RENAL HOMOTRANSPLANTATION: I. THE EFFECT OF CORTISONE ON THE TRANSPLANT; II. THE EFFECT OF THE TRANSPLANT ON THE HOST*

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RENAL HOMOTRANSPLANTATION remains one of the most intriguing problems which faces the urologist. The benefits to be derived from the fulfillment of this ideal and the new surgical horizons that would be opened are obvious. However, it is apparent that to date all attempts to accomplish this have met with failure. Two major factors are to be considered in an attack upon this problem: first, the purely technical, and second, the means for circumventing the profound systemic and local response which manifests itself both in the host and donor organ following homotransplantation. That the first of these problems is immense becomes apparent when one reviews the results obtained by other workers[1-12, 15-17, 19-23] who have performed renal *autotransplants* where the problems inherent in the homologous transplantation of tissues are not present. It is notable that since the classic experiments of Carrel and his coworkers,[1-7] only two other investigators have reported survivals of more than one year of an autotransplant. However, it is also apparent that even in those cases in which there appears to be a successful reconstitution of the pedicle, *i.e.*, where we are, in fact, dealing with a denervated kidney (since the nerve plexuses cannot be reanastomosed and do not regenerate), there appear certain functional peculiarities.

The accumulation of data from the work of several separate investigators[12, 15, 16, 17, 21, 22, 23] shows clearly that the *autotransplant* is unable to concentrate urine, unable to reabsorb sodium and chloride efficiently, and unable to manufacture ammonia. The titrable acidity of the urine excreted is approximately one-third of normal, and urea, urea nitrogen, and creatinine are excreted in copious amounts regardless of the prevailing nitrogen balance of the animal. From these observations, it appears that such kidneys suffer primarily a severe derangement of tubular function. This also holds true for the renal *homotransplant* which, according to Dempster,[12] behaves in exactly the same way until the additional factors associated with homotransplantation appear.

The nature of the disintegration which takes place following homotransplantation is at present still the subject of controversy. The prevailing opinion is that there is a tissue specific reaction which manifests itself in the host organism by a generalized systemic response and in the transplant by a local cellular response. This is thought to be basically an antigen-antibody reaction which is intimately bound up with the reticulo-endothelial system.[13] In homotransplantation of the kidney, however, this reaction appears to be further complicated by the presence of a multi-enzyme system with which, for the greater part, we are still completely unfamiliar. The remarkable specificity of the enzyme systems in general and their susceptibility to even the mildest changes in their environment need no elucidation.

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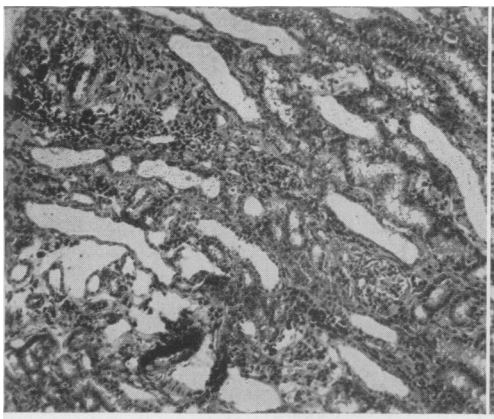

FIG 1

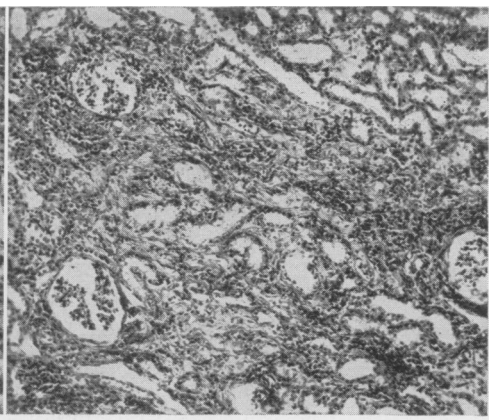

FIG. 2

FIG. 1. Renal homotransplantation to the neck. No cortisone given. Other kidney intact. Twenty days. The small round cell infiltration is apparent (H and E, X 150).
FIG. 2. Renal homotransplant to the neck. No cortisone administered. Only kidney. Twelve days. Note similar appearance to Figure 1 (H and E, X 150).

In this study an effort is made (1) to test the effects of cortisone upon the survival of renal homotransplants and (2) to determine in part the nature of the reaction of the host to the transplant. Because in several earlier pilot studies we were impressed, as was Dempster,[12] by the profound stress to which the animals appeared to be subjected following transplantation, it appeared advisable to study some aspects of adrenal function in the host animals at the time of the homotransplantation.

I. Effect of Cortisone on the Survival of the Transplant

Method

Mongrel bitches selected for equality in size and weight were used. Most of the animals were large, averaging eight to nine kilos. All animals were fed the same diet consisting of Animal Foundation Laboratory pellets varied with Ken-l-Ration. Water was allowed *ad libitum*. After preliminary one-to-two-day acclimatization, during which period daily eosinophil counts and serum sodium and potassium determinations were done, each animal was anesthetized with intravenous sodium pentobarbital and the right kidney removed. Portions of this kidney were preserved in 10 per cent formalin for study.

The dogs were allowed to recover from the right nephrectomy for a period varying from five to ten days, and were then readied for the transplantations. The animals were anesthetized in pairs with intravenous sodium pentobarbital and placed upon the same operating table with their heads adjacent to each other. The usual procedure involved simultaneous transplantation of the left kidney of dog A to the neck of dog B, while the left kidney of dog B was moved to the neck of dog A.

The abdomen and left side of the neck of each dog were shaved and surgically prepared. With two teams at work, each dog was submitted to the following procedure: Through a left-flank incision, the left kidney was exposed and the pedicle defined and stripped of all its adventitia. A wet saline pack was then placed over the incision and attention directed to the left side of the neck where an incision was made directly over the carotid sheath. The external jugular vein was identified and freed up for a distance of one and one-half inches. All tributaries encountered were ligated. The common carotid artery was then defined at a deeper plane and similarly mobilized. Both

TABLE I. *Kidney Homotransplants. Total: 25; 3 Operative Deaths.*

	Total	Length of life in days	Postmortem Findings: Art. Thr.	Ven. Thr.	Infection
(A) OtherKidney intact:	3	4	−	−	++++
		20	Yes	−	++
		12	Yes	Yes	++++
(B) Only Kidney: S Cortisone	16	2	Yes	Yes	−−−−
		2	Yes	−−−−−−	
		3	−−−	Yes	+++
		3	Yes	Yes	+++++
		3	−	Yes	+++
		4	Yes	Yes	+++
		4	"	"	"
		4	"	"	"
		4	"	"	"
		4	−	Yes	++
		6	Yes	Yes	++++++
		6	"	−	++++++
		10	Yes	−−	+++++++
		10	Yes	−−−	+++++++
		12	−−−−−−		+++++++++
(C) Cortisone 10 mg/kilo/day	6	3	Yes	−−−	++++++
		3	Yes	Yes	+++++++
		4	Yes	Yes	++++++++
		5	Yes	Yes	+++++++++
		5	Yes	−−−	+++++++
		21	−−−−−−		+++++++++

The high incidence of thrombosis and infection is apparent. In group A, the survival time refers to the homotransplant, in group B, to the animal.

vessels were then ligated as far cranially as possible and a rubbershod bulldog clamp placed as far caudally as possible. The vessels were then cut as close to the cranially placed ligature as was advisable. The lumens were irrigated with normal saline to prevent the formation of a thrombus between the cut ends and the site of the previously placed bulldog clamps. The adventitial layers were then stripped off the vessels by sharp dissection. Separate 6–0 arterial silk sutures with a swedged-on needle were then placed at opposite ends of the cut edges of the artery and vein and left untied.

Attention was then redirected to the previously exposed kidney and its pedicle. The renal artery and vein were ligated and cut as close as possible to their origin from the great vessels of the abdomen. The kidney was lifted from the abdomen and the ureter cut three to four inches distal to the kidney and allowed to drop back into the wound. The kidney was wrapped in warm saline gauze and transferred to the neck of the other dog where the adventitial layers of the vessels were stripped by sharp dissection. The previously placed arterial silk sutures in the cervical vessels were then passed through the coapting site on the renal vessels and tied. The short loose ends were retained as stays and the anastomosis between the cut ends of the arteries completed by a running everting whip stitch which was terminated at the opposite end by tying it to the stay suture; the time required to reestablish the arterial continuity was usually about eight to ten minutes. Immediately following this, the bulldog clamp on the artery was released for a few seconds and arterial blood allowed to perfuse the kidney. Blood was invariably seen to flow from the cut venous ends. The clamp was then re-applied and a similar anastomosis carried out between the cut ends of the veins.

The total time of anastomosis of the vascular pedicle usually consumed between 20 and 30 minutes. After the kidney was wrapped in warm saline and allowed to distend with blood, a subcutaneous pocket sufficiently large to accommodate it without undue pressure was made in the neck and the kidney was then placed therein. The free end of the ureter, which at this time was usually seen to be putting out urine, was then catheterized with a small polyethylene catheter and brought to the exterior through a stab wound. The cervical and abdominal wounds were then closed in the usual fashion with interrupted silk sutures. Each animal was then given 300,000 units of penicillin and returned to its cage, where no further supportive measures were employed other than the cortisone which was used in the one study group. This group was given cortisone at a dosage level of 10 mg./Kg. body weight per day.

Where possible, the kidneys were sectioned and stained with hematoxylin and eosin for routine postmortem histological

examination, and also with phloxine and methylene blue for specific identification of eosinophils if present.

RESULTS

Twenty-five transplants were completed in both studies and functioned for periods varying from 24 hours to 21 days (Fig. 1). There were three operative deaths. In three cases, the homotransplants were done while one normal kidney remained *in situ*. In 16 cases, the transplant was done following primary nephrectomy but without the benefit of pre- or postoperative cortisone. In this group, which served as controls, the renal homotransplant represented the only renal tissue available to support the life of the animal. In six cases, the animals received pre- and postoperative cortisone following homotransplantation of the sole remaining kidney. The longest survival noted in the group which had not received cortisone was 20 days. The longest survival noted in the cortisone-treated group was 21 days. In only three cases was there no evidence microscopically and macroscopically of thrombosis of the pedicle. Two cases were noted in the group which did not receive cortisone, one of these animals living but four days and dying of intercurrent disease, the other living for 12 days with a single homotransplant and dying of renal failure. One animal treated with cortisone lived 21 days with a single homotransplant, and then died of renal failure without evidence of thrombosis. All untreated animals surviving more than three days developed the marked systemic disturbances noted by other observers, becoming rapidly and progressively lethargic, anorexic, pyrexial, rapidly losing weight and ultimately becoming cachectic and emaciated. These features developed despite an apparently adequate urinary output during the initial period. It is also of interest to note that, in the cases in which the remaining kidney had not been removed, these features also developed and disappeared only after the homotransplant in the neck had ceased to function. In all the cases, the urine from the transplanted kidney maintained a persistently low specific gravity and showed a progressively increasing albuminuria and pyuria. Many of the cases in the initial days gave a positive Benedict test for reducing substances. The ureters soon developed a grayish slough at their ostia which rapidly produced ureteral obstruction unless the lumen was kept open by the polyethylene catheters. In only one case of the cortisone-treated group was the postoperative course different. This animal did not develop the marked systemic changes noted above, the animal appearing healthy and lively for 18 days, after which it developed the features of impending renal failure and died three days later. This animal also showed the presence of marked albuminuria and pyuria. The ureter, however, at all times looked healthy, with red granulations around it, and at no time developed the gray slough noted above.

FIG. 3. Renal homotransplant to the neck. Only kidney. Twenty-one days. Pre- and postoperative cortisone. Note characteristic "tubercle" with healthy-appearing tubules and glomeruli surrounding it (H and E, X 150).

Microscopically (Figs. 2 and 3) the kidneys of the untreated group showed evidence of a progressive small round cell infiltration similar to that described by numerous other observers. This was associated

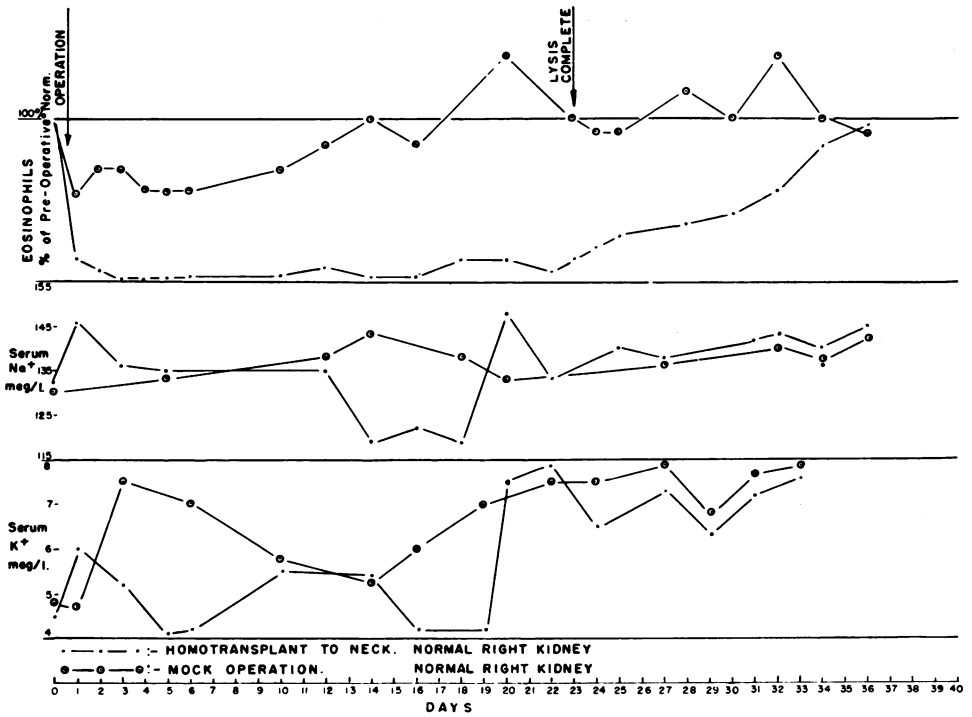

FIG. 4

with marked cytoplasmic vacuolation, desquamation, and loss of definition of all the tubular elements. Those tubules which still appeared to maintain their structure were filled with desquamated material and casts. In contrast to this, the glomeruli appeared singularly free from evidence of disease. In those cases which remained viable for any length of time, there was also evidence of multiple small areas of necrosis and infarction scattered throughout the cortex and, in some cases, in the medulla. In one case in the cortisone-treated group where the animal lived 21 days, the kidney did not show this microscopic appearance (Fig. 4). The glomeruli and tubules appeared histologically normal. The previously noted small round cell infiltration was not present. However, scattered throughout the renal cortex there were numerous "tubercles" consisting of a centrum of "epithelioid" cells surrounded by a layer of small lymphocytes. No multinucleated giant cells were seen. All the homotransplants were also stained for eosinophils. In none of the sections were these cells noted.

II. Effect of the Transplant on the Host.

Method

The untreated control group of cases were used for studies on the eosinophils,

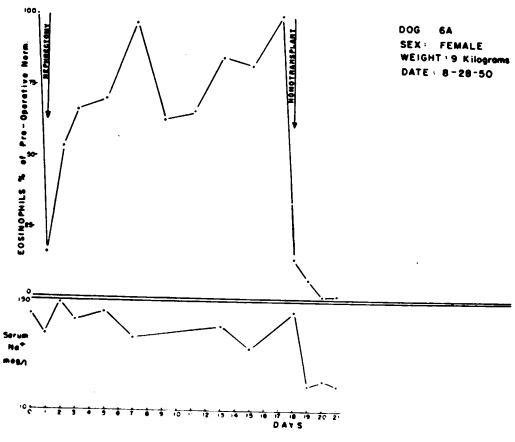

FIG. 5

serum sodium, and serum potassium. Eosinophils were determined according to the method of Randolph,[18] using phloxine and the methylene blue as a counterstain, the results being expressed as a percentage of

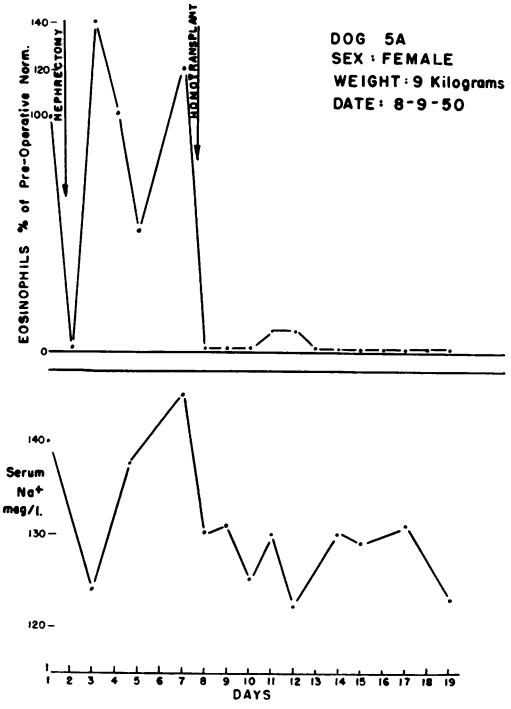

FIG. 6

the preoperative norm. The preoperative norm was the established mean of three readings taken prior to the initial nephrectomy. The serum sodium and potassium were determined upon the Perkin-Elmer Flame Photometer Model 52A, using lithium nitrate as an internal standard, the blood for these readings being drawn at identical times each morning.

RESULTS

Eosinophil response. After the preliminary nephrectomy, there was the usual immediate fall in the eosinophils followed by a subsequent return to normal levels as reported by numerous observers.[8] Following the *homotransplantation,* however, the animals developed an immediate absolute eosinopenia (Figs. 5, 6 and 7). The eosinophils failed to reappear in the blood stream up to the time of death of the animal or transplant. This picture was not limited to those cases in whom a preliminary nephrectomy was done but also observed in the three cases in which the other kidney was preserved (Fig. 5). In one instance where, by virtue of an anatomical deformity of the renal vascular supply, one animal could not be subjected to homotransplantation after the preliminary dissection *in tandem* with another animal, an absolute eosinopenia never developed despite the fact that the animal had been subjected to the same degree of operative trauma over the same period of time as its mate, in whom this eosinopenia developed very rapidly (Fig. 5). Furthermore, in this case after the homotransplanted kidney had commenced to slough, there was a progressive increase in the blood eosinophil level until it ultimately reached the preoperative norm. It appeared that the persistence of the eosinopenia was contingent on the completion of the homotransplantation. That this picture does not develop in autotransplanted kidneys is apparent from Figure 8, where the only kidney of the animal was transplanted to its own neck. It will be noted that throughout a period of 19 postoperative days, severe eosinopenia was never observed until the day of the death of the animal.

Serum Sodium. This also showed a pattern in many respects very similar to that noted with the eosinophils. Immediately following the homotransplant the level fell well below the preoperative norm and remained low until the demise of the animal (Figs. 6 and 7) or the sloughing of the transplant (Fig. 5). However, at no time did the readings reach critical levels.

Serum Potassium. These readings showed no characteristics which could be attributed to the homotransplantation *per se.* All of the animals in the terminal stages of the procedure when the kidney had commenced to

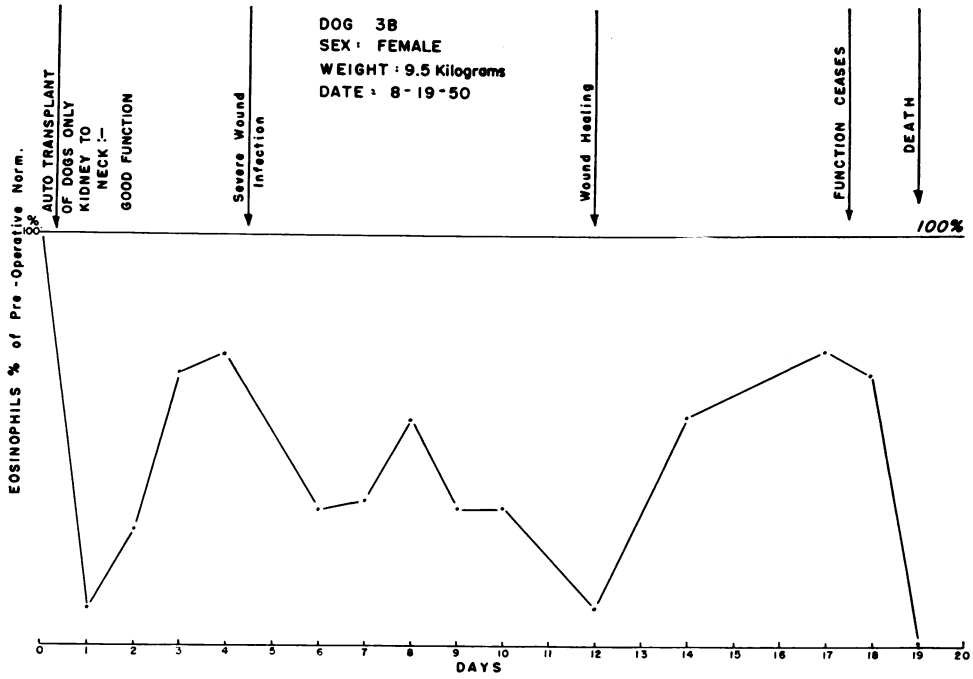

FIG. 7

infarct, showed a progressively increasing hyperkalemia which reached its height shortly before the death of the animal.

DISCUSSION

It is apparent that in the dog the results obtained by renal homotransplantation are extremely difficult to evaluate in the light of the observed technical difficulties associated with the vascular anastomosis. The occurrence of infarction and infection and their effect upon the transplant cloud the picture, and one is hard put to ascribe the histological changes noted to the reaction to the homotransplant *per se* or to the results of infection and/or infarction. However, with at least as severe an infection as in the other cases, there was a marked difference histologically between the kidney of the untreated homotransplant, and the kidney of the cortisone-treated homotransplant, despite the fact that the last-named had survived eight days longer.

The cortisone-treated case surviving 21 days showed large areas of histologically normal appearing tissue and where there were changes, these were well localized into "tubercles." The cortisone-treated homotransplant was, however, still not able to maintain the life of the animal, indicating that the apparent improvement histologically was not reflected in a concomitant improvement of function. In the light of our observations regarding the remarkable specificity of the enzyme system in general and, in all likelihood, in the kidney, too, this is not too surprising. It appears that further progress in the understanding of the changes that occur in the homotransplanted kidney must await a better understanding of these enzyme systems in the normal kidney.

The profound eosinopenia noted in the renal homotransplants appears to be but another feature of the severe stress which the act of renal homotransplantation produces in the animals. This feature was also noted in the renal and perirenal tissues of the homotransplant where specific staining failed to reveal the presence of any eosinophils. This is in contrast to the increasing eosino-

phila noted at the line of junction between a skin homograft and the host tissues. If the eosinopenia of circulating blood is due to the severe stress and, therefore, the result of adrenal hyperactivity, then the hyponatremia which these animals develop is probably due to intracellular sodium retention. The hyponatremia occurs very rapidly after the operation—too soon to be accounted for by loss *via* the urine as noted by Wu and Mann,[23] and too soon to be due to loss *via* the gastric juices. It is known that this intracellular retention can occur to a marked extent without the development of edema, and the onset of the edema could well be masked in the early stages where it has been noted that the animal excretes large amounts of very dilute urine from the homotransplant. The terminal hyperkalemia can well be secondary to the serum sodium depletion but is probably only a terminal feature in the developing renal failure.

If the mere act of renal homotransplantation results in marked adrenal hyperactivity to the detriment of the animal, it appears that some preoperative measures should be instituted to dampen adrenal activity more effectively than we have done. It would seem necessary to administer larger amounts of cortisone over much longer periods of time and to thereby get a more marked "dampening" effect on the adrenal before attempting this procedure.

The use of cortisone over a long preoperative period is a serious factor to contend with when applied to clinical medicine where the majority of the cases which would conceivably come to homotransplantation are those in which there is severe permanent renal damage. Fortunately, resultant upon the work of Hume, *et al.*,[14] who have done a series of well-controlled studies on human homotransplantations in terminal glomerulonephritics, there are indications that the human does not react with such severe systemic changes as have been noted in the dog. It is, of course, conceivable that the reaction was to some extent masked in these patients by the very nature of the severity of their illness, and there is also the likelihood that there had already developed a considerable degree of adrenal cortical exhaustion in these cases and that these patients were just not capable of the response shown in the case of the relatively healthy animals. Hume and his co-workers did not report on the adrenal response in their cases, and this information may well be enlightening.

The ultimate aim of those attempting renal homotransplantation should be to replace the diseased kidney by a healthy one fully capable of performing the numerous complex functions required of this organ and maintaining the health of the host. It is our opinion that the successful resolution of this problem is dependent upon a careful investigation of the fundamental changes that occur in the host and the homotransplant subsequent to the transplantation.

SUMMARY

1. The results following 25 canine renal homotransplantations are reported.

2. The effect of pre- and postoperative cortisone on the function, histologic appearance, and survival of six transplants are presented.

3. The blood eosinophil, serum sodium and serum potassium levels were studies in 16 untreated cases.

4. The difficulty of evaluating the histologic changes of the homotransplanted kidney is noted.

5. The role of the adrenal gland in the response of the host to the renal homotransplant is commented upon.

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