JOURNAL OF PHARMACEUTICAL SCIENCES

FEBRUARY 1981
VOLUME 70 NUMBER 2

RESEARCH ARTICLES

Evaluation of Methods for Producing Renal Dysfunction in Rats

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Received July 3, 1980, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication July 28, 1980.

Abstract □ The following methods for producing renal dysfunction in rats were compared: single-step 5/6th nephrectomy, two-step 5/6th nephrectomy, bilateral ureteral ligation, and uranyl nitrate injection. Control groups consisted of single- and two-step sham-operated animals and animals that received an injection of normal saline solution. The methods were evaluated on the basis of the following criteria, which were assessed daily for 6 days: survival, body weight, hematocrit, serum creatinine concentration, serum urea nitrogen concentration, serum glutamic pyruvic transaminase activity, serum albumin concentration, and serum protein binding of salicylate (determined every other day). Animals with bilateral ureteral ligation survived only 2 days, and single-step 5/6th nephrectomy caused a high incidence of fatalities. Some of the methods were associated with the development of hypoalbuminemia, but no significant elevation of transaminase activity occurred. Serum protein binding of salicylate was reduced in rats with renal dysfunction. A strong positive correlation between the creatinine and urea nitrogen concentrations in the serum of animals with renal dysfunction ($r = 0.91$, $p < 0.001$) and a negative correlation between the serum albumin concentration and salicylate free fraction ($r = -0.71$, $p < 0.001$) were found. Uranyl nitrate injection has the advantages of technical simplicity, a high survival rate (no deaths in this study), and relatively consistent and sustained diminution of renal function (as reflected by serum creatinine and urea nitrogen concentrations).

Keyphrases □ Renal dysfunction—evaluation of methods for producing renal dysfunction □ Uranyl nitrate injection—evaluation for producing renal dysfunction

Renal dysfunction can have pronounced effects on the pharmacokinetic and pharmacodynamic characteristics of drugs (1). Because exploration of these effects in patients may be limited by ethical and practical considerations, it often becomes necessary to perform studies on animals with experimental renal dysfunction (2, 3). Several methods are available for producing renal failure or dysfunction in experimental animals (2, 4–10); they include the administration of various nephrotoxic agents (such as dichromate, uranyl nitrate, certain pigments, mercurials, and glycerol), temporary occlusion of the renal artery or intrarenal infusion of vasoconstrictors, ureteral ligation, and partial nephrectomy.

Since sequence and relative importance of the pathophysiological components of acute renal failure in patients are not clearly defined (2), it is difficult to determine which experimental method for producing renal failure in animals yields results that are most representative of the clinical condition. However, a number of practical considerations should influence the choice of the method for producing renal failure or dysfunction in experimental animals for pharmacokinetic investigations. These considerations include the time, effort, and skill required to carry out the procedure, the stability and reproducibility of renal functional impairment, the general condition and survival time of the animals, the target organ specificity of the method, changes in the hematocrit and serum albumin concentration, and changes in the serum protein binding of certain drugs (which occur clinically and are important for pharmacokinetic studies).

The purpose of this investigation was to compare and evaluate several surgical methods and one chemical method for producing renal dysfunction in rats, namely, bilateral ureteral ligation, single-step 5/6th nephrectomy, two-step 5/6th nephrectomy, and uranyl nitrate injection. Uranyl nitrate was selected because it is not a known hepatotoxin. The development of renal dysfunction was determined by daily measurement of the serum creatinine and urea nitrogen concentrations, and the correlation between these concentrations was examined. Serum glutamic pyruvic transaminase activity was measured as an index of hepatocellular damage. Hematocrit and serum albumin concentrations were monitored because these values are known to decrease in some clinical cases of renal failure (11, 12). Finally, the serum free fraction of salicylic acid was determined because the protein binding of this

0022-3549/81/0200-0117$01.00/0

Table I—Survival of Rats with Renal Dysfunction Induced by Various Methods [a]

Method	Posttreatment Day 0	1	2	3	4	5	6
Bilateral ureteral ligation	6/6	6/6	5/6	0/6	0/6	0/6	0/6
Single-step 5/6th nephrectomy	6/6	5/6	3/6	3/6	3/6	2/6	2/6
Single-step sham-operated controls	6/6	6/6	6/6	5/6	5/6	5/6	5/6
Two-step 5/6th nephrectomy	8/8	8/8	6/8	6/8	6/8	6/8	6/8
Two-step sham-operated controls	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Uranyl nitrate injection	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Saline-injected controls	4/4	4/4	4/4	4/4	4/4	4/4	4/4

[a] Values are given as the number of survivors remaining/original number of animals.

Table II—Body Weight (Grams) of Rats with Experimental Renal Dysfunction and of Their Controls

Method	Posttreatment Day 0	1	2	3	4	5	6
Bilateral ureteral ligation	248 ± 21[a] (6)	260 ± 18 (6)	251 ± 24 (5)	—	—	—	—
Single-step 5/6th nephrectomy	232 ± 19 (6)	234 ± 21 (5)	219 ± 12 (3)	223 ± 16 (3)	220 ± 23 (3)	230, 237 (2)	228, 238 (2)
Single-step sham-operated controls	238 ± 11 (6)	258 ± 13 (5)	264 ± 13 (6)	264 ± 12 (5)	264 ± 17 (5)	268 ± 11 (5)	273 ± 17 (5)
Two-step 5/6th nephrectomy	319 ± 18 (8)	324 ± 17 (8)	316 ± 16 (6)	316 ± 25 (6)	317 ± 31 (6)	318 ± 27 (6)	318 ± 30 (6)
Two-step sham-operated controls	333 ± 22 (6)	356 ± 21 (6)	353 ± 25 (6)	354 ± 21 (6)	350 ± 23 (6)	349 ± 23 (6)	351 ± 23 (6)
Uranyl nitrate injection	204 ± 16 (6)	211 ± 8 (6)	214 ± 15 (6)	228 ± 16 (6)	230 ± 16 (6)	234 ± 14 (6)	237 ± 15 (6)
Saline-injected controls	222 ± 17 (4)	242 ± 18 (4)	241 ± 19 (4)	246 ± 16 (4)	250 ± 17 (4)	266 ± 22 (4)	265 ± 17 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses.

Table III—Hematocrit (Percent) of Rats with Experimental Renal Dysfunction and of Their Controls

Method	Posttreatment Day 0	1	2	3	4	5	6
Bilateral ureteral ligation	46 ± 1[a] (6)	46 ± 5 (6)	44 ± 6 (5)	—	—	—	—
Single-step 5/6th nephrectomy	44 ± 2 (6)	NM[b]	37, 40 (2)	40 ± 6 (3)	38 ± 6 (3)	33, 37 (2)	32, 31 (2)
Single-step sham-operated controls	44 ± 2 (5)	46 ± 2 (4)	42 ± 4 (6)	36 ± 2 (5)	35 ± 2 (5)	35 ± 1 (5)	34 ± 2 (5)
Two-step 5/6th nephrectomy	49 ± 1 (8)	43 ± 4 (7)	39 ± 4 (6)	40 ± 6 (6)	34 ± 3 (6)	32 ± 3 (5)	31 ± 3 (6)
Two-step sham operated controls	49 ± 2 (6)	48 ± 2 (6)	46 ± 2 (6)	41 ± 1 (6)	40 ± 1 (6)	38 ± 1 (6)	37 ± 1 (6)
Uranyl nitrate injection	45 ± 2 (6)	53 ± 5 (5)	46 ± 2 (6)	36 ± 2 (6)	36 ± 3 (6)	31 ± 2 (6)	32 ± 5 (6)
Saline-injected controls	44 ± 2 (4)	50 ± 1 (4)	45 ± 1 (4)	37 ± 5 (4)	36 ± 2 (4)	32 ± 2 (4)	36 ± 2 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses. [b] Not measured.

drug, like that of many other acidic drugs, is decreased in patients with impaired renal function (13, 14).

EXPERIMENTAL

Male Sprague–Dawley rats, 175–400 g, were fasted overnight before treatment. Food and water were allowed *ad libitum* thereafter.

Single-step 5/6th nephrectomy was performed on six rats in the following manner. A midline incision extending from the umbilicus to just below the sternum was made under ether anesthesia. The left kidney was isolated and dissected free of the surrounding tissues. A ligature (heavy silk) was placed tightly around the upper and lower poles of the kidney so that the organ was sectioned into thirds. Care was taken not to include the ureter in the lower ligature. The upper and lower poles then were excised. If necessary, a small square of Gel Foam[1] was used to stop bleeding. The right kidney was isolated and freed by blunt dissection from the surrounding tissues. A ligature (heavy silk) was placed around the renal vessels including the ureter, the renal artery, and vein. The entire kidney then was removed. The abdomen was closed with two layers of sutures.

Two-step 5/6th nephrectomy was performed on eight animals. The procedure was identical to the preceding one except that two steps were involved. The first step, which consisted of the complete removal of the right kidney, was performed 2 weeks before the second step to allow the remaining kidney to hypertrophy. Then two-thirds of the remaining kidney was removed as described for the single-step 5/6th nephrectomy.

Bilateral ureteral ligation was performed on six animals by isolating the ureters, placing two ligatures tightly around each ureter, and cutting between the ligatures.

Uranyl nitrate, 5 mg/kg, was administered once intravenously as a 5-mg/ml solution *via* a tail vein to six rats.

There were three control groups. One group of six animals was sham operated on the same day as the bilateral ureteral-ligated rats and the single-step 5/6th-nephrectomized rats and served as the control group for both of these groups. A second group of six animals underwent two sham operations 2 weeks apart on the days of surgery of the two-step nephrectomized rats and served as their controls. A third group of four control rats was given saline solution, 1 ml/kg iv *via* a tail vein, and served as the control group for the uranyl nitrate-treated rats.

After induction of renal failure, injection of saline, or sham operation, 1 ml of blood was taken from the tail artery of each rat on Days 1, 3, and 5 and 3 ml of blood was taken on Days 0 (prior to induction of renal failure), 2, 4, and 6. Blood was taken after the second step of the procedure from the two-step nephrectomized rats and their controls. The serum was separated immediately and stored in the frozen state pending analysis. About 50–100 μl of serum from each rat was set aside daily before freezing for the determination of serum creatinine concentrations. In addition, the rats were weighed daily and the hematocrit was recorded.

Serum creatinine concentrations were measured by the Jaffe (15) reaction, and serum urea nitrogen concentrations were measured by the method of Crocker (16), both using a commercial kit[2]. Transaminase activity was determined by the enzymatic method of Wroblewski and La Due (17) with a commercial kit[3]. Total protein concentrations were measured by the method of Gornall *et al.* (18), using rat albumin as a standard. The albumin fraction was determined by serum protein electrophoresis[4], and the albumin concentration was calculated by multiplying the fraction of albumin by the total protein concentration.

[1] The Upjohn Co., Kalamazoo, Mich.

[2] Rapid-Stat, Pierce Biochemical, Rockford, Ill.

[3] Calbiochem, La Jolla, Calif.

[4] Gelman electrophoresis system, Gelman Instrument Co., Ann Arbor, Mich.

Table IV—Serum Creatinine Concentrations (Milligrams per 100 ml) in Rats with Experimental Renal Dysfunction and in Their Controls

Method	Posttreatment Day						
	0	1	2	3	4	5	6
Bilateral ureteral ligation	1.22 ± 0.40[a] (6)	5.78 ± 0.47 (6)	9.33 ± 1.01 (5)	—	—	—	—
Single-step 5/6th nephrectomy	0.99 ± 0.14 (6)	NM[b]	2.68 ± 0.77 (3)	2.83 ± 1.59 (3)	5.03 ± 5.41 (3)	1.18, 1.54 (2)	1.56, 2.62 (2)
Single-step sham-operated controls	1.02 ± 0.16 (6)	1.19 ± 0.19 (5)	0.89 ± 0.21 (6)	1.19 ± 0.21 (5)	1.09 ± 0.42 (5)	0.90 ± 0.23 (5)	0.97 ± 0.25 (5)
Two-step 5/6th nephrectomy	1.11 ± 0.36 (8)	3.05 ± 1.08 (7)	2.91 ± 1.52 (6)	2.81 ± 1.67 (6)	2.29 ± 0.88 (6)	1.81 ± 0.19 (5)	1.95 ± 0.62 (6)
Two-step sham-operated controls	0.94 ± 0.32 (6)	1.07 ± 0.15 (6)	0.97 ± 0.25 (6)	1.14 ± 0.07 (6)	1.04 ± 0.17 (6)	0.92 ± 0.11 (6)	0.99 ± 0.17 (6)
Uranyl nitrate injection	1.08 ± 0.31 (6)	1.48 ± 0.53 (6)	1.78 ± 0.34 (6)	2.25 ± 0.20 (6)	3.26 ± 0.57 (6)	4.00 ± 1.01 (6)	6.84 ± 1.49 (6)
Saline-injected controls	1.30 ± 0.20 (4)	1.47 ± 0.27 (4)	0.65 ± 0.16 (4)	1.00 ± 0.38 (4)	1.28 ± 0.34 (4)	1.04 ± 0.28 (4)	1.16 ± 0.32 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses. [b] Not measured.

Table V—Serum Urea Nitrogen Concentrations (Milligrams per 100 ml) in Rats with Experimental Renal Dysfunction and in Their Controls

Method	Posttreatment Day						
	0	1	2	3	4	5	6
Bilateral ureteral ligation	10.8 ± 1.9[a] (6)	149 ± 9.0 (6)	254 ± 21.0 (5)	—	—	—	—
Single-step 5/6 nephrectomy	11.4 ± 2.7 (6)	NM[b]	57.4 ± 31.6 (3)	46.8 ± 27.1 (3)	NM[b]	20.3, 28.0 (2)	32.0, 31.0 (2)
Single-step sham-operated controls	9.8 ± 2.3 (6)	15.3 ± 2.7 (5)	12.2 ± 4.0 (6)	8.6 ± 2.1 (5)	16.1 ± 2.5 (5)	9.8 ± 1.7 (5)	9.9 ± 1.5 (5)
Two-step 5/6 nephrectomy	16.1 ± 6.8 (8)	102 ± 56 (7)	85.5 ± 60.0 (6)	72.0 ± 54.9 (6)	59.6 ± 50.6 (5)	31.4 ± 5.7 (5)	44.0 ± 22.4 (6)
Two-step sham-operated controls	10.7 ± 4.2 (6)	13.8 ± 2.8 (6)	17.3 ± 3.0 (6)	17.4 ± 2.3 (6)	14.4 ± 1.9 (6)	14.0 ± 2.4 (6)	13.7 ± 1.7 (6)
Uranyl nitrate injection	12.1 ± 1.7 (6)	45.0 ± 22.0 (6)	28.7 ± 4.7 (6)	30.4 ± 6.6 (6)	47.6 ± 9.5 (6)	78.2 ± 12.6 (6)	175 ± 24 (6)
Saline-injected controls	10.4 ± 1.6 (4)	21.7 ± 2.7 (4)	17.5 ± 2.7 (4)	11.3 ± 1.8 (4)	10.8 ± 1.8 (4)	9.2 ± 1.1 (4)	5.7 ± 1.8 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses. [b] Not measured.

The serum protein binding of salicylic acid was determined by equilibrium dialysis at 37° in plexiglass cells separated by a cellophane[5] membrane on serum samples obtained from the rats on Days 0, 2, 4, and 6. Approximately 0.5 ml of serum was dialyzed for 4 hr against an equal volume of 0.13 *M* isotonic phosphate buffer (pH 7.4) containing 300 μg of salicylic acid/ml. The salicylic acid concentration in the buffer and serum phases was assayed by the method of Brodie *et al.* (19).

RESULTS

Animals with ligated ureters looked and acted very ill and died by the 3rd day. There was a high incidence of death following single-step 5/6th nephrectomy and a lower incidence after the two-step procedure. The uranyl nitrate-treated animals and all but one control rat survived throughout the study (Table I). Animals with renal dysfunction showed less of a gain in body weight than the controls over the 6-day study (Table II). The hematocrit decreased with time in all animals, probably due mainly to repeated blood sampling (Table III).

Serum creatinine concentrations remained stable at ~1 mg/100 ml in all control groups and increased appreciably in all animals subjected to renal injury (Table IV). The most pronounced effect occurred after ureteral ligation. Animals with particularly elevated serum creatinine concentrations after partial nephrectomy tended to expire; this fact partially accounts for the declining average creatinine concentrations in these groups on Days 5 and 6. Uranyl nitrate-treated rats exhibited a gradual and relatively consistent increase of serum creatinine concentrations with time, except for a sudden, more substantial increase from Day 5 to Day 6 (Table IV). The same tendencies were observed with respect to serum urea nitrogen concentrations, including an abrupt increase from Day 5 to Day 6 in the uranyl nitrate-treated animals (Table V). There was a strong positive correlation between the serum creatinine and urea nitrogen concentrations in the rats with renal dysfunction (Fig. 1).

Serum glutamic pyruvic transaminase activity did not increase above normal levels in any of the animals. In fact, the rats with bilateral ureteral ligation (*i.e.*, the only animals that were unable to produce urine) exhibited a remarkable decrease of transaminase activity (Table VI).

Serum albumin concentrations decreased appreciably after bilateral ureteral ligation and partial nephrectomy but did not change remarkably after uranyl nitrate injection (Table VII). Serum protein binding of sal-

[5] Average pore size of 24 Å, VWR Scientific Co., Rochester, N.Y.

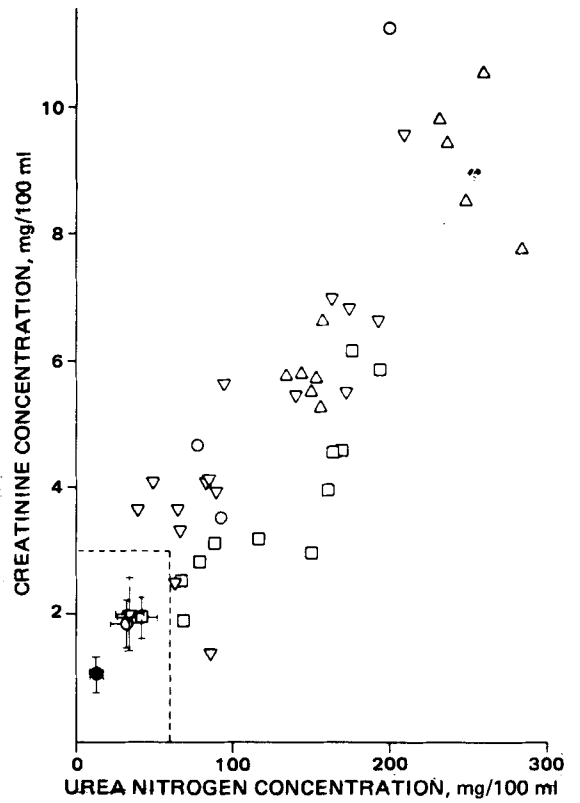

Figure 1—*Relationship between serum creatinine and serum urea nitrogen concentrations in rats with experimental renal dysfunction. Key:* ○, *single-step 5/6th nephrectomy;* □, *two-step 5/6th nephrectomy;* △, *bilateral ureteral ligation;* ▽, *uranyl nitrate injection; and* ●, *data from control animals (sham operated or saline injected) and from all other rats before surgery or injection of nephrotoxin. Data within the rectangle are the mean ± SD values for nine (○), 20 (□), and 20 (▽) determinations, respectively. The correlation coefficient for all except the control values is 0.913,* $p < 0.001$, $n = 90$.

Table VI—Serum Glutamic Pyruvic Transaminase Activity (Milliunits per Milliliter) in Rats with Experimental Renal Dysfunction and in Their Controls

Method	Posttreatment Day 0	1	2	3	4	5	6
Bilateral ureteral ligation	13.4 ± 2.4[a] (6)	5.8 ± 3.2 (6)	1.0 ± 2.3 (5)	—	—	—	—
Single-step 5/6 nephrectomy	17.9 ± 5.0 (6)	NM[b]	18.5 ± 7.2 (3)	10.6 ± 2.6 (3)	20.6 ± 1.8 (3)	15.4, 27.8 (2)	9.3, 24.7 (2)
Single-step sham-operated controls	16.8 ± 2.2 (6)	18.5 ± 1.6 (5)	21.3 ± 4.7 (6)	15.8 ± 2.2 (5)	15.6 ± 3.2 (5)	16.3 ± 2.6 (5)	17.1 ± 2.7 (5)
Two-step 5/6 nephrectomy	14.6 ± 4.2 (8)	10.7 ± 3.8 (7)	10.3 ± 2.5 (6)	12.5 ± 3.6 (6)	16.5 ± 6.1 (6)	20.6 ± 4.4 (5)	23.8 ± 4.2 (6)
Two-step sham-operated controls	11.5 ± 2.2 (6)	17.4 ± 3.6 (6)	23.9 ± 2.6 (6)	23.2 ± 4.6 (6)	23.3 ± 11.8 (6)	23.5 ± 4.7 (6)	26.0 ± 5.9 (6)
Uranyl nitrate injection	11.3 ± 1.1 (5)	11.2 ± 2.4 (6)	17.8 ± 1.1 (6)	17.0 ± 2.3 (6)	21.6 ± 1.8 (6)	20.8 ± 6.1 (6)	10.8 ± 4.9 (6)
Saline-injected controls	12.9 ± 1.3 (4)	12.1 ± 1.8 (4)	22.2 ± 3.0 (4)	15.2 ± 3.7 (4)	14.2 ± 3.6 (4)	17.2 ± 2.5 (4)	15.7 ± 2.1 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses. [b] Not measured.

Table VII—Serum Albumin Concentrations (Grams per 100 ml) in Rats with Experimental Renal Dysfunction and in Their Controls

Method	Posttreatment Day 0	1	2	3	4	5	6
Bilateral ureteral ligation	3.96 ± 0.30[a] (6)	2.73 ± 0.50 (5)	2.58 ± 0.42 (5)	—	—	—	—
Single-step 5/6 nephrectomy	3.92 ± 0.23 (5)	NM[b]	2.41 ± 0.28 (3)	2.44 ± 0.32 (3)	NM[b]	3.13, 2.31 (2)	2.98, 2.02 (2)
Single-step sham-operated controls	3.78 ± 0.41 (6)	3.75 ± 0.40 (5)	3.61 ± 0.47 (6)	3.50 ± 0.37 (5)	3.58 ± 0.42 (5)	3.51 ± 0.34 (5)	3.72 ± 0.34 (5)
Two-step 5/6 nephrectomy	5.11 ± 0.49 (8)	2.54 ± 0.64 (7)	2.46 ± 0.53 (6)	2.51 ± 0.43 (6)	2.57 ± 0.72 (6)	2.72 ± 0.53 (5)	2.87 ± 0.58 (6)
Two-step sham-operated controls	4.31 ± 0.31 (6)	3.53 ± 0.29 (6)	3.34 ± 0.22 (6)	3.30 ± 0.45 (6)	3.26 ± 0.41 (6)	3.35 ± 0.24 (6)	3.51 ± 0.24 (6)
Uranyl nitrate injection	3.99 ± 0.39 (6)	4.41 ± 0.20 (6)	3.94 ± 0.14 (6)	3.52 ± 0.24 (6)	3.49 ± 0.11 (4)	3.28 ± 0.37 (6)	3.46 ± 0.22 (5)
Saline-injected controls	4.10 ± 0.19 (4)	4.55 ± 0.33 (4)	4.79, 4.13 (2)	4.17 ± 0.17 (4)	4.40 ± 0.094 (4)	3.91 ± 0.18 (4)	4.02 ± 0.21 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses. [b] Not measured.

Table VIII—Serum Free Fraction (× 100) of Salicylic Acid in Rats with Experimental Renal Dysfunction and in Their Controls

Method	Posttreatment Day 0	2	4	6
Bilateral ureteral ligation	34.6 ± 1.2[a] (6)	55.3 (1)	—	—
Single-step 5/6 nephrectomy	34.5 ± 5.3 (6)	55.6 ± 16.1 (3)	34.1 (1)	41.5, 27.5 (2)
Single-step sham-operated controls	35.2 ± 2.8 (6)	36.3 ± 4.2 (5)	32.8 ± 5.0 (5)	33.6 ± 2.8 (5)
Two-step 5/6 nephrectomy	32.1 ± 3.4 (5)	51.9 ± 11.0 (5)	59.1 ± 12.4 (6)	52.3 ± 8.8 (5)
Two-step sham-operated controls	43.8 ± 6.1 (5)	40.4 ± 4.0 (4)	39.4 ± 6.7 (5)	43.2 ± 6.7 (5)
Uranyl nitrate injection	33.3 ± 2.8 (6)	33.0 ± 6.1 (5)	43.1 ± 6.0 (6)	47.4 ± 4.6 (6)
Saline-injected controls	29.2 ± 5.5 (4)	24.0 ± 1.9 (4)	23.8 ± 1.8 (4)	25.0 ± 3.6 (4)

[a] Mean ± *SD*; the number of animals is given in parentheses.

icylic acid decreased after renal injury (Table VIII). There was a significant negative correlation between salicylic acid free fraction values and the albumin concentration in the serum, with no discernible distinction as a function of the method employed to produce renal dysfunction (Fig. 2). Weak but statistically significant positive correlations were found between salicylic acid free fractions and serum urea nitrogen concentrations ($r = 0.534$, $p < 0.001$, $n = 118$) and between salicylic acid free fractions and serum creatinine concentrations ($r = 0.477$, $p < 0.001$, $n = 121$).

DISCUSSION

Of the methods studied, uranyl nitrate injection was the simplest for producing renal dysfunction. The method produced a significant and sustained elevation of serum creatinine and urea nitrogen concentrations and, as also found in patients, a decrease in the serum protein binding of salicylic acid. All animals survived for the duration of the study (6 days) and appeared to be in good condition. The surgical methods were more time consuming and slightly more difficult; ureteral ligation was more easily performed than partial nephrectomy. High mortality is a distinct disadvantage of the ureteral ligation and single-step 5/6th nephrectomy methods; two-step nephrectomy was associated with better survival, but the degree of renal functional impairment was more variable than after uranyl nitrate injection.

Uranyl nitrate causes necrosis of the distal portion of the proximal renal tubules. This effect is seen 4 days after injection (20). The results of this study show that renal function begins to decrease within 1 day after uranyl nitrate injection but that pronounced effects become evident on Days 5 and 6 (Tables IV and V). Decreased glomerular permeability also has been reported (21). Uranyl nitrate administration to rats has no significant effect on the cytochrome P-450 concentration and aniline hydroxylase activity in the liver but decreases hepatic aminopyrine *N*-demethylase activity (22). However, the latter also occurs after ureteral ligation and, therefore, is not a specific effect of uranyl nitrate (22). This study showed normal serum glutamic pyruvic transaminase activity after

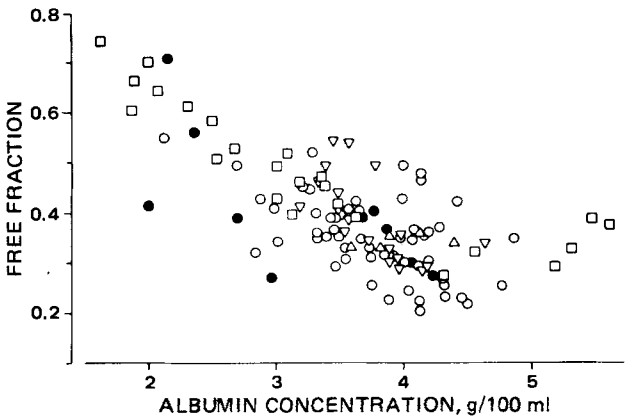

Figure 2—*Relationship between the free fraction of salicylic acid and the albumin concentration in the serum of rats with experimental renal dysfunction. Key: ●, single-step 5/6th nephrectomy; □, two-step 5/6th nephrectomy; △, bilateral ureteral ligation; ▽, uranyl nitrate injection; and ○, control animals. The correlation coefficient is −0.710, p < 0.001, with 113 determinations on 42 rats. This plot contains the free fraction data summdrized in Table VIII and some additional data obtained on days other than those listed in Table VIII.*

uranyl nitrate injection, indicating that there was no gross hepatocellular damage. We have no definitive explanation for the unusual decline of transaminase activity in rats with bilateral ureteral ligation; increased catabolism of the enzyme, decreased binding to serum constituents and consequent redistribution, and assay interference due to endogenous substances in these totally anuric animals may be involved[6]. Other investigators found decreased serum alkaline phosphatase activity in rats after administration of a nephrotoxic dose of cephaloridine and speculated that this result may be due to increased catabolism of the enzyme (23).

Creatinine as well as urea nitrogen concentrations in serum and renal clearances are commonly used indexes of renal function. It has been reported that "blood urea nitrogen results followed a curve virtually identical to that of the serum creatinine" in rats with transient elevation of serum creatinine concentrations associated with gentamicin-induced nephrotoxicity (24). A linear relationship between serum urea and creatinine concentrations was observed in rabbits with renal impairment induced by uranyl nitrate (25). A similar correlation was found in the present study, suggesting that experimental renal functional impairment in rats and rabbits can be assessed by measuring either creatinine or urea nitrogen. However, creatinine and urea serum concentrations or renal clearances are not always interchangeable or alternative indexes of renal function under clinical conditions since urea, but not creatinine, is partially reabsorbed from the renal tubules (26, 27). Serum concentrations of creatinine are affected by the age-dependent synthesis rate of the compound, while those of urea nitrogen may be affected by dietary protein intake. Serum urea was found to be superior to serum creatinine in the degree of the relationship to renal digoxin clearance in patients on digoxin therapy (27).

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ACKNOWLEDGMENTS

Supported in part by Grant GM 20852 and by a postdoctoral fellowship (GM 06774 to S. M. Roberts) from the National Institute of General Medical Sciences, National Institutes of Health.

[6] Assay interference is unlikely since measurement of transaminase activity in mixtures of serum from normal rats and from rats with bilateral ureteral ligation yielded results consistent with the measured transaminase activity of the component serum samples and their volume ratio in the mixture.