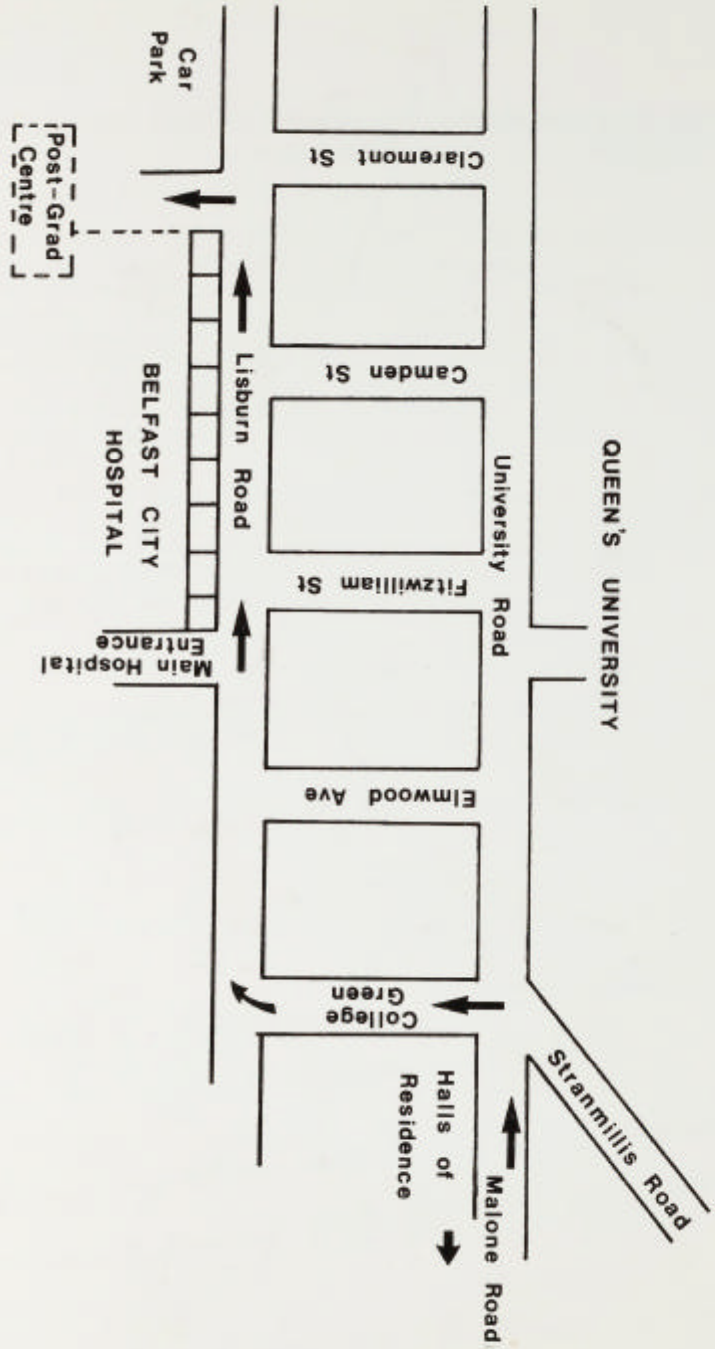

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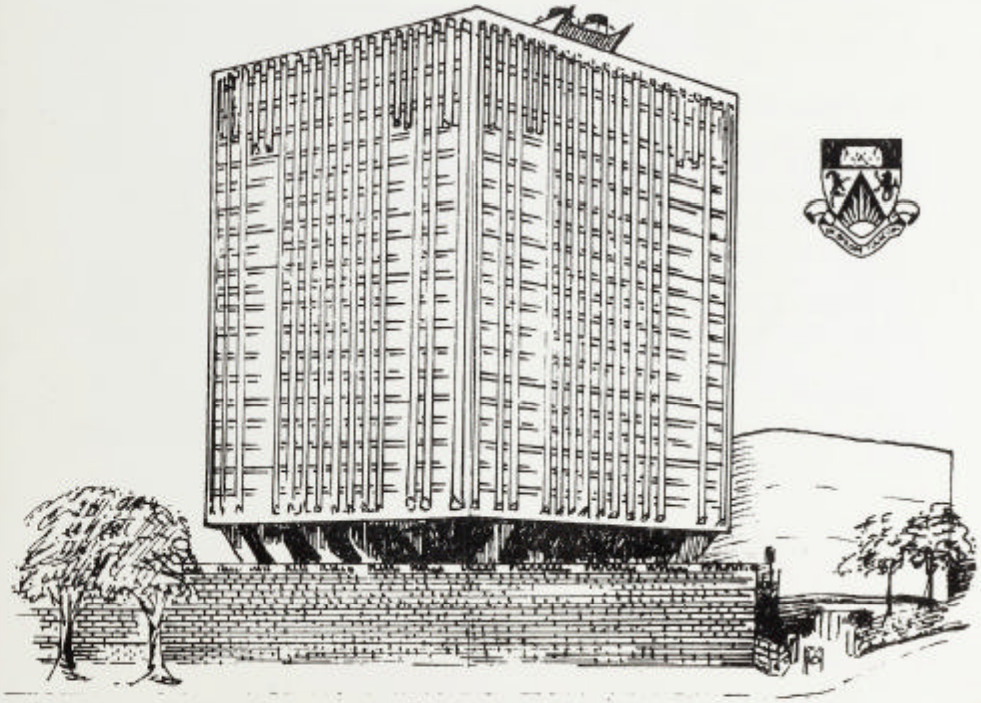

PAPER 1

A REPORT ON THE UK CYCLOSPORIN QUALITY ASSESSMENT SCHEME 1986

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In 1984 the UK Cyclosporin Quality Assessment Scheme was set up to provide laboratories measuring the drug with an external measure of their performance. The scheme now includes 81 laboratories in 16 countries, each centre receiving three samples per month.

From the results of these measurements in 1986 conclusions can be drawn on the relative performance of the analytical techniques in current use. During this year the high performance-liquid chromatographic (HPLC) measurements were prone to a high proportion of falsely positive results, 16/42, when compared to radioimmunoassay (RIA), 18/138. The median level of the falsely positive results were not significantly different, 63µg/1 HPLC (range 20-353), 70µg/1 RIA (21-125). However, since the target concentration range for cyclosporin measured by HPLC is between two and three times lower than that for RIA, this difference may have clinical significance.

For the measurement of cyclosporin in pooled blood samples from patients, HPLC had a significantly higher median coefficient of variation, 27·5%, than the corresponding RIA measurement, 20·5%. Other comparative results of HPLC and RIA blood and plasma measurements will be described. Finally, the performance of a new iodine labelled RIA kit will be compared with the Sandoz Products RIA.

PAPER 2

EXERCISE INDUCED HYPERTENSION IN NORMOTENSIVE RENAL TRANSPLANT RECIPIENTS ON CYCLOSPORIN A

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We have studied the effect on sitting bicycle exercise on the systemic blood pressure (SAP) of normotensive renal transplant recipients. Previous studies (Scott et al, Clin. Sc., Suppl., 1987, **72:** 48-9) suggested patients on Cyclosporin A (CsA) may have a different systemic pressure response to exercise, when compared to patients on conventional Azathioprine and steroid therapy (AzP) suggesting a previously unreported effect of CsA upon the systemic vasculature.

18 renal transplant recipients, with comparable renal function, 10 on CsA, mean age 30·6 yrs. (range: 15-53) and 8 on AzP, mean age 30·7 (range: 19-41) performed graduated cycle exercise with work increasing by 25 watts/4 mins. Mean SAP was measured automatically (Dynamap) and breath by breath expired gases were measured, from which oxygen consumption ($\dot{V}_{O_2}$) was calculated, ECG recorded heart rate (HR) results were analysed for each work period by unpaired student-t test.

Work	Mean SAP (mmHg)			$\dot{V}_{O_2}$ (ml/kg/min)			HR (beats/min)		
(watts)	CsA	AzP	P	CsA	AzP	P	CsA	AzP	p
0	97·7	96·7	NS	7·2	11·1	NS	89·3	95	NS
25	112·8	97·8	<·02	16·6	22·7	<·001	110·8	120·3	<·05
50	119·2	98·7	<·01	20·8	28·7	<·01	119·7	146·9	<·01
75	140·3	112·8	<·001	26·3	42·1	<·001	141·3	163·9	<·1

These results support the view that the systemic pressure response to exercise is altered in renal transplant recipients receiving CsA. The lower $\dot{V}_{O_2}$ and HR responses to exercise in the CsA group emphasise the difference in systemic vascular response between the two groups.

Difficult to find normotensive CyA transplant patients.
Thinks capacitance of the peripheral vas bed is reduced by CyA.

PAPER 3

LOW DOSE CYCLOSPORIN MONOTHERAPY IN RENAL TRANSPLANTATION

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There is conflicting evidence as to whether Cyclosporin (Cy) on its own gives adequate immunosuppression in renal transplantation. Early studies of Cy monotherapy employed doses of 15-17 mg/kg/day which we now know to be excessive. Nephrotoxicity was common and may have adversely affected the results. We report two controlled clinical trials in which Cyclosporin on its own at a lower dose has been compared to combination therapies following cadaveric renal transplantation. In the first trial patients were randomised to receive either Cy 8 mg/kg or triple therapy (Cy 8 mg/kg, Azathioprine 1·5 mg/kg and Prednisolone 0·3 mg/kg) and in the second trial randomisation was between Cy 10 mg/kg or Cy 10 mg/kg plus Azathioprine 1·5 mg/kg. Follow up has been from 3-27 months and the actuarial first cadaver graft survival is shown:

	Cy alone	Combination therapy
1st Trial (43)	78%	75%
2nd Trial (35)	78%	76%

No differences in graft survival were observed. When the two trials were combined it was seen that serious infective complications were significantly more frequent when Cy was used in comination (11 vs 1, p <0·001). Of the 49 patients allocated to Cy monotherapy 34 have still not received any prophylactic steroids. From these two trials we have concluded that Cy on its own gives excellent immunosuppression and is associated with less infection that when used in conjunction with other agents.

Needed to add pred in 28% & some came off again.

Rejection 0·5–1 g mP × 3 If one severe or 2 rejection episodes added steroid, ALG
severe ALG

More rejection episodes and more pred needed in CyA alone gp.
However graft survival seems equally good.
Triple therapy 4 deaths from infection, 2 from GI bleed post surgery.
Second trial Cy 10 mg, or CyA 10, aza 1·5 mg/kg
no diff in graft survival.
No diff in serious infections
Combined both trials still more infection in combination
10 deaths of 6

PAPER 4

DOES METHYLPREDNISOLONE INCREASE PLASMA CYCLOSPORIN LEVELS?

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It has been suggested that RIA plasma Cyclosporin (CyA) levels are increased by high dose methylprednisolone (MP) therapy. Our objective was to determine whether this increase reflected a rise in CyA or its metabolites. Trough plasma CyA levels were monitored by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA) during 17 rejection episodes in 13 renal allograft recipients treated with MP 0·5 G IV daily for 3 days.

The plasma HPLC and RIA CyA levels during and 24 hours after therapy were not significantly different than prior to therapy (Table 1)*. However, 3 rejection episodes displayed a significant increase in HPLC plasma CyA from 71, 122 and 226 ng/mg to 396, 256 and 1147 ng/ml. respectively. The corresponding RIA plasma CyA levels increased from 96, 363 and 520 ng/ml to 865, 785 and 2175 ng/ml.

We have shown that there is an increase in plasma CyA and in CyA metabolites following high dose MP therapy in only 3 out of 17 rejection episodes (17·6%) of which only one required a reduction in CyA dosage for clinical nephrotoxicity. This offers some explanation of combined rejection episodes and nephrotoxicity which have been previously described.

TABLE 1

Plasma CyA ng/ml	Pre-MP	24hrs. Post 1st dose	24 hrs Post 2nd dose	24 hrs Post 3rd dose
HPLC	140(106-238)	160(130-218)*	163(124-250)*	192(120-281)*
RIA	310(240-466)	283(233-450)*	313(246-375)*	362(215-635)*

median (interquartile range)
*Wilcoxon Signed Rank Test Not Significant.

15 mg CyA/kg for 2 wks → 12 → 10 in 4 wks.

Not significantly increased, only one required reduction in dose.

PAPER 5

MONITORING OF CYCLOSPORIN A IN LIVER TRANSPLANT PATIENTS BY DAILY TROUGH LEVELS AND PHARMACOKINETIC STUDIES

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Between November 1984 and August 1986 45 liver transplants were performed in 40 patients. 32 patients who survived for more than 3 months were analysed. Daily trough whole blood CyA levels were measured by RIA. 14 pharmacokinetic studies were carried out following a single oral dose of CyA. Absorption was found to be extemely poor when compared to 8 diabetic uraemic patients.

	Liver transplant	*Diabetic uraemic*
Peak level	285·4 +/— 180	1616 +/— 352
Time to peak level	5·3 +/— 3·3 hrs	2·9 +/— 1·45 hrs
Area-under-curve	905·4 +/— 584	10041 +/— 3290

Four patients had persistent grossly abnormal liver function with bilirubin > 400 umol/1 their trough levels remained low despite increased oral doses (up to 45 mg/kg), yet small iv doses achieved theraputic levels. Clamping of the biliary T-tube increased daily trough levels by > 100% except in 3 patients who had bile production <100 ml/day (2 with grossly abnormal liver function). Biliary leaks (3 patients) and haemolysis (2 patients) produced a sharp rise in levels.

Ten patients also had levels measured by HPLC. RIA/HPLC ratios varied from 1·8-9 being low initially and increasing when liver function was poor. CyA levels in bile varied from 30 ng/ml to 72000 ng/ml when measuring by both RIA and HPLC. Highest levels were associated with iv CyA, good liver function, phenytoin and haemolysis.

In conclusion CyA absorption and elimination seems to be dependent on liver function and the amount of bile production.

White: Astronomical levels in bile of whole blood

PAPER 6

TRIPLE THERAPY IN PATIENTS WITH ATN KIDNEYS FOLLOWING TRANSPLANTATION

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Immunosuppression using azathioprine, prednisolone and low starting dose of Cyclosporin A (CyA), in patients with renal transplants in ATN, offers the possibility of increased graft survival compared to those patients on conventional therapy whilst also reducing the risk of nephrotoxicity associated with CyA therapy alone; although there may be a higher incidence of serious infection. We assess the validity of this concept by reporting our experience using all three immunosuppressive protocols. Conventional therapy consisted of azathioprine 2-2·5 mg/kg and prednisolone 20-25 mg daily. The triple therapy group also took CyA at 4 mg/kg starting dose and increased to full therapeutic levels as ATN resolved. The third group of patients took 17 mg/kg CyA daily.

	Triple (+SE)	**Conventional** (+SE)	**CyA** (SE)
No.	30	33	13
Cold Ischaemic	20·50 + 0·99 hr	19·10 + 0·98 hr	20·40 + 1·30 hr
Length of ATN	13·30 + 2·3 days	18·90 + 3·8 days	15·90 + 3·10 days
No. of Rejection Episodes	0·53 + 0·13	0·80 + 0·12	0·92 + 0·95
Serious Infections	0	2	1
Graft Loss	2[ab]	11[a]	4[b]
a—difference is significant p — 0·003		Chi-square analysis	
b—difference is significant p — 0·02.		Chi-square analysis.	

We conclude that triple therapy does not lead to an increased incidence of serious infection and may produce better graft survival in patients with renal allografts suffering from ATN.

CyA alone 17 mg/kg

ATN rate just under 20%

76 pts had ATN out of 400

PAPER 7

TRANSPLANTING THE HIGHLY SENSITIZED RECIPIENT

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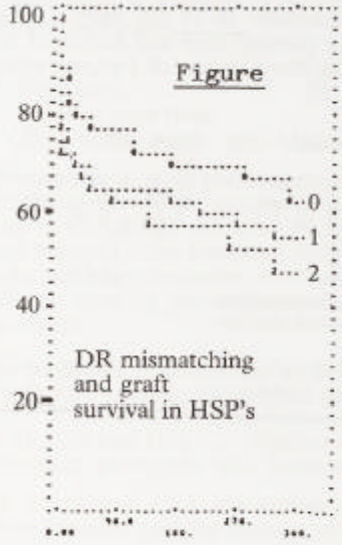
The SOS scheme for transplanting highly sensitized patients (HSP's) with antibody reaction frequency of more than 85% was initiated in February 1984. So far 361 patients have been entered into the scheme. The risks associated with high sensitization are sex and previous graft history. Non transplanted females and transplanted males constitute the largest two groups of HSP's. By 31st December, 1986 transplants have been performed in 115 of the HSP's. The 1 year graft survival was 56%. A high rate of early graft loss or primary non-function was observed. Of the 54 non-functioning grafts 26 (48%) failed within the first 10 days post transplant.



The degree of HLA-A and B matching had no effect on the graft survival. However a positive correlation between graft function and DR matching was observed (see Figure). The striking feature of the 0 DR mismatched group was the low rate of primary graft non-function: 16% compared to 24% and 27% in 1 and 2 mismatched HSP's respectively. Other parameters influencing the graft survival in HSP's were the combination of the sex of the patient and the number of previous grafts. Female 1st grafts and male regrafts had the highest graft survival (73% and 67% respectively at 3 months). First grafts in males and regrafts in females had a graft survival of 60% and 59% respectively at 3 months.

Should now consider introducing DR matching into SOS scheme.

Cycles 1-8 Feb '84 - Aug '86
310 pts 115 (37%) transplanted
Antibodies > 85%
No HLA A B DR matching taken into account but previous mismatch not repeated
1 yr graft survival 57% [14] most of loss is within 10 days, indeed most within 3 days

PAPER 8

PRIMARY RENAL ALLOGRAFT SURVIVAL AND THE EFFECT OF SENSITISATION FOLLOWING DELIBERATE THIRD PARTY BLOOD TRANSFUSIONS

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Since the end of 1982 we have followed a programme of planned transfusions for non-transfused and nuliparous patients prior to primary cadaveric renal transplantation. The protocol is for three units of blood to be given, each at monthly intervals. Serum samples are screened for panel reactive lymphocytotoxic antibodies (PRA) at the outset and two weeks following each transfusion. Altogether, 147 patients have entered the programme: 29 (19·7%) became sensitised but in only seven cases did the antibodies react with more than 10% of the panel (range 15-55%). Eighty seven patients have been transplanted of whom 77% still have functioning grafts. This compares with 78% graft survival for 134 recipients of primary cadaveric renal grafts who were transfused during the same period but were not part of this protocol. Eighteen of the 87 recipients who had had planned transfusions (20·6%) produced PRA post-transfusion and four of those reacted with more than 10% of the panel. All patients received crossmatch negative grafts using the highest positive and current sera. The graft survival does not differ between the groups that did and did not have PRA pre-transplant (77·8% and 76·8% respectively). For 64 of the patients that were unsensitised pre-transplant, there are post-transplant PRA data. Sixteen did and 48 did not produce PRA post-transplant: their graft survival is 62% and 87% respectively (Fisher's $p=0·057$). We conclude that recipients who receive planned transfusions have good graft survival. The sensitisation rate following transfusion is low and the production of PRA does not prevent patients from receiving a transplant nor does it adversely affect subsequent graft survival. However, the production of PRA post-transplant is related to the poorer graft outcome.

1983-86 prior to primary cad transpl.
(all pres non-transpl except multipara)
a) Dons
b) Waiting time
c) graft survival
d) post transplant antibodies
Deliberate v. clinical need no developing antibodies
nor difference, no diff in waiting time.
Random transfused wait longer because the group includes multipara
Those who produce antibodies post graft less good graft survival
Accept max 3 mismatches

PAPER 9

SPECIFICITY OF THE POSITIVE B CELL CROSSMATCH

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A positive B cell crossmatch (+BXm) is ignored by many centres but is thought in some circumstances to predict a poor outcome for renal transplantation. It is possible that the variable results relate to different antibody specificities. We have therefore used monoclonal antibodies directed at monoorphic determinants of HLA Class I (PA2.6), HLA-DR (NDS22), and HLA-DQ (Leu 10), to inhibit the cytoxicity of alloantisera and thus define the molecular specificity of antibodies causing +BXms. Reduction of IgM by dithiothreitol was used to determine the immunoglobulin class.

The +BXms of 33 renal transplant recipients were analysed. 3 were due to anti-HLA-DQ, 2 being IgM both of which failed, and 1 being IgG which is functioning poorly at three weeks. No +BXms were due to HLA-DR antibodies, but this situation was deliberately avoided in donor selection. 2 IgM and 3 IgG anti-HLA Class I antibodies were positive with donor B but not T cells, 2 of the IgG grafts have failed. Only 3 of the 16 grafts with +BXms due to IgM non-HLA (autoreactive) antibodies have failed. 4 of the IgG antibodies had no definable specificity and 5 IgM tests were technically unsatisfactory. 1 year graft survival was 57% in 8 due to IgG and 80% in 25 due to IgM antibodies. In conclusion +BXms due to IgM non-HLA antibodies did not predict poor graft outcome. The roles of +BXms due to IgG non-HLA, HLA Class I, and HLA-DQ antibodies remain uncertain, but these techniques offer better definition of the exact cause of a positive B cell crossmatch.

PAPER 10

FATE OF RENAL TRANSPLANTS IMMUNOSUPPRESSED WITH AZATHIOPRINE AND LOW DOSE PREDNISOLONE 10-18 YEARS AFTER GRAFTING

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The first 100 transplants in Belfast were carried out 10-18 years ago. Ninety seven received cadaver grafts, 3 living donor grafts. There were 91 first, 7 second and 2 third grafts. All except 6 patients received pre-transplant blood transfusion and all were immunosuppressed with azathioprine and low dose prednisolone only. Patient survival for first cadaver grafts at 10 years was 58 out of 88 (65·9%), total patient survival was 60 out of 91 (65·9%). Actual graft survival at 10 years for first cadaver transplants was 48 out of 88 (54·5%), while 40 grafts had failed within the 10 year period. Of these 40 failed grafts, 17 were due to death of the patient with a functioning graft, 8 of the deaths occurring at least 5 years after the transplant. Of the 17 deaths 12 were due to vascular causes and 2 to carcinoma. HLA -A, -B matching had no significant effect in graft or patient survival at 10 years. These results show that the combination of azathioprine and low dose prednisolone permits long term survival of grafts.

PAPER 11

SHOULD WE BE USING KIDNEYS FROM OLDER DONORS?

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The shortage of cadaveric organ donors has resulted in the use of kidneys from older donors than is perhaps ideal. We have analysed the results of 130 consecutive renal allografts performed in recipients aged 13 and over since January 1983, grouping them according to the donors age. 18 (14%) of kidneys were from donors aged 50-59 years and 10 (8%) from donors aged 60 and over (range 61-76, mean 66 years). There were no differences in recipient age, numbers of sensitized patients, HLA matching, ischaemic times and initial immunosuppression between the groups.

Donor age	10 - 19	20 - 29	30 - 39	40 - 49	50 - 59	60 +
Number of patients	27	33	23	18	19	10
% with immediate function	59	61	48	67	21	10
% with stable function (follow-up 3-48 months)	72	69	67	44	39	40
Serum creatinine at six months (mean ± SD umol/1)	131 ±44	137 ±45	170 ±56	172 ±50	187 ±83	153±27

Significantly fewer kidneys from donors over fifty functioned immediately compared to those from donors under fifty ($p < 0{\cdot}001$), and significantly fewer have stable function ($p < 0{\cdot}02$). However, there was no significant difference in serum creatinine after six months in those patients who had successful transplants and received kidneys from older, compared to younger donors.
In view of the chronic shortage of donors we feel that it is reasonable to use kidneys from older donors on low priority patients who otherwise would not have the chance of a transplant.

PAPER 12

PROLONGATION OF RAT FETAL PANCREAS ALLOGRAFT SURVIVAL BY CYCLOSPORIN—AN IMMUNOHISTOLOGICAL STUDY OF ANTIGEN EXPRESSION

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In streptozotocin-induced diabetes in rats, blood glucose may take several weeks to normalise following transplantation of immature fetal pancreata and assessment of rejection based on glucose levels may be misleading. Serial immunohistological examination of the transplanted pancreas may provide an alternative method of assessment.

Initially, the normal distribution of Class I and Class II antigens on fetal, neonatal and adult rat pancreas was determined using an indirect immunoperoxidase method. Following fetal pancreas transplanation to the renal subcapsular site in DA isografts and DA ——> PVG allografts (with and without oral Cyclosporine), rats were sacrificed at intervals from 2 days to 21 days and graft morphology, antigen expression and degree of infiltration assessed.

By day 4, Class I antigens were expressed on duct epithelium and islets in isografts and unmodified allografts and on acinar cells in unmodified allografts. Class II antigens (in isografts only detected on interstitial cells) were expressed on duct epithelium by day 4 in unmodified allografts. Destruction of these allografts progressed rapidly, being complete by day 10.
In cyclosporine-treated allografts, antigen expression closely resembled that of the untreated *isografts* and survival of morphologically intact, insulin producing pancreatic tissue was prolonged to at least day 21 despite heavy infiltration by mononuclear cells.

PAPER 13

PATTERNS OF CLASS II ANTIGEN EXPRESSION IN HUMAN KIDNEY

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We have studied the antigen expression, in particular HLA Class I and II, on cryostat sections from 24 pre-transplant renal biopsies, 4 renal carcinoma biopsies and 18 post-transplant renal biopsies from Cyclosporine A treated patients, using monoclonal antibodies and an indirect immuno-alkaline phosphatase technique. The staining shown by the pre-transplant biopsies is given in the following table:

Monoclonal	No. positive biopsies / No. biopsies			
antibody	Glomeruli	Tubules	Inter-tubular	Large vessel
α—HLA			Structures	Endothelium
class I	24/24	20/24	20/24	13/17
class II DR	21/24	22/24	22/24	5/18
class II DQ	2/15	2/15	2/15	0/7
α—Endothelium	0/22	0/22	20/22	0/15
α—Vimentin	17/23	1/23	7/23	11/16

A similar staining pattern was seen in the biopsies from the normal pole of kidneys from renal carcinoma patients. In 8 biopsies from 5 patients who experienced a rejection episode, occurring at periods from 3 days to 1·5 years post transplant, the general pattern of monoclonal antibody staining was similar to that found in the pre-transplant biopsies although the class II expression on renal tubules appeared more prominent. However, in 11 biopsies from 5 patients, with causes of renal dysfunction other than rejection (ATN, nephrotoxicity) the staining pattern was again similar; the class II expression on renal tubules undiminished. These results suggest that class II antigen is normally expressed on tubules within the kidney and that the level of DR expression cannot be taken as a clear indicator of rejection.

PAPER 14

IDENTIFICATION AND CHARACTERISATION OF TWO DISTINCT WATER SOLUBLE CLASS I MHC MOLECULES IN RAT LIVER AND SERUM

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At the last meeting of the Society, we reported the identification, bulk purification and preliminary studies on donor-specific immunosuppression of a small, water soluble form of the classical class I transplantation antigen (RT1.A) of the DA rat strain. In this study we have extended our search for water soluble class I molecules by combining the use of RT1.A specific monoclonal antibodies with another monoclonal antibody which we have found to be broadly reactive with both RT1.A and non RT1.A class I antigens. Normal DA serum and aqueous extracts of DA liver were passed down RT1.A specific monoclonal antibody affinity columns to remove RT1.A class I molecules, and then passed down the more broadly reactive monoclonal antibody affinity column. Using this approach, we have identified a second water soluble class I molecule in rat liver and serum. This non RT1.A water soluble class I molecule has a heavy chain of molecular weight 41,000 daltons (linked to β2 microglobulin). It is slightly larger than the water soluble RT1.A class I molecule described previously (40,000 daltons). Bulk purification and N terminal amino acid sequencing of the non RT1.A and the RT1.A class I molecules revealed two substitutions in the first 25 amino acids, a Tyr——> His at position 9, and a Ala——> Ser at position 24. These sequencing data establish unequivocally that our second water soluble molecule is the product of a non RT1.A class I MHC gene. The non RT1.A water soluble class I molecule was found in large quantities (approximately 20μg/ml) in the serum of the DA rat strain, and probably represents the rat homologue of the Q10 class I molecule of the mouse. Thus the DA rat strain expresses 2 distinct water soluble class I molecules in its liver and serum, an RT1.A class I molecule and a Q10-like non RT1.A class I molecule, the latter being present in much higher concentrations in the serum.

PAPER 15

SPECIFIC SUPPRESSION OF MIXED LYMPHOCYTE REACTIONS BY ALLOACTIVATED CELL LINES

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Mixed lymphocyte reactions (MLR) activated lymphoblasts can suppress both proliferation and cell mediated lysis (CML) when added to a subsequent MLR. Antigen specificity and the mechanism of MLR induced suppression was investigated with special emphasis on the exclusion of suppression. MLR activated peripheral blood mononuclear cells (PBMNC) were cultured for 10 days and restimulated with the original stimulator cells and interleukin 2 (IL2) for an additional 7 days. The lines that displayed specific suppressor activity upon addition to an MLR, were further studied.

Suppressor lines generated across a HLA class I and LB-Q1 (a DR β-III determinant) difference, suppressed MLR only when the stimulator cell carried the same class I antigen as the original stimulator. When stimulation took place across a class I + D/DR difference, either a D/DR specific suppression or a class I + D/DR specific suppression was observed. When stimulated across a DP difference only, a DP specific suppression was noted.

Furthermore, when analysed on the same panel, suppression correlated linearly with CML activity. While suppression at the stimulator level (A anti-B MLR, A autologous to the suppressor line) could be specific for class I and/or class II, suppression at the responder level (B anti-A) was only class I specific.

These results are all compatible with the hypothesis that suppression is due to lympholysis. The fact that these lines were unable to inhibit the phytohaemaglutinin response of cells carrying the suppressor epitope, is not necessarily in contradiction with this hypothesis.

Based on the similarities of this experimental model to the findings after blood transfusion, we suggest that allospecific CTL might be responsible for the graft enhancement seen after transfusions.

PAPER 16

THE EFFECT OF PRIOR PLASMA EXCHANGE & CYCLOSPORIN A (CYA) ON ALLOGRAFT REJECTION IN A PRESENSITISED HOST

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Recipient PVG (RT1c) rats were presensitised to the donor strain DA (RT1-av1) by implantation of heart fragments into the rectus muscle. Subsequently, cardiac heterotopic allografts were abdominally placed using a standard technique. Rejection was ascertained by abdominal palpation and confirmed histologically. The abrogating effect of intensive pre-transplant plasma exchange (IPE) (70 ml blood/kg body weight on 4 consecutive days) together with immunosuppressive therapy (CYA, Cyclophosphamide and Prednisolone (CYP)), pre- or post-transplant was investigated.

Group	Rejection Time (Days) d = died with functioning graft	Median Rejection Time	No. of Rats
Unsensitised	8, 8, 8, 8, 8, 8, 9, 10	8	8
Unsensitised + IPE	8, 9, 9, 9, 10, 11, 11, 11	9·5	8
Unsensitised + CYP	10, 12, 15, 15, 18, 25, 26, 37	16·5	8
Unsensitised + CYA	d38, d46, d49, > 200, > 200, > 200 > 200, > 200, > 200	> 200	9
Sensitised	2, 2, 2, 2, 2, 2, 2, 3, 4	2	9
Sensitised + IPE	3, 4, 4, 4, 5, 5, 5, 7	4·5	8
Sensitised + CYP	2, 2, 3, 3, 3, 5, 6, 7, 8,	3	9
Sensitised + CYP + IPE	5, 5, 5, 5, 7, 7, 7, 7	6	8
Sensitised + CYA	2, 3, d6, 7, 9, 9, 10, 12, 38, 38 76, 78	10	12
Sensitised + CYA + IPE	11, 13, 20, 52, 84, > 200, > 200, > 200, > 200, > 200	> 84	10

These results clearly demonstrate that even in a previously sensitised animal pre-transplant intensive plasma exchange, when combined with post-transplant CyA is highly effective. A median survival time of over 84 days was achieved in animals treated in this manner and furthermore, 50% of the grafts are still functioning at longer than 200 days.

PAPER 17

ANTI-IDIOTYPIC ANTIBODY ACTIVITY IN POTENTIAL TRANSPLANT RECIPIENTS

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One of the mechanisms responsible for the " transfusion effect " may be the development of anti-idiotypic antibodies. Pre-transplant blood transfusions, however, may stimulate lymphocytotoxic antibodies thus increasing the likelihood of a positive crossmatch test. As renal transplantations are undertaken as long as the current serum is crossmatch negative, anti-idiotypic activity has been sought in non-cytotoxic sera from dialysis patients who once possessed cytotoxic antibodies to target lymphocytes.

Four or more non-cytotoxic sera (AB2) from 6 transfused patients were tested in the short anti-idiotypic antibody assay against normal lymphocytes known to be killed by sera (AB1) from the same patient. Inhibition of $\geq 50\%$ was considered positive provided cell kill in the control wells was $>50\%$. Eighty-seven serum/cell combinations were studied; anti-idiotypic activity was detected in 59 (68%) and only 10/27 (37%) of positive sera were active against HLA tissue typing sera used as AB1 ($p<0{\cdot}01$).

These results indicate that non-cytotoxic sera from highly sensitised patients still possess anti-idiotypic antibody activity.

PAPER 18

TRANSBRONCHIAL BIOPSY IN THE DIAGNOSIS OF PULMONARY COMPLICATIONS OF COMBINED HEART-LUNG TRANSPLANTATION

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The lung is the target organ for many opportunistic infections and is also the major site of rejection in recipients of combined heart-lung transplantation. The clinical features of these complications are often non-specific and difficult to distinguish, but the treatment radically different. We have used transbronchial biopsy performed via the fibreoptic bronchoscope to provide histological material for diagnosis in transplant patients presenting with new respiratory symptoms.

Biopsies were performed on 32 occasions in 14 patients. Inadequate material was obtained in 3 cases (9%) and 1 patient had a pneumothorax (3%). Eight biopsies were diagnositc of opportunistic infection, cytomegalovirus (6) and pneumocystis carinii (2). In 7 biopsies there were features characteristic of rejection and patients responded to augmented immunosuppression (22%). Four patients had lower respiratory tract infections with common pathogens and no evidence of rejection or opportunistic infections (13%). Biopsies were unhelpful in diagnosis in 10 cases (31%) and were particularly difficult to interprete in the early post-operative period.

Transbronchial biopsy is useful in the management of pulmonary complications of heart-lung transplanatation, particularly in distinguishing between opportunistic infection and rejection.

Lungs can regect independently of before heart.

PAPER 19

MONITORING OF ANTIREJECTION THERAPY BY FINE NEEDLE ASPIRATION CYTOLOGY

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The first rejection episode in 35 cadaveric renal allograft recipients immunosuppressed with Cyclosporin A, was monitored by fine needle aspiration cytology (FNAC) to study the effect of antirejection therapy on the cellular infiltrate. Each episode was treated with 0·5Gms Methylprednisolone (MP) IV daily for 3 days. The allograft cellular infiltrate was scored to derive a total cellular increment (TCI). Following MP the TCI decreased to within the normal range of 19 patients (Group A) but remained abnormally elevated in the other 16 patients (Group B). There was no significant difference in the B and DR loci mismatches between these 2 groups. Fifteen Group B patients (93·8%) required further antirejection therapy whereas only 6 Group A (31·6%) patients had a second rejection episode within 28 days. Additional immunosuppression of oral Prednisolone was required for 11 Group B and 2 Group A patients.

Azathioprine was subsequently introduced in 5 and 1 of these patients respectively to prevent further deterioration in function. FNAC in all of these patients demonstrated persistently increased cellular infiltration. Only one of the Group B failed to attain stable function but all other patients have functioning grafts at a follow up of 3-8 months.

We suggest that if FNAC does not demonstrate a response to 3 doses of MP, then the patient is more likely to develop further rejection episodes and may benefit by earlier introduction of additional immune modulation.

	Group A n=19	Group B n=16	Mann Whitney U Test
ATN	6	6	($X^2=0$)
Days to onset of rejection	11·6±0·6	7·5±4·4	pNS
TCI at diagnosis	4·3±1·75	4·83±1·65	pNS
TCI after MP	1·0±0·42	4·53±1·91	$p<0·001$
Further rejection episodes	6	15	$p<0·001$

Perop 0·5g MP.
CyA 15mg/kg → 12 – 7 10 at 4 wks.
(oddoon as pt takes oral fluids)
Rx. 0·5g MP x 3 IV.

PAPER 20

SERUM LEVELS OF ANTI-HUMAN THYMOCYTE IMMUNOGLOBULIN AND THEIR RELATIONSHIP TO SERUM LYMPHOCYTOTOXIC ACTIVITY

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Serum samples were obtained from 18 recipients of renal allografts at the outset, during and/or after their treatment for acute rejection with rabbit anti-human thymocyte Ig (ATG: dosage 2·5 to 5mg/Kg/day). Serum ATG levels were measured in an enzyme-linked immunosorbent assay (ELISA). Swine anti-rabbit Ig was coated onto microtitre plates followed by test serum in serial dilutions or ATG standards: binding was detected with peroxidase conjugated goat anti-rabbit Ig. ATG levels during therapy were in the range 57-90ug/ml with one exception where the peak level was 404ug/ml. After treatment, ATG levels gradually declined to zero within 12 weeks.

All sera and ATG standards were screened for lymphocytotoxic panel reactivity (PRA) using HLA-A, B and DR typed lymphocytes. Positive sera were absorbed with platelets to remove anti-HLA class I antibodies and were also treated with 0·01M dithiothreitol (DTT) to dissociate IgM antibodies. Thus PRA due to anti-HLA class I antibodies, IgM autoantibodies or ATG could be differentiated. ATG standards at >11ug/ml killed all panel cells. Eleven patients, for whom no sera were available until after treatment, had <6ug/ml ATG: 7 had no PRA whilst 4 had anti-HLA antibodies. Seven patients had detectable serum ATG and also PRA. In 3 of these cases the PRA was attributed solely to the ATG; one patient has PRA due to ATG and anti-HLA antibodies; two patients had PRA initially due to ATG and subsequently to the development of autoantibodies; one patient had only autoantibodies.

We have shown that therapeutic levels of ATG can be sensitively quantitated by ELISA. As such levels of ATG can cause *in vitro* PRA it is important that this should be distinguished from anti-HLA or autoantibodies when transplant recipients are being monitored.

PAPER 21

IS PREGNANCY SAFE AFTER RENAL TRANSPLANTATION?

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Since 1979 24 pregnancies have been observed in 17 patients with well-functioning renal transplants, all on conventional immunosuppression. Thirteen patients had 16 actual births (one stillborn): 7 miscarriages and one therapeutic abortion. All deliveries except one were by Caesarean section. Eight out of 16 births (50%) were preterm (normal for a healthy population <10%). Growth retardation occurred in 5 neonates (33%).
Of greatest importance to the outcome is the effect of hypertension (or its treatment) at conception:

	Hypertensive	Not hypertensive
Number of pregnancies	9	14
Live births	4 (44%)	11 (79%)
Miscarriages	4 (44%)	3 (22%)
Preterm deliveries	3/5	5/11
Intrauterine growth retradation	3/6 (incl. mid trimester abortion)	3/12 (incl. mid trimester abortion)
Treatment for BP	4/9	1/14

While blood pressure was normal in all patients at 12 weeks' gestation, hypertensive patients on treatment seemed more at risk from early pregnancy loss, reduced intrauterine growth, and pre-eclampsia. Control of blood pressure was also difficult. Even in patients with no hypertension but on daily steroid therapy the rate of foetal loss and early delivery was increased. One patient with pre-eclampsia had acute renal failure post partum and required dialysis for three weeks after which renal function recovered. One patient developed chronic rejection (6%) and returned to dialysis after a second normal pregnancy. If the patient is requiring hypertensive control at conception, the risk to the foetus is considerable (>50% foetal loss). Risks both to foetus and mother are increased after transplantation, especially in patients with hypertension. Nevertheless pregnancy is generally successful and patients should be warned but not necessarily discouraged.

Johnathan Mydad even ~~higher~~ greater growth retardation with CyA than conventional therapy
Someone needed to raise CyA level during pregnancy — dramatic rise after delivery on the pregnancy dose.

PAPER 22

PREDIALYSIS TRANSPLANTATION IS NOT A RISK FACTOR FOR RENAL ALLOGRAFT FAILURE

Looks Good

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Successful transplantation prior to the need for dialysis in patients approaching ESRF may reduce the morbidity, mortality and financial cost of renal replacement therapy. However, it has been suggested that long-term haemodialysis may be an enhancing factor for graft survival. Possible reasons for this included patient selection, the known beneficial effect of blood transfusion or graft protective-immunological deficiency in the dialysis population.

Since 1975 we have attempted to predict accurately the date of ESRF in our predialysis CRF patients and have considered them suitable for transplantation within one year of expected ESRF. Between January 1975 and June 1986, 742 grafts (551 1st cadaver) have been performed of which 54 (48 1st cadaver) were in previously undialysed patients.

Actuarial patient and graft survival was not significantly different between the predialysis and dialysis groups.

Number			Patient Survival 1 Year	Patient Survival 5 Years	Graft Survival 1 Year	Graft Survival 5 Years
Dialysis	688	724	89·4	80·1	66·8	54·5
Predialysis	54	60	86·6	78·4	61·9	55·4

One regraft before entering dialysis programme

Furthermore if first cadaver grafts alone are considered of the patients are divided into two cohorts—those treated with conventional immunosuppression without planned pretransplant transfusion and those immunosuppressed with Cyclosporin A who also received blood transfusion prior to transplantation the patient and graft survival in the predialysis and dialysis groups remain indistinguishable.

We conclude that predialysis transplantation during the 12 months prior to terminal renal failure is as successful as transplantation from our dialysis population.

10 yr actuarial graft survs. 50% both sets of pts ?
" " - 42%
Min 600 Cr median 850
Several deaths due to infection in the early years

Ross would take PT outs – thinks hypercal is bad for patients Evans: has high incidence suggests 12

PAPER 23

21% prevalence / incidence

HYPERCALCAEMIA AFTER RENAL TRANSPLANTATION

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Hypercalcaemia has been reported as a possible side effect of Cyclosporin (Cy) therapy (1). Since using Cy we have become aware of a relatively high incidence of hypercalcaemia in our patients, a significant number of whom have become symptomatic and have required parathyroidectomy.

Since November 1982 we have performed 256 renal transplants in 247 patients, 159 males and 87 females with a mean age of 43 (4-62 years). All patients were treated with Cy as primary immunosuppression. Fifty three (21%) patients have become or remained hypercalcaemic (serum calcium persistently $>2{\cdot}6$ mmol/1) of which 19 (8%) have required parathyroidectomy. Of 22 patients (9%) who were hypercalcaemic at the time of transplantation, 13 have required parathyroidectomy and only 3 patients have spontaneously reverted to normocalcaemia. Of those patients who were normocalcaemic at the time of transplantation, 31 (12%) have become hypercalcaemic and have remained so, 6 patients requiring parathyroidectomy.

In conclusion, we have observed both a high prevalence and incidence of hypercalcaemia in renal transplant patients treated with Cy. The hypercalcaemia has persisted despite good renal function and a significant proportion of these patients have ultimately required a parathyroidectomy.

1. von Graffenried, B. & Krupp, P.
Transplant. Proc. 1986. **18.** 876-883.

247 treated with Cy 3/12 – 4 yr follow up 53 pts

> 2.6 corrected Ca persistent

6 PTX

25 persistent hypercal but PTH →

22 were hypercal at time of transp', at 6/12

2.65

PTX for bone pain 10 deteriorating renal func / dyspepsia

– cyst 1

Ca > 3-0 4

psych 3

dep. malaise, lethargy

Responders remain at risk of further rejection, esp. 1st 6. rejection
11 remain with stable graft function.

Costs of course about £2000
Never given it twice

PAPER 24

RABBIT ANTITHYMOCYTE GLOBULIN TREATMENT OF STEROID-RESISTENT REJECTION IN RENAL TRANSPLANT RECIPIENTS RECEIVING CYCLOSPORIN A

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Twenty-one of the 120 renal transplant recipients in 1986 developed steroid-resistant rejection (SRR) within 90 days and were treated with rabbit antithymocyte globulin (ATG: Institut Merieux). Initial function had been satisfactory and all were immunosuppressed with Cyclosporin A (CyA). Acute rejection (AR) episodes that failed to respond to conventional high dose Methyl Prednisolone were treated with a 10-14 day course of ATG (2·5-5 mg/Kg/day). Six patients had become dialysis dependent by the start of the ATG and four of these grafts were lost due to ongoing acute rejection. Fourteen of the other 15 episodes were successfully reversed. Adverse reactions were common: fever (7), thrombophlebitis (4), rash (3), septicaemia (1) and leucopenia developed in the majority—necessitating discontinuation of the ATG in one instance. All patients were monitored prospectively for CMV infection, by virus isolation and serology. Five developed CMV, two were asymptomatic and three had a prolonged febrile illness but none had serious clinical sequaelae.

central line / 2 hrs. All had IV meth before the ATG; equiv of 20 mg/day

Five of the 16 responders had further episodes of steroid sensitive acute rejection and 3 have subsequently developed chronic vascular rejection (CVR). Two further patients have also developed CVR and focal segmental sclerosis has occurred in one. One has returned to haemodialysis, four have impaired renal function and eleven have stable graft function (creatinine <200 µml/1) between 3-12 months post-transplant and are immunosuppressed with CyA (11) and Prednisolone (4).

We conclude that rabbit ATG is effective and safe treatment for SRR in patients immunosuppressed with CyA; however salvage is unlikely if patients have become dialysis dependent.

Rabbits Inst. Merieux

21 steroid resist rejection, biopsy pro except one, no resp 3-5 days methyl pred; other causes of graft ↓ excluded

16 revers. 5 lost, more recent Trial.

14/15 not on dialysis, responded.

2/6 on dialysis responded

Infection during or following therapy common 5 CMV

PAPER 25

SHOULD WE USE EUROCOLLINS SOLUTION FOR KIDNEY PRESERVATION?

In situ perfusion

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Not all local donors, some shipped.

It has been suggested that EuroCollins solution is not optimal for kidney preservation. A retrospective analysis of 125 cadaveric kidney transplants performed between January 1984 and December 1985 has been carried out to evaluate the effect of EuroCollins (EC) and hypertonic citrate (HTC) solution. There were 39 patients in the EC group and 86 in the HTC group. Both were comparable in mean age, sex ratio, mean time on dialysis, number of preoperative blood transfusions, HL-A mismatches and mean total ischaemic time of the graft.

54 HTC kidneys had primary function (63%), compared with 12 of the EC kidneys (31%), ($p<0{\cdot}01$). There was no difference in either group in the rate of recovery of kidneys with delayed function, or in the mean serum creatinine of surviving grafts at 6 months. At 6 months, 24 HTC kidneys (28%) had failed, but only 5 EC kidneys (13%) had been lost. However, the losses by rejection were similar in both groups, and the difference is due to an excess mortality in the HTC group. There was no significant difference in 12 month actuarial survival between grafts with primary or delayed function.

Thus HTC preserved kidneys perform significantly better in terms of primary function and early functional recovery, but there is no difference in late graft function.

Eurocollins is a glucose based solution

		Primary.
HTC	86	54
EC	39	12

Start of function as 1st 24 h when Cr ↓ by 100 without dialysis

Marshall prefers hypertonic citrate because increased incidence primary graft function, esp important if using CyA.

Hillis: ? use of dopamine for donors.