Russell Decline in rejection act with time - of pt with rise in urea 5-10 dys again at 45 dys — none for decade since
Part of upper limb can be replaced by bone graft stored in glycerol at -50° C & over months & this is placed in situ allows creeping replacements of new bone saving of limb - No tissue typing or immunosuppression. All 19 limbs saved.
Skin used immunosupp including ALG - measured by rosette test - 4/5 pts with truly lethal burns have skin surviving.
Immunol. privileged sites include cornea, brain, testis (use PT grafts in rats)
HLA grouping adds to success of cornea.
Data to show that frozen blood reduces cytotoxic antibodies the % pts depending have surviving grafts & at 1 yr 50% as non sensitized at 1 yr - same as in common with other centres
However Boston survival of graft at 1 yr greatly enhanced if previously transfused. Now prepared to transfuse a few units of frozen blood just as good results for this effect as whole blood transfusion. But fewer develop cytotoxic antibodies (waiting has greater interest)

OXONIAN REWLEY PRESS OXFORD

Canal Banker's Order
Roy Calne :- Getting better results from liver grafts than with kidney grafts!

JOINT AUTUMN MEETING 1976

Wembley Conference Centre
Harrow, Middlesex

British Transplantation Society	October 20
Joint Sessions	October 21
British Society for Immunology	October 22

Friday, 22nd October Severn Lecture Theatre

5.00 p.m. F.Carswell, J.Oliver (University of Bristol)

'Respiratory response of allergic rats to aerosol challenge'

5.15 p.m. K.Morgan, P.J.L.Holt (University of Manchester Medical School)

'Migration of human lymphocytes in mice'

Abstracts (not for publication)

Functional analysis of T cell subpopulations using alloantisera

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Thymus derived lymphocytes (T cells) participate in a wide variety of (cell mediated) immune reactions. They can act as either "helpers" or "suppressors" in the production of antibody and are active as "killers" against allogeneic target cells. At present there is sufficient data to suggest that a differentiated T cell is required for each effector activity, it is not clear whether one T cell type regulates the differentiation of another, although this has been suggested.

There are at least 9 genetic loci that code for T cell membrane alloantigens in the mouse, including TLa, Thy-1, Ly 1,2,3,5,6,7, and Ia. The use of alloantisera to these surface antigens has facilitated the identification of various T cell subpopulations. It has previously been shown that T "helper" cells bear different Ly antigens than "killers" or "suppressors". Using alloantisera against Ly 5,6,7 and Ia we have shown that suppressor cells and killer cells can be distinguished, and that the killer cell sub-population may contain a variety of subsets.

Studies on B-cell antibodies in human renal transplantation

A.TING, D.O.OLIVER, P.J.MORRIS

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The relevance of B-cell antibodies in renal transplantation is being investigated in several ways.

1. A retrospective screening for B-cell antibodies in pregraft serum samples from 50 patients who subsequently received a kidney transplant was carried out. The results showed that the presence of B-cell antibodies did not influence the outcome of the transplant at either 1 or 3 months post transplant.

2. Serial post transplant serum samples were tested for B-cell antibodies to determine if their appearance correlated with clinical signs of transplant rejection as suggested by Ettenger et al. (New Eng.J.Med., in press). Although B-cell antibodies were detected in a number of patients undergoing rejection they were found also in patients with good graft function. B-cell antibodies were found in the majority of patients after transplant nephrectomy for rejection, and in fact occurred more often than HLA antibodies.

3. A programme of both prospective and retrospective cross-matching for B-cell antibodies has been started and at this time only one patient has been transplanted with a positive B-cell crossmatch. Renal function is good 3 weeks post transplant.

Thus the relevance of B-cell antibodies in renal transplantation is still unclear at the present time. The presence of such antibodies pre-transplantation does not seem to indicate a poor prognosis for a subsequent renal graft, and in fact may enhance graft survival. In contrast B-cell antibodies can be detected in most patients after graft removal suggesting a possible role in graft rejection.

Target determinants in human CML, different from HLA-A, -B, -C and -D

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It is known that GVH and transplantation rejection can occur in HLA-A, -B, -C and -D identical donor recipient combinations. Moreover cell mediated lympholysis (CML) is found in combinations where HLA-A, -B and -C are excluded as the possible target determinants. The aim of our study was to define genes other than HLA-A, -B and -C, located in or outside the MHC region, responsible for the occurrence of this cytolysis.

1. In 9 families cells of the progeny were sensitized _in vitro_ against each parent. 40 such cells were then tested against MLC identical siblings, in search for killing directed against determinants coded for by loci outside the MHC region. With this protocol we never found any killing that indicated that such loci were existing.
2. In 1 family in which one of the parents was homozygous for HLA-A, -B and -C but not for -D a positive CML was found after _in vitro_ sensitization between siblings identical for HLA-A, -B and -C, but different for -D. Segregation studies indicated that the killing was directed against a determinant closely associated with, but different from HLA BW35.
3. Upon _in vivo_ immunization after bone marrow transplantation a direct CML was found between an HLA-A, -B, -C and -D identical sibling combination. Segregation studies suggested that after this _in vivo_ immunization a non MHC target determinant was detectable.

MLC reaction and graft survival in 25 renal transplants

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It is not known whether the mixed lymphocyte culture (MLC) reaction between cadaveric donors and recipients can predict the outcome of renal allografts more precisely than serological HLA typing.

Between January 1975 and July 1976 50 renal transplants were performed in Oxford, and successful experiments were done on 25 of the donor-recipient pairs. In these MLC experiments it was shown both that the recipient's lymphocytes could respond to allogeneic cells and that the donor's lymphocytes could stimulate a response in allogeneic cells.

A relative response index was calculated, based on the median of triplicate or mean of duplicate cultures in one-way MLC's:

$$\frac{(R\ v.\ D^{m}) - (R\ v.\ R^{m})}{(R\ v.\ \bar{X}^{m}) - (R\ v.\ R^{m})} \quad \text{in which}$$

$R\ v.\ D^{m}$ = Recipient response against donor as stimulator (mitomycin-C treated cells)
$R\ v.\ R^{m}$ = Recipient response against self as stimulator
$R\ v.\ \bar{X}^{m}$ = Mean recipient response against all allogeneic stimulators

The strength of these relative responses did not correlate with the degree of mismatch for HLA-A and B antigens (HLA-D typing was not performed) or with graft course as judged by rejection episodes and graft survival. However, it should be noted that HLA-A and B matching in this transplant series was not as close as in other centres where correlations between MLC reaction, degree of matching and graft survival times have been found (Festenstein _et al._, 1976, Lancet, i, 157; Sachs, 1976, unpublished report).

This MLC material is being re-evaluated using a stabilised relative response, similar to that described by Ryder _et al._ (1976, 'Histocompatibility Testing 1975', Munksgaard: 557) which takes account of overall variation in the ability of an individual's lymphocytes to respond or stimulate in MLC.

The immunosuppressive action of niridazole

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Niridazole (Ambilhar) has been used for many years to treat patients with Schistosomiasis, but only recently has this drug been found to be immunosuppressive in mice. We have studied its ability alone and in combination with other drugs to prolong the survival of heart allografts in rats. Grafting was performed from Wistar (AgB^{2}) to AS (AgB^{1}) strains of rats and the hearts were deemed rejected on the day that beating ceased. Treatment was given daily for 20 days then 3 x weekly for another 40 days.

Groups (7 rats in each)	Treatment	* significant prolongation	Survival of heart grafts MST	Range
1	No treatment		7	6-9
2	Niridazole 50mg/Kg (N50)		20*	8-59
3	N50 + Imuran 4mg/Kg (I4)		18*	9->50
4	N50 + Prednisolone 4mg/Kg (P4)		13*	6 ->50
5	N50 + I4 + Prednisolone 4mg/Kg (P4)		55*	42->100
6	N50 on days 5,6,& 7 only + I4 + P4		17*	9-42
7	N25 for 14 days + I4 + P4		12*	10->50
8	Imuran 4mg/Kg + Prednisolone 4mg/Kg		9*	8-31
9	Imuran 8mg/Kg		8	7-11
10	Prednisolone 4mg/Kg		9*	8->16

Niridazole was much more effective than Imuran and Prednisolone in prolonging allograft survival. When used continuously at a dose of 50mg/Kg

in combination with these drugs (Group 5) only two of seven grafts were rejected (42 & 55 days). Two rats died accidentally on days 51 & 52 with beating grafts and the remaining three grafts currently survive (>100 days). White cell depression did not occur. Niridazole also suppressed the GVH response produced when human lymphocytes were injected into irradiated rats, suggesting that the drug is immunosuppressive in man. It is now being considered for use in human renal transplantation.

Inhibition of mixed lymphocyte culture (MLC) reactivity by sera from Niridazole-treated rats

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Niridazole (Ambilhar, CIBA) has been widely used in the treatment of schistosomiasis, but the immunosuppressive properties of this compound, or of its metabolites, have only recently been recognised.

Outbred Wistar rats were dosed with 50 or 100 mg/Kg/day of Niridazole for 1-3 days. Sera were collected at 1-7 days after the final dose and were added to human one-way MLCs at a concentration of 20%. Further untreated rats provided control sera. While the parent compound had no inhibitory effect *in vitro*, MLC reactivity was markedly inhibited by certain sera from Niridazole-treated rats.

It appeared therefore that metabolites of Niridazole were responsible for the *in vitro* immunosuppressive effect and it is believed that the suppression of MLC reactivity will provide a useful model for the identification of the active metabolites. The ability to suppress MLC reactivity might indicate the usefulness of Niridazole in preventing allograft rejection.

The effect of Carragenan on rat heart transplant survival

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Carragenan, a substance derived from marine algae, is known to be toxic to macrophages.

Macrophage function was studied in AS rats using a carbon clearance technique (Biozzi, 1953). While the known effect of *C.parvum* in increasing carbon clearance was confirmed in this model, Carragenan in a single I.V. dose of 5mg/Kg had no significant effect on carbon clearance when compared with controls.

The immunosuppressive effect of Carragenan was then tested in the rat heart transplant model. Hearts from August/AS (F1) hybrids were transplanted into AS recipients.

λ-Carragenan as a single dose of 5 mg/Kg was given intravenously at the completion of the operation. There was a high early post-operative

mortality in the Carragenan treated animals, but transplant survival times in 5 rats are as follows:

1) animal died with functioning transplant 55 days
2) functioning transplant 82 days
3) functioning transplant 22 days
4) functioning transplant 25 days
5) animal died with functioning transplant 7 days

All control transplants were rejected by the eighth day.

Although Carragenan is known to be toxic to macrophages it had no significant effect on carbon clearance in this study. In spite of this it had a marked immunosuppressive action in those rats which survived the early post-operative period.

Biozzi, G. (1953) Brit.J.Exp.Path. 34, 441.

Effects of azathioprine on circulating inhibitory factors of the MLC

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We have shown previously that levels of serum factors which inhibit mixed lymphocyte culture (MLC) correlate with lymphocytotoxic antibodies. As the latter are known to be associated with a poor prognosis for renal allograft survival the aim of this study was to investigate the prognostic significance of circulating inhibitory factors and to observe the effects of Azathioprine on their levels.

Inhibition of one-way MLC was measured by substituting 20% of AB control serum by test serum in 6 combinations of recipient and mismatched lymphocytes. Significant inhibition occurred if the ratio of transformation in control to test culture was 2.5 or more.

Serum from 14 of 23 haemodialysis patients inhibited MLC and in 12 of these early graft rejection followed subsequent renal transplantation. Seven of 9 patients without serum inhibitory factors have surviving, well functioning grafts. The association of graft rejection and inhibitory factors is significant ($p<0.0015$ by Fishers exact test).

Azathioprine, 50mg/day was given to 12 patients on haemodialysis for 1 week. Serum taken before and after the drug showed no significant difference in inhibition of MLC.

We conclude that serum inhibitory factors of MLC are associated with a poor prognosis for renal allografts. Administration of Azathioprine has no effects on the levels of these circulating factors.

Positive and negative typing in human Ia like determinants

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HLA-D specificities are apparently defined by proliferative T cell responses to differences expressed on allogeneic B cells. Such structures may be involved in cellular interactions in immune responses, and presumably are related to the Ia antigens in the mouse. Our objective was to define the heterogeneity of HLA-D using three cellular methods. Firstly, the homozygous typing cell (HTC) method in which zero or low reactions represent clonal deletions to specificities carried by self. 21 HTC's from cousin marriages represented 7 officially recognised HLA-D specificities.

Secondly, the pool-priming method was used in which zero or low responses also represented compatibility, but responders were first primed to a random pool of stimulators in order to amplify responses. After priming, cells were restimulated with all HTC's.

Thirdly, the so called primed lymphocyte typing (PLT) method was employed in which both zero reactions to responder-compatible and positive reactions to primary stimulator-compatible types were analysed. After sensitization responders were rechallenged with all HTC's. 13 unrelated individuals were used for the three methods and all studies were repeated three times. In all methods HLA-D was of predominant influence and appeared to be a simple entity, closely associated to a minor heterogeneous structure.

Bile composition and "bile cast" formation after liver transplantation in man

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Transplantation of the liver in man has been frequently complicated by the development of biliary fistula and obstruction of the biliary tract by "bile casts" (1). While the high incidence of biliary leakage may reflect the poor blood supply at the anastomosis or impaired healing due to immunosuppression, the development of biliary obstruction due to "bile casts" in the absence of fistulae could be due to a basic abnormality in bile flow or composition (2).

Studies of bile flow and composition following orthotopic liver transplantation are reported in 8 patients. During the early post-operative phase while the bile acid enterohepatic circulation is interrupted by T tube drainage, the bile acid pool becomes depleted and the bile becomes supersaturated with cholesterol and potentially lithogenic. Following clamping of the T tube the bile quickly becomes unsaturated with cholesterol. However, no difference in bile composition was noted between those patients developing biliary complications and "bile casts" and those not.

A detailed biochemical and histological analysis of the bile casts in

4 patients shows the major constituent to be an organic protein with relatively small quantities of cholesterol and bilirubin present. The histological features of the protein are those of collagen essentially similar to that of the bile duct wall.

The possible mechanism of bile cast formation will be discussed.

(1) Starzl et al., Surgery, Gynaecology & Obstetrics, 142, 1976.

(2) Waldram et al., Transplantation, 19, 1975.

Treatment of polycystic disease of the kidneys by renal transplantation

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23 patients with end stage renal failure due to polycystic kidney disease have been treated by renal transplantation. There were 14 males (including one boy of nine years) and nine females. The ages ranged from 26-62 years (excluding a child aged 9) two sets of twins have been included in the study.

Most patients presented initially with haematuria or urinary tract infection. 11 had a family history of renal disease. Tissue typing revealed a preponderance of the antigen HLA 9.

The interval between the onset of urological symptoms and the need for substitution therapy averaged five years. 10 patients underwent Rovsings operation before the onset of terminal renal failure and in three deterioration of the kidney function was accelerated by this surgery.

Unilateral pre-transplant nephrectomy was performed on one occasion for urological symptoms and to make space for the transplanted kidneys in a further five patients. Bilateral nephrectomy has been carried out three times for persistent erythremia which developed despite satisfactory transplanted kidneys.

In the past eight years 23 patients received 29 allografts, four of which were from living related donors. 10 recipients are alive with good functioning transplanted kidneys and this includes the four who received live donor allografts. Viral and bacterial infections, unrelated to the polycystic kidneys, accounted for the deaths in 6/12 instances and all of these patients had transplants which were working well. At post mortem examination there were associated liver cysts in 4/12 cases.

We conclude that, despite the late onset of renal failure, kidney transplantation is justified in patients with polycystic disease and in our experience removal of the diseased kidneys is not mandatory.

4 patients died to CVA with berry aneurysm.
3 needed bilateral nephx for post-op erythema — regressed. 18g+ Hb, venesection, radio P failed around polycythaemia
Barnes has had gross infection kidneys – eg 2 gallons pus

The removal of anti-donor immune products using extracorporeal organ perfusion

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The rejection of kidney allografts and subsequent return of the recipient to dialysis has created a group of patients who, because of sensitization induced by the rejected graft, have only a small chance of finding another suitable donor. We have investigated the possibility of removing donor reactive cytotoxic antibodies and cells by extra-corporeal perfusion of recipient's blood through a donor organ.

MHC mismatched pigs were immunized by skin grafting. The blood of the immunized recipient was circulated through either donor liver or lungs at 37°C for periods varying from 1½ to 3 hours. Immediately after perfusion the donor kidney was transplanted. During perfusion blood samples were taken at regular intervals and subsequently tested for anti-donor cytotoxic antibody titres, cytotoxic lymphocytes and mixed lymphocyte culture reactivity. Results showed that perfusion diminished or totally removed cytotoxic antibodies, reduced cell mediated cytotoxicity but did not affect reactivity in mixed lymphocyte culture. None of the transplanted kidneys was hyperacutely rejected (MST 5.8 ± 1.9 days) whereas kidneys transplanted into untreated immune pigs are regularly destroyed in 20-30 minutes.

Litter mates

Transplantation of cadaver kidneys with multiple renal arteries

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22 patients undergoing cadaver renal transplantation in a consecutive series of 212 patients from January 1968 until December 1975 received kidneys with more than one renal artery. 23 received 24 kidneys ē multiple vessels

In the earlier patients, arterial anastomosis between donor and recipient was achieved by pantalooning the donor arteries and anastomising them end-end with the recipient internal iliac artery. Three kidneys failed to function due to thrombosis at the side of anastomosis. The remaining patients were treated by end-end anastomosis of vessels on a patch of aorta. Only one of these kidneys failed to function due to thrombosis in the graft. One patient had a polar vessel ligated resulting in sloughing of the upper pole and subsequent urinary fistula. This patient is alive and well with the same functioning transplanted kidney eight years later.

14 A/W

It is concluded that there is very little increased risk involved in transplanting kidneys with multiple arteries particularly when the anastomosis is performed end-side between donor arteries on a patch and the external iliac artery. End to side ē patch 8 functioned
6 failed to function at any time
18 functioned
8 vascular complications

Linoleic acid in renal transplant recipients

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Prolonged allograft survival has been demonstrated in mice treated with linoleic acid (1). Following promising results in a pilot study, in recipients of poorly matched renal allografts (2), a double-blind controlled trial of linoleic acid as a supplement to immunosuppressive treatment was undertaken.

Naudicelle capsules (72% linoleic acid 8.6% linolenic acid) in a dose previously shown to affect lymphocyte-antigen interaction (3) were compared with placebo (oleic acid). Therapy was continued for six months after transplant. Other anti-rejection therapy was standardized.

Eighty-one patients were included. Forty received Naudicelle and 41 placebo. These groups were similar in terms of:- age, previous blood transfusion, tissue matching, warm ischaemia times.

Immunological failure rate was slightly lower in the Naudicelle group (10/40) compared with placebo (16/41). This difference was not statistically significant. However, in the placebo group all immunological failures were early (0-3 months) compared with the Naudicelle group - 6 early and 4 late (4-6 months). This was a significant difference ($p<0.05$).

There was no difference in the number of clinical rejection episodes (74/40 - Naudicelle; 79/41 - placebo) or in the amount of Solu-Medrone required (Naudicelle mean 6.75G: Placebo 7.5G).

In conclusion linoleic acid, in the dose given, may delay graft rejection in renal transplant recipients, but does not improve long-term results. Now say that it should be tried further. trial.

(1) Ring, J. et al., Lancet, 30th November, 1974.
(2) Uldall, P.R. et al., Lancet, 31st August, 1974.
(3) Uldall, P.R. et al., Lancet, ii, 128, 1975.

Imuran 3 mg / kg Pred 40 mg - for 6 mths down to 20.

Experience with pulsatile perfusion for cadaver kidney preservation

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The aim of this study was to compare the results of cadaver renal transplantation using kidneys that had been perfused on an Ab Gambro machine using a 5% albumin solution with those that were preserved on ice.

During a four year period 105 kidneys were offered for transplantation. Sixty-three underwent pulsatile perfusion. The mean ischaemic time of these was 27 minutes with a range of 6-90 minutes and the mean cold ischaemic time on ice before perfusion was 8 hours with a range of 4 hours 10 minutes to 15 hours. They were perfused for a mean period of 13 hours

23 minutes with a range of 11 hours 24 minutes to 45 hours 58 minutes, giving a mean total cold period of 21 hours 10 minutes.

Forty-two kidneys were preserved on ice. The mean warm ischaemic time was 23 minutes with a range of 1 minute to 61 minutes. The mean total cold ischaemic time was 12 hours 23 minutes with a range of 5 hours 50 minutes to 23 hours 10 minutes.

Of those kidneys on pulsatile perfusion, two were lost because of perfusion damage and a further seven not used because of abnormalities noted on perfusion. Three of these seven kidneys had poor perfusion characteristics with a flow of less than 1 ml/G of tissue/minute. Two had poor perfusion of one pole and another had vascular abnormalities, and the seventh an undiagnosed hypernephroma. A further eight kidneys failed to function because of early clinical problems. Six kidneys had primary non-function because of histologically proven rejection. The remaining forty kidneys all functioned (a urine urea production of greater than 8G/day). The mean onset time of function was seven days with a range of 1-22 days. Renal artery stenosis later occurred in one patient. Of those kidneys preserved on ice, seven failed because of early clinical problems, five failed because of histologically proven rejection and one had irreversible tubular damage. One kidney in this group developed renal artery stenosis. The mean onset of function was 8.5 days with a range of 1-23 days.

We conclude that pulsatile perfusion allows planning of the transplant and the preoperative diagnosis of anatomical abnormalities. The large number of kidneys discarded on pulsatile perfusion may be due to too rigid criteria for only one kidney that was preserved on ice had irreversible ischaemic damage.

due to reduction in no of kidneys lost from rejection

Immunosuppressive effects of ALG in patients undergoing renal transplantation

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The aims of this study were to compare tests of immunological function in patients who had undergone renal transplantation and were receiving either prednisone and azathioprine with tests from those receiving additional antilymphocyte globulin (Burroughs-Wellcome) for 10 days given as part of a double blind clinical trial.

Thirty patients were studied before transplantation, and at 10 days and 42 days after operation.

There was no significant difference in total or differential white count between the groups, although the lymphocyte count was significantly depressed in both groups after treatment. Measurements of IgG, IgM and IgA and complement (C3) were not different. The degree of stimulation by phytohaemagglutinin in the two groups at any time interval and to any dose was not significantly different. The response to pokeweed mitogen was increased in all patients at 10 days but by 42 days was almost normal.

We conclude there was no additive immunosuppressive effect of antilymphocyte globulin using the tests immunological function described.

Most kidney lost for immunological reasons.

Early observations on methylprednisolone blood levels in cadaveric renal allograft recipients

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Introduction Following oral administration of prednisolone there is considerable variation in blood levels of the drug in normal subjects. In situations where microsomal enzyme induction has occurred (e.g. when patients take phenobarbitone in addition to steroids) graft rejection (1) and asthma(2) are more difficult to control. These observations prompted our study, which investigates the blood levels of methylprednisolone (M.P.) after a weight-adjusted oral dose in 8 post-operative renal transplant patients; bio-availability is compared with the outcome of the graft in each case.

Materials & Methods Two groups of patients were studied: I. 5 recipients of cadaver kidney grafts functioning >6 months. II. 3 recipients of cadaver kidney grafts rejected within a week.

All patients were studied >6 months after grafting. After a 12 hour fast, M.P. was given orally (0.5 mg/kg body wt). Blood samples were taken at 1,2,4,6,8,10,12,16 and 24 hours. Serum was removed immediately and frozen, and M.P. immuno-assays performed on batches. Peak levels, half life duration, and areas beneath the blood level curves were computed as meaningful indices of drug bio-availability.

Results	Peak Levels (ng/ml)		½ life (mins)		Area	
	Range	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$
Group I (not rejected)	344-750	516.5	320-360	337	2018-3970	2874
Group II (rejected)	127-302	191.2	140-204	178	500-1181	767

Comment These are preliminary results and the size of the groups precludes statistical analysis. Nevertheless, the consistently low M.P. peak levels, half lives, and areas in those patients who rejected their grafts suggest that the bio-availability of M.P. after oral ingestion may be a significant determinant of net immunosuppression, and hence of graft function.

(1) Wassner, S.J., personal communication.
(2) Brooks, S.M., Werk, E.E., Ackerman, S.J., Sullivan., Thrasher, K. New Neg.J.Med., 286, 1125 (1972).

Specific and non-specific suppression of renal allograft rejection

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In a study of the interaction of immunosuppressive agents on rat renal allograft rejection the following results were obtained when F1 (DA x Lewis) kidneys were transplanted into Lewis recipients. Right nephrectomy was performed at 7 days.

Treatment	No. of Rats	Mean Blood Urea in mg/100 ml at day: 10	14	21	Median Survival (days)
None	7	528	-	-	11
Enhancing serum	6	48	230	271	>30
Azathioprine 30mg/kg	5	565	-	-	10
Prednisolone 16mg/kg	5	541	-	-	10
Azathioprine plus Prednisolone	5	535	-	-	10
Enhancing serum plus Aza and Pred	4	49	54	111	>30
Cyclophosphamide 5mg/kg	5	447	121	162	>30
Cyclophosphamide 10mg/kg	5	79	50	60	>30

Enhancing serum modifies acute rejection by preventing an antibody mediated glomerular necrosis. There is nevertheless an intense cell infiltrate which progresses by week 2 to severe tissue destruction. This functional and morphological deterioration can be prevented by large doses of azathioprine and prednisolone, a finding which is in keeping with the reported lack of effect of passive enhancement on cell mediated immune responses in rat renal allograft recipients.

Cyclophosphamide at 10mg/kg/day prevents the acute glomerular injury but not the cell infiltrate at 7 days. Treatment for a further 7 days eliminates this cell infiltrate so that at day 14 the kidneys are histologically and functionally normal.

These findings suggest important differences in the sensitivity of various effector mechanisms to different immunosuppressive agents and stress the need to assess new immunosuppressive agents in conjunction with other agents.

Donor specific immunosuppression for clinical transplantation

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Tolerance (specifically influencing the host response) has not materialised for clinical use in transplantation because, so far, only the humoral response against transplantation antigens can be suppressed. Enhancement (influencing donor sensitizing cells) is real in the context of transplantation, and has not been used clinically only because of toxicity of the alloantiserum available for passive administration. Auxiliary heart transplant experiments in rats will be

described, showing that enhancing serum is fully effective after removal of cytotoxic "tissue typing" antibodies (SD antibodies) and that the *in vivo* effect is wholly due to residual (Ir region associated, Ia = LD ?) antibody. Supporting evidence comes also from rat kidney grafts, mouse skin grafts, and from a new look at clinical data. Success of clinical kidney transplantation is evidently due in no small measure to active enhancement from previous blood transfusion. Clinical problems to be faced are the choice between passive and active enhancement, and what to do about prospective patients having high SD (graft damaging) antibodies. The relationship of Ia to LD to MLR has not been resolved but clinical passive enhancement need not await that because for safety the serum only needs to be SD negative.

The foetus as a homograft: lymphocyte localisation and division in lymph nodes draining the uteri of pregnant mice.

J.D.ANSELL, Gillian SPEEDY, Catherine M.McDOUGALL, C.J.INCHLEY

Immunobiology Unit, Department of Zoology, University of Edinburgh)

The foetus develops successfully as a homograft, despite evidence that immune responses are initiated in the mother against paternal antigens. This evidence includes the observation that lymph nodes draining the mouse uterus gain weight during first allogeneic pregnancies. To determine whether such weight gains are immunologically related and at what point maternal responses are subsequently suppressed, we have repeated these observations, and investigated concomitant changes in lymphocyte localisation and proliferation in these nodes following injection of ^{51}Cr-labelled lymphocytes and ^{125}I-5-iodo-2'-deoxyuridine.

During CBA (male) x Balb/c pregnancies transient proliferation followed implantation. Increases in lymphocyte localisation were observed later in pregnancy, and post-partum, but were not associated with cell division. Pre-immunisation by paternal allograft or earlier pregnancies further increased localisation, and also gave some late proliferation in the draining nodes.

Localisation and proliferation during pregnancy were compared with that in nodes draining sites of skin allografts or injections of allogeneic or xenogeneic erythrocytes.

Selective H-2 restricted cytotoxic responses to H-Y antigen by F1 female mice

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Recent evidence suggests that the H-2 restriction of cell mediated cytotoxic responses to H-Y, a weak histocompatibility antigen, is the result of an interaction between an H-Y gene product common to all strains and H-2 gene products unique to each strain (1,2). F1 male cells express H-Y antigen in interaction with both parental H-2 haplotypes. Thus, (CBA x B10) female cells sensitized to male cells of either parental haplotype will lyse F1 male cells and male cells of that parental H-2 haplotype (2, unpublished findings).

In the present study we have found that F1 females make selective H-2 restricted responses to H-Y when sensitized to F1 male cells. (CBA x B10) or (B10 x CBA) females, primed in vivo and challenged in MLC with either (CBA x B10) or (B10 x CBA) male cells, will lyse F1 and CBA male cells, but not B10 male cells. Furthermore, (CBA x B10) females, primed in vivo with (CBA x B10) male and challenged in MLC with B10 male, will not lyse F1 male or B10 male target cells, but, if challenged in MLC with CBA male, will lyse F1 and CBA male targets. However, F1 females, primed in vivo with B10 male cells and challenged in MLC with F1 male cells, will lyse F1 and B10 male target cells. Similar findings have been made using (B10 x Balb/c)F1 mice.

We conclude that the interaction of H-Y with different H-2 haplotypes may significantly alter the responsiveness of certain female mice to H-Y. Selective H-2 restricted responsiveness appears to operate at the level of in vivo primary sensitization where precursor cells are likely to be involved, not just at the effector stage where cytotoxic effector cells and target cells only may be involved. Finally, these results provide another example of how the MHC can influence immune responsiveness to non H-2 antigens.

(1) Gordon, R.D. et al. (1975) J.exp.Med., 142, 1108.
(2) Gordon, R.D., et al. (1976) J.exp.Med., in press.

The relative involvement of antigens of different regions of the H-2 complex in passive enhancement of skin allografts

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H-2 alloantisera that passively enhance skin graft survival do not lose their enhancing potency when anti-K,D antibodies are removed from them by absorption with RBC or platelets, suggesting that antibodies of conventional H-2 specificity have no role in enhancement, and that the remaining Ia antibodies are the active moiety. Using antisera raised in a number of H-2 recombinant strain combinations it is found that whereas on their own, Ia antibodies can prolong graft survival by up to twice the normal time, anti-K or D region antibodies give only 2 or 3 days prolongation in combinations involving either K+I+D or K or D incompatibilities alone. Opsonization and endocytosis of graft antigenic material is discounted as a general mechanism of passive enhancement. The possibility is proposed that there are potentiating gene products of both I and K,D regions which stimulate an amplifying T cell population in the hosts immune machinery. The presence of (enhancing) antibodies interferes with the response of these host cells, thus suppressing and delaying the cellular response against the graft.

The effects of anti-H-2 antisera on graft versus host disease (GVHD)

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Anti-H-2 antisera with known immunosuppressive properties for the passive enhancement of skin allograft survival and inhibition of the mixed lymphocyte culture reaction have been tested for their effects upon GVHD in F1 hybrid mice injected with parental strain spleen cells. Antisera were produced in graft strain mice by immunization with tissues from the other parental strain of the hybrid host. Small doses of antisera prepared in this way and included with the inoculum of donor cells produced a massive increase in GVHD splenomegaly, an increase in the proportion of donor cells in the spleen and led to the premature death of the host animals. However other anti-H-2 antisera which were serologically unreactive with either the graft donor or host were equally effective in stimulating splenomegaly. This apparent lack of specificity has important implications for the treatment of GVHD following histoincompatible bone marrow transplantation in man.

Auto-suppression of an auto-antibody response to erythrocytes in mice

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Mice given 3 or 4 inoculations of rat erythrocytes at weekly intervals produce anti-erythrocyte auto-antibodies which can be detected by direct Coomb's tests. Auto-antibody ceases to be detectable after a period of weeks or months (depending on mouse strain , route of injection, and dose of cells, etc.). Thymus, lymph node or spleen cells from recovered mice transferred to previously untreated mice, prevent the induction of auto-antibody in recipients inoculated with rat erythrocytes.

These findings could be explained if rat and mouse erythrocytes share a common antigen which B cells are capable of responding to. Introduction of the antigen on a new carrier (the rat erythrocyte) by-passes or activates T cells, auto-antibody formation ensues, and the potentially damaging reaction is eventually controlled by an autosuppressive response.

Various factors influencing induction of auto-antibody in this model, and the nature of the suppressor cell, will be discussed.

In vitro interaction between lymphoid cell and various intracellular organisms

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Toxoplasma gondii, an intracellular protozoa, is an excellent model for studying *in vitro* macrophage activation against specific and nonspecific microorganisms. Groups of mice were immunized with *Toxoplasma* lysate antigen (TLA) and *Listeria monocytogenes*, and some infected with *Toxoplasma* T strain, and BCG. The peritoneal macrophages of the above groups were challenged with trophozite form of *Toxoplasma* RH strain. The viability of intracellular *Toxoplasma* was detected by autoradiography. Results indicated that macrophages obtained from mice immunized with BCG, TLA and *Toxoplasma* T strain had an enhanced ability to inactivate the intracellular *Toxoplasma* RH strain. Normal and *Listeria*-immune macrophages facilitated the intracellular multiplication of *Toxoplasma* and, eventually, resulted in destruction of macrophage monolayers, *in vitro*.

Various combinations of normal and immune lymphocytes, with or without antigens, were added to the normal and immune monolayers to determine the possible role of the lymphocytes or antigens in the process of macrophage activation. When normal and TLA-immune macrophages were incubated with immune lymphocytes and TLA, a slight activity of of immune macrophages appeared at 24 hours post challenge.

Incubation of normal and TLA-immune macrophages in the presence of normal lymphocytes and TLA resulted in slight cidal activity of immune macrophages at 12 hours after infection with *Toxoplasma gondii* RH strain. No significant difference in the cidal activity was observed when normal and TLA-immune macrophages were incubated with immune lymphocytes alone. However, there was a marked difference between normal and TLA-immune macrophages in killing of *Toxoplasma* if they were incubated in the presence of TLA.

Antibody-dependent attachment of eosinophils to nematodes

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The nematodes *Trichinella spiralis*, *Nippostrongylus brasiliensis* and *Necator americanus*, were cultured with suspensions of inflammatory cells containing varying proportions of eosinophil leucocytes. In the presence of immune serum there is attachment of this cell type to the parasites surface with subsequent flattening of the cells and loss of their cytoplasmic granules. Later in culture there is a pallisading of the cells on top of one another. If macrophages are present in the suspension they are involved with eosinophils in the layering of cells on the parasite's surface. The significance of this cellular interaction with the parasites will be discussed, referring particularly to changes in cell morphology and changes occurring at the parasite surface.

Studies on immunodiagnosis of hydatid disease

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The techniques of gel diffusion (GD), counterimmunoelectrophoresis (CIEP), and crosselectroimmunodiffusion (CEID) were used for diagnosis of hydatid disease. Sera were obtained from suspected patients prior to surgery and the antigens used were either whole human hydatid fluid (WHHF) or whole sheep hydatid fluid (WSHF).

Out of 10 suspected patients, 8 were confirmed for hydatid disease by recovering the cyst(s) from lung (5), liver and spleen (3). The preliminary experimental results showed that the sera of 7 of these confirmed patients gave precipitin line(s) with GD test using WHHF and 6 with WSHF. The technique of CIEP gave precipitin line(s) with 8 sera when WHHF was used and 7 sera were reactive with WSHF. In the case of CEID 7 patients' sera were positive using WHHF and only 2 sera with WSHF. The sera of the two other patients which did not have hydatidosis as well as sera from normal individuals did not demonstrate only precipitin lines with the above tests.

Further experiments with suspected patients sera are in progress; and the sensitivity of these tests will be discussed.

Activated and deactivated macrophages in rodent leprosy

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Spleen cells from CBA mice infected intravenously with *Mycobacterium lepraemurium* showed an enhanced PFC response when cultured *in vitro* in the presence of SRBC. The enhancement was most marked when spleens were taken 4-6 weeks after infection. In contradistinction, spleens taken for culture at later stages of infection showed a depressed response. The nature of the enhancement and the defect were examined using cell fractionation by glass adherence and suitable reconstitution of similar spleen cell suspensions.

Enhancement was macrophage mediated and was also T-cell dependent; spleens from T-cell depleted mice did not give enhanced responses after infection. The depression was also macrophage mediated and probably due to the restrictions placed upon macrophage function by an ever increasing load of intracellular mycobacteria. Responses to DNP-POL, a T-cell and macrophage independent antigen *in vitro*, by the same and similarly prepared spleen cell suspensions were within the normal range throughout infection. The possibility that the capacity to enhance responses in this way may be another function of the activated macrophage will be discussed.

Regression of Lewis lung carcinoma in C57BL mice in response to a lipid extract from anaerobic coryneform bacteria

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In a recent report (Russell et al., 1976) we described a lipid extract from anaerobic coryneform bacteria with a potent and direct stimulatory action on mononuclear phagocytes. The present communication deals with investigations into its anti-tumour activity. The lipid extract when mixed with Lewis lung carcinoma cells and injected subcutaneously into C57BL mice, caused the tumours which were initiated to regress completely and permanently. Similarly, injection of the lipid extract directly into a site inoculated with tumour 24 hours previously resulted in the regression of some of the ensuing tumours. A significantly higher incidence of tumour regression was obtained in mice stimulated 48 hours before with the lipid extract. Direct injection of extract into established tumours produced retarded tumour growth and in one instance, regression. Whole bacteria in comparable doses produced results which were similar to those described for the lipid extract. However, polysaccharide extract from the same bacteria had no development effect on tumour.

The results of further investigations suggest that the lipid extract owes its anti-tumour property to its ability to activate macrophages and attract them into the area around its site of injection.

Russell, R.J., McInroy, R.J., Wilkinson, P.C. and White, R.G. (1976) Immunology, 30, 935.

Suppression of rat adjuvant disease by single doses of cyclophosphamide

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Although rat adjuvant disease is generally considered to be caused by a cell mediated response to disseminated mycobacterial antigen, several observations are difficult to reconcile with this appraisal. Firstly neonatal thymectomy does not suppress adjuvant disease and secondly delayed hypersensitivity to PPD does not correlate well with the onset and severity of the disease. A degree of complement dependence has also been observed.

In this study, male PVG/c rats were dosed intraperitoneally with 100mg/Kg of cyclophosphamide (Cy). Three days later the rats received an intradermal injection of 300µg of Mycobacterium tuberculosis in liquid paraffin. Rats treated in this way showed slightly reduced initial lesions in the injected paw and failed to develop the secondary lesions which usually occur approximately 12 days after adjuvant.

PVG/c rats given similar doses of Cy showed a depressed IgM response to SRBC but skin allograft rejection time was not affected.

These experiments suggest that adjuvant disease involves an important B-lymphocyte component. Reconstitution experiments suggest that antibody is not involved in the development of adjuvant disease and that the disease may be controlled by suppressor B-lymphocytes.

A test for antigen-antibody complexes in human sera using low affinity IgM rabbit antibodies

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A simple semiquantitative test for immune complexes using characterised non-human reagents and permitting analysis of their constituents, has been developed from the agglutination inhibition method of Masson. Low affinity rabbit IgM antibodies to human immunoglobulins of different classes, absorbed on insoluble immuno-absorbents have been prepared. Antigen-antibody complexes are detected by their inhibition of the agglutination of immunoglobulin coated latex particles by these low affinity antibodies. No inhibition is obtained with monomer immunoglobulins. Semiquantitative measurement of complex may be made by counting residual unagglutinated latex particles on a Coulter counter, Model B. 0.1µg of IgG, which has been heat aggregated, gives a positive reaction, using the anti-IgG system. The test is applicable to IgA complexes, C1q binding complexes and presumably all constituents of complexes, antigen, antibody and complement. The test gives positive results for IgG complexes in most of 21 patients with S.L.E.. Results for IgG, IgA and C1q binding complexes will be presented for S.L.E., IgA deposit disease and selected immunodeficiency diseases.

A quantitative latex agglutination inhibition test (LAIT) for the detection of circulating immune complexes

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An LAIT for detecting circulating immune complexes has been described by Luthuma et al. (1976). This has been modified by us to allow rapid quantitation of immune complexes. In principle, agglutination of IgG coated latex spheres by Rheumatoid Factor or C1q is inhibited by test serum containing soluble complexes. These compete for either Rheumatoid Factor or C1q so that less is available for latex agglutination. Unagglutinated particles are counted in a Coulter ZF particle counter at settings adjusted to select 0.8µm spheres.

The LAIT detects immune complexes in sera from patients with SLE, glomerulonephritis, polymyalgia rheumatica, SBE, erythema nodosum, chronic active hepatitis and Crohn's disease and in sera from rabbits with acute serum sickness. EDTA plasma or serum is used in the test since fixation of C3 to the IgG coated latex is itself inhibitory to the LAIT. The rheumatoid factor and C1q versions of the test appear to detect different types

of complex and this combined with its simplicity make the LAIT ideal for screening large numbers of sera.

Lurhuma, A.Z., Cambiaso, C.L., Masson, P.L., and Heremans, J.F. 'Detection of circulating antigen antibody complexes by their inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or C1q.' Clin.exp.Immunol. (1976) 24, in press.

Immune complexes in acute and chronic liver disease

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Sera from patients with alcohol related liver disease (ALD), chronic active hepatitis (CAH, HB_s +ve and -ve) primary biliary cirrhosis (PBC) and acute type A and B viral hepatitis have been examined for immune complexes by anti-complementary (A.C.) assay and by the technique of radio-labelled C1q binding.

Immune complexes were found in all groups. In alcohol induced disease, complexes were only found during episodes of hepatitis. Eight patients with acute viral hepatitis (A and B) had increased C1q binding during the period of elevated transaminases, and one patient with type B hepatitis showed a rise in C1q binding when HB surface antigen titres were falling.

Ultracentrifugation studies on the sera suggest that the C1q binding assay detects large complexes while the A.C. assay detects smaller ones. This may account for the lack of correlation between data obtained with these two assays.

These data suggest that large and small complexes are present in the serum during acute and chronic hepatocellular liver disease of varying aetiology and also in P.B.C.. The presence of large complexes which are normally readily cleared by the hepatic phagocytes suggests impairment of this function in these diseases.

Binding of immune complexes of guinea pig IgG2 to homologous peritoneal exudate cells

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A quantitative study has been performed to correlate the macrophage binding activity of immune complexes of guinea pig IgG2 with the antigen-antibody composition and the size of the immune complex. Binding, measured as (i) uptake at a single IgG concentration, (ii) resistance to IgG2 monomer inhibition and (iii) mean binding avidity of the complex, was maximum at the antigen-antibody ration optimum for immune precipitation. All three parameters declined in value with increasing proportions of antigen

in the complex. Gel filtration of immune complexes revealed that complex size declined with increase in antigen excess. The relationship of binding activity to complex size and the similar inhibitory patterns of IgG2 and its fragments for binding of monomer and complexed IgG2 suggest that binding enhancement results from the co-operative action of multiple Fc-sites in the immune oligomer, and not from conformational change.

Circulating immune complexes following repeated halothane anaesthesia

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Allergic mechanisms have been incriminated in the pathogenesis of the jaundice which occasionally follows the repeated use of halothane as an anaesthetic agent. Indirect evidence in support of such an idea was provided by the demonstration that increased lymphocyte transformation occurred when halothane was added to the lymphocytes obtained from patients suspected of having 'halothane hepatitis'. Other workers, however, have failed to confirm these observations.

Recently we have studied a patient who after receiving three anaesthetics with halothane developed a severe hepatitis. During the course of this illness she also developed a migratory polyarthritis, joint effusions, proteinuria and mild impairment of renal function. Evidence will be presented to show that circulating immune complexes were present in this patient's plasma and joint fluid, and that a metabolite of halothane formed part of this complex.

Elimination of antigen - a function of IgA

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We have investigated the role of IgA antibody in antigen elimination and the subsequent antibody responses by the injection of M.O.P.C. 315 IgA myeloma protein followed by Dnp-ovalbumin, to which the myeloma protein binds with antibody-like affinity. Passive transfer of IgA antibody increases the elimination of antigen from the circulation and increases the subsequent antibody response to that antigen. These data suggest that the humoral IgA response may contribute positively to the handling of ingested antigens.

Altered expression of monocyte Fc receptors in malignant disease

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Several kinds of cell involved in immunity can bind antibody and immune complexes through surface receptors which interact with the Fc portion of IgG (Fc receptors). The expression of such receptors on mononuclear phagocytes (monocytes, macrophages) can change in response to various stimuli and such changes may have a regulatory function in both the induction of an immune response and in immunological effector mechanisms. The expression of Fc receptors on human peripheral blood monocytes was characterized, using a rosette assay, in fifteen patients with solid malignant tumours and in seventeen normal individuals. All patients showed, with one exception, a substantially increased expression of receptors in comparison with normal indicating that in this respect the patients with malignancies possess systemically activated mononuclear phagocytes. Smaller changes in lymphocyte Fc receptor expression also appeared to occur in some patients. The possibility that activated mononuclear phagocytes might exert a net inhibitory effect on anti-tumour immunity will be discussed.

High proportions of lymphocytes binding sheep erythrocytes (E) and complement coated yeasts (C3) simultaneously in some lymphoproliferative diseases

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A combined E and C3 lymphocyte marker test has been developed to study subpopulations of peripheral lymphocytes in patients with lymphoid malignancies. Dead yeasts, sensitised with human C3 and stained with trypan blue, have been used. These are quite distinct from sheep E and enable lymphocytes which bind E and C3 simultaneously to be easily distinguished. Significant increases in the absolute numbers and proportions of such lymphocytes have been found in the peripheral blood of all untreated lymphosarcoma patients (8) and T cell acute lymphoblastic leukaemia patients (4). Such increases were not found in blood from patients with chronic lymphatic leukaemia (40), null cell acute lymphoblastic leukaemia (5), other lymphoid (5) and non-lymphoid (3) malignancies or normal individuals (30). These mixed E and C3 receptor bearing cells were not the malignant cells themselves since they were not found in a suspension from a lymphosarcomatous spleen which contained 62% malignant cells; also they were quite distinct from ALL blasts. They appeared to be Fc receptor positive and represent a separate subpopulation of lymphocytes, possibly "activated" T cells. The relatively high numbers in blood from some patients will enable these cells to be purified and their function studied in closer detail.

Immunoglobulin synthesis in tumours of the breast

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The first aim of the study was to determine whether immunoglobulin synthesis could be detected in breast tumours when cultured in vitro; secondly, to compare the amounts of the three major classes of immunoglobulin produced by malignant and benign tumours of the breast; thirdly, to determine whether immunoglobulin synthesis correlated with clinical and histological features.

Method Tumour tissues were cultured in medium containing 14C labelled aminoacids, and the culture fluid dialysed and concentrated. Following immunoelectrophoresis with appropriate carrier proteins and antisera to human serum, human colostrum, and IgG, IgA and IgM, the presence of radioactively labelled immunoglobulin was detected by autoradiography and graded semiquantitatively (Hochwald et al., 1966).

Results IgA was produced by 26 of 28 malignant tissues and 13 of 18 benign tumours. In contrast, IgG synthesis was found significantly more frequently in cancer tissues: 25 of 28 cancers produced IgG, 8 showing strong synthesis. Nine of 18 benign tissues produced IgG, none of them showing strong synthesis. In 4 breast tumours there was evidence of synthesis of an abnormal IgG with slow mobility on electrophoresis. Although intensity of immunoglobulin synthesis did not correlate well with total plasma cell counts in the tissue, preliminary immunofluorescence studies confirm the presence of IgG containing cells in tumour tissue.

These findings contrast sharply with the pattern of synthesis in normal breast tissue, in which IgG synthesis is very rarely found (Drife et al., 1976). The increased IgG synthesis in tumour tissues suggests that there may be a local immunological response to the tumour.

Drife, J.O., McClelland, D.B.L., Pryde, A., Roberts, M.M., Smith, I.I. (1976) Brit.Med.J. (in press)

Hochwald, J.M., Thorbecke, G.J. and Asofsky, R. (1961) J.exp.Med.114,459.

The mechanism of lymphocyte adherence to endothelium

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The adherence of allergised and non-allergised pig lymphocytes to fresh or cultured endothelial monolayers was studied by allowing lymphocyte suspensions to settle on the monolayers, washing in a standard way and counting the number of adhering cells per unit area. Non-allergised lymphocytes will scarcely adhere to fresh aortic endothelium, but adhere readily to cultured aortic endothelial monolayers. Lymphocytes from skin graft allergised pigs will adhere to fresh donor type endothelium more readily than to control endothelium. The adherence of non allergised lymphocytes to fresh endothelium is increased if the latter is first incubated with an

appropriate alloantiserum. Lymphocyte adherence in this model was temperature sensitive, energy dependent, and inhibited by EDTA, vinblastine or local anaesthetics but not by steroids. Initial adhesion takes place via pseudopodia: appropriately stimulated cells subsequently spread, become motile and penetrate the monolayer. Specificity seems to be manifested at the stage of spreading rather than initial adhesion. Cultured aortic endothelium is an excellent substrate for lymphocyte adhesion, and may in this respect resemble post capillary venule endothelium *in vivo*.

Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse

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It is well established that mesenteric and peripheral lymphoblasts show divergent migration patterns *in vivo* (1,2). Thus, mesenteric lymphoblasts migrate to the lamina propria of the small intestine whereas peripheral blast cells fail to migrate to the gut but accumulate in the spleen. Furthermore we have demonstrated that whereas mesenteric blasts fail to migrate to inflamed ear skin, oxazolone-stimulated peripheral blasts accumulate readily in this site (3).

In the present studies we demonstrate that these differences do not simply reflect the different proportions of T and B immunoblasts in the inocula, and that the migration of mesenteric and peripheral T-blasts is determined to a large extent by their tissue of origin. We also show that although peripheral T blasts do not migrate to the 'normal' small intestine they accumulate significantly in the inflamed gut of mice infected with the nematode *Trichinella spiralis*.

1) Griscelli *et al*., (1969) J.exp.Med. 130, 1427.
2) Guy-Grand *et al*., (1974) Europ.J.Immunol. 4, 435.
3) Parrott *et al*., (1975) in 'Future Trends in Inflammation II'. (J.P.Girond, D.A.Willoughby & G.P.Velo, eds) 32 Birkhäuser Verlag, Basel.

The influence of cell adhesiveness on the migratory behaviour of murine thymocytes

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Thymocytes from CBA mice show a characteristic migratory pattern when infused into syngeneic recipients via the caudal vein. These cells can be fractionated using a nylon wool filtration technique which produces a sub-population of more mutually-adhesive cells that display a different *in vivo* migratory pattern from control cells of the entire thymus. Fewer cells entered all the major organs studied (except the small intestine and kidneys) during the first four hours after infusion, but after 24 hours, the organ distribution of cells from the sub-population was similar to control cells except in the kidneys. Caution is thus necessary when extrapolating from data in which the experimental cells were prepared using this technique. It is suggested that transitory changes in adhesiveness may be responsible for a cell being able to leave one organ and enter another.

Exudate cells, coagulation and delayed hypersensitivity

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The migration of exudate cells is inhibited by lymph draining from an antigen-stimulated node. The mechanism of this inhibition is predominantly by a heat labile, multifactorial system involving fibrinogen and a protease. Studies with antiproteases suggest that the protease is either membrane bound or generated on contact with exudate cells.

The properties of this multifactorial system resemble those of the coagulation system. Results obtained could be explained by production of a procoagulant by exudate cells, leading to activation of clotting system proteases, deposition of fibrin and cell 'trapping'. Exudate cells have appreciable platelet factor 3 activity. (Lymph lacks platelets, the usual source of this procoagulant.) Deposition of fibrin about exudate cells has been demonstrated by immunofluorescence by both our group and another (Colvin & Dvorak (1975) J.exp.Med.142,1377).

In view of the inhibition of delayed reactions by anticoagulants, might this mechanism have some relevance *in vivo*?

Studies on the microcytotoxicity test: evidence that the effects of normal lymphoid cells on the growth/survival of syngeneic tumour cells in microtest plates are caused by non-immunological modifications of the culture medium

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Lymph node cells from normal Wistar rats were found to either inhibit or enhance the growth/survival of syngeneic solid tumour cells in the microcytotoxicity test. Which effect was observed depended on the particular tumour cell used and, in some cases, on the particular *in vitro* subline used. The cells responsible for inhibition were found to be selectively retained on nylon wool columns whereas the cells responsible for enhancement were selectively eluted. When these cell fractions were incubated in the absence of tumour cells the cell-free supernatants mediated the same effects as the cells from which they were derived. The supernatant inhibitory activity was maximal within one hour of cell culture, whereas the supernatant enhancing activity developed over a 48hour period. Furthermore, tumour cells themselves were found to produce growth enhancing activity. It is proposed that interaction between these various supernatant activities accounts for the non-specific effects of normal lymphoid cells in the microcytotoxicity test.

Bioassay of leucocyte migration inhibition factor (LIF) derived by antigen activation of human peripheral blood lymphocytes

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This communication demonstrates the use of a research standard of guinea-pig lymphokine in the bioassay of human LIF. Human peripheral blood lymphocytes from subjects of known tuberculin sensitivity (by skin test and lymphocyte transformation) were cultured with PPD by a modification of the method of Rocklin (1974). The LIF activity of dilutions of the supernatants were assayed on the migration under agarose of purified human peripheral blood polymorphs (Clausen, 1972). Within the same tests dilutions of a standard guinea-pig lymphokine preparation were also tested, and the LIF activities of the human supernatants were expressed in terms of the standard as potency ratio estimates.

The assay allows a reproducible measurement of activity in LIF containing preparations and eliminates the inherent biological variations in the test system. This approach provides a basis for a quantitative assessment of the correlation between different parameters of cellular hypersensitivity.

Clausen, J.E. (1972) J.Immunol. 108, 453
Rocklin, R.E. (1974) J.Immunol. 112, 1461

The effect of protease inhibitors on leucocyte migration inhibition with PPD

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The effect of two protease inhibitors, epsilon amino-caproic acid (E.A.C.A.) and aprotinin on Leucocyte Migration Inhibition with P.P.D. have been studied in Heaf test positive and anergic individuals. Hirschhorn et al. (1971) demonstrated that E.A.C.A. inhibited PHA transformation and Thornes et al.(1974) showed that pre-incubation of lymphocytes with E.A.C.A. diminished rosette formation.

In this study E.A.C.A. and aprotinin have both been found to cause significant increases in the inhibition to P.P.D. in Heaf positive subjects ($p < 0.01$). In Heaf test negative individuals (showing no inhibition to P.P.D.) the addition of either E.A.C.A. or aprotinin had no significant effect on migration index. A dose response to both agents was demonstrated and the effect can be reversed by washing the cells.

The unexpected finding of increased inhibition to P.P.D. may be explained by the reduction in the breakdown of migration inhibition factor (M.I.F.). M.I.F. is sensitive to proteolysis and both E.A.C.A. and aprotinin may prevent this by their known action in inhibiting plasmin.

Hirschhorn, R., Grossman, J., Troll, W. & Weissman, G. (1971) J.Clin. Invest. 50, 1206.
Thornes, R.D., Smyth, H., Browne, O., O'Gorman, M., Reen, D.J., Farrell, D. & Holland, P.D.J. (1974) J.Med. 5, 92.

Isolation and characterization of antigen-dependent MIF

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PPD-specific migration inhibitory factor (MIF) and macrophage activating factor (GUS) produced in vivo in the sheep during a delayed hypersensitivity response to BCG were purified by chromatography on Sephadex-G200 and on Concanavalin-A-sepharose. Final traces of contaminating IgG were removed with anti-sheep IgG sepharose. The activities were thus characterized as 4S glycoproteins. Antigen-dependent MIF and GUS activities were bound to PPD-sepharose and the eluted activity gave one major protein of similar size and electrophoretic mobility to albumin. An antiserum was raised to this material and was shown by affinity chromatography to remove MIF activity. Using indirect immunofluorescence, this antibody was found to react with 13% of sheep peripheral blood lymphocytes. The reaction was not inhibited by sheep albumin or IgG.

Specific cytotoxic cells in picryl chloride painted mice following pre-treatment with cyclophosphamide

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Cytotoxic cells against trinitrophenol (TNP) coupled syngeneic lymphocytes can be readily generated in vitro. It has proved more difficult to detect cytotoxic cells with similar specificity in the lymph nodes of mice painted with picryl chloride. However if mice are given cyclophosphamide (200mg/Kg) 5 days prior to painting with picryl chloride, there is on average a 3-fold increase in the percentage cytotoxicity, measured using TNP coupled syngeneic lymphocytes or tumour cells. The cytotoxicity is T cell mediated, and the specificity is similar to that found for in vitro generated cytotoxicity, in that lymph node cells from CBA mice kill TNP coupled CBA lymphocytes and to a much smaller extent TNP coupled C57 lymphocytes, but not uncoupled lymphocytes of either strain. The increased cytotoxicity can be suppressed by painting the mice with picryl chloride 4 days before administration of cyclophosphamide. In addition normal or immune cells injected intravenously after cyclophosphamide, immediately prior to painting with picryl chloride suppress cytotoxicity. The nature of the cells involved in this suppression and their possible mode of action will be discussed.

Radioimmunometric assay of salivary antibody to C3

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Anti C3 antibodies have previously been demonstrated in human saliva using a haemagglutination assay. A radioimmunometric assay has been developed using C3 coated red cells and ^{125}I labelled antisera to allow the class of antibodies to be assayed at the same time as the quantity. Both IgG and IgA antibodies could be detected in parotid saliva. The binding of the IgA antibodies were not affected. No IgM antibodies were detected. The binding of IgA antibodies could be completely inhibited by pre-incubation of the saliva samples with purified C3. With this assay antibodies could be detected in some salivas at a dilution of greater than 1:1000.

Anti C3 antibodies in amniotic fluid

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The majority of adult parotid salivas contain antibodies to the third complement component, C3. The observation that high titres are present in neonatal saliva collected within a few hours of birth indicates that prior antigenic stimulation with oral bacteria is not required to initiate their production and suggests that they could be produced by the developing foetus. We proceeded therefore to examine 33 samples of amniotic fluid for the presence of these antibodies. Anti C3 activity was found in 32 samples and were demonstrated in fluid obtained as early as 16 weeks gestation. Further examination showed that their properties were similar to the anti C3 antibodies present in saliva.

Serum factors and phagocytic elimination of C.albicans

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Phagocytosis and killing of C.albicans can be objectively measured by ^{3}H-uridine uptake and ^{51}Cr release techniques respectively (1).

Standardization of these techniques for measuring opsonizing activity in serum will be described. The role of complement factors in the opsonization and subsequent killing of C.albicans by PMN leucocytes has been analyzed. While serum depleted of the early complement components (C142) has opsonizing activity in this system, there is a minimal phagocytic killing under such conditions. Similarly, while C.albicans were phagocytosed in the presence of 56°C heated serum, the yeast was not killed.

These findings suggest:

1. Opsonization of C.albicans is not dependent on classical pathway activation.
2. Complement components of the classical pathway are required for effective phagocytic killing of C.albicans.

(1) Yamamura, M., Boler, J. and Valdimarsson, H., J.Immunol. Methods (in press).

Complement in the induction of IgE production

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The IgE response to alum precipitated ovalbumin was measured in NIH mice by PCA in rat skin. Mice which received two doses of antigen separated by 4 weeks produced reaginic antibody in titres of up to 1:2000. Animals treated with cobra factor (CoF) before immunization produced very little IgE antibody, and also failed to show the peripheral blood eosinophilia which developed in control animals. These observations extend to IgE antibody the suppressive effects which have been reported of CoF on the in vivo production of other thymus-dependent antibody classes, IgG and IgA.

C3 in the induction of immunological responses in vitro

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The induction of thymus-dependent, but not thymus-independent antibody responses during in vitro culture of mouse spleen cells is inhibited by both pure IgG and pure F(ab)$_2$ anti-mouse C3 antibodies. More F(ab)$_2$ than IgG anti-C3 is required to produce equivalent inhibition, and this correlates with the shorter survival in culture as functional antibody of F(ab)$_2$ than of IgG. In contrast anti-C3 antibody has no effect on the in vitro induction by antigen of T helper or suppressor cell activity. Incubation of mouse lymphocytes with purified mouse C3 in vitro inhibits C3-dependent rosette formation by complement receptor lymphocytes, and addition of mouse C3 to in vitro cultures suppresses thymus-dependent antibody formation. These results suggest that C3 participates in lymphocyte cooperation leading to T-dependent antibody production after T cells have responded to antigen, and that the B cell C3 receptors may be involved in its action.

Isolation and studies of murine C3

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Murine C3 has been isolated in relatively pure form by precipitation with sodium sulphate and then as euglobulin followed by reverse affinity chromatography. The yield of C3 was about 50%. Results of studies on mouse C3, its activation and its fragments will be presented.

A comparative study of phagocytosis by inflammatory exudate and peripheral blood polymorphonuclear leucocytes in man

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There is little available information on the phagocytic properties of polymorphonuclear leucocytes (PMN) from inflammatory exudates. We have studied phagocytosis by PMN which migrate into the mouth from the soft tissues around the teeth in clinically normal human subjects and compared this to phagocytosis by peripheral blood PMN from the same individual. The phagocytosis and killing of *Candida albicans* (CA) blastospores by both populations of PMN was studied using the method of Schmid and Brune (Infection & Immunity, 1974, 10, 1120). A smaller number of exudate PMN take up fewer CA but kill them with equal efficiency as compared to peripheral blood PMN. Preliminary evidence suggests that this defective phagocytosis by exudate PMN is related to a functional deficiency of the C3b receptor, the Fc receptor function remaining intact.

Immunostimulation by Levamisole therapy promotes gingivitis in man

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The immunostimulatory effects of levamisole on gingival conditions and on cell-mediated immunity were examined in patients with gingivitis and in subjects with clinically healthy gingiva. Treatment with levamisole promoted the development of gingivitis and aggravated the severity of already ongoing gingivitis. The development of gingivitis was associated with increased *in vitro* lymphocyte stimulation by antigens from oral bacteria and by LPS. Lymphocyte *in vitro* responses were amplified by the sera from levamisole treated subjects. The results imply that potentiation of cellular immunity may play a mandatory role in the pathogenesis of gingival inflammation.

Preparation of lymphoid cells from small specimens of human gastro-intestinal mucosa

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There have been many studies of peripheral blood lymphocyte function in patients with disease affecting the mucosa of the gastrointestinal tract; interpretation of the findings is difficult, since there is no reason to suppose that the population of cells studied is representative of those present in the abnormal mucosa. We have therefore studied methods of preparing suspensions of lymphoid cells from gut mucosa to permit characterisation and functional studies of the cells involved in the mucosal lesion.

Several approaches combining mechanical disruption and enzymatic digestion of the tissues have been investigated. The most satisfactory results have been obtained using agitation in a buffered solution of hyaluronidase and collagenase. This procedure gives cell yields of at least 5×10^6 cells/100mg of tissue. The initial cell suspensions contain 65-80% epithelial cells. The remainder consist of lymphocytes (10-15%), plasma cells (5-19%) with a few monocytes, mast cells and eosinophils. Trypan blue is excluded by 80-95% of the cells. Enrichment of the lymphoid cell content is achieved by passage of the suspension through a glass bead column. A high proportion of the lymphoid cells contain large amounts of intracellular IgA demonstrated by immunofluorescence of fixed preparations and IgA synthesis can be demonstrated *in vitro*. In short term cultures for up to 48 hours a high percentage of the cells remain viable, as assessed by dye exclusion and examination of stained preparations.

Demonstration by radioimmunoassay of antibodies to collagen in sera of coeliac patients

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A panel of sera from coeliac patients was examined by radioimmunoassay for the presence of antibodies to human and bovine collagen types I, II and III. The sera were reacted with ^{125}I-labelled collagens, and the antigen-antibody-complex was precipitated with secondary antisera to human immunoglobulins raised in goats. Sera from patients with untreated coeliac disease contained large amounts of antibodies reacting with native human and bovine collagen types I and III. Such antibodies were isolated by immunoabsorption and characterized with respect to antibody specificity, antibody class and reactivity in immunofluorescence. The results are discussed in view of the reactivity, previously detected by other authors, of coeliac sera with reticulin.

Separate autoantibodies reacting with human pancreatic glucagon with somatostatin cells

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Autoantibodies reacting with discrete populations of cells in normal human pancreatic islets were found by immunofluorescence in 17 out of 1279 sera. Using a double immunofluorescence technique, with antisera to pancreatic glucagon, insulin, somatostatin and human pancreatic polypeptide, it was shown that 13 of the sera contained antibodies reacting specifically with glucagon cells, while the other 4 reacted with somatostatin cells. These antibodies were directed against intracellular components and not against the hormones themselves. Both types of antibody occurred independently of the islet cell antibodies (ICA) which have been described in diabetes mellitus. These findings suggest selective damage to individual cell types in the pancreatic islets and raise the possibility of corresponding hormone deficiency syndromes.

Isolation and studies of C-reactive protein

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A number of methods for the isolation of CRP from plasma and pathological fluids, involving affinity chromatography and reverse affinity chromatography have been compared, and the results will be presented. The results of studies on the interaction between CRP and lymphocytes will will be presented.

Further studies on the adjuvant properties of liposomes

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It has previously been reported* that liposomes exert an adjuvant effect upon bovine serum albumin (BSA) entrapped within them when the preparation is injected into CBA/Lac mice. Purified BSA monomer has been entrapped in liposomes and found to produce an immune response. Further studies on the effect of lipid composition of such liposomes on the immune response will be discussed. Investigations have been extended and the immune response to BSA entrapped in liposomes has been examined in two other strains of mouse (C57BL and Porton albino), in rabbits, and in guinea pigs.

Thymectomised, irradiated, bone-marrow reconstituted CBA/Lac mice have been immunised with BSA entrapped in liposomes and we have observed no immune response. Splenectomised mice produce a response to BSA in liposomes. Possible effects of the size of liposomes on the immune response will also be discussed.

* T.D.Heath, D.C.Edwards and Brenda E.Ryman, Biochem.Soc.Trans.(1976) 4, 129.

Genetic control of the response of chicken leukocytes to mitogens

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Peripheral blood leukocytes of the inbred chicken strains WA, CB and GB1 responsd in culture to the mitogens Concanavalin A (Con A) and Phytohaemagglutinin (PHA). The responses of the three strains to PHA, as measured by ^{3}H-thymidine uptake after three days of culture, are of approximately equal magnitudes. However, the response of WA strain leukocytes to various doses of Con A, after three days of culture, is much higher than the response of leukocytes from the CB and GB1 strains; at the optimum dose (about 1µg/ml) of Con A, the response of WA cells is at least 5-fold higher than that of cells from the other strains. All (WA x CB)F_1 birds tested have been high responders. Of 31 (WA x CB)F_2 birds tested, 9 were low, and 22 high responders. Hence, it seems likely that this difference is due to the presence of a single dominant gene for high response to Con A in the WA strain.

Antigens of the chicken major histocompatibility system

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The chicken major histocompatibility antigens (B antigens) are composed of polypeptide chains with molecular weights (in the reduced form) of 39-43,000 and 11-12,000 daltons as deduced from their mobilities in internally calibrated, sodium dodecyl sulphate-containing polyacrylamide gels.

An additional protein or proteins of apparent molecular weight about 30,000 could be precipitated from ^{3}H-amino acid-labelled leukocyte lysates by several anti-B sera. This protein(s) differs from the B major histocompatibility antigens in that it is not associated with the small chain of molecular weight 11-12,000 and does not occur on erythrocytes. Its molecular weight and tissue distribution, together with the fact that its synthesis is controlled by a gene(s) in the major histocompatibility complex, suggest that it is the avian homologue of the mouse Ia antigens.

Immunogenetic control of the response to sperm whale myoglobin in inbred mice

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The immune response to the antigen sperm whale myoglobin has been studied in inbred mice (Young & Ebringer, 1976). The immune response was examined in 7 inbred strains of mice over a dose range from 1µg to 2000µg, and in 12 strains at a dose of 500µg. Antibodies to SWM were assayed by an antigen excess technique employing I^{125} labelled antigen.

The dose response curve obtained to SWM is different for each strain tested, indicating a linkage between the quantitative antibody response and the genetic status of the responder mice. The dose response curves indicate that the dynamics of the AB response is sigmoidal. These sigmoidal dose response curves show a plateau level of AB response. This plateau occurs at a lower level of quantitative AB response in low responder strains than in high responders. It is postulated that the plateau level of quantitative AB response may be used as an index of IR-gene function.

A statistical analysis of the continuous quantitative AB response in the 12 strains of mice at an SWM dose of 500µg suggests that the immune response is under polygenic control. Alternatively, the results could be explained by a cross tolerance hypothesis requiring no IR-genes at all.

C.Young & A.Ebringer, Immunogenetics, 3, 299-304 (1976).

Crosstolerance as a pathogenetic mechanism in HLA-linked diseases

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It has recently been demonstrated that rheumatic fever occurs more frequently in subjects having blood group "A" whilst ankylosing spondylitis occurs more frequently in subjects carrying the HLA-B27 antigen.

The reason for this association is unknown, but either of 2 mechanisms appears possible:

(1) Linked gene hypothesis, in which the gene predisposing to the disease is closely linked to the gene coding for the HLA or blood group marker molecule, but no such predisposing genes have so far been identified.

(2) Crosstolerance hypothesis (Ebringer & Davies, Nature, 241, 144, 1973), in which the HLA antigen or the blood group antigen crossreacts to some degree with microbiological antigens, such that the patient is unable to mount an adequate immune response against these microorganisms.

A rabbit antiserum raised against Streptococcus Pyogenes was found to give a $\log_2$ haemagglutination titre against blood group "A" red cells of 5.88 ± 0.26 (mean ± S.E.) and blood group "O" red cells 3.76 ± 0.05 and this difference is statistically significant ($t = 7.327$; $p < 0.001$).

It is suggested that molecular mimicry between streptococci and blood group antigens is a possible way of explaining the association of blood groups with rheumatic fever and similar mechanisms also appear possible in ankylosing spondylitis.

Tolerance in differentiating B lymphocytes

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B lymphocytes differentiating in vivo were not rendered tolerant by exposure to deaggregated H γG but were by exposure to alum precipitated H γG. In contrast mature B lymphocytes, in the same situation, were primed by alum precipitated H γG. The unresponsiveness induced in differentiating B lymphocytes is specific and does not depend on the presence of T cells. It is concluded that mature and differentiating B lymphocytes differ in their susceptibility to tolerance induction.

Detection and enumeration of lymphocytes in whole blood by immuno-enzyme technique

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We have previously reported an immunofluorescent method for the detection of surface immunoglobulin on human lymphocytes in peripheral blood, which permits the accurate enumeration of absolute numbers of circulating B cells. This method has now been adapted for use with enzyme-labelled antibodies, which has important advantages in yielding permanent preparations which can be viewed in ordinary light. Results obtained with anti-immunoglobulin and other reagents will be presented. This approach may enable the enumeration of human circulating lymphocyte populations to be undertaken in a routine fashion with greater accuracy than hitherto.

Isolation of heterophile antibodies and lymphocytotoxic autoantibodies from infectious mononucleosis sera

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A variety of antibodies are produced in infectious mononucleosis; among these are the heterophile antibodies of Paul-Bunnell type and Forssman type, and autoantibodies with specificities for lymphocytes, red cells and gamma globulin.

With the aid of insolubilized adsorbents made with bovine erythrocyte glycoproteins and guinea pig kidney tissue, we have isolated three antibody

populations from infectious mononucleosis sera. One of these contains lymphocytotoxic autoantibodies.

Immunoglobulin synthesis and expression by neoplastic lymphoid cells in leukaemia and non-Hodgkin's lymphoma

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We have looked at immunoglobulin (Ig) synthesis by lymphocytes from normal and neoplastic tissue using ^{3}H leucine precursor and analysis on SDS polyacrylamide gels. IgM and IgG synthesis was detected in cultures of normal lymph nodes and spleens. In contrast, cultures of peripheral blood lymphocytes synthesised predominantly IgG with little or no detectable IgM. These findings will be discussed with respect to the presence of surface Ig bearing cells and plasma cells in these preparations. The capacity of neoplastic cells to synthesise Ig showed considerable heterogeneity within the CLL and lymphoma groups. Gel analysis patterns were consistent with surface Ig expression as detected by fluorescent antibody staining, except in 'Hairy Cell' leukaemia where the cells expressed predominantly IgM and IgD, and IgG was the only synthesised Ig class. In a proportion of the lymphoma patients studied free light chain synthesis and secretion was detected at the cellular level in the absence of Bence-Jones protein in the urine. The capacity of neoplastic cells to synthesise, secrete and express Ig of varying class will be discussed in relation to normal B cell differentiation.

Respiratory response of allergic rats to aerosol challenge

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Inbred hooded Lister rats were sensitised by injections of DNP-ovalbumen and adjuvant. The respiratory rate and tidal flow were measured using a body plethysmograph. The rats inhaled an aerosol which contained DNP-ovalbumen.

Sensitised rats characteristically showed a slowing of respiratory rate, a reduction of minute volume and a respiratory gas flow pattern suggestive of bronchial obstruction. When some similarly sensitized rats were challenged with DNP-ovalbumen given by stomach tube, they did not give the same response but did so when they were subsequently challenged with the aerosol. The P.C.A. titres of these rats' sera will be reported together with experimental attempts to reproduce this respiratory response in rats passively sensitised by the intravenous injection of allergic sera.

Migration of human lymphocytes in mice

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A method to follow the migration of human peripheral blood lymphocytes in the mouse has been established.

Ficoll/triosil separated lymphocytes are labelled with sodium chromate (^{51}Cr) and are injected intravenously into 8-12 week old, female mice (from a Closed Colony), which have previously been treated with colloidal carbon to remove platelets and macrophages from the blood. Organs and tissue samples are removed and the radioactivity counted.

As compared with killed, non-aggregated lymphocytes, or the products released from dead lymphocytes, active migration of living human lymphocytes to the lymph nodes and spleen has been demonstrated and compared with syngeneic lymphocytes.

Variation between normals, and variation of a normal with time, have been determined. The technique has been used to compare the distribution of lymphocytes from patients with chronic lymphocytic leukaemia, rheumatoid arthritis and systemic lupus erythematosus. The changes in distribution found will be discussed.

POSTERS

Measurement of host defence activity in patients receiving cytotoxic drugs

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Infections seriously limit the success of many promising therapeutic procedures, e.g. 75% of deaths in adult leukaemics treated with cytotoxic agents may be due to infection (1). For this reason we have concerned ourselves with methods for assessing the competence of the host defence system in such patients. We show that present clinical procedure may seriously under estimate impairment of this system, so that patients may become at risk well before individual parameters are markedly affected. This is because the host defence system, even on the simplest analysis, depends on the collaboration of several cellular and humoral components, and, as we show, the activity of such systems cannot be demonstrated reliably from measurements of individual components. With this in mind we suggest a strategy for a host defence screen and illustrate a clinically feasible set of tests.

(1) Chang, H. et al., Medicine, 55, 259 (1976)

The measurement of phagocyte migration for clinical purposes

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We have developed a simple and economical raft technique for chemotaxis suitable for multiple tests during clinical practice (1). This has enabled us to carry out simultaneous tests on neutrophils and monocytes in a variety of patients and we show that the migration of these cells can be affected in different and opposite ways. We suggest that the commonly used methods for measuring chemotaxis which employ positive gradients of casein, activated serum, or complement components, may not provide the most meaningful test of the ability of phagocytes to accumulate at *in vivo* sites of infection. We give evidence and arguments that this is better done using fresh, diluted, glass-activated autologous plasma, in the absence of a gradient.

(1) Addison, I.E. and Babbage, J.W. J.Immunol.Methods, 10, 385 (1976).

B Suppressor cells and contact sensitivity

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Mice immunized in the standard way for the production of contact sensitivity develop B suppressor cells which block the passive transfer of contact sensitivity. In practice mice are painted with picryl chloride on the abdomen. Their lymph node and spleen cells when taken 4 days later passively transfer contact sensitivity when injected into normal mice. However, lymph node and spleen cells taken at 8 days fail to transfer contact sensitivity although the donor mouse is still sensitive. Passive transfer by 4 day immune cells is depressed when 8 day immune cells are also injected into the recipients. The cells responsible are B cells as shown by their resistance to treatment with anti-θ serum and complement, removal by nylon wool filtration and their ability to bind red cells coated with antibody and complement (EAC^{+}).

T-cell depletion in pregnancy

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The thymic involution and decreased phytohaemagglutinin response observed in pregnancy may be reflected in a change in the numbers of circulating T cells. Peripheral blood was obtained from women in various stages of pregnancy, at 6 weeks post-partum and from non-pregnant controls. E-rosette-forming cells (E-RFC), EAC-rosette-forming cells (EAC-RFC) and cells bearing surface membrane immunoglobulin (SmIg) (demonstrable by immunofluorescence) were measured.

There was a reduction in the number of E-RFC throughout pregnancy

with little change in the EAC-RFC and cells bearing SmIg. In the post-partum period the number of E-RFC and cells bearing SmIg returned to non-pregnant levels while the number of EAC-RFC continued to rise to a level higher than that in non-pregnant controls.

The results indicate an increase in or appearance of a population of cells possibly activated T-cells, forming both E- and EAC-rosettes in the post partum period in addition to T-cell depletion during pregnancy.

The effect of phenytoin on the cellular immune response to dental plaque

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Phenytoin therapy has been shown to depress the cellular immune response (Sorrell and Forbes, 1975) and give rise to low serum and salivary immunoglobulin levels (Aarli and Tonder, 1975). Also, patients receiving therapy invariably develop gingival hyperplasia. Despite this increased opportunity for bacterial proliferation there does not appear to be a corresponding increase in tissue destruction in the gingival area.

In this investigation we have examined the effect of phenytoin on the *in vitro* cellular response to autologous dental plaque. The cellular immune response was assessed by comparing the tritiated thymidine uptake of dental plaque stimulated lymphocytes with that of unstimulated lymphocytes in a microculture system. Dental plaque antigen prepared by ultrasonication was added at three concentrations and phenytoin at two concentrations.

Initial results show a depression of the cellular immune response to dental plaque extract at both concentrations of phenytoin.

Aarli, J.A. & Tonder, O, Scand.J.Immunol., 4, 391-396 (1975)
Sorrell, T.C. and Forbes, I.J. Clin.exp.Immunol., 20, 273-285 (1975)

Structural studies of human IgG. The identification of two structural variants having different circular dichroic spectra.

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The C.D. spectral features of a human IgD myeloma protein have been investigated in detail through the preparation and isolation of enzymatic fragments. The tryptic Fab_{δ} and Fc_{δ} fragments yield spectra which in sum are equivalent to the intact protein. Thus no major conformational change is observed on cleavage in the hinge region. On further tryptic digestion of the $tFab_{\delta}$ fragment the $C_{\delta}1$ domain is destroyed and a $V_H V_{\lambda} C_{\lambda}$ fragment is isolated. The C.D. spectrum of this fragment shows very marked differences to that of the $tFab_{\delta}$ fragment and coincides, antigenically, with the revealing of C_{λ} domain determinants. The C.D. spectrum of a second IgD myeloma exhibited a very marked variation by the presence of a large positive maximum at 235nm. This finding confirms the

report of Johnson et al. (1975) and means that of four proteins reported two of each kind have been identified. Through the study of enzyme digestion fragments we have localised the structural feature responsible for this absorption maximum to the Fabδ region of the molecule. Although it would appear that the structural feature responsible for this absorption represents two isotypic forms of the IgD molecule parallel studies have not revealed differences in isotypic antigenicity.

Johnson et al., Febs Letters (1975) 49, 310

Early protection against invasive infection

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Burned mice infected with invasive bacteria (*Proteus mirabilis* and *Pseudomonas aeruginosa*) 2 hours after burning die within 3-6 days after infection. Burned infected mice may be protected against death by a single injection of antigen at the time of burning. These antigens can be made from the cell walls and culture filtrates of bacteria and from bacteria degraded by phagocytes.

From 24 hours after injection of the protective antigens changes in the phagocytic properties of the leucocytes were detected and there was an increase in the number of plaque forming cells secreting specific antibody in the peripheral blood. Raised antibody titres detectable by a passive haemagglutination test were found in the serum of burned immunised mice from 2 days onwards.

Comparison of the cytophilic activities of guinea pig IgG1 and IgG2 antibodies

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Monomeric guinea pig IgG1 binds to homologous peritoneal macrophages, though less effectively than IgG2. It binds to the cells with a lower association constant (Ka = $0.61 \pm 0.22 \times 10^6$ L/M at 20°C) than IgG2 (Ka = $1.44 \pm 0.16 \times 10^6$ L/M), and the number of IgG1 receptor sites per cell ($1.3 \pm 0.18 \times 10^6$) is about one half that for IgG2 ($2.65 \pm 0.45 \times 10^6$). Enhanced IgG1 binding is observed on immune complex formation, though the increase in avidity of the complexes is dependent on their size. Marked enhancement of avidity with increase in complex size occurs only with small complexes (containing less than seven antibody molecules): with larger complexes a plateau in avidity appears to be reached. Immune complex binding is inhibited by monomeric IgG1 indicating the absence of functionally important conformational changes on complex formation. Comparative studies with IgG1 and IgG2 demonstrate that IgG1 is bound to the same cell type as IgG2 (primarily, the macrophage) by a non-identical, though related, receptor.

Eosinophils in the thymus of the equine foetus

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The developmental sequence of the thymus was investigated in the foetal horse. Lymphoid cells appear in the epithelial analage between eight and nine weeks gestation and at this time large numbers of eosinophil leucocytes are also present in this organ. All stages of maturation of the latter cell type are seen, and a possible migration pathway of eosinophils from the outer areas of the thymus to the centre of the organ is proposed. It is likely that these cells are functioning at this tissue site at this time. No accumulations of eosinophils are seen in anywhere else in the foetus at this time of gestation. It is suggested that these findings reflect the intimate association of eosinophils and thymus dependent cells shown by other workers.

DNA synthesis and cell proliferation in the lymph nodes of rats undergoing local graft versus host and host versus graft reactions

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The relationship between DNA synthesis (^{125}Iododeoxyuridine incorporation) and cell proliferation (lymph node weight) was investigated at different times during the course of local HVG and GVH responses in Lewis and Lewis x Brown Norway (LxBN) rats following the injection of varying doses of LxBN and Lewis cells, respectively. Changes in the cellular kinetics during these responses were evaluated by statistical comparison of the slopes of the dose-response regression lines obtained at different times during the course of a response.

The correlation between IUDR incorporation and lymph node weight was linear for HVG and early (pre-peak) GVH responses but exponential for later (peak and post-peak) GVH responses. These changes were accompanied by alterations in the slopes of the dose-response regression lines.

Correlations between lymph node weight and IUDR incorporation in HVG and GVH responses reflect relative differences and alterations in the proliferation of host and donor cells which occur during these reactions.

The effect of transplanting whole bursae into agammaglobulinaemic bursectomised chickens

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A technique for the reconstitution of surgically bursectomised

chickens has been investigated. The mechanism by which the bursa of Fabricius influences the ontogeny of immunoglobulin producing cells is not fully understood. A possible approach to this problem is to study the behaviour of the cells of a donor bursa after transplantation into a B-cell deficient recipient bird. Current evidence suggests that both the lymphoid and epithelial cells are required for bursal function. In this study an attempt has been made to use intact bursae to reconstitute surgically bursectomised birds.

Recipient birds were bursectomised on day one after hatch followed by whole body X-irradiation on day two. Serum samples from the birds were tested for immunoglobulin and antibody levels, before transplantation and at intervals afterwards. At post-mortem the lymphoid tissues and transplant sites were examined histologically. The transplant operation took place when the recipient birds were between four and six weeks of age, each bird received a bursa from a 19 day embryo from chickens of the same inbred line (B2/B2). The whole bursa was placed under the membrane surrounding the anterior end of the kidney.

The majority of bursae were retained and there was evidence of some improvement of the antibody responses of the recipient to antigenic challenge. The significance of this technique will be discussed in relation to other reconstitution attempts.

Differences in the effect of cortisone acetate on the K cells of mice and rats

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Although both the mouse and rat are regarded as 'steroid sensitive' species, a single large dose of cortisone acetate affects their antibody-dependent cytotoxic effector cell populations differently, and the differences are most striking in populations depleted of phagocytic cells. When given to mice, cortisone acetate reduces not only the size of the spleen, but also the cytotoxic capacity of the remaining cells. When given to rats, the drug causes lymphoid depletion, but lymphocytes from spleen and peripheral blood are nearly twice as active per remaining cell so that the total cytotoxic capacity per organ is unaltered.

These results are discussed in terms of the light they throw on the different effector cell populations involved in the two species.

Correlation of clinical findings with neoplastic lymphocyte surface receptors in chronic malignant peripheral lymphocytosis

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Peripheral blood lymphocytes from fifty cases of C.L.L. and lymphoma with overspill have been studied for the presence of surface and intracellular immunoglobulin (Ig) and Fcγ and C3 receptors. The cases can be subdivided on the presence or absence of surface Ig and surface Ig class. More than 80% of surface Ig positive cases expressed k light chain and a small group of surface Ig negative cases had intracellular Ig. The groups have been correlated with clinical findings, serum Ig levels and incidence of Bence Jones protein. Preliminary findings suggest that more than 70% of the surface Ig negative group had lymphadenopathy, needed treatment or had lymphocyte doubling times of less than one year compared with 40% of the rest. These data suggest that surface Ig expression may be an important criteria in the prognosis of C.L.L.