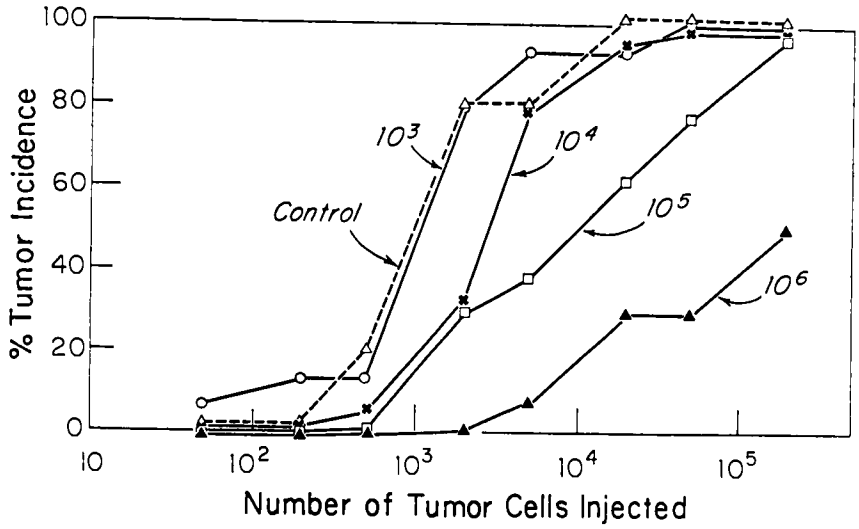

FIGURE 1. Tumor incidence resulting from challenge with various doses of a methylcholanthrene-induced fibrosarcoma 3 weeks after immunization with different numbers of tumor cells. Groups of 15 to 20 mice were immunized in the left hind leg with 10^3, 10^4, 10^5, or 10^6 tumor cells and the leg was amputated 6 days later. After 3 weeks each mouse was given i.d. injections of eight different doses of tumor and tumor development was monitored for 6 weeks. The curves representing immunization with 10^4, 10^5, and 10^6 cells were significantly different than the control curve. $P < 0.05$ by the Smirnov test.

TABLE 1. Comparison of the immunizing dose with the increase in TD_{50} resulting from the immunization[a]

Immunizing dose	TD_{50}[b]	Increase in TD_{50} over control	Ratio
None	1,000	—	—
10^3 cells	1,100	0	—
10^4 cells	3,000	2×10^3 cells	5
10^5 cells	10,000	9×10^3 cells	11
10^6 cells	200,000	2×10^5 cells	5

[a] TD_{50}s were interpolated from the curves in Figure 1. The increase in TD_{50} resulting from immunization was calculated as the difference between the TD_{50} in the immunized groups and that of the control group without immunization. The efficiency of immunization was also expressed as a ratio between the immunizing dose and the increase in the TD_{50} resulting from immunization.

[b] TD_{50}: number (dose) of tumor cells required to cause tumors to appear in 50% of the animals or sites injected with that dose.

defense mechanism other than the thymus-dependent cellular immune response.

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FLUORESCEIN SODIUM INJECTION FOR EVALUATION OF URETERIC VASCULATURE PRIOR TO CADAVERIC RENAL TRANSPLANTATION

Ureteral fistulae are a serious complication of renal transplantation, occurring with an incidence of 4 to 25% (*1*). The major reason for the development of ureterocutaneous fistulae and ureteral necrosis is a deficit in distal ureteral blood flow. This may be attributable to marginal vascularity of the distal ureter or to technical difficulties arising during surgical procurement of the kidney (*2*). A method of detecting compromised ureteral blood flow prior to transplantation, therefore, is desirable if maximal utilization of cadaver kidneys is to be obtained and ureterocutaneous fistulae prevented. We report the use of fluorescein dye injected into the renal artery prior to transplantation to evaluate the integrity of the ureteric vascular supply.

Two kidneys from a 16-year-old donor were received in ice storage preservation. Upon examination, the left kidney appeared to be normal but the right kidney had a double renal artery. The right main renal artery entered the hilum and supplied approximately 75% of the kidney while a smaller artery had been transected to a point 2.5 cm proximal to the hilum and had been skeletonized up to its point of entry into the hilum. Furthermore, a plane of dissection had developed between the medial surface of the inferior pole of the kidney and the lateral aspect of the ureter up to the renal pelvis. As a result

of these findings, the vascularity of the left ureter was believed to be compromised and the kidney had been turned down for transplantation by several transplant centers. Both kidneys were cannulated and placed on a pulsatile portable perfusion machine (Waters Instruments, Inc., Rochester, Minnesota) using an albumin-saline based perfusate. Both kidneys showed excellent perfusion characteristics with a perfusion pressure of 40/20 mm Hg, a flow rate of 133 ml/min, and an excellent cortical "bounce." In an attempt to ascertain the adequacy of the vascular supply of the right ureter, phenolsulfonphthalein dye (Hynson, Westcott, and Dunning, Inc., Baltimore, Maryland) was injected into the lower polar artery. The ureteric blood vessels could be visualized only to a point 1.5 cm cephalad to the lower border of the lower pole. Because of the continued excellent perfusion characteristics, however, we elected to investigate further the ureteral blood supply by the injection of fluorescein. The right kidney was transferred to a separate organ preservation machine and 3 ml of 0.2% solution of sodium fluorescein (Funduscein-10; Smith, Miller, and Patch, San German, PR) in 0.9% sodium chloride was injected into the arterial line leading to the polar artery. The total concentration of fluorescein used was 0.005 mg/ml of perfusate and was much less than that usually used for human fluorescein angiography (0.1 to 0.15 mg/ml of blood) (*3*). The room lighting was extinguished and the preservation cassette illuminated with a long wave ultraviolet lamp (Mineralight; Scientific Products, Harahan, LA). Following injection, the cortex of the kidney supplied by the polar artery immediately demonstrated uniform, intense fluorescence which slowly spread to involve the superficial renal cortex as the dye mixed with the perfusate perfusing the main renal artery. Within 10 sec, dye could also be observed throughout the length of the ureteric vessels and around the distal end of the ureter. Because of these observations, we elected to use this kidney for transplantation. At 21.3 hr total preservation time and 2.5 hr after the injection of fluorescein, the right kidney was placed in the right iliac fossa of a two-antigen match recipient. The cortex of the kidney demonstrated continued fluorescence at the time of removal from the preservation machine. Immediately after completion of the anastomosis to the recipient's vessels, the kidney produced urine and has continued to function well. Technetium renograms performed immediately post-transplant and at intervals during the initial hospitalization demonstrated a homogeneous sharp peak followed by rapid washout of the isotope. Four months after transplantation, the serum creatinine is 1.5 mg/100 ml. Since the successful utilization of this kidney, we have used injection of fluorescein dye to examine ureteric blood supply prior to transplantation in another cadaveric kidney with questionable renal vasculature. That recipient sustained a 3-week period of acute tubular necrosis but is currently doing well 3 months after transplantation with a serum creatinine of 1.2 mg/100 ml. The other kidney from this donor had acute tubular necrosis.

Determining adequate ureteric blood supply prior to insertion of cadaveric kidneys is important if one is to make maximal usage of kidneys, yet prevent development of ureterocutaneous fistulae or ureteral necrosis. Phenolsulfonphthalein dye has been advocated for this use by several transplant centers and is already used in some perfusion solutions as a pH indicator (J.L. Weinerth, personal communication). However, as happened in this case, it is often difficult to observe the progress of the orange color against the blood-stained periureteric tissue. Methylene blue is unsuitable for this use as it has been shown to be toxic to hypothermically perfused canine kidneys (*4*). We know of no other agents routinely used to evaluate ureteric blood flow.

Fluorescein sodium has been extensively used clinically for retinal angiography, assessing viability of skin flaps and testicular tissue, and to determine the adequacy of blood flow to the intestine and lower limbs (*3*, *5–7*).

Fluorescein appears to be nontoxic and there have been relatively few adverse reactions attributed to its use. Stein and Parker (*8*) have determined the incidence of side effects of intravenous fluorescein to be 0.06%. The incidence of adverse reactions may vary depending on the preparation, concentration, and rate of injection used (*9*).

Fluorescein appears to be a rapid, effective, and inexpensive method for determining adequacy of ureteric blood supply and in this case allowed us to utilize two kidneys which we would have otherwise discarded. The procedure is not without potential risks and should not be viewed as a substitute for meticulously performed donor nephroureterectomy. Opening the cassette and manipulating the kidney could predispose to either contamination or injury of the organ. Furthermore, although there is no evidence that fluorescein injection had a detrimental effect on renal function in either case, the toxicity of fluorescein to the transplanted kidney remains to be evaluated before this procedure can be recommended for routine use.[1]

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[1] Since the preparation of this manuscript, Dr. R. L. Burleson has kindly provided us with information demonstrating the safety of intra-arterial fluorescein administration in canine autotransplantation (*10*).