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PAPER NO. 1

The Clinical Significance of Plasma Immunosuppressive Factors in Renal Transplantation

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Blood transfusions are known to be associated with improved subsequent renal graft survival, and have been shown by us to produce raised plasma immunosuppressive activity (1). Does the level of plasma suppressive activity (P.S.A.) in the graft recipient measured at the time of transplantation correlate with subsequent graft survival? Retrospective and prospective studies have been developed. Results are expressed as μl of recipient plasma required to inhibit by 50% the response of allogeneic lymphocytes to antigen; and the P.S.A. levels have been correlated with graft survival.

Results

a) Retrospective Study (22 patients : follow up 6 years)

	mean P.S.A. (μl) ± S.D.
Surviving grafts (13)	13.6 ± 10.6
Rejected grafts (9)	29.0 ± 11.4
t-test	p < .002

b) Prospective study (47 patients ; follow up 3–12 months)

		mean P.S.A. (μl) ± S.D.
a) Functioning grafts	(34)	5.06 ± 2.44
b) All failures	(13)	8.15 ± 6.22
c) Rejected grafts	(7)	11.93 ± 6.4
t-test		a x b p=NS a x c p < .03

Thus in both studies, patients who rejected their grafts had significantly lower P.S.A. than those who did not. The non-specific P.S.A. of plasma may be a reliable in vitro test for predicting the subsequent graft survival and can be performed prior to transplantation as the test can be completed in 4 hours. The development of P.S.A. following blood transfusion varies from individual to individual. The regular monitoring of P.S.A. during dialysis could allow a more carefully controlled blood transfusion policy to be adopted – and may also indicate the optimum time for transplantation.

1) Proud, G. et.al. B.J.S. 66 678–682.

PAPER NO. 2

The Effect of HLA-DR Matching on Results of Renal Transplantation within One Unit

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To assess whether matching for HLA-DR improves renal graft survival, information was analysed on kidney recipients and donors HLA-DR from one unit only, the Sheffield Renal Transplantation Unit at the Royal Hallamshire Hospital. It was possible to obtain mutual HLA-DR typing on 24 pairs transplanted over the past two years. Selection of donor recipient pairs was done on a routine basis of optimum matching of HLA-A, B antigens and not on a basis of HLA-DR. Therefore, HLA-DR matching was random.

Five transplant pairs were direct mismatched for the maximum 2 HLA-DR antigens; 12 pairs were mismatched for 1 HLA-DR antigen; 7 pairs showed no direct mismatch for HLA-DR. All transplanted patients with no direct mismatch for HLA-DR are surviving with good renal function. Two out of the 12 transplanted patients with one direct mismatch for HLA-DR rejected their grafts within 6 months, the remainder still have good graft function, although generally they have had more treated rejection episodes than the no mismatch group. Of the transplanted pairs with 2 direct mismatches for HLA-DR, 2 have rejected their grafts, the remaining three show poor renal graft function.

Although the relatively small number of cases assessed exclude statistical analysis, our opinion is that matching for HLA-DR would appear to have a good prognosis in renal transplantation.

PAPER NO. 3

B Cell Alloantibodies in Renal Transplant Recipient Sera and in Eluates from Rejected Renal Allografts

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In a previous study serum B cell antibodies were found in recipients with failed and successful grafts. In this report B cell antibody activity has been investigated in eluates from six rejected renal grafts with evidence of severe vascular damage. Eluates were prepared by acid elution. I^{125} labelled normal human IgG was added to the kidney homogenate and monitored as an index of the efficiency of the washing procedure. The seventh wash contained no radioactivity. The quantity and class of immunoglobulin in the acid eluate was determined by laser nephelometry and immunoelectrophoresis. Eluates were fractionated into IgG and IgM by AcA 34 ultragel chromatography and by ion exchange on DEAE 52 and tested together with the unfractionated eluate. Eluates were tested against separated donor T and B cells, prepared from frozen cadaver spleens, by an extended NIH microlymphocytotoxicity test. Reactivity to panels of normal peripheral T and B cells and monocytes and to neoplastic chronic lymphocytic leukaemic cells, was also examined.

Two patients had serum and eluate antibodies cytotoxic for donor T and B cells; one of these had demonstrable anti HLA A I (donor) activity in the eluate. Two patients who had donor specific B cell serum antibody alone also had donor B cell activity in their eluates. When tested against panel B cells no apparent DRW specificity was seen. Furthermore the eluate antibodies were different in class and specificity from those in the serum. The two remaining patients had no activity in their serum or eluates to donor or panel T or B cells, but their eluates were cytotoxic for a panel of monocytes. An eluate from a control normal kidney showed no cytotoxic activity. These findings suggest that B cell antibodies are heterogeneous in type and may explain the discrepancy between the appearance of antibodies in long term survivors and in the eluates of acutely rejected grafts.

PAPER NO. 4

Skin Allograft Survival in Mice Pretreated with Blood

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It is not clear why blood transfusions given before the transplantation of human renal allografts improve graft survival. It is possible that suppressor lymphocytes are generated, as they are in skin-grafted mice treated preoperatively with donor strain liver extracts and postoperatively with a short course of procarbazine hydrochloride (PCH) and antilymphocyte serum (ALS). Attempts were therefore made to substitute the tissue extracts with donor blood. 0.5 - 5 µl of DBA/2 whole blood injected intravenously into H-2 incompatible CBA mice 16 days before transplantation substantially increased the proportion of permanently surviving grafts (up to 90%) compared with grafts on control mice treated with only PCH and ALS (10 - 20%). The dose of blood based on the number of white cells was critically important: a dose containing 10^4 or 8×10^3 cells gave excellent results whereas smaller doses became progressively less efficacious. Larger amounts of blood (0.1 ml) given once or repeatedly were ineffective or even counterproductive.

The unresponsiveness was strain-specific; however, preliminary results have shown that sharing of K and D antigens between blood and skin graft was sufficient to ensure prolonged survival even though this was not quite as good as when there was, additionally, sharing of Ia antigens. Although the data suggest the effect was brought about by histocompatibility antigens this is being further studied by independent blood fractionation experiments.

The relevance of these data to the effects produced by blood transfusion in man will be discussed.

PAPER NO. 5

Blood Transfusions at Transplantation Improve Renal Allograft Survival in Otherwise Non-Transfused Patients

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Following the report of Stiller and his colleagues* that transfusions at transplantation might exert a beneficial effect on renal allograft survival, our own retrospective analysis indicated that a 15% improvement in one year-graft survival in patients never transfused before transplantation could be attributed to the effects of peroperative transfusion. In February 1978, therefore, a prospective controlled clinical trial was set up to investigate the effects of peroperative transfusion. Only patients who had never been transfused nor pregnant and who were about to receive a first cadaver allograft were randomized into the trial. Those patients entered into the treatment group (N = 13) were given 2 units of whole blood at operation whereas the control group (N = 14) received no blood. No attempt was made to match the blood for HLA antigens with the recipient, but patients in both groups were given a well HLA-ABC,DR matched kidney where possible, following the standard practice of the unit.

The overall one-year graft survival for first cadaver allografts (N = 175) is 67%. Analysis of the trial results after 23 months showed a 34% one-year actuarial graft survival in the control group (9 failures) compared with an 85% one-year graft survival in the treated group (3 failures), p=0.04, all patients being followed up for at least 3 months. Of the 5 surviving allografts in the control group, 4 were completely HLA-DR compatible with the recipient. Considering all patients transplanted both before and after the start of the trial, the one-year graft survival in patients receiving no pregraft transfusions and no peroperative transfusions is 44% (N = 34) compared with 70% (N = 28) in those given no pregraft transfusions but blood at transplant. In patients receiving one or more pregraft transfusions, the figures are 82% (N = 38) for those given no peroperative blood and 73% (N = 38) for patients given one or more units at transplantation.

Peroperative transfusions appear to be a safe way of prolonging renal allograft survival

*Stiller et al (1978) Lancet i 169-170.

PAPER NO. 6

Prolonged Canine Renal Graft Survival by Peroperative Blood Transfusion is Caused by Immunocompetent Lymphocytes

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The beneficial effect of bloodtransfusionson graft survival is well established, but the mode of action remains unknown. Controversial data exist about the optimal number of transfusions and the best transfusion scheme. In the present experiment with dogs all recipients received moderate postoperative immunosuppression which induced slightly prolonged renal graft survival (group I). It appeared that one transfusion of 100 ml. third party blood induced significantly prolonged graft survival in 11 dogs (group II). One bloodtransfusion given 14 days before transplantation was not effective (group III). We studied the mechanism of one peroperative transfusion. Irradiation of the third party blood with 2500 rad before transfusion appeared to abrogate the effect on graft survival (group IV), which suggests that immunocompetent cells in the blood are responsible. Therefore we tested the effect of third party lymphocytes and injected 1.5-3.5 x 10^9 lymphocytes i.v. peroperatively. This indeed induced enhanced renal graft survival (group V). The incompetence of irradiated blood peroperatively does not favour the concept of an immunosuppresaive effect of lysed cells. This concept was further tested by the i.v. administration of Fe-dextran in a small group which did not appear to be effective. In conclusion one bloodtransfusion is more effective peroperatively than on day -14. Inactivation of the transfusion effect by irradiation of the blood suggests an effectivity of immunocompetent lymphocytes, which could be confirmed with transfusion of third party lymphocytes.

Group	Third party cell transfusion	N.	Survival in days	MST	
I	-	11	6-9-10-11-12-13-14-14-14-15-21	13	
II	day 0, 100 ml blood	12	14-15-15-16-20-20-20-24-25-25-29-29	20	p<0.01
III	day -14, 100 ml blood	7	11-11-13-14-17-21-23	14	N.S.
IV	day 0, 100 ml irr.blood	8	9-11-11-13-15-15-16-19	14	N.S.
V	day 0, lymphocytes	5	9-17-20-29->30		p<0.01

re Tumor expt / results at next BTS

PAPER NO. 7

Relationship between Dinitrochlorobenzene Skin Testing before Transplantation and Azathioprine Dosage Post-transplantation

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Evidence suggests that the cell-mediated immunity of regular dialysis treatment patients (RDTP) as tested with Dinitrochlorobenzene (DNCB) may determine graft survival after transplantation.

We have now tested 66 RDTP with DNCB who have undergone cadaveric renal allografts and the graft survival was assessed at 6 months. Fifty patients with a weak DNCB had a graft survival of 68% whilst in 16 patients with a strong DNCB graft survival was 31% ($p<0.01$). These results suggest that a low DNCB response is associated with good graft survival.

A recent finding is that weak DNCB reactors receive more pre-transplant blood transfusions and this may indicate greater bone marrow depression in this group (Watson et al, 1979). To find if this was reflected in the dose of Azathioprine and its effect on the WBC we have examined 14 RDTP with a strong response to DNCB and 14 RDTP with a weak response who underwent transplantation. The dose of Azathioprine was given to keep the WBC at about 6,000/cu.mm. There was no difference between the groups in the mean dose of Azathioprine, the mean white cell count, onset of renal function or incidence of clinical infection during the first 3 weeks after transplantation.

Thus any differences in marrow depression between weak and strong reactors to DNCB were not seen in the haematological response to the drugs used (i.e. the exogenous immunosuppression). Endogenous immunosuppression, as measured by low DNCB responses, thus is additive to the drugs used. Hence the benefits of low DNCB reponses are not compromised by sensitivity to azathioprine.

M A Watson et al (1979) Lancet ii 1323-1326

PAPER NO. 8

Leucocyte Inhibition Correlate of Delayed Hypersensitivity to Dinitrochlorobenzene (DNCB)

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It has been suggested that reactivity to a primary challenge with DNCB may be a useful prognostic index of graft outcome. [1,2]. Hamilton [1] has suggested that leucocyte inhibition to a DNCB - human red blood cell conjugate (DNCB RBC) may be a satisfactory *in vitro* correlate. We have investigated leucocyte inhibition to DNCB RBC prepared as suggested by Hamilton at concentrations from 320 - 3.2 µg/ml in 8 unsensitised normals, 1 sensitised normal and 29 sensitised haemodialysis patients. Sensitised individuals were tested at 16 days post primary challenge at the time of patch test assessment. Leucocyte migration inhibition was observed in all groups. This inhibition was reversed by puromycin. Inhibition was not seen to DNCB coupled to human serum albumin or to red blood cell membrane fragments alone. There was no correlation between DNCB skin reactivity and the *in vitro* leucocyte inhibition test. These findings are discussed in relation to the usefulness of the leucocyte migration test and its relationship to the skin test findings in haemodialysis patients.

1) ROLLEY et al (1977) Transplantation Proceedings 9, 81-83.
2) Hamilton et al (1976) Lancet ii, 1170.

PAPER NO. 9

Specific in Vitro Suppression of Both the MLC-Responder Function and the Generation of Cytotoxic T-Lymphocytes by Soluble Factor(s) Present in Human and Pig Liver Extracts

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It has been generally observed that in contrast to other organs allotransplanted liver is better accepted by the recipient. The purpose of our studies was to search for an immunosuppressive factor (LF) in liver extracts and to study its immunoregulatory in vitro function. LF was purified from liver by homogenization, sonification, ultracentrifugation and gel filtration; the M.W. was approximately 65.000 daltons. In the presence of LF (100 µg/ml) no blastogenesis was observed in human one-way MLC at day 4, 6 and 8; at time of harvest the viability of the cells was about 85 %. However, when stimulator and responder cells were pretreated separately with LF for 4, 17 and 36 hrs., then washed and mixed, a normal MLC-response was obtained at day 6. Immunofluorecenceoptical studies as well as cytotoxicity assays revealed that HLA and B-2-microglobulin were still present on the cells after the treatment with LF. Corresponding CML-experiments showed that LF prevented the generation of specifically primed cytotoxic T-lymphocytes. No proliferative response occured until day 6 when LF was added at 4 - 36 hrs. after starting the MLC; in contrast, the PHA-stimulation was completely inhibited adding LF at 0 hr. but not impaired when LF was added at 36 hrs. LF seems to delay the proliferation of responder cells: in the MLC lymphocytes were mixed with LF and left at 37° for 3 days; after removing LF the lymphocytes started to respond reaching their maximal proliferative activity at day 8. During this time also cytotoxic T-lymphocytes were formed and detected in the corresponding CML-assays. Extracts from lymphknotes, heart, spleen, kidney and erythrocytes did not have any effect. The immunoregulatory factor(s) studied herein are not species-specific since almost identical results were obtained with an extract from pig liver tested on human lymphocytes. Our in vitro data demonstrate an immunosuppressive effect of LF. LF, released in vivo by a liver allograft in the early postoperative time, may explain the immunological hyporeactivity of pig as well as of human liver recipients.

Adler, A.J. and Friedman, E.A., Transplantation 25: 271-272, 1978

PAPER NO. 10

The Antigenicity of Purified Mouse Liver Parenchyma Cells

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Isolated A strain liver parenchyma cells (LPC) virtually free of other cell types were prepared by *in situ* perfusion of livers with collagenase and hyaluronidase, followed by repeated centrifugation through Ficoll-Hanks. 30 - 40 x 10^6 LPC can be prepared from a single mouse liver, with a viability of 60 - 90% (mean 82%) and less than 0.1% contaminating Kupffer cells.

Studies on the immunogenicity of these cells have shown that they a) have H-2 antigens at roughly the same concentration per unit surface area as lymphocytes, b) lack Ia antigens, and c) are incapable of sensitizing CBA mice against A strain skin allografts when administered intraperitoneally 3 days before skin grafting. It has been shown, further, that this lack of immunogenicity is not due to removal of antigens by the enzymatic treatment, and experiments with sonicated cells indicate that failure to sensitize is not primarily attributable to the fact that these large cells cannot "home" to the lymphoid organs as readily as lymphocytes. Nor are LPC at all efficient in generating humoral anti-H-2 antibodies when used in a hyperimmunization schedule.

The presence of H-2 antigens and absence of Ia could make these cells valuable toleregens in the induction of specific unresponsiveness, and preliminary *in vivo* results will be reported. Thus, hyperimmunization of adult CBA mice with LPC or sonicated LPC fragments, although failing to sensitize, prolonged survival of A strain skin grafts only marginally when no immunosuppression was used. However, in conjunction with antilymphocyte serum substantial prolongation, but not as yet permanent survival, has been achieved.

PAPER NO. 11

The Effect of Manipulation of the Reticuloendothelial System on Passive Immunological Enhancement of Mouse Skin Allografts

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The involvement of macrophages in passive immunological enhancement of mouse skin allografts was examined. Survival of Balb/c ($H\text{-}2^d$) skin allografts on A strain ($H\text{-}2^a$) recipients was prolonged by i.p. administration of 0.1 ml of hyperimmune anti-donor (A anti-Balb/c) serum on the day of grafting. The prolongation obtained was small but statistically significant (MST untreated = 10.5 ± 0.2 days, n = 82; MST enhanced = 12.6 ± 0.2 days, n = 84; △ MST = 2.1 days, $p < 0.01$). The reticuloendothelial system (RES) of recipient mice was stimulated or depressed with a variety of agents including diethylstilbestrol (DES) and dextran sulphate 500 (DXS 500). Enhancing serum had a greater effect in DES treated (RES stimulated) mice (DES MST = 11.0 ± 0.5 days; DES + serum MST = 14.6 ± 0.6 days; △ MST = 3.6 days, $p < 0.001$) while in DXS 500 treated (RES depressed recipients the enhancing effect of anti-donor serum was abrogated (DXS 500 MST = 10.3 ± 0.7 days; DXS 500 + serum MST = 10.8 ± 1.2 days; △ MST = 0.5 days, not significant). Results with other agents which affect RES activity fit the same pattern. Stimulation of the RES augments the enhancing effect of anti-donor serum whereas depression of the RES prevents the induction of enhancement. These results suggest that macrophages play a critical role in the enhancement phenomenon, and support previous studies with kidney allografts, xenogeneic and allogeneic antigens and syngeneic tumours that opsonization of antigen-reactive cells is important in antibody-mediated unresponsiveness. The possible mechanisms by which macrophages may act in enhanced recipients will be discussed.

PAPER NO. 12

Transplantation of Baboon Islet Auto and Allografts

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The aim of this study was to evaluate intraportal transplantation of baboon islet autografts and assess the susceptibility of baboon islet allografts to rejection. Group 1 baboons had a pancreatectomy alone, Group 2 a pancreatectomy followed by islet autotransplantation, and Group 3 a pancreatectomy followed two days later by islet allotransplantation.

The body and tail of the donor pancreas were carefully excised, infiltrated with cold Hanks' solution, diced into small pieces and digested with collagenase for 20 minutes in a waterbath at 37C. Washed pancreatic fragments were then injected into the portal vein of the recipient. Frequent blood sugar estimations and oral glucose tolerance tests were performed on the baboons in Group 2 to assess diabetic control. Blood sugar rising above 10 mmol/L was regarded as evidence of rejection.

Group 1 baboons survived 5,5,5,6,7, and 25 days. Group 2 baboons survived 45,77,>100(6) days. Random blood sugar values fluctuated widely but were usually within normal limits. Glucose tolerance tests, often abnormal immediately post transplantation, showed a steady improvement. Group 3 baboons rejected their grafts within seven days but baboon survival (3,6,8,11,23,26,29,68 and 71 days) was longer than the control group.

In conclusion, experiental diabetes can be controlled using islets harvested from a single pancreas. Islet allografts appear very susceptible to rejection although the recipient survival data suggests total rejection may take some time.

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PAPER NO. 13

Studies on the Mechanism of Action of Cyclosporin A

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In the difficult DA to Lewis rat renal allograft model a short course of cyclosporin A (cy A) is sufficient to prolong graft survival indefinitely. The mechanism of cy A induced immunosuppression was explored by measuring cell mediated immunity (CMI), and antibody response in these rats, and by observing the result of the interaction between cy A, enhancing serum (ES), and anti lymphocyte serum (ALS) on graft survival. Cy A completely abolished the humoral aspect of graft rejection as measured by a ^{51}Cr lymphocytotoxicity assay. The CMI response, measured using ^{51}Cr labelled DA thymocytes as targets, and Lewis peritoneal mononuclear cells as effectors, was only mildly suppressed, but the peak response was delayed.

When combined in subtherapeutic doses with ES, which itself was only mildly immunosuppressive, little effect was seen upon animal survival or graft function. When the same dose of cy A was combined with a subtherapeutic dose of ALS a favourable interaction was observed with prolongation of survival and reduction in blood ureas.

The results of the in vitro testing on cellular and humoral immunity, as well as the results of combination drug testing suggests that cy A might be similar in action to enhancement.

PAPER NO. 14

The Specificity of Cyclosporin A-induced Allograft Acceptance

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Short courses of Cyclosporin A (CyA) produce prolonged survival of organ allografts in several animal species, particularly rats and rabbits. The mechanism of this unresponsiveness is unknown. In rabbits it does not seem to be donor antigen specific but the animals remain healthy and resist infection.

We investigated the phenomenon by giving second kidney grafts to 12 DA rats who had survived with normal renal function for at least 80 days after receiving a single Lewis kidney transplant under cover of an 8 day course of CyA. The original Lewis kidney was replaced by a new kidney from donors of either Lewis, WAG or PVG strain, without further immunosuppression. Untreated first transplants in the same strain combinations were performed as controls (Recipients underwent immediate contralateral nephrectomy. Blood urea was estimated at intervals and histological confirmation of rejection was obtained in animals that died).

Recipient	Recipient survival (days) according to donor strain		
DA (AgB_4)	Lew (AgB_1)	WAG (AgB_2)	PVG (AgB_5)
Untreated controls	All < 8* (=10)	14*, >28*, >220*	All < 7* (=5)
Previous Lewis transplant	>200, >200, >50, >70	>230, >230, >230	9*, 19*, 36*, 40*, >200

* Denotes evidence of rejection episode - blood urea >40mmol/l

These results confirm in a predictable model that the allograft unresponsiveness induced by CyA is not entirely donor specific.

PAPER NO. 15

Pregnancy and Renal Transplantation

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Up to January, 1979, 13 pregnancies have occurred in 8 transplant recipients in our Unit. These eight patients were all well rehabilitated at the time of conception and none were advised against pregnancy beforehand, nor indeed were any advised to undergo therapeutic abortion when pregnancy was confirmed.

The mean age at conception was 24.2 years (range 17 - 29 years) with a mean time from transplantation to conception of 74 months (range 14 - 144 months). The mean dose of Prednisolone was 11.9 mg/day (range 7.5 - 20 mg/day) and of Azothiaprine 128 mg/day (range 100 - 150 mg/day). The mean plasma creatinine was 90 µmol/l (range 60 - 150 µmol/l) and only two patients had proteinuria greater than 0.5 g/day. Four had significant hypertension requiring treatment and five had intermittent urinary tract infections.

The outcome of these pregnancies was as follows:

1. Two surgical abortions - both performed for social reasons.
2. Three spontaneous abortions - at 8, 9 and 10 weeks.
3. Two intrauterine deaths - one associated with rapidly developing eclampsia at 32 weeks.
   - one associated with foetal asphyxia at 37 weeks.
4. Six live births - four normal children, now aged two months, two, three and seven years.
   - one child now aged five years, with cerebral palsy and an abnormal chromosome pattern.
   - one neonatal death at 36 hours due to pulmonary haemorrhage.

Deterioration in transplant function was noted in two patients, one returning to chronic dialysis one year after delivery. Apart from the patient who developed eclampsia without deterioration in renal function, the pregnancies were otherwise well tolerated. However, 13 pregnancies produced only four normal children.

PAPER NO. 16

Tissue Typing and the Sheep

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The sheep has hitherto received little attention in the U.K. and Europe as an animal model for transplantation studies, although it has been extensively used elsewhere. A commonly preferred large animal model, for a number of reasons including the availability of tissue typing, has been the dog, although disadvantages include the high cost of housing and feeding, and the frequency of post-operative distress.

The sheep offers a number of advantages over the dog. Sheep are relatively inexpensive to keep, they may easily be caged and restrained post-operatively, show little concern at being permanently cannulated, and having little lateral flexibility are unlikely to reach a wound site with their teeth. Nevertheless, a deterrent to the use of sheep has been the unavailability of tissue typing, and a common supposition that in any case domestic flocks would be too inbred to use as an outbred model.

We have tested lymphocytes from 160 sheep against up to 140 cytotoxic antisera from parous or otherwise immunised sheep; samples came from six flocks of different breeds. Methods for the separation of lymphocytes and for the cytotoxic test were identical to those used routinely in human HLA typing. Although many of the sera had broad profiles a number had restricted reaction patterns which identify nine provisional specificities. The distribution and frequencies of these 'antigens' show considerable polymorphism within the different flocks and also variation between flocks, certain specificities being absent in particular flocks. A further measure of polymorphism and of the amenability of the sheep to tissue typing is a 50% incidence of lymphocytotoxic antibodies in parous ewes. Reliable breeding data is sparse but family studies from well-documented flocks suggest that the specificities so far identified would fit most comfortably into a two-locus hypothesis. Although the specificities have not yet been demonstrated to be 'transplantation antigens' the likelihood of their being so is high.

We suggest that tissue typing in the sheep presents few problems, and that the animal should receive more serious consideration as a candidate for a large, and outbred, model in experimental transplantation.