PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1074–1080 (1974)

Renal Neomycin Excretion in the Dog[1] (37956)

WILLIAM J. STONE, RICHARD E. BRYANT, A. HOYUMPA, AND S. SCHENKER

Department of Medicine, Vanderbilt University School of Medicine, and Veterans Administration Hospital, Nashville, Tennessee 37203

Neomycin is an aminoglycoside antibiotic first isolated in 1949 (1). Since then, its spectrum of activity and toxic side effects have been well-described (2). Administration of neomycin orally or by enema has become an important adjunct in the treatment of hepatic encephalopathy (3). At present, neomycin is rarely given by im or iv injection, but is still used in topical ointments and in certain irrigating solutions. However, despite good documentation of the ototoxic and nephrotoxic properties of neomycin reports of adverse side effects of this drug continue to appear. In recent years, toxicity has been noted as a complication of the use of neomycin to irrigate wounds, osteomyelitis lesions, decubitus ulcers, and articular or pleural surfaces (4–8). Adverse effects have also occasionally been noted during prolonged oral neomycin therapy for hepatic encephalopathy (9, 10). The continuing problem of toxic reactions to neomycin is probably attributable to lack of appreciation of the ease with which this drug is absorbed from the peritoneal cavity and other sites (i.e., bladder, pleura, and synovial space) and to the importance of renal excretion in the elimination of absorbed neomycin (11). The latter would appear to be especially important in patients with hepatic coma. Although less than 3% of orally or rectally administered neomycin is absorbed (12, 13), the presence of concomitant renal failure may cause an accumulation of the antibiotic in blood leading to further renal impairment and toxic side effects (12). In view of the importance of urinary excretion in the clearance of neomycin from blood and the known nephrotoxicity of this drug, it is surprising that a detailed study of the mechanism of renal excretion of neomycin has not been carried out. The present investigation in dogs was performed to define the factors which affect the clearance of neomycin by the kidney.

Materials and Methods. Renal clearance of neomycin. Twelve mongrel dogs of either sex weighing 16–32 kg were given 1,000 ml of iv isotonic sodium chloride solution prior to study. Under iv sodium pentobarbital anesthesia, both ureters were catheterized through a ventral midline incision. The right femoral artery and vein were catheterized for sampling and infusion, respectively. After priming dosages of 50 mg/kg for inulin and 1–10 mg/kg for neomycin, inulin and neomycin sulfate were infused separately at a constant rate in either 2/3 isotonic sodium chloride solution or 3% dextrose in water. Infusion rates for neomycin sulfate (Squibb) were adjusted to produce plasma levels between 5 and 50 μg/ml. From 6 to 10 clearance periods of 15–30 min duration were performed on each dog under steady-state conditions. Blood samples were taken at the midpoint of each clearance period. Urine was collected throughout each period.

Distribution space of neomycin. Three unanesthetized mongrel dogs weighing 16–27 kg were given iv "bolus" injections of 5 mg/kg of neomycin sulfate. Blood samples were taken at 5-min intervals for 30 min.

[1] This research was supported by grants from the National Institutes of Health, Nos. A1 09624 and MH 1200-02, and V.A. Research Funds.

Curves were plotted to determine neomycin plasma concentration at zero time. This concentration divided into the neomycin dose administered gave the distribution volume of neomycin in liters/kg.

Serum protein binding of neomycin. Protein binding of neomycin was measured by the equilibrium dialysis method. *Procedure 1: Dialysis of serum containing neomycin against Tris buffer.* Four milliliters of dog serum containing neomycin 100 μg/ml were placed into a sealed semipermeable bag fashioned from ⅜-in. dialysis tubing (Arthur H. Thomas Scientific Co.) and dialyzed against 4 ml of M/15 Tris buffer, pH 7.4, without neomycin in a sterile 14-ml Falcon tube. The tube was tightly capped and gently rotated for 18 hr at 37°. A similar dialysis procedure (control experiment) in which neither the serum nor the buffer contained neomycin was simultaneously set up. At the end of the experiment, samples of serum and buffer were separately recovered. Serum containing neomycin during dialysis was diluted with an equal volume of the Tris buffer dialyzed against neomycin-free serum, while the Tris buffer dialyzed against the neomycin-containing serum was diluted with an equal volume of neomycin-free serum recovered from the control experiment. Antibiotic standards were diluted in solutions containing equal volumes of neomycin-free dialyzed serum and Tris buffer. Thus the concentrations of serum and Tris buffer were identical in all assay specimens. The method for neomycin assay is described below. Percent binding was determined from the following formula:

$$\% \text{ binding} = \frac{[\text{neomycin } (\mu g/ml) \text{ in serum}] - [\text{neomycin } (\mu g/ml) \text{ in dialysate}]}{[\text{neomycin } (\mu g/ml) \text{ in serum}]} \times 100.$$

Procedure 2: Dialysis of neomycin-free serum against Tris buffer containing neomycin. The above experiments were repeated but instead of adding the neomycin to the serum it was added to the Tris buffer. Again, measurements of neomycin were done on samples cross-mixed with those obtained from the appropriate control experiments.

Analytical methods and calculations. Neomycin concentrations in serum or urine samples were measured by the modified cup-plate technique (14). Plates were made by mixing 0.3 ml of an 18-hr culture of *Staphylococcus epidermidis* (ATCC strain 110) with 30 ml of melted Penassay broth at 46° and promptly pouring this mixture into 150-mm petri dishes. Wells of approximately 10-ml volume were punched in the agar and removed with a sterile Pasteur pipet attached to a vacuum line. An Oxford automatic 10-ml pipet was used to fill the wells with antibiotic standards or test samples. Both samples and standards were run in duplicate. Specimens in serum or plasma were tested against standard concentrations of neomycin diluted in serum or plasma, whereas concentrations of neomycin in urine were tested against neomycin standards diluted in water (pH 7.0). The diameter of the zone of inhibition attributable to antibiotic was measured in mm after projecting plate images on a screen with a 521 overhead projector (3M Company). Graphs correlating change in zone size with neomycin concentration were constructed and used to determine neomycin concentrations of unknown specimens.

Because of noticeable variation in initial measurements of neomycin excretion rate in dog urine, it was considered necessary to determine the effects of the physical properties of urine on the neomycin cup-plate assay method. To test the possible effect of urine pH, neomycin standards were made up in pH 7.5 TES [*N*-tris(hydroxymethyl)-methyl 2-aminoethane sulfuric acid (Sigma)] buffer. The values obtained at pH 7.5 gave reproducible recoveries of known amounts of neomycin used and were accepted as actual neomycin concentrations. A series of samples were also assayed at 0.5 pH unit increments between pH 5.0 and 8.0. These samples were made up in TES buffer at neomycin concentrations of 25, 50, 100, and 200 μg/ml. The neomycin reading at these pHs, which might correspond to variable urine pH, was taken as the apparent neomycin concentration. The ratio of apparent to actual neomycin concentration, shown in Fig. 1, thus depicts the possible

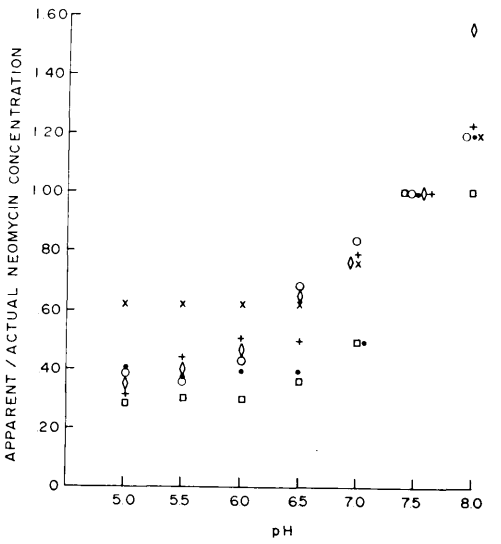

FIG. 1. Effect of pH on cup-plate neomycin assay. Each symbol refers to a separate experiment wherein the neomycin concentration was determined at various pHs. The individual neomycin concentrations are not identified since the effect of pH was independent of concentration within the range used. The number of points at each pH exceeds the number of concentrations used since some neomycin concentrations were assayed more than once. The experimental design and assay procedure are given in the text.

change in bioassayable activity introduced by pH in the dog urine neomycin assay. The data clearly show that irrespective of the neomycin concentration assayed, a pH below 7.5 significantly underestimated the neomycin concentration actually present in urine. A similar group of neomycin samples made up in phosphate buffer over the same pH range gave comparable results. Since sodium and chloride are ubiquitous urine ions and may vary widely in concentration, a range of sodium chloride concentrations comparable to those found in urine was chosen to assess the influence of these electrolytes on the neomycin assay *in vitro*. Standards were diluted in water. A series of solutions between 0 and 550 mEq/liter of NaCl (0 to 250 mEq/liter of Na^+) were used to suspend each of 4 neomycin concentrations—25, 50, 100, and 200 μg/ml. Apparent neomycin concentration for each NaCl solution was compared with the actual neomycin value obtained in water. The ratio of the apparent/actual neomycin observed at different NaCl concentrations is shown in Fig. 2. The data depict an overreading of the actual neomycin concentration at sodium concentrations of 150 mEq/liter or higher. In order to determine whether changes in antibiotic zone size were caused by the osmotic rather than the ionic property of NaCl, a series of studies were performed using sucrose solutions (0–1800 mOsm/liter) as the suspending agent for neomycin. Antibiotic zone size was unchanged by even the highest concentration of sucrose in water. Thus, both pH and ionic strength were shown to alter the neomycin cup-plate assay *in vitro,* but osmolality *per se* had no effect. To minimize the effect of variable urinary NaCl concentrations, 0.1 ml of saturated NaCl solution (pH 7.0) was added to each 0.9-ml dog urine sample and urine standard. Dog urine pH was generally around 7.5, and in this pH range, there was no significant effect on the neomycin assay (Fig. 1). Accordingly, no corrections for pH, or osmolality (see above), were made for these specimens.

Inulin was determined by the method of Schreiner (15). Urine sodium and osmolality were determined by standard methods. Clearances of neomycin and inulin were calculated by dividing the serum or plasma concentration into the excretion rate (urine concentration × urine vol per min). Statistical methods consisted of the *t* test and regression analysis (16).

Results. Renal clearance of neomycin. Table I summarizes the results of 12 dog experiments performed during steady-state infusion of neomycin sulfate. Six to ten clearance periods were averaged to provide clearance values for each dog. Arterial plasma concentration of neomycin ranged from 5.8 to 50.6 μg/ml. The average value for neomycin clearance was 39.6 ml/min ± 15.2 (SD). The average inulin clearance was 60.4 ml/min ± 19.5 (SD). Neomycin clearance correlated with inulin clearance ($r = 0.67$, $P < 0.02$). The actual values for the two clearances, however, were significantly different ($P < 0.01$). The fractional excretion of neomycin (C_{NEO}/C_{IN} ×

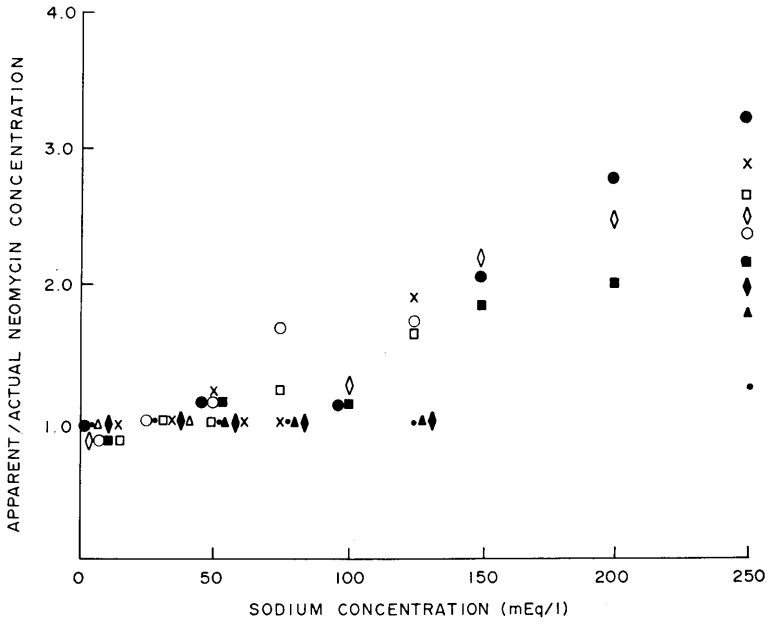

FIG. 2. Effect of NaCl concentration on the cup-plate neomycin assay. Each symbol represents a separate experiment carried out at increasing NaCl concentrations. The effect of ionic strength was independent of neomycin concentration which is thus not identified. Some neomycin concentrations were studied more than once. The methodology is given in the text.

TABLE I. Renal Clearance of Neomycin in the Dog.

Dog	Neomycin sulfate: Infused (μg/min/kg)	Neomycin sulfate: Arterial plasma concentration (μg/ml)	Neomycin sulfate: Excreted (μg/min)	C_{NEO}[a] (ml/min)	C_{IN}[b] (ml/min)	Fractional excretion of neomycin in % $\left(\frac{C_{NEO}}{C_{IN}} \times 100\right)$
1	205	50.6	2570	50.8	85.2	60
2	125	30.0	813	27.1	46.8	58
3	100	24.4	800	32.8	37.2	88
4	67	8.9	472	53.0	61.0	87
5	66	16.6	1185	71.4	69.1	103
6	45	16.5	535	32.4	60.4	54
7	44	18.8	523	27.8	55.1	51
8	36	5.8	119	20.5	23.3	88
9	30	6.4	324	50.6	95.8	53
10	29	7.9	374	47.3	68.7	69
11	24	6.3	140	22.2	56.4	39
12	21	9.0	357	39.7	65.2	61
Mean				39.6	60.4	68
SD				15.2	19.5	19

[a] C_{NEO} = Clearance of neomycin sulfate.
[b] C_{IN} = Inulin clearance.

100) had an average value of 68% ± 19 (SD). No correlation was found between fractional excretion and urinary osmolar excretion, sodium output, urine flow, or arterial plasma neomycin concentration ($P > 0.05$). Values of r were 0.08, 0.19, 0.23, and 0.02 for each of these parameters, respectively.

Distribution space of neomycin. Neomycin distribution space in 3 dogs was 0.11, 0.19, and 0.21 (average 0.17) liters/kg.

Neomycin protein binding. The serum protein binding of neomycin in the dog is shown in Table II. The degree of binding was comparable by both methods used and averaged 38.5 and 44%, respectively. These figures are similar to the values obtained for neomycin protein binding with bovine, ovine (17), and human sera (unpublished data).

Discussion. In the dog, the average fractional excretion of neomycin was 68% and 41.3% of the neomycin was bound to serum protein. The sum of these values is somewhat greater than but not significantly different from 100%. These observations, along with the correlation of neomycin and inulin excretion and the low molecular weight of neomycin (614), suggest that glomerular filtration and subsequent excretion of unbound neomycin is the principal mechanism of renal elimination of neomycin in the dog. While these studies were not designed to examine tubular handling of neomycin, they do not appear to indicate a significant renal reabsorption of neomycin. This is supported by observations that excretion of aminoglycosides like gentamicin, which exhibits virtually no serum protein binding, appears to reflect glomerular filtration alone (18, unpublished data). Our data, however, do not rule out a small contribution of tubular secretion of neomycin. This would be consistent with a somewhat

TABLE II. Dog Serum Protein Binding of Neomycin Sulfate.

Experiment[b]	Neomycin concentration (μg/ml)		% Binding
	Serum	Buffer	
Dialysis of neomycin-containing serum against neomycin-free Tris buffer[a]			
1	74	38	49
2	45	30	33
3	47	32	32
4	60	36	40
Mean ± SD			38.5[c] ± 7.8
Dialysis of neomycin-free serum against neomycin-containing Tris buffer[d]			
1	53	30	43
2	50	34	32
3	52	26	50
4	62	30	51
Mean ± SD			44.0 ± 8.75

[a] Procedure 1 (see text).

[b] Each experiment was carried out in duplicate or triplicate. The individual values given represent a mean of these determinations.

[c] Not statistically different from values obtained by Procedure 2 ($P > 0.05$).

[d] Procedure 2 (see text).

greater than 100% fractional excretion of neomycin after accounting for protein binding in some of the dogs. This possibility will have to be resolved by specific studies of renal tubular neomycin handling utilizing inhibitors of tubular secretion or stop-flow techniques. The possibility that the higher fractional excretion in certain dogs may be related to hypoalbuminemia and lower protein binding cannot be ruled out since serum proteins and protein binding were not determined in these specific animals.

When considered in relation to inulin clearance, neomycin clearance in the dog was unaffected by urine flow, sodium or osmolar excretion, or by serum neomycin concentration. This implies that the only feasible physiologic means to remove excess neomycin from blood is to increase the glomerular filtration rate. Both peritoneal dialysis and hemodialysis have also been employed with success (19).

The present study also points out several problems which may arise in the cup-plate neomycin assay. Our data clearly show that both pH and ionic strength may affect the neomycin measurement in urine. Urine pH below 7 results in a falsely low value for the antibiotic unless the sample and standard are read at the same pH. This effect of pH on the neomycin assay has also recently been observed with plasma neomycin analysis (20). Sodium concentration of urine usually is in a range (0-100 mEq/liter) which does not significantly affect the neomycin measurement. However, in dehydration or with a large solute infusion, as used in some of the dog studies presented here, an appropriate adjustment of the final samples and standard to a uniform ionic strength is necessary to minimize technical errors. The possible effect of other urine constituents on the bacteriologic assay of neomycin by the cup-plate technique remains to be investigated.

Summary. This study examines the mechanism(s) of renal neomycin excretion in dogs. Neomycin is approximately 40% bound to serum protein and the mean neomycin fractional excretion is 68%. Since (a) the sum of protein binding and the fractional excretion of neomycin approximates 100%, (b) the low molecular weight of neomycin should facilitate its filtration, and (c) other aminoglycosides like gentamicin which are not bound to protein have not been shown to be absorbed or secreted by renal tubules, it appears likely that glomerular filtration is the main mechanism responsible for neomycin excretion by the kidney.

The authors express their sincere appreciation for the excellent technical contribution of Miss Bonnie Keith.

1. Waksman, S. A., and Lechevalier, H. A., Science **109**, 305 (1949).

2. Weinstein, L., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gillman, eds.), p. 1269. The Macmillan Co. (1970).

3. Dawson, A. M., McLaren, J., and Sherlock, S., Lancet **2**, 1263 (1957).

4. Trimble, G. X., New Eng. J. Med. **281**, 219 (1969).

5. Editorial, Brit. Med. J. **4**, 181 (1969).

6. Murphy, K. W., Brit. Med. J. **2**, 114 (1970).

7. Myerson, M., Knight, H. F., and Gambarini, A. J., Ann. Thorac. Surg. **9**, 483 (1970).

8. Nilges, T. C., and Northern, J. L., Ann. Surg. **173**, 281 (1971).

9. Last, P. M., and Sherlock, S., New Eng. J. Med. **262**, 385 (1960).

10. Berk, D. P., and Chalmers, T., Ann. Intern. Med. **73**, 393 (1970).

11. Hawkins, J. E., *in* "Pharmacology of Neomycin in Neomycin: Its Nature and Practical Application" (S. A. Waksman, ed.), pp. 130–146. Williams and Wilkins Co., Baltimore (1958).

12. Kunin, C. M., Chalmers, T. C., Leevy, C. M., Sebastyan, S. C., Lieber, C., and Finland, M., New Eng. J. Med. **262**, 380 (1960).

13. Breen, K. J., Bryant, R. A., Levinson, J. D., and Schenker, S., Ann. Intern. Med. **76**, 211 (1972).

14. Grove, D. C., and Randall, W. A., *in* "Assay Method of Antibiotics: A Laboratory Manual," New York Medical Encyclopedia (1955).

15. Schreiner, G. E., Proc. Soc. Exp. Biol. Med. **74**, 117 (1950).

16. Bruning, J. L., and Kintz, B. L., *in* "Computational Handbook of Statistics," pp. 12–15. Scott Foresman & Co., Glenview, IL (1968).

17. Gordon, R. C., Regamey, C., and Kirby, W. M. M., Antimicrob. Agents and Chemother. **2**, 214 (1972).

18. Ziv, G., and Sulman, F. G., Antimicrob. Agents and Chemother. 2, 206 (1972).

19. Schreiner, G. E., and Teehan, B. P., Trans. Amer. Soc. Artif. Intern. Organs 18, 563 (1972).

20. Krumlovsky, F. A., Emmerman, J., Parker, R. M., Wisjerof, M., and Del Greco, F., Ann. Intern. Med. 76, 443 (1972).

Received Sept. 27, 1973. P.S.E.B.M., 1974, Vol.145.