RENAL HOMOGRAFT REJECTION

(with special reference to urinary enzymes and lymphocyturia)

by

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ABSTRACT

The phenomenon and mechanism of tissue and renal homograft rejection is reviewed.

Clinical manifestation of the rejection crisis in human renal homotransplantation is described, and problems of accurate diagnosis delineated. A study of daily stained urinary sediment on transplanted patients at the Royal Victoria Hospital showed significant lymphocyturia occurring in 5 out of 5 major rejection crises studied. It is usually associated with casts and haematuria.

The value of lymphocyturia, serum and urinary enzymes as aids in the diagnosis of rejection was compared in two groups of untreated dogs with renal autografts and homografts. The urinary lactic dehydrogenase and alkaline phosphatase were shown to be better and more specific indices of rejection than the serum lactic dehydrogenase or glutamic oxalecetic transaminase.

Lymphocyturia appears to be a reflection of the degree of lymphocytic infiltration in the transplant kidney.

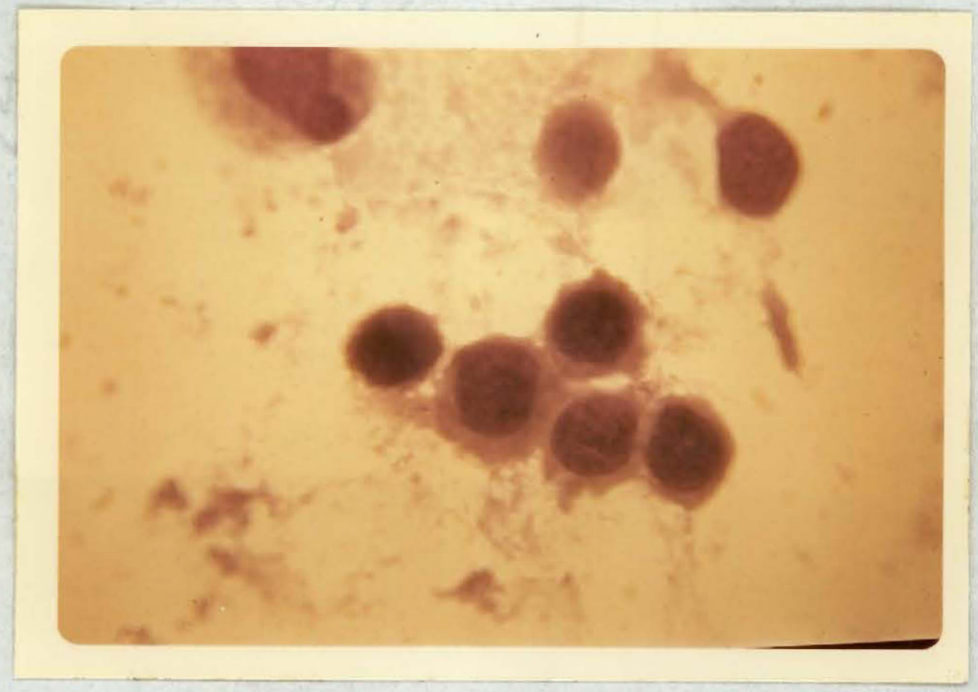
A unique instance of spontaneous remission of homograft rejection crisis with prolonged survival (122 days) in an untreated dog is described, and its implications discussed.

"..... Singular character of the individual entirely dissuades us from attempting this work (homotransplantation) in another person, for such is the force and power of individuality ....."

Tagliacozzi, 16th century.

"..... I am what I am
so don't try to change me
don't try to change me ....."

Pop song by Michael, son of
Charlie Chaplin, 20th century.



LYMPHOCYTES - "The enigma of homotransplantation,
The α and Ω of rejection."

.. as they appear in the urine ..

Dedicated to my wife

June

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PREFACE

In July of 1963, I came over to Montreal from London, England, as part of an exchange programme between the Royal Victoria Hospital and The London Hospital, and in the winter of the same year a programme of human renal homotransplantation, utilising cadaveric kidneys was begun in the hospital. I was immensely interested in this work, and when Dr. L. D. Maclean asked me to stay on another year to work on the problems of transplantation, I gladly accepted his offer.

While technical details of renal transplantation have been more or less well mastered and standardised, the problems of post-operative management, of diagnosis and treatment of rejection crisis, are still bedevilling; and mistakes have been made that proved to be costly and at times tragic. It is on the problem of accurate diagnosis of rejection that my research was centred; first in the detection of lymphocyturia in the transplanted patients, and later in collaboration with Dr. E. D. Monaghan, and Dr. H. M. Gault, we conducted an experimental study in dogs, comparing the usefulness of various aids in the diagnosis of rejection.

I wish to thank Dr. Dosseter for allowing me to work in the renal service and for much knowledge gained from many off-the-cuff discussions. I wish to

thank Dr. L. D. Maclean, under whose department and supervision this work was carried out.

C. G. Koo, June 1965.
Montreal, P.Q.

I.

TRANSPLANTATION - GENERAL CONSIDERATIONS

A. Early History

In the ancient writings of the Hindu Surgeon, Suschruta, is a description of a method of nasal reconstruction utilising a flap of skin that may well have been used in the 6th century B.C.

Celsus (25 B.C. - 60 A.D.) was said to have used autografts of skin.

In the legend of the Saints Cosmas and Damian, recorded by de Voragine in the Golden Legend in the 13th Century, it is written that they miraculously transplanted an Ethiopian's leg to a devoted member of the early church whose own leg was afflicted with a malignant growth. According to the tale, the transplant was a success.

Then, there was the legend of the "Sympathetic Slave". A man, Bianci of Sicily in the 15th Century, possessed of wonderful talent, found how to give a person a new nose which he built from the skin of the arm or borrowed from a slave. A mystical sympathy was alleged to exist between the donor and the graft. When the slave died the nose fell off. Samuel Butler in one of his comic writings was stimulated to write:

"But when the date of the nock was out
Off dropt the sympathetic Snout."

In the 16th century, Gaspare Tagliacozzi achieved remarkable success with pedicle flaps from the arm for rhinoplastics. Of homotransplantation, he wrote: "Singular character of the individual entirely dissuades us from attempting this work in another person, for such is the force and power of individuality." This was one of the earliest observations on the homograft rejection phenomenon.

John Hunter (1771) believed that transplanted tissue would live. He transplanted teeth. He also transplanted a human tooth to a cock's comb and a cock's testis to a hen, without altering the disposition of the hen!

In 1804, Baronio clearly demonstrated that experimental free skin autografts in sheep would survive.

Ullman and Alexis Carrel each reported experimental renal transplants in 1902. In the following decade Carrel and Guthrie demonstrated the feasibility and mastery of technical problems of whole organ transplantation by anastomosis of blood vessels. They also observed that autotransplants were sometimes successful for long periods of time, while homotransplants seldom functioned for more than a few days.

In 1923 Williamson attributed failure of homograft to biological incompatibility between the donor and the recipient: the causative agent being blood borne,

attacking first the glomeruli then the tubules. But the real explanation for this failure was to elude the scientists until over two decades later when Medawar (1944), in his monumental experimental study, provided the immunological basis for homograft rejection. He observed that a second graft of skin from one rabbit to another, after a first graft had been rejected, was now rejected in a more rapid and intense fashion. He concluded that the host had been immunised by the first graft, and that rejection of grafts was an immune response on the part of the host.

B. Terminology

The terms used to designate various types of grafts first proposed by Medawar (1944) have been widely used, although Gorer (1961) has pointed out etymological inconsistencies and proposed a new terminology. These and others are listed in Table I, along with their definitions.

Table I. Terminology of Transplantation

Old Terminology	New Terminology	Definition
Autograft	Autograft	Graft in which donor is also recipient.
Isograft	Isograft	Graft between individuals identical in histo-compatibility antigens.
Homograft	Allograft	Graft between genetically dissimilar members of same species.
Heterograft	Xenograft	Graft between species.
Allostatic grafts	same	Grafts that do not depend upon continued cellular multiplication to fulfil their desired clinical function.
Allovital grafts		Grafts whose cells must continue to grow and reproduce for the graft to be effective

C. The Nature of Homograft Rejection Reaction

Rejection has been defined as the immunological process, mediated by antibody bound and/or unbound to lymphocytis, leading to the dysfunction of the transplant.

This phenomenon was first observed in skin homotransplantation. A skin transplant from one rabbit to another heals in well and has the same appearance as a skin autograft. Capillaries from the host grow across the graft bed and link with those of the graft, and there is minimal inflammatory cells indicating that there is no pre-existing incompatibility of host and graft. Between the fourth and fifteenth day after grafting, biopsies of the graft show collections of lymphocytes, plasma cells, and perhaps cosinophils in the graft-host junction. This is shortly followed by slowing of circulation in some of the capillaries and formation of microthrombi (Tayler 1955). Suddenly, the circulation ceases completely, and the graft becomes black. The incompatibility develops after the graft has been performed. This is the first set reaction. A second skin graft from the same donor will be rejected much quicker, after only three to six days; in fact, the graft may not heal in at all, the white graft of the "second set" reaction.

The evidences for identifying this reaction as an immune response are as follows:

(i) The lag-period required for the development

of the first-set reaction.

(ii) The intensification of the reaction in hosts subjected to a prior exposure to donor tissue.

(iii) The relative specificity of the reaction for one donor over others.

(iv) The sensitive state could be transferred from one host to another by isolated lymph node cells (Mitchinson 1953), affirming the role of immunologically competent lymphocytes in the homograft reaction.

II.

RENAL HOMOGRAFT REJECTION

A. Observations in Animal and in Man

A primary kidney homograft suffers the same fate as a skin graft in an unmodified recipient animal. Initially the kidney is able to produce urine and function well. However, on the average, they cease to function after 4 to 8 days, become swollen, oedematous and haemorrhagic. Cellular infiltration is predominantly composed of lymphocytes and plasma cells. During rejection the animals are ill with fever, anorexia and loss of weight; they die shortly of toxaemia and uraemia.

The second set kidney likewise is rejected more rapidly ranging from a few hours to 4 days. Cellular infiltration is nil or minimal.

Data on renal homograft rejection in unmodified human recipients is understandably scanty, and most of the few that were done utilized cadaver kidneys. However, out of the nine cases done by Hume (1955), four did develop measurable fair function lasting 37 to 180 days. This is considerably longer than on the experimental animal and can be partly accounted for by the fact that human recipients are all terminal uraemics whose immunological responses must surely be attenuated.

B. Mechanism of Renal Transplant Rejection

How is the homograft rejected? What is the pathophysiology involved in this process?

The probable sequence of events in rejection is as follows: Homograft ——— antigen release ——— reticuloendothelial system of recipient ——— production of sensitized lymphocytes and their release into circulation ——— infiltration of homograft by lymphocytes ——— destruction of transplant organ.

IMMUNOLOGICAL PATHWAYS IN REJECTION

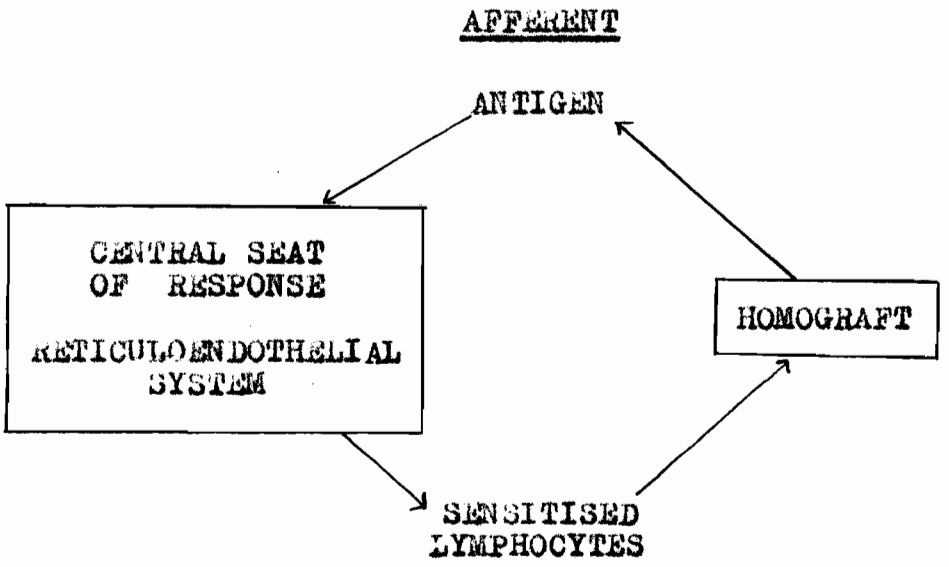

FIG. (1)

(i) Antigen Release. Upon the establishment of circulation between the host and the renal homograft,

antigens are released into the host, inducing sensitization. The degree and rapidity of this accomplishment has been elegantly demonstrated by Paul Nathan (1964), who perfused set homograft kidneys in the animal recipient for intervals of 3 minutes to 24 hours and then removed them. Seven days later, specific second-set kidneys were transferred to the hosts. The degree of host sensitization was assessed by the percentage of second-set rejections that followed. It was shown that when perfusion interval was between 3-6 hours, 40% of the hosts are sensitized; perfusion of 9-24 hours resulted in 75% sensitization. The antigen is transferred to the host lymphoid tissues either "free" or by antigen transporting cells (?macrophages).

The nature of the transplant antigen is still not well understood. It seems to be a lipoprotein, perhaps associated with cell membranes or the enveloping membrane of cell microsomes. The lipid portion of the antigen may have haptenic properties in the determination of specificity, but chemical analyses of the lipoproteins isolated so far has shown a wide variety of lipids in any given preparation (Newton 1965).

(ii) Production of sensitised lymphocytes. It has been shown by serial histological sections of host lymphoid tissues at various intervals following a pelvic renal

homotransplant, that by 48 hours there is slight increase in large lymphocytes in the splenic periarteriolar lymphoid tissue (Porter et al. 1964). By 72 hours, more large lymphocytes and early plasma cells appear in spleen. Over the next few days there is cortical hyperplasia of all lymph nodes, but especially the mesinteric and lumbar nodes.

It seems probable that new sensitized lymphocytes and plasma cells are produced following encounter with the transplant antigen. This is further supported by the fact that host lymphocytes labelled with tritiated thymidine (^{3}HT) before transplantation do not infiltrate the homograft whereas lymphocytes thus labelled in spleen immediately after transplantation do form part of the infiltrate.

(iii) Destruction of the graft. Most of the cells infiltrating the kidney are pyroninophilic and morphologically fell into three categories:

(a) large cells with central or eccentric nucleus, abundant cytoplasm and endoplasmic reticulum;

(b) large lymphocytes with abundant cytoplasm but no endoplasmic reticulum;

(c) small lymphocytes.

From 24 hours, small lymphocytes were found adhering

to endothelial cells of the peritubular capillaries. Later large cells appeared and followed by disruption of the capillary wall and extravasation of red cells and leucocytes into the interstitium. The proximal tubules undergo necrosis but only at a much later date, when the peritubular capillaries are blocked (Kountz et al. 1963).

The above sequence of events suggests that the primary target of the immunological attack is the endothelium of the small vessels of the kidney. Tubular damage and kidney dysfunction or rejection, is mediated by ischaemia following the vascular damage. There is no strong evidence to show any direct damage of renal parenchymal cells by infiltrating lymphocytes.

The "vascular theory" of rejection has been further supported by the following two observations.

(a) Renal haemodynamic in rejection - the renal blood flow has been measured in homograft rejection, the flow decreases with the onset of rejection and runs parallel with the decline of renal function (Kountz et al. 1963)

(b) Horowitz et al. (1963), using immunofluorescent antibody technique, demonstrated gamma globulin carrying lymphocytes in homograft kidney at 24 hours, and by 72 hours droplets of gamma globulin and alpha-2 globulin are found in media of small vessels, whereas none was found in control autotransplant animals.

III.

THE REJECTION CRISIS IN MODIFIED HUMAN RECIPIENTS

The term rejection crisis was first introduced by Hamburger in 1959 to describe acute episodes of renal graft dysfunction by the immunological process in a modified recipient.

In present day renal homotransplantation, grafts are, understandably, only placed in modified recipients whose immunological reaction is "suppressed" by total body irradiation or drug therapy. The latter, being the safer and more effective, is the method of choice at most centres. The current regime of immuno-suppressive therapy, as used at the Royal Victoria Hospital, Montreal, consists of:

(a) Imuran (Azathioprine) 4 mgm./kg./B.W. p.o. daily from the day of operation for the first month or so, then gradually reducing to a maintenance dose of 1 to 2 mgm./kg.

(b) Prednisone starting at 60 mgm./day, and gradually tailing off to 10-20 mgm./day.

(c) Actinomycin C 10 ug./kg. intravenously weekly until transplant function and patient condition stabilizes.

(d) Local irradiation of graft 100 rads. on days 1, 3, and 5.

On this treatment a minority of recipients seem to be

TABLE II

6 EPISODES OF MAJOR REJECTION CRISIS IN 5 PATIENTS

Case	JP_1	S_1	TU_1	L_1	S_2	N_2
Days Post Transplant	4	4	6	20	29	34
Fever‡	xx	xx	xx	xx	xx	xxx
Tenderness	x	xx	x ?	xx	xx	x
Oliguria‡‡	xx	xx	xxx	xx	o	xx
Proteinuria	xx	x	xx	xx	x	x
B.U.N.	xx	xx	xxx	xxx	xxx	xxx
Creatinine	xx	x	xxx	xxx	xxx	xxx
Raised Urinary L.D.H.	-	xx	x	o	xx	x
Raised Urinary Alk. Phos.	-	xx	x	x	xx	xxx
Lympho-cyturia‡‡‡	-	x	x	xx	xx	xx

‡ FEVER
x = fevers under 101°F.
xx = over 101°F. occurring at least 2 days.
xxx = over 103°F. for 2 days.

‡‡ OLIGURIA
x = under 1000 cc./24 hr.
xx = under 500 cc./24 hr.

‡‡‡ LYMPHOCYTURIA
x = at least 1 lymphocyte/H.P.F. x 400.
xx = at least 3 lymphocyte/H.P.F. x 400.

able to tolerate the homograft and go on to prolonged survival without any major rejection crisis.

However, the majority have, during their clinical course, one or more episodes of rejection crises, characterized by fever, oliguria, malaise, swelling and tenderness in the area of the transplanted kidney and deterioration of renal function as indicated by a rising blood urea nitrogen and creatinine, and a falling creatinine clearance. Proteinuria is quantitatively increased and there is an active urinary sediment with casts and red cells.

Table II summarises the clinical findings in six episodes of major rejection crisis occurring in five patients studied.

Fever of over 101°F., lasting at least two days, occurred in all cases; this pyrexia always existed before the deterioration of renal function and therefore always preceded the clinical diagnosis of rejection‡. Thus it is often the earliest single sign of threatened rejection, the only drawback is that it is nonspecific and does not distinguish between infection and rejection. Oliguria of under one litre per 24 hours is also a useful early sign and occurred in all except one case.

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‡ More recently there has been an exception to this in that one major crisis occurred without significant preceding pyrexia.

All these crises occurred within the first six weeks of transplantation. This seems to be the most critical period for the transplanted patient. Late major rejections do occur, but they are on the whole rare. It appears that where there is significant histo-incompatibility between donor and recipient, it declares itself pretty early in the game.

The demonstration by Hamburger (1959), Murray (1962) and Starzl (1963) that rejection crisis is not an all or none phenomenon and that in most instances can be totally reversed by intensive therapy, stimulated the search for methods that will aid or confirm the early diagnosis of rejection, so that prompt treatment can be given. A method was also needed to differentiate rejection and other pathological processes such as pyelonephritis, infarction of kidney, and obstructive uropathies, as the cause of deteriorating renal function.

This is highly important because, whereas increasing the prednisone dosage to such high levels as 160 to 200 mgm. per day is the logical and specific therapy for rejection, it will be unthinkably tragic if the same is given for what turns out to be an infective process. Therefore any aid that will help or lend weight towards making an accurate diagnosis of rejection crisis is not only desirable for our scientific interest, but is also intensely relevant to the safe

and sound conduct of the patient's postoperative management.

IV.

AIDS IN THE DIAGNOSIS OF REJECTION CRISIS

The various special aids that have been advocated for use in the diagnosis of renal homograft rejection can generally be classified as follows.

A. Methods of Measuring the Size of the Transplanted Kidney.

It is a well attested fact that in classical homograft rejection the transplant becomes oedematous haemorrhagic and enlarged. This enlargement is sometimes quite obvious to palpation. However, more precise appreciation of the transplant size can be obtained by:

The use of metal clips (Hume et al. 1964). The placing of radio opaque silver clips, one on each pole and a third at the midpoint of the back of the transplanted kidney, allows the accurate radiological assessment of the kidney size, independent of the state of function of the kidney. This method is especially useful in the very early post-operative period to distinguish between acute tubular necrosis and rejection as the cause of early graft dysfunction. Enlargement would favour a diagnosis of rejection.

The use of radioactive mercuhydrin renal scan. Dossetor et al. (1964) believed that meralluride scan was more specific in determining changes in renal size.. However, because the test depends on the uptake of

mercuhydrin by the kidney. The quality of the scan is very poor in presence of a markedly decreased renal function from whatever the cause is.

B. I^{131} Hippuran Renogram.

Theoretically, renogram would give a detailed pictorial representation of the three phases of kidney function, that of an initial vascular phase followed by secretory and an excretory phase. The function-time relationship is also demonstrated. This method is used widely in the diagnosis of unilateral renal disease, but, as Burbank et al. (1963) have pointed out, the abnormalities so demonstrated did not permit distinction between renal vascular and renal parenchymal disease. Thus in one patient at the Royal Victoria Hospital, Mrs. D.N. who had deteriorating renal function from renal arterial stenosis rather than "pure" rejection, her renogram was unhelpful.

C. The Reactivity of Lymphocytes to Phytohaemagglutinin Stimulation in Vitro.

Hirschhorn et al. (1963b) first demonstrated that when Phytohaemagglutinin (PHA), an extract of the kidney bean, Phaseolus Vulgaris, is added to a culture of lymphocytes of a normal individual, 90 per cent or more of the small lymphocytes are morphologically changed to large lymphocytes, some resembling plasma

cells. They also found that lymphocytes from renal transplant recipients, who were receiving immuno-suppresive drugs, did not respond to phytohaemaglutinin. However, on two occasions (Hirschhorn et al. 1963a), a positive response was observed a few days prior to the development of rejection crisis. They suggested this test as a screening device for the escape from immune-suppression before a rejection crisis occurs. At the Royal Victoria Hospital, we have conducted a trial of this method on our post transplant patients and we have not been able to confirm Hirschhorn's observations (Gordon, 1964). There are still a great deal of technical difficulties and controversies which have to be resolved before this test could be considered as a reliable indicator of impending rejection crisis.

D. Serum and Urinary Enzyme Changes that Relate to Renal Tissue Damage or Necrosis from Rejection.

Serum Glutamic oxalacetic transaminase (S.G.O.T.) and serum lactic dehydroginase (S.L.D.H.). Renal tissue has been shown to contain large amounts of LDH (Wroblewski and Ladue, 1955) and GOT (Cohen and Hekhius 1941). Experimentally induced necrosis of kidney tissue has been shown to produce elevation of serum GOT levels (Rudolph et al., 1957). Serum LDH has been shown to be elevated in over 50% of patients with renal disease

(West et al. 1958). Hume in 1964 reported the use of serum LDH level as an aid in the diagnosis of renal homograft rejection and as an index of prognosis.

Urinary LDH and Alkaline Phosphatase (A.P.)

The urinary LDH like its serum counterpart is raised in renal disease such as acute infarction of kidney, malignant hypertension, lupus nephritis, acute tubular necrosis, and in about 25% of cases of pyelonephritis. It is also raised in malignant tumours of the urinary tract especially the kidney (Wacker et al. 1962, Wacker et al. 1964). The urinary LDH and AP have been used clinically for the detection of occult urinary tract tumours (Amador et al. 1963). More recently, Dr. H. M. Gault and Dr. J. B. Dossetor (Dosseter et al. 1963) advocated the use of urinary LDH and Alkaline phatase levels in anticipating renal homograft graft rejection. Further studies at the Royal Victoria Hospital continued to show a fairly good correlation between these urinary enzyme levels and rejection crisis (H. M. Gault, 1965). The urinary AP is the more specific and rises to very high levels unreached by any other renal disease. The normal value for this enzyme in the urine is usually under 1.5 K.A. units per 100 ml. The urinary LDH tends to be less specific in that it rises with increased urinary red cells and leucocytes and may rise in destruction of urinary tract tissue other than the kidney, e.g. prostate.

E. Lymphocyturia

Studies of stained urinary sediment have shown that the appearance of increased number of lymphocytes in the urine correlates with clinical rejection (Kauffman et al. 1964). The authors reported that in 11 rejection episodes occurring in 7 renal transplant patients, lymphocyturia occurred in 8; and sometimes prior to clinical rejection. They admitted that lymphocytosis in urine is not specific for homograft rejection and is also found in acute type I nephritis and lupus nephritis. But they did not define what is significant lymphocyturia, and no mention was made on its relationship with urinary casts and haematuria which are the time honoured pointers of renal inflammation. I therefore undertook a study of stained urinary sediments on all the renal transplant patients at the Royal Victoria Hospital operated on since July 1964.

Method: Daily early morning specimen of fresh urine is collected. Fifteen ml. of the urine is centrifuged at 2500 r.p.m. for 5 minutes. The supernatant is discarded and 2 drops of human serum is added and sediment redissolved by finger flicking. A small drop of this is then placed on a glass cover slip and two smears are made from it. The smear is air dried and stained with Wright's stain. The staining time should be 75% of that used for blood, otherwise the

cells become too deeply stained (see Appendix I). The stained smear is then mounted permanently on a slide, which is examined under LP x 100, HP x 400, and under oil immersion HP x 800.

The various identifiable cellular elements in the urinary sediment are listed with photomicrographs in Appendix II.

By making differential counts of cases, red cells, and other cellular elements in transplant urines, I made the following observations:

(a) Typical small lymphocytes do appear in urine during rejection.

(b) Along with small lymphocytes are other atypical mononucleated cells which have a central or eccentric darkly stained nucleus with abundant purplish intensely basophilic cytoplasm. The cells are rather pleomorphic, assuming oval, round, or plasma cell shapes. These are large lymphoid cells similar to those seen in the histology of rejected kidney. Because of their large size and abundant cytoplasm, they may be difficult to distinguish from tubular cells. However, the dark staining dense chromatin network in nucleus is quite characteristic and the intensely basophilic cytoplasm is rarely seen in tubular cells.

(c) For practical purposes, it is well to count these large and small lymphocytes together and

regard one cell per HPF x 400 as being significant. However, accurate quantitation on a smear is not easy.

(d) Lymphocyturia is, more often than not, associated with casts and microscopic haematuria, and it occurred with every major rejection crisis studied so far (see Table II and Fig. 2, 3 and 4).

Question arises as to whether the changes of serum and urinary enzymes and that of lymphocyturia are due to immunological rejection or could they be spurious outcomes from the therapeutic and other treatments that a transplant patient gets for threatened rejection, e.g. local irradiation. With this and other uncertainties in mind, it was decided to carry out the following experiment.

V.

THE COMPARATIVE VALUE OF LYMPHOCYTURIA, SERUM AND URINARY ENZYMES IN THE DIAGNOSIS OF RENAL HOMOGRAFT REJECTION (An Experimental Study)..

A. Object.

(i) To observe the changes in urinary sediment, serum and urinary enzymes, during renal homograft rejection in dogs not modified by any drug, steroid or irradiation.

(ii) To compare the value of these parameters as diagnostic aids.

B. Material and Method.

Large adult mongrel dogs of both sexes, weighing between 20-35 kg. were used. The basic experimental model is a bilaterally nephrectomized dog with a single transplanted kidney in the pelvis. Post-operatively sequential bloods and fresh urine samples are studied for enzyme changes and lymphocyturia. These changes are compared between a group of dogs with autotransplants and a group with homotransplants. The serum glutamic oxalacetic transaminase (SGOT) was determined by the method of Reifman and Frankel; serum lactic dehydrogenase (SLDH) by the colorimetric method of Berger and Broida; ULDH by the same method after dialysis; urinary alkaline phosphatase (UAP) by a modification of the King Armstrong method after dialysis; lymphocyturia was

looked for in stained urinary sediment, using the Wright stain technique as described by Kauffman et al.. (1964) (see Appendix I.). Significant lymphocyturia was arbitrarily defined as the presence of at least one lymphocyte per high power field x 400, the reason being that occasionally urine sediments from autotransplanted dogs have up to one lymphocyte per low power field x 100.

Operative Technique. The dog is anaesthetised with intravenous injection of nembutal and is placed on the operating table in the supine position. 20 ml. of blood is withdrawn for determination of BUN, serum creatinine (Cr.), SLDH, and SGOT. An intravenous infusion is set up and the dog is given 500 ml. of glucose-saline with 500 mgm. of tetracycline during the operation. The abdomen is prepped with cetavlon and 2% iodine, and draped. A midline incision is used in all the cases, dividing the linea alba of the rectus sheath from the ziphoid process to the pubis.

Nephrectomy. The left or the right kidney is removed for transplantation. Whenever possible the side with a single renal artery and single renal vein is used. The kidney is first mobilised; the ureter is then gently and carefully dissected out and freed down to the pelvic brim, taking care not to injure the ureteric blood vessels. The renal artery is next approached from behind by lifting the kidney up and towards the

opposite side of the body. The main artery is stripped clean of adventitia down to its origin from the aorta. The renal vein is likewise dissected out neatly. No attempt was made to inject procaine or papaverine around the renal pedicle. The renal artery is first ligated and cut; a few seconds later, the renal vein is ligated and cut. The kidney so obtained is immediately immersed in a kidney basin filled with TIS-U-SOL at 4°C. It is then core cooled manually by perfusing through the renal artery 50 ml. of 10% rhaeomacrodex in saline again at 4°C, using a 10 ml. plastic syringe. The kidney is then ready for transplantation. The use of low molecular weight dextran as perfusate in preference to Ringer-lactate solution is because, with the former, the efflux from the renal vein becomes clear much faster.

Pelvic Transplantation. Using large abdominal packs, the intestines are packed towards the upper abdomen in order to expose the pelvis. The external iliac artery and the common iliac vein are prepared to receive the kidney graft. The renal artery of the graft is anastomozed end to end to the cut external iliac artery, using two continuous running 6/0 cardio-vascular silk sutures. The renal vein is anastomozed end to end, or end to side, to the common iliac vein using the same anastomozing technique. If the common iliac vein (CIV) is small and comparable to the size

of the renal vein, then I use the end to end technique with ligation of the distal cut end of the CIV. If the CIV is big, I use the end to side method. Latterly, I have preferred the end to side technique because ligation of the common iliac vein, although well tolerated by the dog, often results in disability and limping in the first few post-operative days; thus making the collection of bloods and fresh urines difficult. The average ischaemic time is about 25 minutes. The uretero-neo-cystostomy is done by the simple implantation method: the cut end of the ureter is spatulated and drawn into the bladder through a stab wound just above the trigone. The spatulated end is fixed to the bladder wall by a single suture of the 4/0 chromic catgut about 1.5 cm. away from the stab wound. The stab wound is then closed snugly around the ureter by a few interrupted sutures. This method avoids an open cystotomy and mucosa to mucosa suturing of ureter and bladder, or the use of submucosal tunnels. However, it is simple and has proved to be quite satisfactory in the experimental animal. The kidney is then fixed to the posterior abdominal walls by a few sutures.

C. Results and Discussion

The dogs are divided into 3 groups.

(i) Control - unoperated dogs. A series of bloods and fresh urines are taken from apparently healthy unoperated

dogs. This was necessary to determine the normal range of the serum and urinary enzymes under study. It was found that the normal ranges for SLDH and SGOT are similar to those in human subjects. In the majority, the SLDH were below 500 units/ml. and SGOT below 40 units/ml. (Fig.5 and 6). The few elevated ones are possibly from dogs that had some occult sickness. The ULDH was found to be under 10 units/ml, and the UAP to be under 1.5 units/ml. in all the 20 control samples taken (Fig.7 and 8).

(ii) Autotransplanted group. In this group of 8 dogs, pelvic renal autotransplant was done with immediate contralateral nephrectomy. There was an inconsistent small rise of ULDH and/or UAP in the first 48 hours following operation but no significant rise thereafter, even though 4 dogs developed uremia from diverse causes (Table III). The early elevations were attributed to renal damage during transplantation. The serum enzymes, however, were often raised even after the immediate post-operative period and especially in dogs that developed uremia (Fig.5 and 6). There was no significant lymphocyturia in any of these animals. Fig.9 illustrates an autotransplanted dog that had normal renal function post-operatively and went on to live over two months and died from causes unrelated to

the transplanted kidney; Fig.10 illustrates a dog that had extrarenal uraemia from intestinal obstruction and vomiting. The urinary enzymes remained within normal range, despite high levels of BUN and Creatinine.

(iii) Homotransplanted group. (Tables IV and V). In this third group of 11 dogs, pelvic renal allografts were performed with simultaneous bilateral nephrectomy. In 8, definite clinical rejection crises occurred with raised BUN and Cr.; this was associated with elevation of ULDH in 6, UAP in 7, and lymphocyturia in 7 (Table IV). Fig. 11, 12, 14, illustrate three of these 8 dogs. The dog that did not have lymphocyturia died on the 15th post-operative day with BUN of 267 mgm.%, Cr. of 18.4; the transplanted kidney, however, showed some oedema with a mild monocytic infiltrate only (Fig.15, Dog.112). In contrast, the other 7 had moderate to severe cellular infiltrates. In 2 dogs (No.101 and 172), death occurred with elevated BUN but nearly normal serum Cr; rejection was strongly suspected. In one (Dog 172) the UAP rose to very high levels 3 days prior to the animal's death; lymphocyturia was absent and again the transplanted kidney had only a mild round cell infiltrate on microscopy. In the other dog (No.101, Fig.16), the urinary enzymes were negative, but lymphocyturia was positive and was associated with a moderate perivascular round cell infiltrate on microscopy.

The final dog (No.17, Fig.17) was sacrificed after 13 days, when the BUN was only 32 mgm.%, had a tenfold elevation of ULDH in the absence of significant homograft reaction, lymphocyturia or increase in UAP. This could be considered a false positive. The average maximum increases in UAP and ULDH were 27 and 7 times the upper limit of the normal range. In two episodes of rejection, there was only borderline elevations of ULDH (Fig.7). Elevations of serum enzymes also occurred with rejection, but were less consistent. (Fig.5 and 6). Significant lymphocyturia has been encountered with most rejection crises; the few times that it did not appear were in the case where no rejection has yet occurred due to early sacrifice (Dog.No.17) or where rejection had occurred but with mild lymphocytic infiltrate only. The presence of lymphocyturia appears to correlate well with the degree of lymphocytic infiltration of the kidney (Table V), and this important point must be borne in mind when studying lymphocyturia in drug-modified human recipients, where the cellular infiltrate of homograft reaction is considerably attenuated. This possibly explains the absence of significant lymphocyturia in 3 of the 11 rejection episodes reported by Kauffman et al. (1964). The other observations I made on lymphocyturia were (a) its occurrence can be very

sporadic, appearing sometimes only for a day or so at a time, and (b) its accurate quantitation is not easy.

Special mention must be made on Dog 115, which is remarkable in that not only is it the longest survival (122 days) ever reported of an unmodified, homografted dog, but also it had undergone one or probably two rejection crises which were spontaneously reversed. Moreover, the animal died on the 122nd post-operative day from an accident unrelated to the homografted kidney which was still functioning adequately. Fig.17 illustrates the clinical course of the first 60 days of the dog. The initial transient rise of BUN and Cr. during the immediate post-operative period was believed to be from surgical trauma. The dog underwent a major rejection crisis during the 3rd week with BUN rising to a maximum of 63 and Cr. to 2.6. The SLDH and SGOT were elevated during this episode. It is of more interest to note that the UAP rose to very high levels 3 days prior to the rise of BUN. ULDH also rose but to a much lesser degree. Lymphocyturia which occurred sporadically between the 4th and 10th post-operative day, was present again during the latter part of the crisis. An open biopsy of the transplanted kidney immediately following the spontaneous remission of the crisis, showed many foci of typical homograft reaction consisting of peritubular infiltrates

of lymphocytes and plasma cells. A second possible but milder rejection crisis occurred and again was reversed spontaneously. Following this, the renal function became fairly stable BUN around 30 mgm.%, and Cr. around 1.8. However, the rejection crisis did leave its damaging marks: (a) The creatinine clearance on the 91st day was 39.5 ml./min., an adequate but subnormal glomerular filtration. (b) the creatinine urine/plasma ratio which had a wide range of between 20 to 140 before, became limited to a small range of 20-60. (c) The specific gravity became fixed to around 1.007.

The dog was healthy, friendly and gaining weight until the time of its accidental death. The final histology of the transplanted kidney showed, apart from foci of lymphocytic and polymorpho infiltrate, endothelial thickening of the arterioles and membranous glomerulitis.

These changes have been reported previously in homografts of treated dogs with attenuated homograft reaction and prolonged survival (Porter 1964; Zukoski 1965). However, the exact aetiology of these changes has been controversial. Are they caused by the purine analogues, steroids, irradiation, or by an immunological reaction? The fact that both types of changes were observed in this dog's homograft proves conclusively that they can be caused by the homograft reaction alone.

The spontaneous reversal of the rejection crisis is the second one reported in the literature. Jeejeehboy (1965), in studying the effects of acute tubular necrosis on the survival time of renal homografts, encountered a dog that appeared to have some functional and histological reversal of homograft reaction. The dog died on the 30th day. In Dog 115 the ischaemic time during transplantation was only 25 minutes, but the steep rise of BUN and Cr. in the immediate post-operative period suggests that there might be significant acute tubular necrosis. However, its influence on the prolonged survival and reversal of rejection must remain purely speculative.

D. Conclusions

(i) The urinary enzymes ULDH and UAP, with rare exceptions, are elevated during renal allograft rejection and not with autografts. The average percentage of UAP is about 4 times that of the change in ULDH. UAP appears to be the more specific and better rejection index of the two.

(ii) Lymphocyturia occurs regularly with rejection. Sometimes it is sporadic and appears for a day or two only. Accurate quantitation is not easy. It appears to be a reflection of the degree of lymphocytic infiltration in the transplanted kidney, whereas the urinary enzymes rise from parenchymal damage.

(iii) The serum enzymes SLDH and SGOT are

elevated with rejection but less consistently.. They also rise in the presence of autografts from nonspecific causes.

(iv) An unique instance of spontaneous remission of rejection crisis with prolonged survival (122 days) was encountered in an unmodified recipient dog. This is the longest survival in the literature.

(v) Claims on the effectiveness of any immunosuppressive regimen that is based on a few prolonged survivals or the occasional successful reversal of a rejection crisis, should be viewed with caution.

(vi) Changes of obliterative "arteritis" and membranous glomerulitis in long surviving renal allograft are caused by immunological reaction rather than the medications per se.

T A B L E S

CONTROL AUTOTRANSPLANT GROUP TOTAL 8

	No.	Raised* Urinary Alk.Phos.	Raised* Urinary L.D.H.	Lympho-cyturia
Normal Renal Function	4	0	0	0
Uraemic From:				
?Chr. Pyleonephritis	1	0	0	0
Int. Obstruction	1	0	0	0
Haemorrhagic diarrhoea ?cause	1	0	0	0
Unknown	1	0	0	0
	8	0	0	0

*Rise of Alk. Phos. and L.D.H. in first 2 days not included

TABLE III

HOMOTRANSPLANT GROUP TOTAL 11

	No.	Raised* Alk.Phos.	Raised* L.D.H.	Lympho-cyturia
Definite rejection crisis	8	7	6	7
Rejection strongly suspected	2	1	0	1
No rejection at sacrifice	1	0	1	0
TOTAL	11	8	7	8

TABLE IV

* Rise of UAP and ULDH in first 2 days not included

TABLE V HOMOGRAFT GROUP 11 DOGS (DETAILED DATA)

	DOG NO.	SURVIVAL DAY P.O.	REJECTION CRISIS	LAST BUN	LAST Cr.	SLDH*	SGOT*	ULDH*	UAP*	LYMPHOCYTURIA	HISTOLOGY. DEGREE OF LYMPHO INFILTRATE
1)	17	13‡	NO	32	2.0	340	70	140	0.65	NO	MINIMAL
2)	96	11‡	YES	124	18.3	-	-	33.5	0.43	xx	SEVERE
3)	101	9‡	SUSPECTED	85	2.4	1200	90	0.4	1.11	x	MODERATE
4)	102	9‡	YES	105	10.4	-	-	206	37.7	x	SEVERE
5)	105	10‡	YES	146	24.6	-	-	149	7.56	xx	SEVERE
6)	115**	122**	YES	32	1.85	880	200	19.7	9.8	xx	MODERATE
7)	112	15	YES	267	18.4	560	56	12.1	244	NO	MILD
8)	111	10	YES	121	10.0	480	84	12.8	2.9	x	MODERATE
9)	34	9	YES	86	3.0	-	-	4.3	9.8	xx	MODERATE
10)	172	20	SUSPECTED	47	0.98	380	46	6.0	51.3	NO	MILD
11)	171	5	YES	153	3.6	460	64	9.3	7.9	x	MODERATE

* Highest value excluding the first two post-operative days.

‡ Sacrificed.

** Died of an accident unrelated to renal homograft.
Dog had spontaneous remission of Rejection Crisis.

F I G U R E S

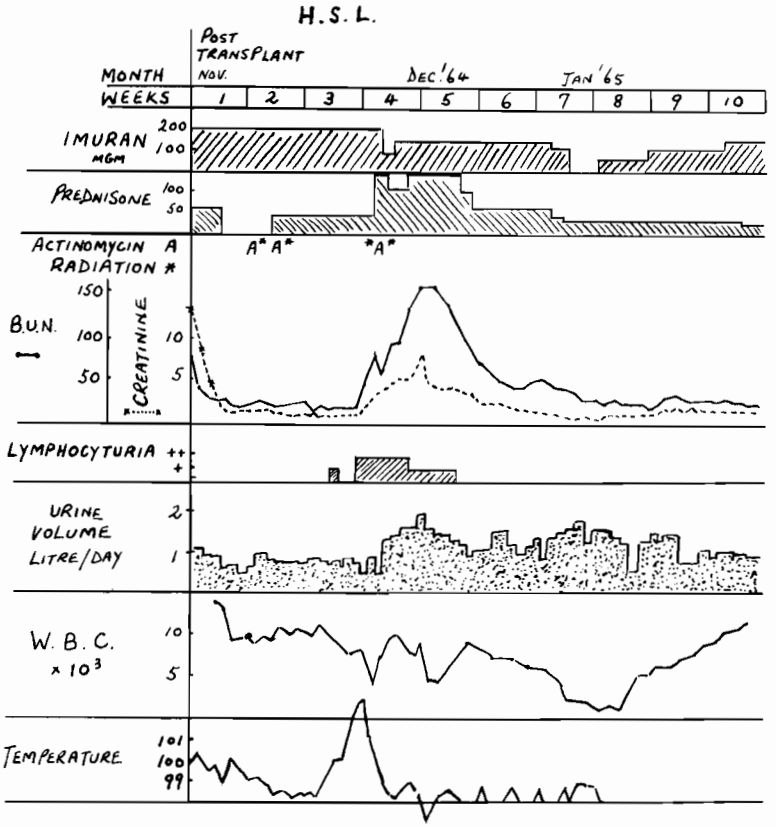


Fig. 2

Mr. H.S.L., a 31 year old Chinese cook, received a cadaveric transplant on 17 Nov. 1964. A major rejection crisis occurred during the 3rd week preceded by fever. Lymphocyturia occurred during and a few days before the rise of BUN and creatinine. The crisis was successfully treated with large doses of prednizone. The patient is alive and well 6 months after transplantation.

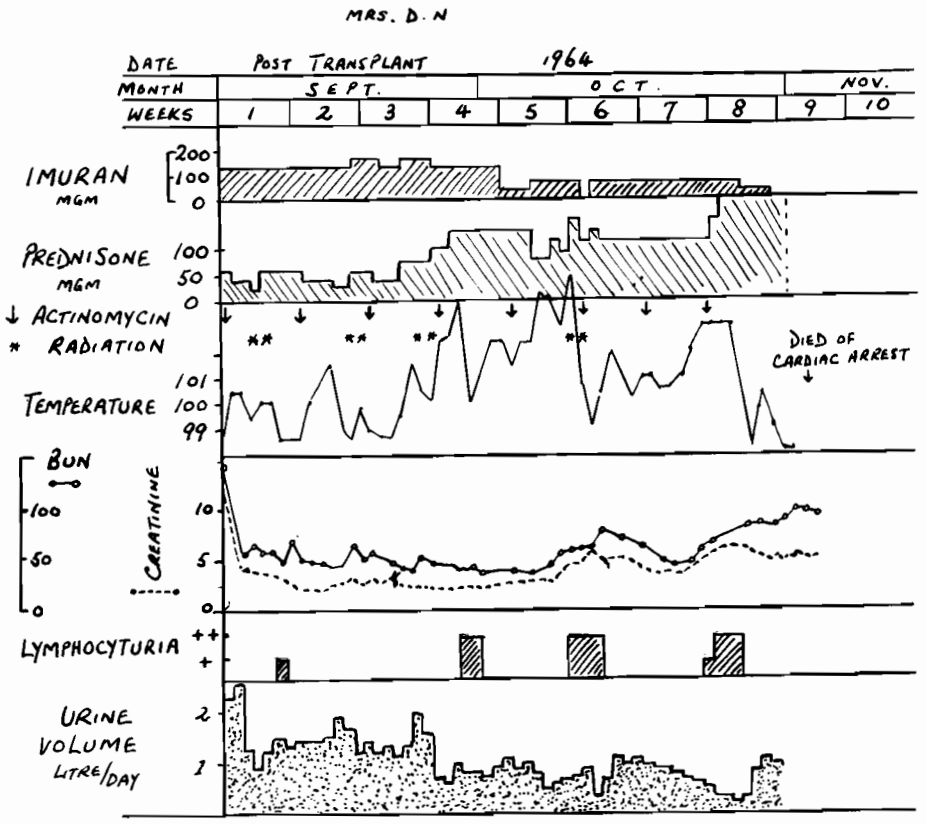


Fig. 3

Mrs. D. N., 28 year old French Canadian, had chronic nephritis following toxaemia of pregnancy. She received a cadaveric kidney on 4th Sept. 1964. A suspected minor rejection was aborted in the first post-transplant week from the 4th week onwards, she developed a severe rejection crisis with chills and rigors, followed by progressive renal failure. Lymphocyturia occurred regularly with each crisis.

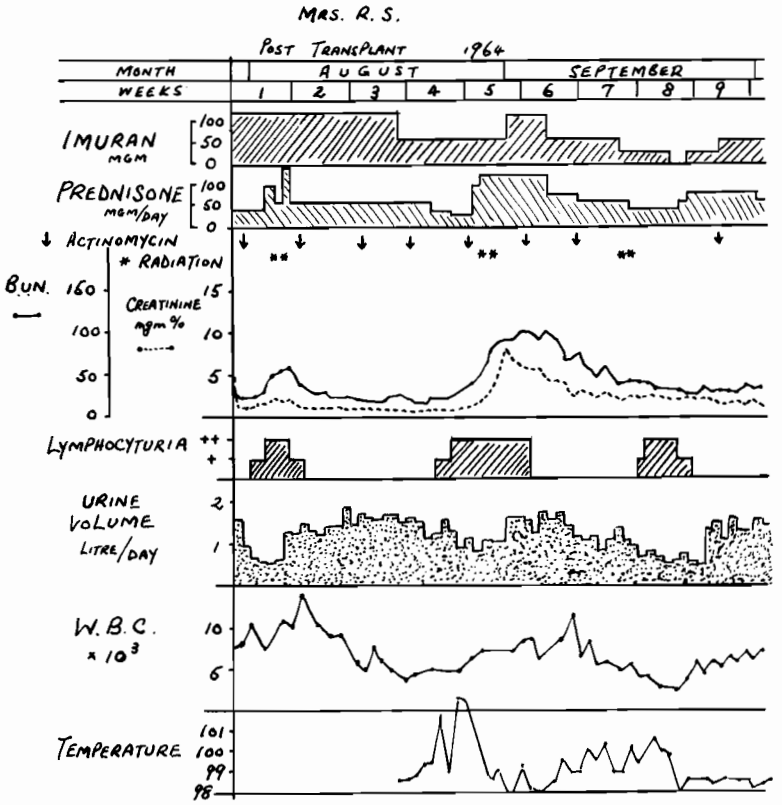


Fig. 4

Mrs. R.S., a 28 year old housewife, received a homograft kidney on the 29th July 1964. She had two major rejection crises, in the 1st and 5th post-operative week respectively. Both were successfully reversed with increased prednizone, Actinomycin C, and local irradation to the transplant. She was well until January 1965 when she developed Jaundice from imuran toxicity and died 194 days post-transplant.

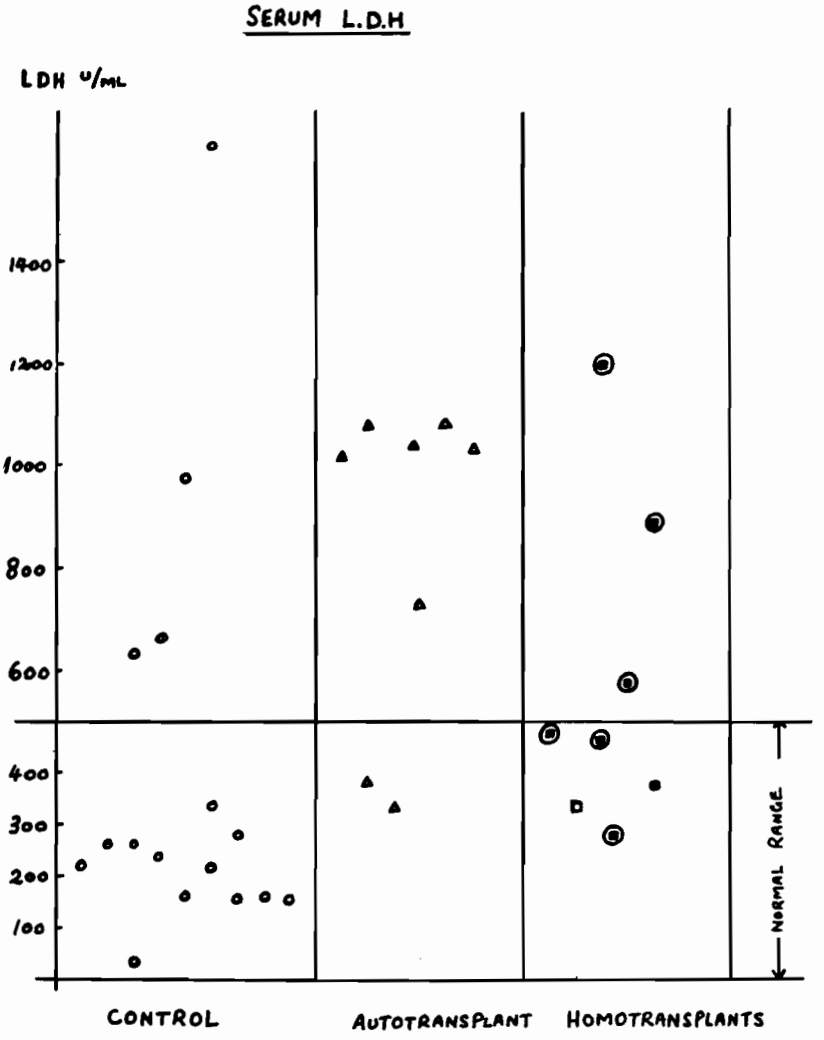


Fig.5

◉ Definite rejection crisis

■ Rejection strongly suspected

▫ Not rejected at sacrifice.

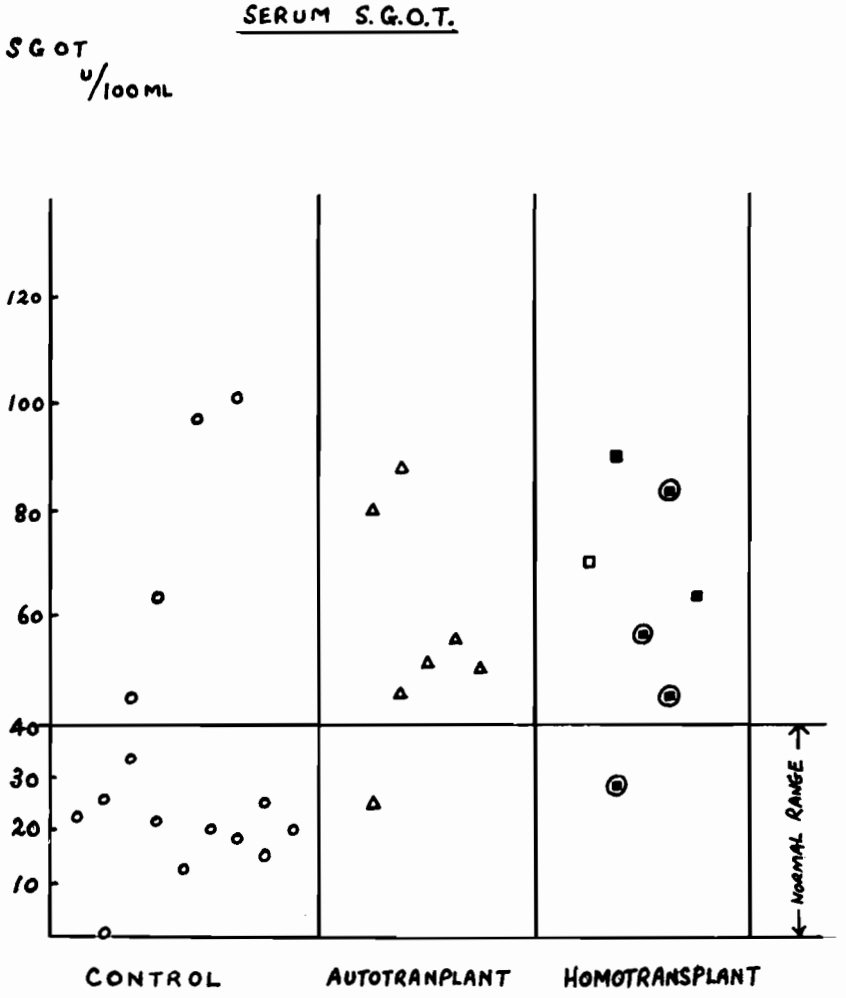


Fig. 6

◉ Definite rejection crisis.

■ Rejection strongly suspected.

▫ Not rejected at sacrifice.

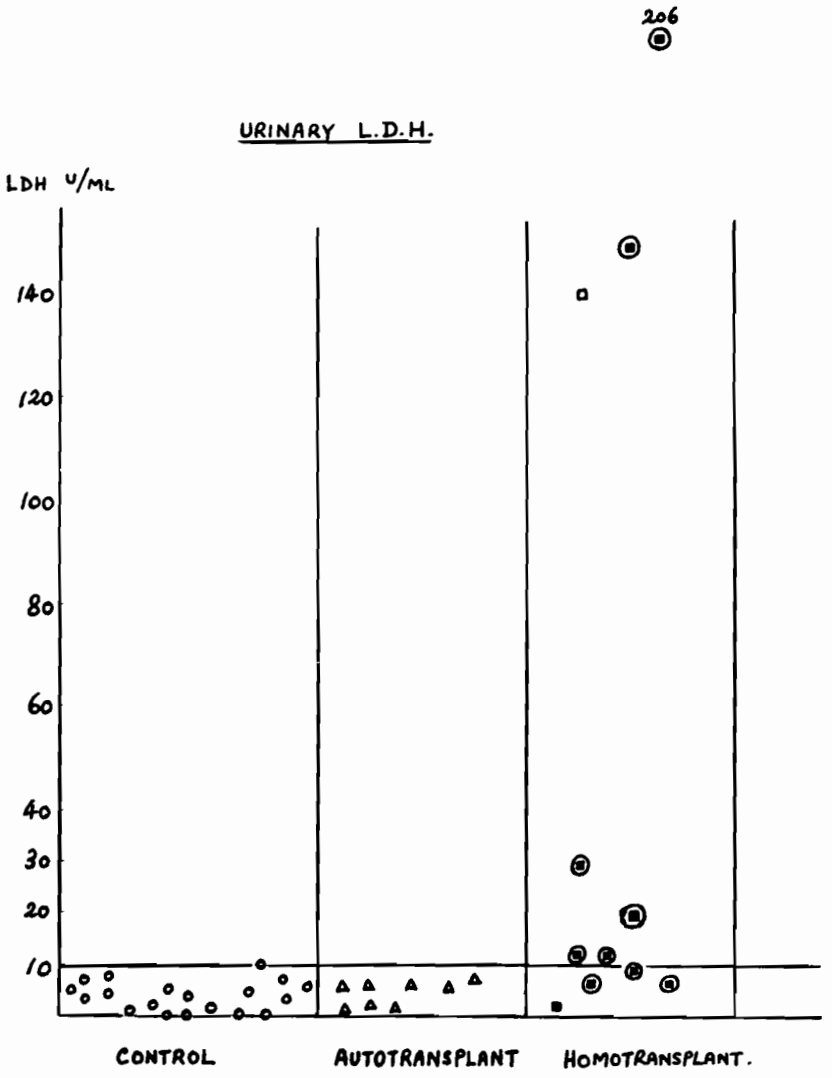


Fig. 7

◉ Definite rejection crisis.

■ Rejection strongly suspected.

□ Not rejected at sacrifice.

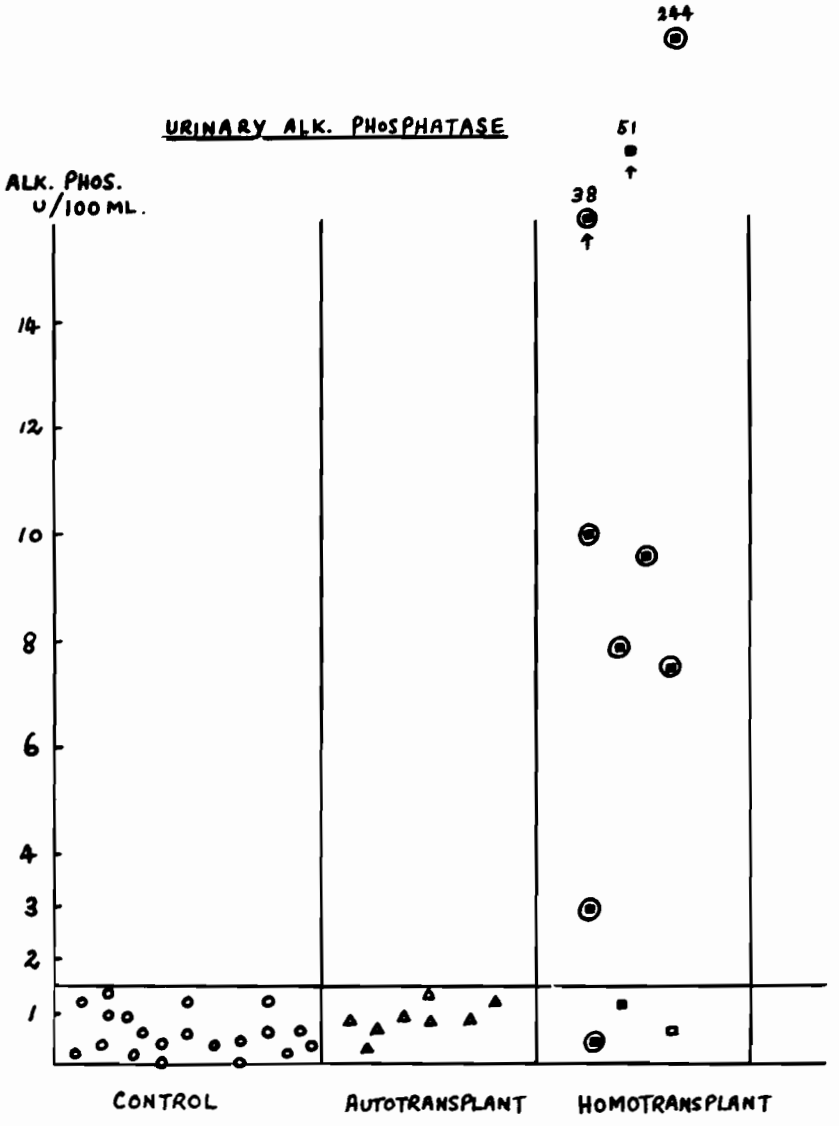

URINARY ALK. PHOSPHATASE
ALK. PHOS.
U/100 ML.
244
51
38
14
12
10
8
6
4
3
2
1
CONTROL
AUTOTRANSPLANT
HOMOTRANSPLANT

Fig. 8.

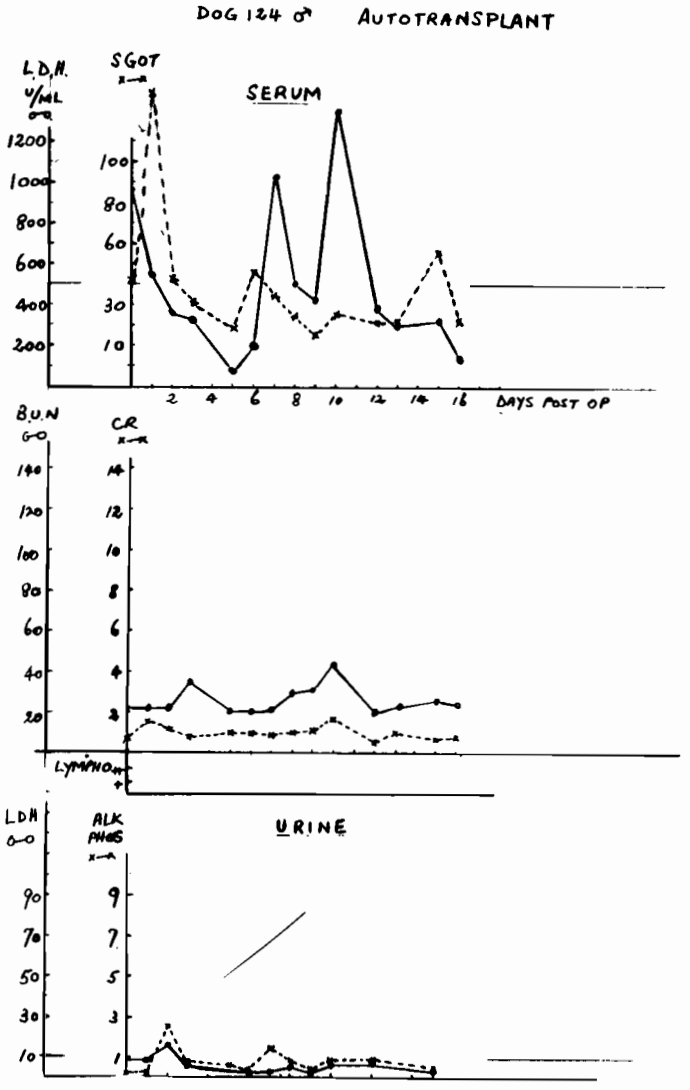


Fig. 9

This dog went on to live for over 2 months and died from causes unrelated to the autotransplanted kidney. Note the fluctuations of serum enzymes.

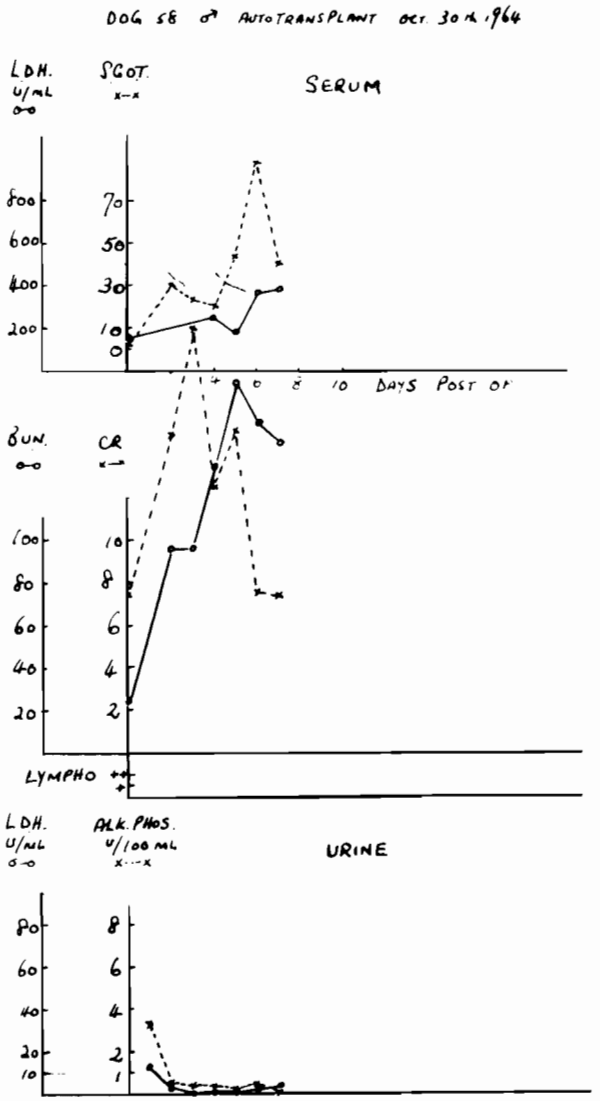


Fig. 10

This autotransplanted dog developed extrarenal uraemia from intestinal obstruction. The urinary enzymes remained within normal range.

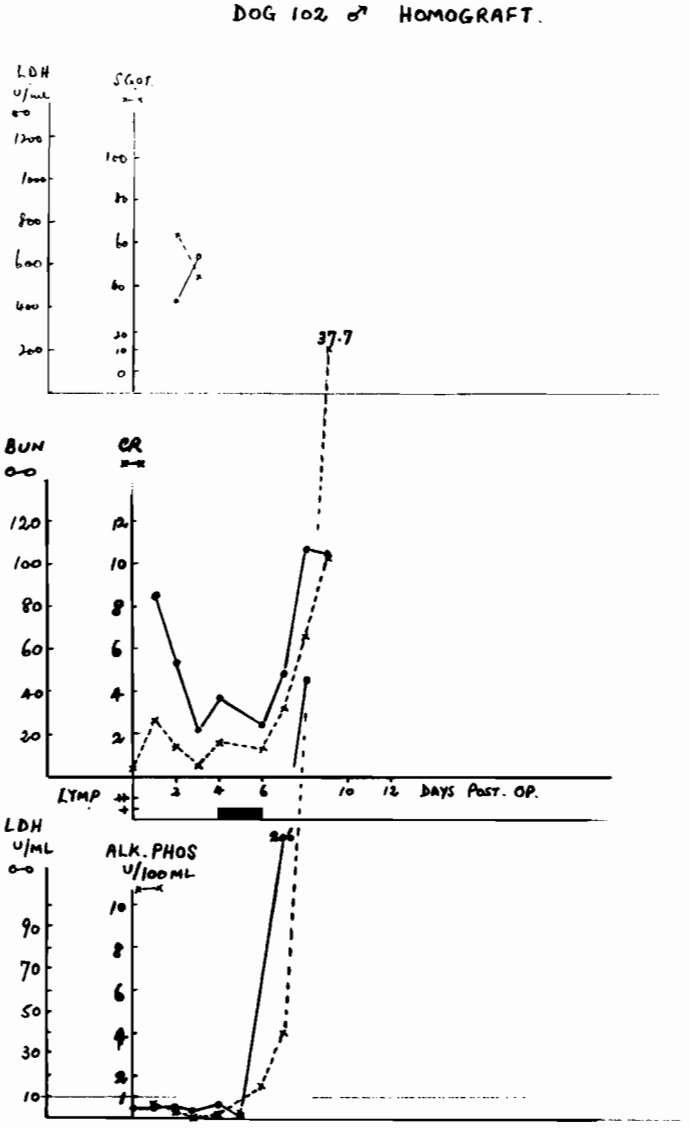

DOG 102 ♂ HOMOGRAFT.
LDH U/ml
SGOT
37.7
BUN
CR
LYMP
DAYS POST. OP.
LDH U/ML
ALK. PHOS U/100 ML
246

Fig. 11

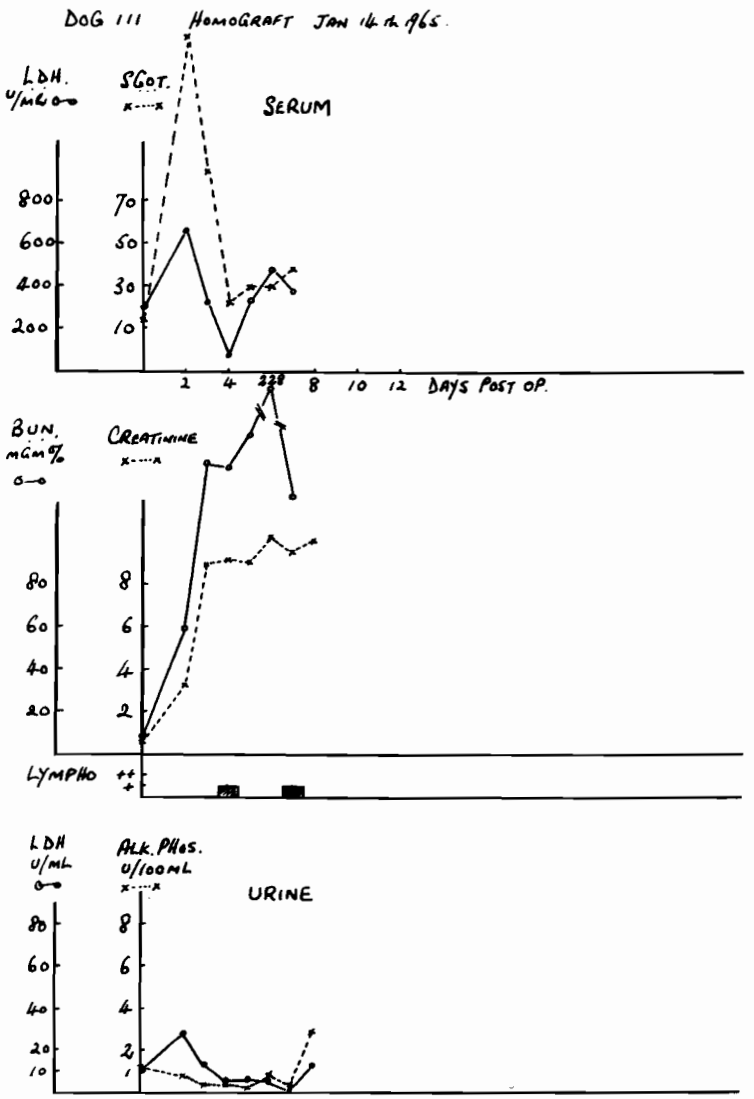

DOG 111 HOMOGRAFT JAN 14th 1965
LDH. U/mL
SGOT.
SERUM
800
600
400
200
70
50
30
10
2
4
8
10
12
DAYS POST OP.
BUN. mGm%
CREATININE
80
60
40
20
8
6
4
2
LYMPHO
++
+
LDH U/mL
ALK. PHOS. U/100mL
URINE
80
60
40
20
10
8
6
4
2
1

Fig. 12

DOG 105 ♂ HOMOTRANSPLANT DEC 14 th 1964

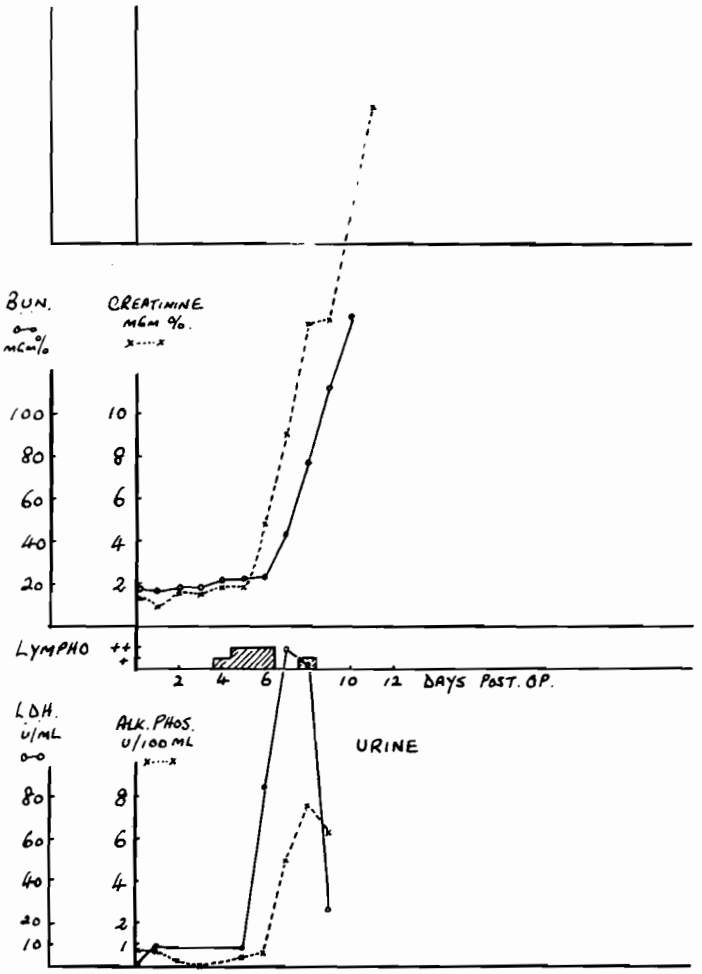


Fig. 14

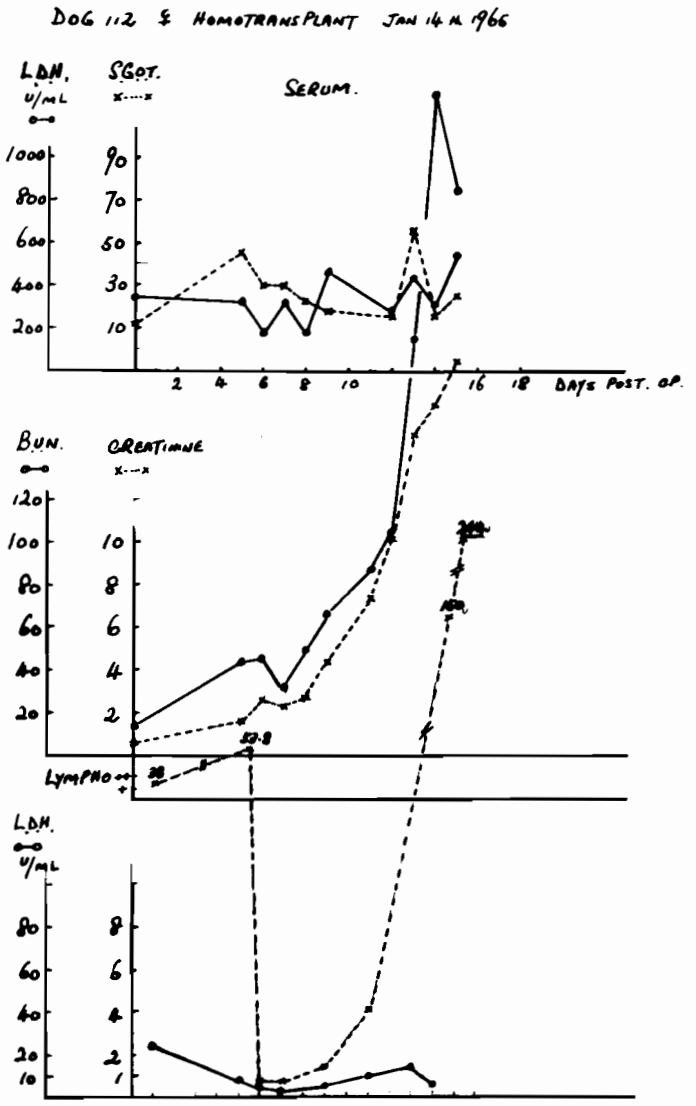

DOG 112 & HOMOTRANSPLANT JAN 14 M 1966
LDH. U/mL
SGOT.
SERUM.
1000
800
600
400
200
90
70
50
30
10
2
4
6
8
10
16
18
DAYS POST. OP.
BUN.
CREATININE
120
100
80
60
40
20
10
8
6
4
2
52.8
LYMPHO
LDH. U/mL
80
60
40
20
10
8
6
4
2
1

Fig. 15

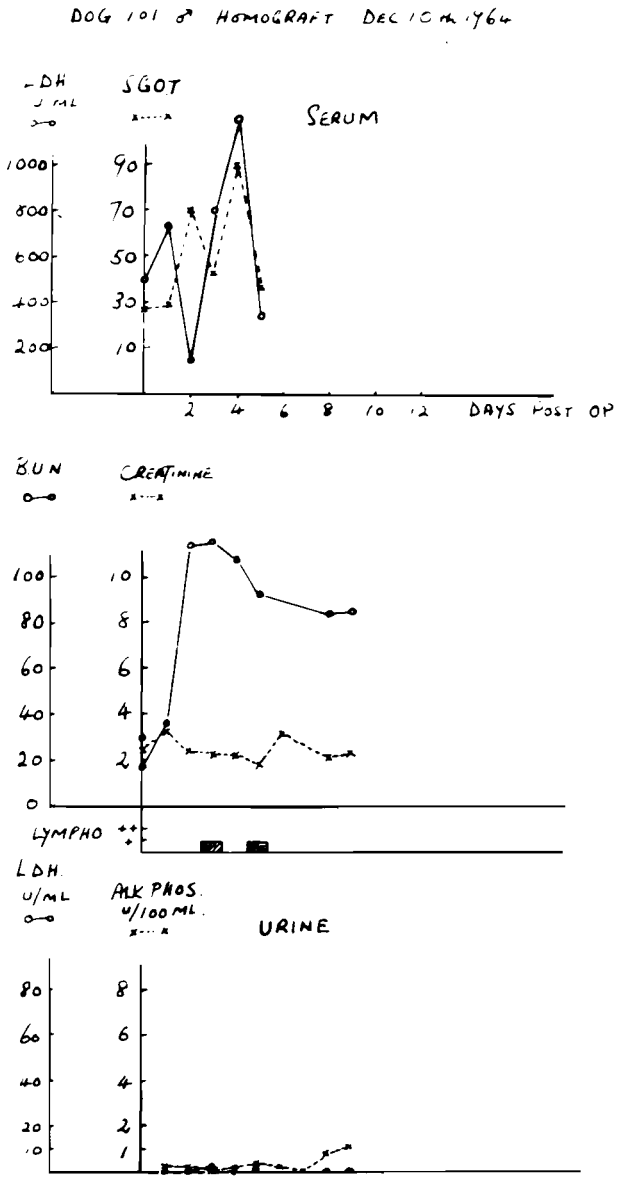

DOG 101 ♂ HOMOGRAFT DEC 10 TH 1964
LDH
SGOT
SERUM
1000
800
600
400
200
90
70
50
30
10
2 4 6 8 10 12 DAYS POST OP
BUN
CREATININE
100
80
60
40
20
0
10
8
6
4
2
LYMPHO
LDH
U/ML
ALK PHOS.
U/100 ML.
URINE
80
60
40
20
10
8
6
4
2
1

Fig. 16

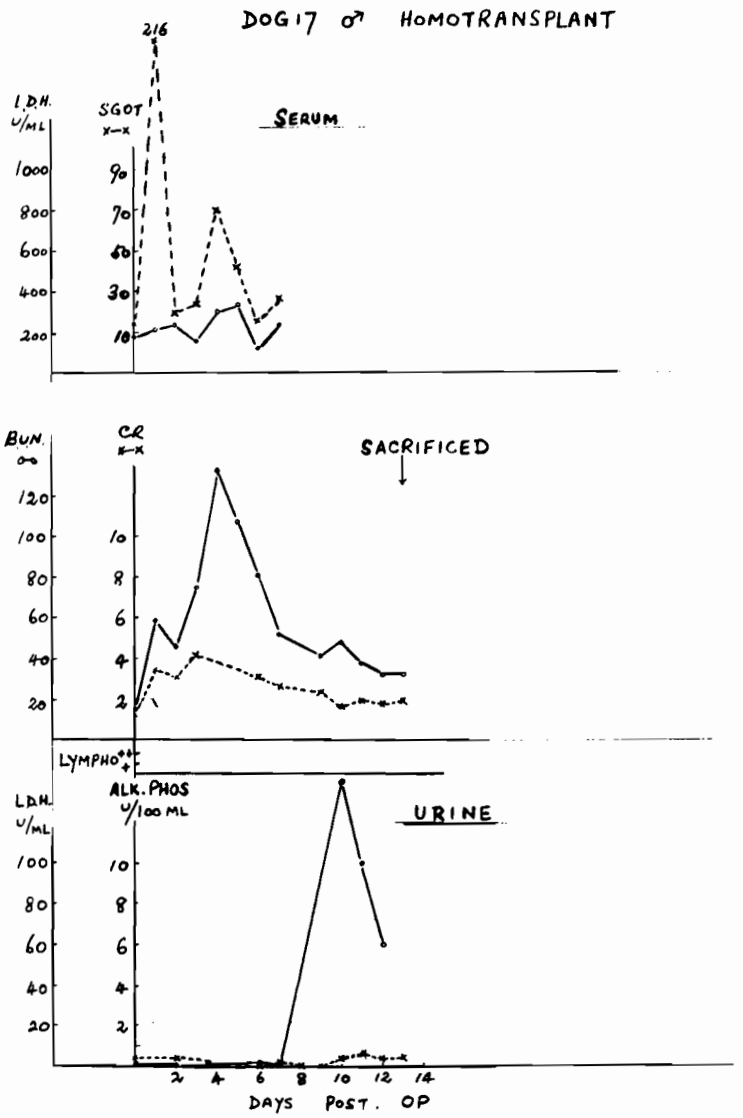

DOG 17 ♂ HOMOTRANSPLANT
216
L.D.H. U/ML
SGOT x–x
SERUM
1000
800
600
400
200
90
70
50
30
10
BUN.
CR
SACRIFICED
120
100
80
60
40
20
10
8
6
4
2
LYMPHO
ALK. PHOS U/100 ML
URINE
2
4
6
8
10
12
14
DAYS POST. OP

Fig. 17

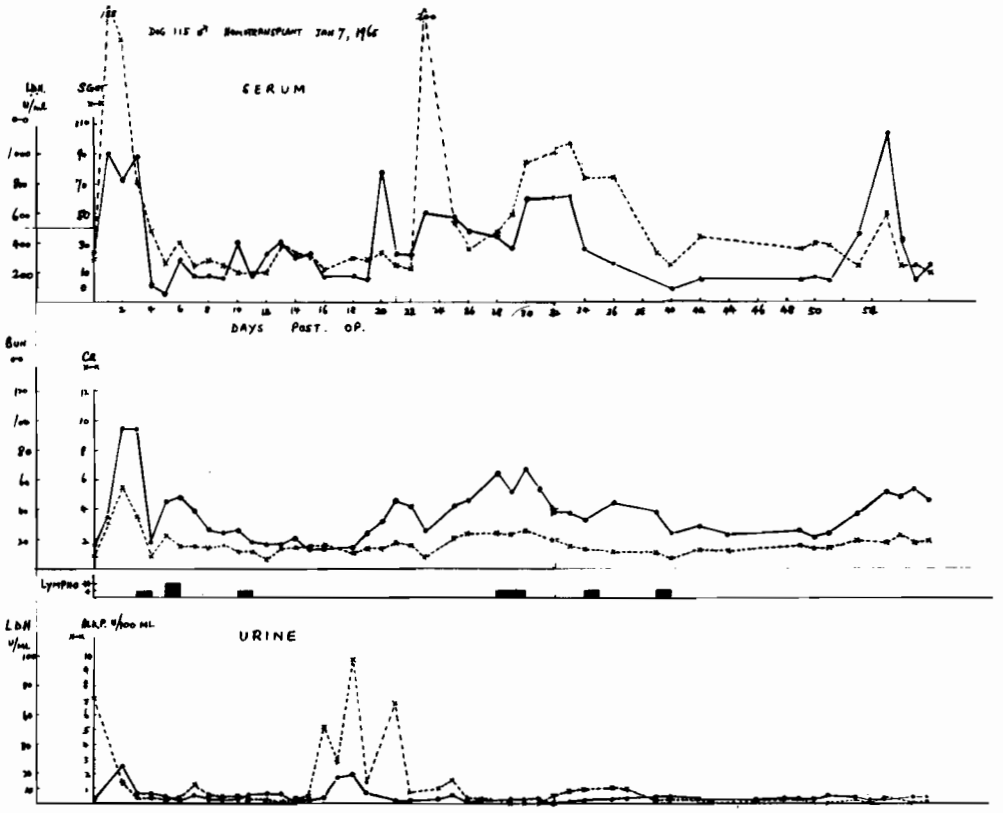


Fig. 18

Dog 115 with spontaneous remission of rejection crisis and prolonged survival.

BIBLIOGRAPHY

AMADOR, E., ZIMMERMAN, A. B., and WACKER, W. E. C. "Urinary alkaline phosphatase activity. I. Elevated urinary L.D.H. and alkaline phosphatase activities for the diagnosis of renal adenocarcinomas" J.A.M.A., 185: 769, 1963.

BAIN, B., VAS, M.R. and LOWENSTEIN, L. Blood, 23:108, 1964.

BERGER, L. and BROIDA, D. "The colorimetric determination of lactic dehydrogenase" Technical Bulletin No.500, 1957.

BURBANK, M. K., HUNT, J. C., TAUXE, W. Newton, and MAHER, F.T. "Radioisotope renography" Circulation, 27: 328, 1963.

COHEN, P. P. and HEKHIUS, G. L. "Rate of transamination in normal human tissues" J. Biol. Chem. 140: 711, 1941.

DEMPSTER, W. J. et al. "Rejection processes in human homotransplanted kidneys" B.M.J., 2: 969, 1964.

DOSSETOR, J. B. et al. "Cadaver renal homotransplants: initial experiences" Canadian Med. Ass. Journ. 91: 733, 1964.

GAULT, M. H. Unpublished data. Department of Medical Chemistry, Queen Mary Veterans Hospital, Montreal.

GORDON, J. Unpublished data. Department of Experimental Surgery, Royal Victoria Hospital, Montreal, 1964.

GORER, P. A., LOUTIT, J. F. and MICKLEM, H. S. "Proposed revisions of 'Transplantese'" Nature, 189: 1024-1025, 1961.

HARRISON, S. P. "Origins of vascular surgery: The Carrel-Guthrie letters" Surgery, 52: 406-418, 1962.

HAMBURGER, J. et al. "Transplantation d'un rein entre jumeaux non homozygotes apres irradiation du receveur" Presse Med. 67: 1771, 1959.

HAMBURGER, J. et al. "Kidney homotransplantation in man" Ann. N.Y. Acad. Sci. 99: 808, 1962.

HIRSCHHORN, K., BACH, F., KOLODNY, R.L., FIRSCHEIN, I.L., and HASHEM, N. Science, 142: 1185, 1963(a).

HIRSCHHORN, K., KOLODNY, R. L., HASHEM, N. and BACH, F. Lancet, 2: 305, 1963(b).

HOROWITZ, R. E. et al. "Immunocytochemical observations on canine kidney homografts" Fed. Proc. 22, Part I, 274, 1963.

HUME, D. M. et al. "Studies of renal homotransplantation in man" Annals N.Y. Acad. Sci., 120: 578, 1964.

HUME, D. M. et al. "Experience with renal homotransplantation in human" J. Clin. Inves. 34: 327, 1955.

KAUFMAN et al. "Lymphocytes in urine as an aid in the early detection of renal homograft rejection" Surg. Synec. and Obstet., 119: 25, 1964.

KOUNTZ, et al. "Mechanism of rejection of homotransplanted kidneys" Nature, 199: 4890, 1963.

JEEJEEHBOY, J. F. "Spontaneous reversal of homograft rejection" Transplantation, 3: 257, 1965.

MEDAWAR, P. B. "Behaviour and fate of skin autografts and skin homografts" J. Anat. 78: 176-199, 1944.

MITCHINSON, N. A. "Passive transfer of transplantation immunity" Nature, 171: 267-268, 1953.

MURRAY, J. E. et al. "Reversibility of the kidney homograft reaction by retransplantation and drug therapy" Am. N. Y. Acad. Sci. 99: 768, 1962.

NATHAN, P. "Antigen release from the transplanted dog kidney" Am. N. Y. Acad. Sci. 120: 458, 1964.

NEWTON, W. T. "The biological basis of tissue transplantation" Surg. Clinic of North America, 45: 393, 1965.

PORTER, K. A. et al. "Rejection of kidney homotransplants" Am. N. Y. Acad. Sci., 120: 472, 1964.

PROUT, G. R., MACALALAG, E. V. and HUME, D. M. "Serum and urinary lactic dehydrogenase in patients with renal homotransplants" Surgery, 56: 283, 1964.

RUDOLPH, L. A., SCHAEFER, J. A., DUTTON, R. E. Jr. and LYONS, R. H. "Serum glutamic oxalacetic transaminase in experimental tissue injry" J. Lab. and Clin. Med. 49: 31, 1957.

STARZL et al. "The reversal of rejection in human renal homografts with subsequent development of homograft tolerance" Surg. Synec. and Obstet., 117: 385, 1963.

TAYLOR, A. C. and LEHRFELD, J. W. "Definition of survival time of homografts" Am. New York Acad. Sci. 59: 351-360, 1955.

WACKER, W. E. C. et al. "Metallo-enzymes and myocardial infarction" New Eng. J. Med. 255: 449, 1956.

WACKER, W. E. C. et al. "Relation of copper to ceruloplasmin activity and zinc to malic and lactic dehydrogenase activity in acute myocardial infarction" J. Clin. Inv. 35: 741, 1956.

WACKER, W. E. C. and DORFMAN, L. E. "Urinary lactic dehydrogenase activity. I. Screening method for detection of cancer of kidneys and bladder" J.A.M.A. 181: 972, 1962.

WACKER, W. E. C., DORFMAN, L. E. and AMADOR, E. "Urinary lactic dehydrogenase activity. IV. Screening test for detection of renal disease dissociated and in association with arterial hypertension" J.A.M.A. 183: 671, 1964.

WEST, M. and ZIMMERMAN, H. G. "Serum enzymes in disease, IV, lactic dehydrogenase and glutamic oxalacetic transaminase levels in renal disease" J. Lab. and Clin. Med. 52: 185, 1958.

WILLIAMSON, C. S. "Some observations on the length of survival and function of homologous kidney transplants" (Preliminary report). J. Urol., 10: 275, 1923.

WROBLEWSKI, F. and LADUE, J. S. "Lactic dehydrogenase activity in blood" Proc. Soc. for Exp. Biol. and Med. 90: 210, 1955.

ZUKOSKI, C. F. Transplantation, 3: 118, 1965.

A P P E N D I X

APPENDIX I

WRIGHT STAIN TECHNIQUE FOR LYMPHOCYTES IN URINARY SEDIMENT

(1) Fresh urine, preferably an early morning specimen, is collected.

(2) 15 ml. of the urine is centrifuged at 2500 r.p.m. for 5 minutes.

(3) The supernatant is discarded.

(4) 2 drops of homologous human serum is added and sediment redissolved by finger flicking.

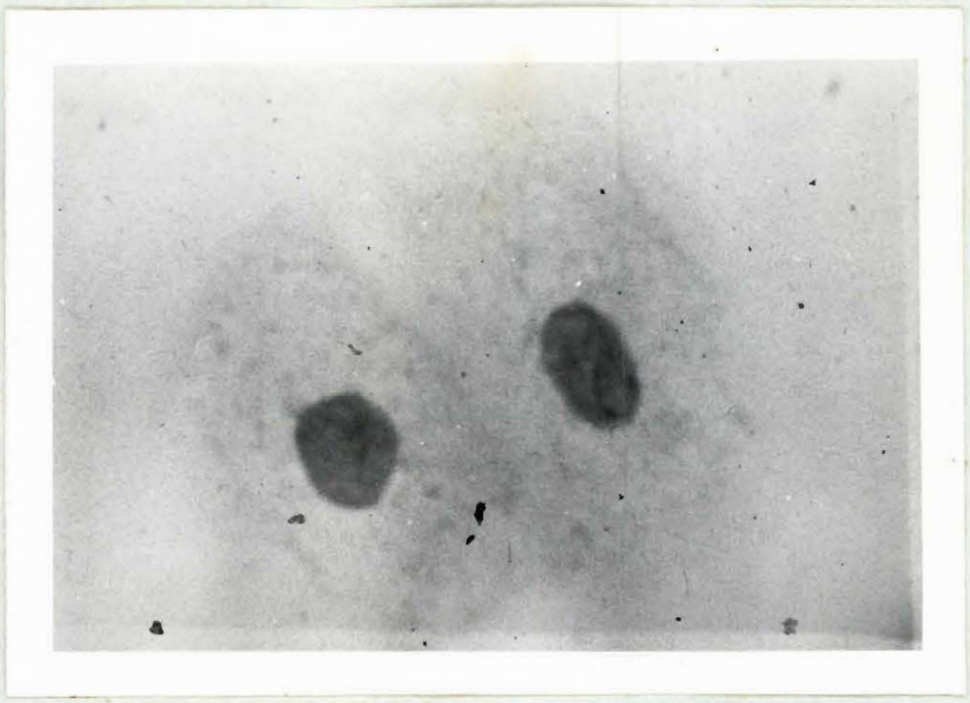
(5) Using a pipette, a small drop of the redissolved sediment is placed on a glass cover slip and two smears are made from it.

(6) The smears are air dried and then stained with Wright's stain. The staining time should be 75% of that used for blood smears; otherwise the cells become too deeply stained.

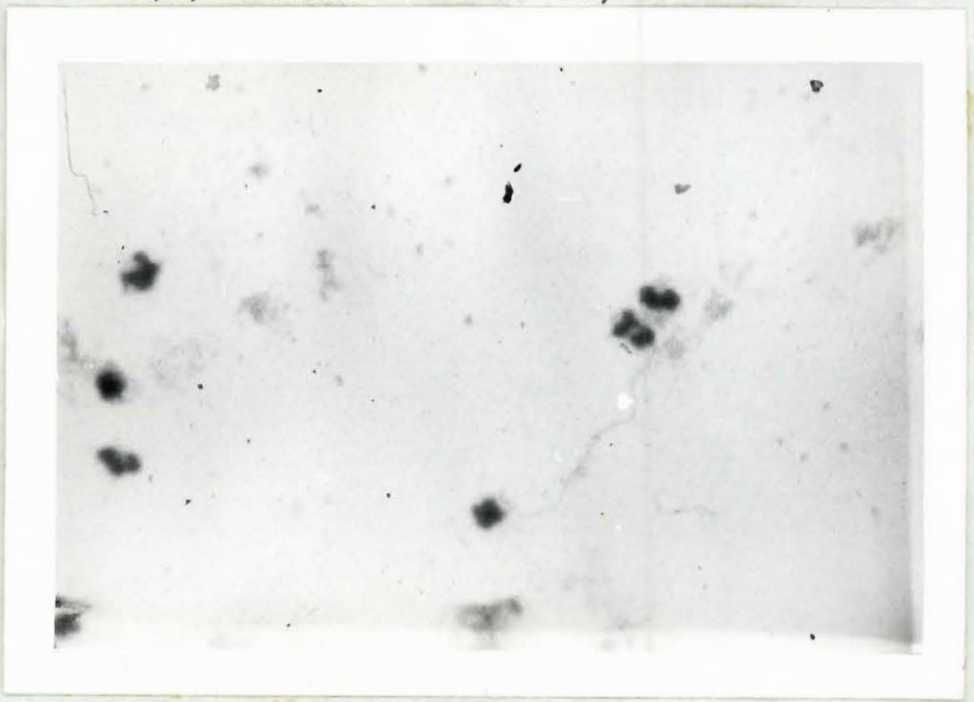
(7) Smears are dried by the use of blotting paper and then mounted on a glass slide.

APPENDIX II

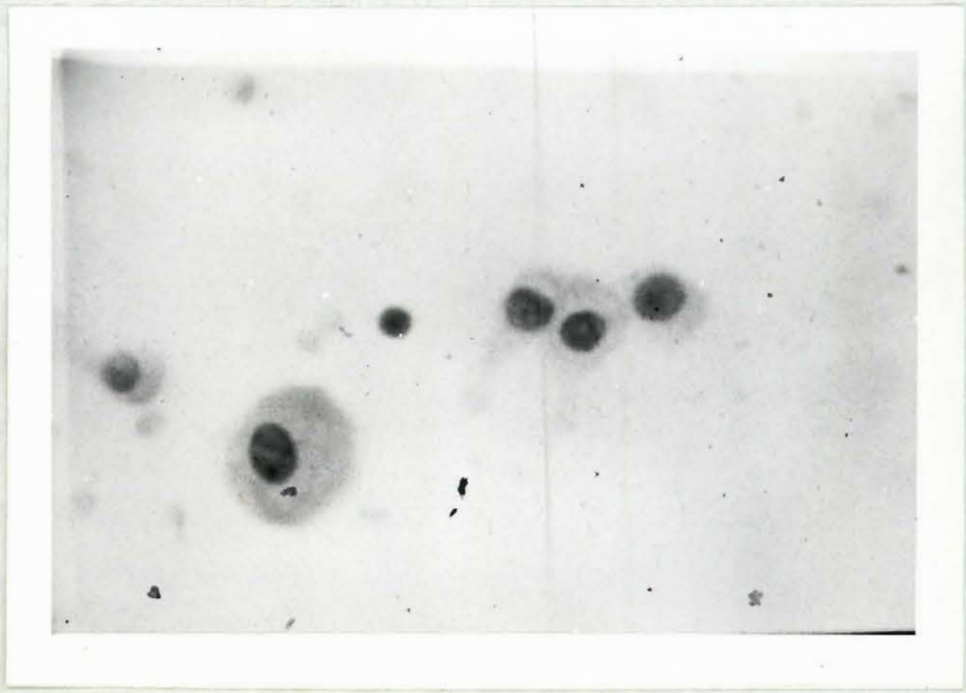
CELLULAR ELEMENTS IN URINARY SEDIMENT



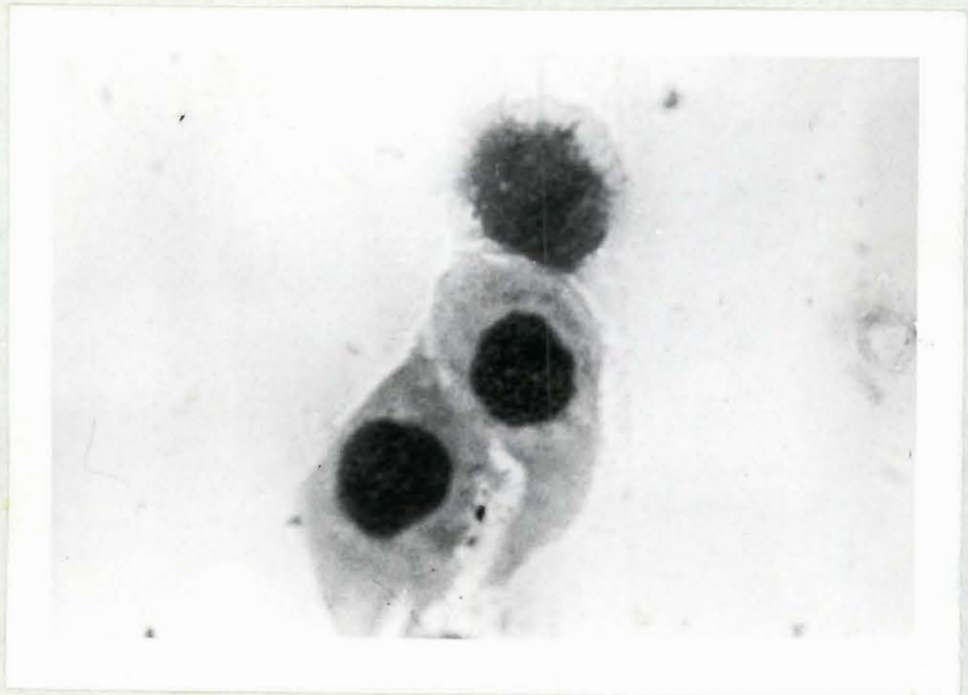
(a) Bladder cells x 800, oil immersion.



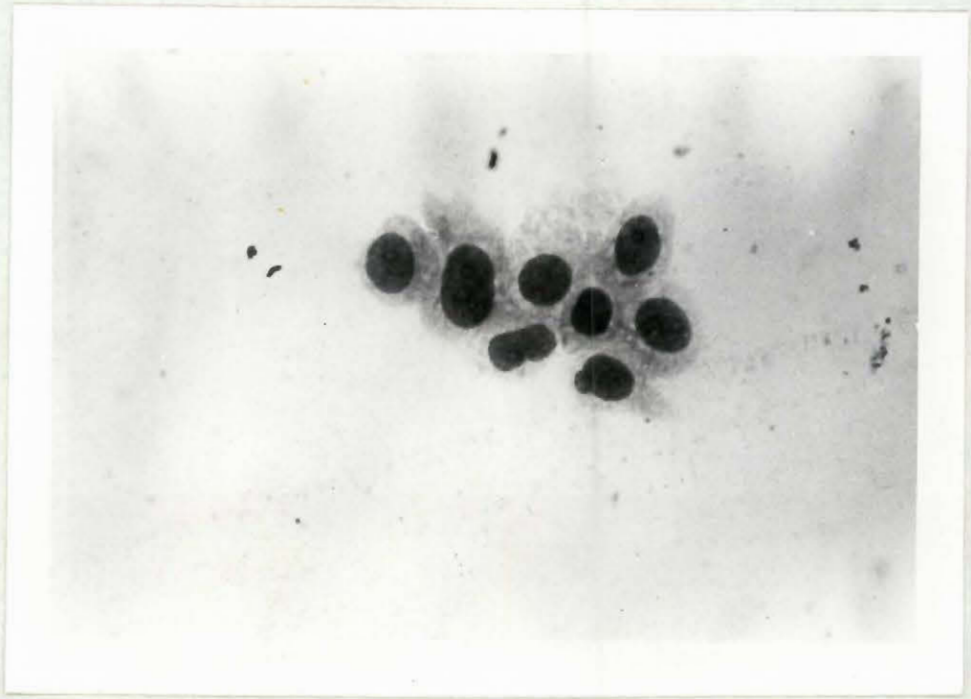
(b) Polymorphs in sediment HPF x 400



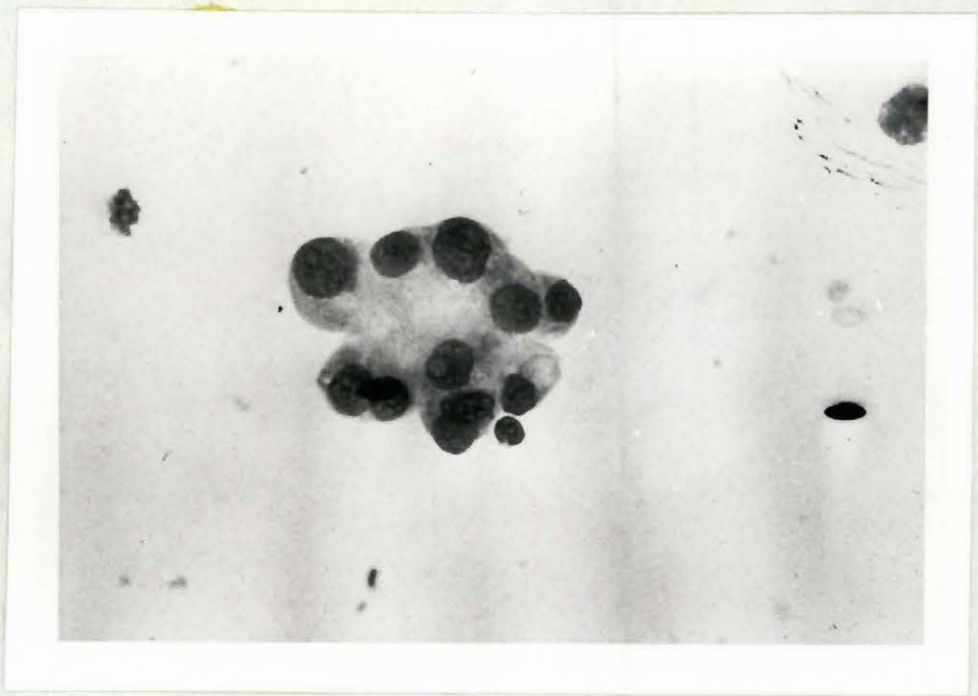
(c) Ureteral cells. Seapings taken from cadaveric ureter HPF x 400.



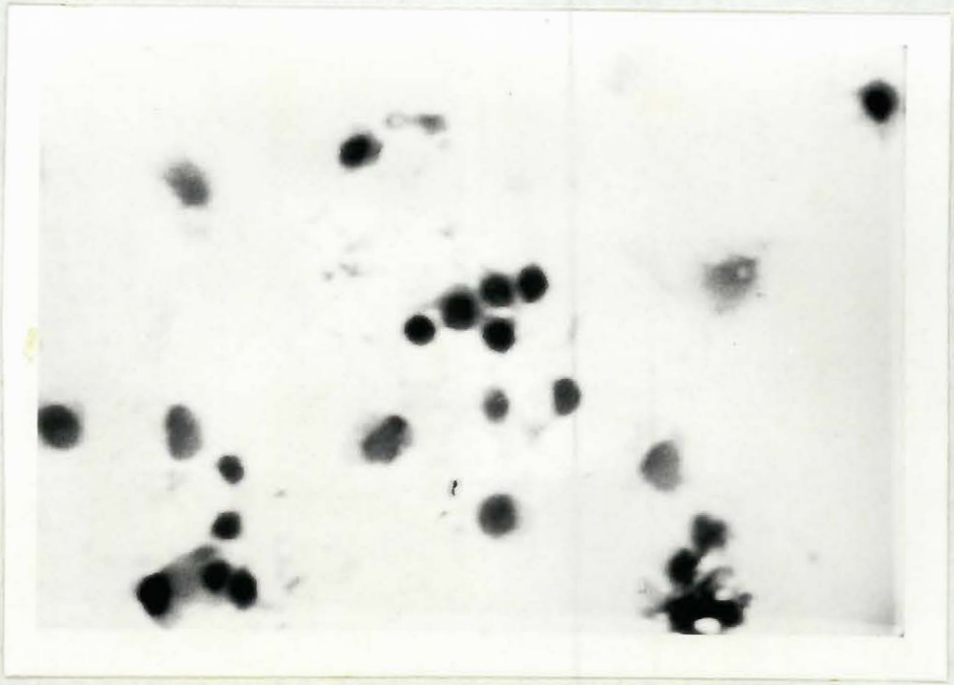
(d) Ureteral cells from sediment x 800 oil immersion.



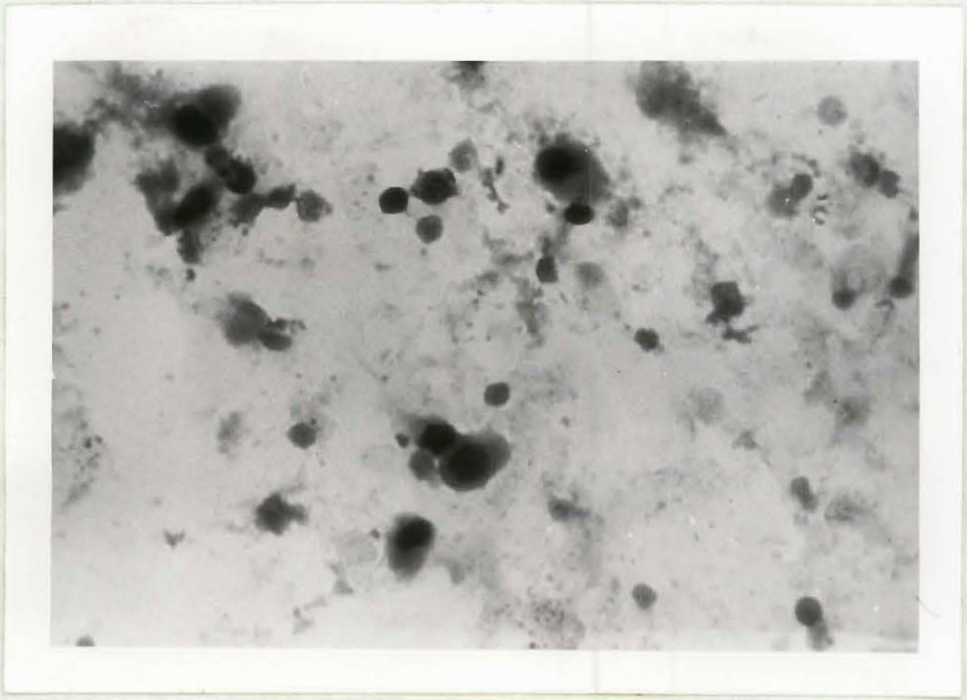
(e) Tubular epithelial cells, cortex scraping taken from cadaveric kidney HPF x 400.



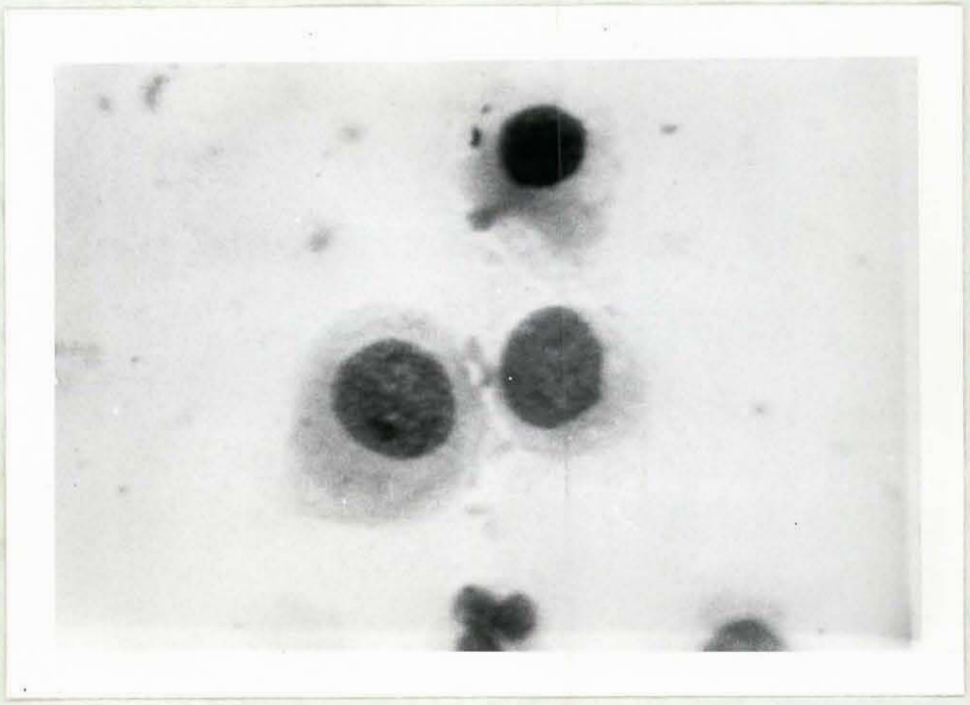
(f) Tubular epithelial cells from sediment HPF x 400.



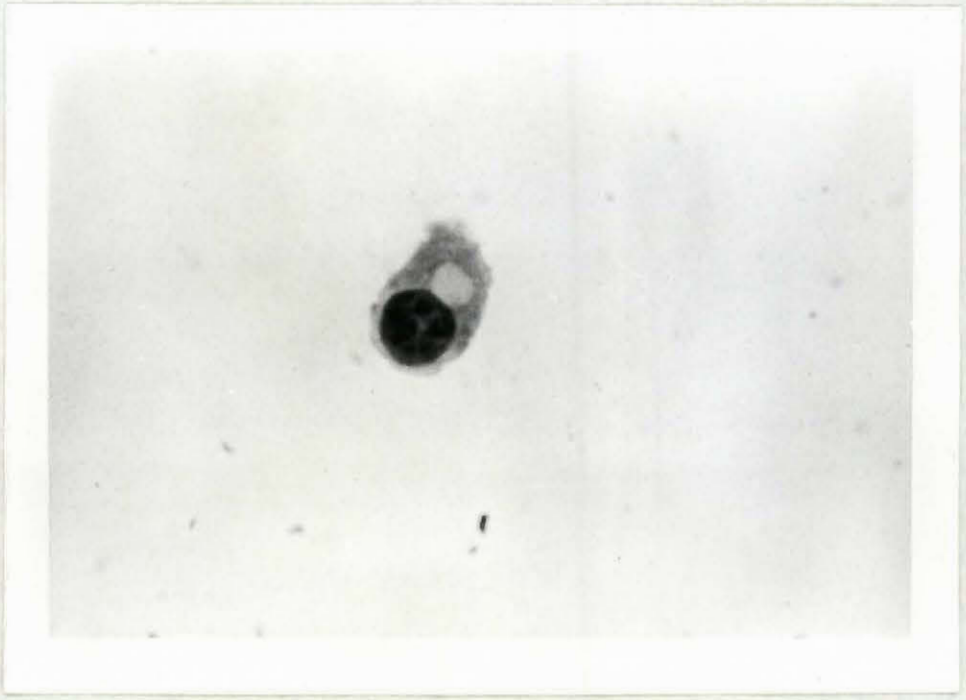
(g) Small lymphocytes in sediment. HPF x 400.



(h) Large lymphocytes in sediment. HPF x 400.



(i) Tubular cells x 800 oil immersion.



(j) A degenerating plasma cell in sediment, x 800 oil immersion.

APPENDIX III

GRADING OF ELEMENTS IN URINARY SEDIMENT

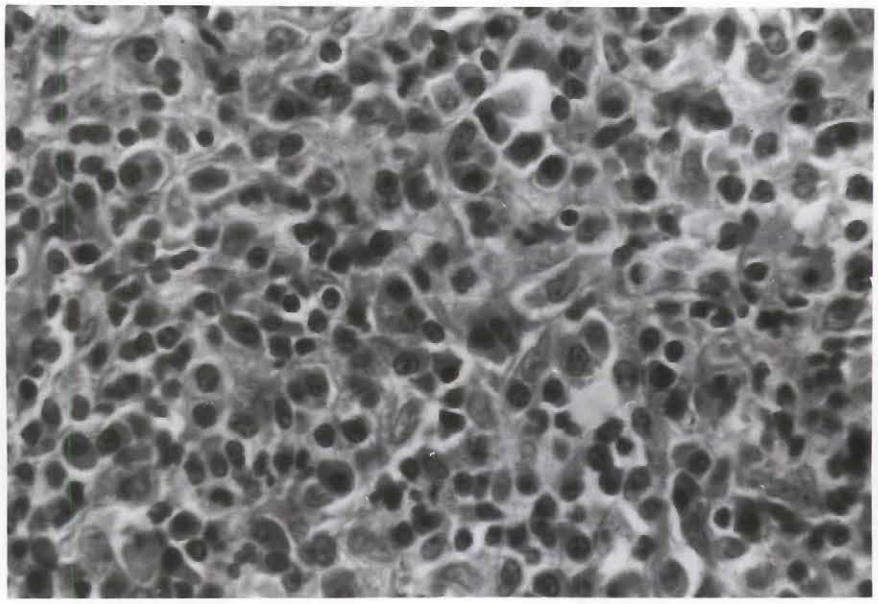
CASTS	occ. =	occasional
	1 =	1 cast/20 HPF
few	1^x =	2 - 3 casts/20 HPF
mod.	2^x =	5 - 10 "
many	3^x =	1 cast/HPF
loaded	4^x =	more than 1 cast/HPF.

RED CELLS	occ. =	occasional
	1 =	10 RBC/HPF
	1^x =	10 - 20 RBC/HPF
	2^x =	moderate
	3^x =	numerous
	4^x =	loaded.

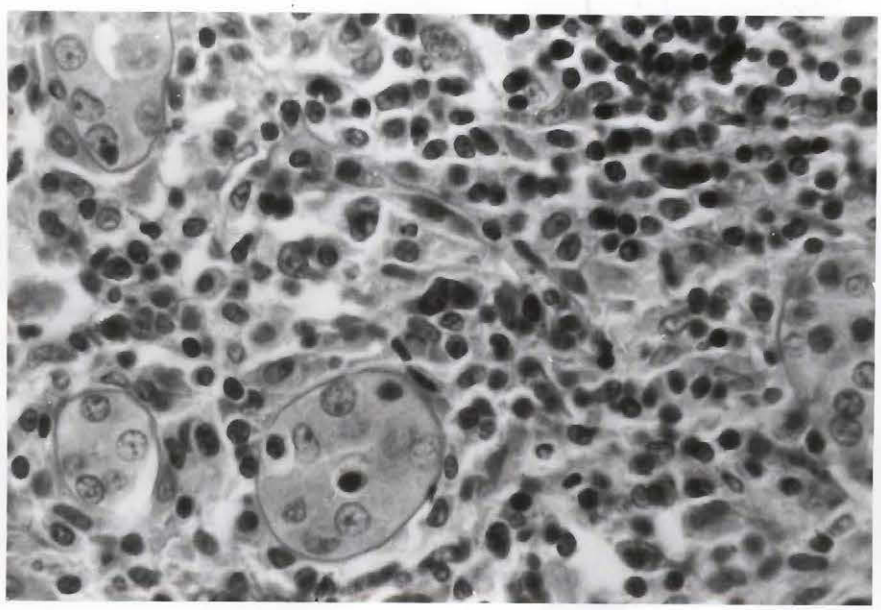
LYMPHOCYTIS	occ. =	occasional
	1^x =	over 1 cell/HPF
	2^x =	more than 3 cells/HPF.

APPENDIX IV

HISTOLOGY OF RENAL HOMOGRAFT REJECTION



A. Infiltration of plasma cells, large and small lymphocytes in rejection kidney HPF x 400.



B. Section showing lymphocyte in the lumen of tubule HPF x 400.

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I wish to thank Dr. E. D. Monaghan for his kind surgical assistance; Dr. M. H. Gault for his expert comments and for his allowing the use of the Department of Medical Chemistry, Queen Mary Veterans Hospital, for performing the biochemical estimation of the experiment. My appreciation goes to the staff of the surgical laboratory of the Royal Victoria Hospital.

C. G. Koo,

June, 1965.