DISTRIBUTION OF PHOSPHATASES IN THE GOLGI REGION AND ASSOCIATED STRUCTURES OF THE THORACIC GANGLIONIC NEURONS IN THE GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS*

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ABSTRACT

The neuronal perikarya of the grasshopper contain sudanophilic lipochondria which exhibit an affinity for vital dyes. These lipochondria are membrane-delimited and display acid phosphatase activity; hence they correspond to lysosomes. Unlike those of most vertebrates, these lysosomes also hydrolyze thiamine pyrophosphate and adenosine triphosphate. Like vertebrate lysosomal "dense bodies," they are electron-opaque and contain granular, vesicular, or lamellar material. Along with several types of smaller dense bodies, they are found in close spatial association with the Golgi apparatus. The Golgi complexes are frequently arranged in concentric configurations within which these dense bodies lie. Some of the smaller dense bodies often lie close to or in association with the periphery of dense multivesicular bodies. Further, bodies occur that display gradations in structure between these multivesicular bodies and the dense lysosomes. Acid phosphatase activity is present in the small as well as the larger dense bodies, in the multivesicular bodies, and in some of the Golgi saccules, associated vesicles, and fenestrated membranes; thiamine pyrophosphatase is found in both the dense bodies and parts of the Golgi complex. The close spatial association of these organelles, together with their enzymatic similarities, suggests the existence of a functional or developmental relationship between them.

INTRODUCTION

Baker (6) originally used the term lipochondrion in 1950 to identify spheroidal or ovoid cytoplasmic bodies that are sudanophilic, show an affinity for vital dyes, and give a positive response to the acid hematein test for phospholipids (7). This term occurs frequently in the literature dealing with invertebrate neurons, including a number of studies made on the cytoplasmic organelles of orthopteran nerve cells (1, 9, 19, 31, 32, 48, 49, 60–62). Recent electron microscopical studies have re vealed that the lipochondria in many invertebrate neurons are membrane-bounded and often have a characteristic granular and lamellar structure (3,

12, 13, 34, 36), each lamella being as wide as a phospholipid membrane (7). The possibility that lipochondria correspond to lysosomes was suggested by the demonstration, in molluscan neurons (24, 26, 27, 36), that they contained reaction product for acid phosphatase activity.

A few ultrastructural studies have been made previously on the abdominal, thoracic, or cerebral ganglia of orthopteran insects, including *Melanoplus* (10), *Periplaneta* (20, 63, 64), *Blaberus* (71), *Leucophaea* (51), and *Laplatacris* (67). These studies show that the lipochondria are quite distinct from the Golgi lamellae or saccules. However, in a fine

structural study of *Locusta* neurons, Ashhurst and Chapman (3) considered that both organelles were a form of lipochondrion and that artificial disruption produced concentrically arrayed saccules resembling Golgi membranes; a rather similar situation in the neurons of the snail *Helix* (12) had been described earlier.

Unlike those of molluscans, the neurons of orthopterans have not been extensively studied for the intracellular distribution of phosphatases. Indeed, the only cytochemical investigation to determine the localization of such enzymes in orthopteran neurons has been a light microscopical examination of *Locusta migratoria* (30). In this study (30) it was concluded that acid phosphatase activity was present, not in the lipochondrial dense bodies, but in the concentric lamellar arrays described by Ashhurst and Chapman (3) as lipochondria "resembling Golgi bodies." The smaller, dense lipochondria, on the other hand, were found to hydrolyze thiamine pyrophosphate, a substrate *not* split by the concentric lamellar arrays. In vertebrate neurons, as well as in many other vertebrate cell types, thiamine pyrophosphatase is highly concentrated in the saccules of the Golgi apparatus (40); it is also so localized in the spermatogonia and the neurons of the snail *Helix* (35, 36). Since the distribution of neither TPPase[1] nor acid phosphatase in locust neurons was the same as in vertebrate or *Helix* neurons, it was concluded that these cytoplasmic organelles in orthopteran neurons were in some respects quite different from those in the nerve cells of other animals (7, 30).

Cytochemical tests for phosphatases can be readily applied at the level of electron microscopy; the results of such tests on orthopteran neurons would make apparent the similarities and differences in enzyme constitution between the lipochondria and Golgi elements. Moreover, such results would also indicate whether these nerve cells differ significantly from those of other animal groups with respect to their intracellular phosphatase distribution. The American genus *Melanoplus* was used in this study because living *Locusta*

[1] The following abbreviations are used throughout this paper: TPP, thiamine pyrophosphate; TPPase, thiamine pyrophosphatase; ADP, adenosine diphosphate; ADPase, adenosine diphosphatase; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; CMP, cytidine monophosphate; CMPase, cytidine monophosphatase.

can be neither collected nor imported in the United States.

This study of the structural features of grasshopper neurons in combination with their intracellular distribution of phosphatases indicates that the lipochondria contain acid phosphatase as well as TPPase and other phosphatases active at neutral pH. Thus the lipochondria are identifiable, like those of molluscan neurons, as lysosomes. Moreover, the Golgi saccules do contain TPPase activity, in addition to acid phosphatase activity, although reaction product for the latter appears more abundant than that for the former under the conditions of these experiments.

A report on this work has been published previously in abstract form (25).

MATERIAL AND METHODS

Adult specimens of *Melanoplus differentialis* (Insecta; Orthoptera) were employed in this study. In a few experiments, adults of another species, *M. femurrubrum*, were used. The animals were maintained in screened cages at approximately 75–80°F and were fed dandelion leaves, spinach, or wheat seedlings; a plentiful supply of water was provided.

The insects were decapitated, the thoracic wall was slit dorsally, and the ventral nerve cord was exposed. As they were dissected out, the thoracic ganglia were covered with cold 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 (56). They were then placed in 2% glutaraldehyde at 4°C for a total fixation period of 60 min. After fixation they were washed overnight at 4°C in 0.1 M cacodylate buffer with added 0.2 M sucrose. For light microscopy, sections were cut on the freezing microtome at 10 μ, or cut unfrozen by hand, and tested for the following enzyme activities: (a) Acid phosphatase, at pH 5.0 by the Gomori (18) procedure by using as substrate sodium β-glycerophosphate, thymidylic or cytidylic acid[2] (39), or AS-TR phosphate[2] (8); (b) thiamine pyrophosphatase or adenosine diphosphatase[2] at pH 7.2, according to the method of Novikoff and Goldfischer (40); and (c) adenosine triphosphatase[2] at pH 7.2 by Wachstein and Meisel's technique (69). Incubation was carried out at 37°C for periods ranging from 5 to 60 min. The sections were subsequently washed in water, and their sites of enzymatic activity were visualized with ammonium sulphide; they were then mounted with glycerogel on glass slides. Control sections, otherwise treated similarly, were incubated in media without substrate. Ganglia fixed in cold formaldehyde-calcium (4) were postchromed and embedded in gelatine; the gelatine

[2] Substrates obtained from the Sigma Chemical Company, St. Louis, Mo.

blocks were sectioned at 10 μ, and the sections were stained for 5 min with Sudan black B for lipids (5). Isolated, unfixed neurons were treated for 10 min with 0.01% neutral red to study the sites of uptake of this vital dye.

For electron microscopical examination, 40-μ sections, cut on the freezing microtome or by hand, were tested for acid phosphatase, TPPase, and ATPase in substrate media with added 5% sucrose, and then washed in 7.5% sucrose and postosmicated for 60 min. In a few cases they were treated briefly with a dilute ammonium sulphide solution in 7.5% sucrose before postosmication. These sections were then dehydrated in a graded series of ethanols followed by propylene oxide and embedded in Araldite. Thin sections were cut on a Porter Blum MT-2 ultramicrotome and stained in uranyl acetate (70) or lead citrate (52). Grids were examined in a Philips EM 200 at 60 kv. Control sections, incubated in substrate-free media, were prepared for electron microscopy in the same way as described above.

For the study of unincubated tissue, ganglia were cut into several pieces and fixed in 1% Veronal-buffered osmium tetroxide for 60 min or in 2% glutaraldehyde for 60 min followed by postosmication. The pieces were washed, embedded, sectioned, and examined as described above for the incubated sections.

OBSERVATIONS

Like those of other orthopterans (63), the thoracic ganglia of *Melanoplus* are ensheathed in a collagenous acellular neural lamella which overlies a perineurium (28). Beneath these sheaths lie the ganglia in which peripheral neurons encapsulated by glial cells surround a central neuropile of axonal processes. The neurons are relatively large cells, round or ovoid in outline. The encompassing glial cells, on the other hand, are attenuated and send thin processes into the peripheral cytoplasm of the larger neurons (Fig. 1). Details of the fine structure
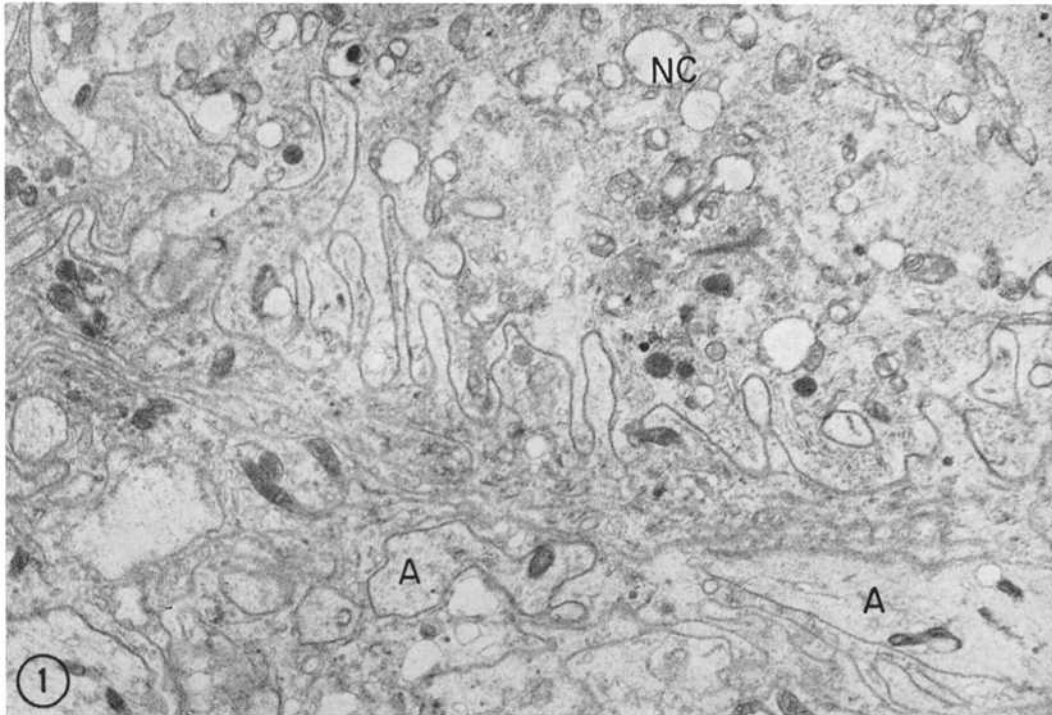
All the electron micrographs shown in these figures are of the thoracic ganglion of the grasshopper *Melanoplus differentialis*; the ganglia have been fixed in cacodylate-buffered glutaraldehyde, postosmicated, and embedded in Araldite.

FIGURE 1   Low-power view of the peripheral cytoplasm of a large nerve cell (*NC*) into which processes of the ensheathing glial cells project. Some of the mitochondria in the neuronal perikarya appear hollow, an artifact typical of glutaraldehyde-fixed tissue. Sections of axons (*A*) can be seen beneath the neurone which are also surrounded by glial cells; these axons contain mitochondria and microtubules. × 9,000.
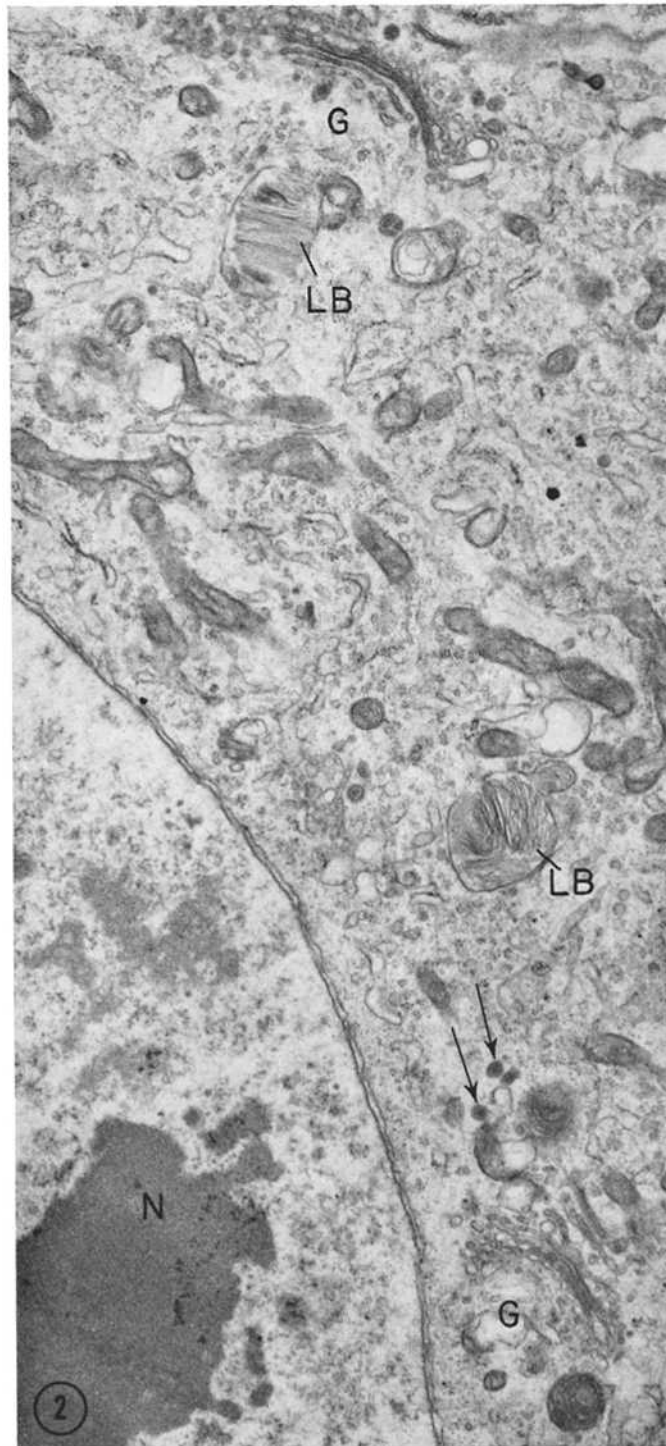
FIGURE 2   Part of a nerve cell body containing a nucleus which displays a prominent nucleolus (N). The cytoplasm contains several Golgi complexes (G) and two lamellated lysosomal bodies (LB). Small dense granules (arrows) are also present. × 20,000.
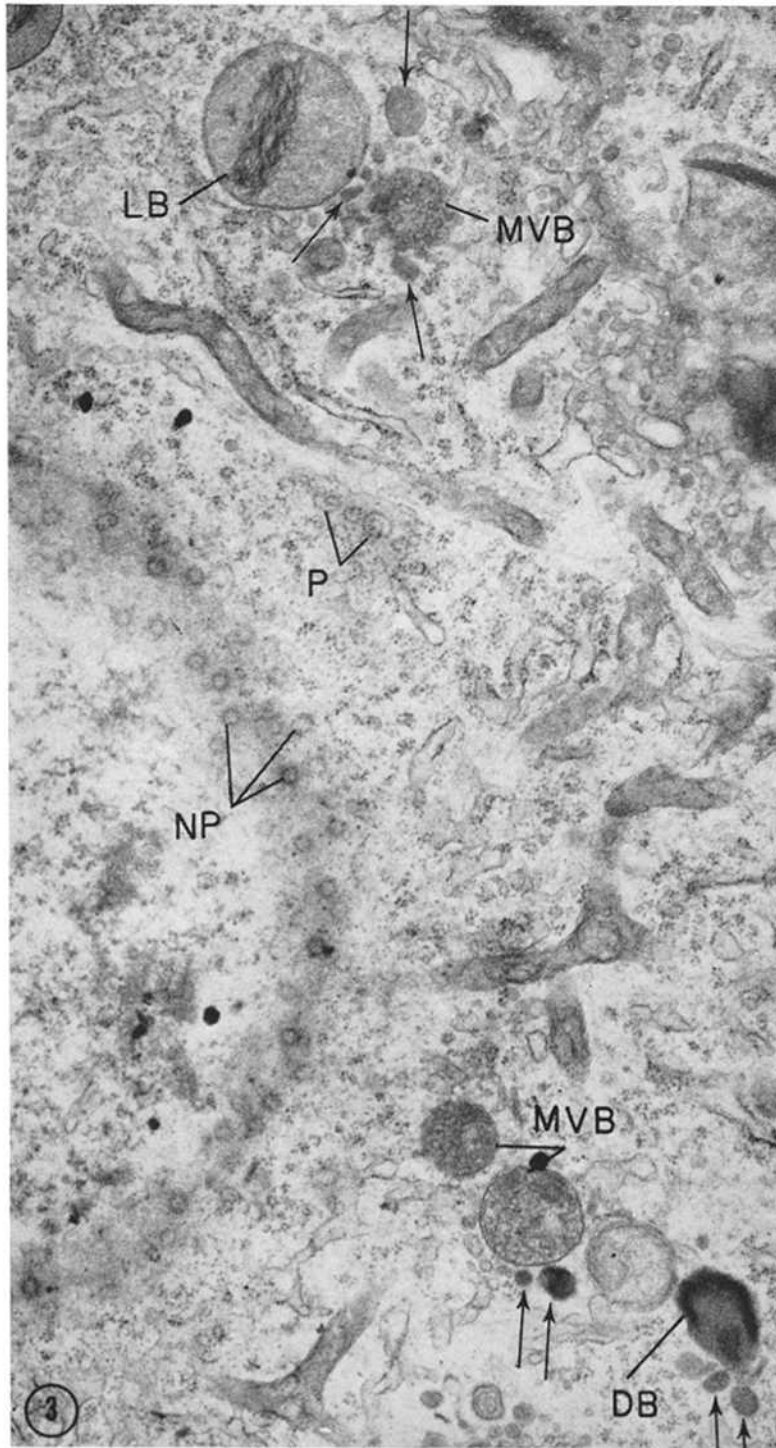
FIGURE 3 Perinuclear region of a neurone, showing nuclear pores (NP) and nearby polyribosomal configurations (P). Lysosomes of various sorts are present including a large dense body (DB), multivesicular bodies (MVB) (of which the one nearest the bottom may be intermediate in form between a dense body and a dense multivesicular body), a lamellated body (LB), and small dense bodies or granules (arrows) which are often in close spatial association with the multivesicular bodies. × 27,000.
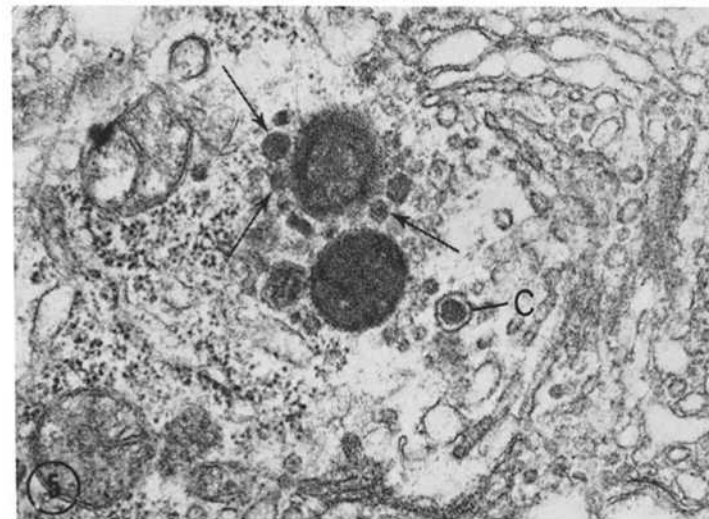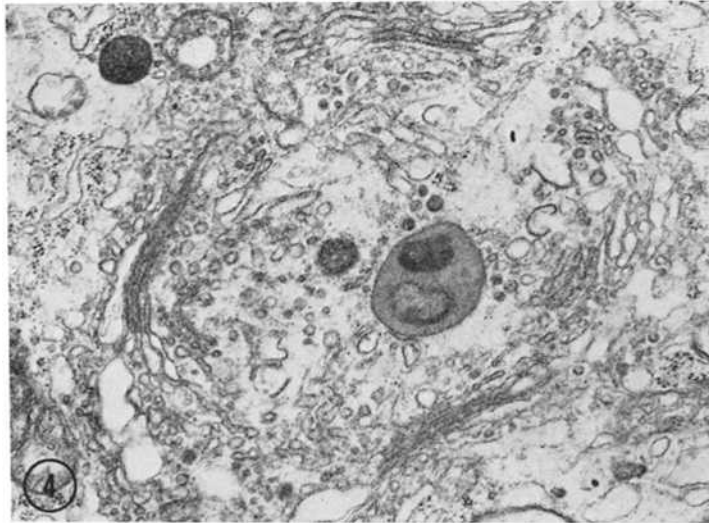
FIGURE 4   Golgi complex showing several stacked saccules encircling a dense body and a multivesicular body; other multivesicular bodies lie on the outer border of the saccules. × 25,000.

FIGURE 5   Several dense bodies in the center of a field of Golgi saccules; their structure suggests that they may have originally been multivesicular bodies. Note the presence of smaller electron-opaque bodies (arrows) around them as well as a granule-containing vesicle (C) with a clearly separated outer membrane. × 37,500.

of the neuroglial cells, with particular reference to the intracellular distribution of phosphatases, are described elsewhere (28).

Morphological Features

The nucleus of each neuron contains one or several nucleoli of varying shapes (Fig. 2) which are composed of granular and fibrillar material.

The chromatin is diffusely dispersed throughout the nucleus and shows occasioral clumping in regions near the nuclear envelope.

The endoplasmic reticulum is composed of vesicles and cisternae which usually have a random distribution, although occasionally the cisternae are regularly arranged in parallel arrays. Spiral configurations of ribosomes, suggesting the pres-
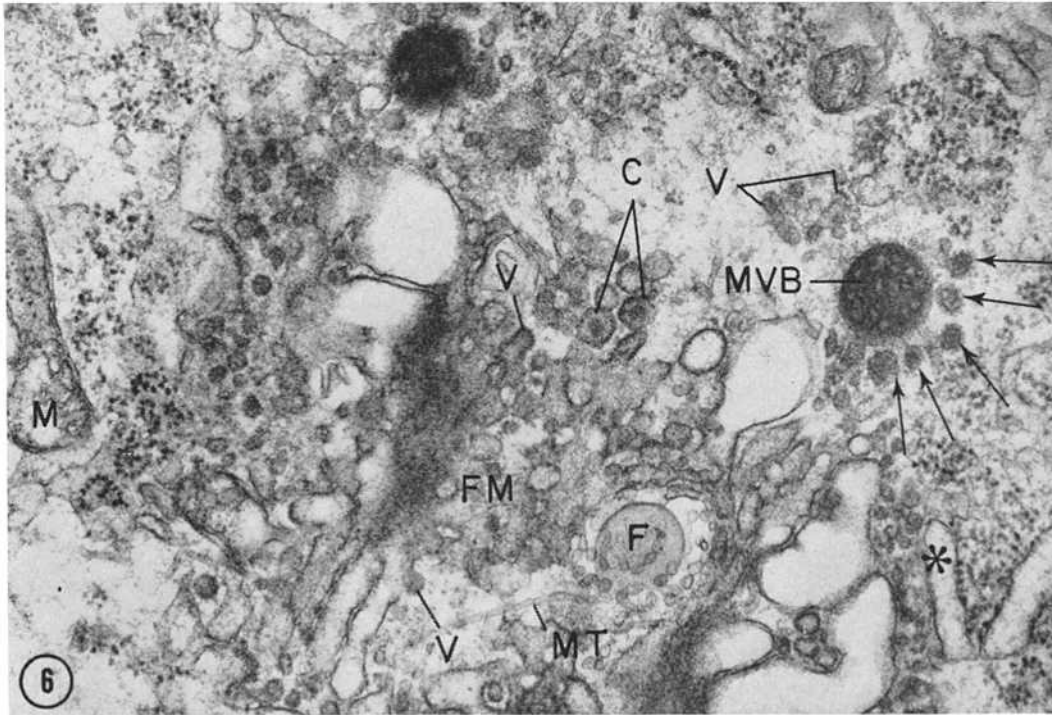
FIGURE 6 A Golgi region and associated structures. The area of fenestrated membranes (*FM*) may be a tangential section of a Golgi saccule, or part of the smooth endoplasmic reticulum. At the periphery of the agranular Golgi membranes, note the rough endoplasmic reticulum, particularly the cisterna (*) with one rough and one smooth surface, the latter facing the Golgi saccules. Small vesicles lie between this cisterna and the dilated Golgi saccules. Small dense bodies and dense coated vesicles are present (arrows) around the periphery of a dense multivesicular body (*MVB*). Other coated vesicles (*V*) may be seen as "buds" in association with the fenestrated membranes. The body at *F* may represent a multivesicular body in the process of forming. *MT*, microtubule; *M*, mitochondrion; *C*, granule-containing vesicles. × 49,000.

ence of polyribosomes, frequently occur (Fig. 3). Cisternae are sometimes found that have one rough and one smooth border, the latter facing the outer border of a region of stacked Golgi saccules (Fig. 6). Small smooth vesicles that may be derived from the smooth border of the cisternae lie between the cisterna and the Golgi saccules.

There are numerous Golgi complexes scattered throughout the cytoplasm (Figs. 2 and 4–7). Unlike the diffusely distributed Golgi complex of vertebrate neurons, each of these complexes has a "dictyosome"-like[3] structure and is composed of

curved stacks of Golgi saccules; these stacks are found as single units[4] or in concentrically arranged groups of two to three. In the latter, the concave curved border of each stack of saccules faces the center of the circle formed by the group (Fig. 4). The component saccules sometimes have dilated lateral edges which may be an artifact of fixation. Golgi vesicles and larger vacuoles occur alongside these lateral edges. A region of fenestrated membranes is associated with the inner face of the Golgi complex (Fig. 6) and may be an integral

[3] The term dictyosome has been used in the past to refer to the scalelike or crescent-shaped objects seen scattered through invertebrate cells under the light microscope, particularly after metallic impregnation procedures. The term suggests that each dictyosome

is a separate entity, but the evidence for this is equivocal.

[4] The "single" unit of stacked Golgi saccules may only appear to be an individual structure due to the plane of sectioning; each unit may actually form part of a concentrically oriented *group* of units.
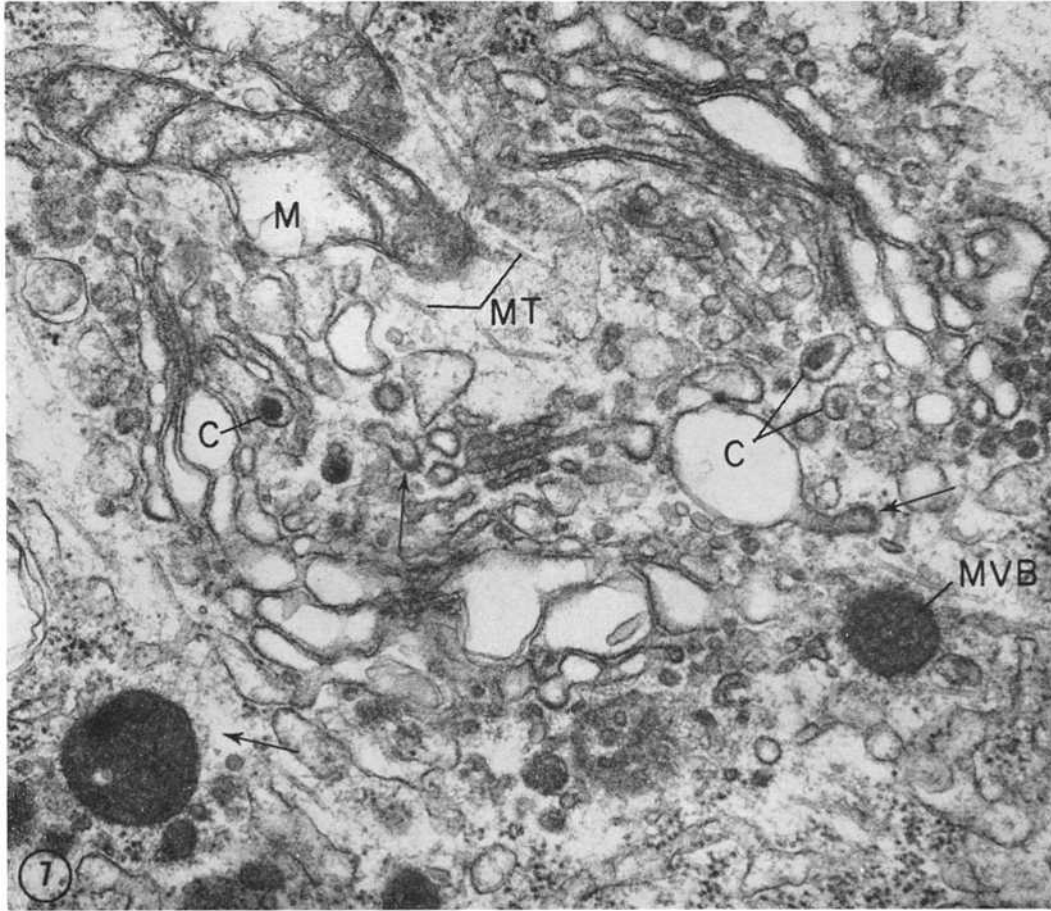
FIGURE 7   Golgi region and associated structures. Golgi saccules and granule-containing vesicles (C) are present. Note the opaque multivesicular body (MVB) as well as dense bodies of various sizes, the largest of which may have developed from a multivesicular body. Coated vesicles are in connection (arrows) with the region of fenestrated membranes. The mitochondrion (M), like that in Fig. 6, contains a number of small particles aligned along the cristae and internal membranes. These particles resemble those described in the mitochondria of cells in other insects (2) and in gastropods (27, 66); it has been tentatively suggested by Ashhurst (2) that they might be particles concerned with electron transport. MT, microtubule. × 49,000.

part of the complex, although alternately it could be part of the smooth endoplasmic reticulum, as it is in the Golgi–endoplasmic reticulum–lysosomal (GERL) region of rat spinal ganglionic neurons (44). These membranes often have swollen bud like edges that have a coated appearance similar to that of the coated or alveolate vesicles (Fig. 7).

A number of different sorts of spheroidal bodies are associated with the Golgi regions; five of them are sufficiently distinct to warrant description. *Dense bodies* are frequently found lying close to or

within a circular area surrounded by Golgi saccules (Figs. 4 and 5). The dense bodies are spheroidal and range in diameter from 0.2 to 1 μ; they are delimited by a single unit membrane (Fig. 13) and contain dense lamellar, granular, or vesicular inclusions (Figs. 2–4 and 13). The larger bodies appear to correspond in size and distribution to sudanophilic granules, the lipochondria, that may be seen scattered through the cytoplasm in light microscopical preparations. Similarly there is a correspondence between these bodies and the
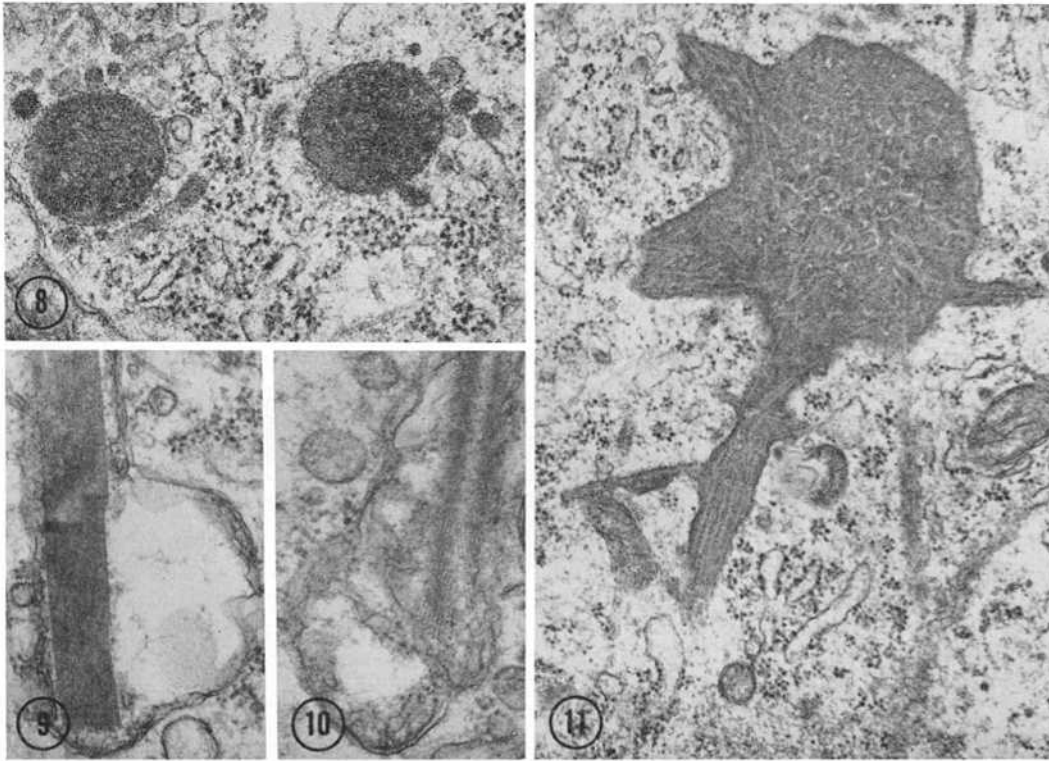
FIGURE 8 Cytoplasm of a nerve cell containing two dense multivesicular-like bodies. These are partially surrounded by small electron-opaque granules which, in some cases, are in connection with the outer membranes of the larger bodies. These bodies appear intermediate in appearance between multivesicular bodies and dense bodies. × 44,000.

FIGURES 9 and 10 "Crystalline" inclusions found in the neuronal cytoplasm. These are surrounded by membranes which bear some resemblance to those of the mitochondria. Fig. 9, × 45,000; Fig. 10, × 59,000.

FIGURE 11 Portion of a neurone containing a large cytoplasmic inclusion partially composed of microtubule-like structures. × 32,000.

vitally colored granules observed in neurons after treatment with neutral red. In addition, however, neutral red induces a faint pink staining in the larger cytoplasmic bodies whose form resembles that of the Golgi dictyosomes.

Multivesicular bodies are also present with a distribution similar to that of the dense bodies. However, as their name implies, they contain vesicles, not lamellae (phospholipid?) such as characterize lipochondria ultrastructurally; in this sense they do not correspond to the typical lipochondrion as defined by Baker (7), although in certain other regards (vital dye uptake, reference 54; phosphatase activity) they resemble them. Some of the multivesicular bodies encountered are like those that have been described in the neurons of other

species (as in reference 20). However, more frequently, they possess a fairly dense background substance (Figs. 3, 6, and 7). In addition, bodies occur which show various degrees of gradation between multivesicular bodies and dense bodies proper (Figs. 3, 5–8).

The multivesicular bodies are often surrounded by much smaller *dense granules* (averaging about 0.1 μ in diameter) and *dense coated or alveolate vesicles* (about 50–80 mμ in diameter) which at times lie close to, or in direct contact with, multivesicular bodies (Figs. 3, 5, 6, and 8). The dense coated vesicles appear to arise by budding from the fenestrated membranes in the Golgi regions (Figs. 6 and 7), as does another type of inclusion, the *dense, granule-containing vesicles* (*GC vesicles*) (about

0.1 μ in diameter). These vesicles consist of a dense granule surrounded by a clearly separated outer membrane (Figs. 5–7).

Microtubules are often present in the Golgi region (Figs. 6 and 7) as well as elsewhere in the cytoplasm. They are, however, particularly evident in the clear patches between clumps of endoplasmic reticulum. In the perikarya they run in different directions, in contrast to those within the axonal processes which are all aligned in one direction along the length of the axon and parallel to the limiting plasma membrane.

In the cytoplasm of a few neurones, large bodies have been observed which are composed of tubules, possibly microtubules (Fig. 11). These bodies have an electron-opaque matrix in which granules occur as well as what may be cross-sections of tubules. They have an outline that is in some ways suggestive of a giant mitochondrion, but they contain no recognizable cristae. The neuronal cytoplasm also infrequently contains crystalline bodies delimited by a double membrane that may represent modified mitochondria (Figs. 9 and 10).

Most of the mitochondria proper are long and narrow and occasionally show branching. Some are spherical in outline, but these may be cross-sections through rod-shaped ones. Their cristae are often longitudinally oriented. Along the inner walls of the cristae, alignments of small, electron-opaque particles are sometimes found (Figs. 6 and 7).

Also present in the cytoplasm are structureless, moderately electron-opaque droplets; these lack a limiting membrane and at times display a crenated outline. They probably represent droplets of triglyceride.

Enzymatic Distribution

Control sections, incubated in media without substrate, contain no lead precipitate when examined by light or electron microscopy, except for the preparations incubated in acidic pH control media. Here a nonspecific precipitate, also present in the same place in substrate-incubated sections, may be found but only at the edge of the tissue sections. It may therefore be assumed that all the intracellular sites of reaction product in substrate-incubated sections represent genuine enzymatic activity.

At the light microscopical level, acid phosphatase activity is localized throughout the perikaryon in the form of scattered solid granules and

ring-like or dictyosome-like granules. The former have the same size and distribution as the sudanophilic lipochondria. Sections incubated in media at pH 7.2 with TPP, ADP, or ATP as substrate contain a number of scattered, phosphatase-rich granules similar to those seen in sections incubated for acid phosphatase. However, in sections treated with these substrates, particularly ATP, there seem to be relatively fewer active sites than in the sections incubated for acid phosphatase; also, the plasma membrane shows a positive reaction for TPPase and ATPase which is not present in acid phosphatase preparations.

At the ultrastructural level, incubation in media with any one of these substrates, CMP, TPP, or ATP, produces reaction product over the dense, spheroidal lipochondria (Figs. 12, 14–16). Since there seem to be no large dense bodies without the reaction product for acid phosphatase, these bodies all appear to be lysosomes; this includes those associated with the center of circular arrangements of Golgi complexes. Reaction product for acid phosphatase is also found in multivesicular bodies and in some of the smaller dense granules (Fig 14). These smaller granules appear to correspond to those indicated by arrows in Figs. 2, 3, and 5 and those shown at higher magnification in Figs. 8 and 13; most of them display depositions of reaction product in incubated preparations. Further, acid phosphatase is demonstrable in one or two of the inner Golgi saccules of which the innermost may in some cases be in the form of fenestrated smooth membranes, as well as in some of the Golgi-associated vesicles (Fig. 12). TPPase is also present in the Golgi apparatus (Fig. 15). However, the reaction product resulting from TPPase activity is somewhat lighter than that resulting from acid phosphatase activity; this might be owing to lower levels of activity or, more probably, could be owing to the fact that glutaraldehyde has a greater inhibitory effect on TPPase than on acid phosphatase (17). ATPase seems not to be associated with the Golgi complex (Fig. 16).

DISCUSSION

In this investigation the lipochondria found in the neurons of *Melanoplus* are identified as lysosomes on the basis of their cytochemically demonstrable acid phosphatase activity and by such fine structural features as their delimitation by a unit membrane and their electron-opaque matrix. In struc-
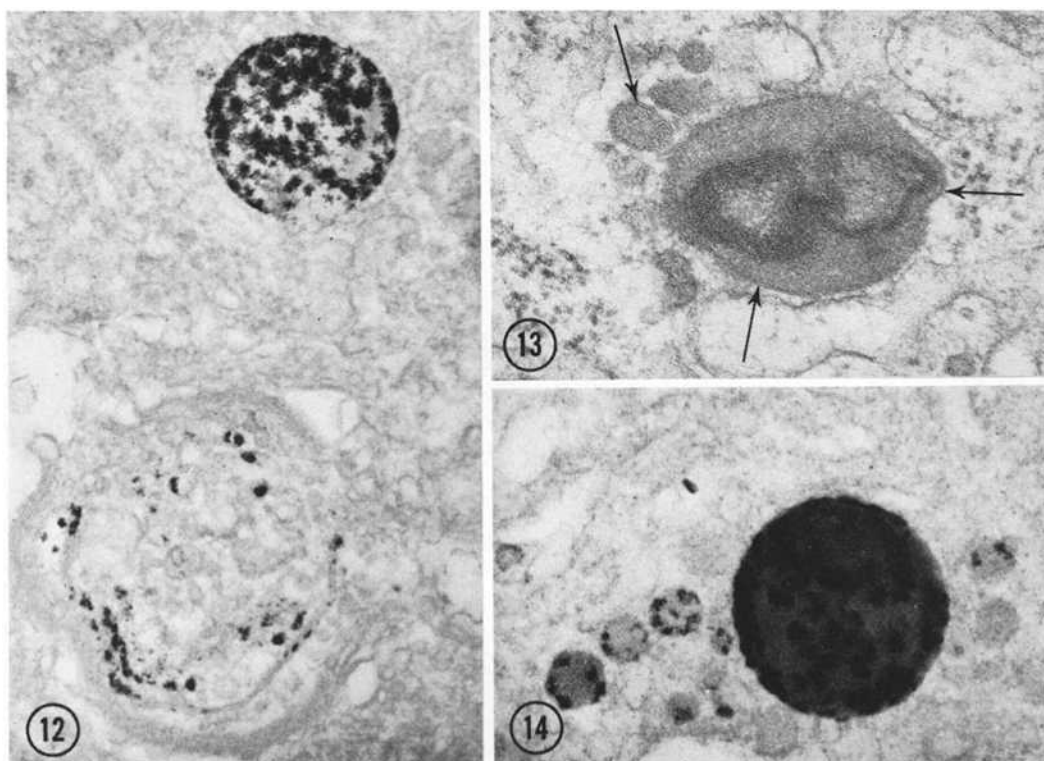
FIGURE 12 Section of a neurone incubated for acid phosphatase for 50 min, with CMP as substrate. Note the reaction product resulting from enzymatic activity in a dense body and several Golgi saccules with associated vesicles. × 42,000.

FIGURE 13 Unincubated section of a neurone showing a dense body with a partially lamellar matrix, surrounded by smaller dense granules. Note that all these structures are delimited by unit membranes (arrows). × 68,000.

FIGURE 14 Section of a neurone incubated for acid phosphatase for 60 min. Note dense bodies rather similar to those in Fig. 13. The reaction product can be seen to be present in both large and small dense bodies (lysosomes). × 52,000.

ture they markedly resemble the lysosomal dense bodies of vertebrate neurons (41). Earlier it had been suggested that the lipochondria or "cyto-somes" of certain other invertebrate nerve cells also correspond to lysosomes; such nerve cells include the neurons of gastropods (24, 26, 27, 36, 38) and annelids (55). Dense bodies containing acid phosphatase activity have also been reported to exist in insect cells other than neurons, such as cells in the salivary glands of metamorphosing larvae (37, 50, 58) and in prothoracic glands (46, 57).

The lysosomes in the neurons of *Melanoplus* have been found to hydrolyze a variety of substrates,

including not only a number of monophosphates at pH 5 but also nucleoside diphosphates and tri-phosphates at neutral pH. A fairly broad range of substrate specificity has also been observed in the lysosomes of the other invertebrate species thus far examined; these include the lysosomes in the cells of the prothoracic glands (46) and glial cells (28) of insects, the intestinal cells of the earthworm (Novikoff, A. B. Unpublished data.), and the cells of the intestine and digestive gland (Lane, N. J. Unpublished data.), as well as the neurons (24, 26, 27, 36), of gastropods. These cytochemical observations could be variously interpreted. The hydrolysis of the various substrates by the
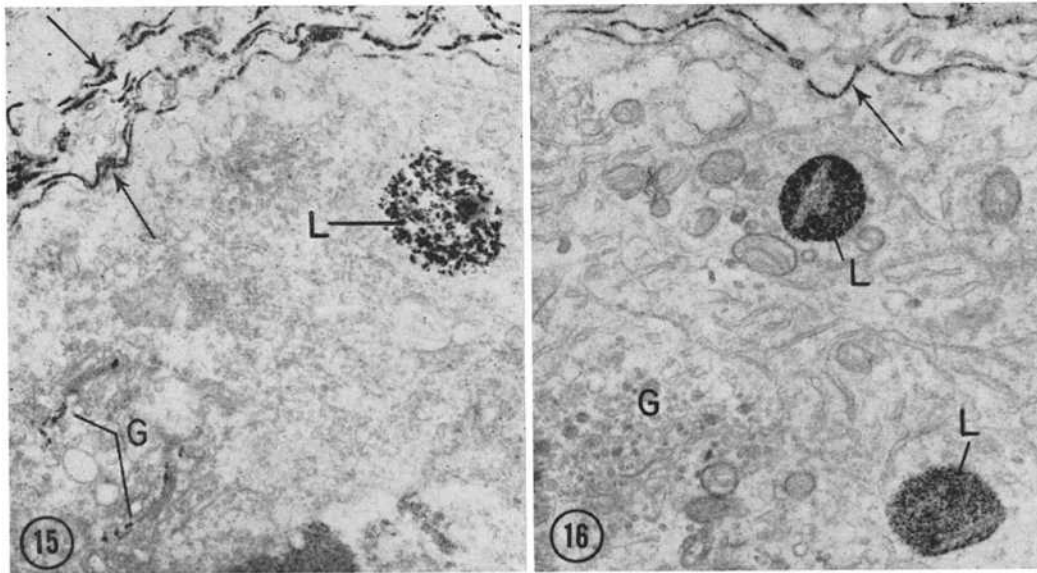
FIGURE 15 Section of a neurone incubated for TPPase at pH 7.2 for 60 min. Reaction product is present in the lysosomal dense body (L) and in the Golgi complex (G) as well as on the plasma membranes of the neurone and the ensheathing glial cell (arrows). × 17,000.

FIGURE 16 Section of a neurone incubated for ATPase at pH 7.2 for 60 min. Reaction product is present in the lysosomes (L) and plasma membrane (arrow), but not in the Golgi complex (G). × 21,000.

lysosomes may all be owing to the action of one, relatively nonspecific phosphatase; alternately, the lysosomes may contain a complex of separate, quite specific phosphatases, each of which splits only one substrate at a particular pH optimum. It is also possible that the population of lysosomes may be biochemically heterogeneous, as is suggested by a comparison of the numbers of reactive sites in cytochemical preparations made by using different phosphates as substrate.

Although the lysosomes of a few vertebrate cells have been reported to display nucleoside mono-phosphatase activity at pH 7.2 (44), alkaline phosphatase (22, 42) or ATPase activity (47, 68), most of the lysosomes in vertebrates contain only acid phosphatases. It is not yet clear whether the differences in substrate specificity between the lysosomes of vertebrates and those of invertebrates are of any special significance.

In contrast to some earlier studies on the lipochondria of insects (1, 3, 31, 60, 61), the observations made in this fine structural examination make it clear that the solid, ovoid lipochondria or lysosomes are quite distinct morphologically from the

saccules and vesicles forming the Golgi complex. Further, there is no morphological evidence to indicate that the Golgi saccules arise by the disruption of other cytoplasmic organelles, such as the lipochondria, as proposed by Ashhurst and Chapman (3). It seems possible that the arrangement of curved stacks of Golgi saccules in concentric arrays may be the ultrastructural basis of the osmiophilic "rings" and "curved rods" which were described in light microscopical preparations by Gresson and his colleagues (19). Such an interpretation differs from that proposed by Shafiq (60, 61) and Malhotra (31, 32) who believed that the metallic impregnation occurred on the cortices of the lipochondria. However, recent ultrastructural studies on the reduction of osmium tetroxide in both invertebrate and vertebrate cells show that the reduced metal is localized along the length of the Golgi saccules (13, 16). Contrary to what might have been expected by extrapolating from the situation in vertebrate cells, the distribution of enzymatic activity in the neurons of *Melanoplus* does not immediately distinguish between lipochondria (lysosomes) and Golgi complex at the

light microscopical level; in most vertebrate cells the lysosomes are fairly specifically characterized by the presence of acid phosphatase, and the Golgi complex by TPPase (17, 39, 40, 43). Electron microscopical examination of *Melanoplus* has shown that both lysosomes and Golgi complex contain both acid phosphatase and TPPase. Hence at the level of light microscopy, the two organelles can be distinguished only by the morphologic appearance of the phosphatase-rich structures, the granules on the one hand and the rings on the other.

Within the Golgi region of *Melanoplus*, at least some of the dense granules and coated vesicles which are found there originate from the fenestrated membranes which lie in association with the Golgi membranes. In this regard, the neurons show similarities to certain neurons in rat spinal ganglia (14, 44) and ganglion nodosum (21), in which the fenestrated membranes of the GERL region of the Golgi complex appear to be the sites of origin of both the lysosomes and the coated vesicles. In addition to this similarity in morphology between rat neurons and *Melanoplus* neurons, similar enzymatic characteristics are displayed by their component Golgi arrays; the same is true of the Golgi dictyosomes in the neurons of the invertebrate snails *Planorbis* (27) and *Helix* (36). Both TPPase and acid phosphatase are present in certain of the Golgi elements in the neurons of each of these snails, in comparison with most vertebrate cells in which the Golgi complex usually contains only TPPase. The possible functional implications of the presence of a lytic enzyme such as acid phosphatase in the Golgi region and associated structures have been considered in detail by Smith and Farquhar (65).

The evidence arising from this study bearing on the mode of formation of lysosomes is equivocal. Some of the electron micrographs suggest that coalescence of certain of the electron-opaque coated vesicles or granules found in the Golgi region may lead to the formation of dense multivesicular bodies; on the other hand, however, it is possible that these vesicles are pinching *off* the multivesicular bodies. Spheroids displaying gradations in structure between dense bodies and multivesicular bodies occur; this suggests the possibility that interconversion between the two may take place. With regard to such a speculation, multivesicular bodies have been described as becoming transformed into dense bodies in a variety of other cells (see bibliographies in references 41, 65). In *Melanoplus* neurons, similarities in enzyme content between the dense bodies, multivesicular bodies, and certain of the Golgi elements may point to a functional or developmental relationship between these organelles. Analogous situations have been reported in various tissues of the rat, including the ganglion nodosum (21), vas deferens (15), and anterior pituitary (65).

The sites of uptake of neutral red in these neurons correspond to those described in the nerve cells of other orthopterans (31, 32, 60); the vitally colored granules appear to represent the lipochondria, which in these cells have been shown to be lysosomes (see reference 26). In vertebrate nerve cells (23) and cultured cells (29, 45, 53) studied by light microscopy, it is claimed that the lysosomes are the foci of vital dye segregation; electron microscopical studies support this contention (11, 33, 54, 59). In light microscopical preparations the coloration observed in the Golgi regions of orthopteran neurons may be owing to dye uptake by the dense bodies intimately associated with the inner border of each Golgi complex. The possibility, however, of uptake by some component of the Golgi apparatus itself cannot be excluded, and only ultrastructural examination of such cells vitally stained will resolve the issue.

In summary, this study indicates that the intracellular distribution of phosphatases in the neurons of orthopteran insects is similar to that in the cells of other invertebrates thus far examined. The lysosomes of these neurons differ from those of most vertebrate cells in the substrate range of their component phosphatases, but seemingly only to a limited degree. It is evident that biochemical studies on the lysosomes of invertebrate tissues are required to clarify the exact number and nature of their constituent enzymes, as well as to ascertain precisely how such lysosomes differ in substrate specificity from the lysosomes of vertebrate cells.

REFERENCES

1. ASHHURST, D. E. 1961. The cytology and histochemistry of the neurones of *Periplaneta americana. Quart. J. Microscop. Sci.* 102:399.
2. ASHHURST, D. E. 1965. Mitochondrial particles seen in sections. *J. Cell Biol.* 24:497.
3. ASHHURST, D. E., and J. A. CHAPMAN. 1962. An electron-microscope study of the cytoplasmic inclusions in the neurones of *Locusta migratoria. Quart. J. Microscop. Sci.* 103:147.
4. BAKER, J. R. 1944. The structure and chemical composition of the Golgi element. *Quart. J. Microscop. Sci.* 85:1.
5. BAKER, J. R. 1949. Further remarks on the Golgi element. *Quart. J. Microscop. Sci.* 90:293.
6. BAKER, J. R. 1950. Studies near the limit of vision with the light microscope, with special reference to the so-called Golgi-bodies. *Proc. Linnean Soc. N. S. Wales.* 162:67.
7. BAKER, J. R. 1963. New developments in the Golgi controversy. *J. Roy. Microscop. Soc.* 82:145.
8. BARKA, T., and P. J. ANDERSON. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* 10:741.
9. BEAMS, H. W., and R. L. KING. 1932. Cytology of orthopteran nerve cells. *J. Morphol.* 53:59.
10. BEAMS, H. W., A. W. SEDAR, and T. C. EVANS. 1932. Studies on the neurones of the grasshopper with special reference to the Golgi bodies, mitochondria and neurofibrillae. *Cellule.* 55:291.
11. BYRNE, J. M. 1964. Acid phosphatase activity in the neutral red granules of mouse exocrine pancreas cells. *Quart. J. Microscop. Sci.* 105:343.
12. CHOU, J. T. Y., and G. A. MEEK. 1958. The ultra-fine structure of lipid globules in the neurones of *Helix aspersa. Quart. J. Microscop. Sci.* 99:279.
13. DALTON, A. J. 1960. Morphology and physiology of the Golgi complex. *In* Cell Physiology of Neoplasia. The University of Texas Press, Austin, Texas. 161–184.
14. DEDUVE, C., and R. WATTIAUX. 1966. Functions of lysosomes. *Ann. Rev. Physiol.* 28:435.
15. FRIEND, D. S. 1966. Peroxidase absorption in the vas deferens of the rat. *J. Cell Biol.* 31:37A.
16. FRIEND, D. S., and M. J. MURRAY. 1965. Osmium impregnation of the Golgi apparatus. *Am. J. Anat.* 117:135.
17. GOLDFISCHER, S., E. ESSNER, and A. B. NOVIKOFF. 1964. The localization of phosphatase activities at the level of ultrastructure. *J. Histochem. Cytochem.* 12:72.
18. GOMORI, G. 1952. Microscopic Histochemistry; Principles and Practice. University of Chicago Press, Chicago, Illinois.
19. GRESSON, R. A. R., L. T. THREADGOLD, and N. E. STINSON. 1956. The Golgi elements of the neurones of *Helix, Locusta* and *Lumbricus. Cellule.* 58:7.
20. HESS, A. 1958. The fine structure of nerve cells and fibers, neuroglia, and sheaths of the ganglion chain in the cockroach (*Periplaneta americana*). *J. Biophys. Biochem. Cytol.* 4:731.
21. HOLTZMAN, E., A. B. NOVIKOFF, and H. VILLAVERDE. 1967. Lysosomes and GERL in normal and chromatolytic neurons of the rat ganglion nodosum. *J. Cell Biol.* 33:419.
22. HUGON, J., and M. BORGERS. 1967. Fine structural localization of lysosomal enzymes in the absorbing cells of the duodenal mucosa of the mouse. *J. Cell Biol.* 33:212.
23. KOENIG, H. 1962. Histological distribution of grain gangliosides: Lysosomes as glycolipoprotein granules. *Nature.* 195:782.
24. LANE, N. J. 1963. Thiamine pyrophosphatase, acid phosphatase, and alkaline phosphatase in the neurones of *Helix aspersa. Quart. J. Microscop. Sci.* 104:401.
25. LANE, N. J. 1965. Phosphatase distribution in lysosomes and cytoplasmic membranes in the thoracic ganglionic neurons and glia of the grasshopper (*Melanoplus differentialis*). *J. Cell Biol.* 27:56A.
26. LANE, N. J. Lipochondria, neutral red granules and lysosomes: synonymous terms? *In* The Interpretation of Cell Structure. S. McGee-Russell and K. Ross, editors. E. Arnold Ltd. London. In press.
27. LANE, N. J. 1966. The fine-structural localization of phosphatases in neurosecretory cells within the ganglia of certain gastropod snails. *Am. Zoologist.* 6:139.
28. LANE, N. J. 1968. The thoracic ganglia of the grasshopper, *Melanoplus differentialis:* Fine structure of the perineurium and neuroglia with special reference to the intracellular distribution of phosphatases. *Z. Zellforsch. Mikroskop. Anat.* 86:293.
29. LANE, N. J., and A. B. NOVIKOFF. 1965. Effects of arginine deprivation, ultraviolet radiation, and X-radiation on cultured KB cells. A cytochemical and ultrastructural study. *J. Cell Biol.* 27:603.
30. LEE, R. S. 1963. Phosphatases in the neurones of *Locusta migratoria. Quart. J. Microscop. Sci.* 104:475.
31. MALHOTRA, S. K. 1955. Golgi bodies in nerve cells of insects. *Nature.* 176:886.

32. MALHOTRA, S. K. 1956. The cytoplasmic inclusions of the neurones of certain insects. *Quart. J. Microscop. Sci.* **97**:177.

33. MCDONALD, T. F., and H. KOENIG. 1965. Ultrastructural changes in neuron lysosomes induced by in vivo staining with neutral red. *Proc. Am. Assoc. Neuropathol.* **15A**.

34. MCGEE-RUSSELL, S. M. 1964. On bridging the gap in cytology between the light and electron microscopes by some combined observations on snail neurones. *Quart. J. Microscop. Sci.* **105**:139.

35. MEEK, G. A., and S. BRADBURY. 1963. Localization of thiamine pyrophosphatase activity in the Golgi apparatus of a mollusc, *Helix aspersa. J. Cell Biol.* **18**:73.

36. MEEK, G. A., and N. J. LANE. 1964. The ultrastructural localization of phosphatases in the neurones of the snail, *Helix aspersa. J. Roy. Microscop. Soc.* **82**:193.

37. MISCH, D. W. 1962. Localization of acid phosphatase in tissues of metamorphosing flesh-fly larvae. *J. Histochem. Cytochem.* **10**:666.

38. NOLTE, A., H. BREUCKER, and D. KUHLMANN. 1965. Cytosomale Einschlusse und Neurosekret im Nervengewebe von Gastropoden. Untersuchungen am Schlundring von *Crepidula fornicata* L. (Prosobranchier, Gastropoda). *Z. Zellforsch. Mikroskop. Anat.* **68**:1.

39. NOVIKOFF, A. B. 1963. Lysosomes in the physiology and pathology of cells: contributions of staining methods. *In* Ciba Foundation Symposium on Lysosomes. A. V. S. de Reuck and M. P. Cameron, editors. Little, Brown and Company, Boston.

40. NOVIKOFF, A. B., and S. GOLDFISCHER. 1961. Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. Natl. Acad. Sci. U. S.* **47**:802.

41. NOVIKOFF, A. B., E. ESSNER, and N. QUINTANA. 1964. Golgi apparatus and lysosomes. *Federation Proc.* **23**:1010.

42. NOVIKOFF, A. B., P. IACIOFANO, and H. VILLAVERDE. 1965. Observations on the staining reactions of hepatic lysosomes. *J. Histochem. Cytochem.* **13**:29A.

43. NOVIKOFF, A. B., E. ESSNER, S. GOLDFISCHER, and M. HEUS. 1962. Nucleoside phosphatase activities of cytomembranes. *In* The Interpretation of Ultrastructure. R. J. C. Harris, editor. Academic Press Inc., New York. **1**:149–192.

44. NOVIKOFF, A. B., N. QUINTANA, H. VILLAVERDE, and R. FORSCHIRM. 1964. The Golgi zone of neurons in rat spinal ganglia. *J. Cell Biol.* **23**:68A.

45. OGAWA, K., N. MIZUNO, and M. OKAMOTO. 1961. Lysosomes in cultured cells. *J. Histochem. Cytochem.* **9**:202.

46. OSINCHAK, J. 1966. Ultrastructural localization of some phosphatases in the prothoracic gland of the insect *Leucophaea maderae. Z. Zellforsch. Mikroskop. Anat.* **72**:236.

47. OTERO-VILARDEBO, L. R., N. LANE, and G. C. GODMAN. 1963. Demonstration of mitochondrial ATPase activity in formalin-fixed colonic epithelial cells. *J. Cell Biol.* **19**:647.

48. PIPA, R. L. 1961. Studies on the hexapod nervous system. IV. A cytological and cytochemical study of neurones and their inclusions in the brain of a cockroach, *Periplaneta americana* (L.). *Biol. Bull.* **121**:521.

49. PIPA, R. L. 1962. A cytochemical study of neurosecretory and other neuroplasmic inclusions in *Periplaneta americana. Gen. Comp. Endocrinol.* **2**:44.

50. RASCH, E. M., and S. GAWLIK. 1964. Cytolysosomes in tissues of metamorphosing Sciarid larvae. *J. Cell Biol.* **23**:123A.

51. REHBERG, S. 1966. Über den Feinbau der Abdominalganglien von *Leucophaea maderae* mit besonderer Berücksichtigung der Transportwege und der Organellen des Stoffwechsels. *Z. Zellforsch. Mikroskop. Anat.* **72**:370.

52. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.

53. ROBBINS, E., and P. I. MARCUS. 1963. Dynamics of acridine orange-cell interaction. I. Interrelationships of acridine orange particles and cytoplasmic reddening. *J. Cell Biol.* **18**:237.

54. ROBBINS, E., P. I. MARCUS, and K. GONATAS. 1964. Dynamics of Acridine orange-cell interaction. II. Dye-induced ultrastructural changes in multivesicular bodies (Acridine orange particles). *J. Cell Biol.* **21**:49.

55. RÖHLICH, P., B. AROS, and B. VIGH. 1962. Elektronemikroskopische Untersuchung der Neurosekretion im Cerebralganglion des Regenwurmes (*Lumbricus terrestris*). *Z. Zellforsch. Mikroskop. Anat.* **58**:524.

56. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**:19.

57. SCHARRER, B. 1966. Ultrastructural study of the regressing prothoracic glands of blattarian insects. *Z. Zellforsch. Mikroskop. Anat.* **69**:1.

58. SCHIN, K. S., and U. CLEVER. 1965. Lysosomal and free acid phosphatase in salivary glands of *Chironomus tentans. Science.* **150**:1053.

59. SCHMIDT, W. 1962. Licht-und Elektronemikros-

kopische Untersuchungen über die Intra-
zelluläre Verarbeitung von Vitalfarbstoffen. *Z.
Zellforsch. Mikroskop. Anat.* **58**:573.

60. SHAFIQ, S. A. 1953. Cytological studies of the
neurones of *Locusta migratoria.* Part I. Cyto-
plasmic inclusions of the motor neurones of the
adult. *Quart. J. Microscop. Sci.* **94**:319.

61. SHAFIQ, S. A. 1954. The lipochondria in the
developing nerve cells of the locust. *J. Roy.
Microscop. Soc.* **74**:207.

62. SHAFIQ, S. A., and W. G. B. CASSELMAN. 1954.
Cytological studies of the neurones of *Locusta
migratoria.* Part III. Histochemical investiga-
tions, with special reference to the lipochon-
dria. *Quart. J. Microscop. Sci.* **95**:315.

63. SMITH, D. S., and J. E. TREHERNE. 1963. Func-
tional aspects of the organization of the insect
nervous system. *In* Advances in Insect Physiol-
ogy. J. W. L. Beament, J. E. Treherne, and
V. B. Wigglesworth, editors. Academic Press
Inc., New York. **1**:401–484.

64. SMITH, D. S., and J. E. TREHERNE. 1965. The
electron microscopic localization of cholines-
terase activity in the central nervous system of
an insect, *Periplaneta americana* L. *J. Cell Biol.*
26:445.

65. SMITH, R. E., and M. G. FARQUHAR. 1966. Lyso-
some function in the regulation of the secretory
process in cells of the anterior pituitary gland.
J. Cell Biol. **31**:319.

66. TAHMISIAN, T. N. 1965. The locus and structure
of mitochondrial elementary particles as seen
in *Helix aspersa* spermatocytes. *J. Cell Biol.*
27:103A.

67. TRUJILLO-CENÓZ, O. 1962. Some aspects of the
structural organization of the arthropod gan-
glia. *Z. Zellforsch. Mikroskop. Anat.* **56**:649.

68. WACHSTEIN, M., and M. BESEN. 1964. Electron
microscopic study in several mammalian
species of the reaction product enzymatically
liberated from adenosine triphosphate in the
kidney. *Lab. Invest.* **13**:476.

69. WACHSTEIN, M., and E. MEISEL. 1957. Histo-
chemistry of hepatic phosphatases at a physio-
logic pH. *Am. J. Clin. Pathol.* **27**:13.

70. WATSON, M. L. 1958. Staining of tissue sections
for electron microscopy with heavy metals. *J.
Biophys. Biochem. Cytol.* **4**:475.

71. WILLEY, R. B., and G. B. CHAPMAN. 1962. Fine
structure of neurons within the Pars Inter-
cerebralis of the cockroach, *Blaberus craniifer.
Gen. Comp. Endocrinol.* **2**:31.