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An ultrastructural study of sinuatrial node cells in the embryonic rat heart

J. M. DOMENECH-MATEU AND J. BOYA-VEGUÉ
Departments of Anatomy and Histology, Medical Faculty, University of Madrid

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INTRODUCTION

Although the myocardium was one of the first tissues to be studied with the electron microscope, the ultrastructure of the specialized conducting tissues has only recently begun to receive attention.

James *et al.* (1966) studied the cytologic characteristics of nodal cells with the electron microscope in the dog and in man, while Yamauchi (1965) studied the ultrastructure of the sinuatrial and atrioventricular cells in human embryos. These studies, and those of James (1970), have shown that the cells initiating the cardiac impulses – the nodal P cells – are characteristically round, small, clear elements arranged in long cellular tracts, the cells being in contact with each other on all sides. Every 'P' cell has its own plasma membrane, but groups of these cells are enclosed within a single basal membrane. Desmosomes are rare. Myofibrils are scarcer than in ordinary cardiac muscle. The mitochondria are also structurally simpler than those of the ordinary myocardial fibres.

In the present work on the ultrastructure of the nodal cells of the embryonic white rat, some interesting details were brought to light.

MATERIALS AND METHODS

Embryos from white rats (*Rattus norvegicus*, var. *albinus*) were used. The day of fertilization was determined by examination of the vaginal contents. Pregnant rats were killed with chloroform on the 15th, 16th and 17th days. The embryos were rapidly removed, liberated from their amniotic sacs and immediately submerged in a solution of 3 % gluteraldehyde in phosphate buffer at a pH of 7·4. Using a binocular microscope the anterior chest wall was opened and the heart and large vessels extracted. Keeping the material submerged in gluteraldehyde, the junctional region between the right atrium and vena cava, the auricular myocardium, and a fragment from the ventricular myocardium were removed.

After further fixation in gluteraldehyde the material obtained was sectioned into fragments approximately 0·5 mm in diameter and post-fixed in 2 % osmium tetroxide for 2 hours.

Following dehydration in acetone the specimens were embedded in Westopal W. Sections 50 nm thick were made with an LKB ultramicrotome and studied with a Philips E 200 electron microscope.

RESULTS

Differentiation between nodal cells and ordinary myocardial fibres was not difficult. The former had a pale, partially empty appearance, because of the paucity of myofibrils. Their hyaloplasm had a low density (Fig. 1).

Nodal cells had a central nucleus with a generally circular, sometimes oval profile. The chromatin was fine and there were usually one or more nucleoli visible.

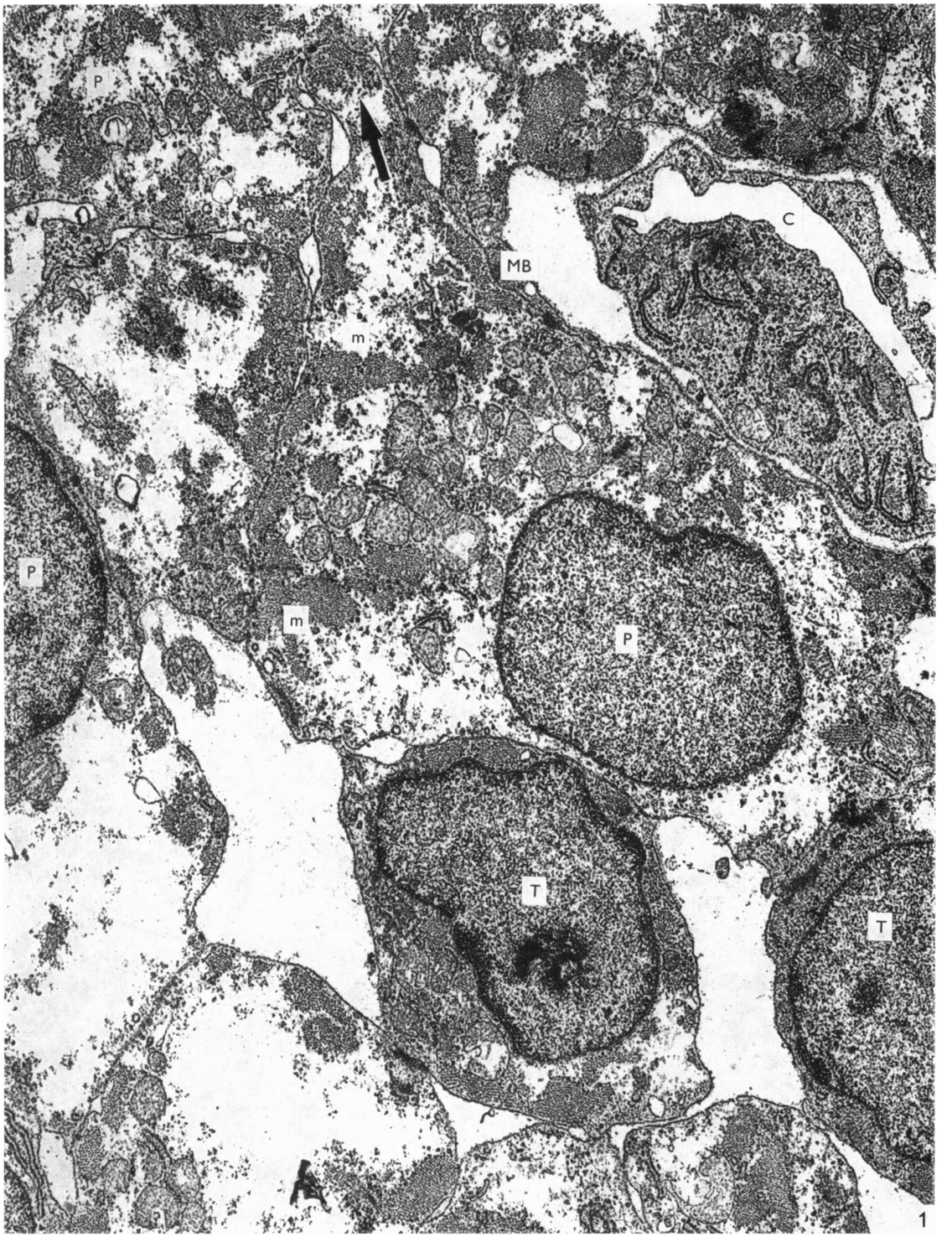
The cytoplasm was abundant in this embryonic phase. There were often thick evaginations penetrating between adjacent cells (Figs. 1–2). Mitochondria were rather abundant and tended to be located in the central zone of the cytoplasm; they tended to be thicker than those of ordinary myocardial fibres, but their cristae were further apart. The endoplasmic reticulum was poorly developed, being represented by a few rough-surfaced vesicles. Ribosomes were abundant, grouped in 'rosettes' and distributed throughout the hyaloplasm. The well-developed Golgi system showed flat vesicles and microvesicles; it was almost always located close to the nucleus. Multivesicular bodies were present.

Striated myofibrils were present in all nodal cells, but their numbers were always considerably smaller than those of the myocardial fibres. They were very scarce in some cells (Figs. 2–3). There was a tendency for them to be located peripherally (Fig. 2), but this was not the case in all nodal cells, for in some the myofibrils were randomly located. The myofibrils of the nodal cells did not show the characteristic orientation that they have in myocardial fibres parallel to the longitudinal axis of the cell (Fig. 3).

Many of these nodal cells showed osmiophilic granules distributed throughout the cytoplasm, although with perhaps a special affinity for the region of the Golgi complex (Fig. 3). They appeared to be membrane-bound and filled with dense structureless material.

A characteristic feature of the cells of the sinuatrial node was that they showed many areas of contact (Fig. 2) where the plasmalemmas of adjacent cells were parallel to and close to each other and the neighbouring hyaloplasm appeared dense. The myofibrils were not oriented towards or related in any special way to these zones of contact. Elsewhere the periphery of the nodal cells showed varying numbers of pinocytotic invaginations and vesicles (Fig. 3).

Fig. 1. Nodal cells (P) from a 16 day old rat embryo. Note their clear appearance due to the low density of the hyaloplasm and the scarcity of myofibrils (m). These nodal cells emit thick prolongations which penetrate between adjacent cells (arrow). Besides the nodal elements, other cells (T) can be observed. These are rich in myofibrils and belong to the contractile myocardium. The basal membrane (MB) surrounds the groups of myocardial fibres, separating them from the connective spaces where capillaries (C) are found.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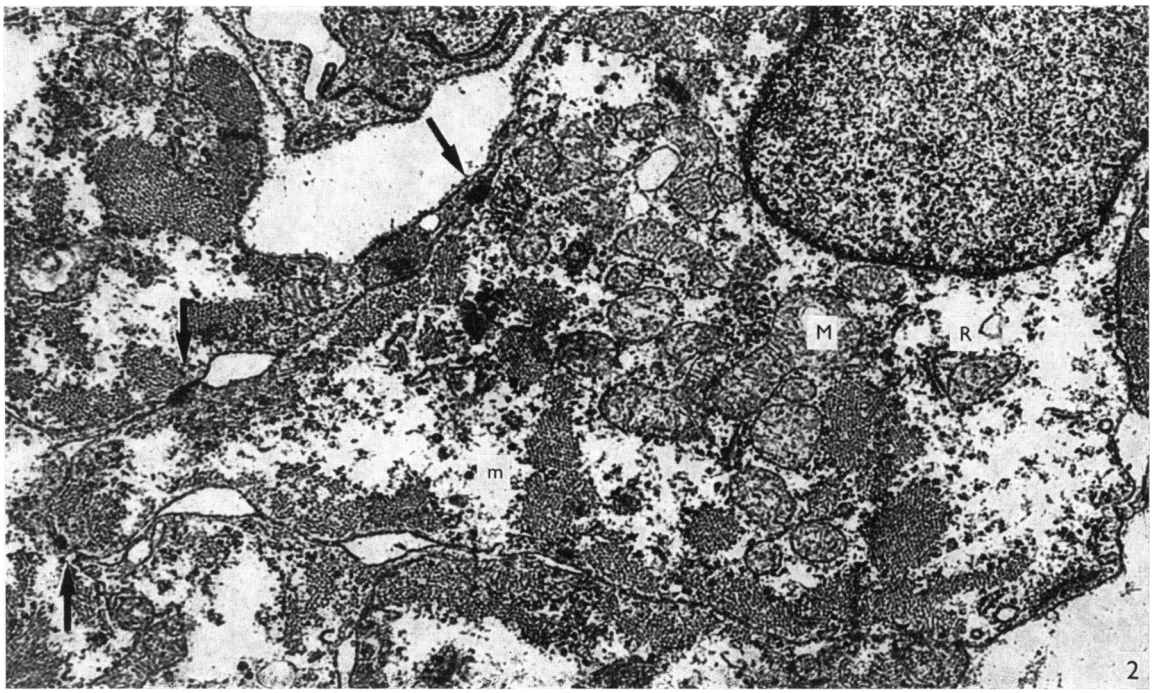


Fig. 2. Portion of a nodal cell from a 16 day old embryo (see Fig. 1) which exhibits various contact points between adjacent cells (arrow). Some vesicles of rough-surfaced reticulum (R), as well as mitochondria (M) and myofibrils (m), can be observed in the cytoplasm.

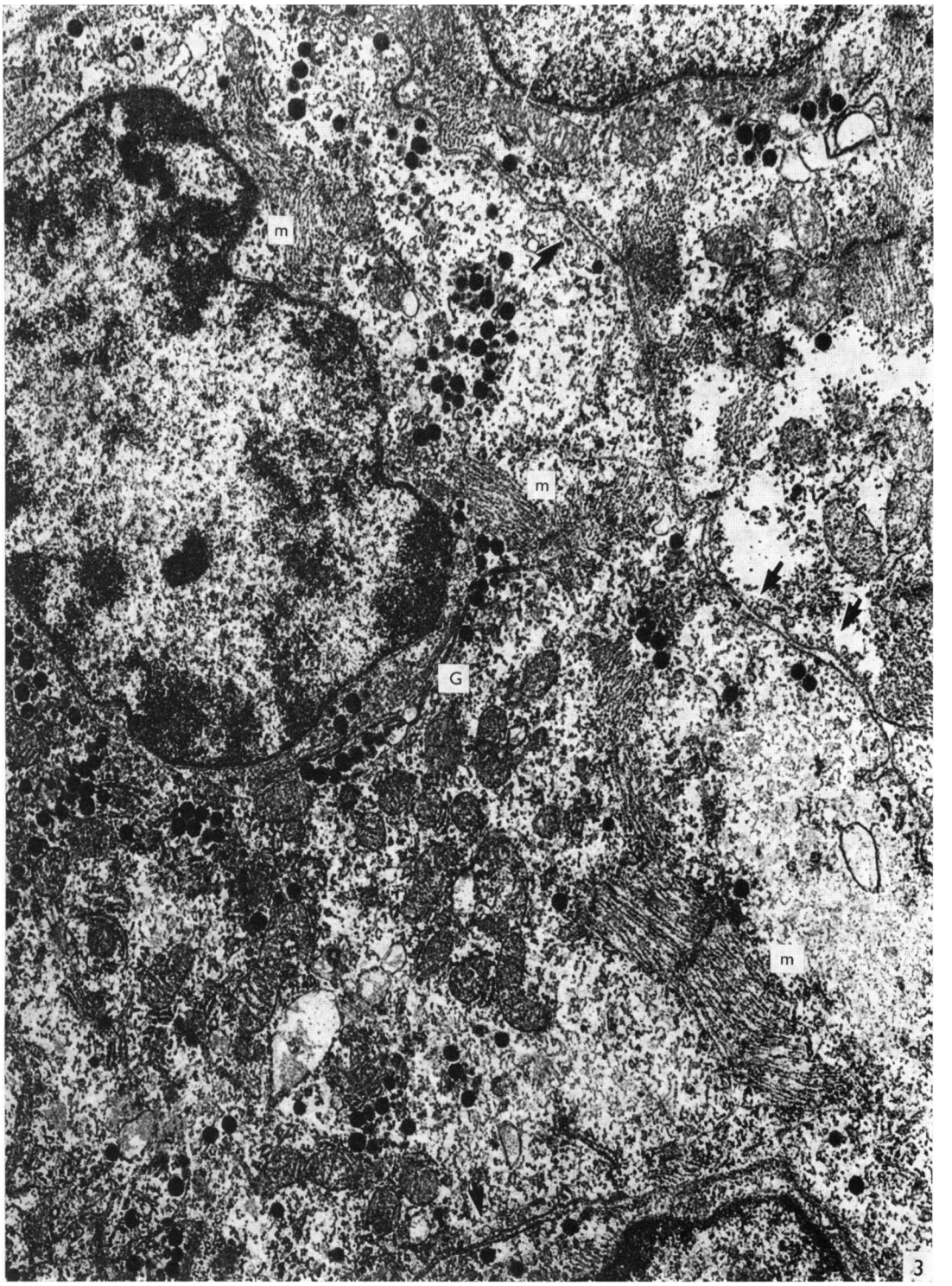
DISCUSSION

In the present study we were interested first of all in the ultrastructure of the embryonic nodal cell and secondly in any differences between nodal cells and ordinary myocardial cells.

The sinuatrial node in the adult rat has been exhaustively studied with the light microscope (Halpern, 1955; Muir, 1955; Domenech Mateu, 1973). We have also studied it (Domenech Mateu, 1973) in rat embryos, where we have verified that the primordium of the node appears in rat embryos at 12 days, in the ventromedial wall of the right common cardinal vein, just above its entrance into the sinus venosus. The node presents a typical mature appearance in rat embryos as early as the 16th or 17th day. It resembles a 'cellular package' located in the ventromedial wall of the right upper vena cava at the atriocaval junction. Knowledge of the precise location of the node in rat embryos has enabled us to isolate the structure for electron microscopy without any difficulty.

Most authors accept that most of the cells of the sinuatrial node are specialized

Fig. 3. Transverse section of a nodal cell from a 17 day old embryo showing many dense granules. Some of these are related to the Golgi complex (G). The few myofibrils (m) do not appear orientated in relation to the longitudinal axis of the cell. In the periphery, a few pinocytotic vesicles can be seen (arrow).


m
m
G
m
3

P cells, although some cells with the characteristics of the myocardial fibres, and possibly some transition cells, may also be observed (James & Sherf, 1968).

Early workers believed that the sinuatrial node contained 'syncytial' cells. However, the electron microscope has shown that the latter are P cells, each with its own plasma membrane and, therefore, there is no syncytium.

James *et al.* (1966), describing the ultrastructure of the P cell, emphasized the paleness of its cytoplasm. This seems to be partly due to the relatively small number and disseminated arrangement of the intracellular organelles and partly to the low glycogen content.

Our description of the P cell agrees in most respects with that of other authors who have studied the adult cell in the atrioventricular node in various species. Kawamura (1961), Torii (1962) and Hayashi (1962) have studied it in dog, rabbit and cow hearts respectively. Maekawa *et al.* (1967) carried out a study on the rat, dog and monkey. Meredith & Titus (1968) studied nodal cells in the human heart and Thaemert (1973) in the rat heart. However, very little work has been carried out on the ultrastructure of the embryonic nodal cells, apart from that of Yamauchi (1965), who studied the sinuatrial and atrioventricular nodes in human embryos and fetuses.

Nevertheless, while the general ultrastructural organization of embryonic P cells was similar to that of adult nodal cells, there were significant differences. First, the cytoplasm of the embryonic nodal cells showed thick prolongations which insinuated themselves between adjacent cells. Secondly, osmiophilic granules were present in the Golgi complex. They resembled only superficially the dense bodies which Yamauchi (1965) observed in the atrioventricular node cells of human fetuses of 4–6 months. Thirdly, the P cells of adult hearts had a high pinocytotic index (James & Sherf, 1968), but our embryonic cells showed a lesser and variable degree of pinocytosis.

To sum up, the chief fact which emerges from this study is that the embryonic nodal cell shows most of the characteristic features of the 'P' cell of the adult heart, which indicates that it begins to function very early on. The differences we have found between embryonic and adult 'P' cells do not seem to be relevant to the cell's role as an initiator and transmitter of the cardiac impulse.

SUMMARY

Sinuatrial nodal tissue, obtained from rat embryos of 15, 16 and 17 days, was examined with the electron microscope.

Embryonic nodal cells were generally similar to adult cells except that (1) they showed thick prolongations of the cytoplasm which insinuated themselves between neighbouring cells; (2) they possessed osmiophilic granules with a predeliction for the region of the Golgi complex; (3) they exhibited a lesser and variable degree of pinocytosis.

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