Maternal Influences on Mouse Embryos and Preservation of Mutant Strains by Freezing

Whittingham et al. (1), in suggesting that frozen mouse embryos may be used to preserve mutant strains, have made no allowance for maternal modifications. Even if prolonged viability is attainable, the ova-transfer strain [distinguishable by the symbol e (2)] would not be expected to be identical to the parental strain from which the fertilized ova were obtained if influences exerted on the developing fetuses by their foster-mothers resulted in phenotypic modifications. Several types of experimental support this contention.

The foster nursing experiments of Bittner (3) exemplify the early investigation of maternal influences. Law reported that foster nursing alone influenced the growth of transplantable leukemias in mice (4). Foster nursing alone was then found to affect both resistance and sensitivity to x-irradiation (5).

The technique for ova transplantation in mice (6) was developed at the Jackson Laboratory more than 30 years ago for the purpose of investigating intrauterine influences. Cloudman demonstrated that tumors of the genotype of the foster-mother grew progressively and killed recipients of the ova-transfer strain, but not recipients of the ancestral strain, even though the tumors were of a different histocompatibility-2 (H-2) genotype from the recipients (7). Mice of the ova-transfer strain C3HeB, exposed to a radiation dose lethal to some but not all animals and then inoculated with bone marrow of the H-2b or H-2d genotype, were found to exhibit changes specific for the H-2 genotype of the foster-mother (8) and to have a shift in radiation sensitivity (9).

The ova-transfer substrains RIIIeB, DBA/2eB, C3HeB, and C3HeD and the foster-nursed strain C3HHeB were investigated for maternal alteration by using marrow transplantation into lethally irradiated recipients. At least one modification was demonstrable in each artificially derived strain. No two ova-transfer substrains exhibited identical modifications. However, the genetic relationship between the transplanted ova and the foster-mother was different in each case; consequently, it was assumed that the type of alteration occurring was a function of the genetic disparity between the two strains. Maternal modifications of several types were documented. (i) RIIIeB mice were more sensitive to irradiation than either the ancestral strain or the strain of the foster-mother. They also had a reduced capacity to respond to tissue antigens of the genotype of the foster-mother, as shown when the immunogenicity of these antigens was quantitatively reduced by hybridization (10). (ii) DBA/2eB mice acquired an increased capacity to respond to certain tissue antigens, their maternal response being intermediate between that of the ancestral and foster lines (11). (iii) C3HeD mice also acquired an increased capacity to respond to certain tissue antigens. The foster-nursed strain C3HHeB, on the other hand, had a decreased capacity to respond to these antigens, apparently acquired from their foster great-great-grandmother C57BL (12). (iv) C3H and the derived strains C3HHeB, C3HeB, and C3HeD were tested for their capacity to recognize C3H as "self" or to be recognized as "self" by the other substrains. The two ova-transfer substrains, C3HeB and C3HeD, had acquired an increased antigenicity as compared with the foster-nursed strain C3HHeB (13). This change in antigenicity was detectable by marrow transplantation but not by reciprocal skin grafts between substrains.

The observed phenotypic alterations are permanent and vertically transmitted from mother to offspring for many generations, since none of the test mice had contact with allogeneic foster parents. From the available evidence it must be assumed that frozen ova transplanted into foster-mothers would be similarly modified by their fetal development in a foreign environment and thus be endowed with subtle differences characteristic for the substrain and absent in the ancestral strain. Since antigenicity and immunogenicity of hybrid tissues are also modified by their maternal environment, it was postulated that this phenomenon represents an immunologic adaptation that prevents rejection of the fetus by the mother (14). The same biological
mechanism that results in the environmental adaptation of a hybrid fetus is responsible for a modified responsiveness induced in its mother (15). In addition, recent experimental manipulations by Jacobs (16) also involved an environmental adaptation and produced results reminiscent of Cloudman's earlier work (4), but in a totally different type of system. The full extent to which this adaptive mechanism influences mammalian systems is unknown. However, the potential impact of this mechanism on evolution is apparent.

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References

11. Ibid., p. 1317.
15. Ibid., ibid. 50, 304 (1973).
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We accept the view that maternal influences on developing fetuses can produce phenotypic modifications in mice derived from transferred ova. Uphoff contends that such modifications result from the influence of the foster-mother and are transmitted to subsequent generations of the ova-transfer strain. But, as he has pointed out, "It is this self-perpetuating nature of these alterations without an altered genome that presents the greatest difficulty in explaining these data on the basis of usually acceptable biological concepts" (1). There are, of course, several well-understood genetic mechanisms that could produce such phenotypic alterations.

Table 1. Substrains derived from transferred ova.

<table>
<thead>
<tr>
<th>Substrain</th>
<th>Ancestral strain</th>
<th>Foster strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHeB</td>
<td>C3H</td>
<td>C57BL/De</td>
<td>(2)</td>
</tr>
<tr>
<td>DBA/2eB</td>
<td>DBA/2</td>
<td>C57BL/De</td>
<td>(7)</td>
</tr>
<tr>
<td>CHeD</td>
<td>C3H</td>
<td>C57BL</td>
<td>(6)</td>
</tr>
<tr>
<td>RIIIeB</td>
<td>RIII</td>
<td>C57BL/De</td>
<td>(8)</td>
</tr>
</tbody>
</table>

1) As a result of selection, the genomes of mice developed from transferred ova and used to establish a substrain may differ slightly from the genome of the ancestral strain as a whole. Uphoff's ova-transfer strain CHeB may be an example of this sort, since it was derived from a single brother-sister pair selected from seven live young resulting from the transfer of 63 fertilized ova (2).

2) Once a substrain is isolated from its ancestral strain, subsequent generations may become subject to phenotypic modification by genetic drift. The ova-transfer substrains that Uphoff has tested and their derivations are shown in Table 1. Some of these substrains were separated from their ancestral strains for as many as 20 generations before both strains were compared. Genetic drift of both the ancestral strain and the substrain may have occurred in that time. The phenotypic modifications that Uphoff has observed are, after all, small ones. For example, substrain RIIIeB was found to be more sensitive to radiation than the ancestral strain RIII in that the survivals after exposure to 565 r were 30 and 65 percent, respectively. But there was virtually no difference in the percentage of survival of the two strains at radiation doses of 510 and 621 r (3). Moreover, substrain DBA/2eB and the ancestral strain DBA/2 responded almost exactly the same to all radiation doses tested (4). Similarly, when marrow samples from DBA/2 and DBA/2eB were used in lethally irradiated (DBA/2 × C57Bl/Ka)F1 recipients to test for antigen response, there was no test in the survivals of the recipients until day 60, at which time they were 41 and 16 percent for those that had received DBA/2 and DBA/2eB marrow, respectively (4).

Some of the evidence that Uphoff presents to demonstrate phenotypic modifications, such as her citation of Cloudman's experiments (5), does not seem pertinent to this discussion. She herself states that "we could not reproduce Cloudman's experiment with our ova-transfer strains and transplantable neoplasms" (1). Uphoff and Deringer (6) were also unable to repeat Cloudman's experiment. Uphoff (1) has pointed out that alterations in these ova-transfer subestrains may have resulted either "during their derivation or subsequent inbreeding." It is inbreeding itself that we believe can be greatly reduced by the use of embryos stored for extended periods in the frozen state. Such storage would reduce genetic drift by greatly reducing the number of generations required to maintain a mutant stock. The ability to store frozen but viable embryos might also provide a more rigorous test of Uphoff's hypothesis by permitting simultaneous comparison of different generations of a substrain with the ancestral strain.

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References and Notes

4. Ibid., p. 1317.
8. Ibid., ibid. 43, 1347 (1969).
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