Papers and Originals

Human Cadaveric Renal Transplantation

Report of Twenty Cases*

J. F. MOWBRAY,† M.B., B.CHIR., M.R.C.P.; S. L. COHEN,‡ M.B., B.S., M.R.C.P., D.OBST.R.C.O.G.; P. B. DOAK,§ M.B., CH.B., M.R.C.P.; J. R. KENYON,|| B.SC., CH.M., F.R.C.S., F.R.C.S.ED.; K. OWEN,|| M.S., F.R.C.S.; A. PERCIVAL,¶ M.A., B.M., B.CH.; K. A. PORTER,** M.D., D.SC.; W. S. PEART,†† M.D., F.R.C.P.

Brit. med. J., 1965, **2**, 1387–1394

In this paper we present the results obtained in 20 patients with terminal renal failure treated by immunosuppression and cadaveric renal transplantation. Eight of these patients were still alive and well on 1 August 1965. An earlier series of 18 transplants performed here has already been reported (Porter *et al.*, 1963, 1964).

It is often assumed that cadaveric renal homografts will be less successful than those from live donors. Admittedly the former generally do not function as well as transplants from close relatives and there are fewer long-term survivors. However, when live donors taken at random are used the success rate is no higher than for transplants from cadavers. In September 1964 35% of 98 cadaveric renal homografts performed in the previous 18 months were still functioning, compared with 17% of 46 kidneys transplanted in the same period from live genetically unrelated donors (Murray *et al.*, 1965).

The ultimate fate of renal homografts is unknown, and although very encouraging results are being obtained with sibling and parental donors (Starzl *et al.*, 1964), in one of the most successful series four of the eight kidneys biopsied at approximately two years after transplantations showed narrowing of the interlobular arteries (Starzl *et al.*, 1965). Furthermore, changes in the basement membrane of glomerular capillaries have been described (Hamburger *et al.*, 1964). Presumably both are manifestations of incompatibility, which may limit the useful life of the transplant and continue to be a problem until identification of the stronger human histocompatibility antigens permits some form of selection of donors.

Because of these uncertainties about the future of renal transplants, even those from closely related living donors, we have continued to use only cadaver kidneys.

Procedure

Selection of Recipients

Patients selected for inclusion were those with irreversible renal failure under the age of 46 who could reliably measure their fluid balance and take their medication. Renal function in these patients was insufficient to maintain them without dialysis. Uncontrollable sepsis was a contraindication to transplantation; hypertension was not.

Pre-operative Treatment

All patients were maintained on dialysis pre-operatively; all but one on peritoneal dialysis. Peritoneal dialysis was performed at night with a rest period in the day. Although some patients had early periods of intermittent dialysis, this was performed nightly after starting azathioprine (Imuran) treatment to diminish the accentuated marrow-depressive effects of that drug. Dialysis catheters were changed only when they became blocked, and were closed with disposable plastic spigots when not in use. Initially tetracycline was used routinely in the dialysis fluid, but later in the series this was omitted. Bilateral nephrectomy before transplantation was carried out in Cases 6 and 14 because of difficulty in controlling hypertension with drugs alone. In Case 17 nephrectomy before transplantation was undertaken because this patient's polycystic kidneys were large enough to interfere with transplantation in the iliac fossa.

Selection of Donors and Removal of Kidneys

The donors of kidneys used in this series were all normotensive patients aged 15–44 and without evidence of kidney disease or known urinary-tract infection. To avoid transplanting diseased kidneys it is essential to have knowledge of the clinical state of potential donors in the few days before death. Prolonged hypotension prior to death led to the exclusion of many potential donors. Although donors with cerebral neoplasms were used, patients with other malignancies were usually avoided because of the possibility of transmission of the tumour to the recipient (McIntosh *et al.*, 1965). The only matching of donor and recipient was the requirement for ABO blood-group compatibility. The majority of donors did not have indwelling urethral catheters, as this was thought to be a source of infection. Where urethral drainage by catheter was used it was always into closed sterile plastic urine-bags. At the moment of death 20,000 units of heparin were given intravenously. On declaration of death by the clinicians in charge of the patient the donor was removed to a suitable operating area. The abdominal skin was sterilized, and, with full surgical asepsis, the abdomen was opened by a transverse supraumbilical incision. Both ureters were divided at the pelvic brim and dissected towards the kidney. As it is difficult to define the blood-supply of the ureter after death, tissue close to the ureter

* From St. Mary's Hospital, London.
† Lecturer, Medical Unit.
‡ Registrar, Medical Unit.
§ Late Registrar, Medical Unit.
|| Surgeon, St. Mary's Hospital.
¶ Lecturer, Department of Bacteriology.
** Reader, Department of Pathology.
†† Professor of Medicine.

was not dissected. The two kidneys and segments of aorta and vena cava were then removed; the great vessels were divided longitudinally and the two kidneys placed in sterile nylon bags, which were then immersed in an ice-and-water mixture. Perfusion of the kidneys with 250 ml. of Ringer's solution containing albumin 12.5 g./l. and heparin 5,000 units/l. at 4° C. was used only in Cases 11 and 19.

The average time for the whole procedure was 18 minutes from the death of the patient. The kidneys were then transported to the operating theatre and kept cold until required.

Operative Techniques

At the time of transplantation bilateral nephrectomy and splenectomy were performed. Splenectomy was undertaken, following the suggestion of Hume and his associates (1964) that this procedure permitted larger doses of azathioprine to be used.

In order to reduce delays to a minimum the recipient was anaesthetized as soon as the removal of the donor kidney began, and surgical exposure of the recipient's right iliac fossa by the conventional oblique incision proceeded when the donor kidney was found suitable for transplantation.

Anastomosis of the renal vessels to the iliac vessels was performed by the standard technique (Küss *et al.*, 1951). The renal capsule was, however, incised in all cases at the time of transplantation. Two methods of ureteric anastomosis were used. One was a mucosa-to-mucosa anastomosis in the region of the trigone after bringing the ureter obliquely through the bladder-wall. The other was to pull the donor ureter through the lower end of the recipient ureter after removing a small cuff of bladder mucosa. The ureter was anastomosed to the bladder by mucosa-to-mucosa sutures.

The left kidney and spleen were removed transperitoneally through a left subcostal incision. The oblique incision in the right iliac fossa was used for the right nephrectomy.

Drainage of both ureters and bladder was carried out, catheters being left in position for 4 to 24 days. This time was made as short as possible because of the danger of infection, and in several patients an attempt was made to omit ureter drainage completely, draining only the bladder for two or three days. In two patients, however, blockage of the ureter with debris during periods of low urine flow required the reinsertion of a ureteric catheter. At present a soft vinyl catheter of 2 mm. bore is left in the ureter, secured in position by a fine catgut stitch to the bladder trigone, and brought out through a stab incision in the anterior abdominal wall. The ureteric catheter is removed when a satisfactory flow of urine has been established, the bladder catheter being removed one or two days later.

Immunosuppression

Azathioprine 1–1.5 mg./kg. was given for 2 to 120 days before transplantation. During operation 200 mg. of hydrocortisone was given intravenously, with a further 600 mg. in the next 12 hours. As soon as possible on recovery from anaesthesia each patient was given 5 mg./kg. of azathioprine and 20–30 mg. of prednisone daily. In the presence of good renal function the azathioprine and prednisone were continued until the first rejection episode. It was found necessary to modify the above regime in patients with acute tubular necrosis. The large doses of azathioprine could not be tolerated in the absence of good renal function, but such patients have recently been maintained satisfactorily on 1.5 mg./kg. until renal function has improved. Peritoneal dialysis was rapidly reinstituted in the absence of good renal function and continued until recovery. The dose of prednisone was not lowered during periods of poor renal function.

Recognition of Rejection Episodes

Several criteria for the recognition of rejection episodes were used. An increase in small mononuclear cells with clear cytoplasm, similar to those described by Kauffman *et al.* (1964) and often associated with cellular casts, was always regarded as a rejection episode. It was absent in only one episode. These cells were recognized in the centrifuged deposit of fresh morning urine stained with aqueous 0.5% methylene blue. An increased number of tubular cells, though often present, was not considered to be diagnostic. The additional presence of two or more of the following was also regarded as evidence of rejection: decrease in urine volume, osmolarity or creatinine clearance, falling urinary urea and sodium concentration, increasing blood urea, proteinuria, fever, tachycardia, and tender enlargement of the kidney.

Treatment of Rejection Episodes

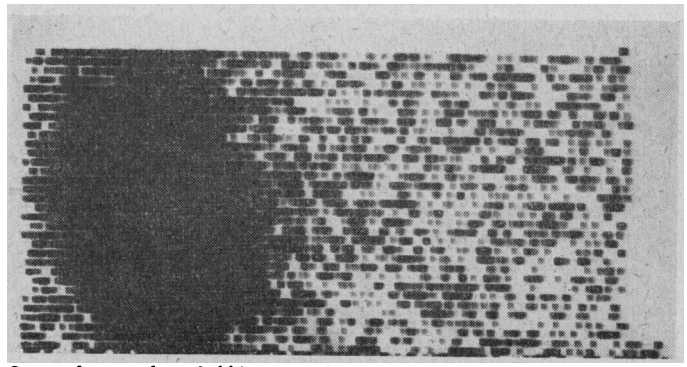
When the first rejection episode occurred the azathioprine was increased to 10 mg./kg. and the prednisone to 200 mg. daily. A single dose of 400 μg. of actinomycin C (Sanamycin) was given intravenously. After five days the azathioprine was reduced to 3 mg./kg. and the prednisone dosage lowered to 50–75% of the previous dose at five- to seven-day intervals. In subsequent rejection episodes the azathioprine was increased to 6 mg./kg. daily for five days and then reduced to 3 mg./kg., but otherwise the second and subsequent episodes were treated in the same way as the first. If the first rejection episode occurred before recovery from acute tubular necrosis the azathioprine was only increased to 3 mg./kg. for five days.

General Post-operative Regime

To diminish muscle-wasting, patients were mobilized as soon as possible, usually on the second or third post-operative day, and were most forcefully encouraged to exercise. They were not confined to their rooms for more than two weeks.

Oliguria

The frequent occurrence of oliguria with cadaver kidneys meant that the differential diagnosis in the early post-operative period between tubular necrosis, obstruction, and vascular thrombosis assumed great importance. We found the use of scintillation scanning of the transplanted kidney, using ^{125}I-labelled Hippuran (see Fig.), of great value in determining the presence of blood-flow. As a routine, intravenous pyelograms were performed within the first few weeks after transplantation for the early detection of obstruction.



Scan of transplanted kidney 72 hours after operation when Case 19 was anuric; taken after 100 μC ^{125}I Hippuran had been given intravenously, showing good concentration, indicating a patent blood-supply.

Control of Infection

Nose, throat, vaginal, and skin swabs, faeces, and a mid-stream specimen of urine were examined upon admission and two to three times weekly thereafter, together with swabs from the peritoneal catheter site and surgical incisions. After transplantation urine and peritoneal dialysate were examined daily. All potentially pathogenic bacteria and yeasts isolated were identified and their antibiotic sensitivities determined by the paper disk technique. For all infecting organisms the minimum inhibitory and bactericidal concentrations of appropriate antibiotics were measured in the presence of 20% pooled human serum by the tube-dilution method.

Patients carrying *Staphylococcus aureus* or *Candida* spp. in the nasopharynx were treated with antibacterial nasal cream or nystatin mouth-washes respectively. The skin surrounding the peritoneal catheter site was treated with antibiotic spray and antifungal dusting powder. Prior to urethral catheterization, chlorhexidine jelly (1/2,000) was introduced into the urethra, and 20 ml. of chlorhexidine solution (1/5,000) was instilled into the bladder afterwards and daily until removal of the catheter. Bactericidal antibiotics were used for the treatment of infection. Dosage was controlled by determination of antibiotic blood levels and adjusted to given peak serum levels at least two to five times the bactericidal level *in vitro*. Because of toxicity, daily estimations were made when using kanamycin or Colomycin (colistin methane sulphonate). When peritoneal dialysis was necessary administration of antibiotics was by incorporation in the dialysate, but systemic treatment was also given for the first two to three days, until the infection was controlled. For two weeks after transplantation patients were barrier-nursed in isolation.

Out-patient Care

After discharge from hospital the survivors were seen at intervals of up to two weeks. This was considered essential, particularly in the first six months, so that rejection episodes could be treated promptly. In patients who lived far from London intermediate attendances at local hospitals were used between visits here. The patients were instructed to telephone us immediately if their urine volume diminished appreciably or they became ill in any way.

Results

The clinical course of the patients is summarized in Table I. Eight patients in this series are alive and well at times ranging from three to twenty months after cadaveric renal transplantation. We present a brief summary of each of them.

Case 2, male aged 17, presented in terminal uraemia due to chronic pyelonephritis. Early post-operative progress was uneventful, but later he developed marrow depression and jaundice (see below). At present, at 586 days, he is very well, working full-time as an optical-lens maker. Recently he successfully competed in a long-distance walk.

Case 3, male aged 37, had been uraemic for three years with severe peripheral neuropathy but had not required blood transfusion, possibly owing to the influence of the polycystic disease. At operation the donor kidney had double renal arteries, which were anastomosed to the common iliac and internal iliac arteries respectively. Right nephrectomy was delayed until 31 days, because its size caused initial technical difficulty. This wound became infected and required resuture. He returned to work but became depressed, a recurrence of a depressive illness which had occurred many years earlier; he was given E.C.T. at days 185 to 201 post-transplantation. He is now successfully managing a shoe-shop, his neuropathy has disappeared, and he is very well at 466 days.

Case 5, female aged 24, presented in 1958 with albuminuria in pregnancy. In 1964 she developed malignant hypertension and uraemia due to chronic pyelonephritis. Operation and post-operative course were uneventful. She had only one rejection episode, and at 412 days is very well, looking after her husband and two children.

Case 13, male aged 37, presented with nephrotic syndrome due to Type II glomerulonephritis, and had been unable to work for four years. After operation he returned home, but at 86 days he developed a transient pulmonary infection of uncertain origin, diffuse mottling being shown in the chest *x*-ray film. Ureteric obstruction associated with *Aspergillus fumigatus* developed at 210 days. This and the results of renal biopsy are discussed below. He was then treated at days 239 to 268 with a total of 164 mg. amphotericin B, given in divided doses intravenously at out-patient visits. Since then there has been no evidence of further renal damage. Tests for precipitating serum antibodies to *Aspergillus fumigatus* were kindly carried out by Dr. I. G. Murray (Mycological Reference Laboratory, London School of Tropical Hygiene and Medicine); they were negative at 100 days, strongly positive at 230 days, but after treatment had fallen at 270 days. At 301 days he is very well and running his building firm.

Case 16, male aged 25, presented with severe uraemia due to Type I glomerulonephritis. Post-operatively there was a long period of anuria and oliguria due to tubular necrosis because the transplanted kidney had a "warm ischaemia time" of 113 minutes. He required peritoneal dialysis for 20 days and was the first patient to be maintained on the lowered dose of azathioprine (1.5 mg./kg.). He left hospital at 60 days, and has subsequently returned to full-time heavy manual labour. He is very fit at 204 days, but has slight hypertension.

Case 18, female aged 22, for a year preceding transplantation required increasingly frequent hospital admissions because of renal failure due to chronic pyelonephritis. On the second post-operative day the ureter became blocked by thrombus, which required exploration and reimplantation into the bladder. Thereafter progress was uneventful. She is working as a shop assistant, and is well at 139 days.

Case 19, female aged 30, had a long history of pyelonephritis, and was the only patient to have been maintained pre-operatively on haemodialysis. At first the kidney produced urine at a rate of 400 ml./hour, but two days later she became anuric. A ^{128}I Hippuran scan (see Fig.) confirmed the presence of blood flow, and a retrograde pyelogram excluded obstruction. After 36 hours urine flow gradually returned, and progress thereafter was uneventful. She is well at 104 days, and has returned to Ireland to care for her aged father.

Case 20, male aged 14, developed acute nephritis in September 1964, rapidly became oliguric, and renal function did not recover. He was maintained on peritoneal dialysis for six months, and became totally withdrawn with anorexia nervosa. Post-operatively he was hypothermic for four days, and needed a respirator for a week. At this time he developed a *Ps. pyocyanea* pneumonia with a positive blood culture while receiving Colomycin (6 mg./kg./day), but this responded on increasing the Colomycin dose to 12 mg./kg./day. Now he is home and well at 97 days.

By reference to Table I it can be seen that 8 of the 20 patients died in the first three weeks after renal transplantation. With the exception of Cases 4, 7, and 14, this was due to infection in those who had acute tubular necrosis or vascular or ureteric obstruction in the transplant. In Cases 16, 18, and 20 the dose of azathioprine was reduced to 1.5 mg./kg. as soon as poor renal function became apparent. Using this modified regime, these patients with acute tubular necrosis survived the early period on peritoneal dialysis until good renal function was restored.

Of a total of 23 renal transplants 14 had poor initial function. In the majority of these cases acute tubular necrosis was responsible. In the presence of a low urine flow obstruction of the ureter with thrombus and debris became an additional problem. Of all the factors which could have contributed to the appearance of acute tubular necrosis in the transplant the total time the kidney was warm without a blood supply and not cooled in ice and water correlated best with the presence of the necrosis. Table II shows that there was only one exception to the observation that total warm times of less than 85 minutes were associated with good initial function. The exception was the second graft in Case 15, transplanted three months

TABLE I.—*Twenty Cases of Cadaveric Renal Homotransplantation performed at St. Mary's Hospital between 4 October 1963 and 26 April 1965*

Case No.	Hosp. No. Sex, Age	Date of Transplant	Ischaemia Time (min.)		Donor		Recipient		Outcome	Present Renal Function		Present B.P. (mm. Hg)
			Warm	Cold	Sex, Age Disease	Blood Group	Blood Group	Disease		Ccr (ml./min.)	Blood Urea (mg./100 ml.)	
1	589710 M 22	4/10/63	46	0	F 40 Cerebral haemorrhage	O+	O+	Bilateral hydronephrosis due to bladder-neck obstruction	Bladder-neck repaired at time of transplant. Oliguria at 101 days: treated as rejection. Died at 116 days. At necropsy rupture of pelvis of transplant. Pulmonary aspergillosis and cytomegalic inclusion disease			
2	592727 M 17	24/12/63	61	49	F 41 Head injury	O+	B+	Chronic pyelonephritis	See text Alive at 586 days	132	50	140/85
3	573806 M 37	21/4/64	125	0	M 43 Rheumatic carditis; water intoxication	A+	A−	Polycystic disease	See text Alive at 466 days	100	52	115/60
4	595121 F 19	16/6/64	40	50	M 21 Ruptured cerebral haemangioma	O+	O+	Chronic type I glomerulonephritis	Ureteric bleeding and obstruction. Re-explored. Died at 4 days. At necropsy A.T.N. with evidence of tubular repair			
5	599188 F 24	16/6/64	64	61	M 21 Ruptured cerebral haemangioma	O+	AB+	Chronic pyelonephritis	See text Alive at 412 days	101	41	130/80
6a	604015 M 19	27/6/64	148	7	M 23 Post-operative cardiac arrest	O−	A+	Chronic type I glomerulonephritis	Pelvis perforated by catheter; transplant removed at 3 days			
6b		30/6/64	83	42	M 33 Subarachnoid haemorrhage	O−			Second transplant functioned well. Perforated acute peptic ulcer on day 24. Candida wound and urinary infections at 50 days. Died from septicaemia and peritoneal abscesses at 74 days. At necropsy transplant showed vascular lesions			
7a	604893 M 26	28/7/64	95	45	M 44 Cerebral aneurysm	A+	A+	Chronic type I glomerulonephritis	A.T.N. Transplant removed at 2 days			
7b		30/7/64	70	30	F 22 Glioma	A+			Massive diuresis from second transplant. Twenty-four hours later developed uncontrollable fits and hyperpyrexia. At necropsy cerebral oedema and staphylococcal bronchopneumonia			
8	608025 M 32	31/7/64	67	38	M 18 Cerebral abscess	O+	O+	Chronic type I glomerulonephritis	Necrosis of tip of ureter on day 4. Repaired by bladder flap. Died from *Ps. pyocyanea* septicaemia at 14 days			
9	595177 F 17	6/9/64	60	25	M 28 Head injury	O+	O +	Chronic pyelonephritis	Two renal arteries: one to upper pole thrombosed at time of transplant. Developed urinary infection with *Candida tropicalis* at 6 days. Died from disseminated candidiasis and *Ps. pyocyanea* septicaemia at 28 days			
10	607920 M 21	6/9/64	74	30	M 28 Head injury		A+	Chronic glomerulonephritis	Anuria. Re-explored at 2 days and clot removed from ureter. At 4 days thrombosed iliac and renal veins. Infarcted transplant removed. Died at 8 days from pulmonary emboli and *Ps. pyocyanea* peritonitis			
11	597660 M 40	16/9/64	114	36	F 41 Carcinoma of stomach	O+	A+	Chronic pyelonephritis	Severe A.T.N. Azathioprine treatment led to marrow hypoplasia. Died at 20 days from haemorrhage due to *Candida albicans* enterocolitis. Transplant showed tubular repair			
12	608981 M 44	4/10/64	57	58	M 22 Head injury	O+	A−	Type II glomerulonephritis	No function. Renal vein thrombosis following ureteric obstruction by blood clot. Transplant removed at 4 days. Patient died at 8 days from pulmonary emboli and staphylococcal peritonitis			
13	609839 M 37	4/10/64	50	73	M 22 Head injury	O+	O+	Type II glomerulonephritis	See text Alive at 300 days	44	52	135/95
14	609708 M 13	31/10/64	104	141	M 40 Carcinoma of bronchus	A−	A+	Chronic type I glomerulonephritis	Severe A.T.N. Became hyperkalaemic and died 12 hours later from cardiac arrest			
15a	609833 M 17	31/10/64	109	59	M 40 Carcinoma of bronchus	A−	A−	Chronic pyelonephritis	Severe A.T.N. followed by renal vein thrombosis. Transplant removed at 12 days. Maintained on peritoneal dialysis for 78 days, with recurrent infection, peritonitis, pericarditis, and an empyema			
15b		29/1/65	41	0	M 16 Pneumococcal meningitis	A+			Second transplant also had A.T.N. At 3 days cardiac arrest. At necropsy large chronic *Ps. pyoancyea* empyema			

TABLE I.—*(continued)*

Case No.	Hosp. No. Sex, Age	Date of Transplant	Ischaemia Time (min.) Warm	Ischaemia Time (min.) Cold	Donor Sex, Age Disease	Donor Blood Group	Recipient Blood Group	Recipient Disease	Outcome	Present Renal Function Ccr (ml./min.)	Present Renal Function Blood Urea (mg./100 ml.)	Present B.P. (mm. Hg)
16	614885 M 25	30/12/64	113	108	M 43 Cirrhosis	O−	A+	Chronic type I glomerulo-nephritis	See text Alive at 204 days	100	64	160/95
17	588125 F 46	10/3/65	38	47	F 41 Astrocytoma	O−	O+	Polycystic disease	Ureter blocked by clot on day 2, necessitating re-exploration. Became pyrexial on day 53 and died from pulmonary emboli at 90 days with good renal function. At necropsy pulmonary aspergillosis and cytomegalic inclusion disease			
18	617860 F 22	15/3/65	106	28	F 15 Head injury	O+	O+	Chronic pyelo-nephritis	See text Alive at 139 days	54	22	120/80
19	619961 F 30	19/4/65	61	83	M 44 Glioma	O+	O+	Chronic pyelo-nephritis	See text Alive at 104 days	65	41	125/80
20	614822 M 14	26/4/65	85	57	F 34 Asthma	A+	A+	Chronic type I glomerulo-nephritis	See text Alive at 97 days	34	22	135/100

A.T.N. = Acute tubular necrosis.

after the first. This kidney was extremely slow to become pink after the blood flow was re-established, and renal arterial spasm was apparent for about 1½ hours afterwards. The contralateral donor kidney did not show evidence of acute tubular necrosis but did show "osmotic nephrosis"—that is marked vacuolation of tubular cells, attributable to intravenous mannitol, fructose, and urea given four hours before death. Despite this exception it seems that acute tubular necrosis will occur if the warm time is in excess of 85 minutes, but rarely if it is below that time.

The causes of failure listed in Table I do not include uncontrolled rejection of the kidney, which was not seen, though multiple rejection episodes, shown in Table III, occurred at time intervals similar to those found by Starzl and his colleagues (1963, 1964), using live donors. The late episode in Case 3, at 310 days, appeared to be rather slow in onset but otherwise had the usual criteria of a rejection episode as cited above. With treatment there was restoration to previous function, but proteinuria persisted. The other late episode, in Case 13, followed a renal biopsy at 210 days when a ureteric stenosis was being resected. Only Case 5 had a single episode, a minor one.

TABLE II.—*Correlation Between Warm Ischaemia Time and Evidence of Acute Tubular Necrosis (A.T.N.) in Cadaveric Renal Transplants*

Renal Homograft No.	Warm Ischaemia Time (min.)	Clinical Evidence of A.T.N.
6a	148	Moderate
3	125	"
11	114	Severe
16	113	"
15a	109	"
18	106	Moderate
14	104	Severe
7a	95	Moderate
20	85	"
6b	83	None
10	74	"
7b	70	"
8	67	Mild
5	64	None
19	61	"
2	61	"
9	60	"
12	57	"
13	50	"
1	46	"
15b	41	Moderate
4	40	None
17	38	"

Fibrous thickening of the intima lining the arterioles and interlobular arteries of the renal homograft was found in the biopsy obtained 210 days after transplantation from Case 13 and at necropsy on Case 6b. In both instances this change was accompanied by rupture of the internal elastic lamina of the interlobular arteries, by tubular atrophy, and by focal interstitial fibrosis and infiltration with mononuclear cells, about 5% of which were pyroninophilic. Similar obliterative changes were present in the arterioles and arteries of the ureters and had caused ischaemia of the ureteric wall. In Case 13 invasion of the ischaemic wall by *Aspergillus fumigatus* had occurred. In patient No. 1, who died 116 days after transplantation, vascular lesions were absent from the kidney but present in the vessels of the ureter and pelvis. Failure in this case was due to extravasation of urine from rupture of the ischaemic pelvis.

All patients, with the exception of Case 3, who had polycystic disease, required frequent blood transfusions while being dialysed awaiting transplantation. Post-operatively there was a brisk reticulocyte response, usually starting between 20–35 days, and haemoglobin rapidly reached normal levels.

Sternal-marrow examinations in five out of seven patients showed the presence of changes of a megaloblastic type, and in four patients this led to the development of anaemia, probably due to folic acid deficiency. Serum folate levels were frequently low and urinary formiminoglutanic acid (Figlu) excretion

TABLE III.—*Timing and Frequency of Rejection Episodes in Patients who Survived the Immediate Post-operative Period after Cadaveric Renal Transplantation*

Case	Time of Rejection Episodes in Days															
	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–150	150–200	200–250	250–300	300–350	350+
1	1		1			1		1								
2	1				1					1						
3			1			1		1		1					1	
5		1														
6		1		1		1										
9		1														
13	1		1			2							1			
16		1			1				1			1				
17	1				1											
18	1							1								
19	1		1		1											
20	1		1	1				1								

after 15 g. histidine was raised (Table IV) (Chanarin, 1963 ; Chanarin and Bennett, 1962). In Case 2 jaundice and anaemia with severe megaloblastic changes in the marrow occurred at five months. Although the serum folate and vitamin B_{12} concentrations were normal, the urinary Figlu excretion was very high at 900 mg. in eight hours. For this reason the dose of azathioprine was reduced and he was given a five-day course of oral folic acid and parenteral folinic acid, with rapid rise in haemoglobin and neutrophil count and disappearance of jaundice but without a rejection episode occurring.

TABLE IV.—*Showing Relationship Between Peripheral Blood Picture, Sternal Marrow, and Folate and Figlu Levels after Transplantation*

Case No.	No. of Days after Transplantation	Figlu mg. in 8 hr. after 15 g. Histidine	Serum Folate (μmg./ml.)	Hb (g.)	Reticulocytes (%)	W.B.C./c.mm.	Sternal Marrow
2	164	900*	9	11·4	2	3,000	
	170			10·4	1·6	2,000	Depressed granulopoiesis. Megaloblastic
	198			10·1	7·6	9,000	Normal
	204	34		11·2	6·3	7,000	
	212		>50	12·9	5·9	3,500	Normal. Active
	416	20		16·2	1·1	9,000	
5	36			11·7	2·7	6,000	Normoblastic. Very active marrow
	43	0	5·5	11·7	1·9	10,000	
6	19	163	5	8·3	1·1	7,000	Megaloblastic
13	26			9·5	2·7	8,000	Normal
	41	32		10·0	8·5	14,000	
	49	38		11·6	4·5	12,000	
	72	46	2·5	10·7	9·7	16,500	Active. Normal
	108	335*	4	10·7	5·1	9,000	
	127			12·2	4·9	8,000	Early megaloblastic change
	144	33	4	12·4	1·9	13,000	
	172	19		12·4	2·6	13,000	
16	41	49		11·2	7·1	15,000	
	54		2	12·1	8·2	12,000	
	100	29		14·6	11·5	9,000	
17	20	374*		9·3	3·0	2,500	
	30	83*		8·6	11·6	5,000	
	61	440*	3	9·3	1·6	2,500	Normoblastic
18	25	54*	2·5	8·3	3·3	1,500	
	56	59	4	8·6	3·5	6,000	Megaloblastic
19	21	13	4	9·0	1·3	22,000	
	81	36		12·1	2·2	14,000	
20	82	76		6·8	2·4	3,000	

* Predominantly urocanic acid.

Raised serum bilirubin levels were seen in five patients. In Cases 1 and 2 this appeared to be related to a high azathioprine dose. Case 1 had marked centrilobular cholestasis at necropsy. Jaundice was reversed in Case 2 with folic and folinic acid —see above.

Arthritic lesions and rashes of the type described by Waller *et al.* (1965) in patients with a renal homograft, treated by azathioprine, were not seen in this series.

A further complication of treatment occurred in patient No. 6, who had a perforated duodenal ulcer while on a high dose of prednisone.

The surviving patients all had hypertension of varying severity before transplantation, but only three out of the eight had mild hypertension subsequently, readily controlled by drugs (see Table I).

Of the 12 who died infection was the main cause of death in Cases 1, 6, 8, 9, and 11 and a contributory factor in Cases 7, 10, 12, 15, and 17. Fourteen patients had infection of the peritoneal cavity, 13 of the urinary tract, 5 of wounds, and 6 of the lungs. Before transplantation peritoneal infections developed in 9 of the 16 patients dialysed for 2–12 weeks, and after transplantation in 9 out of 11 who were dialysed. In four of the latter (Cases 8, 9, 10, and 15), all due to *Ps. pyocyanea,* death followed within a few days despite treatment with Colomycin. Bacteriuria was present in seven patients before operation but was eradicated in six. Thirteen patients survived for more than 14 days with a functioning kidney, and all of these developed a significant bacteriuria. In 12 this originated during the period when indwelling catheters were in use. In Case 9 a *Candida tropicalis* infection disseminated from a polar infarct in the transplant. In three patients the bacteriuria persisted for many weeks despite treatment, and in two others emergency operations for leakage of urine from the ureter were immediately followed by circulatory collapse due to Gram-negative bacteriaemia originating from the urinary tract. Severe and recurrent wound-infection developed in Cases 1, 3, 6, 9, and 15.

In addition to Case 9 systemic fungal infections have been found in a further five patients. In Cases 1 and 17 necropsy revealed the presence of multiple small pulmonary abscesses due to *Aspergillus fumigatus.* Cytomegalic inclusions were also present. In Case 13 an episode of pulmonary infection 86 days after operation resembled that ascribed by Rifkind *et al.* (1964b) to cytomegalovirus infection. This patient later presented at 210 days with invasion of the ureteric wall by *Aspergillus fumigatus* at the site of a stenosis. Patient No. 11, who died from gastro-intestinal haemorrhage, had an ulcerating enterocolitis and a thyroid abscess due to *Candida albicans.* Candida ileitis was also present in Cases 1 and 6.

Most of the bacterial infections, except those due to *Ps. pyocyanea,* were eradicated by treatment with antibiotics, although the administration of kanamycin caused impairment of hearing in two patients. The reasons for the unsatisfactory response of *Ps. pyocyanea* infection to Colomycin may be related to its poor diffusibility. Addition of other antibiotics to the peritoneal dialysate was followed within two to three days by equilibration with the blood, but, with Colomycin, the level of free (non-protein-bound) serum antibiotic remained much lower than that in the dialysate even in the presence of an inflamed peritoneum.

Discussion

The improved survival rate in this series, compared with the report of 1963 (Porter *et al.*, 1963), would seem to justify the continued use of cadaver material. The encouraging accounts of cadaveric renal transplants, some showing survival with good renal function for periods of up to 15 months, that have recently been published from other centres support this view (Merrill *et al.*, 1963 ; Nakimoto *et al.*, 1965 ; Hamburger *et al.*, 1965).

The improvement over earlier results with cadaver donors exceeded the hopes of many of those involved in human tissue transplantation. This may be partly explained by the postulate of Simonsen (1965) that in man there are only a limited number of alleles at a single major histocompatibility locus, and that hence a considerable fraction of cadaver non-related transplants do not transgress a major genetic barrier. Possibly one of the leucocyte antigens described by van Rood and van Leeuwen (1965) may represent such a major histocompatibility locus.

Rejection episodes encountered in this series do not seem to have been more severe or more difficult to treat than those encountered by others using renal homografts from parental and sibling donors. Patient No. 6 died as a consequence of treatment of rejection, but not from irreversible rejection. In this study all the patients received the same treatment for each rejection episode irrespective of its severity.

Obliterative vascular lesions (Porter *et al.*, 1963), which sometimes follow rejection episodes, may not become clinically apparent until one to two years after transplantation. As most of our patients have not been biopsied we cannot yet determine the incidence of this complication in the present series.

The major difficulty with the use of cadaver material, as opposed to that of live donors, is the management of patients who have had transplants with acute tubular necrosis. This has led to the death of a number of patients in the present and

other series. However, the risks of acute tubular necrosis after transplantation can be decreased by limiting the total warm time to 85 minutes and by reducing azathioprine dosage to 1.5 mg./kg. daily while the kidney is oliguric.

Although most groups performing renal homografts from live donors use only urethral drainage, we have found that a ureteric catheter is helpful for the first few days post-operatively because it allows free drainage of the small volume of urine often secreted by kidneys with temporary ischaemic damage, and it can be used to flush out the debris which sometimes blocks the ureter. It also acts as a support for the ureteric anastomosis, which is especially vulnerable in cadaveric transplants because the ureteric blood-supply is poorly defined and easily damaged during removal of the kidney. In addition, retrograde pyelography can easily be performed when obstruction by clot is suspected. On the other hand, early removal of indwelling catheters from the urinary tract is essential. Despite prophylactic measures 12 of the 13 patients who survived the immediate post-operative period of 14 days developed bacteriuria. This infection originated during the period of catheterization, but in most at least six days had elapsed before infection appeared.

Death from septicaemia after renal homotransplantation comprises an appreciable proportion of the early failures in most series despite prophylactic antibiotic treatment or maintenance by haemodialysis (Hume *et al.*, 1955 ; Rifkind *et al.*, 1964a ; Dunea *et al.*, 1965). Uraemia (Shreiner and Maher, 1961), treatment with steroids (Thomas, 1953 ; Torack, 1957) and cytotoxic drugs (Lannigan and Meynell, 1959) have all been thought to reduce host defence mechanisms. These factors may account for the frequency in this series of fungal or viral infection ordinarily seen only in leukaemia or carcinomatosis treated with steroids and cytotoxic drugs. However, our patients had abdominal incisions and indwelling catheters, which offer an easy portal of entry for bacteria. In the absence of a control group of patients submitted to similar procedures but not receiving immunosuppressive therapy, any increased susceptibility to infection cannot be assessed.

Prophylactic antibiotics were not used, because our previous experience had shown that adding antibiotics to the dialysate led to infection by resistant organisms without reducing the incidence. Furthermore, there were 32 occasions in these patients when reinfection occurred by organisms resistant to antibiotics used for treatment of a prior infection. Our results suggest that the great majority of infecting organisms were derived from the patient's own flora. Of 15 patients who were admitted with or acquired carriage of *Staphylococcus aureus* nine subsequently developed a clinical infection with staphylococci of the same phage type. Five non-carriers did not develop staphylococcal infections. *Ps. pyocyanea* was isolated from the stools or nasopharynx of seven patients, and six subsequently developed infection by this organism either alone (Cases 8, 10, 15, and 20) or in combination with others (Cases 6 and 9). The infection was the main or a major contributory cause of death in five. *Candida* spp. were isolated from 10 patients, and disseminated infection occurred in five of these, being unsuspected during life in three. It would seem reasonable to limit chemoprophylactic measures to the eradication of organisms such as *Ps. pyocyanea* and candida from the patients' environment and endogenous flora, because treatment of disseminated infection caused by them is rarely successful.

The importance of surgical technique has been emphasized (Dunea *et al.*, 1965), and in this study most of the severe and fatal infections rapidly followed further operations which had to be undertaken shortly after transplantation in patients who also required the reinstitution of peritoneal dialysis. In patients who had had recent operations and dressings over abdominal wounds, it is difficult to re-sterilize the skin before reintroducing a dialysis catheter. The catheter present before operation is now left *in situ* and used after transplantation, if necessary. If renal function is adequate the catheter is removed without delay. There has not been a fatal infection since this change. The severity of peritoneal infections after transplantation was partly due to the nature of the causative organisms. Infections were mostly due to *Staphylococcus albus* (seven out of nine) before transplantation, but to Gram-negative organisms, usually associated with bacteriaemia, afterwards (seven out of nine). Gram-negative bacteriaemia is associated with a high mortality (McCabe and Jackson, 1962), and successful treatment is difficult. High antibiotic blood levels may be necessary (Brumfitt *et al.*, 1964), and when using kanamycin and Colomycin are toxic. In this study, with the exception of *Ps. pyocyanea*, many severe infections were eradicated, and it is suggested that early diagnosis by continuous bacteriological surveillance and laboratory control of antibiotic treatment is vital.

In Case 2 folinic acid apparently counteracted the effect of an excessive dose of azathioprine when he had marrow depression and jaundice. This treatment did not produce a rejection episode. In a similar patient with marrow depression and megaloblastic erythropoieses, described by Dossetor *et al.* (1964), a marrow response was thought to be due to vitamin B_{12} therapy. In their patient, however, the serum folate was normal and the cell folate low, suggesting a period of dietary folate deficiency in the period preceding transplantation. Both in this patient and in Case 2 of the present series the serum vitamin B_{12} was normal. Azathioprine is chemically dissimilar from folic acid, being an imidazole derivative of mercaptopurine (6-MP), but the structures of methotrexate (amethopterin) and folic acid are very similar. The reversal of methotrexate marrow toxicity by folinic acid therapy is well recognized (Berenbaum, 1965), and it is known that methotrexate acts as a competitive inhibitor of folic acid reductase. This prevents the reduction of folic to folinic acid (tetrahydrofolic acid), a nicotinamide-adenine-dinucleotide (N.A.D.) dependent reaction. After conversion to mercaptopurine *in vivo*, azathioprine may act by interference with the availability of N.A.D.H. (the reduced form of N.A.D.) as a hydrogen donor for the reduction of folic acid. Alternatively, a hypothetical mercaptopurine analogue of N.A.D. could interfere directly with N.A.D.-N.A.D.H. systems. In support of these hypotheses Lambe and Williams (1965) have shown that mercaptopurine interferes with one N.A.D.-dependent dehydrogenase reaction. The high urinary Figlu excretion values we have seen (Table IV) may be taken as further evidence that azathioprine interferes with folic acid metabolism.

Summary

Twenty patients in terminal renal failure were treated between 4 October 1963 and 26 April 1965 by bilateral nephrectomy, splenectomy, transplantation of a kidney from a cadaver, and administration of the immunosuppressive drugs azathioprine and prednisone. With the exception of ABO blood-group compatibility, donor and recipient were unmatched. Eight of the patients are alive and well with transplants that have been functioning for from three months to one year eight months.

Rejection episodes occurred in all patients who survived the immediate period after transplantation but were all controlled by increased doses of the immunosuppressive agents and by the addition of actinomycin C.

Acute tubular necrosis was encountered in 61% of the renal homografts. This was related to the time during transplantation that the kidney was without a blood-supply and not cooled, rather than to the total period of ischaemia. Early function was good in cases where this "warm" time was less than 85 minutes.

When there was poor initial function the toxicity of azathioprine was increased. This toxicity was reduced but adequate immunosuppression still obtained when the dose of azathioprine was lowered to 1.5 mg./kg. daily.

The major cause of death of patients in this series was infection. The nature and source of the infecting organisms and the results of treatment are described.

ADDENDUM.—The renal homograft of Case 2 was recently biopsied at open operation 1 year 10 months after transplantation. Apart from a few foci of superficial cortical scarring, moderate hyperplasia of all the juxtaglomerular bodies, and glomerular hypertrophy, this kidney appeared normal. There were no vascular or glomerular changes visible on light microscopy.

We wish to thank all those clinicians at St. Mary's and other hospitals for their co-operation in providing donors, and Dr. P. Barkham, Dr. I. Chanarin, and Dr. B. J. Houghton for their help and advice. We are indebted to Sister B. Bates and Sister P. Wall and their staffs, and also Mr. Lowe and his staff, for the care and attention given to the patients. We would also like to thank Dr. P. Baker, Dr. H. M. Leather, and Dr. G. R. Steed, of Plymouth, for their help. The Board of Governors are to be thanked for their generous financial support.

REFERENCES

Berenbaum, M. C. (1965). *Brit. med. Bull.*, **21**, 140.
Brumfitt, W., Leigh, D. A., Percival, A., and Williams, J. D. (1964). *Postgrad. med. J.*, Suppl. **40**, p. 55.
Chanarin, I. (1963). *Brit. J. Haemat.*, **9**, 141.
—— and Bennett, M. C. (1962). *Brit. med. J.*, **1**, 27.
Dossetor, J. B., Gault, M. H., Oliver, J. A., Inglis, F. G., MacKinnon, K. J., and MacLean, L. D. (1964). *Canad. med. Ass. J.*, **91**, 733.
Dunea, G., Nakamoto, S., Straffon, R. A, Figueroa, J. E., Versaci, A. A., Shibagaki, M., and Kolff, W. J. (1965). *Brit. med. J.*, **1**, 7.
Hamburger, J., Crosnier, J., and Dormont, J. (1964). *Ann. N.Y. Acad. Sci.*, **120**, 558.
—— —— —— (1965). *Lancet*, **1**, 985.
Hume, D. M., Magee, J. H., Kauffman, H. M., Bower, J. D., Lee, H. M., Cleveland, R. J., and Prout, G. R. (1964). *Transplantation*, **2**, 164.
—— Merrill, J. P., Miller, B. F., and Thorn, G. W. (1955). *J. clin. Invest.*, **34**, 327.
Kauffman, H. M., Clark, R. F., Magee, J. H., Rittenbury, M. S., Goldsmith, C. M., Prout, G. R., and Hume, D. M. (1964). *Surg. Gynec. and Obstet.*, **119**, 25.
Küss, R., Teinturier, J., and Milliez, P. (1951). *Mém. Acad. Chir.*, **77**, 755.
Lambe, R. F., and Williams, D. C. (1965). *Biochem. J.*, **95**, 847.
Lannigan, R., and Meynell, M. J. (1959). *J. clin. Path.*, **12**, 157.
McCabe, W. R., and Jackson, G. G. (1962). *Arch. intern. Med.*, **110**, 847.
McIntosh, D. A., McPhaul, J. J., Peterson, E. W., Harvin, J. S., Smith, J. R., Cook, F. E., and Humphreys, J. W. (1965). *J. Amer. med. Ass.*, **192**, 1171.
Merrill, J. P., Murray, J. E., Takacs, F. J., Hager, E. B., Wilson, R. E., and Dammin, G. J. (1963). Ibid., **185**, 347.
Murray, J. E., Gleason, R., and Bartholomay, A. (1965). *Transplantation*, **3**, 294.
Nakamoto, S., Straffon, R. A., and Kolff, W. J. (1965). *J. Amer. med. Ass.*, **192**, 302.
Porter, K. A., Thomson, W. B., Owen, K., Kenyon, J. R., Mowbray, J. F., and Peart, W. S. (1963). *Brit. med. J.*, **2**, 639.
—— Peart, W. S., Kenyon, J. R., Joseph, N. H., Hoehn, R. J., and Calne, R. Y. (1964). *Ann. N.Y. Acad. Sci.*, **120**, 472.
Rifkind, D., Marchioro, T. L., Waddell, W. R., and Starzl, T. E. (1964a). *J. Amer. med. Ass.*, **189**, 397.
—— Starzl, T. E., Marchioro, T. L., Waddell, W. R., Rowlands, D. T., and Hill, R. B. (1964b). Ibid., **189**, 808.
Rood, J. J. van, and Leeuwen, A. van (1965). In *Histocompatibility Testing*, p. 21. National Academy of Sciences, Washington, D.C.
Schreiner, G. E., and Maher, J. F. (1961). *Uraemia: Biochemistry, Pathogenesis, and Treatment.* Thomas, Springfield, Illinois.
Simonsen, M. (1965). *Lancet*, **1**, 415.
Starzl, T. E., Marchioro, T. L., Porter, K. A., Moore, C. A., Rifkind, D., and Waddell, W. R. (1964). *Ann. intern. Med.*, **61**, 470.
—— —— Terasaki, P. I., Porter, K. A., Faris, T. D., Herrmann, T. J., Vredevoe, D. L., Hutt, M. P., Ogden, D. A., and Waddell, W. R. (1965). *Ann. Surg.*, **162**, 749.
—— —— and Waddell, W. R. (1963). *Surg. Gynec. Obstet.*, **117**, 385.
Thomas, L., (1953). *Ann. N.Y. Acad. Sci.*, **56**, 799.
Torack, R. M. (1957). *Amer. J. Med.*, **22**, 872.
Waller, M., Irby, R., Mullinax, F., and Toone, E. C. (1965). *New Engl. J. Med.*, **273**, 12

Probable Mode of Action of Oral Contraceptives*

EGON DICZFALUSY,† M.D.

"What is past is prologue."—Shakespeare, *The Tempest.*

Brit. med. J., 1965, 2, 1394–1399

At the Fifth International Conference on Planned Parenthood in Tokyo, Pincus (1955) reported an ovulation inhibition by progesterone or norethynodrel[1] taken orally by women. This report indicated the beginning of a new era in the history of contraception. To-day, some 10 years later, oral contraception is effectively used by at least five million women. However, the exact mechanism of action by which these compounds inhibit fertility is still incompletely understood.

Normal Control of Ovulation

A discussion of the probable mode of action of oral contraceptives might be facilitated by a brief recapitulation of the present concepts of the normal control of ovulation in the human species. In my interpretation the available evidence on this can be summarized as follows.

During each menstrual cycle the endometrium is built up gradually in order to be prepared for the nidation of the fertilized ovum. This endometrial development is regulated by ovarian steroid hormones: oestrogen during the first, or proliferative, phase, and progesterone+oestrogen during the second, or luteal, phase.

The synthesis and release of these ovarian hormones is regulated by pituitary gonadotrophic hormones—namely, the follicle-stimulating hormone (F.S.H.) and the luteinizing hormone (L.H.) (also called interstitial-cell-stimulating hormone (I.C.S.H.)). The role of the third gonadotrophin, luteotrophin (L.T.H.) (also called prolactin), in the control of ovarian activity in the human is obscure.

Under gonadotrophic stimulation an array of follicles are brought to considerable development in each cycle. However, only one of these develops into a Graafian follicle; the others become atretic and rapidly degenerate. The mechanism by which this atretic process is regulated is incompletely understood (see Sturgis, 1961).

The maturing follicle shows an increasing rate of growth during the last few days of the proliferative phase. This is associated with the production of increased amounts of oestrogenic hormones, as reflected, among other things, by an increased urinary excretion of the oestrogens produced by the ovary and of their metabolites (Brown *et al.*, 1959; Diczfalusy and Lauritzen, 1961). The increasing amount of circulating oestrogen induces characteristic morphological changes in the endometrium, vaginal epithelium, and cervical mucus (Diczfalusy and Lauritzen, 1961). The mucus becomes thin and watery and is readily penetrable by sperms.

Under additional gonadotrophic stimulation (characterized by an increased L.H. secretion of relatively limited duration) the Graafian

* Based on a paper read at the Annual Clinical Meeting of the British Medical Association, Dundee, 1965.

† From the Hormone Laboratory, Department of Women's Diseases, Karolinska sjukhuset, Stockholm, Sweden.

[1] The systematic names of the compounds mentioned in this review are given in Table I.