Viability and metabolic activity of homozygous Brachyury (T) embryos

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SUMMARY

Some features of metabolic activity of homozygous mutants for the Brachyury (T) mutation were studied. The embryos incorporated tritium-labelled thymidine up to about the '32–36 somite' stage. The total amount of protein per embryo increased until the same stage. T/T cells proved viable in vitro over the in utero lethal period. Several cell lines were established from +/+ and T/T embryonic cells.

INTRODUCTION

The semidominant mutation T (Brachyury) at the T/t locus of the mouse leads to a short-tailed phenotype when heterozygous and is lethal in homozygous condition. The homozygous embryos (T/T) go through the early stages of embryonic development without showing any obvious abnormalities. By 9 days of gestation, T/T embryos are markedly abnormal. Their body ends just posterior to the anterior limb-buds, the somites are diffuse, and the neural tube is severely kinked (Chesley, 1935). To date this mutant has been studied mainly from the morphological point of view and little is known about its metabolic state (Grüneberg, 1958; Bennett, 1964; Spiegelman, 1976). In order to study these embryos at the cellular level (Yanagisawa & Fujimoto, 1977), it was first necessary to know something of their metabolic state.

It is believed that the homozygous mutant embryos are lethal because they fail to establish placental connexions (Gluecksohn-Schoenheimer, 1944). Ephrussi (1935) showed that these embryos could grow in organ culture beyond the time at which they would have died in utero. Gluecksohn-Schoenheimer (1944) reported a similar result by explanting the embryos in the chick host. The present investigation revealed that dissociated T/T cells are also viable in vitro and cell lines have been established from both +/+ and T/T embryonic cell cultures.

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MATERIALS AND METHODS

Mice and embryos. Origin and maintenance are as described by Yanagisawa & Fujimoto (1977).

Analysis of mitotic figures. Essentially the same method was employed as was described by Yanagisawa & Kitamura (1975).

Cell culture. Cells or tissue fragments were cultured in Eagle’s minimum essential medium supplemented with 10% heat-inactivated horse serum and 3% chick embryo extract (MEMHC), in an atmosphere of 95% air and 5% CO₂.

Radioautography. Pregnant females were injected intraperitoneally with 7 µCi/g body weight of [³H]thymidine (specific activity 12.4 Ci/m mole) and killed 1 h later. Embryos were removed quickly, washed thoroughly with Hanks’ salt solution and fixed in Bouin’s fixative. They were embedded in paraplast and serially sectioned at 5 µm. Slides were washed with 5% trichloroacetic acid, dipped in Sakura emulsion NR-M2 (Konishiroku Photo Ind. Co., Tokyo), and stored in a dark box at 4 °C for 6 weeks. They were developed and stained with haematoxylin.

Determination of amount of protein. Embryos were collected in 0.3 ml of 0.002% Triton X-100 and frozen at −70 °C. One tenth millilitre of 4N-NaOH was added to the thawed samples and the amount of protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Although T/T embryos look abnormal morphologically, they develop in a regular fashion. Thus the size and shape of the head of T/T embryos change in a similar way to their normal litter-mates and the forelimb-buds appear at a similar stage (24-somite stage). Even degeneration of the posterior trunk is not random, but starts when the normal litter-mates reach the 32–36-somite stage. It was therefore possible to stage the mutant embryos, though less accurately than the normals. Conventionally, stages of the mutant embryos were indicated by the number of somites of their normal litter-mates.

The number of mitotic figures per unit area (10⁻² mm²) was calculated for embryos of 9 (15–26-somites), 10 (27–36-somites) and 11 days (37–40 somites) of gestation. As shown in Fig. 1 for the level of the forelimb-bud, the number of mitotic figures per unit area in T/T embryos is comparable to that of +/- embryos up to 10 days (30–36-somite stage), but drops to zero at 11 days (37–40-somite stage). The number of mitotic figures per 10⁻² mm² of the neural tube at the posterior end of 10-day T/T embryos (average of 13 alternate sections from the tip) was 2.9 ± 0.4. Although this value is lower than that at the level of the forelimb-bud, mitotic cells existed among necrotic cells at the posterior end of the embryos.
Metabolic activity of T/T embryos

Fig. 1. Number of mitotic figures/10^-2 mm² in the neural tube of wild-type and mutant embryos, at the level of the forelimb-bud. □, +/+; ●, T/T. Number of embryos analysed are as follows: 9-day: +/+ , 5; T/T, 3; 10-day: +/+ , 2; T/T, 4; 11-day: +/+ , 2; T/T, 3.

[³H]Thymidine was incorporated into the nucleus of T/T cells at 10 days but no grains were found over the nucleus of 11-day T/T embryos. The percentage of labelled nuclei in the neural tube and the mesenchyme was counted in 9-, 10- and 11-day embryos. Embryos of each gestational age were siblings. The results are summarized in Table 1. In the homozygous mutant, around 60% of the cells were labelled. Even at the degenerating posterior end of T/T embryos, over 50% of the cells were labelled in 9- or 10-day embryos. These results coincide with those obtained by the analysis of mitotic figures, indicating that the cells of the homozygous embryo have mitotic activity up to 10 days of gestation.
Figure 2 shows the amount of total protein per embryo. $T/T$ embryos have less than their normal litter-mates, but the amount increases up to the 32–36-somite stage.

Although the mutant embryos are resolved by 12 days of gestation, their cells are viable if cultured in vitro. Dissociated $T/T$ and $+/+$ cells have already
Table 1. Percentage of labelled nuclei in the neural tube and mesoderm of normal and mutant embryos exposed to $[^3]H$thymidine

<table>
<thead>
<tr>
<th>Day</th>
<th>Embryo</th>
<th>Level of section</th>
<th>% nuclei labelled</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>+/+</td>
<td>Forelimb-bud</td>
<td>$69.3 \pm 2.8$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$75.8 \pm 2.1$ (mesoderm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior end</td>
<td>$65.6 \pm 4.1$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>Forelimb-bud</td>
<td>$71.2 \pm 2.8$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>Forelimb-bud</td>
<td>$67.3 \pm 4.1$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior end</td>
<td>$56.2 \pm 4.1$ (neural tube)</td>
</tr>
<tr>
<td>10</td>
<td>+/+</td>
<td>Forelimb-bud</td>
<td>$69.6 \pm 2.8$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$79.2 \pm 1.9$ (mesoderm)</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>Posterior end</td>
<td>$64.2 \pm 4.1$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td>T/+</td>
<td>Forelimb-bud</td>
<td>$67.3 \pm 2.8$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>Forelimb-bud</td>
<td>$59.2 \pm 5.2$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$78.5 \pm 1.8$ (mesoderm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior end</td>
<td>$54.0 \pm 4.1$ (neural tube)</td>
</tr>
<tr>
<td>11</td>
<td>+/+</td>
<td>Forelimb-bud</td>
<td>$79.2 \pm 2.1$ (neural tube)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>Forelimb-bud</td>
<td>0 (neural tube and mesoderm)</td>
</tr>
</tbody>
</table>

been passaged over 50 times in 15 months. Preliminary results show that T/T cell lines show some characteristics of freshly isolated T/T embryonic cells. Cloning and further characterization of these lines are in progress.

**DISCUSSION**

Our results suggest that T/T embryos at the 12-36-somite stage are still viable and that dissociated cells from these embryos can serve as experimental material. Erickson & Pedersen (1975) have reported that t/t* embryos can survive beyond the in utero lethal period if they are cultured in vitro. On the other hand, Wudl & Sherman (1976) have recently suggested that t$^{12}$/t$^{12}$ embryos are lethal at similar stages in vitro to in vivo. The present results clearly demonstrate that at least some fraction of T/T cells are viable in vitro. Further study of these lines may shed some light on the action of the T gene.

**REFERENCES**


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