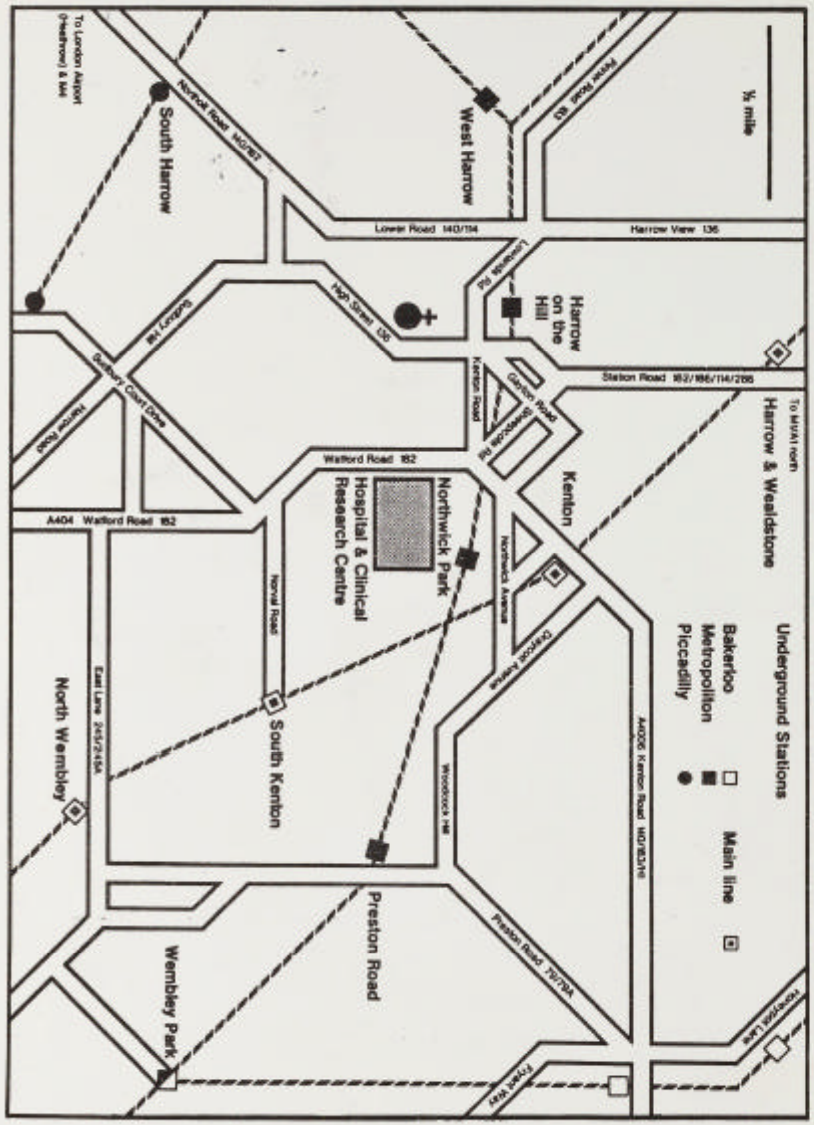

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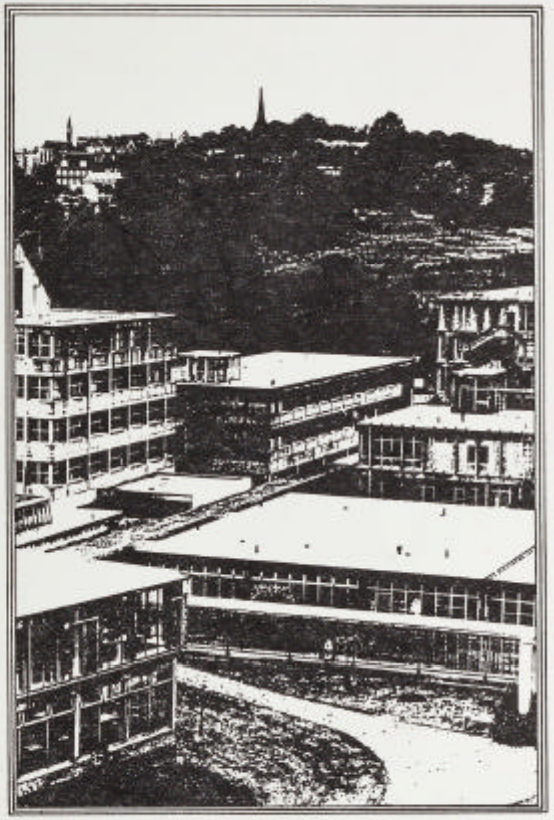

Northwick Park Hospital & Clinical Research Centre

20th November 1984

The following have applied for membership of the
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Simon Lucas
Mark T. Drayson
Wieslaw Adam Jurewicz

PAPER 1

THE USE OF MONOCLONAL ANTILYMPHOCYTE ANTIBODIES IN CLINICAL IMMUNOSUPPRESSION

P.J. Friend, R.Y. Calne, D.J.G. White, G. Hale, H. Waldmann.
Department of Surgery, Addenbrooke's Hospital.

A rat monoclonal antibody, with novel specificity for human leucocytes, has been used in clinical immunosuppression following renal, hepatic and pancreatic transplantation. The antibody, Campath I, is an IgM which recognises T and B lymphocytes and monocytes and, in the presence of human complement, causes the lysis of more than 99% of all peripheral blood lymphocytes.

Campath I has been used prophylactically with low dose Cyclosporin A following renal transplantation and full dose Cyclosporin A following renal and pancreatic transplantation. After hepatic transplantation, it has supplemented treatment with prednisolone and azathioprine. Seven patients have been treated with the antibody for reversal of acute rejection.

The antibody is administered intravenously for ten days at a dose of 25 mg daily (after hepatic transplantation) or 25 mg twice daily (after renal/pancreatic transplantation). Adverse reactions have been noted in a minority of the patients, none of whom were receiving concomitant steroids, and antibody administration was not continued in 6 cases out of a total of 44 cases treated. Anti (Rat Ig) antibodies have been detected consistently in those patients who have received a full course of Campath I.

Four patients, with steroid resistant acute rejection episodes, have been treated with a second antibody, Campath 2, and IgG2b rat monoclonal, which is specific for T lymphocytes and blast cells and which fixes human complement only weakly.

The results of this initial experience with monoclonal antibodies have encouraged us to plan a controlled clinical trial to establish the benefit of this form of treatment.

PAPER 2

PRELIMINARY STUDIES ON THE USE OF ANTI T AND ANTI-LEUCOCYTE COMMON (LC) MONOCLONAL ANTIBODIES FOR THE REVERSAL OF REJECTION IN CLINICAL CARDIAC TRANSPLANTATION

Rosmarie Dalchau°, Marlene Rose*, M. Jacoub*, John W. Fabre°

Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ and *Harefield Hospital, Harefield, Middlesex UB9 6JH.

The optimal class of monoclonal anti leucocyte antibody and the optimal specificity or combination of specificities for immunosuppression remain to be established. Here we report our experience with two monoclonal antibodies, each used (at the time of writing) to treat two patients early in the course of cardiac transplantation. Both antibodies were of the IgG_{2a} subclass, and cytotoxic with rabbit but not human complement.

Following the identification of cellular infiltrates in the graft by endomyocardial biopsy, patients were given an intravenous dose of monoclonal antibody on each of 3 consecutive days. The monoclonal antibody Mifa-7 is a pan T lumphocyte antibody, probably OKT1-like. Prior in vitro assays had demonstrated rapid capping of the target antigen. In vivo, at the end of a 4 hour infusion, all Mifa-7 antigen had been capped off the circulating T lymphocytes with minimal diminution in T cell numbers, as indicated by OKT3 and OKT11 numbers on FACS analysis. Free circulating Mifa-7 antibody was present until 8 days following the first infusion, and throughout this period the circulating T cell numbers were normal, but the T cells lacked the Mifa-7 antigen. In the second patient, a more rapid infusion of antibody was given and circculating T cells were substantially reduced, but only transiently. The Mifa-7 treatment did not reverse the cellular infiltrate in the cardiac allografts. The F10-89-4 antibody is directed at the human leucocyte common (LC) antigen, a highly leucocyte specific membrane glycoprotein found on lymphocytes, granulocytes and other leucocytes. Previous work had established that rabbit antisera to rat LC completely suppressed renal allograft rejection in the rat. Moreover, F10-89-4 did not cap LC from the leucocyte surface in vitro. After treatment with anti LC, all circulating lymphocytes and granulocytes were coated with antibody for at least 6 days, but with no diminution in circulating cells or reversal of the cellular infiltrate in the graft.

We are currently extending these preliminary studies, especially by testing the combined use of two anti LC monoclonal antibodies directed at different sites of the LC molecule.

PAPER 3

INTELLIGENT MISMATCHING FOR HIGHLY SENSITIZED RECIPIENTS

B.A. Bradley, P.T. Klouda and T. Ray.

U.K. Transplant Service, South Western Regional Transfusion Centre, Southmead, Bristol BS10 5ND.

19 centres throughout the UK and Ireland collaborated in an exercise to find transplants for highly sensitized recipients (>85% reaction frequency). Serum sets from these patients were distributed at 3 monthly intervals and experience gained with the first two cycles are reported. 216 donors were tested against these sets and cross-match negative reactions were confirmed by 'blind' duplicates. 27 transplants were performed with kidneys selected primarily on the basis of negative cross-match test and without close regard to HLA matching. Transplants have been followed up for periods ranging between 1 week and 7 months. 18 transplants were functioning and 9 had failed at the time of writing. During the course of this exercise several kidneys were moved on to other centres because negative cross-match tests were not confirmed on arrival at the first chosen centre. However none were wasted as a consequence. Details of further follow-up and cold ischaemia times etc. as well as a separate analysis of first and second transplants will be presented.

Thus, absolute HLA compatibility is not a mandatory requirement to avoid hyperacute rejection in all cases and further evaluation of a policy of selective mismatching for sensitized recipients is warranted.

PAPER 4

ELECTIVE CONVERSION FROM CYCLOSPORIN A (CyA) TO AZATHIOPRINE (Aza) AT 3 MONTHS

P.S. Veitch, T. Horsburgh, S. Parvin, P.R.F. Bell.

Department of Surgery, Leicester General Hospital, Leicester.

The aim of this study was to assess a regimen of CyA plus steroids for the first three months after transplantation followed by elective conversion to Aza and steroids. The overall patient and graft survival of 43 consecutive recipients of cadaver donor grafts (28 first, 15 second) is 97% and 88% respectively (4-15 months follow up). In the first three months 2 grafts were lost and 5 failed to achieve stable renal function leaving 36 patients who entered conversion. Nineteen (53%) patients (O diabetic) converted successfully with a mean decrease in their serum creatinine from 167 umol/l, to 117 umol/l. All have stable renal function without further rejection episodes (1-12 months). Fourteen patients (3 diabetic) failed to convert because of rejection occurring 3-86 days after stopping CyA. The first 8 proved steroid resistant or were followed by re-rejection after 3 x ½g Solumedrone and required re-introduction of CyA. Three grafts have been lost from this group (1 CMV death, 2 rejections). Six subsequent cases were treated by immediate re-introduction of CyA with rejection reversal. The remaining three patients failed conversion because of a viral-like illness (2 proven CMV). Their symptoms resolved with cessation of Aza and steroids and re-introduction of CyA. We conclude that for patients converting successfully there is a significant improvement in renal function. However, conversion at 3 months is potentially hazardous and may be accompanied by early steroid resistant rejection or CMV disease.

PAPER 5

LOW DOSE MAINTENANCE CYCLOSPORIN A (CsA) THERAPY AFTER RENAL TRANSPLANTATION

E.C. Haygood*, S. Dosa, A.M. Thompson and S. Karmi*.

Div. of Nephrol., George Washington Univ. Med. Ctr., Washington D.C.

CsA nephrotoxicity in renal allograft recipients appears to be dose related and in most cases reversible. The effective CsA maintenance dose is reported to be 5-10 mg/kg/day. However, the minimum dose required to maintain good graft function is yet to be established.

Fifteen patients (mean age 38.4 yrs, range 28-60 yrs) with cadaveric renal allografts were treated with CsA and Medrol. CsA was given orally starting at 13-16 mg/kg/day. At 4 wks post-transplant, the mean CsA dose (mg/kg/day) was 8.8 ± 2.7; at 8 wks 6.2 ± 1.62; at 12 wks 4.6 ± 1.13 and at 16 wks 3.65 ± 1.16. Medrol was tapered from the initial mean dose (mg/day) of 144.7 ± 48.2 to 25.7 ± 10.4 at 4 wks, 13.2 ± 4.8 at 8 wks and 8.3 ± 1.62 at 16 wks post-transplant. Trough whole blood CsA levels were measured by HPLC. The mean follow up period post-transplant was 19.4 wks (range 4-35 wks). At present 14/15 patients studied have functioning grafts. The mean serum creatinine (mg/dl) at 4,8,12 and 16 wks was: 1.94 ± 1.13, 1.80 ± .73, 1.65 ± .59 and 1.45 ± .36 respectively. The mean CsA levels (ng/ml) at 4,8,12 and 16 wks were: 221 ± 34; 214 ± 26; 229 ± 49 and 206 ± 27.8. The changes in the CsA blood levels from 4-16 wks were not significant ($p<0.01$).

Our data show that in renal allograft recipients the maintenance dose of CsA used in combination with steroids could be much lower than previously reported. The long term effect of low dose CsA therapy on the incidence of nephrotoxicity and allograft survival requires further studies.

PAPER 6

CYCLOSPORIN A TOXICITY DIFFERENTIATED FROM REJECTION BY OBJECTIVE MEASUREMENT OF RENAL SIZE BY ULTRASOUND

S.D. Parvin, P.S. Veitch, P.R.F. Bell and Y. Rees.

Department of Surgery, University of Leicester, Leicester.

Accurate ultrasound measurement of cross sectional area (CSA) of the transplanted kidney has been used to differentiate between Cyclosporin A toxicity and rejection in both the immediate post-transplant period and at the time of conversion from Cyclosporin A to Azathioprine. Patients were scanned daily after transplantation for three weeks, as outpatients if clinically indicated, and during the conversion period from Cyclosporin A to Azathioprine three months after transplantation. At each scan four measurements of CSA were made and the mean recorded. A rise in CSA of greater than 10% over two consecutive days coupled with a rise in creatinine level of greater than 30mmol/l was taken to indicate rejection, whilst a similar rise in creatinine without the rise in CSA was taken to indicate toxicity.

Forty patients have been studied and twenty one have been converted from Cyclosporin A to Azathioprine.

Figure 1.

	N	Pts	U/S Correct	Biopsy Proven
Early rejection	12	9	12	8
Conversion rejection	7	7	7	7
Toxicity	8	7	7	-

In 5 of the early rejections and in all 7 of the conversion rejections the rise in CSA antedated the rise in creatinine and clinical evidence of rejection by at least 2 days.

PAPER 7

CYCLOSPORINE A FOR TREATMENT OF STEROID RESISTANT ACUTE REJECTION IN RENAL CADAVER GRAFT RECIPIENTS

F.W. Ballardie, C.G. Winearls, D.J. Evans, A.J. Rees, G. Williams.

Royal Postgraduate Medical School, London W12 0HS.

We have used Cyclosporine A to treat acute rejection refractory to repeated high dose steroids, in patients treated initially with azathioprine and maintenance prednisolone. The outcome is compared with that of an earlier group of patients with rejection apparently refractory to this treatment. Morphological changes and prognostic features of rejection were assessed by graft biopsy in each group.

Group I : Seven recipients (8 grafts) were given Cyclosporine A, initially 15 to 17 mg/kg/day orally, after failure to respond to a minimum of 6 grams of intravenous methyl prednisolone. Azathioprine was discontinued in each. Six grafts recovered function and two failed.

Group II: Thirteen recipients (14 grafts) received no further high dose steroids after continuing deterioration in graft function following treatment with 6 to 8 grams of intravenous methyl prednisolone. Eleven grafts failed, but three recovered.

(Group I vs Group II, $P<0.05$, 2 tailed Fisher's exact test).

In both groups, biopsy appearances were similar and scores for unfavourable prognostic indices showed no differences between the groups.

Our experience and that of others suggests that Cyclosporine A is a useful option in patients treated initially with conventional immunosuppression when acute rejection is refractory to high dose steroids, and indicates the need for a controlled trial of its use as a secondary immunosuppressant.

PAPER 8

PERCUTANEOUS ANTEGRADE PYELOGRAPHY (PAP) FOR THE DIAGNOSIS OF URINARY LEAKAGE AND OBSTRUCTION AFTER RENAL TRANSPLANTATION

Johan H. van Bockel[1], Evert H. Overbosch[2], Leen C. Paul[3].

[1]Dept. of Surgery; [2]Dept. of Radiology; [3]Dept. of Nephrology of the University Hospital Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

In order to determine whether deterioration of transplant kidney function has been caused by immunological or urological complications, proper anatomical imaging of the urinary system is often mandatory. For this purpose PAP is used in our institution.

From Oct. 1982 to June 1984 23 pyelograms were performed in 18 patients. The indication for PAP was suspected leakage in 8 pts and obstruction in 10 other patients.

In the presence of a dilated collecting system PAP was performed successfully in all cases, in the absence of dilation PAP was technically impossible in 3/8 patients.

After the procedure microscopic haematuria was observed in all patients, but only in one patient haematuria was severe, resulting in complete urinary obstruction which was successfully relieved by percutaneous nephrostomy.

After technically successful PAP the site and configuration of the ureteral lesion was properly delineated in all cases. In addition, recovery of function after temporary percutaneous nephrostomy in 2 patients with obstruction demonstrated the causative relationship between the obstruction and decreased renal function. Consequently definitive management could be performed. In pts with suspected leakage without confirmation on PAP the subsequent clinical course was uneventful without any specific urological therapy.

We conclude that PAP is an effective method in the precise diagnosis of posttransplant urinary complications. Selective nephrostomy yields essential information about the cause of impairment of transplant function.

PAPER 9

PROSPECTIVE EVALUATION OF THE INDIUM-PLATELET METHOD IN MONITORING RENAL ALLOGRAFTS IN MAN

W.A. Jurewicz, J.G.A. Dykes, B.K. Gunson, P. McMaster, A.D. Barnes.

Renal Unit, Queen Elizabeth Hospital, Birmingham.

Forty three recipients (33-CyA and 10-Aza) were monitored for rejection in the first 2-3 post-operative weeks using the Idium-platelet method.

Based on the criteria established in a previous study, diagnosis regarding the presence of significant rejection was made daily, in a prospective manner.

Fourteen patients showed no clinical evidence of rejection but the remaining 29 suffered at least one acute rejection episode during the Indium-platelet surveillance.

Each patient was followed up for up to 1 year (mean 7.5 ± 3.9 months). Comparison between clinical assessment, the Indium-platelet diagnosis and the long term results is presented in the table.

Clinical DIAGNOSIS / IN-PLATELET DIAGNOSIS	N-R	A-R	GRAFT LOSS	CH-R	F.G.	DEATH
N-R	10	5	0	0	14	1
A-R	4*	24	9	4	13	2
GRAFT LOSS	0	9				
CH-R	2	2				
F.G.	12	15				
DEATH	0	3				

N-R - no rejection during the study.
A-R - acute rejection.
CH-R - chronic rejection on biopsy.
GRAFT LOSS - indicates the loss due to A-R.
F.G. - functioning grafts.

* - 2 out of the 4 patients, who were initially regarded as false positive results, developed chronic rejection during the follow-up period.

Predictability of the positive diagnosis by the Indium-platelet method was calculated at 93%, sensitivity at 84% and specificity at 83%.

PAPER 10

MASSIVE INDUCTION OF DONOR-TYPE CLASS I AND CLASS II MHC ANTIGENS IN REJECTING CARDIAC ALLOGRAFTS IN THE RAT

Andrew D. Milton and John W. Fabre

Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ

There is now a multitude of data indicating that the cellular expression of class I and class II MHC antigens is inducible by the interferons. Given the central role of MHC antigens in immune regulation and allograft rejection, and that the cellular infiltrates involved in rejection almost certainly in most instances release many lymphokines (including the interferons) within the grafted tissue, we set out to study MHC expression during the rejection of cardiac allografts in the rat.

DA (RT1a) hearts were transplanted into PVG (RT1c) or DA recipients, excised on days 1, 3, 5 or 7 after grafting, and examined by immunohistological techniques and quantitative absorption analyses. Allospecific mouse anti rat class I and class II MHC monoclonal antibodies were used, which reacted with donor (DA) but not recipient (PVG) MHC antigens. Cryostat sections stained by the peroxidase technique demonstrated that, in the normal heart, class I antigens were largely restricted to vascular endothelium and interstitial cells, with no observable staining of the myocardial cells except at the intercalated discs. Class II antigens were found only on occasional interstitial dendritic cells. By day 3, however, there clearly was patchy induction of both class I and class II antigens on the myocardial cells, usually in the region of cellular infiltrates. By day 5, class I antigens had been strongly induced throughout the graft, with the myocardial cells being very strongly positive. Class II antigens were also uniformly expressed on myocardial cells at day 5, and at this stage the vascular endothelium was also strongly positive. Quantitative absorption analyses showed a 10-fold increase in class I antigen content in cardiac allografts at day 5 after transplantation when compared to normal DA heart. DA heart isografts showed no increase in class II antigens, but by 5 days after grafting there was weak but definitely increased expression of class I antigens on the myocardial cells. These studies demonstrate that MHC content and distribution changes dramatically during the allograft response, and this might play a critical role in the development of the rejection response.

PAPER 11

INTRA ORGAN PRESSURE CHANGES IN REJECTING KIDNEY, LIVER AND HEART TRANSPLANTS IN THE RAT

B. Vasir, N. Kamada, H.E. Hohmeier, T. Tamaki and J.R. Salaman.

Departments of Surgery and Transplantation, Royal Infirmary, Cardiff and Addenbrookes Hospital, Cambridge, U.K.

We have previously demonstrated to the Society that intra renal pressure is usually raised in patients with kidney transplants that are undergoing rejection. To study pressures in other transplanted organs we have performed the following experiments in rats. In three separate studies kidneys, livers and hearts were transplanted from DA donors to Lewis recipients. Lewis to Lewis isografts were used as controls. The renal and cardiac transplants were anastomosed to the cervical vessels using cuff techniques but the liver transplants were placed orthotopically. The intra organ pressure was measured on every second day using a fine needle, and animals were sacrificed on the 9th or 10th day if they had not already died.

*p<.05		Day 2	Day 4	Day 8
Renal pressure mmHg ± 1SD	Isografts n=8	30.8 ± 6	21.8 ± 4	21.3 ± 2
	Allografts n=13	27.2 ± 2	44.8 ± 15*	63.4 ± 16*
Myocardial pressure	Isografts n=8	25.8 ± 2	27.3 ± 4	22.0 ± 1
	Allografts n=9	25.6 ± 2	44.5 ± 9*	68.5 ± 6*
Hepatic pressure	Isografts n=4	10.1 ± 1	12.5 ± 1	12.2 ± 1
	Allografts n=6	10.0 ± 1	33.0 ± 3*	53.8 ± 15*

A raised pressure was recorded from all allografts, this first becoming significant on days 3, 4 and 3 for renal, cardiac and liver allografts respectively. The test may therefore be useful in the management of patients with cardiac and liver grafts as well as those with renal transplants.

PAPER 12

FUNCTION OF INTESTINAL ALLOGRAFTS IMMUNOSUPPRESSED WITH CYCLOSPORIN-A

A. Dennison, R. Watkins, J. Collin, P. Millard and P.J. Morris.

Nuffield Departments of Surgery and Pathology, Oxford.

Intestinal motility, absorption, intraluminal pressures, electrical activity and histology were evaluated in autografts and allografts of small intestine in dogs. All grafts were 100cm Thiry Vella fistulae with orthotopic vascular re-anastomosis. Cyclosporin-A (CyA) was given to 18 dogs with allografts at 20mg/kg/d p.o., and daily plasma levels measured.

57 dogs received grafts (24 auto and 33 allo) with 14 technical failures. 18 dogs with autotransplants survived indefinitely. 14 non immunosuppressed animals survived 8-15 days (11.26 ± 2.6 mean ± s.e.m.), while survival in 11 dogs receiving CyA was increased to 19-142 days (70.09 ± 13.34; t=4.41, p=0.0002). Earliest histological evidence of rejection occurred at 6.2 ± 0.9 days but differences (cellular infiltrates, lacteal oedema and blunting of villi) between auto and allografts were quantitative, and when unequivocal evidence of rejection (necrosis of the lamina propria) was present, vascular changes were irreversible. A blood stained effluent, the abolition of intestinal electrical activity and reduced water absorption (5.4 ± 1.09 day 6 to 1.77 ± 0.26 day 7; mean ± s.e.m. ml/5min; t=3.22, p=0.0092) followed histological changes by 1-2 days. Dogs with autotransplants lost less of their pre-operative weight (3.9 ± 0.75% mean ± s.e.m.) by day 28, than immunosuppressed dogs (15.1 ± 1.54%; t=6.45, p<.0001). All immunosuppressed dogs had plasma CyA levels of >200ng/ml, but grafts were thickened, granular, pale, and bled little when biopsied.

Function in autotransplants is only minimally disordered compared with non transplanted Thiry Vella fistulae, but immunosuppressed allografts have grossly disordered histology and function, and the clinical condition of the animals is poor.

PAPER 13

THE EFFECT OF EXCHANGE BLOOD TRANSFUSIONS ON ANTIBODY PRODUCTION IN AS RATS

N. Mistry, T. Horsburgh, P.S. Veitch, P.R.F. Bell.

Department of Surgery, Leicester General Hospital, Leicester.

To study the effect of plasma exchange on the antibody response a technique of whole blood exchange was developed. This involved the removal of approximately half the circulating blood volume by cardiac puncture, immediately followed by a transfusion of a similar volume of non-heparinised syngeneic blood. Four exchanges replaced approximately 90% of the circulating blood as determined by ^{51}Cr labelled red cell study. AS rats injected with 1ml August blood, were exchanged transfused four times within 24 hours and the antibody response followed with a ^{51}Cr labelled, complement dependent cytotoxicity assay (CDC). There was a significant increase in anti-August antibody cytotoxicity over the first 10 days, but this effect was only seen when exchange transfusion was performed immediately after immunisation. AS rats primed with August blood, boosted with August blood and exchanged transfused showed no increase in anti-August antibody. However, August primed AS rats immunised with Lou/F blood showed both anti-Lou/F and anti-August antibody activity, which responded to exchange transfusion. We conclude that exchange transfusion only produces a "rebound" effect on cytotoxic antibody production when performed close to antigen administration. These findings may be relevant in the use of plasmapheresis in transplantation.