Ultrastructure of diploid and haploid cells of *Xenopus laevis* larvae

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SUMMARY

A study was made of tissues of diploid and haploid *Xenopus laevis* larvae by electron microscopy. The comparison of the two types of larvae was both quantitative and qualitative. Haploid cells contain fewer similarly sized mitochondria and/or cilia (compared with the diploid), commensurate with their smaller cell size. Cellular differentiation is retarded in haploids and the degree of retardation is reflected in the retention of cellular lipid and yolk. It is suggested that the failure to differentiate adequately is an important component in the development of the haploid syndrome.

INTRODUCTION

When haploid amphibian larvae develop they usually become abnormal and are said to show the haploid syndrome (Fankhauser, 1945; Hamilton, 1963; Gallien, 1967). Abnormality is first seen at gastrulation, which does not start in the haploids until their cells have the same nucleo-cytoplasmic ratio as diploid cells. The delay in the onset of haploid gastrulation approximates to the time taken for all the cells to divide once, so that from the beginning of gastrulation onwards a haploid embryo contains about twice as many cells, stage for stage, as a diploid embryo.

Even though all *Xenopus* haploid embryos are morphologically different from diploids during embryonic stages of development about 5% will develop into slightly abnormal swimming feeding tadpoles. The remaining 95% develop the haploid syndrome (Hamilton, 1963) and are blocked in their development at stage 43⁺ (Nieuwkoop & Faber, 1956). They have, in addition to shortened axial structures and microcephaly, a poorly formed gut and oedema, which latter two signs of the haploid syndrome cannot easily be related to reduced cell size per se.

In this study we have examined both qualitatively and quantitatively the fundamental cellular architecture of haploid and diploid cells, particularly in tissues which might affect the development of oedema (Fox & Hamilton, 1964a). For this reason our ultrastructural investigation concentrated on different regions of the pronephros, the skin and cloaca – together with the pharynx. The

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electron micrographs relating to the cloaca have been published by Fox (1970a) to which reference should be made when this region is described in the present paper.

MATERIALS AND METHODS

Androgenetic haploid eggs of *Xenopus laevis* were obtained using ultraviolet irradiation (Gurdon, 1960; Hamilton, 1963). Growth of larvae was in dechlorinated tap water at about 16–20°C. Specimens were fixed whole in ice-cold osmic acid (Palade, 1952) or occasionally in glutaraldehyde-osmic acid (Hirsch & Fedorko, 1968). (For further details of electron-microscopic methods used, see Fox, 1970a, b; Fox, Mahoney & Bailey, 1970.) Larvae staged according to the scheme of Nieuwkoop & Faber (1956) were investigated at stages 38/39, 43, 44 and 47 for diploids and at stage 43+ and stage 43++ for haploids. [Owing to the impossibility of staging haploid *Xenopus* beyond stage 43, the authors have decided to call haploid larvae in advance of stage 43, 43+ and 43++, which are equivalent in age to diploids of stage 47 and 50 respectively. Haploid stage 43+ and stage 43+++ are equivalent to haploid stages 43 and 43−45 of previous work by Fox (1970a).]

Araldite sections (1–2 μm thick) and paraffin-embedded transverse serial sections (8 μm and 10 μm thick) were examined by phase contrast and light microscopy, in addition to the ultrathin, silver grey sections (60–100 nm thick) used for electron microscopy. Thus in the two types of larvae comparisons of specific regions of selected tissues were made. Ultrathin sections were stained by uranyl acetate and lead citrate and viewed under an AEI EM 6B electron microscope. Mitochondrial profiles were measured with a planimeter. The level of statistical significance used was \( P < 0.05 \).

RESULTS

The pronephros and duct

For morphological and quantitative information on the amphibian (larval) pronephros, including that of *Xenopus*, reference may be made to papers by Fox (1962, 1963, 1970b), Fox & Hamilton (1964a, b) and Christensen (1964).

The pronephros of *Xenopus* includes three nephrostomial tubules, each of which opens at one end into the coelom (nephrostome) and at the other into the proximal, convoluted microvillous tubule (Figs. 3, 4, 5). During later larval development the first two nephrostomial tubules lead into a common nephrostome, with the third not far behind. The lumen is lined by ciliated cells, which presumably sweep coelomic fluid (derived by diffusion through the glomerular membranes of the paired glomi, or from fluid of tissues adjacent to the coelom) into the pronephros. Thenceforth it travels along the pronephric duct to the exterior via the cloaca.
A considerable number of nephrostomial tubule cells of diploids at stage 47, and also those of haploids at stage 43+ of about the same age and older ones at stage 43++ include a large lipid droplet, often as large or larger than the nucleus (Figs. 1–5). Most of the lipid and practically all the yolk, however, have disappeared from the rest of the pronephros and duct in stage 47 diploids, though lipid is still plentiful in these regions in stage 43++ haploids. Though haploid nephrostomial tubule cells are generally smaller than diploids they are however frequently longer (sometimes twice as long) and considerably narrower. Diploid nephrostomial tubule walls vary in thickness from 8 μm to 16 μm and maximum nuclear measurements were 12 μm long \times 8 μm wide. In haploids comparable measurements of walls and nuclei were 5 μm to 10 μm and 9 μm \times 5 μm respectively. Diploid tubule walls often appear more than one cell across, in contrast to a wall only one cell thick in haploids.

In both types the basal lamina of the nephrostomial tubule is enveloped externally by a similar delicate collagenous sheath. Intercellular junctions are straight (Figs. 1, 2), not highly folded, nor are there any elaborate infoldings of the plasma membrane as in the rest of the pronephros (Fox, 1970b). Mitochondria are irregular in shape and variable in numbers in nephrostomial tubule cells; on the whole they are more numerous in the diploid than in comparable haploid areas.

Within the nephrostomial tubule lumen groups of cilia are recognizable, in each of which the cilia are usually sectioned in a similar orientation. It is likely that each ciliary grouping originates from one particular cell (see Figs. 1, 2, 8). In diploids six groups were sufficiently discrete to be measured. They were made up of 85–100 cilia (mean 89/group). In haploids three comparable discrete groups of nephrostomial cilia each comprised 44, 60 and 51 cilia (mean 51/group), a little over half the diploid number. In both haploids and diploids a length of cell margin about 1200 nm long in each case included three ciliary bases, or 400 nm/cilium (Figs. 6, 7). Measurements of 12 cilia in transverse section, from each of a diploid and haploid nephrostomial tubule showed their mean areas not to differ significantly; they average about 250 nm in diameter in both forms and have a similar structure (Table 1).

The pronephric microvillous tubules are well developed in diploids at stage 47 (Fig. 9a, b). A fine collagen layer surrounds it and the plasma membrane has developed some modest peripheral infoldings; intercellular junctions are likewise infolded. Nuclei and nucleoli are prominent. Mitochondria are large, irregular in shape, some extremely elongated, and well differentiated. The cytoplasmic matrix often includes large, roundish less-dense vesicles possibly containing mucous-like substances. Well-developed Golgi cisternae are present. The endoplasmic reticulum comprises a number of irregularly shaped smooth and rough vesicles, and groups of polysomes in small rosettes extend throughout the cell. Large numbers of pinocytotic vesicles are seen, either at their sites of origin at the margin of the tubule between the microvilli, or further within the
cell (Fig. 9b). The whole inner apical margin is bounded by microvilli whose lengths vary between 2 μm and 2.7 μm.

In contrast stage 43+ and stage 43++ haploid microvillous tubules are variable in their degree of development, often less well differentiated than in stage 44 diploids and far less than in stage 47 diploids. Sometimes some cells, or portions of them, appear almost as well differentiated as in diploids (especially in the older haploids), though lipid and occasional small yolk bodies are usually still retained (Fig. 11). In most cases, however, a basal complex of infoldings is not developed, intercellular junctions are less infolded and the mitochondrial populations of these retarded tissues are not as dense as in the diploids. Furthermore, yolk bodies show variable profiles (Fig. 10) and there are numerous large lipid droplets, often situated close together (Fig. 12), or in groups of smaller droplets of varying sizes situated around dense tissue, which may well be remnants of lipid digestion (Fig. 10). Microvilli appear of similar length to those in diploids but pinocytotic vesicles, present at the lumen margins of the cell, are not as profuse in numbers.

Diploid distal pronephric tubules at stage 47 differ from its microvillous tubules of the same stage in the greater complexity of the plasma membranous infoldings, some of which penetrate deeply into the tubule cell (Fig. 13). Inter- cellular junctions are often straight, sometimes highly interfolded or irregular along their course. Mitochondria are large, variable in shape and often extremely elongated (up to 4 μm long); very often they are partially enclosed within the surface infoldings. The inner (apical) lumen margin is bordered by short, stub- like processes, shorter and thicker than the microvilli and fewer in number, though usually they appear transverse in section. No pinocytotic vesicles were found. Occasional lipid droplets are recognized but practically all the yolk has disappeared. Golgi networks are present. On the whole the cytoplasmic matrix

For description of abbreviations on figs. see page 98.

Fig. 1. Haploid specimen, stage 43+. Nephrostomial tubule of pronephros with cilia inside the lumen.

Fig. 2. Diploid specimen, stage 47. Nephrostomial tubule. The blocks of cilia, sectioned at different orientations, probably are each derived from different individual nephrostomial cells and clearly show the greater cilia population per group in the diploid, compared with the haploid of the previous figure.

Fig. 3. Diploid specimen, stage 47. Thick Araldite section, picture by phase contrast. Longitudinal section of a nephrostomial tubule leading from the coelom via the nephrostome. The large round lipid droplets are easily seen in the tubule cells.

Fig. 4. Haploid specimen, stage 43+. A nephrostomial tubule. The illustration was prepared in the same way as in Fig. 3.

Fig. 5. Haploid specimen, stage 43++ (about 13 days old). Junction of the ciliated nephrostomial tubule distally and the microvillous proximal convoluted tubule proximally. Note the large lipid droplets retained in the nephrostomial tubule cells. Normally diploids (and probably haploids too) retain lipid in the nephrostomial tubule longer than in the rest of the pronephros.
EM of haploid and diploid cells
Fig. 6. Diploid specimen, stage 47. Cilia and their rootlets from cells of the nephrostomial tubule.

Fig. 7. Haploid specimen, stage 43+. Cilia and rootlets (from nephrostomial tubule cells), of similar age, size and appearance as in the diploid of the previous figure. The number of rootlets per unit cell length is generally similar in diploids and haploids.

Fig. 8. Diploid specimen stage 47. Ciliary bases and rootlets, in transverse section, of a nephrostomial tubule cell.
is similar to that of the microvillous tubule, though no mucous vesicles are present.

The ultrastructure of the distal pronephric tubules of stage 43+ haploids is generally similar to that of the stage 47 diploid and their walls are of similar thickness; 8 \( \mu m \) to 10 \( \mu m \) across (Fig. 15). The main differences relate to the inferior haploid nuclear and cell volumes, their retention of lipid (see also in stage 44 diploids) and the frequent appearance of cavities between the plasma membranous infoldings. These could well be artifacts, though they were not found in comparable diploid tubules, nor in nephrostomial, microvillous or duct tubules of both types of larvae. Fewer haploid mitochondria appear to be enclosed by the membranous folds. In the older stage 43++ haploids the arrangement is more like that of the diploid.

The hinder pronephric duct in the stage 47 diploid is remarkable for the high degree of infolding or branching of the plasma membrane (Figs. 16, 17), a feature similarly found in *Triturus* larvae (Lehmann, 1967). Infolding is more highly developed in stage 47 diploids than in younger stage 44 diploids or stage 43+ haploids of the same age, or older stage 43++ haploids (Figs. 14, 18). The diploid duct is well developed and only occasionally are lipid droplets found, though large ones and often yolk bodies are still present in the comparable aged or older haploids and in stage 44 diploids (Figs. 17, 18). Intercellular junctions may be straight but usually they are highly folded. The thickness of the wall is variable ranging from 6 \( \mu m \) to 12 \( \mu m \) in diploids and 5 \( \mu m \) to 15 \( \mu m \) in haploids and the lumen wall is almost smooth with merely a few cellular projections. The smaller nuclear areas of haploids, compared with diploids, are clearly seen in low resolution ultrasections of the entire duct (Figs. 17, 18). Golgi networks are present in both types of cell. In general, apart from the differences described, the duct is similar in appearance to that of the distal tubule.

*The cloaca* (and hind rectum) (see Figs. 1–13; Fox, 1970a)

At diploid stages 38/39 the cloacal cells were partially differentiated, in that much yolk and lipid were present but few signs of ciliation. By stage 44 cilia were well developed but some yolk and lipid remained. In stage 47 larval cloacae, most of the lipid and all of the yolk had disappeared. These latter cells may contain up to 150 cilia, which total when compared with the 85–100 in the diploid pronephric nephrostomial cells suggests that in different tissues ciliated cells have a specific mean number of cilia. Non-ciliated vesicular cells, similar to epidermal cells, were also present in the cloaca. Haploid cloacal cells at stages 43+ and 43++ are similar to diploid ones at stages 38/39 and 47 respectively.

*The skin*

In normal (diploid) *Xenopus* larvae the first epidermal cilia appear at stages 20–21, following fusion of the neural folds (Steinman, 1968). In the present work diploids at stages 43 and 44 still retain some cilia in tail fin epidermal cells though
the typical non-ciliated vesicular cells are well developed and now preponderate (Fig. 22). Occasional yolk bodies and large lipid droplets still abound however in these cells as in the case of stage 43+ haploids. Lipids, moreover, are still recognized in stage 47 diploid epidermal cells (Fig. 21). Epidermal cilia are occasionally found in stage 43+ haploids, usually in a degenerate condition, though some normal ones may often be present (Fig. 19). In stage 47 diploids of the same age and older stage 43++ haploids they have disappeared entirely from the skin (Figs. 20, 21). In general the skin ultrastructure of this stage agrees with that described by Steinman (1968). The well-developed surface mucous vesicles of stages 43+ and 43++ haploids and stages 43 and 47 diploids do not seem to differ markedly in size, number or appearance.

The pharynx

The haploid stage 43+ pharynx is only partially differentiated, and the cells usually include one or several large yolk bodies and numerous lipid droplets, of varying sizes (Fig. 23). Nuclei and nucleoli are well developed and some mitochondria have differentiated. The appearance is generally similar in younger stage 43 diploids. In stage 47 diploids, at the same age as the stage 43+ haploids, the pharynx is highly differentiated (Fig. 25). Cell components include those with apical vesicles and in specific regions, particularly laterally in dorsal pouches, there are well-developed ciliated cells (Fig. 26) which are equally well-developed in diploids at stage 44. Goblet cells were recognized in the posterior pharyngeal region where it grades into the oesophagus (Fig. 24).

Quantitative data on mitochondria in pronephros of diploid and haploid larvae (Table 1)

Where there is sufficient pronephric cellular differentiation, so that well-differentiated mitochondria populate a specific region of a cell, the mito-

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Fig. 9. (a) Diploid specimen, stage 47. Transverse section of pronephric microvillous tubule. The less-dense vesicles may possibly represent areas which previously contained lipid granules. Cellular structure is quite well differentiated. (b) Diploid specimen, stage 47. Pinocytotic vesicles at microvillous margin.

Fig. 10. Haploid specimen, stage 43+. A similarly orientated section of a microvillous tubule as in Fig. 9. Compared with the diploid of similar age there is less development and differentiation in the haploid, and many lipid granules and yolk bodies are still retained. Some small pinocytotic vesicles are recognized at the lumen margin (they are numerous in the diploid stage 47), which suggests that in these haploids some incipient functional absorption occurs.

Fig. 11. Haploid specimen, stage 43++. Microvillous tubule older than in specimens of Figs. 10 and 12, showing a greater degree of development and differentiation; the intracellular organelles have profiles similar to those in the microvillous tubule of the younger diploid of Fig. 9.

Fig. 12. Haploid specimen, stage 43+. Microvillous tubule of similar age to that in Fig. 10, showing a greater degree of differentiation but not as much as in the diploid of the same age in Fig. 9, or in the older haploid of Fig. 11.
Fig. 13. Diploid specimen, stage 47. Distal pronephric tubule showing high development of the infoldings of the plasma membrane.

Fig. 14. Haploid specimen, stage 43+. Pronephric duct (hinder region) with lipid still present and only modest infoldings of the plasma membrane.

Fig. 15. Haploid specimen, stage 43+. Distal pronephric tubule. Note the cavities between the cell plasma membranes and the presence of lipid granules, the level of differentiation here being less than in the comparable diploid of Fig. 13.

Fig. 16. Diploid specimen, stage 47. Pronephric duct (hinder region). Note the complex infoldings of the plasma membrane; generally there is an absence of lipid and yolk and a greater degree of cellular differentiation compared with the comparable haploid duct of Fig. 14.
chondrial population density per unit area of cell surface (MPD) in diploid nephrostomial tubules, is generally superior to that of haploids. In microvillous and distal tubules and the hind duct however the MPD of haploids and diploids is practically the same but the MPD of the distal tubule and hind duct is significantly superior to that in the microvillous tubule.

![Fig. 17. Diploid specimen, stage 47. Pronephric duct in transverse section.](image1)
![Fig. 18. Haploid specimen, stage 43+. The haploid duct shows smaller nuclear profiles, less infolding of the cell surface and retains lipid granules compared with the diploid of the same age. The ducts (as are the distal tubules), however, are quite well differentiated in both haploid and diploid specimens, compared with the more slowly developing microvillous tubules.](image2)

The mean mitochondrial profile area (MPA) in microvillous tubules of stage 47 diploids is significantly superior, by about 29%, to that of stage 44 diploids and stage 43+ haploids. The MPA superiority of 18% of stage 43++ haploids over
Fig. 19. Haploid specimen, stage 43+. Tail-skin with some vestigial cilia still recognizable.

Fig. 20. Haploid specimen, stage 43++. Tail-skin. Cilia are absent and the apical mucous vesicles are highly developed.

Fig. 21. Diploid specimen, stage 47. Tail-skin. Cilia are absent; the apical vesicles are well developed and occasional lipid granules and numerous pigment bodies are present.

Fig. 22. Diploid specimen, stage 43. Tail-skin. Cilia are still present in this specimen which is younger than those in Figs. 23-25.
Fig. 23. Haploid specimen, stage 43+. Inner region of the pharynx showing the undifferentiated structure of these cells.

Fig. 24. Diploid specimen, stage 47. Outer wall of pharynx-oesophageal region. Note the high degree of differentiation compared with the haploid specimen of similar age in Fig. 23.

Fig. 25. Diploid specimen, stage 47. Pharynx, in region between the centre and side; the upper region is uppermost. Compare the development with the haploid specimen of Fig. 23.

Fig. 26. Diploid specimen, stage 47. Dorsal wall of the pharynx in the lateral ciliary groove, showing the highly developed cilia at this stage.
Table 1. Comparison of the numbers of mitochondrial profiles (from comparable areas) and of cilia (per cell) in diploid and haploid pronephric cells of *Xenopus laevis*, at various stages of development (stages according to Nieuwkoop & Faber, 1956): Age in days (approximately) of the different groups is recorded.

<table>
<thead>
<tr>
<th>Region of pronephros</th>
<th>Mitochondria</th>
<th>Cilia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diploid</td>
<td>Haploid</td>
</tr>
<tr>
<td></td>
<td>(stage 44)</td>
<td>(stage 43+)</td>
</tr>
<tr>
<td></td>
<td>(6 days)</td>
<td>(8 days)</td>
</tr>
<tr>
<td>Nephrostomial tubule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean no. organelles/cell</td>
<td>Generally higher population density in diploid nephrostomial tubules cells</td>
<td>89 (6 cells) 51 (3 cells) (mean diameter cilium 250nm in both groups)</td>
</tr>
<tr>
<td>Total no. profiles/100 μm² cell surface (from 400 μm²)</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Mean and standard error of individual mitochondrial profile area (× 10⁵ nm²)</td>
<td>±0-24</td>
<td>±0-28</td>
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<tr>
<td>Total mitochondrial area/100 μm² cell surface (× 10⁶ nm²)</td>
<td>101-5</td>
<td>87-5</td>
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<tr>
<td>Total no. profiles actually measured</td>
<td>112</td>
<td>95</td>
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<tr>
<td>Microvillous tubule</td>
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<td>Total no. profiles/100 μm² cell surface (from 400 μm²)</td>
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<td>39</td>
</tr>
<tr>
<td>Mean and standard error of individual mitochondrial profile area (× 10⁵ nm²)</td>
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<td>±0-32</td>
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<tr>
<td>Total mitochondrial area/100 μm² cell surface (× 10⁶ nm²)</td>
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<td>136-5</td>
</tr>
<tr>
<td>Total no. profiles actually measured</td>
<td>291</td>
<td>137</td>
</tr>
<tr>
<td>Distal tubule</td>
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<td>Total no. profiles/100 μm² cell surface (from 400 μm²)</td>
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<td>Mean and standard error of individual mitochondrial profile area (× 10⁵ nm²)</td>
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<td>±0-15</td>
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<tr>
<td>Total mitochondrial area/100 μm² cell surface (× 10⁶ nm²)</td>
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<td>136-4</td>
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<tr>
<td>Total no. profiles actually measured</td>
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<td>243</td>
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<tr>
<td>Hind pronephric duct</td>
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<td></td>
</tr>
<tr>
<td>Total no. profiles/100 μm² cell surface (from 200 μm²)</td>
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<td>3-1</td>
</tr>
<tr>
<td>Mean and standard error of individual mitochondrial profile area (× 10⁵ nm²)</td>
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<td>±0-15</td>
</tr>
<tr>
<td>Total mitochondrial area/100 μm² cell surface (× 10⁶ nm²)</td>
<td>170-5</td>
<td>136-4</td>
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<tr>
<td>Total no. profiles actually measured</td>
<td>109</td>
<td>243</td>
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</table>
stage 47 diploids was not significant. The mitochondria of such haploids often appear somewhat abnormally distorted in shape (though cristae are generally unaltered), amid the relatively under-differentiated tubule. This feature may, however, be a fixation artifact, but again could well represent abnormal structure or function.

The total mitochondrial area per 100 μm² of tubule cell surface (TMA) of haploids is inferior to that of diploids of comparable age, though in the stage 43++ haploids (about 13 days old) this measurement, apart from that of the duct, is now similar to that of the younger stage 47 diploids (8 days old).

In distal pronephric tubules the MPA of stage 44 diploids is significantly inferior by 22% to that of stage 47 diploids and all the haploids. Presumably these early diploid mitochondria are in the process of enlarging to the size reached in the older diploid stage. In the hind duct the MPA of the latter is significantly superior by 45% to that in the hind ducts of all the other groups.

During development within the diploid groups significant increases in the MPA in the microvillous tubule and hind duct show this measurement to reach 450000 nm² at stage 47. In the latter the MPA of the distal tubule of 3.5 x 10⁶ nm² may be its final size but more likely an intermediate stage, perhaps revealing a slower rate of size increase. Within the haploid groups the MPA is unchanged in the distal tubule (3.5 x 10⁶ nm²) and hind duct (3.1 x 10⁶ nm²) or significantly larger than the others in the microvillous tubules (5.3 x 10⁶ nm²).

It is likely therefore that mitochondria of different regions of the pronephric system have different rates of development. The differentiation of the microvillous tubule seems to be slower than in other pronephric regions and even in normal diploids large lipid droplets are retained within nephrostomial tubule cells far longer than in the rest of the pronephros and duct.

In general in *Xenopus* larvae, within the range studied, analysis of the limited data on pronephric mitochondria reveals a slower rate of development and differentiation in haploids. Ultimately from stages 43++ haploid mitochondria attain a similar size (distal tubule) or are smaller (hind duct) or are somewhat larger (microvillous tubule) than in the generally younger diploid stage 47.

In each differentiated specific region the numbers of mitochondrial profiles per unit area are similar in haploids and diploids and thus in the entire haploid cell (half the volume of a comparable diploid cell) the diploid number is reduced by half.

**DISCUSSION**

In assessing the observations made on haploid and diploid *Xenopus* larvae one may pose the following questions.

First, are the cell organelles in a haploid cell scaled down and if so is it volumetrically or numerically, in comparison with those in the larger diploid cell?

Secondly, macroscopic and microscopic studies suggested that the develop-
ment of abnormal haploid embryos is blocked just beyond stage 43 and that after stage 43 is reached, advancing oedema and gradual reduction of yolk are the only observable changes. Can one detect further cell differentiation, after stage 43, using ultra-structural techniques?

Thirdly, is there any evidence from the study of the pronephric system or epidermis to implicate them in any component of the haploid syndrome, i.e. oedema?

1. **The effect of cell size on organelle distribution**

Fankhauser (1945) demonstrated convincingly that amphibian haploids contain more cells than diploid individuals, and that the individual haploid cells were half the size of their diploid counterparts. The haploid nucleus is also half-sized. In this study we have evidence to suggest that lipid or yolk contained in a cell effectively reduces the amount of cytoplasm available to house mitochondria. From Table 1 one can see the effect of residual lipid and yolk, for in all cases (except in the distal tubule where the measurement in the haploid stage 43++ reaches that in the diploid stage 47) standard areas of haploid cells contain a smaller total area of mitochondrial profiles than equivalent diploid areas. As the haploids which are blocked at stage 43++ get older, and the lipid and yolk disappear, this parameter approaches the diploid one.

Cilia, where present, had identical structures and were similarly spaced in the two types of animal but the numbers per cell were not the same. The ratio of surface area of half-sized cells (haploid) to full-sized cells (diploid) is 0.63 to 1, so one might predict that any reduction in cilia number per haploid cell would be 0.63 of the diploid number rather than 0.5. The ratio of numbers of cilia observed was 51 per haploid cell to 89 per diploid cell: that is 0.57:1.

In conclusion one would say that organelle reduction has occurred by number rather than by individual volume, and that this reduction is in proportion to the geometry of the cell. The single haploid nucleus is, of course, volumetrically reduced compared with the diploid nucleus.

2. **Cellular differentiation and larval development**

From the results reported here and by Fox (1970a) it appears that some cells of haploid larvae are retarded in their differentiation even when compared with diploids of a similar stage. Other haploid cell types may approach the level of differentiation seen in diploids of similar age but advanced stage. The key to cellular differentiation appears to lie in the assimilation of lipid and yolk, for the yolkiest cells examined (pharynx) showed the greatest discrepancy between haploids and diploids and the least yolky (epidermis) showed the least difference. The pronephric system approaches the epidermal situation.

From these observations the authors would speculate that the typical *Xenopus* haploid larva is trapped in a vicious circle where for some reason yolk is not metabolized, differentiation is slowed, mitochondrial development is restricted, yolk is not metabolized, and so on.
3. The cause of oedema

Throughout the haploid pronephric system the cells are somewhat retarded in their rate of development and there would appear to be less mitochondrial substance per unit volume of tissue compared with that of the diploid. It is known from studies on mammalian kidneys that efficient tubular resorption of water is highly dependent on the provision of sufficient energy. It may well be the case that owing to mitochondrial deficiency haploids never possess sufficient energy to osmo-regulate in the normal manner. Dalcq's (1932) original view of pronephric abnormality and thus its malfunction being related to oedema in haploid larvae might well be true, though other factors doubtless also contribute. The suggestion that haploids may be unusually permeable to water (Fox & Hamilton, 1964a) remains unanswered by this investigation, since there are no apparent differences between haploid and diploid epidermal cells except in size.

In conclusion the results clearly show that the majority of haploid cells are strikingly retarded in their rate of development, compared with diploids, a feature manifest in many cells by the retention of large numbers of lipid droplets and yolk bodies, when comparable diploid cells of similar or even younger age have already digested them. In addition haploids show delay in developing or in other cases in discarding, specific cellular structures. Within the range of stages studied the half-sized haploid cells, when they include specifically recognizable, well-differentiated organelles such as mitochondria and cilia, and have greatly reduced yolk and lipid content, possess about half the number of mitochondria (or cilia) of similar size compared with diploid cells.

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REFERENCES


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Description of Abbreviations on Figures

bv, blood capillary; c, cilium; cb, ciliary base; cn, collagen layer; co, coelom; cr, ciliary rootlet; cv, cellular vesicle of microvillous tubule; d, desmosome; er, endoplasmic reticulum; gc, goblet cell; go, Golgi complex; ic, inner cell (skin); ij, intercellular junction; is, inner surface of cell (nephrostomial tubule); lg, lipid droplet (granule); ls, lower side (pharynx); lu, lumen; lyb, lipid-yolk complex; m, mitochondrion; mu, mucous vesicles (skin); mv, microvilli; mvt, microvillous tubule of pronephros; n, nucleus; nl, nucleolus; nlu, lumen of nephrostomial tubule; np, nucleopore; nst, nephrostome; nt, nephrostomial tubule of pronephros; oc, outer cell (skin); os, outer surface of cell (nephrostomial tubule); pg, pigment granule; pm, plasma membrane; pmc, cavity between plasma membranes; pmi, infoldings of plasma membrane; pnc, perinuclear cisterna; pt, pronephric tubule; pv, pinocytotic vesicle; pxl, pharyngeal lumen; r, ribosomes; tb, terminal bar; us, upper side (pharynx); yb, yolk body.