LIGHT- AND ELECTRON-MICROSCOPE STUDIES ON THE SPLEEN OF THE NEWT TRITURUS CRISTATUS: THE FINE STRUCTURE OF ERYTHROPOIETIC CELLS

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SUMMARY

The stages of erythrocyte maturation were identified in spleen smears stained with May-Grunwald-Giemsa. The fine structure of sectioned cells from about the basophilic erythroblast stage onwards was investigated in the electron microscope and serial 1-μ sections were examined by microspectrophotometry to ascertain haemoglobin content. The nuclei of basophilic erythroblasts contain large and small blocks of chromatin as well as light-staining zones of unknown composition and ill-defined structure: the nuclear sap contains numerous interchromatin granules, about 400 Å in diameter. During maturation the small blocks of chromatin aggregate and the nuclear light-staining zones tend to disappear, as do the interchromatin granules. Storage lysosomes occur in the basophilic and early polychromatic erythroblasts and during subsequent maturation these lysosomes are probably involved in the degradation of mitochondria. The changes in distribution of ribosomes in cells at the later stages of maturation have been investigated by counting the numbers of single ribosomes and polysomes seen in electron micrographs. During erythropoiesis the ratio of the amount of fibrillar material to the amount of granular material in the nucleolus increases; in the mature erythrocyte the nucleolus consists almost exclusively of fine fibrillar material.

INTRODUCTION

Erythropoietic tissues lend themselves to studies of cell maturation and protein synthesis. Mammalian reticulocytes, which can be obtained in large quantities, have been used extensively for studying the mechanism of protein (globin) synthesis (see review by Allen, 1964). In addition the enzymic pathway of haem synthesis has been determined (see Shemin, 1957; Rimington, 1959; Granick & Levere, 1964). Several microspectrophotometric investigations of the distribution of haemoglobin, nucleic acids and non-haem iron in mammalian and, to a lesser extent, amphibian erythroblasts have been made (Thorell, 1947; Sondhaus & Thorell, 1960; Grasso, Woodward & Swift, 1963) and the histology of mammalian erythropoietic cells from foetal liver, spleen and bone marrow has been repeatedly studied by light and electron microscopy (Maximov, 1924; Bloom, 1938a; Bessis, 1963; Bessis & Breton-Gorius, 1957; Ackerman, Grasso & Knouff, 1961; Jones, 1960, 1962; Grasso, Swift & Ackerman, 1962; Serenson, 1960, 1963; and Orlic, Gordon & Rhodin, 1965).

Although amphibian erythropoietic tissues were examined with the optical microscope by several earlier workers in comparative haematology, notably Jordan and...
Speidel (Jordan, 1932, 1933, 1938; Jordan & Speidel, 1924, 1930; see also reviews by Foxon, 1964 and Andrew, 1965), these tissues have been neglected recently; we are not aware of any published electron-microscopic studies of amphibian erythropoiesis.

This paper reports the results of light- and electron-microscopic studies of erythropoiesis occurring in the spleen of the newt *Triturus cristatus*. We shall describe elsewhere the structure of the spleen, especially that of the non-erythroblast cells (Tooze & Davies, unpublished); the structure of the chromosomes in interphase and dividing erythroblasts has already been described (Davies & Tooze, 1964, 1966).

**MATERIALS, METHODS AND TERMINOLOGY**

The observations were made on the spleen and other organs of adult *Triturus cristatus* and on heart blood of this and two other species, *Triturus granulosus* and *Amphiuma tridactylum*. Observations were also made on oxyhaemoglobin obtained from mature erythrocytes of *T*. *cristatus*

May-Grunwald-Giemsa staining of organ smears

Smears of kidney, liver, spleen and bone marrow were made and air-dried. These were then stained in May–Grunwald–Giemsa stains without prior fixation. The May–Grunwald stain (G. Gurr) was made up as follows: 0.3 g of stain were dissolved in 100 ml of methanol at 50 °C for 15 min; the solution was then filtered and used at room temperature. The staining time was 15 min. The slides were then transferred to Giemsa stain (G. Gurr's Improved Giemsa R 66 diluted 1:20 in triple-distilled water). The staining time was 30 min. The slides were differentiated in distilled water, air-dried and mounted in D.P.X. (G. Gurr). We are indebted to Dr W. Jacobson of the Strangeways Laboratory, Cambridge, for teaching us this technique.

Fixation, embedding and staining for light and electron microscopy

Pieces of spleen were fixed in Zenker formol or Helly's fixative (Lillie, 1954) and embedded in paraffin wax. Sections, 5–10 μ thick, were stained with haematoxylin and eosin. Whole spleens were also fixed in glutaraldehyde, embedded in Araldite, sectioned at 1 μ on a Huxley–Cambridge microtome and variously stained for examination in the light microscope (Grimley, Albrecht & Michelitch, 1965).

For electron microscopy small pieces of spleen were fixed in one of the following fixatives: (a) in 1 % osmium tetroxide in veronal-acetate buffer at pH 7.3 (Palade, 1952), containing 0.14M sucrose (Caulfield, 1957), for 45 min; (b) in 2.5 % or 5 % glutaraldehyde in 0.1M phosphate buffer at pH 7.2 for 1–2 h and then washed overnight in 0.1M phosphate buffer, pH 7.2, containing 0.2M sucrose (Sabatini, Bensch & Barnett, 1963); (c) as for (b) and then post-fixed for 30 min in 1 % osmium tetroxide in 0.1M phosphate buffer, pH 7.2, containing 0.2 M sucrose. After dehydration the blocks were embedded in methacrylate, Epon (Luft, 1961) or Araldite (Glaevert & Glaevert, 1958; or Luft, 1961). Heart blood was fixed in any of the above fixatives and then processed as described by Davies (1961) and Tooze (1964). Sections were cut with either glass or diamond knives and mounted on collodion-coated grids. A small amount of carbon was deposited on the section after staining. Sections were examined either without staining or after staining with a 2 % aqueous solution of uranyl acetate for 4 h or with lead citrate for 5–8 min (Reynolds, 1963), or both.

Sections were examined in either a Zeiss EM 9 or a Siemens Elmiskop I with a 50–μ objective aperture, at magnifications up to 80000 at 80 kV. Thick sections (0.5–1 μ), serial to thin sections, were examined either by microspectrophotometry with a universal microspectrophotometer (UMSP, Zeiss, Oberkocken) or in the light microscope by phase-contrast or, preferably, after staining in toluidine blue by bright-field. There are several variations in the procedure for staining with toluidine blue, but since the method devised by Miss Margaret Blade in our laboratory gives, in a few minutes, very well-stained sections which, after suitable mounting, have not faded after several years, details of the procedure are given here. Sections
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of spleen embedded in Araldite were dried down onto a clean slide and then a few drops of 1% toluidine blue in 1% borax solution diluted 20- to 100-fold were placed on the section and heated over a hot plate for 2–3 sec. The stain was then washed off with distilled water and after drying the sections were mounted in Euparal (G. T. Gurr).

Microspectrophotometry

Alternate thick (about 0.5–1 μ) and thin (about 600 Å) sections were cut of spleen fixed in glutaraldehyde with or without post-fixation in OsO₄. The thick sections were dried on quartz coverslips and mounted on quartz slides with an aqueous solution of glycerol, n = 1.455. Cells interpreted as erythroblasts at various stages of maturation were selected in the electron microscope and were then located on the serial thick sections; the absorption spectra of areas 0.5–1 μ in diameter in the cytoplasm of these cells were determined with the Zeiss UMSP.

Measurements of the distribution of haemoglobin in mature erythrocytes were also made from photographs taken in violet light (λ = 4047 Å). Details of this procedure were given by Tooze & Davies (1963).

Spectroscopic measurements of haemoglobin solutions

A solution of oxyhaemoglobin was prepared by lysing saline-washed erythrocytes of T. cristatus. This was treated with an excess of 5% glutaraldehyde in 0.1M phosphate buffer at pH 7.2, followed by 1% osmium tetroxide similarly buffered. The changes in absorption spectra during the fixation were determined with a Perkin Elmer 137 ultraviolet spectrophotometer.

Identification of cell types: terminology

The different types of blood cells are identifiable according to classical methods, namely by light microscopy of squashes or smears after staining with May–Grünwald–Giemsa (MGG) or Wright’s stain. Even here there are well-known problems in identifying the different stem cells and early stages of differentiation, as well as in terminology, which may partly depend on the author’s adherence to a particular theory of haematopoiesis (Bloom, 1938b). Difficulties of identification of blood cells are enhanced in the electron microscope (Bernhard & Granboulan, 1960) for two reasons. First, there are no comparable specific staining methods, which often depend on subtle colour differences, for thin sections of embedded material. Secondly, only a fraction of the cell is examined in any one section; consequently the data on relative cell size, shape, ratio of nuclear to cytoplasmic volume and presence of specific organelles, important for identification in smears, are frequently not available. Only from favourable cell images can one hope to infer these parameters.

The erythrocyte is thought to arise (see Bloom & Fawcett, 1962) from a stem cell with lymphoid or lymphocyte-like characteristics, the so-called primitive cell (Sorenson, 1960, 1963) or haemocytoblast or lymphoid haemoblast (Jordan, 1938). The stem cell gives rise to the basophilic erythroblasts, an intermediate cell type, the proerythroblast, being described by some workers (see Bloom & Fawcett, 1962). With the continued synthesis and accumulation of haemoglobin, the basophilic erythroblasts mature into polychromatic erythroblasts, normoblasts, reticulocytes and finally mature erythrocytes. In their extensive studies of erythropoiesis in urodeles, Jordan (1932) and Jordan & Speidel (1924, 1930) refer only to haemocytoblasts, proerythroblasts and erythroblasts, the terms ‘basophilic erythroblast’ and ‘polychromatic erythroblast’, with one exception, not being used.

In MGG smears in the light microscope we recognize a few cells with characteristics (see Results) ascribable to haemocytoblasts, and proerythroblasts. Basophilic erythroblasts, and especially polychromatic erythroblasts, are more common. In the electron microscope we have been able to identify a series of cells as erythroblasts at various stages of maturation due to the presence of related intermediate stages. In the earliest stage the number of ribosomes per unit area in thin (~ 600 Å) sections is very high but gradually decreases. For technical reasons we have been unable to examine the fine structure of cells stained with MGG. However, it is likely, judging particularly by the number of ribosomes, that the earliest stages recognizable in the electron microscope as erythroblasts correspond to basophilic erythroblasts or possibly
proerythroblasts seen in stained smears. The term 'polychromatic erythroblast' is used by us to describe stages in which, in thin sections, the ribosomes or polysomes are appreciably separated from their neighbours. Nearly mature or mature erythrocytes contain few ribosomes. A new approach to the problem of defining the erythroblast series, some preliminary results of which are given here, is to measure cellular haemoglobin concentration by microspectrophotometry in thick sections and examine the fine structure in serial thin sections.

**Nuclear morphology: terminology**

As a result of observations with the light microscope on cell nuclei during interphase, Ris & Mirsky (1949) distinguished two states for the chromosomal material. In the so-called extended state the nucleoprotein molecules are relatively far apart. In the so-called condensed state the nucleoprotein molecules are relatively close together. The chromocentres or heterochromatic regions of the interphase nucleus are in the condensed state. The process of condensation or its reverse can be brought about in gels of nucleoprotein by altering the ionic environment. We draw a distinction between **condensing** and **clumping**. We use the term 'clumping' to refer to the process whereby small regions of condensed chromatin aggregate to form large regions, the concentration remaining constant (Fig. 1B–C). If the small regions of condensed chromatin, large compared with the molecular dimensions, are smaller than the resolving power of the microscope (~ 0.2 μ) then the processes of condensing (Fig. 1A–C) and clumping (Fig. 1B–C) would be indistinguishable in the light microscope. They would, however, be resolvable in the electron microscope.

**RESULTS**

The absorption spectra of haemoglobin solutions treated with glutaraldehyde and osmium tetroxide

Since microspectrophotometric studies of the distribution of haemoglobin in thick sections of erythroblasts fixed in glutaraldehyde, with or without post-fixation in osmium tetroxide, were to be made, it was an essential prerequisite to determine the changes in the absorption spectrum of oxyhaemoglobin caused by fixation. When a solution of oxyhaemoglobin is treated with 5 % glutaraldehyde the denaturation, or fixation, is slow, taking 3-4 h to complete at room temperature. During the denaturation the characteristic absorption bands of oxyhaemoglobin at 542 μ and 577 μ arc destroyed and the wavelength of the Soret band maximum gradually shifts from 415 to 410 μ; the absorbance at the Soret band of the denatured haemoglobin—which is, glutaraldehyde-haematin—is only about 50 % that of the original oxyhaemoglobin (Figs. 2, 3). With the pyridine-haemochromogen reaction (Lemberg & Legge, 1949) which is used to measure the total numbers of haem groups present irrespective of whether they are in haemoglobin or haematin, it was shown that fixation in glutaraldehyde does not destroy haem groups. We conclude that glutaraldehyde-haematin has a much lower extinction coefficient at the Soret band peak than oxyhaemoglobin.

The addition of osmium tetroxide to glutaraldehyde-haematin causes instantaneous change: the Soret peak shifts to 397 μ but the optical density of the solution at the peak remains unaltered (Fig. 4), and the product, OsO₄-glutaraldehyde-haematin, has the spectral characteristics of OsO₄-haematin (Tooze & Davies, 1963; Tooze, 1964).
Light microscopy

Smears from various organs, namely kidney, intestine, liver and bone marrow of adult *T. cristatus*, showed no erythropoietic activity. Erythropoiesis was found to occur in the spleen; these results are in agreement with those of Jordan & Speidel (1924, 1930) on *Triturus* (formerly *Diemyctylus*) *viridescens*.

![Diagram of chromatin condensation](image)

Fig. 1. This diagram shows extended and condensed chromatin and the processes of clumping and condensing. In the light microscope nuclei A and B would appear full of chromatin at fairly uniform concentration so long as most of the regions of condensed chromatin in B were below the limit of resolution of the light microscope. Condensing is the process shown in A to C or A to B where the local concentration of chromatin increases. Clumping is the process shown in B to C where the small aggregates form large aggregates but the local chromatin concentration remains the same.

After staining in MGG we found a series of lymphoid cells similar to those seen by Jordan & Speidel (1924, 1930) in various urodeles after staining in Wright's stain. As well as lymphocytes, which were identified by their reddish or slightly reddish-blue nuclei and hardly discernible or slightly basophilic cytoplasm, there were other, large cells with a definitely purple nucleus and blue (basophilic) cytoplasm (Fig. 8). The nucleus of these large cells sometimes appeared fairly homogeneous, sometimes more
Fig. 2. The absorption spectra of solutions, at equal concentrations, of (I) oxyhaemoglobin and (II) oxyhaemoglobin fixed for 233 min in an excess of a 5% buffered solution of glutaraldehyde.

Fig. 3. Changes in the absorption spectrum, in the Soret band region, of oxyhaemoglobin during fixation in 5% glutaraldehyde buffered at pH 7.2 in 0.1M phosphate buffer. The spectra were recorded at 0, 1.5, 5, 11, 22, 37.5, 61, 80, 113, and 233 min after the beginning of fixation. The Soret maximum shifts from 415 μm to 410 μm and the extinction coefficient at the maximum is decreased by about 50%.
particulate. They probably correspond to the haemocytoblasts of Jordan & Speidel; their possible identification in the electron microscope will be discussed elsewhere. Other cells, which occurred infrequently, had a blue-staining nucleus and cytoplasm stained more intensely blue than the haemocytoblasts. These cells might correspond to proerythroblasts.

The cells classified as basophilic erythroblasts had a blue-staining nucleus, smaller than that of the haemocytoblasts and containing better-defined blocks of chromatin, the cytoplasm staining deep blue (Fig. 9). A series of intermediate stages existed between these cells and the easily recognized polychromatic erythroblast (Fig. 10) with its still smaller nucleus and grey-staining cytoplasm, indicating the presence of haemoglobin, which is eosinophilic, as well as basophilic material. During maturation the shape of the cell becomes pronouncedly elongate and in the mature erythrocyte (Fig. 11) the nucleus has shrunk still further and contains a number of well-defined, discrete chromatin masses; the cytoplasm is considerably enlarged and pale orange in colour. In the smears basophilic erythroblasts were seen fairly frequently, although they were less numerous than polychromatic erythroblasts and nearly mature erythrocytes.

**Electron microscopy**

**Basophilic erythroblasts.** The earliest erythroblasts recognizable in the electron microscope (Figs. 12, 13) have a large nucleus, usually approximately round in section,
occasionally elongate (Fig. 13) and sometimes with deep indentations. Peripherally there are dense regions of chromatin with a few large, internally placed blocks of similarly staining chromatin and characteristically many smaller dense chromatin regions scattered throughout the extensive nuclear sap (Figs. 12, 13). These small regions have dimensions at or just below the limits of resolution of the light microscope (approximately 2000 Å). Occasionally fairly large finely fibrillar zones occur, characterized by a low electron density (Figs. 13, 14), lower even than any part of the nucleolus. We do not know what these zones are, but suggest that they might be extended chromatin. We refer to them as the nuclear light-staining zones. The nuclear sap or interchromatin regions are not structureless: they contain ill-defined fibrils, for example. However, the most striking feature of this part of the nucleus is the numerous large granules (Figs. 13, 14), referred to as interchromatin granules (Bernhard & Granboulan, 1963), with diameters ranging from about 400 Å to less than 150 Å. Since the section thickness is comparable to the granule diameter, variation in dimensions due to sectioning would be expected. It is impossible to decide, therefore, whether these interchromatin granules are of fairly constant dimensions or a heterogeneous population. However, since even ribosomes, which are thought to constitute a homogeneous population, vary both in size and contrast in our micrographs, probably most of the variation in size of the interchromatin granules is due to the sectioning. The electron-staining properties of the interchromatin granules resemble those of cytoplasmic ribosomes; both structures are more osmiophilic than the chromatin or cytoplasm in unstained material and both are more heavily stained than the chromatin or cytoplasm with lead citrate alone. As well as interchromatin granules, other granules about 400–500 Å in diameter occur adjacent to and partly surrounded by chromatin but separated by a pale zone. These are similar to the perichromatin granules of Watson (1962a, b) and Swift (1962a).

In basophilic erythroblasts and more mature stages the structure and size of the interchromatin granules after fixation in osmium tetroxide alone is similar to that in material treated with glutaraldehyde with or without post-fixation in osmium tetroxide.

The nuclear envelope of basophilic erythroblasts has the characteristic fine structure and pores are numerous (Figs. 13, 14). The nuclear envelope of basophilic erythroblasts and the other erythropoietic cells examined was, however, often poorly preserved, with large gaps between the inner and outer membranes (Figs. 17, 20). This is probably caused by nuclear shrinkage. It occurred with all the fixatives used, but varied in extent from experiment to experiment even with the same fixative. A characteristic feature of the basophilic erythroblast is its large nucleoli (Figs. 13, 14, 18); thin sections of these cells with two or three nucleoli per nucleus were observed.

The defining feature of the cytoplasm of this cell type is the high density of ribosomes, each about 200 Å diameter, so closely packed together that it is difficult to distinguish individual polysomes (Figs. 13, 14, 30). In silver-coloured sections (approximately 600 Å thick) there are about 600 ribosomes per μ². Doubtless they are responsible for the strong basophilia seen in whole cells stained with May–Grünwald–Giemsa. There is a little granular and smooth-surfaced endoplasmic reticulum. Mitochondria are numerous and frequently occur in close proximity to the nuclear envelope.
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(Fig. 13). Similar observations have been made on erythroblasts from mouse (Orlic et al. 1965) and chick (Schjeide, McCandless & Munn, 1964). Paired centrioles are present and their fine structure is similar to that previously described in other cells (Bernhard & de Harven, 1958; Gall, 1961), except that pericentriolar bodies have not been seen. Golgi material occurs in the neighbourhood of the centrioles (Fig. 14).

Bundles of microtubules which closely resemble mitotic spindle tubules frequently occur in the cytoplasm (Figs. 15, 16), usually lying near the periphery of the cell. The microtubules appear circular or elliptical in cross-section (Fig. 16) and about 230 Å in diameter. In addition, in basophilic erythroblasts, but not in later stages, bundles of fine fibrils each about 70-80 Å in diameter are frequently seen in the cytoplasm (Figs. 13, 19).

The plasma membrane is often thrown into projections and invaginations suggesting intense pinocytotic activity. Since, however, we have not seen the uptake of ferritin by pinocytosis (‘rhopheocytosis’ of Bessis, 1963) it is likely that the uptake of non-haem iron is usually by some other process, perhaps by the transferrin mechanism discovered by Jandl, Inman, Simmons & Allen (1959).

Basophilic erythroblasts almost invariably appear to be associated with the reticular framework of macrophages, reticular cells and reticular fibres (Fig. 12), and these cells abut one another and sometimes interlock. However, there are no distinct cell islands as there are in human bone marrow (Bessis, 1961, 1963), and like Berman (1967) we have not so far found evidence indicating the transfer of ferritin from reticular cells to erythroblasts.

Polychromatic erythroblasts. We use the term ‘polychromatic’ erythroblast to describe cells (Figs. 12, 20) in which the amount of cytoplasm relative to the nucleus has increased due, no doubt, to both an increase in haemoglobin content and nuclear shrinkage; in the cytoplasm discrete polysomes can now clearly be distinguished.

The chromatin (Figs. 12, 20), which is often lobed or indented, is arranged predominantly in large clumps, due presumably to clumping of the small regions present in the nuclei of the basophilic erythroblasts. The structure of the surfaces of the chromatin block (Davies & Tooze, 1966) varies greatly, frequently being irregular with fine feathery projections, but sometimes being relatively smooth (Fig. 20). Nuclear light-staining zones occupying small areas are occasionally seen, but are less extensive than at the previous stage. Well-defined nucleoli and interchromatin granules are present (Fig. 20). The nuclear sap has a similar electron density to that of the cytoplasm, presumably because it contains haemoglobin at a similar concentration.

The bulk of the cytoplasm consists of fairly homogeneous electron-dense material, interpreted as haemoglobin, together with dispersed single ribosomes and polysomes (Fig. 20); most of the polysomes consist of aggregates of 2-6 ribosomes. In material fixed in osmium tetroxide alone the ribosomes are surrounded by a region of low electron density, a ‘peripolysome space’. After fixation in glutaraldehyde, with or without post-fixation in osmium tetroxide, however, the electron-dense haemoglobin continues up to the surface of the ribosomes (compare Figs. 21, 22 with Figs. 23, 24).

Rifkind, Luzzato & Marks (1964, and private communication) observed large polysomes in rabbit reticulocytes fixed in glutaraldehyde followed by osmium tetroxide.
These consisted of 2 or 3 polysomes, each containing 2–6 ribosomes, joined by fine filaments thus producing a large polysome. We have rarely seen such large polysomes not attached to membranes in newt erythroblasts after glutaraldehyde fixation: in erythroblasts fixed in osmium tetroxide alone, however, we have observed more frequently large aggregates consisting of up to about 20 ribosomes (Fig. 21).

Mitochondria are present and often retain their close relationship with the nuclear envelope. Bundles of gently curved microtubules lie around the periphery of the cell. There seems to be an increase in the frequency of tubules, but the fine fibrils present in basophilic erythroblasts are not seen. Golgi material persists (Fig. 20); small vesicles near the plasma membrane are common in all cells. There are scattered fragments of granular endoplasmic reticulum. Although we have observed centrioles in mitotic polychromatic erythroblasts, we have never seen them in interphase cells. In general, cell organelles appear much less numerous than in basophilic erythroblasts; this presumably partly reflects the increase in volume of the cytoplasm. Vesicles containing aggregates of electron-opaque material interpreted as ferritin were seen occasionally in the cytoplasm of polychromatic erythroblasts (Fig. 22). These were expected since Sondhaus & Thorell (1960) showed by microspectrophotometry that amphibian erythroblasts at all stages of maturation contain an excess of non-haem iron.

Nearly mature and mature erythrocytes. In the light microscope (Fig. 11) the nucleus of the mature erythrocyte is smaller than at earlier stages. In the electron microscope the chromatin is seen to be in larger blocks than in immature erythroblasts and the area occupied by the nuclear sap is considerably reduced (Figs. 12, 27). The surface structure of these masses of chromatin, like that of polychromatic erythroblasts, is variable, sometimes being very irregular with numerous projections (Fig. 27), but more often, especially in circulating erythrocytes, the surfaces are relatively smooth. Nuclear light-staining zones are rare. The nuclear sap of mature erythrocytes contains interchromatin granules, but these are much less numerous than at earlier stages (compare Figs. 27, 20 and 13). Small nucleoli are occasionally seen (Figs. 25, 26). We have also observed small nucleoli in mature erythrocytes from circulating blood of the frog Rana esculenta.

Mature erythrocytes have a large amount of haemoglobin-containing cytoplasm (Figs. 12, 27). During maturation the number of ribosomes per $\mu^2$ of cytoplasm decreases (compare Figs. 13, 20 and 27). In the mature cell there are very few, if any, free cytoplasmic ribosomes (Fig. 27), although whorls of ribosomes are sometimes seen attached to the nuclear envelope or small membrane vesicles in the cytoplasm (Figs. 23, 24). A few mitochondria persist (Fig. 12). Very occasionally the remnants of the Golgi apparatus have been seen in nearly mature erythrocytes from the spleen and in the mature erythrocytes from circulating blood of T. cristatus, T. granulosus (Fig. 28) and A. tridactylum. Microtubules are present in the mature cell. They form a marginal band like that described by Fawcett (1959), and Fawcett & Witebsky (1964).

Changes in nucleolar fine structure during maturation. During maturation of the basophilic erythroblasts the nucleolus undergoes an interesting structural change. In basophilic and early polychromatic erythroblasts the nucleoli are large and several occur per cell. After fixation in glutaraldehyde alone, or followed by post-fixation in
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osmium tetroxide, the nucleoli have two distinct zones, a granular and a fibrillar zone (Figs. 13, 14, 18, 20). The fibrillar zone consists of closely packed fine fibrils approximately 40 Å in diameter, together with small granules of similar diameter, and it usually forms a compact central mass completely or partly surrounded by a granular zone. This outer granular zone consists of electron-dense granules about 150 Å in diameter embedded in a matrix material. Associated heterochromatin is prominent; most of it is in the granular zone but some occurs within the fibrillar region. After this fixation a nucleolonema (Estable & Sotelo, 1951; Fawcett, 1955) was not usually visible. In cells fixed in osmium tetroxide alone, however, a nucleolonema consisting of an anastomosing strand of coarse fibrillo-granular material has been seen.

Large nucleoli persist through the early stages of maturation but from the late polychromatic stage onwards they undergo a progressive change. The nucleoli decrease in size and the amount of granular material relative to the amount of fibrillar material decreases. The amount of associated heterochromatin also decreases (Figs. 25, 26).

Measurements have been made of the percentage areas occupied by the granular and fibrillar zones in sectioned nucleoli of erythroblasts at various stages of maturation after fixation in glutaraldehyde and post-fixation in osmium tetroxide. The percentage area occupied by the fibrillar zone has been plotted (Fig. 5) as a function of the numbers of ribosomes per µ² of sectioned cytoplasm, which can be taken as an approximate index of the maturity of the cell, assuming section thickness to be fairly constant. Obviously, sectioning causes considerable variation between the recorded proportions of the granular and fibrillar zones in nucleoli containing both zones; this explains the high scatter of values in immature erythroblasts with high ribosome counts. Throughout the later stages of maturation there is, however, an increase in the proportion of fibrillar zone and a decrease in the granular zone. In cells with less than 50 ribosomes per µ² of sectioned cytoplasm the fibrillar zone never occupied less than 85 % of the nucleolar area and often the granular zone was completely absent.

Fig. 5. The percentage area of sectioned nucleoli occupied by fibrillar zone plotted as a function of the numbers of cytoplasmic ribosomes per µ² of sectioned cytoplasm.
Lysosomes and their association with mitochondria during development. Membrane-bound bodies, usually smaller than mitochondria and containing electron-opaque homogeneous material, are often seen in the cytoplasm in thin sections of basophilic and early polychromatic erythroblasts after staining in uranyl acetate and lead citrate (Figs. 13, 29, 30). Very dense particles, interpreted as ferritin (Fig. 29), and membrane material in myelin-like figures are often seen embedded in the homogeneous material. Although we have not demonstrated cytochemically the presence of acid phosphatase in these bodies, their fine structure is characteristic of storage or 'virgin' lysosomes (de Duve, 1963; Moe, Rostgaard & Behnke, 1965). Frequently these lysosomes are found close to groups of mitochondria. They might be in contact with individual mitochondria (Figs. 29, 30) but we cannot be certain of this because of the problems of overlapping structure. The mitochondria themselves often appear with regular cristae but some contain regions which consist of loosely packed whorls of membrane material (Fig. 30).

In late polychromatic erythroblasts and nearly mature erythrocytes, there were very few storage lysosomes and many larger less-dense bodies containing whorls of membrane material. These bodies occur in clusters in association, and apparently fusing, with mitochondria, which are often of aberrant morphology (Figs. 17, 31).

In nearly mature erythrocytes in the spleen and in mature circulating erythrocytes, most of the mitochondria have disappeared, as have the storage lysosomes, but clusters of so-called polar bodies are present (Fig. 32) that resemble cytolyosomes or autophagic vacuoles and residual bodies as described by Novikoff (1963) and de Duve (1963) and contain acid phosphatases (Tooze & Davies, 1965). We interpret all these observations as suggesting that during erythropoiesis the breakdown of at least some mitochondria is brought about by lysosomes.

Changes in distribution of ribosomes during maturation. During the final stages of erythropoiesis there is a progressive decrease in the numbers of cytoplasmic ribosomes; mature erythrocytes have few, if any. Recently several biochemical and electron-microscopic studies have been made of the changes in distribution of single ribosomes and polysomes during the maturation of reticulocytes from rabbits treated with phenylhydrazine (Marks, Rifkind Danon, 1963; Rifkind, Danon & Marks, 1964; Mathias, Williamson, Huxley & Page, 1964). We have made a limited study of the changes in distribution of ribosomes in maturing newt erythrocytes in the normal spleen. Silver sections of material fixed in glutaraldehyde and post-fixed in osmium tetroxide were cut and counts of the total numbers of single ribosomes and the number of polysomes—that is, aggregates of two or more ribosomes—were made in cells at various stages of maturity. We assumed that pairs of ribosomes separated by the distance which separates ribosomes in polysomes, about 70–80 Å, are in fact part of polysomes. Two ribosomes separated by a greater distance could be either single ribosomes, or parts of polysomes not entirely contained in the section. Marks et al. (1963) estimate that in sections 400–600 Å thick about 15% of the total number of ribosomes would be expected to appear single, because of thin sectioning, even though they were in fact part of polysomes. Table 1 shows the results of our counts of the distribution of ribosomes and polysomes in polychromatic erythroblasts and nearly mature erythrocytes. These data have not been corrected for thin-sectioning error.
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In late polychromatic erythroblasts, containing about 110–160 ribosomes per \( \mu^3 \) of cytoplasm, about 50% of the ribosomes are in aggregates of two or more and 50% are single (see Table 1, cells nos. 1–5). If the correction of Marks et al. (1963) is applied there are about 65% polysomes and 35% single ribosomes in late polychromatic erythroblasts. As the cells mature the total numbers of ribosomes per \( \mu^3 \) of cytoplasm in thin sections decrease. The numbers of single ribosomes and polysomes were counted in nearly mature erythrocytes with less than 25 ribosomes per \( \mu^3 \) of sectioned cytoplasm. Since these cells have approximately the same numbers of ribosomes per \( \mu^3 \) of cytoplasm they could be considered to be at roughly the same stage of maturity. The counts showed the existence of two types of cell. In one (see Table 1, cells nos. 6–11) about 15% or less of all the ribosomes are single. Since about 15% single ribosomes would be expected because of sectioning, these cells probably have no single ribosomes. Cells of the other type (cells nos. 12–16) have about 60–70% single ribosomes or, if the correction for sectioning is applied, 45–55% single ribosomes. The counts also show that in cells with a high percentage of polysomes (cells nos. 6–11) about half of the polysomes are large, being clusters of 4 or more ribosomes, whereas in the cells with a low percentage of polysomes, the small polysomes (aggregates of 2 or 3 ribosomes) predominate (cells nos. 12–16).

These two types of nearly mature erythrocytes exist side by side in the same animal and there is no evidence suggesting any gradient in the distribution of polysomes and single ribosomes in any one cell.

### Table 1. Distribution of single ribosomes and polysomes in maturing erythrocytes

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>Cell stage</th>
<th>No. of ribosomes per ( \mu^3 ) of sectioned cytoplasm</th>
<th>% of ribosomes in total population occurring singly or in polysomes of 2–6 ribosomes</th>
<th>Total of ribosomes counted in each cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Late polychromatophil 1</td>
<td>156</td>
<td>46 22 10 6 10 6 627</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Late polychromatophil 2</td>
<td>121</td>
<td>45 13 13 16 8 5 968</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Late polychromatophil 3</td>
<td>117</td>
<td>51 18 14 9 5 3 770</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Nearly mature erythrocytes</td>
<td>115</td>
<td>45 19 15 10 8 3 830</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nearly mature erythrocytes</td>
<td>110</td>
<td>50 17 15 7 4 7 845</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Nearly mature erythrocytes</td>
<td>21</td>
<td>12 13 27 23 25 121</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Nearly mature erythrocytes</td>
<td>21</td>
<td>14 10 21 21 24 114</td>
<td></td>
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<tr>
<td>8</td>
<td>Nearly mature erythrocytes</td>
<td>12</td>
<td>13 14 27 33 13 133</td>
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</tr>
<tr>
<td>9</td>
<td>Nearly mature erythrocytes</td>
<td>12</td>
<td>16 16 15 20 23 10 106</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Nearly mature erythrocytes</td>
<td>10</td>
<td>13 20 25 28 9 5 101</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Nearly mature erythrocytes</td>
<td>9</td>
<td>15 20 25 16 24 — 79</td>
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<td>Nearly mature erythrocytes</td>
<td>22</td>
<td>70 9 6 5 10 — 117</td>
<td></td>
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<td>Nearly mature erythrocytes</td>
<td>17</td>
<td>73 12 6 5 3 1 518</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Nearly mature erythrocytes</td>
<td>14</td>
<td>61 6 17 11 5 — 107</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Nearly mature erythrocytes</td>
<td>13</td>
<td>67 11 12 7 3 — 220</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Nearly mature erythrocytes</td>
<td>11</td>
<td>72 10 8 4 6 — 114</td>
<td></td>
</tr>
</tbody>
</table>
Microspectrophotometry

The absorption spectra of areas 1 μ in diameter were recorded for the cytoplasm of cells in 1-μ thick sections of spleen fixed in glutaraldehyde and post-treated with osmium tetroxide. Fig. 6 shows the spectra of two cells classified as basophilic erythroblasts (B and C), two polychromatic erythroblasts (E and F) and a mature erythrocyte (G). Micrographs of these cells are shown in Figs. 33–36. Fig. 6D shows the absorption spectrum of the mitotic polychromatic erythroblast seen in Fig. 33. During development there is an increase in the absorption maximum at 395 mμ which can be interpreted as due to an increase in concentration of haemoglobin. These observations support the cell classification we adopt. However, any conclusions about immature erythroblasts that show only small amounts of absorption at 395 mμ must be tentative because a lymphocyte nucleus (Fig. 33 (cell A)) (the cytoplasmic area is small and hence difficult to measure) showed a small but significant absorption maximum at 395 mμ (Fig. 6A), which could be due to the presence of a haem compound, for
example a cytochrome (Granick & Levere, 1965), or to haemoglobin, extracted from neighbouring red cells during fixation, that diffused into the lymphocyte. Clearly, we need more data on cells containing small amounts of absorbing substances.

The concentration of haemoglobin in the nuclear sap regions of mature erythrocytes compared with that in the cytoplasm was determined from absorption measurements at 4047 Å at the two sites using a photographic method (Tooze & Davies, 1963). In a series of sections of decreasing thickness (1 μ–1000 Å) with decreasing chance of over- and underlying chromatin in the measuring beam, the absorption ratio of nuclear sap to cytoplasm increased to about 0.8–1 (Fig. 7). This indicates that haemoglobin occurs in the nuclear sap regions of mature erythrocytes at concentrations equal to but never exceeding the concentration of haemoglobin in the cytoplasm. Davies (1961) drew similar conclusions regarding the nuclear sap regions of mature erythrocytes of Rana pipiens.

Fig. 7. The ratio of the absorption, at 4047 Å, in the nuclear sap, or nuclear pool regions, to that in the cytoplasm of the newt erythrocyte, as a function of section thickness. Each point is the average of three determinations at different sites in one cell. As the section thickness decreases this ratio approximates to unity.

**DISCUSSION**

Jordan & Speidel (1924, 1930) found the spleen in urodeles to be the site of production of haemocytoblasts, cells with lymphocyte-like characteristics, and thought to have the properties of stem-cells. These haemocytoblasts are either liberated to the blood stream, as in Proteus anguineus (Jordan, 1932) where differentiation into erythrocytes largely occurs; or, as in Triturus viridescens (Jordan & Speidel, 1924, 1930) haemocytoblasts are thought to differentiate entirely within the red pulp of the spleen. Our observations reported here on the presence of stages of erythroblast maturation within the spleen of T. cristatus indicate that this organ is the site of erythropoiesis as in T. viridescens. Observations (Davies & Tooze, 1966) on mitotic cells, which showed that in a few animals divisions occurred in cells with little or no haemoglobin, are consistent with the idea of a lymphoid cell being produced in the spleen of T. cristatus. However, in most animals divisions occurred in polychromatric erythroblasts,
haemocytoblasts being rare; this means that proliferation of differentiated erythroblasts is a major factor in maintaining the erythrocyte population.

Our data do not provide any information about the relationship between the cells recognizable in the electron microscope as erythroblasts and a possible lymphoid stem-cell. The fine structure of a cell which might correspond to the haemocytoblast will be discussed elsewhere but it is not recognizably related to the erythroblasts described here by the presence of a series of intermediate stages. In this respect, our material seems less favourable than that available to Jordan & Speidel.

The general fine structure of the newt cells termed 'basophilic erythroblasts' by us resembles that of the mammalian basophilic erythroblasts described by other workers (Grasso et al. 1962; Bessis, 1963; Sörenson, 1960, 1963). From the polychromatic erythroblast to erythrocyte stage, however, amphibian cells differ from their mammalian counterparts. The amphibian cells retain their nuclei and nucleoli and occasionally a few mitochondria and Golgi material.

The microtubules, about 230 Å in diameter, that occur in amphibian erythroblasts have also been observed in the equivalent mammalian cells (J. A. Grasso, private communication). Similar tubules have been seen in undifferentiated and differentiated cell types at interphase (Porter, 1965), and Behnke (1964) considers that they may be a ubiquitous cell organelle.

Despite the numerous studies of mammalian erythropoiesis, which have shown that during maturation the cell organelles are lost, there is little information about how these cellular changes occur. Grasso et al. (1962) simply state that the numbers of mitochondria decrease during erythropoiesis but that the structure of the mitochondria remains unaltered. The polar bodies in mature amphibian erythrocytes have been interpreted as cytolysomes (Tooze & Davies, 1965) or, in the terminology of de Duve (1963), autophagic vacuoles and residual bodies. The present study of erythropoiesis confirms and extends this interpretation. The autolysis of cell organelles by lysosomes has been observed in several types of cell (see for example Novikoff, 1963; de Duve 1963; Behnke, 1963; Napolitano, 1963). These workers describe structures consisting of recognizable cell organelles together with hydrolytic enzymes enclosed within a membrane. They suggest therefore that the cell organelles and the hydrolytic enzymes are somehow screened off from the rest of the cytoplasm by a resistant membrane so that indiscriminate autolysis of the cytoplasm does not occur. The mechanism of the screening off process is, however, unknown. As Novikoff (1963) states, 'the origins of the membranes delimiting the cytolysomes have yet to be established'.

We have not observed recognizable organelles enclosed within a membrane. Instead in basophilic and early polychromatic erythroblasts we observed structures resembling storage lysosomes which often appeared to be in contact with mitochondria, and in late polychromatic erythroblasts and more mature stages we observed mitochondria, often with aberrant morphology, associated and even apparently fusing with lysosomes. We suggest that hydrolytic enzymes may be transferred from storage lysosomes, that have been activated by some unknown mechanism, to mitochondria when they come into contact. The small myelin-like regions in mitochondria might represent localized regions of autolysis. Subsequently, the autolysing mitochondria may coalesce with
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As maturation continues the cytolysomes may assume a polar position and give rise to the polar bodies seen in the mature erythrocytes (Tooze & Davies, 1965). Recently, lysosomal structures were found in mammalian reticulocytes (Lessler & Pack, 1964), and earlier workers using light microscopy showed that structures considered homologous to the polar bodies of amphibian erythrocytes occur in erythrocytes from at least some species of all classes of vertebrates (see reviews by Dawson, 1932; and Shinagawa, 1960). The lysosomal autolysis of mitochondria may therefore be a general feature of vertebrate erythropoiesis.

Recent studies (Marks et al. 1963; Rifkind et al. 1964; Mathias et al. 1964) of the changes in distribution of single ribosomes and polysomes during the maturation of rabbit reticulocytes have produced what appear to be discrepant results. Marks et al. (1963) showed that in thin sections of reticulocytes 80–90% of the ribosomes were in polysomes. Subsequently, Rifkind et al. (1964) found that as reticulocytes mature in vitro there is a decrease in protein-synthesizing capacity, in the total ribosome content, in the percentage of ribosomes in polysomes, and in the size of the polysomes, and a corresponding increase in the percentage of single ribosomes. In electron micrographs of nearly mature cells they found 70% single ribosomes and 30% polysomes. On the other hand Mathias et al. (1964), who also studied the distribution of polysomes in maturing rabbit reticulocytes, found that, although both the protein-synthesizing capacity of the cells and the total numbers of ribosomes decreased, the ‘reticulocytes containing very few ribosomes and presumably nearly mature, are found to contain the bulk of their ribosomes as polysomes’.

In the same newt spleen, cells with very few ribosomes like those studied by Mathias et al. (1964) and Marks et al. (1963) occur side by side. Until all the factors responsible for maintaining the formation and integrity of polysomes and single ribosomes are known it is difficult to interpret this observation. It seems, however, that at these late stages of maturation the total number of ribosomes in the cytoplasm cannot be used simply as an index of the maturity of the cell.

As Berman (1967) has noted, the literature contains conflicting reports about the rhopheocytotic theory of the uptake of iron as ferritin by erythroblasts. The evidence for rhopheocytosis in human bone marrow originally obtained by Bessis (1961, 1963) has been supported by the studies of Sørenson (1962) on bone marrow of humans with sideroblastic anaemia, of Grasso et al. (1962) on human and rabbit foetal liver and Orlic et al. (1965) on mouse spleen. Jones (1964, 1965), however, suggested that in human bone marrow rhopheocytosis is exceptional and the results of Zamboni (1965) and Berman (1967) also fail to support the rhopheocytosis theory. During our study of newt spleen we have seen only one basophilic erythroblast in which rhopheocytosis might have been occurring. We conclude therefore that in amphibian erythropoiesis rhopheocytosis is a very rare event, if it occurs at all.

The occurrence of dense granules in the nuclear sap regions, or interchromatin spaces, in the nuclei of many different cell types has often been reported (for example, Fawcett, 1955; de Robertis, 1956; Swift, 1959, 1963; Bernhard & Granboulan, 1963). These ‘interchromatin granules’ (Bernhard & Granboulan, 1963) are distinct from
the ‘perichromatin granules’ first noticed by Swift (1962a) and subsequently studied by Watson (1962a, b). The interchromatin granules we have seen are probably similar to the large granules, about 300 Å in diameter, that fill most of the interchromatin spaces in the nuclei of salamander pancreas cells (Swift, 1959). Hardly anything is known about the chemical composition and possible function of these granules (see also Davies & Tooze, 1966).

Nucleoli are lost at an early stage in erythropoiesis in mammals; according to Grasso et al. (1962) nucleoli could not be distinguished in polychromatic erythroblasts in mammalian foetal liver. In contrast they persist throughout amphibian erythropoiesis. As Dawson (1932) demonstrated, nucleoli occur in the erythrocytes of urodeles Necturus maculosus and T. viridescens. We have observed in the electron microscope nucleoli in mature erythrocytes of both the newt T. cristatus and the frog Rana esculenta and have shown that during erythropoiesis in the newt the amount of nucleolar granular material decreases until, in the mature erythrocyte, it is virtually absent, although the nucleolar fibrillar material persists. This change in nucleolar structure parallels the decrease in numbers of cytoplasmic ribosomes; the mature erythrocyte lacks ribosomes as well as nucleolar granular material. Our observations are consistent with the suggestions that the nucleolus is the site of manufacture of ribosomal nucleoproteins and that the nucleolar granules are the direct precursors of cytoplasmic ribosomes (Edstrom & Beerman, 1962; Swift, 1962b). However, the nucleolar granules, about 150 Å in diameter, are smaller than cytoplasmic ribosomes which are about 200 Å diameter.

According to recent experiments and current hypotheses, the chromatin of the interphase nucleus which is metabolically active in producing messenger RNA is in the extended state, condensed regions of chromatin being relatively inert in incorporating precursors into RNA (Hsu, 1962; Littau, Allfrey, Frenster & Mirsky, 1964). In the absence of labelling experiments we are not able to interpret directly our observations on the structural changes in the nuclei of newt erythroblasts during maturation. However, we can make some general comments. The predominant experimental observation in our material is that during the maturation of erythroblasts the small blocks of chromatin, in size at or below the resolvable limits of the light microscope, clump into larger units, a change which, if observed in the light microscope, might be interpreted as the condensation of extended chromatin. The question arises which part of the DNA is producing messenger RNA for haemoglobin synthesis, and/or ribosome synthesis. One possibility is that the small (approximately 2000 Å) blocks of chromatin, which probably represent sections through threads, are the active regions; if so they are considerably larger than either single DNA molecules or the active sites described by Littau et al. (1964) in thymus nuclei. However, at the stages we observe, synthesis of messenger RNA may have ceased, there being an earlier stage in which the small chromatin blocks are dispersed into still smaller units, in other words are in an extended state and are metabolically active. Since, however, Grasso et al. (1963) found a high incorporation of cytidine into RNA in basophilic erythroblasts from foetal rabbits this possibility is not very likely. Another possibility is that the light-staining zones of the nucleus represent the active regions of extended-state
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These zones, which do not appear to have been described previously, may show up clearly in this amphibian material because they contrast with the large amount of condensed chromatin present and the large number of interchromatin granules. Finally, it is possible that the actively transcribed DNA is represented by single molecules scattered throughout the nuclear sap, which could hardly be detected by electron microscopy. Further experiments are needed to resolve these problems relating nuclear morphology with activity.

We are greatly indebted to Professor Sir John Randall, F.R.S., for his continued encouragement, as well as to Professor Dame Honor Fell, F.R.S., Dr R. A. Rikkind and Dr B. M. Richards for discussions. We are indebted to Dr W. Jacobson of the Strangeways Research Laboratory for discussion and advice and to Dr L. M. Franks of the Imperial Cancer Research Fund, London. We gratefully acknowledge technical help from Miss Pamela Rush, Miss Margaret Blade and Mrs Freda Collier.

REFERENCES


Erythropoiesis in newt spleen


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Abbreviations on plates

- be: basophilic erythroblast
- c: cytoplasm
- ce: centriole
- ch: chromatin
- e: erythrocyte
- er: endoplasmic reticulum
- fe: ferritin
- fi: fibrils
- fz: fibrillar zone
- gi: interchromatin granules
- go: Golgi material
- gz: granular zone
- he: heterochromatin
- l: lymphocyte
- ly: lysosome
- lz: nuclear light-staining zone
- m: mitochondrion
- mt: microtubules
- nu: nucleolus
- pe: polychromatic erythroblast
- ps: polysome
- r: ribosome
- rc: reticular cell cytoplasm
All cells except Fig. 28 are from newt spleen and unless otherwise stated were fixed in glutaraldehyde and OsO$_4$, stained with uranyl acetate followed by lead citrate and embedded in Araldite.

Fig. 8. Light micrograph of a cell, probably a haemocytoblast, in an air-dried smear of newt spleen, stained with MGG stain. The cytoplasm was stained blue and the nucleus purple. $\times 1200$.

Fig. 9. Light micrograph of a basophilic erythroblast in an air-dried smear of newt spleen stained with MGG stain. The nucleus stained blue and the cytoplasm deep blue. $\times 1200$.

Fig. 10. Light micrograph of a polychromatic erythroblast in an air-dried smear of newt spleen stained with MGG stain. The cytoplasm stained grey. $\times 1200$.

Fig. 11. Light micrograph of a mature erythrocyte in an air-dried smear of newt spleen stained with MGG stain. The cytoplasm stained orange (eosinophilic). The nucleus is very condensed. $\times 1200$.

Fig. 12. Electron micrograph showing a basophilic erythroblast, a polychromatic erythroblast, a mature erythrocyte and lymphocytes side by side in a venous sinus. The basophilic erythroblast is closely associated with part of the reticular framework and reticular cell cytoplasm. $\times 5250$. 
Fig. 13. Electron micrograph of a basophilic erythroblast. The nucleus has a large nucleolus and a nuclear light-staining zone. Interchromatin granules are prominent in the nuclear sap regions. The cytoplasm contains numerous free ribosomes, mitochondria, lysosomes and fibrillar material. Arrows indicate pores in the nuclear envelope. $\times 18000$. 
Fig. 14. Part of a basophilic erythroblast showing a transversely sectioned centriole and associated Golgi apparatus. The nucleus has a large nucleolus, prominent interchromatin granules and nuclear light-staining zones, and there are pores in the nuclear envelope (arrows). × 17000.

Fig. 15. A longitudinally sectioned bundle of microtubules in the cytoplasm of a basophilic erythroblast. × 59000.

Fig. 16. Transversely sectioned microtubules in the cytoplasm of a basophilic erythroblast. The tubules are about 230 ± 20 Å in diameter. × 76000.

Fig. 17. A cluster of lysosomal bodies and mitochondria in the cytoplasm of a polychromatric erythroblast. The nuclear envelope of this cell is poorly preserved. Fixed in OsO₄ alone, embedded in Araldite, stained in uranyl acetate and lead citrate. × 16500.
Fig. 18. Electron micrograph of the nucleolus in the cell shown in Fig. 14. The nucleolus consists of a fibrillar zone made up of fine fibrils about 40 Å in diameter and small granules of similar diameter; this zone is more-or-less surrounded by a granular zone consisting of granules about 150 Å in diameter embedded in a matrix material. There is no visible nucleolonema. × 60,000.

Fig. 19. Detail of the cytoplasm of the cell shown in Fig. 13. This shows a bundle of fibrils, each about 70–80 Å in diameter, in a basophilic erythroblast. × 120,000.
Fig. 20. Electron micrograph of a polychromatc erythroblast. Most of the chromatin is condensed and clumped into large aggregates. A nucleolus is present together with numerous interchromatin granules. In the cytoplasm the ribosomes are dispersed and polysomes can be identified. A remnant of a Golgi apparatus is present. Some of the mitochondria appear closely associated with the nuclear envelope. \( \times 17000 \).
Fig. 21. Electron micrograph of part of the cytoplasm of a polychromatic erythroblast, showing large polysomes, aggregates of up to about 20 ribosomes, which are surrounded by a peripolyosome space. Fixed in OsO$_4$ alone, embedded in Epon, stained in uranyl acetate and lead citrate. $\times 59000$.

Fig. 22. Electron micrograph of a vesicle, containing electron-dense particles interpreted as ferritin, in the cytoplasm of a polychromatic erythroblast. This micrograph also shows ribosomes surrounded by a periribosome space, a mitochondrion, and chromatin consisting of loosely packed, coarsely fibrillar material. Fixed in OsO$_4$ alone, embedded in Araldite, stained in uranyl acetate, and lead citrate. $\times 54000$.

Fig. 23. Electron micrograph of a whorl of 17 or 18 ribosomes on the surface of membrane material, possibly nuclear envelope, in a late polychromatic erythroblast. Some of the ribosomes (arrowed) in this figure and Fig. 24 show substructure, appearing to consist of two subunits. $\times 144000$.

Fig. 24. Micrograph showing another whorl of ribosomes. $\times 144000$.

Fig. 25. Electron micrograph of a nearly mature erythrocyte in the spleen. A small nucleolus is visible. $\times 5100$.

Fig. 26. The nucleolus shown in Fig. 25 at higher magnification. It consists almost exclusively of fibrillar material; there is only a very small amount of granular zone. $\times 54000$. 
Fig. 27. Electron micrograph of a mature erythrocyte in the spleen. There are very few ribosomes in the cytoplasm. The nucleus contains condensed and clumped chromatin. In this cell the surfaces of the chromatin masses are very irregular with numerous fine projections. A few interchromatin granules occur in the nuclear sap regions. $\times 17000$.

Fig. 28. A remnant of the Golgi apparatus in the cytoplasm of a mature erythrocyte from the circulating blood of *Triturus granulosus*. Fixed in OsO$_4$ alone, embedded in Epon, stained in uranyl acetate and lead citrate. $\times 57000$. 
Fig. 29. A lysosome adjacent to a mitochondrion in the cytoplasm of a basophilic erythroblast. Electron-dense particles interpreted as ferritin are present in the lysosome. × 60,000.

Fig. 30. A lysosome adjacent to a mitochondrion in the cytoplasm of a basophilic erythroblast. The mitochondrion contains regions with regular cristae and regions of loosely packed whorls of membrane material (arrow). × 40,000.

Fig. 31. A detail of Fig. 17, showing what appears to be the fusion of a mitochondrion and a lysosomal body. × 57,000.

Fig. 32. Cytolysomes (polar bodies) in a mature newt erythrocyte. Note the almost complete absence of ribosomes in the cytoplasm. × 18,000.
Fig. 33. A light micrograph taken in violet light, 395 μm, of the 1-μ thick section containing cells A, C, D, E and F. The absorption spectra of these cells are shown in Fig. 6A–F, respectively. × 400.

Fig. 34. A light micrograph taken in violet light, 395 μm, of a different field of the 1-μ thick section shown in Fig. 33. Cell B, a basophilic erythroblast, is shown. The absorption spectrum of this cell is shown in Fig. 6B. × 400.

Fig. 35. An electron micrograph of cell F, a late polychromatophilic erythroblast, shown in Fig. 33. × 4800.

Fig. 36. An electron micrograph of part of the cytoplasm of the basophilic erythroblast (cell C) shown in Fig. 33. × 24,000.