Kidney Transplantation in Modified Recipients *

JOSEPH E. MURRAY, M.D.,* JOHN P. MERRILL, M.D.,** GUSTAVE J. DAMMIN, M.D.,***
JAMES B. DEALY, JR., M.D.,† GUY W. ALEXANDRE, M.D.,††
J. HARTWELL HARRISON, M.D.,†††

From the Departments of Surgery, Medicine, Pathology and Radiology, The Peter Bent Brigham Hospital and Harvard Medical School

SINCE the first successful human kidney transplant, in 1954,[16, 18] at least 23 transplants from one to the other of monozygotic, or identical, twins have been performed, 16 by our group in Boston and others in Richmond (discussion of Ref. 19), Montreal, New Orleans, Portland, Oregon, Denver, Palo Alto[6] and Edinburgh, Scotland.[30] Where no immunogenetic barrier between donor and recipient exists, the successful transplants have furnished invaluable data regarding the etiology of renal disease, hypertension, cardiac failure and the function of the denervated kidney and ureter.[22] The rapid, complete and prolonged restoration of health, even to the extent of retaining normal pregnancy, by a successful transplant, is a powerful stimulus in the search for methods to overcome the immunogenetic barrier which separates all other human beings and prevents unlimited transplantation from one human to another.

During these past eight years, several successful methods of breaching the immunological barrier in lower animals have evolved. Genetic principles which govern tumor transplantation have been found to apply to normal tissue transplantation as well. In pure bred strains of mice, genetic disparity of only a single locus on a chromosome can constitute a barrier to transplantation. The modes of therapy used to overcome the genetic barrier include total body irradiation with and without subsequent bone marrow infusion, embryonic or neonatal inoculation with antigen, antigen overloading, and antigen pre-treatment. These methods produce *radiation tolerance, acquired immunological tolerance, immunoparalysis* and *immunologic enhancement,* respectively.

It has been a slow and difficult task to apply these principles to the larger experimental animals and to man, for several reasons: 1) Probably foremost, the genetics

Presented before the American Surgical Association, Washington, D. C., May 9–11, 1962.

Supported in part by grants from the John A. Hartford Foundation, Inc.; the Avalon Foundation; the Medical Research and Development Board; Office of the Surgeon General, United States Army; U. S. Public Health Services; National Heart Institute; National Institutes of Health; Atomic Energy Commission and Winthrop Laboratories and the Clinical Research Center supported by grant OG-6 of the N. I. H.

* Assistant clinical Professor of Surgery, Harvard Medical School; senior associate in surgery, Peter Bent Brigham Hospital.

** Assistant professor of medicine, Harvard Medical School; senior associate in medicine, Peter Bent Brigham Hospital.

*** Professor of Pathology, Harvard Medical School; Pathologist-in-Chief, Peter Bent Brigham Hospital.

† Assistant clinical professor of radiology, Harvard Medical School; Radiologist-in-Chief, Peter Bent Brigham Hospital.

†† Fellow of the Belgian-American Educational Foundation, Inc.; Research Fellow in Surgery, Harvard Medical School; assistant in surgery, Peter Bent Brigham Hospital.

††† Clinical professor of genitourinary surgery, Harvard Medical School; urologic surgeon and chief of service, Peter Bent Brigham Hospital.

TABLE 1. *Peter Bent Brigham Hospital–Harvard Medical School. Human Kidney Transplantation, Total Experience*

	Year	No. Patients	Source of Kidney: Cadaver	Source of Kidney: Living Hydrocephalic Infant	Source of Kidney: Relative	Treatment of Host: X-ray	Treatment of Host: Drug
Original patients	1951–1953	10	8	2	0	none	none
Unmodified hosts	1953–1955	6	4	2	0	none	none
Identical twins	1954–1962	17	0	0	17	none	none
Modified hosts	1958–1962	17*	3	6	6	11	6
including a non-identical twin	1959	1	0	0	1	1	none
Totals		51*	15	10	24	12	6

* 2 Patients died from x-ray treatment prior to kidney transplant.

of no other species have been so well analyzed as those of the mouse; 2) Distinct species variations independent of the genetic background may exist; and 3) the test systems vary: the usual mouse study uses a skin graft or a tumor graft, the usual rabbit antigen is a heterologous whole cell, a purified protein or a skin graft, whereas vascularized whole organ transplants are standard test grafts in the dog and man. These latter grafts constitute a large antigenic mass introduced directly into the circulation: the former are smaller masses, usually introduced topically or subcutaneously. Just as the antigenic response in an individual animal can be altered by the dosage, the route of administration or the timing of the antigen, so might the reaction of homografts be determined or altered by the same factors. The inability to apply the small animal data to large animals and to man may be due to any or all of the above variables.

Therefore, in order to test the various immunologic principles in the study of human kidney transplantation, it seemed prudent to concentrate on that laboratory test system which parallels the human situation as closely as possible, i.e., kidney transplantation in the dog. At the same time, carefully selected human transplants were continued because experience in the handling of these seriously ill patients was yielding pertinent information regarding the natural history of a human transplant, the effect of uremia as a suppressive factor in transplantation immunity,[7, 11] the state of wound healing and the establishment of an aseptic surgical environment.[20]

Concurrent supporting laboratory study in rabbits and mice was also continued. In these smaller animals, skin grafts, purified protein antigens and cellular antigens from isologous, homologous and heterologous sources were tested following total body irradiation with and without bone marrow protection.[26, 27] These small animal studies serve as a guide or screening program for the more time consuming and expensive large animal experiments.

Because of the disappointing results from the use of total body irradiation in man[15] and larger animals[12] in relation to renal homotransplantation, the laboratory and clinical emphasis has shifted over the past two years to the chemical suppression of the immune response. This report is a summary of our experience of the past four years with kidney homotransplants in both man and the dog in recipients modified by total body irradiation or drug therapy.

Human Experience

Seventeen patients have been considered for renal homotransplantation since 1958 *

* The term *homotransplantation* indicates a graft between individuals of the same species of diverse genetic nature. It applies to all transplants between humans except those between identical twins, which are termed *isogenic grafts,* the term applied also to grafts within pure-bred strains of mice.

(Table 1). Twelve have been conditioned by total body irradiation (Table 2) and six by the purine analogue-6-mercaptopurine or its imidazol derivative, Imuran, formerly termed BW-57-322 (Table 3).

Two of the 12 patients treated with total body irradiation had absence of renal tissue following injury or removal of a solitary kidney. The other ten were in a hopeless preterminal state from renal disease and

TABLE 2. *Twelve Recipients Modified by Total Body Irradiation*

Patient Age Sex	Date	Diagnosis	Kidney Source Drug—Dose	Blood Groups Donor—Recipient	Outcome
G. L. 31 F	Mar. 1958	Absent kidneys (rupture solitary kidney)	2.0 mev, 600 r. Pooled marrow from siblings, unrelated adults and kidney donor. Kidney donor was unrelated infant	A pos. AB pos.	Lived 32 days after x-ray, 28 days after transplant. Renal function was present. Death was hematologic and infectious
N. W. 12 M	Jul. 1958	Absent kidneys (rupture solitary kidney)	2.0 mev, 700 r. Bone marrow from mother. Fetal spleen and liver cells. Skin graft from mother	Kidney transplant not done	Lived 25 days after x-ray. Skin graft intact. Death was hematologic and infectious
J. R. 24 M	Jan. 1959	Chronic glomerulonephritis	250 kv, 250 r and 200 r at 7 day interval. No marrow. Di-ovular twin kidney donor	Shared 26 blood group antigens	Living and well, at 3½ yrs. Donor skin graft rejected at 7 month. Abortive kidney rejection at 10 mos. was successfully treated with x-ray 50 r every 7 days × 4. plus cortisone. Has married
R. D. 56 M	Jun. 1959	Subacute and acute glomerulonephritis	250 kv, 250 r and 200 r at 7 day interval. No marrow. Kidney donor unrelated infant	O pos. B pos.	Lived 19 days after x-ray, 11 days after transplant. No urine output. Died bacterial and uremic death
M. C. 15 F	Aug. 1959	Subacute and chronic glomerulonephritis	250 kv, 250 r and 150 r at 7 day interval. No marrow. Kidney donor father	Shared more than 20 blood group antigens	Lived 22 days after x-ray, 14 days after transplant. Good volume output. Lowering BUN. Died bacterial death of ? "humoral" rejection
G. C. 18 F	Sep. 1959	Chronic glomerulonephritis	250 kv, 250 r. Single dose. No marrow	Kidney transplant not done	Died 18 days after x-ray of uremia and infection. Prospective donor developed urinary infection
A. Q. 22 F	Apr. 1960	Chronic glomerulonephritis	250 kv, 120 r, 120 r, 100 r over 8 days. Kidney from mother	Shared more than 20 blood group antigens	Perfect function for 3 days. Kidney had "humoral" rejection. Pt. died in 14 days
T. H. 21 M	Mar. 1960	Hereditary familial nephritis	250 kv, 200 r single dose. Donor was unrelated infant	O pos. O pos.	Survived 18 days. No kidney function
R. R. 15 M	Jun. 1960	Chronic glomerulonephritis	250 kv, 250 r and 200 r at 7 day interval. Kidney from father	Shared more than 20 blood group antigens	4 anastomoses required. Survived 10 days. Infarcted kidney at autopsy. No function
R. L. 21 M	Nov. 1960	Chronic glomerulonephritis	250 kv, 250 r and 200 r at 7 day interval. Kidney was from brother	Shared more than 20 blood group antigens	Perfect function for 4 days. Then "humoral" rejection. Died in 14 days
C. W. 41 F	Dec. 1960	Polycystic disease	250 kv, 150 r and 100 r at 2 day interval. Kidney was from brother	Shared more than 20 blood group antigens	"Humoral" shutdown occurred after 1 hour. Renal cortical necrosis. Died in 18 days
P. M. 23 M	Mar. 1962	Chronic glomerulonephritis	250 kv, 210 r and 190 r on successive days. Kidney was from brother	Shared more than 20 blood group antigens	Perfect function for 5 days. Died in 13 days after "humoral" rejection

TABLE 3. *Six Recipients Modified by Drug Treatment*

Patient Age Sex	Date	Diagnosis	Kidney Source Drug—Dose	Blood Groups Donor—Recipient	Outcome
L. S. 21 M	Apr. 1960	Familial hereditary nephritis	Cadaveric kidney; 6 MP; 3–4 mg./kg.	O pos. O pos.	Good renal function for 26 days. Died of cardiac death with falling BUN. Only minimal rejection in transplant.
R. G. 52 M	Nov. 1960	Nephrosclerosis	Infant "hydrocephalic" kidney; 6 MP; 4–5 mg./kg.	A neg. A pos.	Good function for 7 days. Early rejection reversed with increased drug Died 14 days of drug toxicity with increasing urine output
D. T. 22 M	Mar. 1961	Acute glomerulonephritis	Infant "hydrocephalic" kidney; Imuran; 10–12 mg./kg.	O pos. O pos.	Drug toxicity forced discontinuation from 21st to 26th day. Kidney rejected at day 28. Died day 38
W. F. 38 M	Dec. 1961	Chronic glomerulonephritis	Infant "hydrocephalic" kidney; Imuran 12 mg./kg. Actinomycin C 10 γ/kg.	A pos. A neg.	Excellent function for 3 days which ceased with onset of convulsions. Died at 13 days
E. K. 20 M	Jan. 1962	Agenesis of kidney and chronic glomerulonephritis	Cadaveric kidney: Imuran; 6 mg./kg.; Actinomycin C: 5 γ/kg.	B pos. O pos.	No function. Died at 14 days
M. D. 23 M	Apr. 1962	Chronic glomerulonephritis	Cadaveric kidney: Imuran; 2–4 mg./kg.—Actinomycin 5 γ/kg., weekly	O neg. O pos.	Function began on 5th day. Still functioning on 120th day with 3–4 L of urine daily. Marked clinical and chemical improvement but with depressed hematopoesis

were sustained through the irradiation and transplantation by frequent hemodialyses. Eight had acute or chronic glomerular nephritis, one had polycystic disease and the last had hereditary familial nephritis. All had a life expectancy measured in days and were considered as candidates for transplantation only after standard therapeutic measures had been exhausted. The first two patients were exposed to 600 r and 700 r, respectively, of total body irradiation from a 2.0 million volt source,** followed by bone marrow infusion. The subsequent ten patients were treated by a variety of doses of sub-lethal, non-marrow requiring total body irradiation from a 250 kv constant potential source. A dose of total body irradiation of 200 r to 450 r, measured to the mid-plane of the body, was given in either one, two or three treatments at intervals of no greater than eight days (Table 2). All patients were kept in a surgically aseptic environment as described in a previous communication.[20] The donor kidney was procured from a living hydrocephalic infant undergoing subarachnoid-ureteral shunt, i.e., "Matson" kidneys, in three instances and from a living relative in seven. The kidney transplant was performed on the day of or the day following the last x-ray treatment.

Prospective donors were selected from those sharing the greatest number of blood group antigens,*** even though there is no genetic evidence that the antigens responsible for hemagglutination are related to transplantation antigens. All living donors were carefully screened according to criteria previously reported.[19] Most recipients had received multiple blood transfusions in the course of their medical treatment except the last patient in the x-ray series, P. M., who deliberately was not transfused for 40 days prior to transplantation. He also received reserpine to

** Irradiation performed by Dr. Joseph H. Marks, New England Deaconess Hospital.

*** All blood groupings were performed at the Children's Medical Center, courtesy Dr. Fred Allen, Jr.

deplete his endogenous serotonin production and storage. Cortisone 200 to 400 mg. daily for five days was also given. Both he and his brother shared at least some transplantation antigens because a *second-set* accelerated rejection of a skin graft from the kidney donor was obtained on an indifferent host who had been sensitized by a prior skin graft from the recipient.*

Six patients have been selected for modification by the purine analogue 6 MP or Imuran (Table 3). The drug is started on the day of transplantation, usually with a high *loading dose* for the first three days, dropping back to a sustaining dose of approximately half the original level. The addition of Actinomycin C in two patients was prompted by experimental evidence of longer and healthier survival when the drugs were used synergistically.[3]

* Skin grafting performed by Dr. Richard E. Wilson, Junior Associate in Surgery, Peter Bent Brigham Hospital.

Results in the Human

Of the 12 recipients conditioned with total body irradiation, only one has survived. The first six patients have already been documented[20] but a brief summary will be included at this time to help evaluate the total series.

The first patient died of pulmonary sepsis and hemorrhage, secondary to the total body irradiation, without evidence of rejection of the kidney homograft. The second succumbed to sepsis and hemorrhage prior to the proposed renal transplant. In neither of these two patients was there

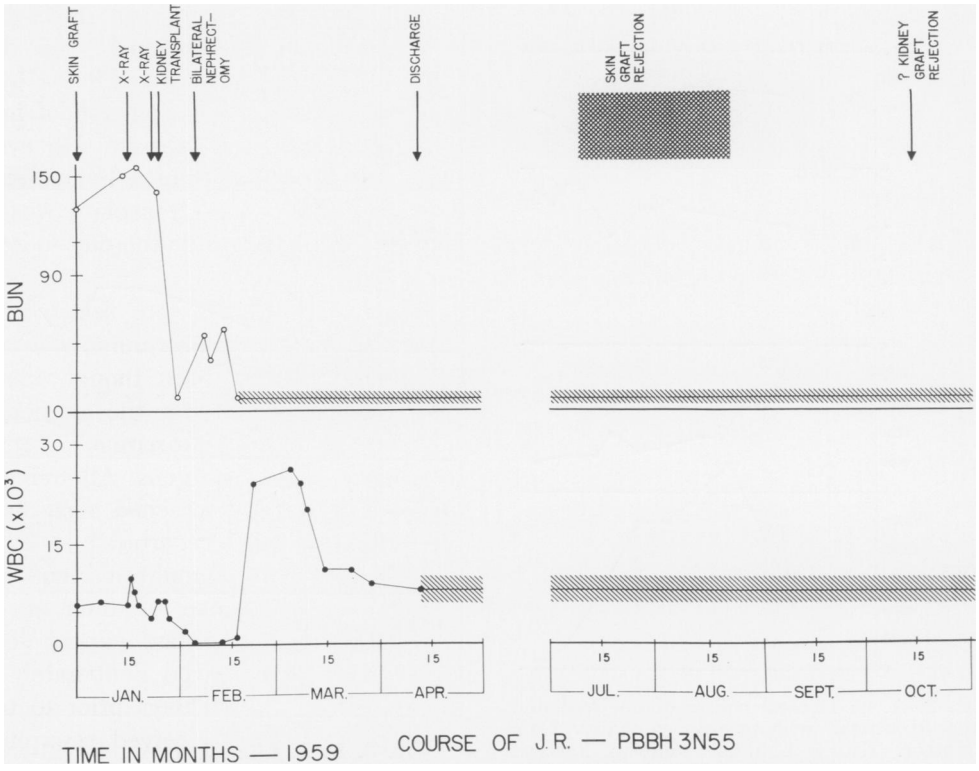

FIG. 1. J. R. Clinical course of irradiated recipient who received kidney from his nonidentical twin brother. Septic postoperative course was complicated by leukopenia and treated surgically by bilateral nephrectomy. Incipient kidney rejection at 10 months treated successfully. Alive and well 3½ years later. (Published courtesy *Surgery* **48**:277, 1960.)

evidence of a successful bone marrow survival. The third patient (Fig. 1), a dizygotic twin who received a kidney from his twin brother, is living and well after three-and-one-half years. A dose of 250 r and 200 r at a seven-day interval without subsequent bone marrow was used. The transplant functioned immediately and a bilateral nephrectomy was performed 11 days later. At six months, the previously well tolerated skin graft from his brother was rejected. Because protein and red cells appeared in the urinary sediment at ten months, a biopsy was performed which showed a beginning rejection process, with periglomerular and perivascular monocellular infiltrate and interstitial edema. He was treated with 60 mg. of Prednisone daily and 50 r of whole body irradiation on four occasions at weekly intervals. His urinary sediment cleared, his creatinine clearance values were maintained at 100 ml./min. and the PSP excretion at 25 per cent in 15 minutes and over 60 per cent in two hours. Since then, he has been maintained on a low sustaining dose of cortisone every other day, his renal function has remained

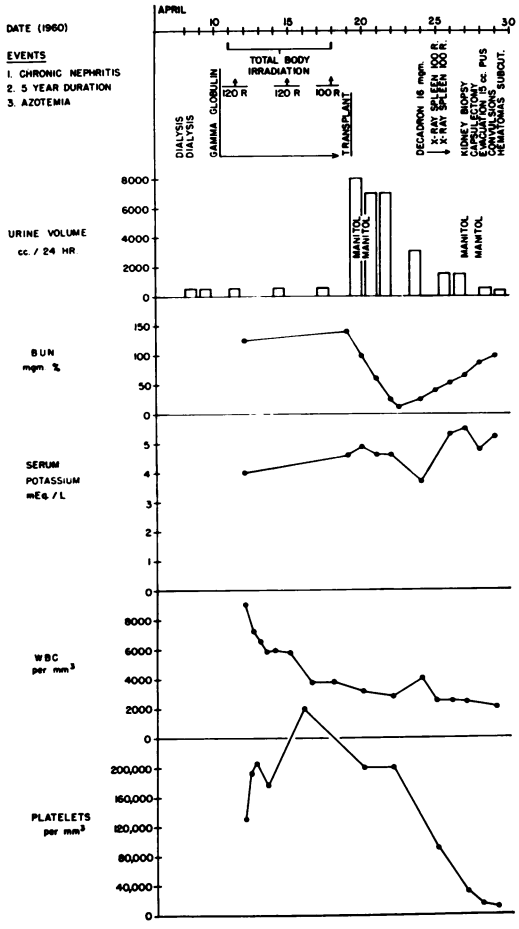

FIG. 2. A. Q. Clinical course of an irradiated 22-year-old woman who received a kidney from her mother. Excellent initial function lowered BUN and improved her clinical condition remarkably. On 6th day, oliguria with proteinuria and red cell casts together with swelling of the transplant indicated a "humoral" rejection which did not respond to vigorous therapy.

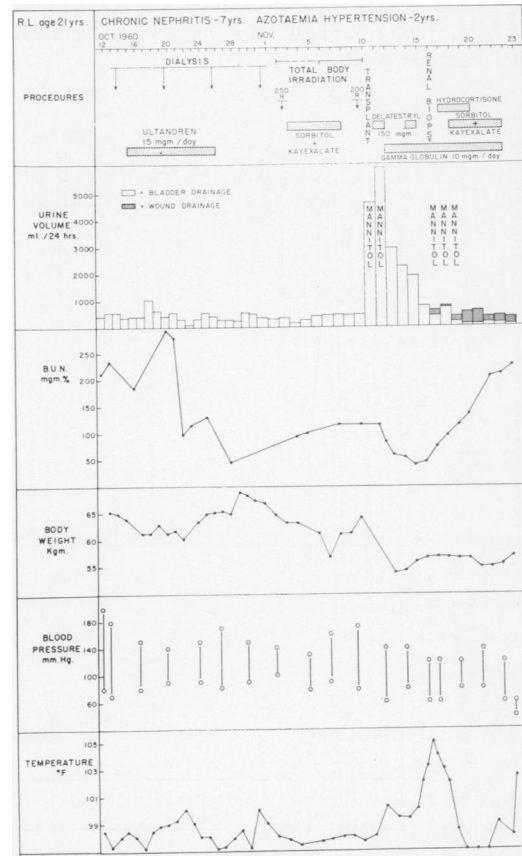

FIG. 3. R. L. Clinical course of a 21-year-old irradiated man who received a kidney from his brother. Similar course to Patient A. Q. is noted with rapid diuresis, fall in BUN and rapid clinical improvement. After 5 days, an inexorable down hill course ensued despite vigorous therapy.

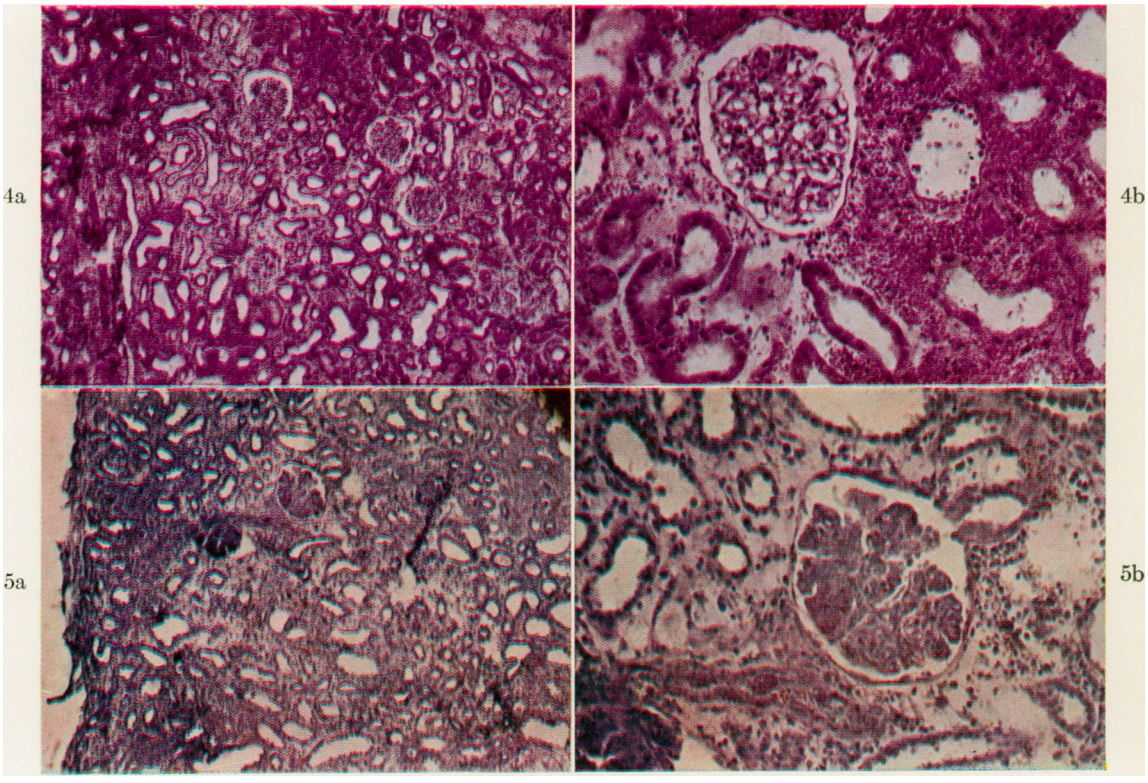

FIG. 4. a) Low power of a biopsy on Patient R. L. (Fig. 3), on the 5th postoperative day. Massive interstitial hemorrhage and marked tubular dilatation with no evidence of mononuclear cell infiltrate are noted. Picture might be confused with ureteral or venous obstruction although no thrombi are noted in vessels. b) Higher power of Figure 4a, with massive hemorrhage between tubules.

FIG. 5. a) Low power photomicrograph of Patient P. M., who received his brother's kidney after irradiation. The 6-day biopsy demonstrates clearly the earliest microscopic indication of the "humoral" rejection with patchy interstitial hemorrhage. b) Higher power of 5a, demonstrating the minute arteriolar thrombi visible in the afferent artery and within one tuft of the glomerulus.

normal, he has completed postgraduate school and was married one year ago.

One patient, G. C., was not transplanted because her mother, the prospective donor, developed bacilluria before the second irradiation treatment. While the mother received therapy, the patient died of uremia and infection with a severe marrow depletion, despite vigorous medical treatment.

Four of the remaining eight patients had perfect function of the transplanted kidney for periods of from four to 14 days only to have function stop very acutely (Fig. 2, 3). Gross swelling and tenderness over the kidney, increasing azotemia along with oliguria and anuria developed within one to three days. Gross and microscopic evidence of intrarenal hemorrhage and swelling was apparent on biopsy and postmortem examination in all (Fig. 4a, b).

There was no evidence of impairment of the vascular anastomoses or of the ureteric outflow. Treatment of this cessation of function by renal decapsulation, high dose of corticoid therapy and by serotonin antagonists was futile.

Patient C. W., transplanted with her brother's kidney, had such a rapid reaction that within one hour of the completion of the vascular anastomoses, the pink, well-

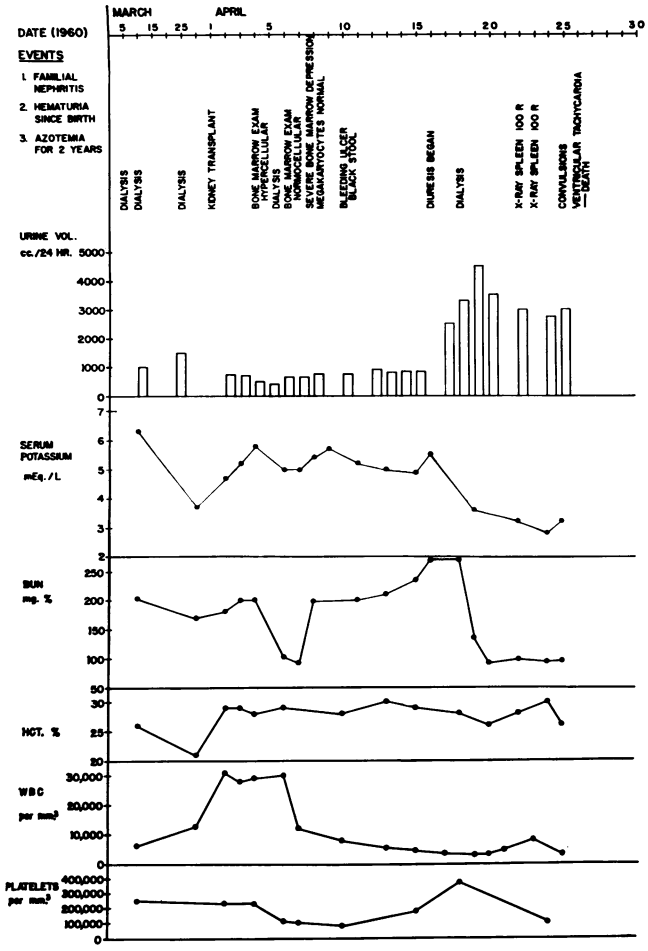

FIG. 6. Clinical course of patient L. S., who received a cadaveric kidney and was conditioned with 6 MP. Note falling BUN and the rising urine output during final week. Death was secondary to a ventricular irregularity possibly related to hypokalemia and/or drug therapy.

oxygenated transplant became cyanotic and dusky. In addition to the other therapy, intravenous mannitol and topical hypothermia were used without success. Microscopy and postmortem examination in this instance revealed complete cortical necrosis with an intact vascular supply to the renal medulla, a picture interpreted as secondary to an intrarenal shunt mechanism.

Of the three remaining failures, one, R. D., was secondary to a major blood group incompatability; another, R. R., had four anastomoses successfully completed within such a confined area that contact and external pressure from one artery to another caused secondary thrombosis. The remaining patient, T. H., had either rejected or thrombosed, the final microscopic pattern being clouded by a total infarct which had wiped out all identifiable cellular structure. Serial biopsies in this instance would have been valuable but were not performed for clinical reasons.

One patient, P. M., will be described in more detail because he is the most thoroughly documented concerning the mechanism of this rapid *humoral*, i.e., noncellular, cessation of function. He was a 23-year-old man suffering from chronic glomerular nephritis.

After 210 and 190 r, respectively, of total body irradiation on successive days, a kidney was transplanted from his brother, who

shared more than 20 blood group antigens. As mentioned earlier, this patient had not been transfused for 40 days, had evidence of antigenic compatability by skin grafting test and had been on serotonin antagonists and high doses of cortisone. Function of the transplanted kidney was immediate, lowering his blood urea nitrogen level from 225 mg.% to normal within five days. With a daily output of over 2,500 ml. of urine, on the fifth day a few red cells were noted in the urinary sediment. The cortisone therapy was immediately increased to 400 mg. daily. The process continued inexorably with the appearance of red cell casts, proteinuria and azotemia within the next four days. Biopsies (Fig. 5a, b) demonstrated at first a normal looking kidney with only a few interstitial red cells and thrombosis of an occasional afferent arteriole. This progressed steadily to extensive tubular destruction and massive intrarenal hemorrhage within a few days. This well documented course eliminates any possibility that impaired vascular or ureteral anastomoses contributed to the process.

Although the results in patients modified by purine analogues have produced no permanent success as yet, progress is encouraging. Five of the six patients had measurable and significant function of the transplant for periods of from three to 35 days. Three patients died of drug toxicity at 24, 14 and 38 days, with minimal to moderate microscopic evidence for rejection. The fourth patient had excellent function for three days but uncontrollable hypertension associated with convulsions occurred. At the same time, the kidney stopped functioning. The fifth transplant never functioned, possibly due to a major blood grouping incompatability. The sixth patient is still alive and well at 120 days. All kidneys were dispensable; from cadavers in three instances and from living hydrocephalic infants in three.

The first patient, L. S. (Fig. 6), died with a functioning transplant, a falling

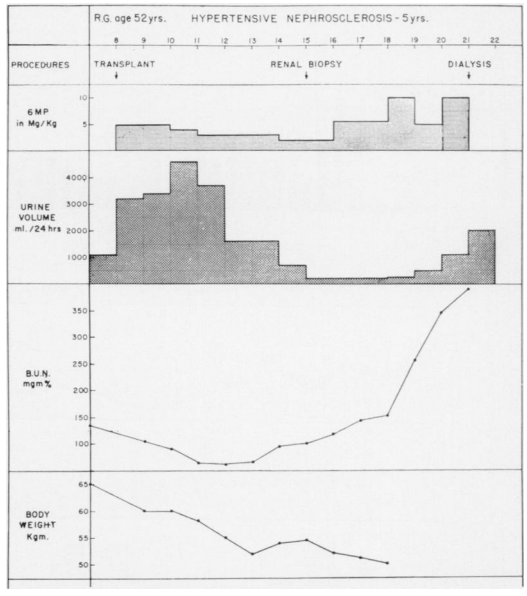

FIG. 7. Clinical course of Patient R. G., who received an infant kidney and 6 MP. Initial good urine output and falling BUN was diminishing on 7th day. The cellular infiltrate seen on biopsy of Day 7 disappeared on Day 14 coincidental with increased dose of 6 MP and rising urine output.

BUN, and improving clinical state. The drug dosage varied from 1.5 to 3.0 mg./kg. of 6-mercaptopurine. No significant depression of hematopoiesis was evident. With an improving appetite, and good urine output, he died rather suddenly of a cardiac irregularity of undetermined cause, presumably related to hypokalemia and the effect of the drug. At postmortem, the transplant had a moderate cellular reaction characteristic of a mild degree of immunologic rejection but there were many uninvolved areas capable of satisfactory function.

The second patient, R. G., previously reported as an example of the reversibility of the transplant reaction [21] was maintained at a slightly higher level of 4.0 to 5.0 mg./kg. of 6 MP for two days, dropping back to 3.0 mg./kg. from day four to day seven. Because of a falling urine output and biopsy evidence of cellular rejection, the dose was increased on day eight (Fig. 7) to 4.0

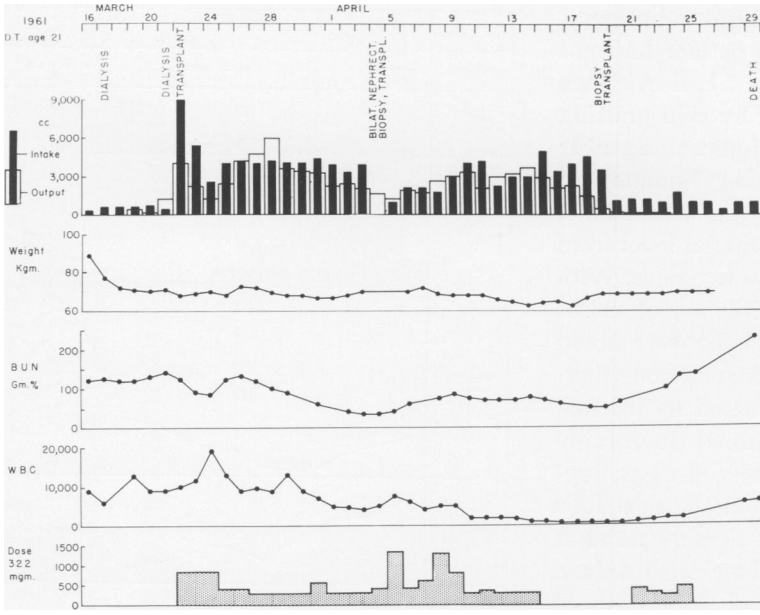

FIG. 8. Chart of Patient D. T., who received an infant kidney and Imuran drug therapy. Prolonged urine output averaged 2,500 ml. daily. BUN fall was gradual. Leukopenia necessitated cessation of drug on 23rd day. A few days later BUN started to rise and urine output fell. Microscopic rejection appeared on 28tth day.

mg./kg. On one day, 7.0 mg./kg. were given. There was a striking increase in urine output but he died at 14 days of sepsis and hemorrhage. Postmortem microscopic study revealed striking diminution in the cellular infiltrate. This was interpreted as a reversal of the rejection process by means of the increased drug therapy.

The third patient, D. T. (Fig. 8), was quite encouraging. The kidney from a six-year-old hydrocephalic girl in a six-foot four-inch 190-pound man had an output averaging 3.0 liters per day for 28 days. The blood urea nitrogen level fell to 39 mg.% and remained in this range for 23 days. The initial Imuran dosage of 8.0 to 11 mg./kg. was lowered to 6.0 mg./kg. on the seventeenth day because of leukopenia. A falling total white blood cell count below 1,000 forced discontinuation of the drug for six days. During this time, the blood urea nitrogen level rose rapidly and the urine output fell and the kidney was rejected. He died at 38 days, the final ten days with anuria. A biopsy at the time the drug was discontinued revealed only minimal cellular infiltrate whereas six days later, hemorrhagic infarction with loss of significant cellular detail was present.

The fourth patient, W. F., was conditioned with Imuran, 12 mg./kg. plus Actinomycin C 10 gamma/kg., the latter drug used for the first three days only. The infant kidney functioned well for two days but stopped abruptly with the onset of convulsions and uncontrollable hypertension as mentioned above. A biopsy on day three revealed viable tubules with interstitial edema and minimal perivascular infiltrate. The Imuran therapy was continued and 6 mg./kg. was given for six days and stopped when a severe leukopenia and low platelet count appeared. He died of sepsis and hemorrhage secondary to the drug therapy at 13 days.

The fifth patient, E. K., whose transplant never functioned because of a major blood group incompatability was given 6 mg./kg. of Imuran and 6 gamma/kg. of Actinomycin C. Even at this low dosage which is half the effective drug dosage in the dog, severe depression of the blood forming elements occurred and he died with infection, bleeding and azotemia in 14 days.

The current patient living at 120 days with a cadaveric kidney is being maintained at 2.0 to 4.0 mg./kg. of Imuran daily keeping the white blood cell count levels above 2,000. His urine output has continued to average 3.0 or 4.0 liters a day, the BUN has fallen from over 200 mg.% to a plateau of 50 mg.%, his appetite is voracious and he is regaining muscle mass. His drug dose is being adjusted at a low maintenance dose to minimize toxic effects on the hematopoetic system. Additional Actinomycin C 5 γ/kg. is given every week.

Laboratory Experience

Over 300 kidney transplants in bilaterally nephrectomized dogs have been performed in the past two years. These comprise 14 separate protocols, some of which have been previously reported.[3, 4] The earlier groups include animals conditioned with 6-mercaptopurine, dinitrophenolthiopurine, Imuran, Imuran in transplants between littermates, Imuran in combination with nitrogen mustard, Imuran in combination with Actinomycin C or Actinomycin D and Imuran in conjunction with excessive antigenic stimulus from the kidney donor. Later groups include the use of Actinomycin C alone, Imuran plus the colloidal blocking agent trypan blue, Imuran plus methotrexate and Imuran plus azaserine. In a final series using Imuran plus Actinomycin C, the donors were kept alive for subsequent skin grafting procedures.

All animals were kept in ordinary animal facilities without special aseptic precautions and are fed regular kennel rations with extra protein as needed. They are followed with daily temperature, urine specific gravity and protein determinations, tri-weekly blood urea nitrogen levels, platelet counts, hematocrit, white cell and differential counts. In most combinations, the basic pattern has been a loading dose of intravenous 6-mercaptopurine on the day of transplantation followed by oral high dose of Imuran in the range of 10 to 12 mg./kg. for the first three days, reducing this to 6.0 mg./kg. daily thereafter. Prolonged sustaining doses vary with individual animals but range between 3.0 to 6.0 mg./kg. daily. When combination drugs are used, the dose of Imuran is reduced and the additive drugs given daily or the dose of Imuran is maintained and the extra drug given once or twice a week. Selection of a drug dosage is a balance between the hematopoetic activity as judged by the total white blood cell count and the renal function as measured by blood urea nitrogen levels. Animals with a rising blood urea nitrogen indicating a beginning rejection are treated with an increased dose of the thiopurine or Actinomycin C or both in order to treat the incipient rejection. All animals dying have microscopic study of the transplant, lymph nodes, spleen, liver, lungs, bone marrow and in some, the adrenals and pancreas.

Skin grafts from indifferent donors or from the kidney donors have been performed on survivors over 60 days to test for the immunologic function of the long-term survivors.

Results in the Laboratory

The usual rejection pattern of the canine kidney is a rapid violent cellular rejection within six to 10 days. Prolongation of a renal homotransplant beyond 20 days can reasonably be considered as demonstrating a beneficial effect of therapy. In the earlier groups[4] 51 of 104 renal transplants in animals with various regimens of treatment survived beyond 20 days, and nine survived more than three months. Two of this group are alive and well over nine months. They are being maintained on a low daily sustaining dose of approximately 4.0 mg./kg. of Imuran and 6.0 γ/kg. of Actinomycin C once a week. These animals have had normal clearance studies and have had no chemical or clinical abnormality which

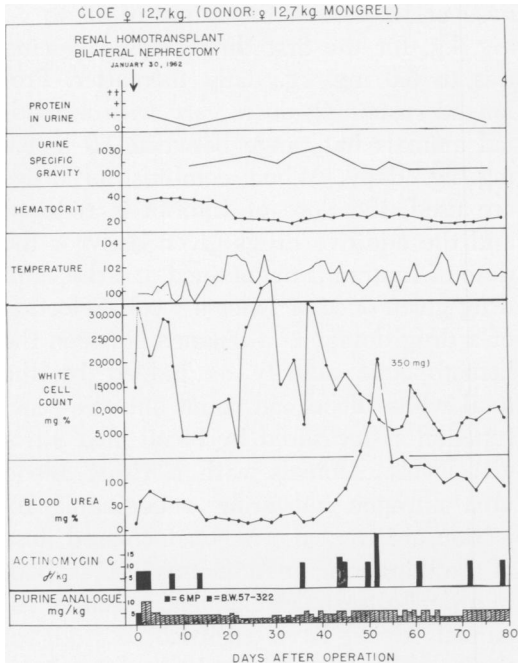

FIG. 9. Dog with bilateral nephrectomy and a kidney transplant whose rejection started on 45th day and was reversed by increased dose of Actinomycin C. Animal's clinical condition improved remarkably with weight gain and return of appetite at this time. Moderately elevated BUN level still present probably reflects residual damage. This animal is still alive over 150 days.

can be detected. In the current group of 16 experimental animals treated with Imuran and Actinomycin C, in whom the donors have been kept alive, 10 lived longer than 20 days and three survived beyond 90 days. A group of 10 dogs treated with Actinomycin alone had no survivors beyond 20 days, most dying of rejection. The combination of azaserine and Imuran has been used in nine dogs. Eight are alive after 20 days, four are alive after 60 days; they are being maintained on a daily dose of azaserine 1.0 to 2.0 mg./kg. and Imuran 4.0 mg./kg. The combination of Imuran and methotrexate 10 to 20 kg. three times weekly is not better than that of Imuran alone, whereas the combination of Imuran and trypan blue was highly toxic with no survivors living beyond 20 days.

One animal demonstrated a striking reversal of the rejection process on the 50th day by the use of extra large doses of Actinomycin C, 10 to 20 γ/kg. (Fig. 9). For approximately 40 days following the transplantation, the dog appeared normal with normal blood urea nitrogen levels, a high specific gravity of the urine and with little or no proteinuria. From the fortieth to the fiftieth day, the blood urea nitrogen rose rapidly to the level of 350 mg.%. The dog became listless, weak and lost his appetite and appeared moribund. A single dose of Actinomycin C 10 γ/kg. was followed by a double dose on the fifty-first day. The dog's condition seemed to improve and with careful feeding and gentle exercising, he regained his appetite and has begun to regain his lost weight. At present, the blood urea nitrogen level has plateaued at 50 to 60 mg.% and his urine specific gravity remains low.

To test the immune capacity of the surviving animals, skin homografts from indifferent, i.e., nonkidney donors, have been tested in four animals. All grafts became well vascularized within four days and behaved initially as autografts. All subsequently were rejected with scaling, erythema and ulceration within 20 days. Three of the animals rejected acutely on the fourteenth to sixteenth day, the other reacted more chronically over a period of six days and rejection was completed by the twenty-first day. One animal grafted with skin from the kidney donor at 60 days rejected 18 days later (Fig. 10). During the skin rejection period, a rise in the BUN occurred which fell to normal after 14 days. Another animal (Fig. 11), was also skin grafted from the kidney donor on the 66th day. Six days later, the BUN was markedly elevated, the urine specific gravity fell, indicating incipient rejection. Massive Actinomycin C was administered to the point of toxicity. The animal died with an intact skin graft 15 days after the

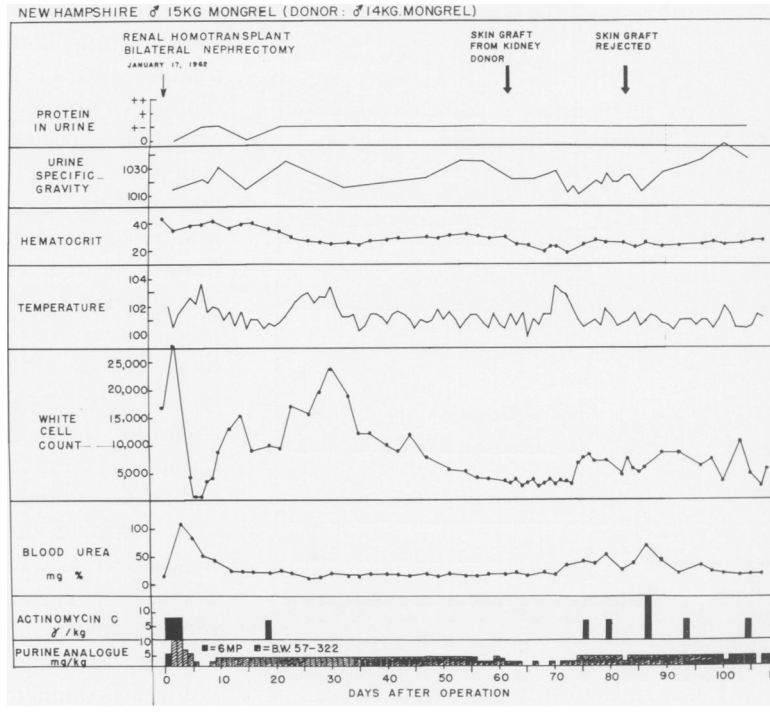

FIG. 10. Animal still alive over 190 days following bilateral nephrectomy and a renal transplant. Imuran and Actinomycin C are still being used as charted. A skin graft placed on Day 60 from kidney donor was rejected 16 days later. Transient elevation in BUN following this rejection probably reflects activation of host's reticulo-endothelial system against skin graft. Animal's BUN returned to normal and he is still alive and well.

skin graft was placed. At postmortem, massive pulmonary infection was present and the kidney appeared normal in gross appearance.

It must be stressed that these surviving animals are not sick, crippled or debilitated. They eat well, they maintain their weight and can resist kennel infection. One animal withstood and healed a saucerization of the mandible required to treat an osteomyelitis arising from an infected tooth.

Discontinuation of the drug in our experience has led to rejection of the transplant even as late as eight months.[4] The success of the transplant is not predicated upon depression of the formed circulating elements because rejection has occurred in many animals with leukopenia and lymphopenia as well as in those with leukocytosis. The variability of the response of these animals of every series could not be related to any measured or observed parameters.

Discussion

Human Experience. In our present state of knowledge, the function of human kidney transplants in recipients modified by total body irradiation is difficult to analyze with any degree of accuracy. It is apparent that there is a contrast between the human and laboratory data regarding the effectiveness of irradiation as a modifying agent for subsequent renal homografts. Thus far the only successful dog kidney graft following irradiation has been reported by Mannick *et al.*[13] who employed bone marrow replacement and a kidney graft from the marrow donor which functioned for 49 days. However, both the Cooperstown group and Hume[12] have tried with great zeal to apply the irradiation-marrow-kidney protocol to large groups of dogs over many years without any further success.

Sub-lethal non-marrow requiring doses of x-ray have never been effective in the laboratory. Yet in the human, between

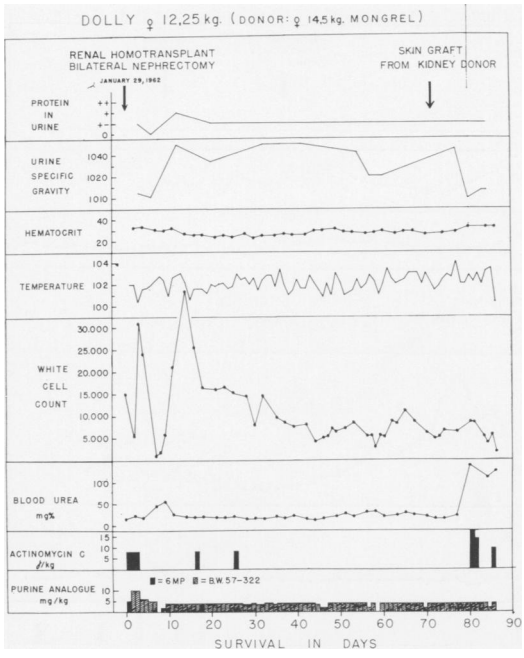

FIG. 11. Dog with a bilateral nephrectomy and kidney transplant treated with Imuran. A skin graft from kidney donor placed on Day 72 was not rejected by Day 87 at which time animal died of severe pneumonia associated with leukopenia, lowering of urine specific gravity and a rise in BUN. Presence of skin graft very likely led to kidney rejection in this instance.

nonidentical twins, two successes, over three and one half years [17] and three years, respectively,[10] have occurred following a mid-lethal dose of x-ray. The exact role of the x-irradiation in these patients has not yet been assessed with certainty because the fate of a kidney transplant between dizygotic twins without the use of total body irradiation is not known. A skin graft from the sick twin to the healthy twin reported above (Table 2) was rejected only after 24 days or twice the normal time, indicating some degree of genetic compatability.[29] In our clinical testing of other dizygotic twins, a normal rejection time of 10 to 12 days has occurred.

Not including the two instances of dizygotic twin transplants, there are five other human instances of *irradiation tolerance* produced following a mid-lethal dose of total body irradiation.[10] Kuss *et al.* have reported a sister-to-brother and an in-law-to-in-law transplants with survival of five months and 17 months, respectively, one dying of chronic rejection and the other of recurrent carcinoma. Another infant hydrocephalic *kidney* is still functioning at five months. Hamburger has reported one mother-to-son transplant still living at 14 months with a failing, presumably rejecting, kidney. His other success was a four-month survivor who died of septicemia following an additional course of x-ray two months after transplantation. Each group reported three failures, two from early *humoral*, i.e., noncellular, type of rejection, the others technical or infectious in origin.

At least 12 other known failures using irradiation are known, making a total of 27 failures including our own. Excluding the nonidentical twins, at least 18 normal kidneys have been removed from healthy volunteer donors to achieve an aggregate of 45 extra months of life for five individuals. This balance sheet should caution everyone contemplating further use of this approach in human transplantation.

In the course of our experience and of others,[15] it was soon apparent that it was not possible to produce with any consistency, successful bone marrow transplantation following heavy irradiation. The failure of host hematopoiesis led to death from sepsis and hemorrhage in the absence of a successful marrow graft. The efficacy of the environmental precaution in excluding extrinisic organisms from the patient is manifest by the serial bacteriological observations which indicated in all instances that the bacteria responsible for mobidity and death originated from the patient himself rather than from the environment.*

* All isolation technics were designed, implemented and controlled by Dr. C. W. Walter and his laboratory which is supported in part by grants from Armour and Co., Fen-Wal Laboratories, Inc., Lehn and Fink Products Corp., Lyman Associates, Posner and Associates, Sterling, Winthrop Research Institute and West Chemical Products Inc.

Possibly some of the differences between the human experience and that of the dog is in the natural history of the kidney transplant itself. In the normal dog, the kidney is rejected violently and acutely within six to 10 days in practically every instance,[5] whereas rejection may be delayed for months in the untreated human.[11] Sublethal x-ray is more effective in breaching the immunogenetic barrier the closer the genetic relationship[25] and perhaps some dizygotic twins, although not identical in their genetic background, are diverse only in respect to weak transplantation antigens like strains of mice which share the same H2, i.e., strong histo-compatability locus, and differ only at the weaker H1 or H3 loci.

Following our one success, it therefore seemed reasonable to attempt to duplicate the experience in other closely related individuals. There is no accurate method of typing individuals genetically, the phenotype not necessarily reflecting the genotype. The matching of donor and recipient by blood groups including leukoagglutinins and by skin testing on indifferent donors are the best methods at present.

The lower dose of total body irradiation employed in the later patients was selected and modified in each to minimize the adverse effect on hematopoiesis and to allow time for the completion of the operation and the early postoperative course prior to the occurrence of the maximum depression of the blood forming elements.

The rapid cessation of function, i.e., the *humoral* noncellular rejection in five patients was unanticipated and recognized only very slowly. In the first two patients, mechanical venous or ureteral obstruction was strongly suspected although not confirmed by biopsy or postmortem examination. With serial observations in Patient P. M., the appearance of red cells in the urine sediment was the first sign of this reaction. The rapid onset of red cell casts, renal swelling, oliguria and anuria could not be prevented by therapy. The microscopic picture differs completely from the usual classical cellular type of rejection which features mononuclear infiltration around the vessels and tubules; instead, the present reaction was quite similar to the accelerated *second-set* reaction seen in a sensitized host. Hemorrhage, swelling, tubular dilatation and the absence of mononuclear cells characterize this picture. Recent evidence strongly suggests that the transplantation antigens are not individually specific in man[14] and in the dog.[24] Furthermore, Altman has produced a very similar gross and microscopic picture by the injection of a specifically sensitized cell free plasma directly into the renal artery of a homografted kidney.[1]

The fact that five of the first six transplants in drug modified patients functioned is encouraging. A kidney from a cadaver or a living hydrocephalic infant whenever available can be used under these conditions and drug therapy commenced during or immediately after the transplantation. The probable mechanism of the thiopurine drugs have been described;[12] their main effect is most likely due to interference with nucleic acid synthesis. Maximum suppression of the antigenic response by the thiopurine occurs during the inductive phase of the antibody formation when the host is first exposed to the graft and specific antibody formation is initiated; later, during the proliferative phase of antibody production, the drug is less effective as a depressant to specific antibody synthesis. Therefore, the rationale for drug therapy i.e., starting the drug co-incident with the first exposure to antigen, meshes well with the clinical planning and care of a patient.

The adaptation of the drug dose to man has been difficult possibly because a large portion of the drug is broken down to thiosulphates and excreted in the urine. In terminal nephritic patients, therefore, the excretion as well as the absorption may be altered. Variability in the dose response between individuals may exist. The first

patient seemed to have a relatively effective suppression of immunity with good renal function for over three weeks, whereas the second showed signs of toxicity with rejection of the graft beginning after one week.

Patient D. T., who was conditioned with Imuran, had excellent function for over three weeks. However, toxicity of the drug was the chief cause of failure in this instance. The drug dose is a balance between hematopoietic depression and rejection of the transplant. Low sustaining doses at present are being tested with encouraging results.

Laboratory Experience. The action and metabolism of the thiopurine has been discussed in previous reports.[2-4] The additional therapeutic effect of Actinomycin C may be a result of its cytostatic effect on the lymphatic system or it may be an additional inhibitor of antibody formation. Actinomycin C is a mixture of three related antibiotics isolated from Streptomyces chrysomallus. They are chromopeptides which share the same chromophoric moiety but differ in the peptide chain. The specificity of the action of Actinomycin D, one of the three components in Actinomycin C, has been demonstrated by Reich *et al.*,[28] who have demonstrated a selective and irreversible suppression of mammalian *cellular* ribonucleic acid biosynthesis without any effect on cellular DNA synthesis or on certain *viral* RNA synthesis. The authors suggest that "Actinomycin appears to block the depression of genetic potentialities by interfering with that portion of RNA synthesis which is dependent on or governed by cellular or viral DNA." These observations are supported by Goldberg and Rabinovitz.[8] The ineffectiveness of Actinomycin C alone in prolongation of renal homografts is interesting. The toxicity from Actinomycin C alone was no greater than when used in combination with Imuran; therefore, the use of the two drugs could produce an additive beneficial effect without additive toxicity.

The combination of azaserine and Imuran was suggested by the marked improvement of the *therapeutic index* when this combination was used assaying the antitumor effect as well as the immune suppressive effect.[23] Azaserine acts on sites in the synthetic pathway of nucleic acid but a major action is to block de novo purine synthesis. Imuran blocks preformed purine. The effectiveness of the two drugs in combination against sarcoma 180 in mice is increased eight-fold compared to either one alone, and at least a four-fold increase is noted against adenocarcinoma in mice.

The rejection of skin homografts by the long-term survivors demonstrates clearly that they are not immunologic cripples and that kidney graft survival is not a result of a generalized suppression of immunity. On the contrary, the health and the stamina of these animals reflects a relatively active state of well being. It is only reasonable to expect the drug to exert some effect against functions other than antibody synthesis and it is evident that these dogs are more susceptible to pulmonary infection especially following a prolonged anesthesia.

The rejection of a skin homograft from an indifferent donor, i.e., other than the kidney donor, was not completely unexpected considering the healthy state of the animal. However, the rejection of a kidney donor skin homograft placed later in the course of the treatment reinforces the potential clinical usefulness of this form of therapy. It has demonstrated the sharpness of the tool. Now possibly we can obtain a specific tolerance to the antigen introduced concurrently with the onset of drug therapy leaving the host functionally intact against other foreign elements of the environment. There is strong evidence that the rejection of indifferent skin homografts and of the kidney-donor skin grafts initiates

some reaction against the homografted kidney, as indicated by the transient rise in the blood urea nitrogen levels in all animals and by the initiation of kidney rejection in one. This process is analogous to the observation of Goldstein, Marshall and Sturgis who produced temporary impaired function of a well-established ovarian homograft in the rat during the rejection of a skin graft from the same donor.[9]

The striking partial reversal of the rejection process by the use of drug therapy can be contrasted to the ineffectiveness of local x-ray over the kidney and high doses of cortisone therapy alone. Both of these latter factors have been studied in many animals without any success. To be effective in reversing the rejection process, drug therapy must be initiated at the earliest indication of rejection.

The observation on these animals compel one to continue the search for better combinations of drugs and to attempt analysis of the site and mode of action. The use of drug therapy is both rational and encouraging.

Summary

This is a clinical report of 18 patients and a laboratory report of over 300 kidney transplants in dogs in whom renal homotransplantation modified by either total body irradiation or drug therapy has been performed. All patients were hopelessly ill from far advanced renal disease with a life expectancy measured in days. The laboratory test system involved renal transplants in bilaterally nephrectomized animals who were started on drug therapy on the day of transplantation.

Only one of the 12 humans subjected to total body irradiation survived permanently. This is a 23-year-old man who is living and well three and one-half years after receiving a transplant from his nonidentical twin brother. Sub-lethal irradiation was used prior to the transplant and again at 10 months when signs of immunologic rejection occurred.

Two patients with heavy irradiation and subsequent bone marrow infusion succumbed to sepsis and infection without evidence of marrow survival. Five patients demonstrated excellent renal function early with remarkable clinical and chemical improvement. However, a rapid cessation of function occurred within three to six days caused apparently by a *humoral,* i.e., noncellular factor. This type of humoral rejection was characterized by marked tenderness and swelling over the transplant, red cells and protein in the urine and a microscopic picture of interstitial hemorrhage without mononuclear cellular infiltrate.

Five of six patients conditioned with thiopurine with or without Actinomycin C have had measurable renal function; one recipient still survives over 120 days. In others, death was due to drug toxicity.

The laboratory experience has been encouraging with the use of drugs. Using various combinations of purine and pyrimadine antagonists, 14 groups of animals have been tested. The most encouraging are those using a combination of Imuran and Actinomycin C and Imuran plus azaserine. The longest survivors are over nine months and still on drug therapy. Stopping the drug even at eight months may lead to rapid rejection of the kidney.

Long-term animal survivors are capable of rejecting skin homografts from nonkidney donors as well as kidney donors if the skin is placed after the transplant is well established. In all instances, the host appears to act against the established kidney transplant during this period of skin rejection. In one animal, rejection of the kidney on the eighty-seventh day apparently was triggered by the skin graft placed 15 days earlier.

Striking reversibility of the rejection process by the use of the drug was noted

in many animals. Local x-ray therapy over the kidney was never effective to initiate reversal of the transplant reaction.

In our present state of knowledge, it seems unwise to pursue further the use of living healthy donors for any type of human kidney transplantation except between twins. Although instances of temporary function in the human following sub-lethal total body irradiation has been reported, in one instance for as long as 17 months, rejection ultimately occurs.

Chemical suppression of the immune response in both the experimental animal and in the human seems promising.

Acknowledgments

The authors wish to thank Dr. F. D. Moore for his constant interest and guidance in every phase of this work. We also wish to thank Drs. R. Y. Calne, B. Altman, T. Hager, W. Retan and R. Burns who participated in the clinical care of these patients as well as in the experimental laboratory studies. The technical help of Mr. William Barry and Miss Gladys Blume is also greatly appreciated.

References

1. Altman, B.: Personal Communication.
2. Calne, R. Y.: Inhibition of the Rejection of Renal Homografts in Dogs by Purine Analogues. Transplant. Bull., **28**:65, 1961.
3. Calne, R. Y. and J. E. Murray: Inhibition of the Rejection of Renal Homografts in Dogs by BW 57–322. Surg. Forum, **12**:118, 1961.
4. Calne, R. Y., G. W. Alexandre and J. E. Murray: A Study of the Effects of Drugs in Prolonging the Survival of Homologous Renal Transplants in Dogs. Fifth Tissue Homotransplantation Conference. Ann. N. Y. Acad. Sc., (in press) 1962.
5. Calne, R. Y.: Observations on Renal Homotransplantation. Brit. J. Surg., **48**:384, 1961.
6. Cohn, R., H. Oberhelman, J. Young and H. Holman: A Successful Case of the Homotransplantation of the Kidney between Identical Twins. Amer. J. Surg., **102**:344, 1961.
7. Dammin, G. J., N. Couch and J. E. Murray: Prolonged Survival of Skin Homografts in Uremic Patients. Ann. N. Y. Acad. Sc., **64**: 967, 1957.
8. Goldberg, I. H. and M. Rabinowitz: Actinomycin D Inhibition of Deoxyribonucleic Acid-Dependent Synthesis of Ribonucleic Acid. Science, **136**:315, 1962.
9. Goldstein, D. P., D. C. Marshall and S. H. Sturgis: Antigenic Relationship between Skin and Free Ovarian Homografts. Surg. Forum, **12**:108, 1962.
10. Hamburger, J.: Fifth Tissue Homotransplantation Conference. Ann. N. Y. Acad. Sc., (in press) 1962.
11. Hume, D., J. P. Merrill, B. Miller and G. Thorn: Experiences with Renal Homotransplantation in the Human: Report of Nine Cases. J. Clin. Invest., **34**:327, 1955.
12. Hume, D., B. Jackson, C. Zukoski, H. Lee, Kauffman and R. Egdahl: The Homotransplantation of Kidneys and of Fetal Liver and Spleen after Total Body Irradiation. Ann. Surg., **152**:354, 1960.
13. Mannick, J. A., H. L. Lochte, C. A. Ashley, E. D. Thomas and J. W. Ferrebee: A Functioning Kidney Homotransplant in the Dog. Surgery, **46**:821, 1959.
14. Marshall, D., E. Friedman, D. Goldstein, L. Henry and J. P. Merrill: Rejection of Skin Homografts in the Normal Human Subject: Part I: Clinical Observation. J. Clin. Invest., **41**:411, 1962.
15. Mathe, G., E. D. Thomas and J. W. Ferrebee: Restoration of Marrow Function after Lethal Irradiation in Man: A Review. Transplant. Bull., **6**:407, 1959.
16. Merrill, J. P., J. E. Murray, J. H. Harrison and W. Guild: Successful Homotransplantation of Human Kidney between Identical Twins. J. A. M. A., **160**:277, 1956.
17. Merrill, J. P., J. E. Murray, J. H. Harrison, E. A. Friedman, J. B. Dealy and G. J. Dammin: Successful Homotransplantation of Kidney between Non-identical Twins. N. Eng. J. Med., **262**:125, 1960.
18. Murray, J. E., J. P. Merrill and J. H. Harrison: Renal Homotransplantation in Identical Twins. Surgical Forum, **6**:432, 1955.
19. Murray, J. E., J. P. Merrill and J. H. Harrison: Kidney Transplantation between Seven Pairs of Identical Twins. Ann. Surg., **148**:343, 1958.
20. Murray, J. E., J. P. Merrill, G. J. Dammin, J. B. Dealy, C. W. Walter, M. S. Brooke and R. E. Wilson: Study on Transplantation Immunity after Total Body Irradiation: Clinical and Experimental Investigation. Surgery, **48**:272, 1960.
21. Murray, J. E., O. Balankura, J. B. Greenberg and G. J. Dammin: Reversibility of the

Kidney Homograft Reaction by Re-Transplantation and Drug Therapy. Fifth Tissue Homotransplantation Conference. Ann. N. Y. Acad. Sc., (in press) 1962.

22. Murray, J. E. and J. H. Harrison: Surgical Management of Ffty Patients with Kidney Transplants. Amer. J. Surg., (in press) 1962.
23. Nathan, H., S. Bieber, G. Elion and G. Hitchings: Detection of Agents which Interfere with the Immune Response. Proc. Soc. Exp. Biol. Med., **107**:796, 1961.
24. Nilsson, C.: Personal Communication.
25. Odell, T. T., Jr. and B. C. Caldwell: Transplantation of Homologous Erythropoietic Elements in Rats after Sublethal Dose of X-radiation. J. Nat. Cancer Instit., **20**:851, 1958.
26. Piomelli, S. and M. S. Brooke: Studies on Homologous Bone Marrow Transplantation in Irradiated Rabbits. Blood, **17**:579, 1961.
27. Piomelli, S., J. O'Connor, D. Behrendt and J. E. Murray: Survival of Skin Homografts in Radiation Chimeras. Transplant. Bull. **27**: 431, 1957.
28. Reich, E., R. M. Franklin, A. Shatkin and E. L. Tatum: Effect of Actinomycin D on Cellular Nucleic Acid Synthesis and Virus Production. Science, **134**:556, 1961.
29. Rogers, B. O. and G. Allen: Intolerance of Dizygotic Human Twins to Reciprocal Skin Homografts. Science, **122**:158, 1955.
30. Woodruff, M. A., J. S. Robson, J. A. Ross, B. Nolan and A. Lambie: Transplantation of a Kidney from an Identical Twin. Lancet, **1**:1245, 1961.

Discussion

Dr. Francis D. Moore (Boston): I believe that this recent work by Dr. Murray and the group marks a milestone in our thinking that is difficult to absorb in a quick ten-minute presentation. It is a milestone in relation to the choice of preferable methods for immune suppression in homotransplantation. Although irradiation, antimetabolites and chelating agents may involve a final common pathway in their cellular action on protein replication and antibody production, their clinical usefulness in the surgery of transplants presents many important contrasts.

Irradiation given in one or two doses has to do its whole job in one sledge-hammer blow. Each cell is hit hard—how hard, we never know—and recovery is slow or may never occur.

In sharp contrast, the antimetabolites can be used gently and with sequential discrimination, altering the dosage required by the sharply opposing needs of the graft (for immune suppression) on the one hand and the host (for survival) on the other.

The essence of this *balance of survival* is shown in several of Dr. Murray's dogs. The animal is holding the new kidney but at the same time he has such an intact immune system that he remains in good health in a normal environment and can reject a skin graft.

Essential to this concept and to its clinical use is the demonstration that the tissue-destroying rejection-sequence is, up to a point, reversible. This makes it possible to adjust the drug dose at a low level without fear that a beginning rejection will be fatal. The dose of the drug is then increased, if required, to abate the rejection response. This is *tough medicine* with whole body irradiation but it is gentle, feasible and practical with these drugs, as Dr. Murray and his group have so nicely shown.

Dr. Joseph E. Murray (closing): I concur with Dr. Moore's analysis. I would just like to reemphasize the nature of the abrupt shutdown that we have seen in these human beings who have been irradiated. It is so rapid that we believed it must be mediated by some humoral, that is, a noncellular antibody, a thesis at odds with the classical thinking of transplantation immunity.

We have been able to duplicate this rapid noncellular cessation of function in the laboratory by sensitizing the recipients with spleen cells, skin grafts, kidney grafts from specific as well as nonspecific, i.e., indifferent, donors.

Although the *humoral* rejection is a different type of reaction microscopically and temporally, it is still lethal to the transplant, and therefore should be thoroughly worked out in the laboratory before again being tried in the human.