Retinal growth in double dorsal and double ventral eyes in Xenopus

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SUMMARY

The growth of normal and surgically produced compound dorsal and ventral retinas in Xenopus laevis has been studied autoradiographically following injections of [³H]thymidine at stages 50 and 58. The animals were sacrificed 3 weeks after metamorphosis. The histogenetic pattern of the dorsal and ventral retinal halves was different at the three time points investigated, i.e. up to stage 50, between stages 50 and 58 and between stage 58 and 3 weeks after metamorphosis. Asymmetrical dorsal retinal growth occurred up to stage 50. From stage 50 onwards the retinal growth tendency reversed so that more ganglion cells were produced along the ventral than the dorsal ciliary margins. The overall preponderance of ventral retinal growth was 32.4% in cell number and 12.4% in retinal length from early embryogenesis to 3 weeks after metamorphosis. The characteristic histogenetic pattern of the dorsal and ventral retinal halves was maintained in an ectopic position in the compound eye, indicating that this particular property of the retinal halves is intrinsically determined.

INTRODUCTION

The eye in lower vertebrates grows continuously throughout the whole life of the animal. Recent observations on the histogenesis of the anuran retina with the use of [³H]thymidine autoradiography have shown (Hollyfield, 1968; Straznicky & Gaze, 1971) that the retina grows by the addition of cells at the ciliary margin. In contrast to the early findings (Glucksmann, 1940; Hollyfield, 1971), no significant loss of retinal cells has been found in Xenopus during development, nor have newly formed retinal cells been seen to migrate from the ciliary margin (Straznicky & Gaze, 1971). Thus in an adult retina there is a well-defined age distribution of cells: the oldest ones are situated at and around the optic nerve head followed by rings of younger and younger cells towards the ciliary margin.

It was described in an earlier paper (Straznicky & Gaze, 1971) that as would be predicted from the adult situation, the retina grows asymmetrically about the optic nerve head. Although in the previous paper the asymmetric growth of dorsal and ventral retina was noted and commented on, insufficient attention

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was paid to the orientation of the retina in some cases and the summary dia-
gram, on page 76 of that paper, is incorrect, as has been shown by the more
recent work of Jacobson (1976). He has studied the histogenesis of the retina
of *Xenopus* and found that after mid-larval stages the majority of newly formed
cells were added to the ventral half of the retina. He in fact claimed the over-all
retinal histogenesis to be strikingly asymmetrical and suggested that the addi-
tion of crescentic bands of retinal cells at the ventral ciliary margin would
assure a linear, rather than concentric retinal growth. The suggested retinal
growth trend could be correlated with the progression of the histogenesis of
the mesencephalic visual centre of the brain, growing rostrocaudally in a linear
fashion (Straznicky & Gaze, 1972). It must however be emphasized here that
the asymmetrical retinal growth appears to be entirely between the dorsal and
ventral halves. None of the recent work on retinal histogenesis has reported
naso-temporal asymmetry; thus, if such a trend exists at all it is very much less
apparent than along the dorso-ventral axis of the eye.

It is interesting to note that compound double ventral eyes, though they
were made of two ventral retinal halves, did not grow unduly large even if the
operated animals were kept alive for one year after metamorphosis (Straznicky,
Gaze & Keating, 1974). That the size of a compound double ventral retina is
maintained within the normal range could be explained by one of the following
possibilities. An intraretinal compensatory mechanism may play a role such
that the transplanted ventral half does not grow at the same rate as that of the
host ventral half; or the ventral asymmetrical growth of the retina diminishes
during the postmetamorphic life; or perhaps both ventral halves grow less
after the operation. In the present paper an attempt has been made to test the
first possibility. \[^{3}H\]Thymidine autoradiography on compound ventral and
dorsal eyes has demonstrated that the characteristic histogenetic activity of
the transplanted retinal halves is maintained in an ectopic position.

**METHODS**

*Xenopus laevis* toads were used, bred in the laboratory and staged according
to the normal tables of Nieuwkoop & Faber (1956). Embryos at stage 32,
after the axial polarization of the eye blastema (Jacobson, 1968), were anaes-
thesitized with tricaine (Sigma) and the right eye blastema cut in half along the
horizontal meridian. The dorsal half was then removed and replaced by the
ventral half of a left eye from another embryo to obtain a compound double-
ventral (VV) eye. In other embryos DD eyes (consisting of two dorsal halves)
were similarly prepared. The DD and VV eye animals were reared separately
and later used for autoradiographic studies. \[^{3}H\]Thymidine (2 \(\mu\)Ci per animal,
per injection in 0.5 \(\mu\)l solution, specific activity 17 Ci/mmol) was administered
intraperitoneally at stages 50 and 58 and the animals sacrificed 3 weeks after
metamorphosis. Before the sacrifice of animals with one VV eye the visual
projections from the compound eye to the tectum were recorded electrophysiologically in order to verify that the embryonic eye operation was successful. The method of electrophysiological recording has been described in detail in a previous paper (Straznicky, 1976). Only animals with the visual field map characteristic of a VV eye (Straznicky et al. 1974) were used for further autoradiographic studies. Because the ventral fissure is missing, the optic nerve failed to form in DD eyes (Straznicky et al. 1974), therefore animals with one DD eye were not subjected to visual field mapping.

The heads of the operated animals were fixed in Bouin’s solution for 24 h. The normal and operated eyes were then dissected from the orbit so that a piece of cartilage from the upper jaw remained attached to the ventral pole of the eye. Eyes were rapidly dehydrated, embedded in paraffin, cut at 10 μm, exactly about the vertical meridian of the globe and the closely spaced serial sections processed for autoradiography according to the method of Rogers (1973). Deparaffinized sections were coated with Ilford Nuclear Emulsion K2, and were exposed at 5 °C for 2 weeks before being developed. The sections were then stained with Harris’s haematoxylin.

The length of the retina and the number of ganglion cells was measured or counted on four subsequent sections at the optic nerve head of five normal and five VV eyes of five operated animals with one VV eye. To avoid the error of double counting, only ganglion cells with their nucleolus in the section were counted. The average of the readings on 20 sections in both normal and VV eyes was established. In five DD eyes only the length of the retina was measured on four subsequent sections at the geometrical centre of the eye.

RESULTS

Because Jacobson’s (1976) recent report on the asymmetrical retinal growth in *Xenopus* is at variance with our previous account (Straznicky & Gaze 1971), it was felt necessary to give a more detailed description of the normal retinal growth before dealing with the histogenesis of the compound eyes.

The growth of the normal retina

The location of the labelled cell groups in the vertical sections of normal retinal autoradiograms can be established quite accurately. It can be seen (Figs. 1A and 3A) that cells labelled at stages 50 and 58 are asymmetrically arranged in the dorsal and ventral retinal halves. The distances between the optic nerve head and the first labelled cell group and that between the second labelled cell group and the ciliary margin as well as the corresponding cell numbers in the dorsal and ventral halves can be established. The actual cell production of the dorsal and ventral ciliary margins at stages 50 and 58 is indicated by the number of labelled ganglion cells in the retina. In order to quantitate the histogenetic pattern of the dorsal and ventral halves and to
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Table 1. The ganglion cell production of the ciliary margin in the dorsal and ventral halves of the retina

The average of the ganglion cell counts with their standard errors and the measurements of retinal length in vertical sections at the optic nerve head/centre of retina in five normal, five VV and five DD eyes is summarized in the Table. The first column gives the number of ganglion cells formed up to stage 50. Second and fourth columns include the number of ganglion cells labelled at stages 50 and 58. Third and fifth columns represents the number of ganglion cells formed between stages 50 and 58 and between stage 58 and 3 weeks after metamorphosis. The total number of ganglion cells in the dorsal and ventral halves of the eye is given in the sixth column. The length of the corresponding part of the retina formed within a certain period of time is also indicated in μm.

<table>
<thead>
<tr>
<th></th>
<th>Up to st. 50</th>
<th>St. 50</th>
<th>St. 50–58</th>
<th>St. 58</th>
<th>St. 58–3 WAM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Cell no.</td>
<td>90.6 ± 15.0</td>
<td>8.1 ± 1.7</td>
<td>44.9 ± 8.7</td>
<td>2.6 ± 1.1</td>
<td>8.2 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>Retinal length (μm)</td>
<td>420</td>
<td>47</td>
<td>358</td>
<td>15</td>
<td>47</td>
</tr>
<tr>
<td>V</td>
<td>Cell no.</td>
<td>50.5 ± 16.9</td>
<td>9.1 ± 2.5</td>
<td>127.1 ± 19.4</td>
<td>4.2 ± 1.9</td>
<td>36.9 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>Retinal length (μm)</td>
<td>207</td>
<td>37</td>
<td>520</td>
<td>25</td>
<td>224</td>
</tr>
<tr>
<td>Vd</td>
<td>Cell no.</td>
<td>62.3 ± 9.0</td>
<td>8 ± 3.6</td>
<td>89.3 ± 6.8</td>
<td>4.3 ± 1.3</td>
<td>68.8 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>Retinal length (μm)</td>
<td>238</td>
<td>33</td>
<td>286</td>
<td>14</td>
<td>273</td>
</tr>
<tr>
<td>Vv</td>
<td>Cell no.</td>
<td>69.0 ± 7.9</td>
<td>8.3 ± 3.4</td>
<td>85.5 ± 11.9</td>
<td>4 ± 0.8</td>
<td>77.0 ± 5.9</td>
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<td></td>
<td>Retinal length (μm)</td>
<td>291</td>
<td>36</td>
<td>280</td>
<td>23</td>
<td>272</td>
</tr>
<tr>
<td>DD eye</td>
<td>Retinal length (μm)</td>
<td>460.7 ± 56.4</td>
<td>421.4 ± 57.3</td>
<td>55.0 ± 19.5</td>
<td>937.1 ± 35.1</td>
<td></td>
</tr>
<tr>
<td>Dv</td>
<td>Retinal length (μm)</td>
<td>460 ± 56.4</td>
<td>433.7 ± 45.4</td>
<td>66 ± 15.8</td>
<td>960.1 ± 31.2</td>
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Fig. 1. Autoradiographs of normal, DD and VV eyes cut along the vertical meridian of the eye. Isotope was administered at stages 50 and 58 and animals killed 3 weeks after metamorphosis (A, B and C). Two labelled cell groups (arrows) are present both in the dorsal and ventral halves of the retina of normal (A), VV (B) and DD (C) eyes. Cells labelled at stages 50 and 58 are asymmetrically arranged in the dorsal (top) and in the ventral (bottom) halves of the normal eye. Notice the symmetrical arrangement of labelled cell groups about the horizontal meridian of the eye in the host and transplanted retinal halves of VV (B) and DD (C) eyes. Cells labelled at stages 50 and 58 are asymmetrically arranged in the dorsal (top) and in the ventral (bottom) halves of the normal eye. Notice the symmetrical arrangement of labelled cell groups about the horizontal meridian of the eye in the host and transplanted retinal halves of VV (B) and DD (C) eyes. Labelled cells at the dorsal (D) and at the ventral (E) ciliary margins of a normal eye. Isotope was administered 3 weeks after the injection and the animal sacrificed 1 week later. Notice that labelled cells (arrow) moved away from the dorsal ciliary margin (D), indicating a continuous addition of cells to the dorsal ciliary margin even 3 weeks after metamorphosis. Bars in these and each of the following pictures represent 50 μm, unless otherwise stated.
contrast the apparent difference between them, the number of labelled and un-
labelled ganglion cells were counted separately both in the dorsal and ventral
halves of the retina. The average of the cell counts and measurements with their
standard errors is summarized in Table 1. The first column includes the number
of ganglion cells from the optic nerve head to the stage-50 labelled group, that
is cells formed up to stage 50. The number of ganglion cells labelled at stage
50 is given in the second column. The third column contains the number of
ganglion cells formed between stages 50 and 58, these cells lie in the retina
between the two labelled cell groups. The fourth and fifth columns include the
number of ganglion cells labelled at stage 58 and the number formed between
stage 58 and 3 weeks after metamorphosis respectively. In addition each of the
columns contains the length of the corresponding retinal sector in the dorsal
and ventral halves. Finally the last column shows the total cell number and
length of the dorsal and ventral halves. The quantitative results demonstrate
that up to stage 50 more ganglion cells are formed in the dorsal than in the
ventral retinal half. After stage 50 there is an apparent slowing down of cell
production in the dorsal half as judged by the smaller number of cells labelled
at stage 58. In contrast to this the number of ganglion cells formed between
stages 50 and 58 or at stage 58 is significantly higher in the ventral than in the
dorsal half, suggesting a substantially enhanced cell production of the ventral
ciliary margin during this developmental period. After stage 58 the number of
cells added to the ciliary margin decreases, more markedly in the dorsal half.
The ratio between the number of ganglion cells in the dorsal and ventral halves
formed after stage 58 suggest that there is still a high preponderance of ventral
retinal growth. On the other hand it is apparent that the addition of ganglion
cells to the dorsal ciliary margin continues after stage 58. To be able to demon-
strate that the cell production of the dorsal ciliary margin is maintained beyond
metamorphosis a third isotope injection was performed 3 weeks after meta-
morphosis in a few experimental animals and they were killed 1 week after the
third isotope administration, that is, 4 weeks after metamorphosis. In these
cases labelled cells were found (Fig. 1D, E) adjacent to the ciliary margin
slightly more in the ventral than in the dorsal half. The relatively few labelled
cells of the ciliary margin following postmetamorphic injection as compared
to their number to stage-58 labelled ganglion cells may be taken as indication
of a slowing down of cell production in the ciliary margin, with an eventual ten-
dency to even out the previous differential mitotic activity of the dorsal and
ventral retinal halves.

It is interesting to mention that higher ganglion cell density was found
in the ventral (2.8) than in the dorsal (17.36) half as it can be expressed
by the number of cells/100 μm retinal length index. This observation
indicates that the linear dorso-ventral retinal asymmetry alone is not a true
representative of the differential addition of cells to the dorsal and ventral
retinal margins.
The growth of VV and DD retinae

The general growth of the VV and DD eyes during the time span of the experiments appeared to be close to normal. None of the VV eyes had grown larger than the intact left eye of the same animal. In a few cases the diameter of the VV eyes seemed to be even smaller than the left normal eye which may be attributed to a side effect of the early embryonic eye operation. Gross anatomical dissection of VV eyes revealed that the optic nerve had occupied an approximately central position in the eye in contrast to the eccentric dorsal position of the optic nerve head of normal eye. Although the DD eyes looked about normal in appearance no optic nerve formation had taken place. Histological examination of DD eyes showed that the ganglion cell layer of the retina was missing and the outer and inner nuclear layers were very thin (Fig. 2 A, B), presumably as a consequence of the retina not being connected with the visual centres of the brain.

The autoradiograms revealed a somewhat symmetrical arrangement of the labelled cell groups about the horizontal meridian of the eye (Figs. 1B, C and 2B, C). The cell counts in VV eyes (Table 1) showed a similar ganglion cell number in the transplanted (Vd) and host (VV) retinal halves. The distances between the optic nerve head and the first, the second labelled group and the ciliary margin correspond well in the two halves. It is worth noting that the cell production up to stage 50 and after stage 58 seems to be slightly higher, conversely the cell production between stages 50 and 58 appears to be lower than in the ventral half of a normal eye though the observed differences are not significant. Although no attempt has been made to count the total ganglion cell number in the normal and VV eye of the same animal, Table 1, clearly shows
Fig. 3. Camera lucida drawings of the neural retina of normal (A), DD (B) and VV (C) eyes starting from the optic nerve head/centre of retina (extreme left section) to the nasal pole. The sections are 80 μm apart. Isotope was administered at stages 50 and 58 and animals killed 3 weeks after metamorphosis.

A higher ganglion cell number in VV than in normal eye. The increased ganglion cell number in VV retina is due to the higher cell production of the transplanted ventral retina as compared to the normal dorsal retinal half. Because the ganglion cells were missing in DD eyes only the length of different retinal sectors could be measured (Table 1). It can be seen that the distances from the geometrical centre of the eye to the first, the second labelled group and to the ciliary margin in the transplanted (Dv) and host (Dd) halves correspond to the data obtained on the dorsal half of normal eyes. In Fig. 4 the linear relationship between the dorsal and ventral half of a normal eye and between the transplanted and host’s half of compound eyes is shown. The position of the cell groups labelled at stages 50 and 58 is also given. The transplanted dorsal and ventral retinal halves reveal similarities in the position of the labelled cell groups and in the linear relationship of the retinal segments for the corresponding normal retinal halves.
DISCUSSION

The present experiments are concerned with the mode of histogenesis of the dorsal and ventral retinal halves. From the quantitative data obtained on the autoradiograms of normal and operated eyes it is apparent that the histogenetic pattern of the dorsal and ventral retinal halves is different at the three time points of the investigation. During early larval stages (up to stage 50), which amounts to about 2 weeks of development, the dorsal retinal half produces about twice as many ganglion cells as the ventral half. From stage 50 to 58 (about 4 weeks duration) a marked acceleration of ganglion cell production occurs especially at the ventral half. This increased mitotic activity of the ciliary margin gives rise to about one-third of the adult retina. After stage 58 retinal cell production progressively decreases; however, the mitotic activity of the ciliary margin is maintained, both in the dorsal and ventral halves 3 weeks after metamorphosis. This observation is in agreement with our former findings (Straznicky & Gaze, 1971) on postmetamorphic retinal growth. It has been shown that in each of the time points of the present investigation there is an excess cell production either in the dorsal or in the ventral half over the other in the retina. The overall difference between the dorsal and ventral halves up to 3 weeks after metamorphosis is 12% in retinal length and 32% in ganglion cell number in favour of the ventral half.

Our present autoradiographic observations on the histogenetic activity of the retina seems to agree with the trend shown by electronmicroscopic studies (Gaze & Peters, 1961; Wilson, 1971) on the composition of the optic nerve from early embryonic stages to adulthood. These observations have demonstrated that by stage 51 only about 6000 nerve fibres are in the optic nerve. During the
relatively short period from stage 51 to 58 the optic fibre number increases to about 20000, corresponding to a similar increase in ganglion cell number (Wilson, 1971). Wilson (1971) also demonstrated a further significant increase of optic fibres and ganglion cells (in the latter case from 28000 to 78000) over a much longer period from the metamorphic climax to full maturity. What proportion of these ganglion cells are added to the ventral or to the dorsal half of the retina is not yet known. Jacobson's (1976) recent findings suggest that a substantial part of the ganglion cells, if not all, formed after metamorphosis, are added to the ventral half of the retina.

The present description of the normal retinal growth involving cell counts represent a considerable shift from our earlier views (Straznicky & Gaze, 1971). In the former paper a preponderance of dorsal retinal growth was advocated. As has been shown here our present observations fully confirm Jacobson's (1976) findings, in that from stage 50 onwards substantially more ganglion cells are added to the ventral than to the dorsal ciliary margin, resulting in an apparent ventral retinal asymmetry. It should be noted at this point that in the 1971 study the emphasis was on early retinal growth, when an excess cell production in the dorsal half is very apparent. The former account given on retinal growth was based on single pulse labelling. In this situation the direction and the rate of retinal asymmetry is very difficult to assess with precision. Multiple isotope administrations to the same animal (introduced by Jacobson) enabled us in the present experiments to follow the pattern of differential addition of cells to the dorsal and ventral ciliary margins at different time points of development and this revealed an overall preponderance of ventral retinal growth, in agreement with Jacobson's (1976) recent account.

It is now quite clear that normal dorsal and ventral halves have different growth rates. The consistent findings on VV and DD eyes were the symmetrical disposition of labelled cells about the horizontal meridian of the compound retina. Both the ganglion cell counts and the measurements of different retinal sectors indicated that the histogenetic activity of the host and transplanted retinal halves were remarkably similar at the three check points of the investigation. Consequently more ganglion cells were found in the W than in the normal eye. Further experiments are in progress in this respect to analyse the ganglion cell and optic fibre number of VV eyes 3, 6 and 12 months after metamorphosis.

The symmetrical growth of the DD and W retinae needs some further comments. It has been well documented (Jacobson, 1968) that there is naso-temporal and dorsoventral polarization of the eye blastema, with respect to what part of the optic tectum the future retinal ganglion cells will connect with and that this occurs during early embryonic stages. This is not, however, the only unique property of the eye which is specified at early developmental stages. One of the distinct features of the amphibian eye is the permanent ventral fissure whose position seems to be determined in the eye at early embryonic stages.
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(Sato, 1933). Another regional character of the retina is the differential growth rate of the dorsal and ventral retinal halves. Proliferation of nerve cell pools has a spatio-temporal pattern and this varies from one area to the other in the CNS. However, the characteristic mitotic pattern of one cell pool appears to be rather invariant, indicating a genetical programming (Jacobson, 1970). The histogenetic pattern of the transplanted ventral and dorsal retinal halves has been found to be similar to the intact ventral and dorsal halves of the compound eye. In other words, the specific histogenetic activities of the dorsal and ventral halves were maintained in an ectopic position. These findings clearly show that this intrinsic property of the dorsal and ventral retinal halves was already present in the eye fragments at the time of the operation. In conclusion it is suggested that the differential histogenetic pattern of the dorsal and ventral retinal halves are determined, presumably at the same time and together with other specific properties of the neural retina.

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REFERENCES


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