

infants in Japan and many other countries during 1963–64, but the highest Q/BW observed in adults was 0.8 nCi/kg in Japan. The high concentration of ^{137}Cs in infants is caused by the high ^{137}Cs content of milk and the low body weight (the biological half-life in infants does not shorten in proportion with their body weight). We are now using these results to calculate the internal dose of bottle-fed and breast-fed infants born in Japan during 1962 and 1967.

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Components of the Genetic Repair Mechanism are not confined to the Nucleus

It has been shown that radiation-sensitive mutants in separate genes, which presumably affect different steps in the repair of damaged DNA, can complement in *Escherichia coli*¹, or yeast² and *Ustilago* diploids. Could such strains complement each other's deficiencies in heterokaryotic cells? Such complementation would not be expected if the repair mechanism was completely confined to the nucleus. Recently, mutants of *Neurospora* two or three times more sensitive to the lethal effect of ultraviolet light than wild type have been reported to complement in heterokaryons³. We have tested this further with three radiation sensitive (*uvs*) mutants of *Ustilago maydis* which are 10–30 times more sensitive to ultraviolet light than wild type cells, and which are believed to be deficient in ability to repair damaged DNA⁴. These mutants are recessive, unlinked and complement in diploid cells. Heterokaryotic cells were obtained using the conditions devised by Puhalla⁵ which allow strains of opposite mating type to fuse to form hyphal cells containing nuclei of opposite mating type, although we used a different medium (*Ustilago* liquid complete medium supplemented with 1.7 per cent Difco cornmeal agar). Groups of heterokaryotic cells were removed with a needle from compatible matings between strains with like or different *uvs* mutations, and spread on a plate of complete medium. The plate was irradiated with 960 ergs/mm² ultraviolet light from a standard germicidal lamp. This dose kills about 20 per cent of wild type cells and at least 98 per cent of *uvs* cells. After overnight incubation, 150 heterokaryotic cells of each type were scored for viability under a low power microscope. On this medium viable heterokaryons grow to form a micro-colony consisting largely of haploid cells. The parent strains each carry complementing auxotrophic markers, and so it is easy to show that the surviving colonies are not diploids by replica plating them to unsupplemented medium on which only diploids will grow. The results of the tests are shown in Table 1.

Table 1. SURVIVAL OF HETEROKARYOTIC CELLS AFTER TREATMENT WITH ULTRAVIOLET LIGHT

$a_2b_1 ad-1 me-15$	$a_1b_2 pan-1$		
	$uvs-1$	$uvs-2$	$uvs-3$
$uvs-1$	0	122	99
$uvs-2$	124	0	110
$uvs-3$	113	95	3

The figures are numbers of surviving cells among 150 tested. [Control survival (wild types $a_1b_2 + a_2b_1$): 125/150.]

We conclude from this result that components of the repair mechanism are diffusible between nucleus and cytoplasm, and cannot therefore be completely confined to the nucleus. From this we surmise that DNA outside the nucleus (for example, mitochondrial DNA) might also be repaired by the same mechanism used for repairing damaged nuclear DNA.

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Microsurgery of Inner Cell Mass of Mouse Blastocysts

MOUSE eggs can be immobilized for injection^{1,2} or for microsuction³, and rabbit blastocysts can be held to permit excision of trophoblast cells⁴. I describe here the use of a microsuction system³ to manipulate the inner cell mass of the mouse blastocyst.

To extract cells from the inner cell mass, a hybrid mouse blastocyst of agouti C3H/HeJ–albino BALB/c was held at the end of a pipette by negative pressure^{1,2}. A suction micropipette, which has a capacity of about 5,000 cubic microns at the terminus and an oblique tip with an orifice of 5–12 microns in diameter, was made to penetrate the zona pellucida, enter the segmentation cavity, and insert into the inner cell mass. Removal of elements of the latter was accomplished by adjusting the screw-syringe or by regulating the finger tip on-and-off control of the microsuction system³ so that the micropipette terminus was filled and refilled. The position of the tip of the suction pipette in the blastocoele was verifiable because liquor could be suctioned back and forth making the cavity close and open repeatedly. When the tip of the micropipette reached the inner cell mass, cell fragments appeared in the terminal part of its lumen (Fig. 1). The suspending medium for the blastocysts was a phosphate buffer saline plus bovine plasma albumin (20 mg/ml.), penicillin (100 U/ml.) and streptomycin (50 µg/ml.); its pH was 6.8–7.0.

After the operative procedure, the blastocysts were usually shrunken and their cavities closed. When they appeared severely damaged, or disorganized, by partial removal of the inner cell mass, they were discarded. The remaining contracted blastocysts were cultured in small drops of sterilized medium⁵ under mineral oil in a Petri dish, and their appearance checked on the following day; survival was accompanied by reappearance of the blastocoele and normal appearance of the cells.