INULIN AND CREATININE CLEARANCES IN DOGS,
WITH NOTES ON SOME LATE EFFECTS
OF URANIUM POISONING*

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The interest which attaches to the study of the rate of renal excretion of inulin in mammals derives from the large molecular dimensions of that substance and from the evidence which permits belief that it is excreted solely by glomerular filtration. The rate of its plasma clearance can be accepted as the equivalent of the rate of glomerular filtration with fewer reservations than are necessary in the case of other substances which have been similarly studied, such as creatinine, xylose, or glucose in phlorhizin poisoning. And the most important outcome of the measurements of the magnitude of its plasma clearance is the evidence which they afford that the volume of the glomerular filtrate in dogs and rabbits is great enough to contain all of the normal constituents of urine except such as are formed within the kidney itself.

Independent work from three laboratories has established the approximate identity of the plasma clearances of injected inulin and creatinine in dogs, thus validating Rehberg's hypothesis (1) as applied to dogs. Our published figures (2) of inulin to creatinine clearance ratios gave a mean of 1.06 with a standard deviation of 0.20; those of Shannon (3) gave a mean of 1.006, standard deviation, 0.034; those of Van Slyke, Hiller, and Miller (4), 0.97, standard deviation, 0.14. While the general conclusion from each of these groups of measurements is the same, the remarkable consistency with which Shannon's ratios approximate to unity

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gives a special significance to his results. They mean not only that the two substances are excreted at exactly the same rate relative to their concentrations in plasma, but also that neither substance is reabsorbed from the tubule, either actively or by diffusion. Our ratios could mean that variable, usually small, fractions of the creatinine of the glomerular filtrate are reabsorbed from the tubule. We became convinced that the technique of Shannon's experiments was more perfect than ours and hence were inclined to accept the more rigid conclusion drawn from his results. But at the same time, in view of the great difference between the diffusibilities of inulin and creatinine (5), we found it difficult to believe that the normal dog tubule is possessed of such constant, specific impermeability as never to permit the escape by diffusion of any of the creatinine from the fluid within its lumen. We have therefore continued to collect data concerning clearance of these two substances in dogs, first however having somewhat modified our analytical methods in accordance with suggestions from Professor Smith and Dr. Shannon in order to lessen chances of addition of errors. In the meantime Shannon has published a second group of observations on dogs the results of which confirm his earlier work and extend its conclusion to include low rates of urine flow (6).

In our experiments ten normal and two abnormal dogs were studied. These two (Dogs SH and RD), loaned by Dr. MacNider, had been poisoned with uranium more than 2 years before, but now look perfectly well. The results obtained in all except one of the abnormal dogs coincide so closely with those obtained by Shannon as to uphold the conclusion drawn from his work. In the experiments on one of the abnormal dogs, however, we have found the inulin to creatinine clearance ratio to be so consistently divergent from 1.00 as to indicate the reabsorption of some of the creatinine from the glomerular filtrate.

1 Dr. Shannon very generously came to Philadelphia and collaborated with us in one of the experiments.

2 Dog SH received 2 mg. of uranium nitrate per kilo by subcutaneous injection on June 3, 1932 and again on February 17, 1933. Our first experiment with Dog SH was on January 24, 1936. Dog RD was injected with 2 mg. per kilo on June 1, 1933. Our first experiment was made on January 30, 1936.
**Methods**

Female dogs, weighing 13.0 to 19.4 kilos, accustomed to the experimental routine of catheterization and venipuncture, were used. During intervals between experiments they were kept on the ordinary mixed diet of the animal house and were given no food for at least 20 hours before an experiment. In all experiments save one, water was given by stomach tube in varying dosage and at varying times before the beginning of the experiment. The largest amount was 140 cc. per kilo in divided amounts during 6 hours before; the smallest, 15 cc. per kilo 1 hour before. Inulin was given by intravenous injection (saphenous vein) in all experiments except one in which injection was subcutaneous. Creatinine was given by subeutaneous injection in seven experiments, intravenously in seventeen. Pfanstiehl's c.p. dahlia inulin was used in twelve experiments; an especially purified preparation from the Bureau of Standards was used in 12. Pfanstiehl's creatinine was used in all.

Urine was collected by catheter; urine periods in different experiments varied from 10 to 30 minutes in length. Complete emptying of the bladder was accomplished by abdominal compression after inflation of the bladder with air, or injection of warm salt solution, or both. Blood was taken from the jugular vein at the midpoint of each urine period and centrifuged immediately. In the majority of experiments dry lithium oxalate was used as anticoagulant; in a few, heparin. Analytical methods for inulin and creatinine were the same as those used by Shannon (3, 7) with the exception that glucose was determined in the iron filtrates (8) by the Shaffer-Somogyi method (9) instead of being removed by fermentation with yeast. Urea in experiments with Dogs SH and RD was determined by the method of Van Slyke and Cullen (10). All determinations were made in duplicate.

**Results**

The clearance measurements were made within a wide range of experimental conditions, as represented by the following summary. Rates of urine flow varied between 0.32 and 10.0 cc. per minute, plasma inulin concentrations between 16 and 1292 mg. per cent, plasma creatinine between 8 and 112 mg. per cent, urinary inulin
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between 0.33 and 29.3 per cent, urinary creatinine between 0.16 and 3.74 per cent.

The inulin and creatinine clearances and their ratios are charted

Fig. 1. Inulin clearances plotted against creatinine clearances for each period in each dog. The inset shows inulin to creatinine clearance ratios plotted against rates of urine flow.

in Fig. 1. Exclusive of the results obtained with Dog RD, the mean of 73 inulin to creatinine clearance ratios derived from eighteen experiments on eleven dogs is 1.016, the maximum variations +0.103 and -0.097, the standard deviation 0.044, the
standard error of the mean 0.005. These figures so closely resemble those of Shannon as to force us to accept his conclusion that in normal dogs neither inulin nor creatinine is reabsorbed from the tubule in significant amounts.

The results obtained with the uranium-poisoned animal, Dog RD, however, are consistently divergent from the rest. The lowest of fourteen inulin to creatinine clearance ratios is 1.02, the mean 1.152, the standard deviation 0.109, the standard error of the mean 0.030. The only meaning which we can attach to this difference is that in this dog, an average of at least 13 per cent of the creatinine contained in the glomerular filtrate is reabsorbed from the tubule. Nothing which we know of the sequelae of uranium poisoning permits belief that active tubular processes are increased by it; hence we ascribe the difference between Dog RD and the others to impairment of the normal impermeability of the tubule in the direction lumen → blood with the result that some escape of creatinine occurs by diffusion. It is obvious from the high inulin clearances that little, if any, escape of inulin occurred.

Other comparisons between Dogs RD and SH (the latter had also been poisoned with uranium) support the above interpretation.

Examination at Biopsy—The kidneys of Dog SH were described as tough and fibrous, those of Dog RD as friable, "sutures cut through easily."

Microscopic. Dog SH—Sections of kidney tissue taken at eight biopsies made at intervals between the first uranium poisoning (June 8, 1932) and the beginning of our experiments (January 24, 1936) show marked progressive glomerular change which has resulted in fibrosis and atrophy (including complete disappearance) of many glomeruli and tubules. Compensatory enlargement of many of the existing nephrons is very evident.

Dog RD—Sections of kidney tissue taken at biopsy 4 months after the uranium poisoning show normal glomeruli, vacuolization of cells of the convoluted tubules, nuclei of tubule cells, normal. Tissue taken 8 months and 1 month before the beginning of our experiments show practically normal kidney, no atrophy of nephrons, or fibrosis.3

3 Dr. Lucké informs us that failure to find visible evidence of abnormalities of the tubule cells is not necessarily a reason for doubting the interpretation which we have given to the clearance results.
**Inulin Clearances**—Those of Dog SH were the lowest of any of the twelve dogs studied. The highest of nine measurements was 40.3; the lowest, 29.0 cc. per minute; average, per sq. m. of body surface, 43.3. The inulin clearances of Dog RD were among the highest; the lowest of fourteen was 78.2; the highest, 126.0; average per sq. m., 104.0 cc. per minute.

**Urea Clearances**—These were measured simultaneously with those of inulin and creatinine. For Dog SH the mean of nine urea to inulin clearance ratios is \(0.60 \pm 0.033\) (the standard error of the mean); for Dog RD the mean of fourteen ratios is \(0.347 \pm 0.020\).

When comparison is limited to periods in which rates of urine flow were approximately the same in both dogs, the mean of seven urea to inulin ratios for Dog SH is 0.56; of seven for Dog RD, 0.35.

**Other Tests**

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<tr>
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<th>Dog SH</th>
<th>Dog RD</th>
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<tr>
<td>Maximum sp. gr. after 48 hrs. deprivation of water*</td>
<td>1.042</td>
<td>1.041</td>
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<tr>
<td>Phenolsulfonephthalein excretion after 6.0 mg. intravenously (2 tests)</td>
<td>34, 45</td>
<td>63, 82</td>
</tr>
<tr>
<td>1st hr., %</td>
<td>44, 59</td>
<td>73, 92</td>
</tr>
<tr>
<td>2nd “ %</td>
<td>1.5</td>
<td>Trace</td>
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* Two normal dogs similarly tested gave urines with specific gravities of 1.050 and 1.052.

From these data we conclude that the kidneys of Dog SH have suffered considerable glomerular damage as a result of which the volume of glomerular filtrate is abnormally low. The capacity of the tubules to retain creatinine and urea is normal; the fraction of filtered urea reabsorbed is 40 per cent. Dog RD, on the other hand, survived the poisoning with no residuum of glomerular damage, detectable either microscopically or by inulin clearance measurements. The capacity of the tubules, however, to retain creatinine is somewhat less than normal; the high fraction (65 per cent) of filtered urea which is reabsorbed gives further evidence of abnormal permeability of the tubule.

The inability of Dog SH to excrete a urine as concentrated as that of normal dogs is to be ascribed to decrease in filtration surface. The similar inability shown by Dog RD is due to diminished impermeability of the tubules.
Grateful acknowledgment is made to Dr. Wm. deB. MacNider for the uranium-poisoned dogs and the sections of kidney tissue from them, to Dr. Balduin Lucké for his histological study, and to Miss Ethel Shiels for the urea determinations.

SUMMARY

Plasma clearances of injected inulin and creatinine have been measured in experiments in twelve dogs, of which ten were normal and two had been poisoned with uranium more than 2 years before these tests. In eleven of these dogs, within wide ranges of rates of urine flow and plasma and urine concentrations, the clearances were found to be so nearly the same as to support the view that in normal dogs, under all ordinary experimental circumstances, these two substances are excreted at the same rate with respect to their concentrations in plasma. The observations add to the volume of existing inferential evidence, not only that the dog kidney excretes both substances solely by glomerular filtration, but also that neither substance is reabsorbed from the tubule, either actively or by diffusion.

In one of the poisoned dogs inulin was consistently excreted at a faster rate than creatinine. This fact is interpreted to mean that the impermeability of the tubule which normally prevents back diffusion was so impaired that a fraction of filtered creatinine, amounting on the average to at least 13 per cent, returned to the blood. The renal damage established a condition in which the greater diffusibility of creatinine was revealed.

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