TISSUE TRANSPLANTATION: CIRCULATING ANTIBODY IN THE HOMOTRANSPLANTATION OF KIDNEY AND SKIN

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by

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SINCE THE WORK of Carrel and Guthrie (1905) on vascular anastomosis made the first renal transplants technically successful, nearly half a century elapsed until Simonsen (1953) and Dempster (1953) independently undertook investigations into the mechanism of rejection of a transplanted kidney.

Medawar (1944) had recently demonstrated that the rejection of tissue homotransplants was immunological and, as a result of his work and that of many subsequent researchers, it is widely believed that this immunological response is due to cell-bound antibody and that circulating antibody is not responsible for the rejection process.

This concept has been based partly on the histological changes in a rejecting transplant and partly upon the findings of numerous workers in the search for humoral antibody formed in response to transplants of tissue.

The results of this search may be summarized by saying that although haemagglutinating antibodies have been demonstrated to follow homografts of both tumours and normal tissue, including skin and kidney (Lumsden, 1937, 1938; Gorer, 1937, 1938, 1956; Mitchison and Dube, 1955; Kaliss and Jay, 1950; Amos *et al.*, 1954; Simonsen, 1953; Muirhead and Groves, 1955; Curtiss and Herndon, 1955; Zotikov, 1958; Rovnova, 1961), the weight of evidence has been against haemagglutinins alone being responsible for the rejection process (Medawar, 1959; Hildemann and Medawar, 1959; Schneweis and Knake, 1958). However, the work of Barrett (1951, 1953, 1958) with tumours and Breyere (1959) with skin grafts suggested that the haemagglutinins produced by immunization with erythrocyte antigen alone may accelerate the rejection process of a subsequent transplant.

There have been numerous attempts to demonstrate an antibody other than haemagglutinin formed in response to normal tissue homotransplants. Using *in-vitro* tests, including cytotoxic, precipitin and complement fixation, negative results were reported by Medawar (1948), Allgöwer and his co-workers (1952, 1957), Harris (1948), Simonsen (1953) and Murray

and his team (1953). Positive results in the form of leucocyte agglutinins were found by Amos in response to tumour transplants (1953) and skin grafts (1954), and by Terasaki to skin grafts and spleen cells (1959). Bollag (1956) claimed to have demonstrated individual specific precipitins, by zonal turbidity reactions, but Merrill (1959, 1961) was unable to confirm these results.

In-vivo testing has included attempts at passive transfer of homotransplant immunity with serum or plasma from recipients of normal tissue. While failure was reported by Billingham, Brent and Medawar using skin grafts (1954), Dempster (1953) and also by Egdahl and Hume (1955) using kidney homotransplants, success was claimed by Stetson and his co-workers in mice and rabbits using serum from animals immunized with

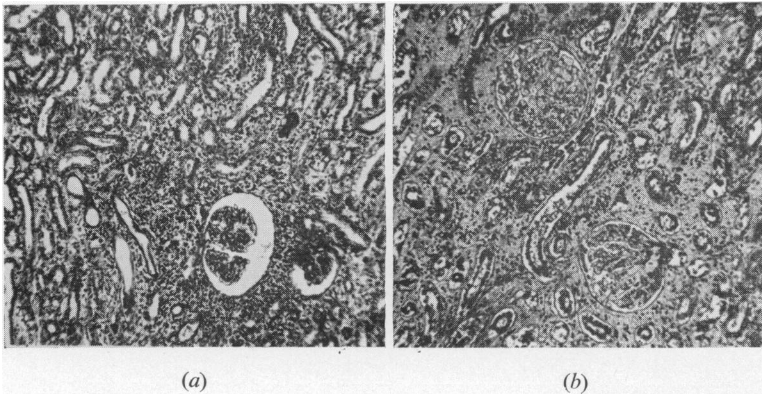


Fig. 1. (*a*) First-set rejection, six days. Dense mononuclear cell infiltration, tubular necrosis and interstitial oedema. (*b*) Second-set rejection, 48 hours. Gross interstitial haemorrhage, vascular stasis and tubular necrosis. Cellular infiltrate is polymorphic.

spleen cells, the serum being tested against donor or donor strain skin grafts (1958, 1960). Only recently has Steinmuller demonstrated passive transfer of immunity with serum in rats after a single skin homograft (1961), but this was strain dependent and time sensitive (1962). Terasaki and his colleagues have also recently produced specific damage to donor kidney with immune sera in mice (1962).

While successful passive transfer of homotransplant immunity has been rare using cell-free serum or plasma, it has been demonstrated frequently with cell suspensions of spleen or regional lymph nodes.

It has therefore been concluded that homotransplant immunity is of the delayed hypersensitive type akin to the tuberculin reaction rather than an immediate type of response. This theory is supported by the histological

evidence of mononuclear cell infiltration in an organ which is undergoing the rejection process after homotransplantation. This infiltrate includes cells which, it is postulated, are responsible for the process either by producing or carrying antibody to the transplanted foreign tissue.

The enigma of the " second-set kidney "

While this last postulate may well apply to a primary kidney homotransplant this is not the case with a second-set kidney, that is, a kidney transplanted after another kidney or some other tissue from the same donor.

It has been recognized for a number of years that a second-set kidney is rejected in an accelerated fashion with a pattern of rejection different from that of a primary transplant. The gross and histological changes in the canine kidney after homotransplantation are well established, having been recorded by Simonsen (1949, 1953), Dempster (1953) and Egdahl and Hume (1956).

A primary renal homotransplant survives on average 5–7 days, the kidney swells and urine secretion ceases at some stage in the rejection process. Histologically (Fig. 1), there is a mononuclear cell infiltration commencing around the small vessels and glomeruli which gradually spreads through the cortex. This contains a large proportion of pyroninophilic cells including plasma cells in varying stages of maturation, large mononuclears with pale staining nuclei and numerous lymphocytes. Typically there is not much evidence of cellular infiltration until the second or third day. Later there develop thickening of the endothelium of the small blood vessels and oedema, later still small thrombi, tubular necrosis, focal areas of interstitial oedema and haemorrhage.

The second-set response is quite different. Here the whole process from transplantation to cessation of function may be complete in 12–48 hours, during which time the kidney swells by up to 300 per cent. and appears grossly congested. The main features histologically (Fig. 1*b*) are interstitial oedema and haemorrhage, tubular necrosis with cast formation but relatively intact glomeruli. The latter show dilatation of the tuft capillaries with blood but little other damage. Blood vessels throughout the kidney are dilated and full of blood. Some are thrombosed. Cellular infiltration is not a feature of the process but when present is polymorphic rather than mononuclear.

Now the speed and histological appearances of the second-set rejection process suggested that mononuclear cells are not responsible for this process which occurs in their absence. If so, does this imply a different mechanism from the primary rejection process, such as a non-cell-bound circulating antibody, and what connection is there with the haemagglutinin demonstrated to follow the transplantation of organs in the dog? Further, will haemagglutinin alone cause accelerated rejection of transplanted tissue?

The investigations about to be described are concerned with attempts to answer these questions.

Canine blood groups

Fortunately for the purposes of these experiments, canine blood groups differ from those in the human because dogs lack naturally occurring iso-antibodies, making it simple to immunize a dog against a specific erythrocyte antigen.

Currently there are five canine erythrocyte antigens recognized (Fig. 2), bearing no relation to similarly named human antigens. " A " is a strong antigen occurring in 63 per cent. of all dogs, leaving 37 per cent. A negative. A_1 cells, which haemolyse in the presence of anti-A, occur in 44.6 per cent. of all dogs. A_2 cells, which agglutinate, occur in the other 19 per cent.

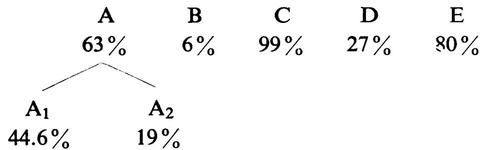


Fig. 2. Distribution of canine blood groups in random mongrel dogs.

The latter can be subdivided into strong A_2 which give direct agglutination and weak A_2 which require the presence of antiglobulin. " C " is the only other strong antigen and occurs in 99 per cent. of dogs.

Only A and C will produce an antibody titre consistently; thus, if no C negative dogs are used then only anti-A will be produced by immunization.

INVESTIGATIONS

Experimental designs

I. To attempt the demonstration of circulating antibody by passive transfer of immunity with plasma from animals hyperimmunized by transplantation of normal tissue (Fig. 3).

In each of these experiments an A negative dog received multiple successive skin grafts from the same A positive donor. After accelerated rejection had been observed and time allowed to elapse for possible antibody formation a kidney was transplanted from the same donor. When it too had been rejected and time allowed for antibody to form the recipient was exsanguinated. The donor's second kidney was then transplanted to a second unimmunized A negative dog and then perfused with plasma from the first recipient. Serial biopsies were taken from the kidney until rejection.

II. To observe the effect of haemagglutinin upon skin homografts the following methods were used:

1. An A negative dog was immunized to a high titre of anti-A by repeated injections of A positive blood. It was then grafted with skin from A positive and A negative dogs (Fig. 4*a*).

2. An unimmunized dog received A positive and A negative skin grafts, the graft beds having been injected with serum containing a high titre of anti-A (Fig. 4*b*).

III. To observe the effect of haemagglutinin upon kidney homotransplants by the following methods:

1. An A positive kidney was transplanted to an unimmunized dog and then perfused with plasma containing a high titre of anti-A.

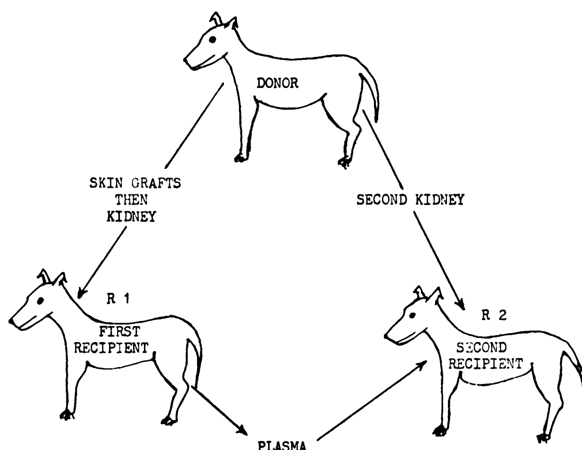


Fig. 3. Experimental design for attempted passive transfer of homotransplant immunity.

2. A positive and A negative kidneys were transplanted simultaneously to a dog which had been immunized to a high titre of anti-A with whole blood from an indifferent A positive dog. The kidneys were observed serially for gross and microscopical changes.

3. An A positive kidney was transplanted to a dog which had been specifically immunized with blood from the same donor.

4. The formation of haemagglutinin was measured by titration at each stage of the passive transfer experiments.

Methods and materials

Mongrel dogs of both sexes were used. They weighed 6 to 16 Kg. for the skin grafting experiment and 14 to 30 Kg. for the renal transplant experiments.

209 dogs were blood typed using the tube method. Of these 66 were suitable for use, all dogs being C positive and being selected for group A according to the requirements of the experiment.

Anti-A serum was prepared by repeated intravenous injection of A_1 blood into A negative dogs. Anti-canine-globulin serum was prepared in rabbits by repeated injections of canine serum. All sera were checked for specificity.

Anaesthesia was with intravenous sodium pentobarbitone, except for very short procedures such as biopsies when sodium thiopentone was used.

Full sterile precautions were taken in all operative procedures.

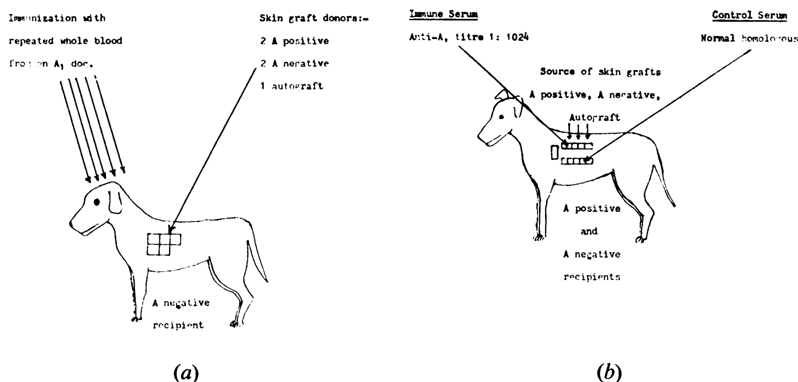


Fig. 4. Experimental design for investigating the effect of haemagglutinin upon skin grafts. (*a*) Grafting after immunization with whole blood to a high titre of anti-A. (*b*) Local injection of serum into graft beds.

Kidney transplants

Kidneys were removed from the donors through an upper midline abdominal incision and placed in the neck of the recipient, a pocket for the kidney being made in the pre-tracheal plane. The renal artery was anastomosed to the carotid artery and the renal vein to the external jugular vein. The ureter was cannulated with a polyethylene tube fixed with two encircling sutures and brought out as a cutaneous ureterostomy. Urine was collected in a plastic bag in the early experiment, but this was later abandoned.

Skin grafts

The lateral chest wall was used for donor and recipient sites. Full thickness grafts were fitted and fixed with a pressure dressing.

Plasma collection and preparation

Under general anaesthesia a cannula was placed in the iliac artery and a sample taken for haemagglutinin titration. The animal was then exsan-

guinated into bottles containing 3,000 units of heparin for each 500 ml. of blood. The heparinized blood was centrifuged at 3,000 r.p.m. for 20 minutes at 0° C. and the plasma pipetted off. Plasma storage was at 4° C. except in the case of the samples for titration and that used in experiments X and Y when storage was at –20° C.

Test aliquots were virtually cell-free, cell counts on four samples for instance revealing only one cell.

Haemagglutinin titration

The tube method was used, serial twofold dilutions of serum in saline being tested against A_1 cells, usually from the donor but sometimes from

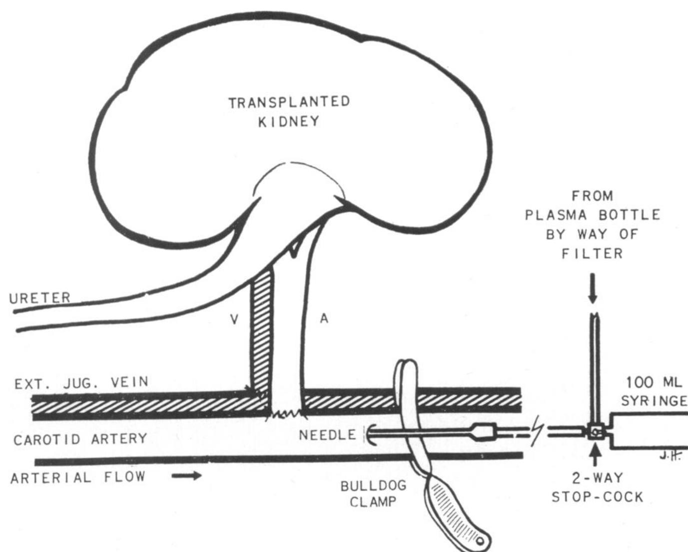


Fig. 5. Perfusion technique. Outflow of blood from the kidney is into the general circulation by way of the renal vein.

an indifferent dog. After incubation at 37° C. for 15 minutes the tubes were centrifuged at 800 r.p.m. for one minute and read for haemolysis and agglutination. In all negative tubes the cells were washed three times with saline, antiglobulin serum was added and after incubating for 15 minutes at room temperature they were read for agglutination.

Perfusion technique (Fig. 5)

The plasma was used immediately or within 24 hours of collection. The kidney was observed for at least 30 minutes to ensure that vascularization and urine secretion were satisfactory and for the kidney to be washed free of donor blood. A bulldog clamp was then placed on the carotid artery distal to the anastomosis so that the kidney continued to receive a

full arterial supply without interruption. The bottle of plasma was suspended above the animal and connected by way of an intravenous administration set with a filter to a 100 ml. syringe with a two-way stop-cock. From here a length of polyethylene tubing led to a gauge 20 or 18 hollow needle which was introduced into the carotid artery proximal to the bulldog clamp. A steady flow of plasma was maintained at a pressure just sufficient to overcome the systolic. At no time was there cessation of pulsation in the renal artery or its branches. The average rate of plasma flow was 15–20 ml./minute.

During perfusion note was made of the colour, size and tension of the kidney, relative rates of flow in the transplant vessels, rate of urine secretion and general systemic changes in the recipient. Some cases showing gross changes were photographed. At the end of the perfusion the bulldog clamp and needle were removed and gelfoam and pressure applied to the arterial puncture. Protamine sulphate was given intravenously.

Biopsies

Kidney biopsies were taken before transplantation, about 30 minutes after ending the perfusion and additionally when gross change was apparent, then each day until rejection or removal of the kidney. They were all open biopsies allowing examination of the kidney and anastomoses. The specimen was divided into two parts, one was fixed in 10 per cent. formalin in normal saline and the other in 90 per cent. methyl alcohol.

Skin biopsies were taken on the 7th day from the centre of the graft to include graft bed whenever possible. At this time a primary graft should be in good condition, not being rejected until the 10th or 12th day, while a second-set graft should be completely rejected.

Sections were cut at a thickness of 5 μ. Those from the formalin fixed tissue were stained with haemotoxylin and eosin or P.A.S.; those from the alcohol fixed tissues were stained with methyl green pyronin.

Clinical assessment of skin grafts

This was on a basis of general appearance, suppleness, degree of oedema and thickening, vascularization and desquamation. Scoring was on an 0 to 4+ basis by comparison with the autograft. 0 indicated a scab or raw wound and 4+ a condition as good as the autograft. Clinical assessments were made blind by Mr. John Anderson, F.R.C.S., and Dr. Joseph Murray, to whom I am indebted. Histological assessment of the skin grafting experiments was made by Dr. Laurence Henry, who examined the sections unaware of origin or treatment received.

Results

1. Attempted passive transfer of homotransplant immunity with plasma

Of seven experiments, six were completed and four showed changes in the perfused kidney unlike the appearances in the control group (Table I).

I will discuss first the experiments which did not show accelerated rejection after perfusion.

TABLE I

PASSIVE TRANSFER EXPERIMENTS. ALL DONORS WERE A + AND ALL RECIPIENTS A − EXCEPT IN EXPERIMENT R WHERE ALL DOGS WERE A −

	First recipient (R 1)					*Second recipient (R 2)*	
Expt.	*Skin grafts Placed on*	*Skin grafts Rejected at*	*Kidney transplant and response*	*Exsanguinated after transplant at*	*Perfusion*	*Kidney transplant and response*	*Result*
A	Day 1 „ 8 „ 11	15 days 10 „ 7 „	Day 18: 48 hours removed cellular response	2 days	Died during attempted exchange transfusion		
B	Day 1 „ 27	13 days 7 „	Day 49: 24 hours haemorrhagic 48 hours removed	6 days	Whole blood 1 litre	Shut-down after 300 ml. at 20 mins. Removed at 1½ hours	Positive
C	Day 1 „ 20 „ 51	14 days 7 „ 7 „	Day 59: 24 hours haemorrhagic 48 hours removed	7 days	Plasma 720 ml.	Removed at 4 days cellular, little haemorrhage and tubular necrosis	Negative
F	Day 1 „ 14 „ 41	9 days 5 „ 4 „	Day 48: Shut-down in 20 mins. 24 hours haemorrhagic 48 hours removed	7 days	Plasma 550 ml.	Haemorrhagic at 24 hours. Removed at 48 hours	Positive
L	Day 1 „ 21 „ 32	10 days 5 „ 5 „	Day 42: 24 hours haemorrhagic 48 hours removed	7 days	Plasma 550 ml.	Shut-down after 50 ml. in 5 mins. Haemorrhagic removed at 48 hours	Positive
O	Day 1 „ 15 „ 25	11 days 5 „ 6 „	Day 35: 4 days removed as first-set rejection	7 days	Plasma 530 ml.	15 mins. diuresis then anuria. Dead at 24 hours. Haemorrhagic	Positive
R	Day 1	11 days	Day 16: 24 hours haemorrhagic removed	9 days	Plasma 450 ml.	3 mins. shut-down then functioned 4 days. Removed as first-set	Negative

Experiment A was a trial of method and although not completed is of interest because even after three skin grafts the kidney showed only a mononuclear response at 48 hours. While this experiment was really abortive both from the viewpoint of timing and the attempted exchange transfusion, which killed the second recipient, it is of interest in that the first recipient was pregnant, which may have had some bearing on the slow reaction to the kidney despite previous immunization with skin grafts.

Experiment C. After three skin grafts, the kidney was rejected as a haemorrhagic second-set. The plasma produced no discernible effect during the period of perfusion and the kidney continued to function for four days. Microscopy showed a little interstitial oedema and haemorrhage at 24 hours with tubular damage indicated by flattened epithelium and protein casts. These changes did not progress, but a perivascular and periglomerular mononuclear infiltration which commenced on the second day increased in density. This was classified as a primary response.

Experiment R. In this, which was the last experiment of the series, all the dogs were A negative and only one skin graft was used to immunize against the first kidney, which was rejected as a haemorrhagic second-set, but with a large number of mononuclear cells in the infiltrate. The second kidney underwent transient vasospasm after 150 ml. of plasma but recovered and continued to function for four days. Grossly and microscopically it appeared to be a first-set rejection.

The other four experiments produced a positive effect together with a phenomenon which requires further explanation. This occurred first in **Experiment B,** which was a pilot experiment using whole blood for the perfusion since it was thought that if there was no effect with this there was little point in trying plasma alone.

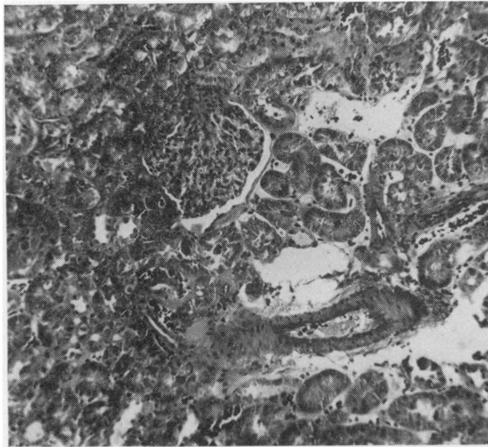
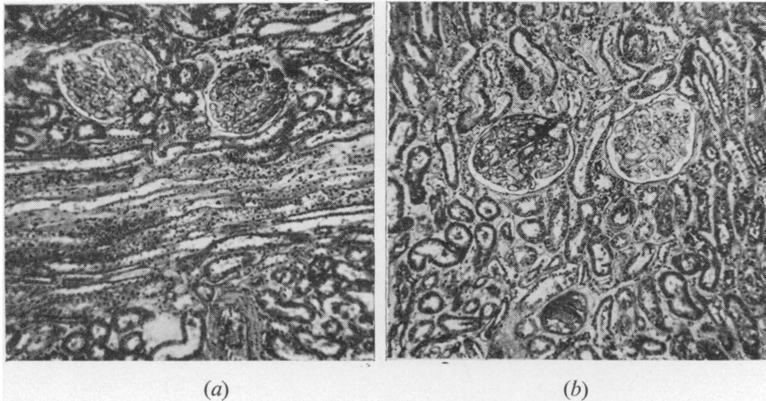


Fig. 6. Experiment B. Second kidney transplant 30 minutes after perfusion with "immune" blood. Increased density of glomeruli and swelling of tubular epithelium.

The first kidney was rejected as a haemorrhagic second-set in 48 hours. The second kidney started secreting almost immediately after 33 minutes' ischaemia. After 300 ml. of blood had been perfused urine secretion stopped, the kidney became flaccid, losing its normal tone, became pale and then cyanosed. These changes progressed so that after 10 minutes the kidney was dark blue in colour, shrunken and quite soft. There continued to be good pulsations in the renal artery and its branches and a continued though diminished flow in the renal vein. A biopsy was taken after 30 minutes while the kidney was still in this state. As there was no improvement in the appearance of the kidney and no return of function it was removed one-and-a-half hours after perfusion commenced and a further biopsy taken. Microscopy showed that the kidney had developed interstitial oedema and tubular damage seen as swelling of the tubular

epithelium and some protein casts. The glomeruli showed an apparent increase in density and there was disruption of endothelium in some of the small arterioles. A few of the cortical vessels contained small thrombi. There was some thickening of basement membrane (Fig. 6). The final biopsy showed similar but more marked changes.

Experiment F again showed this " shut-down ", as I shall now refer to it, but this time in the first kidney, that is the one in the first recipient after three skin grafts. The kidney had been ischaemic for 35 minutes but then became well vascularized and functioned well. After 20 minutes the kidney was shrunken and blue and had stopped secreting, but there was still good blood flow in the renal artery and vein. A biopsy was taken and



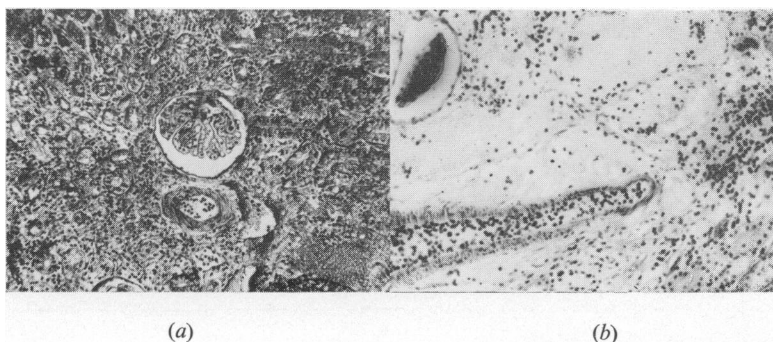
(*a*) (*b*)

Fig. 7. Experiment F. (*a*) First kidney transplant at 20 minutes. Vascular stasis including glomerular tufts, early interstitial oedema and minimal haemorrhage. Commencing tubular damage. (*b*) First kidney at four hours. Interstitial haemorrhage and oedema much increased. Glomerular capillaries dilated, vascular endothelium stripped, tubules dilated with damaged epithelium. Polymorphic infiltration commencing.

the kidney observed for a further four hours. It became darker in colour, remaining shrunken and anuric. It was decided not to remove this kidney but to observe what further changes might occur. A further biopsy was taken and the wound closed. Next morning there were a few drops of heavily blood-stained urine in the collecting bag, the kidney was twice its original size and blue-black. The vessels remained patent with good flow. The kidney was removed with the gross appearance of a haemorrhagic second-set.

Microscopy showed (Fig. 7*a*) at 20 minutes, vascular stasis in vessels of all sizes including the glomerular tufts, very early interstitial oedema and haemorrhage and commencing tubular necrosis seen as loss of brush borders, fragmentation of nuclei and vacuolation. The four-hour biopsy (Fig. 7*b*) showed great increase in the interstitial haemorrhage and there

were now polymorphs infiltrating. Many of the vessels showed endothelial stripping, in the arterioles more than the venules. The glomeruli remained the same but there were more tubules affected than in the earlier sections. The 22-hour biopsy showed great interstitial haemorrhage and a marked increase in polymorphic infiltrate. The vessels had all lost their endothelium, some were choked with polymorphs and some showed polymorphs in their walls as evidence of arteritis. Capillaries in the cortex were distended with blood. There was almost total tubular necrosis, in the medulla as well as in the cortex, the cortico-medullary junction showing the least tubular damage. The glomeruli were mostly distended with blood but a few were shrivelled with protein in Bowman's space (Fig. 8*a* and *b*). The appearances were similar to those seen in previous second-set kidneys.



(*a*) (*b*)

Fig. 8. Experiment F. First kidney at 22 hours. (*a*) Gross interstitial haemorrhage, loss of endothelium in all vessels, widespread tubular necrosis and much increased polymorphic infiltrate. (*b*) Higher magnification to show polymorphic infiltration and loss of vascular endothelium.

550 ml. of plasma from this dog were perfused through the second transplanted kidney, which after 25 minutes' ischaemia, had secreted urine 15 minutes after removing the vascular clamps. The plasma perfusion caused no immediate change in appearance or function. A biopsy was taken and the kidney left *in situ* for a further 24 hours, when it was found to be twice its original size and congested with a dark blotchy appearance, but still a good flow in the vessels. At the end of 48 hours the kidney was removed as a haemorrhagic second-set being nearly three times its original size. The blood flow through the vessels was still good, the rate of outflow from the cut renal vein being 54 ml./minute. Microscopy showed that the kidney, which had a normal appearance before transplantation (Fig. 9*a*), suffered little from the perfusion alone, the immediate post-perfusion biopsy showing some interstitial oedema with a little debris in the tubular lumens, but otherwise no real change from the control section (Fig. 9*b*).

By 24 hours, however, there was gross interstitial haemorrhage with polymorphic infiltrate, all degrees of tubular necrosis from flattening of the epithelium to complete detachment of cells and loss of nuclei. The vessels showed arteritis but the glomeruli were little affected apart from distension with blood (Fig. 10*a*). By 48 hours the changes were more advanced with more widespread vascular necrosis and thrombosis. The picture was strictly comparable with that seen in the haemorrhagic second-set kidneys seen in other experiments (Fig. 10*b*).

Experiment L. The first kidney underwent second-set rejection. The second kidney was ischaemic for 25 minutes but became well vascularized and secreted within 15 minutes. After only 50 ml. of plasma had been perfused in five minutes the kidney shut down and remained in this state.

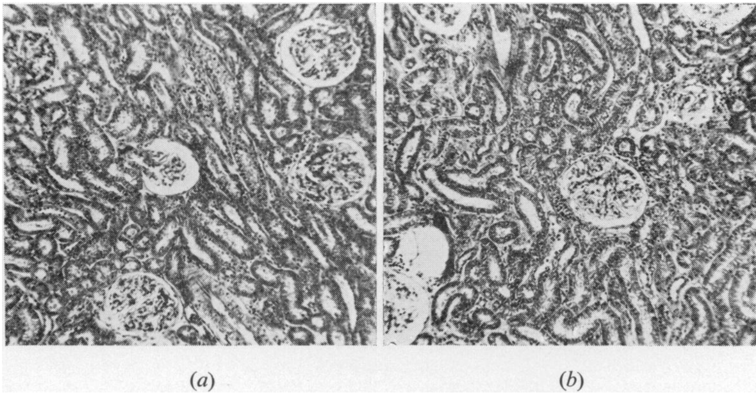


Fig. 9. Experiment F. (*a*) Second kidney before transplantation and perfusion. Normal appearance. (*b*) Second kidney one-and-a-half hours after plasma perfusion. Minimal interstitial oedema and tubular damage, scattered polymorphs, otherwise little change from control.

At 24 hours the kidney was twice its original size and congested but with good blood flow. By 48 hours there were only a few drops of heavily blood-stained urine, the kidney not having changed in size since the previous day. The kidney was removed still with a good blood flow.

The biopsy one hour after perfusion showed vascular damage with endothelial stripping and some thrombi. Tubular epithelium was swollen and glomeruli showed increased density. At 24 hours there was generalized interstitial oedema and patchy haemorrhage. Small vessels showed loss of endothelium and polymorphs in the walls. Some tubules showed complete necrosis while others showed relatively little damage. By 48 hours vessels were blocked with polymorphs and thrombi and the interstitial haemorrhage and tubular necrosis were much more widespread but the changes were not completely uniform.

Experiment O. The first kidney was still functioning at four days when it had to be removed due to damage to one of the two small arterial anastomoses during biopsy. The degree of mononuclear infiltrate at 24 hours was similar to that seen at the third or fourth day in a first-set kidney and progressed to become very dense indeed, but in addition there were widespread interstitial haemorrhage and tubular necrosis. The perfusion of the second kidney produced a diuresis for 15 minutes before secretion ceased, the kidney becoming blue and flaccid but not to the same degree as described earlier. Little urine was secreted during the night and next morning the dog was found dead, apparently from asphyxiation.

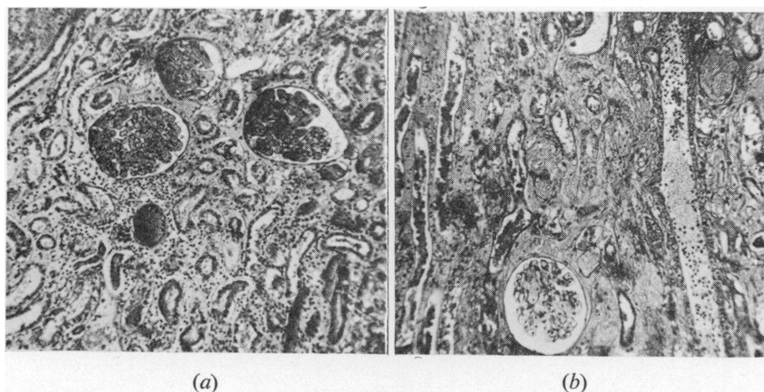


Fig. 10. Experiment F. (*a*) Second kidney 24 hours after perfusion with "immune" plasma. Gross interstitial haemorrhage, tubular necrosis, vascular stasis and polymorphic infiltration. (*b*) Second kidney 48 hours after perfusion with "immune" plasma. Changes as in Figure 10*a* but more advanced. Compare with second-set rejection Figure 1*b*.

Microscopy showed all the changes seen in the earlier sections in experiments F and L, vascular damage, interstitial haemorrhage and tubular necrosis.

Controls (Table II)

Eleven transplanted kidneys were perfused with homologous plasma. Using normal plasma six autotransplants and two homotransplants in bilaterally nephrectomized animals were perfused. Three homotransplants from A positive dogs were perfused with plasma containing a high titre of anti-A.

Of the six autotransplants, four had a profuse diuresis during the perfusion. There were three arterial thromboses at 24 hours and one haemorrhage from the arterial anastomosis. Two continued to secrete, being removed while functioning, one at six days and one at seven days, the latter despite a 10-minute period of vasospasm during perfusion.

Both of the homotransplants in bilaterally nephrectomized animals had a temporary vasospasm during the perfusion which reversed spontaneously. Both transplants continued to secrete well, one for six days and the other for four days when the dog died with a generalized illness of undetermined nature.

Of the three homotransplants of A positive kidneys perfused with anti-A plasma, two had a transient vasospasm during the perfusion. One secreted well for six days and one for three days when the kidney was removed. The third developed a venous thrombosis on the second day.

TABLE II

HOMOLOGOUS PLASMA PERFUSION OF A TRANSPLANTED KIDNEY. CONTROL EXPERIMENTS: SIX AUTOTRANSPLANTS, TWO HOMOTRANSPLANTS PERFUSED WITH NORMAL PLASMA, THREE A POSITIVE PERFUSED WITH ANTI-A PLASMA

Expt.	*Transplant*		*Plasma*		*Response*		
	Donor Blood Type	*Auto or Homo*	*Donor Type*	*Volume*	*Immediate*	*Late*	*Function*
K	A–	Auto	Normal A_2	550 ml.	Diuresis only	Arterial thrombosis	1 day
M	A–	Auto	Normal A_1 A_2	500 ml. 150 ml. 350 ml.	Diuresis only	Died of haemorrhage from anastomosis	—
N	A–	Auto	Normal A_1	500 ml.	Diuresis only	Arterial thrombosis	1 day
P	A_2	Auto	Normal A_2	440 ml.	Diuresis only	Little hydronephrosis	Good at 6 days
S	A_2	Auto	Normal A_2	400 ml.	None	Arterial thrombosis	1 day
U	A_2	Auto	Normal A_1 A_2	500 ml. 300 ml. 200 ml.	Shock with 10 mins. vasospasm	Normal looking, good secretion	Good at 7 days
X	A_2	Homo in A_2 bilat. nephrect.	Normal A–	500 ml.	Temporary vasospasm	Infection but still secreting	Good at 6 days
Y	A_2	Homo in A_2 bilat. nephrect.	Normal A–	500 ml.	Severe vasospasm after 10 mins. lasted 30 mins.	Ill but good secretion	Good at death on 4th day
Q	A_1	Homo in A–	Anti-A 1 : 512	225 ml.	Severe vasospasm after 5 mins. lasted 1 hour	Usual first-set reaction	Still good at 6 days
V	A_1	Homo in A–	Anti-A 1 : 1024	400 ml.	Initial diuresis, 10 mins. later vasospasm—30 mins.	Venous thrombosis	2 days
W	A_2	Homo in A–	Anti-A 1 : 512	400 ml.	None	Normal looking, good secretion	Good at 3 days

The immediate effect of the perfusion differed from the definitive experiments as follows: firstly, the profuse diuresis which was seen in six of the 11 control perfusions was seen only once in the definitive series, transiently in experiment O. Secondly, while a vasospastic response was produced in five of the controls this always reversed spontaneously, unlike the change in the experiments with immune plasma, when what appeared initially to be a similar change became permanent and never reversed but progressed to interstitial haemorrhage. Even when most severe in experiment Y of the control series the rejection pattern was that of a first-set kidney, the only effect of the vasospasm being seen as ischaemic change with patchy vascular damage only in the immediate post-perfusion biopsy. The haemorrhagic picture was never seen in the controls.

These experiments demonstrate that, apart from damage to the carotid artery which in three cases resulted in thrombosis, the perfusion with homologous plasma did not itself result in acceleration of rejection nor any obvious gross alteration of function.

The three A positive homotransplants perfused with anti-A plasma demonstrated that the haemagglutinin directed against the donor's erythrocyte antigen did not result in accelerated rejection, the microscopical pattern being that of a first-set rejection. Fig. 11 demonstrates this well, showing the appearance at 48 hours in experiment Q.

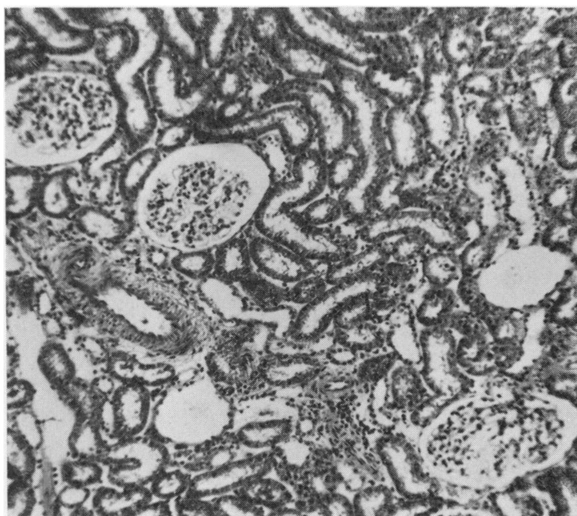


Fig. 11. Experiment Q. An A positive kidney homotransplant 48 hours after perfusion with plasma containing anti-A at a titre of 1 : 512. Commencing perivascular mononuclear infiltration, little oedema but no haemorrhage or vascular damage. Tubules and glomeruli little different from normal.

2. The role of haemagglutinin in skin graft rejection

In the first series of experiments, anti-A serum was injected into the beds of skin grafts from A positive and A negative donors.

In the first group, six dogs, three of each blood type, received two rows of grafts, each row containing one 2 × 2 cm. graft from each of the other five dogs. The first row in each had 1 ml. of 1 : 1024 anti-A injected into and around the bed of each graft. The second row, which acted as controls, received normal serum in four dogs and nothing in two dogs. A 2 × 4 cm. autograft was untreated.

The second group in this series, consisting of two A positive and one A negative recipient, received on the same plan 6 ml. into the bed of each

graft, there being in each row two A positive and two A negative homografts and an autograft.

The total number of grafts in this series was 96, comprising in the first group 30 injected with anti-A, 30 control homografts and six autografts, and in the second group 15 injected with anti-A and 15 controls, each including three autografts.

In the second series six experiments were performed in which grafts from A positive and A negative donors were placed on A negative dogs which had been stimulated to a high titre of anti-A with blood from indifferent A positive dogs. Each graft was 5 cm. square. The same three dogs were used twice (Table III), the first time having titres of anti-A of 1 : 512 in the first and third and 1 : 1024 in the second. Each dog received two A positive and two A negative homografts and an autograft.

TABLE III

SERIES II EXPERIMENTS. SKIN GRAFTING AFTER IMMUNIZATION WITH A POSITIVE BLOOD

Dog number	*Expt. number*	*First stimulation*		*Expt. number*	*Second stimulation*	
		Titre	*Grafts*		*Titre*	*Grafts*
1	SG 7	1 : 512	2 A positive 2 A negative 1 autograft	SG 8	1 : 1024	2 A positive 2 A negative 1 autograft
2	SG 9	1 : 1024	2 A positive 2 A negative 1 autograft	SG 11	1 : 512	2 A positive 2 A negative
3	SG 10	1 : 512	2 A positive 2 A negative 1 autograft	SG 12	1 : 1024	2 A positive 2 A negative

After the grafts had sloughed the dogs were restimulated with blood reaching titres of 1 : 1024 in the first and third and 1 : 512 in the second. They were then regrafted. Different donors were used for the second crop of grafts and at no time did any dog receive more than one graft from the same donor.

The total number of grafts in this series was 28, comprising 12 A positive and 12 A negative homografts and four autografts.

The total number of grafts in the combined series was thus 124.

RESULTS

In both series it became obvious that there was great difficulty in reconciling clinical and histological assessments.

In the first series the clinical assessment had, on the 0 to 4+ scale, shown that in the A positive recipients the A positive grafts which had received anti-A fared worse than the A positive controls and all the A negative grafts, suggesting that the anti-A had accelerated the rate of rejection of the A positive grafts.

But histologically the pattern was much different (Table IV). When each graft was compared with its own control it was seen that only six grafts showed an appearance worse than their controls. Of these six, four were due to ischaemia and only two were due to accelerated rejection. These were an A negative graft in an A positive dog (SG 1–4) and an A positive graft in an A negative dog (SG 4–3).

In the second group at the 6 ml. dose level the only three grafts in a more advanced state of rejection than their controls were A negative (Table V).

TABLE IV

HISTOLOGICAL ASSESSMENT OF TEST GRAFTS COMPARED WITH CONTROLS SERIES I, GROUP 1; BIOPSIES ON DAY 7

Blood type	*Dog number*	A+ *Graft number* 1	2	3	A– 4	5	6	*Positive results (with graft number)*
A+	SG 1	a	=	=	+	-	=	4 rejection; 5 ischaemia
	SG 2	=	a	=	=	+	=	5 ischaemia
	SG 3	=	+	a	=	=	=	2 ischaemia
A–	SG 4	=	=	+	a	=	+	3 rejection; 6 infection
	SG 5	=	+	=	=	a	=	2 ischaemia
	SG 6	=	=	=	=	=	a	

Key: a autograft
= no difference between graft and control
+ test graft worse than control
- control worse than test graft

Similarly in the second series (Table VI), although clinical assessment had suggested acceleration of rejection of A positive grafts in relation to the others this was not confirmed histologically. After the first stimulation with blood, biopsy on the seventh day showed three A positive grafts accelerated (SG 7–1, SG 8–1, SG 8–2), two being white grafts. A white graft is one in which the homograft reaction is so rapid that vascularization never occurs. There were also two A negative grafts showing accelerated rejection (SG 7–3, SG 8–5), one being a white graft. After the second stimulation two A positive (SG 11–2, SG 12–2) and one A negative (SG 11-4) were accelerated. Thus of 12 A positive grafts five were accelerated and of 11 surviving A negative grafts three were accelerated.

Taking the combined results of both series, six out of 33 treated A positive and seven out of 33 A negative grafts showed accelerated rejection, demonstrating that anti-A did not accelerate the rejection of A positive grafts specifically, whether present in the recipients' serum or injected locally.

3. The role of haemagglutinin in kidney transplant rejection

We have already seen from the experiments described under controls in Section 1, that haemagglutinin perfused through a transplanted A positive

kidney does not accelerate the rejection process and this is confirmed by the other experiments in this series.

Firstly, it was found that in a dog which had been immunized to a titre of anti-A of 1 : 2048 the rates of rejection were the same for A positive and A negative kidneys transplanted simultaneously. The serial gross and microscopical appearances in the two kidneys were almost identical over a period of four days when, still functioning, they were removed as first-set rejections.

TABLE V

HISTOLOGICAL ASSESSMENT OF TEST GRAFTS COMPARED WITH CONTROLS
SERIES I, GROUP 2

Blood type	*Dog number*	*Graft number*	A– 1	A+ 2	A– 3	A+ 4	A+ 5	*Positive results (with graft number)*
A–	SG 13		=	=	= a	=	=	
A+	SG 14		+	=	=	= a	=	1 white graft
A+	SG 15		+	=	+	=	= a	1 white graft 3 rejection

Key: a autograft
= no difference between graft and control
+ test graft worse than control
– control worse than test graft.

Secondly, in the passive transfer of immunity experiments in Section 1, the titre of anti-A was measured at each stage of the immunizing procedure (Table VII). Titres varied from nil to 1 : 64 but did not correlate with the quantity of tissue the dog had received nor with the fate of the kidney transplant. For example, while the immunized dog in experiment F had a titre of 1 : 8, only after the first skin graft, this had disappeared by the time of the kidney rejection, yet this kidney was rejected in an accelerated manner and the plasma certainly accelerated the rejection of the perfused kidney. In contrast to this the titre reached in experiment O was 1 : 16 when the kidney was rejected, yet the rejection was cellular.

In the experiment of specific immunization with blood the subsequent kidney transplanted from the same donor showed accelerated rejection which was largely cellular.

Taken together, all the evidence points to the haemagglutinin itself not being responsible for the rejection process of skin grafts or kidney transplants.

Conclusions

Perfusion with homologous plasma from one, or even pooled from two donors, does not have a permanent effect upon a transplanted kidney and

does not accelerate the rejection process. Even when a vasospastic response occurs the kidney recovers quickly and continues to function. This is in contrast to the effect of plasma from an animal hyperimmunized against the donor of a kidney homotransplant. This can produce severe and permanent damage and accelerated rejection of the haemorrhagic type similar to that seen in a second-set kidney transplant. In other

TABLE VI

HISTOLOGICAL ASSESSMENT OF GRAFTS PLACED ON ANIMALS PREVIOUSLY IMMUNIZED TO PRODUCE ANTI-A. SERIES II.

Dog number	*Titre of anti-A*	*Graft number*	*Donor type*	*Microscopical assessment*
		1	A+	White graft
		2	A+	Superficial infection
SG 7	1 : 512	3	A–	Rejecting, superficial inflammation
		4	A–	Good condition
		5	A–	Autograft, good condition
		1	A+	Accelerated rejection
		2	A+	White graft
SG 8	1 : 1024	3	A–	Superficial infection
		4	A–	Autograft, good condition
		5	A–	White graft
		1	A+	Good condition
		2	A+	Ischaemic
SG 9	1 : 1024	3	A–	Autograft, good condition
		4	A–	Early rejection
		5	A–	Early rejection
		1	A+	
		2	A+	
SG 10	1 : 512	3	A–	All in good condition
		4	A–	
		5	A–	
		1	A+	Good condition
		2	A+	White graft
SG 11	1 : 512	3	A–	Ischaemic
		4	A–	Completely necrotic
		1	A+	Good condition
		2	A+	Accelerated rejection
SG 12	1 : 1024	3	A–	Ischaemic
		4	A–	No biopsy, graft gone

words, passive transfer of homotransplant immunity *can* be demonstrated with plasma in the absence of immune cells. Further, this acceleration is not due to haemagglutinin directed against the donor's erythrocyte antigen.

Discussion

Interpretation of these results is difficult in the light of published data and theory.

It is thought likely that in the haemagglutinin experiments when rejection was accelerated this resulted from antigenic similarity between donors of skin and blood and also, perhaps, to sensitization with leucocytes in the blood used for immunization. This might suggest that transplantation immunity in the dog is not individual specific, as has been shown for the human by Friedman and his co-workers (1961).

The immediate vasospastic response is identical with that seen by Calne (1961). In his experiments this occurred twice in reciprocal second-set transplants between dogs and once in a goat-to-dog heterotransplant. He removed the kidney after about an hour as it appeared to be functionless and there was no reason to suspect that the lesion was reversible. This was also the case with the kidney perfused with whole blood in experiment B. It has been shown here by serial biopsies, however, that if a kidney which reacts in this manner is left *in situ* it will progress to a picture identical with that of a second-set response of the haemorrhagic type. Vascular stasis

TABLE VII

HAEMAGGLUTININ TITRES (RECIPROCALS) OF ANTI-A AFTER SUCCESSIVE GRAFTS

Expt.	*Control*	*Skin grafts*			*Kidney*
		1	2	3	
A	—	—	—	—	—
B	—	—	64		32
C	—	—	—	—	8
F	—	8	—	—	—
L	—	1	8	—	4
O	—	1	4	—	16
R	—	—	—	—	—

and oedema are seen as early as 20 minutes after the onset with marked interstitial haemorrhage by four hours. There is increasing polymorphic infiltration with tubular necrosis developing by 24 hours. The smaller vessels show endothelial damage and arteritis, but the larger vessels may appear intact.

Mowbray (1961) has seen a somewhat similar effect when perfusing kidneys *in vitro* with heterologous or pooled homologous plasma. This effect was venous spasm with congestion and gross swelling of the kidney occurring only if five or more homologous plasmas were pooled but never with plasma from a single donor. He is of the opinion that this is due to a naturally occurring antibody in the plasma which reacts with antigen in the kidney releasing 5-hydroxytryptamine. He found the reaction reversible only with serotonin antagonists. He does not explain why, if the effect is due to naturally occurring antibody, it requires the pooling of plasma from at least five donors.

The effect described by Mowbray may well be similar in action to the one seen in these experiments with plasma from the immune animal since the effect was not reversible. It might be implied from this that " tissue immune plasma " contains a factor which normal plasma does

not, and that since this produces permanent damage it is probably an antibody directed against the tissue antigen of the donor.

From these experiments it will be seen that four degrees of intensity of the homograft reaction can now be recognized in the homologous canine renal transplant, with some gradation from one to the next.

In ascending order of severity these are (Table VIII):

1. The mildest, and that generally recognized as the primary homograft reaction, is a mononuclear cell infiltration commencing around the small vessels and glomeruli on the second or third day with later vascular and tubular damage, the process taking on average 5–7 days.

TABLE VIII

GRADATION OF REJECTION PATTERNS OF A RENAL HOMOTRANSPLANT. INCREASING RAPIDITY OF REACTION WITH INCREASING IMMUNITY

Grade	*Rejection pattern*
1.	Primary response, mononuclear infiltration in 2–3 days.
2.	Rapid cellular response, mononuclear cells in 24 hours.
3.	Haemorrhagic second-set without mononuclear infiltration.
4.	Immediate vascular response.

2. An accelerated reaction of the cellular type which may occur after prior sensitization of the recipient with tissue or whole blood from the donor of the kidney. The kidney may develop anuria by the third or fourth day, the histological picture in less than 24 hours showing a degree of cellular infiltration comparable with that seen in a 3–4 day primary transplant.

3. A haemorrhagic second-set response whose main features are interstitial haemorrhage and oedema, tubular necrosis and infiltration with polymorphs rather than with mononuclear cells. The whole process from transplantation to cessation of function may take 12–48 hours, during which time the kidney swells and is grossly congested with blood, although the renal vessels themselves remain patent.

4. An immediate reaction, occurring in 5–20 minutes after transplantation, in which the kidney, which had been well vascularized initially and secreting urine, becomes over the course of a few minutes increasingly cyanosed, diminishes in size and ceases urine production. Throughout this period blood flow continues in the renal artery and vein. This reaction progresses gradually, through vascular stasis to interstitial oedema and haemorrhage, with tubular necrosis and polymorphic infiltration to the picture seen in type 3.

Serial biopsies have established that the first reaction in types 3 and 4 is a vascular one. The small vessels are damaged to a varying degree and may first undergo spasm proceeding to an arteritis or may progress more slowly to an arteritis without spasm. Thrombosis occurs in the

small vessels together with effusion of blood through the walls. Tubular necrosis results from ischaemia. When spasm does occur it would appear to be in the smaller vascular radicles, probably arteriolar rather than venous, since the kidney does not become congested immediately but rather decreases in size. Since blood flow continues there is probably a shunt mechanism in operation, this occurring most likely in the cortico-medullary region.

In searching for an explanation of the changes which take place in a homotransplanted kidney, it is natural, since the process is now undoubtedly accepted as being immunological, to make comparisons with other immunological reactions.

It has been generally accepted that the homograft reaction is similar to a delayed hypersensitivity reaction of the tuberculin type. This reaction is predominantly infiltrative, the cells being mononuclears which first appear around small blood vessels. This type of immunity can be transferred with cells but not with serum.

It is also fairly well accepted that the primary mechanism of the immediate hypersensitivity state is a vascular one and that other effects such as tissue necrosis follow upon this. It is thought that an antigen-antibody reaction occurring in the vicinity of the vessel wall can damage the endothelium, the endothelial cells perhaps taking up the molecular complexes so formed and being damaged in the process. This might act as a " trigger " mechanism for subsequent smooth muscle spasm in the wall of the vessel, although the mode of action is unexplained (McMaster, 1959). It appears to be the smaller arterioles and venules which are affected rather than capillaries, which may be secondarily dilated. The overall effect is of vascular stasis associated with the deposition of leucocytes and platelet thrombi, increase in vascular permeability with diapedesis of cells and oedema fluid, and later rupture of the vessel wall. Depending on the degree of damage will vary the degree of interstitial haemorrhage.

These changes result from a local excess of antigen in relation to antibody, in the " active Arthus phenomenon ", or a local excess of antibody over antigen in the " reversed passive Arthus phenomenon ". One of these factors is in the tissues and the other in the blood stream (Humphrey, 1959).

Typically the Arthus reaction is accompanied initially by a great polymorphic infiltration which over the course of a few days becomes replaced by mononuclear leucocytes, including lymphocytes, mature and immature plasma cells. The presence of circulating antibody is mandatory and serum is effective in passive transfer of this type of immunity.

The passive Arthus phenomenon shows maximal damage, macroscopically, in anything from 2–4 hours to 24 hours depending on species and intensity of the reaction. The delayed type of reaction, although showing a mononuclear infiltration predominantly, may also be observed at its maximum in 24 hours.

Gell and Hinde (1954) showed that the early stages of immunization give reactions of the delayed type, their progressive immunization reaction making this point more obvious. They demonstrated that with increasing antigenic stimulation the reaction on testing gradually changes its character from the delayed type, by way of an intense mononuclear infiltration, through intermediate stages to the fully developed Arthus reaction as the antibody titre rises.

Thus it has been suggested that the delayed response is merely a stage in antibody production and that if the stimulus is continued long enough an antibody titre will eventually result, producing an Arthus type of reaction when tested with antigen.

It is tempting to speculate on the similarity between the progressive immunization reaction and the four stages of the homograft reaction as described above (Table VIII). It may be that these responses represent stages in the production of antibody by the host, directed against donor tissue in a manner similar to the progressive Arthus reaction. That is, the first graft produces a gradual mononuclear response, the accelerated response of the cellular type perhaps indicating a low degree of antibody formation. The haemorrhagic second-set may be looked upon as the expression of a high titre of antibody and the immediate vascular response as the expression of the highest degree of immunity.

We must be careful, however, in attempting to interpret these phenomena in terms of accepted immunological terminology, since the reactions are not rigidly compartmented and there is frequently some overlap from one type to another. We must also remember that the histological sections we examine in the homograft reaction are not the response of host tissue as in most other types of immunological reaction, but are the results of antigen-antibody reactions taking place within the transplanted tissue itself, most of the antibody in a transplanted organ having reached the donor tissue by way of the blood vessels rather than by permeation.

In summary, it is suggested that the homograft reaction is not a delayed hypersensitive state alone, but that circulating antibody to normal tissue may play a part in the accelerated rejection process, and that the accelerated homograft reaction may be of the immediate hypersensitive type and akin to the Arthus phenomenon.

It can be seen that much ground has been covered since John Hunter, whose name and work we remember to-day, transplanted tissues and teeth experimentally and the latter also on a clinical basis.

For enabling me to apply John Hunter's dictum " try the experiment " and to have the honour of presenting the result to-day, I wish to make these acknowledgements.

It gives me pleasure to thank Dr. Joseph E. Murray for facilities, advice and encouragement, Dr. Scott N. Swisher of the University of Rochester for his very kind advice and assistance, and for the initial supply of blood typing sera; Miss Collette Hanau for her technical assistance particularly with antibody titrations; Miss Norma Trabold for her invaluable help with the canine blood grouping techniques; Dr. Gustave Dammin and the Department of Pathology of Harvard Medical School for preparing histological material, and in particular Dr. Laurence Henry for interpreting the skin graft histology; and the Wellcome Trust for a travel grant enabling me to go to the Harvard Surgical Research Laboratory, where most of this work was undertaken.

Finally, Mr. President, I would like to thank the Council of the College for the great honour they have accorded me in allowing me to present this lecture.

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ELECTION TO THE COUNCIL 1963

ON THURSDAY, 4TH JULY 1963, Mr. Harold Edwards was re-elected, and Mr. Norman Tanner, Professor A. L. d'Abreu, Mr. Norman Barrett and Mr. A. W. Badenoch were elected Members of the Council of the College.

The result of the Poll was as follows:

Elected	*Votes*
Norman Cecil TANNER (Charing Cross Hospital)	1,326
Alphonso Liguori d'ABREU, O.B.E. (United Birmingham Hospitals)	959
Harold Clifford EDWARDS, C.B.E. (King's College Hospital)	853
Norman Rupert BARRETT (St. Thomas's Hospital)	688
Alec William BADENOCH (St. Bartholomew's Hospital)	627
Not Elected	
Rodney SMITH (St. George's Hospital)	620
Richard Sampson HANDLEY, O.B.E. (Middlesex Hospital)	602
Richard Harrington FRANKLIN (Postgraduate Medical School of London)	580
W. Frank NICHOLSON, M.B.E. (Royal Infirmary, Manchester)	442
Harvey JACKSON (National Hospital and St. Thomas's Hospital)	428
Thomas Geraint Illtyd JAMES (Central Middlesex Hospital)	391
Richard Arthur MOGG, V.R.D. (Royal Infirmary, Cardiff)	367
George QVIST (Royal Free Hospital)	366
Ronald William RAVEN, O.B.E., T.D. (Royal Marsden Hospital)	332
David Napier MATTHEWS, O.B.E. (University College Hospital)	307
Clifford David Phillips JONES, M.B.E. (Sheffield Royal Infirmary)	307
Henry Reynolds THOMPSON (St. Mark's Hospital, London)	297
Bryan Nicholas BROOKE (St. George's Hospital)	293
Edward Clive Barber BUTLER (The London Hospital)	283
Guy BLACKBURN, M.B.E. (Guy's Hospital)	277
Ralph Owen LEE (Northampton General Hospital)	263
Frederick Thomas MOORE, O.B.E. (Queen Victoria Hospital, East Grinstead, and King's College Hospital)	195
Thomas NICOL (King's College, London)	141

In all 2,497 Fellows voted, and in addition 42 votes were found to be invalid.