Karyotype analysis of teratocarcinomas and embryoid bodies of C3H mice

By S. A. ILES\textsuperscript{1} AND E. P. EVANS\textsuperscript{2}

From the Department of Zoology and the
Sir William Dunn School of Pathology, Oxford University

SUMMARY

Karyotype and capacity for differentiation were determined in four transplantable teratomas, and their embryoid bodies, derived from C3H mouse embryos. An apparently normal karyotype was retained by one tumour and one subline that were able to differentiate into a wide range of tissues, but some chromosomal alterations were found in the two tumours and one subline that showed almost identical restrictions in their capacity for differentiation. Trisomy for chromosome 11 was shared by all three restricted tumours; two of the tumours had similar length changes in the same two chromosome (1 and 14) while the third was generally trisomic for four other chromosomes.

INTRODUCTION

Some primary mouse teratomas, either spontaneous or derived from early embryos in ectopic sites, are transplantable; the basis of such transplantability appears to be the possession of significant numbers of embryonal carcinoma cells (ECC). These transplantable teratomas, or teratocarcinomas, will continue to grow until the host dies, but they do not generally metastasize. Upon transplantation, some teratocarcinomas retain their ability to differentiate into a wide range of tissues, while others show a restricted capacity for differentiation and produce only one or very few differentiated tissues (reviewed by Solter, Damjanov & Koprowski, 1975).

Studies on the karyotypes of mouse teratomas and teratocarcinomas should provide information as to whether transplantability and progressive restriction in the capacity to differentiate are associated with karyotypic changes. Stevens & Bunker (1964) reported that cells of early spontaneous testicular teratomas of 129 mice were indistinguishable in chromosome number and morphology from cells in the normal testis and early embryos; almost all the cells appeared to have a \textit{Y} chromosome. Transplantable teratomas derived from such tumours often had abnormal chromosome numbers and morphology (Bunker, 1966). Dunn & Stevens (1970), in a study of transplantable teratomas derived from 7th day

\textsuperscript{1} Author's address: Department of Zoology, South Parks Road, Oxford, OX1 3PS, U.K.
\textsuperscript{2} Author's address: Sir William Dunn School of Pathology, South Parks Road, Oxford, U.K.
embryos, found normal chromosome numbers in all but one instance; these tumours were considered to be of male or female chromosomal sex, a diagnosis made by reference to the presence of either three (male) or two (female) chromosomes clearly smaller than any of the others.

Until the relatively recent introduction of banding techniques, a few mouse chromosomes could be positively identified from their secondary constrictions. However, it is now possible to identify individual mouse chromosomes with certainty on the basis of their banding patterns. In this study, the karyotypes of four transplantable teratomas of C3H mice (the properties of which are described fully in the preceding paper by Iles, 1977) have been studied over a number of transplant generations; the use of chromosome banding techniques has made it possible to identify karyotypic abnormalities in some of these tumours and their embryoid bodies with some precision, and an attempt has been made to correlate karyotypic abnormalities in the tumours with their capacity for differentiation.

**MATERIALS AND METHODS**

(i) **Induction of tumours and embryoid bodies**

Teratocarcinomas were derived in C3H mice by transplantation of primary teratomas arising from embryos transferred to ectopic sites in syngeneic adult recipients. The derivation and properties of the four teratocarcinomas studied here are described in Table 1.

Intraperitoneal transfer of all four teratocarcinomas resulted in the formation of ascitic fluid containing embryoid bodies (e.g. Stevens, 1959, 1970). Full details of the derivation, maintenance and histology of these tumours and embryoid bodies are given in the preceding paper (Iles, 1977).
(ii) **Chromosome preparations**

The analysis of chromosomes in primary tumours proved difficult because of the low number and poor quality of the metaphases recovered. Chromosome studies were therefore carried out on a number of transplant generations of both solid tumours and the embryoid bodies derived from them. For this purpose, solid tumours were allowed to grow in the transplant hosts until they reached an approximate diameter of 2 cm; embryoid bodies were obtained from hosts when it became visually obvious that they contained peritoneal ascites fluid. To obtain a sufficient number of metaphases, either the host was injected with Colcemid (0.25 ml of 0.04 %) 1 h before killing, when the solid tumour or embryoid bodies were recovered and disaggregated by teasing and pipetting in MEM, or the host was killed and the tumours or embryoid bodies disaggregated and the cell suspensions obtained subjected to 2 h incubation in MEM containing 0.5 mg Colcemid per ml. Following either procedure chromosome preparations were then made by a standard air-drying method (Ford, 1966), but using 0.56 % potassium chloride as the hypotonic solution.

Slides for the scoring of chromosome numbers were stained immediately with toluidine blue (Breckon & Evans, 1969) but slides for chromosome banding were ‘aged’ at room temperature for at least 1 week. From these slides chromosome G bands were obtained by a modification of the method of Gallimore & Richardson (1973). The banded chromosomes obtained were first analysed at the microscope and between 5 and 10 selected metaphases photographed and the chromosomes subsequently arranged according to the nomenclature proposed by Nesbitt & Francke (1973) for the mouse karyotype. When karyotypic abnormalities were recorded they were identified with a confidence which lay within the limitations of the banding technique.

**RESULTS**

(i) **Tumour 17**

(a) **Solid tumours.** Chromosome counts were made on solid tumours between the 4th and 10th transplant generations (Table 2); more than one subline was examined in some generations. The modal chromosome number was always 40 (the diploid number for the mouse). Cells with more than 40 chromosomes were very rare, as were polyploid cells; there was a minor population of cells with 38 or 39 chromosomes (particularly noticeable in the 5th and 6th generations) but it was not determined whether these counts were artifactual and resulting from broken mitoses.

Banding studies on cells with 40 chromosomes, seven in the 8th and six in the 10th generation, showed that there were no detectable departures from the normal mouse karyotype; there were two X chromosomes (Fig. 1). Tumour 17, even at the 10th generation, retained the capacity of the primary tumour to
Table 2. Chromosome counts in solid transplants and embryoid bodies of tumour 17

<table>
<thead>
<tr>
<th>Generation</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
<th>42</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
<th>47</th>
<th>Tetraploid (78–88)</th>
<th>Total no. of counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>4</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>EB†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in columns refer to number of cells with that chromosome count.
† Vesicular embryoid bodies.

differentiate into a wide range of tissues, although ECC rather than the differentiated tissues predominated in later generations.

(b) Embryoid bodies. Embryoid bodies arising from tumour 17 were propagated independently of the solid tumour and initially had a structure typical of most teratocarcinoma-derived embryoid bodies (e.g. Stevens, 1970 and preceding paper by Iles, 1977) but they later came to consist of hollow vesicles of flattened endoderm-like cells, which gave rise to yolk-sac carcinoma when injected subcutaneously. Their modal chromosome number was 41 (Table 2). Seven banded cells with 41 chromosomes were analysed from the same passage. Though all were alike in being trisomic for 17 and monosomic for 19 and X, no two were identical. Four were monosomic for a further chromosome, different in each case, and a fifth was additionally trisomic for 6. Each contained from one to three chromosomes whose origin could not be determined. Even these varied between one cell and another. There were 17 altogether and they included a minimum of 11 different types. Despite the common features the total picture was therefore one of great disorder with no single modal karyotype discernible.

(ii) Tumour 86

(a) Solid tumours. Chromosome counts were made on tumour 86 between the 1st and 6th transplant generations (Table 3). The 1st generation tumour showed a wide range of differentiated tissues and subline (b) of the second generation, although less well differentiated, was still producing ECC and some differentiated tissues, e.g. cartilage, bone, striated muscle and nervous tissue: both these tumours had almost equal numbers of cells with 40 and 41
Fig. 1. G banded karyotype of tumour 17 at the 10th generation. 40 chromosomes, apparently normal female. × 2800.
chromosomes, but the 1st generation tumour had as many near-tetraploid cells as it did near-diploid cells. Subline (a) of the 2nd generation consisted only of ECC and nervous tissue: there was now a strong mode of 41 chromosomes and only a minor population of cells with 40 chromosomes. Tetraploid cells had almost totally disappeared from the 2nd generation tumours. The 3rd generation tumour, with a strong mode of 41 chromosomes, produced mainly ECC and nervous tissue, with only a little smooth muscle and epithelial tissue. In later generations, the mode of 41 chromosomes persisted, and tumours consisted of only ECC and nervous tissue, with a little epithelial tissue. Banding studies of seven cells at the 6th generation showed the following consistent abnormalities: (i) trisomy for chromosome 11, (ii) an approximate 20% addition to the distal end of chromosome 1, (iii) an approximate 30% deletion of the distal end of chromosome 14 (Fig. 2). The length changes in (ii) and (iii) were not obviously reconcilable with a single reciprocal translocation. The observations did not exclude intra-chromosomal duplication as the source of the extra material in (ii).

(b) Embryoid bodies. Embryoid bodies derived from tumour 86 consisted of a central core of ECC often surrounded by endoderm-like cells; their differentiative capacity, when injected subcutaneously, was restricted in a manner similar to that of the solid tumour. Both the embryoid bodies and solid implants derived from them had a strong mode of 41 chromosomes (Table 3), with abnormalities identical to those in the solid tumours.

| Generation | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | Tetr- | Total |
|------------|----|----|----|----|----|----|----|----|----|----|----|----| ploid | no. of |
| 1          | 0  | 0  | 0  | 2* | 12 | 10 | 0  | 0  | 0  | 1  | 0  | 0  | 25  | 50   |
| 2 (a)      | 2  | 0  | 0  | 2  | 13 | 31 | 3  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 51   |
| 2 (b)      | 0  | 0  | 1  | 1  | 8  | 8  | 1  | 1  | 0  | 0  | 0  | 0  | 1  | 22   |
| 3          | 0  | 0  | 0  | 1  | 8  | 31 | 8  | 2  | 0  | 0  | 0  | 0  | 0  | 50   |
| 4          | 0  | 0  | 0  | 0  | 4  | 5  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 9    |
| 5          | 0  | 0  | 0  | 0  | 3  | 11 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 15   |
| 6          | 0  | 0  | 0  | 0  | 1  | 9  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 11   |
| 7          | 0  | 0  | 0  | 0  | 2  | 27 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 30   |
| 8          | 0  | 0  | 0  | 0  | 1  | 28 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 30   |
| Solid from |     | 0  | 0  | 1  | 2  | 17 | 1  | 0  | 0  | 0  | 0  | 0  | 2   | 24   |
| ascites    |     | 0  | 0  | 0  | 4  | 45 | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 50   |
| EB         |     | 0  | 0  | 0  | 1  | 29 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 30   |

* Figures in columns refer to number of cells with that chromosome count.
Fig. 2. G banded karyotype of tumour 86 at the 6th generation. 41 chromosomes, trisomy for 11, extension of 1 and deletion of 14. × 2800.
Table 4. *Chromosome counts in solid transplants and embryoid bodies of tumour 106*

<table>
<thead>
<tr>
<th>Generation</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
<th>42</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1*</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>18</td>
<td>9</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>16</td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EB</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Figures in columns refer to numbers of cells with that chromosome count.

(iii) *Tumour 106*

(a) *Solid tumours.* Tumour 106, when first examined at the 3rd transplant generation, had 42–45 chromosomes in most of the cells and lacked a clear mode (Table 4). Although the primary tumour was well-differentiated, it was now starting to become restricted (see Iles, 1977). By the fourth generation, one sub-line had a mode of 44 chromosomes, another had almost equal numbers of cells with 43 and 44 chromosomes, while the third had a mode of 42 chromosomes with strong sub-populations with 43 and 44 chromosomes; all three tumours contained only ECC and nervous tissue. A mode of 44 chromosomes with a strong sub-population of cells with 43 chromosomes persisted in the 5th and 6th generations (tumours consisting only of ECC and nervous tissue).

Banding studies at the 5th and 6th generation showed the following changes: six and seven cells respectively with 44 chromosomes were trisomic for chromosomes 11, 12, 15, 17 and 19 and monosomic for X (Fig. 3); two cells and one cell respectively with 42 chromosomes were trisomic for 11, 12 and 15 only, with monosomy for X. By chance, no cells with 43 chromosomes were included in the analyses.

(b) *Embryoid bodies.* Embryoid bodies derived from tumour 106 showed a restriction in developmental potential similar to that of the solid tumour; they resembled the solid tumours in the distribution of chromosome numbers, but the chromosomes were not banded (see Table 4). Embryoid bodies arising after intraperitoneal transfer of the 7th generation solid tumour were almost entirely polyploid: of 30 cells examined, one had 42 chromosomes, one had 43 and 28 had 77–191 chromosomes.
Fig. 3. G banded karyotype of tumour 106 at the 6th generation. 44 chromosomes, trisomy for 11, 12, 15, 17 and 19 with X monosomy. × 3000.
Table 5. *Chromosome counts in solid transplants and embryoid bodies of tumour 145*

<table>
<thead>
<tr>
<th>Generation</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
<th>42</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
<th>47</th>
<th>Tetraploid (78–88)</th>
<th>Total no. of counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1 (a)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>3 (a)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>3 (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>4 (a)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>4 (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>43</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>5 (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>22</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>EB (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

* Figures in columns refer to numbers of cells with that chromosome count.

(iv) *Tumour 145*

(a) *Solid tumours.* Tumour 145 was examined in the 1st to 5th transplant generations (Table 5), but it was not possible to obtain chromosome spreads from either of the second generation tumours. The tumour remained well differentiated in the first three transplant generations; there was a mode of 40 chromosomes in the 1st generation. Chromosome counts of 3rd generation tumours revealed two distinct sublines: one (subline (a)) still had a strong mode of 40 chromosomes, while the other (subline (b)) had a mode of 41 chromosomes, with a sub-population of cells with 40 chromosomes. In the 4th generation, the tumours derived from subline (a) were still capable of producing a wide range of differentiated tissues, and the modal chromosome number was still 40, while in the 4th and 5th generations, the tumours derived from subline (b) produced only ECC and nervous tissue (with a little ciliated epithelium in one tumour) and had a sharp mode of 41 chromosomes.

Banding studies on ten cells at the 4th and seven cells at the 5th generation tumours of subline (b) showed abnormalities remarkably similar to those found in tumour 86, i.e. (i) trisomy for chromosome 11, (ii) an approximate 20% addition to the distal end of chromosome 1, (iii) an approximate 30% deletion of the distal end of chromosome 14 (Fig. 4). As in tumour 86, the length changes in (ii) and (iii) were not obviously reconcilable with a single reciprocal translocation and the observations did not exclude intra-chromosomal duplication as the source of the extra material in (ii). Tumour 145(b) also resembled tumour 86 in differentiative capacity. Banding studies on ten cells of the 4th generation tumour of subline (a) showed a normal female karyotype, thus resembling tumour 17 in both karyotype and differentiative capacity.

(b) *Embryoid bodies.* Embryoid bodies from tumour 145(b) showed a
Fig. 4. G banded karyotype of tumour 145(b) at the 4th generation. 41 chromosomes, trisomy for 11, extension of 1 and deletion of 14. x 2900.
restriction in differentiative capacity identical to that found in solid tumours of that subline, and their karyotype was identical to that of solid tumours of that subline (b) (Table 5).

**DISCUSSION**

These results show that karyotypic abnormalities, as detectable by banding techniques, are not a constant feature of transplantable mouse teratomas: tumours with a seemingly normal karyotype can grow upon transplantation and are capable of killing their hosts. Tumour 17 and subline (a) of tumour 145 fall into the latter category, while tumours 86, 106 and subline (b) of tumour 145 showed specific karyotypic abnormalities. The work of Dunn & Stevens (1970) had previously suggested that transplantable teratomas derived from mouse embryos could have normal chromosome counts and no grossly detectable abnormalities. However, since individual chromosomes were not identified by banding, the possibility that the karyotypes of some of these tumours were pseudo-diploid cannot be dismissed.

Transplantable teratomas or teratocarcinomas can only be derived from a proportion of primary teratomas (Stevens, 1958, 1970; preceding paper by Iles, 1977) but since karyotypic abnormalities do not seem to be a prerequisite for the growth of such tumours upon transplantation, it is unlikely that mouse teratomas or teratocarcinomas owe their origin to embryonic cells with abnormal karyotypes. However, karyotypic abnormalities were found in solid tumours and embryoid bodies of tumours 86, 106 and 145(b) and in the vesicle-like embryoid bodies of the karyotypically normal tumour 17. Consideration should be given to (i) why cells with abnormal karyotypes should have come to dominate these tumours, (ii) whether these alterations in karyotype are associated with changes in differentiative capacity in the tumours.

Chromosome counts on early transplant generations showed the gradual replacement of a diploid chromosome number by a hyperdiploid one in tumour 86, and bifurcation into two sub-lines, one with a diploid and one with a hyper-diploid number, in tumour 145; tumour 106 already had abnormal chromosome counts by the time it was first examined at the 3rd transplant generation. The nature of the abnormalities revealed by later banding studies (mostly trisomies) suggests that the same abnormalities were responsible for the earliest abnormal chromosome counts. This appearance of cells with abnormal chromosome counts during transplantation, coupled with the normal chromosome numbers found by Stevens & Bunker (1964) in primary testicular teratomas and in three embryo-derived primary teratomas by the present authors, suggests that cells with abnormal karyotypes are selected during transplantation, i.e. the abnormalities are secondary rather than primary and are not essential for such progressive growth (see tumours 17 and 145(a)). Primary tumours in mice, rats and hamsters may have normal or abnormal karyotypes, but the normal karyotypes tend to become lost upon transplantation: transplantation may
select strains of aneuploid cells which are more vigorous and better adapted for
growth than cells of normal karyotype (Jean & Bois, 1967; Levan, 1969). It
therefore seems likely that the domination of some of the tumours studied here
by cells with abnormal karyotypes is the result of selection for cells with rapid
growth during transplantation.

In all cases examined here, changes in karyotype were associated with restric-
tion of differentiative capacity. Full differentiative capacity was retained only in
tumours with a diploid chromosome number and apparently normal banding
patterns (tumours 17 and 145(a)). Abnormal hyperploid karyotypes were
associated with a restriction in differentiative capacity in the tumours and embry-
oid bodies of tumours 86, 106 and 145(b). In tumour 86, changes in chromosome
number could be correlated with changes in tumour morphology in different
sublines before the final restriction in differentiation of the tumour was seen,
but in tumour 106, differentiative capacity was already restricted when its (ab-
normal) karyotype was first investigated. In tumour 145, there was a good asso-
ciation between karyotype and differentiative capacity: one subline (a) in the
4th generation retained a normal karyotype and the ability to differentiate
fully, while the other had an abnormal karyotype and a restricted capacity for
differentiation. Although tumour 17 had a normal karyotype, the vesicular
embryoid bodies derived from it had highly abnormal karyotypes and reduced
differentiative capacity.

It is important to know if karyotypic abnormalities, when they do develop, are
consistent in transplantable mouse teratomas, and if they are related to a par-
ticular restriction in developmental capacity. Tumours 86, 106, 145(b) and their
embryoid bodies all showed a similar restriction in developmental capacity; tumours consisted mainly of ECC and nervous tissue, with occasional epithelial
tissue, so any common karyotypic feature might be related to this common
restriction. Tumours 86 and 145(b) and their embryoid bodies were trisomic for
chromosome 11, with alterations in one chromosome of pairs 1 and 14 and had
two X chromosomes. Attention has already been drawn to the close similarities
of the karyotypic changes in these two tumours and it is appropriate to consider
whether they could be one and the same tumour. This could have been effected
by the contamination or accidental mixing of animals or cells during and after
transplantation of the tumours. The authors have considered these possibilities
and conclude that there was no greater chance of mixing than during the course
of any other experiment and that 86 and 145(b) are two different tumours. Tumour
106 was also trisomic for chromosome 11, but it was additionally trisomic for
chromosomes 12, 15, 17 and 19, and monosomic for X. The vesicular embryoid
bodies of tumour 17 had a highly abnormal differentiative capacity, forming
yolk-sac carcinoma when injected subcutaneously: their karyotype was more
abnormal than that of the other teratocarcinomas and embryoid bodies (see
Results) but they had trisomy for chromosome 17 and monosomy for X in
common with tumour 106. It is possible that simple primary trisomy was
involved in the abnormal chromosome numbers found in one transplantable teratoma by Dunn & Stevens (1970) and in two sublines of the teratoma transplanted by Bunker (1966). One X chromosome appears to have been lost in the embryoid bodies of tumour 17 (solid tumour is XX), but an X or a Y chromosome could have been lost in tumour 106; apparent loss of the Y chromosome in teratocarcinomas has been demonstrated by Bunker (1966).

Since these teratocarcinomas were all derived from mouse embryos, it is of interest to compare the growth of the trisomic ones with the in utero development of trisomic mouse embryos. Most of these embryos die early in gestation; few persist to term but none survive after the first day of birth (White, Tjio, Van de Water & Grandall, 1974; Ford, 1975; Gropp, Kolbus & Giers, 1975). On the other hand, although trisomic teratocarcinomas are restricted in their differentiation, they are successful in their growth. A comparison of these two trisomic situations implies that they are disadvantageous to the mouse embryos but are of some advantage to the teratocarcinomas.

Specific primary trisomies are associated with certain tumours in the rat (e.g. Mitelman, 1971) and in a number of haematologic conditions in man (e.g. Rutten, Hustinx, Scheres & Wagener, 1974; Wurster-Hill et al. 1976). Banding studies should now make it possible to identify specific trisomies in mouse tumours; it has recently been shown that trisomy 15 is a common characteristic of spontaneous leukaemias in AKR mice (Dofuku, Biedler, Spengler & Old, 1975). More investigations of the karyotype of transplantable mouse teratomas should be carried out, so as to determine whether trisomy 11 is indeed a common abnormality in these tumours, and to find out if particular karyotypic abnormalities are associated with specific restrictions in developmental capacity in teratocarcinomas.

We should like to thank Drs C. E. Ford, C. F. Graham and M. D. Burtenshaw and Miss Hilary Clegg for their help and advice, and S. R. Bramwell for expert technical assistance.

This work was supported by the Cancer Research Campaign (S.A.I.) and the Medical Research Council (E.P.E.). S.A.I. was partly supported by a Mary Goodger Scholarship.

REFERENCES

Karyotype analysis of mouse teratocarcinomas


(Received 22 April 1976)