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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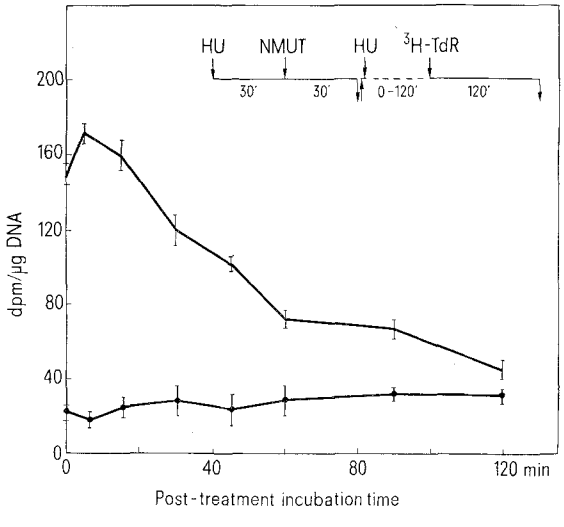
from 0 to 120 min in a fresh medium with HU, followed by a 2 h incubation in 10 μCi/ml ^{3}H-methyl-thymidine (^{3}H-TdR; 5 Ci/m*M*, Amersham-Searle, Arlington Hts., Ill.). Control slices were treated similarly except that equivalent amount of 50% ethanol was added instead of NMUT. Finally, slices from test and control incubation mixtures were homogenized (1:5, w/v) in sucrose-phosphate buffer, and DNA was isolated from triplicate samples of the homogenate, as described previously[4-6], for colorimetric[7] and radioactivity determinations.

The HU-insensitive ^{3}H-TdR incorporation into DNA, as illustrated in the Figure, represents the kinetics of DNA repair synthesis in the slices, following NMUT exposure. It is clear that there was a marked increase over HU-control value in HU-insensitive ^{3}H-TdR incorporation into DNA immediately following NMUT exposure; a further increase was observed when NMUT-exposed slices were incubated for 5 min in a fresh medium with HU, before ^{3}H-TdR labeling. However, with subsequent incubation of these slices in the fresh HU-containing medium, there was a time-dependent decline in ^{3}H-TdR incorporation, reaching approximately control values (+HU) by 120 min. Addition of cold dTTP (upto 10^{-6} *M*) did not produce any significant effect on the levels of ^{3}H-TdR incorporation in the NMUT-exposed slices. In the control slices, the levels of ^{3}H-TdR incorporation in the presence of HU, were consistently low at all intervals during post-incubation. Histologically, no significant differences were observed between test and control slices; however, at the end of ^{3}H-TdR incubation, both test and control slices showed minor toxic changes, as evidenced by loss of zymogen granules and infranuclear basophilia.

The low levels of ^{3}H-TdR incorporation in the presence of HU, in the control slices, and the marked induction of HU-insensitive ^{3}H-TdR incorporation immediately following NMUT exposure, are consistent with our previous results[4-6]. We also demonstrated that suppression of DNA replicative synthesis by HU did not interfere with DNA repair synthesis following in vitro exposure of pancreatic slices to NMUT. The resolution of DNA repair synthesis by HU appears to be particularly enhanced in organs and cells, such as pancreas[5,6], and peripheral blood lymphocytes[8], which are mostly free of S-phase cells, and consequently possess low levels of DNA replicative synthesis.

The present studies indicate that incubation of NMUT-exposed slices in fresh medium with HU allows DNA repair to proceed; hence, ^{3}H-TdR incorporation at various intervals during post-incubation of slices may be taken as a measure of the kinetics of DNA repair synthesis. The steady decline in ^{3}H-TdR incorporation to approximately control values (+HU) suggests that most of the NMUT-induced DNA damage was repaired within 120 min following NMUT-exposure. Additionally, this decline in ^{3}H-TdR incorporation does not appear to be an artefact resulting from the depletion of intracellular deoxyribonucleotide pool, since addition of cold dTTP did not significantly influence the level of ^{3}H-TdR incorporation.

It has been demonstrated that DNA damage due to base alkylation by various alkylating agents, including N-nitroso carcinogens, can be repaired in cultured mammalian cells[8-12], and in liver in vivo[13,14], and that, in most cases, this type of repair is reported to involve the excision of lesions from parental DNA. The present studies on the kinetics of DNA repair synthesis indicate that most of the NMUT-induced DNA damage in pancreatic slices is repaired within 2 h. The extent of repair, and the nature of underlying mechanisms involved in this process are currently under investigation using alkaline sucrose density gradient analysis.



Kinetics of DNA repair synthesis in guinea-pig pancreatic duodenal slices following in vitro exposure to NMUT. 4 or 5 duodenal slices were incubated at 37 °C in separate flasks with 5 m*M* HU for 30 min, and then NMUT in 50% ethanol was added to a final concentration 20 m*M* (1:50 dilution). At the end of a 30 min exposure to NMUT the slices were washed thoroughly with saline, and were incubated in a fresh medium, with 5 m*M* HU, for 0, 5, 15, 30, 45, 60, 90, and 120 min, before the addition of ^{3}H-TdR to 10 μCi/ml final concentration for 2 h. Control slices (● — ●) received similar treatment but were exposed to 50% ethanol instead of NMUT. Data, based on 3 replicate experiments, are expressed as mean dpm/μg DNA ± SD, with bars representing standard deviation.

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