Excretion of Exogenous Creatine by the Guinea Pig Kidney* (33923)

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In the search for substances suitable for measurement of glomerular filtration rates, tubular secretion of exogenous creatinine was discovered in the aglomerular fish (1, 2), dogfish (3–5), frog (6, 7), snake (8), chicken (9), kangaroo rat (10) as well as the albino rat (11–14), sheep and goat (15), dog [under the special condition of stopflow analyses (16, 17)], primate (18, 19), and man (20, 21). In earlier work on guinea pigs, the present authors noted a disparity between inulin and creatinine clearance. Examination of this revealed that the guinea pig kidney tubules also secreted exogenous creatinine. Oyen and Boylan (22) had also obtained evidence for tubular secretion of exogenous creatinine by this species, but a curious depression of the clearance of inulin by creatinine observed by these investigators had vitiated an analysis of the tubular mechanism involved. In the present investigation, a detailed analysis of the mechanism of excretion of exogenous creatinine was made. In particular, the role of tubular secretion was analyzed in a wide range of plasma creatinine concentrations.

Methods. Experiments were performed on 58 male and 9 female guinea pigs of mean body weight for these experiments of 892 (544.5–1172). A total of 223 clearance observations was made. In reporting the results, females were separately grouped, while the males were distributed throughout other groups according to specific experimental maneuvers or procedures used in analyses. Food and water were allowed *ad libitum*. All animals were anesthetized with Nembutal (24–48 mg/kg ip). The left external jugular, right common carotid, trachea, and ureters (suprapubically) were catheterized. Ringer's solution containing 6% inulin and varied concentrations of creatinine was constantly infused intravenously at 0.08 ml/min. Blood pressure was recorded at 5-min intervals from the common carotid with a mercury manometer fashioned from PE100 polyethylene tubing. A three-way stopcock placed in series with the manometer and carotid catheter permitted blood sampling. Finally, body temperature was recorded with a small animal thermometer inserted into the suprapubic incision.

Protocols of the experiments may be described in general with variations which will be noted in discussions of each group. All animals were infused 1 hr prior to the start of the first urine collection period. Bood samples were collected at midpoints. Reasonable steady state conditions with regard to plasma concentrations were believed to exist, with maximal deviation from the mean of 17% in the longer experiments. Filling the arterial catheter with 2.5% mannuronate or 1% heparin in Ringer's sufficiently heparinized the animal through interchange with the arterial blood so that additional anticoagulant was unnecessary. Clearance periods were 15 or 30 min in length. At the conclusion, the kidneys were removed, cleared of fat and fascia, blotted dry, and weighed.

Analytical methods. Creatinine was determined by the technique of Bonsnes and Taussky (23) and PAH by the method of Smith *et al.* (24). Inulin was determined by the technique of Schreiner (25), and as the carboxyl-labeled ^{14}C compound. Recovery experiments with unlabeled inulin indicated a plasma blank of 0.24 mg/ml in 102 determinations on plasma from 22 male animals. Where the chemical method was used, the plasma inulin values were corrected for the clearance expression. Analyses were completed within 24 hr after each experiment.

To circumvent the high plasma blank encountered in inulin determinations, carboxyl-labeled ^{14}C inulin, incorporated into the infusion along with "cold" inulin, was used to

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TABLE I. Relationship of C_{IN} and C_{CREAT} to Plasma Creatinine Concentration.[a]

Stage	No. obs	P_{CREAT} (mg/100 ml; range)	P_{CREAT} (mg/100 ml; av)	C_{IN} (ml/min/g of KW)	C_{IN} (ml/min/100 g of BW)	C_{CREAT} (ml/min/100 g of BW)	C_{CR}/C_{IN}
A	27	0–20	10.3	0.384 ± 0.17	0.250 ± 0.11	0.468 ± 0.03	2.17 ± 1.00
B	25	20–40	29.0	0.502 ± 0.17	0.369 ± 0.14	0.427 ± 0.15	1.20 ± .21
C	15	40–60	51.6	0.500 ± 0.12	0.422 ± 0.09	0.465 ± 0.08	1.23 ± .11
D	9	60–80	74.4	0.445 ± 0.17	0.274 ± 0.08	0.368 ± 0.09	1.39 ± .21
E	4	80–100	88.3	0.462 ± 0.16	0.375 ± 0.15	0.468 ± 0.12	1.29 ± .29
F	8	100–120	110.4	0.454 ± 0.15	0.388 ± 0.13	0.429 ± 0.12	1.15 ± .18
G	8	120–140	130.0	0.406 ± 0.17	0.350 ± 0.15	0.380 ± 0.14	1.20 ± .21
H	9	140–160	151.0	0.328 ± 0.11	0.276 ± 0.10	0.320 ± 0.10	1.19 ± .13
I	8	160–180	175.0	0.360 ± 0.14	0.300 ± 0.10	0.325 ± 0.13	1.10 ± .10

[a] Values are averages ± SD. Data presented here appear in Fig. 1, exclusive of animals given mannitol.

quantitate filtration rates in another series. Counting was done with a proportional-flow converter (Nuclear Measurements Corporation) and a proportional counting system (Nuclear Chicago). Samples were plated on bare planchets cleaned with acetone. Demineralized water was added to the plasma samples to insure even surface distribution, while 0.02% Sterox was added to the urine samples for the same purpose. The results of 10 replications of plasma and urine samples counted as were experimental samples, gave coefficients of variation of ±2 and ±4%, respectively, using Nuclear Measurements Corporation equipment, and ±4 and ±6% for plasma and urine samples counted with Nuclear Chicago equipment.

For presentation of results, animals were classified according to the following scheme:

Group I includes 131 observations on 44 adult male animals of a mean body weight (BW) of 930 g (581–1157). Up to four consecutive 30-min periods constituted an experiment. This group included 6 male animals in which a total of 18 periods were obtained with details the same as the remainder of Group I excepting that the infusion included 6% mannitol. Chemical determination of inulin was made in this group.

Group II includes 61 clearance periods on 14 male animals of mean weight 844 g (616.5–1065.5) in which filtration rates were measured with labeled inulin. All periods were 15 min in length. In addition, Group II includes the results of 31 clearance periods in 9 adult female guinea pigs of 780 g (544.5–940) BW. These animals were treated as the males of Group II, in an effort to uncover a possible sex difference.

Results. The principle purpose of the first experiments (Group I) was to gather data on creatinine to inulin clearance ratios over a wide range of plasma creatinine concentrations. A major consideration in this group is the validity of the estimated glomerular filtration rate. From consideration of the clearance ration, C_{CREAT}/C_{IN}, it is obvious that if the filtration rate is erroneously low, the value of this ratio would be spuriously large. This was considered of particular significance because of the observation of Oyen and Boylan (22) that an average exogenous creatinine plasma concentration of only 3.1 mg/100 ml was able to depress C_{IN} from a mean value (±SD) of 0.387 ± 0.139 ml/100 g of BW/min to 0.256 ± 0.10. The creatinine had been given by the sc route (1 mg/g of BW, 15–20 hr before its determination).

In Table I, data for C_{IN} are shown over a wide range of exogenous creatinine plasma concentrations. As shown, no systematic relationship existed between C_{IN} and the plasma levels of creatinine, and that the overall average, 0.328 ± 0.126 (SD) ml/100 g of BW min fell well within the range of values supplied by Oyen and Boylan. Considering all animals studied, the mean value of C_{IN} was 0.35 ml/min/100 g of BW (±0.13, SD). Figure 1 indicates the variation of clearance ratio for this group (○), with evident self-depression of the clearance ratio. The highest value was 5.12 at a plasma creatinine con-

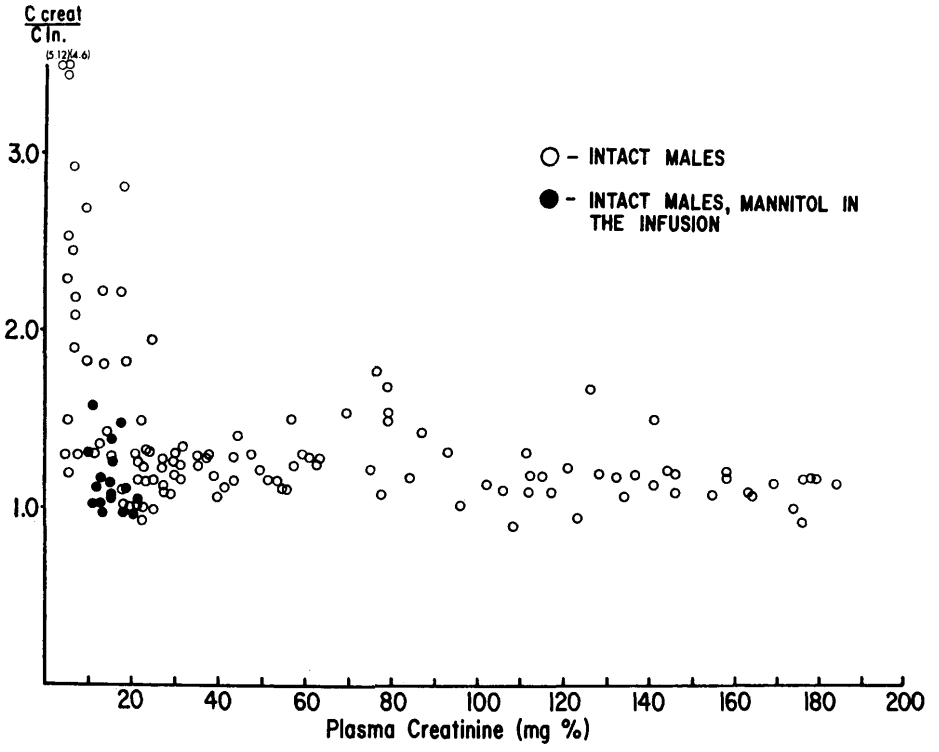

FIG. 1. The relationship of C_{CREAT} to C_{IN} in the range of ca. 4–180 mg/100 ml: (○), non-diuretic animals; (●), with mannitol diuresis (6% mannitol infused at 0.08 ml/min). Inulin clearances calculated by chemical analysis of urine and plasma, latter corrected for inuloid blank (see "Methods").

centration of 4.0 mg/100 ml. From 4.0 to 20 mg/100 ml, the ratio fell rapidly towards unity, changing little thereafter.

At attempt was made to extend the data of this group to an estimation of a secretory T_m. The secretion rate (T_{CREAT}) was evaluated as the total *excretion rate* (UV) minus the amount filtered ($C_{IN} \times P_{CREAT}$). The secretion rate was less than 1.0 mg/min in all but seven periods for five animals, where the rates varied between 1.0 and 1.4 mg/min. Another four animals showed negative results, i.e., reabsorption, in one or more periods. In general, secretion rates varied extensively at elevated plasma creatinine levels even when they were corrected for differences in kidney weight, so that an exact estimation of T_m was not possible; however, a rough estimate would put the value at 0.1 mg/g of kidney weight (KW), or 25 mg/100 ml GFR. At the secretion rates observed, small errors in chemical analyses or passive reabsorption of all or part of the secreted fraction could account for the variability of the findings.

During the course of the experiments, it was noted that the urine volume increased with rising plasma creatinine as the experiment progressed or at high infusion rates of creatinine, probably an osmotic diuretic effect (0.002–0.004 ml/min g of KW to 0.008–0.013). To test whether changes in urine volume affected the results of Group I, six animals were treated with an infusion including mannitol to attain urine volumes in the range of 0.0065–0.013. The results are given (●) in Fig. 1. While the clearance ratios tend to be lower, they distribute themselves among similar values for the other animals.[1]

[1] A statistical analysis using a formula for computation of t when comparing groups of unequal size (26) shows the mannitol clearance ratios as a group are significantly lower than the group not given mannitol ($p < .05$). Although the vertical compari-

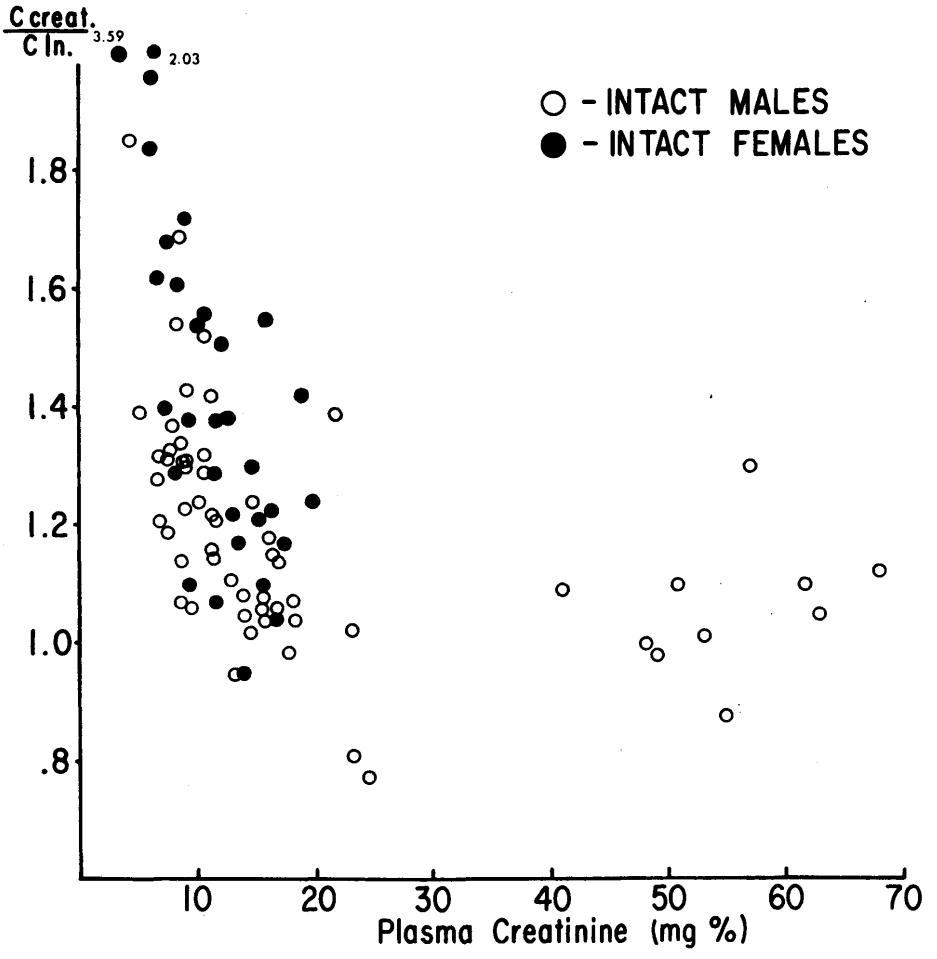

FIG. 2. The C_{CREAT}/C_{IN} ratio related to plasma creatinine concentration, in which kidney function in the male is compared to the female. In this group, C_{IN} was calculated by the use of ^{14}C labeled inulin.

In addition to the data presented, further information was desired regarding the form of the clearance ratio curve, and particularly the plasma levels at which the curve approached unity. For this, 14 male animals comprising a part of Group II were examined, using labeled inulin clearances throughout. Results are shown in Fig. 2. While the plotted values do not encompass the 20 mg/100 ml plasma creatinine level, as they do in Fig. 1, 8 of 11 points are within $\pm 10\%$ of unity within the plasma creatinine range of 15–20 mg/100 ml, verifying Group I results. At more elevated plasma creatinine titers, the ratios distribute themselves about unity. In this shorter series, the highest clearance ratio observed was 3.59 at a plasma creatinine level of 3.4 mg/100 ml. Note that 3 observations fell definitely below unity, again signifying passive reabsorption.

Figure 2 includes data on 9 female animals examined for a sex difference of the kind

son ignores the exponential nature of the relationship relating C_{CREAT}/C_{IN} to the plasma creatinine concentration, it suggests an influence of mannitol on the ratio of an undisclosed nature. This could not involve dead-space errors or influence on back-diffusion of creatinine, for one would anticipate the C_{CREAT}/C_{IN} ratio to rise, not decrease, under the influence of mannitol diuresis. Since mannitol is filtered only, competition with creatinine secretion seems unlikely.

reported in dogs by Swanson and Hakim (16) and O'Connel *et al.* (17); the results (●) in Fig. 2 give no indication of this difference in guinea pigs.

Discussion. The ratios of C_{CREAT} to C_{IN} higher than unity are indicative of tubular secretion of exogenous creatinine, and the depression of the clearance as the plasma concentration is elevated above saturation of the transport system corresponds to known behavior of other substances actively transported by the nephrons. However, although net secretory transport was usually seen, "negative" values (passive reabsorption) were seen often enough to lend caution to the interpretation that the calculated values represented a T_m for creatinine. It seems wisest to presume at this time that a three component system for excretion exists in the guinea pig, viz., filtration, tubular secretion, and passive back-diffusion somewhere in the nephron.

The observation that exogenous creatinine caused a reduction in C_{IN} (22) could not be confirmed in a range 60-fold that used in the reported work. Oyen and Boylan, excluding vasomotor influences and analytical artifact, were unable to account for this reduction in C_{IN} by administered creatinine. Their work was done in unanesthetized animals, and the creatinine was administered differently; perhaps herein lies the basis for difference in results.

Summary. Tubular secretion of exogenous creatinine was demonstrated in a wide range of plasma concentrations, by ratios of C_{CREAT} over C_{IN} with a combined average of 1.48 at low plasma levels ($<$ mg/100 ml) with reduction toward unity at elevated concentrations. Tubular secretion averaged ca. 0.1 mg/g of KW, and 25 mg/100 ml GFR, but evidence was obtained that some reabsorption (passive back-diffusion) occurred, complicating the calculation of a secretory T_m. No sex differences were observed. Combined average values found for C_{IN} were 3.0 ml/min $\pm$ 1.18 (SD) per animal; 0.49 ml/min/g of KW $\pm$ 0.19 (SD); and 0.35 ml/min/100 g of BW $\pm$ 0.13 (SD).

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