A cytological study
of Mauthner’s cells in *Xenopus laevis* and
*Rana temporaria* during metamorphosis

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This paper discusses cytological changes which occur during anuran metamorphosis in a pair of large hind-brain neurones, the Mauthner cells or M-cells which, in many teleosts and amphibians, innervate the tail musculature via ventral horn cells. The M-cells of fishes, urodeles and anuran larvae are unusually large neurones of particular interest, and according to Stefanelli (1951) they constitute a ‘true functional system of nervous activity’. The value of a cytological study is enhanced by the fact that amphibian M-cells have not yet been extensively analysed biochemically (Deuchar, 1966).

In teleosts, the abundant synapses, apparently of a special kind, that terminate on M-cells have been studied by Bodian (1937), Furshpan & Furukawa (1962), Furukawa & Furushpan (1963), Furshpan (1964), Furukawa (1966), Robertson (1963), and Robertson, Bodenheimer & Stage (1963) and have been discussed by Eccles (1964). It is probable that the M-cells of fishes and amphibians are concerned with conveying impulses from head sensory organs (eyes, ears, tactile receptors) and their associated neurones to ventral horn cells to innervate caudal musculature, contraction of which provides for escape or other rapid movements in tailed, cold-blooded aquatic vertebrates (Retzlaff, 1957; Wilson, 1959; Sims, 1962; Aronson, 1963; Rodgers & Melzack, 1963; Furukawa, 1966). The M-cell axons, which are heavily myelinated and of large diameter, appear to play a role analogous to that of giant nerve fibres in some invertebrates, transmitting impulses rapidly from one part of the body to another (Berkowitz, 1956). It is of considerable interest that bilateral asymmetries in cell size and number, axonal size and length, and cell location are not uncommon in the Mauthner system (Moulton & Barron, 1967).

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The M-cell system may also provide for directional orientation to sound in fishes (Moulton & Dixon, 1967), but insufficient physiological data are at present available to extend this hypothesis to amphibians. In bufonid larvae (Bufo vulgaris and B. viridis) the M-cells are absent (Stefanelli, 1951), a result confirmed in Bufo bufo during the present study. The occurrence of M-cells in non-bufonid anurans has been regarded as a special adaptation for larval life in highly aquatic species (Baffoni & Pinacci, 1958). There is evidence that the M-cells normally play a role in tail movement in Amblystoma (Detwiler, 1927, 1933, 1947) and in Xenopus larvae (Sims, 1962); however, in Rana larvae, normal swimming may still be found following removal of cells (Stefanelli, 1951).

In the goldfish (Carassius auratus) large VIIIth nerve fibres (probably auditory in function) synapse on the lateral dendrites of the M-cells (Furukawa, 1966). The possibility of similar connexions in larval anurans, and thus of interesting changes in the auditory nerve during metamorphosis, encouraged us to examine carefully the Mauthner system in Rana and Xenopus. Stefanelli (1951) found the M-cells to outlast metamorphosis, though they gradually involute over a considerable period. However, an impression is still prevalent that loss of the M-cells does occur during metamorphosis (Weiss, 1955a; Etkin, 1964). In agreement with Stefanelli, we find that the M-cells of Rana temporaria and of Xenopus laevis are sites of active synthesis throughout metamorphosis. An investigation of histochemical changes in the M-cells may throw some light on the changes in synthetic activity which occur during metamorphosis.

**Light microscopy**

Xenopus laevis. Mauthner cells were studied in tadpoles obtained from the Institute of Animal Genetics, Edinburgh, from the initiation of feeding to the completion of metamorphosis (stages 45 and 47, 50–66 inclusive, and in a 2-month post-metamorphic toad (Nieuwkoop & Faber, 1956). Larvae were fixed for 45 min in a solution of 5% trichloroacetic acid containing 1.37% lanthanum acetate; tadpoles of younger stages (45 and 47) were fixed whole. In each older stage an area including the hind brain, the inner ears and surrounding tissue was fixed. The material was embedded in paraffin wax, sectioned transversely at 8 μ and stained with methyl green/pyronin (Brachet, 1960; Jurand & Bomford, 1965).

In addition, the effect of treatment with ribonuclease upon the staining reaction was compared using two parallel sets of serial sections at stages 50 and 54. One set was treated for 1 h with ribonuclease type 1-A (RNase) (Sigma, London), 1 mg/ml in phosphate buffer at 37.5 °C; the other control set treated at each stage with distilled water only for 1 h. Both sets were then stained with methyl green/pyronin.
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Serial sections of *Xenopus* (stages 54–56, 60, 66 and a 3-year-old toad) were also stained with haematoxylin and eosin.

*Rana temporaria*. The M-cells were first studied in serial sections of normal larvae from Fox's collection staged as described for *Rana dalmatina* (Cambar & Marrot, 1954). Tadpoles studied throughout metamorphosis (stages 29, 31, 41, 45, 47, 49–54) were fixed in Bouin's fluid, embedded in paraffin wax, sectioned transversely at 10 μ and stained with haematoxylin and eosin. In addition four similarly prepared normal stages, 51–54 inclusive, previously used as controls in other experiments, were also examined.

RNA distribution in M-cells was investigated in stages 41–43 and 52 of larvae of *R. temporaria* obtained from a commercial dealer and prepared as described for *Xenopus*. Sets of serial sections at stage 47 were similarly treated with RNase and controls with distilled water.

Comparable stages of Nieukwoop & Faber (1956) for *Xenopus* and of Cambar & Marrot (1954) for *Rana* provide a basis of comparison:

<table>
<thead>
<tr>
<th>Xenopus</th>
<th>47</th>
<th>49</th>
<th>53</th>
<th>56</th>
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<tr>
<td><em>Rana</em></td>
<td>39</td>
<td>41</td>
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Tadpoles of *Bufo bufo* (obtained at Dunsapie Loch, Edinburgh, in late May 1967) at stages corresponding to 39, 42 and 46 of the Cambar & Marrot series were studied. Specimens were fixed either in trichloroacetic acid/lanthanum acetate and stained with methyl green/pyronin (stages 39, 46), or in Bouin's fluid and stained with haematoxylin and eosin (stage 42), after embedding in paraffin wax and sectioning at 8 μ.

Regions of the brain including the cerebellum and anterior part of the medulla oblongata of specimens of *Carassius auratus*, between 7·5 and 11 cm in total length, were used and were prepared in the same way as in the case of stage 42 *Bufo*.

Electron microscopy

Tadpoles of *Xenopus* in which the cranial cavity and spinal canal had been opened at stage 49 were fixed overnight at 2–4 °C in 2·5 % glutaraldehyde buffered with 0·2M phosphate buffer at pH 7·2. The region of the medulla oblongata directly beneath the posterior edge of the cerebellum was dissected free in fixative and rinsed at 2–4 °C in buffered 2·4 % sucrose solution for the next 24 h, the rinsing solution being changed several times. Tissues were then post-fixed in 1 % osmic acid buffered with phosphate and embedded in Araldite epoxyresin (Jurand, 1965).

In order to localize the Mauthner cells, tissue was first sectioned at 1 μ using a Porter–Blum ultramicrotome. Successive sections were mounted on glass microscope slides, stained for 15 min with toluidine blue (toluidine blue 0·5 g, sodium tetraborate 1 g, distilled water 100 ml), and examined with a light microscope. When the level where decussation occurs was reached, sections at
60–80 μ of the rest of the material were mounted on collodion-carbon-coated grids, stained with 1 % potassium permanganate and 2.5 % uranyl acetate, and viewed with an AEI EM6 electron microscope.

The medulla of *Xenopus* embedded in methacrylate (stage 49) or in Araldite (stage 56) was studied by electron microscopy at the level of the Mauthner axons.

The part of the medulla oblongata of a 5 cm *Carassius auratus* which includes the M-cells (Robertson *et al.* 1963) was isolated in buffered glutaraldehyde and embedded in Araldite after post-fixation in osmic acid, as described for *Xenopus laevis*.

**RESULTS**

The M-cells in *Xenopus laevis* lie ventral to the caudal border of the cerebellum, in the walls of the medulla oblongata, near the mid level of the roots of the VIIIth nerve (Plate 1, fig. A). The cells lie somewhat more dorso-laterally in *Xenopus* than in the *Rana temporaria* tadpoles (Plate 1, fig. B). In *Rana* the cells are situated between 250 and 400 μ posterior to the caudal border of the cerebellum in the floor of the medulla oblongata, behind the roots of the VIIIth nerve. Although other large nerve cell bodies are adjacent to the M-cells, the latter are usually large enough to be immediately recognizable. In doubtful cases, they can be identified by tracing forward from the spinal cord or posterior medulla their large, heavily myelinated axons, which are readily apparent in the floor of the medulla and spinal cord posterior to the Mauthner cell bodies themselves. The Mauthner axons exceed in size all other neighbouring axons. The left axon generally passes over the right below the neurocoel in the medulla oblongata, gradually in the goldfish and abruptly in anurans. In an 8.1 cm goldfish the axons lie vertically aligned, 130 μ behind the M-cells. In *Xenopus* the vertical alignment occurs closer to the M-cells, 20 μ behind in one case (stage 54). The M-cell axons normally continue to the tip of the tail (Stefanelli, 1951).

Topographical and morphological variations of the Mauthner system between the two sides of *Carassius*, *Xenopus* and *Rana* have been noted, which may be explained by failure to achieve perfect transverse sections (see Plate 1, fig. A). One *Xenopus* larva of stage 49 prepared for electron microscopy showed the axon of the right Mauthner cell to extend merely a few hundred microns along the medulla oblongata. Likewise in a brain of *Carassius* (5 cm long) the left Mauthner axon (of the right cell) could not be distinguished at any level. The single axon present had a maximum measurement of 54 μ in diameter in the medulla. Both cells were present and of similar size. The diameters of Mauthner axons of the two sides differ at times, most notably in *Carassius*, where axon diameter far exceeds that in anurans. In another specimen of *Carassius* (5 cm long) prepared for electron microscopy, at the level of decussation the left axon (including myelin) measured 46 μ, the right 28 μ.

Duplication bilaterally of the M-cell system sometimes occurs (Weiss, 1955b).
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Unilateral duplication was observed in *Xenopus* at stage 64, where two left M-cells and their respective axons on the right occurred; all three cells and axons were apparently normal. Axon measurements (including the myelin sheaths) at the level of the spinal cord were 25 $\mu$m for the unduplicated cell and, at the same level, 14 $\mu$m for each of the duplicated cells. In transverse section the largest nuclear diameters for the duplicated cells were 18 $\mu$m and 22 $\mu$m respectively, and 18 $\mu$m for the unduplicated cell. Axons of a normal pair of M-cells in a stage 63 *Xenopus* at the level of the spinal cord measured 18 and 14 $\mu$m in diameter respectively, with their nuclei each measuring 22 $\mu$m in their greatest diameter, both in transverse section.

Frequently in *Xenopus* and *Rana* there is some degree of variation in antero-posterior and dorso-ventral levels between M-cells of opposite sides, to judge from their positions relative to adjacent parts of the brain and ear. The orientation of M-cells relative to the transverse section also varies; in anurans the perikaryons may span from 10 to 40 $\mu$m in serial preparations due to variations in orientation. In one stage 52 *Rana* (Plate 1, fig. B; Plate 2, fig. F) the left M-cell showed a more vesicular appearance than the right.

The two types of amphibian M-cells in *Xenopus* and *Rana* may be compared with the M-cells of *Carassius* (Plate 1, figs. C, D; Plate 2, fig. F). M-cells in the goldfish are much larger than those of the anurans at any stage; it has been possible to isolate those of the former by microdissection (Edström, Eichner & Edström, 1962). The axon cap of the goldfish M-cell (Plate 1, fig. C) is lacking in amphibians, although large neurones grouped at the base of the axon in *Xenopus* (Plate 1, figs. D, E; Plate 2, fig. A) occupy an analogous position. The axon of the M-cell of *Carassius* (Plate 1, fig. C) emerges quite separately from the lateral and ventral dendrites, which are not usually seen at the level of the nucleus in transverse sections. In *Xenopus*, the ventro-medial dendrite and axon emerge together (Plate 1, fig. D), and, in *Xenopus* and *Rana*, transverse sections emphasize the alignment of the dorso-lateral dendrite (Plate 1, figs. B, E), perikaryon, ventro-medial dendrite and axon (Plate 1, fig. D; Plate 2, fig. F).

The nucleus of the amphibian M-cell frequently is eccentric (Plate 1, figs. D, G, H) while that of the goldfish is located almost centrally (Plate 1, fig. C). It may be centrally arranged in *Xenopus* (Plate 1, fig. F), and often is in the smaller M-cells of *Rana temporaria* (Plate 2, figs. G, H). The strikingly numerous and variable synaptic endings surrounding the perikaryon (Plate 1, fig. C), axon and dendrites of *Carassius* (Bodian, 1937) are far less evident in anurans.

Cytoplasmic staining is generally homogeneous in *Carassius* (Plate 1, fig. C) and *Rana* (Plate 2, figs. C–H), whereas in most stages of *Xenopus* there is a pronounced excess of basophilia peripheral to the nucleus (Plate 1, fig. H). In all three species studied, the nucleus is highly vesicular and the nucleolus (Plate 1, fig. E) is large (5–6 $\mu$m in diameter), a condition which persists throughout metamorphosis in the two anurans. In the latter particularly, the M-cells and the bases of their dendrites appear fibrous (Plate 1, fig. E; Plate 2, fig. F).
Shortly after the initiation of feeding in *Xenopus* at stage 47, the M-cells show a dense concentration of pyronin-positive material around the nucleus (Plate 1, fig. D), details of which are obscured by its density and by the limits of light microscopy. Beyond the perinuclear concentration, similar material occurs, but in a curiously aligned fashion. Here and elsewhere in the M-cells and dendrites, the cytoplasm stains a light pink with methyl green/pyronin. The strongly pyronin-positive material does not extend into either axon or dendrites (Plate 1, figs. D, E), but these processes are quite strongly coloured in haematoxylin and eosin preparations (Plate 1, fig. H; Plate 2, figs. F, G).

In haematoxylin and eosin preparations of *Xenopus* larvae, strong basophilia, concentrated peripherally to the nucleus and scattered elsewhere in the perikaryon in early metamorphosis, corresponds in position to the distribution of the pyronin-positive material. Early in metamorphosis and at its conclusion (Plate 1, fig. H) the M-cells present a picture of strong perinuclear basophilia with a lighter field beyond marked by streaks of basophilic material aligned with the long axis of the cells. In both staining methods the lighter field is usually

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**EXPLANATION OF PLATES**

Abbreviations on plates: *a*, axon; *ac*, axon cap; *dd*, dorso-lateral dendrite; *er*, endoplasmic reticulum; *fm*, fibrillar matrix; *fv*, fourth ventricle; *G*, Golgi complex; *ler*, linearly arranged endoplasmic reticulum; *IMc*, left Mauthner cell; *In*, large neurone; *lpn*, linearly arranged pyronin-positive material; *ly*, lysosome; *m*, melanin granules; *mi*, mitochondrion; *N*, nucleus of Mauthner cell; *n*, nucleolus of Mauthner cell; *pb*, perinuclear basophilia; *per*, perinuclear endoplasmic reticulum; *po*, polysome; *ppm*, perinuclear pyronin-positive material; *r*, ribosome; *rMc*, right Mauthner cell; *s*, synaptic endings; *sb*, streaked basophilia; *sv*, smooth vesicle; *v*, vesicle; *vd*, ventro-medial dendrite. Abbreviations in legends: *H./E.*, haematoxylin/eosin preparation; *M.G./Py.*, methyl green/pyronin preparation.

**PLATE 1**

Each scale mark represents 50 μ.

Fig. A. Transverse section of the hind brain showing both M-cells in *Xenopus* stage 50. *M.G./Py.* × 100.

Fig. B. Transverse section of the hind brain, showing both M-cells in *Rana temporaria* stage 52 on the ventral border of the grey matter. (25 mm.) *H./E.* × 96.

Fig. C. Right M-cell of a 7-9 cm goldfish in transverse section. *H./E.* × 550.

Fig. D. Left M-cell of *Xenopus* (stage 47). Perinuclear pyronin-positive material is contrasted with sparser linearly arranged pyronin-positive material in the rest of the cell. *M.G./Py.* × 550.

Fig. E. Left M-cell of *Xenopus* (stage 53), showing accumulation of perinuclear pyronin-positive material. *M.G./Py.* × 550.

Fig. F. Left M-cell of *Xenopus* (stage 56). Perinuclear pyronin-positive material has accumulated heavily. *M.G./Py.* × 550.

Fig. G. Right M-cell of *Xenopus* (stage 59), showing re-emergence of linearly arranged pyronin-positive material. *M.G./Py.* × 550.

Fig. H. Right M-cell of *Xenopus* (stage 66). Compare with Plate 1, fig. D, and note that linearly arranged material again occupies a substantial part of the cell. *H./E.* × 550.
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bordered peripherally by a dark, strongly staining rim (Plate 1, figs. D, G, H); basophilic, pyronin-positive material appears to adhere to the cell membrane in belt-like fashion around the perikaryon.

In both *Rana* and *Xenopus* the distribution of basophilia in haematoxylin and eosin preparations agrees with the distribution of pyronin-positive material, but in the more restricted cells of *Rana* there is a less clear demarcation between perinuclear material and linearly arranged basophilic, pyronin-positive material (Plate 2, figs. E, F).

In the intermediate stages of *Xenopus*, distribution of basophilia is more uniform (Plate 1, figs. E, F). As metamorphic climax approaches (stage 56 approximately), the dark perinuclear stain extends farther into the perikaryon to occupy it fully (Plate 1, fig. F). Later (stage 59), pyronin staining is again more restricted, and the linear arrangement of pyronin-positive material again becomes apparent beyond the perinuclear material (Plate 1, fig. G). At the completion of metamorphosis (stage 66), distribution of pyronin-positive material has reverted to the condition recognizable at initiation of feeding (Plate 1, fig. H). The widths of two *Xenopus* M-cells sectioned through the nuclei at stages 47 (Plate 1, fig. D) and 66 (Plate 1, fig. H) are 23 and 32 $\mu$m respectively. Throughout the metamorphic series, tiny vesicular bodies occur in M-cells of *Xenopus* (Plate 1, figs. D, F) associated with perinuclear pyronin-positive material.

In a *Xenopus* 2 months after metamorphosis, the M-cells again colour rather homogeneously with methyl green/pyronin, but in a manner different from that in larvae at stage 56. The left M-cell of the former specimen appears somewhat shrunken and wrinkled. In cells of each side, the linearly arranged pyronin-positive material predominates, streaking the left cell so heavily as to partially obscure the nucleus in a section through the nucleolus. This contrasts with the heavy pyronin staining of the M-cells of *Xenopus* at stage 56 (Plate 1, fig. F) in

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**Plate 2**

Each scale mark represents 50 $\mu$m.

Fig. A. Left M-cell of *Xenopus* (stage 54) treated for 1 h with distilled water. *M.G./Py.* × 450.

Fig. B. Left M-cell of *Xenopus* (stage 54) treated for 1 h with RNase. *M.G./Py.* × 450.

Fig. C. Right M-cell of *Rana temporaria* (stage 45, 16 mm). *H./E.* × 550.

Fig. D. Right M-cell of *R. temporaria* (stage 45, 28 mm). *H./E.* × 550.

Fig. E. Right M-cell of *R. temporaria* (stage 51, 32 mm). Note accumulation of vesicles, which reaches a climax in the next stage. *H./E.* × 550.

Fig. F. Left M-cell of *R. temporaria* (stage 52, 25 mm), showing strongly vesicular cytoplasm. *H./E.* × 550.

Fig. G. Left M-cell of *R. temporaria* (stage 53, 14 mm), showing regression of vesicles. *H./E.* × 550.

Fig. H. Right M-cell of *R. temporaria* (stage 54, 14 mm) at completion of metamorphosis. *H./E.* × 550.
which the perinuclear arrangement of pyronin-positive material predominates. In the two-month post-metamorphic *Xenopus*, the right cell is fully expanded (25 μ in width through the nucleolus); the slightly shrunken left M-cell (18 μ in width) is still clearly recognizable among other neurones in the region. Cellular processes are still well developed, and the axons are present in sections of the medulla oblongata. In a 3-year-old adult female *Xenopus* neither M-cells nor their axons could be identified.

In *Xenopus* at stages 50 and 54, the staining response of the pyronin-positive material was eliminated by treatment for 1 h with RNase; control sections similarly treated with distilled water stained normally (Plate 2, figs. A, B).

The M-cells of *Rana* are smaller than those of *Xenopus*. The widths of two cells at stages 42 and 52 at the level of the nucleolus are 7 μ and 14 μ respectively. Cytoplasmic basophilia and pyronin-positive material increase during the early part of metamorphosis (Plate 2, figs. C–H) in parallel with a decrease in melanin granules. In *Rana* differentiation within the more restricted perikaryon into perinuclear and linearly arranged material so clearly recognizable in *Xenopus* is less apparent, although it still occurs to some extent (Plate 2, fig. E). In all specimens of *Rana* examined, beginning with stage 41, clear unstained vesicles appear in the M-cells, at first near the nucleus and later elsewhere in the cytoplasm. They progressively enlarge and reach a peak at or near stage 52 in our series (Plate 2, fig. F), but vesiculation moderates in late metamorphosis (Plate 2, figs. G, H). Vesiculation of the *Rana* M-cell nucleus is also particularly marked at stage 52. In *Xenopus* tiny vesicles occur occasionally in the cytoplasm (Plate 1, figs. D, F), and comparatively large vacuoles occur in the nucleolus during much of metamorphosis (Plate 1, figs. E–G).

**Electron microscopy**

The cytoplasm of the M-cell of *Xenopus* (Plate 3) contains abundant rough endoplasmic reticulum in the form of short single profiles. These profiles demonstrate (a) rather irregularly arranged cisternae (perinuclear endoplasmic reticulum) closely crowded together, which predominate in the immediate vicinity of the nucleus (Plate 3; Plate 4, fig. A), extending outwards in a conical accumulation toward either pole in the long axis of the cell, and (b) cisternae arranged throughout the rest of the perikaryon (linearly arranged endoplasmic reticulum), predominantly in the long axis of the cell (Plate 3; Plate 4, fig. A).

At a magnification of 5000 the highly granular nature of the rough endoplasmic reticulum (Plate 3) is imparted by single ribosomes and abundant polysomes (Plate 4, fig. C). Each polysome includes a minimum of 4 and a maximum of 8 loosely grouped ribosomes. Collectively, polysomes predominate markedly over single ribosomes. Where both occur, the latter occur in a row on the cisternal membrane, and a row of polysomes lies beyond them (Plate 4, fig. C).

Amid both types of rough endoplasmic reticulum, but more abundant in the perinuclear, are apparently typical active Golgi complexes, about 12 per section.
Electron micrograph of a part of an M-cell on the side of the nucleus toward the dorso-lateral dendrite of a *Xenopus* larva (stage 49). The space marked with an asterisk (*) is probably due to separation of the Mauthner neuron from surrounding tissue during preparation. × 5000.

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(Plate 3; Plate 4, figs. A, B, D), each consisting of a few lamellae of cisternae and membranes. The complexes are frequently marked with vesicular swellings (Plate 4, figs. B, D), some of which may be responsible for the finely vesicular structure of *Xenopus* cells under light microscopy (Plate 1, figs. D, F). At the periphery of a Golgi complex there is an admixture of endoplasmic reticulum, smooth vesicles and Golgi cisternae (Plate 4, fig. B).

Lysosomes also are found between the endoplasmic reticulum profiles. They are somewhat irregular or oval-shaped electron-dense bodies ranging from 0.6 to 0.1 μ or less in diameter (Plate 3). Each lysosome possesses a unit membrane (Plate 4, figs. C, D), and is packed with electron-dense, finely granular material. Some lysosomes include myelin-like configurations (Plate 4, fig. C) similar to membranes found elsewhere as lysosome inclusions (Rhodin, 1963, p. 13). These bodies occasionally appear to be cavitated. Usually they are topographically near to a Golgi complex. An apparent continuity of the cisternal membrane and the delimiting membrane of one of the bodies has been observed (Plate 4, fig. D).

Mitochondria are abundant in Mauthner neurones in the perikaryon and dendrites (Plate 3; Plate 4, fig. A); they are somewhat less abundant in the axoplasm. Their somewhat atypical, highly vesicular structure would suggest poor preservation if it were not that they appear so consistently well preserved in adjacent structures.

The ground substance (Porter, 1961) of the M-cells of *Xenopus* and *Carassius*, throughout the perikaryon, axon and dendrites, is characterized by a fine fibrillar matrix (Plate 3; Plate 4, figs. A, C, D) which comprises a fine meshwork of small beaded filaments surrounding spaces 0.08-0.17 μ in diameter. The filaments, approximately 8 μ in diameter, are interwoven so as to leave clear spaces in the interstices of the network. Although smaller, the fibrils bear some resemblance morphologically to collagen fibrils. In the axon, they are arranged predominantly along the long axis of the process.

The M-cell nucleus in *Xenopus* (Plate 3; Plate 4, fig. A) has a relatively smooth membrane, slightly undulating in contour, unlike the same structure in *Carassius*, where the membrane is highly folded. In both species, the electron-dense nucleolus is a prominent feature (Plate 3), sometimes vacuolated in *Xenopus*. In

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**Plate 4**

Each scale mark represents 1 μ.

Fig. A. Perinuclear and linearly arranged endoplasmic reticulum of an M-cell of *Xenopus*. × 12000.

Fig. B. Golgi complex, endoplasmic reticulum and smooth vesicle formation in *Xenopus*. × 60000.

Fig. C. Lysosome and endoplasmic reticulum with polysomes. × 60000.

Fig. D. Golgi complex with adjacent lysosomes. × 40000.
Carassius the axon hillock is prominent at the base of the M-cell axon (Plate 1, fig. C), whereas a hillock is not noticeable in Xenopus or Rana. Again, the nucleus of Carassius is more electron-dense and homogeneous than that of Xenopus (Plate 3) and its M-cell lacks the fibrous cytoplasm and does not differentiate into linear and perinuclear endoplasmic reticulum as in Xenopus. Neurotubules, abundant in the M-cells of Carassius, occur singly and in small groups, as well as in large bundles; the latter bundles are absent in the M-cells of Xenopus and, unlike other neurones, neurotubules are extremely scarce.

DISCUSSION

The electron-microscopic structure of the Xenopus M-cells of stage 49 may be correlated with the observations made by light microscopy. There can be little doubt that the perinuclear concentration of basophilia and basophilic cytoplasmic streaks corresponds to the pyronin-positive material. Comparison of appropriate stages of Xenopus with electron micrographs of stage 49 shows clearly that the haematoxylin- and pyronin-coloured streaks seen in light microscopy are, in fact, sections of linearly arranged endoplasmic reticulum with the cisternae arranged predominantly in the long axis of the M-cell. The cloud of basophilia and of pyronin-positive material around the nuclei of M-cells of Xenopus is perinuclear endoplasmic reticulum, the cisternae being arranged in irregular fashion, the spaces between them occupied by ribosomes, polysomes and fibrillar matrix. The M-cell of Carassius does not show this kind of differentiation, while the M-cell of Rana is similar to that of Xenopus.

One may suspect that proliferation of endoplasmic reticulum during metamorphosis in Xenopus M-cells is characterized by replacement of the linearly arranged endoplasmic reticulum with the more irregular perinuclear kind, and that this change leads to the more homogeneously staining cell of stage 56, which may be interpreted as demonstrating a peak of synthetic activity. The validity of this interpretation is suggested by the presence of abundant polysomes distributed along the cisternae of the reticulum, frequently distal to a row of ribosomes that is immediately adjacent to the cisternal membranes. The results of staining with methyl green/pyronin and of treatment with ribonuclease show that the pyronin-positive material of light microscopy is in fact ribosomal and polysomal ribonucleic acid. At stage 49 of Xenopus, this ribonucleic acid of the M-cells mainly occurs in polysomes, each comprising 4–8 ribosomes. The peak of endoplasmic reticulum development in Xenopus M-cells is reached at a time when growth is nearly complete (stages 56–58), for the tail begins to atrophy at stage 58.

It might be easier to explain the relationship between the endoplasmic reticulum and growth if we were certain what role the M-cells play in anurans. A multiple role is not impossible. In addition to providing high-speed pathways for nerve impulses, a function of large myelinated neurones, from head sensory
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organs to the tail, the Mauthner neurones may also respond to, and participate in, the growth of the tail prior to the metamorphic climax. Thus the increase in M-cell innervated tissue might require an increasing flow of secretory material along the large axon, and it is not unlikely that preparation for resorption of the tail might precede overt indications of resorption and be accompanied by a decrease in synthetic activity at the perikaryon of the M-cell. This might explain the increasing pyronin reaction of *Xenopus* M-cells up to stage 56, followed by its regression. Some aspects of amphibian histogenesis, including auditory development, are enhanced by ribonucleic acid (Finnegan & Biggin, 1966), and there is good evidence that RNA plays a role in spinal cord regeneration in fishes (Batkin, 1966). Further, Tata (1966) has shown that even tail resorption in anuran larvae requires RNA production, and is accompanied by synthesis of both RNA and protein. Non-ribosomal RNA is present in the goldfish Mauthner axon (Edström *et al.* 1962), but ribosomes and endoplasmic reticulum are lacking in axons of both *Carassius* and *Xenopus*. *Xenopus* M-cells remain well developed during metamorphosis, though there is a reduction in perinuclear endoplasmic reticulum. Thus, although intimately related to the development and function of tail musculature, M-cells differ considerably from the latter in their subsequent fate. The picture of active synthesis which the M-cells of *Xenopus* and *Rana* present suggests a possibility that in anurans these cells have assumed a glandular function, and produce a specialized kind of neurosecretion.

Duplication of the M-cell in one case yielded two cells with axons of equal diameter, both substantially smaller than the axon of the contralateral undivided cell. Such an arrangement would seem to support the idea of a transport role for the Mauthner axon, in addition, perhaps, to that of nerve impulse transmission, for though reduction in axonal size would be detrimental to high-speed functioning of the Mauthner system, multiplication of axons would presumably increase the effectiveness of transport of secretory substances along such restricted pathways.

The M-cells of *Rana* synthesize protein without proliferation of endoplasmic reticulum. Although extremely fine clear spaces are discernible in the M-cells of *Xenopus*, nothing like the enlarged spherical vesicles of the *Rana* M-cells is recognized. These vesicles could represent further intracellular synthesis, reaching a peak after the increase in amount of endoplasmic reticulum, which in turn has reached a high level in *Rana* at stage 51. Although the vesicles are largest in one of the *Rana* cells at stage 52, tail resorption having just begun at stage 51 (Camber & Marrot, 1954), they are visible throughout the metamorphic climax, although they are smaller in earlier and later stages. The M-cell nucleus of *Rana* also shows more variation in vesiculation than does that of *Xenopus* (compare Plate 1 and Plate 2, figs. C, F, H). It seems likely that both *Xenopus* and *Rana* have a similar accumulation of endoplasmic reticulum during early metamorphosis, but that the large cytoplasmic vesicles of *Rana* indicate some basic difference in the function of the cells, perhaps in the protein synthesized.
That the cells are entirely lacking in *Bufo* further emphasizes the variability of
the Mauthner system in anurans.

During the period of active synthesis, there are indications that the M-cells
are preparing for post-metamorphic involution. The swollen appearance of the
mitochondria and the poor definition of their cristae correspond to changes that
take place at the beginning of metamorphosis in tadpole tail muscle (Weber,
1963), or under unfavourable conditions generally (Jurand, 1966). That cells so
far removed in time and place from the site of future tail atrophy should so
strikingly share a change with tail tissue further emphasizes the close relation-
ship of the M-cells with the caudal appendage (Stefanelli, 1951).

Although no tests for acid phosphatase were made in *Xenopus* M-cells at
stage 49, it is extremely likely that the electron-dense bodies scattered chiefly
through the endoplasmic reticulum are lysosomes. The bodies show the typical
lysosome unit membrane and densely granular interior, occasionally cavitated
or characterized by a myelin-like figure (see also Novikoff, 1961; Novikoff,

The Golgi complexes at the same stage, scattered similarly through the endo-
plasmic reticulum, appear to be actively associated with secretion, and in some
cases lysosomes are immediately adjacent to them, an arrangement to which
Novikoff (1963) has called attention. In one case continuity of the Golgi and
lysosome membranes may be inferred.

In *Xenopus*, the relations between the endoplasmic reticulum, smooth vesicles,
Golgi membranes and lysosomes seem to present a fairly classic example of the
kind of sequence from one to the other postulated by Novikoff *et al.* (1962).
The endoplasmic reticulum is accompanied by smooth-membraned vesicles
which, in turn, are adjacent to Golgi cisternae. In other places lysosome forma-
tion appears to proceed from a Golgi complex. Whether lysosomes represent a
resting stage in autocytoytic bodies or ‘cytolysomes’ (Novikoff, 1963) or a
stage in enzyme secretion by the Mauthner cells is uncertain. The intracellular
digestive role of lysosomes is now well known (de Duve & Wattiaux, 1966) and
intracellular digestion by lysosomes has recently been described in neurones
following axon section (Dixon, 1967).

The scarcity of neurotubules in the Mauthner neurones of *Xenopus*, and
evidence in anuran M-cells of secretion and of preparation for breakdown, both
suggest that these large neurones are nerve cells with a very special function.

The synaptic connexions of *Xenopus* M-cells are considerably less elaborate
and less well defined than those of the goldfish, which have been more thoroughly
studied (Bodian, 1937; Robertson *et al.* 1963; Furukawa, 1966). Although many
small neurones probably terminate on the dendrites and perikaryon of *Xenopus*
M-cells, unusually large or otherwise striking synapses are absent. While it is
conceivable that the cluster of large neurones near the base of the M-cell axon
in *Xenopus* could represent the analogue of the goldfish axon cap and play a
role in contralateral inhibition (Eccles, 1964), the prominent club-shaped syn-
aptic endings on the goldfish M-cells are lacking. We have no evidence for the high-speed neural pathway from ear to caudal musculature, via the M-cells, which can be demonstrated in the goldfish (Furukawa, 1966; Moulton & Dixon, 1967), apart from the relatively large diameter of the M-cell processes themselves and some incidental behavioural observations (Sims, 1962). Among teleosts, fishes that have eel-like swimming movements have smaller M-cells than those that swim with strong beats of the tail (Stefanelli, 1951). Since eel-like movements characterize caudal function in tadpoles, this may help to explain the small size and different form of anuran M-cells as compared with those of teleosts.

The involution of anuran M-cells following metamorphosis is not always perfectly synchronized on the two sides (Stefanelli, 1951). That the asymmetries between the Mauthner neurones of the two sides may have a functional significance has been suggested elsewhere (Moulton & Dixon, 1967; Moulton & Barron, 1967).

In view of the differences in anatomy and physiology between teleost and anuran M-cells, it seems possible that M-cells in anurans play a quite different role. A synthetic function may have partly superseded the neurophysiological one.

The role of the thyroid gland in metamorphosis, recently reviewed by Etkin (1964) and studied anew by Fox (1966) and Fox & Turner (1967), suggests that M-cell involution might be induced by a raised level of thyroid hormone secretion, and indeed repression of cells in anuran larvae immersed in dilute thyroid extract has been reported (G. M. Baffoni & G. Catte, cited by Weiss & Rossetti, 1951). Several workers have agreed that in anuran tadpoles the M-cells respond differently to thyroid hormone than do other brain cells, and that M-cells are caused to regress precociously on the same side as unilaterally implanted thyroxin-agar pellets (Weiss & Rossetti, 1951; Pesetsky & Kollros, 1956). Tadpole caudal nerves generally are reported to be resistant to breakdown at metamorphosis (Brown, 1946; Wright, 1951). Pesetsky (1962), however, has proposed that, rather than being induced to degenerate by raised thyroid secretion, degeneration of M-cells is, in fact, initiated by thyroid withdrawal following the metamorphic climax. This is not at variance with the timing of thyroid activity in *Xenopus* (Saxén, Saxén, Toivonen & Salimaki, 1957) and *Rana* (Etkin, 1964; Fox, 1966; Fox & Turner, 1967).

During metamorphosis amphibian M-cells reach a maximum size (Stefanelli, 1951), accumulate glycogen (Janosky & Wenger, 1956), and show an increase in endoplasmic reticulum. It may well be that these aspects of M-cell physiology are influenced by an increasing rise in the circulatory concentration of thyroid hormone during the metamorphic cycle, and that M-cell growth and increased synthesis, followed by some reduction in size and activity at the end of metamorphosis, are part of the metamorphic syndrome.
SUMMARY

1. Cytological changes in the M-cells of *Xenopus laevis* and *Rana temporaria* were studied throughout the metamorphic cycle. The ultrastructure of the M-cell of *Xenopus* was also examined by electron microscopy, and the results compared with the cytological changes recognized by light microscopy.

2. In anuran larvae there is an increase in basophilia and in pyronin-positive material during metamorphosis. In *Xenopus* these increases are actually in perinuclear and linearly arranged endoplasmic reticulum and in RNA; the same is probably true of *Rana*. In *Rana*, but not in *Xenopus*, the peak of basophilia is followed by maximum occurrence of large clear vesicles. In *Xenopus*, polysomes are abundant in the endoplasmic reticulum and lysosomes are recognized in the cytoplasm by stage 49. Analysis of the topographical relationships of the Golgi complex and endoplasmic reticulum shows a transition from endoplasmic reticulum through smooth vesicles and Golgi cisternal membranes to lysosomes.

3. The anuran M-cells show a decrease in synthetic activity during the metamorphic climax. Nevertheless they remain of large size and rich in endoplasmic reticulum and show no signs of regression throughout the metamorphic cycle.

RÉSUMÉ

*Etude cytologique des cellules de Mauthner chez Xenopus laevis et Rana temporaria au cours de la métamorphose*

1. On a étudié les modifications cytologiques des cellules de Mauthner de *Xenopus laevis* et *Rana temporaria* tout au long du cycle métamorphotique. On a également examiné l’ultrastructure de la cellule de Mauthner du Xénope en microscopie électronique et les résultats en ont été comparés aux modifications cytologiques reconnues en microscopie optique.

2. Chez les larves d’Anoures, un accroissement de la basophilie et du matériel pyroninophile a lieu pendant la métamorphose. Chez le Xénope, cet accroissement survient dans le réticulum endoplasmique périmucléaire, et disposé linéairement, et dans l’ARN; il en est probablement de même chez la grenouille. Chez celle-ci, mais non chez le Xénope, le maximum de basophilie est suivi du nombre maximum de grandes vésicules claires. Chez le Xénope, les polysomes sont abondants dans l’ergastoplasm et on reconnaît des lysosomes dans le cytoplasme au stade 49. L’analyse des relations topographiques entre le complexe golgien et l’ergastoplasm montre une transition à partir de l’ergastoplasm vers des vésicules lisses et des membranes cisternales golgiennes jusqu’aux lysosomes.

3. Les cellules de Mauthner des Anoures présentent une diminution des activités de synthèses au cours du climax métamorphotique. Néanmoins, elles restent de grande taille et riches en réticulum endoplasmique et ne présentent pas de signes de régession tout au long du cycle métamorphotique.
Maughn's cells during metamorphosis

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