bits was similar to the half-life of homologous albumin. The discrepancy between the present observations and those of Cinader and coworkers cannot be explained. However, the validity of the present data is supported by the fact that similar results were observed with 2 other heterologous serum proteins.

Summary. Rates of elimination of heterologous serum proteins during the exponential phase are similar in normal, x-radiated and immunologically tolerant rabbits and do not appear to be immunologically determined.

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Antibody Formation in X-Irradiated Rats Protected with Rat or Rabbit Hematopoietic Cells. (23995)

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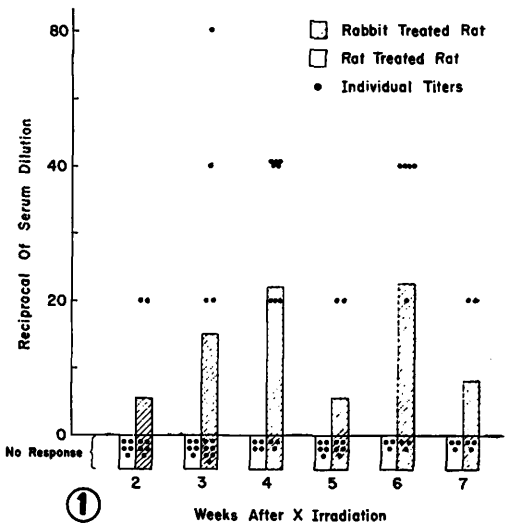
It is now well-established that injection of hematopoietic cells into lethally-irradiated rodents, even of widely divergent genera, results in colonization of the host by the injected cells. Congdon(1) has recently reviewed the status of such colonization experiments. Furthermore, it is apparent that the colonizing cells, even though living in the milieu of their foreign host, maintain their original physiologic properties(2,3,4). Indeed, one of the early proofs for continued existence of such introduced cells was the demonstration(2,3,5) that following injection of rat bone marrow into mice, there is progressive appearance of cells (presumably of rat origin) that stain positively for alkaline phosphatase. Mouse cells do not exhibit this positive reaction. Gengozian and Makinodan(6,7) have tested recovery of antibody-forming ability in lethally-irradiated mice supported with mouse cells and also with rat cells. One can only speculate, however, about whether the antibody-forming capacity in such recovering animals is that of the injected cells or that of the animal's own cells whose recovery is being hastened by supportive treatment.

The present experiments were performed to find out which cells were forming the antibody, using a new technic. Earlier work had shown(8) that embryo liver, which contains predominantly mesenchymal cells, is capable of protecting lethally-irradiated rodents. It is a well-established fact that while rats do not form precipitating antibodies to soluble antigens, rabbits will respond very actively to injections of such antigen(9,10). It is thought that by "protecting" lethally-irradiated rats with rabbit mesenchymal cells and then stimulating them with a soluble antigen one might obtain information on whether injected rabbit cells rather than cells of host rat took part in formation of antibody.

Materials and methods. Male Sprague-Dawley albino rats, weighing 350 to 400 g, were given 750 r total-body X irradiation while confined in a circular aluminum chamber fashioned to hold 12 rats. Delivery source was a G.E. Maxitron-250, operated at 250 KVP and 30 m.a., with 0.5 mm Cu plus 1.0 Al filtration. The half-value layer was

1.43 mm of Cu. Target to center of rat distance was 82 cm, and the delivery rate averaged 28 r/min measured in air at the position to be occupied by the center of the rat's body. Within 4 hrs after irradiation, these rats were injected *via* tail vein, with 1 ml of cell suspension of 28- to 30-day-old rabbit embryo liver, obtained from Swift's snuffle-free rabbits. Livers were removed from freshly-killed rabbit embryos and minced in Locke's solution with sterile scissors. Cells were separated by repeated passage through a 10-ml syringe. The suspension was filtered through gauze to remove coarse clumps. Fatty material, which rose to the surface upon standing, was also removed. The remaining cells were counted with a haemocytometer, and the cell count adjusted to 100 million cells/ml. Two to 7 weeks after liver cell transfer, groups of these rats were injected intravenously with 100 mg of BSA (Bovine Albumin Powder, Fraction V from bovine plasma)* dissolved in 1 ml of *Salmonella typhi* vaccine, prepared by the method of Cannon *et al.*(11). The purpose of the double antigenic stimulus was, on one hand, to obtain information on recovery of antibody-forming capacity to typhoid antigen, to which the rat is normally responsive, and on the other, to search for any response to BSA which normally does not occur in the rat. Rats were bled 7 days after antigenic stimulation, and sera from each group frozen until all samples had been obtained for a simultaneous assay. The method described by Cannon *et al.*(11) was used to carry on agglutination titrations. Since antibody response to BSA might be slight, especially since antigens had been administered intravenously, the anti-BSA titer of sera was determined by the collodion particle method as described by Cannon and Marshall(12), rather than by quantitative precipitation technics, because of greater sensitivity of the former method. Similar groups of irradiated rats were given the same dose of total body irradiation and injected according to the same schedule with 100 million 18- to 20-day-old embryo rat liver cells for control purposes. Representative groups were stressed with BSA plus typhoid antigen at weekly intervals as described above. These controls, of course, served as a check on recovery of capacity to form anti-



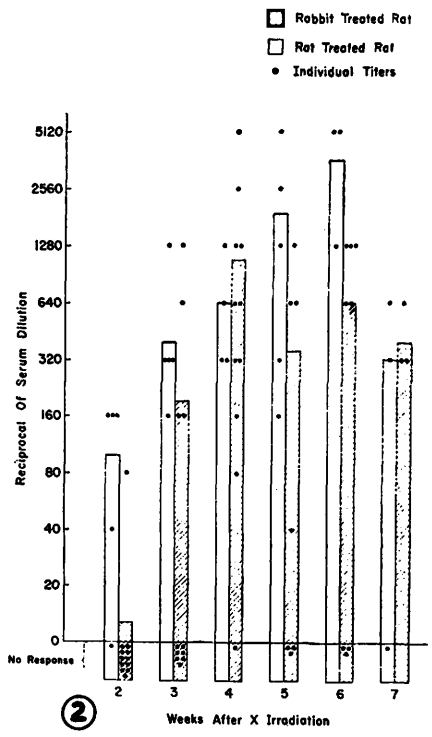


FIG. 1. Anti-BSA serum titers of X-irradiated rats receiving rat embryo liver or rabbit embryo liver cells.

FIG. 2. Anti-typhoid serum titers of X-irradiated rats receiving rat embryo liver or rabbit embryo liver cells.

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body following irradiation.

Results. Average titers to BSA are shown in Fig. 1. Rats irradiated and given rat cells at no time responded to the stimulus of BSA in forming precipitating antibody, nor have normal rats in this laboratory ever done so. Rats which had been given rabbit cells, however, showed a gradual increase in titer, starting as early as first assay at 2 weeks and increasing 4-fold by 4 weeks.

Agglutinin titers to *Salmonella typhi* vaccine on the same rats (Fig. 2) showed that ability to respond to this antigen was fairly well recovered by 2 weeks in rats given rat cells, but in rats injected with rabbit cells, response to the vaccine did not appear to any appreciable degree until 3 weeks. The antibody titer of individual animals is indicated in the 2 Figures by points on each bar representing mean titer of the group. It is apparent that there is some variability in magnitude of titers and that among the rabbit-treated rats there are animals showing no response to either BSA or *Salmonella typhi*. Odell *et al.*(13) have shown that success of recolonization process is not consistent in a group of animals given heterologous cells. Variability in titers and occurrence of negative titers in several rats, apparent in the present work, may reflect variation in recolonization. The fact that the peak of antibody titer occurs at different times after administration of antigen, may also help to explain the variability observed in magnitude of antibody response. Blood was drawn only on 7th day after antigen injection rather than on several days during which peak titer is known to occur in these animals.

Discussion. Irradiated rats injected with rabbit-embryo liver cells formed precipitating antibody in response to BSA. No such response has ever been observed in this laboratory in normal rats or in irradiated rats protected with rat-embryo liver cells. The rabbit responds strongly to stimulation with BSA.

Appearance in these rats of an immune response pattern completely unlike their own and similar to that of donor animal could be due either to presence of rabbit cells capable of antibody formation, or to the fact that rabbit cells administered to save the rat's life following irradiation somehow endowed the immune mechanism of the rat with capabilities typical of the rabbit. Considerable evidence has accumulated in recent years(1) to support the first possibility, but we know of no convincing evidence that cells injected into an irradiated animal have ever resulted in transduction of their traits to cells of the host's body.

In recent independent publications by Weyzen and Vos(14), Grabar *et al.*(15), and Trentin(16) there is speculation on the possibility of colonized cells responding to antigenic stimulation from host's own body proteins to form antibody against the host, thus leading to its death. The results reported above suggest that cells of a colonized heterologous graft are capable of synthesizing a type of antibody that the host's cells cannot manufacture. Thus colonized cells of either isologous or heterologous origin may be considered as surviving and being active in the host's body for a long period of time.

Gengozian and Makinodan(6,7) have shown that agglutinin production in response to antigenic stimulation of sheep RBC returns more slowly when irradiated mice are protected with rat bone marrow than when they are given injections of bone marrow of their own strain. The response observed in rats given typhoid antigen is in keeping with their findings. Agglutinin production in rats protected with rat cells was evident as early as 2 weeks after irradiation and continued to increase markedly at 3rd and 4th weeks, with only 2 rats giving a negative response. On the other hand, rats protected with rabbit cells did not respond to typhoid vaccination at 2 weeks; only 4 out of 11 animals showed an appreciable response by the 3rd week; and not until 1 month was almost entire restoration of antibody response observed. Animals showing no measurable titer were also very common. Presumably, fewer heterologous cells capable of antibody formation are successful in colonizing initially than is true of isologous cells. On the other hand while the initial "take" may be exactly the same in both cases, the subsequent growth rate may be slower in the case of heterologous cells. It is interesting that though this difference is true

for recovery of antibody-forming ability, Jacobson(17) has shown that there is essentially no difference if the recovery pattern of total leucocytes in irradiated mice given mouse marrow *vs.* rat marrow.

Heretofore, colonization studies have been most successful within the Rodentia, using mice, rats, or guinea pigs as donors and recipients(1). Jacobson(18,19), however, recently reported that mouse cells successfully enhanced survival in rabbits given 900 r, although the actual presence of these cells in bodies of surviving rabbits has not been demonstrated. Similarly rabbit cells have not been demonstrated in the rat's tissues in our experiments. In this respect, it may be of interest to mention the following preliminary experiment. The only available rabbit that had received 315 million newborn rat-liver cells following 900 r 1 yr previously was given 2 injections of BSA spaced several months apart. Two control rabbits which at that time had been given rabbit-embryo liver cells were treated similarly. These animals were bled 1 week after the 2nd injection.

Whereas the rabbit-treated rabbits responded with a normal precipitin formation, the rat-treated animals gave no response that could be measured even by employing very sensitive technics. Experimental groups are now being studied to expand the observations on this latter approach. Such experiments may aid in establishing just how soon ability to form precipitins is lost, whether it is ever regained by the rabbit following irradiation. The evidence presented above seems to indicate that, under the conditions described, embryonic (mesenchymal) cells other than the rat's own, can survive in the rat body and, after appropriate stimulus, can actively engage in antibody formation for a long period of time after their introduction.

Summary. Rats given lethal doses of total-body X-irradiation were treated with liver cells obtained from rat and rabbit embryos. Two to 7 weeks after this treatment, groups of animals were stimulated with a mixture of Bovine Serum Albumin and *Salmonella typhi* vaccine intravenously. Rats were bled 1 week after antigen injections and titers were performed for detection of antityphoid and anti-BSA antibody. Rabbit-treated rats showed a positive titer to BSA and a delay in recovery of antibody-forming capacity to both antigens administered. Although rat-treated rats recovered their antibody-forming capacity earlier, they did not show any response to BSA. Possible implications of these phenomena are discussed.

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