Uranyl Nitrate Induced Acute Renal Failure in the Rat: Effect of Varying Doses and Saline Loading (37305)

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(Introduced by Robert J. T. Joy)

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The administration of uranium salts, such as uranyl nitrate, has been demonstrated to result in severe renal insufficiency and renal tubular necrosis in the dog (1), the rat (2), and the rabbit (3). Previous studies have suggested that passive backflow of a normally formed glomerular filtrate across necrotic tubular epithelium may be the mechanism responsible for renal insufficiency in uranyl nitrate induced acute renal failure (1, 3). Alternatively, a primary hemodynamic alteration resulting in diminished effective glomerular perfusion has been proposed as the pathophysiologic abnormality responsible for renal dysfunction in acute renal failure (4–9). The renin–angiotensin system has been suggested as the effector mechanism responsible for the alteration in renal hemodynamics (10–12).

The present study was designed to determine if there is any correlation in the rat between the extent of tubule damage and degree of renal dysfunction after various doses of uranyl nitrate. To evaluate the role of the renin–angiotensin system in this model of acute renal failure, an additional group of rats was studied after suppression of the renin–angiotensin system by saline loading.

Materials and Methods. All studies were performed on male Sprague-Dawley rats[1]. Six groups, each with eight rats, were placed in individual metabolic cages designed to separate urine from feces and allow accurate determinations of 24 hr urine volume and fluid intake. After a period of adaptation, data were collected for a 24 hr control period and each group was injected with one of the following doses of uranyl nitrate: 2.5, 5.0, 7.5, 10.0, 15.0 and 20.0 mg/kg body weight. All injections were made into a tail vein with a 26 gauge needle. The uranyl nitrate was dissolved in physiological saline in concentrations such that each animal received 1 ml of intravenous solution/kg body weight. Following injection, the animals were returned to the metabolic cages and urine volume and fluid intake were measured for the next 48 hr.

An additional group of 8 rats was given free access to the standard laboratory diet and 1% saline as the sole source of drinking fluid. After 3 wk of sodium chloride loading, the animals were placed in individual metabolic cages and given intravenous uranyl nitrate, 10 mg/kg body weight in a volume of 1.0 ml/kg body weight. All other measurements and procedures were the same as for the other 6 groups.

Blood urea nitrogen (BUN) concentrations were determined on the control day as well as 48 hr after uranyl nitrate (13). At 48 hr the animals were sacrificed, the kidneys were rapidly removed, longitudinally sectioned and fixed in buffered 10% formalin for histological study. Urine osmolality was measured on all urine samples (control, 24 and 48 hr) using a Fiske osmometer (Model G-66A, Uxbridge, MA). Sodium and potassium concentrations of the urine samples were determined with an IL flame photometer (Model 143, Lexington, MA).

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

TABLE I. Course of Body Weight, Urine Volume, Fluid Intake and BUN Concentration After Various Doses of Uranyl Nitrate.

Dose of uranyl nitrate, iv: (mg/kg body wt)	2.5 (8)[a]	5.0 (8)	7.5 (8)	10.0 (8)	15.0 (7)	20.0 (6)
Body wt (g)						
Control	274 ± 18	240 ± 12	264 ± 4	235 ± 4	210 ± 5	263 ± 5
24 hr	271 ± 16	228 ± 11	249 ± 4[b]	227 ± 5	206 ± 4	256 ± 4
48 hr	266 ± 18	225 ± 10	245 ± 4[b]	221 ± 5[b]	201 ± 5	248 ± 4[b]
Urine vol (ml/24 hr/100 g body wt)						
Control	6.3 ± 0.8	8.5 ± 1.2	5.8 ± 0.5	7.1 ± 0.6	9.6 ± 0.6	10.0 ± 1.0
24 hr	8.5 ± 0.8[b]	10.1 ± 1.3	8.3 ± 0.5[b]	10.2 ± 1.1	14.0 ± 1.8	14.8 ± 0.9
48 hr	7.4 ± 0.6	13.2 ± 1.1[b]	13.8 ± 0.8[b]	15.6 ± 0.9[b]	17.8 ± 1.1[b]	18.1 ± 1.2[b]
Fluid intake (ml/24 hr/100 g body wt)						
Control	11.7 ± 0.6	14.6 ± 2.1	12.0 ± 0.9	11.3 ± 0.9	16.8 ± 1.2	14.9 ± 1.9
24 hr	12.4 ± 0.6	14.5 ± 1.8	13.1 ± 1.7	14.3 ± 1.4[b]	21.5 ± 3.0	19.3 ± 1.2[b]
48 hr	11.1 ± 0.7	16.4 ± 0.9	16.6 ± 1.4[b]	19.7 ± 1.3[b]	24.4 ± 1.5[b]	23.8 ± 0.6[b]
BUN concn (mg/100 ml)						
Control	18 ± 2	17 ± 3	16 ± 2	15 ± 3	17 ± 2	16 ± 3
48 hr	28 ± 2[b]	44 ± 3[b]	50 ± 3[b]	56 ± 5[b]	68 ± 8[b]	59 ± 14[b]

[a] The numbers in parentheses represent the number of animals in each group.

[b] Significantly different from control at the 5% confidence limit or below.

All results are reported as the mean ± standard error of the mean (SEM) and, where applicable, have been presented per 100 g of body weight. Statistical analyses were performed according to Snedecor and Cochran (14).

Results. Tables I and II contain the data obtained during the control period and after intravenous uranyl nitrate. All groups had a tendency towards diminished body weight during the 48 hr period after uranium (Table I). The weight loss was statistically significant only in the groups receiving 7.5, 10.0, and 20.0 mg/kg body weight of uranyl nitrate. Urine volume and fluid intake showed a tendency to increase which was more marked at the higher doses of uranyl nitrate. At 24 hr, the percentage increase in urine volume ranged from 34.1 and 18.3% in the groups receiving 2.5 and 5.0 mg/kg body weight, respectively, to 45.8 and 48.4% in the groups receiving 15.0 and 20.0 mg/kg body weight. At 48 hr, statistically significant increases in urine volume were observed in all groups except animals receiving 2.5 mg/kg body weight of uranyl nitrate.

There were no significant differences in control BUN concentrations with a range of 15 ± 3 to 18 ± 2 mg/100 ml. At 48 hr, the mean BUN concentration in the group receiving 2.5 mg/kg body weight of uranyl nitrate had increased to 28 ± 2 mg/100 ml from a control value of 18 ± 2 mg/100 ml ($p < .05$). In the remaining groups, despite doses of uranyl nitrate from 5.0 to 20.0 mg/kg of body weight, the 48 hr BUN concentrations were not statistically different from each other and were elevated to a similar degree above control values. This azotemia has been demonstrated, in preliminary studies, to be associated with parallel alterations in both glomerular filtration rate and renal blood flow (15). In the present study the extent of renal failure was gauged by the degree of azotemia, even though glomerular filtration rate and renal blood flow were not measured.

Significant depressions in urine osmolality were observed 24 and 48 hr after uranyl nitrate in all groups except those animals receiving the lowest doses of uranium (Table II). The depression in urine osmolality was least (15.7%) in the 5.0 mg/kg body weight and most marked in the 15.0 and 20.0 mg/kg body weight groups (45.7 and 45.5%, respectively). No significant decrease in sodium excretion was noted below the 10.0 mg/kg body weight dose of uranyl nitrate. The decrease in sodium excretion observed at the three highest doses were significantly different from control values ($p < .05$) at 24 and 48 hr and were of a similar magnitude. Although variations in potassium excretion occurred, they were sporadic and of a lesser degree.

The saline loaded group had significant increases in urine volume and fluid intake 24 and 48 hr after uranyl nitrate (Table III). Mean urine sodium excretion during the control period was 3.0 ± 0.2 mEq/24 hr/100 g body weight, significantly greater than the control values obtained in the animals on the routine diet. Sodium excretion did not change significantly after uranium. Control urine potassium excretion was in the same range as the animals on the routine diet and did not vary after uranyl nitrate. During the control period, mean urine osmolality was 1008 ± 87 mOsm/liter, significantly lower ($p < .01$) than control urine osmolality in the nonsaline loaded rats. A 43.6% depression in mean urine osmolality to 569 ± 56 mOsm/liter ($p < .001$) was noted 48 hr after uranium. Forty-eight hours after uranyl nitrate, the mean BUN concentration was 24 ± 3 mg/100 ml, not significantly increased ($p > .05$) over the mean control value of 18 ± mg/100 ml and significantly lower than the value obtained in the nonsaline loaded animals receiving the same dose of uranyl nitrate ($p < .001$).

Histologic sections of the kidneys were reviewed without prior knowledge of the dose of uranyl nitrate received by each animal. An overall estimate was made of the number of tubules with evidence of a lesion as well as the extent and severity of the damage to the tubules. In general, a proportional relationship was observed between the extent of proximal tubule damage and dosage of uranyl nitrate. A focal proximal tubular lesion was apparent in the inner portions of the renal cortex in rats receiving 2.5 mg/kg body weight, and was characterized by swell-

TABLE II. Course of Urine Osmolality and Electrolyte Excretion After Various Doses of Uranyl Nitrate.

Dose of uranyl nitrate, iv: (mg/kg body wt)	2.5 (8)[a]	5.0 (8)	7.5 (8)	10.0 (8)	15.0 (7)	20.0 (6)
Urine osmolality (mOsm/liter)						
Control	1304 ± 128	1500 ± 117	1858 ± 80	1532 ± 110	1798 ± 70	1401 ± 144
24 hr	1281 ± 122	1379 ± 132[b]	1466 ± 77[b]	1358 ± 113[b]	1298 ± 97[b]	857 ± 142[b]
48 hr	1353 ± 122	1265 ± 103[b]	1124 ± 45[b]	1202 ± 110[b]	976 ± 127[b]	764 ± 69[b]
Urine sodium excretion (mEq/24 hr/100 g body wt)						
Control	1.3 ± 0.1	1.8 ± 0.1	1.4 ± 0.2	1.9 ± 0.1	2.51 ± 0.1	2.0 ± 0.1
24 hr	1.2 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	1.6 ± 0.2[b]	2.0 ± 0.1[b]	1.7 ± 0.2[b]
48 hr	1.2 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	1.6 ± 0.1[b]	1.6 ± 0.1[b]	1.5 ± 0.1[b]
Urine potassium excretion (mEq/24 hr/100 g body wt)						
Control	0.7 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	1.3 ± 0.1	0.9 ± 0.1
24 hr	0.8 ± 0.1	1.0 ± 0.1[b]	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
48 hr	0.7 ± 0.1	1.0 ± 0.1[b]	0.9 ± 0.1	1.2 ± 0.1[b]	1.2 ± 0.1	1.0 ± 0.1

[a] The number in parentheses represents the number of animals in each group.
[b] Significantly different from control at the 5% confidence limits or below.

TABLE III. Various Parameters Studied After Intravenous Uranyl Nitrate in Eight Saline Loaded Rats.

Time after uranyl nitrate:	Control	24 hr	48 hr
Body wt (g):	299 ± 4	287 ± 4[a]	286 ± 5[a]
Urine vol (ml/24 hr/100 g body wt)	10.4 ± 0.4	12.4 ± 1.5[a]	13.2 ± 0.6[a]
Fluid intake (ml/24 hr/100 g body wt)	11.2 ± 0.7	16.5 ± 1.6[a]	17.5 ± 1.1[a]
BUN concn (mg/100 ml)	18 ± 2	—	24 ± 3
Urine sodium excretion (mEq/24 hr/100 g body wt)	3.9 ± 0.2	4.3 ± 0.3	3.9 ± 0.2
Urine potassium excretion (mEq/24 hr/100 g body wt)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Urine osmolality (mOsm/liter)	1008 ± 87	762 ± 81[a]	569 ± 56[a]

[a] Significantly different from control at the 5% confidence limits or below.

ing of cytoplasm, nuclear pyknosis and focal shredding of proximal tubular epithelial cells into the tubule lumina. An estimated 10% of proximal tubules were involved by this lesion (Fig. 1A). Animals receiving 5.0 mg/kg body weight demonstrated a similar tubular lesion, primarily involving the inner cortex. The histopathologic change was slightly more extensive and occurred in an estimated 15–20% of tubule profiles. Much more extensive epithelial necrosis was apparent in rats receiving 10 mg/kg body weight of uranyl nitrate (Fig. 1B). The great majority of involved tubules revealed only eosinophilic granular cellular remains within these lumina. In other areas, pyknotic nuclei could be recognized. There changes involved an estimated 30–40% of proximal tubules and the damage extended into outer cortical tubules. Although the distal tubules were intact, protein and granular casts were seen in the pars recta. Animals receiving 20 mg/kg body weight of uranyl nitrate demonstrated similar necrotic changes in 80% of proximal

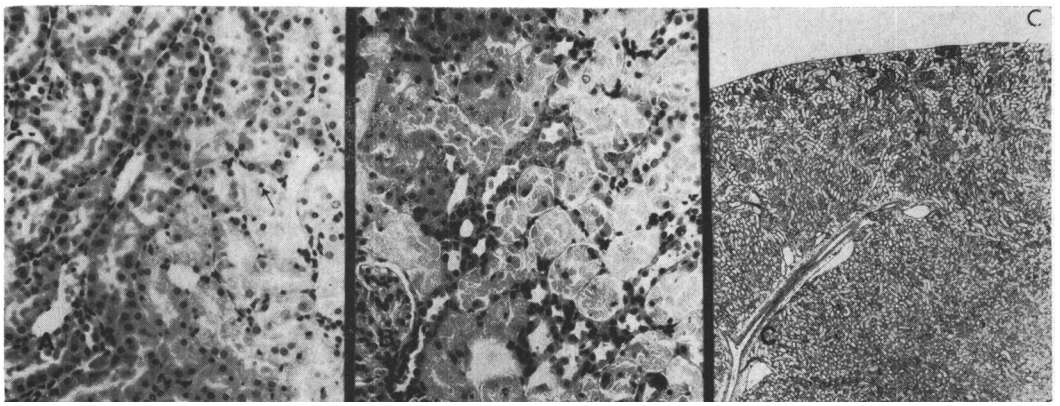

FIG. 1. A. Renal cortex from rat receiving 2.5 mg/kg uranyl nitrate. The majority of proximal tubules are normal; however, to the lower right, there is focal necrosis characterized by focal swelling of cells and nuclear pyknosis (arrow). H and E ×300. (B) Renal cortex from rat receiving 10 mg/kg uranyl nitrate. Note necrosis of proximal tubules characterized by a spectrum of change from cellular swelling and nuclear pyknosis to complete obliteration of cellular detail. Distal tubules are normal and there are some proximal tubules which appear relatively normal. H and E ×300. (C) Renal cortex from rat receiving 20 mg/kg uranyl nitrate. Note extent of necrosis which involves the majority of the proximal tubules. Casts are present in the distal tubules. H and E ×30.

tubules with extension throughout the cortex (Fig. 1C). In addition, nearly every distal tubule and collecting duct contained proteinaceous or granular casts.

The saline loaded animals receiving 10 mg/kg body weight of uranyl nitrate had a highly variable degree of tubular epithelial necrosis. The extent of damage ranged from 15 to 60% of proximal tubule profiles in the cortex. Significant qualitative or quantitative differences in the cellular response to the injury could not be detected by light microscopy in saline loaded versus nonprotected rats receiving 10 mg/kg of uranyl nitrate.

Discussion. Previous studies of uranyl nitrate induced acute renal failure have suggested that back diffusion of glomerular filtrate through the damaged tubular epithelium might explain the observed depression in renal function (1–3, 16). This mechanism has also been proposed to operate in mercuric chloride induced acute renal failure (17, 18). A recent study of uranyl nitrate induced acute renal failure in the dog showed that marked alterations in both glomerular filtration rate and renal blood flow occurred prior to any significant histopathologic change in tubular epithelium (19). The present study was undertaken to determine if the greater extent of tubular damage resulting from increasing doses of uranyl nitrate was associated with more marked renal dysfunction as estimated by the BUN concentration.

Uranyl nitrate induced acute renal failure in the rat is characterized by a relatively stable degree of azotemia 48 hr after doses of uranyl nitrate ranging from 5.0 to 20.0 mg/kg body weight. The severity and extent of renal tubular epithelial necrosis increased as the dose of uranyl nitrate was increased. If tubular necrosis *per se* was responsible for the azotemia, there should be differences in the 48 hr BUN concentration related to low and high doses of uranium since marked differences in tubular necrosis were observed. Although minimal necrosis and mild azotemia occurred at the lowest dose (2.5 mg/kg body wt), a fourfold increase in dose from 5.0 mg/kg body weight to 20.0 mg/kg body weight was not associated with a marked change in the degree of azotemia even though major histopathologic differences were observed. On the other hand, the degree of urine osmolality depression and, to a lesser extent, diminution in sodium excretion tended to parallel the dose of uranyl nitrate. This observation is consistent with previous reports describing inhibition of renal tubular function after uranium (3, 16, 20), mercuric chloride (4, 25) and glycerol injection (7). If only passive backflow occurred after uranium administration, then severe oliguria and prompt decreases in urine sodium excretion should be observed. The well-maintained urine volume, depressed urine osmolality, and mild diminution in sodium excretion relative to the degree of azotemia is more indicative of a functional rather than a structural alteration in renal tubular epithelium. Additionally, a uranyl nitrate induced abnormality in the distal nephron, where light microscopy shows only minimal changes, may participate in the observed alterations in urine osmolality. The influence of other variables, such as renal blood flow or filtered load of sodium, cannot be evaluated as they were not measured in the present study.

Chronic saline loading protects against the development of azotemia after uranyl nitrate in the rat. Were the azotemia a direct result of tubular epithelial necrosis, saline protection would be associated with prevention of epithelial damage. Saline loading, however, did not significantly alter the histopathologic changes after uranyl nitrate. A similar lack of correlation between tubular necrosis and saline protection has also been demonstrated in mercuric chloride induced acute renal failure (21). In addition, a poor correlation between histopathologic changes (*i.e.*, "acute tubular necrosis") and acute renal failure has been observed in man (22, 23). It seems unlikely, therefore, that the azotemia in uranyl nitrate induced acute renal failure in the rat is primarily due to the passive backflow of glomerular filtrate through necrotic tubular epithelium. Despite amelioration of the azotemia, saline loaded rats had changes in urine volume and urine osmolality after uranyl nitrate like those of animals on a regular diet. This also suggests that the tubular necrosis resulting from uranyl nitrate is associated with functional epithelial changes rather than being a direct cause of azotemia,

and that saline loading cannot prevent these changes.

Recent studies of a variety of models of experimental acute renal failure suggest that the renal insufficiency is the result of an alteration in renal hemodynamics such that glomerular filtration is markedly diminished (4–7, 24–26). In addition, it has been suggested that the renin–angiotensin system is the effector mechanism responsible for the alteration in renal hemodynamics (4–6). The observation that increased renin–angiotensin system activity is demonstrable in acute renal failure lends support to this suggestion (10–12). We have previously shown that saline loading, performed as in this study, results in a 25% decrease in plasma renin activity and a renal renin content less than 1% of control values (27). The association of plasma renin depression and renal renin depletion with protection against the development of acute renal failure is additional evidence for the role of the renin–angiotensin system in the pathogenesis of acute renal failure (27). It is of note that the prevention of acute renal failure by saline loading is independent of saline induced alterations in serum and urine sodium, urine osmolality, plasma and urine volume and urine pH (6, 28). While saline loading ameliorates the development of azotemia, it does not protect against the renal epithelial necrosis or renal epithelial dysfunction (21). These observations are consistent with a dual action of uranyl nitrate which may or may not be interrelated: (a) induction of an abnormality in effective glomerular filtration pressure presumably by activating the renin–angiotensin system; (b) induction of a metabolic abnormality in renal tubule epithelium such that tubule fluid sodium reabsorption is limited, urine volume is maintained, urine osmolality is depressed. Therefore, although these additional physiologic alterations might contribute to the renal insufficiency, they apparently are not solely responsible for the azotemia in uranyl nitrate induced acute renal failure in the rat. Similarly, although a role in the development of acute renal failure for passive blackflow through necrotic tubular epithelium cannot be totally excluded, the results of this study indicate that other factors predominate.

Summary. Increasing doses of intravenous uranyl nitrate above 2.5 mg/kg of body weight result in more extensive renal tubular necrosis without increasing azotemia in the rat. Despite a uniform level of azotemia, renal tubular epithelial dysfunction tended to parallel the changes in renal tubular pathology. Saline loading, designed to suppress the renin–angiotensin system, ameliorated the azotemia but not the tubular necrosis or tubular dysfunction after uranyl nitrate. These results suggest that passive backflow of glomerular filtrate through necrotic tubular epithelium is not responsible for the azotemia after uranyl nitrate in the rat. The protection afforded by saline loading further suggests a role for the renin–angiotensin system in the development of uranyl nitrate induced acute renal failure.

1. Bobey, M. E., Longley, L. P., Dicks, R., Price, J. W., and Hayman, J. M., Jr., Amer. J. Physiol. **139**, 155 (1943).

2. Bencosme, S. A., Stone, R. S., Latta, H., and Madden, S. C., Arch. Pathol. **69**, 470 (1960).

3. Nomiyama, K., and Foulkes, E. C., Toxicol. Appl. Pharmacol. **13**, 89 (1968).

4. Flanigan, W. J., and Oken, D. E., J. Clin. Invest. **44**, 449 (1965).

5. Henry, L. N., Lane, C. E., and Kashgarian, M., Lab. Invest. **19**, 309 (1968).

6. McDonald, F. D., Thiel, G., Wilson, D. R., DiBona, G. F., and Oken, D. E., Proc. Soc. Exp. Biol. Med. **131**, 610 (1969).

7. Oken, D. E., Arce, M. L., and Wilson, D. R., J. Clin. Invest. **45**, 724 (1966).

8. Hollenberg, N. K., Epstein, M., Rosen, S. M., Basch, R. R., Oken, D. E., and Merrill, J. P., Medicine **47**, 455 (1968).

9. Ayer, G., Grandchamp, A., Wyler, T., and Truniger, B., Circ. Res. **29**, 128 (1971).

10. Kokot, F., and Kuska, J., Nephron **6**, 115 (1969).

11. Tu, W. T., Circulation **31**, 686 (1965).

12. Brown, J. J., Gleadle, R. I., Lawson, D. H., Lever, A. F., Linton, A. L., Macadam, R. F., Prentices, E., Robertson, J. I. S., and Tree, M., Brit. J. Med. **1**, 253 (1970).

13. Marsh, W. H., Fingerhut, B., and Miller, H., Clin. Chem. **11**, 624 (1965).

14. Snedecor, G. W., and Cochran, W. G., "Statistical Methods." Iowa State Univ. Press, Ames

(1967).

15. Flamenbaum, W., Huddleston, M., and McNeil, J., Proc. Int. Congr. Nephrol., 5th. p. 67 (1972).

16. Eisner, G. M., Slotkoff, L. M., and Lillienfield, L. S., Amer. J. Physiol. **214**, 929 (1968).

17. Bank, N., Mutz, B. F., and Aynedjian, H. S., J. Clin. Invest. **46**, 695 (1967).

18. Steinhausen, M., Eisenbach, G. M., and Helmstadter, V., Pfluegers Arch. **311**, 1 (1969).

19. Flamenbaum, W., McNeil, J. S., Kotchen, T. A., and Saladino, A. J., Circ. Res. **31**, 682 (1972).

20. Bowman, F. J., and Foulkes, E. C., Toxicol. Appl. Pharmacol. **16**, 391 (1970).

21. DiBona, G. F., McDonald, F. D., Flamenbaum, W., Dammin, G. J., and Oken, D. E., Nephron **8**, 205 (1971).

22. Brun, C., and Munck, O., Lancet **1**, 603 (1971).

23. Finck, E. S., Jeremy, D., and Whyte, H. M., Quart. J. Med. **31**, 429 (1962).

24. Barenberg, R. L., Solomons, S., Papper, S., and Anderson, R., J. Lab. Clin. Med. **72**, 473 (1968).

25. Flamenbaum, W., McDonald, F. D., DiBona, G. F., and Oken, D. E., Nephron **8**, 221 (1971).

26. Ruis-Guinazu, A., Coehlo, J. B., and Paz, R. A., Nephron **4**, 257 (1967).

27. Flamenbaum, W., Kotchen, T. A., and Oken, D. E., Kidney Int. **1**, 406 (1972).

28. Thiel, G., Wilson, D. R., Arce, M. L., and Oken, D. E., Nephron **4**, 276 (1967).

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