Tissue degeneration: an electron microscopic study of the pronephros of *Rana temporaria*

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**SUMMARY**

The structure of the non-degenerate and degenerating pronephros, of larval *Rana temporaria*, at different stages of the metamorphic cycle, was examined by electron microscopy.

Extra-renal cellular components in the circulation were likewise investigated, and particular consideration was given to their relationships with the pronephric tubules whose regression would seem to be bound up with them.

The activity of lysosomes in pronephric tubule degeneration suggests that they are an important component of the degeneration process, which probably embraces autolysis and phagocytosis.

**INTRODUCTION**

The structure of the adult amphibian mesonephros was the first to be investigated by electron microscopy in *Xenopus laevis* and *X. muelleri* (Bargmann, Knoop & Schiebler, 1955), *Rana pipiens* (Karnovsky, 1962, 1963), *Batrachoseps attenuatus* (Christensen, 1963) and *R. esculenta* (Linss & Geyer, 1964; Geyer & Linss, 1964). Soon afterwards the larval functional pronephros was similarly described by Christensen (1964) in *Ambystoma opacum* and briefly by Lehmann (1967) in *Triton alpestris*; the larval mesonephros of *X. laevis* has recently been examined by Spannhof & Jonas (1968).

Pronephric degeneration in *R. pipiens* and *R. temporaria* has earlier been described, using light microscopy (Jaffee, 1954; Fox, 1962a, b, 1963). The present account, however, may be the first to deal, by electron microscopy, with pronephric cell death. Such an organ is relatively less complex than, say, a whole tail, and would seem ideal for a study of cellular degeneration.

The investigation here deals with the ultrastructure of the functional and the degenerating pronephros throughout ontogeny. Pronephric necrosis was found to be a disorganized process, of variable rate and time of origin in different regions of the tubules and throughout metamorphic climax, though well-recognizable patterns of degeneration are common. Furthermore, a similar study was made on the relationship which exists between the pronephros and certain extra-renal circulatory leucocytes, together with other ‘dark cells’, probably macrophages of unknown origin. It would seem likely that autolysis and phagocytosis are jointly implicated in the necrotic process.

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MATERIAL AND METHODS

Pronephroi of *Rana* larvae were investigated when (a) in a well-developed, functional, non-degenerate condition and (b) at the stage just preceding the onset of degeneration, and (c) at the height of the period of pronephros cell death (normally lasting about 1 week) (larval stages 45, 48/49 and 51-54 respectively, Cambar & Marrot, 1954; see Fox, 1962a, 1963). The pair of pronephroi (extirpated from non-anaesthetized larvae in tap water, whose brains were destroyed), were fixed (ice cold) immediately after operation for about 2 h in either 2 % osmium tetroxide, buffered with veronal acetate at pH 7·4 (Palade, 1952), or in potassium permanganate (Luft, 1956). All other fixatives tested after extended trials (glutaraldehyde, acrolein, paraformaldehyde-glutaraldehyde of Karnovsky, 1965) were found to distort or damage pronephric tissue to a greater or lesser extent. After washing with buffer for 10 min, specimens were dehydrated over a period of 3 h in successively increasing concentrations of cold alcohol (at least two changes, at 8 °C), and then embedded in Araldite. For preliminary investigation by ordinary light or by phase-contrast microscopy, Araldite sections cut at approximately 1 μ were stained with toluidine blue. Ultra-thin, silver-grey sections (60–90 μ thick) were obtained on a Huxley ultramicrotome, cut with a Dupont diamond knife and mounted on uncoated Athene new 200 mesh (Coslett design) or on hexagon grids. Sections were stained for 3 h with saturated uranyl acetate in 50 % alcohol, followed by lead citrate for 10 min (Reynolds, 1963), and viewed under an EM 6B AEI electron microscope.

The localization of acid phosphatase (used as a lysosome marker) in pronephros tissue was determined by the method of Miller (1962). Even though there is some distortion or tissue damage due to the use of glutaraldehyde in this method, nevertheless various organelles within the pronephric cells, especially in degenerating regions, are in many cases still clearly recognizable.

RESULTS

Pronephric tubules at stages 45 and 48/49

Cells of the pronephros of larvae of *Rana temporaria* are strikingly similar to those of *Ambystoma opacum* (Christensen, 1963) and *Xenopus laevis*. All clearly resemble amphibian larval and adult mesonephric tubules (Linss & Geyer, 1964; Geyer & Linss, 1964) and kidney tubules of higher vertebrates (Anderson, 1960; Maunsbach, 1966; Salzgeber & Weber, 1966; Ericsson, 1967).

In *Rana* cross-section of a proximal (microvillous) tubule reveals an outer layer of collagen fibres (not always present in younger stages but well developed in older ones) which extend in three dimensions (Fig. 1B). Within this the plasma membrane forms a network of infoldings (or intussusceptions of the
Electron microscopy of Rana pronephros

Electron microscopy of Rana pronephros; see Pease, 1955) whose compartments may contain mitochondria or small vesicles (Figs. 1B, E, 3A). This network presumably provides a large peripheral surface area which may facilitate transfer of substances of essential nature into the post-cardinal circulation, thenceforth to be retained by the larva.

Intercellular membranous junctions, variable in width from 400 to 1200 Å, lead inwards from the periphery. Sometimes they are almost straight but often they are extremely infolded and wavy in outline. Rows of small vesicles frequently associated with them (Fig. 1A, E) may be either 'breakdowns' or merely sections of the infoldings (Rhodin, 1963). Doubtless the latter enlarge the surface area between adjacent pronephric cells. Large numbers of mitochondria, rounded, irregular or elongated in form, occupy the more peripheral regions of the tubules. Mitochondrial population density tends to diminish on proceeding towards the lumen; in the denser regions calculation showed over 15000 per mm² of cell surface. Their average diameter, when in a non-degenerate, functional state is about 1-3 μ, although elongate or dumb-bell-shaped examples up to 3-5 μ long were recognized.

Each mitochondrion has a structure similar to that described by Whittaker (1966). Cristae are about 500 Å wide and extend in various directions; they may be seen extending practically right across the mitochondrion or merely as small rounded vesicles in transverse section (Fig. 1A, B).

The pronephric intercellular junctions separate nuclei whose largest diameter recorded was about 11-7 μ (see Fox, 1961). The finely granular nucleoplasmic chromatin is in continuity with the cytoplasm via numerous small nucleopores, each about 800–1600 Å wide, and separated from each other by distances of from 4000 to 8000 Å. Spaces separating the peripheral nuclear membranes may be fixation artifacts (Fig. 1A).

Within the nucleus a central nucleolus, of denser granular material, is frequently recognized. Golgi networks are rarely seen alongside the nucleus with any degree of clarity. In Xenopus larval tail skin, preserved and stained the same way, they are well developed and clearly distinguishable. Perhaps they are not a pronounced feature of Rana pronephric cells. They are described but not figured prominently by Christensen (1964) in the pronephros of Ambystoma. Desmosomes are often seen, especially near the lumen of the tubules, separating adjacent cells.

Bounding the lumen and extending into it are the microvilli (Figs. 1D, 4A, F), about 6000 of them situated side by side per mm length of the tubule margin. They are about 2 μ long and about 1000 Å wide, shorter than the average of 3 μ for the microvilli of Ambystoma, though Christensen (1964) did emphasize the variable height of the brush border; microvilli contain similar endoplasmic reticulum to the rest of the cell. Frequently two of them originate proximally from a common base. Between villi are seen the micropinocytotic invaginations (averaging about 3500 Å deep by 700–3000 Å wide), which ultimately become rounded cytoplasmic vesicles by pinching off of the lateral membrane; see Pease, 1955) whose compartments may contain mitochondria or small vesicles (Figs. 1B, E, 3A). This network presumably provides a large peripheral surface area which may facilitate transfer of substances of essential nature into the post-cardinal circulation, thenceforth to be retained by the larva.

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EXPLANATION OF FIGURES

All illustrations, either by electron or light microscopy, are of larvae of *Rana temporaria*. Figures 2 (A–C) and 4 (A) are of Araldite sections (1μ thick approx.) stained with toluidine blue. Figures 1 and 3–6 are of ultra-thin sections (60–90μ thick), stained with uranyl acetate (Fig. 5B) and all the others uranyl acetate and lead citrate. All illustrated specimens were those fixed in Palade fixative.
cytoplasmic walls (Fig. 1D). This is probably a method of absorbing water and other molecules into the cell from the lumen fluid (Pease, 1955).

Small electron-dense membrane-bound bodies (about 4000 Å across) are often seen between the mitochondria. Possibly they are emulsified fat droplets (Palay & Karlin, 1959) (Fig. 1A, C). The rest of the cell comprises profiles of rough and smooth endoplasmic reticulum. Small groups of ribosomes, or polysomes, are present in all Palade-fixed material, but the ribosomal content is not high. The overall appearance is of a tissue concerned with absorption and of energy expenditure but not with protein synthesis or secretion.

The distal tubule is generally similar in appearance to the proximal tubule (without the microvilli), though the intercellular junctions seem to be less infolded (Figs. 1E, 2A). Dense small granules, probably glycogen, were often recognized at the lumen margin.

A short intermediate ciliated segment is situated between the common proximal and the distal tubules (see Christensen, 1964) (Fig. 1C). Ciliary striated rootlets show a web-like appearance in the subluminal cytoplasm (Fig. 1C). The average transverse diameter of pro- and mesonephric tubule cilia is about 2500 Å. Sometimes, in transverse section, all the nine pairs of peripheral strands in each cilium appear hollow, or light in texture, but in other cases all the pairs may each comprise a dense solid and a hollow strand. The central pair of strands is always hollow. These descriptions could, however, represent different

ABBREVIATIONS ON FIGURES

bb, Brush border; bm, basement membrane; c, cytolsyme; cl, cilia; co, collagen layer; cr, crista of mitochondrion; eve, crista vestige; db, degeneration body; dc, dark cell; den, degenerating nucleus; dn, dark cell nucleus; dt, distal tubule of pronephros; e, erythrocyte; ef, emulsified fat droplet; en, endothelial cell nucleus of blood vessel; er, endoplasmic reticulum; g, granulocyte; gr, granule of granulocyte; ij, intercellular junction; lc, lymphocyte-type cell; lg, lipid granule; lu, lumen of pronephric tubule; ly, lysosome; m, mitochondrion; mv, microvillus of tubule; mve, mitochondrial vestige; n, nucleus of tubule; nbv, nucleus of blood vessel; ng, nucleus of granulocyte; nl, nucleus of lymphocyte; np, nucleopore; pi, plasma infolding of basement membrane of tubule; pm, polymorphonuclear leucocyte; ps, pronephric strand; psc, pronephric strand cortex; psm, pronephric strand medulla; pt, pronephric tubule; pv, pinocytotic vesicle; pve, pronephric vestige; pxt, pronephric proximal tubule; v, vacuole.

FIGURE 1

A, at stage 48/49; B, D, E, at stage 45/46; C, at stage 51

(A) Region of wavy intercellular junction in between adjacent pronephric cells.

(B) Peripheral plasma membranous infoldings, below collagen layers of two adjacent pronephric tubules. The lower one is a ciliated and the upper a microvillus tubule.

(C) Ciliated tubule, probably of the intermediate segment.

(D) Brush border showing micropinocytotic vesicles.

(E) Cross-section of region of pronephric distal tubule.
regions, or levels, along a homogeneous population of cilia. Each central strand is about 250 Å in diameter; the outer pairs, situated closer together, have an overall diameter of about 400 Å. Their background tissue is less dense, with no clear structural differentiation. Cilia are seen either in whorls or parallel to one another within the lumen. Whether the former is a normal arrangement at some stage of ciliary activity cannot yet be decided.

The three nephrostomal tubules (Fox, 1962a), which lead from the coelom, via nephrostomes, to the proximal microvillous tubules, are usually damaged when the pronephros is extirpated. However, they would seem to be generally similar to those of the intermediate segment.

Pronephric degeneration at stages 51–54 (climax)

Degeneration of the pronephros begins after stage 49 (Fox, 1962a) when the level of circulatory thyroid hormone reaches its peak (Etkin, 1964) (Fig. 2 A–C). Some of the tubule regions of older larvae are often rather less degenerate than those at earlier stages of climax. Again at climax some tubules have a lumen when others are solid strands; adjacent renal cells differ in their degree of necrotic progress; even within the same cell similar organelles are hardly or wholly degenerate (Figs. 3D, 5D).

A typical feature of pronephric tubule cells at climax is the presence of degeneration bodies (Figs. 3A–D, 5A) which may correspond to either enlarged cytosomes or, more probably, groups of them (Novikoff, 1963; Novikoff & Essner, 1962); that is, to vacuoles in which mitochondria and other organelles are undergoing lysosomal hydrolysis (Fig. 3C). They are occasionally present at stage 48/49 (late prometamorphosis of Etkin, 1964) (Fig. 3A), and small cytosomes can occasionally occur in younger, non-degenerate, functional tubules.

Degeneration bodies occur at all levels of the pronephric cell, often in close vicinity to the cell nucleus (Fig. 3D). They represent specific centres of intense, chronic cellular necrosis, of variable form and size. The criterion is qualitative and arbitrary, being the degree to which various cellular components have retained their recognizable structure and organization in a particular cell region. Usually a degeneration body is rounded or oval in outline, often apparently quite discrete and enveloped by several peripheral membranes. They may measure up to 8-5 μ in diameter, while some of the smaller ones comprise merely a few degenerating mitochondria, i.e. are typical small cytosomes.

Figure 2

(A) Stage 45/46. Functional non-degenerate pronephric tubules and inter-tubular blood cells.

(B) Stage 51/53. Degenerate pronephric strand and some almost digested pronephric tissue amid extra-rerenal cells.

(C) Stage 51/53. Highly degenerate vestigial pronephric tissue submerged amid masses of extra-renal circulatory cells. The recognizable granulocytes are particularly abundant.
Electron microscopy of Rana pronephros
former often include several separate and clearly recognizable smaller degeneration bodies, whose individuality is enhanced by the presence of their own bounding membranes (Fig. 3C), an arrangement almost akin to emboitement. It is likely that as a necrotic area becomes more extensive, it can incorporate at its periphery other cytolysomes or intercellular junctional membranes or those of the tubule peripheral complex of infoldings, or indeed those from degenerating mitochondria or from the endoplasmic reticulum. In contrast Ashford & Porter (1962) suggested that the limiting membranes of cytolysomes may form de novo, in order to separate off such centres of necrosis from the rest of the cell.

A degeneration body usually includes mitochondria in various stages of degeneration (similar to those which may degenerate independently). Sometimes the less degenerate ones are situated at the periphery of the necrotic area (Fig. 3C); at other times they are hardly recognizable (Fig. 3B). Vesicles, myelin figures and a variable number of round, electron-dense, osmiophilic bodies (sometimes adjacent ones are fused together) are also present, but without separate recognizable membranes; they stain heavily with lead. The degeneration body includes a background matrix of more or less disorganized granular composition. Though Miller's technique for acid phosphatase (because of its glutaraldehyde content) was not entirely satisfactory in the case of the pronephros of Rana, nevertheless extensive tests were sufficient to suggest strongly that the dense inclusions are rich in acid phosphatase, especially at their periphery (Fig. 5B). They are thus probably centres of lysosomal enzymes (Novikoff, 1963; de Duve, 1963), known to degrade mitochondria (Tappel, Sawant & Shibko, 1963).

Free lipid granules (0.6–0.7 μ in diameter) of irregular outline occur, especially near degeneration bodies (Figs. 3B, 5A). Though not seen in large numbers at any time they are also present in functional pronephric tubules (stage 48/49), sometimes closely associated with neighbouring mitochondria. Within a degenerating tubule and apart from the degeneration bodies some mitochondria still retain cristae (Fig. 5D), others are rounded membrane-bound bodies of undifferentiated tissue and others are shrunk and often devoid of inner tissue but still retain remnants of cristae as peripheral projections, though the outer double membranes are still present. Mitochondria of similar appearance tend to occur in groups. Many large vacuoles may well be derived by fusion of adjacent degenerate mitochondria.

Pronephric nuclei, often highly degenerate, dense and shrunken (Fig. 4B), may appear normal even in degenerate tubules (Fig. 3D). Likewise in degenerating proximal tubules the brush border and pinocytotic vesicles are often still present and apparently normal in appearance, at least in the first half of climax (Fig. 4F), and cilia too are retained at climax stages (Fig. 1C). The basement membrane, together with its overlying collagen layer, tends to remain until near the final stages of tubule degeneration when it disappears. The peripheral
Electron microscopy of Rana pronephros

FIGURE 3

A, at stage 48/49; B–D, at stage 51/53

(A) Large degeneration body in the peripheral region of a pronephric tubule of a late prometamorphic larva. The tubule was sectioned at a solid end region, but it is probably microvillous. Parts of a ‘dark cell’ and lymphocyte-type cell are seen outside and against the tubule surface.

(B) Large degeneration body. At least some of the electron-dense bodies are lysosomal.

(C) Large degeneration body showing aggregation of cytolysomes (arrowed); some of the small, round, dense bodies are probably lysosomes. Less degenerate mitochondria are situated in the peripheral region.

(D) Topographic relationships of a degeneration body to its cell nucleus.
(A) Portion of the brush border of a microvillous tubule.
(B) Pronephric nuclear degeneration.
(C) Lymphocyte-type cell against a pronephric tubule.
(D) Erythrocyte. (E) Granulocyte.
(F) Non-degenerate brush border of a proximal tubule still present at climax, emphasizing the variability of the rate and onset of necrosis.
(G) Lymphocyte and polymorphonuclear leucocyte, between degenerating pronephric tubules.
(H) Azurophilic lymphocyte.
Electron microscopy of Rana pronephros 149

plasma infoldings are greatly reduced in number in older tubules (Figs. 5A, 6A, B), and eventually are lost. Degenerate tubules often still show a lumen; others become solid strands, comprising a less dense ‘cortical’ region and a denser ‘medulla’ of cellular debris and poorly organized structure (Figs. 5C, 6B). Possibly the latter originates by breakdown of the inner tubule wall and accumulation within the lumen of products of necrosis. The failure to recognize cellular organelles in these strands suggests that here we have tissue in an advanced state of degeneracy. The scarcity of degeneration bodies may perhaps be explained by the fact that peripheral bounding membranes, which help to give them form, are not present.

Ultimately pronephric tissue becomes unrecognizable amid a mass of extra-renal cells; gradually it is reduced in amount and finally by the end of climax it has disappeared (Fig. 2B, C).

Extra-renal cells associated with the pronephros

In young larvae pronephroi are enveloped by vessels of the post-cardinal sinus, whose walls are composed of extremely flattened, delicate endothelia, closely apposed to the outer surfaces of the pronephric tubules, simulating an outer tubule membrane. In older larvae, and in particular at climax, these vessel membranes are not recognizable and they may have broken down to degenerate simultaneously with the pronephric system. Various blood components are thus in close association with and have access to the tubule outer surface, either with or without the intervention of a blood vessel wall.

The fact that large numbers of blood cells are present between tubules after surgical extirpation of the pronephros implies that this procedure does not seriously disturb any anatomical pronephric-vascular relationships which existed in vivo (Fig. 2A–C).

The nucleated erythrocytes are distinguished primarily by their electron-dense nucleus and cytoplasm, the latter containing only few mitochondria (Fig. 4D); erythrocyte cytoplasm of the adult frog consists predominantly of haemoglobin (Davies, 1961). They are flattened cells whose round nucleus bulges outwards on either side. Nucleopores provide communication between nucleus and cytoplasm. The maximum nuclear diameter recorded was 8.5 μ. Blood smears from old larvae and of adult Rana and Xenopus confirm the high preponderance of erythrocytes to leucocytes (see Foxon, 1964); the ratio of erythrocytes/leucocytes is between 20 and 70 and adult Rana has 400000 erythrocytes per mm³ of blood (Stephan, 1954). In Rana temporaria there are about 300000 erythrocytes/mm³ of blood in adult females and 450000/mm³ in adult males, with comparable differences, though the totals are slightly smaller, in Bufo vulgaris (Arvy, 1947). Nevertheless numerous leucocytes are found amid young (stage 45) and climactic pronephric tubules, and the ratios of red to white cells here would appear at variance with those described for the general circulation (Fig. 2A, C).
All Figs. from specimens at climax; B (stages 49–53); A, C–E (stages 51/53); F (53/54).

(A) Several centres of intense necrosis (degeneration bodies) in a degenerating pronephric strand; probably replete with lysosomes. A highly vacuolated 'dark cell', together with its nucleus, envelopes the outer region of the degenerating strand.

(B) Lysosome bodies (after incubation, Miller, 1962) showing positive reaction for acid phosphatase, especially at the periphery. They were located in the central (medulla) region of the pronephric strands, where heavy necrosis occurs. Control pronephros, incubated without $\beta$-glycerophosphate did not show any acid phosphatase deposition.

(C) Microvillous tubule now an almost solid strand and highly degenerate, with an outer cortical and inner medullary region. The electron-dense bodies may well be lysosomes.

(D) Degenerating mitochondria.

(E) Detail of portion of vacuolated 'dark cell' cytoplasm seen in Fig. 6B.

(F) 'Dark cell' from region between mesonephric tubules of larva.
Electron microscopy of Rana pronephros

FIGURE 6

(A) Stage 51/53. Lymphocyte-type cell fused with the peripheral collagenous surface of a degenerating tubule. $lcp =$ lymphocyte cytoplasmic process.

(B) Stage 51/53. 'Dark cell' cytoplasm within a pronephric strand in the 'cortical region' below the surface.
Numerous lymphocyte-type cells (found between pronephric tubules) have a clear vacuolated cytoplasm with small rounded mitochondria and a large nucleus. Many of them, which include small dense azurophilic granules (Galle & de Montera, 1962), could well be young or intermediate stages of granulocytes. The largest found measured about $12 \times 9 \mu m$ and the nucleus $8 \times 6 \mu m$ (Fig. 4H).

The granulocytes, probably the most common leucocyte, have large, round, extremely dense granules (which seem to be hollow or at least have a less dense core) situated within cytoplasmic vesicles (Figs. 4E, 6A). The largest granules in both stage 45 and at climax were up to $1.5 \mu m$ in diameter, and cell size is about the same as that of the azurophiles.

Agranular lymphocytes with a large nucleus (size $8.5 \times 7.5 \mu m$) and relatively less cytoplasm ($10 \times 9 \mu m$) (Figs. 4C, G, 6A) often appeared in most intimate tactile association with young functional and older degenerating pronephric tubules, though numerous granulocytes and erythrocytes are also found alongside the latter. In some cases lymphocyte pseudopodia are closely bound against the tubule outer collagenous layer, and here the surface membrane of the lymphocyte seems to merge with that of the tubule, so as to be indistinguishable (Fig. 6A). The other lymphocyte-type azurophiles are also seen in close association with the tubules.

Characteristic 'dark cell' tissue, often nucleated, is recognizable mainly at climax. The flattened, dense, almost tentacular cytoplasm contains mitochondria and many digestive vacuoles enclosing dense inclusions. The tissue is charged with numerous small granules, perhaps of ribosomal nature (Figs. 3A, 5E, 6B). Cytoplasmic extensions are seen either alongside the outer periphery, or at climax situated at all levels within pronephric cells or between adjacent ones—possibly one route of invasion. Frequently 'dark cell' tissue is found situated in close association with centres of intense pronephric degeneration. It was never seen within younger preclimactic, non-degenerating pronephric cells. A large nucleus of a 'dark cell' found between mesonephric tubules of a late-stage larva measured $8.5 \mu m \times 3.5 \mu m$ wide (Fig. 5F).

Other recognizable extra-renal circulatory components include polymorphonuclear leucocytes (whose cytoplasm is generally similar in appearance to that of the azurophilic cells, Fig. 4G), fibroblasts presumably derived from associated connective tissue of the body wall and mesentery, and cells with spherical nuclei and little cytoplasm, which could be haemocytoblasts but more likely are nuclei of ruptured blood vessels.

**DISCUSSION**

The pattern of pronephric necrosis in larvae of *Rana* is generally similar, though variable, throughout metamorphic climax. Different tubules, or even regions of the same tubule, often appear only slightly degenerate, while others show intense necrosis. The degeneration bodies likewise are variable in form,
Electron microscopy of *Rana* pronephros

doubtless due to a lesser or greater degree of organelle destruction. Similar ‘corps denses’, composed of membranous elements and other more electron-dense homogeneous material, were described by Salzgeber & Weber (1966) in mesonephric tubules of the embryo chick. Only the ‘corps dense à structure membraneuse’ gave a positive reaction for acid phosphatase however; a positive reaction for particulate inclusions in the degeneration bodies of *Rana* is also strongly indicated.

Mitochondria would seem to degenerate in a similar manner in the anuran pronephros, tail muscle of *Xenopus* (Weber, 1964), the mesonephroi of chick soon after hatching (Salzgeber & Weber, 1966) and in hamster cancer cells infected with Krebs’ virus (Thomas, 1965). There is disorganization of the cristae with vestiges of dense granules at the periphery. The fact that tissue within the mitochondrial bounding membranes regresses before the membranes suggests that such membranes are more resistant than cristae to hydrolysing enzymes. Such intra-mitochondrial degeneration may implicate autolytic phenomena, mediated perhaps by lysosomal enzymes in the vicinity, which would diffuse into the mitochondria.

Pronephric cellular necrosis, though a widespread activity throughout the tubules, seems to originate (at least in many cases) from a number of separate and localized areas, seen particularly in the case of degeneration bodies. This feature could well be the result of accumulations of catabolic enzymes, derived from lysosomes, originating primarily at a number of separate loci, each centre of activity spreading outwards rather than diffusing haphazardly throughout the cell. Perhaps some centres overlap, giving rise to the degeneration complexes previously described. The less-degenerate mitochondria would therefore be expected to be found at the periphery of such degeneration centres. The origination and further activity of a degeneration centre would therefore be governed by the stability of the lysosomal membrane and the release of lysosomal enzymes, the former able to be influenced by stimuli from a variety of sources (see Fox, 1965). Weber (1964), however, considered that in tail degeneration of *Xenopus* primary lysosomes are not recognized during initial regression. He envisages muscle first undergoing autolysis without the intervention of lysosomal enzymes.

It is known that thyroxine can modify the structure and cause swelling and affect the functional activity of mitochondria, at least in some mammalian tissues (see Pitt-Rivers & Tata, 1959, pp. 120–123). Whether regressing mitochondria in degenerating pronephroi of *Rana* larvae are swollen is a moot point; most of them do not appear so when compared with non-degenerate ones. The question as to whether the circulating thyroid hormonal level is the primary stimulus eliciting pronephric necrosis cannot be settled in this investigation. The fact that thyroid hormones, at different circulatory concentrations, are responsible in some way for so many diverse tissue reactions in anuran larvae suggests that their action could well activate other metabolic processes or enzyme
systems during larval development. Until further experimental evidence is available the view that diverse changes of metamorphosis are predetermined, and are triggered off by thyroid hormone (Pitt-Rivers & Tata, 1959), is probably the most reliable hypothesis still extant in amphibian larval development. All pronephric tubules are closely associated with extra-renal cells, which in most cases have access to their outer surfaces. Light and electron microscopy show a high proportion of granulocytes, together with numerous lymphocyte-type agranulocytes, azurophilic leucocytes and erythrocytes in the inter-tubular spaces, leucocytes seeming especially more numerous amid older degenerating pronephric tubules. Only the ‘dark cells’ often associated with centres of intense pronephric regression were seen within climactic pronephric cells, at least when the pronephric tissue retained a tubule or strand-like appearance. Such invasive cells may well be able to digest living tissue, but more likely they phagocytose necrotic cells and ingest cell debris.

Weber (1964) recognized macrophages in degenerating tails of *Xenopus*, probably derived from mesenchymatous cells of connective tissue. They contained well-marked inclusion bodies, those showing a positive reaction for acid phosphatase being regarded as phagosomes.

The appearance of the degenerating pronephros and its involvement with leucocytes resembles the homograft reaction which is known to occur in amphibian larvae (Hildemann & Haas, 1959, 1962; Hildemann & Cooper, 1963; Cooper, Pinkerton & Hildemann, 1964). In *Rana pipiens* larvae lymphocytes congregate in profusion below a foreign graft during rejection (Bovbjerg, 1966). It has been suggested that the small lymphocytes and perhaps eosinophil granulocytes are mainly responsible for homograft recognition and destruction in larvae of *Rana catesbeiana* when the latter develops an iso-immune response (Hildemann & Haas, 1959; Hildemann & Cooper, 1963). Anuran larval lymphocytes (and perhaps granulocytes) can thus become immunologically competent. It is indeed tempting to suggest that during normal larval ontogeny, in prometamorphosis, *Rana* in addition to developing an immunity mechanism also acquires a specific auto-immunity to its own pronephric system, which at climax is rejected in some way: perhaps involving among other things autolysis, phagocytosis and the activity of lysosomal enzymes, mediated in some way through the agency of sensitized leucocytes and macrophages.

Such a hypothesis, though no more than tentative, may provide a basis for any future vigorous experimental analysis on this particular aspect of organ necrosis.
Electron microscopy of Rana pronephros

RÉSUMÉ

Dégénérescence tissulaire: étude en microscopie électronique du pronephros de Rana temporaria

On a examiné, en microscopie électronique, la structure du pronephros normal et en dégénérescence de tétrads de Rana temporaria, à divers stades du cycle de métamorphose.

On a de même examiné les composants cellulaires extra-rénaux dans la circulation, et on a considéré en particulier leurs relations avec les tubules pronéphrétiques dont la régression semblerait leur être liée.

L’activité des lysosomes dans la dégénérescence des tubules pronéphrétiques suggère qu’ils représentent une composante importante du processus de dégénérescence, qui englobe probablement autolyse et phagocytose.

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REFERENCES


Electron microscopy of Rana pronephros


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