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Orthotopic Kidney Transplantation in the Rat with Non-splinted End-to-end Ureteric Anastomosis: Details of a Technique

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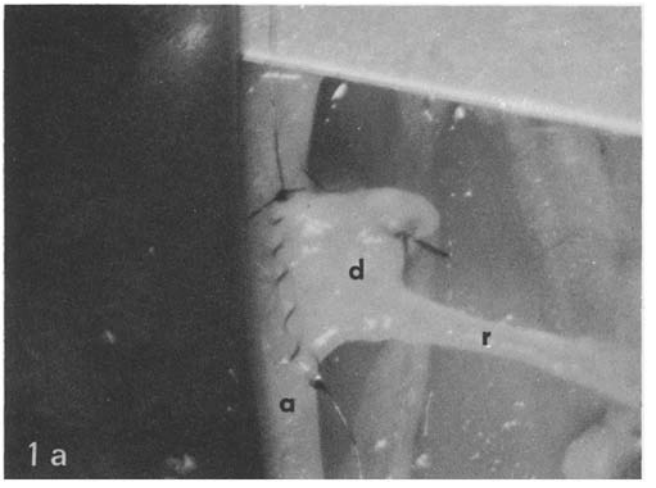
Summary. A detailed description is presented of an improved technique of orthotopic rat kidney transplantation with end-to-end anastomoses of the renal vessels, non-splinted end-to-end ureteric anastomosis and simultaneous bilateral nephrectomy. Forty-four consecutive transplantations are analysed. No failure due to complications of the vascular anastomoses occurred. The ureteric anastomoses were followed by stenosis in only two cases (5%), one of which was reconstructed. The operation times for the vascular anastomoses were 16–24 min, with an average of 20 min, and for complete transplantation 50 min. Therefore, our method seems to be less time consuming than other common techniques of renal transplantation. The technique described has produced a success rate of 95% and guarantees a standardised and reproducible organ quality.

Key words: Rat kidney transplantation, Microsurgical technique, Ureteric anastomosis.

Introduction

Kidney transplantation in the rat is a model frequently used in transplantation research and experimental microsurgery [12, 14]. This model covers most aspects of transplantation biology. The technique of heterotopic rat kidney transplantation was developed by Fisher and Lee [7] in 1965 (Fig. 1a, b) and the orthotopic technique was described by Daniller et al. [3] in 1968. These methods are now standard procedures. Modifications of these original techniques mainly deal with their most vulnerable aspect – the method of ureteric reconstruction [12].

In the present paper an improved microsurgical method for orthotopic kidney transplantation in the rat is described in detail.



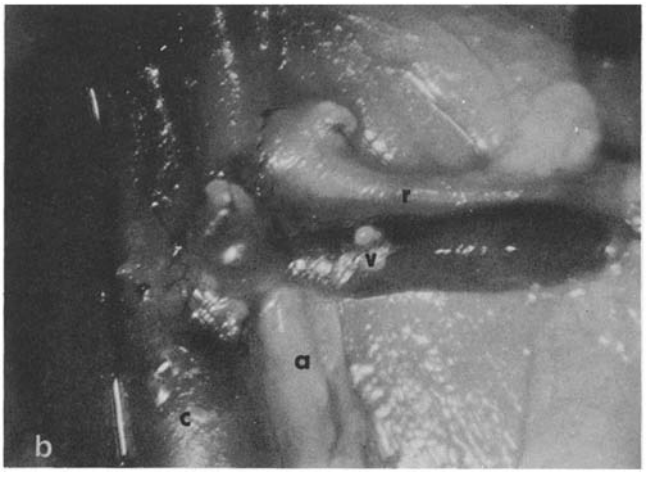


Fig. 1. Heterotopic kidney transplantation. a) Arterial anastomosis end-to-side, x22 (*d* – donor aortic segment, *r* – renal artery, *a* – recipients' aorta). b) Arterial and venous anastomoses completed, after recirculation, x10.7 (*r* – renal artery, *v* – renal vein, *a* – recipients' aorta, *c* – recipients' vena cava)

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Material

Male rats of two different strains were used for transplantation, 10 allografts were from Sprague-Dawley donors to BD IX recipients, 22 allografts were from (Sprague-Dawley x BD IX) F_1 hybrids to BD IX recipients and 12 isografts were from BD IX donors to BD IX recipients. The rats were fed with a standardised laboratory diet and kept under conditions as uniform as possible. They were starved for at least 12 h before the operation but had free access to water.

The study comprised a total of 44 consecutive transplantations.

In all these experiments the complications from vascular and ureteric anastomoses were analysed. Serum creatinine levels after transplantation were studied in all rats in this series. Blood samples were drawn from the medial angle of the eye.

The following microsurgical instruments were used for the operation: Yasargil needle holder, curved scissor, microvascular clamp, jewellers' forceps No. 4 and No. 5. The suture material was Ethilon® (10-0/BV 4 needle, 11-0/BV 6 needle). For maximum speed and precision of transplantation an operating binocular microscope (OpMi 310, Carl Zeiss Jena) was used.

Methods

a) Donor Kidney

Donor rats were anaesthetised with Pentobarbital® (Spofa, Prague) given intraperitoneally in a dose of 50 mg/kg body weight. They were placed on an operating table with a heating pad which kept the body temperature at 37 °C. Heparin, 100 IU/animal, was given intravenously via the jugular vein 15 min before the vessels from the donor kidney were occluded [8]. The operations were performed under clean but not sterile conditions [4, 10] in the following steps:

The abdomen was opened through a midline incision and the left kidney was exposed.

Xylocitine® infiltration around the renal pedicle to prevent vasospasm.

With 7x to 12.5x Magnification. Division of the suprarenal vein; atraumatic preparation and separation of the renal artery and vein from each other and the surrounding fat; mobilisation of the kidney and the ureter from the surrounding tissue; the left ureter was divided on the middle between the kidney and urinary bladder; simultaneous occlusion of the renal vessels before their junction with the aorta and vena cava, the renal artery and vein were cut and the kidney removed.

In these experiments no perfusion was performed. The transplant was placed in 0.9% NaCl solution at 8 °C during the time that the recipient was prepared.

b) Transplantation

The transplant recipient was prepared in the same fashion as the donor, except heparin was not given:

The renal vessels were occluded close to their origin with the aorta and vena cava using a microvascular clamp. The renal artery and vein were cut and the kidney removed.

Care was taken to avoid bleeding from the ends of the blood vessels due to inaccurate placing of the clamp, since this could easily have given rise to thrombosis in the area of the anastomosis [10].

The donor kidney was placed on the posterior abdominal wall of the recipient. The artery was anastomosed first. It was the more difficult anastomosis because of its small size (Ø 0.5–0.8 mm). Attempts were made to handle the artery only by the adventitia [4].

With 12.5x to 22x Magnification. The ends of the arteries were trimmed by removing the adventitia for a distance of 0.5 mm and slight dilation of the arteries with a jewellers' forceps No. 5 was performed. Two stay sutures of 10-0 Ethilon® were placed 120° apart [2] and the anastomosis of the front wall was completed with 2–3 further single sutures between the two stay sutures (Fig. 2a). For suturing the posterior wall the artery was twisted 180° by means of the stay sutures. 3–4 single sutures were placed on the dorsal wall. Thus the arterial anastomosis was completed usually with 8–9 single sutures (Fig. 2b).

The vein was much larger than the artery (Ø 1.0–1.5 mm) and size was not a problem. However, the major difficulty was the extreme thiness and fragility of the vein walls [4].

Two stay sutures of 10-0 Ethilon® were placed 180° apart and the anastomosis completed with two semicircular continuous sutures on both ventral and dorsal walls. For suturing the dorsal wall the vein also was twisted 180° similar to the artery by means of the stay sutures. One end of the sutures on both ventral and dorsal walls respectively was left untied so that the anastomosis could be widened when the renal vein filled with blood ([10], Fig. 2c). Slight compression was applied over the anastomosis with a small piece of gauge for one minute after recirculation. This was usually sufficient to secure haemostasis [4, 12].

With 22x to 40x Magnification. Magnification was used while trimming the ends of the transplant and recipient's ureter by removing the periureteral tissue for a distance of 1 mm and dilating the lumina (Ø 0.2–0.3 mm) with a jewellers' forceps.

Two full-thickness stay sutures of 11-0 Ethilon® were placed at 180° apart. The anastomosis of the front wall was completed with a third stitch between the two stay sutures (Fig. 2d). The ureter was then twisted and a fourth suture was put on the back wall.

We were able to examine intraoperatively the patency of the uretic anastomosis by observing undisturbed peristaltic waves and urine passage through the anastomosis.

Finally, a right nephrectomy was performed on the recipient. The kidney was separated from the perirenal fat and suprarenal gland and removed after placing a single tie around the renal pedicle [4]. The abdominal wound was closed in two layers.

Results

In this series of 44 consecutive transplantations no failure due to complications (e.g. bleeding, stenosis, thrombosis) of the arterial of venous anastomosis occurred. The non-splinted ureteric anastomoses were complicated by stenosis in only two cases in the allograft group. In these animals – in contrast to all other recipients – an increase of the serum creatinine concentration on the 2nd postoperative day was noted and ureteric stenosis suspected. One ureter was re-anastomosed successfully. The other animal was sacrificed after exploration on the 3rd day.

The operation times for the vascular anastomoses were 16–24 min, with an average of 20 min. The total operative time for transplantation ranged from 45 to 60 min, with an average of 50 min.

All the recipients of kidney isografts are alive between 8 and 17 weeks after transplantation with normal serum creatinine levels. The patency of the ureteric anastomoses was investigated 2 months after operation and in no case was ureteric obstruction or hydronephrosis found. The recipients of allografts from Sprague-Dawley donors died 5–7 days

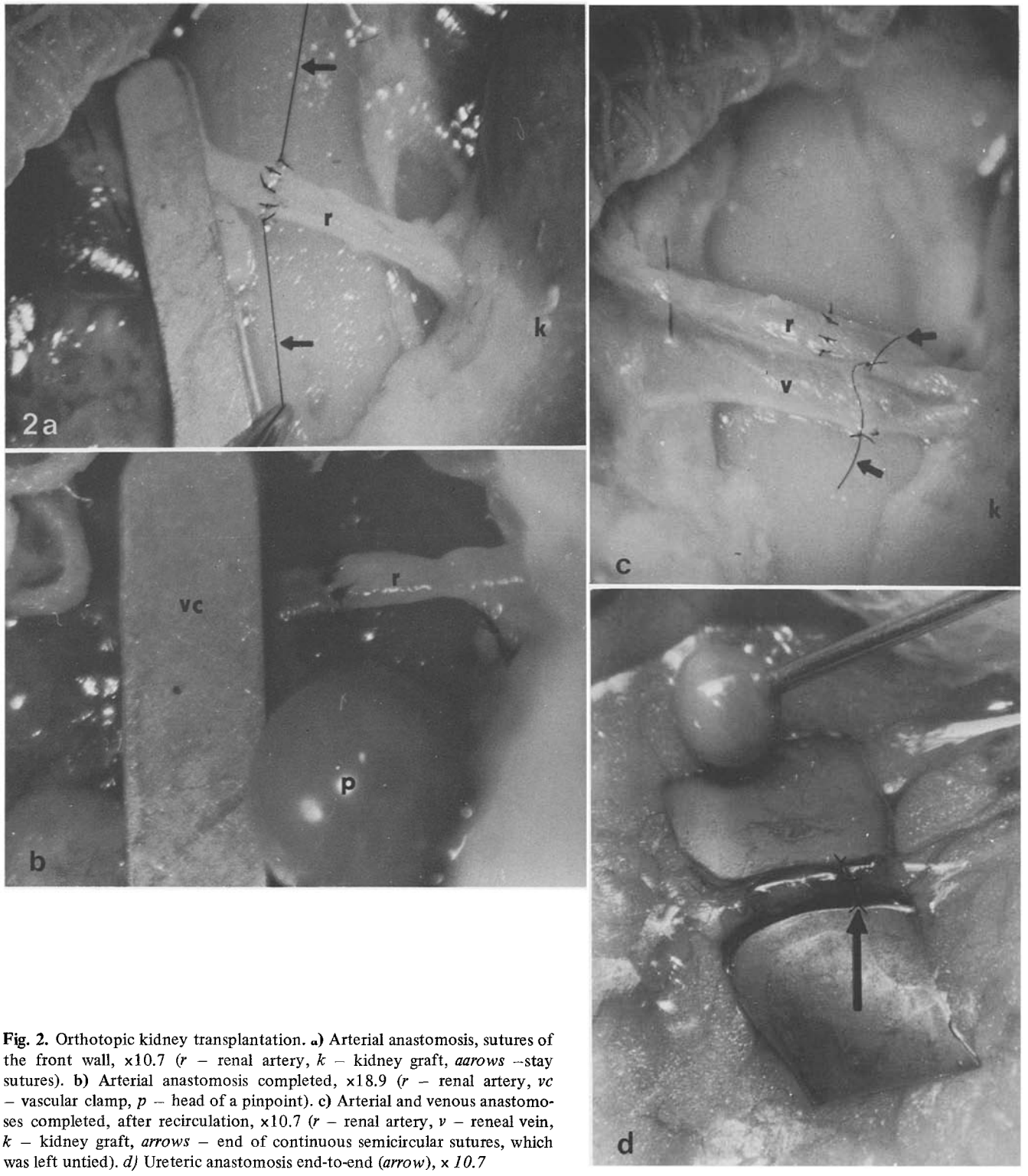

Fig. 2. Orthotopic kidney transplantation. a) Arterial anastomosis, sutures of the front wall, x10.7 (*r* – renal artery, *k* – kidney graft, *aarows* –stay sutures). b) Arterial anastomosis completed, x18.9 (*r* – renal artery, *vc* – vascular clamp, *p* – head of a pinpoint). c) Arterial and venous anastomoses completed, after recirculation, x10.7 (*r* – renal artery, *v* – reneal vein, *k* – kidney graft, *arrows* – end of continuous semicircular sutures, which was left untied). *d)* Ureteric anastomosis end-to-end (*arrow*), x *10.7*

and those with allografts from F_1 hybrids 11–16 days after transplantation from rejection. The post-mortem examinations showed no signs of urinary outflow obstruction. In one animal of the latter group some degree of tolerance or enhancement has been induced and it is alive 6 weeks after transplantation.

Discussion

The technique of rat kidney transplantation originally described by Fisher and Lee [7] involves a right heterotropic transplant with end-to-side anastomoses of the renal artery and vein with patches of the aorta and inferior vena cava

respectively to the aorta and inferior vena cava of the recipient. This technique required about 30–40 min occlusion of the circulation to the lower half of the body during transplantation. When the clamp is released undesirable metabolic and circulatory phenomena may appear [10].

This problem was solved by the orthotopic technique introduced by Daniller et al. [3], described in detail by Fabre et al. [4] and more recently published with superior results by Harvig and Norlen [10]. The transplantation was performed by only closing off the renal vessels of the recipient, which were then anastomosed end-to-end to the vessels of the kidney transplant. The numerous modifications [11, 13, 14, 16] and complications [1, 12, 15] of the ureteric reconstruction indicate that this part of the transplantation procedure remains the major stumbling block in rat kidney transplantation [12]. In the orthotopic technique the ureteric reconstruction is mostly performed as an end-to end anastomosis over a silastic catheter which is left in situ [3, 5, 9, 10, 14]. Although using such a technique ureteric complications can be reduced, complications include dislocation of the splint upwards to the renal pelvis with resulting obstruction and hydronephrosis [12], blockage of the catheter with blood clot and perforation of the ureteric wall [10]. The non-splinted end-to-end ureteric anastomosis reduced that incidence of urine leakage and hydronephrosis [17]. Only four 10-0 nylon sutures are necessary, as small leaks will heal spontaneously [6]. This technique, though technically more difficult, is much faster and less laborious [4]. Our results confirmed these experiences.

Finally, it should be stressed that for optimal results a skilled assistant and adequate magnification for both operator and assistant in the form of an operating microscope are essential [4].

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