indication that with shorter intervals the survival of third-party grafts also may be extended.

As to the relevance of the additional antigen, it may well be true that the antigen constantly released from the surviving grafts suffices to preserve the tolerant state. However, the administration of additional antigen was a prerequisite to lasting tolerance. Whereas the kind of additional antigen administered did seem to affect the outcome less conspicuously in the A ← CBA combination (Table 2), it may well be that still stronger antigenic differences, like the C57Br ← A pairing, are needed to demonstrate the superior efficacy of intravenously applied spleen cells. Both the possible occurrence of chimerism and the importance of the cell dose are now being investigated. The data relating to the role of additional donor antigen for the maintenance of tolerance need further amplification.

Analogous conditioning with other immunosuppressive drugs may produce equal tolerance to allografts. Earlier studies of the chemical suppression of transplantation immunity showed the methylhydrazine derivatives to be the most effective, with cyclophosphamide as the next best (*10, 11*). The described method of inducing tolerance seems to have the advantage over currently available alternatives (actively acquired tolerance, neonatal thymectomy, and lethal irradiation), that the possibility of clinical application should not be ruled out as impracticable.

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Protection through Parabiosis against the Lethal Effects of Exposure to Large Doses of X-Rays

Abstract. *Parabiotic rat pairs with a skin-vascular anastomosis were used to test whether shielding of one member of the pair would protect the irradiated partner against exposure to very large doses of x-rays (1200 to 2400 roentgens). Except with the lowest dose, all unshielded, irradiated pairs or single irradiated animals died before 5 days had elapsed. In contrast, irradiated rats that had a shielded parabiont partner survived the 5-day period and many survived beyond 30 days. This is interpreted as protection against the acute intestinal death that normally occurs in the dose range investigated.*

During the course of a study aimed at clarifying some of the gastrointestinal disturbances (gastric stasis and decreased food intake) that occur after irradiation, we observed what appears to be a remarkable degree of survival for irradiated members of parabiotic rat pairs if the partners were shielded. This exceptional survival was observed in irradiated rats that had sustained radiation doses reported by others (*1–4*) to cause "acute intestinal radiation death" within 3 to 5 days of exposure in single animals.

These observations led us to formalize an investigation that was designed to test, in unrestrained parabiotic rat pairs, whether shielding of one member of the pair would lengthen the survival time of the irradiated partner exposed in the supralethal dose range.

Male littermates of the specific pathogen-free strain of Sprague-Dawley rats bred at the U.S. Naval Radiological Defense Laboratory were paired by weight on removal from their litter at 24 days of age. The parabiosis operation on certain pairs was usually performed 1 to 2 days later. A skin-to-skin anastomosis was made with the Bunster-Meyer technique (*5*), modified by deleting the abdominal muscle union. Losses among the anastomosed animals, amounting to about 35 percent, occurred during the first 3 weeks from parabiosis intoxication (*6*), with no additional losses following this period. The remaining pairs were maintained, with ad libitum access to food and water, up to the age of 100 days, before use in the experimental design.

At 100 days of age, the pairs were subdivided into the following three treatment groups: parabiont pairs with one animal x-irradiated and the partner shielded (X-C); parabiont pairs with both partners irradiated (X-X); and pairs of animals physically tied together by sutures (but with no anastomosis) with one member irradiated and the other shielded (X/C). Each treatment group contained 15 pairs of animals.

For irradiation purposes, the rats were placed in double-chambered radiation exposure units that were oriented radially on a radiation exposure table at isodose distances from the x-ray target. Members of each experimental group were represented in each exposure run. X-rays were generated by a 250-kv-peak unit operated at 250 kv peak, 25 ma, with 0.5 mm Cu and 1.0 mm Al filter (half-value layer = 1.49 mm Cu). The unshielded partners and the X-X pairs were irradiated with whole-body exposures to 1200, 1500, 1800, or 2400 r at a dose rate of approximately 25 r/min. During exposure, the shielded partner received less than 30 r at the highest exposure level used, primarily through scatter.

After irradiation, the animals were returned to the home cages and maintained with ad libitum access to food and water. All pairs were checked for deaths at 12-hour intervals for the first 10 days and at 24-hour intervals thereafter.

The criterion used in this study to assess protection against acute intestinal radiation death was the percent survival after the 3- to 5-day period of mortality. The percent survival for the period of intestinal death and also for the 30-day postirradiation survival period are shown in Table 1.

It can be observed that a dose of 1200 r does produce some acute (3- to 5-day) intestinal deaths among the X members of the X/C group and in the X-X group. Although not shown in the table, a bimodal distribution of death occurred at this dose level, which would

Table 1. Protection afforded by parabiosis against the lethal effects of exposure to large doses of x-rays; percent survival during the acute intestinal death phase (*A*) and the 30-day survival rate (*B*). Fifteen animal pairs per treatment group at each exposure dose.

Treatment group*	Exposure dose (r)			
	1200	1500	1800	2400
A. Percent survival at 120 hours (5 days)				
X/C	87	0	0	0
X-X	53		0	
X-C	100	100	100	60
B. Percent survival at 30 days				
X/C	0	0	0	0
X-X	0		0	
X-C	100	94	13	0

* Treatment: X, irradiated; C, shielded; X/C, pairs that are sutured together without vascular anastomosis; X-X, parabiont pairs with both animals exposed; X-C, parabiont pairs with one partner exposed and the other shielded.

indicate that not all of these animals died from acute intestinal injury. However, at 1500, 1800, and 2400 r, all of the irradiated animals that did not have a shielded, anastomosed partner died before 5 days had elapsed. In contrast, 1200, 1500, and 1800 r produced no acute intestinal deaths (3 to 5 days) in any irradiated rats that had a shielded, parabiotic partner. At the 2400-r exposure level it may be observed that, although some deaths did occur among the irradiated members that had a shielded, parabiotic partner, a remarkable survival rate was still apparent even at this extremely high radiation exposure dose.

In addition to the protection afforded by vascular anastomosis against intestinal death, attention is directed to the 30-day survival rates among these groups of parabionts. Table 1 shows that, with the exception of those animals irradiated with 2400 r, there were survivors in all the other groups of irradiated animals that were anastomosed to a shielded partner.

Over a span of about 20 years, numerous studies have been reported on the subject of acute intestinal radiation death. Although there is still no agreement on the mechanism of death, it does appear that there is general agreement among investigators that the small intestine is the target organ which initiates the syndrome. The various hypotheses which have been frequently proposed for the mechanism or mechanisms leading to death include the following: (i) denudation of the intestinal lining as a result of crypt cell destruction, (ii) excessive fluid and electrolyte loss, and (iii) bacteremia. These hypotheses are supported by the work of Quastler and others (*2, 7*) who demonstrated an almost immediate destruction of crypt cells and intestinal villi. Direct measurements of electrolyte and fluid loss have been made by several investigators (*8*) and survival time was shown to be increased markedly by massive infusion of electrolyte solutions and plasma (*4*). Others (*9*) have demonstrated that treatment with antibiotics also leads to increased survival time.

In the present study, we have no direct evidence to support any specific hypothesis that has been discussed. It would seem reasonable to speculate, however, that there is undoubtedly a substantial loss of cells with denudation in the small intestine of the irradiated parabiont at these extremely high doses of irradiation, yet these animals were observed to survive not only the period for acute intestinal radiation death but for 30 days beyond, depending on the dose. It would appear to us that the supportive action of the shielded partner on the irradiated parabiont is probably through maintenance of fluid and electrolyte balance during the critical period. If bacteremia is a factor in the syndrome, the shielded partner may also lend support to the irradiated partner. With regard to the 30-day survival rate, it is conceivable that the shielded partner is capable of providing stem cells in peripheral blood that have the potential for division. These cells then provide hematopoietic support to the irradiated rat and make possible its ultimate survival.

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Ultraviolet Irradiation of DNA in vitro and in vivo Produces a Third Thymine-Derived Product

Abstract. *A new thymine-derived product was separated from DNA irradiated with utlraviolet light in vitro and in vivo. This compound was mistaken to be thymine homodiner (T=T) by other workers because it is chromatographically indistinguishable from T=T in most eluents. It has absorbancy maximums at 312, 312, and 300 millimicrons in neutral,* p*H 2, and* p*H 11 aqueous solutions, respectively. When it is irradiated in aqueous solution with wavelengths of 360 and 313 millimicrons its spectrum reverts to one similar to that of thymine. Therefore, at least three thymine-derived products can be detected in ultraviolet irradiated DNA, namely the homodimer, a material with absorbancy maximum at 312 millimicrons, and a "minor" product suggested by others to be a dimer of cytosine and thymine. In cells, the latter two are formed in about equal amounts. While these three products were shown to exist in the acid hydrolyzates of ultraviolet irradiated DNA, a material with absorbancy maximum at about 310 millimicrons was demonstrated to form in ultraviolet irradiated DNA without further treatment. The magnitude of this spectral increase varied directly with the increase in the adenine-thymine contents in the DNA as shown by differential transmittance spectra of the irradiated* Micrococcus lysodeikticus, *calf thymus,* Bacillus cereus, *and* Hemophilus influenzae *DNA.*

Two chromatographically separable thymine-derived products have been obtained from acid hydrolyzates of DNA irradiated with ultraviolet light (*1*). The minor product (P_1) (*2*) was taken to be a cyclobutyl type heterodimer (*3*) of cytosine and thymine (*4*) (C=T), while the major one (P_2) (*2*) was considered a homodimer of thymine (T=T) (*5*). Both dimers, C=T and T=T, are con-