SPECIFIC GRANULES IN ATRIAL MUSCLE CELLS

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ABSTRACT

Large populations (up to 600/cell) of spherical, electron-opaque granules ~ 0.3 to 0.4 μ in diameter are characteristically found in muscle fibers of mammalian atria. They are absent in muscle fibers of the ventricles. The granules are concentrated in the sarcoplasmic core and occur in lesser numbers in the sarcoplasmic layers between myofibrils and under the plasma membrane. Their intimate association with a central voluminous Golgi complex and the frequent occurrence of material reminiscent of the granular content within the cisternae of the Golgi complex suggest that the latter is involved in the formation of the atrial granules. Atrial granules are larger and more numerous in smaller species (rat, mouse), and generally smaller and less numerous in larger mammals (dog, cat, human); they are absent from the atrial fibers of very young fetuses (rat) but are present in those of newborn animals. A small population of bodies containing glycogen particles and remnants of the endoplasmic reticulum and mitochondria occurs in the sarcoplasmic cores of atrial as well as ventricular muscle fibers in the rat; they contain acid phosphatase and thus appear to be residual bodies of autolytic foci. Their frequency increases with the age of the animal. Typical lipofuscin pigment granules, which are known to contain acid phosphatase and are found in the sarcoplasmic cores in old animals (cat, dog and human), are presumed to arise by progressive aggregation and fusion of small residual bodies.

INTRODUCTION

The fine structure of mammalian ventricular myocardium has been described in several reports (1–3) which have placed special emphasis on the organization of myofibrils and disposition of sarcoplasmic reticulum in its muscle cells. Mammalian atrial myocardium has been less extensively studied: only a few reports (4–10) on the fine structure of its muscle cells have appeared. The presence of a population of dense granules within atrial muscle cells was mentioned by some of these authors and studied in more detail by Bompiani *et al.* (8) in the rat heart. In 1961, Palade (11) reported that the number of atrial granules appeared to decrease in the rat upon reserpine treatment and suggested that they may be intracellular storage sites for catecholamines. Since then, we have undertaken an extensive survey of atrial muscle cells from a wide variety of mammalian species, placing particular emphasis on their granules. The inquiry was conducted in order to select a favorable material for the future chemical and functional characterization of these granules.

MATERIALS AND METHODS

Preparation for Electron Microscopy

Hearts of a variety of laboratory animals (rat, mouse, hamster, guinea pig, bat, cat, rabbit, dog,) and of the pig, ox, and human were used in this study. Large animals were anesthetized with ether or intravenous Na pentobarbital; smaller animals were sacrificed by a blow to the head. The chests were quickly opened and portions of the atria and

ventricles removed for fixation. In many cases, especially with smaller animals, the heart was perfused and distended with fixative which was allowed to react *in situ* for several minutes to minimize contraction and distortion of the muscle. Human left atrial appendage was obtained during open heart surgery performed on patients with mitral stenosis. In another instance, human material was obtained 3 hours postmortem.

The specimens were fixed at ~0° for 1 to 3 hours in 1 per cent OsO_4 buffered to pH 7.6 with 0.1 M K phosphate; in some instances, the tissue was prefixed at 0° for 1 to 3 hours in 6.25 per cent glutaraldehyde in the same buffer (12), washed overnight in the buffer with sucrose added to restore osmolarity, and finally fixed for 1 to 3 hours at 0° in 1 per cent OsO_4 buffered to pH 7.6 with 0.1 M K phosphate. Following dehydration in the cold in a graded series of ethanol solutions, the tissue was embedded in Epon 812 according to the method of Luft (13), or in methacrylate cross-linked with 5 per cent divinylbenzene (14). Sections were cut on a Porter-Blum microtome equipped with a diamond knife, collected on naked copper grids, and stained with 0.5 per cent aqueous uranyl acetate for 30 minutes followed by alkaline lead or with alkaline lead solution alone (15). The sections were strengthened by a thin coat of carbon and examined in an RCA EMU 2B electron microscope or a Siemens Elmiskop I equipped with a 50 μ objective aperture and operated with a double condenser at 80 kv.

Cytochemical Procedures

The Gomori reaction for the detection of acid phosphatase (16) was performed on 50 μ frozen sections of rat atrium and ventricle fixed in the cold for 2 hours with 4 per cent glutaraldehyde in 0.05 M Na cacodylate (pH 7.1) and subsequently washed for 2 hours at 0° in buffer to which 8 per cent sucrose was added. The sections were incubated at 0° for 1 hour in 3 per cent Na-β-glycerophosphate in 0.1 M Na acetate at pH 5.0; the reaction mixture contained 0.12 per cent $PbNO_3$ to precipitate liberated inorganic phosphate as electron-opaque lead phosphate. Following a brief wash in acetate buffer, the sections were fixed at 0° in 1 per cent OsO_4 in 0.1 M Na cacodylate (pH 7.1) for 1½ hours. Routine dehydration and embedment in Epon 812 followed.

The chromaffin reaction for the detection of norepinephrine and epinephrine in thin section was carried out according to the method of Wood and Barrnett (17).

Autoradiography

Osmium tetroxide-fixed tissues were embedded in methacrylate or Epon 812 and sections prepared for light and electron microscopic autoradiography according to the method of Caro (18).

OBSERVATIONS

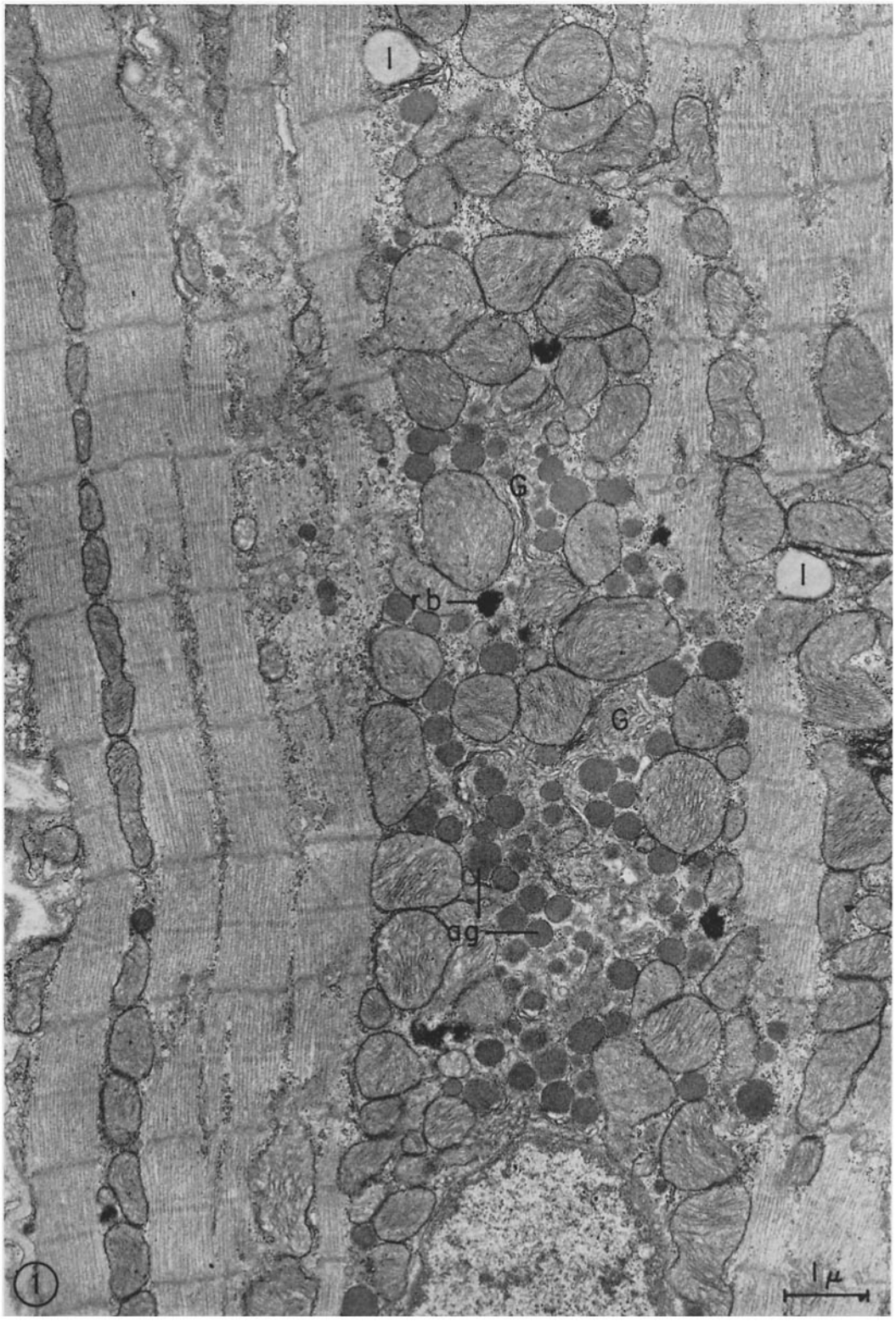
General Description of Atrial Muscle Cells

The general organization of these cells is similar to that described by others in both atrial and ventricular muscle fibers (1, 2, 8). Of particular interest for us were the structures comprising the central sarcoplasmic column or core (Figs. 1 and 2). Centrally placed within this core is a single elongate nucleus with numerous large mitochondria packed around each of its poles. Adjacent to one, and occasionally to both, of the poles of the nucleus, the sarcoplasmic core contains a large population of spherical, electron-opaque granules intimately interspersed among the elements of a voluminous Golgi complex. Similar granules are found in much smaller numbers in other loci in the cell as discussed later. In addition to these predominant structures, the sarcoplasmic core contains elements of rough endoplasmic reticulum, accumulations of glycogen particles, a few lipid droplets, and, scattered among all these components, a variable number of polymorphic bodies which could be described as dense bodies, residual bodies, or lipofuscin pigment granules.

The sarcoplasmic core is enveloped by bundles typical myofibrils which occupy the remainder of the cell volume. These bundles are separated by sarcoplasmic layers containing mitochondria, elements of the sarcoplasmic reticulum, atrial granules, and glycogen particles. The volume of the cell occupied by the myofibrillar envelope varies according to the region of the atrium examined, tending to be small in cells of the nodal and conductile tissue and relatively large in the rest.

Atrial muscle fibers are bounded by a usual plasma membrane covered externally by a base-

FIGURE 1 Longitudinally sectioned muscle cell illustrating general features of atrial muscle cell. Anterior wall of the right atrium (rat). The sarcoplasmic core contains a population of spherical granules (*ag*), profiles of the Golgi complex (*G*), numerous mitochondria, an occasional residual body (*rb*), and a few lipid droplets (*l*). Sarcoplasmic layers between myofibrils contain an occasional granule. × 13,000.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ment membrane; the intercellular junctions consist of typical intercalated discs.

The structures in the sarcoplasmic core, specifically the atrial granules and their relationship to neighboring elements, will be discussed in detail below. As indicated, many mammalian species were examined but, as the heart of the rat was most extensively studied, the description and illustration will be based primarily on this material.

Detailed Description of Atrial Granules

1. General Intracellular Distribution of Granules

As already mentioned, most of the "specific" granules of the atrial muscle cells occur within the sarcoplasmic core where they are packed in large masses (up to 600 per cell) (Figs. 2 and 3) adjacent to one pole of the nucleus. Occasionally a few granules are found on the sides and adjacent to the other pole of the nucleus. Because of the spindle shape of the sarcoplasmic core, the granular mass occupies a roughly conical portion of the cell. Specific granules are also scattered in small numbers throughout the sarcoplasmic layers separating adjacent myofibrils (Fig. 4). Further, similar granules are seen in sarcoplasmic pockets immediately beneath the plasma membrane, again in association with other cell components. In this location, they may occur singly or in relatively large masses (Fig. 5).

2. Morphology of Granules

The atrial granules of the rat are spherical structures ranging in diameter from 0.25 to 0.50 μ. They are bound by a limiting membrane $\sim$ 80 A thick (Fig. 6) of the unit membrane type whose electron-opaque leaflets appear symmetrical in OsO_4-fixed, uranyl acetate-alkaline lead stained preparations. In many cases, the opaque leaflets are thickened and stain intensely on one side of the granule, forming a "plate" which appears to be relatively rigid (Figs. 2 and 7). External to the plate a layer of fuzzy material with a radial or helical orientation is frequently seen (Fig. 7). At high magnification, the limiting membrane appears to have points of discontinuity which may represent a preparative artifact.

The opaque content of the granules is often separated from the limiting membrane by a thin light zone $\sim$ 250 A wide (Fig. 7). The texture of the material contained in the granule varies with the type of fixative, the embedding medium, and the staining procedures employed on the section. After OsO_4 fixation, Epon embedment, and alkaline lead staining, the content is electron-opaque and finely granular. Embedment in methacrylate following OsO_4 fixation tends to enhance this granularity, as does staining the tissue block with potassium permanganate prior to embedding (Fig. 9). If glutaraldehyde fixation precedes osmium tetroxide fixation, the content of the granules is much less opaque and generally more homogeneous (*cf.* Fig. 5). Finally, the content becomes extremely opaque in sections stained with uranyl acetate.

3. Relations of Specific Granules to Components in the Central Core

a. GOLGI COMPLEX: The large masses of specific granules are intimately associated with a voluminous Golgi complex whose elements appear scattered throughout the sarcoplasmic core. The complex consists mainly of flattened, stacked, smooth-surfaced cisternae and associated small vesicles. Large vacuoles are rare or absent. Favorably oriented sections show that the flattened cisternae are extensively fenestrated and have festooned margins which suggest that small vesicles either bud from or coalesce with the cisternal periphery (Fig. 8). Some of the vesicles in continuity with or in the immediate vicinity of the stacked cisternae have thickened membranes surrounded by a halo of condensed cytoplasmic matrix (Fig. 8) reminiscent of features described by us in connection with some atrial granules and by others in connection with certain pinocytic vesicles (19, 20).

In many instances, the cisternae of the Golgi complex are filled with a fine granular material, and local cisternal dilatations contain masses comparable in density and texture with the content of the specific granules (Fig. 9). These findings, together with the fact that the limiting membranes of the elements of the Golgi complex and of the granules are similar in width and staining characteristics (Fig. 10), suggest that the latter are formed within the complex. The assumption is also supported by the fact that ventricular muscle cells, which do not produce comparable granules, have a simple Golgi complex which occupies little space in the corresponding sarcoplasmic cores (Fig. 2 *a*). The relatively

large volume of the Golgi complex in atrial muscle cells is particularly striking in fetal and newborn animals in which the cisternal elements of the complex are frequently distended.

b. ROUGH-SURFACED ENDOPLASMIC RETICULUM: Rough-surfaced elements of the endoplasmic reticulum frequently occur in the central sarcoplasm of the atrial muscle cells of dog, guinea pig, and cat. They are less common in rat and mouse. Most of them are relatively large cisternae disposed usually in stacks of 6 to 10 parallel elements and occasionally in more complex circular or concentric patterns. As a rule, the cisternae are flattened (small diameter $\simeq$ 40 mμ) and do not contain any well defined material. Typical ribosomes stud the outer cisternal surface, and grazing sections show that they are arranged on it in spiral, circular, or rosette-like patterns (Fig. 11). The rough-surfaced cisternae are generally located near the Golgi complex but do not appear to have any preferential relationship to it or to the atrial granules. It should be noted that similar elements are rarely seen in the sarcoplasmic core of ventricular muscle cells.

c. MITOCHONDRIA: Most of the space in the sarcoplasmic core of atrial muscle cells is taken by large (diameter $\sim$ 1.5 μ) mitochondria, distributed at random and forming an envelope around the Golgi complex and the masses of specific granules. Most mitochondrial profiles are circular and show parallel, tightly packed, and occasionally concentrically arranged cristae. Quite often the profiles have narrow tails with few or no cristae inside. In three dimensions they probably represent long, fine, finger-like projections of the outer and possibly inner mitochondrial membranes.

d. CENTRIOLES: One or two centrioles of typical structure (nine peripheral tubular doublets) are occasionally found in the immediate vicinity of the nucleus, usually between it and the Golgi complex (Fig. 12).

e. GLYCOGEN PARTICLES AND LIPID DROPLETS: Small (250 to 300 A) dense particles in variable but normally large numbers are scattered among the other elements of the core (Figs. 3, 12, 13). They are identified as glycogen particles because of their morphological similarity to the β-glycogen particles studied by Drochmans (21), their stainability with alkaline lead solutions (22),[1] and their similarity to the particles described in the muscle cells of turtle atria by Fawcett and Selby (23). Glycogen particles are particularly numerous in the atrial muscle cells of dogs (Fig. 13), pigs, and guinea pigs where they frequently form large compact masses. They are also numerous in atrial muscle cells of fetal and newborn animals (rats and mice).

Lipid droplets also occur usually in small numbers among the other elements of the sarcoplasmic core (Fig. 1).

f. RESIDUAL BODIES, LIPOFUSCIN GRANULES: At the periphery of the masses of specific granules or scattered among them, there is a small population of irregularly shaped bodies. Each measures $\sim$ 0.5 μ in diameter and is limited by a unit membrane of symmetrical type whose dense leaflets appear thinner and less dense than those of the limiting membrane of the atrial granules (Fig. 6). The content of most of these bodies is dense, homogeneous, and finely granular (Figs. 1, 2, 3, 6), but some of them also contain recognizable subcellular components such as mitochondria, glycogen particles, and elements of the endoplasmic reticulum altered to a varied extent.

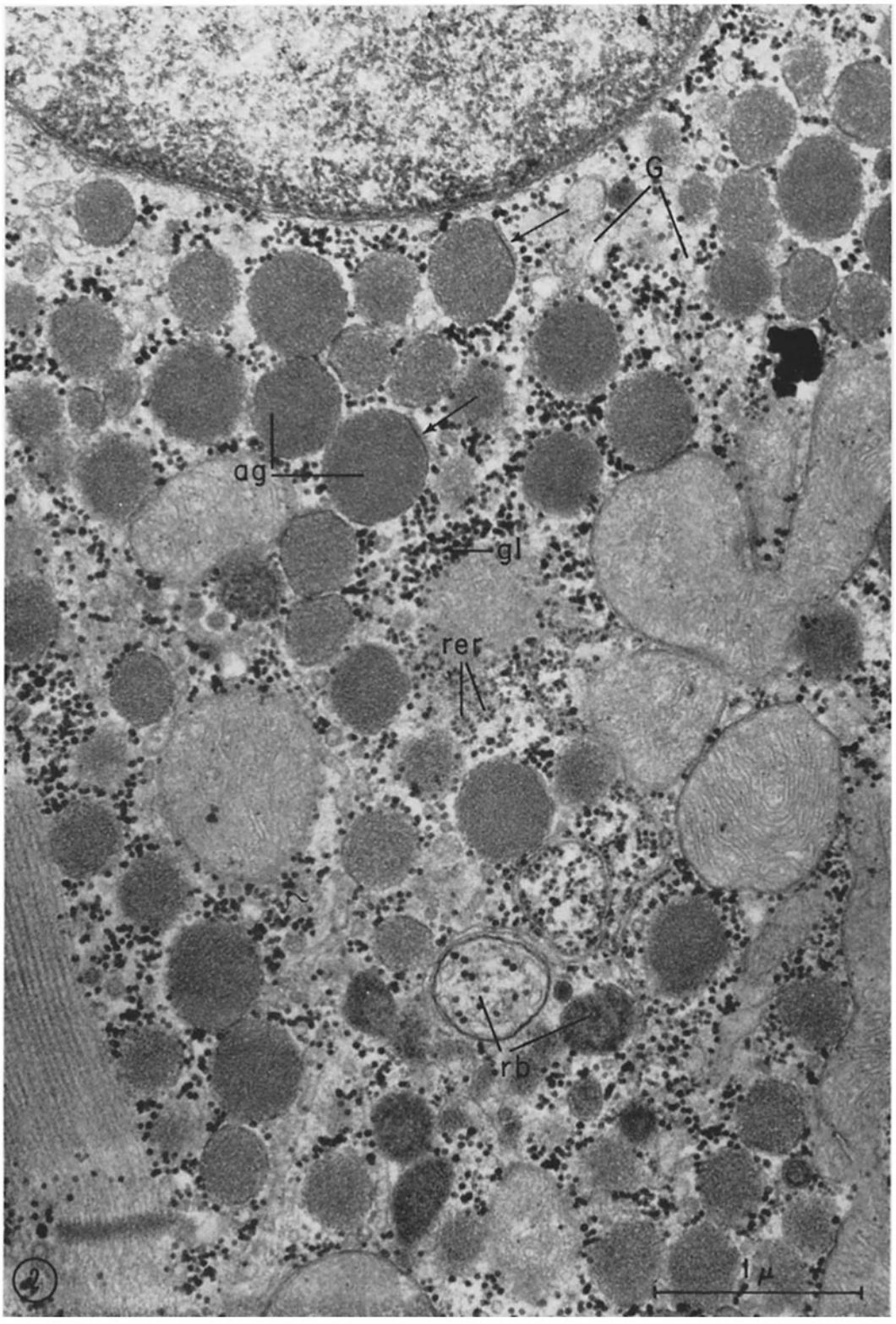
In this respect, these bodies resemble residual bodies described by others (24, 25) in rat liver and nerve cells. Such bodies are absent in newborn rats and mice, and, according to our survey, increase in number and size with the age of the animal. In older animals, *i.e.* cat, dog (Fig. 13), and human, they come to occupy a relatively large

[1] It should be pointed out, however, that this staining reaction is not specific and not understood.

FIGURE 2 Longitudinally sectioned muscle fiber from anterior wall of right atrium (rat). Numerous atrial granules (*ag*), elements of the rough-surfaced endoplasmic reticulum (*rer*) and a few residual bodies (*rb*) are shown. *G*, Golgi complex; *gl*, glycogen particles; arrows indicate thickened "plates" on granules. Compare this figure with a corresponding section of ventricular muscle (Fig. 2 *a*). × 32,000.

FIGURE 2 *a* Longitudinally sectioned muscle fiber. Anterior wall of the right ventricle (rat). The sarcoplasmic core is devoid of "atrial" granules; it contains a small Golgi complex (*G*) and numerous mitochondria with frequent intramitochondrial granules (*mg*). *gl*, glycogen particles; *sr*, elements of the sarcoplasmic reticulum. × 32,000.

For figures, see following pages.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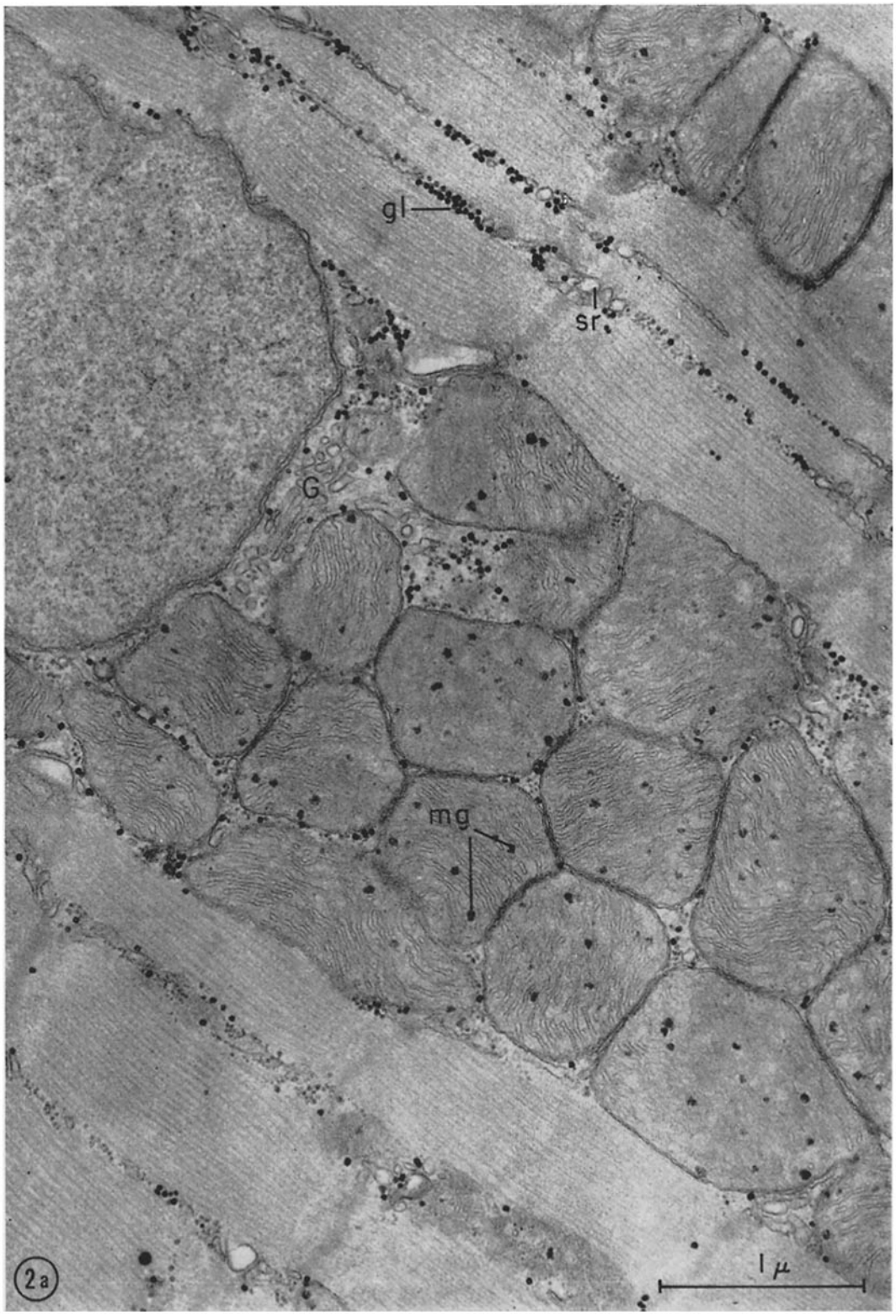
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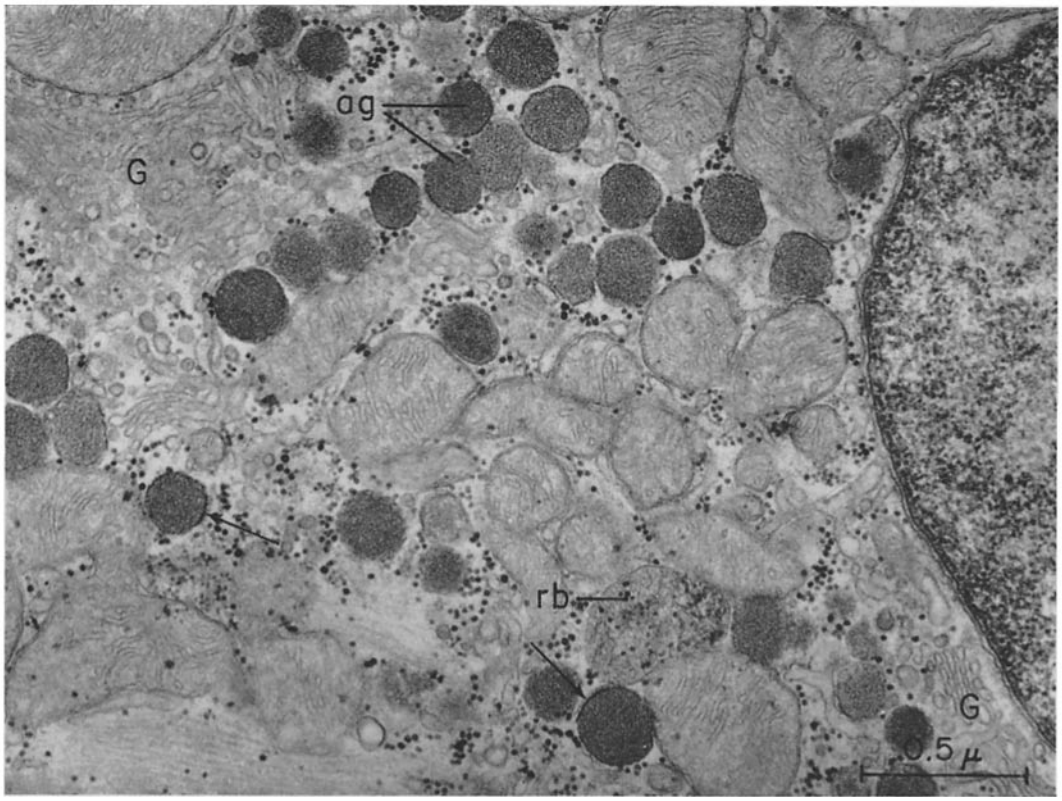

FIGURE 3 Central core of atrial muscle fiber (rat) illustrating the extensive stacks of Golgi cisternae (*G*) associated with specific atrial granules (*ag*). Several granules show asymmetric thickenings of their bounding membrane (arrows). A residual body containing membranous material and glycogen particles is marked *rb*. × 44,000.

part of the sarcoplasmic core. In the same time, the morphology of their content seems to change gradually, for the largest bodies appear as conglomerates of dense granular material and less dense droplets of varied sizes (Fig. 13). Such large bodies are identical with the lipofuscin pigment granules whose morphology and chemistry has been the object of a number of recent studies (26, 27).

We did not find any indication that these bodies are related to atrial granules. In this respect, it should be mentioned that residual bodies and lipofuscin granules occur with equal frequency in atrial and ventricular muscle cells.

4. Relation of Granules to Structures Elsewhere in the Cell

Though most of the population of atrial granules is located in the central sarcoplasm, a few similar granules commonly occur individually or in small groups in the sarcoplasmic ribbons between myofibrils. Here they are closely associated with mitochondria, glycogen particles, and elements of the sarcoplasmic reticulum but do not bear any systematic relationship to either the latter or to the bands of the adjacent myofibrils (Fig. 4). Larger accumulations of atrial granules are often seen in sarcoplasmic pockets between the plasma membrane and the most peripheral myofibrils, where they are again randomly scattered among mitochondria and profiles of the superficial sarcoplasmic reticulum (Fig. 5). Occasionally, these peripheral accumulations are associated, as in the central sarcoplasm, with Golgi complexes (Fig. 14). Although many of these peripheral granules are separated from the plasma membrane by as little as 100 A, their bounding membranes were never seen to fuse with the plasma membrane

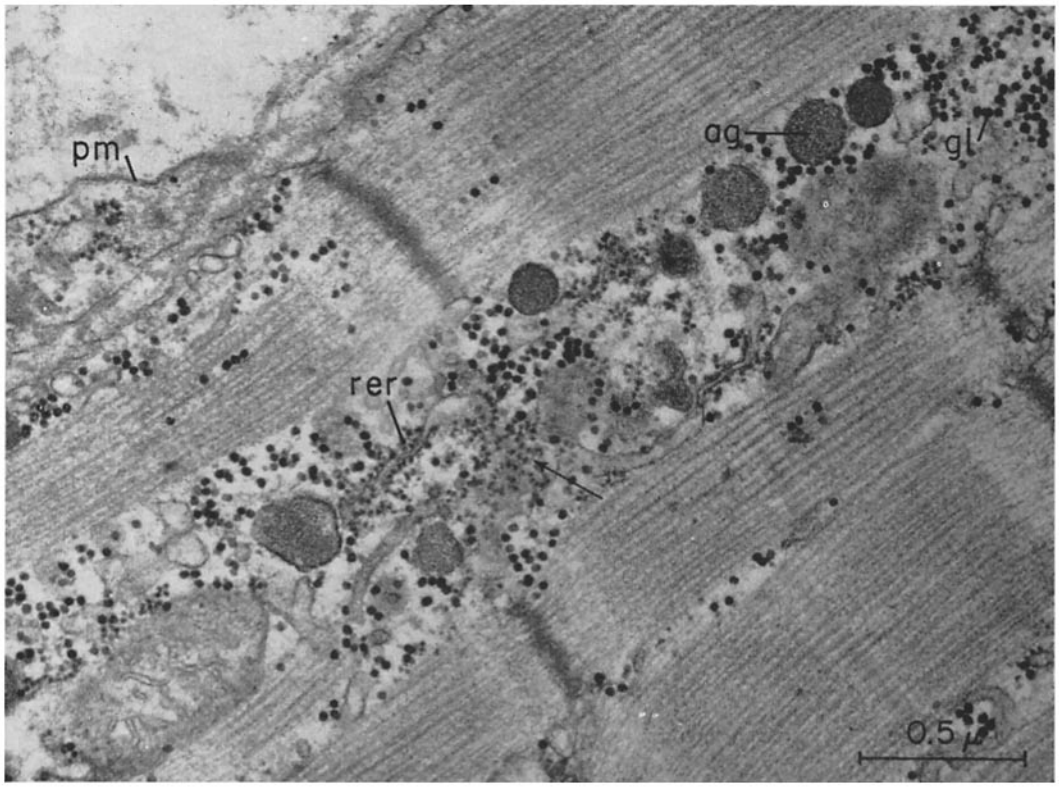

FIGURE 4 Sarcoplasmic layer between superficial myofibrils in an atrial muscle cell (rat); it contains specific granules (*ag*), elements of the rough surfaced endoplasmic reticulum (*rer*), and glycogen particles (*gl*). The plasma membrane is marked *pm*. Arrow denotes ribosomes in a whorl on the surface of a cisterna. × 44,000.

leading to the discharge of the granule contents by a process observed in many glandular cells.

5. Distribution Throughout the Myocardium

In order to determine the distribution of the granules throughout the rat myocardium, regions from the anterior and posterior walls of both atria and ventricles were sampled. In general, muscle cells from both right and left atria contain specific granules. The largest numbers were found in cells from the anterior wall of the right atrium and the smallest in cells near the sulcus terminalis. Similar regional variations were found in the atria of other species. In no instances were specific granules seen in ventricular muscle fibers (*cf.* Figs. 2 and 2 *a*).

6. Age Variations

A survey was also carried out on atria of rats of various ages to determine whether differences in the intracellular distribution of granules exist at various stages of development. The youngest animal studied, a 10 mm rat fetus, possessed no granules in atrial fibers though the Golgi complexes were prominent. In late fetal and neonatal rats the sarcoplasmic core of atrial muscle cells contains relatively large populations of granules closely associated with prominent and distended Golgi cisternae. Similar granules also occur, as in the adult, between bundles of myofibrils and in sarcoplasmic pockets beneath the plasma membrane. In these young animals the granules are, on the average, one half the diameter of those of adults.

7. Variation with Species

An estimate was made of the extent of the granule population and the sizes of granules in the atria of various species. The rat and other small mammals (mouse, hamster, bat) possess as many

as 600 granules in the sarcoplasmic core of the atrial fibers. With increasing size of the mammal (rabbit, cat, dog, human) the granule content per cell is generally smaller.

In the rat, the granules range in diameter from 0.2 μ to 0.5 μ (average diameter, 0.42 μ). Measurements in other species are given in Table I. As a first approximation, they show that atrial granules with the greatest average diameter are found in small mammals while granules from larger mammals tend to be smaller.

TABLE I

Average and Maximum Diameters of Atrial Granules from Anterior Wall of Right Atrium

All material prefixed in glutaraldehyde, post-fixed in OsO_4, and embedded in Epon 812.

Specimen	Average diameter	Maximum diameter
	μ	μ
Rat (adult)	0.42	0.53
Rat (newborn)	0.20	0.27
Mouse (adult)	0.38	0.45
Mouse (fetal)	0.22	0.30
Hamster	0.26	0.31
Bat	0.17	0.20
Guinea pig	0.23	0.26
Rabbit	0.23	0.26
Cat	0.24	0.28
Dog	0.18	0.22
Pig	0.22	0.26
Human	0.25	0.33

8. Attempts to Define Chemically the Granules

Several attempts to identify the nature of the granular content were carried out. Since reserpine treatment appears to deplete the rat atrium of its granules, suggesting that the latter may contain catecholamines, a chromaffin reaction for the detection of epinephrine and norepinephrine in thin sections (17) was applied to rat atrial muscle. It was negative in atrial granules, though positive in the granules of the rat adrenal medulla, known to contain epinephrine and norepinephrine. H^3-dopamine, the immediate precursor of norepinephrine, was not accumulated in the granules as studied by autoradiography (18), though nerves in the atrium and ventricles became highly labeled.

The Gomori test for acid phosphatase at the electron microscopic level (16) was also carried out on rat hearts. The reaction product, lead phosphate, was present in residual bodies (Figs. 15 and 16) but absent from adjacent atrial granules.

DISCUSSION

Granules identified as small mitochondria, dense microsomes, microbodies, lipid droplets, or *dichte Körper* have been casually mentioned in general surveys of the atrial muscle of guinea pig, ox (Kisch, 1956, references 4 and 5), and man (Poche, 1958, reference 6; Battig and Low, 1960, reference 10). As *corps denses* they have been described in more detail in the conductile and contractile tissue of the right atrium of the rat by Bompiani *et al.* (8), who speculated that these granules are related to the special excitability of the bundle of His. Our findings confirm the general morphology and intracellular distribution reported by these authors, except for the limiting membrane of the granules which they found absent. Viragh and Porte (7) observed similar granules in the atrial muscle of the rat, recognized that they were not present in all myocardial fibers, and suggested that they evolve into lipofuscin granules, a view which, as discussed later, is probably incorrect.

In lower vertebrates, morphologically similar granules have been mentioned in the atrial muscle fibers of the eel (Couteaux and Laurent, 1957, reference 28) and studied in detail in the atrial and ventricular fibers of cyclostomes (Östlund *et al.*, 1960, reference 29; Bloom *et al.*, 1961, reference 30). The hearts of the latter also contain interstitial cells with a large number of granules of similar appearance. Cyclostome hearts, particularly their atria, store catecholamines in concentration 100 times greater than mammalian atria (30). Granule fractions containing up to 18.5 μg/gm catecholamines have been isolated from such hearts and found to account for 50 to 70 per cent of the total catecholamines of the original tissue (30). Cyclostome hearts also are remarkably sensitive to *in vivo* reserpine treatment which releases 70 to 85 per cent of their catecholamines (30). These results indicate that the catecholamines of cyclostome hearts are stored in granules, but do not identify the source of the catecholamine-containing granules. It could be the interstitial cells, the muscle cells, or both.

In the present study, specific granules have been found exclusively within atrial muscle fibers of all the species examined, though interspecies variations have been detected. In general, the atria of small mammals contain many granules of relatively large size (0.4 μ), whereas both granule size and frequency tend to decrease with increasing core, the presence in the Golgi cisternae of material similar in appearance to the granule content, and the fact that the unit membranes surrounding both the granules and the Golgi cisternae are similar in width and staining properties, suggest that the granules form by progressive condensation of a cell product in the vesicles or cisternae of the

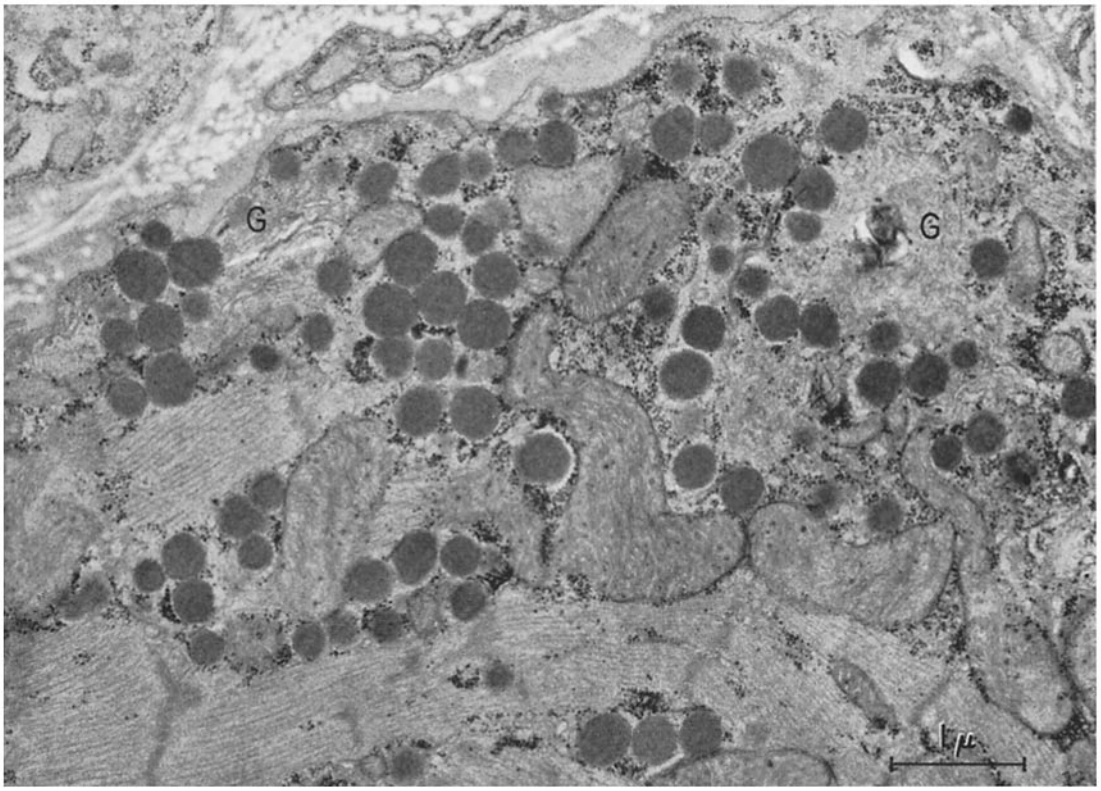

FIGURE 5 Accumulation of specific granules at the periphery of an atrial muscle fiber (rat). *G*, peripheral extension of Golgi complex. × 18,000.

size of the animal. Some exceptions to the generality of their occurrence are suggested by the available literature: no comparable granules were noted in the conductile tissue of steer (9) or sheep (31) atria, and in the muscle cells of the turtle atrium (23). Age variations have also been encountered: no granules have been found in the functional, but not fully differentiated muscle cells of young fetuses (10 mm rat); they were detected in late stages of fetal development, but were smaller and less numerous than in adult animals.

The intimate relationship of the atrial granules to the extensive Golgi complex in the sarcoplasmic Golgi complex. The relationship of granules to the other components of the sarcoplasmic core seems of little functional significance: it is more likely a reflection of the necessity of packing tightly many structures into a limited space.

The frequent occurrence of granules immediately beneath the plasma membrane suggests that the muscle cell discharges its granules or their content through the membrane, but evidence supporting this view has not been obtained.

The presence of catecholamines in high concentrations in granules isolated from cyclostome hearts (30), the fact that these compounds are

more concentrated in the atria than in the ventricles of rats and other mammals (32), and the finding that reserpine treatment, known from other studies (33) to deplete atria of catecholamines, also reduces the population of atrial granules in rats (9), suggested to us that mammalian atrial granules may contain catecholamines. The evidence thus far obtained is against this assumption: (*a*). Ventricular muscle contains catecholamines but its cells have no granules (this result, however, may be accounted for by stores of catecholamines in adrenergic nerve endings). (*b*). The chromaffin reaction failed to demonstrate catecholamines in atrial granules. (*c*). Autoradiographic studies failed to demonstrate any uptake of H^3-dopamine, the immediate precursor of norepinephrine, into atrial granules though the nerves of the heart as well as the granules of adrenal medullary cells became specifically labeled. (*d*). Wolfe *et al.* (34) have demonstrated by electron microscopic autoradiography that atrial granules fail to accumulate injected H^3-norepinephrine though sympathetic nerves do. (*e*). Potter and Axelrod (35) have shown that H^3-norepinephrine is concentrated in the microsomal fraction of rat hearts which we know not to contain granules. It is unlikely that the atrial granules contain 5-hydroxytryptamine because this compound is present only in small amounts in rat hearts (36), and because a labeled precursor of 5-hydroxy tryptamine, H^3-5-hydroxytryptophan, is accumulated in only small amounts in the heart (37). Without excluding it, these findings render unlikely the possibility that the granules contain either catecholamines or other monamines. Isolation and purification of the granules from atrial homogenates is required for the final identification of their content, yet mass isolation may prove

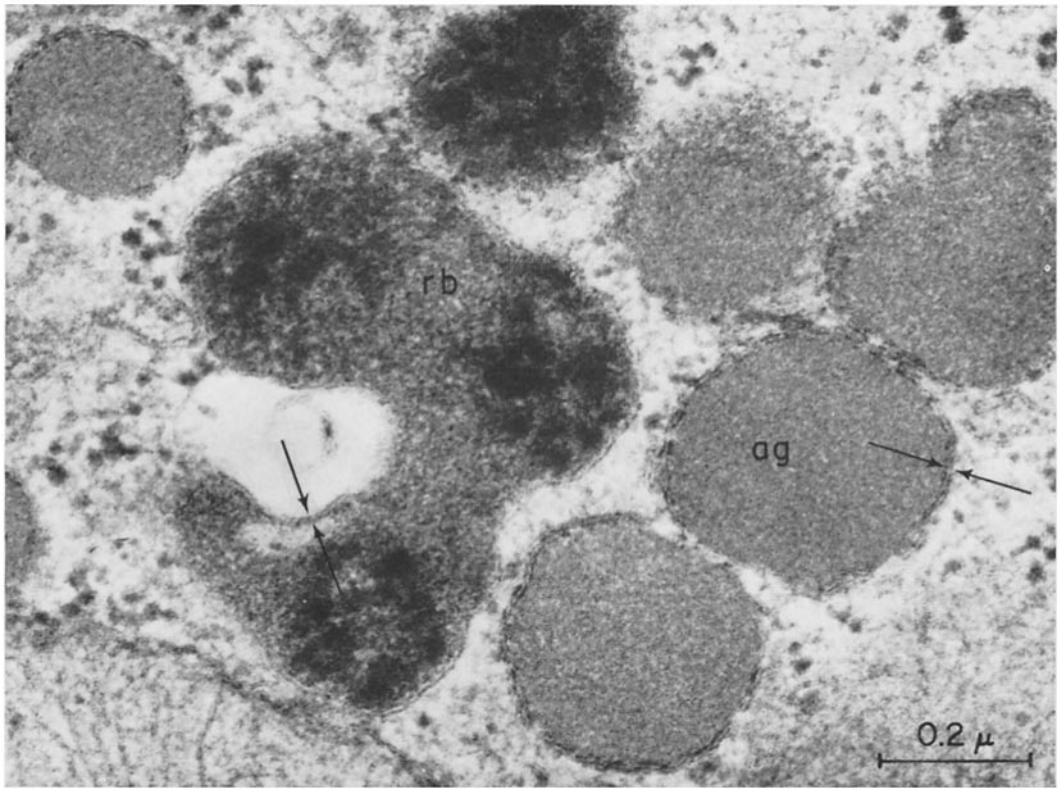

FIGURE 6 Unit membranes (apposed arrows) of atrial granules (*ag*) and a residual body (*rb*). The membrane bounding the latter stains less intensely than the limiting membrane of the granules. Note the heterogeneous content of the residual body. × 100,000.

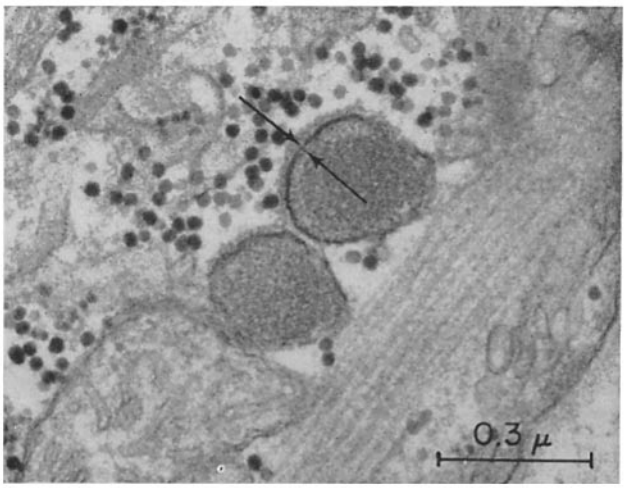

FIGURE 7 Atrial granules showing the symmetrical thickenings of their unit membrane which forms a "plate" (apposed arrows). A layer of fine fibrillar material is seen external to the plate of the upper granule. Its fibrils seem to be helically oriented. Right atrium (rat). × 66,000.

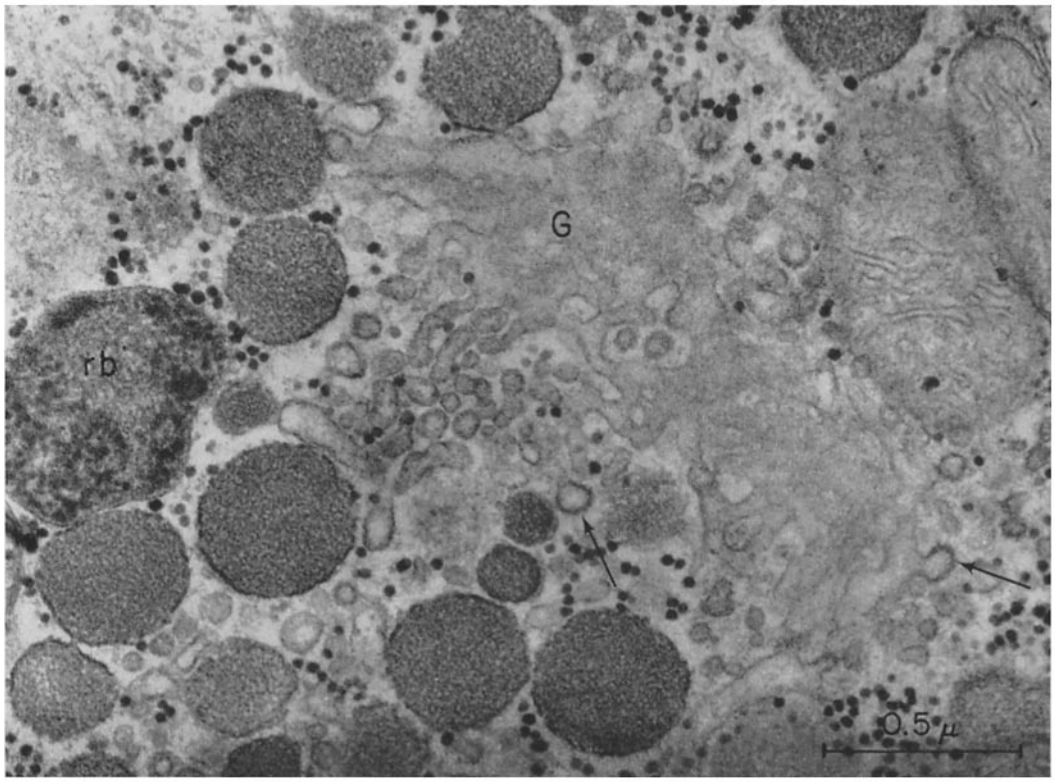

FIGURE 8 Full-faced view of a fenestrated Golgi cisterna (*G*). Its margin shows interconnecting tubules and "budding" Golgi vesicles. The arrows point to the thickening of the membrane on certain "buds." A residual body is marked *rb*. × 57,000.

difficult because the granules are only a minor component of atrial myocardium.

The pleomorphic dense bodies found in the sarcoplasmic core of atrial and ventricular muscle fibers of adult rats are similar to those already described in other cell types, especially in rat neurons (25). We have identified these entities as residual bodies because they frequently contain altered, yet still recognizable subcellular components (mitochondria, elements of the sarcoplasmic reticulum, and glycogen particles). Such bodies are currently assumed to be foci of intracellular autolysis (24). The assumption is supported by the fact that they contain acid phosphatase, a well established lysosomal enzyme (38). Though absent in muscle fibers of young rats and mice, residual bodies seem to accumulate as the animal ages, as already observed for other cell types (39). In old individuals from other species (cat, dog, human), the sarcoplasmic core of atrial and ventricular muscle cells contains large (2 to 3μ in width) membrane-bounded bodies with a dense, heterogeneous content which occupy a large volume of the cell. Such bodies are identical with the lipofuscin pigment granules described by Malkoff and Strehler (26) in electron micrographs of human heart. It has been known for some time from light microscopic studies (40) that the lipofuscin granules of human myocardium contain acid phosphatase, and Strehler and Mildvan (27) have isolated therefrom a fraction of pigment granules rich in the same enzyme. This common enzymatic activity suggests that the large lipofuscin granules arise by the progressive coalescence of the small residual bodies found in young animals. In this connection there is evidence that the amount of lipofuscin pigment increases linearly with age in the muscle cells of human heart (41).

The specific granules of rat atria were not found to contain acid phosphatase as detected by the Gomori test (16); accordingly, though closely associated with the acid phosphatase-positive re-

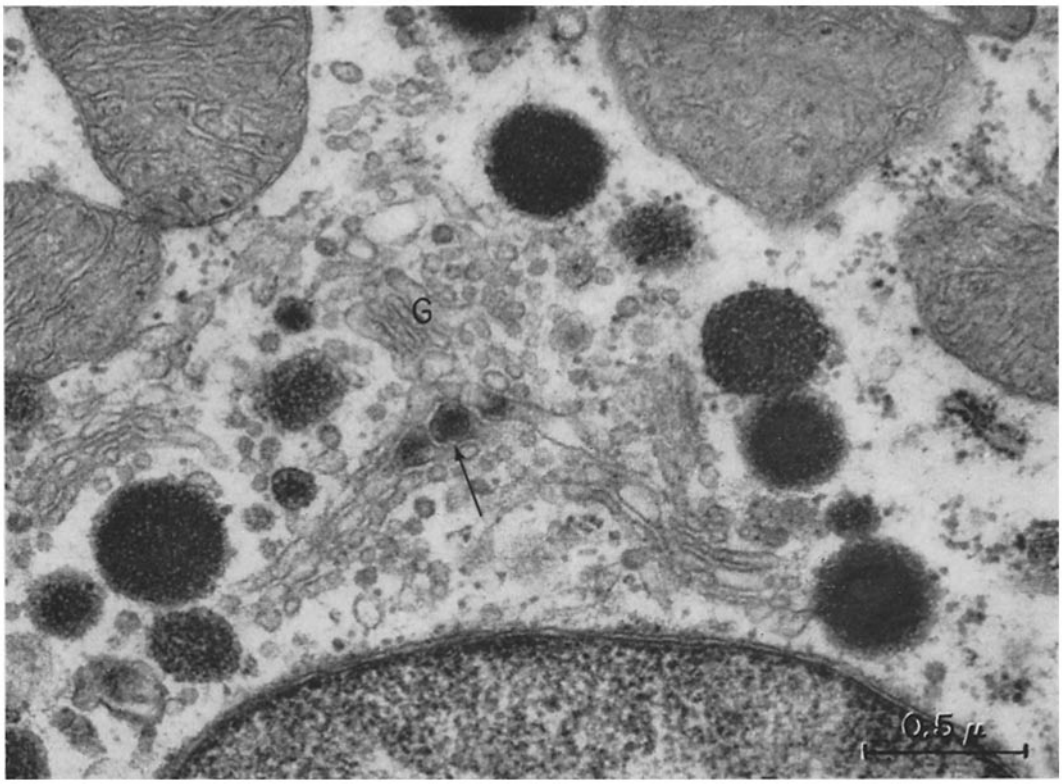

FIGURE 9 Golgi cisternae (*G*) containing material similar in appearance to the content of the atrial granules (arrow). Rat atrium. × 44,000.

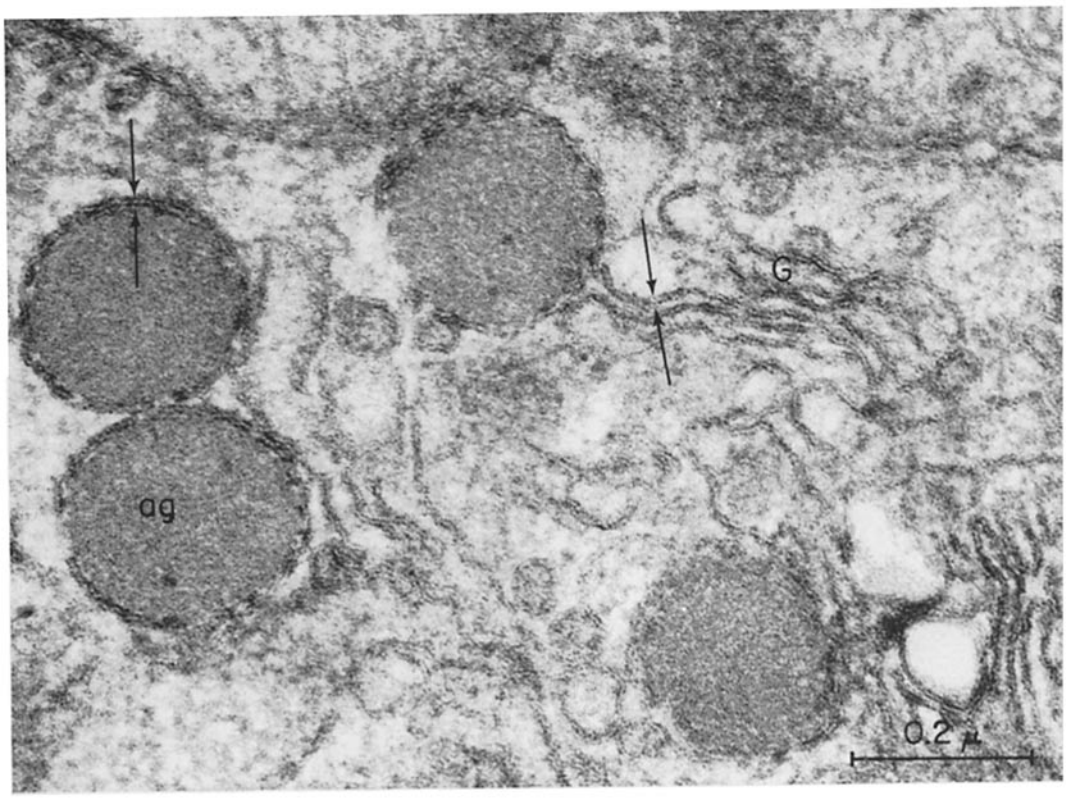

FIGURE 10 Unit membranes (apposed arrows) of Golgi cisternae (*G*) and atrial granules (*ag*). Note that these membranes are similar in total thickness and in the staining properties of their leaflets. Rat atrium. × 120,000.

sidual bodies, they do not appear to be the precursors of the latter as postulated by Viragh and Porte (7). Ventricular muscle cells, free of specific granules in all species studied, contain as many (if not more) residual bodies as do atrial muscle cells. These findings suggest that specific granules and residual bodies are different in origin and function.

Finally, the presence of specific granules within mammalian atrial muscle fibers is a rare example of multiple cellular specialization. In this case, a highly differentiated contractile cell appears to possess a second specialized function in its ability to form and store a population of granules presumably secretory in nature.

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For References, see page 171.

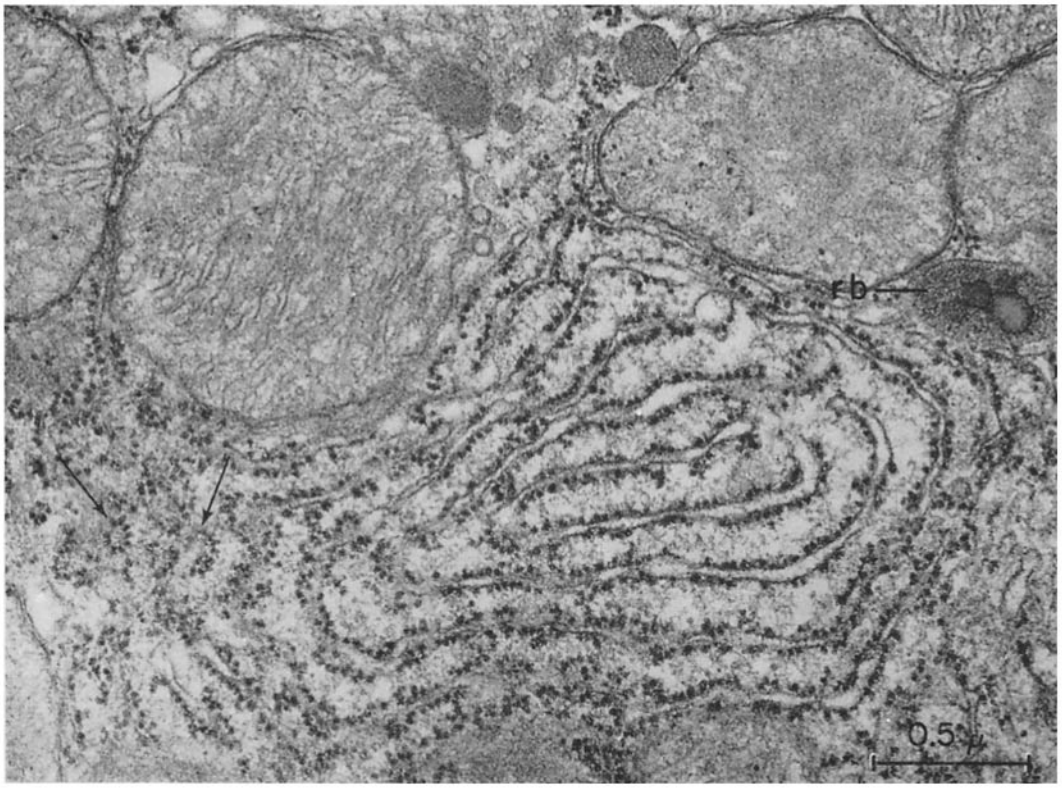


FIGURE 11 Complex pattern of anastomosing, rough-surfaced cisternae of the endoplasmic reticulum in the central core of an atrial muscle fiber (guinea pig). At arrows, the section grazes the cisternae and reveals the patterns formed by the ribosomes attached to their limiting membrane. *rb*, residual body. × 42,000.

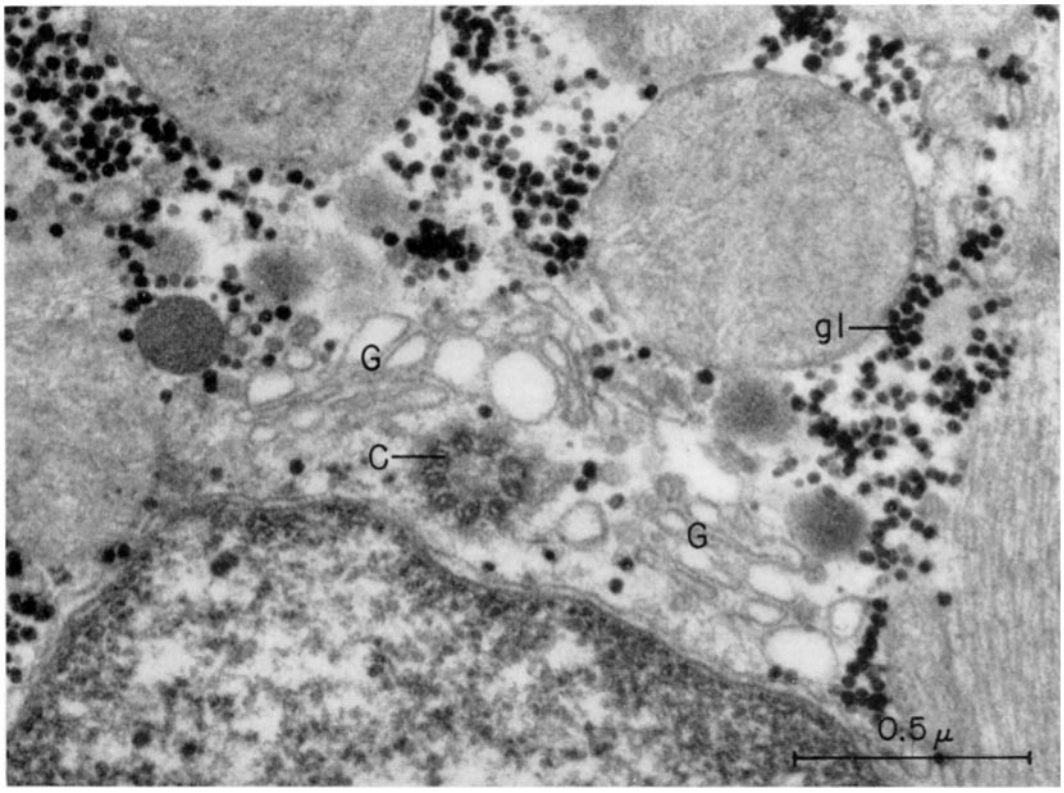


FIGURE 12 Central core of atrial muscle fiber (guinea pig) illustrating a centriole (*C*) composed of 9 pairs of tubules. *G*, Golgi complex; *gl*, glycogen particles. × 62,000.

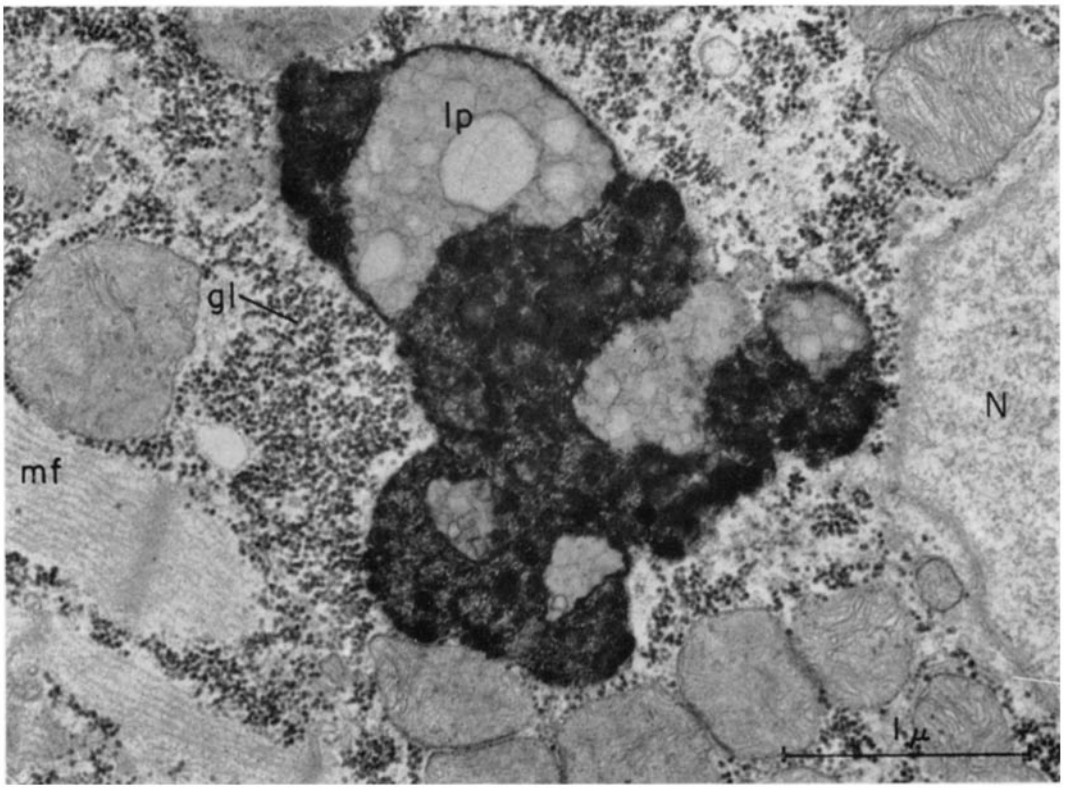


FIGURE 13 Lipofuscin pigment granule (*lp*) in the sarcoplasmic core of an atrial muscle fiber (dog). *N*, nucleus; *gl*, glycogen particles; *mf*, myofibrils. × 32,000.

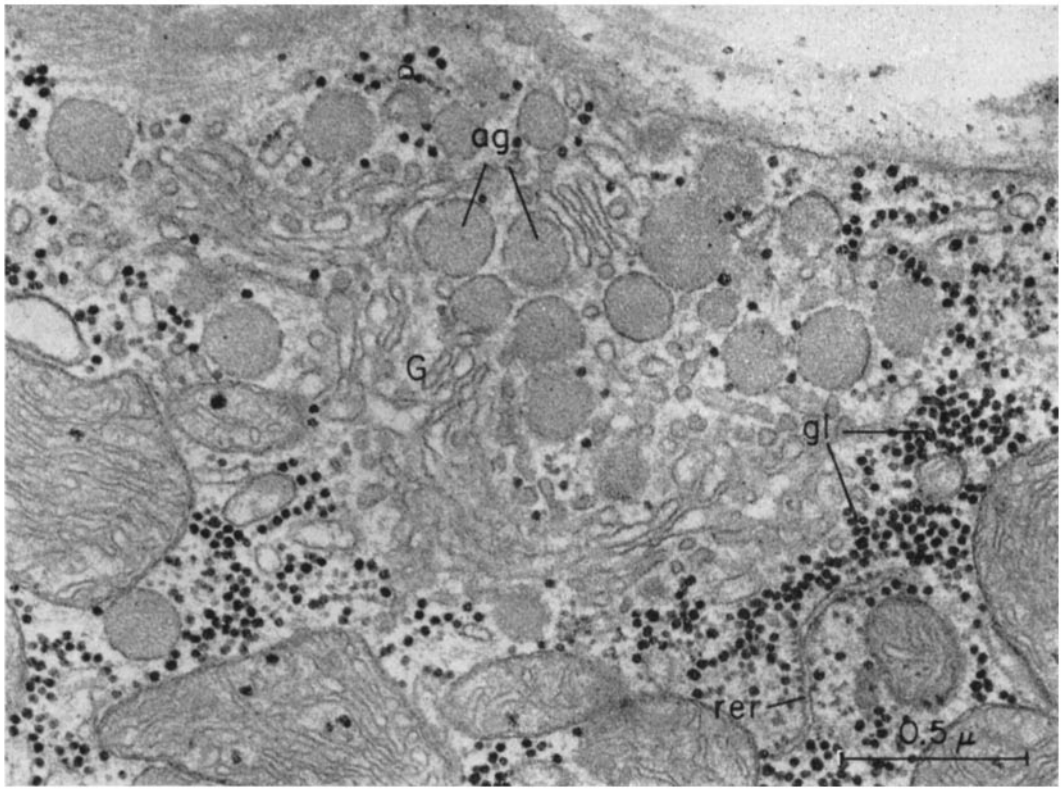
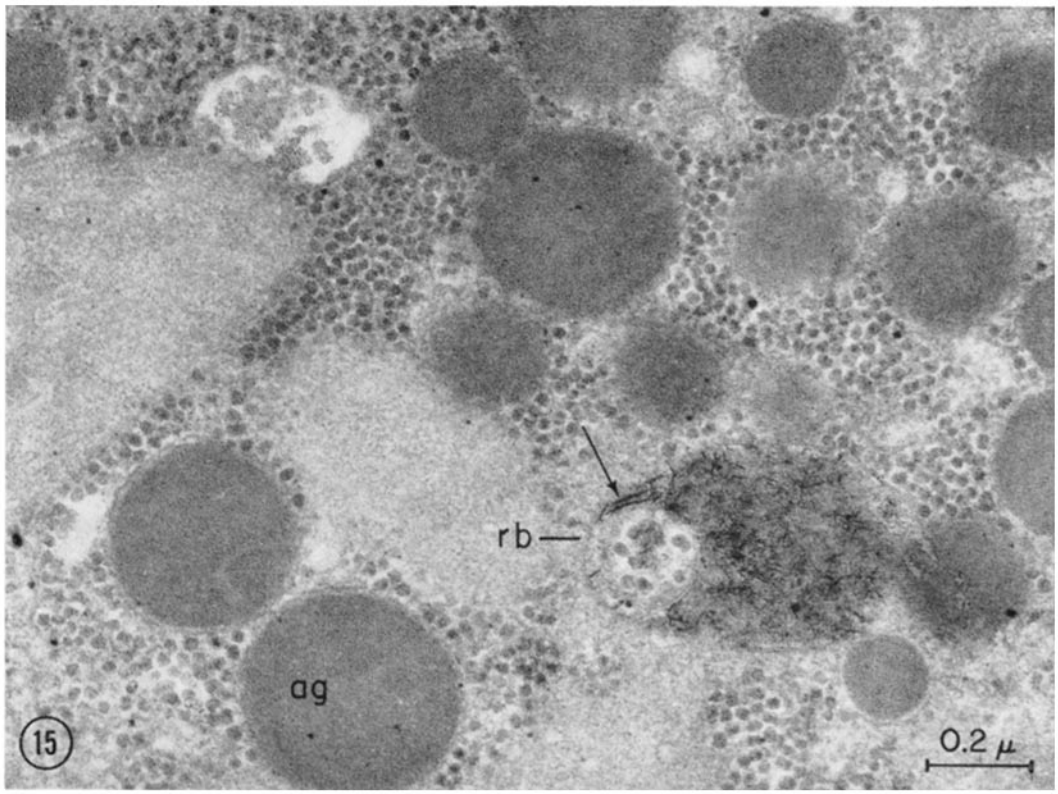
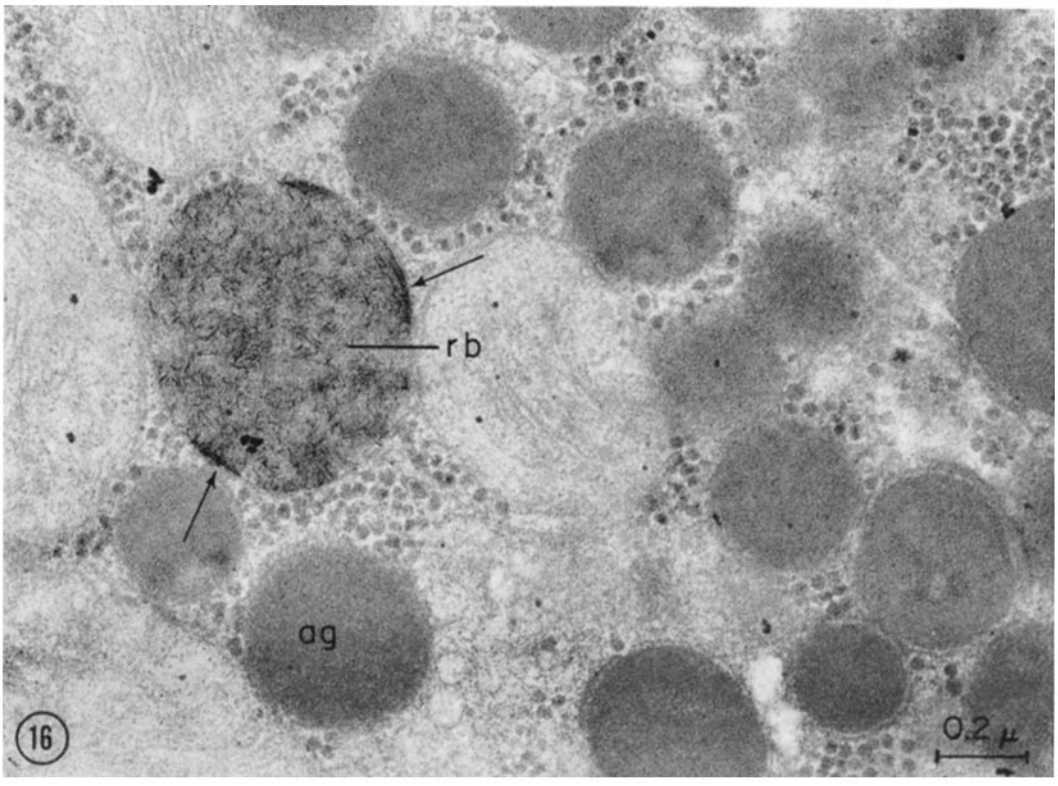


FIGURE 14 Peripheral Golgi complex in an atrial muscle fiber (rat). *rer*, rough-surfaced endoplasmic reticulum; *gl*, glycogen particles; *ag*, atrial granules. × 50,000.


rb
ag
15
0.2 μ


rb
ag
16
0.2 μ

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FIGURES 15 and 16 Sections of atrial muscle fibers (rat) reacted for acid phosphatase. Note the residual bodies (*rb*) which contain recognizable glycogen particles and are marked by dense crystals of reaction product (lead phosphate) (arrows). Adjacent atrial granules (*ag*) are free of reaction product and presumably lack acid phosphatase activity. Fig. 15, × 72,000; Fig. 16, × 70,000.

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