Although the discrepancy between the predicted depth for the disappearance of bubbles and the observed depth is not great, it might be accounted for by errors in the hydrate data, the ice temperature, density, or depth. The hydrate might also have decomposed to form bubbles in the 800- to 1200-m part of the core when the pressure was released on the ice because of the way the ice crystals were packed. The discrepancy is more likely to result from the pressure in the bubbles being less than the hydrostatic pressure. When air hydrate forms, the pressure in the bubble decreases. The restoration of the air pressure in the bubble to the value of the hydrostatic pressure will be slow because of the high viscosity of ice (*11*). The observation that gas is released only when the deep ice melts implies that the structural strength of the ice crystal mass is greater than the dissociation pressure of the hydrate (*12*).

The amount of hydrate can be calculated on the assumption that 10 percent of the volume of the ice is gas bubbles when the snow is compacted into ice. On the basis of the formula $(N_2,O_2)\cdot 6H_2O$, 0.06 percent of the ice would be in hydrate form. This amount of cubic crystals of air hydrate would not be easily detected in the presence of hexagonal ice.

This is the first example of the natural occurrence of clathrate hydrates on the earth (*13*), although methane hydrate has long been known to occur in natural-gas pipelines (*14*).

STANLEY L. MILLER
Department of Chemistry,
University of California,
San Diego, La Jolla 92037

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7. This measurement was made with finely divided ice, and the dissociation pressure was approached from both the high- and low-pressure side of the equilibrium. Equilibrium was approached with a $t_{1/2}$ of about 10 hours. Most of the measurements on N_2 and O_2 hydrates are above 0°C (*6*, *8*) for the equilibrium involving water + hydrate + gas. The measurements below 0°C for the equilibrium involving ice + hydrate + gas extend to only −5°C and are not sufficiently accurate to permit extrapolation to −30°C.
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12. A. J. Gow (personal communication) has noticed that the deep cores have developed small cracks in the otherwise clear ice after storage for several months at −30°C.
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Potentially Lethal Radiation Damage: Repair by Mammalian Cells in Culture

Abstract. *Less than optimum conditions with regard to cell division after x-irradiation provide the necessary environment in which mammalian cells can repair potentially lethal radiation damage. The kinetics of repair suggest that, during the repair process, a transient, unstable cellular state occurs which prevents cell division in complete growth medium. The capacity for repair appears to be dependent on cell age.*

When mammalian cells are exposed to ionizing radiation, molecular perturbations occur which, if of sufficient magnitude, result in the registration of damage. This damage results in disturbances in the economy of the cell, the eventual expression of which is dependent upon the quality and quantity of radiation damage and the capacity for repair by the cell. In general, three classes of damage may be defined: (i) lethal, when registered suppression of colony formation occurs under any circumstance; (ii) potentially lethal, which may be repairable to a nonlethal level if suitable conditions are provided; if repair does not take place, conversion to a lethal state occurs and colony formation is suppressed; and (iii) sublethal; cells having this level of damage eventually form colonies.

The kinetics of repair of sublethal radiation damage have been extensively studied (*1*), and this repair capacity appears to be present in a variety of cells (*2*). To study sublethal damage repair, one must irradiate cells a second time. Survival, measured as a function of time between irradiations, is compared with survival found with the total dose delivered in one exposure; the kinetics observed are those for the repair of damage which would have been expressed as nonlethal had not the second dose been applied, and as modified by the progression of surviving cells through the cell cycle after irradiation.

Repair of radiation damage which would have been expressed as lethal has been less extensively studied. If the initial registration of damage produces injury states which are either nonlethal or lethal and the latter cannot be converted to a nonlethal injury state, survival will be independent of environment after irradiation. However, if survival is dependent upon the environment after irradiation, some part of the registered damage may exist in a labile injury state which may be fixed to a lethal event (*3*) or repaired permitting colony formation. There is evidence (*3–5*) that survival of mammalian cells irradiated in culture is dependent upon culture conditions after irradiation.

We now demonstrate the repair capability dependent on cell age of cells having experienced potentially lethal radiation damage. The cell line used was a clone (V79-103A) derived from lung cells of a female Chinese hamster (*6*). Surface-attached cells were maintained in Eagle's minimal essential medium supplemented wtih Eagle's nonessential amino acids, glutamine, NCTC-109 (4 percent), penicillin, streptomycin, and fetal calf serum (15 percent). Maintained in this medium (FS-15) at 37°C in a humidified atmosphere of 3 percent CO_2, cells grew with a doubling time of 8.5 to 9 hours.

Exponentially growing cell populations were dispersed with 0.05 percent trypsin in Puck's saline A and diluted with FS-15; the cells were then counted in a hemacytometer. Appropriate numbers of cells were plated onto 9-cm glass petri dishes to yield about 200 colonies in control and experimental dishes.

After overnight growth (colony multiplicity $\bar{N}$ = 2.5 to 3.0), the medium was removed, and the dishes without their covers were placed in a holder which allowed for temperature control below and gas control above the dish. Attached cells were irradiated in room air with an OEG-60 Machlett end-window x-ray tube operated at 50 kv-peak and 20 ma. At a distance of 18 cm from the end window, the dose rate at the cell-glass interface was 2170 r/min. The beam, filtered with 0.11-mm aluminum, had a half-value layer of 0.11-mm aluminum.

Cells were irradiated at either 0° to 3°C or 37° to 39°C. Temperature was controlled by melting ice or warm water in the bottom portion of the plate holder. Immediately after exposure to a single dose, cells were overlayed with fresh FS-15 or Earle's balanced salt solution (EBSS); plates were incubated at 37°C. For incubation in EBSS, CO_2 was increased to 6.5 percent in order to maintain *p*H 7.0 to 7.2. After intervals in the balanced salt solution (up to 6 hours after irradiation), sets of dishes were removed, buffer was discarded, and cells were provided with FS-15 for colony formation. After 10 to 12 days of growth, colonies were stained with 1 percent methylene blue solution and counted; corrections were made for plating efficiency, and survival fractions were determined. Incubation of unirradiated cells in EBSS for up to 6 hours did not influence the plating efficiency.

Survival rapidly increased to a maximum after 1 hour in the balanced salt solution (Fig. 1). Survival at zero time for cells irradiated at 3°C was higher than that for cells exposed at 37°C because temperature modifies the radiation response (*7*). When cells were irradiated at 37°C, the initial survival increase in buffer was followed by a decrease and a second rise. In contrast, cells irradiated at 3°C demonstrated a less-prominent decrease, and survival remained at or near that level for at least 4 to 6 hours.

The initial increase observed in both curves suggests that in the presence of buffer, mammalian cells repair potentially lethal damage. This finding is not artifactual: plating efficiency for unirradiated cells did not change for comparable incubation times in buffer. Further, the observation is similar to that which is typical for liquid holding recovery in bacteria; for example, survival increases when cells are placed in solutions of minimum salt content after irradiation and before plating on nutrient agar for colony formation.

However, the subsequent fall in survival suggests that, with continued repair, an unstable cellular state prevents unlimited division. This is a reasonable suggestion, since part of the repair process may be degradative, and, if interrupted at this point by an environment in which other cell functions (including division) must proceed, radiation damage will be expressed as a lethal event. We interpret the second survival increase to mean that this unstable state exists transiently and is converted to a stable, nonlethal condition with further repair in buffer. That similar repair kinetics are not observed with cells irradiated at 3°C may be indicative of a qualitative difference in damage registered at low temperature; during the repair of this particular radiation lesion, the transient, unstable state postulated above may not occur as a component of the repair sequence.

Because survivors to a given radiation dose represent cells which were in resistant response states at the time of exposure, the observed repair kinetics are predominantly for those cells. Thus, the capacity for repairing potentially lethal radiation damage may depend on cell age. To test this we used synchronous cells. Prior to harvesting cells with trypsin, we incubated monolayers with 2 m*M* hydroxyurea in complete medium for 2 hours. Hydroxyurea selectively kills cells which are synthesizing DNA and prevents progression of nonsynthesizing cells into the synthetic phase of the cell cycle. Consequently, after short exposures to this drug, cells accumulate in the presynthetic phase (G_1) of the cell cycle.

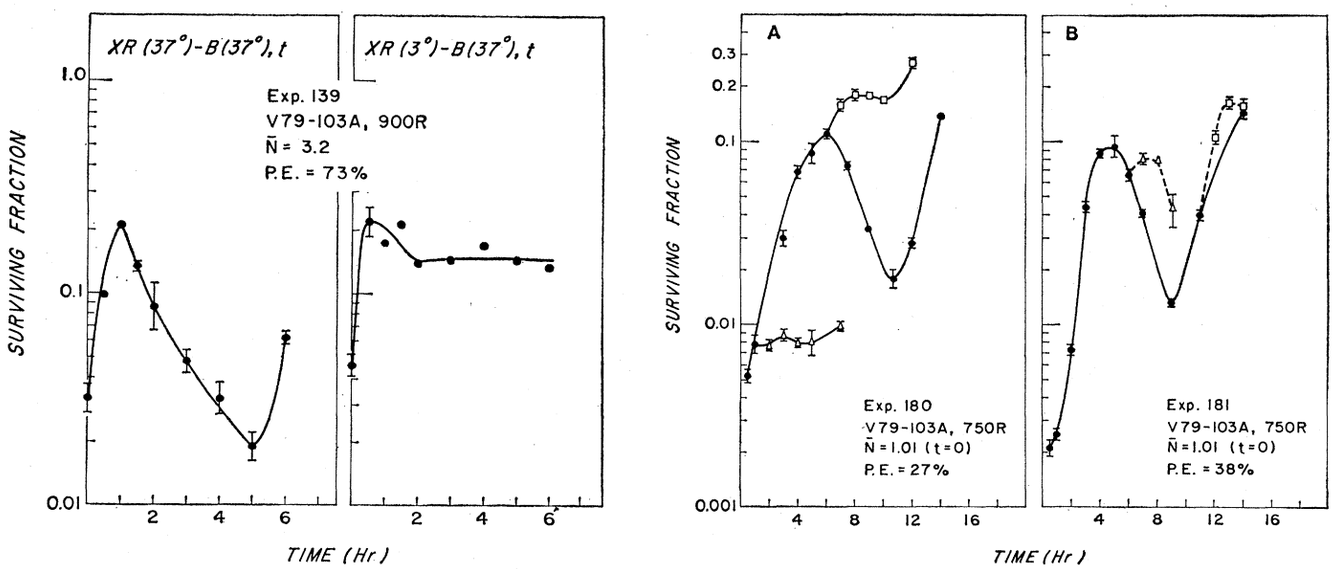

Fig. 1 (left). Survival of surface-attached Chinese hamster cells as a function of time in buffer after irradiation with 900 r at 3° or 37°C. Incubation in buffer was at 37°C, *p*H, 7.0. Abbreviations: $\bar{N}$, average colony multiplicity at the time of irradiation; *P.E.*, plating efficiency. Fig. 2 (right). (A and B) Two experiments showing survival to 750 r as a function of time after synchronization with 2.0 m*M* hydroxyurea, 2 hours. Closed circles show survival without buffer treatment after irradiation. Open symbols represent survival as a function of time in buffer after irradiation at times shown. The low plating efficiencies reflect the killing of S cells by hydroxyurea in the original cell population.

After exposure to hydroxyurea, monolayers were rinsed with complete medium and harvested with 0.05 percent trypsin. The resulting single-cell suspension was counted, and appropriate numbers of cells were plated onto petri dishes.

At intervals after 0.5 hour at 37°C (to permit cell attachment), groups of three dishes were irradiated with 750 r. After irradiation at 1 hour, 6 hours, and 11 hours after synchrony, cells were incubated in EBSS for periods up to 6 hours. Cells were then re-fed with FS-15 for colony formation. There is increased response when cells are in the presynthetic (G_1, 0 to 2 hours), postsynthetic (G_2, 7 to 9 hours) and mitotic (M, 9 to 10 hours) phases, and decreased response during the DNA synthetic (S, 2 to 7 hours) phase (Fig. 2). The ability to repair potentially lethal damage is dependent on cell age. Cells which are in middle or late S appear to have substantial capacity for repair, but cells in early or late G_1 do not appear to have this capacity. Because repair of potentially lethal radiation damage is apparently accomplished most efficiently by cells in the process of replicating their DNA, it is not unreasonable to expect that repair mechanisms and the replicative process are associated.

Any environment which is not optimum with regard to division may provide a milieu which allows repair to be functionally effective. The following should provide such environments: (i) low temperature, (ii) hypoxia, and (iii) certain metabolic inhibitors. Whitmore and Gulyas (*3*) have shown survival increase in synchronous mouse L cells when placed at 5°C after irradiation; an effect similar to that reported here has been observed in late (hypoxic) P-388 ascites tumors when allowed to remain hypoxic after exposure (*8*).

Phillips and Tolmach (*5*) have reported survival enhancement in HeLa S3-3 cells exposed to cyclohexamide after irradiation. Therefore, we feel that EBSS provides an environment permitting repair while preventing or slowing progression toward the first division after irradiation.

JAMES A. BELLI*
MERIJEAN SHELTON
Section of Radiation Biology, Department of Radiology, University of Texas Southwestern Medical School, Dallas 75235

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* Present address: Department of Radiology, Harvard Medical School, 50 Binney Street, Boston, Massachusetts 02115.

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Indole(ethyl)amine N-Methyltransferase in the Brain

Abstract. *An enzyme which* N*-methylates various indole(ethyl)amine substrates was isolated from brain, separated from the pituitary and the pineal glands. It appeared localized in the supernatant and synaptosomal areas after discontinuous sucrose-gradient ultracentrifugation. This is the first demonstration of the enzyme in brain.*

Axelrod (*1*) has described a nonspecific *N*-methyltransferase in lung adrenal, kidney, spleen, heart, and liver of rabbit, but not in the brain of rabbit or the lung of other species. We have now found a similar enzyme in the brain of the chick. Our enzyme appears, however, to be more specific than that found by Axelrod in that it fails to *N*-methylate phenylethylamine substrates.

Table 1. Substrate specificity of chick brain indole(ethyl)amine *N*-methyltransferase. Purified enzyme (10 μg of protein) from chick brain was incubated with 5 μmole of substrate and *S*-adenosylmethionine-methyl-^{14}C. After 90 minutes of incubation, the ^{14}C-methyl-labeled metabolites were isolated from the reaction mixture and the radioactivity measured.

Substrate	Relative activity (%)
5-Hydroxytryptamine	100
5-Methoxytryptamine	88
Tryptamine	60
N-Methyltryptamine	47
Norepinephrine	0
5-Hydroxy-*N*,*N*-dimethyltryptamine	0

White Leghorn cockerels (5 days old) were decapitated, and the whole brain was dissected free from the pituitary stalk and overhanging pineal. The brains were homogenized in five volumes of 0.32*M* sucrose. The enzyme content of whole-brain homogenates and supernatants from centrifugation at 100,000*g* in 0.25*M* phosphate buffer (*p*H 7.9) was estimated with 5-hydroxytryptamine (5HT) as a substrate and *S*-adenoxylmethionine–methyl-^{14}C as a methyl donor. This mixture was incubated for 90 minutes at 37°C. The reaction was stopped by the addition of borate buffer (*p*H 10); radioactive methylated products were extracted into water-saturated isoamyl alcohol and the radioactivity was determined in a liquid scintillation counter.

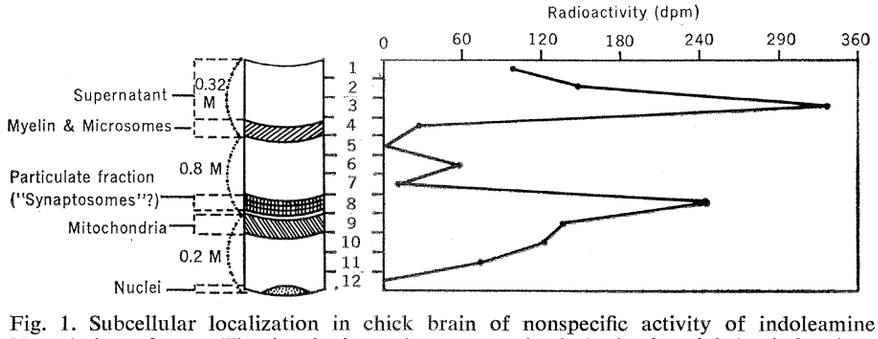

Fig. 1. Subcellular localization in chick brain of nonspecific activity of indoleamine *N*-methyltransferase. The incubation mixture contained 5 μl of serial 3-ml fractions from the sucrose gradient; 5-hydroxytryptamine was the substrate. The results are expressed in disintegrations per minute (dpm).