Stable programming for map orientation in fused eye fragments in *Xenopus*

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**SUMMARY**

Compound eyes were formed in *Xenopus* embryos at stages 32/33 by fusion in the right orbit of (1) two right naso-ventral halves, (2) two right ventral halves, (3) two right temporo-ventral halves, (4) one right and one left naso-ventral half and (5) one right and one left temporo-ventral half. The contralateral visuotectal projections from the operated eyes later showed abnormalities reflecting the anatomical arrangement of the fused fragments. The experiments thus revealed considerable stability of the developmental programme leading to the later development of map orientation, in the face of operative disturbance of the types used.

**INTRODUCTION**

The normal visuotectal (and thus retinotectal) map in *Xenopus* is continuously ordered in that neighbourhood relations between ganglion cells in the retina are preserved in the tectal projection, and is also properly oriented on the tectum.

It has recently been shown (Gaze, Feldman, Cooke & Chung, 1979) that the developmental programme in the *Xenopus* eye anlage, concerned with the eventual orientation of the visuotectal map, is determined by stage 21/22 (Nieuwkoo & Faber, 1956) of embryonic life. Surgically formed conventional compound eyes (double-nasal, NN; double-temporal, TT; double-ventral, VV) project to the tectum in such a way that each half-retinal projection spreads out, eventually, across the whole tectum, with an orientation which is correct with respect to the nature of the half-retina (Gaze, Jacobson & Székely, 1963; Straznicky, Gaze & Keating, 1974). Since the absolute tectal distribution of the projection changes with time, both for normal projections (Gaze, Keating, Ostberg & Chung, 1979) and for those from compound eyes (Straznicky, Gaze & Keating, in preparation), the nature of compound eye projections is best characterised by their orientation.

Operations to form NN, TT and VV eyes have usually been made around stage 32 and the projections later mapped from such eyes suggest that the retinal...
developmental programme, in so far as it relates to the eventual orientation of the map, is preserved following the operation to form a compound eye. Each retinal fragment projects to the tectum with a map orientation that corresponds to the orientation of the retinal fragment.

There are, however, other circumstances in which projections resembling NN, TT and VV projections can be obtained. If half an eye *anlage* is surgically removed at stage 32 in *Xenopus*, the remaining half-eye normally rounds-up by itself and eventually produces a complete normal eye with a normal retinotectal map. A small proportion of such operations, however, produce eyes giving mirror-reduplicated projections (Berman & Hunt, 1975; Feldman & Gaze, 1975; Feldman, 1978). A residual nasal half-eye may thus give an NN projection, a residual temporal half-eye may give a TT projection and a residual ventral half-eye may give a VV projection.

Thus it is possible that the NN, TT and VV maps obtained after operations involving the surgical fusion of the appropriate half-eyes may not reflect the stability of the developmental programme within the eye, but rather the converse; the results could imply that the operations had led to the initiation of pattern-reduplication in the host eye fragment.

The present experiments were designed to demonstrate the stability or otherwise of the developmental programme within the eye. This we have done by constructing a variety of new forms of compound eye, including combinations where the grafted fragment is rotated 180° with relation to the host fragment and the map predicted on the basis of a stable programme cannot be produced by mirror-reduplication.

The various types of compound eye can usefully be illustrated by means of clockface diagrams (Fig. 1). The normal retina may be arbitrarily divided into sectors numbered from 1 to 12 in a clockwise direction. The dorsal pole will be represented by 12, the nasal pole by 3, the ventral pole by 6 and the temporal pole by 9. Figure 1 shows the composition of normal and of conventional NN, TT and VV eyes in terms of this model, as well as the composition of the varieties of compound eye used in the present experiments.

The results of these experiments show that the developmental programme relating to the orientation of the visuotectal map is stable in the face of operations of the type used. Thus the partial map formed by the tectal projection from each retinal fragment has an orientation which is the same as the orientation of that fragment in the compound eye.

The compound eyes described here are of types not previously made. The general principles which emerge from study of these results, however, apply also to other forms of compound eye. In the next paper of this series (Gaze & Straznicky, 1980) we describe certain further varieties of compound eye operation, which have also been studied by other workers; and we there compare the nature of the results obtained in the different laboratories.
Fig. 1. Clockface diagrams illustrating the composition of a normal eye, the three conventional compound eyes (NN, TT, VV) and the five varieties of compound eye discussed in this paper. The normal right retina is shown subdivided into segments numbered from 1 to 12 in a clockwise direction. Thus nasal is 3, ventral is 6, temporal is 9 and dorsal is 12. The curved arrows surrounding the diagrams indicate orientation. These clockface diagrams are intended to represent positional values within the eye in a comprehensible manner. The diagrams do not refer to the clockface model of pattern formation (French, Bryant & Bryant, 1976).
Embryos of *Xenopus laevis* were obtained from laboratory breeding pairs. Under anaesthesia with MS222 (tricaine methane sulphonate, Sandoz, 1:5000) the right eyes were operated on at stages 32/33 in full strength Niu-Twitty solution (Rugh, 1962). An eye from a donor embryo at the same stage was dissected out, surrounding mesenchymal tissue was cleaned away and the unwanted retinal half was removed by suction with a Spemann pipette. Particular care was taken to avoid damage to the donor eye fragment during manipulation. The right eye of the host embryo was exposed by cutting open the overlying ectodermal layer and the ectoderm was reflected aside. Half the eye anlage was removed by suction and substituted by the donor retinal fragment. The ectodermal layer was then replaced over the eye and a small splinter of cover glass was placed on top of the operated eye. Five types of eye recombination were carried out (Figures 1 and 2).

**Type 1.** The dorso-temporal half of the right eye was substituted by a right naso-ventral half, to obtain a 2NV_R eye.

**Type 2.** Two right ventral halves were combined to give a 2V_R eye.

**Type 3.** The dorso-nasal half of the right eye was substituted by a right temporo-ventral half, to form a 2TV_R eye.

**Type 4.** The dorso-temporal half of the right eye was substituted by a left naso-ventral half, to form an NV_R NV_L eye.

**Type 5.** The dorso-nasal half of a right eye was substituted by a left temporo-ventral half, to form a TV_R TV_L eye.

Thirty minutes after the surgery, operated embryos were transferred from the operating dish to a small container with fresh full strength Niu-Twitty solution. Two days after operation the success of the surgery was checked upon and all the animals with apparent eye abnormalities other than those intended were excluded from further experiments. Throughout larval life animals were kept in Stearn’s rearing solution and fed on Heinz’ beef and vegetable puree. Young toadlets were given tubifex worms, and from 3 months after metamorphosis, beef heart at twice weekly intervals.

The method of mapping the visuotectal projections was similar to that previously described for adults (Straznicky, Gaze & Keating, 1971) or for tadpoles (Gaze *et al.* 1974). In short, Wood’s metal microelectrodes were used with tip diameters of 10–15 μm and impedances of 0·1 MΩ. Tadpoles were anaesthetized with 1:3000 MS222 and recording was done in a Perspex globe filled with oxygenated Niu-Twitty solution containing MS222, 1:15000. Adult animals were anaesthetized with intra-peritoneal injections of 0·1 ml of 0·2% MS222, decerebrated with watchmaker’s forceps and immobilised with tubocurarine (0·2 mg intramuscularly). The tectum was exposed, single or multi-unit action potentials were recorded from terminal arborisations of optic fibres at predetermined tectal positions, and the corresponding receptive fields in the visual field were mapped. Maps were based on about 30 different tectal positions in each animal.
Fig. 2. The types of compound eye investigated. In each diagram the grafted fragment is hatched.

After the visuotectal mapping, the heads of the animals were fixed in Susa fixative. In some cases serial sections cut transversely at 15 μm were taken and stained with Holmes’s silver method.

RESULTS

After operation the fused eye fragments healed together within 24 h and further growth and differentiation of the operated eyes proceeded normally. Visuotectal mapping of the operated animals was carried out at various intervals after eye surgery, ranging from late larval stages to 4 years after metamorphosis (Table 1). Thirty-six animals with successful operation and completed mapping were included in this study. Since all the transplanted eye fragments contained part or all of the ventral retina, in 27 out of 36 cases a second ‘ventral’ fissure was present, the position of which could be correlated with the nature of the operation. In the other nine animals, for reasons unknown, a second fissure was not seen. The operated eyes, with or without a second fissure, showed gold pigmentation on both the dorsal (transplanted) and ventral (host) poles, in conformity with the embryonic origin of the fragments.

(1) The projection from normal and conventional compound eyes

For comparison with maps from the present experiments, a short description is given of normal projections and conventional compound eye projections. In normal animals, the nasal visual field projects to rostral tectum, temporal visual
Table 1. Summary of operations and results

T, N, Temporal, nasal; MAM, months after metamorphosis; YAM, years after metamorphosis; DV, dorso-ventral; NT, naso-temporal.

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>Position of eye fissures</th>
<th>Time of recording</th>
<th>Nature of visuotectal map</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1: 1</td>
<td>N⊙T</td>
<td>18 MAM</td>
<td>Dorsal half-field properly oriented; ventral half-field disorganised</td>
</tr>
<tr>
<td>2NV₁</td>
<td>2</td>
<td>4 YAM</td>
<td>DV reduplicated; ventral field NT reversed; rotated as eye; messy.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6 MAM</td>
<td>Dorsal field only</td>
</tr>
<tr>
<td>Type 2: 1</td>
<td>O</td>
<td>6 MAM</td>
<td>DV reduplicated; NT reversed (Fig. 5)</td>
</tr>
<tr>
<td>2V₁</td>
<td>2</td>
<td>6 MAM</td>
<td>DV reduplicated; NT reversed. Rotated as eye</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>St. 60</td>
<td>DV reduplicated; NT reversed</td>
</tr>
<tr>
<td>Type 3: 1</td>
<td>O</td>
<td>St. 57</td>
<td>Normal from temporo-dorsal field + some double points</td>
</tr>
<tr>
<td>2TV₁</td>
<td>2</td>
<td>St. 56</td>
<td>Normal from dorsal field; some double points ventral.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>St. 57</td>
<td>Normal + group of naso-ventral points.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed. (Fig. 6)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4 YAM</td>
<td>Dorsal half-field + few ventral disorganised points.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed, messy.</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4 YAM</td>
<td>DV reduplicated; NT reversed</td>
</tr>
<tr>
<td>Type 4: 1</td>
<td>O</td>
<td>St. 56</td>
<td>Resembles NN</td>
</tr>
<tr>
<td>NV₁NV₂</td>
<td>2</td>
<td>St. 57</td>
<td>? VV rotated (Fig. 11)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>St. 56</td>
<td>Resembles NN (Fig. 9)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3 MAM</td>
<td>Reoriented as retina, but compressed into narrow band.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>18 MAM</td>
<td>Resembles VV</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4 MAM</td>
<td>Dorsal, normal; ventral reoriented as retina</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4 YAM</td>
<td>Dorsal, normal; ventral reoriented as retina (Fig. 8)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4 YAM</td>
<td>Dorsal, normal; ventral reoriented as retina</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4 YAM</td>
<td>Resembles VV (Fig. 10)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4 YAM</td>
<td>Dorsal, normal; ventral reoriented as retina</td>
</tr>
</tbody>
</table>
11  ○  4 YAM  Dorsal, normal; ventral re-oriented as retina (Fig. 7)
12  ○  18 MAM  Normal map

| Type 5: | 1  | ○  | St. 57 | Normal |
| TV<sub>R</sub>T<sub>V</sub> | 2  | ○  | 3 MAM | Resembles TT (Fig. 12) |
| 3  | ○  | 3 MAM | ND field normal; TV field re-oriented as retina (Fig. 13) |
| 4  | ○  | 18 MAM | Resembles TT |
| 5  | ○  | 18 MAM | Resembles TT |
| 6  | ○  | 3 MAM | Dorsal field only; normal + few double points |

Field to caudal tectum, superior visual field to medial tectum and inferior visual field to the lateral part of the tectum (Fig. 3). In conventional compound eyes each of the similar half-retinas projects in a retinotopically ordered fashion, and with the orientation appropriate to that half-retina, across the whole extent of the tectum; thus the projection from both temporal halves in a TT eye spreads out caudally, the projection from both nasal halves in a NN eye spreads out rostrally and the projection from both ventral halves in a VV eye spreads out laterally on the tectum.

(2) Types 1, 2 and 3 operations (2NV<sub>R</sub>, 2V<sub>R</sub>, 2TV<sub>R</sub>).

The structure of 2NV<sub>R</sub>, 2V<sub>R</sub> and 2TV<sub>R</sub> eyes is different from that of conventional compound eyes, which are combinations of similar half-eyes of opposite handedness, in that they are all combinations of similar half-eyes of the same handedness, and thus involve a further axial reversal, along the line of fusion of the eye fragments (Fig. 1). These operations thus form a separate class of compound eyes; and in accord with this the results of these three types of operation were essentially similar to one another. Each type of eye gave maps which showed a reduplication of the visual field projection about the line of fusion of the eye fragments; but, reflecting the nature of the operations, the components of the visual field projection originating from the host and graft retinal fragments were rotated 180° with respect to each other. Thus the reduplicated visual field positions projecting to one tectal electrode position showed diagonal or point symmetry about the centre of the visual field, rather than mirror-image symmetry about the axis of the operation, as is the case with conventional compound eyes.

In Type-1 operations (2NV<sub>R</sub>) two right naso-ventral fragments were combined. Four out of six such eyes gave maps of the type described above (Fig. 4). One of the remaining animals gave a map from the host retinal fragment (dorsal half-field) together with some disorganised responses from the graft (ventro-nasal field), while the other animal gave a projection from the host retinal fragment only.

In Type-2 operations (2V<sub>R</sub>) two right ventral fragments were combined. Three out of three such animals gave maps as described above (Fig. 5).
Fig. 3. Summary of the normal visuotectal projection and the projections from TT, NN and VV eyes. The numbered arrows on the left tectum (centre) represent rows of electrode positions. The numbered rows in each of the (right) visual field charts represent corresponding stimulus positions in the visual field. The diagrams are greatly simplified; in particular the tendency for the rows of stimulus positions in the temporal part of the VV field to incline towards the temporal pole, has been ignored.

In Type-3 operations (2TV\textsubscript{R}) two right temporo-ventral fragments were combined. Five out of nine such eyes gave maps of the type described (Fig. 6). The other four animals gave organised projections from the host retinal fragment (dorsal field) only, with a few disorganized field positions ventro-nasally.
Fig. 4. Visuotectal projection from a 2NV<sub>R</sub> eye, recorded 4 years after metamorphosis. The projection is compound, showing two separate partial maps. That from dorsal field (host retina) is normally oriented while that from ventral field (graft retina) is rotated 180° as is the graft retinal fragment (inset). In this and the following maps, the top diagram shows the dorsal surface of the left tectum with the midline indicated by a heavy black arrow pointing rostrally. Rows of numbered electrode positions are indicated. The bottom diagram represents the right visual field with rows of numbered stimulus positions corresponding to the tectal electrode positions. The inset shows the nature of the compound eye, with grafted fragment hatched. The arrows on the tectum and the visual field chart indicate the orientations of the parts of the map. N, Nasal; V, ventral; T, temporal; D, dorsal.

(3) Types-4 and -5 operations (NV<sub>R</sub>NV<sub>L</sub>, TV<sub>R</sub>TV<sub>L</sub>)

These two types of operation, like conventional NN, TT and VV eyes, involve combining similar half-eyes of opposite handedness (Fig. 1).
In Type-4 operations \((NV_{R}NV_{L})\) one right and one left naso-ventral fragments were combined. Six out of 12 such eyes gave maps that reflected the intended nature of the operation. Thus host retina (temporo-dorsal field) gave an approximately normally oriented map whereas graft retina (naso-ventral field) gave a map that was oriented in correspondence with the orientation of the transplanted retinal fragment (Fig. 7). A further example of this type of result is shown in Fig. 8. In all cases the projection to the tectum was discontinuous (Figs. 7, 8) in that some regions of tectum received input from the host fragment only, some of the graft fragment and some from both.
The nature of the operation to form an NV_{H}NV_{L} eye is such that slight inclination of the planes of section one way will give a conventional NN eye while inclination of the planes of section the other way will lead to the formation of a conventional VV eye (Fig. 1). It is therefore not surprising that two of the 12 operated animals gave maps resembling that from a conventional NN eye (Fig. 9).

Two of the 12 animals gave maps resembling that of a conventional VV eye (Fig. 10). Another gave a projection somewhat like a conventional VV map,
Fig. 7. Visuotectal projection from an NV<sub>R</sub>NV<sub>L</sub> eye, recorded four years after metamorphosis. The partial map from dorsal field (host retina) is normally oriented while that from ventral field (graft retina) is re-oriented in accordance with the graft (see Fig. 14).

rotated through 90° anti-clockwise (Fig. 11); we are unable to account for the orientation of this map. Lastly, one animal gave a normal map.

In Type-5 operations (TV<sub>R</sub>TV<sub>L</sub>) one right and one left temporo-ventral halves were combined. The nature of the operation is such that slight tilting of the planes of section one way will give a conventional TT eye while tilting in the other direction will give a conventional VV eye (Fig. 1). Three out of six such operations gave projections similar to those from conventional TT eyes (Fig. 12) except that the orientation of the axis of symmetry for the field positions re-
Fig. 8. Visuotectal projection from an NV₂NV₁ eye, recorded four years after metamorphosis. The partial maps are oriented as in Fig. 7.

reflected the orientation of the line of fusion of the two temporo-ventral eye fragments (this is not obvious in the case illustrated).

One animal gave a projection where the orientation of the two half-maps reflected the intended orientations of the two half retinas (Fig. 13). In two further cases the projection was essentially normal, with a few doubled field positions.

**DISCUSSION**

The original descriptions of the projections from conventional (NN, TT, VV) compound eyes showed abnormalities in the visuotectal maps directly reflecting the nature of the operations (Gaze, Jacobson & Székely 1963; Straznicky et al.)
Fig. 9. Visuotectal projection from an NV<sub>R</sub>NV<sub>L</sub> eye, recorded at stage 56 of tadpole life. Because the innervated part of the tadpole tectum tends to be elongated rostrocaudally, tadpole maps are usually presented with the rows of electrode positions running rostrocaudally on the tectum, as here. The map resembles a distorted NN projection.

1974). The common features of NN, TT and VV maps are that the projection from the host retinal half spreads across the entire dorsal surface of the tectum in a normally oriented fashion, and the transplanted half-eye projects in a mirror-image manner over the same area of tectum (Figs. 1 and 3). The present experiments extend the previous observations and show that the eye fragments used
Fig. 10. Visuotectal projection from an NV_RNV_L eye, recorded four years after metamorphosis. The orientation of the map resembles that of a VV projection.

Here retain their original programmes, relating to map orientation, when put together to form compound eyes.

From Fig. 1 it is immediately apparent why 2NV_R, 2V_R and 2TV_R eyes produced mostly maps with reduplication dorso-ventrally and with naso-temporal reversal of the half of the map corresponding to the grafted retinal fragment. Given the nature of conventional NN, TT and VV maps (Gaze et al. 1963; Straznicky et al. 1974), the present finding of dorso-ventrally reduplicated, naso-temporally reversed maps from 2NV_R, 2V_R and 2TV_R eyes is to be expected; and it provides further evidence for the autonomous behaviour of the retinal fragments in relation to the orientation of the maps that result from them.
Fig. 11. Visuotectal projection from an NV_r NV_L eye, recorded at stage 57 of tadpole life. The parts of this map, while messy, have an orientation that resembles a VV map, rotated 90° anti-clockwise.

What a map looks like will depend on the conventions used in mapping. In the retinotectal system it has become customary to arrange electrode positions in rows running either latero-medially or rostro-caudally across the tectum. Because of the orientation of the normal visuotectal map in Xenopus, this traditional method of electrode placement leads to conventional compound eye maps which are fairly simple and easy to comprehend.
Fig. 12. Visuotectal projection from a TV\textsubscript{R}TV\textsubscript{L} eye, recorded three months after metamorphosis. The projection resembles that from a conventional TT eye.

In NV\textsubscript{R}NV\textsubscript{L} and TV\textsubscript{R}TV\textsubscript{L} eyes, however, the plane of operation is such that the line of symmetry conforms neither to latero-medial nor rostro-caudal tectal rows; and it is not immediately obvious, intuitively, what sort of a projection should result (in terms of orientation) if the fragments behave autonomously. Figure 14 is intended to clarify this. Starting with the usual array of electrode positions on the tectum, the field and retinal positions that correspond to these in a normal animal are shown. Using these retinal positions, NV\textsubscript{R}NV\textsubscript{L} and TV\textsubscript{R}TV\textsubscript{L} retinas are constructed, and from these, corresponding field maps. It may then be seen that the result of the NV\textsubscript{R}NV\textsubscript{L} combination, deduced on the basis of autonomy of the retinal fragments for map orientation, is closely similar.
Fig. 13. Visuotectal projection from a TVR,TVL eye, recorded three months after metamorphosis. The partial map from naso-dorsal field (host retina) is normally oriented while that from temporo-ventral field (graft retina) is reoriented in accordance with the graft (see Fig. 14).

Twenty-six out of 36 recorded animals gave results expected on the basis of fragment autonomy. Nine of the ten remaining animals gave approximately normal maps, with a few reduplicated points. One animal (NVr,NNvL) gave a result which resembled a rotated VV map and which at present seems incomprehensible. These results argue strongly that the fused eye fragments retained intact their programmes for the development of properties relating to map orientation.
Fig. 14. The derivation of the predicted patterns of map orientation for NV_rNV_l and TV_rTV_l eyes. The top diagram shows a right and left tecta with orientations as shown in previous figures. The second row shows right and left visual field. The third row shows right and left retina. The fourth row shows how the two varieties of compound retina are put together and the bottom row shows the expected field orientations for TV_rTV_l and NV_rNV_l eyes. For the purposes of this figure the order and orientation of the arrows are significant, not their extent.

It is worth emphasizing, however, that what is preserved in these experiments is the orientation of the partial map from each retinal fragment; the tectal positions to which each fragment projects are quite abnormal, in that the projection from each fragment tend to spread across the entire extent of the tectum, albeit in a somewhat discontinuous fashion. The tectal distribution of compound eye
projections will be treated elsewhere (Straznicky, Gaze & Keating, in preparation). The results of confronting a nasal or temporal fragment with a ventral fragment are presently being investigated and the projections from further varieties of compound eyes are discussed in the next paper in this series (Gaze and Straznicky, 1980).

We are grateful to Dr D. Willshaw for helpful discussion of these experiments. Fig. 1 was suggested by these discussions and our views on the interpretation of some of the results have been profoundly affected by Dr Willshaw's analysis, which will be published elsewhere. We are grateful also to Mr J. Steedman for help in clarifying the argument presented. We wish to acknowledge the skilled histological assistance of Mrs J. Colville.

REFERENCES


(Received 6 June 1979, revised 2 August 1979)