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Splenectomy and Thymectomy in Human Renal Homotransplantation.* (28534)

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Renal homotransplantation has been given a limited clinical trial in several centers using either cytotoxic drugs or irradiation to weaken the host immunologic potential. The results of these efforts have been discouraging, with a high early failure rate due to rejection. In a recent world survey of experience with renal homotransplantation, Goodwin and Martin found that less than 10% of the 176 known cases had survived for as long as 3 months(1). Only 6 cases had lived for as long as a year.

During the past 6½ months, 5 patients have received kidney transplants, using a somewhat different approach to the problem of host preparation. The thymus and spleen of the recipient patients were removed surgically, either prior to or on the day of renal homografting for the purpose of reducing the antibody producing tissue of the host. In addition, the patients were provided with standard anti-rejection therapy, using total body irradiation or cytotoxic drugs.

Methods. The underlying disease was chronic glomerulonephritis in 4 cases, and polycystic kidney in the fifth (Table I). In each instance, the disease had progressed to a terminal phase, and multiple hemodialyses were required in the weeks or months before transplantation. Two patients had bilateral nephrectomy before transplantation to control severe hypertension. A third patient had one kidney removed before and the other at the time of transplantation (Table I). One of the living patients still retains one of his own kidneys.

Thymectomy was performed 13 to 31 days before transplantation (Table I) through a midline sternum splitting incision, with a transverse cervical collar extension. Care was taken to remove the entire thymus, particularly the inferior bilobed portion and the superior portion which often extends to the thyroid gland. In 2 patients, splenectomy was performed at the same time as thymectomy by another surgical team. In the other 3, splenectomy was performed 19 days before, 7 days before, and on the same day as transplantation.

In addition to thymectomy and splenectomy, standard techniques to prevent rejection were used (Table II). In principle, all patients except the first one were treated in the same way. The first patient was treated with 300 R total body irradiation preoperatively, delivered with a 250 KV unit. Fifty R were given on the 17th, 14th, 11th and 5th days before transplantation, culminating in a final dose of 100 R on the day before operation. On the 4th and 6th days after transplantation, additional doses of 50 R were given, shielding the renal transplant on the second of these occasions. Despite the resultant suppression of the white count to

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TABLE I. Temporal Relation to Transplantation Date of Removal of Thymus, Spleen and Kidneys.

	Date of					
	Transplantation	Thymectomy	Splenectomy	Right nephrectomy	Left nephrectomy	Renal disease
1.	11-24-62	10-23-62	11- 4-62	11- 4-62	11- 4-62	Chr. GN
2.	1-31-63	1-17-63	1-31-63	Not done	1-31-63	" "
3.	2- 9-63	1-24-63	2- 2-63	2- 9-63	2- 2-63	" "
4.	2-25-63	2-12-63	2-12-63	2-12-63	2-12-63	" "
5.	3-26-63	2-26-63	2-26-63	Not done	Not done	Polycystic kidney

less than 500, vigorous rejection began after 2 weeks. Therapy was changed to azathioprine (Burroughs-Wellcome 57-322, "Imuran"), actinomycin C and prednisone at this time with apparent reversal of the rejection. Subsequent therapy for this patient has been similar to that described below for the other cases.

Therapy in the last 4 cases was similar, although there was some variation in the details (Table II). The basic treatment was with oral azathioprine(2). The drug was started 1 to 7 days before transplantation in a dose of 3 to 6 mg/kg/day. On the day of operation and for 2 or 3 days after, the dose was increased to 8 to 15 mg/kg/day. Thereafter, the daily dose was 3 to 6 mg/kg/day. The most stringent indication for reducing the dose was suppression of the white count.

If evidence of rejection developed, the patients were given 8 μg/kg of actinomycin C (3) intravenously, divided into 2 doses, 12 hours apart. At least 3 days were allowed to pass before giving a second course. At the time of threatened rejection, prednisone (200-300 mg/day) was also used. Two patients first received steroids with the threat of rejection, and the other 3 who were already receiving steroids had the dose increased. As soon as the threat of rejection had passed, the steroid doses were reduced to 15 to 20 mg/day, and the treatment with actinomycin C was discontinued.

All 5 kidneys were provided by living donors (Table III), either from the family (Cases 1-3) or from genetically non-related sources (Cases 4, 5). In 2 instances, the major blood groups of the donor and recipient were different. The donor patients were usually cooled to 32°C at the time of their nephrectomy. Cooled lactated Ringer's solution containing procaine and heparin was used to wash out the kidney in the 2 cases with mismatched donor-recipient blood types.

The grafts were implanted in the iliac fossa, using the technique of Murray and Harrison (4). The periods of graft ischemia in the 5 cases were 34, 28, 24, 36, and 71 minutes. The period of ischemia occurred in Case 5 as the result of cardiac arrest, which happened while performing the vascular anastomoses, and which necessitated cessation of the transplantation until cardiac resuscitation had been carried out. Ureterocystostomy was done with the method of Paquin(5). Careful attention was paid to fluid electrolyte replacement during the diuretic phase in the period immediately after transplantation. The patients were kept in an isolated room for the first few weeks after operation.

Results. Four of the 5 patients are alive, from 105 to 198 days after transplantation

TABLE II. Non-Surgical Therapy to Prevent Rejection.

	Total body irradiation		Imuran	Intermittent actinomycin C	Prednisone
	Preop.	Postop.			
1.	300 R	100 R	Starting 7th postop. day	18th postop. day	Starting 16th postop. day
2.	0		Yes	23rd & 27th postop. days	Pre & postop.
3.	0		"	None	None
4.	0		"	22nd & 23rd postop. days	Pre & postop.
5.	0		"	4th & 5th postop. days	Starting 4th postop. day

TABLE III. Results with Renal Transplants as of June 10, 1963.

	Survival (on 6-10-63)	Rejection	Renal function	Genetic relation and age of donor and recipient	Donor to recipient blood groups
1.	198 days	Severe, 15 days; reversal	Normal, except mild proteinuria	Mother (35) to son (12)	B+ —— B+
2.	130 "	Severe, 27 days; reversal	Normal	Sister (32) to brother (38)	B+ —— A+
3.	122 "	None	"	Fraternal male twins (21)	A+ —— A+
4.	105 "	Mild, 18 and 42 days; reversal	"	Wife (30) to husband (34)	A+ —— AB+
5.	Died 10 days of generalized sepsis	None evident	Improving before death	Unrelated male (42) to male (50)	A+ —— A+

(Table III). The genetic relationships of Cases 1-3 may have contributed to the prolonged homograft survival, but in Case 4 there was no blood relationship. Case 5 died of mediastinal sepsis 10 days postoperatively. This patient had required thoracotomy and cardiac massage at time of transplantation. The surviving patients all have normal renal function, except Case 1 who has proteinuria of 3 to 4 g/day. The blood urea nitrogen (BUN) values are less than 20 mg% in each case, and the creatinine clearances are greater than 60 cc/min. in all 4 patients.

Three of the 4 surviving patients had overt evidence of rejection from 15 to 27 days after transplantation (Table III). This ranged in intensity from an asymptomatic rise in BUN (Case 4) to a syndrome of fever, oliguria and uremia (Case 2). A return to good renal function occurred with addition of actinomycin C and prednisone to the azathioprine therapy, with complete subsidence of the crisis in 3 to 20 days.

Two of the 4 living patients developed femoral thrombophlebitis on the side of the graft. One (Case 2) was treated with anticoagulants. The other (Case 4) passed a massive pulmonary embolus, one month after transplantation, and underwent a successful pulmonary embolectomy with the aid of cardiopulmonary by-pass.

Discussion. The unique feature of the antirejection therapy in these cases was the use of thymectomy and splenectomy. These ancillary operative procedures are adjuvant in scope, since neither can potentiate homograft survival in the adult experimental animal when used alone(6-8). The central agents of therapy were total body irradiation or preferably azathioprine, measures which have a strong and easily demonstrable deterrent effect upon the rejection process.

The value of splenectomy and thymectomy in the preparation of patients for the receipt of homografts will remain speculative until more practical experience is acquired with their use. However, considerable experimental information stimulated this program to test the value of removal of these reticuloendothelial masses. For example, it has been known for decades that the antibody response to many antigens is obtunded after splenectomy (9-11). Wissler and his colleagues(12, 13) have recently obtained evidence that the spleen is the most active portion of the lymphoid system in responding to intravenous antigens. They have suggested that migrant cells from the sensitized spleen can populate other lymphoid tissue and establish specific clones in their new location.

The role of the thymus in establishing immunologic reactivity in the newborn state appears to be unquestioned(14,15). Thymectomy performed shortly after birth results in immunologic crippling, and the consequent inability to reject homografts in a normal manner. The absence of the thymus at this stage of development results in generalized lymphoid hypoplasia, due either to absence of an organizer substance which promotes lymphoid development, or to absence of thymocytes which migrate to and populate distant areas of the lymphoid system(15).

In adult life, thymectomy has no demonstrable effect on antibody response or upon homograft survival. However, it has been

shown that thymectomy in adult mice, combined with total body irradiation, can result in homograft tolerance of a high degree(16). This finding suggests that the thymus gland may resume its perceptor function in adult life under circumstances in which there is temporary suppression of the lymphopoietic system.

Summary. Five patients with terminal renal failure have been treated with renal homografts. Total body irradiation and cytotoxic drugs were used to prevent rejection. In addition, the thymus and spleen were surgically removed prior to the homotransplantation. Four of the 5 patients are alive with good renal function after 105 to 198 days. The role of thymectomy and splenectomy in conditioning patients for the receipt of homografts is highly speculative at present. However, the early success rate in this group of patients exceeds that generally attained with renal homografts, and appears to justify further clinical evaluation of this approach under carefully controlled experimental conditions. The data are insufficient to allow a recommendation for the general use of these adjuvant procedures.

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Arterial Angiotensin Levels in Edematous Patients.* (28535)

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The relationship between the renal renin content, the granularity of the juxtaglomerular cells and the width of the adrenal zona glomerulosa in rats has been reviewed by Tobian(1) and by Hartroft *et al.*(2). Our findings of a direct stimulatory effect of valine-5 angiotensin II aspartic β-amide on aldosterone, both in normal subjects and in patients with arterial hypertension(3-6), have been confirmed by many other groups(7-11).

Bock *et al.* demonstrated the sodium retaining effect of angiotensin when infused into normal subjects(12). Subsequently, Nijenson (13), Peart *et al.*(14) and Genest *et al.*(3,4, 6) showed its natriuretic effect, when infused into hypertensive patients. Laragh also brought evidence of the same natriuretic effect in some patients with cirrhosis of the liver and ascites(15).

Merrill *et al.* reported an increase in "renin content" of renal venous blood from patients with congestive heart failure(16). Tobian *et al.* recently demonstrated a high degree of correlation between the ascites in rats rendered nephrotic by administration of aminonucleoside and the granularity of the juxtaglomerular cells(17).

Our findings of normal arterial angiotensin blood levels in 75% of patients with essential

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