THE RENAL MECHANISM FOR URATE EXCRETION IN THE DALMATIAN COACH-HOUND*

By WILLIAM Q. WOLFSON, ‡M.D., CLARENCE COHN, M.D., AND CLARICE SHORE

(From the Department of Biochemistry,§ Medical Research Institue, Michael Reese Hospital, Chicago)

(Received for publication, May 1, 1950)

Three findings characterize the unusual purine metabolism of the Dalmatian dog (1): (*a*) Unlike other dogs, the urine of the pure bred Dalmatian contains large amounts of urate and relatively small amounts of allantoin, (*b*) per unit body weight, the urinary urate excretion of the Dalmatian is several times that of man, (*c*) although the Dalmatian excretes more urate than man per unit weight, this is accomplished at a plasma urate level much lower than that of man. In other words, the renal clearance of urate per unit weight per minute is much larger in the Dalmatian than in man.

From the available literature, it is not clear whether the urate clearance of the Dalmatian exceeds the glomerular filtration rate or is merely equal to the glomerular filtration rate. Schaffer, Dill, and Stander (2) estimated urate and urea clearance simultaneously during a study of the renal effects of renin. In three clearance periods, when no renin had been given or after its immediate action had terminated, urate clearance was found to average 435 per cent of the urea clearance. These urate clearances were estimated with a colorimetric total urate method, but the same authors also studied enzymatically determined true urate values in other experiments on Dalmatians. If an average correction is made for the differences found between total urate and true urate values, the urate clearance in their experiments averaged 539 per cent of the urea clearance, a value far in excess of the glomerular filtration rate. Friedman and Byers, however, have presented data which they interpret as indicating an identity of urate clearance and glomerular filtration rate in the Dalmatian (3).

It is possible that this discrepancy in results may be methodological in origin. In neither of the studies summarized above was a well accepted pro-

* These studies were assisted by grants-in-aid from the Committee on Scientific Research of the American Medical Association and from the Committee on Research in Endocrinology of the National Research Council.

‡ Present address: Rackham Arthritis Research Unit, University Hospital, Ann Arbor, Michigan.

§ The Department of Biochemistry in part is supported by the Michael Reese Research Foundation.

cedure used to estimate glomerular filtration rate nor an accurate enzymatic method used for the estimation of true urate. The relationship of urate clearance to glomerular filtration rate has, therefore, been restudied in two pure bred Dalmatians using a uricase procedure for the estimation of true urate and exogenous creatinine clearance for the determination of glomerular filtration rate. As in the study of Schaffer and associates, our results show urate clearance considerably to exceed glomerular filtration rate in the Dalmatian.

SUBJECTS AND METHODS

Dog 1 was a 7 kilo female Dalmatian. Her plasma true urate concentrations and minute output of urate under forced diuresis are somewhat larger than those ordinarily seen in this breed, probably because of the rapid correction of an antecedent dehydration. Dog 2 was a fine healthy 17 kilo male Dalmatian. Both dogs were given priming doses of creatinine intravenously and thereafter received a maintenance solution of creatinine in normal saline, given at a rate of approximately 0.5 ml. per kilo per minute. Creatinine was estimated by the Folin-Wu method.

The uricase method used is conventional in that the chromogenic material is compared in untreated samples with those incubated with uricase at alkaline pH. However, the procedure differs from previous uricase methods except that of Leone (4) in that true urate is estimated directly and not calculated by subtracting urate chromogen from total urate values. Uricase-treated samples and control samples are both alkalinized and incubated. Strict identity of procedure, except for the presence or absence of uricase activity is maintained in order to eliminate certain difficulties noted when samples contain such alkali-labile chromogenic substances as gentisate and when only the uricase-treated samples are alkalinized and incubated (5, 6). Details of the method follow.

Method for the Direct Estimation of True Urate in Serum or Urine by Differential Enzymatic Colorimetry

REAGENTS.—

Uricase Solution.—As a starting material, use the kidney powder of Buchanan, Block, and Christman (7) or commercial, dried, defatted beef kidney powder, viobin UK-21.[1] To 100 gm. of either powder, add 1.0 liter of pH 9.2, M/15 borate buffer and extract for 2 hours on a Conn shaking machine. Decant off and save the supernatant, and repeat the extraction with a second portion of buffer. Combine the extracts and centrifuge at 3000 R.P.M. to remove gross turbidity, then filter. If kidney powder UK-21 was used, transfer the extract to a hot plate and *with continuous stirring* warm the extract to 55–60°C. for *exactly* 5 minutes, then immediately place in an ice box for 4 hours and filter off the precipitate. Using either a pH meter or pH paper as a guide, adjust the pH to 7.0 by the cautious addition of glacial acetic acid, drop by drop, with continuous stirring. Add an equal volume of 4.1 M ammonium sulfate and place in an ice box overnight. Filter off the precipitate onto a fluted paper, preferably in the cold. Take up the precipitate in 250 ml. of pH 9.2, M/15 borate buffer and transfer to a cellophane dialysis bag. Dialyze against three 2.5 liter changes of pH 9.2, M/15 borate buffer, for 24 hour periods. Filter off residual turbidity. Assay the enzyme solution for activity; 0.5 ml. of satisfactory enzyme solution catalyzes the complete conversion of 0.5 ml. of a 16.8 mg. per cent urate solution to non-chromogenic end-products in 1.5 hours at 45°C. Serial dilutions of the enzyme should be assayed and the results used as a guide to the permissible dilution

[1] We wish to thank Dr. A. Kaplan of the Viobin Corporation for his kindness in supplying us with liberal amounts of kidney powder UK-21 and of other materials screened for suitability as starting materials for the preparation of uricase.

of the enzyme. Following dilution of the enzyme with pH 9.2, M/15 borate buffer, the solution is divided into convenient portions and stored in the deep freeze until use.

Buffered Albumin.—Dilute commercial human albumin with pH 9.2, M/15 borate buffer, until its protein concentration equals that of the uricase solution. Store in the deep freeze until used.

pH 9.2, M/15 Borate Buffer.—Add sufficient distilled water to 13.5 gm., of anhydrous sodium tetraborate to make 1 liter of solution. (If the 10 H_2O salt is used, use 25.3 gm.)

1 per cent sodium tetraborate solution.

1.0 N sulfuric acid.

7.5 Per Cent Sodium Tungstate Solution.—Use Merck sodium tungstate free of molybdate (according to Folin).

Arsenophosphotungstate Reagent.—Place 50 gm. of Merck sodium tungstate, free of molybdate (according to Folin) in a 1 liter Erlenmeyer flask. Add 300 ml. of distilled water and dissolve. After solution is accomplished, add 25 gm. of arsenic acid (As_2O_3) and dissolve. Add 12.5 ml. of 85 per cent phosphoric acid and 10 ml. of concentrated hydrochloric acid. Cover the mouth of the flask with a beaker and boil for 20 minutes. Let the solution cool to room temperature and transfer to a 500 ml. volumetric flask. Dilute to volume and filter.

Urea-Cyanide Reagent.—Place 25 gm. of sodium cyanide, Merck, in a 1 liter Erlenmeyer flask and add about 400 ml. of distilled water. If necessary, warm to complete solution. Cool to room temperature and add 75 gm. of urea. Transfer to a 500 ml. volumetric flask and dilute to volume with distilled water. Filter. *Discard this solution after 2 months—old urea-cyanide is the usual cause of errors.*

0.5 Ml. Pipettes, Folin.—To insure accuracy, always use the same pipette to transfer aliquots of a sample to the two reaction and two control tubes. In addition, it is advisable to rinse the pipette once with the sample before beginning.

STANDARDIZATION.—

Place 168 mg. of uric acid in a 1 liter volumetric flask. Add 200 ml. of distilled water and just enough 1 N NaOH to bring all the urate into solution. Dilute to volume with M/15, pH 7 phosphate buffer. This standard solution must be used on the day it is prepared.

Prepare 1:10, 1:20, 1:40, and 1:80 dilutions of the standard, using distilled water. They will represent urate concentrations, respectively of 16.8 mg. per cent, 8.4 mg. per cent, 4.2 mg. per cent, and 2.1 mg. per cent.

Set up each dilution in triplicate as follows: Place 2.5 ml. of 1 per cent sodium tetraborate into a 10 ml. diluting cylinder. Add 2.0 ml. of standard urate solution, 0.5 ml. of urea-cyanide reagent, and 0.2 ml. of arsenophosphotungstate reagent. Dilute to volume with 1 per cent sodium tetraborate and mix by inversion. Prepare a blank with 0.5 ml. of urea-cyanide reagent, 0.2 ml. of arsenophosphotungstate reagent, and 9.3 ml. of 1 per cent sodium tetraborate.

Decant the mixed solutions into Evelyn cuvettes. Let stand exactly 30 minutes, from the time of mixing, and read on the Evelyn photoelectric colorimeter at 690 millimicra, using the 10 ml. aperture.

PRELIMINARY TREATMENT OF URINE SAMPLES.—

Urine for the estimation of urate should always be worked up immediately, frozen until used, or at least collected with a preservative. The urinary sediment from a cold, acid urine contains most of the urate. Therefore, when the urine is removed from the ice box or deep freeze, warm it under a hot water tap and shake well to redissolve the precipitate.

Place 10 ml. of well mixed urine in a 25 ml. diluting cylinder. Add 1 N NaOH, drop by drop, until the urine is neutral to nitrazine paper. Dilute to 20 ml. with distilled water and mix. Then carry out the preliminary determination of urate concentration, according to the procedure of the next paragraph, if the urine is of avian, human, or Dalmatian origin.

Place 0.2 ml. of neutralized, diluted urine in a 10 ml. diluting cylinder. Add 0.5 ml. of urea-cyanide reagent, 0.2 ml. of arsenophosphotungstate reagent, and make up to volume with 1 per cent sodium tetraborate. Mix and decant into an Evelyn cuvette. After 30 minutes, read on the Evelyn photoelectric colorimeter at 690 millimicra with the 10 ml. aperture, with distilled water as a blank. If the color is too intense to read, add successive 10 ml. aliquots of distilled water until a readable color results. The number of 10 ml. water aliquots required indicates the degree of additional dilution of the urine which is necessary.

Dilute an aliquot of the neutralized, diluted urine according to the results of this preliminary survey, and mix well. This sample is referred to below as the *final neutralized diluted urine.*

TABLE I

Renal Excretion of True Urate in Two Pure Bred Dalmatian Dogs

Period	Serum urate	Urate clearance	G.F.R.*	G.F.R. / Urate clearance	Glomerular urate excretion	Tubular urate excretion
	mg. per cent	*cc./min.*	*cc./min.*		*mg./min.*	*mg./min.*
Dog 1, female, 7 kg.						
1	1.10	79.6	24.8	0.31	0.27	0.60
2	1.15	96.2	40.0	0.42	0.46	0.65
Average	1.13	87.9	32.4	0.37	0.36	0.62
Dog 2, male, 17 kg.						
1	0.64	231.0	114.7	0.50	0.73	0.74
2	0.58	210.0	102.9	0.49	0.60	0.72
Average	0.61	220.5	108.8	0.49	0.66	0.73
Average of both subjects, per 10 kg.	0.87	129.6	56.1	0.43	0.49	0.64

* Glomerular filtration rate.

PROCEDURE.—

1. For Urine.—Set up control and reaction tubes, in duplicate, as follows:—

Control Tubes: 0.5 ml. of final neutralized diluted urine, 0.5 ml. of pH 9.2 borate buffer, 0.5 ml. of buffered albumin, 3.0 ml. of distilled water.

Reaction Tubes: 0.5 ml. of final neutralized diluted urine, 0.5 ml. of pH 9.2 borate buffer, 0.5 ml. of uricase solution, 3.0 ml. of distilled water.

Mix all tubes well and cork securely. Incubate for 2 hours at 45°C. To each tube, add 0.3 ml. of 1 N sulfuric acid. Mix and add 0.2 ml. of 7.5 per cent sodium tungstate. Centrifuge to obtain a protein-free filtrate.

2. For Serum.—Set up duplicate control and reaction tubes as follows:—

Control Tubes: 0.5 ml. of sample, 0.5 ml. of pH 9.2 borate buffer, 0.5 ml. of buffered albumin, 2.5 ml. of distilled water.

Reaction Tubes: 0.5 ml. of sample, 0.5 ml. of pH 9.2 borate buffer, 0.5 ml. of uricase solution, 2.5 ml. of distilled water.

Mix and cork securely. Incubate for 2 hours at 45°C. Then, to each tube add 0.5 ml. of 1 N sulfuric acid and mix. Add 0.5 ml. of 7.5 per cent sodium tungstate and mix. Centrifuge to obtain a protein-free filtrate. If the supernatant is cloudy, add 0.5 ml. of $\frac{2}{3}$ N sulfuric acid *to each of the control and reaction tubes for the particular sample* and recentrifuge.

3. Place about 2.5 ml. of 1 per cent sodium tetraborate solution into a suitable number of 10 ml. mixing cylinders. Add 2.0 ml. of protein-free filtrate and mix. (If an extra 0.5 ml. of acid was needed, use 2.2 ml. of protein-free filtrate.) Add 0.5 ml. of urea-cyanide reagent and mix. Add 0.2 ml. of arsenophosphotungstate reagent and mix. Dilute to 10 ml. with 1 per cent sodium tetraborate and mix by inversion.

4. Decant the solutions into Evelyn cuvettes and let stand exactly 30 minutes. Then read each *control* tube against *each* of the two *reaction* tubes as blanks. Thus, for each sample four readings will be obtained, since there are two control and two reaction tubes for each, sample. Average the four readings to obtain the value for true urate.

5. If sufficiently large samples are available, it is advisable when using Dalmatian serum, to set up step 2 with doubled quantities of all reagents, and to use 4.0 ml. of protein-free filtrate in step 3. If this modification is used, the value obtained in step 4 is twice the actual true urate concentration.

RESULTS

The simultaneously estimated true urate and exogenous creatinine clearances for our two Dalmatians are summarized in Table I. In both, true urate clearance considerably exceeded glomerular filtration rate, thus indicating that urate was cleared both by glomerular filtration and active tubular secretion. The average ratio of glomerular filtration rate to urate clearance is 0.43, which is rather too high for an exact equivalence of urate clearance and renal plasma flow. However, the urate clearance in the Dalmatian, as in birds, obviously approaches the general order of magnitude of the renal plasma flow.

DISCUSSION

True urate clearance greatly exceeds simultaneously determined exogenous creatinine clearance in the Dalmatian, indicating urate to be cleared both by glomerular filtration and by active tubular secretion. These observations differ from those of Friedman and Byers (3) who believed that the Dalmatian clears urate at the glomerular filtration rate. These investigators used the poorly established allantoin method for the estimation of glomerular filtration rate and did not estimate true urate enzymatically. The latter seems more probably responsible for their findings since the serum of the Dalmatian contains appreciable amounts of urate chromogen while his urine contains virtually none (6). Thus, in this species, total urate clearance, estimated colorimetrically, is considerably below the true urate clearance.

Even though the urate clearance exceeds the glomerular filtration rate in the Dalmatian, it is still not sufficiently high to equal the renal plasma flow. Ordinarily, when a substance is cleared at a rate well above that of glomerular filtration and when its clearance does not exactly equal renal plasma flow even at low plasma concentrations, the cause for the discrepancy will be found in plasma binding of a portion of the substance. If one assumes that the filtration

fraction in the dog is approximately 0.30 to 0.35, plasma binding of about one-third of the true urate would explain the failure of urate clearance exactly to equal renal plasma flow. Thus it is not surprising that Byers and Friedman (8) found some selective hindrance to the entrance of urate into Dalmatian cerebrospinal fluid from the blood stream, as compared to creatinine. It is, moreover, not surprising that salicylate does not increase the uric acid clearance in the Dalmatian, any more than in birds (9). Elsewhere we shall show that salicylate probably increases urate excretion by a selective depolymerization of the biologically non-diffusible urate fraction of plasma, the circulating polymeric urate (10). Although birds may clearly be shown to have circulating polymeric urate under some circumstances (11), in these species there appears to be no glomerular hindrance to its passage. Since the Dalmatian kidney appears to handle urate in a manner identical with that of the bird, a drug which causes selective depolymerization of a plasma urate fraction which already passes the glomerulus easily could not be expected to increase the excretion of urate.

The present results somewhat simplify the biological picture of urate excretion. It now appears that, at least in birds and mammals, there are only two varieties of excretory mechanism for urate. Man, the common dog, and most other mammals clear urate at only a fraction of the glomerular filtration rate. The ultimate reason for this remains disputed. We believe that it arises largely from the non-diffusibility of most of the plasma urate, at least in man (12), while many others believe that filtration and reabsorption are responsible. A second excretory mechanism is that common to the bird and Dalmatian dog. Here, urate is cleared both by glomerular filtration and by tubular secretion and urate clearance approaches or equals renal plasma flow.

Some clarification of the physiological processes responsible for the Dalmatian's anomalous purine metabolism is now possible. Unlike man, who produces urate only in his liver (13), all dogs—including Dalmatian dogs—produce urate in peripheral tissue. Thus, in all canine species, evisceration-nephrectomy results in a steady rise of plasma urate concentration and the cessation of allantoin production. In all dogs, urate formed in the periphery is transported to the liver. There it meets the hepatic uricase mechanism and is converted to allantoin. However, a portion of the mixed central blood leaving the heart reaches the kidney before ever reaching the liver. In the common dog, because of his low urate clearance, most urate entering with renal arterial blood leaves through the renal vein. However, in the Dalmatian, most of the urate entering the kidney is excreted into the urine. Thus, in this breed, the kidney constitutes a large prehepatic sluice through which urate of peripheral origin is diverted to the exterior without ever passing the liver. It is, therefore, unnecessary to assume as did Klemperer, Trimble, and Hastings (14) that the hepatic uricase of the Dalmatian is somewhat sequestered from contact with circulating urate. The uric acid found in the Dalmatian urine has never been exposed to hepatic uricase.

It is now clear that the urine urate plus allantoin represent the end-products of purine metabolism in all canine species, including the Dalmatian. The demonstration by Friedman and Byers of identity between Dalmatians and non-Dalmatians in the amount of urate plus allantoin excreted now makes it necessary to consider why dogs excrete so much more purine end-product per kilo than do men. From recent isotope studies, we now know that this problem cannot be dismissed by reference to possible destruction of urate in man, since in man urate is a true end-product of nucleoprotein metabolism and is not further metabolized. The amount of purine end-products which a given species excretes per kilo per day may or may not actually represent a fundamental measure of a basic aspect of nucleoprotein metabolism; but, obviously, this possibility requires further exploration.

SUMMARY

1. The renal mechanism for urate excretion in the Dalmatian dog resembles that in birds. Urate is cleared at a rate considerably greater than the glomerular filtration rate, indicating that its excretion is the result of both glomerular filtration and active tubular secretion. The basic physiological mechanisms involved in the anomalous purine metabolism of the Dalmatian are indicated and their possible biological significance briefly discussed.

2. A new enzymatic method for the direct estimation of true urate in biological fluids is described, which does not require the estimation of total urate and urate chromogen, followed by the calculation of true urate as a difference.

Note Added in Proof.—Praetorius and Kirk (*J. Lab. and Clin. Med., 1950*, **35**, 865) have recently reported the first known instance in man of an avian-Dalmatian type of tubular excretory mechanism for urate. Their subject's extremely low plasma urate levels were associated with high plasma oxypurine levels, reminiscent of the high circulating oxypurines which occur when plasma urate values are lowered by the administration of ACTH (Wolfson, W. Q., and Cohn, C., *Proc. 1st Clin. ACTH Conf.* 1949, 241).

BIBLIOGRAPHY

1. Benedict, S. R., *Harvey Lectures*, 1915–16, **10,** 346.
2. Schaffer, N. K., Dill, L. V., and Stander, H. J., *Endocrinology*, 1941, **29,** 243.
3. Friedman, M., and Byers, S. O., *J. Biol. Chem.*, 1948, **175,** 727.
4. Leone, E., *Quaderni nutriz.*, 1947, **10,** 13.
5. Yü, T. F., and Gutman, A. B., *Fed. Proc.*, 1949, **8,** 267.
6. Unpublished data.
7. Buchanan, O. H., Block, W. D., and Christman, A. A., *J. Biol. Chem.*, **1945, 157,** 181.
8. Byers, S. O., and Friedman, M., *Am. J. Physiol.*, 1948, **157,** 394.
9. Friedman, M., and Byers, S. O., *Am. J. Physiol.*, 1948, **154,** 167.
10. Wolfson, W. Q., data to be published.

11. Levine, R., Wolfson, W. Q., and Lenel, R., *Am. J. Physiol.*, 1947, **151,** 186.
12. Wolfson, W. Q., and Levine, R., *Fed. Proc.*, 1948, **7,** 136.
13. Wolfson, W. Q., Cohn, C., Levine, R., Rosenberg, E. F., and Hunt, H. D., *Ann. Int. Med.*, 1949, **30,** 598.
14. Klemperer, F. W., Trimble, H. C., and Hastings, A. B., *J. Biol. Chem.*, 1938, **125,** 445.