

An Electron Microscope Study of Embryonic Heart Muscle Cells Grown in Tissue Cultures.*

BY H. MEYER AND L. T. QUEIROGA. (From the Instituto de Biofisica, Universidade do Brasil, Rio de Janeiro).‡

Cells in tissue cultures usually leave from the mass of tissue and migrate to the periphery of the medium where they may extend and flatten considerably. Under these conditions they have been widely used for the study of their inner structure under the light microscope, *in vivo* as well as in fixed and stained preparations. In the present paper some results will be given of an electron microscopic study of thin sections of such cells grown from explants of the heart muscle of chick embryos.

Materials and Methods

Heart muscle of chick embryos of 5 to 9 days' incubation was cultured in hanging drops consisting of a clot of diluted (1:3) chick plasma and diluted (1:3) embryo extract, in equal parts. In some instances the rollertube method was also used. The time of cultivation varied from 24 hours to 30 days.

Fixation was carried out in 1 per cent osmic acid, and dehydration in methyl alcohol, following the method advised by Palade (1), however, with shorter time periods. During the fixation and the dehydration, the cultures remained on the coverslips or in the rollertubes. After one night in 90 per cent methyl alcohol the clot was usually hardened to such a degree that the cultures could be cut out with a sharp cataract knife and transferred easily through the higher alcohols and the methacrylate, which was used for the final embedding. The sectioning was done with a Porter-Blum microtome (2), and the sections were observed with a Philips electron microscope.

OBSERVATIONS

Among the fibroblast-like cells in the growth area of the tissue cultures, many muscle cells were found with the electron microscope. They can be distinguished from the fibroblasts by the myofibrils which they contain. These myofibrils do not always have the rigid parallel course that they show in preparations of the uncultured heart muscle. Their position changes according to the movements of the migrating cells. Often they are found branching or even running in several directions. They are of different sizes and shapes (Figs. 1 to 6), and their structure is not so complex as in preparations of the heart muscle fixed *in situ*.

* This work has been partly supported by the National Research Council of Brazil.

‡ Received for publication, July 21, 1958.

The telophragma Z and the thin 100 to 150 A myofilaments, which run from one Z to another, are always present. In uncontracted fibrils, the lighter I band can be distinguished from A. Only very seldom are H or M observed. Never are these structures as sharply distinguished from each other as in preparations from the uncultured heart muscle. In most of the myofibrils in the tissue cultures, only A can be seen; the fixative seems to provoke contraction in the cultured cells. In the contracted cells I has disappeared and Z is often enlarged (Figs. 3 and 4).

All transition stages from relaxed to contracted can be found. The space between two Z bands measures, in the relaxed fibrils, from 1.8 to 2.3 μ , and in the contracted ones approximately 1.2. The myofilaments run parallel, showing no periodicity or lateral connections. The Z band is characterized by its higher electron density. Its shape varies from a thin line to large, irregular bands (Fig. 4). In many fibrils it is seen as a double line or a ring-like formation, and even with fragments extending from its mass (Figs. 3 and 4).

The very much flattened cells of the growth area offer good conditions for the study of the interrelationship of the myofibrils with the other components of the sarcoplasm, such as mitochondria, sarcoplasmic reticulum, Golgi apparatus, and centrosome. No membrane separates the myofibrils from the surrounding sarcoplasm of the cell. No direct contact or continuity has been found with the mitochondria, which are always abundant (Fig. 1). In a few images the Z band seems structurally related to the sarcoplasmic reticulum (Fig. 3), but in most of the preparations it ends abruptly and independently in the sarcoplasm. The Golgi apparatus or the centrosome has not been identified with certainty. The myofibrils have never been found to pass beyond the cell borders when these are visible; they have always been found restricted to one unit. In extremely flattened cells fragments only were found, composed of one or two Z bands with bundles of free filaments at both ends (Figs. 5 and 6).

DISCUSSION

The results described above confirm, in general, those obtained by authors as Weinstein (3), Hibbs

(4), Edwards, Santos, and Vallejo-Freire (5), and Moore and Ruska (6), who examined heart muscle cells with the electron microscope—especially in cases in which the measurements of the singular units are concerned. The liberation of single cells and their considerable spreading in tissue cultures give the opportunity of observing the greatly extended sarcoplasm of the muscle cells which is not possible in the compressed masses of cells in the heart tissue used by the other investigators. Were there any structural continuity as, for instance, between myofibrils and mitochondria, or between Z bands and the sarcoplasmic reticulum, this would be more visible here than in tissue preparations. On the other hand, these conditions are not quite normal, and it is possible that the more simple structure of the myofibrils, which we find in the cultured cells, is due to the great spreading and extension of the cells.

Even a loss of differentiation has to be considered since we know that heart muscle cells *in vitro* lose their specific morphological and physiological characteristics. They do not contract indefinitely and have, under the light microscope, the aspect of undifferentiated fibroblasts. These facts have been known and discussed since the beginning of tissue culture studies, and opinion on the fate of the heart muscle cells *in vitro* has always been divided. One group (A. Carrel, A. Fisher, and others) have thought that the muscle elements in the heart cultures degenerate and only the fibroblasts survive. The other (G. Levi, W. Lewis, Olivo, etc.) assumed that a loss of structure, a “de-differentiation” takes place *in vitro* (7).

In the material which has been examined by us with the electron microscope, myofibrils were still present after more than 30 days' cultivation. It is possible that a loss of structure, a de-differentiation was taking place in those cells where we saw the short myofibrils consisting of one or two Z bands only (Figs. 5 and 6). However, we do not know whether, under more favorable conditions, these cells would not be able to re-assume their original structure. The heart muscle cells in culture can be easily submitted to experi-

mental (chemical, physiological, or pathological) conditions. Their study with the electron microscope under these conditions, might help to solve some of the questions that are still unanswered as to their fine structure and function.

SUMMARY

Tissue cultures of heart muscle from 5 to 9 day chick embryos have been observed in thin sections with the electron microscope. Their fine structure has been described.

Myofibrils have been found in the cells after more than 30 days' cultivation. They are present in different numbers, sizes, and contraction stages, and running in several directions, often branching. They are limited to one cell unit. Their structure is not so complex as it is in preparations of the uncultured heart muscle. Always present are Z and A bands, and in the relaxed fibrils I bands and the myofilaments which run between the two Z bands. Very seldom only are H or M bands observed.

In highly extended cells of the growth area, only fragments of myofibrils have been found and the possibility of a “de-differentiation” process is discussed.

No continuity of myofibrils with mitochondria has been observed. In most of the examined preparations, the Z band appears independent of the structures in the sarcoplasm, but a few images suggest a continuity with the sarcoplasmic reticulum.

REFERENCES

1. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
2. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117**, (4), 685.
3. Weinstein, H. J., *Exp. Cell Research*, 1954, **7**, 130.
4. Hibbs, R. G., *Am. J. Anat.*, 1956, **99**, 17.
5. Edwards, G. A., Santos, P. S., and Vallejo-Freire, A., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 143.
6. Moore, D. H., and Ruska, H., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 261.
7. Levi, G., *Ergebn. Anat. u. Entwicklungsgesch.*, 1934, **31**, 125.

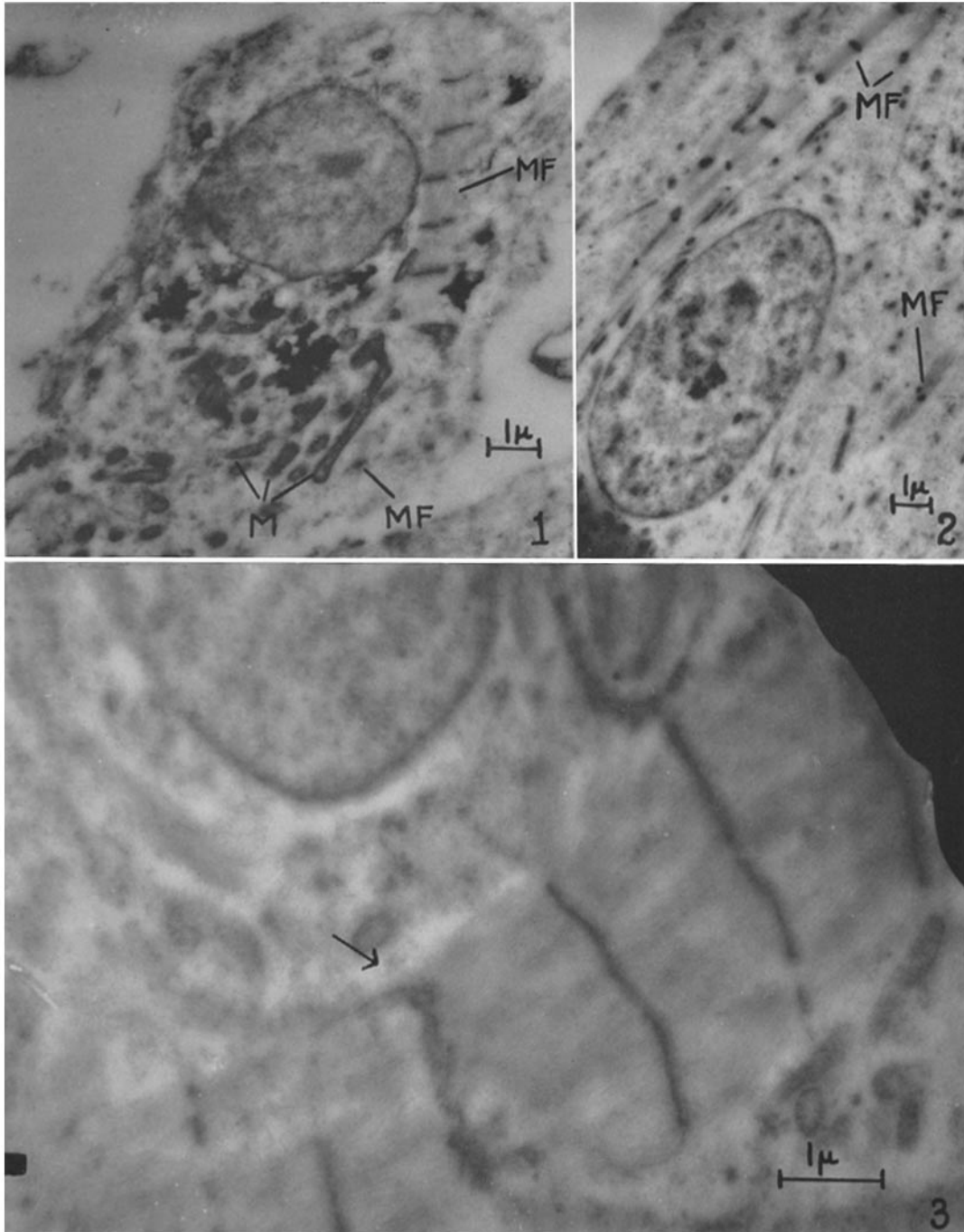
EXPLANATION OF PLATES

PLATE 81

FIG. 1. Heart muscle of 5 days' chick embryo, 2 days *in vitro*, in hanging drop culture (Plasma 1:1 Extract 1:3 in equal parts). Cell in growth area. Long myofibril (MF) in upper right part of cell, apparently in contraction state. Fragment of myofibril in lower part of cell (MF). The many mitochondria (M) show no contact with myofibrils. $\times 7,500$.

FIG. 2. Heart muscle of 9 days' chick embryo, 4 days *in vitro* in hanging drop culture (Plasma 1:1 Extract 1:3 in equal parts). Cell in growth area with one long and several short myofibrils (MF). $\times 15,000$.

FIG. 3. Same as in Fig. 4. Portion of cell with large myofibril in contraction state. One of Z bands (arrow) seems to continue into sarcoplasmic reticulum. $\times 14,200$.



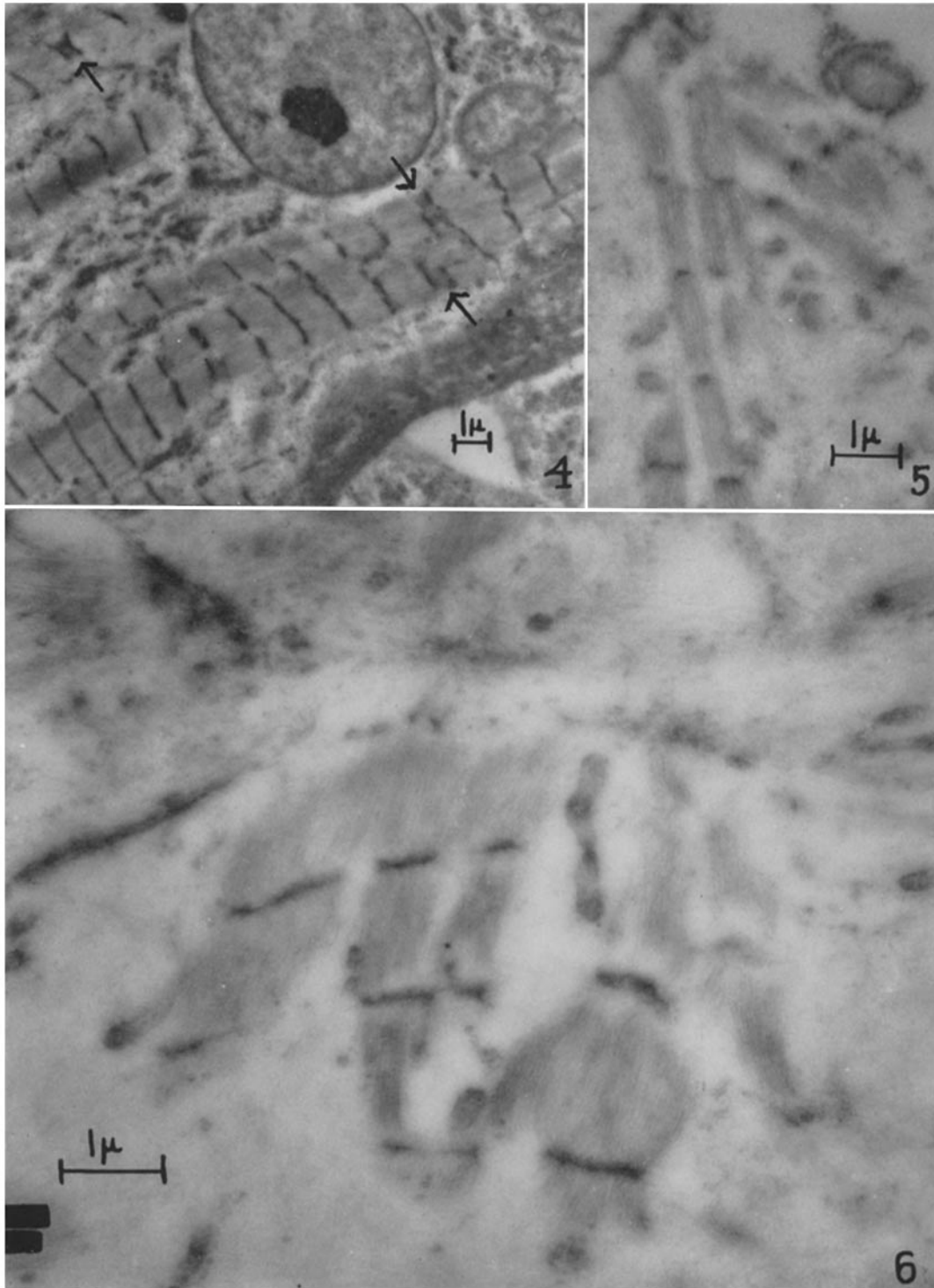
(Meyer and Queiroga: Embryonic heart muscle cells)

PLATE 82

FIG. 4. Heart muscle of 5 days' chick embryo, 30 days *in vitro*, in hanging drop culture first, later in roller tube. Cell with many long myofibrils, apparently all in contraction state. Some of Z bands seem to split (arrows). \times 5,000.

FIG. 5. Heart muscle of 5 days' chick embryo, 22 days *in vitro*, in hanging drop culture (Plasma 1:3 Extract 1:3 in equal parts). Portion of much extended cell with myofibrils, running in several directions. \times 9,600.

FIG. 6. Same as in Fig. 4. Portion of much extended cell with short myofibrils perhaps in beginning of "de-differentiation." \times 14,800.



(Meyer and Queiroga: Embryonic heart muscle cells)