MEETING OF
BRITISH TRANSPLANTATION SOCIETY

17th October, 1973

THE WELLCOME BUILDING, EUSTON ROAD, LONDON

9.45 a.m. **D. N. H. Hamilton, D. G. Gilmour and Catherine E. Hamilton** (*Department of Surgery, Western Infirmary, Glasgow*):

'Problems in the restoration of 'B' mice by thymus grafts.'

10.00 a.m. **J. W. Fabre and P. J. Morris** (*Queen Victoria Hospital, East Grinstead*):

'Specific suppression of renal allograft rejection in rats presensitised by skin allografts.'

10.15 a.m. **N. A. Staines, K. Guy and J. H. Creedy** (*Searle Research Laboratories, High Wycombe*):

'Relative immunogenicity of H-2 and other membrane components in Xenoimmunisation.'

10.30 a.m. **Saroj Sengupta and J. F. Mowbray** (*St. Mary's Hospital Medical School, London*):

'Detection and characterisation of soluble HLA-alloantigens in normal human plasma.'

10.45 a.m.-11.00 a.m. Coffee.

11.00 a.m. **D. N. Fernando, S. P. Newman, V. M. Hird, D. G. Sampson, H. S. Williams, J. P. Hopewell, P. R. Read and J. F. Moorhead** (*The Royal Free Hospital, London*):

'The effects of Frusemide on the blood flow of transplanted kidneys in dogs.'

11.15 a.m. **Valerie C. Joysey, J. H. Roger, D. B. Evans and B. M. Herbertson** (*Addenbrooke's Hospital and Department of Medicine and Pathology, Cambridge*):

'Kidney graft survival and matching for HL-A and ABO antigens.'

11.30 a.m. **J. D. Briggs, D. Jackson and P. R. F. Bell** (*Western Infirmary, Glasgow*):

'Infection following renal transplantation.'

11.45 a.m. **H. V. Price, H. Langmaid and J. R. Salaman** (*Cardiff Royal Infirmary*):

'Renal transplantation and pregnancy—the effects of immunosuppressive drugs on the foetus.'

12.00 noon **G. A. Coles, H. O. White and A. D. Barnes** (*Cardiff Royal Infirmary, Southmead Hospital, Bristol and Queen Elizabeth Hospital, Birmingham*):

'A controlled trial of anticoagulants in cadaveric renal transplantation.'

12.15 p.m. **A. G. Clarke and J. R. Salaman** (*Cardiff Royal Infirmary*):

'Methyl prednisolone in the treatment of rejecting renal transplants.'

12.30 p.m. **Judy Lipscombe and R. J. Hamshere** (*Urological Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London*):

'An *in vitro* test of renal viability.'

12.45 p.m. **R. Y. Calne** (*Addenbrooke's Hospital, Cambridge*):

'Collaborative investigation into kidney viability.'

1.00 p.m. LUNCH.

2.00 p.m. WORKSHOP—'Predictive Tests for Clinical Transplantation.'
Organised by P. R. F. Bell and H. Festenstein.

3.15 p.m. TEA.

3.30 p.m. Workshop continued.

4.30 p.m. ANNUAL BUSINESS MEETING OF THE SOCIETY.

FUTURE MEETINGS

3rd JANUARY, 1974, the day preceding the Surgical Research Society Meeting, Charing Cross Hospital (Fulham). Open papers and Workshop on 'The potential role of tolerance and enhancement in the management of human organ transplants.' (Organiser: J. R. Batchelor.)

17th APRIL, 1974. Spring Meeting, London.

16th OCTOBER, 1974. Autumn Meeting, London.

ABSTRACTS (not for publication)

D. N. H. Hamilton, D. G. Gilmour and Catherine E. Hamilton

'B' mice (thymectomised, irradiated bone marrow reconstituted CBA mice) can be restored to normal levels of cell-mediated immunity (CMI) by an adult or foetal thymus lobe placed subcutaneously in the groin. 'A' strain skin allografts placed at intervals after thymus grafting showed return of CMI after 3 weeks with the foetal graft and more slowly with the adult lobe.

If, however, thymus grafting was carried out **after** skin allografting, progressive difficulty in rejecting these grafts was encountered with time. Newly placed skin grafts were rejected at about 20 days, but well-established grafts proved difficult or impossible to reject, the mice thus becoming tolerant.

The mechanism of this phenomenon will be discussed.

W. Fabre and J. Morris

These studies were initiated largely to test the effectiveness of passive enhancement of renal allografts in sensitised recipients, so as to better define the role that passive enhancement might play in clinical immunosuppressive regimens.

Prospective renal graft recipients were sensitised by two donor-strain skin grafts 6 weeks apart and the renal allograft was performed 4 weeks after the second skin graft. The immune response against the renal graft was followed by weekly blood ureas and lympho-cytotoxin titres, and by animal survival. Untreated and passively enhanced groups were studied in both normal and sensitised recipients, and all experiments were performed in both the (DA x Lewis)F_1 to Lewis and (DA x Lewis)F_1 to DA models.

The results were as follows:

Surprisingly, skin graft sensitised recipients showed suppressed renal allograft rejection. This was only barely detectable in the F_1 to Lewis model, but was very marked in the F_1 to DA model, with the majority of sensitised recipients surviving indefinitely after a delayed rejection episode.

The addition of enhancing serum to the sensitised recipients made no difference, and this was shown to be due to the fact that the sensitisation procedure had already induced adequate quantities of enhancing antibody in the sensitised recipients.

A comparison of the results of passive enhancement in normal and sensitised recipients showed that the sensitised recipients did worse, especially in the F_1 to Lewis model, where the effect of passive enhancement was only barely detectable in sensitised recipients.

It is reasonable to interpret these findings as showing that skin graft sensitisation had increased the reactivity of the cellular immune system but that it had also induced the production of enhancing antibodies to which the sensitised cellular immune system was susceptible. One can draw two main conclusions. Firstly, sensitised clones are susceptible to specific immunosuppression. Secondly, when considering second and third set grafts one cannot assume that rejection will be more vigorous. The result depends on the nature of the test graft and the strain combination used.

From the clinical point of view, these results show that passive enhancement is only partially effective, and sometimes virtually ineffective in strongly presensitised individuals and this defines a limit in the use of passive enhancement. Further experiments in the F_1 to Lewis model have shown that a short post-graft course of ALS, in addition to enhancing serum, delays but does not prevent rejection.

N. A. Staines, K. Guy and J. H. Creedy

The cytotoxic antibody response in rabbits against mouse spleen cell membranes was shown to be predominantly directed against determinants associated chromographically with H-2. Gel filtration of complexes of H-2 and xenoantibody F(ab')2 fragments showed that the dominant determinants reside on H-2 molecules. Only minor serological reactions of non H-2 membrane components were detectable. The immunogenicity in rabbits of soluble membrane components separated from H-2 by ion-exchange chromatography was examined. All components were immunogenic, several more so than H-2 antigen itself. Sera against these components were used to analyse antigen distribution after ion-exchange chromatography. Sera were classified in 3 groups on the basis of their reaction profiles. First, sera against H-2 which only reacted with H-2 active fractions. Second, sera which reacted with antigen(s) distributed across the column. Third, sera which revealed areas of activity not coincident with either H-2 or major protein peaks. A paradox exists: H-2 molecules are immunodominant in the intact membrane but separated soluble membrane components can be more immunogenic than H-2 on its own. A parallel is drawn between this and the alloimmune response to H-2 and other membrane antigens, but no model can yet be proposed to explain this phenomenon.

Saroj Sengupta and J. F. Mowbray

Earlier reports by Van Rood *et al.* and Charlton and Zmizewski have shown the presence of HLA 2, HLA 7 and 7b in the normal sera. Using inhibition of ^{51}Cr release and fluorochromasia cytotoxicity techniques, a number of other antigenic specificities present in normal human plasma have been studied. The antigenic activity is present in the euglobulin fraction of plasma and can be concentrated by euglobulin precipitation. Gel filtration of plasma or euglobulin yields fractions of 40-60,000 apparent molecular weight which carry the various antigenic specificities. Evidence will be presented that the antigenic specificities belonging to the first and second series in these fractions may be separated by ion exchange chromatography on DEAE cellulose.

References

1. Van Rood, J. J., Van Leewen, A. and Van Santen, M. C. T. (1970). Nature 226, 366.
2. Van Rood, J. J., Van Leewen, A., Kuck, C. T. and Frederika, E (1970). In P. I. Terasaki (ed.). Histocompatibility testing 1970, p. 483. Munksgaard, Copenhagen.
3. Charlton, R. K. and Zmilewski, C. M. (1970). Science, 170, 636.

O. N. Fernando, S. P. Newman, V. M. Hird, D. G. Sampson, H. S. Williams, J. P. Hopewell, P. R. Read and J. F. Moorhead

The restoration of normal blood flow to a cadaver kidney immediately after completion of vascular anastomosis is desirable but often impossible.

Phenoxybenzamine and procaine hydrochloride have been added to the cold solution used to perfuse kidneys to procure a dilatation of renal vasculature. Experiments were performed on dogs to determine whether by the addition of Frusemide to the perfusate the blood flow to transplanted kidneys could be increased. Blood flows were measured using a ^{133}Xe technique and clearance curves were analysed into cortical and medullary components. Kidneys perfused with cold perfusate containing Frusemide showed an 11% reduction in the total renal blood flow and 19% reduction in the cortical blood flow while the distribution of blood to the ureter and medulla was unchanged. In contrast control kidney (i.e. perfusate only) showed a 47% reduction in total blood flow and a reduction of 55% in cortical flow. These results show that the addition of Frusemide to the perfusate used in clinical transplantation may help restore a blood flow similar to that before donor nephrectomy.

Valerie C. Joysey, J. H. Roger, D. B. Evans and B. M. Herbertson

Results of 124 first kidney transplants performed over 6 years at one centre are analysed. HL-A typing was done prospectively and retrospectively with cryogenically stored cells, using sera of 23 specificities. Rejection was assessed histologically. Actuarial curves were computed at 3 monthly intervals.

HL-A identity of donor and recipient was associated with improved graft survival, but this did not reach statistical significance.

Compatibility of ABO antigens according to blood transfusion practice is normally exercised in choice of donors for kidney transplantation. The present data were analysed to examine this practice. Actuarial curves showed superior allograft survival in group O recipients, and the failure rate due to rejection was less than in non group O recipients ($P<0.05$). In group O recipients no effect of HL-A compatibility was demonstrable. In the non-group O recipients (i.e. groups A, B and AB) no relationship was evident between graft survival and incompatibilities at the first segregant series. However, compatibility at the second segregant series was associated with improved graft survival ($P<0.05$), confirming the greater importance of the second segregant series (Oliver *et al.* 1972).

The possible implications of these findings will be discussed.

Oliver, R. T., Sachs, J. A., Festenstein H., Pegrum, G. D. and Moorhead, J. F. (1972) Lancet ii, 1381.

J. D. Briggs, D. Jackson and P. R. F. Bell

Serious infections, in particular fungal infections, are relatively common following renal transplantation (Bach *et al.*, 1973). This paper reports our experience with 49 transplants in 48 patients with a minimum follow-up of one year. Thirty-four patients suffered no infection, while 15 patients suffered from systemic or local infection. There were 3 cases of pneumonia, 6 pelvic abscesses, and single cases of sonnei dysentery, pyogenic arthritis, perianal abscess, herpes zoster, wound infection, scrotal abscess and serum hepatitis. In addition, the 2 deaths in the series were due to infection, in one cerebral moniliasis, while in the other the precise nature was not determined. Of the 39 transplants which functioned for more than 3 months, 27 had no bacteriuria at any time. In six cases, there was less than one episode of bacteriuria per year, while the six remaining cases had more than one episode per year. The low rate of serious infection may be related to the fairly small doses of azathioprine and oral prednisolone with the use of intravenous prednisolone to treat rejection episodes. This immunosuppressive regime has resulted in a graft survival of 63% (12-52 months) and mortality of 4%.

Bach, M. C., Sohroun, A., Adler, J. L., Schlesinger, R. M., Bremen, J., Macras, P., Fang-Ku, Peng, and Monaco, A. P. (1973). Lancet, 1, 180.

H. V. Price, H. Langmaid and J. R. Salaman

Pregnancy after renal transplantation is uncommon. Although experimental evidence would suggest that the taking of immunosuppressive drugs by the mother could result in foetal abnormalities[1] few gross deformities have been seen.[2] We have studied two infants born to recipients of cadaveric renal transplants, and have found evidence of chromosomal damage in both.

The first infant was born by caesarean section at 37 weeks at which time foetal growth had ceased, and the mother was exhibiting pre-eclamptic toxaemia. The second child was delivered prematurely at 30 weeks whilst the mother was suffering from SH negative hepatitis. Both infants were grossly normal and are currently 22 and 4 months old.

Investigations showed that the first child had a small thymus, and that both had lymphopoenia during the first two weeks of life. PHA transformation was normal. Serial cortisol estimation were low-normal but a good response was obtained with A.C.T.H. The mothers' and babies' lymphocyte chromosomes have been serially analysed and chromatid gaps, deletions and acentric fragments have been regularly found.

Abnormal chromosomes have been previously reported in patients receiving Azathioprine[3] but these have not been sought in their progeny. The abnormalities found in our infants have persisted for up to 22 months and we consider that some permanent chromosomal damage must have occurred. The significance of these findings will be discussed.

1. Rosenkranz, J. G. *et al.*, Amer.J.Obstet.Gynae 97, 387.
2. Penn, I. *et al.*, J.A.M.A. 126, 1755 1971.
3. Jensen, M. K., A.C.T.A., Med.Scand. 183, 445. 1967.

G. A. Coles, H. O. White and A. D. Barnes.

One of the prominent pathological findings in rejecting kidney transplants is the deposition of fibrin in the vessels of the graft and it has been suggested that anticoagulation treatment might limit this phenomenon (Kincaid-Smith 1969).

A randomised controlled trial has been performed on seventy-six patients who received cadaveric renal transplants. Seventeen of the transplants failed to function, fifty-nine patients entered the trial proper, twenty-nine received Warfarin and thirty acted as controls. Anticoagulation was commenced early in the post-operative period on the decision of the clinician in charge and was maintained for six months or until complications necessitated its being stopped. The findings were that in ten patients anticoagulation was discontinued because of dyspepsia, bleeding or uncontrolled prothrombin time. One patient died from widespread bleeding despite stopping Warfarin. One patient in the controlled group died of a pulmonary embolus. At the end of six months follow-up there was no statistical difference in the survival of grafts between anticoagulant and control groups.

A. G. Clarke and J. R. Salaman

Since 1967, 139 renal transplants have been performed in Cardiff and until recently all rejection episodes were treated with oral prednisone commencing at 200-400 mg/day with subsequent doses tapered gradually to maintenance levels (Group A). In 1971 "pulse" therapy with intravenous methyl prednisolone was introduced (1g 12 hrly for three doses). At first the oral prednisone dose was briefly raised as well (Group B), but in the last 18 cases the prednisone dose was not altered (Group C). The three groups have been compared as regards transplant survival, mortality and overall complication rate.

Group	No. of Transplants	Rejection Episodes/ Transplant	Rejections Reversed	1 year Transplant Survival	Complications/ Patient	Deaths
A	50	1.5	67%	34%	1.0	26 (52%)
B	19	1.7	69%	47%	0.9	5 (26%)
C	18	1.4	72%	61%	1.2	3 (17%)

Since the introduction of methyl prednisolone there has been a striking reduction in mortality. In Group A, most deaths were due to sepsis although 5 patients succumbed to gastro-intestinal bleeding. Of the 5 patients dying in Group B, one developed pneumonia, and another secondary haemorrhage. The three others died on dialysis 11-12 months after all immunosuppression had been discontinued. In Group C one patient died of cardiac failure and two others died of a wasting syndrome 1-2 months after returning to dialysis.

The overall complication rate was not altered, but in Groups B and C the complications were less serious than in Group A. A similar rate (1.2/patient) was observed in 52 recipients who had no recorded rejection episodes.

Methyl prednisolone on its own was effective in reversing rejection in 72% of cases, with a low accompanying mortality and we consider this to be the best form of therapy at the present time.

Judy Lipscombe and R. J. Hamshere

As a corollary to renal transplantation, a test that predicts the quality of cadaveric donor kidneys is urgently needed.

A test is described that depends upon the ability of a kidney biopsy actively to absorb I^{125} Hippuran against a gradient.

Thin slices of kidney are incubated in an oxygenated buffered saline medium for one hour. Counts of the radioactivity of the medium and slices are determined and expressed as a ratio (S/M ratio).

Rat and dog kidney tissue with minimum warm ischaemic injury gave a mean S/M ratio of 6.1 and 9.2 respectively, while tissue exposed to 24 hours warm ischaemia gave a value not exceeding 1.2 ($p = > 0.001$).

With increasing warm ischaemic times, the S/M ratio fell from 9.2 to 4.4 over 2 hour periods ($p = < 0.001$).

Survival experiments have shown that kidneys with S/M ratios less than 4 after warm ischaemic injury were not able to support life even when contralateral nephrectomy was delayed for 2 weeks.

The method described is quick and easy to perform. It does not seem to be influenced by solutions used for preservation and can be used as an index of viability of a kidney during preservation.

R. Y. Calne

At a recent CRC meeting at Northwick Park, one field of research that was felt to have been neglected in the past was the assessment of kidneys prior to preservation. This is particularly pertinent in the U.K. where, due to shortage of donor kidneys, organs are often removed under relatively unsuitable circumstances, resulting in a primary failure rate from ischaemic damage of around 25%.

This is a great waste of effort and a cause of unnecessary suffering to patients given useless kidneys. An *ad hoc* committee was formed to discuss methods of assessment and it was felt that the following perameters should be studied:

1. Assessment of donor status.
2. Organ biopsy for intracellular electrolytes.
3. Perfusion characteristics.
4. Effluent composition.

It was felt that although this involved considerable extra work, the collaboration of as many centres as possible should provide data fairly rapidly and point to whether or not this project was worthwhile. The National Tissue Typing Reference Laboratory at Bristol under Dr. Tovey has agreed to collect and process the data and I wish to solicit as many collaborators as possible.

AGENDA FOR THE ANNUAL BUSINESS MEETING

TO BE HELD ON WEDNESDAY, 17th OCTOBER, 1973

1. Minutes of Business Meeting held on October 18th, 1972 (see below).
2. Matters arising from the Minutes.
3. Election of Committee members.

Present Committee	*Elected*	*Due to retire*
General Secretary: Leslie Brent	1972	1974
Meetings Secretary: Anthony D. Barnes	1972	1974
Treasurer: John Hopewell	1972	1975
Richard J. Batchelor	1972	1973
Roy Y. Calne	1972	1974
Hilliard Festenstein	1972	1975
Eugene M. Lance	1972	1975
Sir Peter Medawar (Chairman)	1972	1974
John R. Salaman	1972	1973
John L. Turk (B.S.I. representative)	—	—

Two Committee member vacancies: the following nominations have been received:

Nominated	*Proposer*	*Seconder*
HEATHER M. DICK, Department of Bacteriology, Royal Infirmary, Glasgow.	P. R. F. BELL	D. N. H. HAMILTON
A. J. S. DAVIES, Chester Beatty Research Institute, London.	L. BRENT	J. R. BATCHELOR
G. WILLIAMS, Charing Cross Hospital (Fulham), London.	R. M. R. BARNES	G. D. PEGRUM
R. A. SELLS, Department of Surgery, University of Liverpool, Liverpool.	R. Y. CALNE	B. BRADLEY

Note: The retirement of Committee members has been arranged by the Committee so as to preserve a balance between "experimental" and "clinical". Rules 15 and 16 of the Constitution are to be found below, as required by the Constitution.

4. Election of new members of the Society.
   The applicants listed below have been considered and approved by the Committee.
5. Resignations: J. B. Morton, Edinburgh; B. Nolan, Edinburgh.
6. General Secretary's Report.
7. Meetings Secretary's Report.
8. Treasurer's Report: Statement of accounts up to May 31st, 1973.
9. 5th International Congress of the Transplantation Society, Jerusalem, 1974.
10. 2nd International Congress of Immunology, Brighton, 1974.
11. Any other business.