Cadaver Renal Homotransplants: Initial Experiences

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ABSTRACT

Four renal homotransplants were carried out between cadaver donors and four recipients, all of whom were in terminal chronic renal failure. Immune suppression was attempted with azathioprine (Imuran), actinomycin C and prednisone; no radiation was used, nor were the recipient's kidneys, spleen or thymus removed. One patient died with disseminated histoplasmosis at two weeks; another with irreversible homograft rejection at 30 days; a third patient died of septicemia after 9½ weeks with stable renal function. The fourth patient, whose transplant had been ischemic for 190 minutes and had not functioned for 2½ weeks thereafter, eventually achieved good function which remained unchanged to 7½ months. Changes in urinary enzyme excretion and in the I^{131} renogram and meralluride scan were of value in assessing homograft rejection.

SOMMAIRE

On a procédé, chez quatre sujets qui en étaient tous à la phase terminale d'insuffisance rénale chronique, à l'homœogreffe de reins prélevés sur des cadavres. On a tenté de supprimer les réactions d'immunité au moyen d'azathioprine (Imuran), d'actinomycine C et de prednisone. Chez aucun des receveurs, on n'a employé de radiations ni enlevé les reins, la rate ou le thymus. Un des malades est mort, après deux semaines, d'histoplasmose généralisée, un autre, à 30 jours, de rejet irréversible de l'homœogreffe, un troisième de septicémie, après 9½ semaines, alors que sa fonction rénale était stable. Quant au quatrième malade, dont la greffe était demeurée ischémique pendant 190 minutes et dont le rein n'avait pas rempli sa fonction pendant les 2½ semaines subséquentes, il a finalement eu une bonne fonction rénale qui est restée telle depuis 7½ mois. Pour évaluer la possibilité du rejet de l'homœogreffe, il est utile d'étudier les changements de l'excrétion urinaire d'enzymes, les modifications du rénogramme à l'I^{131} et l'excrétion du meralluride.

SIGNIFICANT advances have been reported in the field of renal homotransplantation in the last several years. In particular, it has been established that the homograft rejection in man can be controlled to a large extent by immunosuppressive drugs, especially azathioprine (Imuran), thereby avoiding the use of sublethal total-body irradiation. It has also come to be recognized that rejection is not an all-or-none phenomenon, nor should it be considered irreversible. Furthermore, the intensity of rejection of a specific graft appears to subside with time. A form of host adaptation occurs after a period of several months. With respect to man, the foregoing observations have been supported by a number of publications.[1-6] In dogs, it has even been shown that more than two years following a renal homotransplant, rejection may not occur when all immunosuppressive medication is withdrawn.[7, 8]

Despite these encouraging signs of progress, the results of renal homotransplantation to date do not justify its acceptance as an established form of treatment for chronic uremia.

These results were summarized at a Transplantation Conference held at Washington, U.S.A., in September 1963 and recently published.[6] If the results in identical twins are excluded, a total of 23 out of 96 patients in whom renal transplants were performed in situations where the donor was a blood relative have survived up to six months. At the time of the conference report,[6] only seven of the 23 patients had survived more than one year after transplantation. When live non-relatives acted as donors, only three out of 52 patients survived up to six months and none were alive after one year. After 68 cadaver transplants, only four patients survived up to six months and only one was alive after one year. Furthermore, there has been a growing awareness of several obscure syndromes that may develop in patients who survive for six months or more after kidney trans-

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Preliminary Definitions

Rejection: The immunological process, mediated by antibody bound to lymphocytes, leading to dysfunction of the transplant. *Second rejection*: The same process as above occurring as a second wave of immunological response; that is, a second episode of tissue rejection as evidenced by decreasing function of the transplanted organ. *Immunosuppression*: The suppression by drugs of the reactivity of the recipient reticuloendothelial (R-E) system. *Period of graft ischemia*: duration of interruption of blood supply to the transplanted kidney. *Histoincompatibility*: The dissimilarity of antigens between the tissues of the donor and recipient.

plantation. These include splenomegaly and hypergammaglobulinemia,[9] obliterative vascular changes,[10] hypersplenism with decreased red-cell survival,[11] and glomerulonephritis in the transplanted kidney developing in patients whose original disease had been glomerulonephritis or, in one case, pyelonephritis.[12]

After considering all of the aspects of this problem, a series of renal homotransplantations were undertaken at the Royal Victoria Hospital, Montreal, without putting a healthy donor at hazard. We have accepted the decreased chance of success in using the cadaver technique only. This report concerns the first four cadaver transplants in this series. Of the four patients, one is alive and in good health, seven and one-half months after transplantation.

Selection of Recipient

The four recipients were all patients in terminal uremia in whom life could not be maintained without either peritoneal dialysis or hemodialysis. Two patients had terminal glomerulonephritis, another had pyelonephritis and the fourth polycystic renal disease; all were in the "end-stage" of their diseases. Other criteria for selection were that the recipient be under middle age (although an exception was made in the case of V.T.); and that the renal disease not be a manifestation of a diffuse systemic disorder such as might subsequently affect the transplant, for example, lupus nephritis or active glomerulonephritis. Other criteria that are important in the selection of patients for programs of intermittent hemodialysis, namely, freedom from cardiomegaly, or significant atherosclerosis and its complications, and the patient's psychological stability, were not considered to be important in selection of patients for transplantation.

Preparation of the Recipient

The patients had all been moribund on admission and were revived to some degree through dialysis and transfusion. At the time of transplantation, it was necessary that recipients be free of significant fluid overload and infection. Three of the four patients were prepared for the procedure by the administration of immunosuppressive drugs. In one patient, azathioprine (Imuran) was given two weeks before operation in a dose of 2 mg./kg./day, and the other two were given this drug only two days before operation. Irradiation was not used on any of these recipients.

Selection of Donors

The selection of donors was of necessity conditioned by chance. The donor, in each instance, was another hospital patient who had died, under the age of 40, in circumstances in which the kidney had not been subjected to prolonged hypotension and had not been the site of chronic renal disease. The criteria for this decision were evidence of normal urinary sediment, normal blood urea nitrogen (BUN) and a urinary specific gravity greater than 1.020 on a random specimen. All four patients had died from neurological causes: one from hemorrhage into the mid-brain, another from severe brain trauma, a third at a terminal phase of an inoperable astrocytoma, and the fourth from a hemorrhage from an inoperable intracranial hemangioma.

OPERATIVE PROCEDURES

Removal of Donor Kidney

In three of the patients the donor of the kidney died in a ward side-room. Death was sudden and not immediately anticipated in one instance but was expected and accurately predicted in the two other instances. In the last two patients, death was accepted on neurological grounds and confirmed by the failure of the initiation of spontaneous respiration during the period of one minute after discontinuation of an artificial respirator. This maneuver was carried out on several occasions in both subjects. The donor kidney, in these cases, was removed within 20 minutes of the last spontaneous or assisted respiration. The removed kidney was perfused at 120 mm. Hg with saline or Krebs-Ringers-bicarbonate, precooled to 4° C.; the organ was then stored at the same temperature in a mobile refrigerator for transfer to the operating room. In the fourth instance the donor died in the operating room where the kidney could be removed rapidly and after a minimum period of anoxia or ischemia. This donor kidney was the only kidney which responded with an immediate diuresis and exhibited normal function (creatinine clearance of 70 ml./min.) immediately after transplantation.

THE TECHNIQUE OF TRANSPLANTATION

The recipient was taken to the operating room when it was apparent that the prospective renal donor was *in extremis*. An epidural anesthetic was instituted. The site of the renal implant was the right iliac fossa in three patients and the left in the fourth. The iliac vessels were exposed through a Gibson incision. Arterial anastomosis was carried out in an end-to-end manner between the patient's internal iliac artery and the renal artery of the donor kidney. In one patient (V.T.) a double renal artery was encountered on the donor kidney and end-to-end anastomosis was carried out between the renal vessel of the donor kidney and the superior and inferior gluteal arteries of the host. Venous anastomosis was carried out end-to-side between the donor renal vein and the common iliac vein. The donor ureter was reimplanted into the bladder by means of a tunnel and mucosa-to-mucosa anastomosis. In one patient (D.B.) the host ureter was retained and a pelviureteric anastomosis was performed. The incision was closed with drainage.

Neither the patient's spleen nor thymus was removed or irradiated. On only one occasion was one of the recipient's kidneys removed at the transplant operation. The transplanted kidney was not irradiated prior or subsequent to implantation in any instance.

OUTLINE OF POSTOPERATIVE MANAGEMENT

A special unit was constructed, consisting of three rooms, a dressing room, a nurses' room or access room, and a room for the patient. Rigid surgical antiseptic technique was observed throughout. The room was sterilized at least once every 48 hours, pure air entering under pressure. A draft of air was therefore leaving the transplantation suite through the various exits at all times. All trays or equipment were double-wrapped in sterile dressings and the outer wrap was removed as each article was placed into a manual pass-through section where it was exposed to ultraviolet light.

Immunosuppressive drugs: Azathioprine (Imuran) was used in all four patients; it was administered preoperatively in three (as mentioned above). From the day of operation onwards the dose was 4 mg./kg. per day until a change of dose was made necessary by a fall in the peripheral total white blood count to a level below 5000 per c.mm. Actinomycin C was administered by intravenous injection to all four patients as a second agent to prevent kidney rejection. The reason for commencing this drug differed. Actinomycin C was started in V.T. because the development of leukopenia necessitated a reduction in the other immunosuppressive drug, azathioprine (Imuran); in J.P., actinomycin C was added to azathioprine and prednisone at the time of the second episode of incipient transplant rejection. Patient C.B. was given actinomycin C on the tenth day postoperatively, and D.B. was given this drug only at the terminal stage.[7] The dose administered was 6-10 μg./kg. intravenously (i.v.) once weekly. Prednisone was given orally as treatment for rejection in doses of 100-200 mg. daily. The duration of prednisone administration was limited as much as possible. "Tailing-off" was not undertaken if the duration of corticosteroid treatment was less than five days.

Antibiotics were not administered routinely or prophylactically after the first postoperative week. Subsequently antibiotics were selected on the basis of sensitivity tests,[7] bactericidal agents being used whenever possible.

Patients were placed on a full electrolyte and fluid balance regimen and were weighed daily on a bed scale. Urine, blood, and gastrointestinal drainage, if indicated, were analyzed for electrolytes. Daily creatinine and urea-nitrogen concentrations were determined in blood and urine. Renal function was followed by estimations of BUN, serum creatinine, urine/plasma (U/P) creatinine ratios or creatinine clearance. Quantitative proteinuria and the changes in urinary microscopic sediment were closely observed. The differential and total white blood count was done each day. I^{131} renogram and radio-meralluride renal scans were done every one to three weeks. Aliquots of 24-hour urine were collected in one patient and measured for lactic acid dehydrogenase (LDH) and alkaline phosphatase in order to determine if these were related to the development of rejection (Fig. 8). Each patient was examined daily for evidence of change in size or tenderness of the transplanted kidney.

RESULTS

The clinical course of each of these patients should be considered with special reference to the parameters charted in Figs. 1, 3 and 5. The time of transplantation is indicated by a heavy vertical arrow. Arrows (in lighter print) indicate hemodialyses (H) or doses of actinomycin C. The blocks at the top of each section of the diagram indicate administration of prednisone and azathioprine. The upper part of these three figures shows the daily volume and changes in serum creatinine and BUN. In the lower part of each figure the ordinate represents the total white blood count for each patient, the change in body weight, and in one patient (J.P.) the mean temperature. The abscissa in each patient is the time, in weeks.

Case Histories

CASE 1.—The first patient (J.P., Fig. 1) had chronic pyelonephritis and had two peritoneal dialyses before receiving a renal transplant. Preoperatively, she was severely oliguric and moderately hypertensive. The donor was a victim of severe cranial injury. Neurosurgical exploration had indicated that specific treatment was impossible. Respirations ceased spontaneously soon after admission and the donor was maintained on a respirator, isoproterenol and transfusions until the recipient reached the operating room. Artificial respiration of the donor was discontinued and the kidney excised, employing the technique described above. The duration of total ischemia of the grafted kidney was 90 minutes. Moderately good renal function was obtained postoperatively, although the urine volumes were not greater than 1½ litres per 24 hours. The BUN and creatinine fell almost to normal; creatinine clearance was 25-30 ml./min. Subsequently a fairly rapid rise in BUN and creatinine occurred, reaching a peak toward the end of the second week. Proteinuria increased and a high swinging fever developed. She was treated with high doses of prednisone because transplant rejection was suspected. Subsequently diuresis occurred with a fall in the two parameters of nitrogen retention, BUN and creatinine.

From the third to sixth week, renal function was stable with urine volumes above 2 litres, creatinine below 2.5 mg. %, and creatinine clearances in the region of 20 to 30 c.c. per minute. Intermittent unexplained fever persisted and became worse at the beginning of the sixth week. Serum creatinine and blood pressure began to rise again. Red blood cell casts appeared in the urine; proteinuria increased and urine volume decreased. As it was believed that a second episode of transplant rejection was occurring,

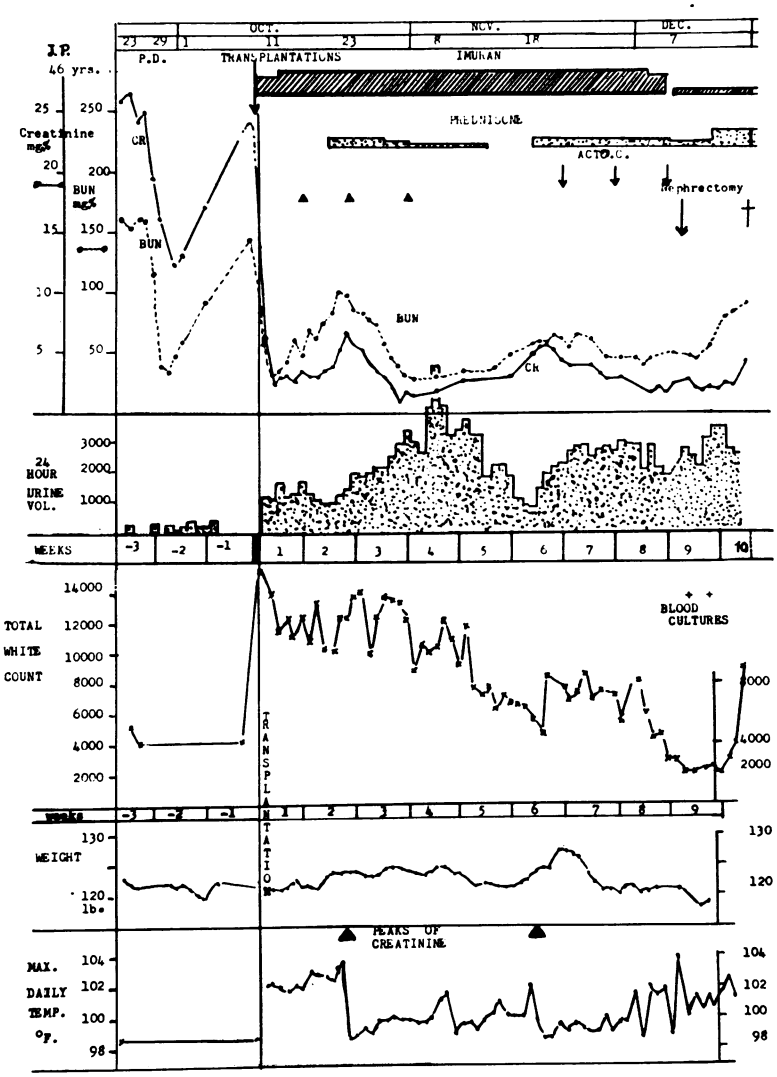

Fig. 1.—Transplantation data in recipient J.P. from three weeks preoperatively to her death 9½ weeks postoperatively. Administration of immunosuppressive drugs is indicated across the top (for doses, see text). Ordinates include BUN, serum creatinine, urine volume, total leukocyte count, weight, and maximum recorded daily temperature. The three symbols ▲ in the upper part of figure refer to the renograms and renal scans shown as A, B and C in Fig. 7.

prednisone (100 mg./day) and actinomycin C (10 μg./kg./week i.v.) were started. With this therapy, the urine volume increased to 2 litres per day and renal function returned. However, intermittent fever continued. It was suspected that infection might be present in the right kidney, so right nephrectomy was performed, but renal infection was not found. She lived another 10 days, during which the fever continued and *E. coli* was cultured from her blood. She developed gastrointestinal hemorrhage for several days and multiple convulsions occurred on the day before her death. At autopsy she was found to have a small *E. coli* abscess of the lower end of her incision, in the retropubic space.

The changes in this patient's white blood cell count (Fig. 1) drew attention to the absence of any close correlation between immunosuppression and the degree of leukopenia induced by azathioprine administration. Over a nine-week period the total white count was greater than 4000 per c.mm., yet this long period was presumably characterized by immunosuppression. The initiation of treatment with prednisone increased the leukocyte count temporarily but did not obscure the gradual downward trend (in leukocyte count) over this whole period. Probably azathioprine in a dose of 4 mg./kg./day was continued for too long a period in this patient. It is perhaps significant that blood stream invasion from the pelvic abscess occurred only when the white blood count was falling toward leukopenic levels.

At autopsy the kidney weighed 150 g. and its surface was smooth and brownish-grey. On section, the kidney was reddish-brown. The corticomedullary junction was well preserved and the cortex averaged 12 mm. in thickness. There was no gross evidence of papillary necrosis. All anastomoses were patent and intact. At the junction of the ureter with the bladder there was a small abscess pocket, containing approximately 10 c.c. of purulent exudate. Another abscess of approximately the same size was localized at the inferior pole of the transplanted kidney.

Microscopic examination of the donor's (transplanted) kidney showed that the transplant was viable. The arteries and veins were patent, showing slight intimal thickening. The most striking changes were (1) homogeneous hyaline-like thrombi within the glomeruli and focal necrosis of glomerular tufts (Fig. 2); (2) infiltration of the interstitial tissue by mononuclear cells, associated with slight edema. The mononuclear cells were predominantly lymphocytes and plasma cells the cytoplasm of which contained pyraninophilic material; (3) focal degenerative changes of the tubular epithelium with glandular and hyaline casts; and (4) necrotizing papillitis with presence of fungus which microscopically could be identified as hyphenated and yeast forms of *Candida albicans*.

The spleen was enlarged and the follicles were decreased in size. There was associated hypocellularity; small lymphocytic cells and nuclear debris were present and the littoral cells were markedly prominent.

CASE 2.—V.T., a 55-year-old man, had a history of hypertension for over five years, and symptoms suggestive of uremia for eight months. Polycystic renal disease had been previously diagnosed. He was admitted in advanced uremia: his BUN was 270 mg. %; hemoglobin, 8 g. %; and CO_2-combining power, 12 mEq./l. He had marked asterixis and was stuporous.

His urine volume was 2 l./day and the urine culture was sterile. He was submitted to two periods of peritoneal dialysis prior to transplantation. He was the only patient of the four who had appreciable urine volume prior to transplantation. Azathioprine was started two days prior to transplantation. He received a kidney from a patient who had died from an inoperable astrocytoma. The total period of ischemia of the grafted kidney was 190 min. of which at least 20 min. occurred

between the moment of death and the removal of the kidney and its subsequent cooling.

The recipient's right iliac vessels were exposed *via* a Gibson incision while the patient was under epidural anesthesia. On mobilizing the internal iliac artery, it was obvious from palpation that it contained many atherosclerotic plaques. Because of the double renal arteries in the donor kidney, it was necessary to mobilize the gluteal division of the internal iliac artery; the superior gluteal artery was prepared for the major renal anastomosis and the inferior gluteal artery was used to anastomose the vessel leading to the lower pole of the kidney. It was necessary to remove several atherosclerotic plaques in order to obtain vessels suitable for suturing. During this time the donor kidney was kept refrigerated. Anastomosis for the major renal artery was satisfactory but that for the lower pole was not. In addition to atherosclerosis, the vessel to the lower pole was only 4 mm. in diameter; mottling of the lower pole was still present at the completion of the operation. Postoperatively, leakage of urine through the operative site was considerable. Bladder urine was presumed to consist of a mixture of urine from the transplanted kidney and from the patient's own kidneys. The higher concentration of creatinine in the leakage urine and the fact that phenosulfonphthalein (PSP) injected into the bladder did not stain the leakage urine indicated that only urine from the transplant was draining through the wound. Later, the volume of bladder urine fell to only about 200 ml./day and the transplanted kidney's urine all drained from the wound, as indicated in the shaded area in Fig. 3. Renal function was very poor initially as a result of the long period of ischemia, the age of the recipient and the fact that difficulty was encountered in establishing anastomosis between the inferior gluteal artery and the lower of two donor renal arteries.

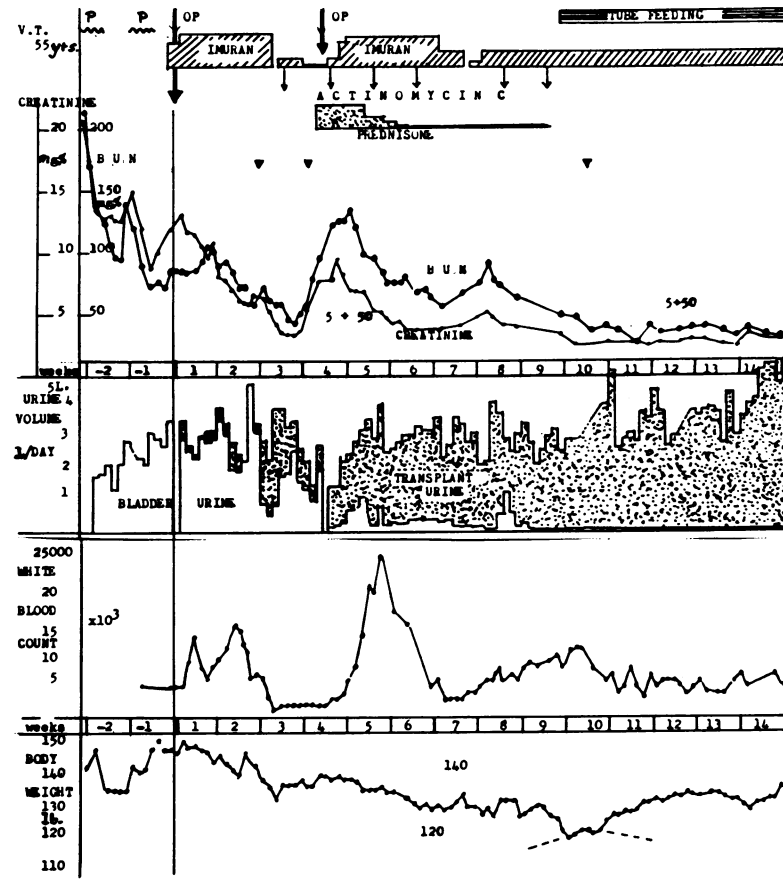

Fig. 3.—Transplantation data in recipient V.T. from two weeks preoperatively to 14 weeks postoperatively. The periods of drug administration are shown across the top. The shading in the middle of the figure refers to urine volume from the urinary fistula. Also shown are the leukocyte count and the body weight. The three symbols ▼ refer to renograms and scans D, E and F of Fig. 7.

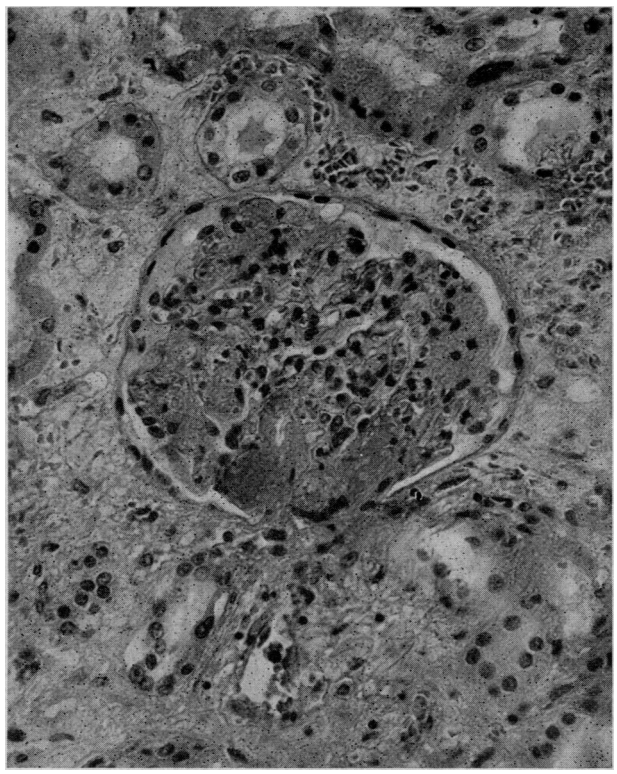

Fig. 2.—Glomerulus from transplanted kidney of J.P. at autopsy (× 400).

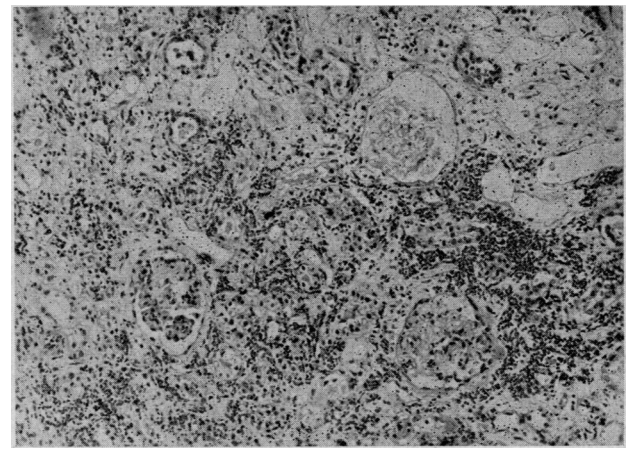

Fig. 4.—Section of transplanted kidney of V.T. at time of second operation in fourth post-transplant week (× 175).

TABLE I.

Age (years) sex	Recipient (preoperative)					Donor			Min. of ischemia to cooling	Recipient (postoperative)				
	Date of transplant	Renal disease	Blood group	Preop. dialysis +	Preop. azathioprine 2 mg./kg./d	Age, sex	Disease	Blood group		Min. of total ischemia	Renal function 1st week	Renal function 3rd month	Rejection crises *	Course
1. J.P. ♀ 46	Oct. 11, 1963	Chronic pyelo-nephritis	A; pos.	2P	0	♂ 37	Brain injury	O; pos.	24	90	Fair	Good	10 d.: + 3 wk.: ++	Died: septicemia after 9½ weeks Creatinine 3 mg.%
2. V.T. ♂ 55	Nov. 7, 1963	Polycystic kidneys	A; pos.	2P	1 d.	♂ 37	Astro-cytoma	O; pos.	28	190	Poor	Good	4 wk. ++	Well at 4½ mos. Creatinine 2.5% Urinary fistula
3. D.B. ♂ 39	Nov. 10, 1963	Chronic glomerulo-nephritis	A; pos.	8H 1P	1d	♀ 38	Bleeding into brain stem, thrombo-cytopenia	AB; pos.	20	75	Bad	—	10 d.: ++++ Complex (see text)	Died: 14 days renal necrosis Thrombo-cytopenia
4. C.B. ♀ 21	Dec. 6, 1963	Chronic pyelo-nephritis	A; pos.	4H 1P	14d	♂ 21	Thalamic hem-angioma	A; pos.	0	45	Very good	—	2½ wk.l ++++ No reversal	Died: 30 days Anuria Pneumo-nia

*Rejection graded + to ++++ on clinical data.
+H—Hemodialysis; P—Peritoneal dialysis.

By the early part of the fourth week it was evident that the patient's renal function was deteriorating and he was febrile. Urine from the bladder and the flank was grossly infected. It was necessary to reduce the dose of azathioprine because leukopenia developed, and actinomycin C (10 μg./kg., i.v.) was started. An exploratory operation revealed that infarction had occurred in the lower pole of the transplanted kidney and a line of demarcation was apparent. The lower pole of the kidney was excised and a T-tube placed in the ureter because some degree of ureterovesical obstruction was present. The general external appearance of the remainder of the kidney was mottled and unhealthy. It was biopsied (Fig. 4). The biopsy showed glomeruli with varying degrees of necrosis and considerable round-cell infiltration; the changes were patchy in distribution. Prednisone (200 mg./day) was started.

During the early postoperative phase of the second operation the total white count rose sharply to 25,000 per c.mm. and the dose of azathioprine was increased to 4 mg./kg./day again.

There was a slow and gradual improvement in the degree of uremia. Prednisone was discontinued gradually. Following the second period of leukopenia, azathioprine in a maintenance dose of 2 mg./kg./day was started and has been continued since that time. Despite stable renal function, the patient had a steady weight loss. This may have represented the combined antianabolic effect of the infected urinary fistula and the immunosuppressive drugs. Continuous nasogastric intubation was instituted and 3 litres of protein milk shakes were given daily *via* a fine polyethylene catheter; this resulted in a steady increase in weight.

One unexpected feature in the twelfth to sixteenth postoperative weeks was the development of macrocytosis, megaloblastic erythropoiesis, reticulocytosis of

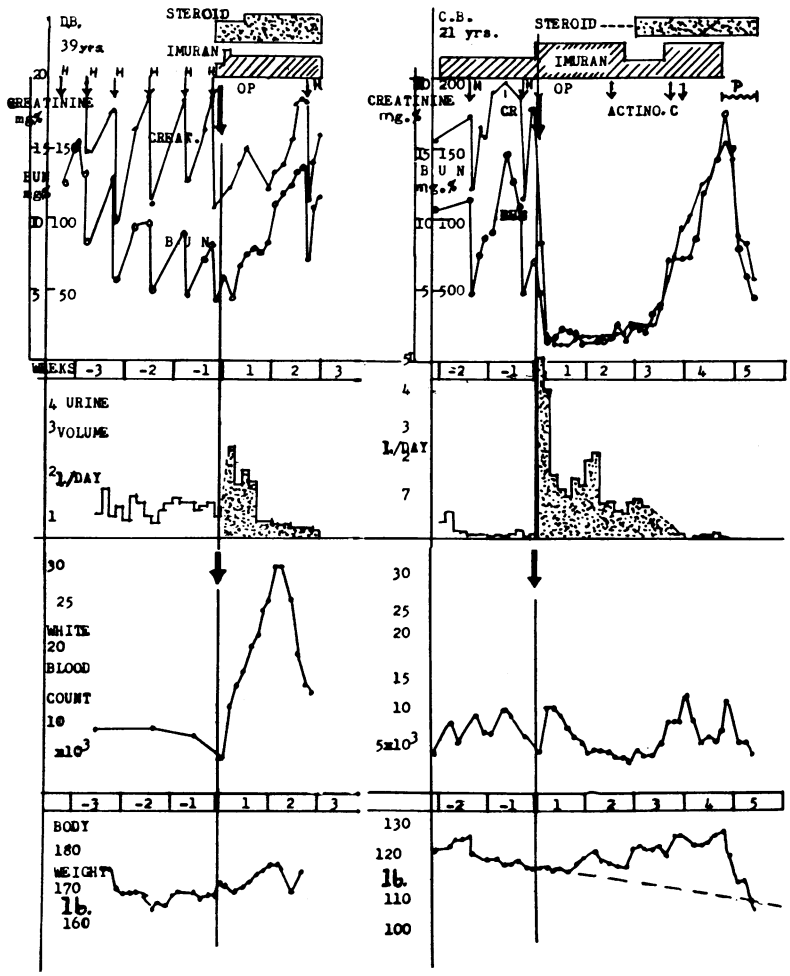

Fig. 5.—Clinical data for transplant recipients D.B. and C.B. H=hemodialysis; P=peritoneal dialysis. Ordinates are the same as for Fig. 3.

6-12% and a decreased RBC survival. The following were normal: precursors of the neutrophilic granulocytes, platelets, gastric acidity, vitamin B_{12} absorption, serum B_{12} (1.12 μg./ml.) levels and serum folic acid, but RBC folate was low. The mechanism of these hematologic changes is discussed below. This patient has had prolonged survival (see Addendum).

CASE 3.—D.B., a 39-year-old man with chronic glomerulonephritis, had been submitted to eight hemodialyses in preparation for transplantation. The donor of the kidney was a 29-year-old patient who died of mid-brain hemorrhage thought to be due to idiopathic thrombocytopenic purpura. The blood group of the recipient was incompatible with that of the donor (Table I), but because the patient had been waiting for a considerable period for the transplantable kidney, and because of reported success in transplants between subjects with blood group incompatibility,[13] it was decided to go ahead. One of the recipient's diseased kidneys was removed during transplantation.

The period of total renal ischemia on this occasion was 75 minutes. The function of the transplanted kidney was very poor (Fig. 5) although the urine volumes were significantly increased. There was no fall in BUN and creatinine. At no time did the patient recover a sense of well-being, and by the twelfth day he was severely oliguric. Shortly thereafter thrombocytopenia was evident and diffuse purpural eruption occurred. The patient's appearance was similar to that of the donor prior to her death. He died with diffuse ecchymoses and petechiae presumably from intracerebral hemorrhage.

The leukocyte count in this patient showed changes that normally would not be anticipated with a leukopenia-inducing drug. At autopsy the parenchyma of the kidney and its capsule were markedly hemorrhagic; it weighed 150 g. and appeared to be infected. The anastomosed artery and the vein were patent but marked perivascular hematoma formation was noted, which constricted the lumen of the blood vessels. Microscopically, the kidney was necrotic and hemorrhagic. There was a minimal scattering of mononuclear cells (lymphocytes and a few identifiable plasma cells) in the interstitial spaces without any special arrangement around the vascular channels.

An outstanding and unusual feature was present on microscopic examination of the liver. Within the Kupffer cells there were innumerable PAS-positive spherical structures which also stained with Grocott's stain. These bodies were identified histochemically as *Histoplasma capsulatum* (Fig. 6). Similarly, the reticuloendothelial cells of the spleen were densely invaded by these organisms. The spleen, in addition, showed a decrease in follicle size, hypocellularity and marked prominence and swelling of littoral cells. *Histoplasma capsulatum* was also obtained by culture from the recipient's blood immediately prior to his death.

The postmortem examination of the donor had revealed, in addition to the brain hemorrhage resulting from thrombocytopenic purpura, a granulomatous lymphadenitis of the mediastinal nodes with miliary spread of granulomatous lesions in the liver, spleen, and bone marrow. Subsequent microscopical study with PAS and Grocott's stains identified, within the lymph nodes, spherical structures similar to those seen in the recipient. Although these structures were not identified by culture, they were very probably Histoplasma organisms. The hematological features of this case will be the subject of a separate report.

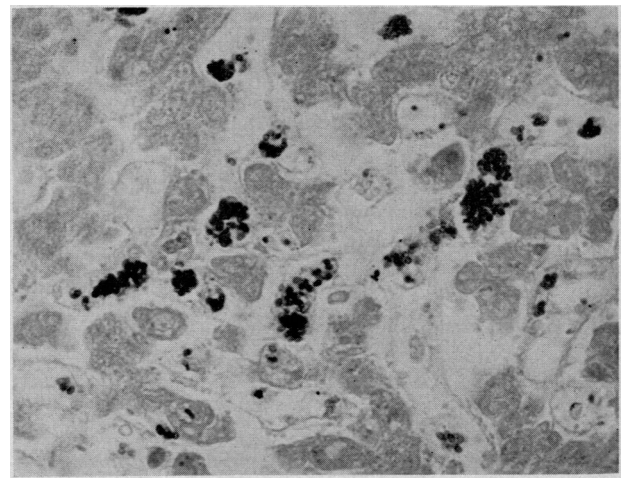

Fig. 6.—Section of liver of patient D.B. showing Histoplasma organisms (× 600).

CASE 4.—C.B., a 21-year-old schoolteacher, had had one peritoneal dialysis and four hemodialyses prior to transplantation. She had also received azathioprine 2 mg./kg./day for two weeks preoperatively. She was oliguric (Fig. 7) but had no significant infection or hypertension, and was in good condition at the time of surgery.

The kidney was obtained from a donor who died of a nervous-system lesion. Circulation, respiration and renal function were maintained until the kidney was excised. The organ was immediately cooled by perfusion and transplanted at once. The function of this kidney was excellent and a good urine output was evident before the end of the operation. The total period of ischemia was 45 minutes. In the first 24-hour period the urine volume was 5 litres and BUN and creatinine levels became normal. Creatinine clearance during the remainder of the first and the early part of the second week was between 50 and 70 c.c. per min. The patient received azathioprine (4 mg./kg./day) and three days of hydrocortisone (Solucortef) 200 mg./day i.v. postoperatively.

Towards the end of the second postoperative week the patient's condition deteriorated. She had a fever of a remittent type, her urine volume was reduced to one litre per day, and a slow rise of serum creatinine and BUN took place. The area over the transplant became very tender and the organ was obviously enlarging. Actinomycin C (10 μg./kg., i.v.) and prednisone (100 mg./day) were administered. Despite this therapy she entered upon a severe rejection crisis with a rapid rise of BUN and creatinine, and an increase in protein and hemegranular casts in the urine. The fall in BUN and creatinine in the last few days of her life was due to peritoneal dialysis. During this period and to the time of her death, she was unable to ingest azathioprine. Hydrocortisone was given in massive doses, 400 mg./day, by the intravenous route. The kidney remained large, tender and anuric. No reversal in the downhill course occurred. Unfortunately, permission for autopsy could not be obtained and the nature of graft rejection was not established.

In this patient, enzyme excretion in the urine was closely studied during the postoperative period. In

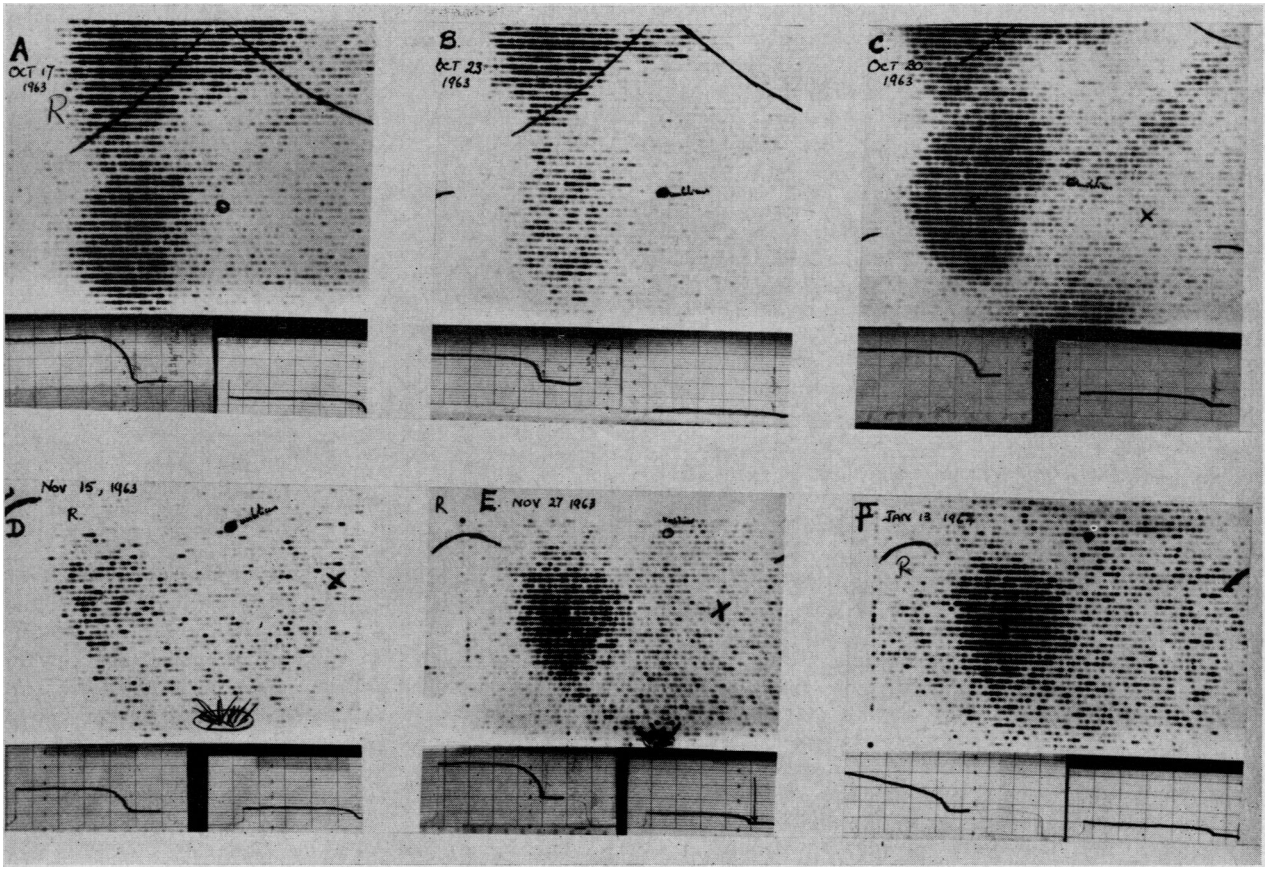

Fig. 7.—I^{131}-Hippuran renograms and meralluride renal scans of patient J.P. (A, B and C) and V.T. (D, E and F) at the points indicated by the symbols ▲ on Fig. 1. and by ▼ on Fig. 3, respectively.

Fig. 8 the changes in BUN and creatinine are plotted above the excretion values of urinary lactic dehydrogenase (LDH) and alkaline phosphatase in the urine. It is interesting to note that the level of urine alkaline phosphatase rose before the clinical diagnosis of graft rejection was made and before the rise of serum creatinine. These urine enzyme measurements were made on the suggestion of Dr. M. H. Gault. The total leukocyte count did not fall below 5000 per c.mm. in this patient despite a long period of administration of azathioprine at two dosage levels. The severity of rejection may have been due to decreased responsiveness to the drug or, more probably, to a greater degree of histoincompatibility between the randomly selected donor and this recipient's tissue.

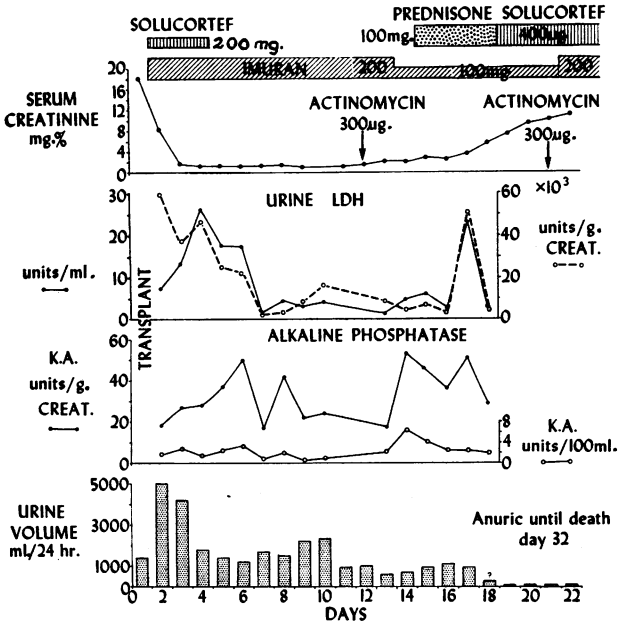

Fig. 8.—Changes in urinary lactic dehydrogenase (LDH) and alkaline phosphatase excretion in patient C.B. during the period from transplantation to subsequent rejection. Collections became impossible once oliguria was marked, although the patient survived for another 10 days. A rise in alkaline phosphatase excretion occurred when renal tenderness was noted and several days prior to significant rise in serum creatinine (see Day 13).

Some features of these four patients are compared in Table I. It can be seen that the fourth patient (C.B.) had the most immediately successful transplantation and was the only patient who had excellent renal function in the immediate postoperative period (creatinine clearances of 50 to 70 c.c. per min.). She had the longest period of preoperative azathioprine therapy and five preoperative dialyses and was in good physical condition. Rejection in this patient was extremely severe nevertheless and completely irreversible. The clinical course of the third patient (D.B.) was unexpectedly complicated. The donor of the kidney that he received was presumed to have died as a result of idiopathic thrombocytopenia, but probably had unrecognized disseminated histoplasmosis at

the time of his death. Disseminated histoplasmosis developed in the recipient (D.B.) during the period of immune suppression.

The first patient (J.P.) did well for over three months although she had an intermittent fever. She died from *E. coli* septicemia secondary to a pelvic abscess at a time when her serum creatinine was only 2 mg. % and renal function was good. The second and oldest patient (V.T.) had the smallest number of preoperative dialyses; his graft was subjected to the longest period of ischemia with very poor immediate renal function. He suffered from two postoperative complications, an infarct of the lower pole of the transplanted kidney due to thrombosis of the lower branch of a double renal artery, and a urinary fistula, which developed following the partial nephrectomy performed 3½ weeks after the original transplant. Despite these hazards he had done better than the others.

DISCUSSION

Permission for the removal of the donor kidney was obtained from relatives prior to the death of the prospective donor. A special "Permission for Autopsy" form was utilized. In the case of accident victims, the coroner of the City of Montreal has permitted removal of the kidney in the hospital before removal of the body to the morgue for coroner's autopsy (for example, the donor in Case 1, J.P.) when the relatives of the deceased agree to this procedure.

The experience herein reported emphasizes the probability that differences in histocompatibility between donor and recipient are of greater importance than the period of ischemia in the donor kidney.

Kidneys from cadavers have a poor survival record, owing to prolonged ischemia with irreversible damage to the organ as well as greater genetic difference. In the present series the kidney with the longest period of ischemia at the time of transplantation is functioning six months after transplantation. In contrast, a kidney removed in the operating room from a decerebrate patient who had a normal blood pressure and urine output was rejected irreversibly after 21 days; this reaction suggests that termination of function is related predominantly to differences in histocompatibility. However, as seen in Table I, the possibility that hemodialysis (using blood that still contains many of its original leukocytes) was a cause of sensitization in these individuals cannot be excluded because the two who survived longest were those who had only had peritoneal dialysis.

Preoperative matching of tissue antigens between donor and recipient is not possible at the present time. Immunosuppressive therapy is more effective when slight differences in histocompatibility exist, but its effect is not sufficient to overcome the reaction to great differences in compatibility or to act after non-specific sensitization has occurred. While the human red blood cell does not contain transplantation antigens, a major blood group difference between donor and recipient is presumed to be an index of major differences in tissue histocompatibility which makes successful transplantation unlikely, using present immunosuppressive therapy.

We are unable to explain the difference in response of the total leukocyte count in these three patients. In the first (J.P., Fig. 1), administration of azathioprine in a dose of 4 mg./kg./day was associated with a persistent, prolonged period of elevation of the white blood count, without disproportionate lymphopenia, although there was a gradual decline in total leukocyte count over a nine-week period. In patient V.T. (Fig. 3) the same dose of azathioprine (4 mg./kg./day) induced a severe leukopenia in the third week. In the fourth patient (C.B.) even though it was preceded by two weeks' treatment at the 2 mg./kg. level, the 4-mg./kg. dose of azathioprine was not associated with any significant change in the leukocyte count. Despite the difference in white-cell response in the first and second patients, both have shown evidence of immunosuppression.

Experience in these four patients provides evidence that rejection of the graft of a randomly selected cadaver kidney is reversible in a way comparable to that following renal transplantation between relatives. We believe that corticosteroid is the best agent for reversal of rejection when it occurs, but should be withdrawn as soon as possible thereafter.

When this investigation was initiated, it was thought likely that the recipients could be improved again by dialysis even if the homografted kidney was rejected. This has not been the case. Two patients in whom the graft was irreversibly rejected died because of complications of the therapy or as a direct result of necrosis of the transplanted kidney. A third, in whom the transplanted kidney was functioning, died of septicemia related to the suppressive therapy necessary to maintain kidney function. Prompt removal of rejected transplants is necessary, as soon as it appears that reversal is not going to occur, if reinstitution of dialysis is planned.

Another important need is a means of anticipating imminent graft rejection. We have used three approaches to this problem: (a) attempts to detect circulating antibodies to donor renal tissue, (b) the use of I^{131}-hippuran renogram and meralluride (Mercuhydrin) scan to detect changes in size and function and (c) the measurement of enzyme excretion in the urine (Fig. 6).

In three of the four patients reported here, a diligent search for circulating antibody was made. Antigen was prepared from the donor's opposite kidney, and blood from recipients was obtained at frequent intervals. The highly sensitive tanned-cell hemagglutination test[14] was used for detection of circulating antibodies. In neither patient who had

prompt rejection, nor in the patients with long survival, were antibodies against kidney detectable.

Fig. 7 shows a series of renograms in the first and second patients. In J.P. it can be seen, from the meralluride scan, that the kidney gradually increased in size, especially with respect to the upper pole. This enlargement was confirmed at autopsy. The renogram becomes less normal with the passage of time. In V.T., infarction of the lower pole could be diagnosed in retrospect by the appearance of the scan; the rest of the kidney shows increasing function with time. Hume[11] has suggested the use of metal clips to diagnose changes in renal size. We believe that the meralluride scan is more specific.

Fig. 8 shows a correlation between the changes in clinical events and the urinary excretion of two enzymes: LDH (Day 16) and alkaline phosphatase (Day 13) rose before significant elevation of the blood urea and creatinine, or reduction in urine volume, although renal tenderness suggested that rejection was about to occur. It was at this time that cortisone was restarted. Cortisone alone would not have caused this change. The urinary LDH did not rise until several days later. The significance of this observation cannot yet be assessed. The initially elevated excretion of these two enzymes is assumed to be due to the non-immunopathologic damage to the kidneys at the time of transplantation.

As mentioned earlier, the second patient, V.T., in whom prolonged survival was achieved, had a megaloblastic marrow between the twelfth and sixteenth weeks with normal serum vitamin B_{12} folate, but he had reduced RBC folate and therefore decreased RBC/serum folate ratio.[15] Furthermore, this ratio was restored to normal by a rise in RBC folate (from 63 to 250 mμg./l.) without significant change in serum folate (4.1-3.2 mμg./ml.) after administration of 2000 μg. (2 mg.) cyanocobalamine (vitamin B_{12}) as part of a Schilling test. The result of the Schilling test was 20% (normal: greater than 12%).

It is known that vitamin B_{12} is concerned in the conversion of methyl tetrahydrofolate to tetrahydrofolate and it is speculated that azathioprine (a purine analogue) may have impeded this step, although the impediment has been overcome by a large dose of vitamin B_{12}. It is not known how purine analogues cause this impairment in B_{12} action. (We are indebted to Dr. A. A. Cooper and Dr. N. K. M. de Leeuw, of the Department of Hematology, Royal Victoria Hospital, Montreal for assistance in elucidating this problem and initiating further studies.) It should be noted that correction of the marrow change by vitamin B_{12} administration was not associated with any change in immunosuppression, as judged by renal function.

SUMMARY

Four cases of cadaver renal homotransplantation are presented. Two patients obtained prolonged acceptance of the graft and only in these two was the process of graft rejection reversible. Azathioprine (Imuran) was used as the principal immunosuppressive drug; prednisone and actinomycin C were used for treatment of rejection of the transplanted kidney. Only one patient is still alive and has moderately good renal function at the time of this report (June 1964).

Methods of anticipating graft rejection are reported and discussed.

ADDENDUM (September 20, 1964)

The prolonged survivor of these four transplants, V.T. (Case 2), had a serum creatinine between 2 and 3 mg. % at 10 months; he is back at work and feels well.

Five more cadaveric transplants have been done since June 1964; on one occasion both kidneys were successfully transplanted from one cadaver to two separate recipients (both the latter are still doing well). One of the five died at 2½ weeks of infection of the transplant site; the other four are doing well (all had serum creatinine values of less than 2.5 mg. %) at 12, eight, eight and two weeks, respectively.

We wish to acknowledge the help given by Dr. S. Helle, who supervised the hemodialysis of these patients; and by Dr. H. Blondal, who obtained and interpreted the radioactive renal studies. We wish also to acknowledge the technical assistance of Mrs. S. B. Day.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

THE USE OF GENERAL PRINCIPLES

Dangers common to all wounds are shock, hæmorrhage, and sepsis. The use of a knowledge of general principles is emphasized by a story Mr. Saint told me of one of his students, a clever, but somewhat idle young man. In his final examination one of the questions asked was: "What are the symptoms produced by and the dangers following a penetrating stab wound of the chest wall in the mid-axillary line?" The student knew nothing of it, but proceeding on general lines he first described the symptoms produced by shock, then he followed with hæmorrhage from the lung and from an intercostal artery, and ended up his answer with the conditions resulting from sepsis in the wound and in the pleura and in the lung. He knew that the dangers of every wound arose from shock, hæmorrhage, and sepsis, and applied this knowledge to the case. He did not know that the escape of air from the lung might cause emphysema and pneumothorax, but his answer otherwise was so good that his examiner credited him with almost full marks, thinking that he must have forgotten to put down the matter concerning air escape.—R. Morison, *Canad. Med. Ass. J.*, 4: 681, 1914.