DEVELOPMENT OF TWO-CELL MOUSE EMBRYOS IN THE ABSENCE OF A FIXED-NITROGEN SOURCE

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Mouse embryos developed from the eight-cell stage to expanded blastocysts in a defined medium in which glycine was the only source of fixed nitrogen (Whitten, 1957). These findings were confirmed by Bunim (1960), who also showed that glycine could be replaced by Versene and suggested that the function of the glycine was not for protein synthesis but to chelate ions of copper or zinc which might have contaminated the medium. However, Brinster (1965) found that only an occasional two-cell embryo developed in the presence of single amino acids, though he subsequently demonstrated that 80% formed blastocysts when glutathione was the only source of fixed nitrogen (Brinster, 1968).

Incorporation of labelled leucine by early mouse embryos *in vitro* was reported by Mintz (1964) and by Monesi & Salfi (1967) but neither Greenwald & Everett (1959), nor Weitlauf & Greenwald (1967), could demonstrate uptake of methionine *in vivo*. It is not clear if this difference was due to the amino acid studied or to a difference between the conditions *in vitro* and those *in vivo*. Nevertheless, Brinster (1967) demonstrated a 26% loss of protein during the first 3 days of development which indicates that the embryo draws heavily on its own stores at that stage.

Preliminary observations (Cholewa, unpublished), using the more refined techniques of embryo culture (Whitten, 1969, 1970), indicated that substantial numbers of two-cell embryos developed in media without any amino nitrogen. The experiments described here fully confirm those findings. The possibility remained, however, that the embryo could obtain fixed nitrogen from degenerated spermatozoa, polar bodies, or other débris within the perivitelline space or even from the zona itself. Therefore, we also examined the development of zona-free eggs in medium free of available nitrogen and again observed significant development.

Spontaneously ovulated eggs from (SJL/J × C57BL/10Dg)F₁ hybrid females mated to similar males were cultured in tubes in the basic medium described by Whitten & Biggers (1968) but which contained polyvinylpyrrolidone (PVP) in place of the bovine plasma albumin. The gas phase was 5% O₂, 5% CO₂, and 90% N₂ (Whitten, 1969). All of the eggs from two or three females were collected on the morning following that on which the copulation plug was found. The eggs were taken into a mechanically controlled pipette with about

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1 μ l of fluid, transferred to a cavity slide containing 1 ml of medium and any cells or débris removed. Three such washings were made before the embryos were placed in the culture vessel with 1 ml of medium. Naked eggs were obtained by dissolving the zonae pellucidae with pronase. They were washed twice to remove the pronase and this treatment dislodged most of the polar bodies.

The proportion of embryos developing into morphologically normal blastocysts with distinct inner-cell masses is given in Table 1. Forty additional embryos which had been cultured from the two-cell stage to early blastocysts in fixed nitrogen-free medium were transferred to suitable pseudopregnant

TABLE 1
THE NUMBER OF BLASTOCYSTS
DEVELOPING FROM TWO-CELL MOUSE
EMBRYOS IN THE ABSENCE OF AMINO
NITROGEN

<i>No. of two-cell embryos cultured</i>	<i>No. of blastocysts observed</i>
17	17
16	15
20	20
19	18
29	27
22*	13†
21*	9†

* Zonae removed.

† Estimate because of fusion of embryos.

females. Twenty-two young were born and all appeared normal when weaned 3 weeks later. This proportion of 55% is not less than that which we obtained when uncultured embryos were transferred and indicated that those cultured without amino nitrogen did not have a reduced potential for development. The number of zona-free eggs developing to blastocysts (Table 1) is only an estimate because many of the embryos fused to form complex chimaeras, while some of the blastomeres separated and formed miniature blastulae or uncavitated masses. There was no evidence of lysis of any of the cells.

Our results indicate that the two-cell mouse embryo does not require any external source of fixed nitrogen. However, a beneficial effect may be obtained from amino acids, peptides, or albumin if toxic metallic ions are present in significant amounts (Whitten, unpublished data). Albumin also functions as a macromolecule to prevent embryos from sticking to the glassware and it has been credited with the property of stabilizing membranes. Albumin does appear to have a beneficial effect on mouse embryos because we have not been able to obtain, consistently, more than a single cleavage when one-cell zygotes are cultured without it, whereas full development takes place in its presence (Whitten, 1969). Even with two-cell embryos, when the period of culture is extended for 5 days, fewer of the embryos grown without albumin undergo 'hatching' and a greater proportion of them collapse.

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REFERENCES

- BRINSTER, R. L. (1965) Studies on the development of mouse embryos *in vitro*. III. The effect of fixed-nitrogen source. *J. exp. Zool.* **158**, 69.
- BRINSTER, R. L. (1967) Protein content of the mouse embryo during the first five days of development. *J. Reprod. Fert.* **13**, 413.
- BRINSTER, R. L. (1968) Effect of glutathione on the development of two-cell mouse embryos *in vitro*. *J. Reprod. Fert.* **17**, 521.
- BUNIM, L. (1960) *In vitro* development of mouse blastulae in Versene-Ringer solution. Summer Student Report, Jackson Laboratory.
- GREENWALD, G. S. & EVERETT, N. B. (1959) The incorporation of S³⁵ methionine by the uterus and ova of the mouse. *Anat. Rec.* **134**, 171.
- MINTZ, B. (1964) Synthetic processes and early development in the mammalian egg. *J. exp. Zool.* **157**, 85.
- MONESI, V. & SALFI, V. (1967) Macromolecular synthesis during early development in the mouse embryo. *Expl Cell Res.* **46**, 632.
- WEITLAUF, H. M. & GREENWALD, G. S. (1967) A comparison of the *in vivo* incorporation of S³⁵ methionine by two-celled mouse eggs and blastocysts. *Anat. Rec.* **159**, 249.
- WHITTEN, W. K. (1957) Culture of tubal ova. *Nature, Lond.* **179**, 1081.
- WHITTEN, W. K. (1969) The effect of oxygen on the cleavage of mouse eggs *in vitro*. *2nd Ann. Meet. Soc. Stud. Reprod. Davis, California*, 1969. Abstracts p. 29.
- WHITTEN, W. K. (1970) Nutrient requirements for the culture of preimplantation embryos *in vitro*. *Advances in Biosciences* (in press).
- WHITTEN, W. K. & BIGGERS, J. D. (1968) Complete development *in vitro* of the pre-implantation stages of the mouse in a simple chemically defined medium. *J. Reprod. Fert.* **17**, 399.