# CYTOLYSOMES IN AMPHIBIAN ERYTHROCYTES

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The presence of clusters of granular and vacuolar bodies in the cytoplasm of mature erythrocytes of many, but not all, species of urodeles is well established. They were extensively studied with vital staining techniques, but no reliable evidence about their cellular function was obtained. Various authors have described them as parasites, artefacts, centrosomes, nuclear emissions, Golgi bodies, and segregation apparatus (Dawson, 1932). Dawson and Charipper (1929) made a survey of urodele erythrocytes in an attempt to correlate the presence or absence of these bodies with the

phylogenetic relationships of the various species; they found no such correlation. Consequently, they suggested that the occurrence of these structures may only reflect the maturity of the individual erythrocytes.

Beams and Anderson (1960) studied the fine structure of these granules in the erythrocytes of *Necturus maculosus* but were unable to decide whether they were inclusion bodies characteristic of fish and amphibian erythrocytes or some organelle that had undergone morphological change during the maturation of the erythrocytes so that its identity could no longer be determined.

This note reports studies, made with the light and electron microscopes, on the structure of these bodies in the erythrocytes of the newts *Triturus cristatus* and *Triturus granulosus* and also of *Amphiuma tridactylum*. These bodies have the characteristic morphology of the lysosomal structures described as cytolysomes by Novikoff (1960, 1963) and as autophagic vacuoles and residual bodies by de Duve (1963). In addition, the bodies in these erythrocytes show acid phosphatase activity which confirms their lysosomal nature.

## METHODS

### 1. *Light Microscopy*

Living erythrocytes mounted in serum were examined with phase contrast microscopy in green light λ5461 A, and with brightfield microscopy in violet light λ4047 A and also green light λ5461 A. The brightfield microscopy at λ4047 A indicated the distribution of haemoglobin in the erythrocytes.

Air-dried blood smears were fixed for 20 seconds in ice cold citrate-buffered acetone pH 4.2 (Kaplow and Burstone, 1963). They were then incubated in an acetate-buffered naphthol A. S. B. I. phosphate solution at pH 5.2 (Burstone, 1962) for 15, 30, 60, and 120 minutes at 37°C. to reveal sites of acid phosphatase activity.

### 2. *Electron Microscopy*

Blood from adult animals was fixed for 30 minutes either in buffered 1 per cent $OsO_4$ solution (Palade, 1952) containing 0.14 M sucrose (Caulfield, 1957) or in 3 per cent glutaraldehyde in 0.1 M phosphate or cacodylate buffer at pH 7.2 followed by 1 per cent $OsO_4$ similarly buffered (Sabatini, Bensch, and Barrnett, 1963). After ethanol dehydration, cells were embedded in Epon or Araldite (Luft, 1961) or methacrylate. Thin sections, mounted on carbon collodion grids, were stained for 4 hours in 2 per cent aqueous uranyl acetate followed by lead citrate (Reynolds, 1963) for 10 to 30 minutes. They were examined in a Zeiss EM9 electron microscope.

## RESULTS

With phase contrast microscopy, clusters of granular bodies and vacuoles undergoing Brownian movement were seen in about 90 per cent of the erythrocytes. Typically, these bodies were in unipolar clusters (see Fig. 1) although in some erythrocytes, especially those of *Amphiuma tridactylum*, bipolar clusters and occasionally cells with scattered granules were seen. In a fresh preparation, the bodies were usually less refractile than the surrounding haemoglobin-containing cytoplasm, but within some of the larger bodies there were small regions of high refractive index. As the preparations aged, the erythrocytes haemolysed, the refractive index of the cytoplasm decreased, and the bodies then became more refractile than the cytoplasm; as haemolysis progressed, the Brownian movement of the bodies increased and the clusters tended to disperse.

With brightfield microscopy in green light (λ5461 A) the granules and vacuoles were barely visible, but in violet light λ4047 A they were easily seen as discrete non-absorbing bodies; this shows that they do not contain haemoglobin. Light microscopy of $OsO_4$-fixed erythrocytes showed that the bodies were intensely osmiophilic. It was for this reason, and because the bodies stained vitally with neutral red, that Dawson (1928) and others suggested they were Golgi apparatus.

In erythrocytes incubated for the demonstration of acid phosphatase activity a faint trace of the pink reaction product was found after 30 minutes' incubation, and after 60 minutes' and 120 minutes' incubation there was a considerable amount of reaction product entirely localised in the bodies. There was no reaction in any other region of the erythrocytes. This result demonstrates that all the acid phosphatase in these cells appears to be localised in the granules and vacuoles.

In the electron microscope, the bodies appear to be roughly spherical or ovoid and have a single or, much less frequently, a double outer membrane. They vary greatly in size, having diameters ranging from less than 0.5 to 3 to 4 $\mu$. The outer membrane encloses a matrix of variable electron opacity, and embedded in this there are varying amounts of membranous material and small particles resembling ferritin in density and size

(see Figs. 2 and 3). The extent of the enclosed membrane system is highly variable; small circular profiles, most probably sections of vesicles or tubules, swirls of parallel sheets of membrane, and myelin-like figures are all common (see Figs. 2 and 3). The aggregates of ferritin-like material are seen less frequently.

In addition, bodies typically with a single bounding membrane and homogeneous matrix of low electron opacity, and with little or no enclosed membranous material or ferritin, are often present (see Fig. 4). These structures may represent the final stages in the breakdown of enclosed material; they probably correspond to the vacuolar bodies seen in the light microscope, whereas the membrane and ferritin-containing structures correspond to the more granular bodies seen in the light microscope.

The structures described above have been seen in both $OsO_4$-fixed and glutaraldehyde-$OsO_4$–fixed material.

## DISCUSSION

The fine structure of these bodies together with the demonstration that they contain acid phosphatase indicates that they are cytolysomes. Novikoff (1963) suggested that cytolysomes may be involved in a slow degradation of organelles in normal, non-lytic cells, and Napolitano (1963) has described cytolysomes in metabolically active brown adipose cells. On the basis of the present study, we suggest that cytolysomes are involved in the degradation of organelles during the normal maturation of erythrocytes.

It is noteworthy that most erythrocytes from the circulating blood of adult urodeles have, in addition to cytolysomes, a few mitochondria, whereas the red cells from adult frogs of the species *Rana temporaria*, *Rana pipiens*, and *Rana esculenta* typically have neither cytolysomes nor mitochondria. A possible interpretation of these observations is that, in adult anuran amphibia, erythrocytes are liberated from the erythropoietic organs after the degradation of their cytoplasmic organelles is completed, whereas urodele erythrocytes are liberated at an earlier stage, before this process is completed. This view supports Dawson and Charipper's suggestion that the presence or absence of the segregation apparatus, which we now interpret as cytolysomes, reflects the degree of differentiation of the individual erythrocyte.

Cytolysomes containing recognizable organelles, *e.g.* mitochondria, have not been seen although mitochondria, often with aberrant morphology, are frequently found in close proximity to the cytolysomes (see Fig. 2). A study of amphibian erythropoiesis, which is now in progress, may well yield further information on the origin and role of cytolysomes in these cells.

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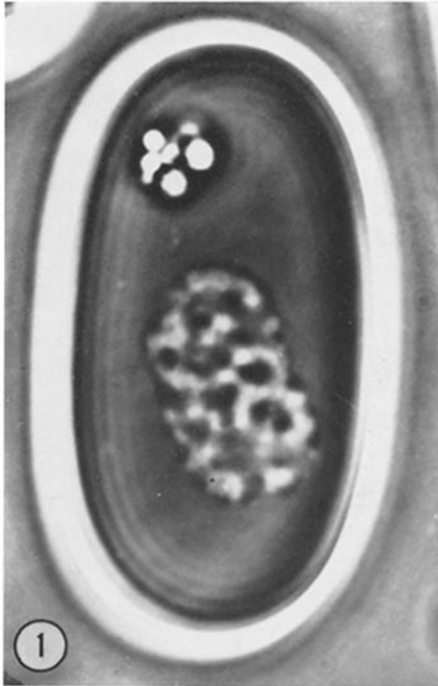
FIGURE 1 A phase-contrast micrograph of a living erythrocyte of *Triturus cristatus*, mounted in serum, showing a unipolar cluster of cytoplasmic bodies. × 2,000.

FIGURE 2 An electron micrograph of a cytolysome in an erythrocyte of *Triturus cristatus* containing enclosed membranous material (*M*) and electron-opaque, ferritin-like particles (*F*). Mitochondria, some with aberrant morphology, are near the cytolysome. $OsO_4$-fixation; methacrylate embedding. × 40,000.

FIGURE 3 An electron micrograph of cytolysomes in an erythrocyte of *Amphiuma tridactylum* showing enclosed membranous material (*M*) in one body and ferritin-like particles (*F*) in another. $OsO_4$-fixation; Araldite embedding. × 40,000.

FIGURE 4 The structures in this micrograph of an erythrocyte from *Triturus cristatus* are probably the final stages in the breakdown of material within the cytolysomes. $OsO_4$-fixation; Epon embedding. × 24,000.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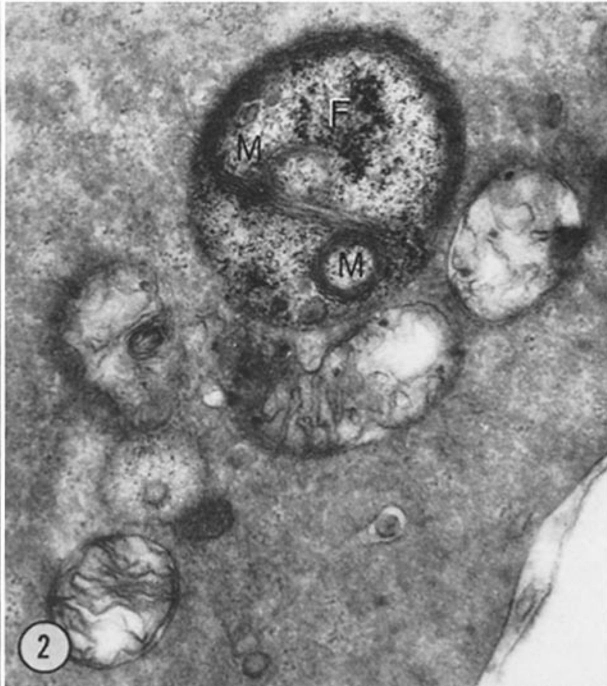
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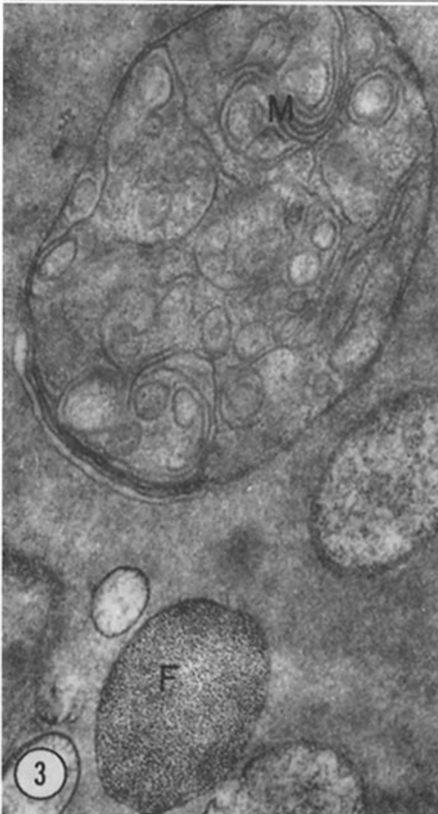
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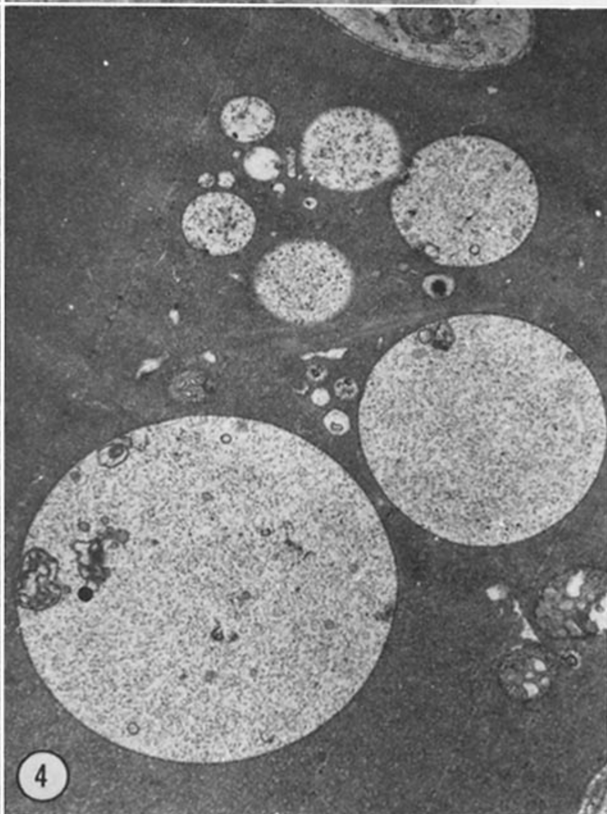
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