Distortion of patches of retinal degeneration in chimaeric mice

By J. D. West

From the Department of Genetics, University of Edinburgh, Scotland

SUMMARY

Estimation of the proportion of degenerate retina in rd/rd and +/+ (or +/rd) chimaeras, using a linear analysis of histological sections, suggests a considerable preponderance of the normal phenotype, while analyses based on the area of the surviving tissue suggest that the proportion of each component is similar to that in other tissues. This argues against the validity of the linear analyses used by other authors. It is suggested that cell movement occurs in the outer nuclear layer, following the degeneration of the rd/rd population, and results in a distortion of the original patches of normal cells.

INTRODUCTION

The recessive gene retinal degeneration, rd (Tansley, 1954) provides a histological marker for examining patches in the neural retina of chimaeric mice. Mice, homozygous for the recessive allele (rd/rd), develop a normal retina but from ten to twenty days after birth the layer of photoreceptor cells (outer nuclear layer) degenerates and eventually disappears.

Both Mintz & Sanyal (1970) and Wegmann, LaVail & Sidman (1971) demonstrated patches of degenerate and normal retina in chimaeric mice and also reported regions of intermediate phenotype, where degeneration appeared to be incomplete. Mintz & Sanyal noted that completely degenerate regions commonly contributed a small proportion to the outer nuclear layer, and they interpreted this as implying selection favouring +/+ over rd/rd.

It is suggested here that the apparent low proportion of completely degenerate regions is an artifact and results from cell movement within the outer nuclear layer. This is misinterpreted in a linear analysis, where classification rests on the width of the outer nuclear layer.

MATERIALS AND METHODS

1 Mice

Eight-cell morulae from rd/rd and +/+ (or +/rd) stocks were aggregated to produce chimaeric mice as described by Bowman & McLaren (1970). Adult

1 Present address: Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263, U.S.A.
chimaeras of three strain combinations were used: C3H/BiMcL ↔ C57BL/McL, C3H/BiMcL ↔ Recessive and pigmented-Q ↔ unpigmented-Q. Recessive is the multiple recessive strain described more fully by McLaren & Bowman (1969) and is uniformly +/+ as is C57BL/McL. C3H/BiMcL is rd/rd and the Q-strain mice are members of a closed random-bred stock and may be +/+ rd/+rd or +/rd and either pigmented or unpigmented. It is not known whether the rd/rd cells were derived from the pigmented-Q or the unpigmented-Q aggregant in those pigmented-Q↔unpigmented-Q chimaeras which were also found to be chimaeric for retinal degeneration.

(2) Histological methods

Standard histological methods were used. After fixation overnight in Bouin's fluid, an incision was made in the cornea of each eye and the lens removed. The eyes were dehydrated in graded alcohols, cleared in toluene, embedded in wax, sectioned at 6 μm and stained with Ehrlich's haematoxylin and eosin. If the eye is compared to a globe with the cornea in a pole position, the two planes of section considered are equivalent to 'latitudinal', parallel to the equator, and 'longitudinal', perpendicular to the equator.

(3) Analysis of the neural retina

The mid-sections from the right eyes of six chimaeras were used to compare three methods of estimating the proportion of the rd/rd component in the outer nuclear layer. Firstly, the outer nuclear layer was classified into two types in a linear analysis. Lengths where no outer nuclear layer remained were assumed to be rd/rd and the remaining intermediate and normal regions were grouped together as normal. The length of each patch was measured using a calibrated eye-piece micrometer and the proportion of rd/rd calculated. The other two estimates were based on the area of the outer nuclear layer in the section, rather than the relative lengths of two classes. Camera lucida drawings were made from the six chimaeric sections and appropriate +/+ and rd(rd controls. The lengths and areas of the outer nuclear layer (ONL) and the inner nuclear layer (INL) were measured with a map measurer and planimeter. First, the ratio of the ONL area to the INL area in each chimaera was compared to the mean ratio for control +/+ retinas. Second, the mean depth (area/length) of the ONL was compared to the mean depth in +/+ controls. The first estimate assumes retinal degeneration does not significantly alter the overall area of the INL, which seems reasonable from the eyes studied. Whereas the second method does not rely on this assumption it is inaccurate for oblique sections, or sections taken from different parts of the eye, where the width of the ONL may vary.

Other markers were used, as described elsewhere (West, 1976), to estimate the proportions of the two cell populations in other tissues and organs. Pigment markers were used to distinguish two populations of retinal epithelium cells and
Table 1. Estimates of relative proportions of two phenotypic classes in the neural retina and retinal epithelium of the right eyes of six chimaeras (also shown are the relative proportions of two cell populations in the coat and brain)

<table>
<thead>
<tr>
<th>Plane of section</th>
<th>Chimaera</th>
<th>Proportion of degenerate retina</th>
<th>Corresponding proportions in other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Linear estimates</td>
<td>Area estimates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p) (p^1) (p^2)</td>
<td>(p) (1−p)</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>C3H&lt;-&gt;C57BL (XQ12)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>C3H&lt;-&gt;Recessive (X35)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Pig-Q&lt;-&gt;Unpig-Q (XQ19)†</td>
<td>0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>Latitudinal</td>
<td>C3H&lt;-&gt;C57BL (XQ18)</td>
<td>0.60</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>C3H&lt;-&gt;Recessive (X34)</td>
<td>0.01</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Pig-Q&lt;-&gt;Unpig-Q (XQ26)†</td>
<td>0.03</td>
<td>0.53</td>
</tr>
</tbody>
</table>

p^1, Estimate of proportion of degenerate retina in section calculated as:

\[
\frac{\text{mean depth of ONL in chimaera}}{\text{mean depth of ONL in normal controls}}
\]

p^2, Estimate of proportion of degenerate retina in section calculated as:

\[
\frac{\text{ONL area/INL area in chimaera}}{\text{ONL area/INL area in normal controls}}
\]

* No variants were present in the retinal epithelium of the C3H<->C57BL chimaeras or for glucose phosphate isomerase in the pigmented-Q<->unpigmented-Q chimaeras.

† The rd population could be derived from either the pigmented-Q (p) or the unpigmented-Q (1−p) component.

RESULTS

For each of six chimaeras three estimates of the proportion of the rd/rd component, together with estimates of the proportions of the two cell populations in other tissues, are shown in Table 1.
DISCUSSION

In all the chimaeric eyes studied the proportion of the \textit{rd/rd} component estimated by the linear analysis is lower than the proportion of the equivalent cell population in other tissues, and in some cases this difference is considerable. This is in agreement with the findings of Mintz & Sanyal (1970) and could result from cell selection against \textit{rd/rd} in all three strain combinations, as these authors suggest for their chimaeras, or could result from survival of some \textit{rd/rd} cells in chimaeric tissue, if retinal degeneration is not cell-autonomous.

However, the close agreement between the estimates of the proportion of the \textit{rd/rd} component, based on area measurements, and the proportion of this population in other tissues provides evidence against these interpretations and suggests that the one-dimensional method of analysis is inappropriate to the system. The phenotypes scored in the linear analysis may not reflect the original distribution of the genotypes but the true proportion of the \textit{rd/rd} component would still be related to the number of nuclei in the outer nuclear layer. While no cell counts were made, the cell density in the surviving regions of outer nuclear layer in chimaeras appeared similar to that in the \textit{+/+} controls: it is therefore assumed that the proportion of \textit{rd/rd} in the chimaeras can be directly estimated from the surviving volume of the outer nuclear layer (or its area in a section). The depth of the neural retina in the chimaeras studied was fairly uniform; where the outer nuclear layer was very thin or absent the underlying inner nuclear layer was correspondingly thicker. This suggests that either local proliferation or cell movement occurs in the inner nuclear layer. The second alternative seems more likely as adjacent areas of the inner nuclear layer underlying normal regions of outer nuclear layer, in sections of chimaeric retina, often appeared thinner than usual. It seems likely, therefore, that cell movement occurs at least in the inner nuclear layer. It is suggested that retinal degeneration acts intracellularly and that movement of surviving \textit{+/+} (or \textit{+/rd}) cells within the outer nuclear layer follows the degeneration of \textit{rd/rd} cells, distorting the original pattern of patches. This would explain the low proportion of 'completely degenerate’ retina, scored in the linear analysis, and the presence of regions of ‘intermediate degeneration’ seen here, as well as by Mintz & Sanyal (1970) and by Wegmann, La Vail & Sidman (1971). If cell movement does occur in this way, retinal degeneration is clearly not a suitable marker for the analysis of clonal development in the chimaeric neural retina, using adult eyes, and Mintz & Sanyal’s conclusion that ‘the basic plan consists of 10 radiating sectors per retina’ is open to question. Mintz (1974) also refers to an experiment by Noell and Mintz where the physiological assessment of the degree of degeneration in a group of \textit{rd/rd} \leftrightarrow \textit{+/+} chimaeras was always lower than would be expected from a linear relationship between the ‘area of functional retina’ and electroretinogram amplitude. Although experimental details are not given, if the histological analysis of the proportion of each cell population was based on
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linear measurements of ‘completely degenerate’ regions in sections, the proportion of rd/rd is again likely to be underestimated and so the physiological response may be more directly related to the true proportions of the two cell populations.

I wish to thank Dr Anne McLaren for valuable supervision and discussion. I also thank Drs Anne McLaren, Patricia Bowman and Mrs Janet Carter for providing the chimaeras and Mrs Katrine West for helpful discussion. This work was done while I was in receipt of an S.R.C. studentship. I am also grateful to Ford Foundation for financial support for this work.

REFERENCES


(Received 13 January 1976)