

THE THREE-KIDNEY RAT MODEL¹

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ABSTRACT

A simplified technique of renal transplantation in rats is presented. Using this technique to study the effect of three normal kidneys in a host, we have created a model with no hypotrophy of the transplanted kidney. The remaining kidney hypertrophies in response to nephrectomy on the other side, but kidneys do not hypotrophy in response to being in excess.

When one kidney is removed from an animal, the other rapidly increases its size and function. However, it is not clear what would happen if an extra kidney were given to an animal. Highly inbred strains of rats are available that do not reject a renal graft. By transplanting a third kidney into these rats, a model could be available for studying the reverse phenomenon of compensatory renal hypertrophy (1). Previous methods of transplantation in rats involved a difficult pair of vascular anastomoses in which the kidney had to be flipped back and forth while the renal artery and vein were retracted out of each other's way (2-4). With such delicate structures, there were many problems. Vascular complications were numerous, hydronephrosis and hydroureter occurred 20 per cent of the time, up to 30 per cent of the animals died, and the transplanted kidneys underwent atrophy when the host's original kidneys were not removed. We have used a simplified technique to create the first three-kidney rat model that is not hampered by the artifact of hypotrophy.

METHODS

An extra kidney was transplanted into 20 male Lewis rats weighing 300 to 450 g from littermate donors. A 2× set of glasses and the simplest ophthalmologic instruments are used. For a vascular clamp, the prongs of a Hartman mosquito are filed down to 1 mm and Silastic tubing is fitted over them to protect the vessels. 9-0 nylon is used for the anastomoses. Sterile technique was never used.

The aorta, vena cava, right renal artery and vein, right adrenal artery, and lumbar arteries of the donor are isolated (Fig. 1). The adrenal artery

is tied and divided and the kidney and ureter are freed up to the bladder, leaving a thick envelope of periureteral and perirenal fat. A liberal cuff of bladder is excised where the ureter enters.

The aorta and vena cava are then tied above and below the renal artery and vein, and after a small cut is made in the vena cava for outflow, the artery is perfused with 10 ml of warm saline (Fig. 2). A wide cuff of aorta and vena cava are taken with the renal artery and vein, and by grasping only the perirenal fat, the kidney is placed upside down in the right flank of the recipient (Fig. 3). The donor kidney is never touched or moved from this position. The vascular clamp is applied to the vena cava, and an end to side anastomosis of donor vein to vena cava is easily accomplished by suturing the posterior row inside, and the anterior row outside (Fig. 4).

Then a similar anastomosis is made to the aorta, without ever moving or retracting the kidney (Fig. 5). The clamps are removed and the kidney makes urine in 1 to 10 min. Clamp is usually well under an hour, but 1½-hr clamp times were used in five of the 20 rats. Cooling was never employed. The donor bladder cuff is sutured to the dome of the recipient bladder with 7-0 chromic (Fig. 6). Ten milliliters of saline are left in the peritoneal cavity for fluid and electrolyte replacement.

Pre- and postoperative renal size was ascertained with intravenous pyelography and direct measurement. Blood was drawn by cardiac puncture for serum creatinine preoperatively and 2 months postoperatively. Creatinine was determined by the method of Hare (5). Determinations for each rat's pre- and postoperative serum creatinine were done simultaneously on frozen specimens. Each specimen was run twice and agreed within 5 per cent. The average is reported.

RESULTS

One of the rats developed ureteral obstruction where the periureteral fat envelope was inade-

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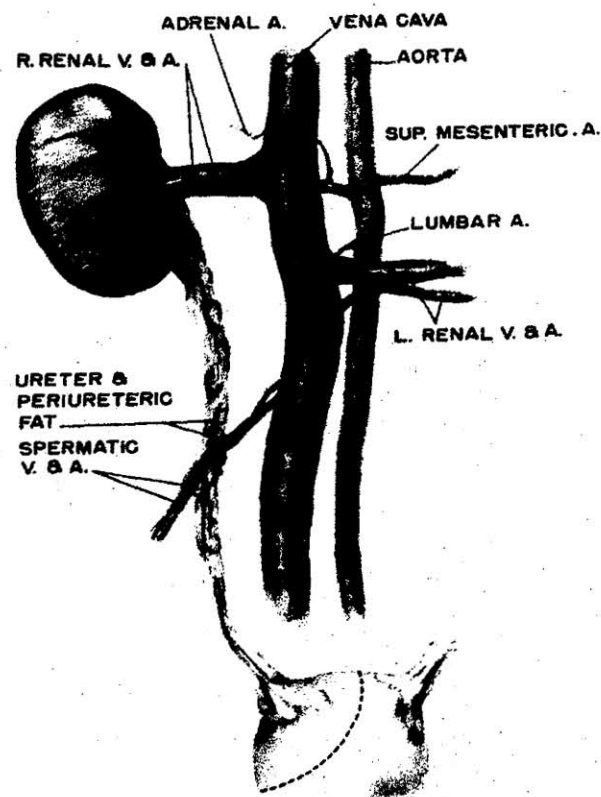


FIG. 1.

FIGS. 1 TO 6. The steps in performing renal transplant in rat.

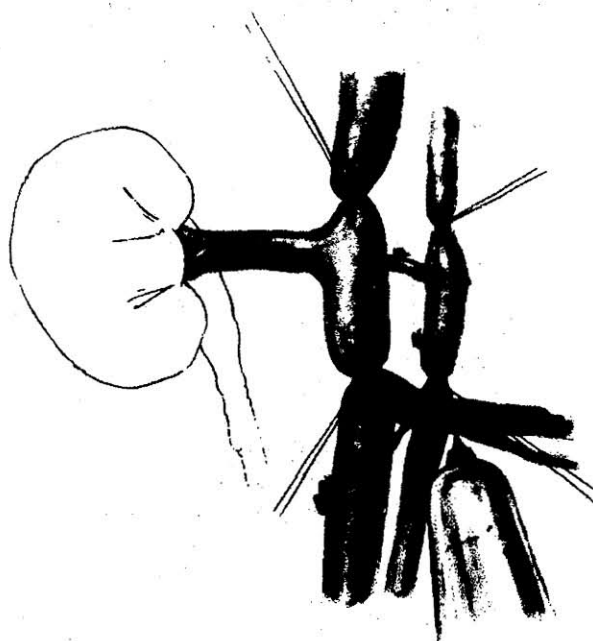


FIG. 2.

quate. None of the other 19 rats experienced graft failure, stones, or vascular complications. In every case all three kidneys remained their original size. Review of all of the pyelograms indicated that there was no atrophy, hypotrophy, or other abnor-

malities in any of these 19 rats (Fig. 7). Neither the transplanted nor the original kidneys decreased in size (Table 1). These results were the same for both long and short ischemia times.

The mean serum creatinine decreased 33 per cent of what it was when the rats had only two kidneys (Table 2). The paired *t* test was applied to these values and *P* was less than 0.001.

DISCUSSION

The phenomenon of renal compensatory hypertrophy has been a very heavily studied enigma for many years, and its eventual understanding may have enormous importance. It is well known that when one kidney is removed, the other quite rapidly increases its size and function to make up for the loss of its partner. In rats, blood flow to the remaining kidney increases in minutes, and its glomerular filtration rate (GFR) increases 30 per cent in 3 days, 60 per cent in 1 week, and 76 per cent at 2 to 3 weeks (6-9). In dogs GFR and effective renal plasma flow double in the 1st day (10). Human renal donors gain about 70 per cent of their original GFR within a few days to a week (11, 12).

Our findings are rather striking in that one might predict an atrophy or decrease in function in each kidney in an animal given three of them. Since nephrons respond to a decrease in their number by an increase in size and function, one would presume that they would respond to an increase in their number by a decrease in size and function. This did not happen. Each kidney maintained its previous size, giving these three-kidney

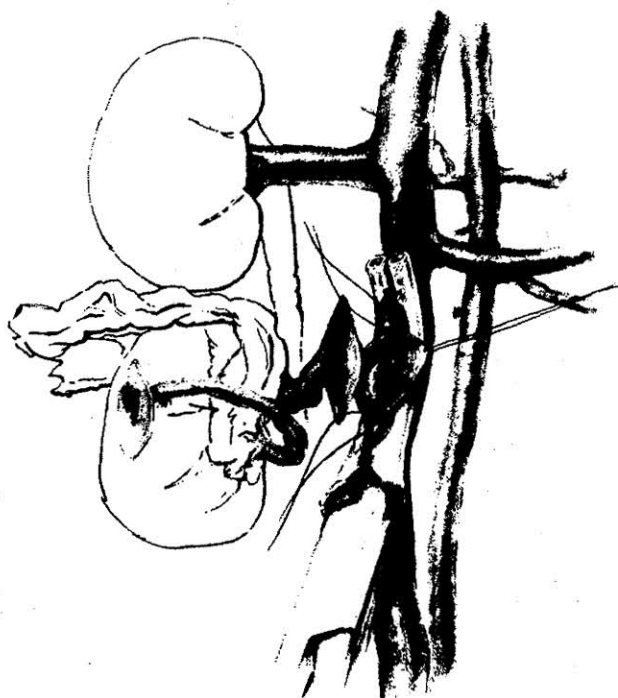


FIG. 3.

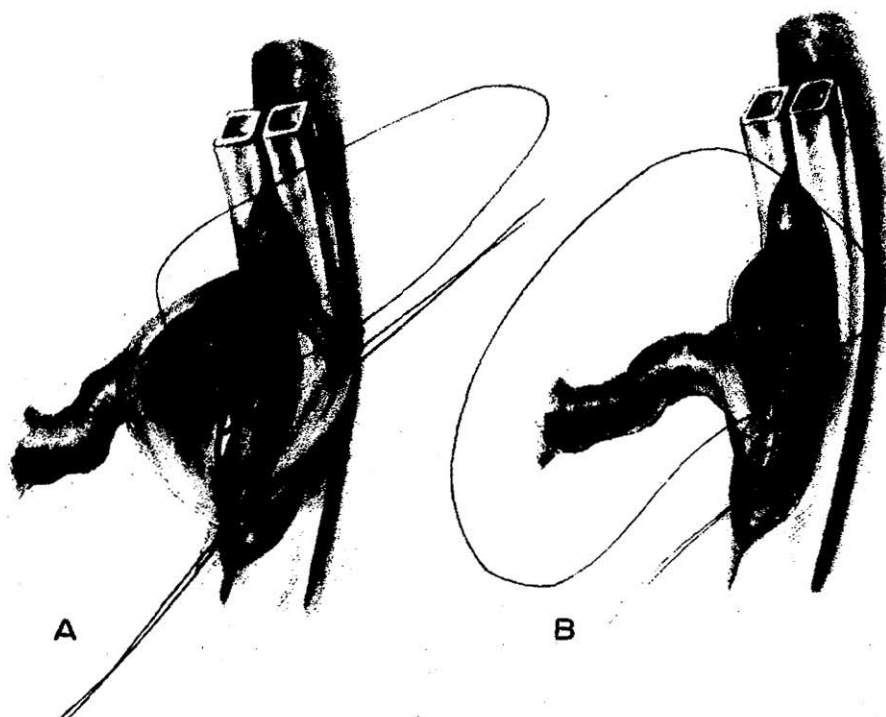


FIG. 4.

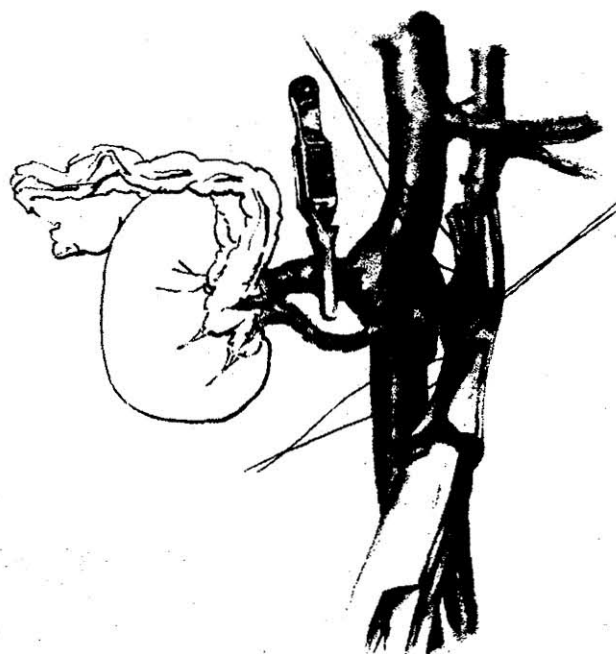


FIG. 5.

animals a large increase in total renal mass.

Of course, serum creatinine is not an altogether reliable indicator of renal function, and these data need to be corroborated with clearances. However, the decrease in serum creatinine of 33 per cent, that is the mean difference between pre- and postoperative serum creatinine, was statistically significant. This suggests an increase in total renal

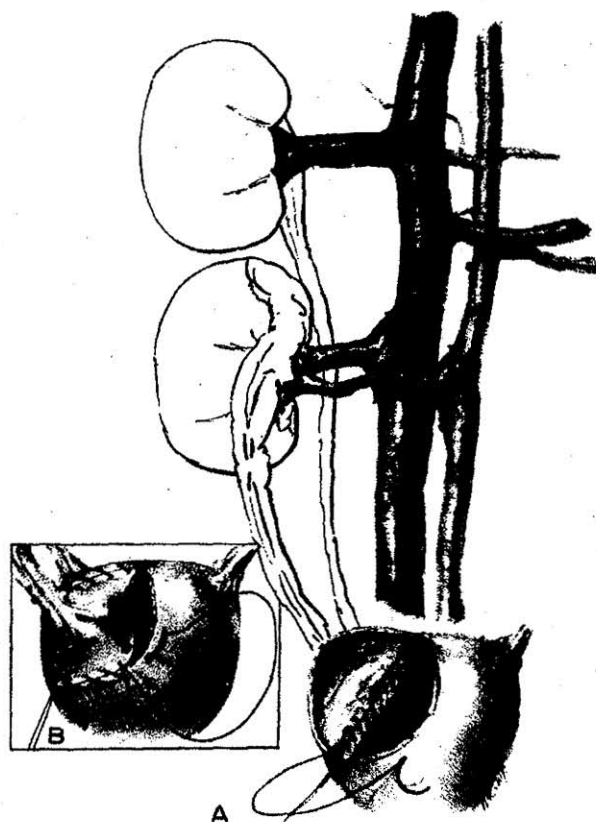


FIG. 6.

function in the animal of one-half of its original function with two kidneys.

In similar studies by Klein and Gittes (4),

varying degrees of atrophy were noted in the transplanted kidney which could be prevented by removing the recipient's other two kidneys. With less ischemia time, less atrophy was noted, but as long as the original kidneys were left in, there was some atrophy. The discrepancy between our findings and theirs needs to be explained.

Since many of our rats had warm ischemia times as long as those of Klein and Gittes and yet experienced no hypotrophy, or atrophy, ischemia time itself does not account for the difference in our results. Our technique involves absolutely no renal manipulation or flipping back and forth. This could account for the "weakened" condition of their transplanted kidney which might have caused it to atrophy in the face of two normal host kidneys. However, even kidneys that go into acute failure from prolonged transplant ischemia times do not generally atrophy.

The experiments of Masson and Hirano lead to another possible explanation (13). They con-

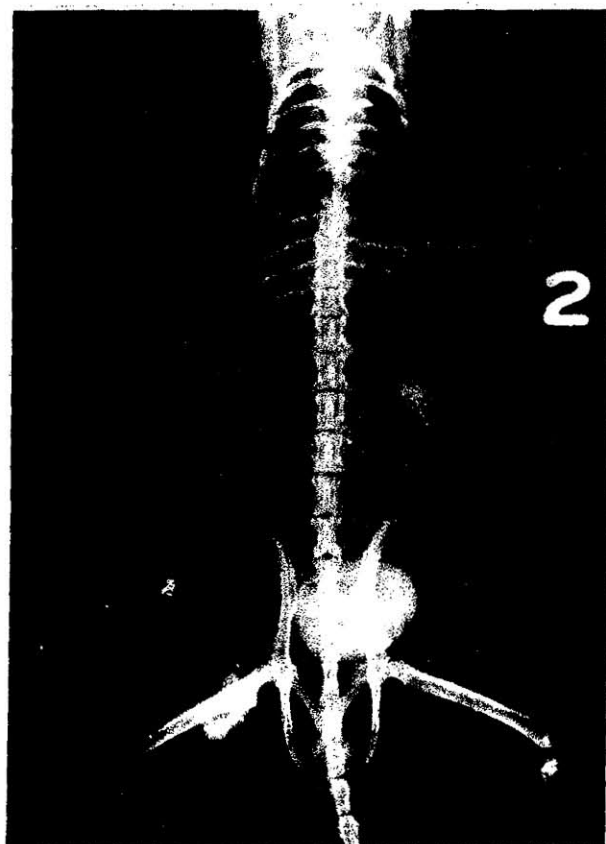


FIG. 7. Intravenous pyelogram of three-kidney rat demonstrating no hypotrophy and prompt equal visualization.

TABLE 2. Serum creatinine (mg per 100 ml) before and after extra kidney transplanted from littermate^a

	Preoperative	Postoperative
Mean	0.51	0.35

^a N = 19; mean difference, 0.16; standard error of mean difference, 0.02; P less than 0.001.

stricted the blood flow to one kidney, producing atrophy on the constricted side and hypertrophy on the contralateral side. When the contralateral kidney was removed, however, constriction of the arterial supply resulted in no atrophy of the ipsilateral kidney. In fact, this kidney hypertrophied as normal. Klein and Gittes experienced the same problems with the technique of transplant that others have reported—vascular blockage, leakage, hydronephrosis, and bladder stones. It is quite conceivable that all of their rats had some degree of vascular compromise to the transplanted kidney, and as with Masson and Hirano, the kidney could function normally only when the others were removed.

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TABLE 1. Kidney size (cm longitudinal axis) before and after extra kidney transplanted from littermate^a

Ischemia Time	Preoperative			4 Months Postoperative		
	Right	Left	New kidney	Right	Left	New kidney
Short (<1 hr)	2.16 ± 0.10	2.19 ± 0.09	2.18 ± 0.11	2.15 ± 0.12	2.17 ± 0.10	2.19 ± 0.08
Long (1½ hr)	2.20 ± 0.11	2.20 ± 0.12	2.17 ± 0.08	2.19 ± 0.12	2.17 ± 0.10	2.20 ± 0.12

^a ± 1 standard deviation; N = 19.

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Due to circumstances beyond our control, corrected proofs of this article were never received from the author.