Renal Handling of Nitrofurantoin in Hydropenic Rats.* (28784)

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The effectiveness of a chemotherapeutic agent in treatment of pyelonephritis is related not only to the concentration achieved in tubular and final urine but also to that achieved in the renal interstitial tissue. Investigations of renal concentrating mechanisms have demonstrated the existence of a solute concentration gradient, increasing from cortex to papilla under conditions of hydropenia(1-4). Participating in this solute concentration gradient are such substances as sodium, chloride, urea and to a lesser degree creatinine and amino acids. However, potassium, a substance known to be excreted almost entirely by tubular secretion, does not participate in this gradient.

If the degree of renal impairment in pyelonephritis is not sufficient to impair the establishment of a solute concentration gradient (*i.e.*, not sufficient to preclude production of concentrated urine) then a chemotherapeutic agent that participated in this gradient might be more effective than one that did not. To take advantage of this greater effectiveness there would be required moderate restriction of fluid intake in patients instead of the conventional heavy water loading usually provided. Moderate water restriction to provide higher concentrations in urine of the chemotherapeutic agents has already been proposed by at least one investigator(5).

As type substance we have chosen nitrofurantoin (Furadantin®) an agent widely used in treatment of pyelonephritis. In the present study, efforts were directed at a determination of the extent of its participation in the solute concentration in hydropenic rats.

Methods and procedures. The following special materials were used: 1-C^{14}-labelled *p*-aminohippurate, 0.627 mc/100 mg (Merck Radiochemicals, Montreal, Canada); C^{14}-labelled inulin carboxylic acid, 0.93 mc/g (New England Nuclear Corp., Boston, Mass.), and sodium nitrofurantoin C^{14}-labelled 1.08 mc/mM (Eaton Laboratories, Norwich, N. Y. No. 1430-11).

Albino male rats (Charles River brand) weighing between 300 and 600 g were deprived of water for 48 hours prior to each experiment. After intraperitoneal pentobarbital anesthesia (5 mg/100 g), the kidneys were exposed by a midline incision. Injection of a C^{14}-labelled solution of either *p*-aminohippurate, nitrofurantoin or inulin was made into the inferior vena cava just cephalad to the junction of the iliac veins. Bleeding was controlled by means of Oxycel®. An equilibration period of 7-10 minutes was allowed. Each kidney was then excised and immediately frozen in liquid nitrogen. A 2-3 mm freehand coronal slice of each kidney (containing cortex, medulla and papilla) was used for analysis. The frozen slices were divided into 3 areas of approximately 3 mm each, progressing from cortex to papilla. These areas were arbitrarily denoted as cortex, medulla, and papilla, respectively. Each of the 3 areas was further subdivided into 2 portions. The first portion, weighing 150 mg or more, was placed in a previously dried and weighed aluminum foil boat and the sample weight was determined by difference. This sample was pulverized in a mortar with a pestle and suspended in 2.5 ml of distilled water. The homogenate was then divided into 2 portions. The portion for sodium and potassium analysis was heated to 100°C for one hour. Aliquots of the remainder we used for analysis of chloride, urea and the C^{14}-labelled injectate. The second portion of tissue, weighing

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approximately 10 mg, was placed in a previously dried and weighed 10 ml vial and the actual sample weight determined by difference. The vials were then sealed with rubber stoppers and metal crimp caps and stored in a freezer for eventual determination of moisture content.

Trichloroacetic acid was added to the samples for sodium and potassium analyses; concentrations of these ions in the filtrates were determined by flame photometry. Chloride was determined potentiometrically after the method of Sanderson(6). Urea analysis was accomplished by the microdiffusion method of Conway(7). Compounds labelled with C^{14} were planchetted as described(8). Radioactivity was determined with a thin window flow detector with an automatic sample changer and recorder. Samples were counted for at least 5 minutes or for 1000 counts. Background was consistently in the range of 2 counts per minute. The moisture content of renal tissue was determined manometrically with a CaLab-Heckley Aquamometer (Unit 1242). The procedure suggested by the manufacturer was modified only in that samples were distilled at 60°C for 30 minutes. Results of all analyses were expressed as microequivalents, micromoles or micrograms of substance per gram of tissue water. The kidneys of 5 Kangaroo rats which had not been made hydropenic were also analyzed in the manner described. Standards for each of the C^{14}-labelled compounds injected were prepared by serial dilution of aliquots of the solutions injected. From the standards, the specific activity of the C^{14}-labelled compounds, and the experimental data values for the μg or micromoles of injectate present were calculated.

The actual amount of *p*-aminohippurate injected was approximately ½ the calculated TmPAH(9). Ten minutes before each experiment with nitrofurantoin, a carrier dose of 25 mg/kg of a 1% solution of unlabelled sodium nitrofurantoin was injected into the tail vein of each rat. Actual volumes injected in each experiment were between 0.30 and 0.50 ml. The time required for each injection was 30-40 seconds.

The sections designated cortex, medulla and papilla were examined histologically. The first 3 mm of the sections were predominantly cortical tissue, the next 3 predominantly medullary tissue and the last 3 predominantly papillary tissue. Each section or zone contained collecting ducts, urine and blood vessels in addition to renal interstitial tissue. No attempt was made to correct for the test substances present in these structures.

TABLE I. Relative Water Contents of Renal Zones.*

	Albino rats (n = 29)	Kangaroo rats (n = 10)
Medulla/cortex	1.01; s.d. .05	1.02; s.d. .06
Papilla/cortex	1.04; s.d. .06	1.05; s.d. .05

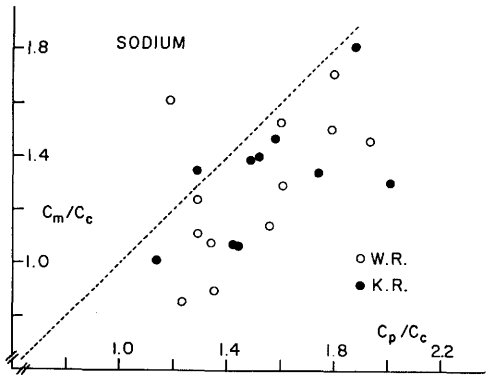
* Number of kidneys analyzed in each group denoted by "n." Values given are means followed by standard deviations (s.d.).

Results. For convenience in reporting the results, we have compared the ratios, Cm/Cc, of the concentrations in the medulla (Cm) to the concentrations in the cortex (Cc) with the ratios, Cp/Cc, of the concentrations in the papilla (Cp) to the concentrations in the cortex.

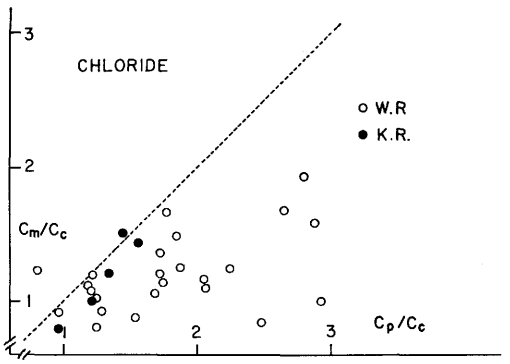
The relative proportions of water in the several zones of renal tissue are shown in Table I. The slightly larger water contents in the papillae of both white rats and Kangaroo rats are probably due to the higher urine content of these zones.

The concentration ratios of the several test substances are indicated in Fig. 1–3. As expected the ratios for sodium (Fig. 1a) and for chloride (Fig. 1b) were higher in the papillae than in the medulla. Nearly all the Cm/Cc ratios are greater than unity. Significant and increasing gradients from cortex to papillae therefore obtained for sodium and chloride. Even larger gradients obtained for urea and for inulin (Fig. 2a). Again as expected, no gradient of potassium concentration was demonstrable (Fig. 2b). Similarly, significant gradients were not found for *p*-aminohippurate and for nitrofurantoin beyond those anticipated from the high concentrations of these substances in the urine of the tubules and of the collecting ducts.

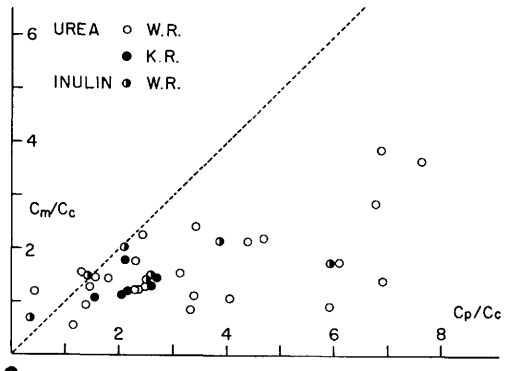
Although the ratios of concentrations of each substance analyzed in the Kangaroo rat



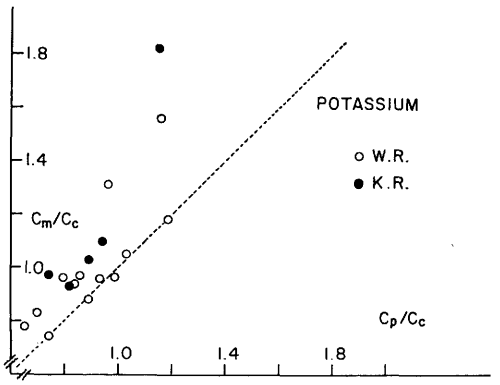
1a



1b



2a



2b

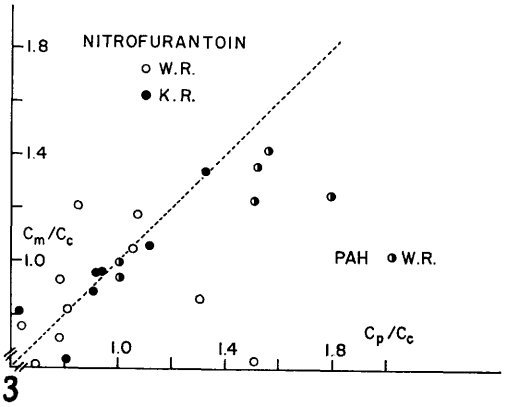


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FIG. 1. Comparison of concentration ratios of medulla/cortex and of papilla/cortex. a) Results with sodium. b) Results with chloride. Ordinates: ratio of concentration in medulla to concentration in cortex. Abscissae: ratio of concentration in papilla to concentration in cortex. W. R. denotes data from white rats; K. R. denotes data from Kangaroo rats. Diagonal line gives locus of 1:1 correspondence.

FIG. 2. a) Results with urea and inulin. b) Results with potassium. Two data for inulin with coordinates (1.83, 14.5) and (1.55, 15.5) have been omitted from figure.

FIG. 3. Results with PAH and nitrofurantoin. One datum for PAH with coordinates (1.31, 4.36) has been omitted from figure.

did not differ significantly from those found in hydropenic albino rats, the actual concentration of urea in the papillar of the Kangaroo rats was almost 3 times that found in the albino rats. Sodium, chloride, potassium and water concentration ratios were nearly identical in the 2 species.

Discussion. Nitrofurantoin(10), potassium and *p*-aminohippurate are all 3 excreted primarily by tubular secretion in the rat. None of these substances participates significantly in the solute concentration gradient of hydropenia under the conditions of these experiments. It seems probable that the uptake by the tubule cells prevents accumulation of sufficient amounts in the medullary interstitial fluid to permit the establishment of gradients for secreted substances. We have examined

the excretion of nitrofurantoin administered by constant intravenous infusion in a series of patients with endogenous creatinine clearances between 22 and 160 ml per minute. The average of clearance of nitrofurantoin was greater by a factor of approximately 2.8 than the simultaneously determined clearance of endogenous creatinine. Nitrofurantoin in humans appears, accordingly, to be excreted primarily by tubular secretion. In that event participation of nitrofurantoin in a solute concentration gradient appears to be unlikely in man. Nitrofurantoin may, therefore, be less satisfactory as a chemotherapeutic agent in treatment of pyelonephritis in humans than an analogue that would participate in the solute concentration gradient.

Restriction of water intake to limit urine flow and to provide higher concentrations of the chemotherapeutic agents at least in the final urine may have important clinical drawbacks. Chernew and Braude have recently demonstrated that phagocytosis in urine is decreased in the presence of high solute concentrations(11). In addition, medullary blood flow is less in antidiuresis than in diuresis (12). The relative importance of concentration of the chemotherapeutic agent, of the effect of solute concentration on phagocytosis, and of changes in medullary blood flow in pyelonephritis has not been assessed. The time-honored procedure of water-loading patients with pyelonephritis may be far more effective than has been recognized.

Summary. Increasing renal tissue concentration gradients have been demonstrated in hydropenic albino and Kangaroo rats for sodium, chloride and urea. No significant gradients could be demonstrated for water, potassium, *p*-aminohippurate and nitrofurantoin. Non-participation of nitrofurantoin in the solute concentration gradient may limit its effectiveness as a chemotherapeutic agent in pyelonephritis. However, phagocytosis as affected by solute concentration and medullary blood flow as related to diuresis may be of greater importance clinically.

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Studies on Ester Sulphates. 18. Ester Sulphate Formation in the Germfree Rat.* (28785)

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The occurrence of various phenol sulphates in the urine of mammals has long been known(1,2). These compounds which, under physiological conditions, probably represent the main portion of the ester sulphate fraction are believed to reflect the detoxication in the liver of toxic phenolic compounds formed by bacteria in the gut(3,4,5). Other

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