THE ORGANIZATION AND INNERVATION OF THE LUMINESCENT ORGAN IN A FIREFLY, *PHOTURIS PENNSYLVANICA* (COLEOPTERA)

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ABSTRACT

The organization of the luminescent organ of an adult firefly has been studied with the electron microscope, and particular attention has been given to the disposition of nerve terminals within the organ. The cytological structure of the cells of the tracheal system, the peripheral and terminal axons, the photocytes and the cells of the dorsal ("reflecting") layer is described. Previous observations on the peripheral course of nerve branches alongside the tracheal trunks at the level of the dorsal layer and photocyte epithelium have been confirmed, and specialised nerve endings containing axoplasmic components structurally identical with "synaptic vesicles" and "neurosecretory droplets" have been identified, not in association with the surface of the photocytes, but lying between the apposed surfaces of two components of the tracheal epithelium: the tracheal end-cell and the tracheolar cell. These cytological findings are discussed in terms of available biochemical and physiological evidence concerning the mechanism of light emission in the firefly, especially with respect to the possible role of chemical "transmitter" action in triggering a response in a luminescent effector system.

INTRODUCTION

It is well known that normal or non-pathological luminescence in animals may either be associated with the presence of luminescent bacteria in the body, or it may be an intrinsic function involving more or less elaborate organs, within which the components of the light-producing reaction are produced. Among the latter category, luminescence may occur (as in the crustacean *Cypridina*) when the reactants are mixed after they have been secreted into the external medium, while in other instances light is produced intracellularly. Of the variety of forms showing intracellular bioluminescence, the lampyrid and elaterid beetles known collectively as "fireflies" and "glow-worms" have, by virtue of their abundance and widespread distribution, been most extensively studied, both from the anatomical and physiological standpoint.

It would be inappropriate here to attempt to review the work on this subject in detail: the extensive literature on the anatomy of the light organs of Lampyridae and other Coleoptera has been fully surveyed by Buck (1948), while recent information on the biochemical and physiological aspects of the luminescent reaction in these insects may be found in the work of Buck (1948, 1955), Harvey (1952), McElroy (1957), McElroy and Hastings (1955, 1957), and McElroy and Seliger (1961). Preliminary accounts of the fine structure of the light organs of *Photinus pyralis* and *Photuris pennsylvanica* have been given by Beams and Anderson (1955) and Kluss (1958), respectively: these authors were able to confirm and amplify many earlier conclusions, based on light microscopic studies, concerning the organization of the

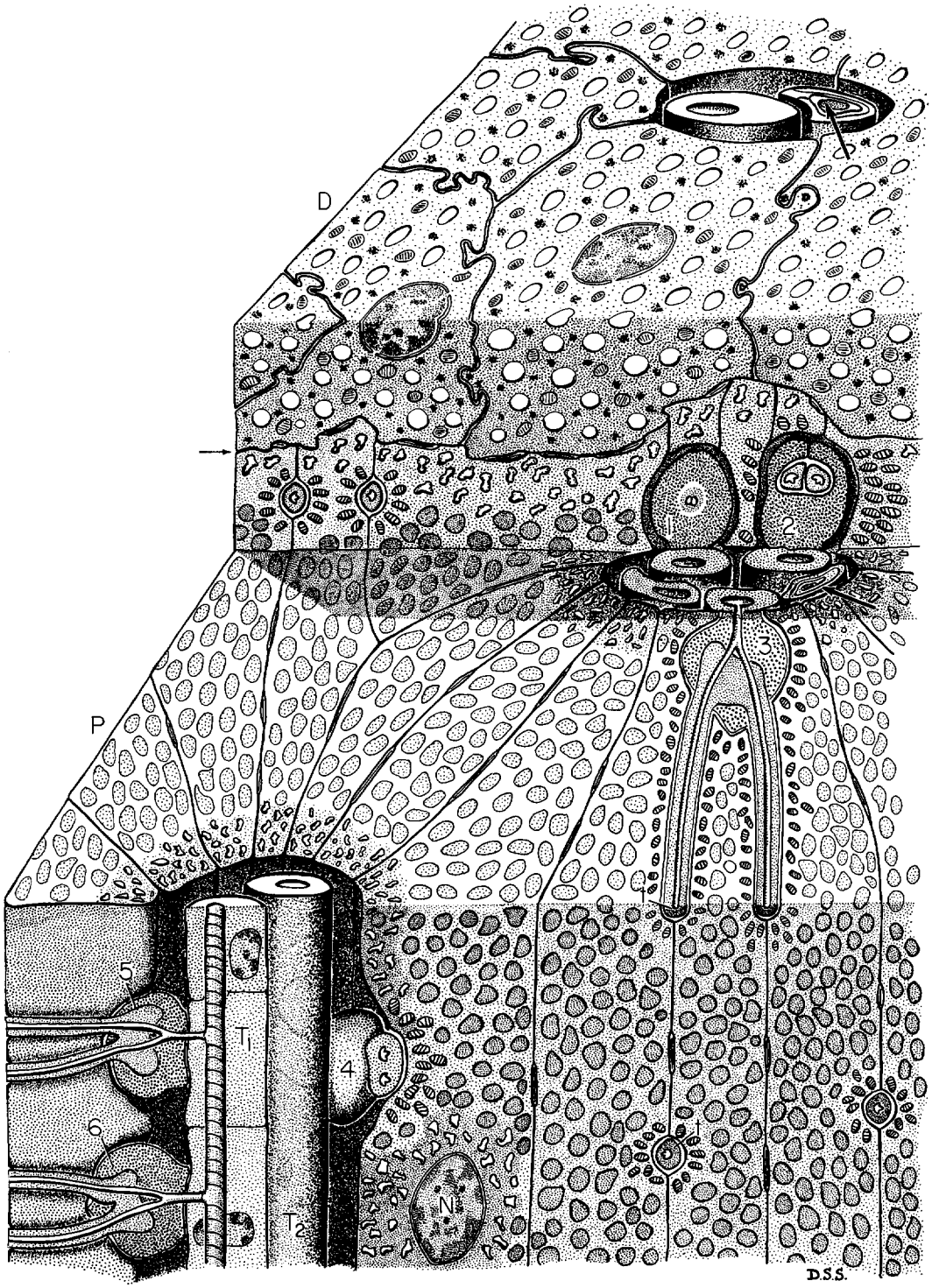

FIGURE 1 (see facing page)

cell types taking part in the construction of these complex organs.

The aims of the work reported here have been to examine the cytological structure of the cells comprising the firefly lantern more extensively, and with the advantage of improved techniques, and, in particular, to investigate the most salient but hitherto obscure problem of the structural basis of nervous control of light production; for while it has been shown that the light-organ may be supplied with motor innervation from the central nervous system (Kluss, 1958; Hanson, 1962, etc.), no detailed information has hitherto been available on the nature and distribution of nerve terminals in this tissue. Much of the controversy and multiplicity of hypotheses that have centered around the control mechanism involved in flashing in fireflies (Buck, 1948) is attributable to this shortcoming in structural descriptions of the light organ.

The cell types comprising the light organ of *Photuris pennsylvanica* will first be described, and, after this morphological evidence has been presented, the possible functional inferences which may be drawn will be discussed. To serve as a background for the descriptive portion of this work, the main features of the origin, location, and organization of the firefly lantern must be mentioned briefly. In the North American species of *Photinus* and *Photuris*, in which light is characteristically emitted in flashes, the lanterns are compact organs which lie against the ventral sclerites of the 6th and 7th abdominal segments, in the male, while in the female they are smaller and often restricted to one segment. The variation in distribution of the lanterns in these and other forms has been reviewed fully by Buck (1948). In the larva, two similarly situated but very small lanterns are present in the 8th abdominal segment; according to Hess (1921) these originate from mesodermal fat-body cells which differentiate locally to produce a two-layered organ, consisting of the ventral "photogenic epithelium" backed by the cells of the "reflecting layer." The same basic anatomical divisions are seen in the adult organs which are elaborated during the pupal instar, after their larval equivalents have degenerated, by, or soon after, the time when the adult emerges. The photocytes, abutting onto the transparent cuticle of the sternite, are arranged in rosette form (see Fig. 1), and are backed by a layer of irregular cells containing large numbers of granules reported to consist of urate, constituting the so called "reflecting layer," though since, as Buck (1948) points out, the evidence that they reflect the light emitted by the photocytes is slender, these cells will here

FIGURE 1

Semidiagrammatic reconstruction of a "stepped" block of tissue, illustrating the spatial relationship between the cell types occurring in the lantern of the firefly *Photuris pennsylvanica*, based on electron micrographic evidence.

Portions of cells of the dorsal layer (*D*) are included, abutting onto the dorsal surface of the photocyte epithelium (*P*) at the level indicated with an arrow (*cf.* Figs. 8, 28). Three "cylinders" containing tracheal trunks are shown: two of these are in transverse profile at the level of the dorsal and photocyte layers, respectively, while the third contains one trachea in longitudinal section (T_1) and another in surface view (T_2). The plate-like photocytes, largely filled with characteristic granules of complex structure (Figs. 6, 9, 11 through 16), extend in "rosette" arrangement between adjacent cylinders. Note the differentiated zones, lacking these granules but containing large numbers of morphologically distinct bodies, situated in the cytoplasm of the photocyte bordering on the cylinders around the nuclei (*N*) and in the region adjoining the dorsal layer cells (*cf.* Figs. 7, 8). Profiles of six tracheal end-cells are shown, associated with the tracheal trunks: at *1* and *2*, respectively, sectioned proximally and distally to the point of origin of the tracheoles (*cf.* Figs. 25, 26, 32); at *3*, *5*, and *6* the association between end-cell and tracheolar cell and the extensions of the latter between the photocytes is indicated, and at *4* most of the end-cell lies below the plane of section in the cylinder, which passes through the tracheolar cell. (*cf.* Figs. 9, 10, 28). Transverse sections of tracheolar cell prolongations are shown at *t*; the adjacent photocyte cytoplasm is rich in mitochondria (*cf.* Fig. 17). Peripheral nerve branches accompanying the tracheae in two of the cylinders are included (arrows) but details of the nerve terminations are omitted here, and are indicated in Fig. 2.

Note that in this figure the size of the photocyte granules and the diameter of the tracheoles lying between them has been exaggerated. Note also that the contours of the photocytes are somewhat irregular, and that the adhesion plates between them are, in fact, very narrow (*cf.* Fig. 7).

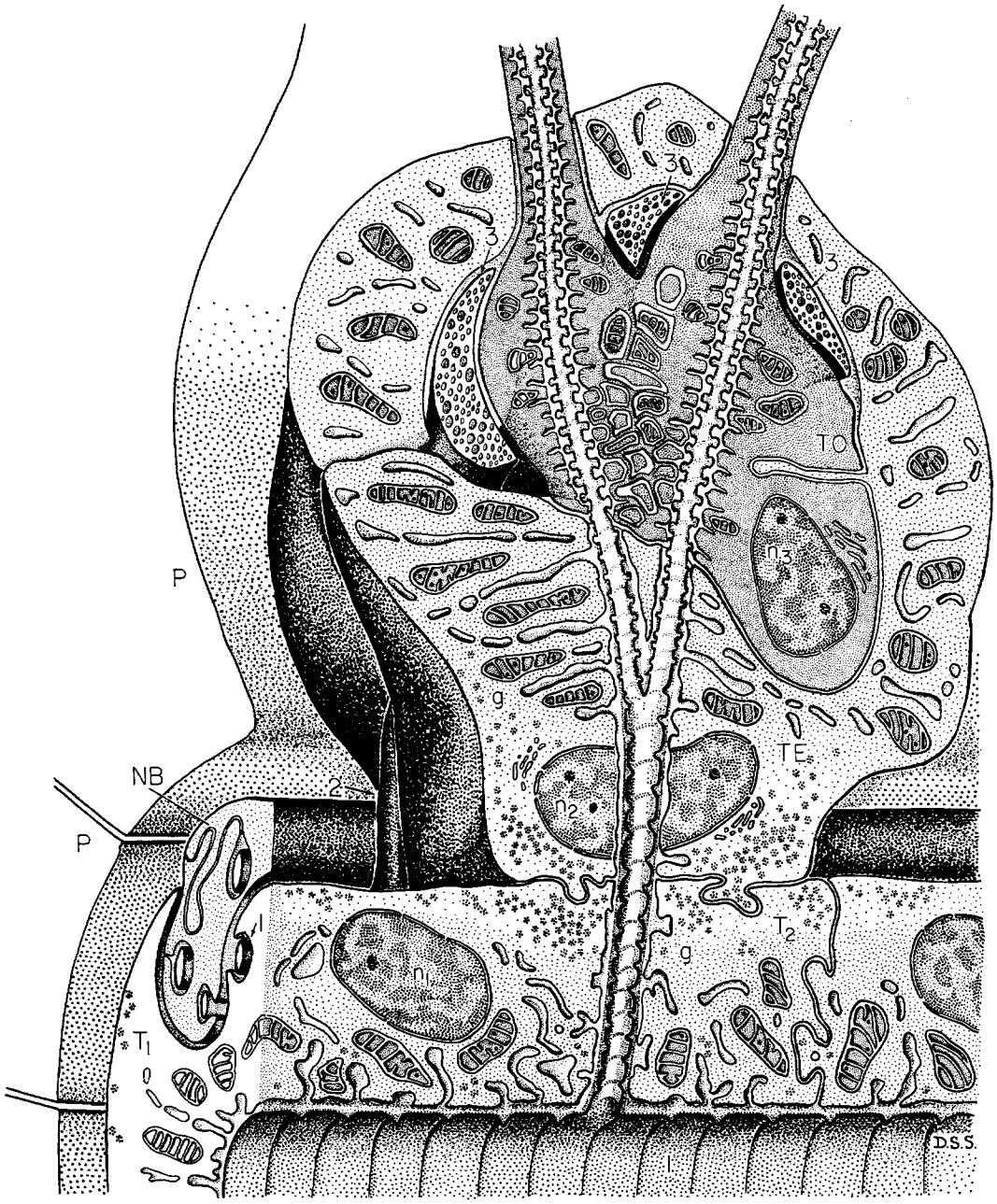

FIGURE 2

Semidiagrammatic reconstruction illustrating the relationship between nerve tissue and the cells of the tracheal system in the lantern of *P. pennsylvanica*, based on electron micrographs.

Portions of two tracheal epithelial cells (T_1, T_2) are shown, sectioned transversely at lower left and longitudinally elsewhere: the margins of transversely sectioned photocytes (*P*) are indicated along the left of the figure. The cells of the tracheal trunk surround the cuticle-limited lumen (*l*), a lateral branch of which divides into two. Shortly afterwards the tracheolar tubes thus formed leave the tracheal end-cell (*TE*) and enter the tracheolar cell (*TO*), and cytoplasmic processes of the latter surround

be referred to non-committally as the "dorsal layer cells."

Both the photogenic epithelium and the dorsal layer of the light organ are traversed by perpendicular tubular cavities (or "cylinders"), along which run branches of the abdominal tracheal trunks. The tracheae lying in the cylinders pass through the dorsal layer without modification, but on their course through the photogenic epithelium give rise to an extensive system of lateral tracheal twigs, the origin of which, in many species of firefly, is associated with a morphologically well defined tracheal end-cell, within which division of the twig into tracheoles takes place. The tracheal end-cells thus form the anatomical link in the chain between the epithelial cells surrounding the main tracheal trunks, and the tracheolar epithelial cells, delicate processes of which accompany the tracheoles throughout their path between the photocytes. The electron microscopic observations of Kluss (1958) confirmed earlier reports that peripheral nerve branchs follow the course of the tracheae along the cylinders (Geipel, 1914; Hasama, 1942, *a*, *b*, and *c*, etc.; see Buck, 1948 for further references), but he did not find specialized nerve terminations in the lantern; however, both Geipel and Hasama believed that the nerves end in association with the end-cells.

The organization of the firefly lantern is very complex, and we are not yet in a position to integrate fully the structural and biochemical features of the system. The work described here is a contribution to the former aspect, and, while the ultimate solution to the problem of intracellular luminescence must involve the synthesis of information on the localization of the reactants in the photogenic system and on the physiological mechanisms initiating and controlling light production, an essential first step must be the characterization of the structural organization of the intact tissue.

MATERIALS AND METHODS

Adult specimens of *Photuris pennsylvanica*[1] (DeGeer) were obtained from Georgia. The abdomens were removed and opened in 1 per cent OsO_4 buffered at pH 7.6 with glucose-containing phosphate buffer (Millonig, 1961 *a*). The intact light organs were then gently separated from the cuticle of the sternites, to allow access of the fixative to the ventral surface of the photocytes, fixed for 90 minutes, and embedded in Epon according to the procedure of Luft (1961). Figs. 32 and 33 represent light organs of *Photinus pyralis* (Linn.) obtained from New Jersey: this material was fixed in veronal-acetate-buffered 1 per cent OsO_4 and embedded in methacrylate. Sections were cut on Porter-Blum and Huxley microtomes using glass knives, and were examined in a Siemens El-

[1] These specimens, collected in Marietta, appear to represent an unusually large race of this species: both males and females up to 19 mm. in length were found in this locality, though LeConte (1881) gives the size range as 10.5 to 15 mm.

The customary spelling of this species, originally described as *Photuris pensylvanica*, has been adopted here.

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the tracheolar branches as they emerge from the encompassing end-cell to penetrate between the photocytes (*cf.* Figs. 1, 9, 10, etc.). Certain cytological features of the tracheal cells are indicated here: note the complex folding of the membrane (continuous with the superficial plasma membrane *via* the mestracheons) around the tracheal lumen, the nuclei, deposits believed to be of glycogen (*g*) in the tracheal epithelial cells and the end-cell; the structural details and proportions of these components is seen in the accompanying electron micrographs. (n_1, n_2, and n_3 denote, respectively, the nuclei of the tracheal epithelial cell, the end-cell, and the tracheolar cell).

A peripheral nerve branch (*NB*) is shown in transverse section (*cf.* Figs. 5, 20, 22, 23), indenting the tracheal cell at lower left. The progressively more intimate association between axons and tracheal cells is indicated semidiagrammatically: an axon becomes freed from the lemnoblast (arrowed *1*) and follows a lateral course alongside the end-cell (arrowed *2*) and ends in dilated vesicle-packed "terminal processes" (arrowed *3*) around the "cell body" of the tracheolar cell, and tightly inserted between the surface of the latter and the concave distal portion of the end-cell (*cf.* Figs. 25 through 30). (Note that neither the point at which the axon becomes divested of the lemnoblast nor the distance from the termination at which vesicles first appear in the axoplasm has been precisely determined.)

This figure is intended to indicate only the spatial relations between the cells, rather than the relative size of the cells and of their cytological components. In particular the size, relative to that of the end-cell, of the terminal processes of the axon and of the tracheolar cell processes has been exaggerated for clarity, and the actual proportions may be seen in the electron micrographs.

miskop I and a Philips EM 200, after they had been stained with a solution containing a lead-tartrate complex according to the method of Millonig (1961*b*) or (Figs. 32 and 33) with aqueous uranyl acetate.

Note that all the micrographs included in this paper represent sections of the light organ of male fireflies, though during the course of this work it was ascertained that in the two species examined the organs of males and females, while differing markedly in size, are cytologically similar.

THE STRUCTURE OF THE PHOTOGENIC ORGAN

(a) *The "Dorsal Layer" Cells*

The fine structure of the cells comprising the dorsal layer of the light organ has been described briefly by Beams and Anderson (1955) and Kluss (1958). The suggestion that this region provides a reflecting surface for the light emitted by the underlying photocytes was first put forward by Kölliker (1858) who believed that the refractile bodies found in the cytoplasm of these cells are crystals of urate. Buck (1948) points out that the chemical nature of these bodies has yet to be clearly established, and furthermore that the reflecting properties of these cells is an unsupported assumption.

The nuclei of the dorsal layer cells are usually centrally placed in the cytoplasm, the components of which appear to be similar in all parts of the cell. The most striking cytoplasmic feature is the presence of profiles of spherical cavities, each about 0.5 to 1.0 μ in diameter, which do not appear to be limited by a membrane (Fig. 8). The cavities sometimes contain traces of residual material, though their appearance in osmium tetroxide-fixed material certainly suggests that the bulk of their original content has been dissolved out of the tissue during preparation. Ovoid profiles of small mitochondria, showing parallel transverse cristae, are evenly distributed throughout the cytoplasm, and these are accompanied by a sparse population of profiles of cisternae of the rough-surfaced endoplasmic reticulum. In material fixed in phosphate-buffered osmium tetroxide and "stained" with lead salts, a further, and hitherto undescribed, component of these cells is visualized: granules *ca.* 200 to 300 A in diameter, associated in clusters each *ca.* 0.1 to 0.15 μ in diameter. These bodies are identical in appearance with the material identified as glycogen in several vertebrate tissues by Revel and his coworkers (1960) and others. As will be mentioned later, similar granules, generally arranged in smaller clusters, are present in the cytoplasm of the tracheal epithelial cells (Figs. 3, 4).

The junctions between cells of the dorsal layer exhibit profiles of complex interdigitations, and adhesion plates are present between these cells and also along the region of apposition between them and the underlying photocytes (Fig. 8).

As has often been described in the past, the tracheal trunks do not give off lateral branches at the level of the dorsal layer, and as described by Kluss the tracheae traversing the cylinders at this level are accompanied by numerous nerve branches, containing one or several axons enclosed within a common sheath or lemnoblast. As will be described in due course, terminating axons are only found at the level of the photocyte layer.

(b) *The Photocytes*

The ventral portion of the light organ of the firefly comprises an epithelium of large cells of unusual cytological organization, which are generally recognized to be responsible for light emission. The photocytes are large slab-like cells extending between the surface of the cells of the dorsal layer and the thin hypodermis overlying the abdominal cuticle. As is well known from light microscopic studies, the photocytes are arranged in rosette form around the tubular spaces or cylinders occupied by the tracheal trunks; each photocyte typically abutting onto two adjoining cylinders. The spatial relationship between the photocytes, the cylinders, and the dorsal layer cells is indicated in Fig. 1. The bulk of the cytoplasm of the photocytes is occupied by profiles of the so called "photogenic granules" to which term "photocyte granules" is to be preferred, since their function in the luminescent reaction is not known. These are, in this material, subspherical or ovoid in form (Figs. 6, 9). It is possible that the wide separation between, and the irregularity of outline of the granules described by Beams and Anderson (1955) and Kluss (1958) reflects some shrinkage or polymerization artefact incurred by methacrylate embedding, since this appearance has not been found when Epon is employed. In the present material, the photocyte granules are apparently of variable size, attaining a length of up to 2.5 μ. It is clear in approximately diametric profiles (Fig. 11) that each granule is limited by a membrane,

ca. 75 A in width, surrounding a finely granular matrix, and many profiles include a restricted, and often peripherally located, region of higher density (fig. 9). If a field including a large number of granules is examined, it is found that a number of the profiles show a further structural component: a membrane-limited cavity lying in the matrix. Some of these profiles indicate that the cavities are flask-shaped, and are produced into a narrow neck which may lie within the plane of section for some distance (Fig. 11), while small circular profiles of the cavity necks (*ca.* 300 A in diameter) also occur in the matrix (Fig. 13) either singly or as a consequence of branching of the tubules (Fig. 16) in groups of two or three. Occasionally, direct continuity between a cavity and the extragranular cytoplasm via a tubular neck is observed (Figs. 12, 15). A number of survey fields were examined, and it was found that cavity profiles were present in about 10 per cent of the granule sections, suggesting that each granule generally contains a single cavity, since the approximate ratio of the cavity-granule diameter is 10:1. Many of the cavities have a light granular content, unlike the material in the surrounding matrix (Figs. 11, 12), but occasionally contain deeply "staining" bodies (Fig. 14).

Mitochondria are sparsely distributed throughout the bulk of the cytoplasm of the photocytes, except in those peripheral regions of the cell either adjoining a tracheal end-cell (Fig. 28) or flanking each tracheole in its passage between adjacent photocytes (Fig. 17), where mitochondria are locally present in very large numbers. These are subspherical or elongate, and show an unusual arrangement of the cristae, which sometimes divide the mitochondrial matrix into a central cavity with irregular peripheral chambers.

Certain well defined regions of the photocyte were recognized, in light microscopic studies (Lund, 1911; Dahlgren, 1917; Hess, 1921), as lacking photocyte granules, designated as the "differentiated zones," described by Buck (1948) as containing "an extremely fine-grained and compact-looking cytoplasm." In the present material, photocyte granules are virtually absent from the extreme dorsal and ventral regions of the cell, from the lateral regions bordering on the cylinders, and from the cytoplasm immediately surrounding the nucleus. These regions are somewhat better supplied with mitochondria, and are characterized by the presence of bodies, recognized by Beams and Anderson (1955), that are distinguishable from the photocyte granules by their smaller size and irregular outline (Fig. 7). Each is limited by a single membrane, enclosing a matrix with a light central area, surrounded by denser material. Sinuous profiles of rough-surfaced cisternae of the endoplasmic reticulum are more evident in the differentiated zone cytoplasm than elsewhere in the photocyte (Figs. 7, 8). In the region bordering on the cells of the dorsal layer, the differentiated zone granules contain dense material surrounding a clear central region, which presumably represents, as in the dorsal layer cells, a substance dissolved out of the tissue during preparation. From the location of this soluble material in the layers of the light organs, it is tempting to suppose that the dorsal layer cells serve as a store for a product passed to them from the cytoplasm of the photocytes. Lund (1911) stated that a "crystalline nitrogenous deposit," similar to that in the cells of the dorsal layer, is found in the peripheral regions of the photocytes and around the nuclei, though according to Buck (1948), several workers failed to confirm Lund's claim that products of decomposition resulting from photogenesis are transferred from their site of origin in the photocytes, to the cells of the dorsal layer.

One further cytoplasmic feature of the photocytes deserves mention: the presence of elongated adhesion plates situated at intervals along the apposed faces of the cells (Fig. 7). In these regions the plasma membranes separate for some distance, and one or two delicate layers of dense material is interposed between them. Adhesion plates also occur between the dorsal surface of the photocytes and the cells of the dorsal layer (Fig. 8), while a thin sheet of basement membrane material is always present between the surfaces of the photocytes and of the tracheal end cells lying at the periphery of the cylinder, and also between the photocytes and the prolongations of the tracheolar epithelial cells accompanying the tracheoles (Figs. 10, 29).

(c) *The Tracheal Supply to the Lantern*

Buck (1948) gives a comprehensive survey and classification of coleopteran luminescent organs, primarily based on the topographical variation of the tracheal supply. It has been suggested that certain aspects of this variation, notably the apparent absence of morphologically well defined

tracheal end-cells in some instances, may have important physiological implications. Before this question is discussed, however, the cytological organization and distribution of tracheae and tracheoles in *Photuris pennsylvanica* must first be described.

The insect tracheal system consists of cuticular tubes, ultimately open to the exterior *via* the segmental spiracles, and enveloped at all times by cells of the tracheal epithelium. While it is convenient to regard the tracheal cells as forming a single arborizing epithelial system, the precise relationship between the cuticular intima and the epithelium varies as the diameter of the former decreases through repeated branching, a feature that is well illustrated in the material described here. The lumen of a trachea close to its origin at a spiracle is large in relation to the cells of the epithelium, portions of several of which may thus be seen in a transverse section of the trachea. By the time the tracheal branches reach the dorsal surface of the light organ of *P. pennsylvanica*, each contains a lumen *ca.* 1 to 2 μ in diameter, enclosed within a single row of epithelial cells stacked end-to-end, forming a hollow cylinder (Figs. 1, 3). No lateral branching of the tracheal trunks occurs within the cylinders at the level of the dorsal layer of the light organ, and the branching that occurs as the tracheae traverse the photocyte layer will be described in due course; we are at the moment concerned only with the cytological structure of the main tracheal stem, which is similar throughout the organ.

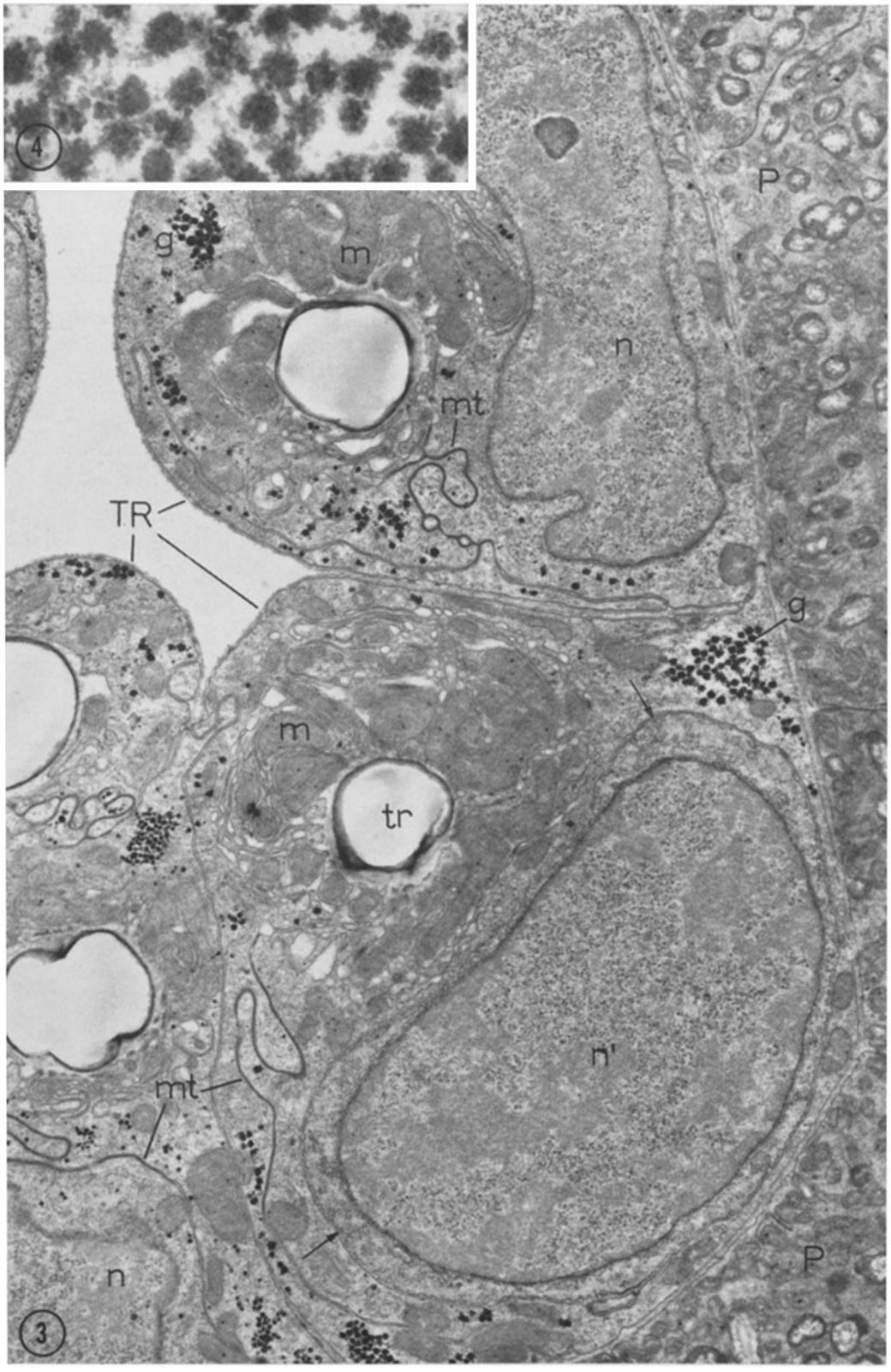
Fig. 3 illustrates, in transverse section, a group of tracheae within a cylinder at the level of the photocyte layer, and this section happens not to include any lateral tracheal twigs. The plasma membranes of adjacent epithelial cells are very closely applied, but each tracheal lumen is associated with a single encompassing cell. Sinuous paired membrane profiles are seen, originating as invaginations of the superficial membrane and traversing the cytoplasm to surround the cuticular tracheal tubes. These are evidently to be regarded as "mestracheons" analogous with the mesaxons of the nerve sheath: the cuticular lining of the trachea at this level, as well as in the larger trunks, is an extracellular product, secreted by the cells that surround it. The mestracheons (*cf.* Edwards, Ruska, and De Harven, 1958) consist of paired membranes separated by a space of *ca.* 100 A and their path may be tortuous, before the membranes diverge as they approach the tracheal lumen. The termination of the mestracheon is not simply lapped around the tracheal tube, but is reflexed back into the cytoplasm, which is thereby dissected into a complex lattice-work seen in section as a series of elongated or circular profiles of

FIGURE 3

A survey electron micrograph including a portion of a cylinder at the level of the photocyte layer, in the lantern of *P. pennsylvanica*. The organ has been sectioned frontally (*i.e.* parallel with the ventral surface) and the tracheal trunks are thus seen in transverse profile, *TR*. At this level, each tracheal tube is associated with a single encompassing epithelial cell containing a laterally placed nucleus (*n*). The nucleus at *n'* is surrounded at a short distance by a pair of membrane profiles (arrows) and represents an interdigitation or invagination of one tracheal cell with its neighbor. The plasma membrane of the tracheal cell is infolded to form a "mestracheon" (*mt*) which gives rise to a complex series of folds around each tracheal tube (*tr*) (*cf.* Figs. 31 through 33), and numerous mitochondria (*m*) lie within the cytoplasmic processes thus formed. Groups of densely stained granules (*g*) occurring in the tracheal cell cytoplasm are believed to represent deposits of glycogen. Along the right hand margin of this field lie the peripheral regions of several photocytes (*P*); the detailed organization of these cells is illustrated in Figs. 6 through 17. × 12,000.

FIGURE 4

An electron micrograph of the granular deposits within the cytoplasm of a tracheal cell, believed to represent glycogen. In these cells each such cluster of granules is *ca.* 0.1 μ in diameter, and similar, though characteristically larger clusters occur in the cells of the dorsal layer in the lantern of *Photuris* (*cf.* Figs. 8, 28). × 60,000.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cytoplasm (Figs. 28, 31, 33) which often contain elongated mitochondria, exhibiting subparallel arrays of cristae, and small dense granules lying in the mitochondrial matrix. In addition to the membrane profiles derived from the mestracheon, are found clusters of small vesicles which may be organized into a well defined Golgi zone near the nucleus. Particle-bearing cisternae are uncommon, though small unattached particles occur throughout the cytoplasm. Beyond the limits of the invaginated membrane system surrounding the tracheal lumen, notably in the peripheral regions of the cell (Fig. 28) lie clusters of deeply "staining" particles, probably of glycogen, resembling, except for their smaller size, the bodies described in the dorsal layer cells (*cf.* Figs. 3, 8, 23).

The large ovoid or reniform nuclei of the tracheal epithelial cells are situated peripherally: the nuclear envelope bears pores at frequent intervals.

(d) *The Tracheal End-Cells*

The presence of a specialized cell associated with the origin of the lateral branches of the tracheal trunks in the light organ of many species of beetle has often been recognized in earlier accounts of the structure of the light organ, and the much debated possibility that these cells are of physiological importance in light production has

FIGURE 5

A field similar to that shown in Fig. 3, but including a peripheral nerve branch (*NB*) containing a large axon (*a*), lying between the tracheal cells. A small portion of photocyte cytoplasm (*P*) limiting the cylinder is included at lower left. Another axon, accompanied by a very narrow lemnoblast sheath and invaginated into the surface of a tracheal cell, is indicated by an arrow (*cf.* Figs. 20, 22, 23). × 14,000.

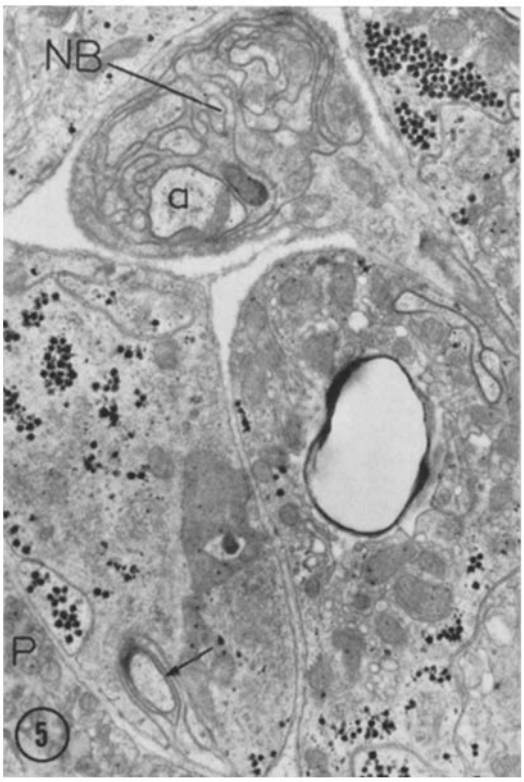
FIGURE 6

A low-power survey field of a frontal section through the photocyte layer in the lantern of *P. pennsylvanica*, illustrating the spatial relationship between photocytes (*P*) and tracheal cells (*TR*). This field represents a lateral extension of the aspect shown in Fig. 3. The bulk of the photocyte cytoplasm is filled with membrane-limited granules (*pg*) but these are absent from the differentiated zones bordering on the cylinder (dz_1) and around the nucleus (dz_2). Photocyte nucleus, *np*. Each photocyte is a plate-like structure, and the field included here represents a transverse profile of about one-half of a photocyte, which extends symmetrically between adjacent cylinders (*cf.* Fig. 1). One lateral cell margin of the photocyte is indicated by an arrow; the other lies just to the right of this field. The structure of the photocyte is illustrated, at higher magnification, in Figs. 7 through 17. × 7,500.

FIGURE 7

Illustrating the organization of the perinuclear cytoplasm in a photocyte (dz_2 in Fig. 6). Photocyte granules are virtually absent from this region and from the cytoplasm bordering the cylinders, and these differentiated zones are characterized by the presence of smaller bodies, the differentiated zone granules (*dg*) which are irregular in profile and contain a dense matrix with a lighter central region: they appear to have an internal tubular system (arrow) as in the photocyte granules, but they lack the well-defined flask-shaped cavities present in the latter (*cf.* Fig. 11). In the dorsal region of the photocytes, bordering on the cells of the dorsal layer, are found similar bodies (Fig. 8) in which, however, the central region is very distinct, and appears to represent a soluble component, lost during preparation of the material. A small portion of the photocyte nucleus is seen at *np*: note also the adhesion plates (*ap*) that occur at frequent intervals between the photocytes.

The differentiated zone cytoplasm contains mitochondria (*m*) similar in appearance to those occurring elsewhere in the cell, and sinuous profiles of the rough-surfaced endoplasmic reticulum (*er*) are especially evident in these regions (*cf.* Fig. 8). × 25,000.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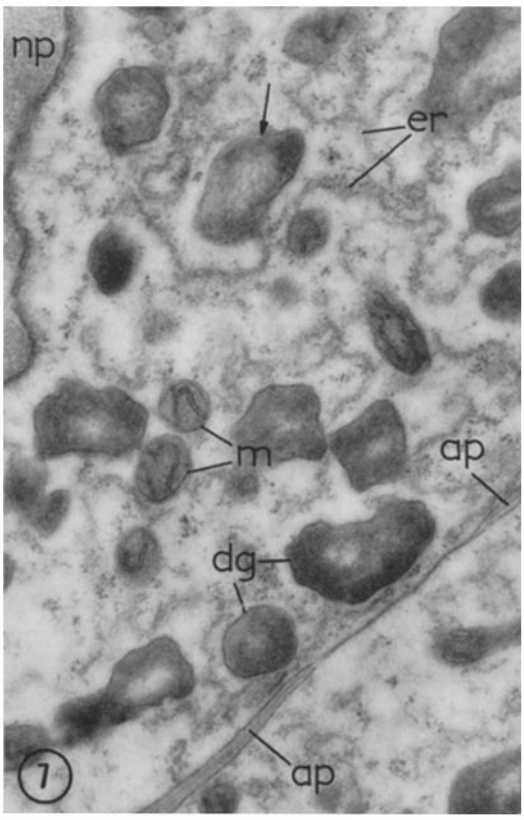
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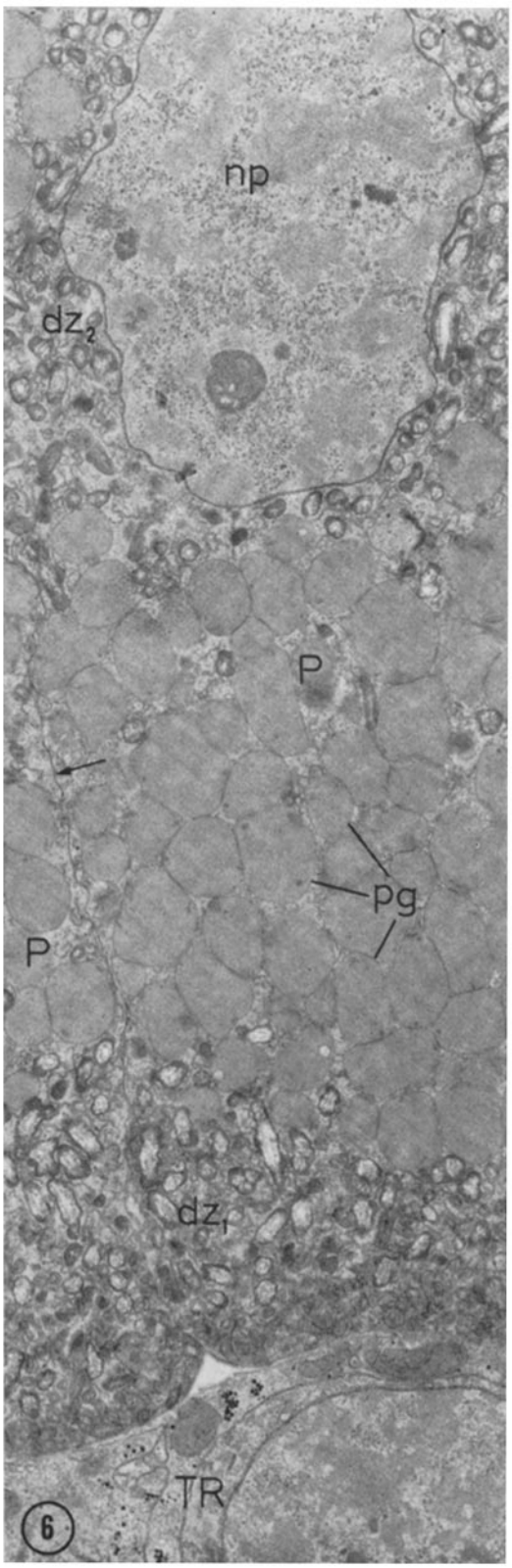
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TR
6

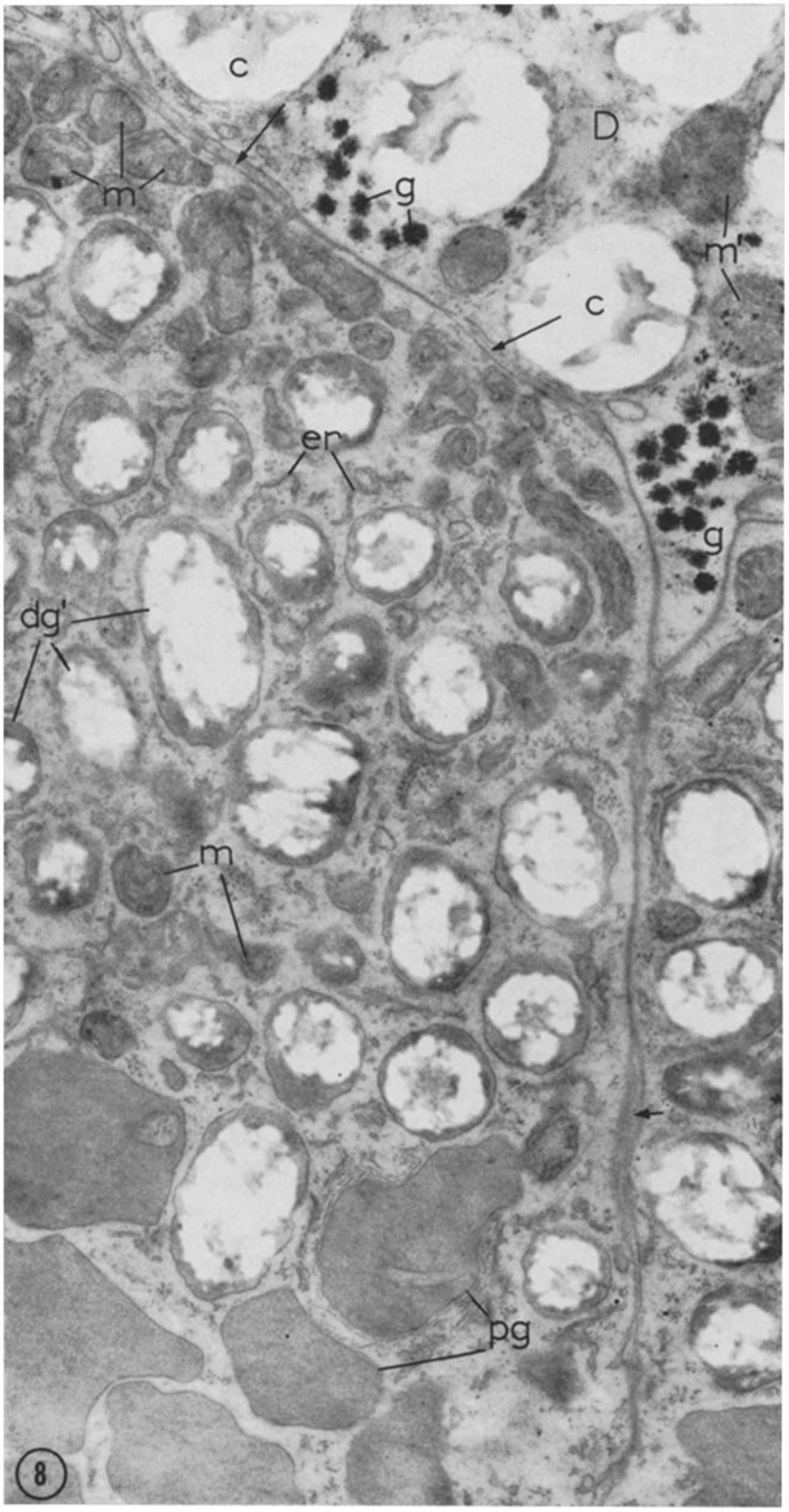
been reviewed by Buck (1948). While the suggestion (Dahlgren, 1917, etc.) that the tracheal end-cells contain contractile fibrils acting as a sphincter mechanism limiting the passage of oxygen to the photocytes was refuted by the electron microscopic observations of Beams and Anderson (1955) and Kluss (1958), the preservation of the material described by these authors did not permit a full description of the cytology of the tracheal end-cell, and its relationship with the adjoining tracheal system. The term "tracheal end-cell" has been widely used, in light microscopic descriptions, to describe the region of the tracheal system where a tracheal branch abruptly divides into two or more tracheoles. Its use has been attended by some confusion: it has been sometimes employed to imply the supposed termination of the tracheal epithelium and the origin of naked tracheolar branches (Williams, 1917), though alternatively, it has been suggested (Wielowiejski, 1882) that very fine cytoplasmic processes of the end-cell always accompany the tracheoles: neither of these alternatives is strictly correct, as is clearly seen in the present material. In *P. pennsylvanica*, as in many lampyrids, lateral branches are absent from the cylinders at the level of the dorsal layer cells, but arise in large numbers throughout the photocyte layer. Initially the lateral branch is *ca.* 1 μ in diameter, and this continues for a short distance before dividing into two (or occasionally three) tracheoles, the lumen of each of which is *ca.* 0.3 μ in diameter: these represent the ultimate branches of the tracheal system in the luminescent organ. The branching of the tracheal twig occurs within the confines of the end-cell, as has been described by many authors (Buck, 1948; Beams and Anderson, 1955, etc.) and in this region the sharply defined wall of the tracheal lumen is surrounded by a sheath of homogeneous material (Figs. 31 to 33). Longitudinal sections through the end-cell indicate that it is closely applied to the neighboring cells of the tracheal epithelium associated with the main tracheal trunk and that the proximal region of the end-cell, containing the nucleus, lies within the cylinder, while the distal portion of the cell indents the margins of the adjacent photocytes. Although the tracheal end-cell has often been regarded as a specialization present in some lampyrid light organs and absent in others (Buck, 1948), it seems probable that this supposed variation merely reflects size differences in the end-cell among the various forms; in the instance described here, the end-cell basically resembles other cells in the tracheal epithelium, from which it is demarcated by its position, rather than by any

FIGURE 8

Illustrating the cellular organization of the junction between photocyte and dorsal layer cells in the lantern of *P. pennsylvanica*. The cytoplasm of the latter (*D*) contains profiles of spherical cavities (*c*) from which a soluble substance appears to have been removed during preparation of the material. (It has been suggested that urate granules are stored in these cells, and this region of the lantern has often been referred to as the "reflecting layer" as a consequence of the supposed reflecting properties of these cells. However, since the function of this region of the lantern and the nature of the soluble products present within it remains in doubt, the non-committal term "dorsal layer" has been employed here.)

Note that the bodies present in the photocyte cytoplasm bordering on the lower surface of the dorsal layer cells also appear to have lost a soluble material: these structures (*dg'*) resemble, except for the greater extent of the "empty" central area, the differentiated zone granules found in the cytoplasm adjoining the cylinders and around the nucleus of the photocyte (*cf.* 6, 7).

The cytoplasm of the dorsal layer cells contains mitochondria (*m'*) and clusters of deeply staining granules (*g*) similar in appearance to those observed in the tracheal cells (Figs. 3, 4, 28): these are believed to represent glycogen. There is a rather sharp demarcation between the differentiated zone granules (*dg'*) and the photocyte granules (*pg*) in this region, and the cytoplasm surrounding the former is richer in mitochondria (*m*) and in cisternae of the endoplasmic reticulum (*er*). Note the adhesion plates between adjoining photocytes (short arrows) and between the photocytes and cells of the dorsal layer (long arrows). × 27,000.


c
D
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m'
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dg'
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pg
8

fundamental cytological peculiarities, although it is true that the end-cell shows to a particularly marked degree certain features already described in the cells of the general tracheal epithelium. As in the latter, the end-cell exhibits an invaginated mestracheon (Figs. 31, 33) and the membranes of the mestracheon-fold likewise give rise to a very complex system of plications surrounding the tracheal tube; indeed, this system is much more elaborate than in the main tracheal epithelial cells, and occupies much of the volume of the cell (Fig. 28). Circular or elongated profiles of mitochondria are abundant in the cytoplasmic areas defined by these membrane folds, and these mitochondria contain dense granules *ca.* 250 to 500 A in diameter (Fig. 29). The predominantly radial disposition of the mitochondria in the end-cell is more striking here than in the case of the tracheal epithelial cell; otherwise there seems to be little to distinguish the cytological architecture of these two cell types, since the disposition of clusters of agranular membranes and glycogen clusters appears to be similar in each, and their respective nuclei are similar. The arrangement of these folds and mitochondria presumably misled Dahlgren (1917), as Beams and Anderson (1955) suggest, into postulating the existence of radially arranged, supposedly contractile fibrils within the end-cell, a suggestion that was often seized upon to construct hypotheses of control of light emission involving limitation of oxygen access to the photocytes by a sphincter mechanism situated in the end-cell. In *Photinus pyralis*, the species investigated by Beams and Anderson, the folds continuous with the inner surface of the mestracheon are more regular than in the present instance, and the cytoplasm of the end-cell is effectively dissected into cytoplasmic tubules, many of which contain filiform mitochondria. This feature is illustrated in Figs. 32, 33 which represent a field parallel with and close to the ventral face of the lantern of P. *pyralis*, at the base of a cylinder, and include a number of end-cells with rather regular cytoplasmic arborizations, and in addition demonstrate stages in the division of lateral tracheal twigs.

The spatial relationship between the tracheal end-cell and the main tracheal stem in the present

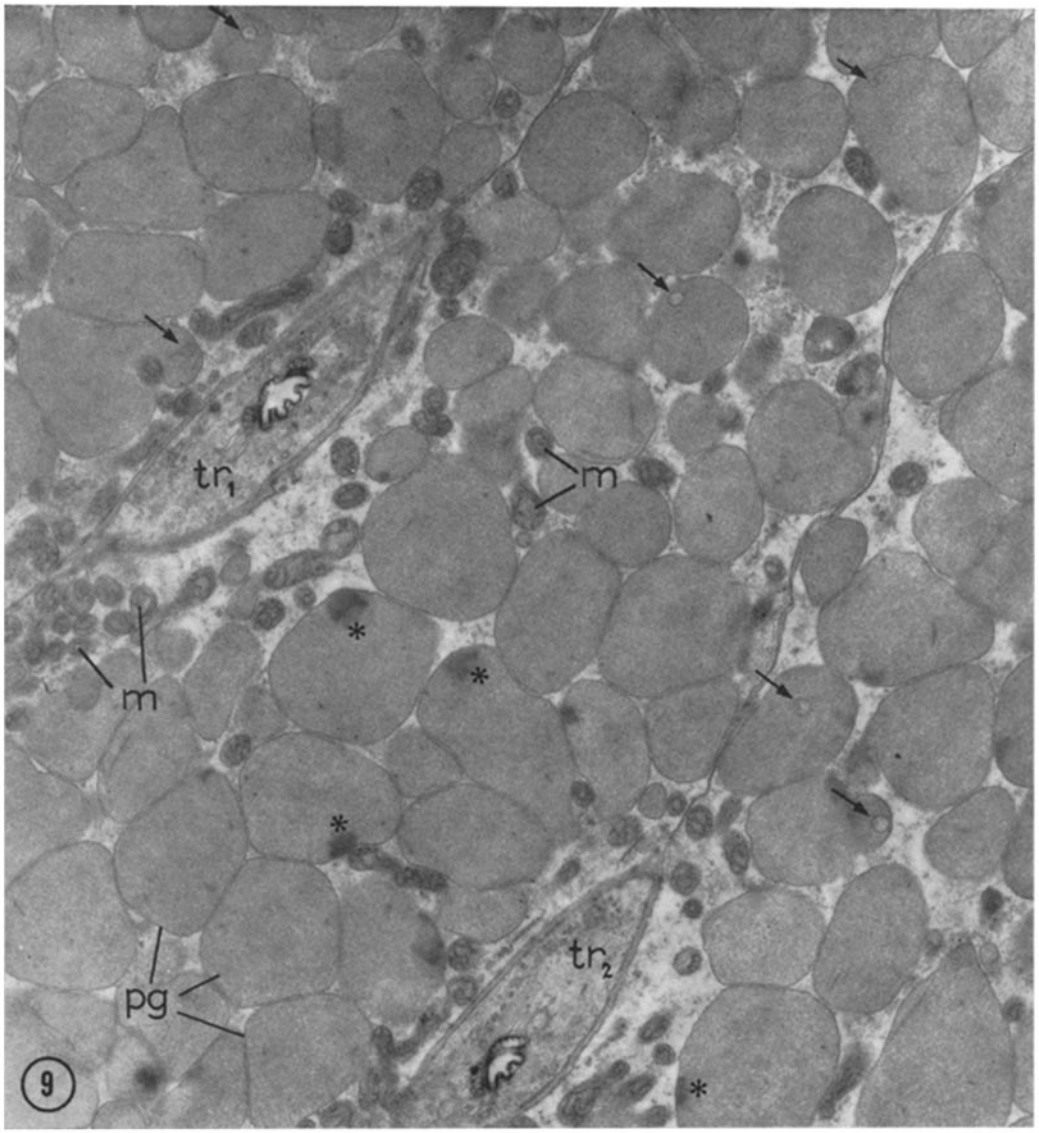
FIGURE 9

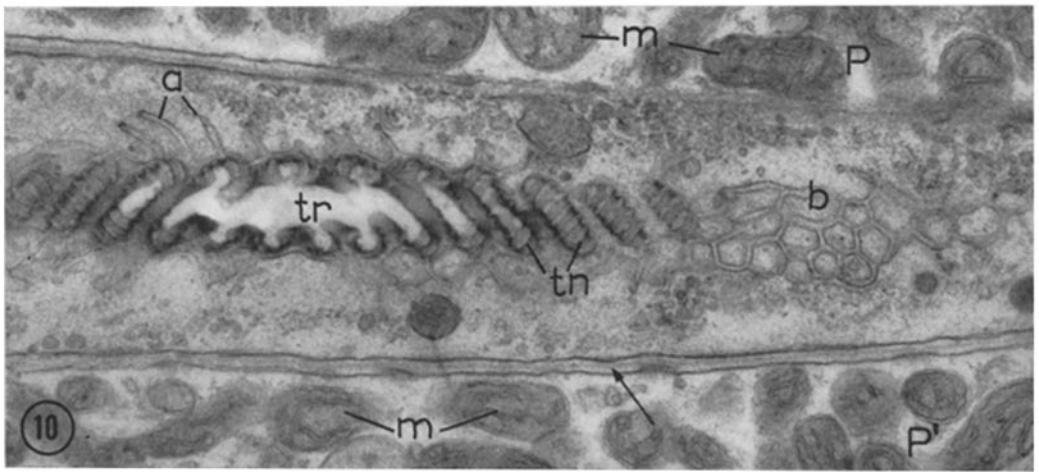
This field includes portions of three longitudinally sectioned photocytes, with two tracheoles (tr_1, tr_2) interposed between their lateral surfaces. The bulk of the photocyte cytoplasm is filled with profiles of photocyte granules (*pg*), each of which contains a granular matrix limited by a single membrane, and these profiles often show localized (usually peripheral) regions of greater density (*) and in some instances internal cavities are included in the plane of section (arrows). The structural details of the photocyte granules are illustrated further in Figs. 11 through 16. Small mitochondria (*m*) occur sparsely throughout the photocyte cytoplasm, but these are abundant in the regions opposite the intercellular tracheoles (Fig. 17). × 12,000.

FIGURE 10

An oblique longitudinal section through a tracheolar branch (*tr*) situated between two photocytes (*P*, *P'*). A thin layer of basement membrane material is interposed between the plasma membranes of these cells (arrow). The cuticular lining of the tracheolar lumen takes the form of helically arranged taenidia (*tn*) each of which bears a series of transversely oriented thickened bars. As was described by Beams and Anderson (1955) and Kluss (1958), the tracheoles of the firefly lantern are unusual in that the membrane adjoining the cuticular tube is produced into a series of lateral or circumferential projections (*a*) which define polygonal "pockets" of cytoplasm arranged in honeycomb fashion, as seen in surface section at *b*. An analogous situation, though on a larger scale, is found in the end-cell and tracheal epithelial cell, where the cytoplasm around the tracheal tube is dissected into a complex system of processes, many of which contain mitochondria (Figs. 29 through 33).

Note the mitochondria (*m*) in the photocyte cytoplasm adjoining the tracheole; these may be present in very large numbers in this region of the cell, as in Fig. 17. × 32,000.


tr1
m
m
pg
tr2
9


m
P
a
tr
b
tn
m
P'
10

species is illustrated in the reconstruction shown in Fig. 1. Excellent light micrographs of these components are illustrated by Buck (1948), Beams and Anderson (1955), and Kluss (1958), and Buck figured isolated tracheae surrounded by clusters of end cells, obtained by macerating lanterns of *Photinus pallens*.

(e) *The Tracheoles*

Beams and Anderson (1955) and Kluss (1958) showed that the tracheoles resulting from the division of the lateral tracheal twig within the end-cell are throughout their course invested with a cytoplasmic sheath. Kluss ascertained that the "cell body" of the circumtracheolar extensions, that is, the portion of the tracheolar cell in which the nucleus is situated, is overlapped by or inserted into the end-cell, in the configuration indicated diagrammatically in Fig. 2. Sometimes, in fortunate sections (Fig. 26), the nucleus and surrounding cytoplasm of the tracheolar cell, invaginated into the end-cell, are included, and the connection between the former and the cytoplasmic sheath surrounding the tracheoles is evident. In many profiles of the end-cell, however, the tracheolar cell body, or its connection with the investment of the tracheolar tubes, lies out of the plane of section (Figs. 28, 31).

The cuticular tubes situated within the processes of the tracheolar cell are *ca.* 0.25 to 0.3 μ in diameter, and this size is maintained throughout their course between the photocytes. The tracheolar taenidia are spiral and the turns are sharply angled with respect to the long axis of the tracheole (Fig. 10), and each taenidium bears transverse thickened bars. Bordering the tracheolar tube, and closely applied to it, lies a regularly folded membrane, defining "pockets" of cytoplasm: frontal sections through these are arranged around the intima in honey-comb fashion, as was described by Beams and Anderson, and this arrangement persists throughout the length of the tracheole. In the proximal portion of the tracheolar cell, these cytoplasmic pockets often contain mitochondria (Fig. 29), but beyond the level of the end-cell, mitochondria are infrequent. No mestracheon is present along the extensions of the tracheolar cell: the cuticular tube is limited by, and is presumably secreted across, the membranous sheath just described, which is continuous with the superficial plasma membrane. The plasma membranes of end-cell and tracheolar cell are separated throughout by a gap of *ca.* 100 A and the region of apposition between them shows convolution and interdigitation (Figs. 26, 30). The proximally placed cytoplasm of the tracheolar cell is restricted to a rather narrow zone around the nucleus (Fig. 26); the Golgi region is represented by compact and well ordered arrays of agranular cisternae, and the cytoplasm, as elsewhere in the tracheal epithelium, is rich in unattached particles, possibly of ribonucleoprotein. However, no glycogen deposits have been here identified in any part of the tracheolar cell.

The lateral arms of the end-cell (Fig. 28) terminate at the surface of the neighboring photocytes, and the tracheoles issue forth, unaccompanied, between these cells, their respective plasma membranes being separated by a space of *ca.* 400 A within which is interposed a narrow layer of diffuse material. The cytoplasmic sheath surrounding each tracheolar intima contains occasional mitochondria, multivesicular bodies, and clusters of very small smooth-membraned vesicles (Fig. 17). Transverse sections of the light organ (traversing the dorso-ventral aspect of the photocytes) indicate that the tracheolar branches issuing from the limits of the end-cell in general run a lateral course between the photocytes, approximately parallel to one another, with a vertical separation between each of about 10 to 15 μ. Since the width of each photocyte is *ca.* 10 μ, it is evident that, throughout this tissue, the maximum distance over which diffusion of oxygen must take place to supply the photocyte cytoplasm cannot be more than a few microns. Such richness in the tracheal supply is unusual and, so far as is known, is exceeded only in the case of insect fibrillar flight muscle where, *e.g.* in *Tenebrio*, it was found that the internalized tracheoles within the fiber reduce the distance over which oxygen must diffuse to reach the site of utilization to about 3 μ (Smith, 1961). In the firefly light organ, oxygen is not only utilized in the general processes of oxidative metabolism of the photocyte, but is also a reactant in the photogenic mechanism. Synthesis of ATP, a reactant in the light-producing system, presumably takes place in the mitochondria of the photocyte, which are strikingly restricted to the regions of cytoplasm flanking the intercellular tracheoles (Fig. 17).

The Innervation of the Photogenic Organ

Buck (1948) points out that in the extensive literature on the firefly, a connection between the

nervous system and light emission is generally recognized. Nevertheless, as he states, "the least known major anatomical feature of the photogenic organ is its nerve supply." The material described here provides a more complete description of the structure of the peripheral nerves supplying the organ than that given by Kluss (1958), and also reveals details of the site and organization of the nerve terminations within the lantern, a prerequisite for any consideration of the role of the nervous system in the initiation or control of light production.

The present study is concerned only with the distributions of peripheral axons, *within the light organ*: the origin of the nerves within the abdominal ganglia has not here been followed, and the experimental study of Hanson (1962) provides valuable information on the latter subject. Buck (1948) reviews the light microscopic evidence on the distribution of nerves within the light organ in lampyrids, and concludes that "the nerves generally follow the tracheal system rather closely. . . and are distributed in roughly the same fashion," an observation that has been confirmed and extended here.

The tracheae lying in the cylinders at the level of the dorsal layer of the lantern of *P. pennsylvanica* are accompanied, as reported by Kluss (1958), by nerve branches, illustrated in Figs. 5, 18, 19. In fig. 19, four axons are present enclosed within a common lemnoblast, and each axon is lapped around by loose turns of the irregular branching mesaxon folds, in the "tunicated" arrangement characteristic of insect nerves (Edwards, Ruska, and de Harven, 1958; Smith, 1960 etc.). Kluss (1958) states that the nerve branches in *P. pennsylvanica* contain either one or two axons, though in the present material as many as six axons have been observed within a branch. The axons shown in Fig. 19 are about 0.3 μ in diameter and, except where local dilatations are present, establishing lacunae within the mesaxon system, the paired membrane profiles of the mesaxons are separated by a gap of variable width (Fig. 21). The nerve branch shown in Fig. 18 contains two axons only, and the section includes profiles of what are probably two horns of a single reniform lemnoblast nucleus, separated by a strip of cytoplasm containing a mesaxon fold. The cytoplasm of the lemnoblast contains scattered mitochondria, and, while the axons have not here been found to contain a well defined system of neurofilaments, the axoplasm in this region contains occasional vesicle profiles and small mitochondria. Immediately outside the superficial plasma membrane of the lemnoblast lies a layer of basement membrane material similar in appearance to that surrounding the tracheal epithelial cells (Fig. 22).

The first indication of the establishment of a connection between the tracheal and nerve supply in the lantern is signalled when the basement membranes of these two cell types coalesce, a situation corresponding to the fusion of lemnoblast and fiber basement membranes in the presynaptic innervation of *Tenebrio* flight muscle (Smith, 1960). Examination of the cells lying within the cylinders of the lantern of *Photuris* at the level of the photogenic epithelium reveals an increasingly more intimate relationship between the tracheal cells and the lemnoblasts (Figs. 5, 20, 22, 23), where the respective cell membranes are found to be closely apposed for some distance, without the interposition of basement membrane material. The lemnoblast ensheathing the axons becomes increasingly tenuous, again paralleling the pattern observed in the myoneural junction, until it appears that axons, finally divested of their sheaths, continue their path, lying in indentations of the tracheal epithelial cell, a sequence diagrammed in Fig. 2. The axons ultimately traverse the laterally directed tracheal end-cells, as they approach their terminations (Fig. 25), *and end in specialized dilatations or "terminal processes," inserted between the apposed plasma membrane surfaces of the tracheolar epithelial cell and the surrounding end-cell, virtually encompassing the proximal region of the former.*

The spatial relationship between the terminal processes of the axon and the cells of the tracheal system with which they are associated is illustrated in Figs. 25 through 30. In Figs. 25 through 29, the terminations are sectioned more or less transversely, while in Fig. 30 the spatulate terminal processes invaginated within the end-cell are seen in tangential or frontal aspect. In Fig. 25, a single axon branch is present, lying near the surface of an end-cell, presumably sectioned some distance before its division into the terminal processes. In each instance, the terminating axoplasm is filled with large numbers of vesicles: the point along the presynaptic path at which these first appear has not been determined; indeed, this seems to be somewhat variable.

FIGURES 11 TO 16

Electron micrographs illustrating the structure of the "photocyte granules" in the lantern of the firefly *Photuris pennsylvanica*. Each of these bodies appears to contain at least one subspherical or flask-shaped cavity, continuous with the surrounding cytoplasm *via* narrow membrane-limited tubes. The function of these granules is not known, but their abundance in the photocytes suggests that they have an active role in the light-producing mechanism, perhaps through the synthesis of luciferin which is believed to be irreversibly oxidized during photogenesis.

FIGURE 11

A section through a portion of a photocyte granule, including a profile of an intragranular cavity (*c*) produced into a narrow tubular neck (*t*) which lies within the plane of section for some distance. Note the light contents of the cavity, and the denser matrix of the granule. × 55,000.

FIGURE 12

A peripheral region of a photocyte granule, illustrating the continuity between the interior of a cavity (*c*) and the cytoplasm outside the granule, *via* a short tubular neck (arrow). Transverse profiles of two other necks are also included in this field: although each granule appears to contain only one cavity, each cavity is probably associated with a number of tubules. × 90,000.

FIGURE 13

A group of three transversely sectioned necks situated in the matrix of a photocyte granule. Each of these structures is limited by a membrane *ca.* 75 A in width continuous with, and similar in appearance to that surrounding each granule. The over-all diameter of each tubular neck is *ca.* 300 A, and it is believed that these tubes radiate out from the cavity to the surface of the granule, and they appear to branch along their path (*cf.* Fig. 16). × 120,000.

FIGURE 14

In most instances the granule cavities appear to contain a poorly defined, irregularly clumped content (*cf.* Fig. 12), but occasionally deeply staining bodies are seen within them as in the instance illustrated here. The nature of this material is unknown; it has the same appearance as glycogen deposits in this tissue (*cf.* Fig. 4) except that the characteristic morular organization is absent. × 55,000.

FIGURE 15

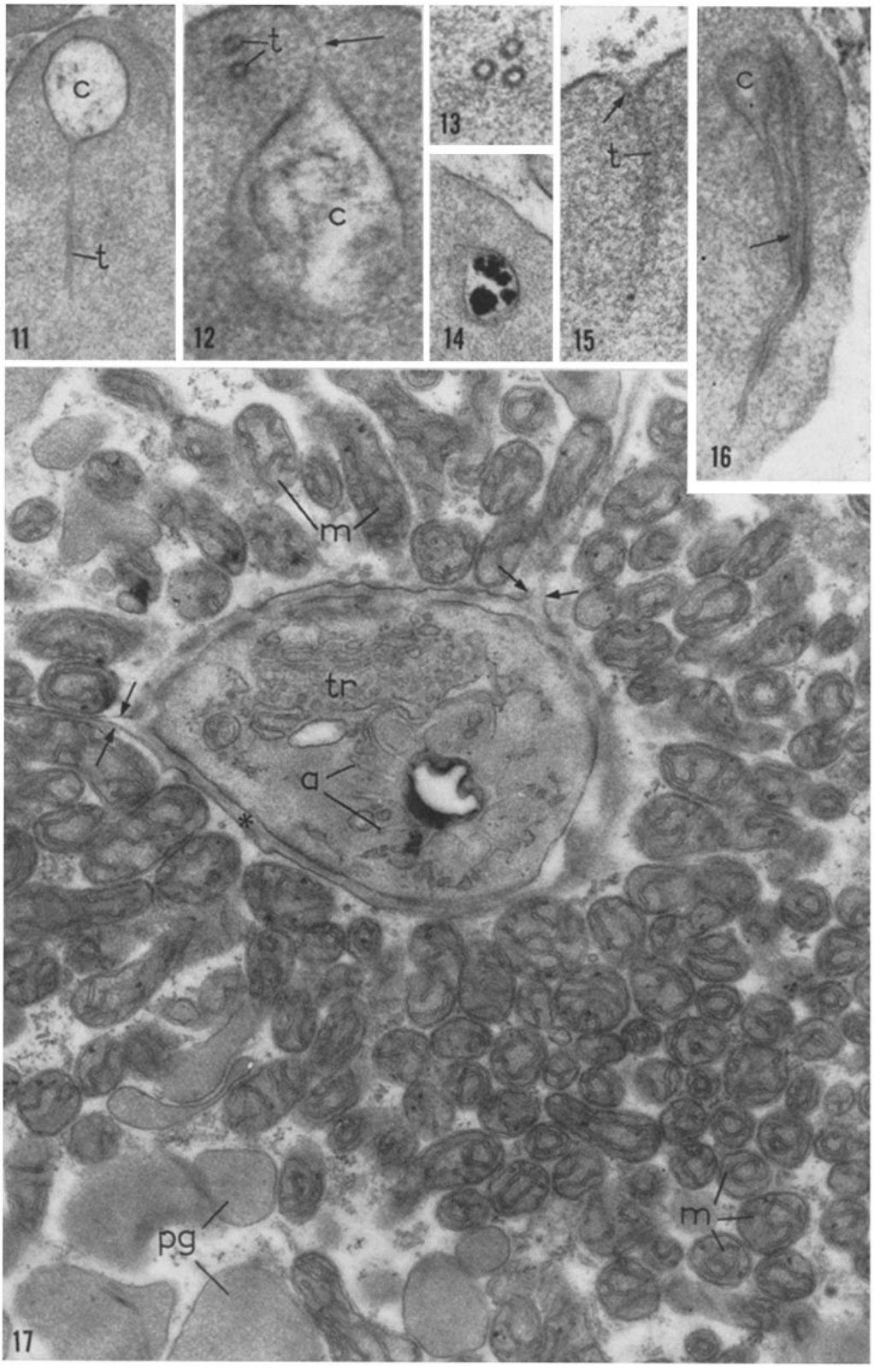
A region at the periphery of a photocyte granule showing the continuity of a tubular neck (*t*) with the superficial membrane limiting the granule (arrow). × 90,000.

FIGURE 16

A profile of a portion of a photocyte granule including a cavity (*c*) and longitudinal profiles of three tubular necks: branching of the latter appears to occur at the point indicated with an arrow. Transverse sections of areas such as this provide the grouped tube-profiles illustrated in Fig. 13. × 45,000.

FIGURE 17

Electron micrograph illustrating the organization of the photocyte cytoplasm in the vicinity of an intercellular tracheole. The adjoining plasma membranes of the photocytes diverge (arrows) to accommodate the prolongation of the tracheolar cell (*tr*), and the surface membranes of these two cells are separated by a gap containing a layer of basement membrane material (*). The cuticle-limited intima of the tracheole is surrounded by a cytoplasmic sheath, and the membrane adjoining the intima is folded in a complex fashion (*a*), a feature more clearly seen in Fig. 10. Large numbers of mitochondria (*m*) occur in the photocyte cytoplasm surrounding the tracheole, and also adjoining the end-cell (*cf.* Fig. 28): these mitochondria are small and have a characteristic internal structure, often containing concentrically arranged cristae. This concentration of mitochondria is very localized; at a short distance from the tracheole, photocyte granules (*pg*) appear, and mitochondria are only sparsely interposed between these (*cf.* Fig. 9). (ATP is a reactant in the light-producing mechanism, and it is probable that the presence of large numbers of mitochondria in the cytoplasm bordering the tracheoles, through which oxygen is supplied to the photocytes, indicates the main site of oxidative phosphorylation in these cells.) × 33,000.


c
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11
t
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13
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c
16
m
tr
a
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pg
m
17

Although vesicles are almost always very sparsely distributed in axons still accompanied by the lemnoblast sheath, such axons, at some distance from their terminations, are occasionally found to contain abundant vesicles similar in appearance to those in the terminal processes (Fig. 24).

Palay (1956) points out that most central and peripheral nerve terminations or synapses, whether in vertebrates or invertebrates, have certain morphological features in common: notably, an intimate juxtaposition of the pre- and postsynaptic membranes; a greater concentration of mitochondria than elsewhere in the axon; and the presence of large numbers of small vesicles in the centripetal axoplasm. In insects, the transsynaptic gap at the myoneural junction seems to be unusually small (Smith, 1960), and this is paralleled in the present instance: the plasma membrane of the axon termination is separated from that of the end-cell and tracheolar cell by a

FIGURES 18 TO 21

Electron micrographs illustrating the organization of the peripheral nerve trunks associated with the lantern of *P. pennsylvanica*.

FIGURE 18

Two axons (a_1, a_2) are present in this field, enclosed within a common lemnoblast (*LB*), the analogue, in insect nerves, of the Schwann cell. This section includes two mesaxon origins (*); one mesaxon (*mx*) lies between two nuclear profiles of the lemnoblast cell (n, n') which probably represent horns of a single reniform nucleus. Note that this nerve branch is situated between photocytes (*P*) and a tracheal epithelial cell (*TR*), and that the lemnoblast is here separated from the cells bordering it by basement membranes (arrows). × 22,000.

FIGURE 19

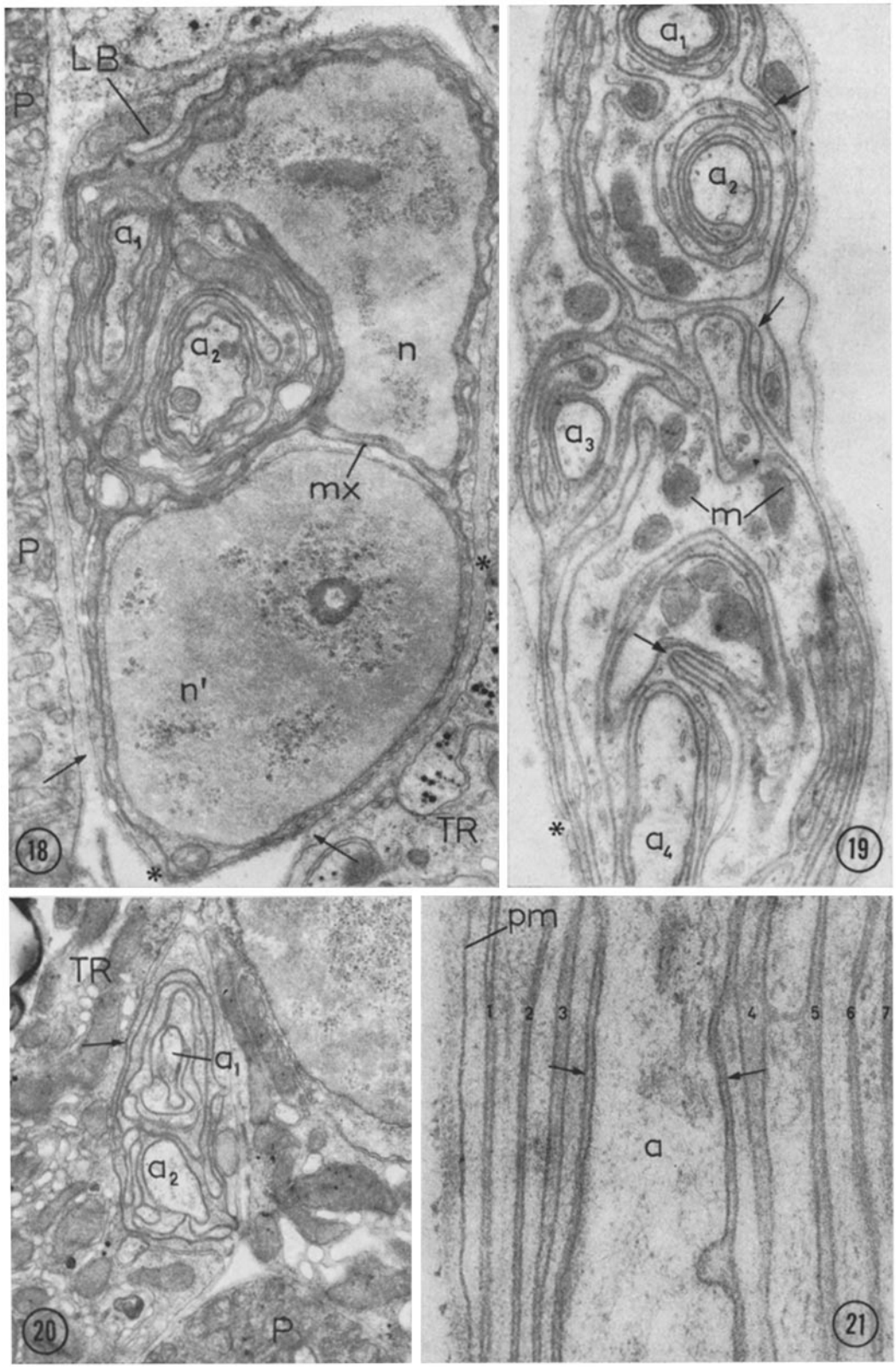
A nerve branch, in transverse section, including profiles of four axons (a_1 to a_4). The irregular mesaxon folds, the origin of two of which are seen here (*), are arranged in a more or less spiral fashion around the axons, and profiles of mesaxon-branches are often seen (arrows). This "tunicated" arrangement appears to be characteristic of insect peripheral nerves. The lemnoblast cytoplasm contains small mitochondria (*m*): mitochondria are rarely seen in the presynaptic axoplasm in the light-organ, but are fairly abundant in the terminal processes of the axons (*cf.* Fig. 30). × 28,000.

FIGURE 20

Illustrating the preterminal association between nerve and tracheal tissue in the lantern of *P. pennsylvanica*. In this field, a nerve branch containing two axons (a_1, a_2) indents a tracheal epithelial cell (*TR*): the surface of the latter is very closely applied to the lemnoblast (arrow) and no basement membrane material is present between them. The lemnoblast sheath, still present here, is lost before the axons terminate in vesicle-filled processes between the end-cell and tracheolar cell (Figs. 28 to 30). *P* indicates the margin of a photocyte. × 18,000.

FIGURE 21

A longitudinal section through a peripheral nerve in the lantern, illustrating the irregularity of the mesaxon folds. On the left (*pm*) is seen the superficial plasma membrane of the lemnoblast (Schwann cell) and in the center of the field lies an axon (*a*) the surface of which is surrounded by a membrane-sheath (arrows) continuous with the superficial plasma membrane *via* the mesaxons. Profiles of seven of the latter are included (*1* through *7*): note the inconstancy of the space between apposed pairs of membrane profiles; apparently this is a characteristic feature of insect "tunicated" nerve. × 80,000.


LB
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TR
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TR
a1
a2
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pm
1 2 3
4 5 6 7
a
21

space of only *ca.* 100 A (Fig. 29) from which basement membrane material is excluded. Mitochondria are infrequent in axons still enclosed by the lemnoblast sheath, but are present in large numbers within the terminal processes. The vesicles present within the terminating axoplasm appear to comprise two distinct populations: small profiles *ca.* 200 to 400 A in diameter, without any electron-opaque content, are interspersed with profiles of membrane-limited vesicles *ca.* 600 to 1200 A in diameter, usually containing a droplet of dense material. The former correspond in size and appearance to the "synaptic vesicles" recognized in the presynaptic junctional axoplasm of many central and peripheral synapses, and believed to represent the site of sequestration or release of a chemical "transmitter" (acetylcholine or a physiological analogue), initiating depolarization of the postsynaptic membrane. The larger profiles within the axoplasm of the terminal processes in the firefly light organ, on the other hand, resemble the structures described elsewhere as neurosecretory droplets. The organization of the vesicular components in the nerve terminals within the light organ has a *morphological* parallel in the terminations within the neurohypophysis, described by Palay (1957) as containing clusters of "synaptic vesicles" 250 to 300 A in diameter, together with larger profiles 1000 to 1500 A in diameter containing dense neurosecretory material.

Fig. 2 illustrates semidiagrammatically the suggested topographical relationship between the axons and innervated structures in the firefly

FIGURES 22 TO 24

Electron micrographs illustrating further details of the preterminal association between nerves and tracheal cells in the firefly lantern.

FIGURE 22

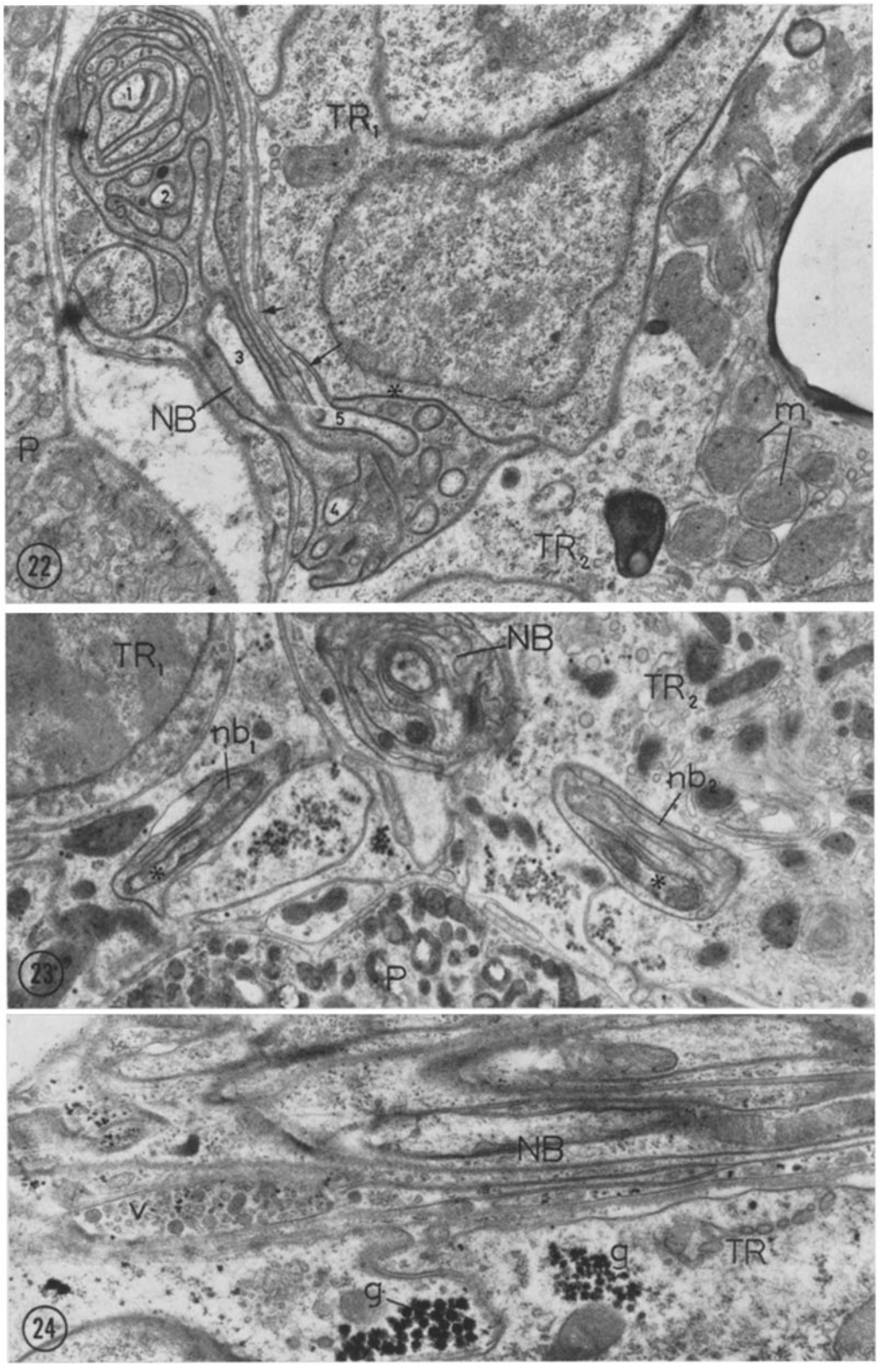
In this field a nerve branch (*NB*), in transverse profile, lies alongside two tracheal cells (TR_1, TR_2). The basement membranes of the lemnoblast and a tracheal cell coalesce (short arrow), and beyond this point the respective plasma membranes become tightly apposed (*). Several axons are present within this lemnoblast profile: those at *1*, *2*, *3*, and *4* are still surrounded by mesaxon folds, while a portion of the axon *5* lies directly against the surface of the tracheal cell (long arrow). In the lemnoblast cytoplasm at lower center are circular profiles of axons apparently enclosed within concentric mesaxon sheaths, the connections of which with the superficial plasma membrane presumably lie out of the plane of section. At the extreme left of the field are included the margins of two photocytes (*P*). Note the mitochondria (*m*) arranged in cytoplasmic processes around the cuticular tracheal tube. × 25,000.

FIGURE 23

In this micrograph a transversely sectioned nerve branch (*NB*) is seen at upper center, situated between two tracheal cells (TR_1, TR_2), within each of which lies a small invaginated nerve branch (nb_1, nb_2) the axons of which (*) are still accompanied by a narrow sheath of lemnoblast cytoplasm. Subsequently, the axons become freed from the sheath and terminate between the surfaces of the end-cell and of the "cell body" of the tracheolar cell (*cf.* Figs. 2, 28, 29). *P* denotes the margin of a photocyte bordering the cylinder in which lie the tracheal trunks. × 17,000.

FIGURE 24

The terminal processes of the axons invariably contain a mixed population of vesicles of varying size (Fig. 30). However, the point at which these vesicles first appear seems to be inconstant; they are occasionally seen (as at *v* in this figure) within an axon in a nerve branch (*NB*) prior to its invagination into a tracheal cell (*TR*). (Note the clusters of deeply "stained" granules in the tracheal cytoplasm *g*, believed to represent glycogen. × 35,000.


TR1
1
2
3
NB
5
4
P
m
TR2
22
TR1
NB
TR2
nb1
nb2
P
23
NB
v
g
TR
g
24

light organ: the presynaptic axons enclosed by the lemnoblast, the free axons indenting the surface of the tracheal epithelial cells, and the lateral course of the axon across the end-cell to the endings, situated apparently impartially between the base of the tracheolar cell and the concave surface of the end-cell into which this is inserted. It should be noted that in no instance has a nerve termination been found to extend beyond the confines of the end-cell, along the projections of the tracheolar cytoplasm lying between the photocytes. *Thus it is the tracheal cells associated with the lateral tracheal branches, and not the photocytes themselves, that appear to be the initial recipient of the motor impulse in this photogenic organ.*

Discussion

In a review of the physiological control of bioluminescence in animals, Nicol (1955) points out that in the majority of instances of intrinsic bioluminescence, the light is produced in a discontinuous or controlled fashion. The precise nature of the control mechanisms are undetermined however, although evidence that the central nervous system is involved has been obtained in several instances. In Coelenterates (pennatulids, Scyphomedusae) and Ctenophora, the pattern of light emission affords evidence for photocyte activation *via* non-polarized neural transmission from the stimulated area, and, as in the case of

FIGURE 25

A transverse section through a tracheal end-cell in the lantern of *P. pennsylvanica*, proximal to the point of bifurcation of the tracheal twig and of the insertion of the "cell body" of the tracheolar cell (*cf.* Fig. 26). Note the single tracheal lumen (*tr*) and the large mitochondria (*m*) in the end-cell cytoplasm. A portion of the end-cell nucleus is included at *n*. An axon profile is present (arrow) traversing the end-cell (*cf.* Fig. 2); this contains vesicles (*v*) similar to those occurring in the terminal processes, as illustrated in Figs. 26 through 30. (The extensive branching and dilatation of the axon seen at the termination appears to commence just before the tracheolar "cell body" is reached, and the final processes of the axon lie between the apposed surfaces of the tracheolar cell and the end-cell.)

This field includes the margin of a photocyte adjoining the end-cell: large numbers of small mitochondria (*m'*) occur in this region (*cf.* Fig. 28). × 23,000.

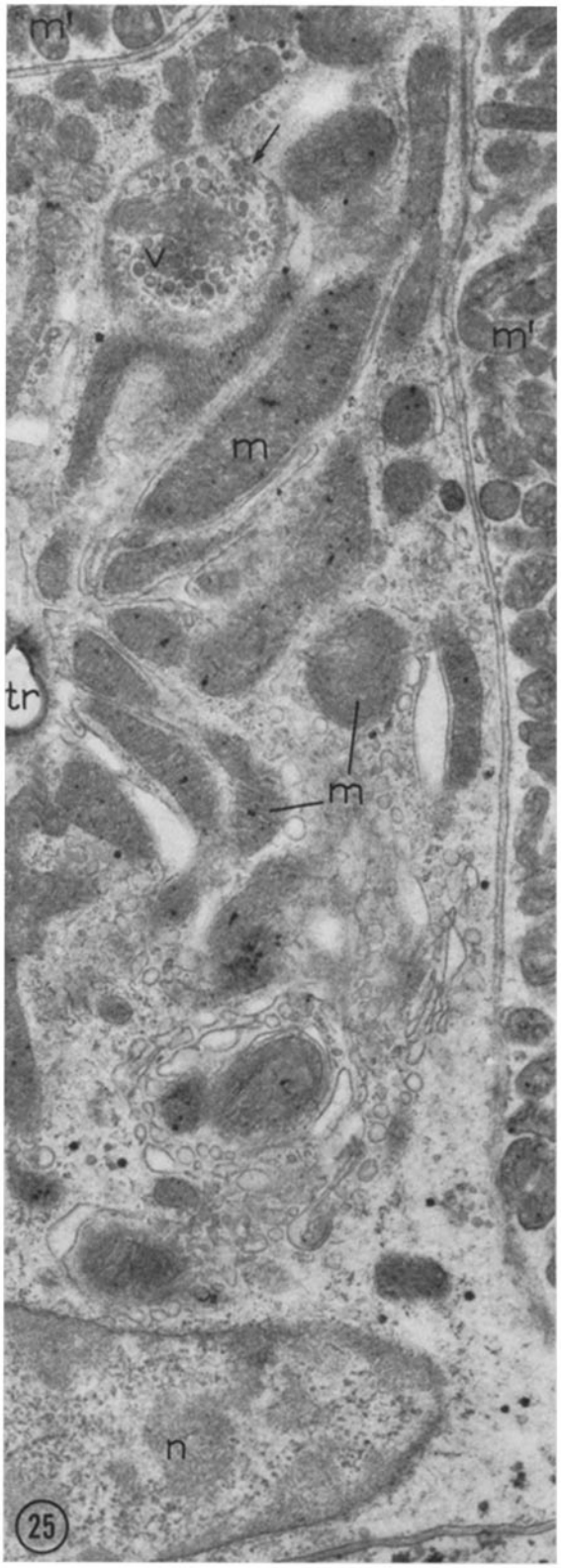
FIGURE 26

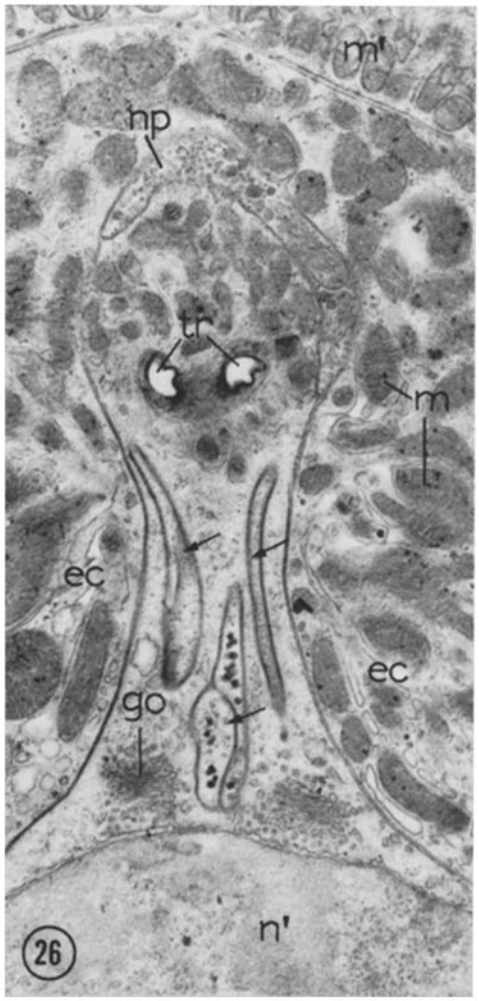
In this figure, the plane of section passes through the tracheolar branches (*tr*) just dista to their point of origin at the bifurcation of the tracheal twig (*cf.* Figs. 2, 25), and a transverse or oblique profile of the tracheolar "cell body" is included. A portion of the nucleus of the latter is shown (*n'*), close to the surface of which lie stacks of smooth-membraned cisternae representing the Golgi complex (*go*). The "neck" of tracheolar cytoplasm continuing from the perinuclear region to surround the tracheoles contains elongated profiles of interdigitations between this cell and the surrounding end-cell (arrows): the respective cell membranes are separated by a gap of *ca.* 100 A from which basement membrane material is absent.

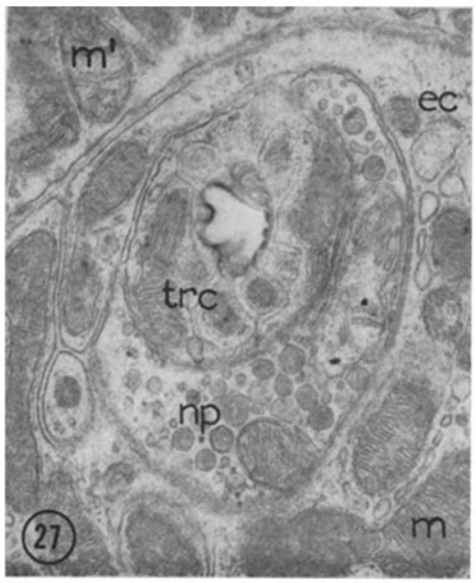
At *np* is seen a transversely sectioned terminal nerve process, situated between the tracheolar cell and end-cell (*ec*) surfaces. The edge of a photocyte is included at the top of this field: note the mitochondria in the end-cell (*m*) and in the adjoining photocyte cytoplasm (*m'*). × 15,000.

FIGURE 27

A transversely sectioned process of a tracheolar cell (*trc*) still ensheathed by the end-cell (*ec*). A crescentic profile of a terminal nerve process (*np*) containing vesicles, lies between the cell surfaces of these two tracheal components. The plane of this section is approximately perpendicular to that illustrated in Fig. 28 and passes through the tracheole just proximally to its point of exit from the end-cell. Note the mitochondria in the end-cell (*m*) and in the adjoining photocyte cytoplasm (*m'*). × 35,000.


m'
v
m'
m
tr
m
n
25


m'
np
tr
m
ec
ec
go
n'
26


m'
ec
trc
np
m
27

myoneural response in these animals, facilitated response to repetitive stimulation occurs in the initiation or amplification of light production. A full account of this work, with detailed references, is given by Nicol (1955). In higher animals, with a centralized nervous system, neuro-effector control of light production from specialized photophores has been demonstrated by Nicol (1955) in a polynoid worm *Acholoë astericola*, while amongst those teleost fish in which light production is an intrinsic feature not associated with luminescent bacteria, evidence of neural control, possibly *via* the sympathetic nervous system, has also been obtained (references in Nicol, 1955).

It has often been suggested that the central nervous system is involved in light production in the firefly. This conclusion has been based on the observed gross innervation of the lantern, because decapitation or section of the nerve cord anterior to the ganglia supplying the lanterns either prevents luminescence or permits only a faint glow in place of the flash, because mechanical or electrical stimulation of the lantern elicits light production, and also on the grounds that the pattern of normal light emission appears to be a specific mating signal. The literature on these aspects of firefly luminescence has been reviewed by Buck (1948) and Harvey (1952). The innervation of the light organs of *Photuris versicolor* and *Photinus pyralis* has recently been investigated by Hanson (1962), who showed that the organ is a physiological "mosaic" divided into areas corresponding to the branched motor nerve supply, electrical stimulation of each branch eliciting a response from the area of the light organ it supplies. Hanson demonstrated, in addition, that the origin of this localized control is within the abdominal ganglia supplying the light organs in addition to the abdominal musculature, rather than in the more anterior portion of the central nerve cord.

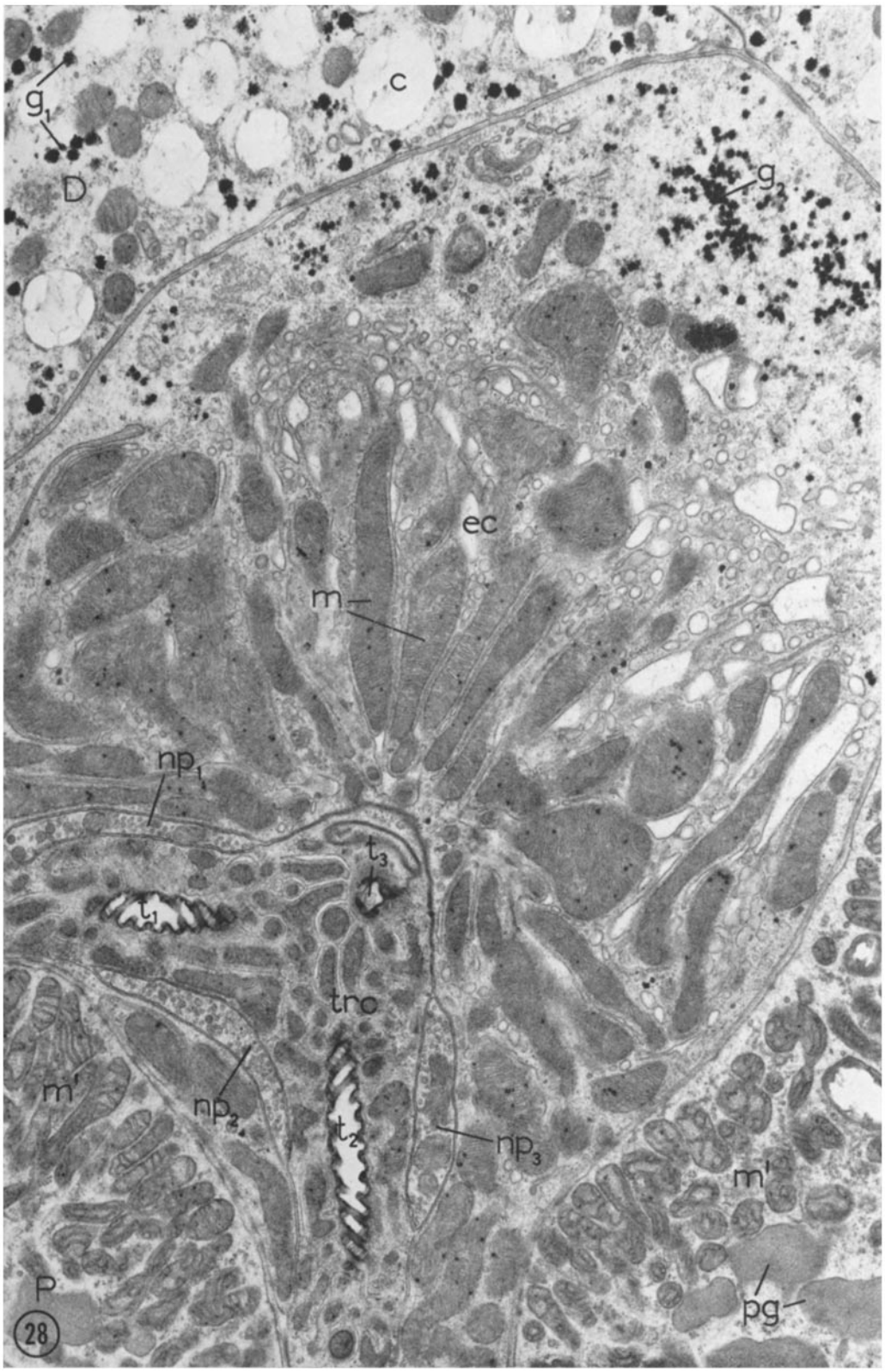
Perhaps the most interesting aspect of the cytological results described here is the establishment of the site and organization of the motor nerve terminations within the lantern. Earlier investigations that touched on the innervation of the organ were hampered by the small size of the nervous structures involved, as well as by the difficulty of interpreting the results of staining methods employed to visualize the nerves. However, Geipel (1914) and Hasama (1942 *a*, *b*, *c*) concluded that in *Photinus marginellatus* and *Pyroceolia rufa*, respectively, the fine nerves end at the tracheal end-cell, a conclusion close to that demonstrated here: that the terminal processes of the axons are insinuated between the "cell-body"

FIGURE 28

A survey field representing an area of junction between cells of the dorsal layer (*D*) and the photocyte epithelium (*P*), including profiles of a tracheal end-cell (*ec*) and a tracheolar cell (*trc*). Note the concentration of photocyte mitochondria (*m'*) along the margin of the end-cell, and also the photocyte granules (*pg*). Empty cavities in the dorsal layer cells (*c*) are thought to represent a soluble material lost during preparation of the material (*cf.* Fig. 8): the deeply "stained" deposits in these cells (g_1) are believed to be of glycogen, and smaller clusters of similar appearance occur in the cytoplasm of the end-cell (g_2).

Much of the cytoplasm of the end-cell (occupying the bulk of this field) is filled with more or less radially arranged mitochondria (*m*) situated within a complex system of membrane-limited processes (*cf.* Figs. 31 to 33). The plane of this section passes through the end-cell distally both to the point of branching (in this instance trifurcation) of the lateral tracheal twig (*cf.* Fig. 2) and also to the cell body of the tracheolar cell, though processes of the latter, including the tracheolar tubes, are seen. Three tracheolar branches are present: two of these (t_1, t_2) are sectioned obliquely, and the third (t_3), transversely. The cytoplasm of the tracheolar cell (*trc*) contains mitochondria, lying in pockets defined by the membrane surrounding the tracheolar tube (*cf.* Figs. 10, 29), and at lower center a tracheolar cell process is seen as it is about to leave the confines of the end-cell, to pass between the photocytes (*cf.* Fig. 9).

Three terminal nerve processes (np_1, np_2, np_3) are seen in transverse profile, insinuated between the end-cell and tracheolar cell surfaces: the detailed organization of these structures is seen to better advantage in Fig. 29. × 16,000.


g1
c
D
g2
ec
m
np1
t3
t1
trc
m'
np2
t2
np3
m'
P
pg
28

of the tracheolar cell and the surface of the adjoining end-cell.

Buck (1948) has reviewed the various hypothetical mechanisms whereby intermittent light production might be achieved: through the controlled synthesis of an essential reactant in the photogenic system; through the regulation of oxygen supply to the photocytes; or by other means. Although it was established that the pattern of normal light emission is controlled by the nervous system, the controversial question of whether the nerves act directly on the photocytes or, secondarily, *via* the end-cell (often pictured as regulating the oxygen supply to the photocytes), was not answered by the light microscopic studies.

Chang (1954) has pointed out the close similarity between neuromuscular relations and light production in the ctenophore *Mnemiopsis*: similarities were noted in the time relations of the two series of events, and both appear to show analogous properties of tetanus and summation. Nicol (1955) accepts the similarity between nerve-muscle and nerve-luminescent effector response and believes that "it is probable that neuro-effector control was established initially for muscular systems and extended secondarily to other effectors, including luminescent organs" and that "it is not surprising, therefore, to find certain modes of regulation common to muscular and luminescent systems." In the neuromuscular junction, the plasma membrane of the terminating axon is closely apposed to that of the fiber, generally in a circumscribed end-plate region. In the light organ of *P. pennsylvanica* described here, a similar intimacy between pre- and postsynaptic surfaces occurs, but in this case no muscle tissue is involved, and the axon terminates between the tracheal end-cell and the tracheolar cell. This finding defines more clearly the parameters to be considered in attempting to relate nervous stimulation and light emission, and at the same time poses important and puzzling physiological problems.

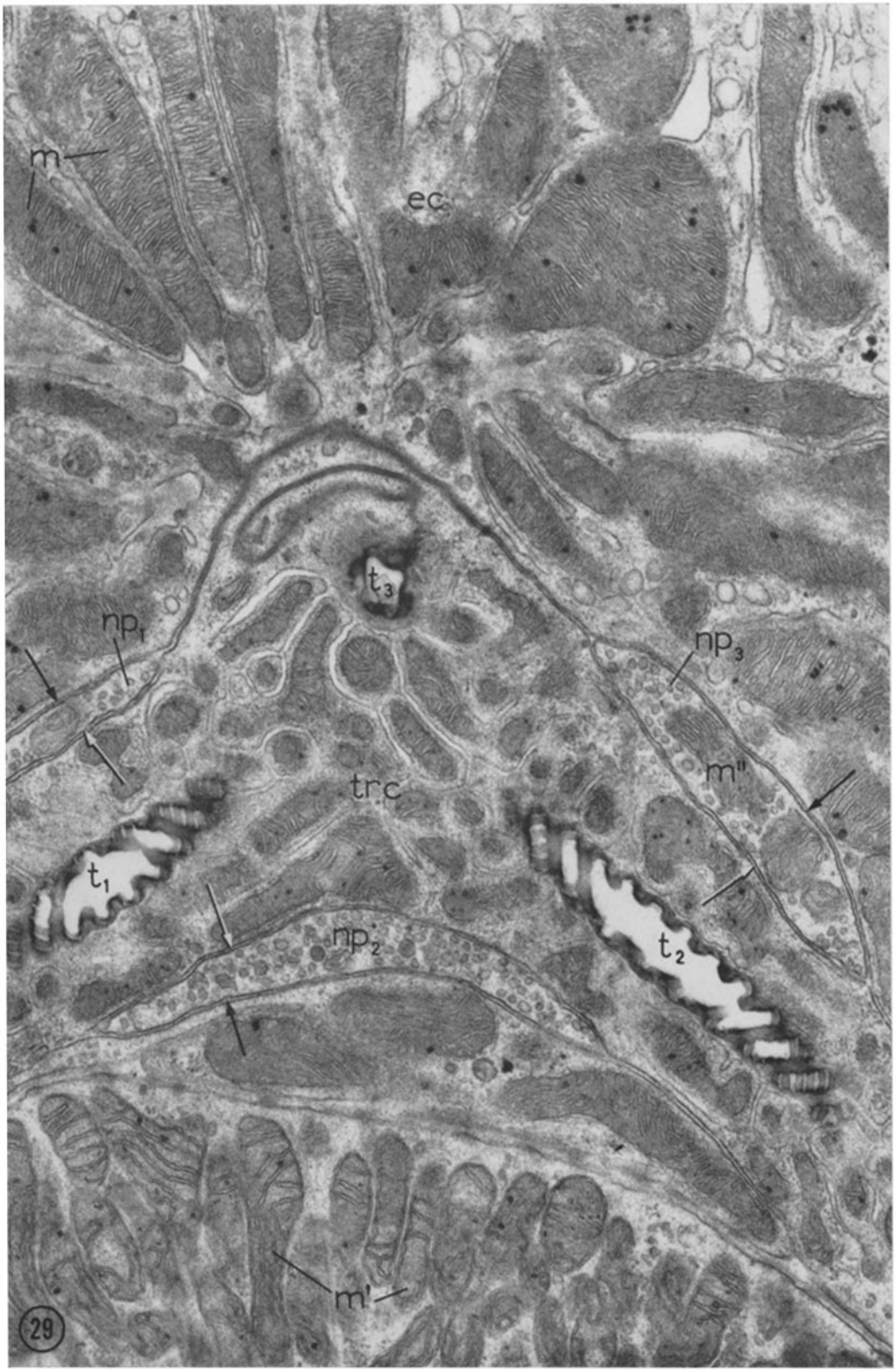
The precise localization within the photocyte of the reactants of the light-producing system has not yet been demonstrated. While it seems likely, on circumstantial grounds, that the characteristic granules occupying the bulk of the photocyte cytoplasm are concerned in the production or storage of one or more of the reactants, this has not been investigated. However, the complex system of cavities and tubules, within the matrix of the granules and leading to the exterior, may represent channels along which a product secreted within the matrix is passed to the surrounding cytoplasm. Since luciferin appears to be irreversibly expended during light production, while *in vitro* at least, luciferase is recovered from the product of the light-emitting reaction (McElroy and Hastings, 1955, 1957; McElroy and Seliger, 1961), the role of the photocyte granules may prove to be the continuous production of luciferin.

Much is now known about the factors controlling the *in vitro* production of light in the firefly system, notably through the work of the authors mentioned above. In addition to (firefly)

FIGURE 29

A portion of the field shown in Fig. 28, at higher magnification: the same labelling has been employed. Note the close-packed subparallel arrays of cristae within the mitochondria of the end-cell, which also contain deeply stained granules lying within the matrix. The mitochondria in the peripheral region of the photocyte (at the bottom of this figure) are smaller, and have a more open arrangement of the cristae. Note the mixed population of vesicles, and the axoplasmic mitochondria (*m''*) within the profiles of the terminal nerve processes (*np*): the larger vesicles have a dense content (*cf.* Fig. 30). The polygonal pockets of tracheolar cytoplasm limited by plications of the membrane surrounding the tracheolar tubes (*cf.* Fig. 10) often contain mitochondria in this region of the cell, though along the narrow extensions of the cell, between the photocytes, the pockets are present but usually lack mitochondria.

The membrane limiting the terminal processes is separated from that of the end-cell on the one side (black arrows), and of the tracheolar cell on the other (white arrows) by a gap of *ca.* 200 A: there is no morphological indication in these preparations that the terminal processes are preferentially associated with either one or other of the cells flanking them. × 30,000.


m
ec
t_3
np_1
np_3
trc
m''
t_1
np_2
t_2
m'
29

luciferin and (firefly) luciferase, magnesium ions, adenosine triphosphate and molecular oxygen are known to be utilized in the photogenic reaction. According to McElroy and Seliger (1961), the role of the enzyme is to catalyze the interaction of luciferin and ATP (with magnesium ions as a co-factor) to produce an enzyme-luciferyl-adenylate complex, which in presence of molecular oxygen is oxidised to a corresponding enzyme-oxyluciferyl-adenylate complex, with the emission of one light quantum per molecule. The latter complex is believed to be very stable, and to act as an efficient product-inhibitor, curtailing light emission, but which is believed to react with pyrophosphate (which stimulates light production *in vitro*), releasing the free enzyme from the inhibitory complex, a reaction that also produces ATP and oxyluciferin. The latter product takes no further part in light emission, and apparently is not reduced to luciferin in the cell; hence luciferin must be continually resynthesised, presumably in the photocytes.

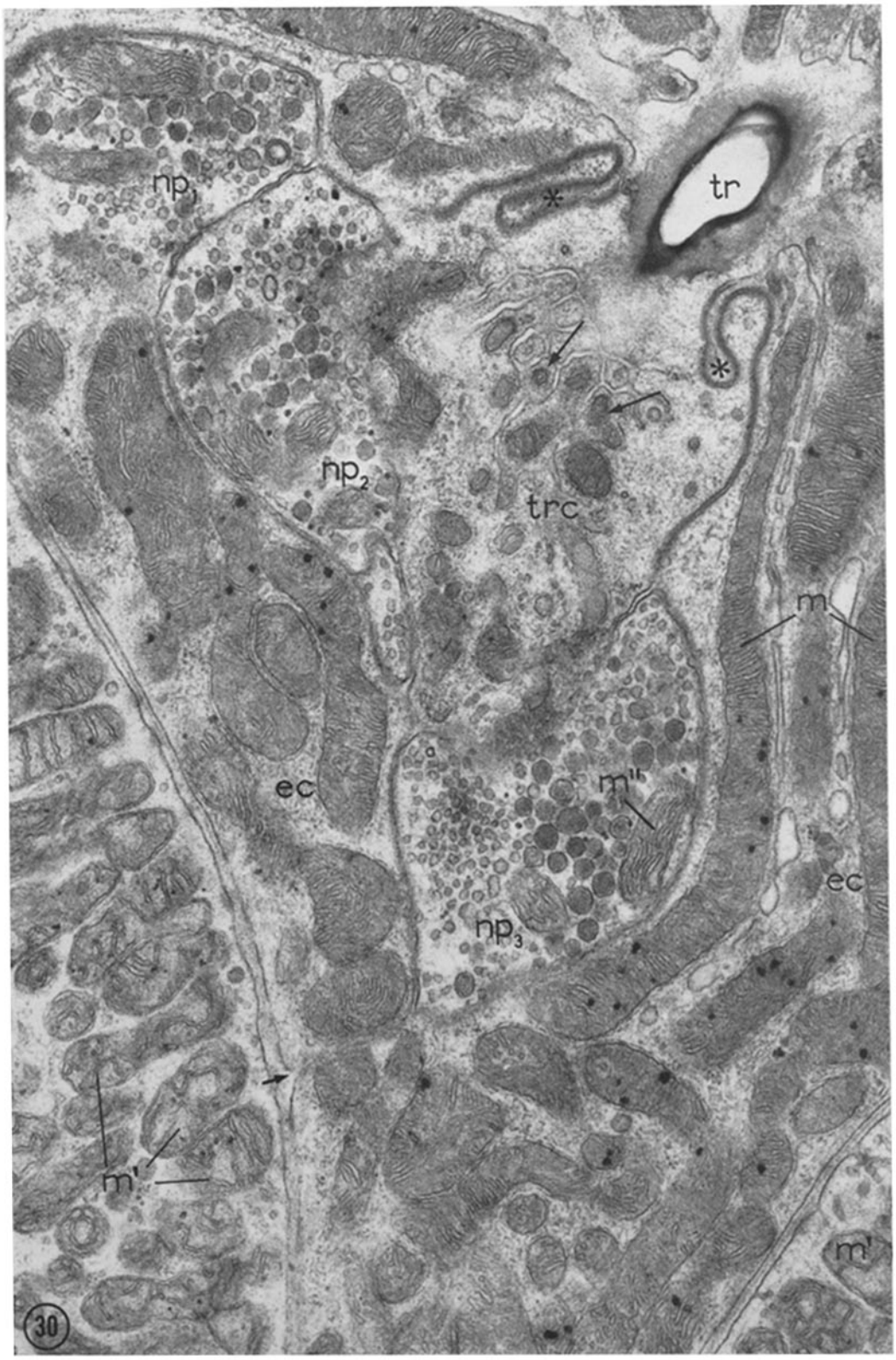
McElroy and Hastings provide evidence for the *in vitro* build-up of the active complex under anaerobic conditions, which is oxidized on rapid addition of oxygen to the system, to produce a flash. A striking *in vivo* analogy to this phenomenon has been described by Buck (1955): in low oxygen tensions (pO_2 about 4 mm), intact fireflies that normally flash exhibit instead a prolonged "hypoxic glow," giving way to a brief bright "pseudoflash" when the oxygen tension is suddenly increased (Snell, 1932; Alexander, 1943; Buck, 1948, 1955). However, Buck points out that while the "pseudoflash" may well represent the rapid oxidation of an active complex produced during hypoxic conditions (as in the *in vitro* system), it is most unlikely that under normal conditions the photocytes could ever be segregated in the body at the required initial level of oxygen lack for the operation of this mechanism in the normal flash, and that, even if this hypoxic condition were to be attained, the rate of oxygen diffusion from the tracheoles to, and in solution through, the photocyte cytoplasm would probably be too slow to be reconciled with the observed rapidity of the normal flash. In addition, McElroy and Seliger (1961) have pointed out that the light emission curves for the "*in vitro* flash" and the "pseudoflash" are similar, both being much longer than the normal flash, which has not been reproduced *in vitro*, and they conclude that both these experimental systems are only models of the normal flash. This is a most important conclusion, since

FIGURE 30

An oblique section through an end-cell region in the lantern of the firefly *P. pennsylvanica*. An undivided tracheal twig is present at upper right (*tr*), and much of the area of this field is occupied by end-cell cytoplasm (*ec*) containing elongated mitochondria (*m*) with subparallel cristae and deeply stained granules (*cf.* Fig. 29). This section has grazed the tracheolar cell (*trc*) invaginated into the end-cell, and the tracheolar cytoplasm contains characteristic membrane-limited "pockets" (long arrows) often surrounding small mitochondria, and seen here in surface aspect (*cf.* Figs. 10, 29). Sinuous profiles of the closely apposed cell membranes of tracheolar- and end-cell are indicated by asterisks.

Three terminal processes of the nerve are present (np_1, np_2, np_3), sectioned parallel to their broad faces; *i.e.* approximately perpendicular to the profiles shown in Fig. 29. These contain small mitochondria (m'') and large numbers of circular profiles of varying size, representing a mixed population of vesicles, the largest of which have a dense content. The functional significance of this size variation is not known, but it should be noted that the populations of large and small vesicles fall respectively into the size range of "neurosecretory vesicles" and "synaptic vesicles" described in nerve terminals in vertebrates and invertebrates. The axon (with its terminal processes) is invaginated into the end-cell (*cf.* Fig. 2) and the level at which the flanges of the latter rejoin above the axon is indicated by a short arrow: the cell membrane is here sectioned tangentially.

Portions of photocyte cytoplasm are included at the left and lower right; note the abundant mitochondria (m') within this cell, aligned opposite the end-cell surface. Similar concentrations of mitochondria also occur in the regions of the photocyte bordering the intercellular tracheolar branches (Fig. 17). × 38,000.


tr
np1
np2
trc
np3
m
m''
ec
ec
m'
m'
30

it is the physiology of the latter that must ultimately be related to the cytological organization of the light organ.

The difficulties met with in relating a model system of light production to the occurrences in the intact organ have been stressed by Buck (1955), who points out, firstly, that in view of the evident biochemical complexity of the light-producing reaction, the establishment of the *in vivo* rate-limiting portion of the reaction, a prerequisite for the analysis of the control mechanism, is a very difficult task. Secondly, he points out that to draw inferences about the bioluminescent mechanism from the time-intensity relations of light produc-

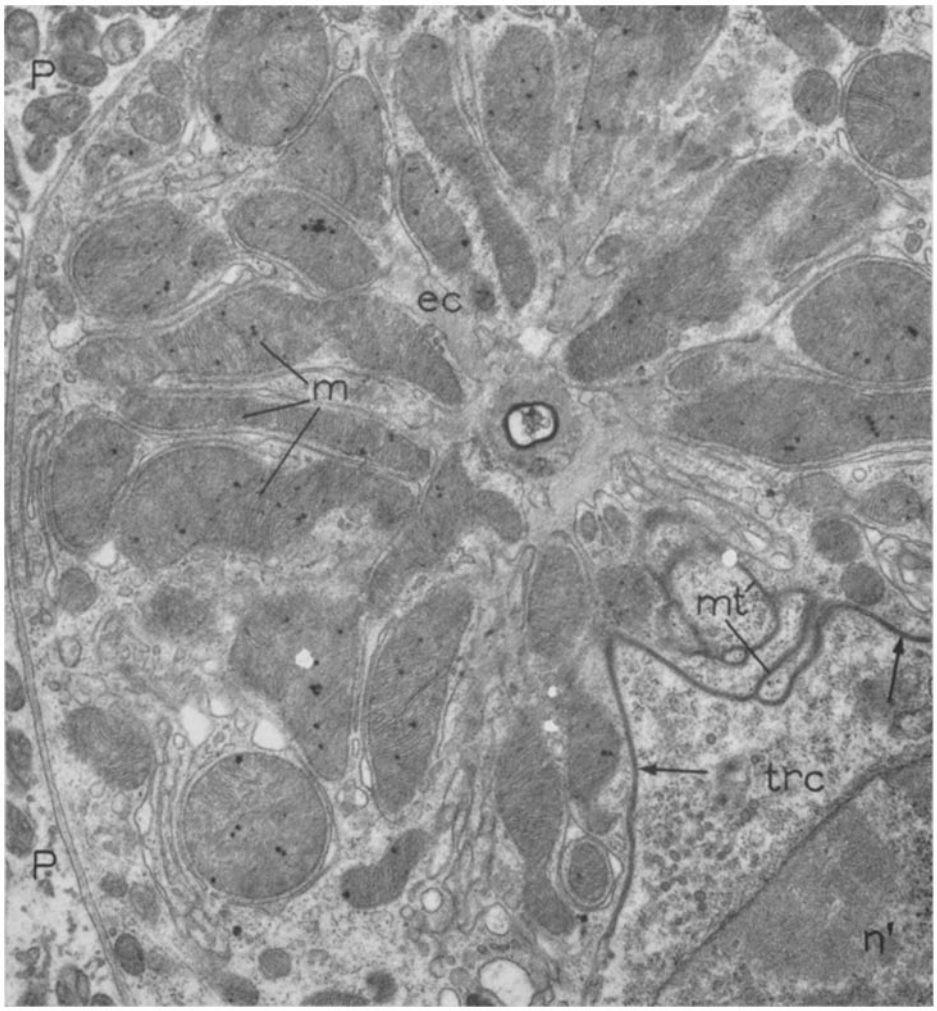


FIGURE 31

An end-cell profile (*ec*) sectioned proximally to the point of division of the tracheal tube (*cf.* Fig. 25) and including an oblique profile of the tracheolar cell-body (*trc*) the nucleus of which appears at *n'*. Processes of the tracheolar cell envelop the tracheolar branches (as in Fig. 26) at a level above or below that included in this section. Note the closely apposed cell membranes of end-cell and tracheolar cell (arrows) and the infolding of the former to form the mestracheon (*mt*), which gives rise to the folded membrane-system dissecting the cytoplasm of the cell surrounding the tracheal tube into a complex meshwork (*cf.* Figs. 32, 33), containing radially arranged mitochondria (*m*). Margins of photocytes (*P*) are included on the left of the figure. × 20,000.

tion in the intact insect is unsafe, because of the structural complexity of the light organ, which obscures the relationship between the response of the whole organ and that of the individual photocytes. Buck (1948) states that in male *Photinus pyralis*, the two lanterns together contain about 15,000 photocytes, arranged around 6,000 cylinders, each of which is traversed by tracheae giving off 80 to 100 end-cells per cylinder. Buck observed that the cells surrounding each cylinder may glow independently, since "The surface of the organ may show minute isolated 'doughnuts' of light which glow on and off independently," and suggests that the flash duration of 0.1 to 0.2 sec. found by Brown and King (1931) and Snell (1932) represents the "statistical result of the firing of units slightly out of phase," a possibility supported by the "resemblance of the time-intensity curve to the normal-distribution curve." Buck suggested that the flash duration of the individual luminescent unit may be of the order of 10 msec. The observations of Hanson (1962) imply that the physiological unit of the flash is the group of cylinders supplied by each nerve branch.

The tracheoles in the light organ described here form an extremely rich and rather regular array, arranged in subparallel fashion between the photocytes, principally perpendicular to the cylinder axis containing the end-cells within which they arise. Since the slab-like photocytes are only *ca.* 6 to 10 μ in width, and have tracheoles interposed between them at intervals of *ca.* 10 μ (Fig. 1), the maximum distance separating any point in the photocyte cytoplasm from tracheolar surface is evidently considerably less than 10 μ. The present study confirms the conclusions of Beams and Anderson (1955) that no mechanical sphincter exists in the light organ, whereby the oxygen supply might periodically be cut off, and the *morphological* indications are that the photocytes are continually and efficiently supplied with oxygen, whether by rapid diffusion if the tracheoles are gas-filled during life, or more slowly but no less constantly, if these are fluid-filled. In short, the detailed architecture of the light organ seems to preclude the possibility that oxygen availability is rate-limiting, under normal conditions.

It has been suggested (McElroy and Hastings, 1955, 1957; Buck, 1955; McElroy and Seliger, 1961) that, in the intact insect, the immediate control of flashing may be *via* the rate-limiting formation of the active intermediate, possibly through the pyrophosphate-mediated release of enzyme (luciferase) from the inhibitory oxyluciferyl-adenylate complex, and that the triggering of light emission by the motor nerve impulse may reflect the sudden release of pyrophosphate in the photocyte cytoplasm. McElroy and Hastings (1955, 1957) and McElroy (1957) proposed that controlled release of pyrophosphate may take place *via* an acetylcholine-Coenzyme A-ATP cycle[2] if acetylcholine is released from the nerve ending as in the vertebrate cholinergic myoneural junction. In insects, acetylcholine has not been found in the myoneural junction (Wigglesworth, 1958), but is present in high concentrations in the insect central nerve cord (Mikalonis and Brown, 1941; Tobias *et al.*, 1946), and, in view of the evident diversity of transmitter substances in invertebrates (Welsh, 1957), it would not be surprising if this substance proves to be present in the nerve terminals in the firefly lantern. The topography of the terminal processes in the light organ constitutes a more serious objection to the suggestion that a transmitter passes from the axons directly to the photocytes, since the nerves have been found to end between the end-cell and the "cell body" of the tracheolar cell, the latter lying between the nerve terminals and the surface of the photocytes. From the spatial relations between the endings and the tracheal and photocyte cells, it may be inferred that the triggering action of the nerve impulse may act in one of two ways. Either (1) the photocytes may be stimulated to produce light by the secretion of a substance from the axon which, chemically altered or otherwise, reaches the photocytes via the intervening end-cell cytoplasm, or (2) the role of the nerve terminals in the lantern may be analogous to the situation in striated muscle: the arrival of the nerve impulse initiating a depolarization of the membrane of the tracheolar cell which might then be channeled between the photocytes.

With regard to the second alternative, it should be remembered that the plasma membrane of the

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[2] (Acetylcholine)

$$\text{AcCh} \dashrightarrow \text{Ch} + \text{Ac} \xrightarrow[\text{CoA}]{\text{ATP}}$$

$$\text{pyrophosphate} + \text{Ac.CoA} + \text{Ad}$$

$$\downarrow$$

(splits inhibitory complex)

(from McElroy and Hastings, 1955, 1957)

tracheolar cell prolongation is separated from that of the photocyte by an extracellular gap containing a layer of basement membrane material, and thus the photocytes could only secondarily be affected by any electrical disturbance propagated along the tracheolar membrane, *via* alteration in the ionic content of this extracellular space. In view of what is known of the biochemistry of light emission, and following the principle of conservation of hypothesis, the first alternative seems to offer the more promising line of approach. If pyrophosphate supply is indeed critical in triggering light emission, it is possible that this is passed in a "pulse" to the photocyte following the arrival of the nerve impulse at the nerve ending, *via* the liberation of acetylcholine into the end-cell cytoplasm, in which the acetylcholine-Coenzyme A-ATP cycle may take place. It is possible that the unusual complexity and high mitochondrial content of the end-cell is connected with this role.

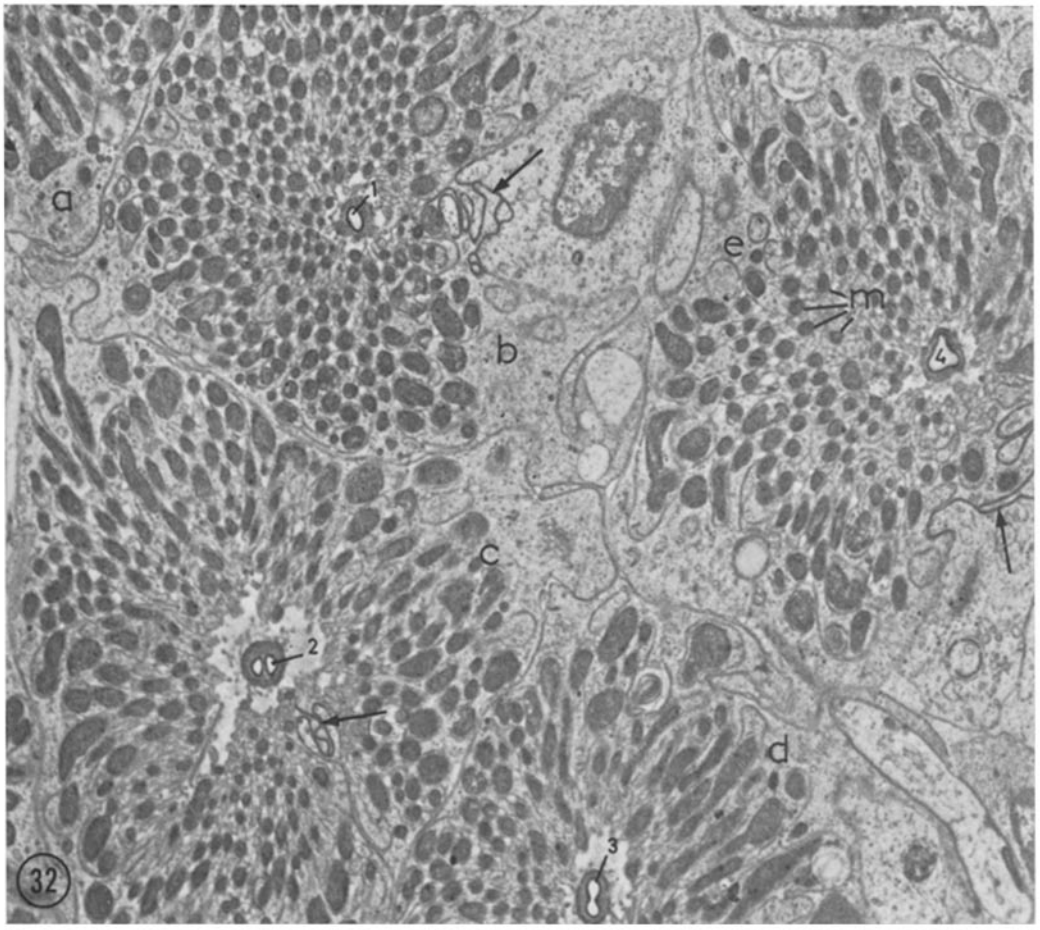
Several authors (see Buck, 1948 and Harvey, 1952) have observed that in species in which the photocytes are arranged around cylinders (Buck's "Type 6 organ") the surface of the lantern during submaximal light emission induced by toxins, injury, or hypoxia, shows a pattern of minute luminescent rings, each of which appears to represent a response from the peripheral cytoplasm of the photocytes bordering each cylinder. Of special interest here is Lund's (1911) suggestion that the bright "angular spots" of fixed position, into which the luminescent rings may be resolved, do not correspond to the end-cells themselves, but are situated in the photocyte cytoplasm immediately adjoining the origin of the tracheoles, those regions, that is, that have here been shown to be closest to the terminal processes of the nerves. In addition, the spread of luminescence across the photocyte cytoplasm from the borders of the cylinders has been described (Buck, 1948) and it is possible that these observations, admittedly made on abnormally luminescing insects, may give an indication of the normal sequence of events in light emission, which are not otherwise directly observable because of the uniformly brilliant appearance of the surface of the light organ during the normal rapid flash. According to the hypothesis adopted here then, the initial pulse of pyrophosphate (or other stimulating material) is supplied to the photocytes shortly after the arrival of the nerve impulse, and initiates light production first in the regions of the photocyte cytoplasm adjoining the end-cells. The liberation of further pyrophosphate during the initial photogenic reaction (McElroy and Seliger, 1961) may then allow the reaction to proceed progressively throughout the photocytes.

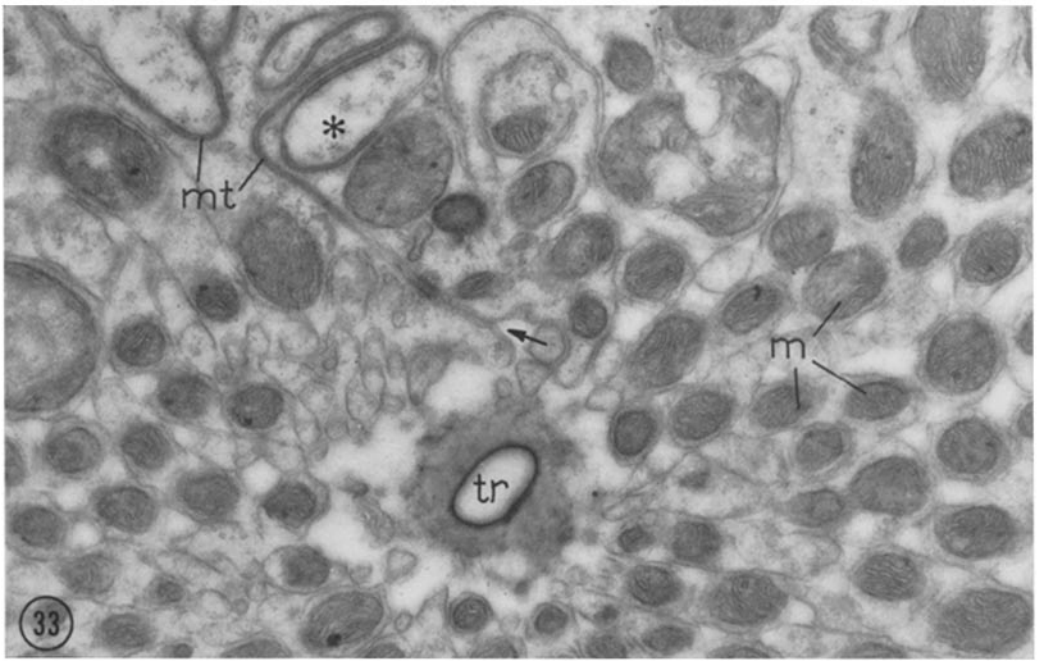
FIGURES 32 AND 33

Electron micrographs of material from a methacrylate-embedded lantern of the firefly *Photinus pyralis* (male). In this species the arrangement of the cytoplasmic processes and enclosed mitochondria within the end-cell is more regular than in *Photuris pennsylvanica*, illustrated in Figs. 1 through 31.

In Fig. 32 is seen a group of five transversely sectioned tracheal end-cells (*a* through *e*) situated at the ventral surface of the lantern, adjoining the hypodermis, in which region the "cylinders" containing the cells of the tracheal system are wide, in this species. Note the sinuous mestracheon profiles (arrows) and the levels of section illustrating the division of the tracheal twig within the end-cell into two tracheolar branches (at *1*, *2*, and *3*). The profile at *4* appears to represent a tracheal twig close to a point of trifurcation. Note the elongated mitochondria (*m*), here seen for the most part in transverse section, arranged rather regularly in the end-cell cytoplasmic processes, as has been described by Beams and Anderson (1955).

Fig. 33 represents an enlargement of a portion of the end-cell profile seen at *b* in the last figure. The mestracheon (*mt*) follows an irregular course across the cell, sometimes affording profiles of finger-like processes (*), before the two membrane surfaces of the mestracheon diverge (arrow) close to the tracheal lumen (*tr*). The cytoplasmic tubular processes in the end-cell, many of which contain mitochondria (*m*), are limited by an elaborate (and in this species regular) membrane system, ultimately continuous with the superficial cell membrane via the mestracheon invagination. Fig. 32, × 7,500. Fig. 33, × 32,000.


a
1
b
c
2
d
3
e
m
4
32


*
mt
m
tr
33

It should be remembered however, in considering this hypothesis, that pyrophosphate would not be expected to pass readily across membranes, and that, in the above scheme, two cell membranes and an intercellular space separate end-cell and photocyte cytoplasm. On the other hand, according to the scheme proposed by McElroy and Hastings (1955) and McElroy and Seliger (1961), only a small amount of pyrophosphate may be required to trigger the light reaction, since the subsequent production of this substance during the reaction may allow the spread of luminescence from the initial foci, adjoining the tracheal end-cells. Moreover, Chase and Buck (1959) and Hanson (1962) found that weak stimulation of the nerves supplying the light organ elicits a neurally mediated flash with a latency of about 65 to 75 msec., while very strong stimulation produces a flash with a latency of about 15 msec., possibly representing a response via direct excitation of the photocytes. The neurally mediated latency is far longer than that occurring at a myoneural junction, suggesting that the events taking place between the arrival of the motor impulse at the nerve terminal in the light organ and the activation of the photocytes are unusually slow.

From a subject of considerable complexity, it is possible to isolate certain points of outstanding interest, the solution of which promises to advance our understanding of the mechanism of the initiation and control of light production. First, one may consider the relation between the structure of the luminescent organ and the pattern of light emission. Buck (1948) points out that fireflies that emit light in brief flashes (as opposed to those which glow or pulse) alone exhibit well defined end-cells associated with cylinders, as in the species described here. If the end-cell in flashing species is the site of localized production and sudden release of pyrophosphate or other material to the photocytes, then information on the relationship between nerve endings, tracheal cells, and photocytes, in species in which end-cells are either absent or are not sufficiently well elaborated to permit easy histological identification, might reveal some cytological basis for the variable patterns of light production. It seems probable, on the other hand, that this variation may be attributable, in part at least, to differences in the pattern of motor impulse supply.

The presence of large numbers of mitochondria in the photocyte cytoplasm adjacent to the intercellular tracheoles presumably indicates the site of production of ATP, a reactant in the luminescent system, since these structures are sparsely distributed elsewhere in the cell. The "differentiated zones" around the nuclei, in the ventral region and especially in the dorsal region of the photocytes bordering on the cells of the dorsal layer, are cytologically distinct, but the significance of the bodies located in these regions of the photocytes has not been established. However, the fact that the cells of the dorsal layer and the granules in the immediately adjacent photocyte cytoplasm are characterized by similarly soluble inclusions possibly indicates, as has often been suggested, that the dorsal layer may function as a repository for waste material from the photocyte epithelium, perhaps for oxyluciferin, believed to be produced irreversibly during the luminescent reaction (McElroy and Hastings, 1955; McElroy and Seliger, 1961). A further and more active role for the dorsal layer cells is suggested by the presence of large amounts of material believed to be glycogen in their cytoplasm, which is possibly mobilized for use in the underlying photocyte epithelium.

It is clear that valuable information on the localization of the reactants taking part in the luminescent reaction might be afforded by combined cytological and biochemical studies on the light organ: for example, the role of the "photogenic granules" might be definitely established by cell fractionation studies. While the suggestion that acetylcholine liberation may be involved in triggering the light reaction has been accepted as a working hypothesis, the presence of structures resembling "neurosecretory droplets" in addition to the "synaptic vesicles" within the terminal axoplasm in the light organ may indicate that a more complex transmitter mechanism is operative in triggering the light reaction.

The work described here defines more clearly the details of the intact organization and innervation of this effector system, an important point to be considered in any attempt to establish the physiological sequence of events involved in light production. The organization of the neuro-effector junction in striated muscle shows a striking degree of similarity in a wide range of animals. A comparative investigation of the neuro-effector junction in those forms exhibiting intracellular luminescence in innervated photo-

phores would not only contribute to a clearer understanding of the physiology and control of this type of light production, but perhaps also to the more general problem of the mechanism of transmitter release from the terminating axon.

Results similar to those described in this paper have been obtained by Dr. and Mrs. Bruce Wetzel, who are, in addition, currently investigating the structure of the larval firefly light organ, at the National Institutes of Health Laboratories, Bethesda, Maryland.

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