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Metabolism of the Transplanted Dog Kidney. (27746)

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Organs and tissues transplanted from one dog to another are usually rejected by the recipients in 5 to 10 days. In the present work, a search was made for a correlation between biochemical changes and the course of tissue rejection in homologous transplants of dog kidneys to provide some insight into the mechanism of homotransplant destruction. The kidney was selected for study because well-established methods are available for determination of renal function and metabolism. Our results show that several sensitive measures of cellular function and metabolism in tissue from transplanted kidneys remain relatively unaffected by the rejection process. Even during accelerated rejection of a kidney in a specifically immunized animal, the metabolism of tissue measured *in vitro* remains within the range observed for normal tissue. Because damage to the parenchyma by a cytotoxin would very likely manifest itself in changed metabolic activity, another cause for tissue rejection is postulated. It is suggested that interference with cell nutrition might constitute the primary mechanism responsible for rejection of homologous kidney transplants.

Methods. Adult mongrel dogs from different sources were employed in these experiments. Vascular anastomoses were performed between the renal vessels of freshly removed donor kidneys and the iliac artery and vein of the recipient (first-set transplant). The kidney was placed in the abdominal cavity and its ureter exteriorized through a stab wound. These transplants were removed for metabolic studies one day after the operation.

Specific sensitization was produced in a series of animals by allowing the homologous transplants to remain in place until urine flow ceased. This usually occurred within a week after transplantation. If routine inspection showed an abscess, these kidneys were removed; otherwise, they were left undisturbed. Within 4 weeks of rejection of the primary transplant, the second kidney from the donor was transferred to the specifically immunized recipient (second-set transplant). These second-set transplants were then removed for the metabolic studies 20 to 24 hours after the operation, when the rejection appeared to be well advanced as evidenced by gross hemorrhage in the kidney tissue and a reduced flow of urine.

Primary sensitization was also achieved in 5 dogs by cross perfusion for one hour between donor and recipient(1). One week later the second-set kidney was introduced into the recipient.

Control autotransplants were prepared by

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removing one of the dog's kidneys and reconnecting the renal vessels to the iliac artery and vein of the same animal. The ureter was exteriorized as in the homotransplant operation.

The para-aminohippurate (PAH) accumulation by slices of renal cortex was measured by the method of Cross and Taggart(2). The tissue slices were incubated for one hour at 37°C. The results are expressed as the slice-to-medium concentration ratio (S/M), where S equals micrograms PAH per gram wet weight of tissue and M equals micrograms PAH per milliliter of incubation medium.

Respiration studies of renal cortical slices were carried out by the Warburg technic at a temperature of 37°C in an atmosphere of oxygen. The suspending medium consisted of Krebs-Ringer phosphate to which was added 10 mM acetate as substrate.

In 3 experiments the recipients received an intravenous injection of 0.5 to 1.0 millicurie P^{32} as NaH_2PO_4 after the surgical completion of the second-set operation. Twenty-four hours later tissues were analyzed for incorporation of P^{32} into the acid and lipid soluble and the nucleic acid fractions(3). The specific activities obtained in the transplanted organ were compared with normal kidneys obtained from the specifically immunized host.

Results. Table I presents data for *in vitro* accumulation of PAH by dog kidney tissue removed one day after both first and second-set types of transplantation. Seven kidneys transferred to specifically immunized hosts, after primary renal transplants had been rejected, gave an average S/M value for PAH of 7.2 while that for the slices of normal kidney was 9.3 ($P = .05$). S/M for 7 first-set homotransplant kidneys was 9.5. A comparison of the means of the second-set kidneys mentioned above to first-set homotransplants again gives a P value of 0.05. The S/M of 2 second-set kidneys in cross-perfused animals were practically identical with those of first-set homotransplants. Autotransplants in 7 experiments gave an average S/M value of 6.7, similar to the mean observed with second-set organs.

The average S/M ratio for first-set kidneys is 9.5, compared to 6.7 for the autotransplants ($P<0.01$). The lower activity of the autotransplant, as compared to the first-set transplants, may be due to the greater trauma of the autotransplantation procedure which requires that the kidney be removed and reanastomosed as compared to the homotransplant operation in which the recipient simply receives a kidney.

TABLE I. Accumulation of PAH by Dog Kidney Slices Expressed as Slice-to-Medium Ratio (S/M). Transplants were removed one day after operation.

	No. of kidneys	S/M Avg*	S/M Standard deviation
Normal kidneys	38	9.3	2.4
First-set homotransplants	7	9.5	1.8
Autotransplants	7	6.7	1.4
Second-set to primary kidney transplant†	7	7.2	2.1
Transplantation after cross perfusion‡	2	10.6	—

* P values: first-set *versus* autotransplants $<.01$; first-set *versus* second-set .05; and autotransplant *versus* second-set $>.05$.

† The first kidney from a specific donor was rejected before second kidney was transplanted.

‡ Donor and host were cross-perfused a week before kidney was transplanted.

Table II presents results of respiration measurements on renal cortical slices. Average oxygen consumption values for second-set kidneys, 205 μl O_2/g dry wt/minute, do not differ from first-set homotransplants, 207 μl O_2/g dry wt/minute. Second-set kidneys to cross-perfused hosts also gave a mean value of 207. The oxygen metabolism in 2 autotransplants was slightly higher than the homotransplants, but their mean of 225 was within the range observed for homotrans-

TABLE II. Oxygen Consumption in μl O_2/g Dry wt/min of Renal Cortical Tissue. Transplants were removed one day after transfer to the host.

	No. of transplants	O_2 consumption Avg	O_2 consumption Range
Normal kidneys	12	266	247-312
First-set homotransplants	4	207	164-233
Autotransplants	2	225	223-226
Second-set to primary kidney transplant*	7	205	148-229
Transplantation after cross-perfusion†	3	207	130-233

* First kidney from a specific donor was rejected before second kidney was transplanted.

† Donor and host were cross-perfused a week before kidney was transplanted.

TABLE III. Specific Activity (Counts/min/μg Phosphorus) in Second-set and Host Kidneys One Day After Transplantation.*

	Exp 1		Exp 2		Exp 3	
	Transplant	Host	Transplant	Host	Transplant	Host
Acid soluble phosphorus	30	23	13	16	37	40
Lipid " "	21	19	13	13	23	24
Nucleic acid "	9	8	13	8	11	7

* (0.5 to 1.0 millicuries of P^{32} as NaH_2PO_4 were injected into each recipient at conclusion of the second-set operation. Plasma phosphorus values in counts/min/μg phosphorus: first exp 24; second exp 13; third exp 27.)

plants. The mean oxidative metabolism in slices from normal control kidneys, 266 μl/g dry wt/minute, is higher than that observed with the transplants.

Table III presents data from 3 experiments in which each recipient was injected with radioactive phosphorus at the time of the second-set transplantation. Specific activities of the acid soluble and the lipid soluble fractions were similar for transplants and host kidneys. The combined ribonucleic acid and desoxyribonucleic acid phosphorus showed a slightly elevated activity in the homotransplants, perhaps reflecting the invasion of the transplant by white cells of the host.

Discussion. Biochemical measurements on renal tissue from kidneys transplanted to specifically immunized dogs demonstrated an unexpected freedom from metabolic damage. A highly sensitive and specific renal function test, the accumulation of PAH, indicated that the kidney slices from the second-set transplant could readily take up PAH. This accumulation of PAH is known to depend on oxidative metabolism of the tissue slices. It is interesting, therefore, that oxygen consumption of renal cortical slices from the transplants was substantially the same as that of the control autotransplants. Other tests showed that the specific activity of lipid or acid soluble phosphorus, measured 24 hours after second-set transplantation, remained at normal levels. At that time the nucleic acid phosphorus was only slightly increased in the transplant relative to the value in the host's own kidney.

The relatively small changes in PAH uptake and nucleic acid phosphorus turnover, as well as the absence of changes in oxygen consumption and lipid and acid soluble phosphorus turnover, all contrast sharply with the almost complete *in vivo* rejection of second-set homotransplants one day after transfer of the kidney to sensitized recipients. At that time, the second-set kidneys have become anuric, and show edema, hemorrhage and capillary damage(4,5). The present experiments suggest that tissues from second-set kidney transplants retain their capacity for normal function *in vitro,* in spite of the severe loss of function observed *in vivo.* Cellular infiltrate 24 hours after the operation was not marked in second-set kidneys in contrast to the extensive white-cell invasion seen several days after first-set operations. The rapid progression of the second-set reaction is indicated by the complete cessation of blood flow through all such kidneys within 2 days of the operation.

In a previous study with first-set transplants of the rabbit kidney(6) we showed that a partial blood supply can be retained in organs undergoing homologous transplant reactions. Some tubules could survive in such transplants for as long as a month; this occurred in the kidney undergoing rejection with massive invasion by lymphoid cells. It is of interest to recall Woodruff's thesis that after a critical period of survival, some homografts may continue to function indefinitely(7). He observed that portions of a skin graft may retain their blood supply and remain viable although part of the graft is rejected. In view of these observations on cell survival in homologous tissues heavily infiltrated by lymphoid elements, it is suggested that the invading cells kill the kidney cells by interference with the *in vivo* nutrition of the parenchyma. Others have also previously proposed that rejection depends on the loss of the blood supply

to homografts(8,9). Those cells which survive the lymphoid invasion and retain a blood supply survive for prolonged periods. Were a toxic agent involved in this reaction, one would expect destruction of all the cells within the first-set transplant.

The present experiments also indicate that generalized cytotoxicity is probably not involved in the destruction of parenchymal cells during kidney transplant rejection in second-set kidneys. Interference with nutritional exchanges here also may be the primary cause of cellular damage. The abrupt destruction of second-set kidneys, occurring before massive invasion by white cells, favors the view that interruption of the blood supply is the primary cause of second-set rejection. Were the blood supply to remain intact, the *in vitro* studies suggest that kidney function would be prolonged even in second-set kidneys.

Summary. Oxygen consumption and para-aminohippurate accumulation have been studied in slices of renal cortex from second-set kidney transplants in dogs. The organs were removed for evaluation 24 hours after transplantation, at a time when they show gross hemorrhagic reactions and reduced rates of urine secretion. In spite of advanced rejection, these second-set transplants still accumulated PAH *in vitro* to the same high level as autotransplant controls. Oxygen uptake also remained within normal limits. Further, the turnover of phosphorus in the acid and lipid soluble fractions remained unaffected by the acute rejection process. A small increase in the specific activity of the nucleic acid phosphorus was observed. We interpret these observations to indicate that cytotoxic reactions are not a major factor in transplant rejection. An interruption of the vascular supply, or a mechanical interference with the nutritional exchange of the tissue, are suggested as more likely causes of transplant destruction.

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Effect of Angiotensin II on Adrenal Ascorbic Acid. (27747)

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Hog renin and synthetic asparaginyl 1, valine 5 angiotensin II stimulate secretion of adrenal cortical steroids in the dog(1,2). Saline extracts of dog kidneys stimulate dog adrenal steroid secretion, and it has been postulated that it is the renin present in the renal extract that stimulates steroidogenesis(3). There is indirect evidence in the rat, as shown by widening of the adrenal zona glomerulosa, that hog renin and saline extracts of rat kidneys stimulate steroidogenesis(4). As ACTH is also bound in significant amounts to kidney tissue(5), the role of renin in saline extracts of kidney cannot be assessed unless ACTH can be measured at the same time. Accordingly, we have compared the effects of ACTH with those of the renin-angiotensin system on adrenal ascorbic acid in the rat. The results