Plating Efficiency of Mouse Embryo Cells as a Function of Gestational Age

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Summary. Mouse embryo cells can be plated directly from single-cell suspensions of fresh embryos. For randomly bred Swiss mice, there is an increase in plating efficiency as a function of gestational age. Colony-forming units appear at about day 10 and there is an exponential increase in colony-forming ability up to day 16, after which there is no further increase.

Cultured rodent embryo cells have been utilized for a variety of purposes. Primary and secondary cells are used for carcinogenesis studies[3–6], for feederlayers[6] and for a number of other purposes. Ordinarily, 'mid-stage' embryos are taken and single cell suspensions are made, plated at high multiplicity, incubated for several days and then replated. Since 'mid-stage' could mean anything from day 13 to day 18 for mice, rats and hamsters, it is conceivable that different populations of cells are represented depending upon which day of gestation the embryos have reached when they are plated. Furthermore, the colony-forming potential of individual cells as a function of gestational age has not heretofore been described. In this paper, we report that the plating efficiency of mouse embryo cells is an exponential function of the gestational age of the animal, and that, at early stages especially, colony-forming ability is enhanced by the presence of a feeder-layer. Direct plating of mouse embryo cells also provides a suitable system for the study of chemical carcinogen induced cellular transformation.

Materials and methods. Pregnancies of Swiss white mice were timed by placing 1 male with 3 to 5 females for 1 night. Embryonic stage was verified by comparing the embryos with diagrams in a standard text[7] with respect to length and stage. Single cell suspensions of embryo cells were prepared after removal of embryos which were freed of loose membrane material and rinsed in sterile Hank's basic salt solution without calcium and magnesium. The number of embryos pooled for each point depended on the stage of gestation. Before day 13, all the embryos (usually 9 to 11) in the mouse were used. For days 13 and 14, 2 embryos were used, and thereafter, 1 embryo was used. The whole embryos were minced fine with scissors in a small amount of 0.25% trypsin (Gibco). The method of progressive trypsinization was used to obtain a single cell suspension. Trypsinization was continued until clumps no larger than 0.25 mm in diameter remained. After each trypsinization, cells were collected in a tube containing MEM-α (Gibco) with 10% heat-inactivated horse serum (Gibco) (αHS). After completion of the trypsinizations, the pooled cells were pipetted vigorously and left to stand for 15 min. The top 2/3rds of the suspension was removed, diluted and plated in αHS. The resulting single cell suspension had better than 99% viability by trypan blue exclusion. Mouse L-cell feeder layers (HRLcells) were prepared by irradiating a suspension of exponentially growing spinner cells with 3,000

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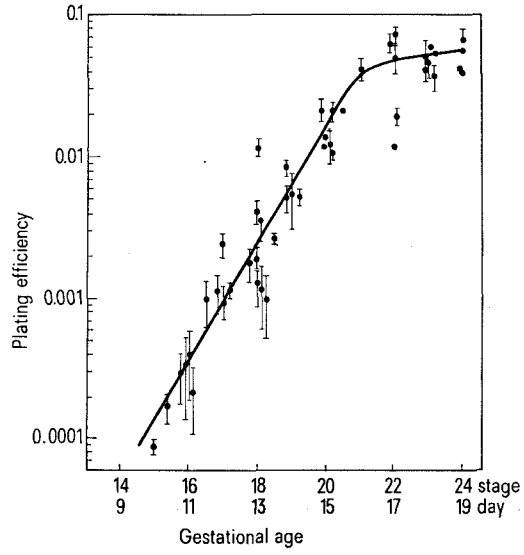

Plating efficiency of mouse embryo cells as a function of gestational age. Each open circle represents the plating efficiency of a single embryo suspension plated in quintuplicate. Error bars denote SD.

Feeder effect on mouse embryo cells

Gestational age (days)	Feeder-layer		Mouse embryo cell plating efficiency (± SD)
	No. per dish	Type	
14	10^5	HRLCells	0.00116 ± 0.000207
14	10^5	HRHela	0.00139 ± 0.000225
14	10^5	HRCHO	0.00136 ± 0.000351
14	10^5	HRMEC (primary)	0.00108 ± 0.00257
14	10^6	HRMEC (primary)	0.000400 ± 0.00236
14	None		0.000769 ± 0.00632
19	10^5	HRLCells	0.0488 ± 0.0100
19	10^5	HRHela	0.0836 ± 0.0144
19	10^5	HRMEC (secondary)	0.00993 ± 0.00798
19	None		0.0635 ± 0.0142

HRLcells were prepared as described in the text. At day 14, primary mouse embryo cell feeder layers (HRMEC) were prepared from the same embryo suspension that was used to determine plating efficiency. At day 19, the secondary HRMEC were from day 13 embryos that had been passaged once. Hela (HRHela) and Chinese hamster ovary (HRCHO) cells were harvested from plates by trypsinization. All feeder cells received 3,000 rads before plating, and were plated at the same time as the mouse embryo cells.

rads from a ^{137}Cesium γ-ray source. These were added to the embryo cell plates at 10^5 per 60 mm dish. Plates were fed on day 7 and fixed and stained on day 10.

The plating efficiency (number of colonies/number of cells plated) of mouse embryo cells as a function of gestational age is shown in the Figure. There is an exponential increase between stages 16 and 21 (days 11 to 16). At stage 14 (day 9), less than 1 colony per embryo is found, thus there are esentially no colony-forming units at this time. Some time between stages 14 and 15, that is during organogenesis, colony-forming units appear. As the plating efficiency increases, the morphologies of the colonies change and there is an increase in variety of cell types. At early stages, the colonies, after 10 days of incubation, are still quite small, containing 25 cells or less (colonies with less than 15 cells were not counted). The cells in many of the early colonies are spindle-shaped, fibroblast-like and tend to be well separated from each other. After stage 18 (day 13), the fibroblasts in the colonies are more densely packed. The various morphologies seen for mouse embryo cells generally agree with those described by DiPaolo et al.[8] for hamster embryo cells, except that in our studies, macrophage colonies were scored and these were found to comprise about 2 to 10% of the colonies seen from stage 18 on. Macrophages were detected by incubating plates before staining with 5×10^7 autocalved yeast particles in 0.4 ml of phosphate-buffered saline (PBS) containing reconstituted guinea-pig complement (Gibco) for 30 min at 37 °C. The plates were then washed with PBS and stained. Macrophages contain ingested yeast particles. Macrophage colonies were not seen if the mouse embryo cells were plated at low densities in the absence of the L-cell feeder layer (HRLcells). They were not as numerous on high density plates, although they did arize on such plates in the absence of HRLcells. Presumably the numerous fibroblasts on these plates both replace the feeder effect and crowd out the macrophage colonies.

The embryo cells grew equally well with 10% fetal calf serum in place of 10% horse serum. After stage 22, the effect of the feeder-layer was not as marked, and in some experiments, the cells grew equally well without it. However, consistently high plating-efficiencies were only obtained when the feeder-layer was present. The effect of the concentration of feeder-layer cells was determined for stage 18 (day 13) embryo cells. Maximum feeder effect is obtained at 3×10^4 to 10^5 HRLcells per plate. 3×10^5 and 10^6 cells are inhibitory. Other types of cells were examined for their feeder effect and these are shown in the Table. Interestingly enough, secondary mouse embryo fibroblasts at 10^5 cells per plate inhibited the plating of stage 23 cells. Cultured embryo fibroblasts are frequently used by other investigators as feeder-layers.

In preliminary experiments, we have injected pregnant mice on days 17 to 19 with carcinogens and found an increase in colonies with piled, non-contact inhibited morphology consistent with neoplastic transformation.

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Kinetics of DNA Repair Synthesis in Guinea-Pig Pancreatic Slices Following in vitro Exposure to N-Methyl-N-nitrosourethane

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Summary. In vitro exposure of guinea-pig pancreatic slices to NMUT resulted in an increase in hydroxyurea-insensitive ^{3}H-TdR incorporation into DNA; this represents DNA repair synthesis following NMUT-induced DNA damage. The kinetics of this hydroxyurea-insensitive ^{3}H-TdR incorporation suggest that the NMUT-induced DNA damage is largely repaired within 2 hours.

Chronic administration of N-methyl-N-nitrosourethane (NMUT) induces exocrine pancreatic cancer in guinea-pigs[2]; however, little is known about the underlying mechanism involved. Our previous studies have demonstrated the uptake of NMUT in the guinea-pig pancreas, following oral administration, and also the alkylation of pancreatic DNA[3]. We have also demonstrated suppression of normal replicative DNA synthesis in pancreatic slices in vitro by hydroxyurea (HU), without suppression of DNA repair synthesis, following exposure to NMUT[4–6]; ^{3}H-TdR incorporation into DNA, in the presence of HU, represented DNA repair synthesis. We report here on the kinetics of DNA repair synthesis in guinea-pig pancreatic slices exposed in vitro to NMUT.

The methods for in vitro exposure of pancreatic slices to NMUT, and for subsequent determination of DNA synthesis have been previously described[4–6]. Briefly, 4 or 5 duodenal pancreatic slices (1 mm, 40–50 mg) from male Hartley guinea-pigs (400–500 g; Litton Bionetics, Ft. Detrick, Md.) were incubated for 30 min in 10 ml Eagle's modified minimum essential medium (Flow Laboratories, Inc., Rockville, Md.) in the presence of 5 m*M* HU; NMUT (Starks Associates, Inc., Buffalo, N.Y., Lot No. ETl–148–1; NSC No. 2860, NCI, Bethesda, Md.), dissolved in 50% ethanol, was added (0.2 ml) to a final concentration of 20 m*M*, and the mixture was incubated for a further 30 min. Extra slices were included for histological examination. Slices were thoroughly washed with saline and incubated for various periods

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