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Experimental assessment of ureteral free autograft

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Abstract *Objective:* To establish the sequence of ureteral events under ischemic and desvascularized conditions but without immunologic interference.
Materials and methods: Sixty-two rats were divided into four groups: NU group (*n* 20) control group, normal ureter; SC group (*n* 15) only surgical control ureterolysis; NIAFG group (*n* 13) ureter used as non integrated autologous free graft; IAFG group (*n* 14): ureter used as integrated autologous free graft. Urographic, histologic and histomorphometric studies were performed.
Results: We established ureteral changes in the NIAFG and IAFG groups, compared to the control groups (NU, SC). Surgical findings and urographic assessment revealed normal peristalsis with no ectasia in some cases, and no ureteral fistulas or extravasations were found in the IAFG group. Histologic findings showed preservation of the architecture of the three normal layers. Histomorphometric studies showed that ureterolysis caused edema in the lamina propria, while changes in ureter free graft depended on whether the ureter was integrated or not. In the NIAFG group only the urothelial layers showed differences and in the IAFG group the ureteral wall appeared thicker. Histomorphometric studies showed preservation of the normal histologic structures in all cases.
Conclusions: The rat ureter can be used as a free autologous graft and represents an experimental model for immunologic events. We may assume that the necrosis and fibrosis observed in transplanted ureters are secondary to rejection in some cases.

Key words Ureter · Transplantation
Urologic surgery · Models · Theoretical

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Introduction

Renal transplantations are frequently complicated by stenosis or fistulas, which are often attributed to secondary phenomena of necrosis. This is due to a lack of ureter vascularization rather than to rejection from secondary vasculitis. It is necessary, however, to search for new and improve existing experimental models that enable us to study these immunological phenomena. Previous studies have demonstrated the possibility of autotransplanting the ureter as a non-pediculated free graft in rats [11] and dogs [3].

Materials and method

The use of the rat ureter as a nonpediculated free graft implanted in the same animal (autologous) and anastomosed at the ureteral end (integrated) has been defined as an integrated autologous free graft (IAFG). On the other hand, the use of a ureteral segment as a nonpediculated free graft which is placed in the interior of the same animal (autologous), but not anastomosed to any organ or system (nonintegrated), has been defined as a nonintegrated autologous free graft (NIAFG).

Rats were randomized into four groups. The surgical procedure was performed on the right side in all cases. The NU group (n = 20) comprised healthy rats in which the ureter was extracted in one operation in order to establish a normal standard. The SC group (n = 15) comprised rats in which the ureter was disected and

freed during the first operation, without sectioning or anastomosing, and later on extracted during a second operation. This represented the surgical control group. The NIAFG group ($n = 13$) comprised animals in which the ureter was dissected and extracted from the animal during the first operation and then abandoned in the retroperitoneum after a nephrectomy was performed, as a nonintegrated autologous free graft (NIAFG). In a second operation the ureter was extracted for study (1 week to 6 months after the first operation). The IAFG group ($n = 14$) comprised rats in which in the first operation the ureter was extracted from the body of the rat, without nephrectomy, and then reimplanted or autotransplanted as an integrated autologous free graft (IAFG), with the use of two anastomoses (juxtapyelic and juxtavesical with 10–0 nylon). In a second operation (from 1 week to 6 months after the first one) the whole ureter and kidney were removed.

Anaesthesia was administered intramuscularly to all the groups using ketamine, diacepan and atropine. Surgical examination, urographic controls, and histologic and histomorphometric studies were all performed. All interventions were visualized pictured and video, recorded. The surgical methods used have been described in previous reports [2,12]. To perform IVU (intravenous urography), a perpendicular incision was made in the inguinal ligament, the femoral vein was dissected and then cannulated with a 24G endovenous catheter, leaving it tied to the vein. A 2-cm^3 bolus of iodized contrast medium (20-cm^3 Pielograf 70 containing 1.85 g sodium salt and 12.15 g methylglucamina salt of 2-4-6 tri-iodobenzoic 35 di-acetamide) was administered. Plain X-rays were made at 10 and 15 min, the later ones depending on previous findings.

For the histologic and histomorphometric studies, transverse sections were made to divide the ureter into three segments, each corresponding to one-third of the ureter (A proximal, B middle and C distal). The kidney (K) was studied in the NU, SC and IAGF groups. In the IAFG group, additional transverse sections were made in the ureter above the proximal suture (D), underneath the distal suture (E), and two longitudinal sections were ,ade in both surgical anastomoses (F and G). Histologic sections were stained using hematoxylin-eosin and Masson's trichrome. The following data were recorded for every section: (I) number of glomeruli per microscopic field, (II) renal evaluation (duct dilatation), (III) degree of mucosal denudation, (IV) fibrosis in the lamina propria and muscular layer, (V) vascularization in the lamina propria, muscular layer and adventitia, and (VI) number and diameter of blood vessels in the different layers.

In the histomorphometric studies, measurements were made using a micrometer at × 25 magnification. To determine the area, light micrographs from each segment were measured using a Koizumi KP-90 digital planimeter. For each of the 72 variables studied (36 thicknesses and 36 areas), means and standard deviations were obtained, and a linear correlation was performed for rat weight in both the first and second surgical procedures, as well as for the time lapse between them and, finally, differences between the three segments (A, B and C) were investigated by analysis of variance with the Kruskal-Wallis and chi-square tests.

A new nonparametric analysis of variance was used to compare again the results with the Kruskal-Wallis test using the mean values from the three segments for all the recorded variables. Subsequently, the results were contrasted with the Wilcoxon test, which allows group matching.

Principles of laboratory animal care and Spanish law were followed.

Results

NU group results were used to define the characteristics of the normal ureter in the Sprague-Dawley rats. In the SC group an unchanged ureter was observed in all cases, although with the presence of slight periureteral fibrosis. The IVU studies performed showed an identical morphology of the upper tract to the one seen in the NU group (Fig. 1). Mild edema was noticed in the lamina propria in some sections. None of the three ureteral layers showed any fibrosis or other lesions. Histomorphometric studies showed a greater thickness of the mucosa and lamina propia layers in the SC group than in the NU group. No significant differences were found between the different levels of ureter.

The abandoned ureters in the NIAFG group showed no significant alterations. In some specimens (15%) the ureter appeared to be of thiner caliber, perhaps due to a lack of urine in its interior. Periureteral fibrosis was noted in all cases. Vessels were distributed similarly as observed in the NU group. In some cases a vascular framework stood out, covering the ureter and surrounding fat. All ureters appeared to be permeable to an intubator (4-0 nylon). Ureters showed spontaneous contractions in seven cases (53.8%) and contractions after mechanical stimulus in all cases. Cyst formation, necrosis or lumen stenosis was not observed in any rat of this group. IVU was not performed for obvious reasons. Histologic and histomorphometric studies revealed preservation of all layers. The urothelium showed assorted findings, from mild hyperplasia (Fig. 2) to wide denudation, with a mean of three to five cell layers. The lamina propia showed mild fibrosis in some sections. Almost all sections showed an increase in the amount of blood vessels compared to the NU group. No significant correlations were found between any variable and time of surgical procedure. When the ureter was used as NIAFG there was a slight but nonsignificant reduction in diameter, and a significant increase in urothelium thickness percentage (35% vs 29%), both compared to NU.

In the IAFG group, the ureter segment used as IAFG was easily recognized as a tube with spontaneous mobility over which was drawn a vascular framework crossing all of its length. Its appearance through the surgical microscope showed a normal state. In all cases, a moderate degree of periureteral fibrosis was observed, as well as a vascular framework on the fat immediately adjacent to the ureter. We believe that the presence of the vascular framework in the ureter was due to revascularization arising from the periureteral fat, similar to the subcutaneously implanted revascularization observed by Vistnes [17].

Spontaneous contractions were observed in 13 out of the 14 operated cases. Ureteral segments used as IAFG were catalogued as not having alterations in ten cases. A greater or lesser stenosis of the proximal suture with supra-anastomotical dilation was found in eight cases. In regard to the kidney, its macroscopic appearance did not display macroscopic alterations in eight cases. IVU showed a functioning kidney (Fig. 1) in ten cases, and silent kidney in three cases. Of the former ten cases, proximal ectasia was observed in seven cases, with no relation to the time passed between the first and second operation. Regarding the segment of the ureter reimplanted as IAFG, it was observed in its totality in nine cases. All the IAFG ureters that were qualified as viable

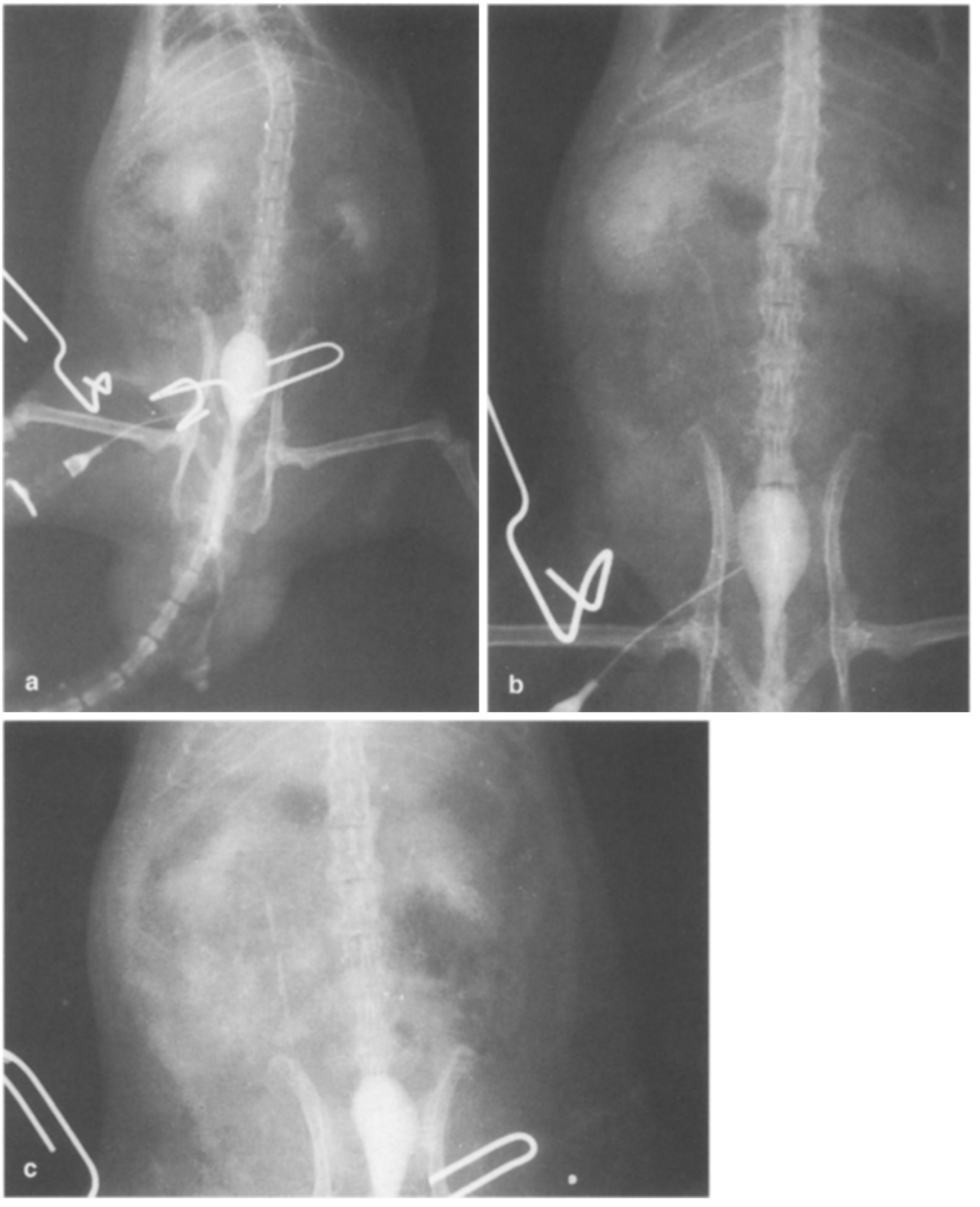

Fig. 1a–c Urographic results. Urography of the NU group is used as a reference. **a** IVU of NU-1 rat. Sprague-Dawley rats have a pyelocaliceal system that may be likened to "silhouette of a bird with extended wings" and the ureter is observed as a "line" scarcely 1 mm wide. In the SC group, the IVUs performed showed a morphology of the upper tract identical to that seen in NU group. In the IAFG group, IVU was performed in all rats in the group, with the exception of rat IAFG-17, in which it was impossible to carry out the IVU due to technical problems. In ten cases the IVU showed a functioning kidney. **b** IVU of rat IAFG-23, 56 days after the first operation. The whole length of the ureter is visualized. Ectasia, necrosis, stenosis, and extravasation are not seen. **c** IVU of rat IAFG-9, 218 days after the first operation. Neither dilation of the IAFG nor stenosis of the sutures was observed

and functioning under the surgical microscope showed neither moderate nor severe ectasia. Comparing the groups, we observed absolute normality in the SC group, and in some cases of the IAFG group.

In the IAFG group, correct functional results were observed in seven cases, in which no stenosis was present (IAFGa subgroup) (Table 1). In these cases, the three-layer structure was preserved (Fig. 2), though some sections showed fibrosis and increased vascularization. Some ureteral segments showed abundant subepithelial blood vessels, and there was an overall increased vascularization. The muscular layer was also preserved, with increased vascularization and, in three cases, with foci of fibrosis. Longitudinal sections across the suture area showed a foreign body reaction with giant cells around the stitches. In these sections, the three ureteral layers could be identified at both ends of the suture. In six cases of the IAFG group, functional results were poor. Although all the ureteral layers could be identified, they showed a disorderly appearance. The urothelium was composed of three to five-cell layers in some cases, while in other instances, it was thinner or hyperplastic. Some histologic sections showed very hyperplastic areas which seemed to contain blood vessels, this appearing in relation to the infoldings of the lamina propria that occur when chronic stimulation results in increased epithelization. Variable degrees of fibrosis were present in 75% of histologic slides. One of the slides from the suture zone showed metaplastic bone formation (Fig. 2). The origin of this phenomenon is still obscure. Several mechanisms have been proposed for its development: ischemia, microtrauma, metaplasia of blood vessel endothelium and contact between urothelial mucoproteins and mucosal connective tissue.

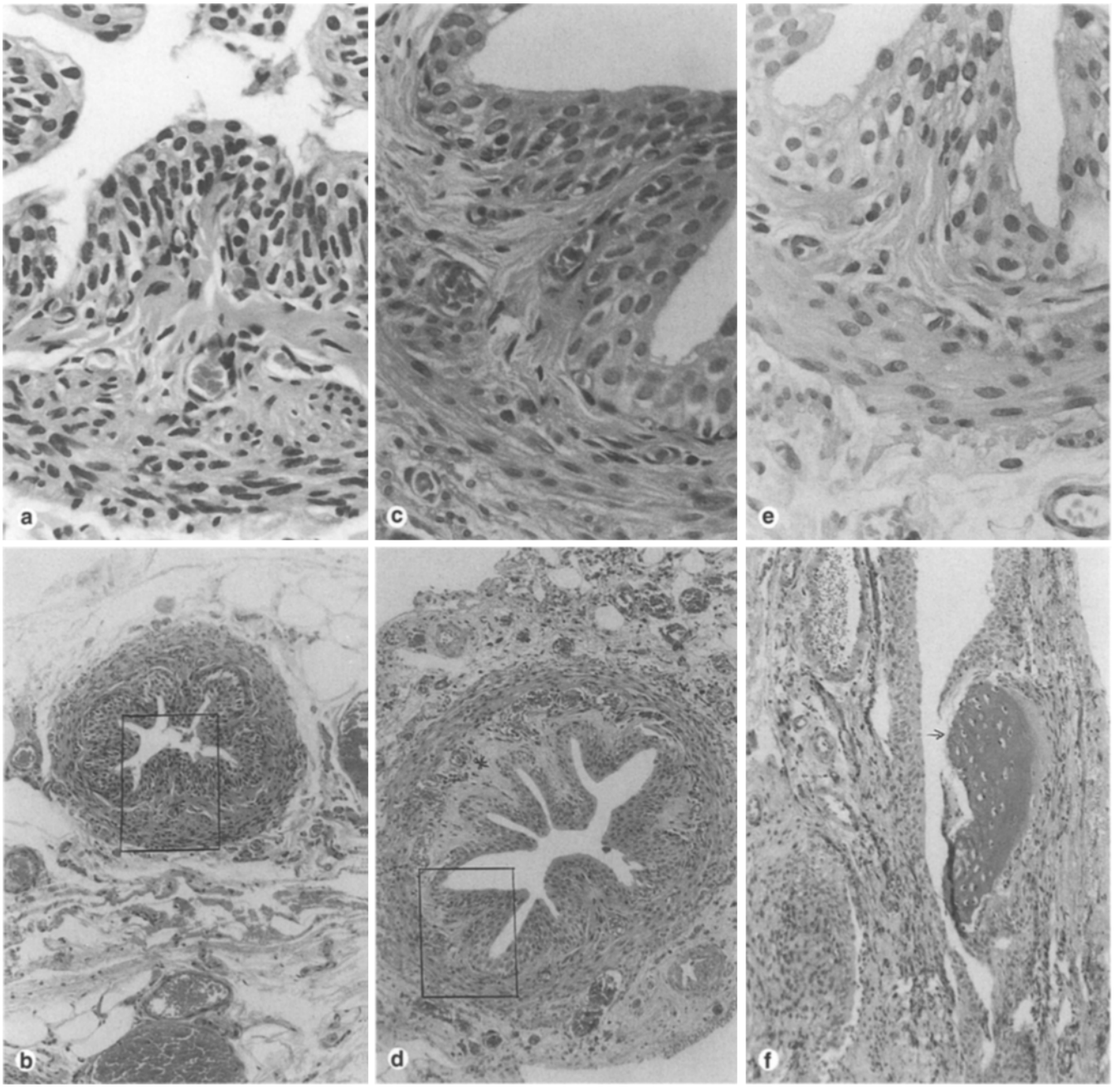

Fig. 2 a,b NIAFG group, Urothelium shows a range of appearances similar to those of the NU group (**e**). In some areas, cell debris may be seen in the ureteral lumen. In some sections, the lamina propia shows a reduction in the number of cells (30% of cases). In less than 5% of histologic sections, a mild fibrosis is observed in the muscular layer. In this layer also, the number of blood vessels is 3–5 times greater than in the NU group, and their diameter is twice that of the latter. Usually, neither necrosis, fibrosis, or loss of ureteral lumen was observed. **c,d** IAFG group. In cases with correct functional results, a three-layer structure is preserved, though some sections show some ureteral disorganization, with the separation between different layers becoming focally blurred, and fibrosis and increased vascularization being observed. However, there are no relevant foci of necrosis or other findings that could challenge ureteral viability (**d**). The urothelium is composed of three to five cell layers (**c**). Three cases show foci of fibrosis in the lamina propria. Ureteral segments such as that in rat IAFG-15 (**d**) display slight edema in the lamina propria(*). Some ureteral segments show abundant subepithelial blood vessels (**d**), and there is an overall increase in vascularization. The muscular layer is also preserved, with increased vasculature and, in three cases, foci of fibrosis. Only one case (**f**) (rat IAFG-22) shows metaplastic bone formation(→). **a** Hematoxylin-eosin slide of rat NIAFG-6 (175 days after NIAFG), segment B, x 40. **b** Idem, x 10. **c** Hematoxylin-eosin slide of IAFG-15 (42 days after IAFG), segment A, x 40. **d** Idem, x 10. **e** Hematoxylin-eosin slide of rat NU-16, x 40. **f** to hematoxylin-eosin slide of rat IAFG-22 (28 days after IAFG), segment G

Table 1 All values are means of A, B and C ureteral segments in micrometers ± SD (*MajD* major diameter, minD minor diameter, *UT* urothelial thickness, *LPT* lamina propria thickness, Mt muscular thickness, *TLT* three layer thickness, *TA* total area, *TLA* three-layer area, *UL* ureteral lumen, *UA* urothelial area, *LPA* lamina propria area, *MA* muscular area, *IAFGa* IAFG subgroup with good functional results)

	NU group	SC group	NIAFG group	IAFG group	IAFGa group
MajD	472.24 ± 69.42	466.29 ± 53.56	412.12 ± 91.80	1206.00 ± 637.02 (*)	578.00 ± 106.54
MinD	349.61 ± 49.27	390.80 ± 32.57	318.79 ± 74.47	709.56 ± 341.67*	433.33 ± 24.51*
UT	21.16 ± 2.50	25.53 ± 4.11	29.82 ± 10.70	24.92 ± 7.32	22.68 ± 5.67
LPT	19.69 ± 5.16	28.11 ± 7.32	18.88 ± 5.20	19.35 ± 4.09	18.45 ± 4.52
MT	33.81 ± 6.93	34.65 ± 3.67	33.17 ± 7.40	49.27 ± 14.51	42.56 ± 8.39
TLT	74.67 ± 7.70	88.29 ± 7.26*	81.87 ± 21.97	93.54 ± 23.33	83.68 ± 16.63
TA	131 108 ± 30 896	151 325 ± 32 623	121 999 ± 58 210	410 882 ± 342 491*	205 136 ± 39 960*
TLA	109 212 ± 23 745	131 601 ± 24 799	101 074 ± 49 413	236 403 ± 99 706*	149 199 ± 24 771*
UL	21 896 ± 12 407	19 723 ± 9 041	20 924 ± 12 699	174 478 ± 287 083*	55 936 ± 44 948
UA	31 044 ± 8 150	39 162 ± 10 079	30 219 ± 16 804	66 829 ± 32 756*	43 442 ± 7 221
LPA	32 462 ± 10 685	42 869 ± 12 287	25 049 ± 12 942	53 713 ± 22 232*	33 684 ± 9 157
MA	45 706 ± 12 485	45 569 ± 8 977	45 806 ± 20 867	115 861 ± 52 867*	72 072 ± 17 112*

* $P < 0.001$ compared to NU group

Compared to NU, all groups showed mild fibrosis and/or edema in the lamina propria and muscular layers. In addition, there was also a mild to moderate increase in blood vessels in the IAFG and NIAFG groups. There were no significant differences in number of cell layers of the urothelium, number of folds in the transverse section of the ureter, number of glomeruli per photographic field (except for the malfunction result in the IAFG group), number of glomeruli with sclerosis, collecting tubule diameter (except for the malfunction result in the IAFG group). However, there was an increase in vascularization affecting both number and diameter of blood vessels in the NIAFG and IAFG groups.

When the IAFG group with good functional results was compared with the NU group, the following statistically significant differences were seen: increased major diameter, greater total area, greater three-layer area and greater muscular layer area in the IAFG than in the NU group, with $P < 0.001$.

Discussion

The ureter itself has been used as a free graft, in autologous and homologous form, either lyophilized or not [4,6,10,15]. The results of these studies which were all performed in dogs but not in rats, were not very satisfactory, and the ureteral changes were not established. The length of the ureteral segment replaced was only 1–4 cm of the dog ureter.

Regarding ureteral vascularization, the description of the juxtal and intramural organization [13] are perfectly accepted by us, based on the findings of the vessels in the transversal sections. Previous studies [14] observed a decrease in vascularization after dissection of the ureter over its whole length, with a delicate moment on the 3rd day, which we did not observe either after 21 days or from that moment on until the 182nd day.

Contractions were observed after pinching in all the studied rats, which is normal in well-vascularized and innervated ureters. There is no doubt that an autonomy exists that allows the presence of contractions, which agrees with the myogenic theory and with the presence of an electrical focus of low resistance [5]. Barastegui believes that rats have a rich intramural innervation [1], which explains the existence of contractions. In the IAFG group, as ureteral contractions were present in rats after waiting 21 days before the second operation, the myogenic theory is confirmed, as well as in the other groups, in which the second operation was performed after 6 months.

We did not observe cysts, lumen stenosis, or necrosis in the NIAFG group. This would contradict the various authors who believe that desvascularization is the cause of transplanted ureteral necrosis. The function of the ureter used as IAFG depended on the anastomosis. When no stenosis was observed, there was no IAFG dilatation on IVU, and thus it was indistinguishable from the normal IVU.

Some studies have reported that exfoliation could be an index of rejection or the result of inadequate irrigation. The present study contributes to resolving this issue, as immunologic mechanisms are not involved. In some studies [7,8], a true ureteral rejection with prominent destruction of the urothelium has been advocated, which was not obvious in the NIAFG group. Texter et al [16] transplanted dog ureters after discontinuing steroid treatment that had been given since the beginning of the experiment. Their findings included muscular layer atrophy and edema in the whole ureteral wall, as well as fibrosis and loss of ureteral lumen, which has not been found in our study.

Histologic sections of untouched ureteral segments (D, E) showed ureteral tissue at both ends of the giant cell foreign body reaction against the nylon suture. In respect to the urothelium, this finding agrees completely with the results of Lamesch and Dociu [9], who identified urothelium over the suture 7 days after uretero-ureterostomy in rats, and complete re-epithelization of the same area 3 weeks later.

In general, the use of the ureter as a free graft gives rise to foci of fibrosis in different layers and increased vascularization, probably as a result of the sudden ureteral ischemia that takes place in these groups. The absence of differences between the three ureteral segments in all groups is additional evidence for the viability of free ureteral graft in the rats, as it reflects the lack of any predilection in the process of revascularization.

Conclusions

We believe that the two experimental models studied, the NIAFG and the IAFG, have enabled us to study the changes undergone by the denervated and desvascularized ureter. These models may be systematically used. No ureteral wall necrosis was observed. Both models permit the performance of homologous grafts in the future, in which immunologic ureteral phenomena may be studied. The IAFG graft allows the repeatable integration of the ureter, although the final result depends on the anastomosis, which in turn is seen to be affected by ischemical processes.

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