
**THE APPEARANCE OF GRANULES IN THE GOLGI
COMPLEX OF EMBRYONIC CARDIAC MYOCYTES**

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INTRODUCTION

During embryogenesis, cardiac myocytes have many cytological characteristics of secretory or glandular cells. Early embryonic ventricular myocardial cells have a large amount of granular endoplasmic reticulum and a well-developed Golgi complex, and these cells have been implicated in the elaboration of cardiac jelly (7). Indeed, evidence is accumulating that indicates that cardiac muscle has a secretory role in the adult, as well as in the embryo. For example, Lockett (6) has provided physiological evidence for the secretion of a humoral agent by mature myocardium.

In an earlier electron microscopic study, Jamieson and Palade (4) described the presence of membrane-bounded granules associated with the Golgi region of mature atrial muscle cells of several mammalian species. More recently, Hibbs and Ferrans (3) reinvestigated rat atrial granules, employing histochemical as well as electron microscopic techniques. During the course of a comparative study, Trillo et al. (11) noted that both atrial and ventricular muscle cells of the chick contain approximately the same number of secretory granules. They have also been described in ventricular muscle cells of toads (8), cyclostomes (1), and frogs (10), and, in fewer numbers, in rat ventricular muscle (11).

Little is known about the time of appearance and distribution of myocardial granules during embryonic development. Jamieson and Palade (4) noted their presence in late fetal and neonatal rats, but not in a 10 mm embryo, and Pager (9) con-

firmed their presence in late embryonic rats. No other observations of these structures in embryos of other species were found in the literature.

MATERIALS AND METHODS

Fertile, white Leghorn eggs were incubated at 39°C. Embryos were removed and placed in a dish of glutaraldehyde-formaldehyde fixative (5) buffered to pH 7.6 with 0.2 M cacodylate buffer. Hearts were dissected free and placed in fresh fixative, at 0°C, for periods of time ranging from 10 to 30 min. In order to facilitate penetration of the fixative, they were cut in half longitudinally, at right angles to the developing interventricular septum. Following a brief buffer rinse the tissue was osmicated at 0°C for 2 hr. Following dehydration in a graded series of alcohols, the half-hearts were embedded either in Araldite or Epon and sectioned with glass knives. Sections were mounted on uncoated copper grids, single stained with lead citrate (12), and examined with either an RCA EMU-3F or Hitachi HU-11E electron microscope operated at 50 kv.

RESULTS AND DISCUSSION

The Golgi complex of immature cardiac myocytes is prominent during embryonic development. It occupies a juxtannuclear position, and is usually comprised of several stacks of lamellae and a large number of smooth-surfaced vesicles (Fig. 1). Occasional short profiles of granular endoplasmic reticulum are present within the complex, and often a few profiles of smooth-surfaced cisternae have a number of ribosomes apposed to them. Characteristically, these structures have ribosomes only on one side of the profile (Fig. 2). The outer membrane of the nuclear envelope in the

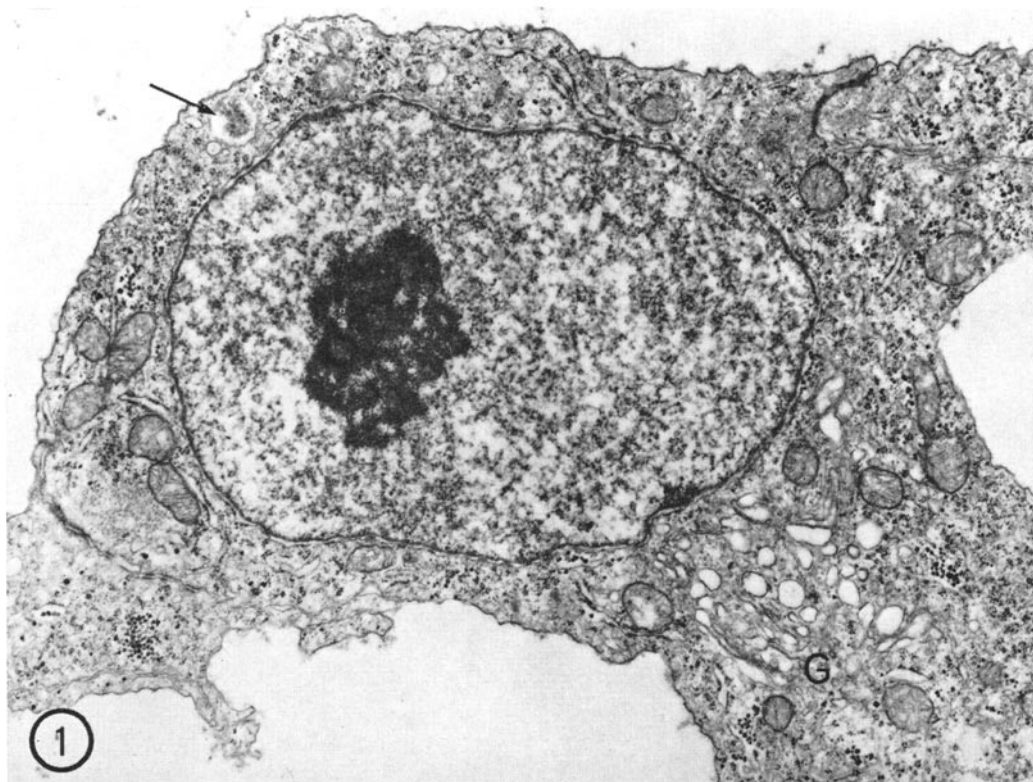


FIGURE 1 The contents of the Golgi lamellae and vesicles (*G*) in this cardiac myocyte from the heart of a 10-somite chick embryo are electron lucent. Dense granules are not seen this early in development. A multivesicular body (*arrow*) is seen in the upper left. $\times 17,200$.

region of the Golgi complex commonly demonstrates blebbing (Fig. 2), suggesting that it is either contributing to, or receiving material from, the Golgi complex.

Large multivesicular bodies are often present in the region of the Golgi complex (Fig. 2). These structures, present throughout embryonic life, are not restricted to the Golgi region but may also be scattered throughout the cytoplasm (Fig. 1).

By the fourth day of development, occasional dense granules are demonstrable within the Golgi apparatus. As development continues, these dense inclusions become more frequent (Figs. 2, 3). They are characterized by a small (0.08μ), electron-opaque particle commonly occurring in the ends of the Golgi lamellae (Fig. 2) and within vesicles (Figs. 2, 3). The dense particles appear to have a fairly uniform diameter and are often seen occupying a peripheral location within much larger (0.2 to 0.3μ) vesicles (Fig. 3). By this cri-

terion, these granules are unlike those described in the hearts of other species (1, 4, 8, 10).

Prior to the appearance of inclusions, the contents of both Golgi lamellae and vesicles are electron lucent (Fig. 1). In an earlier study (7) it was suggested that the empty appearance of these structures might be the result of poor fixation. However, identical preparative techniques preserve the granules in later stages, suggesting that their absence in earlier stages is not artifactual. Thus, it appears that the onset of the type of secretory activity characterized by these granules does not occur until well after the cells have begun synthesizing myofibrils, and that the two specialized functions can occur contemporaneously. Indeed, the absence of these granules in earlier stages (Fig. 1) suggests that developing cardiac myocytes may secrete different substances during different periods of development, an hypothesis that is currently under investigation.

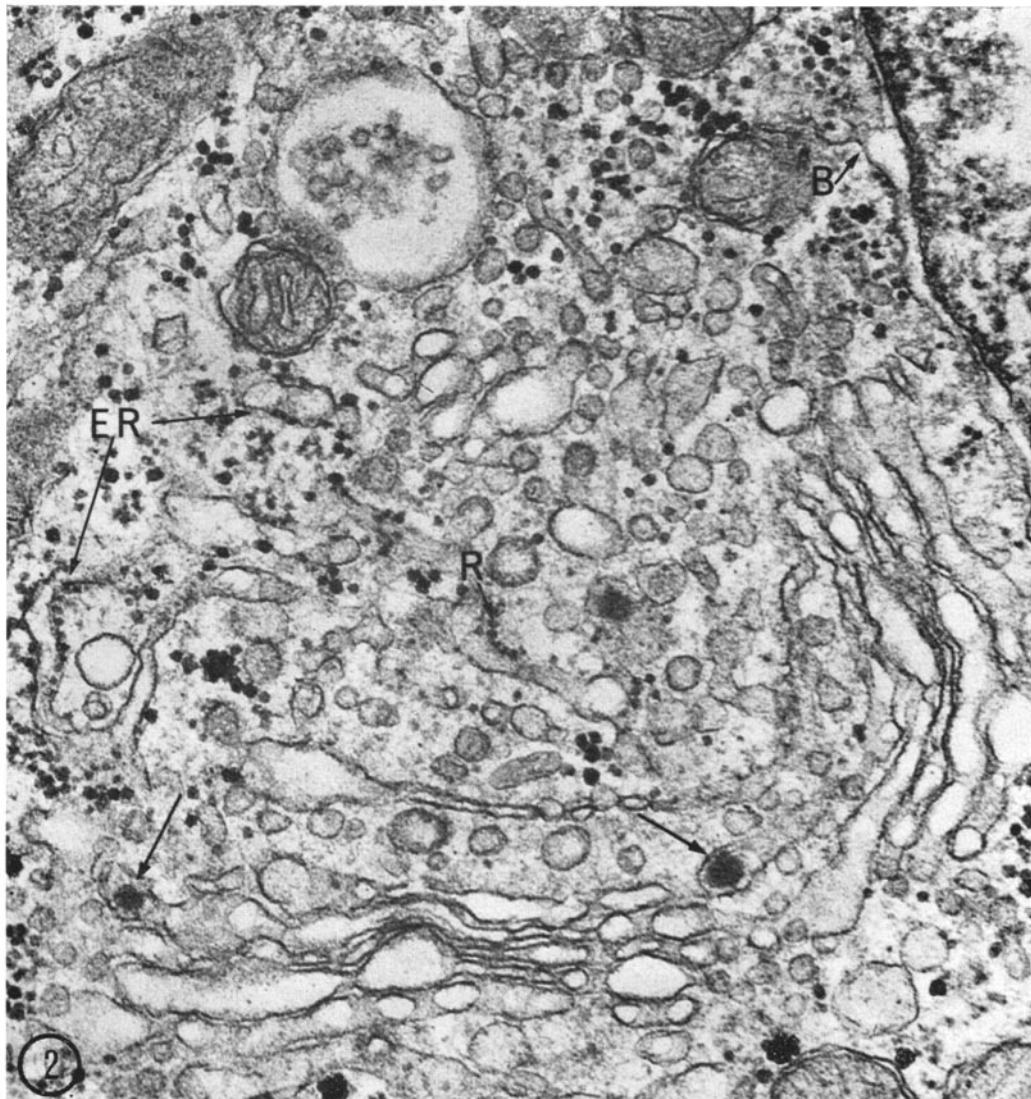


FIGURE 2 The Golgi complex of a myocyte in the ventricle of a 6 day old chick embryo is seen in this electron micrograph. Several stacks of lamellae and a large number of smooth vesicles are prominent. Two dense granules are seen in the terminal portion of the lamellae (*arrows*) and another granule is seen within a vesicle near the center of the field. Short elements of granular endoplasmic reticulum are present (*ER*) and several ribosomes are apposed to short segments of otherwise smooth membranous profiles (*R*). A large multivesicular body is conspicuous. The nucleus is at the upper right and the nuclear envelope exhibits a bleb (*B*). $\times 60,900$.

Ventricular granules, similar to those present in embryonic cells, are also seen in hearts of newly hatched chicks (Fig. 4) and are also present in mature tissue (11). Although the dense secretory-like granules seen in mature cardiac muscle cells of other species (1, 4, 8, 10) have been compared to

neurosecretory granules, on the basis of their close structural similarity (10), biochemical evidence for any similarity is lacking.

It seems certain, however, that cardiac muscle has a multiplicity of specialized functions. The presence of specific secretory granules in mature

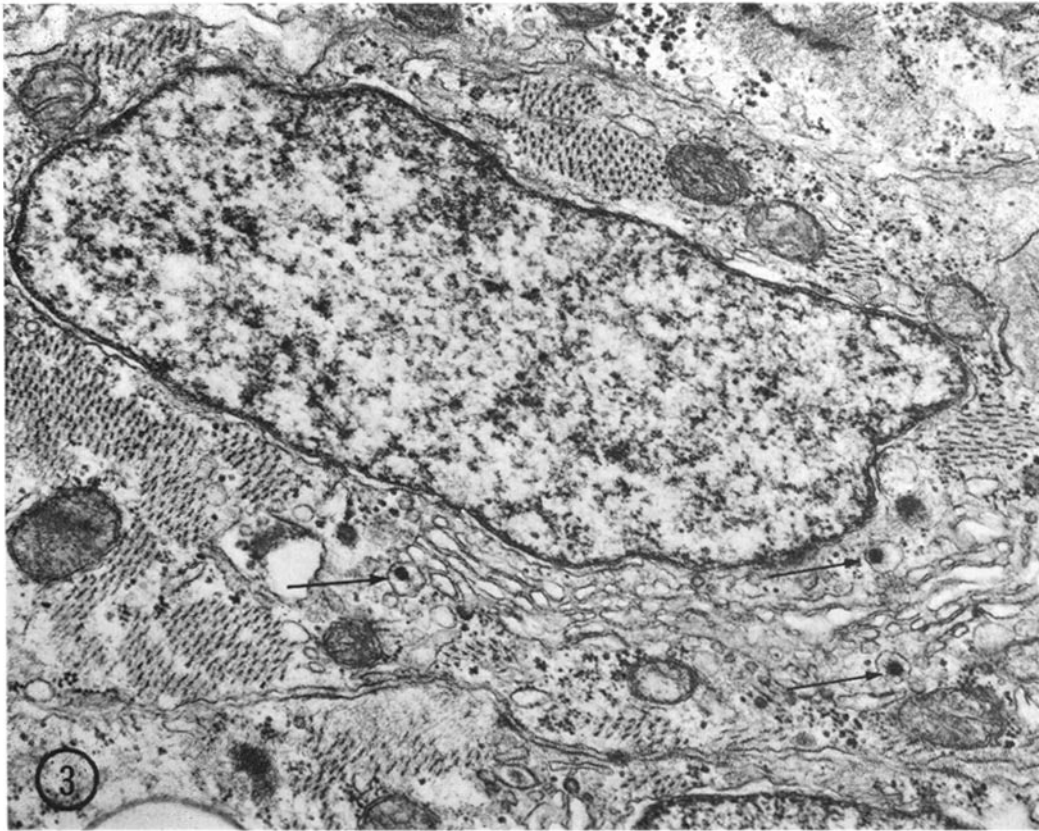


FIGURE 3 Embryonic chick ventricular myocytes retain a prominent Golgi complex throughout development. In this electron micrograph of a cross-section through a ventricular myocyte from an 8 day old chick embryo, several dense granules can be seen in vesicles within the Golgi region (arrows). $\times 30,800$.

cells suggests that the cells have a secretory as well as a contractile function (3, 4). Similar evidence of secretion during the developmental stages discussed in this study suggests that the immature cardiac myocytes are capable of synthesizing a secretory product while they are still producing the specialized proteins of the myofibrils. Certainly, the complexity and apparent divergence of the specialized functions of cardiac muscle stress some of the unique characteristics of this cell type compared to skeletal muscle cells. In addition, the pronounced differences between cardiac muscle

and skeletal muscle in their embryonic development (2, 7) emphasize the need both for caution in making generalizations about myogenesis, and for further elucidation of the events of cardiac myogenesis.

I would like to thank Doctors James D. Ebert and Don W. Fawcett for their critical readings of the Manuscript, and Mr. Richard Grill for his assistance in preparing the micrographs.

Received for publication 26 February 1969, and in revised form 23 July 1969.

REFERENCES

1. BLOOM, G. D. 1962. Fine structure of cyclostome cardiac muscle cells. *Z. Zellforsch. Mikroskop. Anat.* 57:213.
2. HAY, E. D. 1968. Organization and fine structure

of epithelium and mesenchyme in the developing chick embryo. In *Epithelial-Mesenchymal Interactions*. R. Fleischmajer, editor. The Williams & Wilkins Co., Baltimore 31.

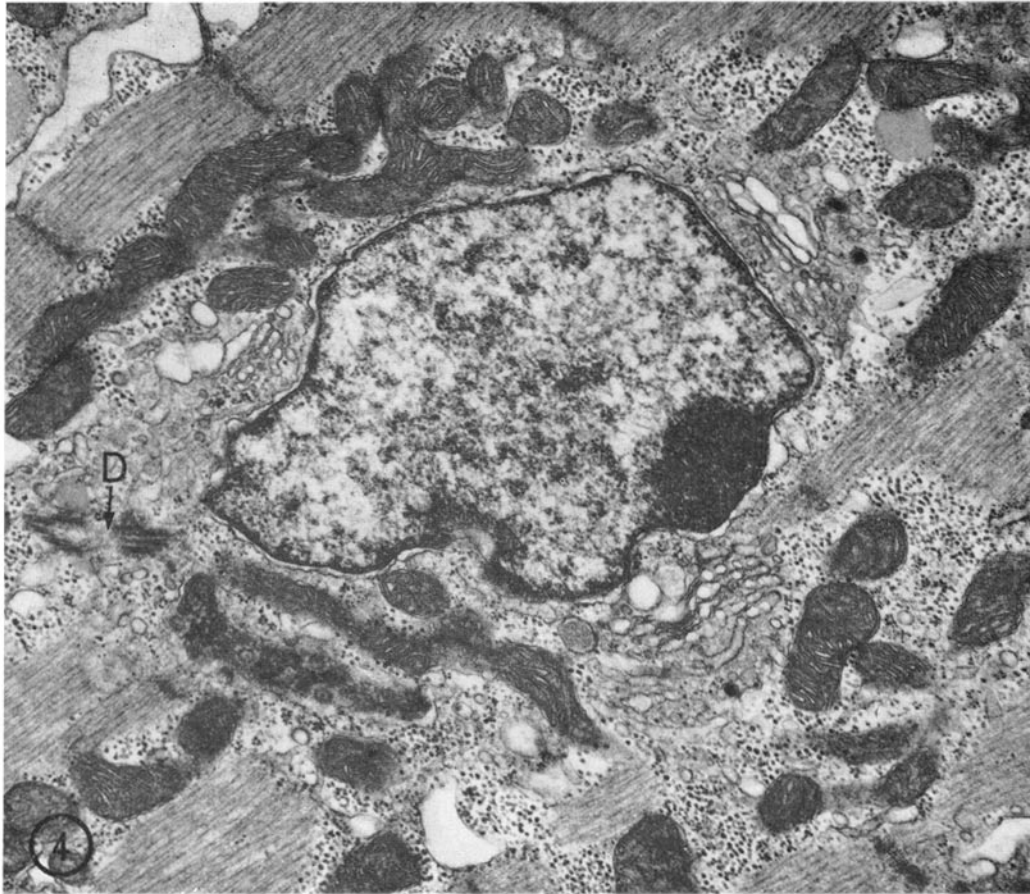


FIGURE 4 Ventricular myocytes of newly hatched chicks also demonstrate extensive Golgi regions. Dense membrane-bounded granules similar to those seen in earlier stages are present. A tangentially sectioned diplosome (*D*) is seen in the lower left. $\times 25,000$.

3. HIBBS, R. G., and V. J. FERRANS. 1969. An ultrastructural and histochemical study of rat atrial myocardium. *Amer. J. Anat.* **124**:251.
4. JAMESON, J. D., and G. E. PALADE. 1964. Specific granules in atrial muscle cells. *J. Cell Biol.* **23**:151.
5. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**:137A.
6. LOCKETT, M. F. 1967. Hormonal actions of the heart and of the lungs on the isolated kidney. *J. Physiol. (London)*. **193**:661.
7. MANASEK, F. J. 1968. Embryonic development of the heart. I. A light and electron microscopic study of myocardial development in the early chick embryo. *J. Morphol.* **125**:329.
8. NAYLER, W. G., and N. C. R. MERRILLEES. 1964. Some observations on the fine structure and metabolic activity of normal and glycerinated ventricular muscle of toad. *J. Cell Biol.* **22**:533.
9. PAGER, J. 1968. Evolution structurale et ultrastructurale du tissu cardiaque en développement chez le foetus de rat. Doctorate Thesis. Faculty of Sciences, University of Lyon. France.
10. STALEY, N. A., and E. S. BENSON. 1968. The ultrastructure of frog ventricular cardiac muscle and its relationship to mechanisms of excitation-contraction coupling. *J. Cell. Biol.* **38**:99.
11. TRILLO, A., A. MARTINEZ-PALOMO, and S. A.

- BENCOSME. 1966. Estudio ultraestructural del musculo cardiaco en relacion a los granulos especificos. *Arch. Inst. Cardiol. Mex.* 36:45.
12. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* 25:407.