Observations on the Fine Structure of the Turtle Atrium*

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ABSTRACT

The general fine structure of the atrial musculature of the turtle heart is described, including; the nature of the sarcolemma; the cross-banded structure of the myofibrils; the character of the sarcoplasm, and the form and disposition of its organelles. An abundant granular component of the sarcoplasm in this species is tentatively identified as a particulate form of glycogen.

The myocardium is composed of individual cells joined end to end at primitive intercalated discs, and side to side at sites of cohesion that resemble the desmosomes of epithelia. Transitional forms are found between desmosomes and intercalated discs. Both consist of a thickened area of the cell membrane with an accumulation of dense material in the subjacent cytoplasm. This dense amorphous component is often continuous with the Z substance of the myofibrils and may be of the same composition. The observations reported reemphasize the basic similarity between desmosomes and terminal bars of epithelia and intercalated discs of cardiac muscle.

Numerous unmyelinated nerves are found beneath the endocardium. Some of these occupy recesses in the surface of Schwann cells; others are naked axons. No specialized nerve endings are found. Axons passing near the sarcolemma contain synaptic vesicles, and it is believed that this degree of proximity is sufficient to constitute a functioning myoneural junction.

Investigations with the light microscope have failed to produce a generally accepted interpretation of the nature of the intercalated discs of cardiac muscle. Those investigators who considered the myocardium to be a syncytium, interpreted the intercalated discs as sites of formation of new sarcomeres (15); as special devices for coordination of the contraction of the myofibrillae (10); or as irreversible contraction bands (16, 17). Those who questioned the syncytial character of heart muscle, regarded the intercalated discs as accumulations of intercellular cement (41) or tendinous junctions between individual cells (21). In recent years cardiac muscle has been examined with the electron microscope by Kisch *et al.* (19), Beams *et al.* (4), van Breemen (8), Weinstein (39), Sjöstrand and Anderson (37), Poche and Lindner (31), Moore and Ruska (22), and Muir (23). The most significant result of these studies has been the demonstration that cardiac muscle is not a syncytium and that the intercalated discs are specialized junctions between cellular units of the myocardium. This study of the atrial musculature of the turtle heart supports these conclusions and presents new observations on the origin of the relatively simple intercalated discs of this species and further clarifies the relationship of these structures to desmosomes, terminal bars, and other specializations of the cell surface that serve to maintain cell cohesion. It also reports the occurrence of a particulate form of glycogen in the sarcoplasm that has hitherto gone unrecognized, and provides additional information on the structure of the sarcolemma and the nature of the myoneural junctions in cardiac muscle.

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Materials and Methods

The observations are based upon the study of cardiac muscle from one turtle of the species *Sternotherus odo-*

ratus, and three *Pseudomys elegans*. For electron microscopy, small blocks of tissue (1 x 1 x 2 mm.) were cut from the atrium of the excised heart and fixed by immersion for 2 to 3 hours in 1 per cent osmium tetroxide adjusted to pH 7.6–7.8 with veronal acetate buffer (25). After very brief washing in distilled water, the tissue was dehydrated in increasing concentrations of ethanol (60, 80, 95, and 100 per cent) and infiltrated 3 hours in three changes of *n*-butyl methacrylate containing 2 per cent luperco CDB as catalyst. Polymerization of the plastic was carried out in an oven at 50–60°C. Sections approximately 30 mμ in thickness were cut on a Porter-Blum microtome (32) and examined directly without removal of the plastic. Micrographs were made on an RCA electron microscope, model EMU-3B, at original magnifications of 2000 to 15,000 and enlarged photographically to the desired final magnifications.

For light microscopy, blocks of tissue were fixed in Stieve's fluid, and 5 μ paraffin sections were stained with hematoxylin and eosin. Other blocks were fixed in Rossman's fluid and stained by the periodic-acid–Schiff reaction for the demonstration of glycogen. Control sections were similarly stained after preliminary treatment with saliva. Nerves were studied in tissue fixed in alcohol-formol-acetic and stained by the Bodian method or were preserved in 25 per cent chloral hydrate in 50 per cent alcohol and impregnated by Ungewitter's urea-silver carbonate method (38).

Discussion of surface specializations of non-cardiac tissues are based upon previous electron microscopic studies of epidermis (35), oral and intestinal epithelium (13), and renal adenocarcinoma (12).

OBSERVATIONS

The wall of the turtle atrium is composed of a continuous outer layer of muscle lined by a delicate network of interlacing trabeculae carneae (Figs. 7 and 8). Each of these slender muscular bands is covered by an endocardium consisting of a single layer of flattened endothelial cells resting upon a thin basement membrane. The cytoplasm of the endocardial cells is of very low density and contains relatively few organelles. The inner aspect of the cell membrane on both the free and the attached surfaces of these cells is studded with numerous minute vesicles (400 to 600 A). Some of these are attached to the cell membrane by a narrow neck through which their lumen appears to communicate with the extracellular space. The vesicles thus resemble those first described by Palade (26) in capillary endothelium and later observed in several other cell types of mesenchymal origin.

The endothelium is separated from the underlying muscle by a subendocardial space of variable width (Figs. 1, 14, and 17) filled with a ground substance that appears homogeneous and has considerable density (Fig. 5). Embedded in the ground substance are collagenous fibers and unmyelinated nerves, the two often running side by side (Figs. 12 and 17).

The muscle fibers are in close contact with one another over much of their surface but at intervals they diverge, leaving narrow intercellular spaces (Figs. 22 and 23). The clefts between groups of muscle fibers apparently communicate with one another and with the subendocardial space and are probably important pathways for the diffusion of metabolites to and from the interior of the avascular muscle trabeculae.

The Sarcolemma.—The term sarcolemma, as commonly used in the histological literature, apparently included the cell membrane and an associated layer of ground substance and reticular connective tissue fibers. For reasons that will be discussed later, "sarcolemma" is used in the present paper to designate the thin smooth membrane (80 A) that corresponds to the plasmalemma of other cell types. Where it is exposed to the subendocardial space or to an intercellular space, the sarcolemma is covered by a distinct layer (400 to 500 A) of material that is amorphous at the best resolution attained in the present study (Figs. 15, 16, 22, and 23). In electron micrographs, this extraneous coating is of low density immediately adjacent to the sarcolemma, but is distinctly more dense or osmiophilic at a distance of 150 to 200 A from the membrane, where it appears as a dark line running parallel to the cell surface (Figs. 22 and 23). Beyond this dark zone the density falls off gradually, and the exact outer limit of the layer is poorly defined. Spaced at irregular intervals along the outer aspect of the sarcolemma are small bundles of collagen fibrils (Figs. 12 and 22) that correspond to the "reticular" fibers observed with the light microscope in silver-impregnated sections of cardiac muscle. The surface of muscle cells that border on intercellular spaces tends to have many small vesicles just beneath the sarcolemma identical to those previously described in association with the membranes of the endothelial cells (Fig. 23). Similar vesicular structures were observed by Poche and Lindner (31) in mammalian cardiac muscle. Their presence suggests that an active interchange of material takes place between the sarcoplasm and the extracellular fluids by means of pinocytosis.

Extensions of the subendocardial space pene-

trate deeply into the bundles of muscle fibers. Except for these intercellular clefts, the cells are as closely associated with one another as are the cells of epithelia, and where the adjacent muscle cells are in intimate contact, the extraneous coating previously described, is lacking or is reduced to a very thin layer (150 A) between the confronted membranes. At irregular intervals along the cell boundaries there are fusiform densities (Figs. 17 and 21) which very closely resemble the so called *"adhesion plates"* observed with the electron microscope on the surfaces of contact between epithelial cells (Fig. 18). It is now generally accepted that these structures correspond to the dark bodies seen with the light microscope in the "intercellular bridges" of stratified squamous epithelium, and known to histologists as *desmosomes*, or *nodes of Bizzozero*. These bodies were formerly interpreted as granules situated within the intercellular bridges or as deposits of intercellular cement-substance between the ends of connected cell processes. Neither of these interpretations has proved to be correct. Electron micrographs reveal that the desmosomes of stratified squamous epithelium are local specializations of opposing cell surfaces, and thus have a bipartite structure. Each half consists of a thickened area of the cell membrane with an accumulation of dense material in the subjacent cytoplasm. The two halves are separated by a narrow space 150 to 200 A wide that usually appears empty in electron micrographs, but in life is probably occupied by an amorphous intercellular substance of low density that is not well preserved by osmium fixation. Although no visible structure traverses the narrow space between the cell surfaces, it is believed that the desmosomes are points of especially firm attachment between cells. The terminal bars of epithelia have likewise been regarded as rod-like accumulations of intercellular cement, but the electron microscope has shown that they have the same fine structure as desmosomes, differing from them only in their shape. While the desmosome is a round plaque or disc, the terminal bar is a strip or band that may extend the full width of the cell (Fig. 20).

The desmosomes in epithelia are irregularly distributed over the cell surface and have no particular relation to organelles or inclusions of the underlying cytoplasm. In the cardiac muscle described here, the desmosomes generally occur opposite Z bands of myofibrils located in the peripheral sarcoplasm. Moreover, the dense substance of the Z band is often continuous with the dense cytoplasmic component of the desmosome, an appearance which may be responsible for the common misconception that the Z "membranes" of the myofibrils are continuous with the cell membrane.

The lateral surfaces of the muscle fibers are not straight throughout their length, but have step-like offsets where the sarcolemma runs transverse to the axis of the muscle fiber for a distance of a micron or so and then turns to continue parallel to the axis (Fig. 26 and 27). Although such offsets occur at irregular intervals along the length of the fiber, their distribution is not random with respect to the periodic structure of the contractile elements. They invariably cross the myofibrils at a position that would normally be occupied by a Z band. At each such offset the transversely oriented segment of the opposing cell membranes is specialized in the same manner as is the membrane of epithelial cells at desmosomes. The myofilaments of the two adjoining cells terminate in a dense substance immediately subjacent to the membranes (Fig. 26). This dense material has the same appearance and density as that forming the Z bands and is probably of the same composition. These narrow offsets on the surface of adjacent muscle cells appear to be a primitive form of *intercalated disc*. Numerous transitional forms are found between typical desmosomes where the muscle cells are in side to side contact, and simple intercalated discs where they meet end to end (Figs. 24 to 26). The width of the discs is variable, but is seldom greater than that of a single myofibril, whereas their counterparts in higher forms may traverse the greater part of the width of the cell. The fact that these simple intercalated discs in the turtle are not detected with the light microscope is doubtless attributable to their small size and to the fact that they are relatively straight and thus very little thicker than a Z band while in the more highly developed mammalian intercalated discs, the opposing cell surfaces are highly irregular and the over-all thickness of their interdigitated junction is great enough to make them readily visible.

Myofibrils.—In skeletal muscle the myofilaments are assembled in myofibrils of rather uniform cross-sectional area. In turtle cardiac muscle they usually do not form a large number of myofibrils of the same size, but a few of quite variable size. In cross-section the myofilaments often appear to form confluent masses having a highly irregular

shape (Fig. 1). Transverse sections through the A band generally show regular close packing of the myofilaments, but defects and irregularities in the pattern are quite common (Fig. 3). In longitudinal section (Figs. 15 and 16) the myofibrils branch freely and vary greatly in width.

The pattern of cross-striations is essentially the same as that of skeletal muscle. The sarcomere length is 1.5 to 1.7 μ and the A and I bands are clearly demarcated (Figs. 2 and 6). The myofilaments are very distinct in the A bands, but the thinner filaments in the I band are resolved with difficulty. In favorable preparations they can be seen where they cross the Z band (Fig. 6). The A band is traversed by a distinct H, but no M band is seen. The I band is bisected by a dense Z band, and about a third of the distance between it and the A–I junction a faint N band can sometimes be detected.

Sarcoplasm.—The myofibrillar components of the sarcoplasm in turtle heart muscle are relatively few compared to those of mammalian cardiac or skeletal muscle and the interfibrillar sarcoplasm is relatively abundant (Fig. 1). In it are found mitochondria, a Golgi complex, scattered lipide droplets, clumps of lipochrome pigment, small vesicles, and a profusion of small dense granules. The mitochondria are numerous and are found throughout the cell, but they are especially plentiful in the conical regions of sarcoplasm at the poles of the nucleus. They are elongated in form and have the usual disposition of internal membranes, but the latter are often obscured by an unusually dense mitochondrial matrix. Although the mitochondria are commonly aligned in rows in interfibrillar clefts, their spacing shows no particular relation to the pattern of cross-banding in the adjacent myofibrils. The small Golgi complex is situated close to one pole of the nucleus and has essentially the same fine structure as that described for other non-secretory cell types. A sarcoplasmic reticulum is lacking or at best very poorly developed in the turtle heart. It appears to be represented only by small vesicles that occur singly or in small clusters. These seem to be discontinuous and bear little or no resemblance to the labyrinthine system of interconnecting tubules that surrounds the myofibrils of mammalian cardiac and skeletal muscle (33). The interfibrillar sarcoplasm is crowded with small dense granules 150 to 250 A in diameter (Figs. 15, 16, and 4). These bear more than a superficial resemblance to the particles seen in electron micrographs of liver and pancreas and identified as ribonucleoprotein (25, 26). However, the presence of ribonucleoprotein in any considerable amount imparts a strong basophilia to the cytoplasm. The granules in the sarcoplasm of turtle heart muscle are so abundant that, if they were ribonucleoprotein, the cells would almost certainly show a strong affinity for basic dyes. Actually, cardiac muscle is markedly acidophilic. Thus it appears unlikely that the sarcoplasmic particles described here are the same as the nucleic acid-rich particulate described by Palade (27, 29). Careful examination of the micrographs discloses that the sarcoplasmic granules are somewhat larger than ribonucleoprotein granules, more variable in their size and shape, and have less sharply defined margins. Their chemical nature has not been determined, but circumstantial evidence suggests that they may be a particulate form of glycogen. Atrial muscle examined with the light microscope after fixation in Rossman's fluid and staining with the periodic-acid–Schiff reaction, shows the intense magenta color characteristic of glycogen (Fig. 9). This staining reaction is abolished by prior treatment of the sections with salivary amylase or malt diastase (Fig. 10). Sections of osmium-fixed, methacrylate-embedded tissue also exhibit a strong glycogen reaction, with nuclei, myofibrils, and mitochondria appearing as negative images in the homogeneously stained sarcoplasm. The observation with the light microscope of an intense staining reaction for glycogen in the same distribution as the population of small dense granules seen in electron micrographs, constitutes presumptive evidence that the granules are a particulate form of glycogen. More definite evidence must await isolation and chemical characterization of these granules.

Nerves.—Many unmyelinated nerves are observed in the subendocardial space, and some extend into the clefts between muscle fibers. The larger ones are made up of several axons completely enveloped by a common Schwann cell sheath. More numerous than these are the smaller fibers that consist of several axons lodged in shallow recesses in the surface of Schwann cell processes. There may be more than one size class among the axons associated with the same Schwann cell. This is apparent in Figs. 15 and 17, where there are two or three axons 0.5 to 0.8 of a micron and several smaller structures that appear to be axons measuring 0.3 μ or less. The finding of nerves that are at or below the limits of resolution of the light

microscope was unexpected, but this observation does not stand alone. Gasser (14) reported fibers 0.3 μ in diameter in the peripheral nerves of the cat. The axons are often covered by an attenuated Schwann cell process on one side, but exposed on the side adjacent to the muscle fibers (Fig. 13). The surface of the Schwann cell and the exposed portion of the axon are both enclosed in a continuous layer of an amorphous material similar to that coating the sarcolemma. In addition to fibers that retain their contact with Schwann cells, there are a considerable number of axons that have no associated sheath cell. No morphological criteria have yet been defined for distinguishing between sympathetic and parasympathetic nerves at the electron microscope level.

No specialized terminations of the nerves upon the muscle cells are observed. The slender axons (0.3 to 0.6 μ) simply run in close proximity to the sarcolemma. The axoplasm and sarcoplasm are separated from one another at all points by the axolemma and sarcolemma and the combined thickness of their associated layers of amorphous "basement membrane" material (Fig. 17). No evidence was found of interdigitation of the surfaces or of firm attachment of one to the other.

The axoplasm of the nerve terminals, as a rule, is more dense than the cytoplasm of the Schwann cell and contains filiform mitochondria, and variable numbers of minute vesicles 200 to 300 A in diameter. The latter apparently correspond to the *synaptic vesicles* described by De Robertis and Bennett (9) in nerve cells of the earthworm and observed by Palade (28) and Palay (3) at synapses in various parts of the nervous system of higher animals. In the turtle material under discussion here these vesicular elements are few or absent in axons of larger cross-sectional area, but in the smaller axons the axoplasm is often completely filled with closely packed vesicles of uniform size (Figs. 5 and 16). The correlation with the diameter of the axon is not entirely dependable, for where two axons of similar size are associated with the same Schwann cell, one may be crowded with vesicles while the other contains none (see Fig. 15). The naked axons almost invariably contain abundant synaptic vesicles.

It is tentatively concluded that specialized intracellular (hypolemmal) or extracellular (epilemmal) myoneural junctions do not exist in turtle atrial muscle, but that numerous terminal nerve processes of small size course through the subendocardial space and between the muscle bundles and these, simply by passing near the muscle fibers can apparently function without establishing intimate contact with the sarcolemma.

DISCUSSION

After fifty years of controversy over the syncytial versus the cellular theory of cardiac muscle, and of speculation concerning the nature of the intercalated discs, modern methods of microscopy are finally leading to interpretations that may soon be accorded universal acceptance. The results of the present investigation of turtle atrium are in agreement with the earlier electron microscope studies of mammalian heart by van Breeman (8), Sjöstrand and Andersson (37), Poche and Lindner (31), Moore and Ruska (22), and Muir (23), all of which support the conclusion that intercalated discs are specialized junctions between cellular units of the myocardium. Of particular interest in relation to the results reported here is the recent work of Muir, who followed with the electron microscope the development of intercalated discs in rabbits, from embryos to adults. In the early stages, dense plaques were observed on the membranes wherever the surfaces of contact between the adjacent spindle-shaped cells passed obliquely across the myofibrillar axis. These specializations of the adhering membranes were interpreted as the forerunners of intercalated discs. In the newborn, the specialized regions of the membrane were oriented at right angles to the myofibrillar axis and formed a series of simple, step-like discs. Later in postnatal life, there was an increase in complexity of the intercalated discs leading to the condition in the adult mammalian heart, where the surfaces of adjoining cells are highly irregular in contour and deeply interdigitated. It was suggested that traction exerted by the myofilaments, where they insert on the specialized areas of membrane, might be responsible for the step-like offsets of the cell surface; and that the increasing complexity of the mammalian intercalated disc with age may be a response to the greater mechanical force developed in contraction (17, 18). The simple intercalated discs depicted by Muir (23) for mammalian embryos and newborn resemble very closely those described here in the adult turtle atrium. It is not clear whether this is to be interpreted as an example of ontogeny recapitulating phylogeny or whether the contraction in the turtle atrium is simply not vigorous enough to produce mechanical conditions

favoring further elaboration of the cell surfaces at the intercalated discs.

In all of the species that have been studied, the myofilaments of each cellular unit terminate at the intercalated discs in a conspicuous layer of dense material on the inner aspect of the membrane. Poche and Lindner (31) referred to this material as the inner cement substance of the intercalated disc, implying that it serves to bind the myofilaments to each other and to the cell membrane. Muir (23) was of the opinion that it occurs only at sites where cell membranes cross myofibrils. Such is not the case in the turtle heart. In this species there are local accumulations of this dense material along the cell surfaces parallel to the myofibrillar axis. It has been pointed out here that the dark plaques so formed are identical in fine structure to the desmosomes of epithelia. The presence of such structures on the lateral surfaces of cardiac muscle cells and the occurrence of transitional forms between them and simple intercalated discs, clearly establishes the fact that *desmosomes, terminal bars,* and *intercalated discs,* that were formerly regarded as distinct entities, are basically the same kind of specialization of the cell surface. It is also noted in the electron micrographs that the dark substance on the inner aspect of the membranes at desmosomes and intercalated discs has the same density as the Z bands of the myofibrils. Furthermore, the desmosomes tend to occur opposite the Z bands and their dense inner layer is often continuous with the Z substance of the neighboring myofibrils. These observations lead us to speculate that the dense cytoplasmic component of desmosomes, terminal bars, and intercalated discs is probably the same as the Z substance of muscle.

Observations with the light microscope have led to conflicting interpretations as to the composition of the sarcolemma. It has been regarded by some workers as a structureless membrane up to a micron in thickness formed by the muscle cell. According to others, it is not the cell membrane and neither is it a product of the muscle cell (1), but an extracellular layer of connective tissue origin consisting of a network of reticular fibers embedded in ground substance (2). Disagreement over its structure has been largely resolved by the electron microscopic studies of the past few years, but inconsistencies in the use of the term "sarcolemma" persist. Barer (3) defined it in skeletal muscle, as a membrane less than 0.1 μ in thickness, devoid of fibrillar structure. Bennett and Porter (6), in their excellent paper on avian breast muscle, described it as a thin membrane 150 to 250 A in thickness enclosed by a closely adherent collagenous network. A similar structure was reported by Weinstein for cardiac muscle, but the thickness was estimated to be only 100 A. In these earlier electron microscope studies the layer of amorphous substance investing the cell membrane may not have been preserved by the fixation employed. In the more recent studies of Poche and Lindner (31) the sarcolemma in the dog heart is reported to consist of two dark lamellae separated by a lighter intermediate layer. Moore and Ruska (22) also refer to the "outer layer of the sarcolemma." The outer layer of these authors apparently corresponds to what has been interpreted here as an amorphous extraneous coating. The positive periodic-acid Schiff reaction observed with the light microscope in the "sarcolemma" of cardiac muscle (34) is doubtless attributable to the presence of this layer. Its observation adds to the accumulating morphological and histochemical evidence indicating that many cell types have on their exposed surfaces, a thin layer of a mucoprotein or mucopolysaccharide. In electron micrographs of well fixed tissue such a layer can be observed not only at the base of epithelia, where it constitutes the "basement membrane," but also on the smooth muscle cells in the walls of blood vessels, on the Schwann cells of unmyelinated nerves, on the axolemma of naked autonomic nerve endings, et cetera. Whether it is to be regarded as a condensation of the intercellular ground substance of connective tissue or a coating elaborated by the cells themselves, has not been established. It now seems likely that the "sarcolemma," as it was visualized with the light microscope, included at least three distinct components: the cell membrane; an extracellular layer of amorphous material; and a network of collagen fibrils. The sharper definition of the tissue components afforded by the electron microscope makes it possible to assign more precise meaning to many of the descriptive terms in common use in histology. The limiting membrane of the cytoplasm of cells in general is often referred to as the *plasmalemma.* It is logical that the term *sarcolemma* be used to specify the membrane limiting the sarcoplasm of muscle and that *axolemma* be applied to the limiting membrane of the axoplasm of the nerve cell. Since most membranes encountered in electron micrographs of tissue sections appear as sharply defined, dense lines approximately 80 A

thick, the continued use of the term "basement membrane" to describe a relatively thick extracellular layer with ill defined outer limits, no longer seems appropriate but the selection of a more suitable term for this and for the corresponding layer that invests the sarcolemma of cardiac muscle and the exposed membranes of various other cell types must await further study of its origin and function.

Recent publications of Bennett (5), Porter and Palade (33), Moore and Ruska (22) have drawn attention to the presence of a continuous system of membrane-limited vesicles and tubules closely investing the myofibrils. It is assumed that this *sarcoplasmic reticulum* may facilitate and direct diffusion of metabolites within the cell or that it may function in the intracellular propagation of the excitatory impulse. The presence of such a system in the heart muscle of the turtle could not be established. Small vesicles or tubules that might be interpreted as elements of a sarcoplasmic reticulum were seen only very rarely, and there was no indication that these rudiments formed a continuous canalicular network. Such an intracellular circulatory or conducting system may well play an important role in the physiology of skeletal and cardiac muscle of higher warm-blooded animals, but the present findings indicate that a well developed sarcoplasmic reticulum is not essential for the normal functioning of the turtle heart.

The small sarcoplasmic granules tentatively interpreted as a particulate form of glycogen have not been identified, as such, in previous electron microscope studies. This is probably due to the fact that there is relatively little glycogen in either the heart or skeletal muscle of the common laboratory mammals, and sarcoplasmic particles in this size range (150 to 250 A) were assumed to be ribonucleoprotein. It is to be noted, however, that Palade (27), in his classical paper describing the nucleic acid–rich cytoplasmic particles stated that "... a special situation is encountered in striated muscle fibers in which the small granules, together with the endoplasmic reticulum, are found restricted to the thin layers of sarcoplasm that separate the myofibrils. Although close to the membrane of the reticulum, the granules do not appear to be attached to it. Besides this feature, they are also characterized by larger sizes, *i.e.* 200 to 300 A, and a more polymorphic appearance, rods and granules with a light core being a frequent occurrence." This account closely parallels the description given here of the granules in turtle heart muscle, and in retrospect, it is possible that the particles of exceptional size and appearance which Palade noted in muscle, were not nucleoprotein, as he supposed, but glycogen. Definite identification of these sarcoplasmic granules must await their isolation and chemical characterization. It will be recalled that Lazarow (20) isolated by centrifugation of liver homogenates a fraction which he called particulate glycogen. On chemical analysis this consistently contained a small amount of protein (1 to 1.4 per cent) that was believed to play a role in maintaining the particulate structure of the glycogen. In electron micrographs of mammalian liver, the glycogen-rich areas of the cytoplasm often do not show resolvable particles, but instead have a diffuse cotton-wool texture of low density. In the liver, kidney, and skeletal muscle of frogs, and cardiac muscle of turtles, the density (osmiophilia) of the glycogen is greater and its particulate nature more evident. If one may assume that the osmiophilia of the particles resides in the protein moiety of a glycogen-protein complex, one might expect to find a greater proportion of protein in the glycogen fraction isolated from those tissues which display rather dense, discrete particles. Correlated morphological and biochemical studies are now being undertaken to explore the suggested differences in chemical composition and state of aggregation of glycogen in various tissues and in different animal species.

The literature of light microscopy abounds in studies on the innervation of the heart, but the exact nature of the junction of the nerves with the muscle fibers is still poorly understood. Some investigators described end-bulbs or terminal loops (Fig. 11) ending intracellularly in the region of the nucleus (40, 7). Others believed that the nerve endings did not penetrate the cell but terminated on the sarcolemma (24). A number of workers failed to find any special nerve endings and reported instead a rich plexus of exceedingly fine nerve filaments surrounding the muscle fibers (11, 36). This latter interpretation was subject to the criticism that the silver staining methods used were capricious and the appearance of a nervous terminal reticulum might have resulted from the incomplete impregnation of a fine network of argyrophilic connective tissue fibers. The identification of axons and collagen fibers in electron micrographs depends upon clear cut morphological differences instead of silver staining methods of

questionable selectivity. The preliminary observations on the turtle atrium have revealed a large number of axons of varying size running in the subendocardial space and penetrating between the muscle fibers. Some of these are wholly or partially ensheathed by Schwann cells, but the majority are naked axons. No hypolemmal endings were seen and no special epilemmal endings were identified. The small nerves pass near the sarcolemma but apparently are not firmly attached to it at any point. It is recognized, however, that the problem of sampling in electron microscopy is such that one cannot rule out the possibility that more intimate contact is established at sites not visualized in this study. The axoplasm in the bare axons is usually filled with small vesicles of the kind described by other workers at axodendritic synapses. If the presence of these "synaptic vesicles" can be taken as an indicator of sites of chemical transmission of nerve impulses, then one must conclude that the majority of the free axons in the turtle atrium are active and that their proximity to the sarcolemma suffices to constitute functioning myoneural junctions.

This paper has presented certain general observations on the fine structure of the turtle atrium, including a description of the structure of the intercalated discs and a preliminary account of the nature of the myoneural junctions. The old question as to whether there is a specialized conducting system in the turtle heart remains unsettled, and the anatomical relations and functional significance of the abundant smooth muscle in the myocardium of this species are still to be elucidated. Because the turtle is still much used in teaching and in research on the physiology and pharmacology of cardiac muscle, it is to be hoped that an adequate morphological basis for the interpretation of the experimental results will soon be provided.

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EXPLANATION OF PLATES

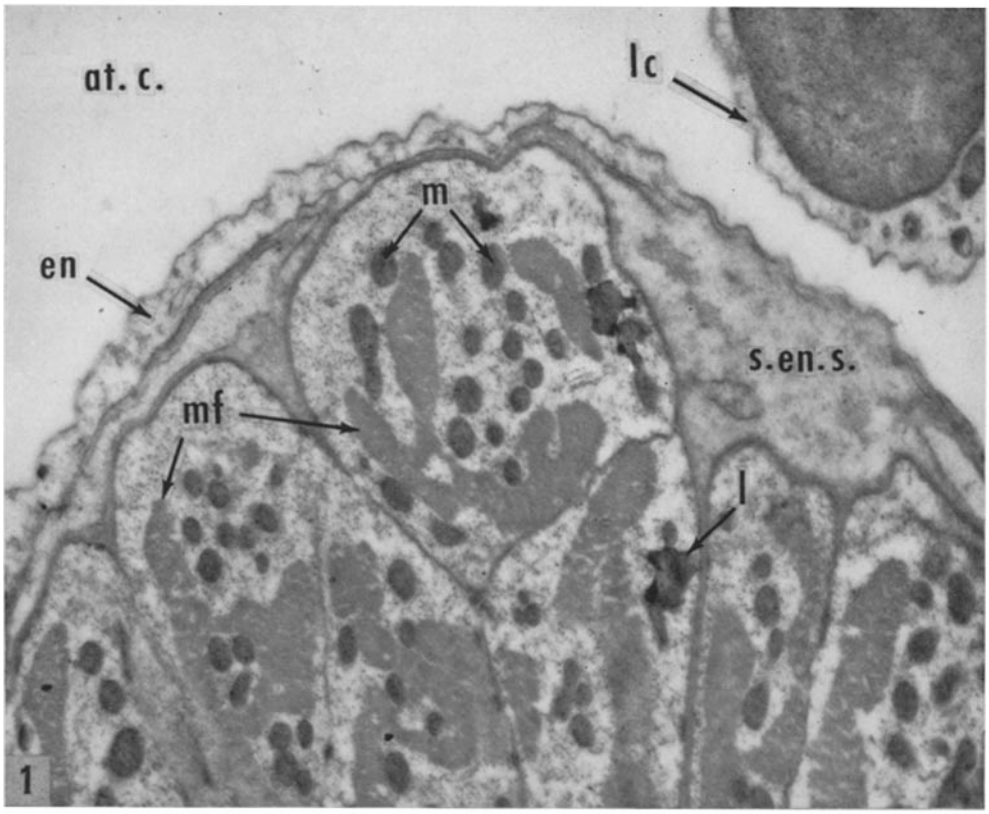
Abbreviations Used in the Figures

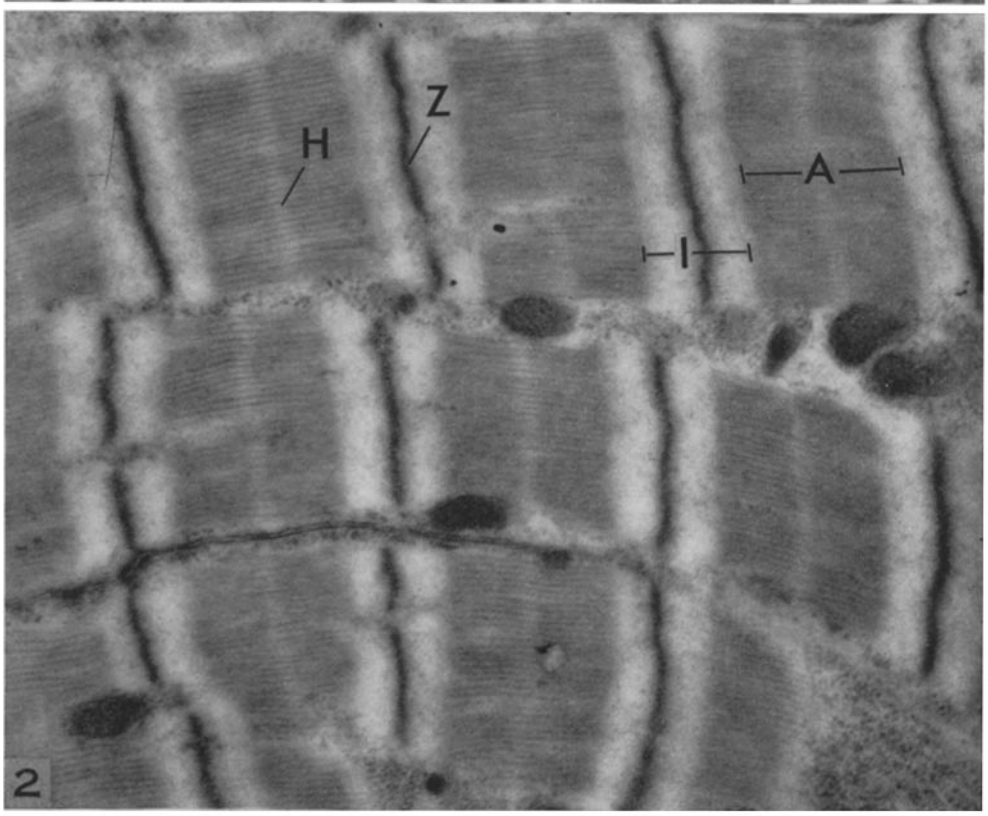
at. c., atrial cavity.
en, endocardium.
lc, leucocyte.
s. en. s., subendocardial space.
nc, nucleus.
m, mitochondria.
ncl, nucleolus.
mf, myofibril.
mfl, myofilament.
g. s., ground substance.
ax, axon.
s. v., synaptic vesicles.
gl, particulate glycogen.
cl, collagen.
sl, sarcolemma.
s. c., Schwann cell.
dm, desmosome or adhesion plate.
b. m., extraneous coating of sarcolemma.
g. c., Golgi complex.
lp, lipide.
s. r., sarcoplasmic reticulum.

PLATE 23

FIG. 1. Electron micrograph at relatively low magnification of a transverse section through one of the trabeculae carneae of the turtle atrium. For orientation see Fig. 7. At the upper part of the figure is the atrial cavity (*at. c.*) containing a leucocyte (*lc*). The trabecula is covered by a thin continuous layer of endocardium (*en*). Beneath this is a subendocardial space occupied by a ground substance of moderate density. Sections of several muscle cells are included. These have an abundant granular sarcoplasm containing numerous dense mitochondria (*m*) and occasional lipide droplets. The myofibrils (*mf*) are few in number and tend to form confluent masses with a highly irregular profile. × 14,000.

FIG. 2. Longitudinal sections of myofibrils show distinct A and I bands. The I band is bisected by a dense Z band and an H band is discernible in the A band. Myofilaments are clearly visible only in the A band. × 30,000.





(Fawcett and Selby: Fine structure of turtle atrium)

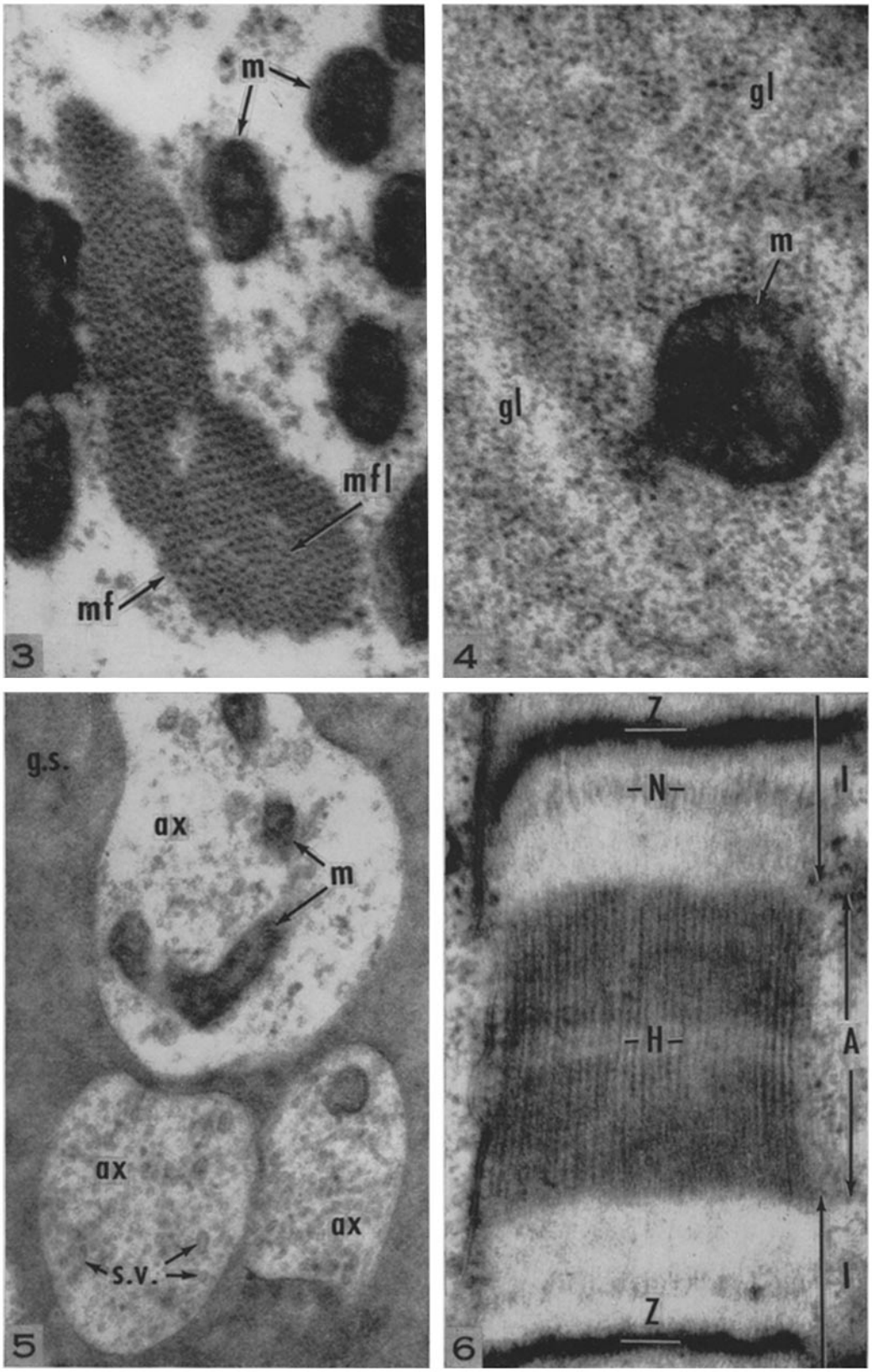
PLATE 24

FIG. 3. Transverse sections through the A band of a myofibril at high magnification showing a regular close-packing of the myofilaments, but irregularities and defects in the pattern are not uncommon. × 63,000.

FIG. 4. The interfibrillar sarcoplasm contains very large numbers of moderately dense granules 150 to 300 A in diameter. These are more variable in their size, more irregular in shape, and less distinct in their outline than ribonucleoprotein granules, and are tentatively interpreted as a particulate form of glycogen (*gl*). × 50,000.

FIG. 5. Cross-sections of three naked axons (*ax*) embedded in rather dense ground substance (*g.s.*) in the sub-endocardial space. The axoplasm of the larger axon contains mitochondria (*m*) and a small number of small vesicles. The smaller axons contain large numbers of axoplasmic vesicles. × 50,000.

FIG. 6. One sarcomere in longitudinal section. The sarcomere length is 1.5 to 1.7 μ. Myofilaments 100 to 120 A are visible in the A band. More delicate filaments can be made out with some difficulty in the I band where they are crossed by the Z band. An H band is present, and a faint N band is shown near the Z band. × 59,000.



(Fawcett and Selby: Fine structure of turtle atrium)

PLATE 25

FIG. 7. Photomicrograph of a transverse section through the wall of the atrium. At the top of the figure is the lumen of the atrium and cross-sections of the trabeculae carneae. At the bottom is the epicardium. The rectangle encloses an area such as that shown in the electron micrograph in Fig. 1. × 250.

FIG. 8. Photomicrograph of a longitudinal section of turtle heart muscle. The rectangle encloses an area such as that shown in the electron micrograph of Fig. 27. × 400.

FIG. 9. Turtle heart muscle stained for glycogen by the periodic-acid–Schiff reaction and counterstained with hematoxylin. The intense staining, which appears black here, illustrates the abundance of glycogen present in the sarcoplasm of this species. Periodic-acid–Schiff reaction. × 400.

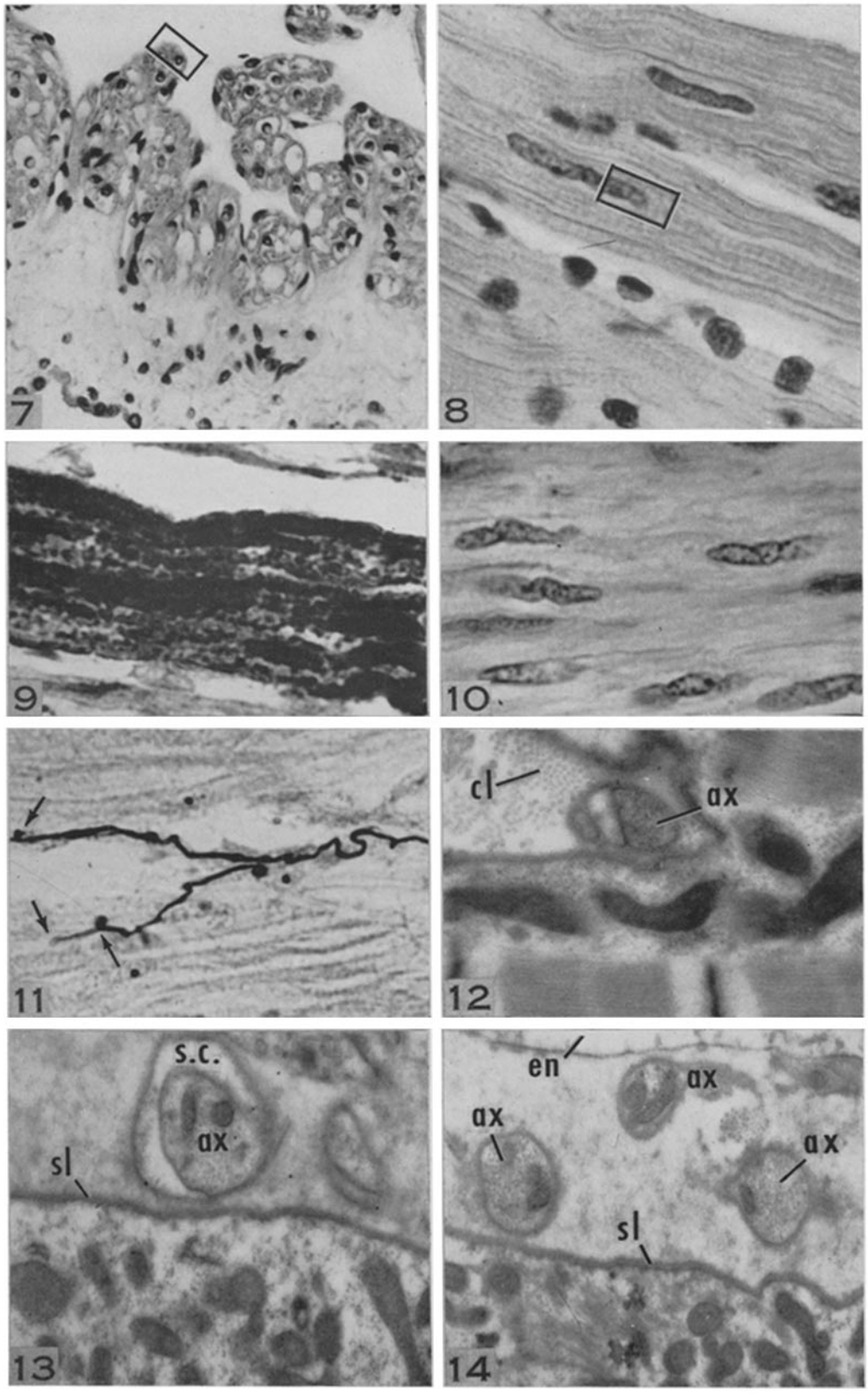
FIG. 10. A section of muscle stained in the same manner as that in Fig. 9 but after digestion by salivary amylase. The sarcoplasmic staining has been abolished, thus indicating that the reacting substance was glycogen. Periodic-acid–Schiff reaction. × 400.

FIG. 11. Photomicrograph of two small nerves in the atrial muscle. The arrows indicate loops of the sort that may have been mistaken in the past for special nerve endings. × 400.

FIG. 12. Electron micrograph of a small nerve lodged in the bifurcation of a muscle cell. The darker cross-section on the right is an axon (*ax*) containing numerous axoplasmic vesicles. The adjoining lighter structure is probably the tip of a Schwann cell process. The identity of the small ovoid body at the left is questionable, but it is probably a smaller axon. × 24,000.

FIG. 13. Electron micrograph including a cross-section through an axon (*ax*) that is exposed on the side toward the sarcolemma (*sl*) but covered by a Schwann cell process on the other side. × 18,000.

FIG. 14. Three cross-sections of naked axons situated between the endocardium (*en*) above and the sarcolemma (*sl*) below. The axoplasm contains mitochondria and numerous minute vesicles. × 16,000.

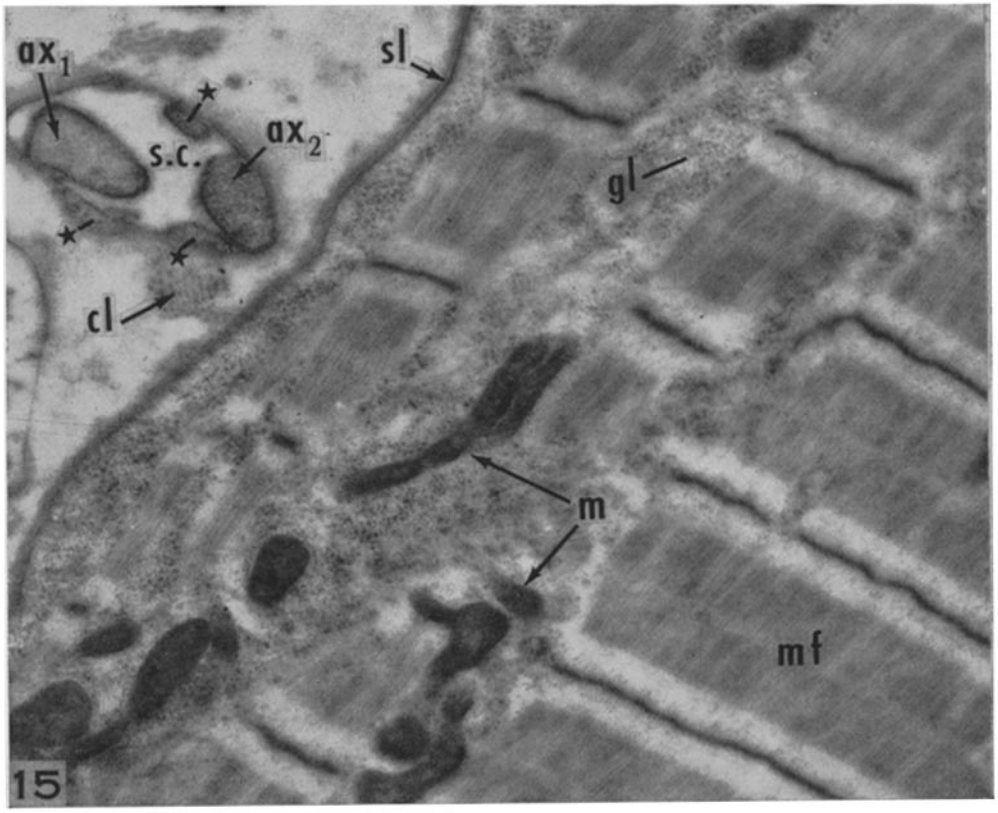


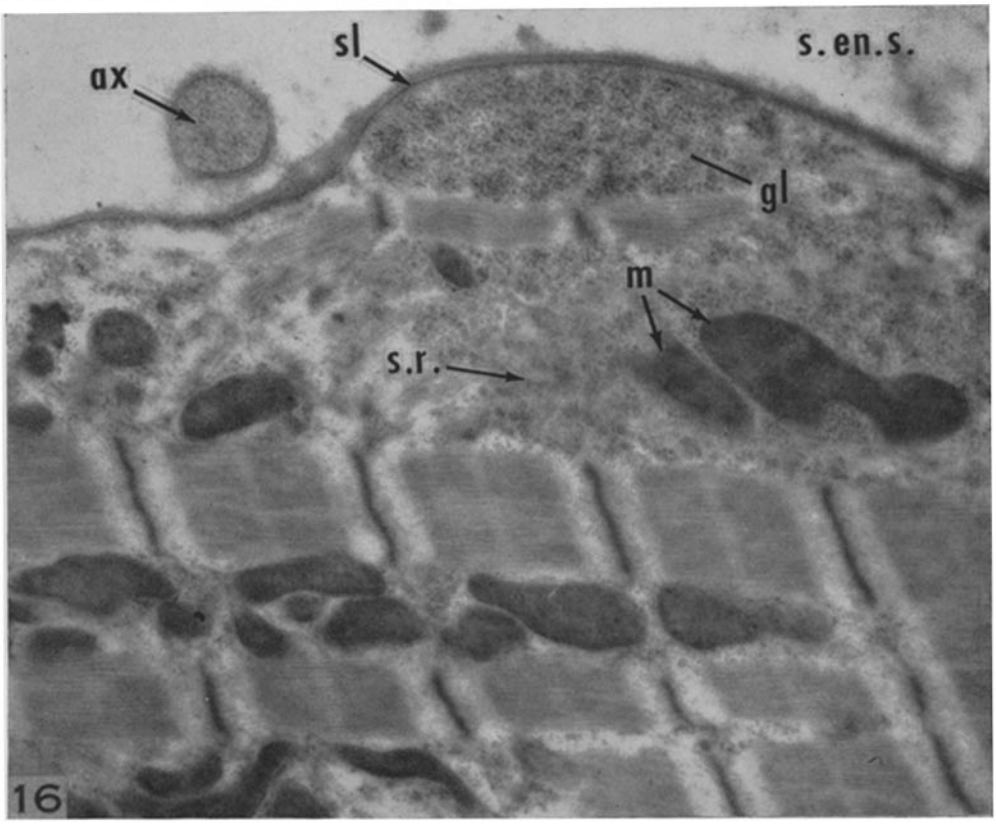
(Fawcett and Selby: Fine structure of turtle atrium)

PLATE 26

FIG. 15. Turtle myocardium in longitudinal section. Notice the varying width of the myofibrils (*mf*) and the granular character of the interfibrillar sarcoplasm. A small nerve is shown in cross-section in the subendocardial space at the upper left. Two axons (ax_1, and ax_2) are recessed in the surface of a Schwann cell process (*s.c.*); one of these (ax_2), containing numerous axoplasmic vesicles, is exposed on the side toward the sarcolemma (*sl*). The other (ax_1), containing no axoplasmic vesicles, is almost completely ensheathed by the Schwann cell. The three oval profiles indicated by asterisks (*) appear to be axons of very much smaller size. A small bundle of collagen fibrils (*cl*) runs parallel to the nerve. × 27,000.

FIG. 16. A longitudinal section of muscle from the turtle atrium. The mitochondria (*m*) are quite variable in size and shape and have a dense matrix that obscures their internal structure. The sarcoplasm is packed with small granules believed to be glycogen and contains a cluster of small membrane-limited vesicles (*s.r.*) that may represent the sarcoplasmic reticulum. The sarcolemma (*sl*) is coated by a moderately dense amorphous substance forming a continuous layer with an ill defined outer limit. At the upper left is a nerve axon, naked save for a thin layer of the same material that covers the sarcolemma. × 27,000.





(Fawcett and Selby: Fine structure of turtle atrium)

PLATE 27

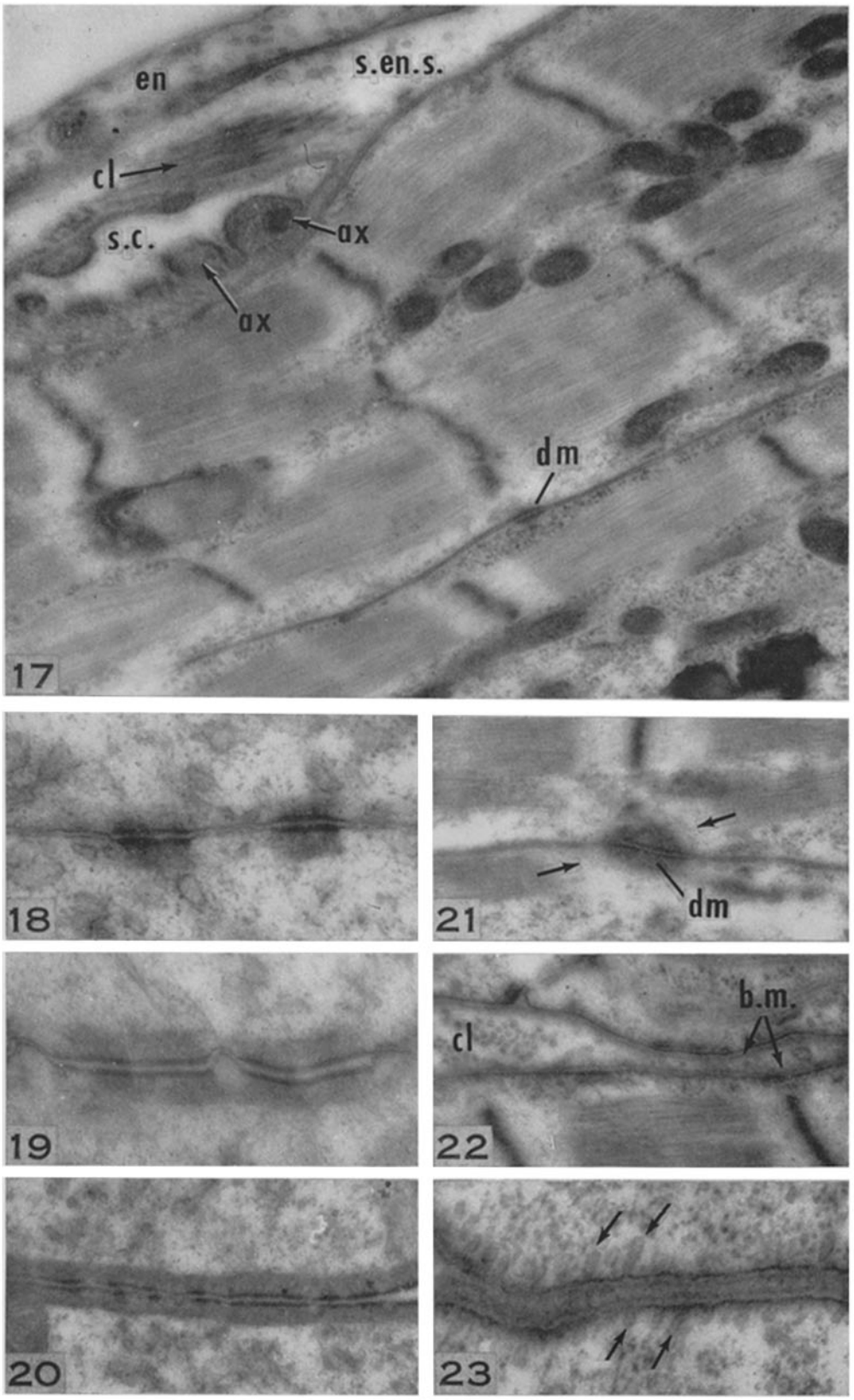
FIG. 17. Turtle cardiac muscle in longitudinal section. The endocardium (*en*) crosses the figure at the upper left. Beneath this, in the subendocardial space, is a group of collagen fibrils (*cl*) and a small nerve situated in a slight depression in the surface of the neighboring muscle cell. The nerve consists of several axons occupying grooves in the irregular surface of a Schwann cell (*s.c.*). Two of the axons (*ax*) are of the order of 0.5 μ in diameter, the others range downward in size to less than 0.2 μ. Notice on the interface between the two adjoining muscle cells an elliptical dense body (*dm*) that has the same fine structure as the desmosomes of epithelia (see Fig. 18). × 27,000.

FIGS. 18 to 20. Specializations for cell attachment found on the membranes bounding adjacent epithelial cells in a renal adenocarcinoma, presented here for comparison with the sites of lateral adhesion and the primitive intercalated discs of turtle heart muscle. Figs. 18 and 19 show typical epithelial desmosomes. Fig. 20 is an example of a terminal bar. × 40,000.

FIG. 21. A desmosome (*dm*) on the lateral surface of two cardiac muscle cells. Note (at the arrows) that myofilaments, diverging from neighboring myofibrils, terminate in the dense substance of the desmosome. × 35,000.

FIG. 22. The surface of two muscle cells separated by a narrow intercellular cleft. Wherever the muscle cells are exposed to such an extracellular space, the sarcolemma is coated by a conspicuous layer of amorphous substance (*b.m.*) resembling that which forms the basement membrane of epithelia. × 27,000.

FIG. 23. Where muscle cell surfaces are not in intimate contact, numerous small vesicles (arrows) are associated with the sarcolemma, some opening onto its surface. Their presence suggests that pinocytosis is involved in the uptake of metabolites from the extracellular spaces of the myocardium. × 40,000.



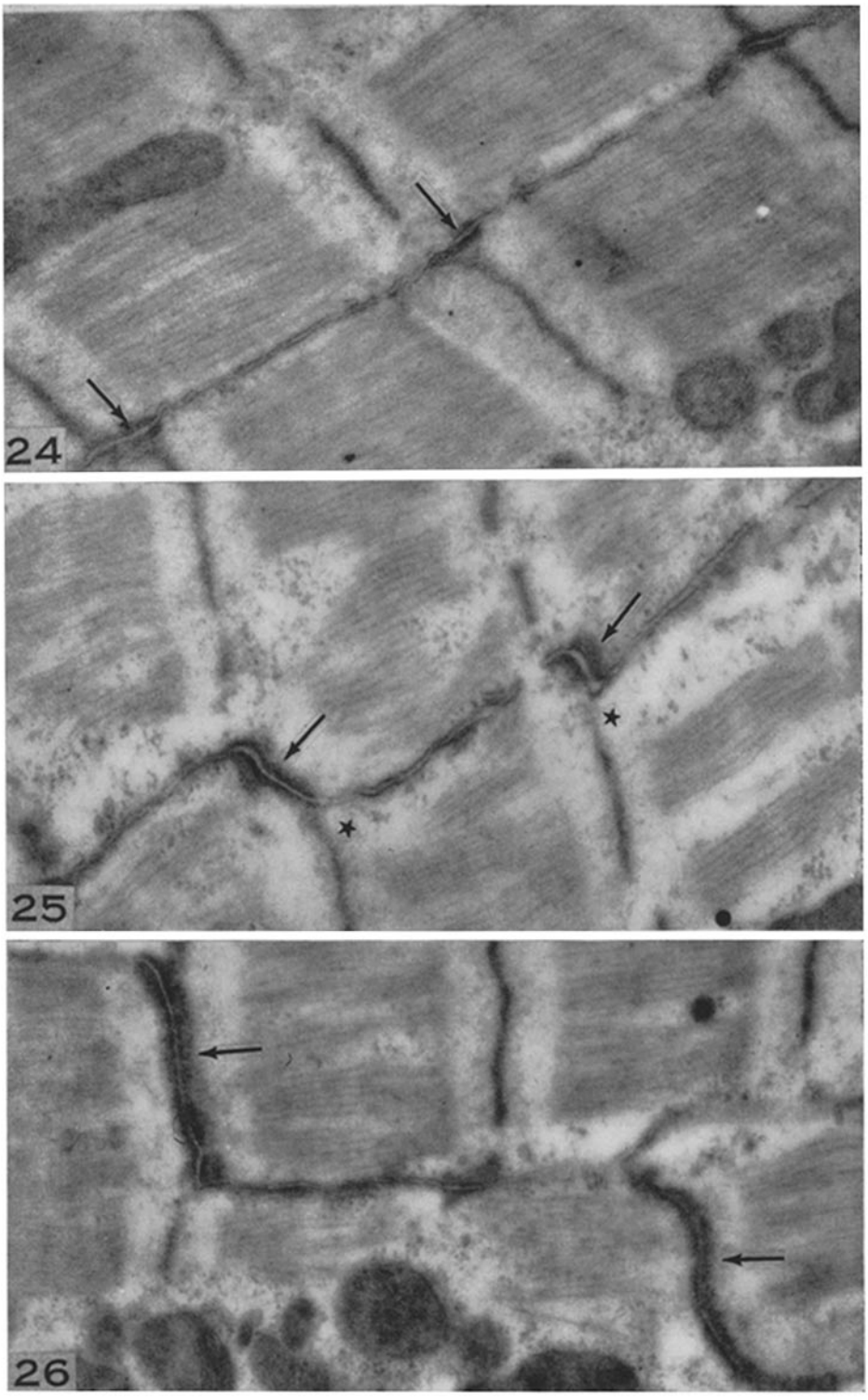
(Fawcett and Selby: Fine structure of turtle atrium)

PLATE 28

FIG. 24. Lateral surface of contact between cardiac muscle cells, showing (at arrows) desmosomes opposite the Z bands. Notice the continuity between the Z substance and the dense cytoplasmic component of the desmosomes. × 40,000.

FIG. 25. A transitional stage between desmosomes and intercalated discs. The specialized portions of the cell surface have been turned ninety degrees, possibly by the pull of myofilaments terminating in the dense amorphous material beneath the membrane. At the asterisks (*) the Z substance can be seen extending beyond the limits of the myofibril to join the dense component of the two desmosomes. × 40,000.

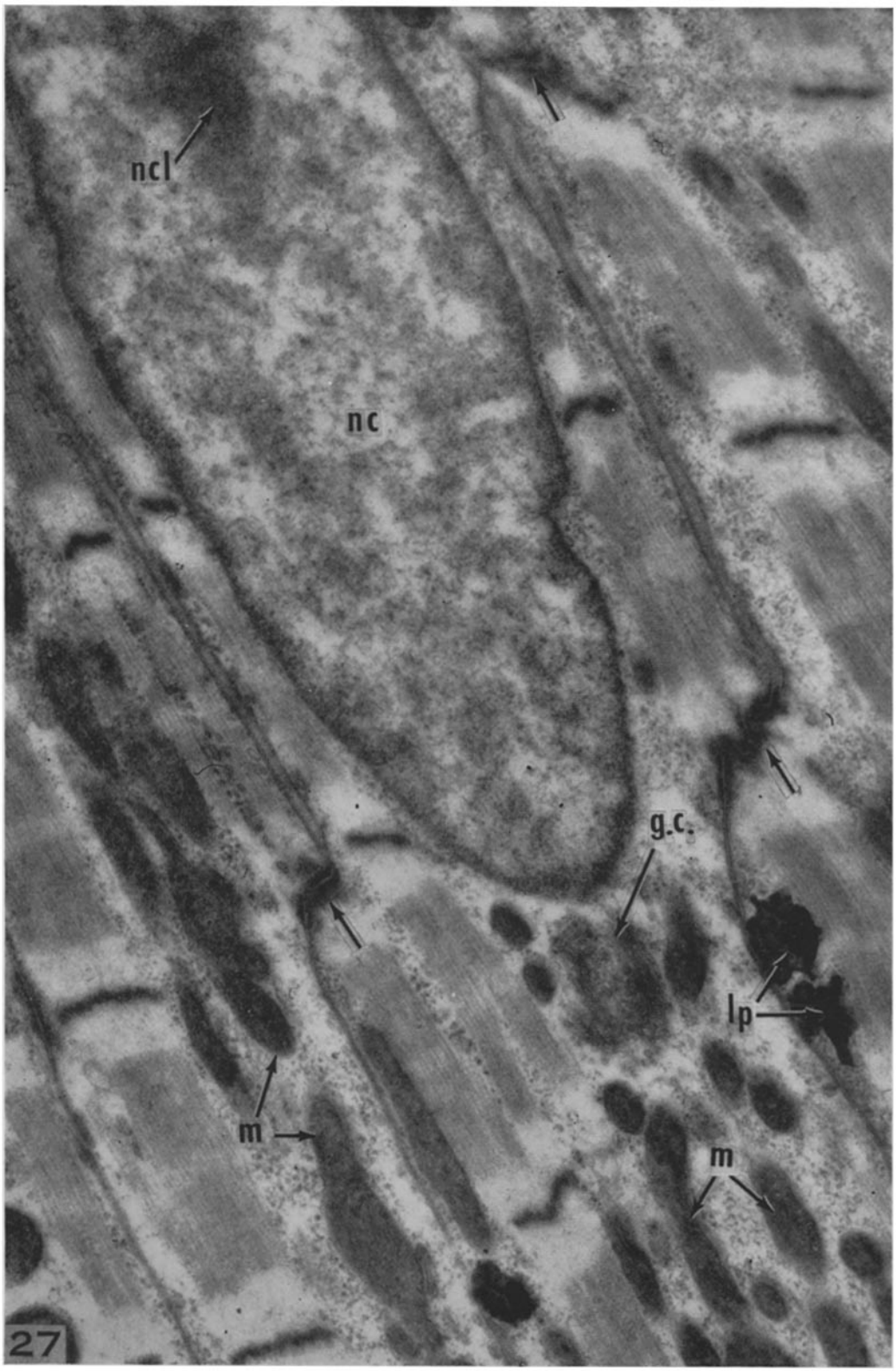
FIG. 26. An electron micrograph showing two of the simple intercalated discs typical of turtle heart muscle. They consist of step-like offsets in the cell surface where myofibrils of adjacent cells meet end to end. The surfaces normal to the myofibrillar axis have an accumulation of dense material immediately subjacent to the membranes that is apparently the same as the dense component of desomsomes and terminal bars. × 40,000.



(Fawcett and Selby: Fine structure of turtle atrium)

PLATE 29

FIG. 27. A general view of turtle atrial muscle including portions of four cells (For orientation see Fig. 8). Extending downward from the upper left corner is a portion of a nucleus (*nc*) containing an irregularly shaped nucleolus (*ncl*) with ill defined limits. In the sarcoplasm of this cell, near the lower pole of its nucleus, is a small compact Golgi complex (*g.c.*). In the peripheral sarcoplasm of the right hand cell is a pair of lipide droplets (*lp*). The arrows on either side of the end of the nucleus and at the top of the figure, point to simple intercalated discs 0.5 to 1 μ in width, in which the cell surface is offset and the pull of a myofibril in one cell can be transmitted end-on, to a myofibril in the next cell. $\times$ 30,000.



(Fawcett and Selby: Fine structure of turtle atrium)