

ULTRASTRUCTURAL CHANGES IN REGRESSING TAIL MUSCLES OF *XENOPUS* LARVAE AT METAMORPHOSIS

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INTRODUCTION

Previous studies on the changes in both the activity and localization of acid hydrolases in tails of *Xenopus* larvae during growth and metamorphosis have led to the conclusion that the increase in activity of cathepsins and acid phosphatase in the regressing tissue reflects the synthesis of acid hydrolases by activated macrophages, rather than the release of such enzymes from preformed lysosomes in the regressing tissue itself (13, 14). In order to elucidate the mechanism that could account for the progressive stimulation of phagocytic activity, it appeared of relevance to follow

the regressive changes in the fine structure of the tadpole tail. This study was also promoted by the recognition of the fact that, in spite of a voluminous literature on the histological aspect of tail regression, a number of important problems have remained unsolved. In fact, there exist divergent views, *e.g.* on the relative importance of autolysis and phagocytosis, or on the origin of macrophages in the regressing tadpole tail (2).

The present report, based on a very extended electron microscope study, is chiefly concerned with some conspicuous early changes in the fine

structure of tail muscle cells. The significance of these changes in relation to the mechanism of tissue regression is briefly discussed.

METHODS

Experiences with different procedures of fixation and embedding revealed the following method to be the most satisfactory one: small pieces of tail tissue, dissected from narcotized (MS 222, tricain metanesulfonate, Sandoz AG, Basel, Switzerland, 1:7000) tadpoles, were fixed in a mixture of 1 per cent OsO₄ plus 0.1 M sucrose made up in 0.014 M acetate-Veronal buffer adjusted to pH 7.4. After dehydration by increasing concentrations of acetone, the tissue was embedded in Durcupan AMC (Fluka AG, Buchs, Switzerland). Ultrathin sections were cut on a Porter-Blum ultramicrotome and mounted on copper grids coated with Formvar or nitrocellulose films. As electron stain, Reynolds' (11) lead citrate method gave excellent results. For the electron microscope demonstration of acid phosphatase in macrophages, the method of Miller (7) was followed, except that the embedding procedure described above was used.

OBSERVATIONS

As a standard for evaluating the regressive alterations, the organization of tail muscle from growing tadpoles will be mentioned first. Although a difference exists in the relative abundance of sarcoplasm and myofibrils in muscle cells at the surface and the interior of myomeres, the ultrastructural characteristics of the subcellular components are identical. As illustrated in Fig. 1, the myofibrils are straight and show the typical sarcomere pattern of alternating A and I bands, the latter being characterized by the Z disc. The

sarcoplasmic reticulum (1) consists of anastomosing tubular elements which line the myofibrils. Mitochondria are very numerous in the peripheral sarcoplasm, but also occur between myofibrils. The ground substance of the sarcoplasm contains numerous small granules, presumably representing both ribosomes and glycogen particles, as well as irregularly distributed smooth-surfaced vesicles.

The regressive changes are illustrated by three typical stages, which were consistently encountered upon examination of more than 500 electron micrographs taken from tissue preparations of initial, middle, and final stages of tail atrophy. Alterations in the fine structure of muscle cells are detectable as early as 3 days after the onset of metamorphosis (as indicated by the eruption of the forelimbs), before any measurable reduction in tail length occurs. Especially in the peripheral muscle cells, these changes are characterized by the zig-zag-like folding of the myofibrils in which the I bands are already less distinct (Fig. 2). Furthermore, the mitochondria, both in the peripheral sarcoplasm and between the myofibrils, show unmistakable signs of structural disintegration as demonstrated by the progressive loss of internal cristae (Fig. 3).

In tadpoles in advanced stages of tail atrophy, the degree of structural disintegration in the muscle cells varies considerably. The most profound changes are noticed in the sarcoplasm which has lost its small granules and instead is now filled with numerous smooth-surfaced microvesicles. Mitochondria are still present, but in the greater part the internal cristae have undergone

FIGURE 1 Portion of a tail muscle cell of a growing tadpole. Straight myofibrils (*MF*) with typical sarcomere pattern and sarcoplasmic reticulum (*SR*). Granular appearance of sarcoplasm (*SP*) with large mitochondria (*M*) and a few smooth-surfaced vesicles (*SV*).

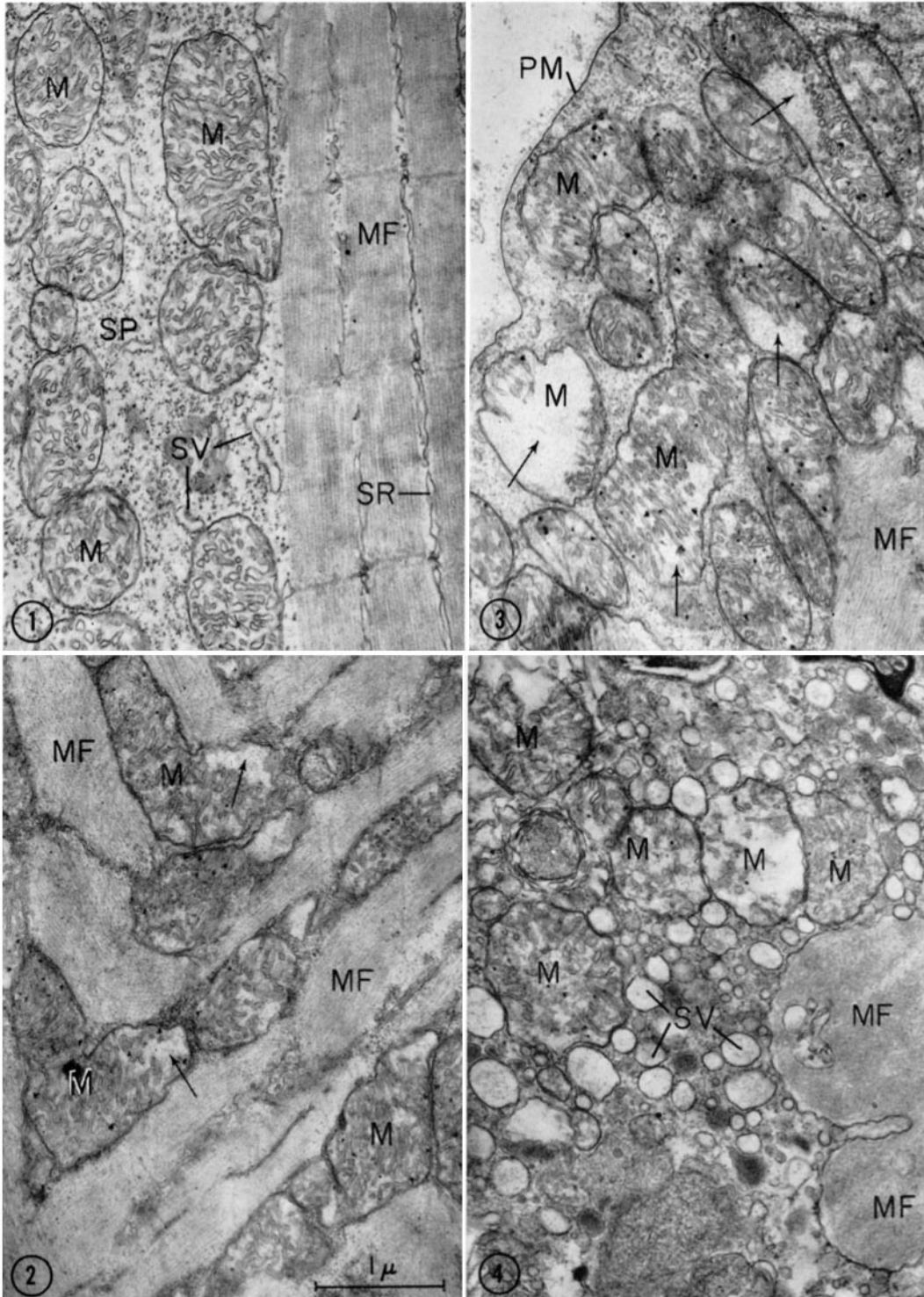
FIGURE 2 Portion of tail muscle cell of a tadpole 3 days after the onset of metamorphosis, showing early signs of regression: Folding of myofibrils (*MF*), fading of cross-striation, and erosion (arrows) of mitochondria (*M*).

Black spots, at least in part, represent intramitochondrial granules. It is noteworthy that such granules are most abundant in disintegrating muscle cells (*cf.* also Figs. 3 and 4), but are far less conspicuous in the growth stage (Fig. 1).

FIGURE 3 Similar stage as Fig. 2, showing peripheral mitochondria (*M*) at different stages of erosion (arrows). Plasma membrane (*PM*) still intact.

FIGURE 4 Sarcoplasmic portion of tail muscle cell of a tadpole at advanced metamorphosis. Mitochondria (*M*) and sarcoplasm with marked structural disintegration, appearance of smooth-surfaced vesicles (*SV*) and patches of disintegrating myofilaments (*MF*).

FIGURES 1 TO 4, $\times 19,500$.



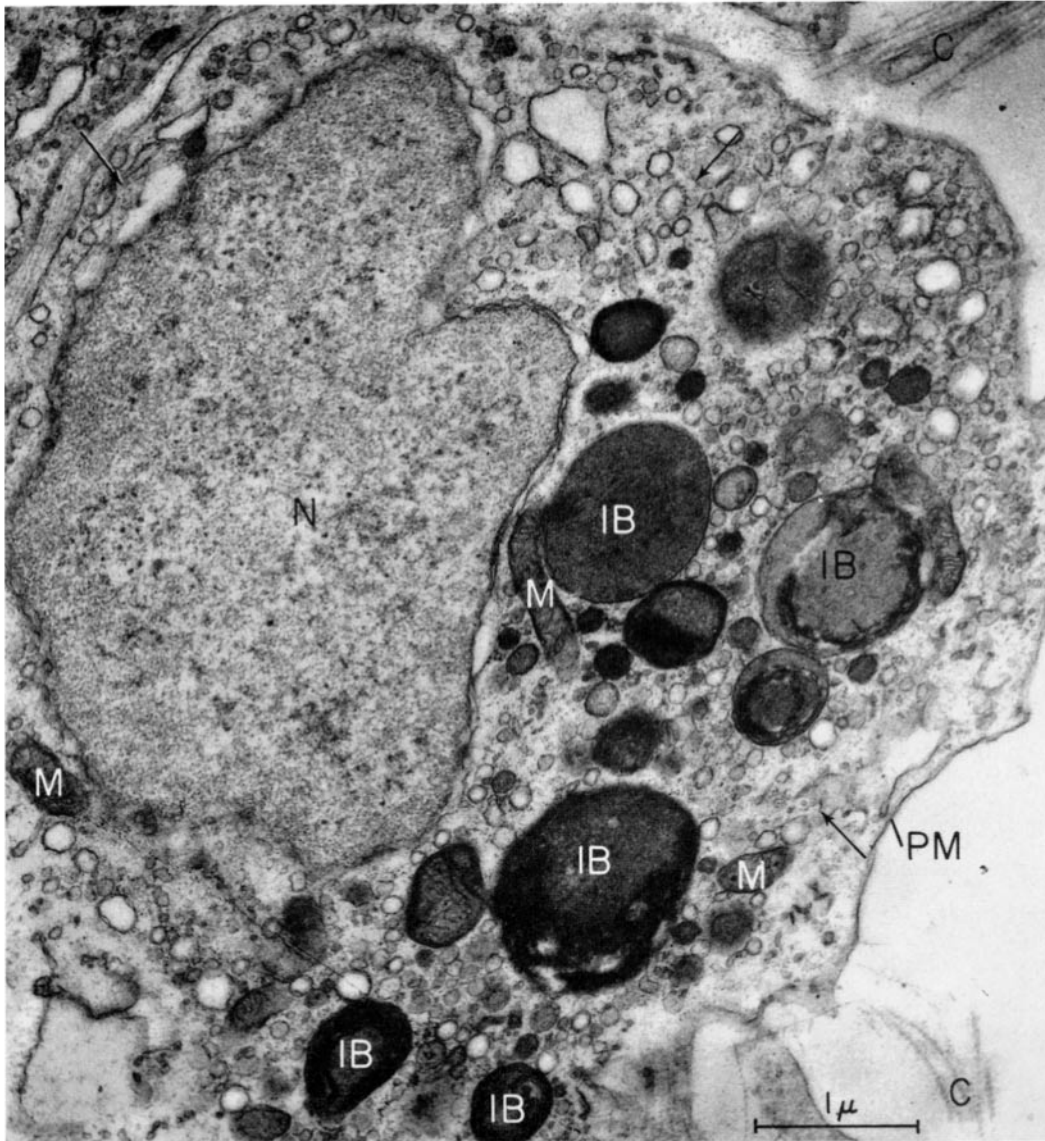


FIGURE 5 Early macrophage, probably derived from a mesenchymatous cell of the connective tissue. Cytoplasm with various types of inclusion bodies (*IB*) with outer membranes, rather few small and elongated mitochondria (*M*); around the nucleus (*N*) various components of the endoplasmic reticulum (arrows): rough-surfaced and smooth-surfaced vesicles and free ribosomes. Plasma membrane (*PM*) partially in contact with collagenous fibrils (*C*) of the intercellular ground substance. $\times 21,500$.

marked erosion. There is also evidence of an extrusion or shedding of the peripheral sarcoplasm, including mitochondrial remnants and nuclei, into the intercellular space (Fig. 4). As a result of this process, bundles of curled myofibrils appear, which are covered by a variable number of

concentric lamellae. These conspicuous bodies, consisting exclusively of myofibrillar material, probably correspond with the so called "sarcolytes" mentioned in the earlier literature (5).

Macrophages are best recognized in preparations of tail rudiments of small frogs shortly before

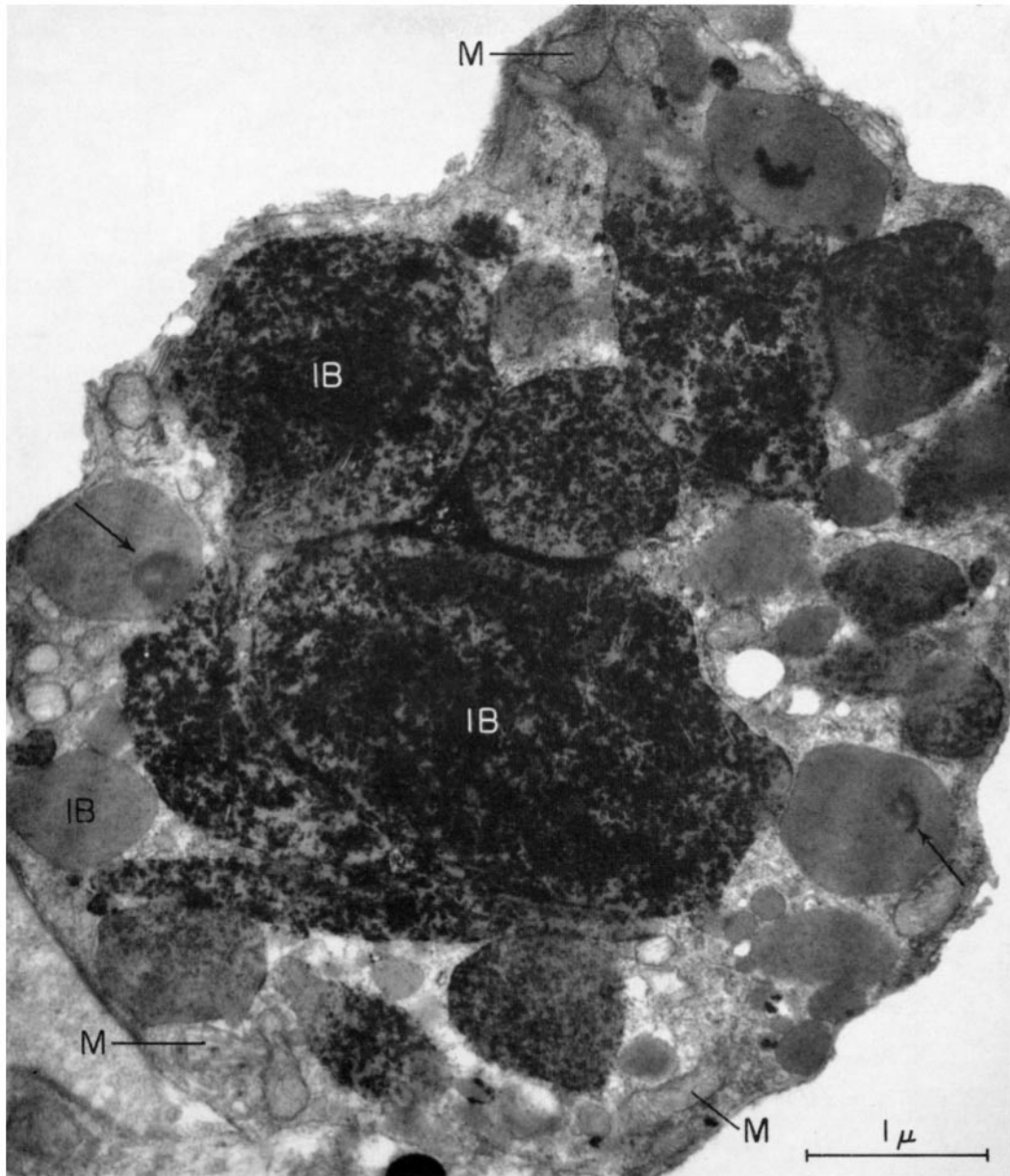


FIGURE 6 Well developed macrophage from tail rudiment stained for acid phosphatase activity. Inclusion bodies (*IB*) differ in size and enzyme content; those with a positive reaction for acid phosphatase may be regarded as "phagosomes." Some inclusion bodies contain myelin figures (arrows). Small mitochondria (*M*). $\times 24,800$.

completion of metamorphosis, when the greater part of the tail tissues has already been resorbed. Because of their large size, only parts of macrophages are usually found in ultrathin sections. Yet they may be distinguished from extruding

sarcoplasm by specific structural characteristics, such as the presence of a plasma membrane, relatively small and elongated mitochondria with intact cristae, typical Golgi membranes, and numerous inclusion bodies very different in size

and electron opacity, most of them possessing a distinct outer membrane. An early stage of macrophage formation, probably at the expense of a so called mesenchymatous cell of the connective tissue, is shown in Fig. 5. Apart from having numerous inclusion bodies, this cell contains a well developed endoplasmic reticulum comprising rough-surfaced vesicles and free ribosomes. With the aid of histochemical staining techniques it is possible to localize acid phosphatase activity on the membranes as well as in the interior of many inclusion bodies. An example of a well developed macrophage is given in Fig. 6. As regards the fate of myofibrillar material, it may be mentioned that in tail rudiments there are still patches of loose fibrillar elements occurring either free or ingested in inclusion bodies of macrophages.

DISCUSSION

The present findings clearly demonstrate that definite changes in fine structure occur in the muscle cells of tadpole tails before any external signs of tissue atrophy are detectable. In fact, the peculiar folding of the myofibrillar bundles and the concomitant loss in cross-striation appear to be the first signs of cellular regression. However, according to an earlier electron microscopic observation reported by Weiss (15; and personal communication) the regression of gill muscles in amphibian larvae shows a somewhat different picture. In this case, the actin filaments (I band) seem to be the first to disappear during metamorphosis, whereas the myosin filaments (A band) persist for some time as protein plates, thus suggesting a differential chemical erosion. Among the early alterations, the disintegration of the mitochondrial cristae also deserves attention. Considering that the metamorphic reaction in tadpoles is elicited by thyroid hormones, and that, in addition, thyroxine has been found to impair both the structural and the functional integrity of mitochondria (10), the question arises as to whether the erosion of mitochondrial cristae in the tail muscle represents a direct response to the circulating hormones, but as yet no experimental proof is available.

In his pioneer study on anuran tail atrophy Metchnikoff (6) expressed the view that phagocytic cells or "sarcoplasts" arise from the fragmentation of the sarcoplasm. Recently, as a result of an electron microscope study on muscle dedifferentiation in regenerating urodele limbs, Hay (3) has

postulated an analogous process, *viz.* the separation of mononucleated cells from the sarcoplasm, which in this instance are assumed to contribute to the formation of the regeneration blastema. Similar observations on limb regeneration (in adult urodels) by Salpeter and Singer (12), however, disclosed the presence of typical macrophages, which, as may be judged from the conspicuous inclusion bodies, are apparently involved in the elimination of cellular debris.

In view of the massive structural disintegration which electron microscopy has revealed in the sarcoplasm of the regressing tadpole tail, it seems more likely that phagocytes are formed at the expense of mesenchymatous cells, an alternative already discussed by Metchnikoff (6). In fact, mesenchymatous cells, which are characterized by a well developed endoplasmic reticulum and abundant free ribosomes, are frequently found between muscle fibres and particularly between myomeres in growing tails (R. Weber, data unpublished). In this context it should be noted that, by direct observations on the tails of living *Xenopus* tadpoles, Lehman (4) was able to disclose two varieties of phagocytes, *viz.* cells entering from adjacent blood capillaries and stellate mesenchymatous cells from the connective tissue, both participating in the ingestion of melanophore fragments and pigment granules.

The extrusion of sarcoplasm, by which cellular debris accumulates in the intercellular compartment, may play a decisive role in stimulating the phagocytic activity of mesenchymatous cells which thus would acquire the characteristics of macrophages. As was shown by histochemical staining reactions for lysosomal enzymes, this process coincides with the appearance of inclusion bodies which, as judged from their content in acid hydrolases, must be regarded as "phagosomes" (13). On the other hand, active macrophages are exceedingly scarce in the tails of growing tadpoles, and also cytoplasmic structures which would correspond to the definition of primary lysosomes (8) are not detectable during the initial regression of muscle cells. It is, therefore, conceivable to consider the early fine structural changes in muscle cells as a result of an autolytic process which apparently does not require the intervention of lysosomal enzymes. This conclusion is supported by very recent electron microscope observations reported by Novikoff (9) who found that the regression of lymphocytes in the thymus after cortisone treatment or irradiation

tion by x-rays was accompanied by profound changes in the mitochondria and the cytoplasm without involving marked activity of lysosomes.

SUMMARY

In tails of *Xenopus* tadpoles specific changes in fine structure of muscle cells are among the earliest signs of tissue regression at metamorphosis. These changes, which are already detectable prior to any appreciable degree of tail atrophy, comprise the folding of myofibrils with concomitant loss of cross-striation, and the erosion of intramitochondrial cristae. Subsequently, the sarcoplasm itself undergoes massive structural disintegration and is shed into the intercellular space. It is concluded that the early changes in muscle cells reflect an autolytic process, whereas the extrusion of the sarcoplasm is assumed to induce phagocytic activity in mesenchymatous cells of the connective tissue, which probably constitute an important source of macrophages found in more advanced stages of tissue regression.

This work was supported by a grant from the Swiss National Science Foundation (Project No. 2546).

The able technical assistance of Mr. T. Wyler is gratefully acknowledged. The author is also indebted to the Laboratory of Electron Microscopy of the University of Bern for the opportunity of using the Siemens Elmiskop I.

Received for publication, February 24, 1964.

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