phocytes of short life span and their generation or replacement time has been shown to be 3 to 5 days(4,5). In fact, if rats were thymectomized prior to daily injection of ARLS, no periodic rises in the lymphocyte count were observed. Therefore, it is possible that the thymus releases small lymphocytes into the circulation when lymphopenia develops, although the majority of these cells may die within the organ if peripheral lymphoid tissues are intact(6).

In animals treated with ARTS the uridine uptake of thymocytes was increased and the increase continued over the 9 days as indicated by the uptake ratio (Fig. 4). However, the thymidine uptake remained lower than that of the control group and the lymphocyte count in the blood also stayed depressed despite an increased production of a thymic humoral factor in thymus as suggested by the uridine uptake. It is possible that ARTS contains antibody against a thymic humoral factor and continuously inactivates it in the circulation. Because of decreased level of a thymic humoral factor in the blood, lymphocyte production in thymus remains depressed despite compensatory increase in the production of a thymic humoral factor in thymus glands. The possibility that in rats receiving ARTS depletion of thymic small lymphocytes was mediated by a decreased level of a thymic humoral factor in the blood is further supported by the findings that no rabbit serum was detected in the imprints of the thymus of these animals by indirect immunofluorescent techniques and there were no signs of inflammatory reaction in the thymus histologically to account for the depletion of small lymphocytes by direct toxic effect of the antiserum.

Summary. Thymidine-H^3 uptake of thymocytes from rats injected with antilymphocyte serum was increased when the blood lymphocyte count was low whereas it was decreased when the count returned to normal. Thymocytes from rats injected with antithymus serum showed increased RNA metabolism despite lowered thymidine-H^3 uptake and sustained lymphopenia. The increased RNA metabolism may indicate a compensatory increase in the production of a thymic humoral factor which was inactivated by antithymus serum in the blood resulting in sustained lymphopenia.

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Demonstration of an Early Host-Graft Incompatibility Reaction In Radiation Chimeras with the Jerne Plaque Technique.* (32385)

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It is well documented(1,2) that mice can be protected from the effects of lethal total-body irradiation by injection of isogeneic, allogeneic, and xenogeneic bone marrow shortly after exposure. A variety of techniques has been used to demonstrate that this protection is due to transplantation of a complete donor hematopoietic system into the radiated host. With foreign-marrow treatment, a major

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complication in the subsequent recovery of the animal has been the secondary disease syndrome that is attributed to an immunologic incompatibility reaction between host and donor tissue. This syndrome, which usually begins during the third or fourth week post-treatment, is characterized grossly by severe loss in body weight, diarrhea, a hunched appearance, and roughness of the fur. Evidence points strongly to a graft *vs.* host etiology although some host reaction to the graft has not been ruled out. If a graft *vs* host mechanism is the primary factor, one would expect the reaction to begin almost immediately after infusion of the donor's immunologically competent cells. They should react promptly against the host environment, far earlier than the recognized clinical manifestations of secondary disease are seen(1). Biochemical studies concerning amino acid metabolism have revealed differences between animals treated with allogeneic and isogeneic marrow and have been noted as early as 5 and 9 days post-treatment(3). The present report concerns our effort to use the Jerne plaque technique for identification of an early graft *vs* host or host *vs* graft reaction in tissues obtained from lethally irradiated animals treated with foreign marrow.

Materials and methods. Animals. Male $C3BF_1$ [$(C3H \times C57Bl)F_1$] mice approximately 14-16 weeks old were used in all experiments. They were given food and water *ad libitum.*

Radiation. A constant potential Westinghouse X-ray machine was used as a radiation source with the following conditions: 250 kv; 15 ma; inherent filtration 0.5 mm aluminum; added filtration 0.5 mm copper and 1.0 mm aluminum; and 100 R per min at 50 cm. For irradiation, the mice were placed in a circular, perforated lucite container attached to a revolving turntable, and a single total-body exposure of 950 R was given.

Bone marrow treatment. Xenogeneic marrow was obtained from either rats (Carworth Farm Nelson) or hamsters (Syrian). Rat marrow was obtained from the femurs and humeri and hamster marrow from the femurs only. Marrow-cell suspensions were made in TC-199 and 1 ml, containing 100×10^6 nucleated cells, was injected intravenously within 2 hours after irradiation.

Jerne plaque technique. The agar plaque technique was essentially that described by Jerne *et al*(4) with only a few modifications. The bottom agar layer, which served as a base for the overlying agar layer containing the test and target cells, consisted of a 0.7% agar solution made in Eisen's(5) medium and contained 1 mg/2ml of DEAE (di-ethyl-amino-ethyl)-dextran. The agar (Difco Special Agar Noble) and DEAE-dextran were placed in a beaker containing Eisen's medium and heated very slowly to a boil until in solution. The solution was brought to pH 7.4 with a few drops of 1 N NaOH and 3 ml of the hot agar was pipetted into plastic petri plates already warmed to 45°C in an incubator. The plates were then covered and allowed to gel at room temperature. The agar for the overlying layer was made in the same manner and 2 ml was pipetted into test tubes and maintained in a 45°C water bath. With prewarmed pipettes, 0.1 ml of a 10% suspension of target red cells was added to the test-tube agar and mixed thoroughly. After this, 0.1 ml of the experimental spleen cell suspension containing the desired number of cells was added to the tube; the contents were again mixed thoroughly, and then decanted into the prewarmed agar plates having the base layer. The plates were gently swirled to obtain an evenly distributed agar layer, allowed to gel at room temperature, and then placed in a 37°C incubator for 1 hour. After incubation, 10 ml of Eisen's medium was added to the plate and incubation was carried on for an additional 10 minutes at 37°C. The medium was then decanted and the agar plate was rinsed twice. Fresh guinea pig complement, previously absorbed at 0-2°C with the target-type red cells to remove any heteroagglutinins, was diluted 1:7 and added to the agar plate. The plate was then reincubated for 1 hour at 37°C. After incubation, the complement was decanted and the plate was scored for hemolytic plaques. Scoring was done macroscopically and any questionable plaques were examined microscopically.

Preliminary studies utilizing sheep, rat, and hamster red blood cells as antigens in either

mice, rats, or hamsters were performed. Spleen cells from normal unirradiated animals immunized with the foreign antigens were tested in Jerne plates to demonstrate the feasibility of detecting an immune response to the specific red cell antigen by this technique. This was accomplished satisfactorily in all combinations, although it should be noted that in mice immunization with sheep red blood cells gave consistently greater numbers of plaque-forming cells per spleen than immunization with either rat or hamster red cells. This latter observation will be the subject of another report.

Tissue preparation. The irradiated mice treated with xenogeneic marrow were killed at varying intervals after treatment and their spleens were removed. The spleens were placed in a small petri plate containing Eisen's medium and gently teased apart with a fine forceps and scalpel. The resulting cell suspension was gently aspirated through successively smaller gauge hypodermic needles to yield a uniform cell suspension free of large particles and debris. Since the cellular content of such spleens obtained from 950 R mice was low, it was necessary to use a pooled cell suspension obtained from 3-5 spleens for each time interval studied. When the animals were killed identification of donor granulopoietic cells in the spleens was made by alkaline phosphatase staining of touch preparations of transverse sections of each spleen.

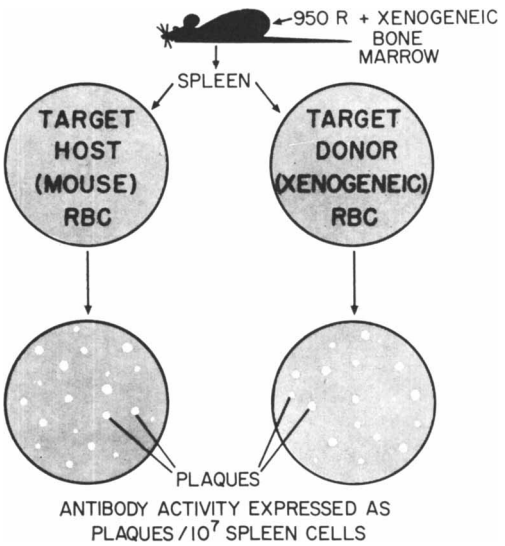

FIG. 1. Experimental design showing the use of the Jerne plaque technique for detection of an anti-host or anti-donor reaction in spleen cells obtained from xenogeneic radiation chimeras.

Experimental design. To determine the presence of reciprocal host-graft immunologic activity in the irradiated marrow-treated host, the splenic cell suspension was divided into two aliquots, one part used in agar plates containing host-type red cells as the target system, and the other in plates containing donor-type red cells as the target system. This procedure permitted identification of immunologic activity of either one or both cell systems simultaneously. The scheme is shown diagrammatically in Fig. 1.

Results. A series of experiments was performed with irradiated mice treated with rat bone marrow cells. Spleen cells were obtained from these animals on consecutive days from 1 through 18 post-treatment and tested as outlined in Fig. 1. No anti-donor activity, *i.e.*, plaque formation in agar plates containing the rat-type target cells, was observed in a total of 36 individual tests, each involving a pool of 3-5 spleens from the experimental animals. In the reciprocal tests there was only minimal evidence suggestive of anti-host activity. When present this consisted usually of 1-5 plaques per 10^7 spleen cells; a maximum number of 14 was observed on day 5 postirradiation. Similar results were obtained with spleen cells obtained from animals 29, 32, and 39 days post-treatment.

Failure to obtain meaningful data with the rat as the xenogeneic donor prompted us to perform another series of experiments with lethally irradiated mice treated with hamster bone marrow. That such xenogeneic radiation chimeras can be obtained with this host-donor combination has already been demonstrated (6). It has also been shown that with a greater phylogenetic distance between host and donor, there is an earlier and more intense immunologic incompatibility reaction, the components of which may be more readily identifiable with the present technique. The results of a series of experiments from 1-12 consecutive days post-treatment are shown in Fig. 2. Both anti-host and anti-donor activity was evident in these xenogeneic radiation chimeras. A host anti-graft component reac-

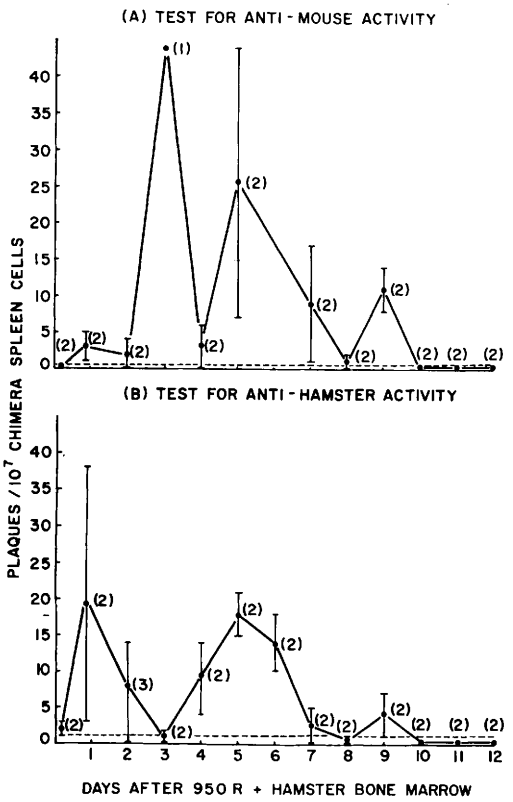

FIG. 2. Plaque formation as a function of time in lethally irradiated mice treated with hamster bone marrow. A. Target cells in agar plates were mouse (host) red blood cells, plaque formation therefore indicating the presence of anti-mouse activity in the chimera spleens. B. Target cells in agar plates were hamster (donor) red blood cells, plaque formation therefore indicating the presence of anti-hamster activity in the chimera spleens. Numbers in parentheses indicate number of spleen cell pools (3-5 spleens per pool) tested at the various time intervals. Each point represents the mean number of plaques obtained from each pool and longitudinal bars indicate the spread in number of plaques counted. Horizontal dashed lines represent the mean background number of plaques obtained from normal spleens of hamsters (A) and mice (B) for the reciprocal target red blood cells.

tion was evident within 20 hours postirradiation and marrow treatment and persisted throughout the first 6 days. Maximum graft anti-host activity occurred within 3 days posttreatment and was also evident throughout the first week. Unfortunately, the great variation observed in the different spleen cell pools prevents any definitive statement about the time course of either host or graft reactivity, but apparently, for the 12 day period studied, the greatest reaction for both cell components occurred within the first week.

Discussion. The objective of the present report, to demonstrate the existence of an early immunologic incompatibility reaction between host and donor tissue in irradiated animals receiving foreign bone marrow, was accomplished. This was demonstrated only equivocally with the rat as the xenogeneic donor, but the results with hamster bone marrow were more definitive, although variable and of low magnitude. This variability and low-order reaction, however, are not surprising considering the biologic factors involved: (1) the irradiated mice had received 950 R and their response to a foreign antigenic stimulus could be anticipated to be very weak or even nonexistent; indeed, we are not aware of any other study demonstrating any antibody formation in normal animals immediately after such a high dose of radiation; (2) the transplantation and proliferation of foreign marrow in an irradiated host still apparently capable of manifesting a response to an antigenic stimulus would preclude any uniformity of a graft *vs* host reaction during this period of recovery; furthermore, any immunologic attack mounted by the grafted cells would be subject to a variety of physiologic stresses unrelated to rejection mechanisms, resulting in variation in expression of any graft *vs* host reaction; and (3) the detectable hemolysin used as the end point of the assay here is a humoral entity that is not the primary immunologic vector operating in tissue incompatibility reactions. This latter point warrants emphasis since it is well recognized that humoral antibody to red cell antigens plays no role in tissue-rejection mechanisms. Thus, the antibody detected in this study may have been directed specifically against red-cell antigens, or against transplantation antigens having a finite degree of cross-reactivity with red cells. Another factor contributing to the weakness of the immune reactions observed is that the assay procedure was largely limited to detection of 19S antibody, 7S antibody having been demonstrated as having a poor hemolytic efficiency when compared to the larger protein(7). In view of these considerations, therefore, the orders of response observed are not surprising. Inability to dem-

onstrate a convincing host or graft reaction with rat bone marrow in the present study was unexpected and may relate to the reservations noted. Uyeki†, using the same technique reported here, noted a strong graft anti-host response in some 950 R x-irradiated LAF_1 hosts given marrow cells from hamsters pre-immunized to LAF_1 antigens. Some of the irradiated hosts, not preimmunized to donor tissues, also showed a response, although much weaker, when their spleen cells were tested in the agar plates containing hamster red cells as the target cells.

The significance of these findings relative to the etiology of secondary disease in foreign marrow radiation chimeras can only be speculated upon at present. Although there is a preponderance of evidence favoring a graft *vs* host mechanism(1,2) only a few studies have indicated reaction of the host's immune system as a factor in the syndrome(8-10). Of immediate relevance to the present concept of an "early" host reaction are the studies of Gengozian *et al*(11) demonstrating the greater probability of obtaining a functional marrow graft with higher radiation exposure to the host. Shekarchi and Makinodan(12), using the irradiated mouse as the recipient, demonstrated that the "take" of foreign marrow in these animals was related to the phylogenetic distance between host and donor as well as the amount of radiation exposure to the host. Thus, the greater (usually supralethal) the radiation dose, the greater the probability of transplanting the more distantly related bone marrow. This effect was attributed to the host's immunologic reaction to the more foreign marrow even after such high doses of radiation. Histologic evidence suggestive of a residual immune function in mice after supralethal doses of 950 R-2500 R has been presented by Congdon and Goodman(13). Another factor relating to this early reaction concept is the greater number of foreign bone marrow cells required to establish a radiation chimera as compared to that in a syngeneic system. We had previously suggested that this may be because the irradiated host is capable of mounting an immediate immune reaction, albeit a minimal one, to the foreign marrow cells(1). The inactivation and destruction of some of the infused cells therefore would result in an increase in the number of hemopoietic cells necessary to protect the animal from radiation death. A reduction of donor hemopoietic cells, however, could also result from the "early" graft *vs* host reaction, an increase in proliferation of donor antibody-forming cells occurring at the expense of proliferation of precursors for the hemopoietic cells necessary to promote recovery. In view of these considerations, this early reaction concept suggests a cause and effect phenomenon that may be implicated in the subsequent recovery of the xenogeneic chimera leading to the secondary disease syndrome.

Summary. The Jerne plaque technique was used to demonstrate the presence of an early immunologic reaction between host and donor tissues in lethally irradiated mice treated with rat or hamster bone marrow. Evidence for both host and graft activity was obtained from spleen cells of mice that had been treated with hamster bone marrow. The responses obtained were of low magnitude and variable, and were evident primarily through the first 6 days post-treatment. A cause and effect relationship of this early immune reaction to the etiology of secondary disease in foreign marrow radiation chimeras is suggested.

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Viruses Recovered from Laboratory Dogs with Respiratory Disease. (32386)

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Respiratory disease with high morbidity and mortality has occurred repeatedly in newly acquired mongrel dogs at this institute. The disease occurred notwithstanding canine infectious hepatitis (ICH) and canine distemper virus (CDV) prophylaxis with attenuated vaccines. Previous attempts to relate bacteria to the respiratory disease episodes were unrewarding. Moreover, the outbreaks could not be controlled by intensive antibiotic treatments. Therefore, attention was directed to the possible role of viruses in respiratory disease in dogs.

Prior to 1962, CDV was the only known viral agent that was unequivocally associated with respiratory disease of the dog. In 1962, a new canine adenovirus was isolated from dogs with laryngotracheitis(1) and in 1963(2) and 1966(3) reovirus type 1 was isolated from dogs with respiratory disease signs. In addition there is also serological evidence of canine herpes virus infection in dogs with similar signs(4). These findings suggest that in addition to CDV other viral agents may be involved in respiratory disease in dogs.

Initial studies were conducted to determine the presence of viruses in throat specimens and post-mortem tissues of dogs with respiratory disease. The isolation of canine adenoviruses, canine herpes virus and a virus serologically related to SV-5 from dogs with respiratory disease are described here. The serological reactions of test dogs and pathogenicity studies on isolated viruses will be presented separately.

Materials and methods. Dogs. Seventy-five mature dogs of mixed breeds were purchased from a commercial vendor. At time of arrival, each dog was examined by a veterinarian and only those which appeared to be free of overt disease signs were accepted. All dogs were given inactivated phenolized rabies vaccine and half the dogs received inactivated ICH vaccine.‡ The CDV vaccination was omitted to avoid interference with serological testing to determine the occurrence of CDV infection. The 75 dogs were kept in the same room in 19 runs. Groups of 4 dogs were kept in 4′ × 12′ runs with 1-1/2′ × 6-1/2′ pallets raised 1-1/4′ above the concrete floor. The dogs were fed a commercial ration which was always available. With the exception of cats, no other species was kept in this building. The dogs were observed daily for clinical signs of respiratory disease and their rectal temperatures were recorded. Sick dogs were isolated and treated symptomatically and with antibiotics.

Specimens. Specimens for virus isolation were taken from each dog the first day signs of respiratory disease were detected. A cotton

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