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The Homotransplantation of Kidneys and of Fetal Liver and Spleen after Total Body Irradiation *

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HOMOGRAFT rejection is generally believed to be a consequence of antibody formation by the host against the antigens of the transplanted foreign tissue. If a sufficiently large dose of total body radiation is administered to mice [6, 28, 65, 56, 66, 80, 96] antibody formation and hematopoiesis can be suppressed and bone marrow homotransplants will take successfully. The repopulation of the hematopoietic system by the grafted tissue leads to the production of an antigenic mosaic pattern which is characteristic of the donor rather than the recipient. Radiated mice with bone marrow homotransplants will therefore accept skin homografts from members of the donor strain.[57, 95]

The use of adult bone marrow transplants after radiation has led to the appearance in a significant percentage of animals of the "runt syndrome" in which failure to grow and eventual death appear to be related to a reaction of the mature graft against the host.[8, 95] Uphoff [100] found that if fetal hematopoietic tissues (liver and spleen) were used instead of adult marrow the runt syndrome did not appear. This is presumably because the fetal tissue, by virtue of its immunologic immaturity, was able to acquire a tolerance to the host's antigens and thus did not react against the host.

The rejection of homografted kidneys in the dog is accompanied by the appearance of lymphocytes and plasma cells in the interstitial spaces of the cortex, edema, tubular destruction, and cessation of urine formation.[31, 43, 84] It has been postulated by Simonsen [84] and Dempster [31] that the round cells which appear in the interstitial spaces are of donor origin and represent a reaction of the donor against the host.

In the present study an answer was sought to three questions: 1) whether infusions of homologous fetal liver and spleen cells would permit indefinite survival after lethal doses of total body irradiation (TBR) in the dog, 2) whether irradiation of the host prior to renal homotransplantation would prevent the appearance of the usual homograft rejection phenomena, and 3) whether irradiation of the donor prior to transplantation would eliminate the homograft rejection phenomena. By virtue of the answers to questions 2 and 3 a fourth question would be answered, namely whether the lymphocytes and plasma cells seen in the interstitial spaces of the transplant were of donor origin, representing a reaction of the graft against the host as claimed by Simonsen and Dempster, or of host origin, representing a reaction of the recipient against the graft.

Methods

A total of 71 unrelated mongrel dogs were used for the experiments. They were dewormed, immunized against canine distemper and splenectomized. The animals

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were divided into six groups as follows: 1) 28 dogs received 600 r total body irradiation (TBR) without transplantation of hematopoietic tissue, and served as controls; 2) 16 dogs received 600 r TBR followed within 24 hours by an infusion of fetal hematopoietic tissue (liver and spleen) from one or more donors unrelated to the recipient; 3) five dogs received renal homotransplants taken from donors who had received 1,000, 1,200, or 1,500 r TBR 24 hours before transplantation; 4) 14 dogs received renal homotransplants from nonirradiated donors 24 hours after receiving 1,000, 1,200, or 1,500 r TBR; 5) seven dogs received renal homotransplants from irradiated donors (600, 1,000, or 1,500 r TBR) 24 hours after receiving 1,000, 1,200, or 1,500 r TBR; 6) one dog received a renal homotransplant without radiation of either host or donor. The transplant functioned five days, and was removed on the seventh day. On the thirteenth day the animal received 1,500 r TBR, and on the same day a secondary homotransplant was carried out using the remaining kidney of the original donor. The animal was sacrificed after 24 hours.

As controls for the renal homotransplantation experiments we utilized data from our previous experiences with over 150 renal homotransplants in nonirradiated animals.[33, 34, 43]

Total body radiation was administered from one of two sources. The first source was a 1,000 KVP G-E x-ray machine. The dogs were placed one meter from the source and were held in a special cage so that they could be radiated on both sides. The rate of radiation was 37.8 r per minute. The second source was a multiple cobalt-60 machine specifically designed for TBR in which the cobalt is contained in the walls of a cylinder which completely surrounds the animal. This radiation source was made available to us at the Naval Medical Research Institute of the National Naval Medical Center.* The rate of radiation was 32 r per minute and radiation was uniform throughout the entire field in which the animal was confined. In the early experiments animals were placed under pentothal anesthesia during irradiation with the 1000 KVP machine, but not during irradiation with the cobalt source. In the later experiments anesthesia was not used during irradiation with the x-ray source either. The animals were maintained on daily injections of penicillin and I-V fluids after irradiation.

The fetal hematopoietic tissue was prepared by removing the liver and spleen asceptically from a fetus which in turn had been removed by cesarian section. The gallbladder, major bile ducts, and vessels of the hilum were removed. The parenchymal cells were then scraped from the stroma with the back of a scalpel. The cells were passed through a tea strainer with an aperture size of approximately 1,000 microns. In the earlier experiments the cells were suspended in an equal volume of isotonic saline and were injected slowly intravenously in one dose. Intra-arterial and intra-marrow routes of injection were found to be less satisfactory than intravenous. All work was done with sterile glassware in an ice bath. In the later experiments the cells were suspended in an equal volume of homologous dog serum, which was also kept in an ice bath. The cells were not injected all at one time, but were injected in several aliquots. The portion of cells to be injected at the time of preparation was kept in the ice bath except for that small amount which was in the syringe at any given time. The remainder of the liver-spleen cell suspension was stored in the deep freeze at $-90°$ C.[35] after adding an equal volume of 30 per cent glycerol in saline.

In some animals death occurred during the injection of the liver-spleen cell suspension. That this was not due entirely to

* With the help and coöperation of Captain F. W. Chambers, MSC, USN.

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TABLE 1. *Total Body Irradiation and Renal Homotransplantation*

X-ray Donor
1,000 r donor
* (1) KT-13 (10 D)—reaction
1200 r donor
* (1) KT-46 (7 D)—no reaction
1500 r donor
* (1) KT-47 (7 D)—reaction
(2) KT-23 (6 D)—reaction
(3) KT-50 (5 D)—reaction
X-ray Host
1,000 r to host
* (1) KT-8 (10 D)—reaction (F)
(2) KT-37 (5 D)—reaction
(3) KT-38 (5 D)—reaction
1,200 r to host
* (1) KT-45 (7 D)—reaction
(2) KT-44 (7 D)—no reaction
(3) KT-27 (6 D)—no reaction
(4) KT-29 (6 D)—reaction
(5) KT-34 (5 D)—reaction
(6) KT-41 (8 D)—no reaction
1,500 r to host
* (1) KT-48 (8 D)—no reaction
(2) KT-10 (13 D)—no reaction
(3) KT-35 (8 D)—no reaction
(4) KT 20 (3 D)—no reaction
(5) KT-40 (4 D)—no reaction
X-ray Host and Donor
Host 600 r
(1) KT-3—11 days (600 r donor) R—reaction (F)
(2) KT-4— 9 days (600 r donor)—reaction (F)
Host 1,000 r
* (1) KT-49—6 days (1,000 r donor)—no reaction
* (2) KT-43—4 days (1,000 r donor)—no reaction
Host 1,200 r
(1) KT-39—4 days (1,000 r donor)—no reaction
Host 1,500 r
(1) KT-7 —9 days (1,500 r donor) R—no reaction (F)
* (2) KT-11—4 days (1,500 r donor)—no reaction (F)
Secondary Transplant
(1) KT-312—primary transplant functioned 5 days
day 7 kidney removed
day 13 1,500 r TBR to host
day 13 secondary kidney transplant
day 14 sacrificed—no reaction

F—received fetal hematopoietic tissue.
R—radiation of host after transplantation.
*—cobalt-60 irradiation source.
The figures in parentheses indicate the period of survival of the animal in days; or, in the case of radiation of the donor, the time when the kidney transplant was removed after it had ceased functioning. The only exception to this is KT-46, which was still functioning when removed.

embolic phenomena was born out by the fact that a Waring blender homogenate of liver in which particulate matter had been centrifuged down and the cell free supernatant portion injected proved to be lethal in a dose range (4 Gm. of liver per kg. of dog) which was similar to that seen with whole-cell suspension injections. The lethal dose of liver cell suspension fell into a fairly well defined range but sometimes very small doses caused rapid demise of the animal. In order to inject a sufficient number of cells, injections were given slowly and a period of several hours was allowed to intervene between injections.[49] During the injection the animals were monitored on an EKG machine. It was found that before death inversion and peaking of the T wave, and, later on, S-T segment depression occurred. In some cases the animal would survive if the injection was stopped when EKG changes first became evident, but other animals died even if the injection was discontinued.

The average last trimester-dog fetus yields roughly 4.0×10^{10} nucleated cells per liver. Cell counts were done on all injected fetal-liver and spleen-cell suspensions, and in addition the liver cells were weighed before the serum was added in order that the lethal dose could be calculated in terms of Grams of liver per kilogram of body weight. An animal was sometimes given an injection of fetal liver and spleen from a single fetus and at other times from several fetuses of the same uterus.

Renal homotransplants were carried out as described by Hume and Egdahl,[43] the transplant being placed in the renal fossa with the vessels anastomosed to the host renal artery and vein. A polyethylene catheter was placed in the ureter and the ureter was brought out through the skin. Daily collections of urine were made. The dogs were maintained until death or until death appeared imminent. The renal homotransplant was taken from an animal who was not related to the host, to the pregnant

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animal donating the fetuses, or to the fetuses.

When female fetal tissue was administered to a male host the female leukocyte tags were searched for by the method of Davidson and Smith.[29]

In some cases fetal liver and spleen injections were given to irradiated animals receiving kidney transplants, but in most cases no hematopoietic cell injections were made, the transplants being carried out primarily for the purpose of determining the dose of irradiation necessary to prevent the renal homograft reaction during the period for which the animal survived. The non-irradiated host animals receiving irradiated kidney transplants were sacrificed after renal transplant function had ceased—which, with a single exception, fell within the period of time the irradiated animals survived.

In two instances in the group in which both host and donor were irradiated, the irradiation of the donor kidney was accomplished immediately after transplantation in the host (KT 3 and KT 7—Table 1). In all other cases the host and donor were irradiated separately.

Results

A. Total Body Irradiation with and without the Infusion of Fetal Hematopoietic Tissue. All 28 splenectomized dogs receiving 600 r total body irradiation died within a period of seven to 17 days. Of the 16 dogs receiving infusions of fetal liver and spleen following 600 r total body irradiation, four animals are surviving 12, 16, 18 and 22 months later. They appear in normal health. One male animal receiving female cells had persistent female leukocytes eight months after injection (Fig. 1). These have since disappeared from the circulation.

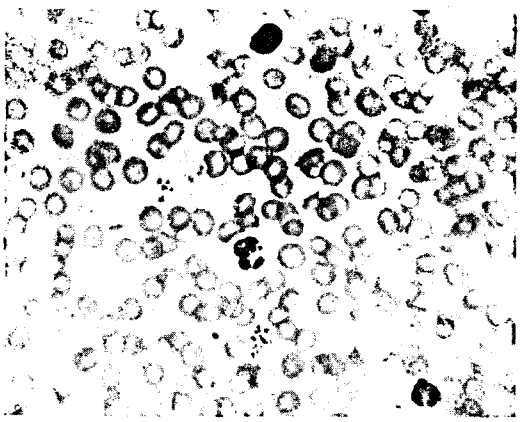

FIG. 1. A female leukocyte in the peripheral smear of an irradiated male host receiving cells from the liver and spleen of a single female fetus 8 months before.

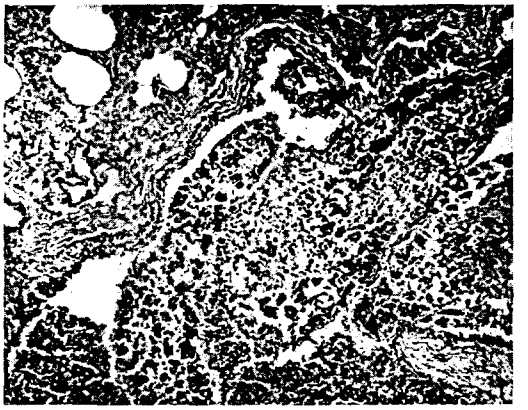

FIG. 2. Liver and hematopoietic cells in the lung of a dog who was irradiated and infused with fetal liver and spleen cells 8 days before.

Following an intravenous injection of fetal liver and spleen cells, some of the injected cells are caught up in the capillary bed of the lungs, as shown in Figure 2. However many of the cells make their way to the bone marrow, which ultimately becomes the site of the most active proliferation. The course of the cells is easy to trace for some time after the infusion because the hematopoietic cells are accompanied by easily recognizable liver parenchymal cells. In a single clinical case, we injected fetal human liver intravenously and recovered healthy hepatic cells in the recipient's bone marrow ten days later.

B. Total Body Irradiation plus Kidney Transplantation. The results of these experiments are listed in Table 1. They may be summarized as follows:

1. *Irradiation of the donor:* In four of five cases in which the donor animal was

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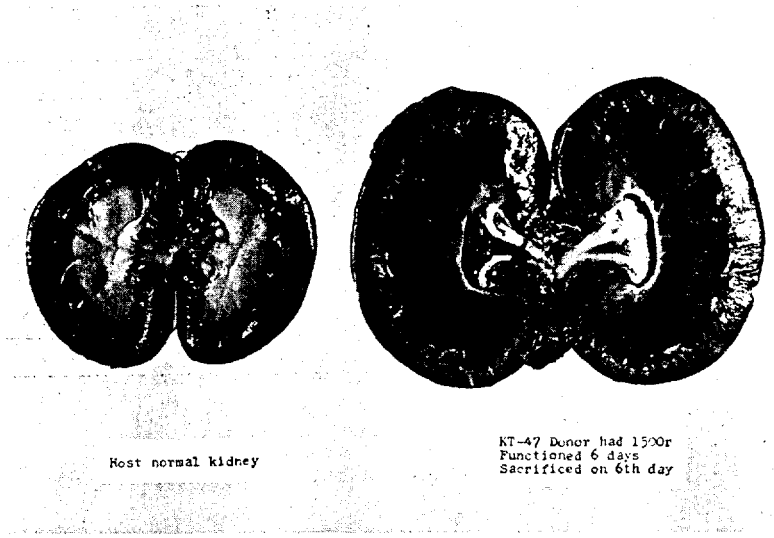

FIG. 3. Dog KT-47. The transplanted kidney is on the right and the host's own kidney on the left. The donor received 1,500 r TBR. The transplant functioned for 6 days and was removed on the 7th. Note the enlargement of the kidney, and the edema and ischemia of the cortex which suggest a typical transplant rejection pattern.

irradiated prior to transplantation with doses of 1,000, 1,200 or 1,500 r, a typical transplant rejection pattern was seen. After a few days of good function, urinary flow of the transplant ceased, or declined very sharply, usually a day or two before the removal time shown on the chart. The renal artery and vein were patent at the time of removal. All transplants from donors who had received 1,500 r showed prompt cessation of function and a typical transplant rejection pattern. A representative gross picture is shown in Figure 3, and a microscopic view of the same kidney (KT 47) in Figure 4. A photomicrograph of a normal dog kidney and of a renal homotransplant between nonirradiated dogs is shown in Figures 5 and 6 for comparison. In one instance (KT 46) in which the donor received 1200 r the transplant appeared normal at seven

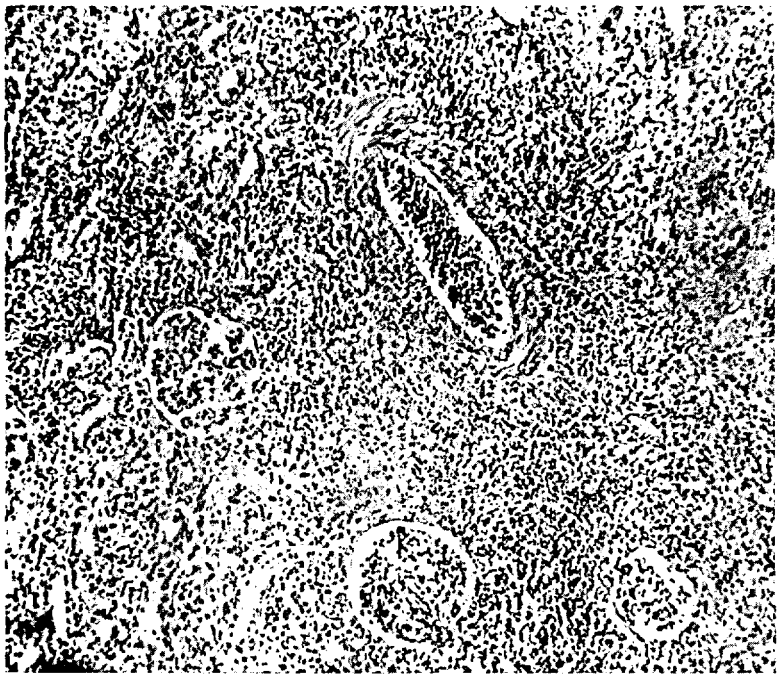

FIG. 4. Microscopic view of the transplant shown in Figure 3. There is tremendous lymphoid infiltration, virtual destruction of the tubular elements, and plugging of a small artery of the cortex seen in cross section (× 100).

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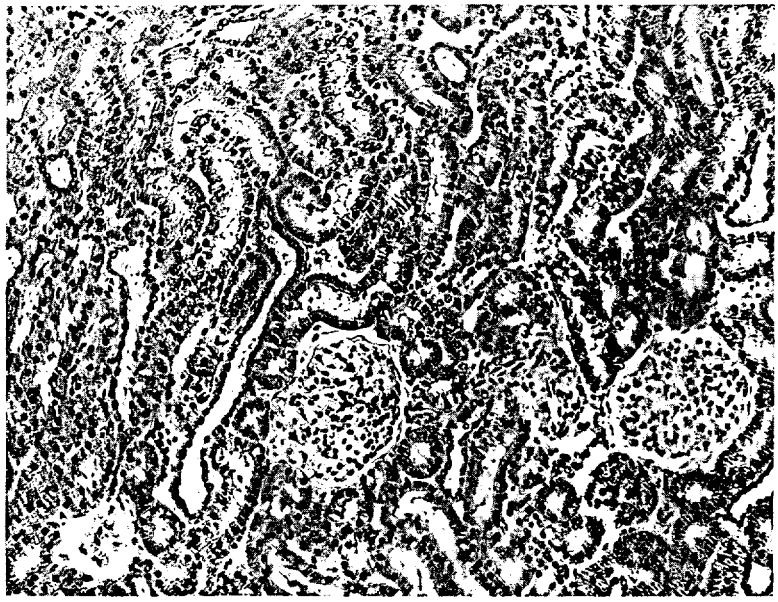

FIG. 5. Microscopic view of a normal canine kidney for comparison with the previous figure (× 200).

days. Occasional prolonged survivals are sometimes seen in a series of transplants [33, 43, 76] and it is probable that this kidney would have been rejected had it remained with the host somewhat longer.

2. *Irradiation of the host:* When a dose of 1,000 r TBR was administered to the host prior to transplantation, the usual homograft rejection pattern appeared—the radiation having failed to alter it. Of the six animals who received 1,200 r TBR, three showed a rejection pattern and three appeared normal. When a dose of 1,500 r TBR was administered to the host all five transplants continued to function for the entire period of survival of the animal (3 to 13 days), and all transplants appeared normal grossly and microscopically. An example (KT 48) is shown in Figures 7 and 8.

3. *Irradiation of the host and donor:* When

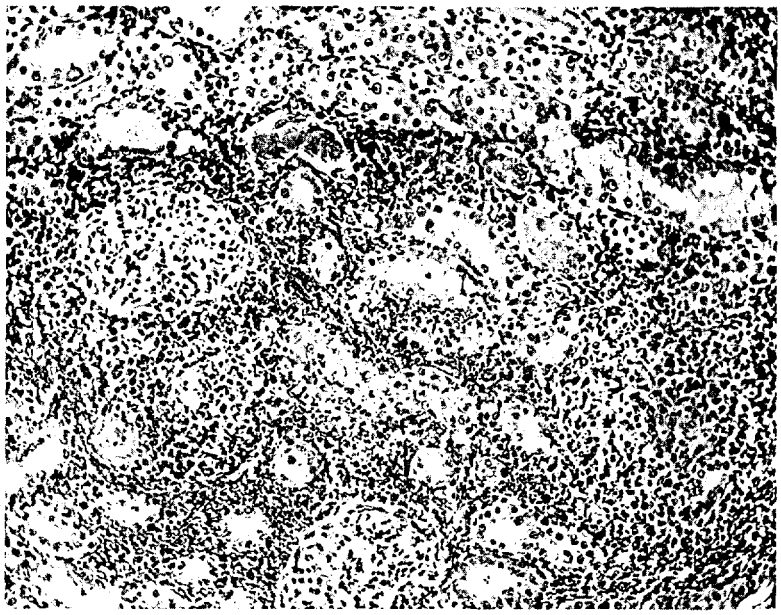

FIG. 6. Microscopic view of a renal homotransplant between nonirradiated pairs 5 days after transplantation (× 200).

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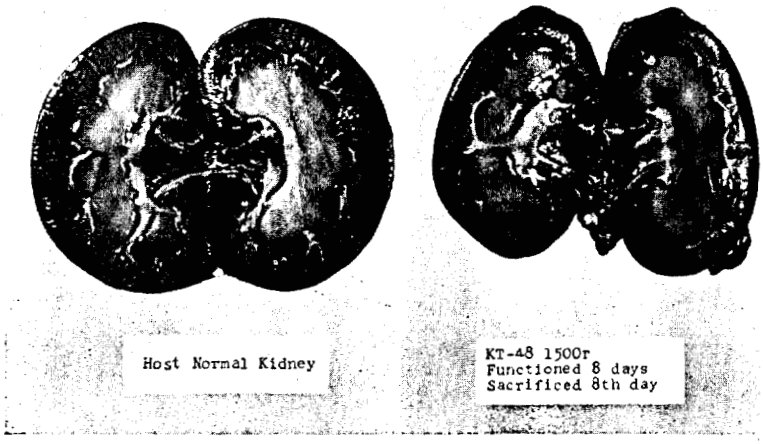

FIG. 7. Dog KT-48. The transplanted kidney is on the right and the host's own kidney on the left. The host received 1,500 r TBR. The transplant was removed while still functioning 8 days after transplantation. It appears normal grossly, in marked contrast to the transplant in Figure 3.

both the host and donor received 600 r TBR the renal transplants showed a homograft reaction. When doses of 1,000, 1,200, or 1,500 were administered to both host and donor, the transplants continued to function and appeared normal grossly and microscopically. Fetal liver and spleen was administered to two cases (KT 7 and KT 11) immediately following transplantation and irradiation. These kidneys appeared normal as well.

4. *Irradiation of the host after primary but before secondary transplantation:* In one case, a primary renal homotransplant was carried out between two nonirradiated dogs. The transplant ceased functioning on the fifth day and was removed on the seventh day. A severe homograft reaction was present. Six days after removal of the transplant, the animal was given 1,500 r TBR, and a secondary renal transplant was carried out employing the other kidney of the original donor. This was removed 24 hours later while still functioning. A photomicrograph of the primary transplant is shown in Figure 9, and that of the second-

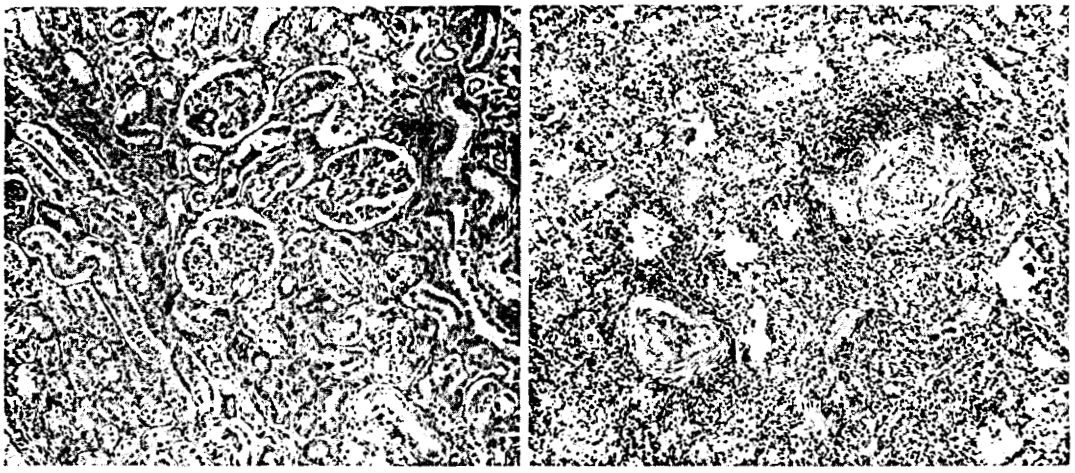

FIG. 8. (left) Microscopic view of the transplant shown in Figure 7. The kidney appears normal (× 100). FIG. 9. (right) Dog KT-312. Microscopic view of the primary transplant. The appearance is that of a typical transplant reaction. The kidney functioned for 5 days and was removed on the 7th day (× 100).

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FIG. 10. (left) Dog KT-312. Microscopic view of the secondary transplant removed after 24 hours in the host. Six days after the primary transplant had been removed the host was given 1,500 r TBR, and the secondary transplant was done on the same day. The appearance of the kidney is normal (× 100). FIG. 11. (right) Microscopic view of a lymph node taken from a non-irradiated host who received a renal transplant from a donor who had received 1,500 r TBR. The lymphoid follicles appear normal, and the architecture is undisturbed (× 40).

ary in Figure 10. It may be seen that the primary transplant shows virtually complete destruction by the homograft reaction while the secondary transplant appears normal despite its residency in an immunized host. In almost all cases a severe reaction with extensive destruction takes place in a dog secondary kidney transplant within 24 hours.[33]

Discussion

In recent years a good deal of investigation has been directed toward a characterization of homotransplantation immunity. Without reviewing the derivation of current concepts in detail, it might be pertinent to the present discussion to outline those recent data which form a background for or relate directly to the experiments presented herein.

It has been shown that homografted tissue incites an immune response in the host which leads to the destruction of the graft (Homograft reaction). The survival time of the graft is an expression of the severity of this response. A second graft from the same donor, or an inbred animal of the donor strain, is rejected more rapidly than the first (Second-set reaction).

The antigens of the graft reach local lymph nodes first in the case of tissue grafts,[69] but later reach other nodes.[64] In the case of organ transplants with functioning arterial and venous anastomoses there is a wide dissemination of antigens which does not depend upon primary transport to the regional lymph nodes[34, 43] despite the fact that a contrary impression is sometimes given in the literature.[18] Once the transplant antigens reach the lymphoid tissue in the lymph nodes, spleen,[26] and other areas throughout the body, these cells produce antibodies against the transplant antigens. The antibodies remain closely associated with the lymphoid tissue from which they arose, and are apparently transported to the graft, at least in part, via circulating lymphoid cells. This cellular association, the difficulty in demonstrating antibodies in the serum with standard immunological technics, and the ability to demonstrate a skin sensitization phenomenon when injecting immunized host lymphoid cells, or cell extracts but not serum,[20, 21] intradermally into the donor are consonant with the hypothesis that homograft immunity is mediated through cells, like drug and bacterial allergies.[17] Recent work by Lawrence *et al.*[51]

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showing that leukocyte extracts are capable of passively transferring homograft sensitivity to another patient, while serum is not, strongly suggests that homograft rejection is related to delayed bacterial hypersensitivity of the tuberculin type. Nevertheless, under certain circumstances, serum antibodies are said to be detectible against the lymphocytes of the donor.[91]

Once the animal has been immunized to the donor, this immunization can be passively transferred to another inbred animal of the same strain by transplanting living lymphoid cells to the donor,[69] and by leukocyte extracts.[51] It has been claimed that immunity can also be transferred by host serum [90] although other investigators have not yet been able to duplicate these results.[20, 51] Transplantation immunity can be induced by living cells, dead cells, or some cellular extracts thought at first to include desoxyribonucleoproteins,[17] but later shown not to.[41, 65] At present, effective cell extracts are thought to represent amino-acid polysaccharide complexes.[64]

The discovery of blood chimera in fraternal twins with common placental circulation [74] led Billingham *et al.*[14, 16] to produce acquired tolerance to foreign cells by intrauterine injections of the fetus. This state persisted into adult life and permitted subsequent grafts from the original cell donor to survive without prejudice. Acquired tolerance can also be produced by injecting foreign cells into the newborn animal in the first day or two of life in some species [39] including the dog.[52, 82] So far acquired tolerance can be produced with certainty only with living cells. Isolated spleen cell nuclei are said to be effective by some investigators [15] and not by others,[40] and some success has been claimed with RNA.[1, 2] In the sheep, by contrast, intrauterine injection of foreign splenic cells is followed by a high fetal mortality and failure of the surviving fetuses to develop tolerance to subsequent grafts of donor's skin or kidney.[67, 68] Acquired tolerance can be transferred by parabioses.[63]

It has often been supposed that developmental immaturity likewise invested fetal tissues with a reduced or absent ability to act as a sufficient antigenic stimulus to lead to rejection when homografted into the adult.[103] Although a prolonged survival of embryonic skin grafts was said to occur in the rabbit [94] no prolongation over the survival of adult grafts was seen in the mouse,[40] dog,[12] or the normal human [12] and only moderate prolongation in the burned patient.[12] Very nice evidence of embryonic antigenicity was provided by experiments showing that the injection of embryonic mouse tissue into an adult mouse of a second strain produced accelerated rejection of a skin homograft of the embryo strain.[15] Even better evidence was provided by showing that embryonic cells can produce tolerance when injected into embryos of another strain.[19, 92] Thus fetal tissue has the capacity to provide an antigenic stimulus when injected into an adult animal of different genetic composition, but because of its immunological immaturity, does not itself act to form antibodies against the antigens of the new host.

When acquired tolerance is induced by the injection of lymphoid cells of an adult animal into the embryo or newborn, certain changes may occur. These include lymphoid involution, splenic enlargement, and failure to grow normally (runt syndrome).[13, 86, 88, 89] It is now generally believed that this represents a reaction of the graft against the host.[22] This "homologous disease" or "secondary disease" usually leads to the demise of the host. Under these circumstances the embryonic or newborn host accepts the homotransplant because of its immunologic immaturity, but the transplant, being composed of adult immunologically competent cells is able to form antibodies against the host.

Jacobson and co-workers showed that shielding the spleen during fatal doses of

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TBR, or performing intraperitoneal splenic implants, protected the animal from the lethal effects of the irradiation.[47, 48] Cole *et al.*[23] showed that intravenous infusions of spleen cell homogenates also conferred protection from lethal irradiation. This was thought to be due to a humoral factor by Jacobson, and for a time by others.[27] Barnes and Loutit showed that the protective effect of spleen cells could be achieved only with living cells and was not due to a humoral or chemical agent.[6-8] Lorenz *et al.*[55] showed bone marrow infusions would also protect against irradiation injury. Many other workers have confirmed the observation that animals could be protected from lethal TBR by infusions of isologous or homologous spleen or bone marrow.[9, 28, 66, 95] These animals proved to be tolerant of skin grafts from the marrow donor or other animals of the same strain.[57, 95] The tolerance to the grafted skin and hematopoietic tissue was lost if lymph node or spleen cells from an isologous nonirradiated mouse were injected into the animal bearing the graft.[97] The closeness of genetic relation of the irradiated host and the marrow donor was found to have a great effect on the per cent survival and duration survival.[6, 9, 46, 95] Nevertheless, it was possible to achieve survival of irradiated mice given rat bone marrow [24, 25, 36, 58, 59, 60] although in a much smaller percentage. Rat cells repopulate the bone marrow and lymph nodes of the mouse with appropriate cells, produce circulating rat cells[58] and rat gamma globulin.[106]

As work progressed in the treatment of irradiated mice, it became apparent that late deaths occurred in a high percentage of cases.[18, 24, 27, 56, 58, 60, 80, 95, 96] The transplantation of immunologically competent cells into an adult irradiated host, like transplantation in the fetus, may lead to secondary disease [7] with disastrous late results for the host. This never occurred with isologous marrow, but was seen when homologous marrow was used, particularly if the genetic disparity of host and donor was great, and was common with heterologous marrow.[25] Secondary disease is characterized by diarrhea, wasting, leukocytosis, bone marrow hyperplasia, lymph node involution, and plasma cell infiltration of the spleen. Although evidence has been presented to suggest that late homotransplant deaths and the changes of secondary disease are a consequence of continued host versus graft reaction,[24, 58, 59, 60] most investigators now agree that this represents a graft versus host phenomenon.[8, 18, 46, 80, 95, 96, 99] Evidence for this view is that lymphoid tissue of the host is repopulated by donor marrow cells,[4, 70] appropriate antibodies are present when immunized donor marrow is transplanted into irradiated rabbits,[42] serum gamma globulin of irradiated mice transplanted with rat bone marrow is of the rat type,[106] and irradiation chimeras who receive skin grafts from the marrow donor do not reject the graft when secondary disease appears.[95, 107]

To avoid the graft versus host reaction. Uphoff [100] and others [5, 28, 32, 39, 50, 53, 77, 102, 103] have used fetal liver and spleen to repopulate the host hematopoietic system instead of adult tissues, thus achieving an acquired tolerance of the graft for the host. In mice, fetal liver and spleen cells can be utilized up to full term [32] or even in the newborn state for 24 hours after birth,[103] but in the rabbit newborn cells [79] are apparently no better than adult cells,[80] though fetal cells are.[77] Fetal heterologous infusions are no better than adult heterologous cells.[102, 103]

Recent experiments using F1 hybrid offspring of two inbred lines of mice would seem to offer very strong evidence that the late deaths after homotransplantation in irradiated hosts occur as a consequence of graft versus host reaction, since they occur only in combinations in which the grafted tissue is capable of reacting against the host, while the host is incapable of reacting against the donor's tissues.[11, 97, 98, 99]

Nevertheless there is evidence that in

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secondary disease donor lymphoid cells are ultimately destroyed,[37] and that host versus graft reaction may sometimes play a role in the demise of the host.[75, 87]

Two other points may be worth mentioning at this time. First, that bone marrow homotransplants fail to provide grafts which completely replace the host's defences, and bone marrow transplanted animals are "immunological cripples" to a greater or lesser degree,[10, 60] despite their ability to form antibodies under some circumstances.[73] Although lymphoid repopulation of the host from the injected marrow occurs,[4] it is less certain than when lymphoid elements are injected along with the marrow. Fetal liver and spleen provide a source not only of erythroid and myeloid leukocytic cells, but also elements of the lymphoid series. Secondly, that infused cells "seek out" their proper anatomical location.[4, 66, 70] Furthermore, infused lymphoid cells seek out lymph nodes and repopulate them.[4, 70]

It thus appears that embryonic liver and spleen cells offer advantages over adult marrow as replacement for the hematopoietic system of the irradiated host: 1) The graft versus host reaction is obviated and 2) full immunological replacement is achieved.

It should be emphasized that while secondary disease in mice given homologous bone marrow is usually fatal, it is not necessarily so. Some mice don't develop secondary disease, and others develop it but manage to recover from it, and continue to carry the foreign cells.[60] Uphoff[101] has been able to modify it by the administration of A-methopterine.

Despite encouraging results of homologous or even heterologous bone marrow transplantation in mice, no such rewarding experiences have occurred with bone marrow transplants following TBR in the dog[78] or man. No long-term survivals are reported in either species, despite many attempts, save in one case where litter mate dogs were used,[93] (a special circumstance), or in identical twins, an isograft rather than a homograft. Thomas and his group now have one male beagle who received 1,800 r TBR and a bone marrow transplant from a female beagle who is in excellent health four months later.* He passed through a period at two months during which he was very sick, (? secondary disease) but managed to recover with the use of hyperimmune serum, but without the use of methotrexate. The other long term survivor, mentioned above, using a litter mate donor, received methotrexate during the period when he seemed to be getting secondary disease. Almost all of the dogs receiving adult bone marrow have encountered a period of late difficulty, once they survived the initial take, three or more weeks after the transplant. This illness proved fatal in all cases except these two—usually from intercurrent pneumonia.* This probably represents secondary disease, and was not seen in our series of embryonic liver and spleen cell transplants.

It has been suggested by Simonsen[84, 85] and Dempster[30, 31] that the lymphoid cells in renal homografts are of donor and not host origin, and that they represent a reaction of immunologically competent cells of the donor against the host.

A few experiments have been reported in which attempts were made to abbrogate renal homograft rejection by means of irradiation. Baker and Gordon[3] using 225 r found no significant prolongation of renal homograft survival. Murray and Holden[71] likewise reported no benefit from irradiation, but gave no data. Dempster[30] stated that he was able to abolish the lymphoid cell infiltration of the interstitium of the renal homograft by irradiating the homograft prior to transplantation, but not by irradiating the host. The irradiation did not alter the pattern of homograft rejection. These results were the basis for his con-

* Personal communication from Dr. E. D. Thomas.

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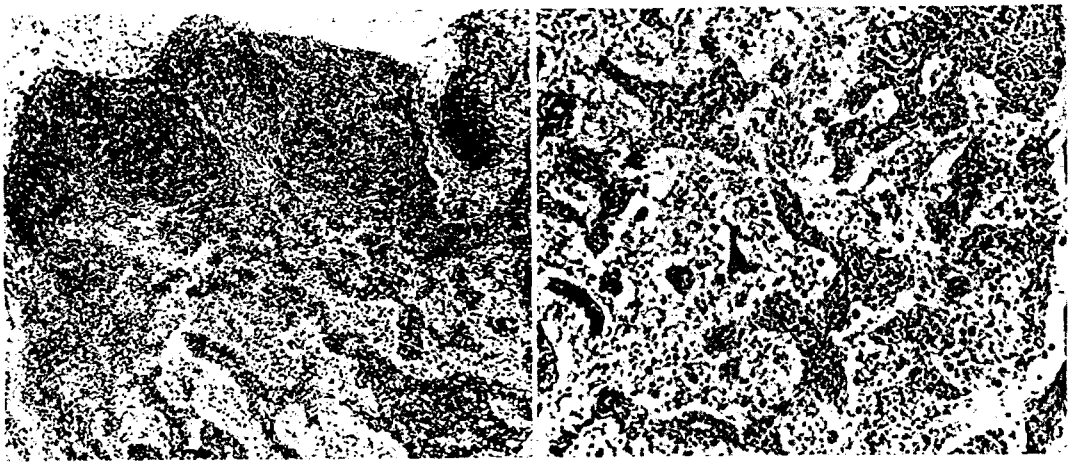

FIG. 12. (left) Dog KT-37. Lymph node taken from a host who had received 1,000 r TBR prior to transplantation. There is considerable destruction of the architecture of the lymph node but a few follicles remain in the outer portion of the node. The kidney transplant was rejected in 5 days (× 100). FIG. 13. (right) Dog KT-35. Microscopic view of a lymph node taken from a host who received 1,500 r TBR. The transplant functioned well for a period of 8 days, and appeared normal, microscopically and grossly. There is complete destruction of the follices of the lymph node.

tention that the proliferating lymphoid cells seen in the homograft were of graft, not host, origin. No dosage was mentioned and detailed results are lacking. Hahn *et al.*[38] tried to prevent renal homotransplant rejection by giving radioactive gold to host or donor without success.

Recently Mannick *et al.*[61] reported a prolonged survival of a kidney transplant in the dog. The animal, a male beagle, was given 1,300 r TBR, followed in eight days by an infusion of homologous bone marrow from an unrelated female beagle. Female leukocytes appeared in the peripheral blood. Sixteen days after the marrow graft, a kidney was transplanted from the marrow donor into the recipient. Ten days later the recipient's own kidneys were removed. The transplant functioned normally until the dog's death from pneumonia 49 days later, 73 days after irradiation. The kidney appeared normal histologically. The bone marrow showed marked hyperplasia and the lymph nodes showed aplasia—both features of secondary disease. Unlike the experiments of Dempster and Simonsen, this transplant showed no plasma cell infiltration, and the authors suggested three possible explanations for this: 1) The graft versus host reaction postulated by Simonsen and Dempster may be transient and may have subsided by the time the first biopsy was taken (10 days); 2) a kidney versus host reaction may not take place in the presence of a successful marrow take from the same donor; or 3) the plasma cells seen by Dempster and Simonsen may have been of host origin and the concept of a graft versus host reaction as applied by them may have been in error.

It was of interest in the present experiments that when a kidney transplant from an irradiated donor was placed into a nonirradiated host the lymph nodes of the host appeared normal at the time of kidney transplant rejection (Fig. 11). When the host had received 1,000 r TBR and the transplanted kidney came from a nonirradiated donor there was partial but not complete destruction of the lymph nodes of the host, and some active follicles were found in the host lymph nodes at the time of transplant rejection (Fig. 12). When the host had received 1,500 r TBR, and the transplant was obtained from a nonirradiated donor, complete destruction of the fol-

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licles of the lymph nodes of the host was found at post-mortem. The kidney transplant appeared normal. There was thus good correlation between the degree of lymph node follicle activity and the presence or absence of homotransplant rejection.

Answers to questions raised at the beginning of this paper may now be set down: 1) Infusions of homologous fetal liver and spleen cells do permit indefinite survival after lethal TBR in the dog. In only one instance in the four long-term survivors were cells from female fetuses transplanted into a male recipient. Female markers were present for eight months, but had disappeared by one year. It is, therefore, likely that the recipient's marrow has finally regenerated again. The dose of radiation used (600 r) was not enough to destroy the host homograft immune response completely, as judged by later experiments. No long term survival has yet been achieved using TBR doses of 1,000 r or greater, but these experiments have sometimes been carried out in conjunction with a kidney transplant which complicates the recovery, and relatively few experiments have been done. Secondary disease did not occur, the deaths being early radiation deaths rather than late "secondary" ones.

2) Irradiation of the host prior to renal transplantation at doses of 1,000 r failed to prevent the homograft reaction. Irradiation at 1,200 r prevented it in half the cases, and irradiation at 1,500 r prevented it in all cases. These experiments were conducted only for the length of time the irradiated animals survived (3 to 13 days). When both the host and donor were irradiated, no homograft reaction was seen at doses of 1,000 r or greater, but with doses of 600 r to both host and donor homograft rejection occurred. Although the number of dogs in each category are too few to draw definite conclusions, there is some evidence that irradiation of both host and donor may prevent the homograft reaction with a smaller total body dose to the host than if the host alone were irradiated.

3) Irradiation of the donor at doses up to 1,500 r TBR did not prolong homograft function nor prevent the usual picture of homograft rejection when the kidney was examined after cessation of function in five to ten days.

4) The lymphocytes and plasma cells seen in the interstitial spaces of the transplant are of host origin and probably represent a reaction of the host against the graft, and not of the graft against the host as claimed by Dempster [31] and by Simonsen.[84, 85] Infusions of fetal blood-forming cells in a few cases did not alter the general pattern of the experimental results.

Of further interest was the observation that in a single case, a secondary transplant from the original donor into an irradiated, previously immunized host showed no evidence of rejection after 24 hours in the host. Further experiments of this nature are under way.

Perhaps the seemingly smaller host dose of irradiation necessary when both host and donor are irradiated is related to a subtle donor versus host reaction or, as Simonsen suggested,[84, 85] a graft reaction to host antibody. This is unlikely in view of the results showing that 1,500 r TBR to the donor failed to alter the normal pattern of homograft rejection. Irradiation of the kidney prior to transplantation poses the problem of the development of radiation nephritis in the transplanted kidney. The subject has recently been reviewed by Redd [83] and the conclusion was reached that radiation nephritis can occur if 2,500 r is given over five weeks. Renal changes have been reported with doses as small as 600 r and we noted minor changes consisting of tubular dilatation and atrophy in some host kidneys receiving 1,500 r. It seems likely that the necessity for irradiating the donor kidney —which appeared to be essential from Dempster's work—can be avoided.

The demonstration that a TBR dose of

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600 r will permit survival of homologous fetal cells for at least eight months, with subsequent disappearance of these cells, might suggest that even sub-lethal doses of irradiation would permit prolonged homograft survival. In the case of renal transplants, this seems unlikely, because supralethal doses of 1,500 r were necessary for predictable prevention of the homograft response. There is no reason to suppose that a sublethal dose of irradiation which allows regeneration of the host hematopoietic system would exempt a resident homografted kidney from ultimate rejection. Although the presence of uremia contributes to prolonged renal homograft survival in the human [45] and in the dog,[62] ultimate rejection is the rule. There is some evidence to suggest that temporary interference with antibody production will prolong graft survival [54] and that ultimate graft "adaptation" to the host may occur,[105] but the evidence is sparse, and the circumstances unusual. It must be remembered that, in the human, renal homografts have been virtually free of lymphoid infiltration at 37 days and have persisted and functioned up to six months [45] without any irradiation. On the basis of presently available data it would seem that the use of a closely related donor is more apt to permit fortuitous long term survival than sublethal TBR.

The results reported here demonstrate that prolonged survival of fetal blood forming cells can occur in hosts receiving a dose of irradiation which is lethal to the unprotected animal, but still too small to permit successful renal homografting. This may explain the failure to produce tolerance with doses of irradiation that permit successful marrow takes.[73] There is evidence to suggest that a successful marrow take will permit successful skin grafts from the same donor even though the dose of irradiation is too small to permit successful skin grafts without a marrow take.[72, 104] Perhaps this is because the marrow graft adds to the destruction of the host's antibody producing cells by forming antibodies against them. In any event, this means of reducing irradiation dosage needed for successful renal transplantation would not be operative in fetal blood-forming-tissue supported irradiated animals, because the blood cell donor's kidney cannot be used, and graft versus host immunological changes would not be expected to occur. Combinations of antimarrow agents and irradiation may ultimately prove useful, but have so far been of limited value.[81]

Summary and Conclusions

Total Body Irradiation with and without Fetal Liver and Spleen Infusions

1. Twenty-eight dogs were given 600 r TBR from a 1,000 KVP x-ray or multiple cobalt-60 source. All dogs died within seven to 17 days.

2. Sixteen dogs were given 600 r TBR plus infusions of fetal liver and spleen cells. Four of this group are surviving 12, 16, 18 and 22 months later. In one of the cases in which a male host was given female embryonic blood-forming cells female leukocyte markers could be readily detected in the peripheral blood of the host up to 8 months, but had disappeared by 12 months.

3. No long-term survival was achieved with the use of fetal tissues in a few cases in which TBR doses of 1,000 r or greater were used.

Renal Homotransplantation Following Irradiation of Donor, Host or Both Donor and Host

1. Renal homotransplants were carried out in a series of 27 dogs. Irradiation of the donor in doses up to 1,500 r did not prevent the appearance of the usual homotransplant rejection phenomena in the usual time. Irradiation of the host at doses of 600 or 1,000 r likewise failed to prevent rejection. Irradiation of the host at doses of 1,200 r prevented rejection in half the cases, and

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irradiation at doses of 1,500 r prevented rejection in all cases.

2. Irradiation of both host and donor prevented rejection at doses of 1,000 r or greater in all cases, but not at doses of 600 r.

3. Long term survival of the renal homografts was not sought in most cases, the present study being directed at the appearance of functional and histological evidence of rejection three to 13 days after transplantation.

4. The greater the degree of destruction of the lymphoid follicles of the host lymph nodes by irradiation, the greater was the likelihood that the renal transplant would persist without rejection.

5. Evidence is presented to suggest that the lymphoid cellular infiltration of renal homografts is of host, not donor, origin.

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Discussion

Dr. J. Englebert Dunphy: I congratulate Dr. Hume on this important and beautiful study and wish to comment on just one phase of it.

In the approach to homotransplantation which he describes it is assumed that the host bone marrow is knocked out and then repopulated by the donor cells. It is also assumed that bone marrow is extremely sensitive to irradiation.

We have been irradiating autotransplants of bone marrow and find that 600 r has no demonstrable effect upon the regeneration of autologous marrow. In fact after 2,000 r autologous marrow will regenerate quite well.

We've been particularly interested in finding plasma cells present at levels of as high as 4,000 r. I would ask whether or not infusions of homologous marrow do not in some way block an indirect effect of total body irradiation rather than repopulate the bone marrow itself.

For example, if one shields the spleen the response to total body irradiation is quite different than without shielding the spleen. Could the infusion of homologous cells simply be acting in this same fashion rather than by producing new marrow? I think that if this is the case it introduces a new aspect to this problem.

Dr. Francis D. Moore: I, too, would like to thank Dr. Hume for his discussion. I believe that the work going on in his department in Richmond is some of the most exciting and beautifully planned work in this field.

As to radiation abating immunogenetic rejection, our experience in man tends to corroborate the suspicion that Dr. Dunphy raises since in one patient at 650 r at 28 days there was no histologic evidence of kidney rejection. The patient died essentially of the radiation injury. That brings me to the point I'd like to ask Dr. Hume—I know he didn't have time to discuss it initially—what about long-term survivors in his high-dose dogs? Has he had some good long-term survivors at dosages over 1,000 r?

Dr. Herbert Conway: This is a brilliant presentation of Dr. Hume's of some very accurately controlled work and it ties in excellently with the thoughts expressed in Dr. Cole's Presidential Address this morning.

I want to take advantage of this scientific presentation to bring to this audience a thought that there are not enough of immunologists in this country or in the world. This suggestion was made a few years ago by Eichwald at the Third Tissue Transplantation Conference in New York in the presentation entitled "The Might of Immunology."

Eichwald pointed out that the number of articles in the scientific literature on immunological subjects by immunologists has been diminishing.

Now those of us who are engaged in transplantation research know that we are up against the obstruction—the almost impenetrable obstruction—of the immunologic phenomenon. That it can be solved is suggested by the optimism of Dr. Cole's address this morning and by the fact that other gargantuan objectives have been met successfully or defeated.

The time has come for complete and accurate cooperation between surgeons engaged in research and immunologists. I am one of those who met obstruction from immunologists without decrying their efforts at all because always their laboratories are too small to accommodate a surgeon who may be research minded; they are not the aggres-

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sive type of research investigator—this is in general, not specifically—as are surgeons. I think that those members of this Association who serve on educational committees such as the National Research Council and the National Institutes of Health, should keep this in mind. Always a research grant is blocked by immunologists because there is no immunologist directing it.

Other facets of ancillary fields of medicine have been mastered by surgeons and I believe that a properly-minded research surgeon can master the technics of immunology sufficiently to meet the criteria for successful scientific investigation.

This work of Dr. Hume's is another step forward and he and his group in Virginia are certainly to be congratulated upon it.

Dr. David Hume (Closing): In answer to Dr. Dunphy's comments, which I enjoyed, as usual, I should like to say that this is a very complex problem and that it is difficult to go into all the ramifications of it in such a short time. There is a tremendous variation in response depending on the type of animal employed, the type of radiation used and so on.

I think, though, there is incontrovertible evidence that under certain circumstances one can give total body irradiation followed by an injection of foreign bone marrow cells which do, in fact, completely repopulate the bone marrow. This has been most clearly demonstrated in the case of mice given total body x-ray followed by rat bone marrow. The rat bone marrow cells go into the bone marrow of the mouse, produce rat gamma globulin, and give rise to circulating rat cells (which are different from mouse cells) for the entire future life of the animal. I think this is convincing evidence that you can destroy bone marrow with radiation and that it can, in fact, be repopulated by foreign cells. The biopsies of the bone marrow on our dogs show complete absence of cells unless they've been given bone marrow; I think this may account for the differences in your experiments and our experiments.

It is of interest, actually, that the cells that are injected from a donor tend to seek out their proper site. If one gives an animal bone marrow only, without spleen cells, he is an immunological cripple. The bone marrow cells gravitate to the host's bone marrow and multiply there, but there is a decreased production of the lymphoid series.

If you give him lymph cells too, these cells will seek out the host's lymph nodes and will grow and produce foreign lymphocytes.

In answer to Dr. Moore's questions: First of all as far as the two long-term survivals in the Cooperstown group are concerned I believe both these animals were litter mate animals, and littermate puppies are a different proposition from unrelated mongrel dogs.*

Certainly the genetic closeness of the donor and host has a lot to do with the ability of the host to accept the graft from the donor, and littermates are more prone to accept long-term grafts than nonrelated animals are.

In the long-term survival of the kidney transplant which Dr. Mannick reported from there this Fall the animal went 73 days after his x-ray and died of pneumonia. Upon his death he had a complete absence of the germinal centers in his lymph nodes. This strongly suggests two things: First, that he was an immunological cripple because he didn't receive any spleen cells; and secondly, that there may have been a reaction of the transplanted bone marrow against the host which destroyed the germinal centers in the lymph nodes. Secondly, as far as the human case getting 650 r and having a normal appearing kidney at 28 days is concerned, Dr. Moore, as you know, in some instances just by chance even in unrelated humans the kidney will function for a period as long as six months and I don't think that one can say after 28 days that the lack of histological evidence of rejection is related to the fact that the host received 640 to 650 r of irradiation.

Certainly in the experiments reported here, only those doses which destroyed the bone marrow and the lymph germinal centers were capable of producing a tolerance to a foreign graft, and this may also possibly be true in the human.

I'd like to thank the discussants for their comments.

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* I have since learned that at least one fairly long-term survival was a graft between two animals of the same stock but not litter-mates.

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