Renal Clearance in the Unanesthetized Guinea Pig: Depression of Inulin Clearance by Creatinine. (27759)

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The only published renal function studies of guinea pigs are those of Dicker and Heller (1) in which values are given for inulin clearance in 32 adult animals. The present study was undertaken to obtain further basic renal function data for the unanesthetized guinea pig. During the investigation we noted that depression of the inulin and urea clearance followed administration of creatinine.

Material and method. Adult female guinea pigs were catheterized, using a No. 8F Bard USCI catheter cut to about 6 cm in length. The following protocol was adopted as offering the minimum of manipulation at the time of clearance periods.

Initial experiments were conducted to determine the time after a single subcutaneous injection of inulin and/or creatinine when blood levels of these substances became sufficiently stable. Subcutaneous injections of 2 mg of inulin/g B.W. 16 hours before samples were taken produced satisfactory plasma levels which changed less than 1 mg % during the subsequent hour. Creatinine, 1 mg/g B.W., was given to some animals in the same manner at the same time.

Animals were allowed food and water until the time of the tests. Approximately 16 hours after giving inulin or inulin and creatinine the animal was catheterized and the bladder emptied by suprapubic pressure followed by air washout. The catheter was removed and the animal lightly restrained on a padded board for the first timed collection. After 8 to 12 minutes the catheter was again inserted and the bladder emptied into a graduated cylinder. The time between each emptying of the bladder was recorded. Catheterization was repeated after a second timed period, then the guinea pig was lightly anesthetized with ether and blood was drawn by ventricular puncture.

The blood was allowed to clot and the serum separated by centrifugation. The total urine sample for each period was diluted to 10.0 ml in a volumetric flask and clearances were computed from total excretion of each substance using the standard formula without correction for dead space.

Serum creatinine was determined on Folin-Wu filtrates by the method of Langley and Evans(2) as modified for use with a photoelectric colorimeter. The same method was used for suitably diluted samples of urine.

Inulin was estimated by the method of Hubbard and Loomis(3) on 1-ml aliquots of Somogyi filtrates of the serum, urine and standards. Urines were first diluted to an inulin U/P ratio of approxmately 1.

Urea was determined in serum and urine by the method of Friedman(4) modified by Langley.

Inulin blanks, for 11 guinea pigs of the same stock, showed a mean and standard deviation of 5.82 $\pm$ 0.96 mg %. We therefore subtracted 5.8 mg % from all plasma values before calculating the inulin clearances. When the corrected plasma value was less than 10 mg %, that inulin clearance was excluded from the study.

Results. Normal serum values. Forty determinations of serum urea in 19 animals yielded a mean and standard deviation of 19.7 $\pm$ 4.97 mg % (range of values 9.9 to 29.6 mg %). Endogenous serum creatinine, in 18 determinations showed a mean and standard deviation of 0.78 $\pm$ 0.27 mg % (range of values 0.5 to 1.2 mg %).

Inulin clearance at very low urine flow. (Below 0.005 ml/min.) In the usual range of urine flow no correlation between this function and GFR is found. However, extremely low urine flow rates are associated with smaller clearances of inulin. This is shown in Fig. 1, in which urine flows below 0.01 ml/min are plotted against the corresponding inulin clearance.

Clearance and clearance ratios. Table I summarizes 79 clearance studies on 19 animals. Separation of the clearances done with

TABLE I. Renal Clearance Data from Normal Unanesthetized Guinea Pigs. ml/min/100 g B.W.

	C_{IN}	C_u	C_{CR}	C_{CR}/C_{IN}	C_u/C_{IN}
Endogenous creatinine	.387 ±.139 (24)	.236 ±.091 (38)	.366 ±.131 (34)	.95	.61
Exogenous "	.256 ±.10 (35)	.192 ±.094 (41)	.417 ±.142 (43)	1.63	.748

Values are mean and S.D. Number of clearance periods is shown in parentheses.

endogenous and exogenous creatinine was elected because exogenous creatinine depressed both inulin and urea clearance. The mean inulin clearance (± S. D.) determined simultaneously with that of endogenous creatinine was 0.387 ± 0.139 ml/100 g B.W./min whereas inulin clearance determined in presence of exogenous creatinine was 0.256 ± 0.10 ml/100 g B.W./min. The means are significantly different at the 5% level (t = 4.06).

The clearance of creatinine given subcutaneously (0.417 ± 0.14 ml/100 g B.W./min) was slightly but not significantly greater than that of endogenous creatinine (0.366 ± 0.13 ml/100 g B.W./min).

Table I shows that urea clearance also is depressed when it is measured simultaneously with exogenous creatinine. The value falls from 0.236 ± 0.09 (determined without exogenous creatinine) to 0.192 ± 0.09 ml/100 g B.W./min (t = 2.10).

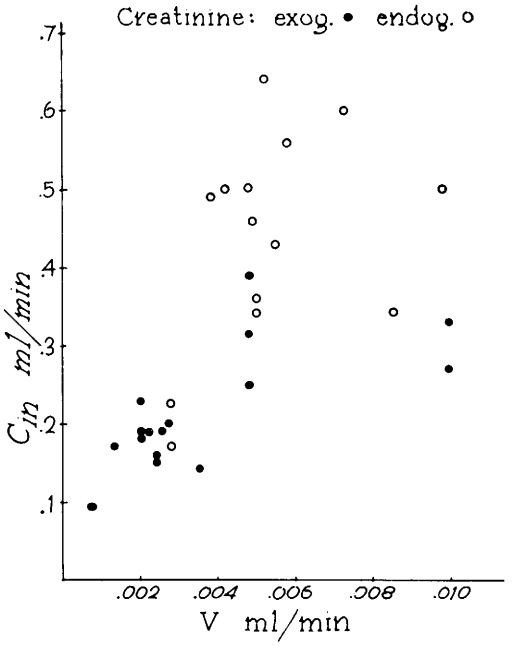

FIG. 1. Relationship of inulin clearance (C_{IN}) to urine flow (V) at values for V below 0.005 ml/min. No relationship exists for urine flow values of 0.006 and above.

In Fig. 2 each value for endogenous or exogenous creatinine clearance is plotted against the simultaneously determined inulin clearance. The broken line represents the regression which would be obtained if inulin and creatinine clearances were identical. It is apparent that all points determined with exogenous creatinine lie to the left of this line indicating that when creatinine is given, simultaneously determined inulin clearances are invariably lower. In 10 animals inulin clearance was measured at different times with and without administration of creatinine. These data are presented in Table II. With one exception inulin clearance *in the same*

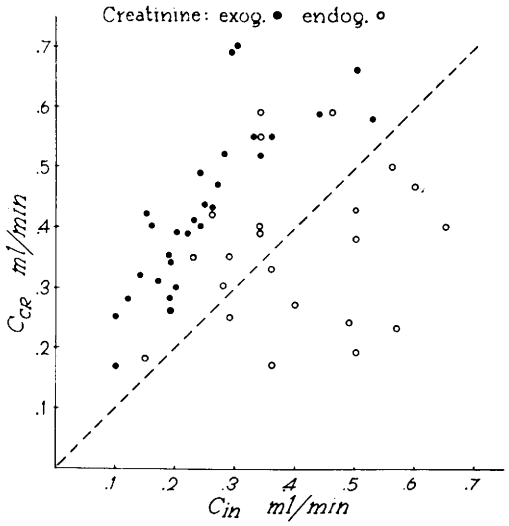

FIG. 2. Depression of inulin clearance by administered creatinine. Endogenous (○) and exogenous (●) creatinine clearances plotted against simultaneously determined inulin clearance.

TABLE II. Data in 10 Guinea Pigs in Which Inulin Clearances Were Done With and Without Administered Creatinine.

	C_{IN}	
	Without creatinine	With creatinine
	ml/min/100 g B.W.	
1	.22	.19
2	.29	.16
3	.34	.43
4	.36	.22
5	.40	.19
6	.43	.16
7	.51	.26
8	.55	.20
9	.54	.48
10	.58	.27

animal was significantly lower when creatinine was given than when it was not.

In Fig. 3 the urea to inulin clearance ratios (% of filtered urea which is excreted) of both groups are plotted against the inulin U/P ratios (measuring water reabsorption) in the manner of Shannon's data for the dog(7).

Discussion. Inulin clearance. The mean value 0.387 ± 0.139 ml/100 g B.W./min obtained for 24 inulin clearance periods compares satisfactorily with published data for this function in other small mammals. For the rat Smith(5a) reviewed the results of several workers who found inulin clearances ranging from 0.27 ± 0.06 to 0.66 ± 0.028 ml/100 g B.W./min with an average for the collected series of 0.52 ml/100 g/min. The last figure is quite similar to that found by Fingl(6) for 25 rats. Dicker and Heller(1) obtained the much lower average of 0.23 ± 0.006 ml/100 g B.W./min in 32 guinea pigs during water diuresis. Their methods of inulin administration and urine collection differed markedly from ours.

Inulin clearance in the guinea pig was independent of plasma conc. at U/P ratios ranging from 5 to 140. As shown in Fig. 1 the clearance of inulin was also independent of urine flow except at very low flow rates (< 0.005 ml/100 g/min), when it was depressed (<0.2 ml/100 g/min). The change in relationship here is abrupt, for at urine flows of 0.005 ml/100 g B.W./min, the upper range of inulin clearance values was found. Depression of GFR at very low urine flows has been noted in the dog(7) and to a lesser degree in man(8). It is interesting that these extremely low urine flows (Fig. 1) occurred mostly in animals which received creatinine.

Inulin clearance in this study was consistently reduced by subcutaneous administration of creatinine (1 mg/g B.W.) 15 to 20 hours before its determination. Thirty-five such measurements gave a mean C_{IN} value (0.256 ml/100 g B.W./min) significantly below that found when creatinine was not given to the animal (Table I). The t value for this difference is 4.06, $p<0.001$. The individual values presented in Fig. 2 and the selected group of animals in Table II are confirmatory.

We are unable to account for this reduction of C_{IN} by administered creatinine. A vasomotor effect was suggested by Lippman to account for his finding(9) that increasing plasma concentrations of PAH were associated (in the rat) with a progressive fall in inulin clearance. No such effect is known to follow administered creatinine. The question cannot be resolved in the present study. To exclude an analytical artifact, experiments were done *in vitro* to determine whether a lessened inulin recovery obtained by our methods in the presence of added creatinine. No such interference was found.

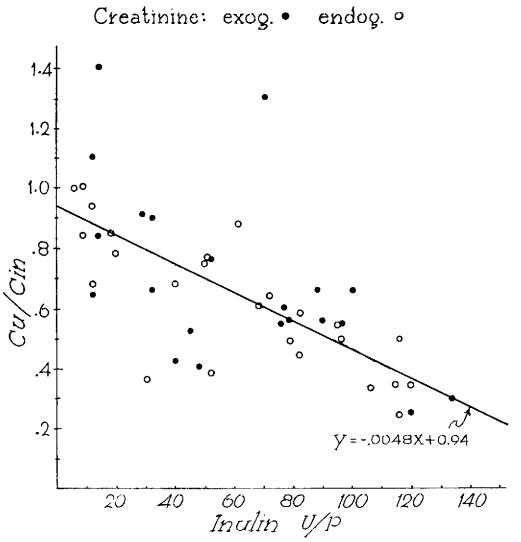

FIG. 3. The excreted fraction of filtered urea plotted as a function of urinary concentration (inulin U/P). The straight line is constructed from the estimating equation determined by the method of least squares.

Creatinine clearance. Endogenous creatinine, determined by the dinitrobenzoate method used in this study, is stated(2) to give essentially the same values as the more tedious procedure of adsorption onto and elution from Lloyd's reagent(10). We consider that our values for endogenous creatinine are close to "true" plasma creatinine and that a minimum of extraneous chromogenic material is being measured.

Clearance of endogenous creatinine in our guinea pigs (Table I) averaged 0.366 ± 0.131 ml/100 g B.W./min in 34 determinations. Therefore, the ratio of endogenous creatinine clearance to that of inulin is 0.95 (Table I). This ratio is in accord with published figures for most subprimate mammals (5b) and supports the identity of endogenous creatinine clearance with GFR in this species as well.

Our average value for endogenous creatinine clearance (0.366 ± 0.131 ml/100 g B.W./min) may be compared with that of Brod and Sirota(11) for the rabbit (0.312 ± 0.058 ml/100 g B.W./min) and with data on 67 dogs assembled by Smith(5c) from many authors which gives an average of 0.384 ml/100 g B.W./min. Reported data for the rat are higher when compared on the basis of body weight(5,9,12,13).

Exogenous creatinine plasma levels averaged 3.1 mg % (range 1.15 to 4.18) or 4 times the average endogenous level. This is a lower value than has been used by most investigators.

In our animals the clearance of exogenous creatinine (0.417 ± 0.142, Table I) is larger than the clearance of endogenous creatinine. The ratio exogenous to endogenous clearance is 1.14. The corresponding endogenous and exogenous creatinine clearances to that of inulin are 0.95 and 1.63, a difference which appears significant when considered alone. The interpretation of this observation, however, is complicated by our finding (see above) that inulin clearance is depressed by previous administration of creatinine.

Since administered creatinine has depressed the glomerular filtration rate without diminishing its own rate of excretion (in fact somewhat enhancing it) then some fraction of exogenous creatinine must be excreted by tubular activity in the guinea pig.

Tubular excretion of exogenous creatinine has been demonstrated in many species, man included(5b). Published data on *endogenous* creatinine clearances are complicated by the inclusion of methods which measure variable amounts of non-creatinine chromogen. The subject has been extensively reviewed by Smith(5b,14), the analytical technics by Owen *et al.*(15), and certain features of methodology are thoughtfully discussed by Ladd(16).

Urea clearance. The mean of 38 determinations of urea clearance in animals which did not receive creatinine was 0.236 ± 0.091 ml/100 g B.W./min (Table I). The average urea to inulin clearance ratio is therefore 0.61, a well established figure for most mammals at relatively high rates of urine flow.

The urea clearance, like the inulin clearance, appears to be reduced by administration of creatinine. Urea clearance estimated in the group of animals receiving creatinine averaged 0.192 ± 0.094 ml/100 g B.W./min in 41 experiments. The difference is barely significant ($t = 2.10$) and represents an 18.6% reduction of the non-creatinine group average, whereas in the case of inulin clearance the reduction following creatinine is 33.8%.

Finally, in Fig. 3 all urea to inulin clearance ratios are plotted against the inulin U/P ratios at which they were obtained. Although there are 4 clearance ratios greater than 1.0, the straight line describing the regression equation determined from the points intercepts the ordinate at 0.94. A similar finding led Shannon to conclude that there was no tubular secretion of urea in the dog. The present evidence favors a like conclusion for the guinea pig. However, Schmidt-Nielsen(17) has pointed out the importance of graded nitrogen intake as a means of uncovering tubular regulation of urea. The occasional C_u/C_{IN} greater than 1.0 which we have seen here (Fig. 3) and elsewhere(18) suggests that such studies should be carried out in guinea pig.

Summary. Renal function studies were conducted in unanesthetized guinea pigs. 1. The mean value for inulin clearance obtained

compares satisfactorily with published data in other small mammals. Inulin clearance is independent of plasma concentration over a wide range of U/P ratios and independent of urine flow except at very low flow rates, when it is depressed. 2. A ratio of endogenous creatinine to inulin clearances of 0.95 indicates that in the guinea pig, endogenous creatinine clearance is a measure of glomerular filtration rate. 3. The average ratio of urea to inulin clearance obtained here (0.61) agrees well with the established figure for most mammals. 4. Inulin and urea clearances were consistently reduced by prior administration of creatinine. The mechanism of this reduction is beyond the scope of this study. 5. The clearance of exogenous creatinine is greater than that of endogenous creatinine. This, with the foregoing, suggests that in this species there is a tubular component in excretion of exogenous creatinine.

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Corticotropin-Induced Hyperlipemia in Rabbits.* (27760)

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The injection into rabbits of crude pituitary extracts or of certain peptides derived from pituitary glands results in a prompt increase in circulating non-esterified fatty acids (NEFA) and in development of hyperlipemia (1-5). Corticotropin, and to a lesser extent several others of the presently recognized pituitary hormones, have been shown to stimulate release of NEFA from rat adipose tissue when incubated *in vitro,* but demonstrations of corresponding *in vivo* effects of ACTH have hitherto not been conclusive(6). In the present experiments, purified corticotropin A_1 was administered to rabbits by repeated subcutaneous injection or by intravenous infusion. There ensued the following sequence of events: prompt and striking increases in circulating NEFA, increase in lipid content of the liver and kidneys, hyperlipemia, and appearance in the plasma of an inhibitor to lipoprotein lipase activity.

Materials and methods. Corticotropin A_1 was purified by chromatography on IRC 50 according to the procedure of Li(7). The chromatographically homogenous and well resolved effluent peak corresponding to A_1 was

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