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High osmolality-low pH flush solutions improve renal transplant function in rats

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Summary. Although transplanting rat kidneys is an established microsurgical technique, inulin clearance is abnormally low, due to rejection and/or warm ischemia-induced damage. In the present studies, rejection was avoided by using inbred Brown Norway rats as donors and recipients. Donor kidneys were flushed with ice-cold solutions of various composition (saline, saline + 200 or 400 m*M* mannitol) and pHs (5.7, 6.4, and 7.4), and the kidneys were kept cold during transplantation into unilaterally nephrectomized recipients. Renal function was assessed by clearance techniques 1 week later. In control rats, with both native kidneys intact, the ratio of inulin clearance, left kidney to right kidney, was 0.99 ± 0.02. In rats with a native right kidney and a transplanted left kidney that had been flushed with saline, the ratio was considerably lower (0.46 ± 0.09). Adding 200 m*M* mannitol to the saline flush solution increased the ratio (0.89 ± 0.09). In comparison, adding 200 m*M* mannitol and 5 m*M* phosphate buffer at pH 7.4 resulted in a somewhat lower ratio (0.80 ± 0.09), whereas adding 200 m*M* mannitol and 5 m*M* phosphate buffer at pH 5.7 resulted in a higher ratio, one that was indistinguishable from control (0.97 ± 0.09). Thus, in this latter group, the inulin clearances of the transplanted kidneys were identical to those of the contralateral native kidneys.

Key words: Kidney ischemia – Kidney preservation – Kidney transplantation – Glomerular filtration rate

Transplanting rat kidneys is a well-established microsurgical procedure [27]. Rats with transplanted kidneys have been used to help elucidate the mechanisms of compensatory renal hypertrophy [7, 14, 21, 24, 25] and the role of the kidney in genetic hypertension [4, 8, 13]. This model also has provided information concerning organ preservation [15, 23], rejection [6, 19, 22], and immunosuppression [10]. However, in the vast majority of these studies, the function of the transplanted kidney has received little or no attention. There are only a few reports of using clearance techniques to assess function [6, 7, 14, 16–18, 21], and without exception, subnormal clearances of para-aminohippuric acid (PAH), inulin, and creatinine were found, especially in the presence of a functioning native kidney [7].

Subnormal function can result from rejection and/or ischemia-induced acute renal failure. Rejection can be avoided by using genetically identical rats as both donors and recipients, but there is no way to avoid ischemia during the vascular anastomosis. Even a 30-minute period of warm ischemia produces as well-characterized syndrome of acute renal failure in rats, and longer periods result in almost proportionate increases in its severity [9]. Using ice-cold solutions, particularly hypertonic solutions, to flush the donor kidney [1–3] and keeping the kidney cold during the anastomosis procedure [16–18] are claimed to have beneficial effects, but there have been no previous attempts to use all of these methods together to optimize the function of the transplant. This was the goal of the present studies. Rejection was avoided by using inbred Brown Norway rats as both donors and recipients. The recipients were unilaterally nephrectomized; the donor kidneys were flushed with one of five ice-cold solutions, and the kidneys were kept ice-cold during transplantation. Split renal function was assessed 1 week later – left transplanted kidney versus contralateral native kidney. We found that with a flush solution consisting of isotonic saline (150 m*M* NaCl) plus 200 m*M* mannitol plus 5 m*M* sodium phosphate buffer at pH 5.7, the renal function of the transplant was indistinguishable from that of the contralateral native kidney in the same animal.

Materials and methods

Adult Brown Norway rats were used as kidney donors and recipients. They were cared for in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (Department of Health, Education and Welfare No. NIH 80-23). The rats were

Table 1. Body weight, blood pressure, plasma Na and K concentrations, and ischemia times in six groups of rats

Group	(*n*)	Body weight (g)	Blood pressure (mmHg)	Plasma Na (m*M*)	Plasma K (m*M*)	Ischemia (min)
Control	(18)	233 ± 10	110 ± 3	143.0 ± 0.4	4.1 ± 0.1	-
S pH 6.4	(7)	243 ± 18	109 ± 6	143.4 ± 0.6	4.0 ± 0.1	228 ± 5
200M pH 7.4	(8)	222 ± 7	103 ± 4	142.1 ± 0.7	3.9 ± 0.1	199 ± 15*
200M pH 6.4	(7)	237 ± 10	102 ± 4	143.4 ± 0.4	4.0 ± 0.1	193 ± 12*
200M pH 5.7	(6)	245 ± 11	103 ± 2	142.0 ± 0.7	3.9 ± 0.1	209 ± 13
400M pH 6.4	(7)	246 ± 12	102 ± 2	143.3 ± 0.4	4.0 ± 0.1	196 ± 11*

Values are Means ± SEM. The measurements were made in control rats (both native kidneys intact) and in five groups of experimental rats, each with a native right kidney and a transplanted left kidney. Prior to transplantation, kidneys were flushed either with saline alone (S) or with saline containing 200 or 400 m*M* mannitol (200M and 400M, respectively), at various pHs.
* $P < 0.05$ compared with the saline group (S pH 6.4)

housed in a room with constant temperature and a 12-h light and 12-h dark cycle, and they had free access to tap water and Purina Rodent Chow except that food was withheld during the night before surgery.

The techniques described by Waynforth [27] were used for harvesting and transplanting rat kidneys. In brief, donors were anesthetized and maintained on a surgical plane of anesthesia with sodium pentobarbital (initial dose ≈45 mg/kg body weight, given via a tail vein). A midline incision was made to expose the left kidney. After heparinizing the rat (100 mU in 0.1 ml, administered via a tail vein), the left kidney was flushed via the aorta with 5 ml of an ice-cold solution over a 1–2 min period. Then the kidney was removed after transecting the ureter near the bladder, the renal vein near the vena cava, and the aorta so as to leave intact the segment containing the origin of the renal artery. The kidney was rinsed with ice-cold flush solution and transferred to a Petri dish filled with ice-cold flush solution. Before killing the donor, 2 ml of its heparinized blood was taken and kept on ice for later use.

Five flush solutions were used: saline pH 6.4 ($n = 7$), saline containing 200 m*M* mannitol at pH 6.4 ($n = 7$), saline containing 400 m*M* mannitol at pH 6.4 ($n = 7$), saline containing 200 m*M* mannitol and 5 m*M* sodium phosphate at pH 5.7 ($n = 6$), and saline containing 200 m*M* mannitol and 5 m*M* sodium phosphate at pH 7.4 ($n = 8$). Rats were assigned one of these five flush solutions in such an order that any differences between the groups which might be attributable to gradual improvements in surgical technique would cancel out.

The recipient was anesthetized as described above. A midline incision was made, and the left kidney was removed after transecting the ureter near the hilum, the renal artery near its origin, and the renal vein near the kidney, leaving the adrenal and spermatic veins patent. A cooling coil made of thin-walled copper tubing was placed in the renal fossa. The coil was connected by rubber tubing to a reservoir, and ice water was continuously circulated through it by a roller pump. The donor kidney was placed on the coil, and cotton wool, soaked in ice-cold saline, was draped over it to prevent drying. With the kidney in place, an end-to-end anastomosis of the donor and recipient renal veins was performed, using 10-0 nylon suture on a 75-μ needle. Then, with atraumatic microvascular clamps in place, a slit in the aorta was made and an end-to-side anastomosis was performed, attaching the renal artery cuff of the donor kidney to the aorta of the recipient. The cooling coil was removed, and the kidney was superfused with room temperature saline for 1–2 min before releasing the vascular clamps. Lidocaine (2%) was applied to the vessels after unclamping. The cut ureters were clamped to prevent bleeding, opened with dilators, and anastomosed end-to-end using four interrupted, equally spaced, 10-0 nylon sutures. After removing the ureteral clamps, the recipient was transfused with donor blood, and the abdominal wall was closed with a continuous fine nylon suture.

Clearance experiments were performed 7 days later on these rats and on 18 age- and weight-matched control rats (both native kidneys intact). Rats were anesthetized as described above. The ureters, a femoral artery and vein, and the left renal vein were cannulated with polyethylene tubing as described previously [5]. The arterial cannula was connected to a pressure transducer, and mean arterial blood pressure was monitored on a polygraph. All rats received a priming injection of inulin and PAH (2 ml/kg body weight of 10 g% inulin and 400 mg% PAH dissolved in saline), followed by a continuous i.v. infusion of inulin and PAH (0.055 ml/min of 3.6 g% inulin and 290 mg% PAH dissolved in saline). The hour following the priming injection was allowed for equilibration. Then, two consecutive 40-min clearance periods were begun. Urine was collected in pre-weighed micro test tubes, and arterial and renal venous blood samples (< 0.5 ml total) were collected at the clearance midpoints. The blood was centrifuged (4°C; 8,000 × *g*; 5 min). Plasma and urine were frozen until analyzed as described below. Both kidneys were removed, stripped of perirenal tissue, blotted dry, and weighed.

Plasma sodium and potassium concentrations were measured by flame photometry using internal lithium standardization. PAH and inulin concentrations in urine and in plasma filtrates were determined by spectrophotometric methods [5]. Standard formular were used to calculate urine flow rates and the clearances of PAH and inulin of both kidneys, and PAH extraction and renal plasma flow of the left kidneys. Except for PAH extraction, these parameters were normalized per kilogram body weight. Values for the two clearance periods were averaged to obtain single values of each parameter for each rat. All results are expressed as means ± SEMs. ANOVA and Scheffe contrast were used to assess the statistical significance of differences in means among the groups, and the paired *t*-test was used to test for differences between the two kidneys within given groups [26]. *P* values below 0.05 were considered statistically significant.

Results

It can be seen in Table 1 that there were no significant differences among the six groups of rats with respect to body weights, blood pressures, and plasma sodium and potassium concentrations at the time of the clearance experiments. Although there were some significant differences in ischemia times among the transplant groups, the differences were relatively small (for example, mean values of ischemia time differed by 15% maximum, group S pH 6.4 versus group 200M pH 6.4), and it is unlikely that they explain the observed differences in renal function or that they detract from our conclusions (see below).

Table 2. Clearance data in control rats and in rats with transplanted left kidneys

Group	(*n*)	PAH extraction (%)	Plasma flow (ml min^{-1} kg^{-1})	PAH clearance (left/right)	Inulin clearance (left/right)
Control	(18)	91 ± 1	14.0 ± 0.7	1.03 ± 0.02	0.99 ± 0.02
S pH 6.4	(7)	75 ± 3*	10.5 ± 1.0	0.64 ± 0.10*	0.46 ± 0.09*
200M pH 7.4	(8)	84 ± 3**	12.6 ± 0.8	0.87 ± 0.07**	0.80 ± 0.09**
200M pH 6.4	(7)	87 ± 1**	13.6 ± 1.1	1.00 ± 0.08**	0.89 ± 0.09**
200M pH 5.7	(6)	92 ± 1**	11.9 ± 0.9	1.03 ± 0.09**	0.97 ± 0.09**
400M pH 6.4	(7)	80 ± 3*	12.4 ± 1.5	0.79 ± 0.13*	0.70 ± 0.12*, **

Values are means ± SEM. Clearances of para-aminohippuric acid (PAH) and inulin were measured in both kidneys; PAH extraction was measured in left kidneys only and the renal plasma flow of the left kidney was calculated as PAH clearance/PAH extraction. Control group, both native kidneys intact; experimental groups, right native kidney and left transplanted kidney. Prior to transplantation, kidneys were flushed either with saline (S) or with saline containing 200 or 400 m*M* mannitol (200M and 400M, respectively) at various pHs.
* $P < 0.05$ compared with control group; ** $P < 0.05$ compared with saline group (S)

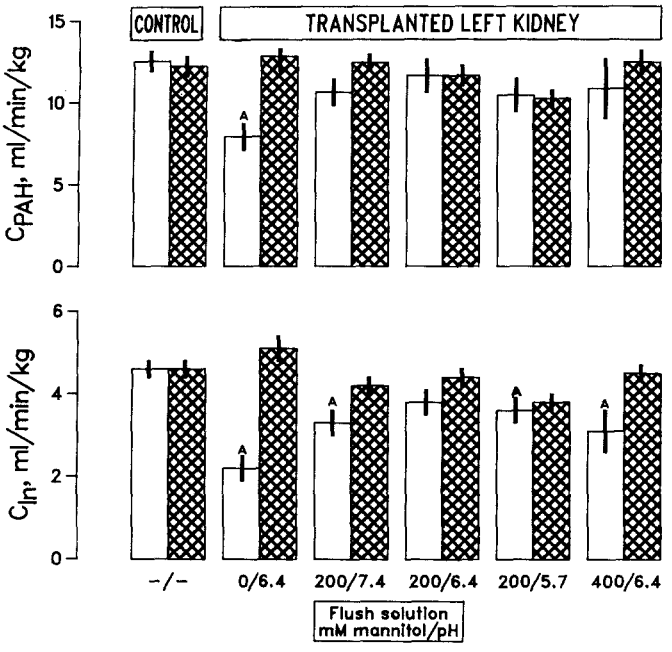

Fig. 1. Para-aminohippuric acid (C_{PAH}) and inulin clearances (C_{In}) in control rats (both native kidneys intact) and in five groups of experimental rats (right native kidney, left transplanted kidney). Prior to transplantation, kidneys were flushed with one of five solutions: aline and no mannitol at pH 6.4 (*0/6.4*), saline plus 200 m*M* mannitol at pH 7.4 (*200/7.4*), saline plus 200 m*M* mannitol at pH 6.4 (*200/6.4*), saline plus 200 m*M* mannitol at pH 5.7 (*200/5.7*), and saline plus 400 m*M* mannitol at pH 6.4 (*400/6.4*). The kidneys of control rats were not flushed (−/−). Means ± SEMs; $n = 18$, 7, 8, 7, 6, and 7 in groups from left to right. $P < 0.05$ compared with the corresponding value in the control group is indicated by *A*. *Open bars*, left kidney; *hatched bars*, right kidney

Mean PAH and inulin clearances of left and right kidneys are presented in Fig. 1. Both kidneys of the control group were virtually identical with respect to these parameters, and the left kidney to right kidney ratios were not significantly different from unity (Table 2). There were no significant differences among the six groups with respect to PAH clearance or inulin clearance of the right kidney. However, there were significant differences in left kidney clearances, either in comparison with the control rats (Fig. 1) or in comparison with the contralateral kidneys of the same rats (left-right ratios in Table 2). These differences suggest three conclusions. First, comparisons between groups 200M pH 7.4, 200M pH 6.4, and 200M pH 5.7 (same osmolality of the flush solution; no significant differences in ischemia times) indicate that the flush solution with the lowest pH (5.7) was superior to the one with the highest pH (7.4). In fact, as assessed by the left-right ratios in Table 2, PAH and inulin clearances of transplanted kidneys flushed with saline plus 200 m*M* mannitol at pH 5.7 were indistinguishable form PAH and inulin clearances of the contralateral native kidneys. Second, comparison of group S pH 6.4 with all the other transplant groups suggests that a flush solution of saline alone was inferior to a mannitol-containing flush solution. Values of left kidney PAH clearance, left kidney inulin clearance, and left-right ratios of PAH and inulin clearances were all lower in group S pH 6.4 than in any other transplant group, including group 200M pH 6.4 (both of the flush solutions had the same pH) and group 200M pH 5.7 (the ischemia times were not significantly different). Third, similar comparisons between groups 200M pH 6.4 and 400M pH 6.4 (ischemia times were not significantly different) suggest that a flush solution containing 200 m*M* mannitol was superior to one containing 400 m*M*.

Means of PAH extraction and renal plasma flow of the left kidneys are presented in Table 2. PAH extraction was lower than control in transplanted kidneys that had been flushed either with saline or with saline plus 400 m*M* mannitol. All three groups with transplanted kidneys that had been flushed with saline plus 200 m*M* mannitol had PAH extractions that were not significantly different from control but that were significantly higher than that of transplanted kidneys flushed with saline alone. Although there were no significant differences in mean renal plasma flows among the six groups, the lowest mean value was found in the group with transplanted kidneys that had been flushed with saline alone.

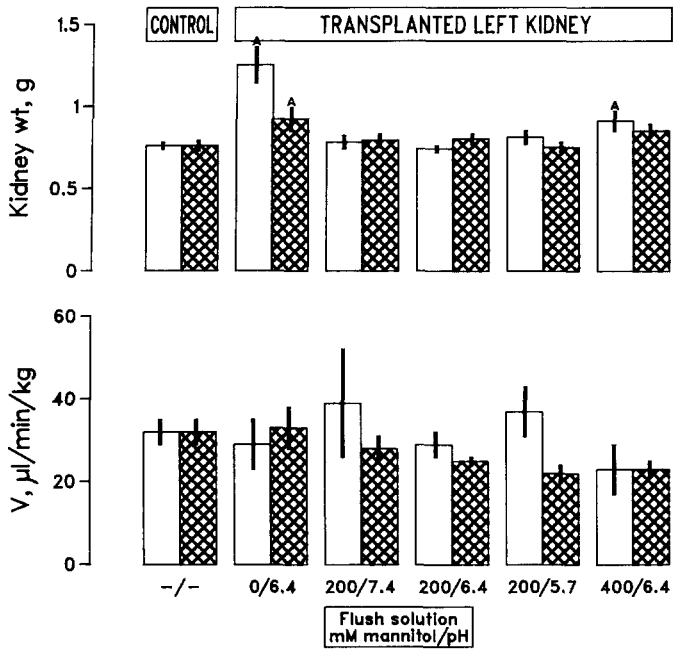

Fig. 2. Kidney wet weight and urine flow (V) in control rats (both native kidneys intact) and in five groups of experimental rats (right native kidney, left transplanted kidney). Prior to transplantation, kidneys were flushed with one of five solutions: saline and no mannitol at pH 6.4 (*0/6.4*), saline plus 200 mM mannitol at pH 7.4 (*200/7.4*), saline plus 200 mM mannitol at pH 6.4 (*200/6.4*), saline plus 200 mM mannitol at pH 5.7 (*200/5.7*), and saline plus 400 mM mannitol at pH 6.4 (*400/6.4*). The kidneys of control rats were not flushed (−/−). Means ± SEM; $n = 18, 7, 8, 7, 6$ and 7 in groups from left to right. $P < 0.05$ compared with the corresponding value in the control group is indicated by *A*. *Open bars*, left kidney; *hatched bars*, right kidney

Means of kidney wet weights are shown in Fig. 2. In control rats, weights of left and right kidneys were virtually identical. Similarly, weights of left and right kidneys were nearly identical, and not significantly different from control, in the three groups of rats with transplanted kidneys that had been flushed with saline plus 200 mM mannitol. The transplanted kidneys that had been flushed either with saline alone or with saline plus 400 mM mannitol weighed significantly more than control. Interestingly, the native right kidney of group S pH 6.4 also weighed significantly more than control, probably due to compensatory hypertrophy in response to diminished function of the transplanted left kidney [14], since this was the group with the most compromised function of the transplanted kidney.

Means of urine flow of left and right kidneys are presented in Fig. 2. There were no statistically significant differences among the groups with respect to either the left or the right kidneys.

Discussion

The above results demonstrate that PAH and inulin clearances in transplanted rat kidneys are affected by the composition of the flush solution. Kidneys flushed with mannitol-saline solutions had significantly higher inulin clearances than kidneys flushed with saline alone, and of the two concentrations of mannitol that we used, 200 mM gave better results than 400 mM. Moreover, lowering the pH below 7.4 had a beneficial effect, since the compositions of the flush solutions in groups 200M pH 7.4 and 200M pH 5.7 were identical except for the pH (150 mM NaCl, 200 mM mannitol, 5 mM phosphate buffer), but the left to right kidney ratios for PAH and inulin clearances were 0.87 and 0.80 in the former group and 1.03 and 0.97 in the latter group. In the latter group, PAH and inulin clearances of the transplanted kidneys were indistinguishable from those of the contralateral native kidney. These differences between the groups were found 7 days after surgery, and since renal function was assessed only at this point in time, the rate at which these differences developed is unknown. It is also unknown whether these differences between the groups would be maximal 7 days after transplant.

It is interesting that wet weights of left transplanted kidneys and native right kidneys were nearly identical in the three groups with normal or near normal PAH and inulin clearances (groups 200M pH 7.4, 200M pH 6.4, and 200M pH 5.7), whereas the transplanted kidneys with the poorest function had the highest wet weights (groups S pH 6.4 and 400M pH 6.4). Perhaps the increased wet weight of the native right kidney in group S pH 6.4 could be taken as evidence of compensatory renal hypertrophy, triggered by reduced function of the transplanted left kidney [14], although the clearances of PAH and inulin in these native right kidneys were not significantly increased above control levels (i.e. right kidneys in control rats). Also it is interesting that, in the same three groups with normal or near normal PAH and inulin clearances of the transplanted kidneys, the urine flow of the transplants tended to be higher than the urine flows of the contralateral native kidneys. This could be the result of denervation diuresis [11]; the transplanted kidneys were denervated, and urine flow and sodium excretion are frequently higher in denervated than in innervated kidneys, since the renal sympathetic nerves enhance tubular reabsorption of sodium and water by an alpha-adrenergic mechanism [11].

Several solutions have been used to flush and preserve human and animal donor kidneys. Probably the most frequently used solutions are those of Collins, Sachs, and Ross and Marshall, or modifications of these [reviewed in 12, 20]. All are "intracellular solutions" in which sodium and chloride are largely replaced by potassium and by phosphate, sulfate, or citrate, respectively. There is evidence to suggest that the efficacy of these solutions is attributable not to the low concentrations of sodium and chloride per se, but rather to their hypertonicity and/or the presence of slowly permeating solutes such as the anions cited above and/or mannitol [12, 20]. In fact, a simple 0.1 M sodium phosphate buffer, made hypertonic by adding mannitol (500 mOs/kg total osmolality), is more effective than Collins solution in perserving the structural integrity of the renal tubules [2]. In brief, hypertonicity [12, 20], the presence of impermeant solutes [1–3, 12, 20], and a pH less than 7.4 [20] have all been shown to be important factors in kidney flush solutions. Our results are consistent and provide the first direct

functional evidence, since kidneys flushed with 200 m*M* mannitol in saline (osmolality ca. 500 mOs/kg) had significantly higher posttransplant inulin clearances than kidneys flushed with saline alone (osmolality ca. 300 mOs/kg), and at the same osmolality, a pH of 5.7 gave better results than a pH of 7.4. The mechanisms of the protective effects of impermeant solutes and low pH are unknown, but may be related to prevention of cell swelling and enzyme pH optima, respectively [12, 20].

The rat kidney transplant model has been used in many experiments, but there are only a few in which renal function was assessed directly [6, 7, 14, 16–18, 21], and in all of these, the glomerular filtration rate was subnormal due to rejection and/or ischemia-induced acute renal failure. In the experiments of Coffman et al. [7], both factors played roles, rejection by design since Lewis rats were donors and Brown Norway rats were recipients. On the first day after surgery, there was no evidence of rejection, but there was evidence of acute renal failure in that inulin clearance was less than half than of an intact native kidney. The kidneys were rejected during the next 5 days, and inulin clearance declined to near zero. In a more recent study, Coffman et al. [6] used Lewis rats as donors and either Brown Norway or Lewis rats as recipients. Again, Brown Norway rats rejected Lewis rat kidneys, and inulin clearance was negligible 1 week after transplantation. In contrast, Lewis recipients did not reject Lewis donor kidneys, but if the contralateral native kidney were left untouched, the transplanted kidney's inulin clearance was only about 10% of that of an intact native kidney 1 week after surgery. In the experiments of Muller-Suur et al. [16] and of Norlen et al. [17, 18], a random sample of Sprague Dawley rats was used both as donor and recipient. A few hours after transplantation, inulin, clearance of the transplants was approximately 60% of that of a normal native kidney in a control rat. Since so little time elapsed between transplantation and the clearance experiments, probably ischemia-induced acute renal failure, rather than rejection, accounted for the decreased glomerular filtration rate.

Our experiments were most similar to the experiments of Coffman et al. [6] in which inbred rats of the same strain were used as donors and recipients: the donor kidneys were flushed with ice-cold solutions, the osmolality of the flush solution they used (75 m*M* NaCl plus 556 m*M* mannitol) was similar to the osmolality of one of the flush solutions we used (150 m*M* NaCl plus 400 m*M* mannitol), the recipients were unilaterally nephrectomized, and renal function was measured 1 week after surgery. However, since even a short period of warm ischemia impairs renal function, one potentially significant difference is that whereas Coffman et al. [6] did not prevent the donor kidney from warming up during surgery, we did. In their experiments, inulin clearance of the transplanted kidney was 10% of control, whereas in our group 400 M pH6.4, the inulin clearance of the transplanted kidney was about 70% of the inulin clearance of the contralateral native kidney. Moreover, in the present studies, even better results were obtained in group 200 M pH 5.7.

In conclusion, flushing donor rat kidneys with 200 m*M* mannitol in saline at a low pH and keeping the kidney cold during surgery results in posttransplant clearances of PAH and inulin that are indistinguishable from those measured in the contralateral native kidney. This is the first direct proof that adding mannitol and lowering the pH of flush solutions results in improved posttransplant renal function in rats.

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