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Elimination of Heterologous Proteins from Blood of Normal, X-Radiated and Tolerant Rabbits.*† (23994)

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Disappearance of serum protein antigens from blood of rabbits proceeds through the following phases: 1) *equilibration*—a rapid initial decrease during first 2 or 3 days as a result of equilibration between intra- and extra-vascular fluid spaces, 2) *exponential elimination*—relatively slow decline which appears to be the result of catabolism of foreign protein in absence of detectable antibody, and 3) *immune elimination*—a rapid decrease, which is the result of combination of antigen with antibody and subsequent catabolism of the complex. Rate of exponential elimination of antigen usually has not been considered to be affected by an immune mechanism(1). However, Cinader and co-workers reported that rate of elimination of human serum albumin (HSA), in normal rabbits, during the exponential phase, was more rapid than the rate in HSA tolerant rabbits, the latter being comparable to the rate of elimination of homologous albumin(2). This would suggest that the exponential elimination of HSA, in the normal rabbit, may be, in part, the result of an immune mechanism which does not necessitate circulating antibody. Such a mechanism would presumably not be operative in the immunologically tolerant animal. To test this possibility, the behavior of serum antigens, in normal, x-radiated and immunological unresponsive rabbits was determined.

Materials and methods. Bovine gamma globulin (BGG), Armour and Co., Lot No. C-904, bovine serum albumin (BSA), Armour and Co., Lot No. P67908, and human serum albumin (HSA), Cutter Laboratories, were used as antigens. These proteins were trace-

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labelled with I^{131} (I*) according to the method of Talmage *et al.*(3). All rabbits were albino males from a single herd. Three groups of immunologically unresponsive rabbits were prepared by several injections, during the first 5 days after birth, with total of 500 mg of either BGG, BSA or HSA(2,4,5). These rabbits were kept without further treatment until they were 3-4 months of age, and weighed 2.0-2.5 kg, at which time they were injected with 10-15 mg of an I* labelled preparation of the same protein. X-radiated§ and or normal adult rabbits, which were the same age and weight as the above rabbits, were injected with 10 mg of either I*BGG or I*HSA. All rabbits were bled periodically and aliquots of sera, or the trichloroacetic acid precipitates thereof, were counted in well-type gamma counters and the counts converted to % of injected I* protein remaining in the total blood volume. Sufficient counts were obtained to insure a maximum counter error of less than 5%. Half-lives of rates of disappearance of I* protein from blood were obtained by graphic analyses.‖ These analyses were based on exponential elimination of I* proteins which followed equilibration between intra- and extra-vascular fluid spaces.

Results. Eight out of 13 rabbits, injected at birth with BGG, did not show an immune elimination of I*BGG, when I*BGG was injected 3-4 months later, whereas no rabbits injected at birth with HSA or BSA showed an immune elimination of antigen when injected later with the respective I* antigen. All rabbits which did not give an immune elimination of the antigen remained unresponsive when again injected with antigen 1-6 months later. The half-lives of BGG, HSA and BSA in these tolerant animals during the exponential phase were 2.3, 4.0 and 4.3 days respectively. These half-lives were similar to those found for BGG, HSA and BSA in x-radiated and/or normal rabbits (Table I).

TABLE I. Half-Lives of Heterologous Serum Proteins in Normal, X-Radiated and Tolerant Rabbits.

Rabbits	Antigen	Interval of measurement in days after inj.	Avg half-life, days
6 Normal	BGG	2– 5	2.1 ± .2*
6 X-radiated		3– 9	2.2 ± .2
8 BGG-tolerant		3–16	2.3 ± .2
8 Normal	HSA	3– 7	4.1 ± .4
8 X-radiated		3– 9	3.9 ± .3
3 HSA-tolerant		3–17	4.0 ± .4
5 Normal	BSA	3– 6	4.3 ± .3
6 BSA-tolerant		3–20	4.3 ± .2

* Stand. dev.

Discussion. Since rabbits, in which the immune mechanisms have been abolished or circumvented by either x-radiation or induction of tolerance, eliminated BGG, HSA and BSA during the exponential phase at the same rate as do normal rabbits, it would appear that immune factors play no role in elimination of these proteins during this period. This conclusion is in agreement with earlier observations, that half-lives of the exponential elimination of heterologous serum proteins have no relationship to degree of their antigenicity (6,7). However, that these heterologous proteins are recognized as foreign on an apparently non-immunologic basis, is evident from the fact that their half-lives during the exponential phase, in both normal and treated (x-radiated and tolerant) rabbits, were shorter than the half-lives of either homologous albumin or globulin. This can be most readily seen with BGG, which has a half-life of 2.2-2.3 days in either normal, tolerant or x-radiated rabbits, whereas, the half-life of the homologous globulin, in the normal rabbit, is 5.0 days(8). Also, the half-lives of HSA and BSA, in normal and treated rabbits were 3.9-4.3 days, whereas, the half-lives of homologous albumin, in normal rabbits, is 5.7 days(9).

In contradiction to the present observations, Cinader and co-workers(2) reported a significantly longer half-life for exponential elimination of HSA in tolerant than in normal rabbits. Their half-life of HSA in tolerant rab-

§ To prevent an immune response to BGG and HSA, some normal adult rabbits were given 400 r wholebody x-radiation 48 hours prior to injection. X-radiation was done at 200 kvp and 15 ma with a filter of 1 mm Al and 0.25 mm Cu delivering at the target distance.

‖ Rate of elimination of BSA from 4 BSA - tolerant rabbits was also calculated from data obtained by duplicate immunochemical determinations. These values agreed, within errors of technic, with those calculated from I* activity.

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