COMPARATIVE EVALUATION OF RENAL TRANSPLANT REJECTION WITH RADIOIODINATED FIBRINOGEN, ^{99m}Tc-SULFUR COLLOID, AND ^{67}Ga-CITRATE

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The diagnostic accuracy, ease, and technical feasibility of imaging with ^{131}I- or ^{125}I-fibrinogen, ^{99m}Tc-sulfur colloid, and ^{67}Ga-citrate in renal transplant rejection are compared. Radiofibrinogen data resulted from literature review, radiocolloid data from 125 studies in 52 transplant patients, and gallium citrate data from 24 examinations in seven renal transplant patients performed simultaneously with the radiocolloid studies. Specificity of graft labeling during rejection appears to be similar with radiofibrinogen, ^{99m}Tc-sulfur colloid, and ^{67}Ga-citrate. For routine clinical use ^{99m}Tc-sulfur colloid surpasses radiofibrinogen and radiogallium because of its better imaging qualities with a permissible radiation dose, leading to better separation of positive and negative results. The ^{99m}Tc-sulfur colloid accumulates in areas of intravascular fibrin thrombosis in acute and chronic rejecting renal transplants. Hence, the mechanisms for accumulation of ^{99m}Tc-sulfur colloid and labeled fibrinogen in rejecting transplants would seem to be similar. Such physiologic properties as rapid blood clearance and such physical properties as short physical half-life combine to produce reliable graft visualization with adequate definition, thus favoring ^{99m}Tc-sulfur colloid as the single agent of choice for clinical evaluation of renal transplant rejection at this time.

Recognition of renal graft rejection (and specifically early rejection) remains a diagnostic challenge. In spite of recent advances in the management of renal transplant patients and donor graft selection, the diagnosis of rejection is particularly difficult when associated with acute tubular necrosis, a frequent but temporary complication of cadaver kidney grafting. While selective renal arteriography and renal biopsy are excellent tools to establish a diagnosis of rejection, they are not applicable, however, to serial examination, which is mandatory for therapeutic management of renal transplant patients for the first few weeks after grafting and during episodes of rejection. The technical complexity and repeated trauma of these procedures limits their diagnostic utility. A nontraumatic technically uncomplicated procedure that yields quick acceptable diagnostic results is needed. In this communication we compare the diagnostic accuracy and technical feasibility of ^{131}I- or ^{125}I-fibrinogen, ^{99m}Tc-sulfur colloid, and ^{67}Ga-citrate imaging in renal transplant rejection.

Since fibrin is deposited in the vascular system and interstitial tissue of renal transplants undergoing acute or chronic rejection (*1,2*), radioactive fibrinogen has been used to detect rejection of human renal transplants (*2–5*). Further evaluation of radiofibrinogen has been carried out in an animal transplant model (*6–8*). We have had no opportunity to study these uses of radiofibrinogen but will refer in this report to results from the literature. Recently we have reported our experience with ^{99m}Tc-sulfur colloid in the diagnosis of human and canine renal transplant rejection (*9,10*). Sulfur colloid is laid down in fibrin thromboses present in rejecting renal transplants (*10*). Localization of gallium citrate in the polymorphonuclear granulocytes of bacterial, parasitic, and sterile inflammatory lesions (*11–15*) suggested that this agent might also localize in the acute inflammatory vasculitis and interstitial inflam-

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TABLE 1. SERIAL EXAMINATIONS OF SEVEN ALLOGRAFTED DOGS*

Dog No.	Days after grafting	Radiocolloid accumulation	Graft "vascular" transit	Creatinine (mg/100 ml)	Pathology at sacrifice
1	1	0	+	1.25	Acute rejection
	2	0	+	1.5	
	3	+	+	1.4	
	4	+	+	1.35	
2	1	0	+	2.8	Acute rejection
	2	0	+	2.5	
	3	+	+	1.58	
	4	+	+	1.8	
3	2	+	+	1.2	Acute rejection
	3	+	+	1.05	
4	2	+	+	0.8	Acute rejection
	3	+	+	1.3	
5	3	+	+	1.1	Acute rejection, anemic necrosis
	4	+	+	0.9	
	5	+	+	1.4	
	6	0	0	1.4	
6	2	+	+	1.55	Acute rejection, hemorrhagic necrosis, and renal vein thrombosis
	4	0	0	4.5	
	5	0	0	9.5	
7	1	0	0	3.7	Marked anemic necrosis, renal artery and vein thrombosis
	2	0	0	16.4	
	3	0	0	11.4†	
	4	0	0	12.5†	

* Three autografted control dogs did not accumulate ^{99m}Tc-sulfur colloid up to 7 days after grafting, while graft transit was consistently shown.
† Receiving intravenous infusions.

mation of rejecting renal transplants. To date we have evaluated seven such renal transplants serially after intravenous administration of ^{67}Ga-citrate.

MATERIALS AND METHODS

Radioiodinated fibrinogen studies (from literature review). Human transplant studies with iodinated fibrinogen (^{131}I and ^{125}I) have been performed with external probe counting, half-time blood clearance rates (*2–5*), and scintillation camera visualization of renal grafts with ^{131}I-fibrinogen (*4*). Examinations were carried out for several days serially to determine optimal graft activity concentration and clearance of peripheral blood activity.

Radiotechnetium sulfur colloid studies. All patients were examined with an Anger scintillation camera (the Pho/Gamma III camera, Searle Radiographics, Des Plaines, Ill.) 15–30 min after intravenous administration of 1–3 mCi of ^{99m}Tc-sulfur colloid (Tesuloid, E. R. Squibb and Sons, New Brunswick, N.J.). Paper chromatography indicated that the pertechnetate was over 99% bound. In all instances, 50,000 counts were accumulated from the transplant site in the right or left iliac fossa, and the time of exposure was recorded. The field of view included portions of the pelvis and vertebral column, occasionally the tip of the right lobe of the liver or the inferior pole of the spleen. Accumulation of radiocolloid by the renal transplant was evaluated subjectively: transplant activity appreciably greater than bone marrow activity was graded as marked accumulation; transplant activity comparable to bone marrow uptakes was graded as slight. Clinical diagnoses (acute rejection, acute tubular necrosis, nephrotic syndrome, chronic rejection, or normal function) were based on clinical criteria, the clinical course, time since transplantation, and in selected cases, renal biopsy, selective renal arteriography, or pathologic examination of the removed transplant. Transplant accumulation of radiocolloid (or absence of accumulation) was correlated with the clinical diagnosis at examination time. Iodine-131-Hippuran and ^{99m}Tc-pertechnetate studies were also performed in each instance (*16*).

Further evaluation of ^{99m}Tc-sulfur colloid was carried out in an animal rejection model. Ten immunologically unmodified female adult mongrel dogs (average weight 19 kg) were used. Seven dogs received renal allografts following bilateral nephrectomy, and three dogs received renal autografts to

serve as controls. The dogs were studied daily for 2–7 days. The scintillation camera, fitted with a diverging collimator, was placed over the transplant site and a bolus of 3 mCi of ^{99m}Tc-sulfur colloid was injected intravenously. To evaluate graft vascularity, kinetic data were first recorded on videotape for 10 min, then 50,000 counts were accumulated for a static image, and then the kinetic data were recalled from an electronically integrated window placed over the transplant site in the pelvis or the neck and displayed as time–activity curves at 10-sec intervals. The first intravascular renal graft transit during the bolus phase of ^{99m}Tc-sulfur colloid (Table 1) was evaluated as to the presence or absence of peak activity and washout (a flat time–activity curve indicated absence of vascular transit of the tracer through the graft). Venous blood samples were obtained during each study to determine serum creatinine levels. In selected instances, contact gross (*17*) and microscopic (*18*) autoradiographs were obtained from renal grafts excised after the death of the animal recipient.

Radiogallium citrate studies. Seven renal transplant patients received an intravenous dose of 5 mCi of ^{67}Ga-citrate (New England Nuclear Corp., North Billerica, Mass.) immediately after the examination with ^{99m}Tc-sulfur colloid, ^{99m}Tc-pertechnetate, and ^{131}I-Hippuran. Following high colonic enemas whenever feasible, patients were scanned 48, 72, or 96 hr after the administration of ^{67}Ga-citrate. A Pho/Gamma III scintillation camera, fitted with a diverging collimator, was used with a discriminator setting of 300 keV and a 20% window; 50,000–100,000 counts were accumulated for each image. All positive ^{67}Ga-citrate images were carefully evaluated for urinary bladder activity; patients had been instructed not to urinate for 2 hr before the scanning in order to evaluate urinary excretory activity. Transplant accumulation of radiogallium was considered positive only in the absence of demonstrable urinary bladder activity.

RESULTS AND DISCUSSION

Iodinated fibrinogen (^{131}I or ^{125}I) originally appeared to be the primary specific agent for the diagnosis of renal transplant rejection (*2–5*). This use of radioiodinated fibrinogen, however, has been hampered by (A) the potential hazard of serum hepatitis in humans; (B) the necessity of at least a 24-hr delay after administration for optimal imaging; (C) the poor quality of images; (D) the narrow range between normal and abnormal transplant uptakes (*2–8*); (E) the false-positive results obtained with wound hematomas (*3*) and urinary leaks; and (F) the negative results reported with chronic rejection (*5*).

Our investigation of ^{99m}Tc-sulfur colloid accumulation in rejecting canine allografts showed definite parenchymal transplant labeling starting as early as the first day after grafting, but usually on the second or third day. Transplants grafted to the internal carotid artery provided better transplant definition when visualized with ^{99m}Tc-sulfur colloid because the liver and spleen did not obscure the graft activity (Fig. 1).

Table 1 summarizes the results from the serial examinations of the seven allografted dogs. Uncomplicated acute rejection (Dogs 1–4) was associated with delayed but persistent vascular transit in the graft and accumulation of ^{99m}Tc-sulfur colloid. Rejecting transplants could be visualized before any significant changes of serum creatinine levels took place. Graft vascular transit and uptake was absent, however, when graft necrosis followed rejection (Dogs 5 and 6) or was an immediate postoperative grafting complication (Dog 7). In contrast, a total of 13 examinations of the three autografted controls provided no evidence of transplant accumulation of radiocolloid at any time from the second to the seventh day after grafting. All studies in these dogs showed a normal vascular transit phase of ^{99m}Tc-sulfur colloid and all autografts were normal on postmortem examination.

After an autopsy diagnosis of uncomplicated rejection, contact gross autoradiographs of excised grafts presented an outline of the larger intrarenal vasculature including interlobar, arcuate, and interlobular vessels (Fig. 2). Grossly visible radioactive thrombi were seen focally in interlobular vessels, sub-

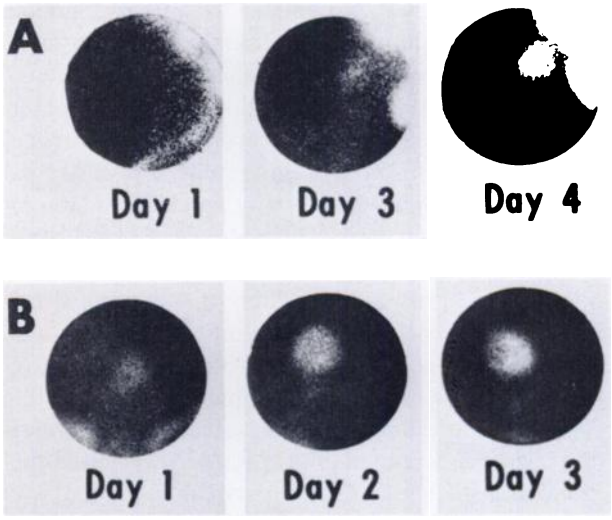

FIG. 1. Serial scintigraphs of two hyperacute rejecting renal allografts with ^{99m}Tc-sulfur colloid. Initial transplant visualization is present as early as 1 day after grafting. (Series A) transplantation in iliac fossa, (B) transplantation in carotid area.

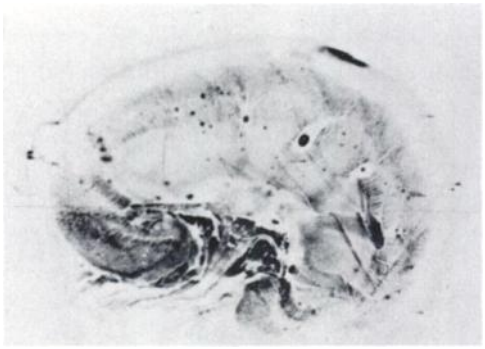

FIG. 2. Gross autoradiograph of rejecting canine renal transplant. Radioactivity is concentrated diffusely in renal vasculature.

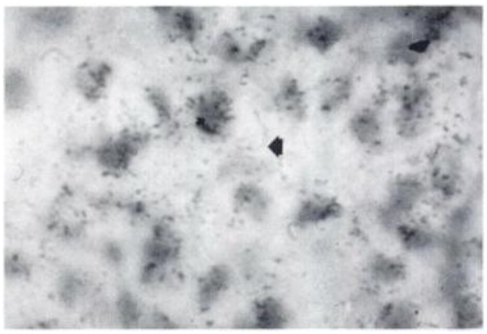

FIG. 3. Microautoradiograph of glomerular capillaries showing fibrin thrombosis and scattered exposed photographic grains (× 1000). Large arrow points to focus of fibrin thrombosis separating capillary loops. Small arrow points to clusters of grains associated with swollen capillary endothelial cells.

capsularly, and at the site of surgical anastomosis at the transplant hilum. Microscopically, radioactivity concentrated in glomerular fibrin deposits (Fig. 3) and in areas of fibrin thrombosis associated with vasculitis (Fig. 4). No radioactivity was found in tubular lumina, in tubular epithelial cells, or in interstitial connective tissue or inflammatory infiltrate.

In our patient series, only those renal transplants undergoing either acute or chronic rejection accumulated ^{99m}Tc-sulfur colloid (Table 2). Most patients with normally functioning renal transplants or acute tubular necrosis did not accumulate sulfur colloid within the renal transplant. Patients showing positive transplant accumulation of ^{99m}Tc-sulfur colloid with normal graft function (5/25) or with acute tubular necrosis (5/26) had had a prior rejection episode or developed clinical rejection within 7 days of the examination. Instances of acute rejection without accumulation (10/45) included necrotic transplants (6/10), transplants treated with high-dose heparin therapy (2/10), or those exhibiting only equivocal clinical signs of rejection (2/10). Transplants with chronic rejection that did not visualize with ^{99m}Tc-sulfur colloid (3/27) included cases of endstage chronic rejection with absent vascularity (shown on the pertechnetate study) and absent renal function (shown with ^{131}I-Hippuran). The instances of nephrotic syndrome with renal transplant accumulation of radiocolloid (2/2) most likely represented chronic rejection (*19*).

In summary, there were essentially no unexplained instances of transplant accumulation of ^{99m}Tc-sulfur colloid in patients with normally functioning renal transplants or transplants with acute tubular necrosis. Acute and chronic rejection, with evidence of residual transplant function and with preservation of blood supply, were characterized by transplant accumulation of ^{99m}Tc-sulfur colloid, which appeared to be a more sensitive indicator of rejection than clinical or biochemical evidence. Rejecting patients treated with high dosages of heparin did not accumulate ^{99m}Tc-sulfur colloid in the transplant; nevertheless, the pertechnetate and Hippuran patterns were abnormal.

The comparison of ^{67}Ga-citrate and ^{99m}Tc-sulfur

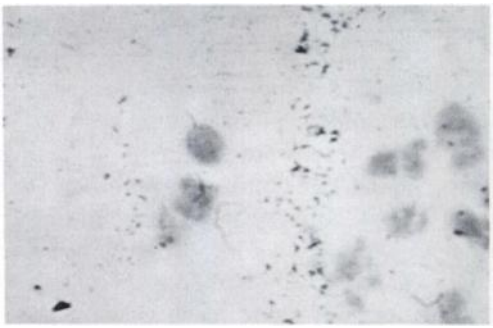

FIG. 4. Microautoradiograph of radioactive thrombus in interlobular artery (×1000). Note embedded grains and polymorphonuclear inflammatory cells.

TABLE 2. RENAL TRANSPLANT ACCUMULATION OF ^{99m}Tc-SULFUR COLLOID IN 125 EXAMINATIONS IN 52 PATIENTS

Diagnosis	Absent accumulation	Positive accumulation	Number of examinations
Normal function	20	5*	25
Acute tubular necrosis	21	5*	26
Acute rejection	10†	35	45
Chronic rejection	3‡	24	27
Nephrotic syndrome	0	2	2

* Developed or recuperated from clinical rejection within 7 days of examination.

† Six instances of transplant necrosis, two instances of heparin therapy, and two instances of equivocal clinical signs of rejection.

‡ Endstage chronic rejection.

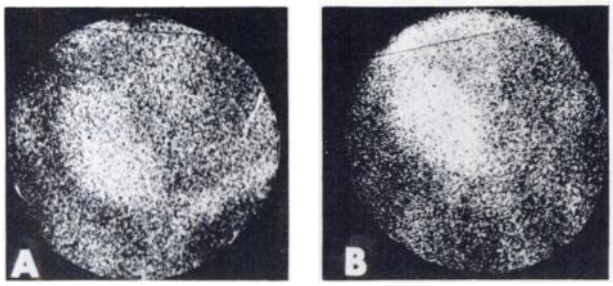

FIG. 5. Scintigraphs of acutely rejecting renal transplant, taken with ^{67}Ga-citrate (A) and ^{99m}Tc-sulfur colloid (B).

TABLE 3. COMPARISON OF ^{99m}Tc-SULFUR COLLOID AND ^{67}Ga-CITRATE IN 24 DUAL-AGENT EXAMINATIONS OF SEVEN PATIENTS

Diagnosis	Number of dual-agent examinations	^{99m}Tc-sulfur colloid		^{67}Ga-citrate	
		Positive	Negative	Positive	Negative
Normal	5		5		5
Acute tubular necrosis	3		3	1*	2
Chronic rejection	3	3		2	1†
Acute rejection	13	9	4‡	8	5‖

* Anuric patient.
† Unexplained (1/2).
‡ Necrotic transplant (1/4), heparin therapy (3/4).
‖ Necrotic transplant (1/5), heparin therapy (3/5), unexplained (1/5).

colloid accumulation in renal transplants (Fig. 5) is presented in Table 3. From these preliminary data it appears that similar diagnostic information may be obtained from both radioagents in a clinical setting. Neither accumulated in normally functioning transplants (5/5). Grafts with acute tubular necrosis were negative for transplant ^{99m}Tc-sulfur colloid (3/3) and ^{67}Ga-citrate accumulation (2/3) except for positive gallium uptake in a patient anuric during the course of the procedure (1/3). Hence, anuria between the time of ^{67}Ga-citrate administration and transplant imaging may produce gallium accumulation by the renal transplant with acute tubular necrosis. The mechanism of gallium accumulation in the renal graft in this situation may be similar to the Hippuran uptake seen in anuric transplants with acute tubular necrosis.

Graft uptake of ^{99m}Tc-sulfur colloid and ^{67}Ga-citrate correlated well in most instances of acute (8/13) and chronic rejection (2/3). Failure to accumulate either radioagent during acute rejection occurred in instances of acute graft rejection necrosis (1/13) and in patients receiving high doses of heparin (3/13) for concurrent problems. The latter finding suggests that heparin interrupted the intravascular coagulation cycle that leads to fibrin thrombosis and may have exerted a potent anti-inflammatory effect, thus inhibiting the formation of assumed specific accumulation sites for ^{99m}Tc-sulfur colloid and ^{67}Ga-citrate in rejecting renal transplants.

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