large process which unfortunately could not be followed for any significant distance in the thin sections. The perinuclear cytoplasm, the ventral part of the cell and the base of the process contained ribosomes, glycogen, mitochondria, and some yolk granules, microtubules, filaments, a few scattered profiles of endoplasmic reticulum and some immature Golgi complexes. The dorsal part of the cell however, contained predominantly ribosomes, glycogen, yolk and lipid with an occasional mitochondrion, microtubule and filament.

The nucleus was enclosed by a nuclear envelope of two unit membranes with numerous pores (Fig. 36, Po). The envelope contained a number of deep infoldings (Fig. 36, X), usually on its ventro-medial side. Dense particles were seen accumulated on the nuclear side of the envelope (Fig. 36, D), while others, probably ribosomes, adhered to the cytoplasmic side. Glycogen like particles were also concentrated in these areas. The presence of particles was most marked in the vicinity of the indentations and in the region of the nuclear pore (Fig. 36, X). Some dense unidentified material was seen in the pore region itself (Fig. 37, Po).

The nucleolus was roughly spherical and occupied a slightly eccentric position within the nucleus.

Mitochondria were found in large numbers throughout the cytoplasm, particularly near the nucleus (Fig. 37) and

in areas which seemed to be narrowing into a process (Fig. 39). The mitochondrial profiles were round, elongated or "Y" shaped (Fig. 37). The cristae were usually oriented perpendicular to the long axis of the mitochondrion. The matrix appeared denser than the cytoplasm and in some cases one or more dense particles of varying diameter were found in the matrix (Figs. 36, 37, DP). A few mitochondria were found associated with a variety of membranous or vesicular components of the cytoplasm (Fig. 36, My). The latter may have resulted from poor fixation, or, as some workers have suggested (Wallace and Linnane, 1964; Pannese, 1965), they could be involved in certain stages of mitochondrial formation. Some mitochondria contained (Fig. 39, My) or were contained within these profiles (Fig. 36, Mx) while others seemed to be in direct continuity with them (Fig. 36, My).

Small particles varying in size from approximately 150 A to 350 A occupied a large part of the cell's cytoplasm. Usually, though not always, the larger particles were more densely stained with lead than the smaller ones and at high magnifications appeared stippled.(Fig. 36, Inset, Gl). These two features have been ascribed to beta glycogen particles (Revel, Napolitano, Fawcett, 1960; Perry, 1967). However, the distinction between ribosomes and glycogen was not that clear cut because there was a large degree of overlap in the size and density of the two particulate species, and beause dense particles seemed to adhere to the membranes of the endoplasmic reticulum and to subsurface

cisternae. Although ribosomes have been repeatedly described as the granular component of the rough endoplasmic reticulum (Porter, 1953; Palade, 1955; Palay and Palade, 1955), beta glycogen has been reported in close association with these membrane profiles (Perry, 1967). Therefore a precise definition of each particle would not be accurate on a purely morphological basis without histochemical verification.

Single profiles of granular endoplasmic reticulum (GER) were scattered throughout the cytoplasm. Most of the membranes were not associated with any particular part of the cell. A few, however, were found beneath the plasma membrane (Figs. 37, 40, 41, SSC). These subsurface cisternae (SSC, Rosenbluth, 1962) were opposite points where other cells or, more commonly, cell processes came into contact with the M cell. There was no way of tracing the origin of these neurites. Only a few of the processes that approximated the plasma membrane had SSCs associted with them at this stage. An SSC was never seen under an identifiable synaptic region. The SSC consisted of a flattened sac of GER completely devoid of ribosomes on the side apposed to the plasma membrane. Some of these sacs were very short and adjacent to the membrane along their entire length (Fig. 37, SSC, 1). Others were longer and only partially apposed the surface, while the rest dipped down into the cytoplasm below (Fig. 37, SSC,2). All parts of the SSC not in direct apposition to the membrane were covered with ribosomes.

One or two immature Golgi complexes, containing three

or four flattened sacs and many tubules and vesicles of varying sizes, were found medial to the nucleus (Fig. 36, 37) oriented toward the emerging ventral process in the long axis of the cell. The vesicles were predominantly smooth, although a number of coated (Figs. 36, 37) and a few dense cored vesicles (Fig. 37) were also present. Both were near to, or in some cases continuous with, the membranes of the Golgi complex (Figs. 36, 37). Multivesicular bodies containing both vesicles and short tubular membrane segments were also often found in this region as well as in the ventral parts of the cytoplasm (Fig. 36).

Microtubules (250 A) and neurofilaments (100 A) were present in these early cells. An occasional microtubule or neurofilament could be seen anywhere in the cytoplasm (Fig. 38), however, large numbers of them were oriented parallel to the long axis of the cell converging towards the ventral process (Figs. 39, 40). The parts of the cytoplasm where the microtubules were found in greatest quantity were conspicuously devoid of heavy concentrations of ribosomes or glycogen.

A peculiar finding in these embryonic neurons was the presence of three kinds of projections extending from the surface of the cell body and from the large ventral process. The projections had the appearance of three morphological entities, but they may be merely three forms of the same structure viewed at different levels or at different periods during their differentiation and growth.

The first form (Fig. 41), also seen extending from the

dorsal part of the cell at this stage was well as from the cell body and ventral process, was thick and filled with ribosomes, glycogen, and to a lesser extent with some very fine filamentous material without any specific orientation. The fibrils were finer than the typical neurofilaments. Its distal portion contained only a number of vesicles and smooth membranous profiles (Fig. 41, P1). Sometimes this latter configuration was encountered as a bulge directly on the cell surface similar to the structures described in developing rat cerebellum by Del Cerro and Snider (1968). The second form (Figs. 36 P, 39, P2; 41, P2) was comparatively thinner than the first (similar to that described in developing mammalian nervous system (Tennyson, 1965) and extended for relatively long distances among the small axonal and denritic processes running longitudinally through the medulla. These thin projections contained a few ribosomes or glycogen particles and a large amount of fine randomly oriented filamentous material. Some visicles and mirotubules and tubular membrane profiles were also included (Fig. 41, P2). The third form (Fig. 40, P3) was actually a combination of the first two types. A wide bulge of the cell surface, filled with ribosomes and/or glycogen from which one or more projections identical to those described as form two, branched off.

The earliest identifiable synapses on the cell body and base of the axon were found on the projections (Figs. 40, S, 41, Sx). These were defined by an aggregation of synaptic vesicles in the presynaptic terminal and at least some

increase in density of the post synaptic membrane. The thickening was usually accompanied by some delicate fibrillar material traversing the cleft (Fig. 41, inset). Synapses were found on both parts of the type three projections (Fig. 41, S1 and S2).

The ventral narrower portion of the perikaryon (Fig. 39) contained the same organelles found in the major portion of the cell body but oriented more longitudinally. It also had a larger microtubular and neurofilament population (Fig. 39). From electron micrographs, there was no way to define the nature of the process, since it could not be traced far enough. On the basis of the light microscopic information at this stage, it seemed likely to be axonic.

Large lipid inclusions, similar to those described in mammalian nervous system on the basis of their ractions with osmium tetroxide and oil-red-O (Nakamura and Tigges, 1970) were identified in the M cell. Inclusions, morphologically identical to those lipid droplets were also found in all cells of Xenopus' neural tube at this stage. They were often closely associated with yolk granules, ribosomes, the nuclear envelope and mitochondria. The droplets did not have any regular subunit structure that could be detected at original magnifications up to 40,000x. They had no outer limiting membrane. Occasionally a row of fine granular material could be seen as its border.

Yolk granules, investigated and described by other workers utilizing electron microscopy, biochemical analysis, and X-ray defraction patterns (Sung, 1962; Ward, 1962; Karasaki,

1963; Jurand and Selman, 1964; Honjin, Nakamura, Shimasaki, 1965; and Lanzavecchi, 1965) appeared in a variety of configurations within the same M cell. The granules in general were round or oval, with or without accessory structures. In its simplest form a yolk granule consisted of a membrane bound dense body (Fig. 36, Y2), which at higher magnifications was seen to be made up of a regular array of subunits arranged in a crystalline lattice structure (Inst Fig. 36, Y). Fine granular material was usually discernable at higher magnifications between the main body and the outer membrane. A more complex yolk granule was formed by one or more irregularly granular segments of medium density separating the main body into two or more distinct components (Figs. 36, Y1; 39, Y1). Another structural variation was a yolk granule with a reduced central body surrounded by either a large and dense granular layer with one or more membranes (Fig. 38, Y2) or a small granular area surrounded by a number of concentric membranes (Fig. 39, Y1).

3. Stage 29/30

The ultrastructure of the M cell at stage 29/30 remained essentially the same as it was at stage 28 except for an increase in the number of Golgi bodies, mostly in the perinuclear region (Fig. 42, G).

One of the M cells observed electron microscopically contained two nucleoli of similar appearance. The rest of the organelles were comparable to the contralateral M cell and to

the other neurons of the medulla at this stage.

4. Stage 31

At stage 31 the M cell was distinctly larger than adjacent cells. It had a broadly tapering dorsal segmant and a ventrally emerging axon that could easily be traced in the silver stained paraffin sections (Fig. 10).

The dorsal cytoplasm was relatively undifferentiated, containing primarily glycogen, ribosomes, yolk, lipid, an occasional mitochondrion and a few scattered single profiles of granular endoplasmic reticulum. Small processes similar to the type 2 projections described for stage 28 protruded at roughly right angles from the dorsal perikaryon (Fig. 43). These projections contained ribosomes, glycogen, fine filaments and sometimes a mitochondrion (Inset Fig. 43).

During stages 31 through 35/36, the nuclear membrane invaginated very deeply at one or two points into the karyoplasm trapping some cytoplasmic components within its folds. This phenomenon was revealed in cress sections as circular cytoplasmic inclusions bounded by two unit membranes which were interrupted by pores (Fig. 43, C.I.). At all stages the nuclear membrane was irregular with many undulations, but not to the extreme depth found in these early stages.

A thin layer of granular endoplsmic reticulum was arranged circumferentially around the nucleus and extended from there for a short distance dorsally and ventrally. A string of Golgi bodies with their associated vesicles formed an arc

around the lateral side of the nucleus (Fig. 43, 44). Synapses were seen on the cell body itself as well as on the small projections (Figs. 44, S, 45, S).

Ventrally, the M cell narrowed into the presumed axon which appeared less dense than the perikaryon (Fig. 45, Ax, 46, Ax). Its major constituents were microtubules and filaments, glycogen, ribosomes, smooth endoplasmic reticulum, vesicles, and multivesicular bodies interspersed among the tubules. Stacks of granular endoplasmic reticulum were absent from the process but subsurface cisternae would often be seen for a few microns from the origin (Fig. 46, SSC). The plasma membrane in this region was not any more or less dense than in any other part of the cell and did not have any underlying granular material associated with it. The probable axon was traced through several serial sections but was finally lost among some other similar neural tube components. In a few ponts along the process, a bulge was seen directly under the membrane containing an aggregate of glycogen, delicate filaments, tubules and a few ribosomes. Sometimes a synapse was located on it (Fig. 46, S1). Synapses were seen on the main part of an axon as well (Fig. 46, S2).

Light microscopically, at this stage an axon was definitely seen emerging from the M cell, but only a suggestion of a ventral dendrite was implied by an extension of fibrillar material into the ventral lateral part of the cell body. Electron microscopically, none of the processes seen in M cells from stage 28 to stage 39 exhibited any features

specific for axons or dendrites. The nature of a particular process was determined by careful comparison of sections both with the light and electron microscopes, of early differentiating and mature M cells, with systematic analysis of the composition and orientation of each process at each developmental stage. The process seen at stage 31 (Fig. 46, Ax), was therefore recognized as the axon.

Lateral to the process described above, the perikaryon curved around a group of small neurites and extended ventrally, roughly parallel to the tentatively identified axon (Fig. 45, D). This large ventro-lateral cytoplasmic expansion (Fig. 45 D), was filled primarily with glycogen, and ribosomes, and ocntained some short finely filamentous projections (Fig. 45, P). Some had one or more synapses on either the main bulge or on the small extension. In tadpoles of later stages, a dendrite occupied the same relative position.

5. Stage 32

At stage 32, a distinct dorsal dendrite and a short ventral dendrite were discerbable with the light microscope along with the ventral medial axon. A larger part of the cytoplasm of the cell body took up the silver stain at this time than had done so at earlier stages.

The nucleolus of the M cell at this stage contained a number of small areas of medium density enclosed within or located on the edge of the denser regions (Fig. 47, N1).

Electron microscopically there was an increase in the amount and complexity of the granular endoplasmic reticulum and the first appearance of small randomly distributed patches of neurofilaments in the cell body (Fig. 47). The primitive or glycogen-rich cytoplasm was restricted to the most dorsal region of the cell body. The single short profiles of granular endoplasmic reticulum were replaced by small groups of branched and longitudinally aligned GER cisternae (Fig. 47).

Subsurface cisternae were also more conspicuous due to an increase intheir frequency and length. Some extended as far as 5-6μ and apposed four consecutive processes before dipping down into the cytoplasm (Fig. 48, SSC). Less commonly subsurface cisternae were found under the cell membrane at sites where the M cell body was apposed to the perikaryon of another cell. In this case they could sometimes be seen on the intracellular sides of both adjacent plasma membranes. (Fig. 49)

The ventral cytoplasm extended into the ventro-medial dendrite and the axon. Several Golgi complexes were aligned at the base of the dendrite, while numerous microtubules, mitochondria, agranular vesicles and smooth tubular membrane profiles extended down into it.

6. Stage 33/34.

At stage 33/34 the second ventral dendrite, located lateral to the axon and original dendrite made its appearance

for the first time.

Beginning with stage 33/34, the body of the nucleolus appeared to hollow out resulting in a central region whose density was intermediate between that of the nucleolus proper and the surrounding nucleoplasm. This configuration was seen in all subsequent stages examined, up to and including stage 55 (Fig. 74).

There was a definite increase in membranous components of the cytoplasm at stage 33/34, particularly in the Golgi complexes. They appeared as isolated units oriented towards and entering the base of both the ventral dendrites. The cytoplasm of the ventral dendrites at stage 33/34 was less dense than at stage 32, and contained a larger amount of longitudinally oriented tubules and filaments. SSCs and synapses were much more common on the cell body and the processes. At several points along the dendrite, fine branches (Fig. 52, B) emerged containing long tubules as their primary constituents, with some very fine granular material scattered between them. Several synapses were seen on their surfaces.

7. Stage 35/36

By stage 35/36, the dorsal and ventral dendrites and axon were all present. The cytoplasm of the dorsal part of the cell was similar in content to that of the rest of the perikaryon, although it still had small regions of immature cytoplasm. Projections of various dimensions, already

described for earlier stages protruded and branched from several points along the dorsal borders of the perikaryon. The longer ones contained 250 A tubules and 100 A filaments while the shorter branches still contained ribosomes and fine diffusely filamentous material.

Dendrites were not sharply delineated from the perikaryon proper. They often had Golgi complexes, glycogen, ribosomes and granular endoplasmic reticulum running into their bases, while the more distal segments contained progressively more microtubules and neurofilaments (Fig. 53, VLD, VMD).

A centriole was seen in one M cell at stage 35/36 in the ventral cytoplasm approximately midway between the ventro-medial and ventro-lateral dendrites (Fig. 53, Ce). At this same stage in a different embryo, an atypical cilium was found protruding from the medial side of the M cell (Fig. 54, C).

The nucleolus of the M cell contained a central region of medium density and in some sections a second dense region in its center (Fig. 55, Nl). After this stage all the nucleoli of all the M cells examined conformed to the basic configurations described.

8. Stage 37/38 through stage 55

The most striking morphological alteration that occurred in the M cell from stage 37/38 to stage 55 was the progressive increase in the amount of filamentous (particularly neurofilaments of 100 A) material. This increase was so

extensive that the filaments and, to a lesser degree, the microtubules, occupied a considerable proportion of the perikaryal cytoplasm, and were the major constituents of the axon and dendrites.

At first the filaments and tubules were restricted to small patches in the cytoplasm (Figs. 47; 56) which were less dense in ribosomes and granular ER. The filamentous patches ran for the most part, parallel to the long axis of the cell. It was not possible to determine the actual length of individual tubules and filaments because of the thinness of the sections and the undulating course of the filaments and tubules. The microtubules found in the initial segment of the axon began to show some signs of aggregation by stage 35/36, which became more apparent at stage 41 (Fig. 58) until by stage 47 (Fig. 65, Inset) they were assembled into longitudinal bundles with some fine dense granular material dispersed between the tubules. The rest of the axon contained a fairly homogeneous distribution of longitudinally oriented filaments and microtubules. The microtubules were most often found in small groups rather than as single units. (Figs. 58; 70). In addition some agranular vesicles were seen in the axoplasm closely associated with the microtubules (Fig. 58)

The dendrites were almost entirely filamentous (Fig.71) although they contained some microtubules and perikaryal type cytoplasm.

The granular ER began to proliferate at stage 31

in the vicinity of the nucleus and remained the most concentrated in this region throughout the developmental periods studied (Figs. 56, 62, 64,74), particularly in the lateral part of the cell. From here the ER became progressively less dense as the distance from the nucleus increased. Occasionally the granular ER appeared as an extension of the outer membrane of the nuclear envelope (Figs. 56, 61) In general long profiles were arranged circumferentially around the nucleus and in parallel stacks longitudinally oriented in the rest of the cytoplasm. Several degrees of branching and looping were present (Figs. 56;57) The axons were devoid of granular ER but some extended down into the proximal portions of the dendrites (Figs. 61; 80; 71; 73). The subsurface cisternae, prominent at stages 31-39 (Figs. 45, 48, 51) began to decrease in frequency by stage 39 so that by stage 47, they were absent from many regions of the cell, particularly from under the axonal membrane. The frequency of subsurface cisternae decreased as the number of synapses increased in any given part of the cell.

Although direct communication with the granular ER system was commonly observed, subsurface cisternae were not specifically related to any other organelle, as previously described for a number of cell types (Rosenbluth, 1962). Subsurface cisternae as a rule were not seen under wll developed synapses, although other workers have seen them in such a position (Gray, 1963; Pappas and Purpura, 1961). One was seen under a terminal filled with typical synaptic

vesicles (400 A - 500 A), but no membrane modifications were distinguishable.

Golgi bodies were very conspicuous at stages 32 to 37/38, the period when the dendrites were first differentiating. After this time their relative concentration in the M cell decreased, although their absolute numbers did not appear to diminish to any significant extent. They were present throughout all the developmental stages studied and quite often were localized at the base of the dendrites or actually penetrated into them for some distance (Figs. 53, 72, 73, 75).

Ribosomes were found as free clusters in large numbers even at the later developmentas stages where they were restricted to regions between the fibrous patches. The remaining ribosomes were seen attached to the membranes of the granular endoplasmic reticulum.

Glycogen was present, distributed in areas rich in ribosomes, and was also scarce in the fibrous areas.

Mitochondria were abundant in all the stages studied. They were found in the perikaryon, dendrites and axons, exhibiting no preferential distribution. They were found with equal frequency among the ribosomes and endoplasmic reticulum and in the midst of filamentous patches. The mitochondria were usually elongated, sometimes up to 3μ-4μ in length within one section (Figs. 51, 53). Profiles of smooth endoplasmic reticulum were scattered throughout the cytoplasm of the cell body, dendrites and a few dilated profiles were

were sometimes seen along the length of the axon.

In later stages, beginning at about stage 40, a few membrane bound dnese bodies began to appear in the cytoplasm of the perikaryon and dendrites, and increased in frequency with the age of the M cell (Figs. 57, 61, 71, 72, 75, DB). The dense bodies often contained inclusions such as vesicles, concentric lamelli and granules, and werecommonly assiciated with the Golgi apparatus and multivesicular bodis.

The dorsal and ventral dendrites followed the same developmental pattern although the latter began to differentiate earler. By stage 35/36 all three dendrites had established their basic structures and from there on continued in the same pattern, emitting some more branches and replacing most of their membranous and ribosomal structures with filaments and tubules (Figs. 61, 65, 72). By stage 42 (Fig. 61) the non-fibrous components of the dendrites were restricted to discrete units containing ribosomes, glycogen, mitochondria, vesicles (including multivesicular bodies), and granular ER. These groups were surrounded by the tubules and filaments. By stage 52, the main bodies of the dendrites were primarily filamentous with few additional components.

The axon at stage 37/38 still contained a number of ribosomes, mitochondria, glycogen particles and membranous profiles. By stage 39 a sharp transition from a granular and membranous cytoplasm to one that was primarily filamentous could already be seen between the perikaryon and the axon, although some particulate material still extended down into

it. At stage 41, (Fig. 58) the filaments and tubules were more organized. By stage 44 mitochondria and a few profiles of smooth ER (primarily vesicular) were the only other organelles found with any significant frequency. Examination of the M cells at stage 47, at which time the axon was clearly myelinated, revealed a considerably elongated initial segment with almost its entire surface contributing to synaptic associations (Fig. 65).

From stage 37/38 to stage 55 progressively more synapses covered the surface of the M cell body, dendrites and unmyelinated intial portions of the axon (Fig. 65). All the pre-synaptic terminals contained aggregates of rounded synaptic type vesicles (400 - 500 A in diameter). Occasionally, a terminal was seen with a few flat or dense cored vesicles along with the rounded ones, but the latter were never the predominent vesicular type. Synapses varied as to the distribution and amount of dense material associated with the cleft and with the membranes.

The presynaptic terminals were usually bouton or en passant and each terminal entered into either one synaptic contact (Fig. 67) or into many (Figs. 65, S, 66, 68, 69), with a small cluster of vesicles at each specialized site. Often one of the synaptic vesicles could be seen as in invagination of the pre-synaptic membrane (Fig. 45, S), but it was impossible to decide whether it was fusing with the membrane or forming from it. Coated vesicles were most often found near the Golgi apparatus (Fig. 57) but occaionally

they were seen as an invagination of the plasma membrane (Figs. 53, 67, CV).

There were no club endings or recognizable electrical synapses present anywhere on the surface of the M cell during stages 28 to 55, and it was not possible to differentiate between excitatory and inhibitory synapses (if there are both ypes on Xenopus Mauthner's cells) on the basis of ultrastructure alone.

The M axon was first seen encircled by glial type processes at stage 44. By stage 47, about ten recognizable myelin layers were deposited. The nodes of Ranvier were easily recognized and had the same characteristics previously described for anurans (Robertson, 1959). Only a few nodes were examined and there was no evidence of synaptic contacts located in these regions.

Most of the embryos examined were fixed first with glutaraldehyde and then post fixed in osmium tetroxide. This method worked reasonably well for the earlier stages but was rather destructive from about stage 41 on. The mitochondria tended to swell (Figs. 62, 63), and there was a large medial portion of the cell that was completely devoid of any cytoplasmic constituents (Fig. 62). The medial side however did support a number of synaptic contacts comparable to those on other regions of the perikaryon.

Variations in buffer concentration, or pH did not appreciably improve preservation. Fixation in osmium without any

aldehyde pre-treatment was also unacceptable. In this case there was no "empty" area, and the mitochondria looked normal, but the microtubules were not fixed at all and the neurofilaments appeared in most parts of the cell as hazy fibrous aggregates (Fig. 60, F.). In addition the nucleolus was only a vague circular outline in the nucleus (Fig. 60). The small uniformly rounded mirotubular profiles were replaced by uneven, irregularly rounded and tubular profiles in the small axons and dendrites of the marginal layer (Fig. 60, V). The membranes of the synaptic regions (Fig. 60) which were identified as such by the aggregation of synaptic vesicles close to the membrane, were somewhat denser than the adjacent membranes but not nearly as dense as those fixed with glutaraldehyde. They did not contain the associated filamentous extensions and membrane thickenings. Some very vague material could be seen in the synaptic cleft.

Simultaneous fixation with glutaraldehyde and osmium yielded still another view of the M cell somewhat intermediate between that produced by the first two treatments (Figs 64, 71). The mitochondria and other constituents of the marginal layer appeared normal. The well preserved medial cytoplasm of the M cell which was "empty" with glutaraldehyde primary fixation, appeared much less compact than the rest of the cell and contained only some fine filamentous material (Figs. 69, 71, 73), and some finely granular substance. In almost all parts of the cell there was an overall increase of ground sub-

stance, perhaps precipitated protein that for some reason was not extracted with this procedure. The dendrites and axon appeared moderately dense and the mitochondria appeared better preserved with this treatment than with glutaraldehyde alone. Some did swell, particularly in the distal parts of the processes. However, many of the granular and smooth endoplasmic reticulum cisternae shoed swelling (Fig. 64). The degree of enlargement varied with the age of the embryo and with the length of fixation. It could not be completely eliminated by varying the fixation time, the buffer or the pH.

Fixation with glutaraldehyde and acrolein with osmium tetroxide post fixation was somewhat better than either agen alone, and intermediate between glutaraldehyde alone and glutaraldehyde with osmium tetroxide post fixation. Some mitochondria were still poorly preserved but the medial cytoplasm, instead of being completely empty or normal, appeared to be shrunk away from the plasma membran but still connected to it by some cytoplasmic strands (Fig. 74).

In summary, each fixative caused a certain amount of artifact within the nervous tissue at the various developmental stages studied, some more than other. However, in comparing the results of each technique, (while recognizing its limitations) a relatively logical sequence of events emerged describing the development of the M cell. At the same time it emphasized the basic changes within the cell that drastically influenced the chemical and/or osmotic reactions of the M cell and other neural tube components to various fixatives.

IV. DISCUSSION

A. Three phases in the development of Mauthner's cell

During its differentiation, Mauthner's cell appeared to go through three major periods defined by (1) the origin and growth of its axon (2) the emergence of its three dendrites and (3) an extended period of generalized growth and maturation of the perikaryon and its processes (Fig. 76).

1. Phase I

When the M cell was first identifiable (Stage 27-28), the axon was already present, hence morphological events leading up to its origin could not be investigated. The larvae at this time did not show any indications of a true "startle" reflex and responded only to direct stimulus to their surface by local twitchings and later by feeble swimming movements away from the source of irritation.

The M cell at the end of stage 1, (Fig. 76A) contained a reunded nucleus with a prominent nucleolus. The cytoplasm was filled with ribosomes and beta glycogen particles. Many mitochondria were dispersed throughout the cell body particularly in the perinuclear region. Yolk granules and lipid droplets were also abundant with a few microtubules and filaments randomly distributed in the perikaryon and longitud-

inally aligned at the base of the axons. A few small Golgi complexes were seen lateral to the nucleus.

The most interesting feature of the M cell at this time was the presence of small projections emanating from its surface (Fig. 76 A, F). They were thin (3μ) and contained fine filaments. Other projections also extending from the perikaryal surface were wider with an accumulation of large smooth agranular vesicles in their distal portion (Fig. 76, P). Some subsurface cisternae were seen apposed to the plasma membranes of the filopodia, the wider extensions and the cell body. A few recognizable synapses were also observed in these regions but never at the same site as a subsurface cistern.

As the dendrites appeared and as the cell continued to grow, the filopodia and the other extensions decreased in number until by stage 35/36 (end of Phase II, Fig. 76 B) they were no longer seen in electron micrographs.

2. Phase II (29/30 - 32)

Just before the appearance of the dendrites, an increase in the size and number of Golgi complexes was observed in the perinuclear region. When the ventro-medial dendrite was seen at the beginning of Phase II, one or two Golgi complexes were found at its origin. This correlation was again made when the dorsal and ventro-lateral dendrites emerged. As the dendrites began to grow, more Golgi complexes accumulated at these locations. In some thin sections a row of Golgi complexes were observed extending from the lateral perinuclear

region down into the proximal portion of a dendrite.

Towards the end of Phase II, the tadpoles exhibited a clearly definable "startle" reflex. A sharp tap on the side of their dish elicited rapid swimming away from the stimulus. The initiation of this response occurred only after the dendrites had appeared, and coincided with the early part of the growth phase. Electron microscopically, this period was characterized by several changes in the cell body of the M cell.

3. Phase III. (33/34-55)

At the onset of Phase III, the round and dense nucleolus seemed to "hollow' out revealing a less dense central region. GER cisternae were found continuous with the outer membrane of the nuclear envelope and as stacks of either single or branched profiles in the rest of the cytoplasm, particularly around the nucleus. Several profiles did extend into the proximal portion of the dendrites and occasionally, during the early part of phase III, one or two single GER cisternae were observed in the axon.

Small patches of filaments were first seen close to the nucleus and groups of microtubules and filaments were observed near the origin of all the processes. As development proceeded, these patches increased in size and became much more abundant, so that by late Phase III, the neurofilaments and to a lesser extent, the neurotubules were the major constituents of the dendrites and axon, and occupied a large portion of the soma. Golgi complexes, which were

found as small islands among the masses of neurofilaments and microtubules, were composed of few stacks of flattened cisternae towards the latter period of Phase III (Fig. 76, C) than during the earlier periods. In addition they contained many more vesicles and cisternae with greatly swollen ends. Quite often a Golgi apparatus had multivesicular bodies and dense bodies associated with it.

Subsurface cisternae, prominent in the early periods of Phase III, gradually declined in number with a concomittent increase in the number of synaptic contacts. The latter eventually became so numerous that they covered almost the entire surface of the cell body, dendrites and axon hillock of the M cell.

B. Summary of developmental patterns observed in Mauthner's neuron

Comparison between the developmental changes described in other vertebrate nerve cells, and the above observations on Mauthner's neuron, led to the conclusion that despite its unusual size, the M cell is a morphologically and embryologically typical neuron. Therefore, it becomes an invaluable tool as a model for the study of nerve cell differentation; where all the cytological changes occurring throughout most of the life cycle of a neuron could be accurately traced in one specific cell.

Several developmental relationships became apparent during this investigation of Mauthner's cell. Briefly, it was observed that (1) the appearance of filopodia preceeded

the differentiation of the dendrites and the expansion of the perikaryon, and gradually retracted as the dendrites emerged and enlarged, (2) Golgi complexes appeared to be intimately related to the growth and maturation of the dendrites, (3) subsurface cisternae prevelent during Phase II, began to decline in an apparently inverse relationship to the increase in the number of synaptic connections made by the M cell and the neurites of the medulla, (4) "vacuolization" of the nucleolus was accompanied by the elaboration of granular endoplasmic reticulum and by the production of microtubues and neurofilaments.

C. Specific cell structures and their role in nerve cell development

1. Somatic and dendritic projections

Filopodia, receiving synaptic contacts were found in areas from which dendrites originated, and also emerging from the growing dendrites themselves. Several workers have, on the basis of experimental evidence (eg, transections of afferent fiber connections proposed that dendrites could not develope normally unless they made the proper synaptic connections, and that early synaptic connections permitted and promoted the formation of subsequent ones (Larsell, 1931; Barron, 1943; Levi-Montalcini, 1949; Filogamo, 1950). It therefore seems possible that these filopodia seen on the M cell, and reported in other nerve cells (Larramendi, 1965; Larramendi and Victor, 1967; Morest, 1968, 1969a,b) could be involved in early intercellular connections that would permit further differentiation

of the dendrites. The filopodia, projecting out of the cell body in advance of the dendrites, could perhaps "choose" a favorable path for subsequent cellular growth and expansion. This process could be mediated by the formation of early synaptic contacts between the M cell and adjacent neurites. The synapses would then perit these neurites to influence the maturation of the dendrite and also the formation of new synaptic relationships. Extirpation of the otic vesicle before the appearance of the VIIIth nerve in Ambystoma (Piatt, 1947, 1969) revealed that the development of its M cell was influenced to some extent, though not completely, by the presence of the VIIth and VIIIth nerves. The nature of this influence was not explained, although in the light of the foregoing discussion it does not seem unlikely that direct contact between the M cell and these fibers is a necessary prerequisite to normal differentiation.

The second projection emerging from the M cell surface, contained large agranular vesicles. These projections were considered growth cones by some workers (Bodian, 1966; Del Cerro and Snider, 1968). Observations on the M cell in Xenopus, however, do not support this contention. This type of projection never extended more than 0.75u from the cell surface, but was most often encountered as a cytoplasmic bulge under the plasma membrane. Filopodia or 60 A filaments, which have been associated with growth cones in tissue cultures of dorsal root ganglia (Yamada, Spooner, Wessells, 1971) were not seen extending from this process. It there-

fore seem more likely that these projections, rather than being actively involved in elongation, serve as storage sites for various materials, such as membranes, to be utilized during the rapid growth phase of the M cell.

2. Golgi Complex

Golgi complexes were observed, in the neural tube of birds and mammals, first in the apical portion of the ventricular cells (Lyser, 1964; Tennyson, 1965). As the cells differentiated into young neurons, Golgi complexes were seen at the base of the axon, and then dispersed throughout the cytoplasm. Several workers also noted the presence of Golgi bodies at the base of newly developing dendrites (Tennyson, 1965; Lyser, 1968; Fisher and Jacobson, 1970) but did not attach any particular significance to their finding.

In Mauthner's cell at stage 28, when the axon was already present, Golgi complexes were found primarily in the perinuclear region, with one or two distributed ventrally. There was a significant increase in their number at the next stage (29/30), which preceeded the emergence of the denrites. As each dendrite appeared, Golgi complexes were consistently found at their base. In some instances they could be seen extending from the perinuclear region into the base of the dendrites. This same pattern was observed histochemically by Dvorak (1968, 1970) who postulated a role for Golgi bodies in the differentiation and growth of the dendrites. Since the Golgi apparatus has been recognized as a site of polysaccharide synthesis and transport (Bainton and Farquhar, 1966;

Hicks, 1966; Neutra and Leblond, 1966; Berlin, 1967; Jamieson and Palade, 1967; Beams and Kessel, 1968; Whur, Herscovics, and Leblond, 1969) it may produce and/or transport some components into the dendrites that are essential for their growth and maintainance.

During the developmental periods observed, the Golgi apparatus underwent considerable morphological changes. Curing Phase I, the perinuclear Golgi contained one or two flattened sacs with some scattered agranular, coated, and dense cored vesicles. Once the dendrites appeared (Phase II) the Golgi matured until it was composed of several long flattened cisternae surrounded by relatively few vesicles. Towards the end of the growth phase, the flattened sacs were usually very swollen, particularly at each end and many vesicles of several sizes occupied a major portion of the Golgi region. At this time also, large dense bodies similar to lysosomes (Novikoff, 1961) began to appear in close association with the vesicles of the Golgi. Moulton, Jurand, and Fox (1968) in the only other ultrastructural study of Xenopus Mauthner's cell, also noted the presence of a large vesicular population, towards the onset of metamorphosis. (This period corresponds to the end of Phase III). They considered it indicative of a high degree of synthesis.

3. Nucleolus and the granular endoplasmic reticulum

Just before the onset of the growth phase in Xenopus (stage 33/34), a central light region became apparent in the nucleolus. This has been recognized in other nerve cells as

a nucleolar vacuole, an intranucleolar body or nucleololus (Hopker, 1953; Dutta, Siegesmund, and Fox, 1963; Busch and Smetana, 1970; Hardin and Spicer, 1970) and was associated with a high degree of nucleolar synthesis.

Coincident with nucleolar changes, there was a gradual increase in the amount of granular endoplasmic reticulum, particularly in the region around the nucleus. Several granular endoplasmic reticulum cisternae were seen continuous with the outer membrane of the nuclear envelope while others were observed in various branched configurations also in the vicinity of the nucleus. This lends some support to Pannese's (1965) proposal that at least some of the granular endoplasmic reticulum originates as an outgrowth of the nuclear membrane. This pattern of granular endoplasmic reticulum development was also seen in mammalian and avian nerve cells (LaVelle, 1956; LaVelle and LaVelle, 1969; Wechsler, 1966)

4. Mitochondria and fixation

Beginning with stage 40, the mitochondria were poorly preserved by fixatives which were quite adequate up to that point. The mitochondria were swollen and their cristae were disrupted. A similar condition was reported by Weber (1963) in tail skeletal musle with the onset of metamorphosis.

Moulton, Jurand and Fox (1968) interpreted these mitochondrial alterations as specific preparations for the involution of the M cells after metamorphosis. However, in

this study all the cells after stage 40 in the neural tube were refractive to glutaraldehyde fixation and manifested swollen mitochondria as well as a more general disruption of their cytoplasm. This in the M cell appeared as a clear medial region. Many other neurons showed the same features. According to Moulton et. al. (1968) this would imply that all the cells were preparing for post metamorphic involution. However, only the M cell degenerates.

The reaction of the M cell to glutaraldehyde has been modified to some extent by comining glutaraldehyde and osmium tetroxide. Then this simultaneous fixation was used, all the neurons including the M cell showed a considerable imporvement in their overall ultrastructural preservation. It, therefore, seems likely that whatever is altering the M cell's physiology is also influencing the adjacent cells. Once again, this stresses the similarity of Mauthner's cell to other neurons, and emphasizes its value as a model for overall nerve cell differentiation. However, later on in its development, the M cell does involute while others remain normal. At that point the M cell becomes more sensitive to a particular substance. In this case it appears to be thyroid hormone. It has been shown that the M cell is highly sensitive to the level of thyroxin. With the decrease in thyroxin towards the end of metamorphosis, the M cell begins to degenerate, probably because there is not enough hormone available to maintain the M cell. The "hypersensitivity" of the M cell in this instance could perhaps be volume related. Its larger

size may require a higher concentration as its maintainance level than the smaller adjacent cells. However, the possibility always exists that there is somthing intrinsically different in the M cell which causes its involution. Just as some factors either extrinsic (input from VII, VIII and IX nerves or feedback from the periphery), intrinsic, or more likely a combination of both, causes a particular neuron to develope into a giant Mauthner's cell.

5. Subsurface cisternae

Subsurface cisternae were seen in M cells in early stages of development. They have been seen in several neurons during developmental stages (Caley and Maxwell, 1968; Tennyson, 1969), and in the adult (Pappas and Purpura, 1961; Rosenbluth and Palay, 1961; Rosenbluth, 1962; Herndon, 1963; Hama, 1965; Hartmann, 1966; Siegusmund, 1968).

Although several functions have been proposed for subsurface cisternae in adult cells, no correlations have been forthcoming for embryonic neurons. In the M cell a roughly inverse relationship exists between the number of subsurface cisternae and the number of synaptic contacts. This is suggestive of a role for subsurface cisternae in intercellular communication.

Glycoproteins have been localized at cell surfaces (Pease, 1966; Bondareff, 1967; Rambourg and Leblond, 1967) and at growing tips of axons (Young and Abood, 1960). They have also been shown to be present in relatively high amounts

during periods of neuronal recognition (Barondes, 1969, 1970).

Perhaps a subsurface cistern is the means by which a "recognition" molecule, such as a glycoprotein, is deposited on the surface of the M cell. It may not be necessary for ervery neurite to be associated with an SSC (although it is impossible at this point to estimate how many actually are present) since certain neurites may be sensitive to a specific glycoprotein gradient. It is conceivable that the SSCs found on different parts of the cell may also deposite different types of glycoproteins and therefore "attract" different neurites.

6. Synapses

Synapses were first seen in Xenopus M cells at very early stages (stage 28), primarily on the small filopodial projections. Although synapses were present on the soma at all the stages studied, they appeared in largest numbers first on the dendrites and then on the perikaryon.

No specific sequence of synaptic development could be detected with any certainty. All membrane thickenings that were seen with true synaptic characteristics were always accompanied by synaptic vesicles. And the presence of synaptic vesicles alone could have been due to the plane of section rather than to any actual developmental progression.

Mauthner's cell in goldfish has been a favorite model for the study of synapses. Several different types of synapses are present and can be correlated with specific

physiological responses. Such morphological variation does not exist in Mauthner's cell in Xenopus laevis. Club endings or an axon cap were not seen on the Xenopus M cells. Although there were several larger synaptic endings located in the axon hillock region that some workers considered analagous to the terminals within the goldfish axon cap (Moulton, Jurand and Fox, 1968), none of them were morphologically comparable to even the simplest of the cap cells. No physiological data is available on the Xenopus M cells that would permit a direct correlation to be made between a spcific synaptic configuration and a specific neural pathway. There is no direct eveidence for a high-speed reflex pathway from the vestibular system to to the innervation of the tail. The only evidences implicating the M cells in this response are some anatomical observations and one behavioral study (Sims, 1962).

7. Neurofibrils, microtubules, and neurofilaments

Silver, which specifically impregnates the constituents of nerve fibers, was utilized to correlate the earliest appearance of the dendrites with ultrastructural changes occurring within the perikaryon.

Unfortunately, these techniques were found to be inconsistent, unreliable, and extremely sensitive to the slightest variation in staining methods or conditions of fixation. For example, Holmes' silver nitrate technique, although adequate for embryonic chick neural tube, was a very

poor agent for the staining of amphibian nerve cells.

Bodian's Protargol method was then resorted to, and after numerous modifications, a relatively satisfactory method was worked out. However, yolk, a major constituent of amphibian neural tube, consistently interfered with fixation and staining. It was therefore impossible to obtain a slide of an early embryo (stage 22) that did not have a muddy appearance. By stage 28, the yolk decreased enough so that the M cell could be recognized in the silver stained sections. However by that time its axon had already appeared. Beginning with stage 28, the M cell was found to stain very deeply with silver, but individual fibrils were vague and difficult to discern. This period was followed by a gradual condensation of the argentophilic material into the classical neurofibrils.

The ultrastructural counterparts of the neurofibrils has been interpreted as being the microtubules and the neurofilaments. However early neurons, the M cell included, contain very few tubules and filaments, and yet stain very darkly with silver. A number of explainations can be offered for this phenomenon. It seems pollible that enough neurofilaments and microtubules were present in the cytoplasm in these early stages to account for the initial density, but, they were either poorly preserved or not detectible with either glutaraldehyde or osmium fixation. Alternatively, unpolymerized fibrous proteins which were already present as part of the ground substance of early stages became impreg-

nated resulting in a diffuse distribution of silver grains. At later stages, the tubules and filaments were assembled and became visible as the neurofibrils.

Neurofilaments and neurotubules have been found as prominent constituents of all types of nerve cells (Cravioto, 1966), with their relative distributions varying somewhat in different parts of the cell. In general the ratio of neurofilaments to microtubules has been found to be high in axons while the inverse has been reported for dendrites (Schmitt and Samson, 1968) The anterior horn cells of the rat are among the exceptions to this generalization (Wuerker and Palay, 1969). Although they were apparaently less numerous than the microtubules, the neurofilaments were found as larger proportions of the dendritic cytoplasm than in the adjacent smaller cells. Mauthner's cells in goldfish (Robertson, Bodenheimer and Stage, 1963) and in Xenopus laevis revealed a preponderance of neurofilaments in the dendrites with relatively few neurotubules scattered throughout. These findings indicate a possible relationship between cell size and neurofilament distribution in the dendrites.

Both neurotubules and neurofilaments have been postulated to be responsible for the maintainance of cell shape and to be involved in the transport of materials from the cell body to the distal processes (Schmitt and Samson, 1968).

Once proposal involving both microtubules and neurofilaments has been recently proposed by Ochs (1972). In his

scheme either neurofilaments or microtubules or both remain stationary while an additional "transport filament", which is composed of or binds various cytoplasmic material, is transported down the process. The mechanism by which this occurs is very similar to the sliding filament hypothesis of muscle contraction.(see Huxley, 1965). It would therefore be interesting to examine the distribution of microtubules and neurofilaments in various sized cells, and correlate differences or similarities in their mechanisms of transport. A giant cell such as Mauthner's particularly in the goldfish would be an ideal system.

8. Comparison of Mauthner's cell size in fish and amphibians

It is interesting form an evolutionary point of view that the size of the M cell has been related to the type of swimming an animal engages in (Stefanelli, 1951). For example, teleosts that swim with strong beating strokes have larger and better developed M cells than fish who swim with eel-like movements. Tadpole, whose swimming patterns would fit into the latter category, have a much simpler M cell, morphologically, as compared with the homologous neurons in goldfish. The M cells in teleosts are retained throughout the entire life of the fish, while in Xenopus, as in other amphibian tadpoes, the M cells are present only for a relatively short time. This pattern is carried even further in Bufo. This genus is almost entirely terrestrial and their M cells are at best, vestigial.

D. Conclusion

This investigation of Mauthner's neurons, the first such detailed developmental study on a single cell has strongly emphasized the value of this particular system. It enabled a progressive study to be made on a unique cell which was not only easily and accurately identifiable, but was also found to follow the same general pattern of differentiation that has been outlined in various systems.

Filopodia preceeded the appearance of the dendrites and the growth of the cell body. Golgi complexes were closely related to the growth and maturation of the dendrites. The nucleolus enlarged and became vacuolated at the same time that there was a significant increase in the amount of granular endoplasmic reticulum, and the beginning of a tremendous increase in the amount of neurofilaments and to a lesser extent, microtubules, in the perikaryon and in the processes. As the number of subsurface cisternae, which were very common at the onset of the cell growth phase, decreased with development, the number of synapses increased until the entire M cell surface was studded with synaptic contacts.

All these events were followed through several stages in the development of the M cell. A gradual progression in the number and distribution of organelles was clearly seen to culminate in the formation of a mature, differentiated neuron.

The M cell showed itself to be a typical nerve cell in all respects, except in its size. It therefore becomes available and desirable as a model for the systematic analysis

of consecutive developmental stages. Further investigations of the biochemical and environmental factors that influence the differentiation, growth and maturation of an individual neuron can now be carried out on one specific, identifiable neuron.

PLATE I

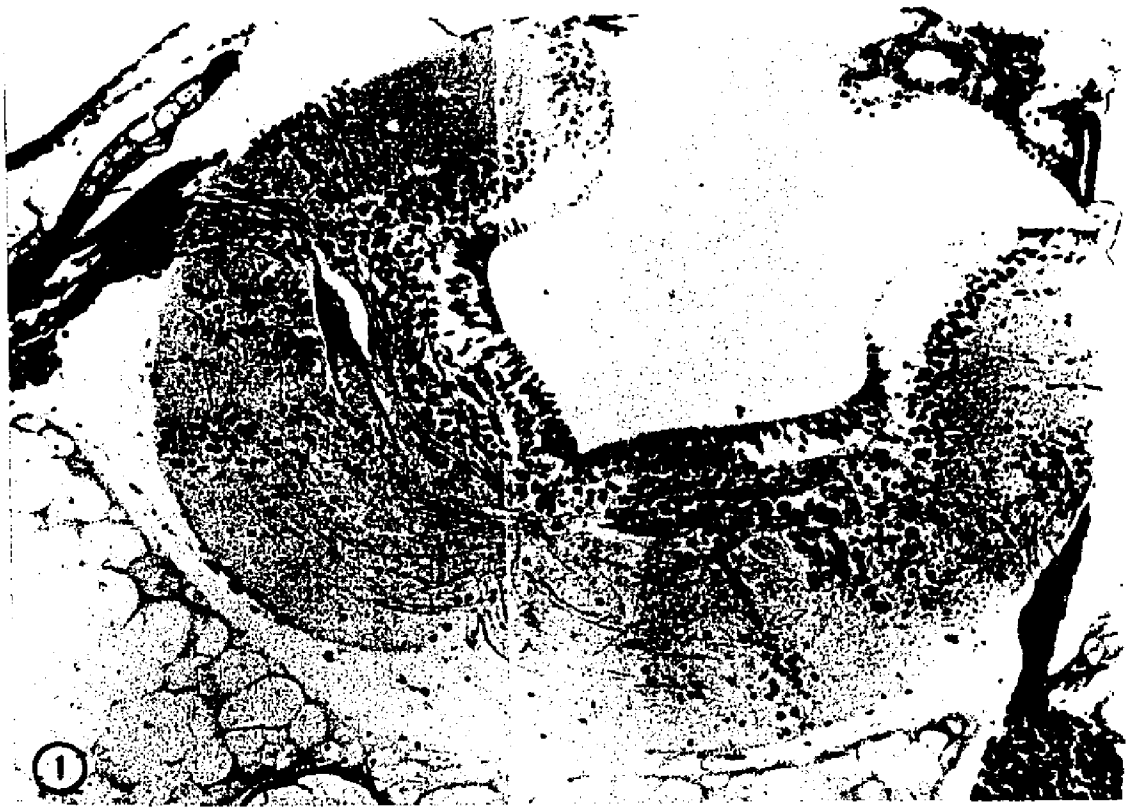
Figures 1-35. Light micrographs of transverse sections through the hindbrain at the leve of entry of the eighth nerve.

Fig. 1. Stage 54. Showing Mauthner's cell (M) and part of the M cell axon (Ax); its histological relationships to incoming fibers of the eighth nerve (VIII) and other cells and fibers of the neural tube. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with amidol. x180

Fig. 2-18. Fixative: Lavdowsky's mixture, Stain: Bodian's protargol, reduced with amidol.

Fig. 2. Stage 22. Neural tube at level of ear placode containing primarily ventricular cells (V), with a large quantity of yolk (Y). Nucleus (N). x338

Fig. 3. Stage 22. Outer margin of neural tube, outer right. Cell with round nucleus (a). Yolk (Y). x1325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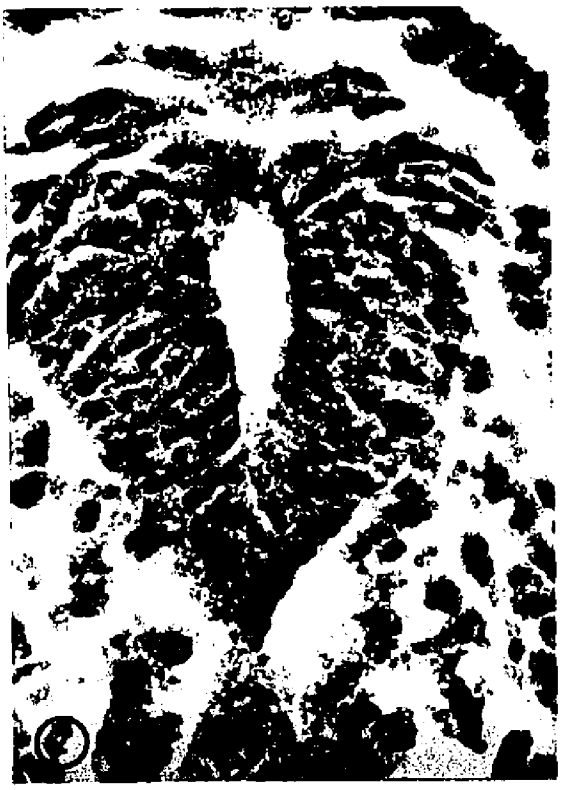

2

PLATE II

Fig. 4. Stage 25. Survey of neural tube, left side. Neurocoel (NC). x753

Fig. 5. Stage 27. Outer margin of neural tube, upper right. Possible M cell (?) located at periphery. Fibrous ventral region (Fb) and globular dorsal region (Gl). x1325

Fig. 6. Stage 28. Survey of neural tube and its relationship to otic vesicle (OV), and early ganglion cells (GC). x338

Fig. 7. Stage 28. Early M cell with distinct nucleolus (Nl) and axon (Ax). x3293

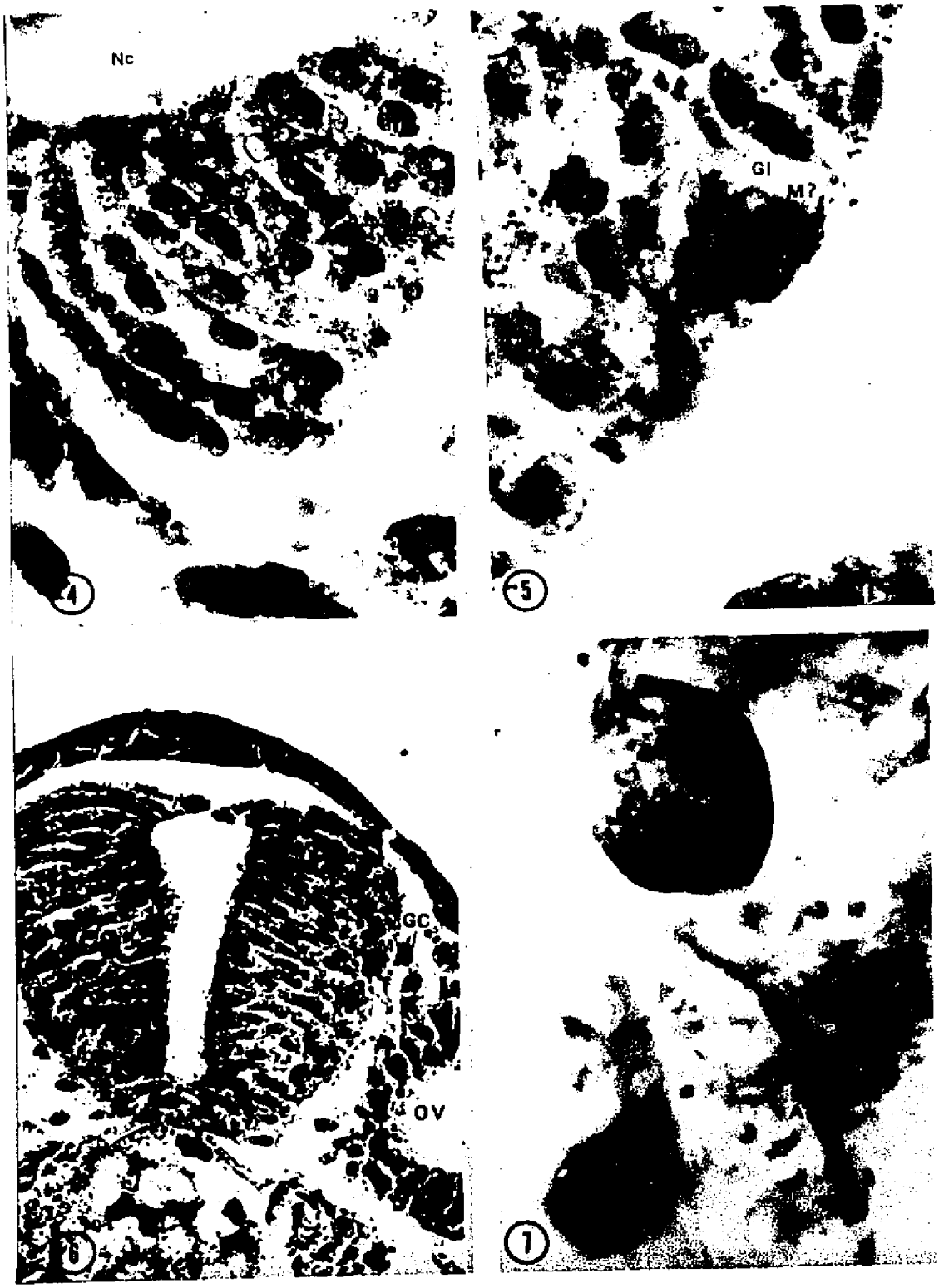

Nc
4
Gl
M?
5
GC
OV
6
A
7

PLATE III

Fig. 8. Stage 29/30. M cell with prominent nucleolus. A process with fibrils (Fb) from the cell body towards fibers of the eighth nerve (VIII). Ventral dendrite (VMD). x1325

Fig. 9. Stage 29/30. M cell and its axon. x1325

Fig. 10. Stage 31. Normal M cell. x1325

Fig. 11. Stage 31. Left M cell (Embryo #312) containing two nucleoli (Nl) in a very elongated nucleus (N). Abundant globular material occupying most of cytoplasm. x1020

Fig. 12. Stage 31. (Embryo #312) Right cell, containing only one seemingly fragmented nucleolus (Nl) and globular material. Dorsal Dendrite (DD). x1020

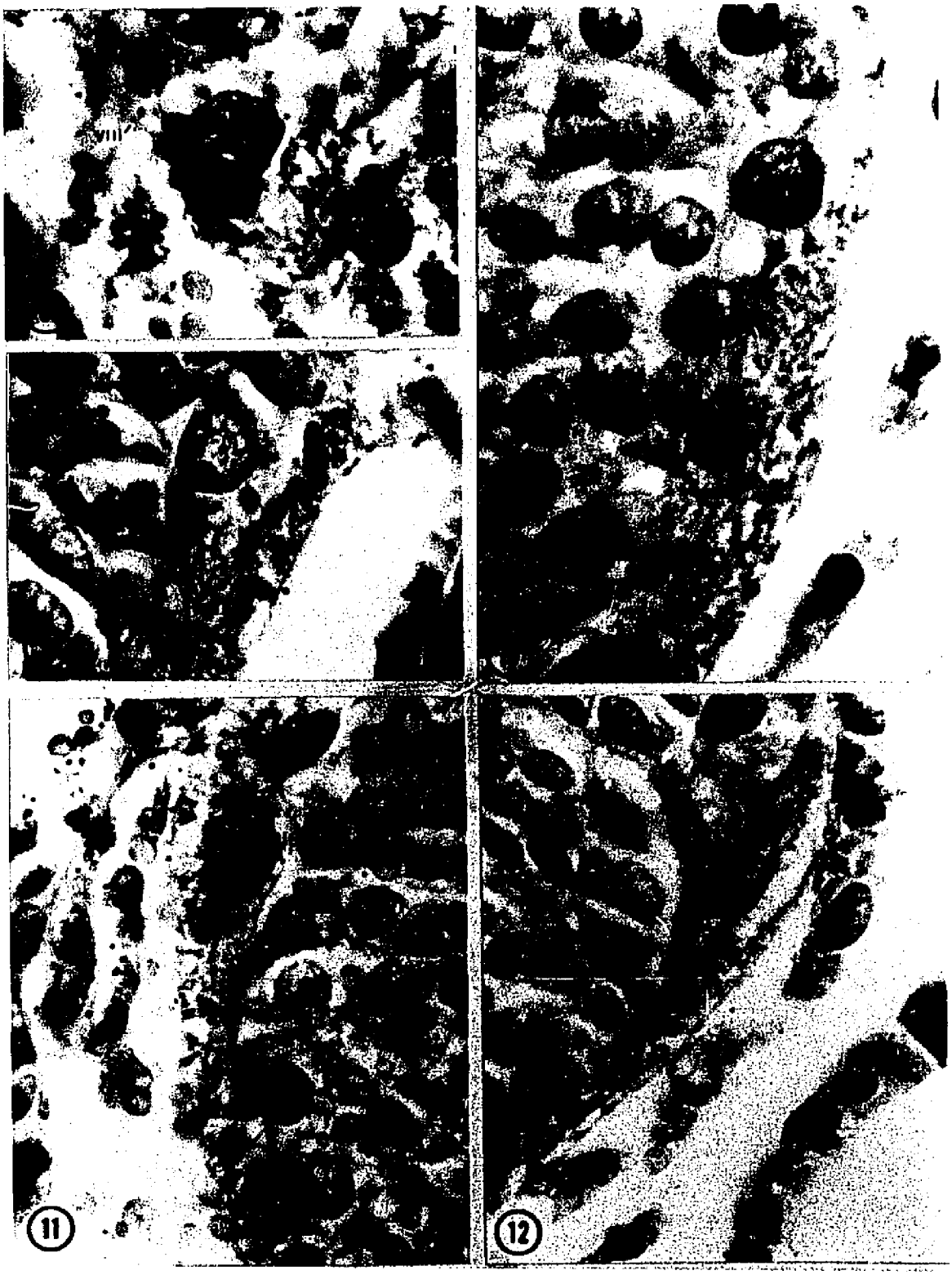

11
12

PLATE IV

Fig. 13. Stage 32. Right M cell with two ventral dendrites: ventro-lateral dendrite (VLD), ventro-medial dendrite (VMD), and an axon (Ax). x1325

Fig. 14. Stage 32. Micrograph of section serial to the section in fig. 13. Dorsal dendrite (DD), fibers of unknown origin (F). x1325

Fig. 15. Stage 33/34. Survey of neural tube. Otic vesicle (OV), ventricular zone (v), intermediate zone (i), marginal zone (m), Mauthner's cell (M). x338

Fig. 16. Stage 33/34. Relationship of M cell to various fiber groups (F1, F2, F3, and circle, see text for description). x1325

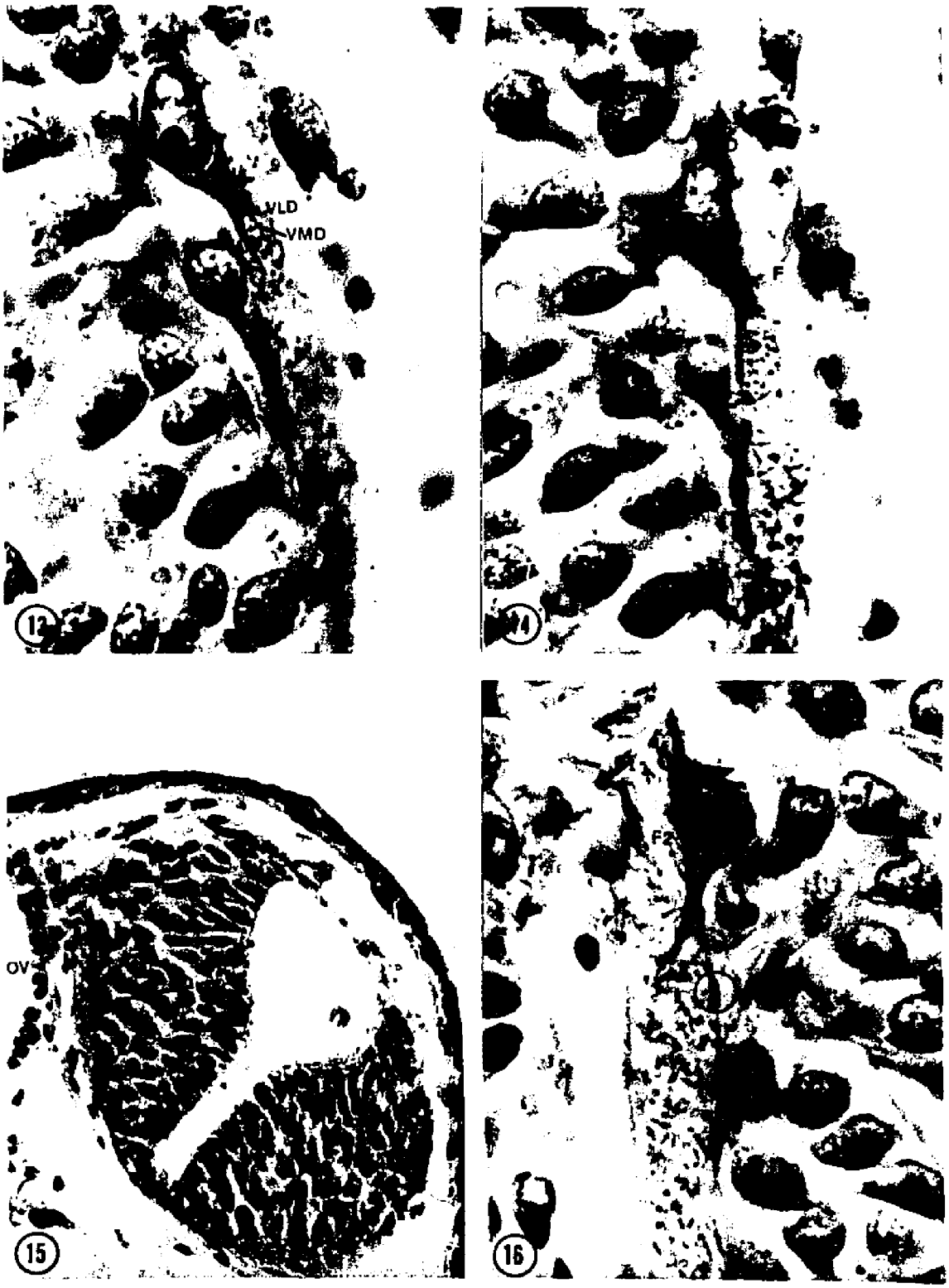

VLD
VMD
F
OV
F2
13
14
15
16

PLATE V

Fig. 17. Stage 37/38. M cell with lobulated nucleolus (arrow) x1325

Fig. 18. Stage 39. M cell containing axon (Ax), ventromedial dendrite (VMD) x1325

Fig. 19. Stage 40. M cell with nucleolus (Nl) containing a central "vacuole" (arrow) x3293


17
VMD
N

PLATE VI

Fig. 20. Stage 44. Slightly oblique section in which parts of M cells of both sides are displayed relative to each other. Ventral, lower left. Fixative: Bodian's protargol, reduced with amidol. x208

Figures 21, 22, 23. Stage 46. Micrographs of three serial sections through an M cell displaying relationships of the various processes to its cell body. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with hydroquinone.

Fig. 21 Ventral portion of M cell containing distinct fibrils. Axon (Ax) can be traced to ventral midline of the neural tube. Ventral, bottom x338

Fig. 22. Dorsal dendrites (DD). Dorsal, upper right. x338

Fig. 23. Axon and ventral dendrites with fibers passing over cell body but not in direct contact with it (a,b). Ventral, left x338

Fig. 24. Stage 47. Fixative: Bodian's #5, Stain: Bodian's protargol, reduced with amidol. Dorsal, right. x208


20


22


Б
23


А
21

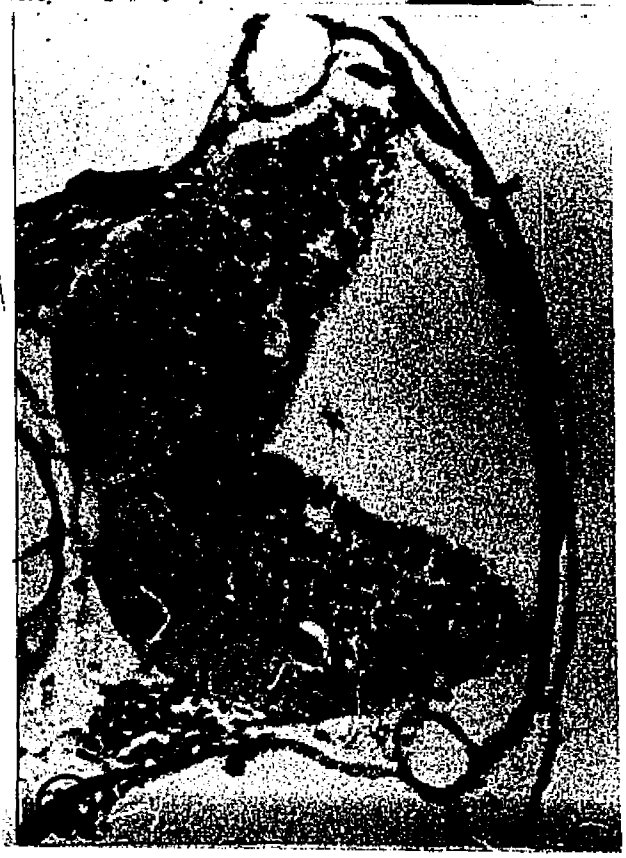


PLATE VII

Figures 25-29. Stage 48. Embryo #230 containing two M cells on left side. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with hydroquinone.

Fig. 25. First left cell (L-1). x338

Fig. 26. Right cell. x338

Fig. 27. Second left cell (L-2). x338

Fig. 28. Two left axons (AxL-1, AxL-2) decussating with one right axon (AxR). x1325


L
25


R
26


L
27


R
AC

PLATE VIII

Fig. 29. Stage 46. Fixative: Bodian's #9, Stain: Bodian's protargol, reduced with hydroquinone. x338

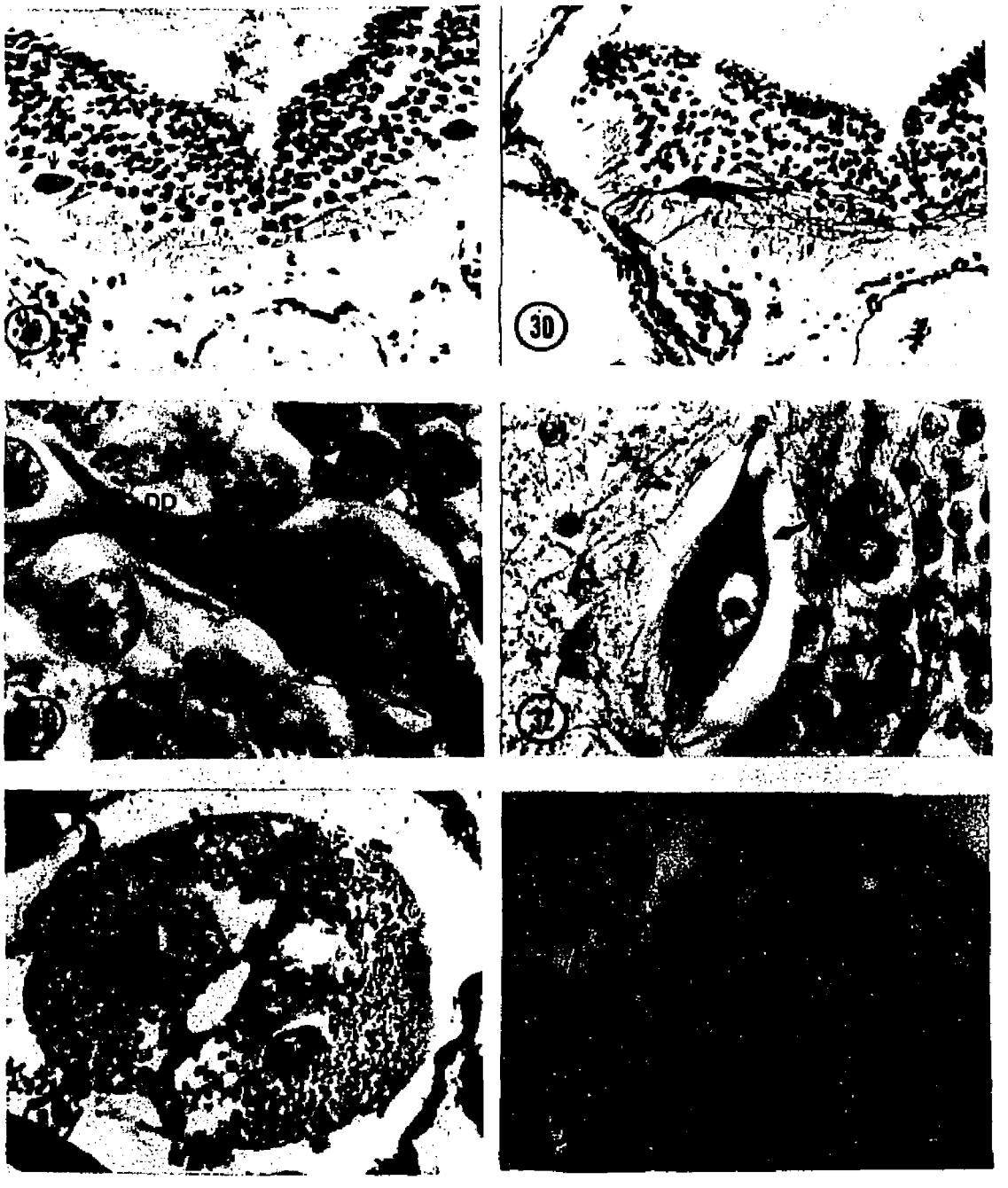
Fig. 30. Stage 46. Fixative: Bodian's #1, Stain: Bodian's protargol, reduced with hydroquinone. x338

Fig. 31. Stage 49. Bifurcation of dorsal dendrite (DD) of M cell. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with amidol. x1075

Fig. 32. Stage 50. M cell. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with amidol. x735

Fig. 33. Stage 54. Primary motor neuron at level of hind limb bud. Fixative: Bodian's #4, Stain: Bodian's protargol, reduced with amidol. x208

Fig. 34. Stage 44. M cell. Fixative: Lavdowsky's mixture, Stain: Holmes reduced silver nitrate. x1325


30
OD

Figures 35-75. Transverse sections of the hindbrain which cut the M cell longitudinally. All specimens were fixed in glutaraldehyde, post-fixed in osmium tetroxide.

EXPLANATION OF ABBREVIATIONS

Ax	axon
CV	coated vesicle
D	dendrite
DB	dense body
DC	dense cored vesicle
DP	dense particle
F	neurofilaments
G	Golgi complex
GER	granular endoplasmic reticulum
Gl	glycogen
L	lipid
M	mitochondrion
MVB	multivesicular body
N	nucleus
Nl	nucleolus
P	process
Po	nuclear pore
S	synapse
SSC	subsurface cistern
T	microtubule
VLD	ventro-lateral dendrite
VMD	ventro-medial dendrite
Y	yolk

PLATE IX

Fig. 35. Stage 27. Upper left neural tube at the level of the otic vesicle showing parts of four cells (1-4); extracellular space (*); lipid (L); yolk (Y). Lateral is at left, ventral at bottom. x6000

PLATE X

Fig. 36. Stage 28. Ventro-medial part of the M cell showing nuclear indentation (X) with dense particles (D) adjacent to the inner membrane of the nuclear envelope, and ribosomes and glycogen close to the outer membrane. The latter are concentrated particularly in pore regions and in the nuclear indentation. Projection (P) extending from the cell surface. Mitochondrion contained within vesicle (Mx). Mitochondrion continuous with membrane (My). Ventral is top right. x35,000

Inset. Detail of yolk granule (Y) and glycogen particles (Gl). x86,000

PLATE XI

Fig. 37. Stage 28. Perinuclear cytoplasm showing mitochondrial concentration (M), nuclear pores (Po), Golgi bodies (G), and subsurface cisternae (SSC). Lateral is to right; dorsal is top. x30,000

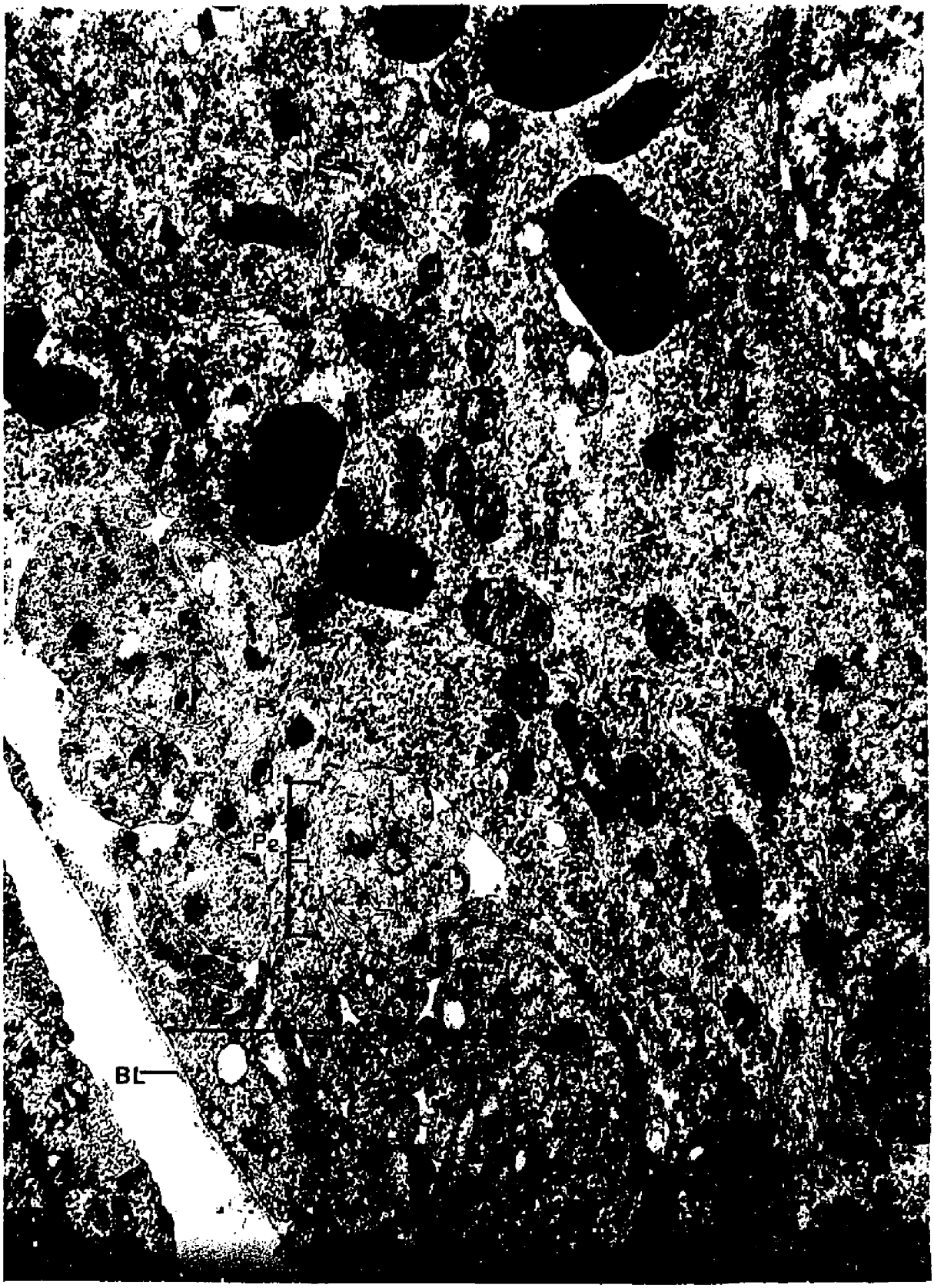

N

PLATE XII

Fig. 38. Stage 28. Dorso-medial portion of cell showing different yolk configurations (Y1, Y2). Dorsal is bottom; ;atera; is to left. x60,000

PLATE XIII

Fig. 39. Stage 28. Ventral part of cell and base of possible axon with microtubules (T) and filaments (F). Type 2 projection on ventro-lateral border of soma (P2). x8,000


Pe
BL

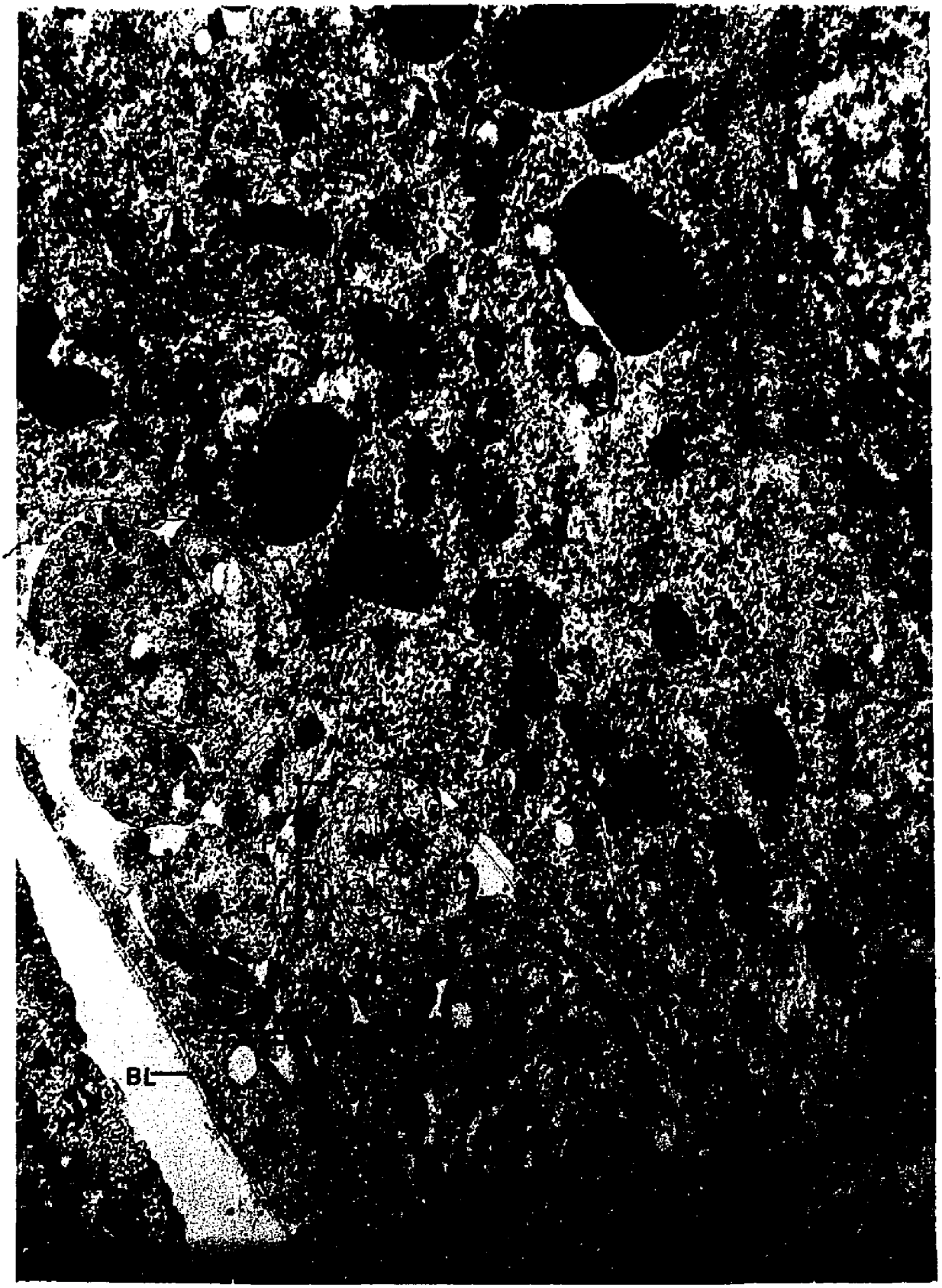

BL

PLATE XIV

Fig. 40. Stage 28. Type 3 projections (P3) from the ventro-lateral perikaryon with synapses (S). Cytoplasm near edge of cell contains many microtubules (T), neurofilaments (F) and subsurdace cisternae (SSC) x60,000


F
M
40

PLATE XV

Fig. 41. Stage 28. Type 1 projection from dorso-lateral perikayon (Pi) and Type 2 projections from dorso-medial perikaryon (P2). x 50,000

Inset. Synapse (S) on type 2 projection. x75,000

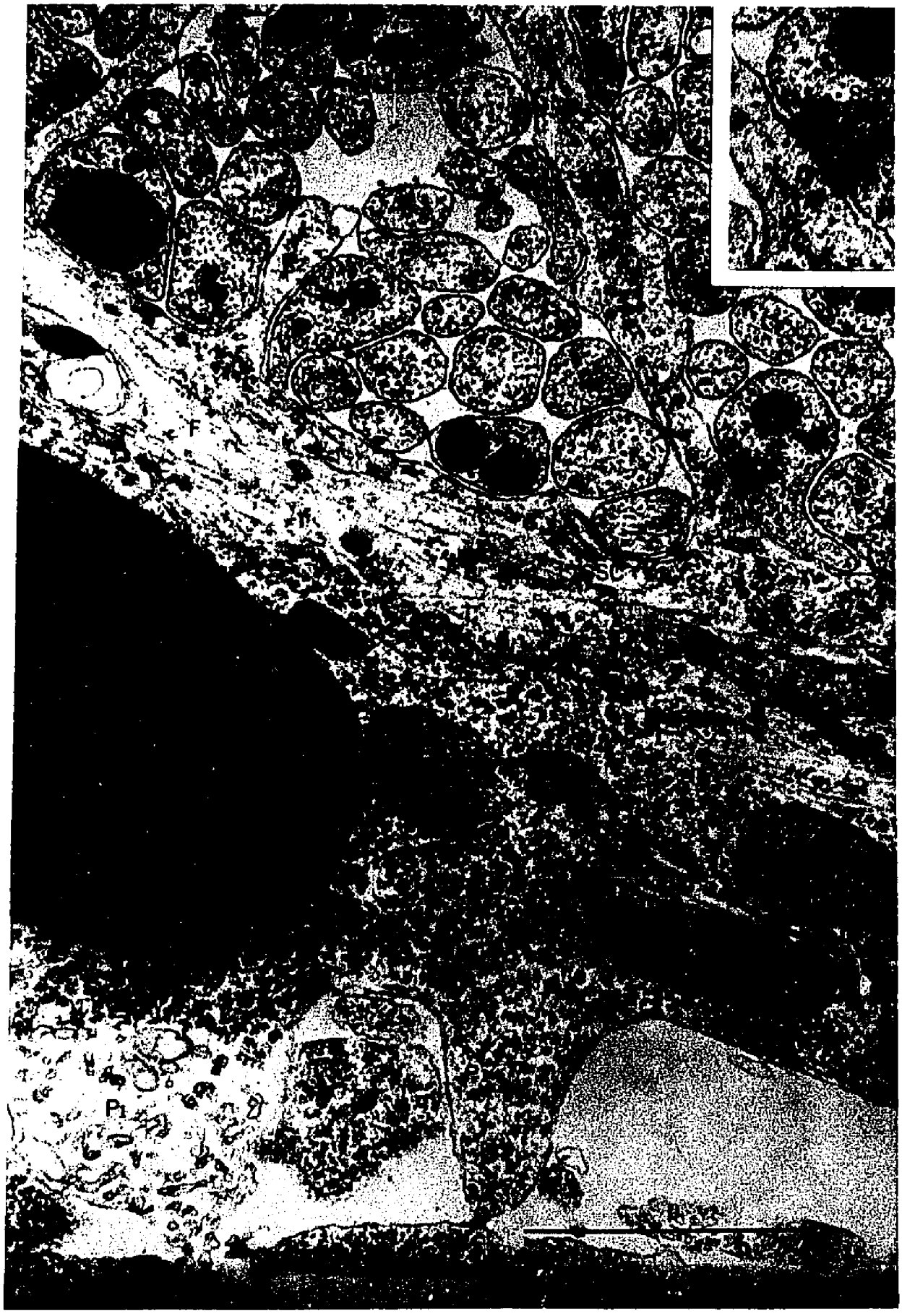

F
SC
Pi

PLATE XVI

Fig. 42. Stage 29/30. Medial part of cell showing abundant Golgi membranes (G), lipid (L), nucleus (N). x60,000


N
D
L

PLATE XVII

Fig. 43. Stage 31. Survey of M cell with cytoplasmic inclusion (DI) in nucleus, and two projections (P) extending from the dorso-lateral border of the cell. x5,000

Inset. Detail of a projection (P) from dorso-lateral part of cell. x30,000

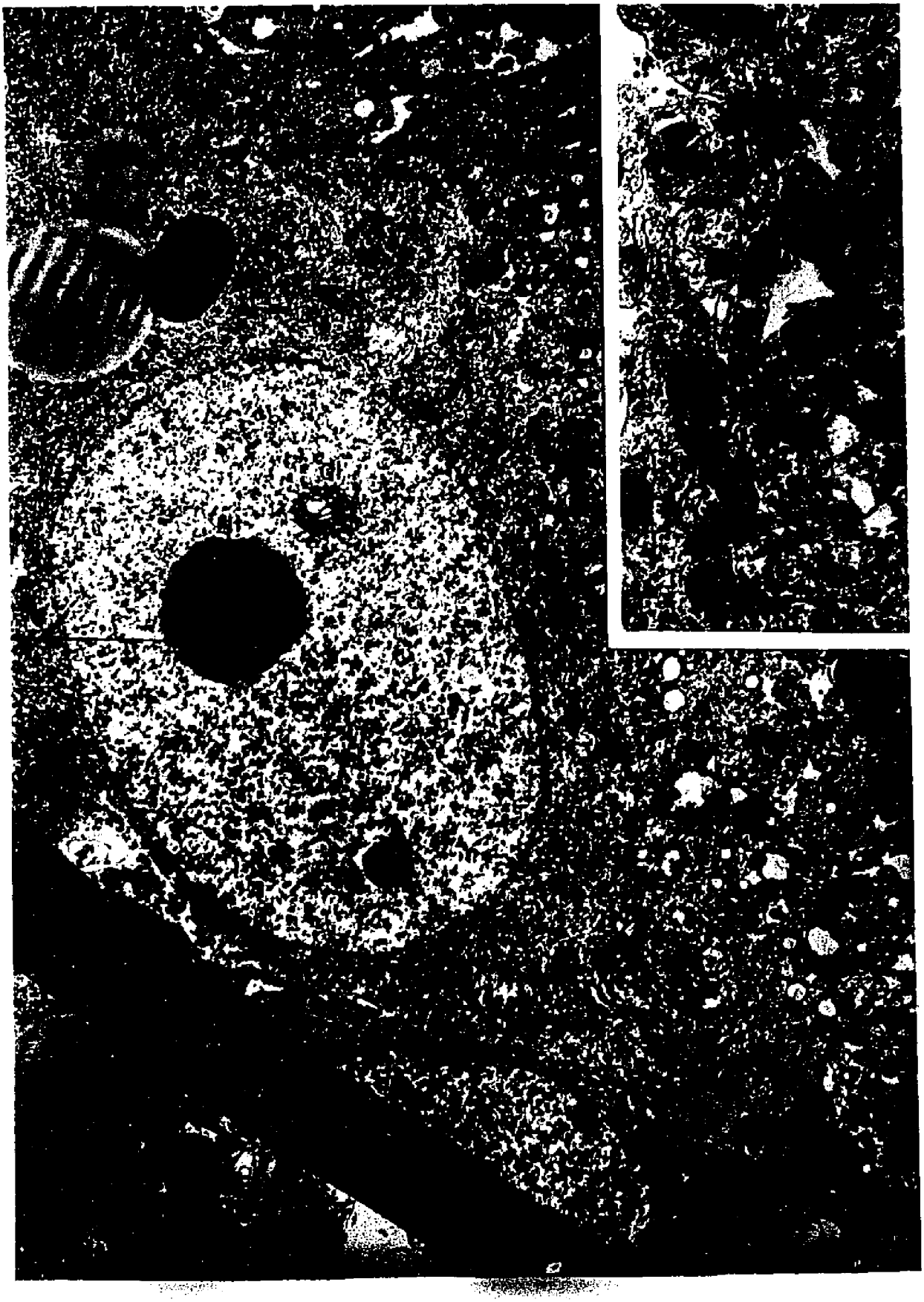


PLATE XVIII

Fig. 44. Stage 31. Ventro-lateral part of cell with synapses (S) on cell body and Golgi complex (G) distributed perinuclearly. Lateral is upper right. x17,500

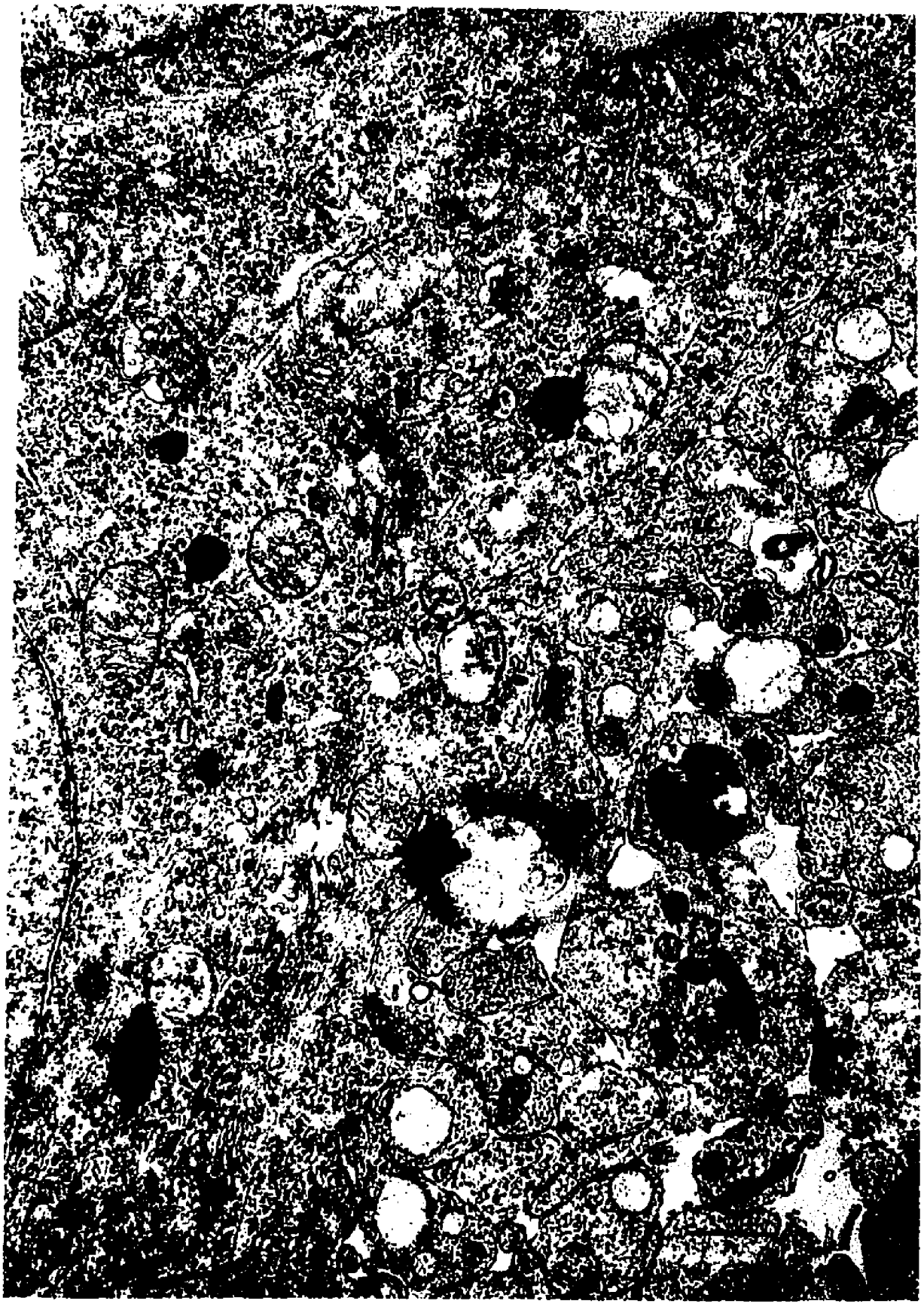


PLATE XIX

Fig. 45. Stage 31. Ventral end of M cell showing probable axon (Ax) and ventral projection (D) which is probably an early ventro-medial dendrite, with a synapse (S) on it. x27,000


AX

PLATE XX

Fig. 46. Stage 31. Presumed axon (Ax) of M cell with bulge on its lateral aspect, synapse (S), subsurface cisterna (SSC), filaments (F), microtubules (T). x27,000

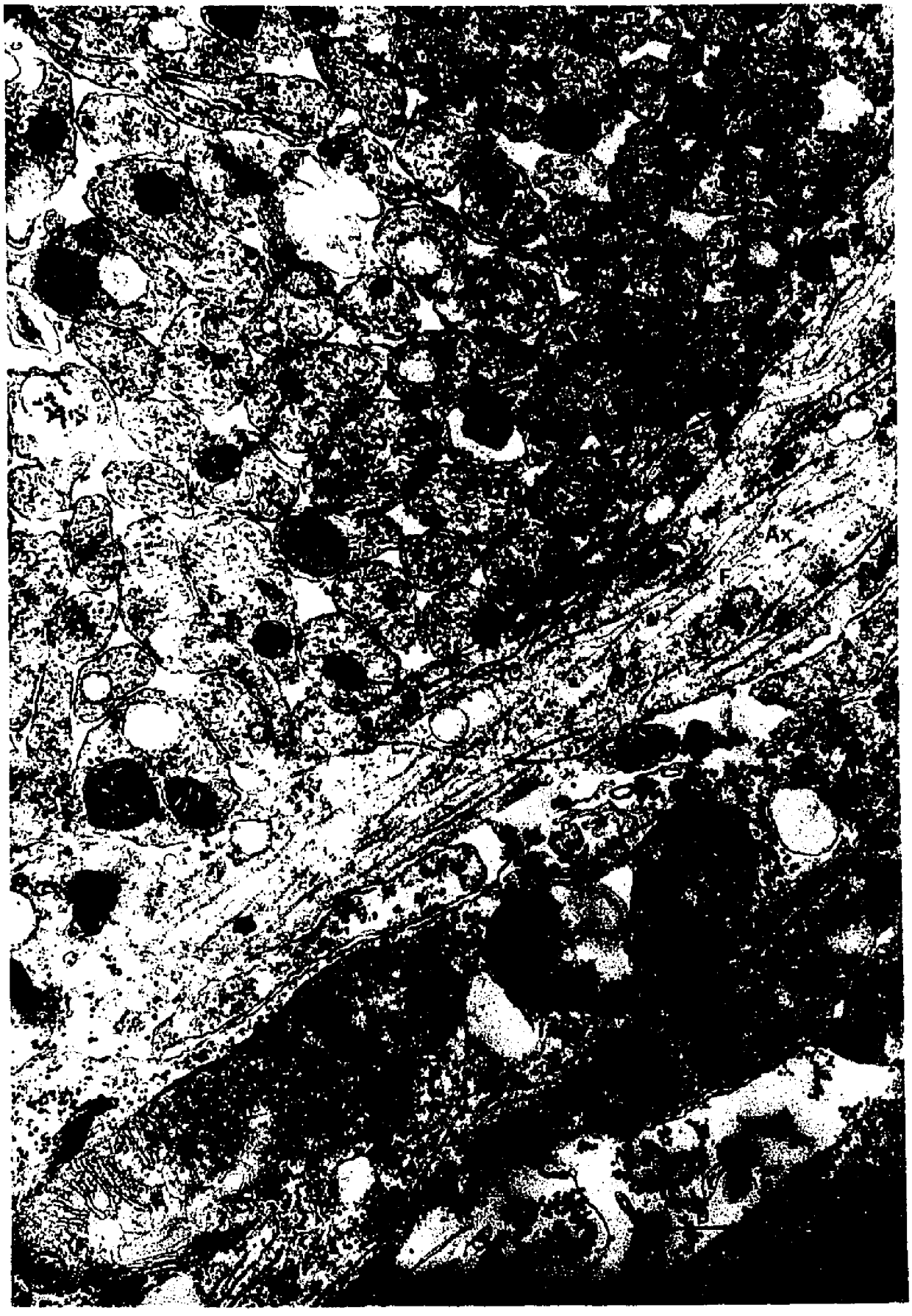

Ax
F

PLATE XXI

Fig. 47. Stage 32. Survey of M cell. Note: a) small less dense regions within the nucleolus; b) long lateral dendrite (D). x4,000

Inset. Detail of dorsal cytoplasm and synapse (S). x14,100

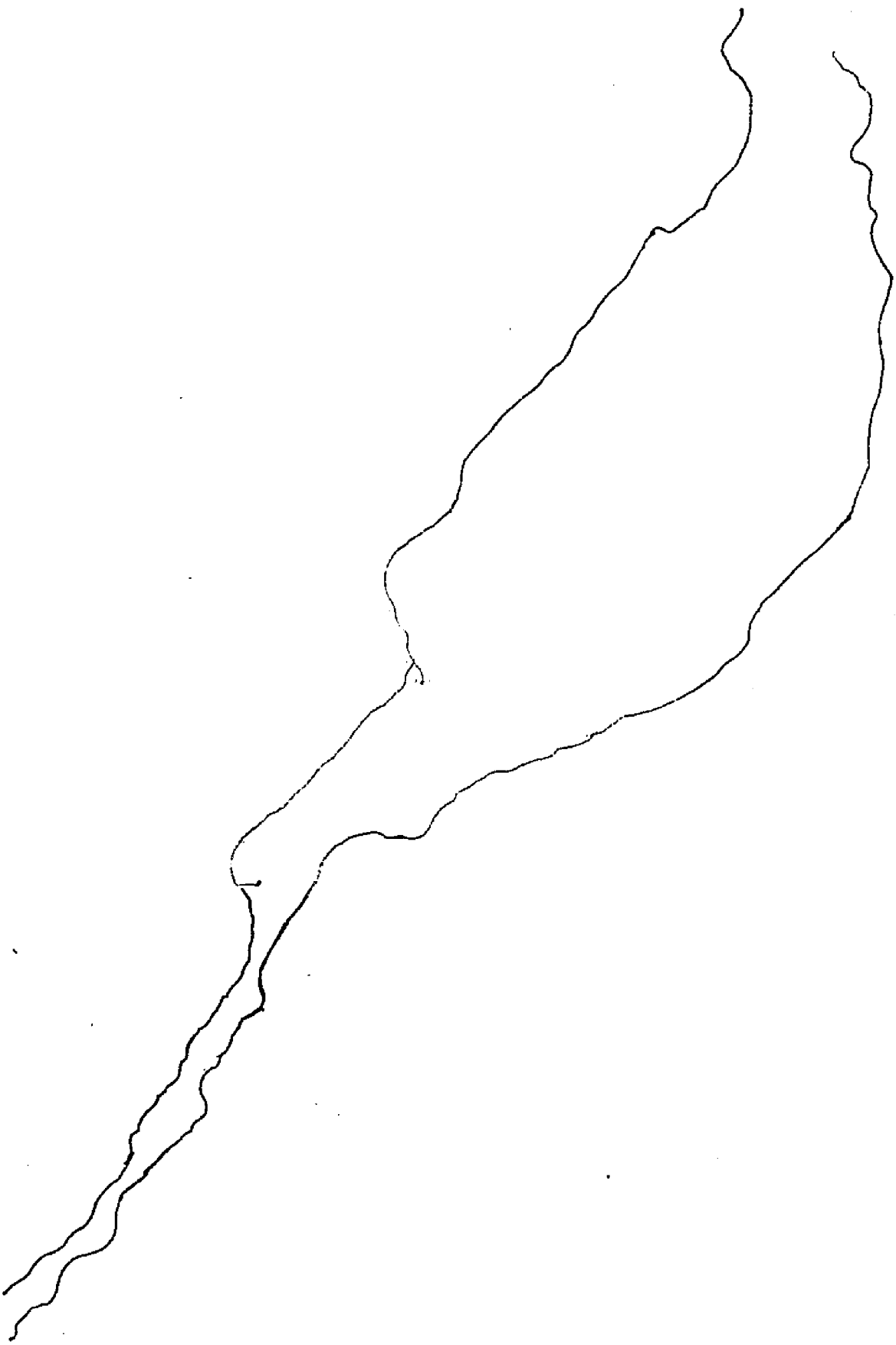
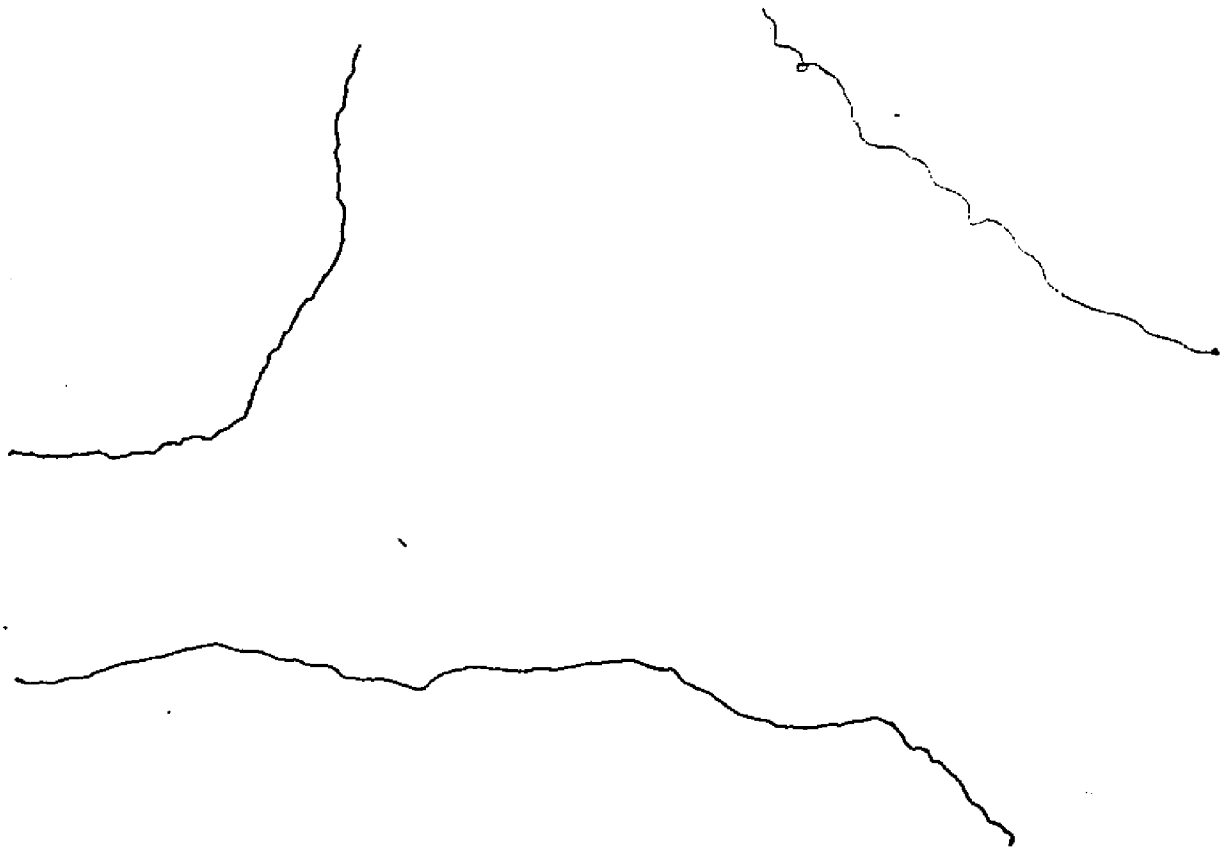


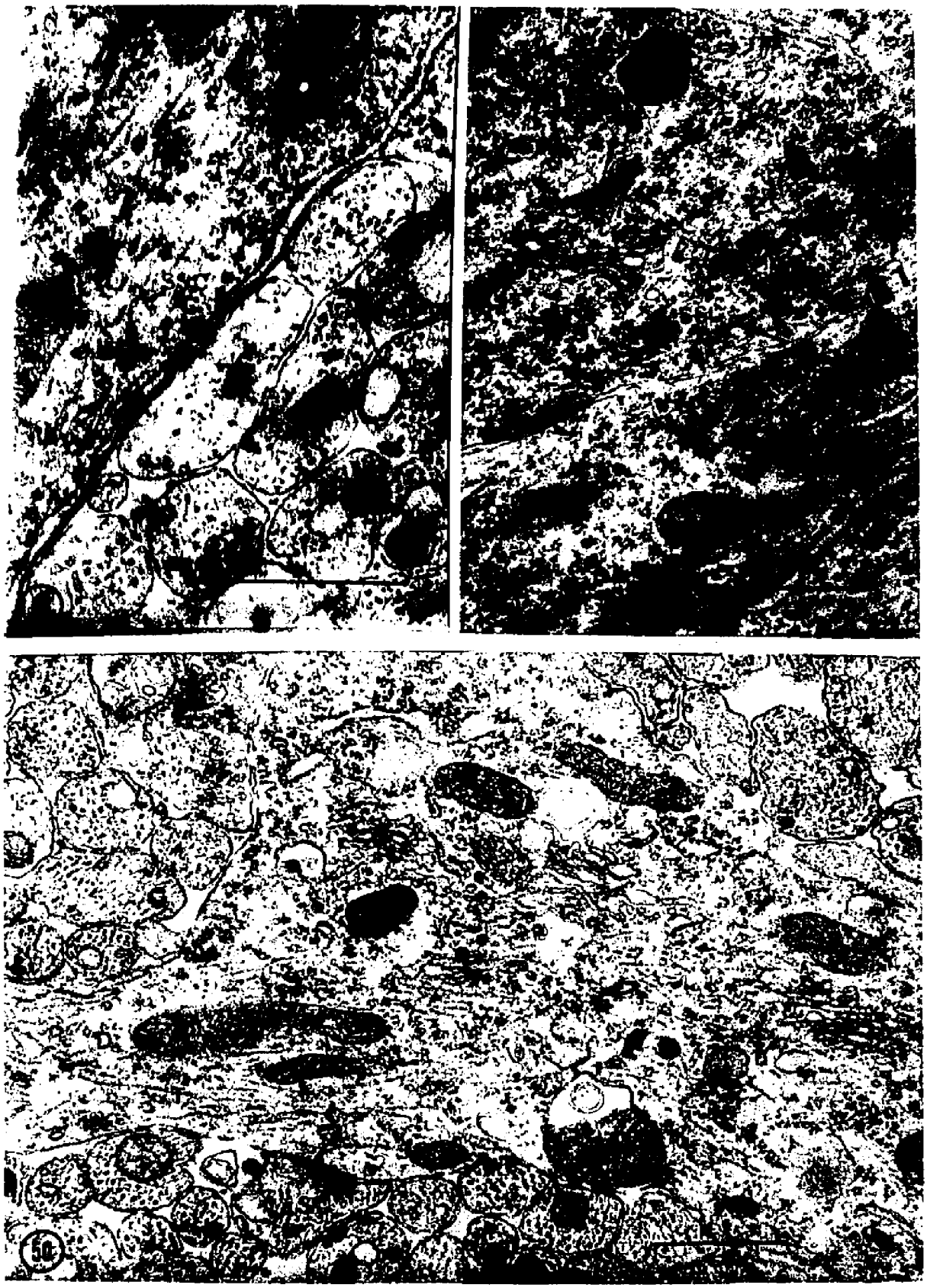
PLATE XXII

Fig. 48. Stage 32. Subsurface cisterna (SSC) apposing four neurites. x32,000

Fig. 49. Stage 32. Subsurface cisternae in M cell (M) and adjacent cell. x20,000

Fig. 50. Stage 32. Ventral part of cell showing Golgi accumulation toward dendrite and medial accumulation of microtubules (T) and neurofilaments (F) towards the medial axon (Ax) x20,000




5c

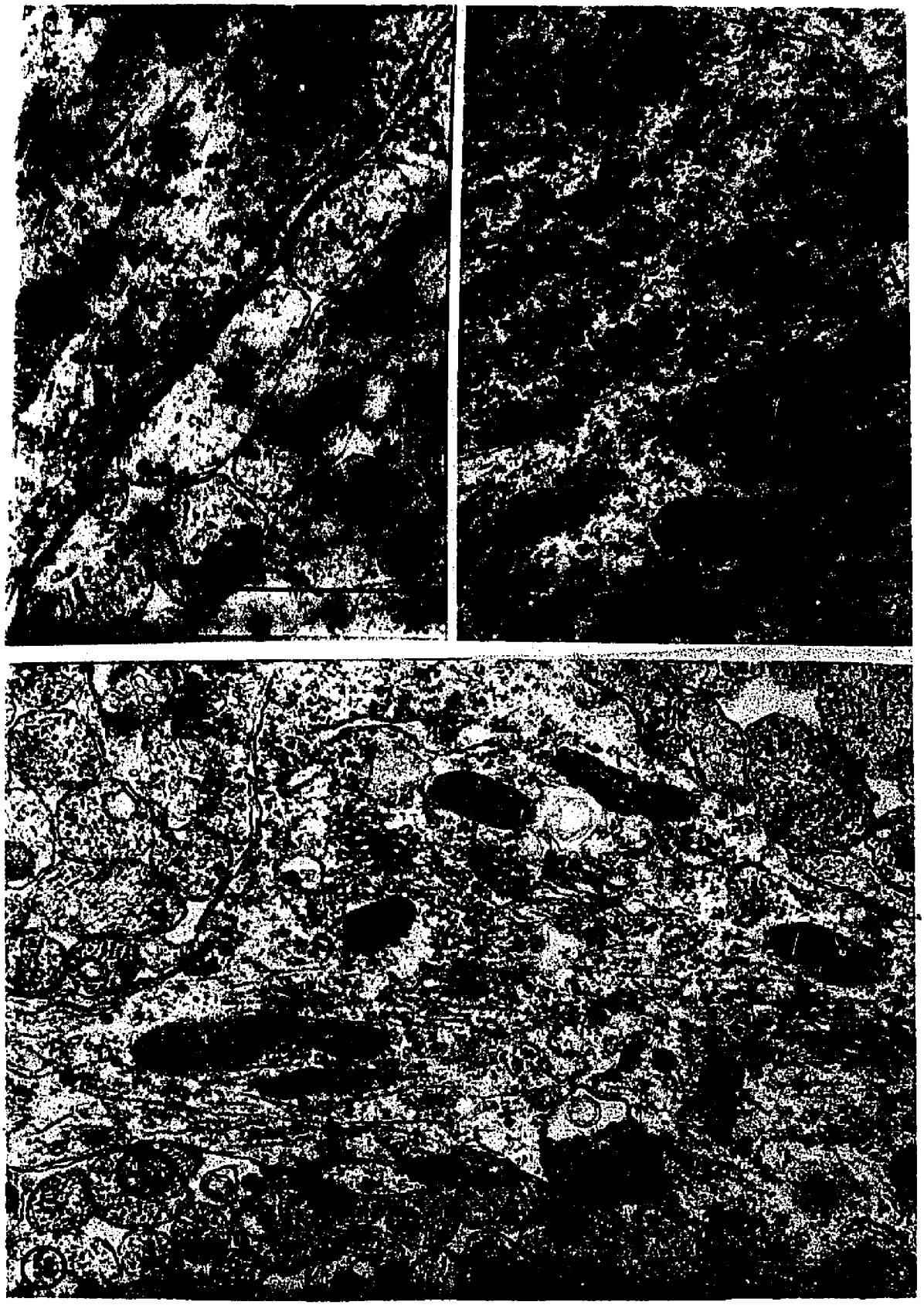
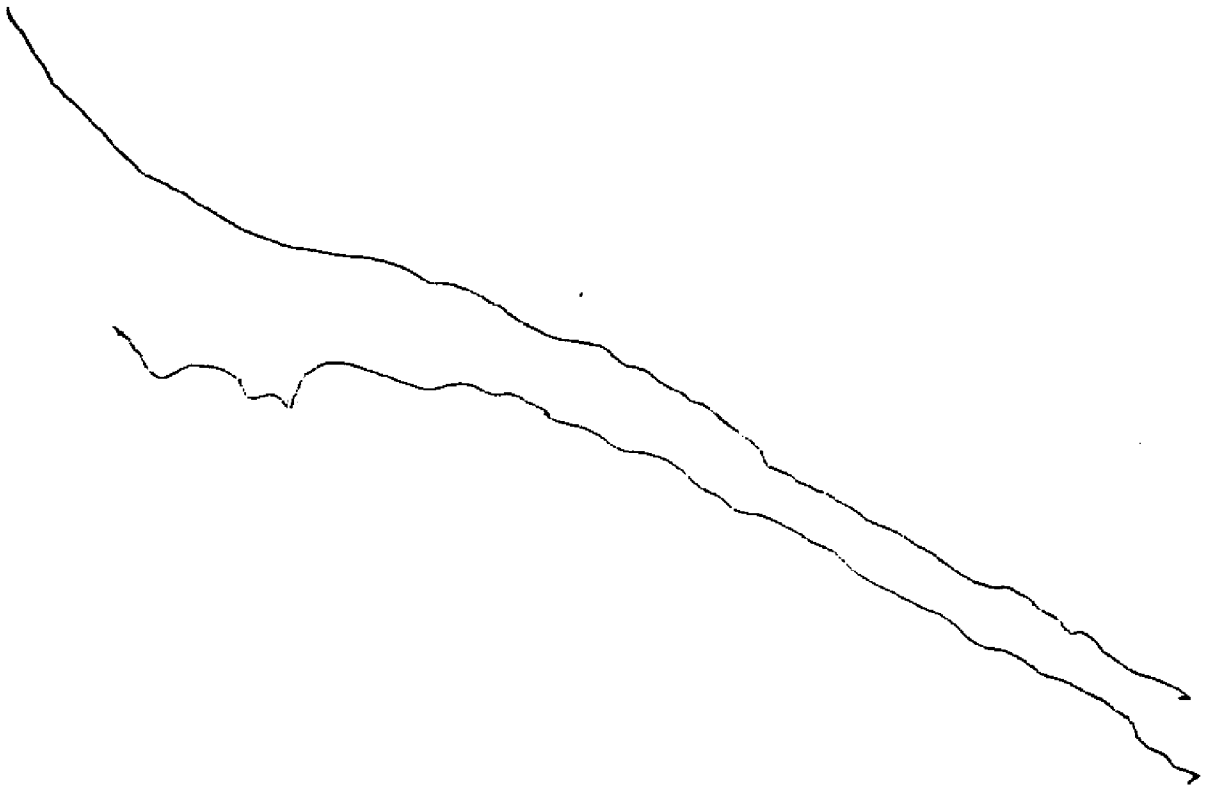


PLATE XXIII

Fig. 51. Stage 33/34. Ventro-medial cytoplasm converging to ventro-lateral dendrite. Subsurface cisternae (SSC). x10,000

PLATE XXIV

Fig. 52. Stage 33/34. Branch from large ventro-medial dendrite (DB) containing long microtubules (T). x10,000




DB
32

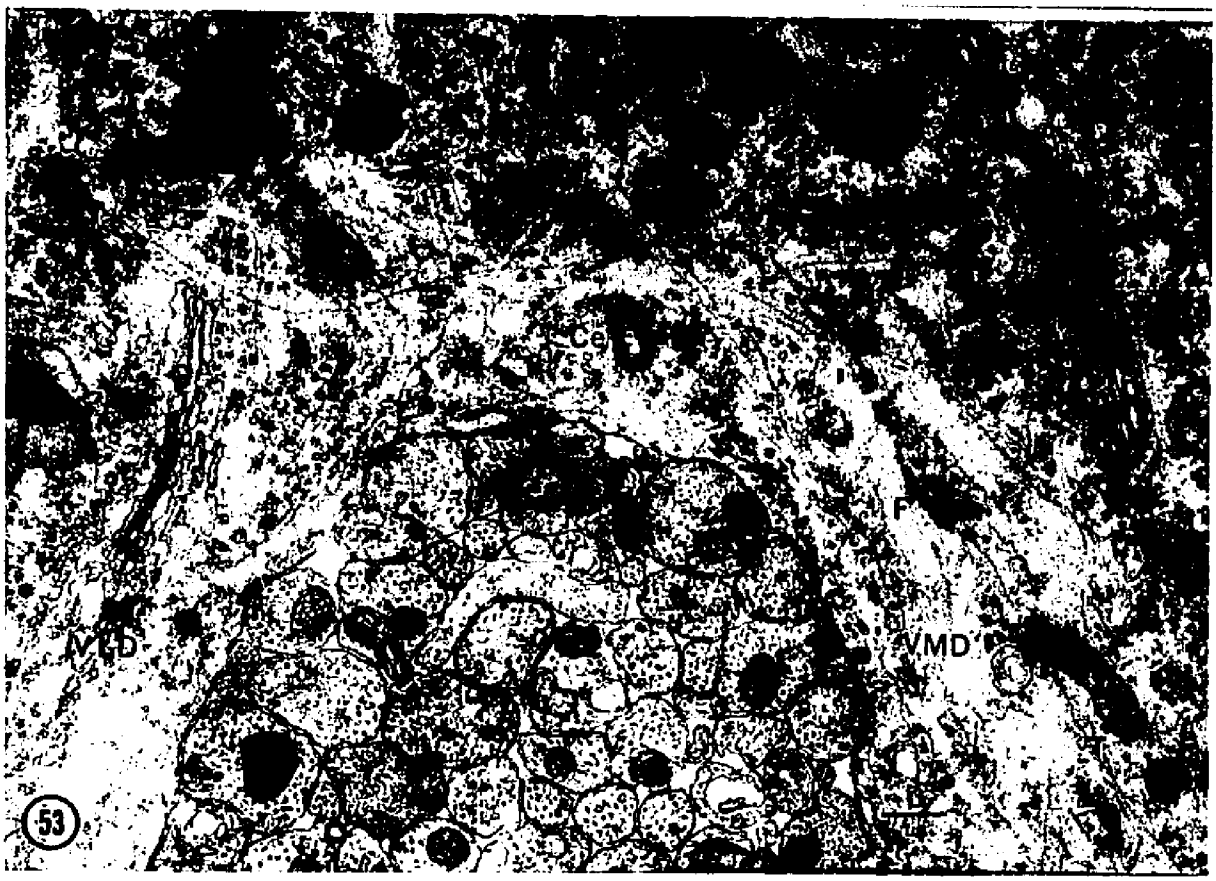

B
N

PLATE XXV

Fig. 53. Stage 35/36. Divergence of ventral portion of the cytoplasm into two ventral dendrites (VMD) and VLD) and Golgi complexes (G) are seen entering the basal portion of the denrites. Centriole (Ce) is located approximately mid-way betwen the two processes. x8,000

Fig. 54. Stage 35/36. Cilium in a Mauthner's cell. x30,000

Fig. 55. Stage 35/36. Nucleolus (Nl) with less dense core containing a central density. x20,000


VMD
VMD
F
53


N
54


NU
55

PLATE XXVI

Fig. 56. Stage 37/38. Lateral cytoplasm with filamentous patches and granular endoplasmic reticulum branching from the nuclear membrane and from other GER cisternae. x30,000

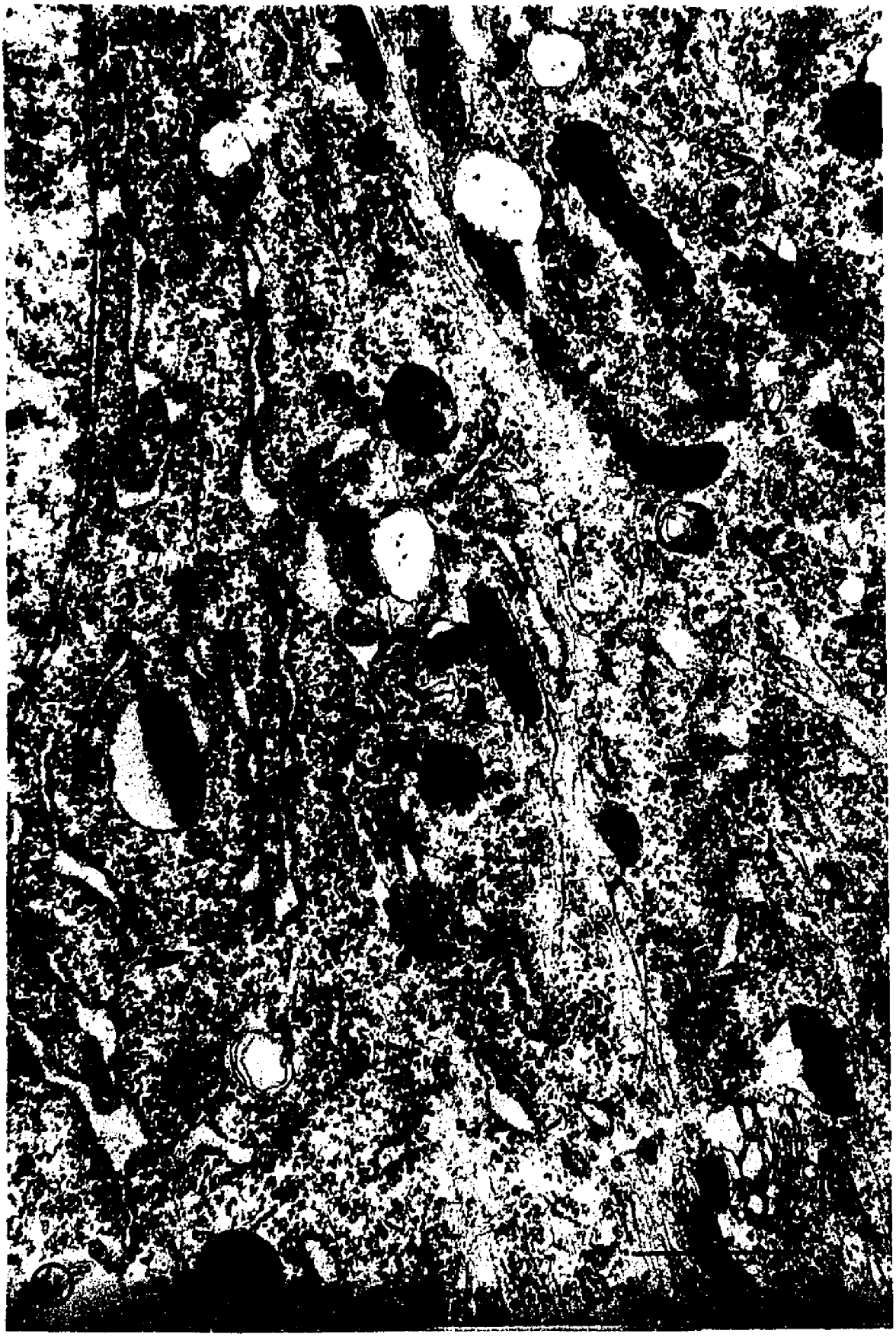


PLATE XVII

Fig. 57. Stage 40. Ventral cytoplasm. Coated vesicle (DV), dense body (DB), filaments (F), vesicles (V), microtubules (T). x11,800


F
F

PLATE XXVIII

Fig. 58. Stage 41. Initial segment of axon (Ax) with prominent microtubules (T), neurofilaments (F), and large agranular vesicles (V). x25,000


Ax

PLATE XXIX

Fig. 59. Stage 42. Origin of the ventro-lateral dendrite (LD) containing dense bolies (DB), many vesicles (V), and multivesicular bodies (VB). x17,000


F
M
MVB
F

PLATE XXX

Fig. 60. Stage 41. Nucleus of an M cell fixed in osmium tetroxide. Not: pale nucleus (N) and vague outline of nucleolus (Nl). x12,500

Fig. 61. Stage 41. Medial edge of M cell with a few hazy fibrous patches (F) and atypical neurites. Fixed in osmium tetroxide. x22,000


11

PLATE XXXI

Fig. 62. Stage 46. Survey. Note large medial vacant area (*) and swollen mitochondria (M). x4,000

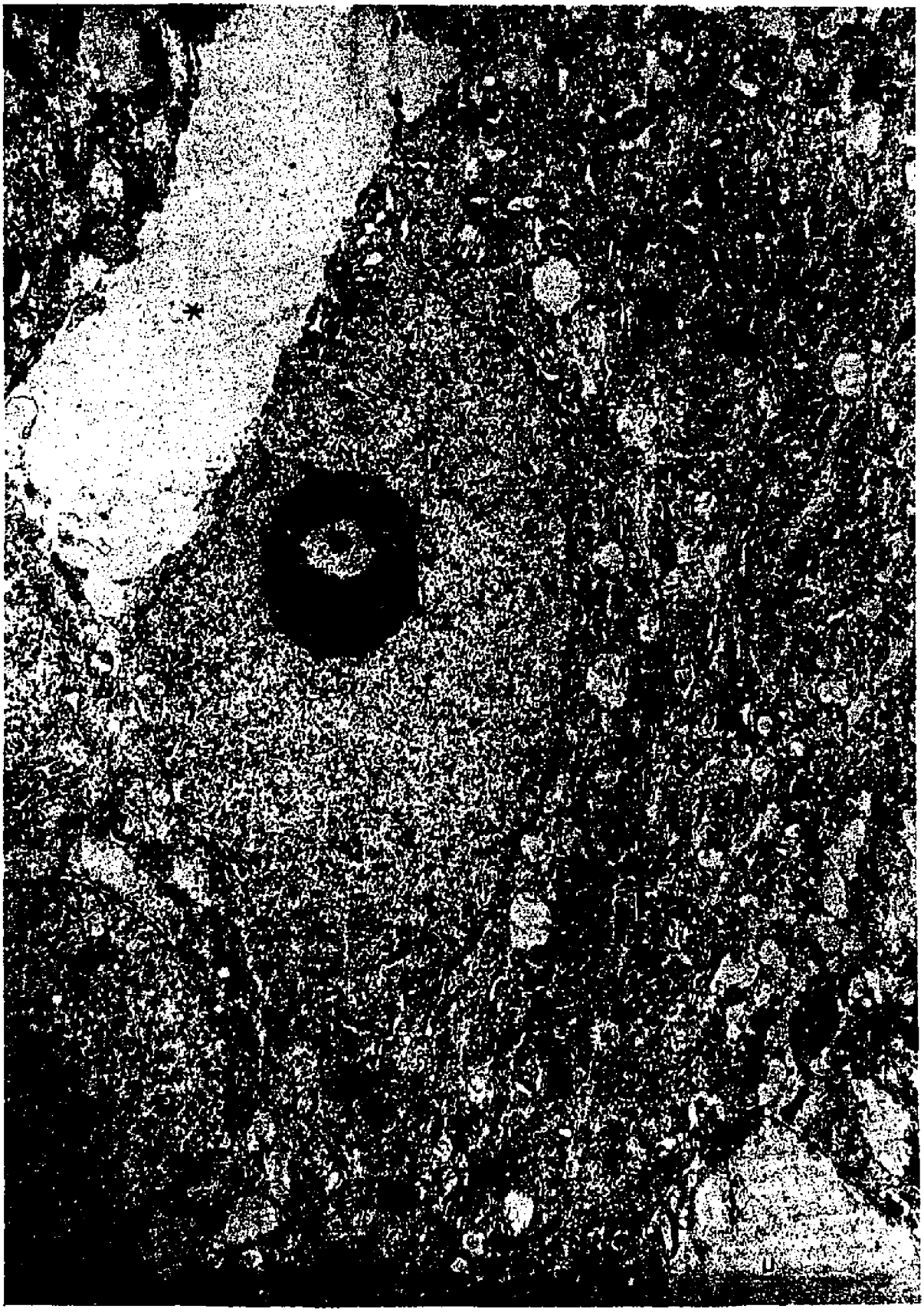


PLATE XXXII

Fig. 63. Stage 46. Lateral edge of nucleus, clearly defined filamentous areas (F), and swollen mitochondria (M). x33,000


F
F
M
N
F
M
F
93

PLATE XXXIII

Fig. 64. Stage 47. Survey of M cell fixed simultaneously with glutaraldehyde and osmium tetroxide. Note the distinct fibrous (F) and membranous regions. The latter are composed of discrete units of granular endoplasmic reticulum (GER) and Golgi complexes (G). (*) medial region well preserved with this fixative.

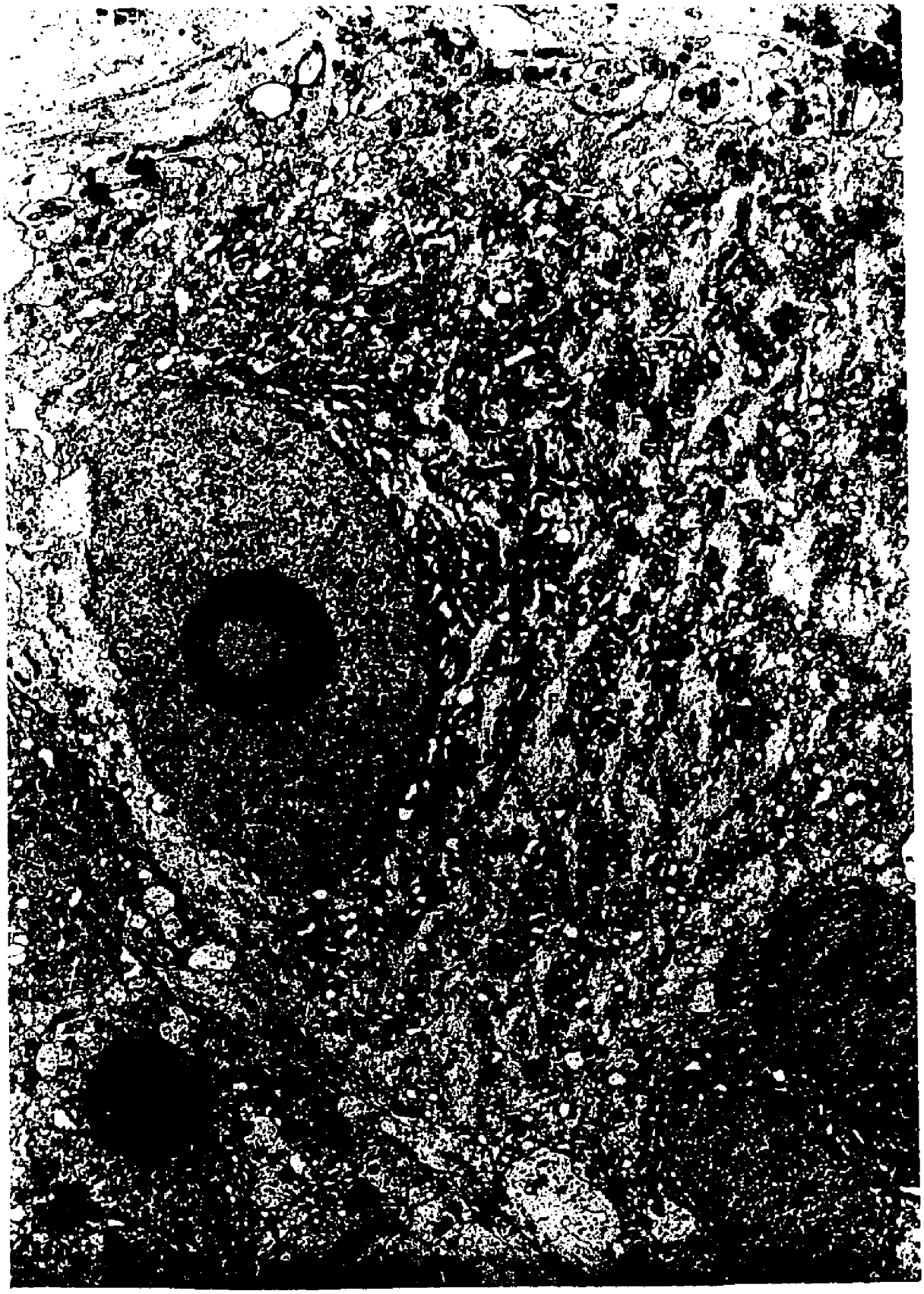


PLATE XXXIV

Fig. 65. Stage 47. Ventral part of M cell showing axon, ventro-medial dendrite and ventro-lateral dendrite. x5,000

Inset. Microtubular bundles corresponding to T in axon. x20,000

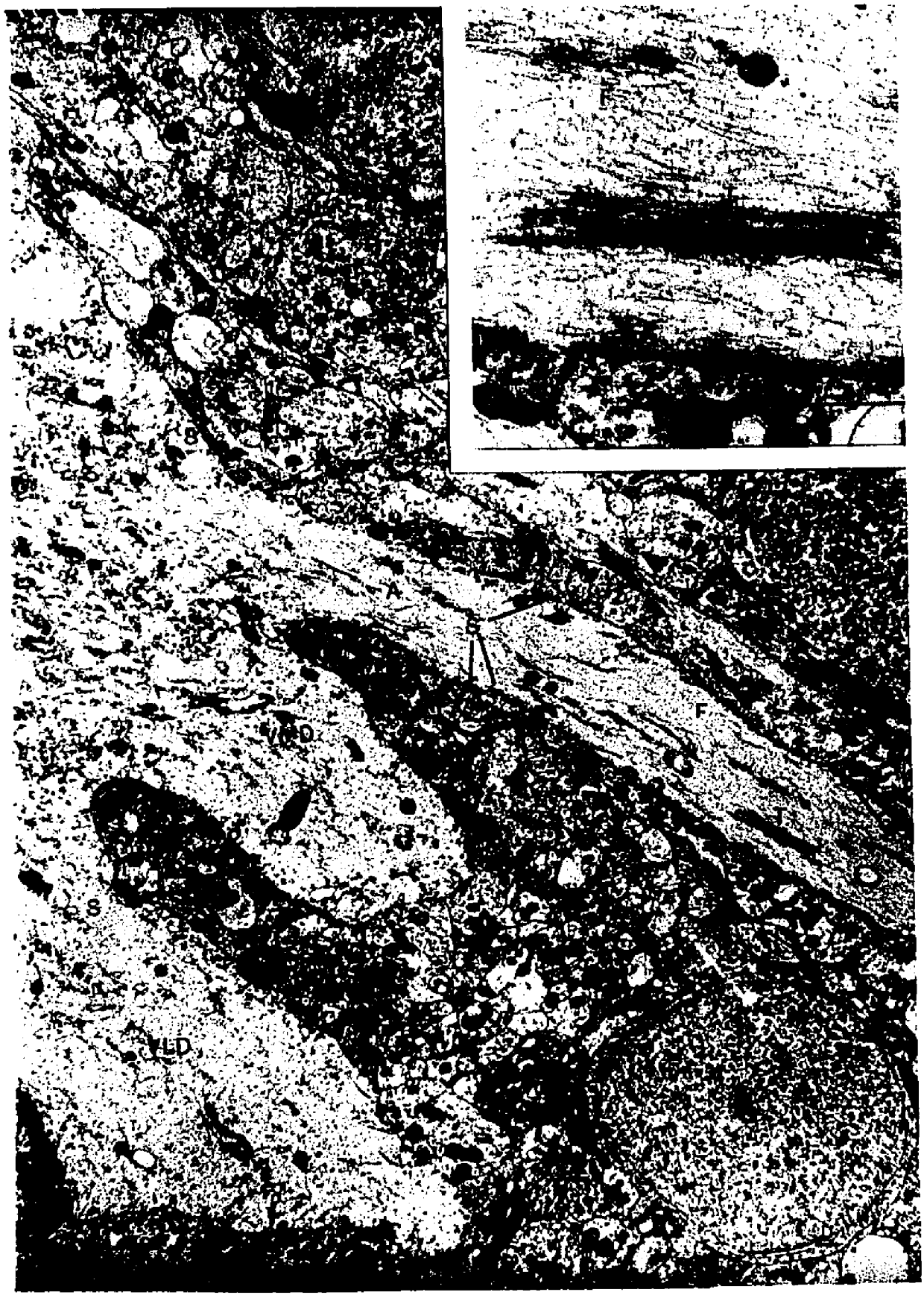

B
A
F
VLD
S
VLD

PLATE XXXV

Figures 66 to 69. Various types of synapses found on the surface of the M cell.

Fig. 66. Stage 35/36. Synapse on dendrite. x20,000

Fig. 67. Stage 37/38. Synapse on ventro-lateral border of perikaryon, near axon hillock region. x24,000

Fig. 68. Stage 47. Synapse on medial surface of cell body, simultaneous fixation with glutaraldehyde and osmium tetroxide. x24,000

Fig. 69. Stage 46. Synapse on medial surface, glutaraldehyde followed by post fixation in osmium tetroxide. x63,000

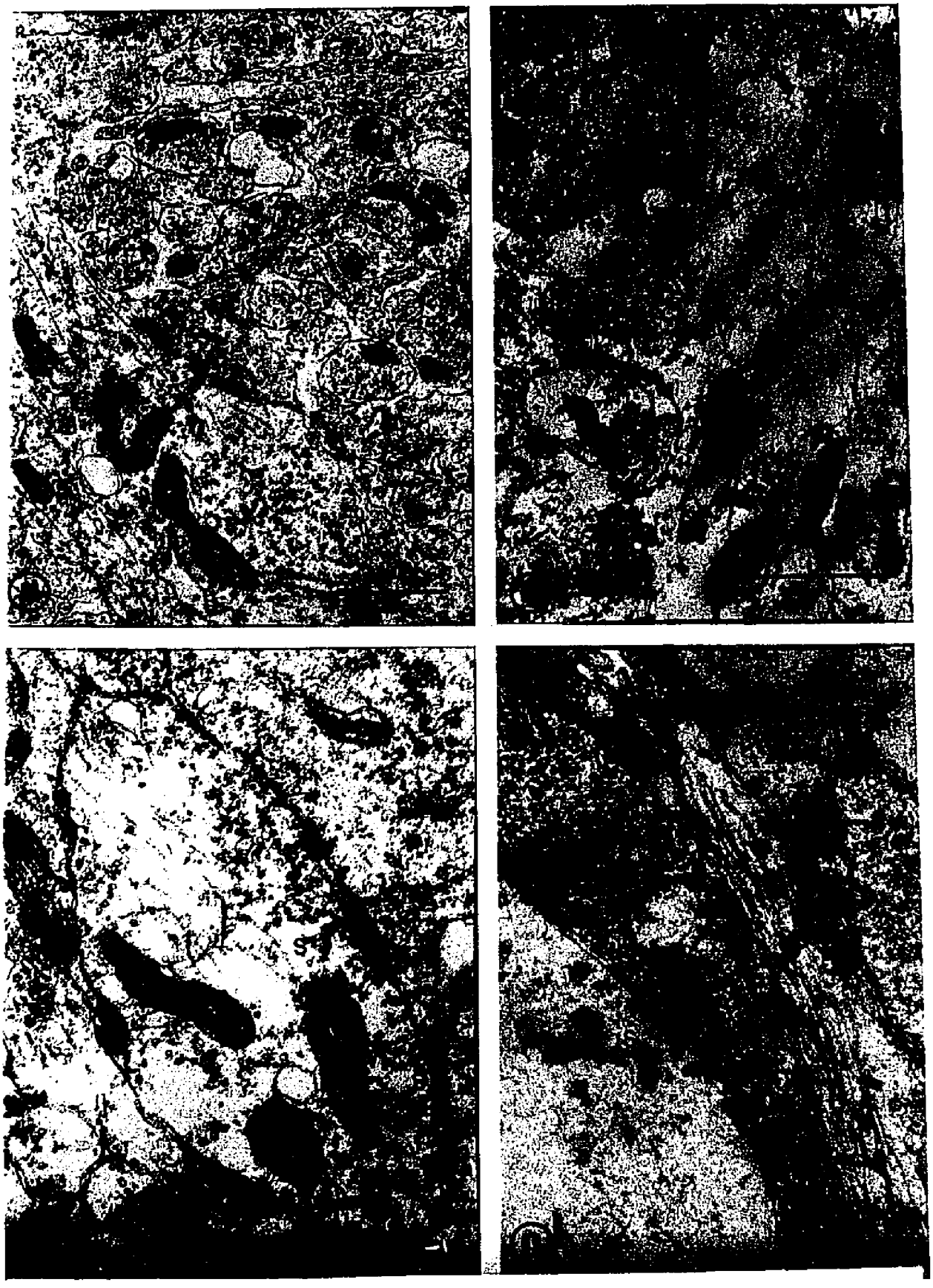

S

PLATE XXXVI

Fig. 70. Stage 70. M axon (Ax) Node of Ranvier (RN), simultaneous glutaraldehyde and osmium tetroxide fixation,. neurofilaments (F), microtubules (T). 30,000

PLATE XXXVII

Fig. 71. Stage 47. Ventral dendrite with large neurofibril population (F) and a few microtubules (T), simultaneous glutaraldehyde and osmium tetroxide fixation. x14,000

PLATE XXXVIII

Fig. 72. Stage 52. Dense bodies (DB) and Golgi complex (G) in cytoplasm of M cell. Glutaraldehyde and osmium simultaneous fixation. x31,000


F
DE
D
U

PLATE XXXIX

Fig. 73. Stage 52. Same M cell as in Fig. 72, dorsal dendrite, with numerous neurofilaments (F) and microtubules (T). x19,800

PLATE XL

Fig. 74. Stage 55. Survey, not concentration of granular endoplasmic reticulum around lateral part of the nucleus (GER), strands of medial cytoplasm still attached to plasma membrane in medial clear area (*). Fixed in acrolein, glutaraldehyde and post-fixed in osmium tetroxide. x7,800

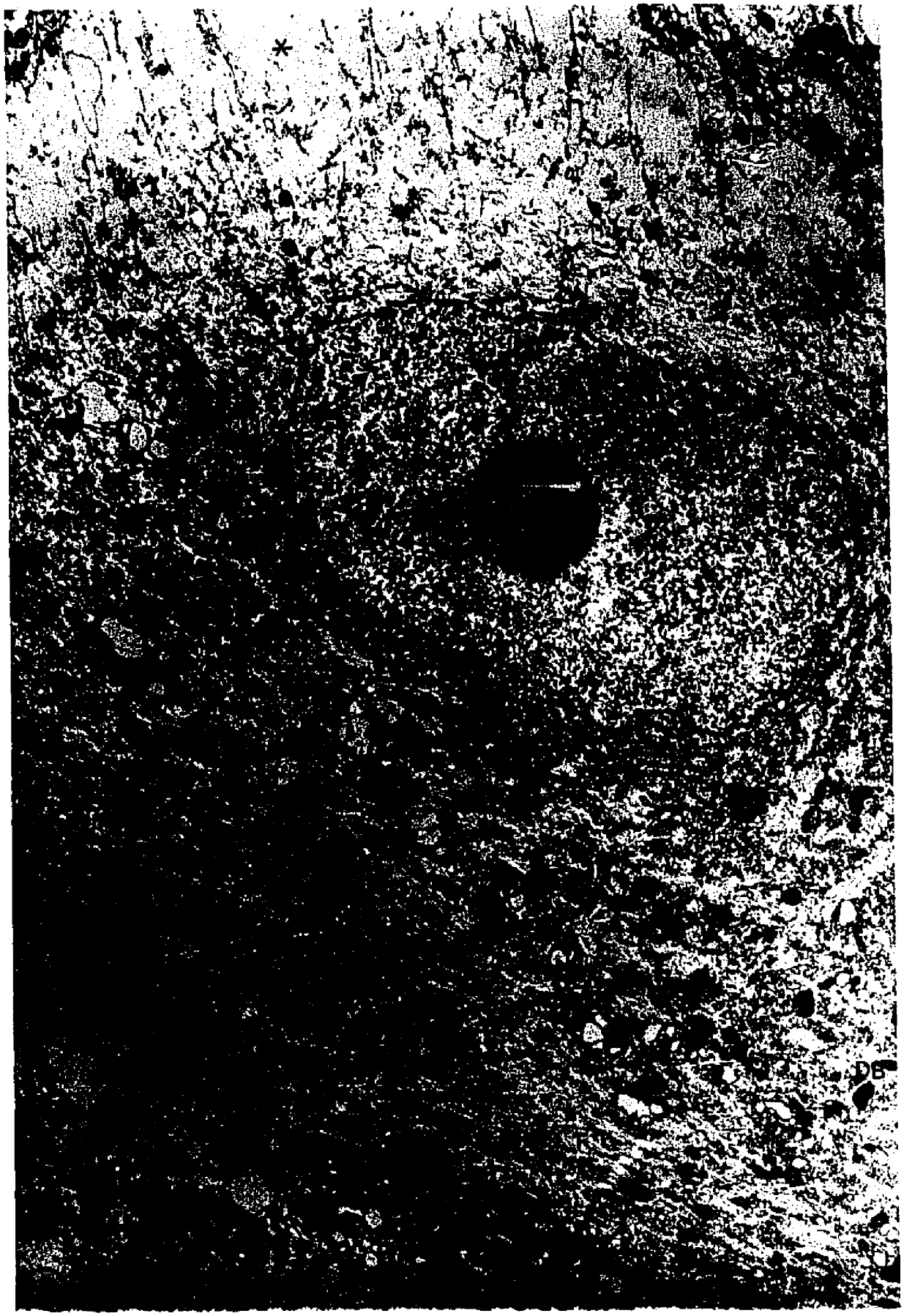

*
DB

PLATE XLI

Fig. 75. Stage 55. Ventral cell body at the base of the dendrite, simultaneous fixation in glutaraldehyde and osmium tetroxide. x28,000


DB
DB
F
F
G
15

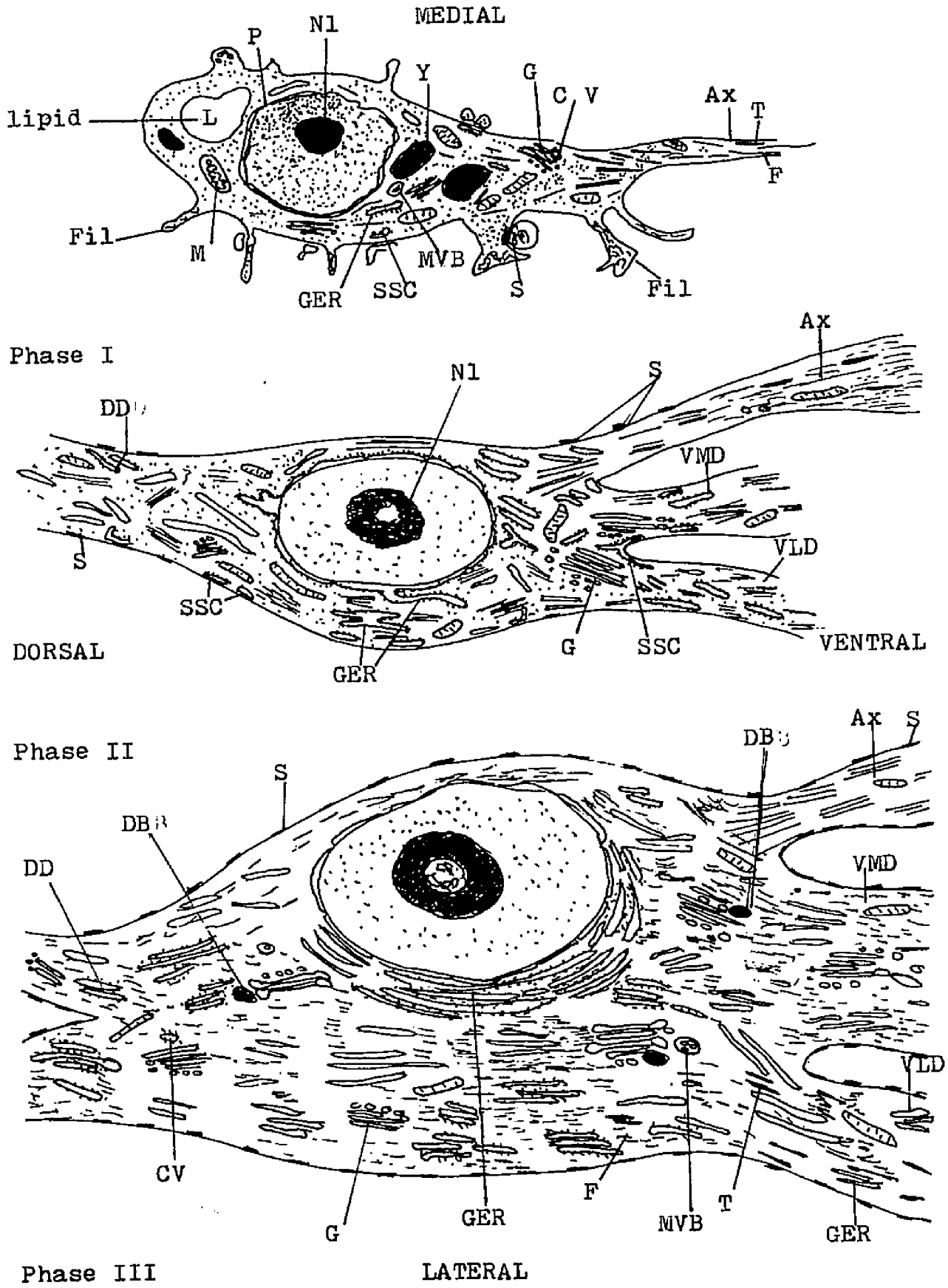
SUMMARY OF DEVELOPMENTAL PHASES IN MAUTHNER'S NEURON

Ax	axon	M	mitochondrion
CV	coated vesicle	MVB	multivesicular body
D	dendrite	N	nucleus
DB	dense body	Nl	nucleolus
DC	dense cored vesicles	Po	nuclear pore
F	filaments	S	synapse
Fil	filopodia	SSC	subsurface cisternae
G	Golgi complex	T	microtubules
GER	granular endoplasmic reticulum	VLD	ventro-lateral dendrite
		VMD	ventro-medial dendrite
L	lipid	Y	yolk

Phase I Stage 28 - presence of axon

Phase II Stage 33/34 - appearance of dendrites

Phase III Stage 55 - period of active growth


MEDIAL
P
N1
Y
G
C
V
Ax
T
F
lipid
L
Fil
M
MVB
GER
SSC
S
Fil
Phase I
Ax
N1
S
DD
VMD
VLD
S
SSC
DORSAL
G
SSC
VENTRAL
GER
Phase II
Ax
S
DB
S
DB
DD
VMD
VLD
CV
G
GER
F
MVB
T
GER
Phase III
LATERAL

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