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Observations on Uric Acid Transport in Man, the Dalmatian and the non-Dalmatian Dog*

Howard Duncan, M.D.** and April S. Curtiss, B.S.**

The urinary output of uric acid from the purebred Dalmatian is similar in amount to that in man. Also, this breed of dog has a higher plasma uric acid level than other dogs and, like man, this hyperuricemia is accompanied more frequently with renal and bladder lithiasis. Allopurinol is effective therapy in both man and dog. Study of the fate of uric acid in the Dalmatian shows that the liver does not oxidize the available uric acid completely although it is capable of doing so when liver homogenates are studied. Consequently, the hepatic cellular membrane appears impermeable, or partially so, to uric acid. The possibility of a general membrane transport problem similar to that encountered at the liver cell has not been confirmed with studies of red cells in the Dalmatian. This dog shows some similarities to certain rare clinical human diseases with deafness, cardiac arrhythmias and renal tubule leak of uric acid, all of which offer ample opportunity for close and detailed examination as clinical models in biochemical, physiological, pathological and genetic studies.

Uric acid, the end product of purine metabolism in man, and also the higher apes, is a highly insoluble and biologically benign material. It introduces a problem of excretion because its solubility in water at 40°C is only 50 mg per liter. In urine the solubility is slightly greater and the concentration is usually near saturation level. The clinical problems of uric acid stone formation and obstructive uric acid nephropathy develop as a consequence of this limited solubility. Uricosuric drugs (probenecid, salicylates, sulfinpyrazone, etc.) and more recently allopurinol (an enzyme inhibitor of uric acid oxidation) for the first time have successfully controlled many of these clinical hazards. However, once a patient has been identified as a former of uric acid stones, particularly in gouty arthritis or with primary hyperuricemia, therapy aimed at correcting these problems has to be continued indefinitely.

Man is not as fortunate as the lower animals, which are capable of handling uric acid in a more efficient manner. Further down the evolutionary scale, the fish and amphibia excrete most of the nitrogen of protein metabolism as urea and ammonia and the mammals have also maintained this mechanism as a major means of nitrogen excretion (Fig 1). However, the reptiles and the birds developed a very clever water-conserving system whereby the last phases of breakdown from protein nitrogen to urea were eliminated, and uric acid is the end-product. This allowed twice the number of nitrogen atoms to be excreted per molecule of
available water. In the bird the plasma uric acid level is similar to man (3-7 mg/100 ml) and the uric acid is concentrated three-thousand times in the tubular urine of the bird and secreted into the cloaca. From here, the water is reabsorbed by the cloacal membrane, and the uric acid in solution, already supersaturated crystallizes and, with this, becomes osmotically and finally biologically inactive. The insolubility of this white uric acid paste can be seen on the superior aspects of our public buildings and statues, which have been visited by pigeons and starlings.

In the dog and similar lower mammals uric acid is effectively oxidized by the hepatic enzyme uricase, which converts the insoluble uric acid to the more soluble allantoin (Fig 1). Man, however, has not shared this bequest from aquatic ancestors. Partly because of these basic metabolic differences between man and dog, research into the control of the uric acid problem has been slow. These biological differences result in man having a serum uric acid level of around 3-7 mg/100 ml and he excretes some 500-700 mg of uric acid per day in the urine while the dog with a plasma uric acid level of less than 0.3 mg/100 ml has a daily 24-hour urine output of uric acid of 10-60 mg. Consequently, the solubility problems for the dog are negligible and uric acid lithiasis is rare.

**PATHWAY OF PURINE DEGRADATION**

**GLYCINE**

**NUCLEOPROTEIN**

**PURINES**

**ADENOSINE**

**GUANOSINE**

**HYPOXANTHINE**

**XANTHINE**

**URIC ACID**

**ALLANTOIN**

**UREA, AMMONIA**

**FISH**

**MAMMALS**

**MAN, APES**

**BIRDS**

**REPTILES**

*Figure 1*
Uric Acid Transport in Man, the Dalmatian and the non-Dalmatian Dog

The purebred Dalmatian (Fig 2) is distinguished biochemically by its extremely high uric acid output (400-600 mg/24 hours in a 10 kgm dog). The plasma uric acid level is two to four times that of the mongrel dog and as a consequence the Dalmatian* pays the penalty for his genetic abnormality with a very high frequency of renal and bladder stones. During the past four years we have used allopurinol in the Dalmatian to control the uric acid production and reduce the uric acid output in the same manner as we have in humans with the same problems.

In this particular breed we recognize its intermediate position, in the handling of uric acid, with man on the one hand incapable of oxidizing uric acid and on the other the non-Dalmatian breeds oxidizing uric acid most efficiently. For this and other reasons to be enumerated, this purebred dog serves us with a remarkable opportunity to study the physiological, biochemical and pharmacological actions and reactions of uric acid. Even though this biochemical attribute was recognized specifically in 1916, the amount of such investigation has been singularly little. Only in recent times has there been sufficient interest in this particular breed to bring about the establishment of the Dalmatian Research Foundation in 1969.**

Aside from the basic uric acid dis-

*The reason that the dog has been called by this name is uncertain. There is no reason to believe that it derived from Dalmatia (Yugoslavia) anymore than it was first bred in England or Bengal although it has often been called an English Coach Hound and the Bengal Harrier.

**Through the efforts of a Dalmatian fancier: J.C. Lowery of York, Pennsylvania.
Duncan and Curtiss

turbances, there are other areas of physiological, pathological, clinical and genetic significance known to be more common in this breed than others.

The main biological aberrations in the Dalmatian include:

1. Unique urate metabolism in:
   a. liver
   b. renal clearance
2. Congenital deafness
3. Cardiac arrhythmias and arrest
4. Heterochromia of the iris, china eye and walleye
5. Renal calculi, hematuria, pyelonephritis
6. Pigment "patches"
7. Recurrent dermatitis

While many of these characteristics are also seen in other pure-bred dogs and reflect the result of close inbreeding—the uric acid disturbance is unique to the purebred Dalmatian. The high level of uric acid in the urine and plasma falls dramatically in the offspring of a cross between a pure Dalmatian bred to any other strain. Even by careful "back breeding" of such offspring into the original pure strain, the uric acid abnormality reacts as a true recessive; even though the characteristic polka-dot pattern of the coat can be reconstituted, the uric acid abnormality fails to reappear. The essence of this study reveals that the spotting phenomenon of the Dalmatian and the uric acid abnormality are coded on different genes.

In 1967, James at the Henry Ford Hospital reviewed the cardiograms of 32 normal and 9 deaf purebred Dalmatians and found a higher incidence of cardiac arrhythmias in the deaf dogs compared with the normals in the same breed. Whereas it is our experience that the sudden death may occur in deaf Dalmatians, it was noteworthy that this "syndrome" in the Dalmatians resembled an unusual clinical picture also seen in man. In this syndrome, offspring of cousin marriages developed combinations of congenital deafness, pigment patches, bizarre electrocardiograms, renal disease, and sudden death. To our knowledge, uric acid of these children is not abnormal.

The deafness in these dogs is reported to be due to VIIIth nerve damage, but degeneration of the organ of Corti and collapse of the cochlear duct and saccule have been described. These defects seem unrelated to the uric acid abnormality.

Although the full uric acid story is not yet known, it is clear that in the Dalmatian at least two distinct abnormalities exist. In the mongrel dog and in the human, uric acid is filtered through the glomerulus and approximately 98%-100% of this is reabsorbed by the proximal tubules and that which ultimately reaches the urine is thought to be almost entirely secreted by the distal tubule (Fig 3). In the Dalmatian, there is no resorption of uric acid at the proximal tubule and a small amount is secreted by the distal tubule. Hence in the Dalmatian, the uric acid clearance may be equal to or even greater than the creatinine and insulin clearances in the same animal.

This renal abnormality in the Dalmatian allows the uric acid to be rapidly excreted and would tend to produce the low plasma uric acid level (0.5-1.0 mg) seen in this dog compared with that of man as we noted earlier. The high urine uric acid output from a 10 kgm dog equals that of a 70 kgm man. A counterpart appears in human patients with Wilson's disease or hepa-
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URATE TRANSPORT IN THE KIDNEY

Non-Dalmatian

100% reabsorption

3% estimated filtrate

Dalmatian

No reabsorption

98% reabsorption

3% estimated filtrate

Man

Glomerulus

Proximal Tubule

Distal Tubule

Figure 3

Urate transport in the kidney

 tolenticular degeneration with a modified deToni Fanconi syndrome wherein the proximal tubules fail to resorb uric acid and the uric acid clearance approaches that of creatinine. In these young people it is usually noted that the serum uric acid is in the range of 0.5 to 1.5 mg/100 ml. If the Dalmatian kidney is capable of excreting 10-100 times more uric acid per day in the urine than the mongrel dog, why then is its plasma uric acid level 3-4 times higher rather than lower than that of the mongrel dog? It is important to understand at the same moment that there is no evidence that there is increased nitrogen turnover in the Dalmatian compared with the non-Dalmatian dog. This being so, some other aberration in the metabolism of uric acid must exist in this animal.

It has been shown that in addition to the renal differences mentioned, the Dalmatian fails to oxidize uric acid as efficiently as the mongrel. In all dogs uric acid is oxidized by the enzyme uricase which is abundantly present in the liver including that of the Dalmatian. There is no significant amount of uricase or other uric acid oxidizing enzyme in any other tissue. Several studies have shown that the hepatic oxidation of uric acid in the Dalmatian differs from that of the non-Dalmatian. One series of observations showed that when the ureters of the mongrel dog were clamped, the serum uric acid level remained low despite the increasing levels of creatinine and other nitrogenous end-products. This was felt to be due to efficient oxidation of the excess urate load by hepatic uricase. In the Dalmatian the serum uric acid level rose rapidly together with the
serum creatinine and urea when the same studies were performed. Another study\(^1\) showed that the liver could be implicated in the Dalmatian's failure to oxidize an excess intravenous load of uric acid. The evidence was that although the intact kidney excreted 52% of the intravenous load in 60 minutes, the plasma uric acid level was still six times the normal figure whereas a similar load given to non-Dalmatians was completely oxidized within 60 minutes—even though only 12% of the load was recovered in the urine and the plasma level was normal. These studies appeared to confirm the premise of Klemperer et al\(^9\) who in 1938 stated that the Dalmatian liver contained uricase which was quantitatively capable of oxidizing as much uric acid as the mongrel dog, but the liver cell wall in some way prevented this action. His evidence depended upon the astute observations that when liver tissue from Dalmatian and from non-Dalmatian dogs was homogenized and incubated with uric acid, the rates of oxidation of uric acid by these homogenates was the same for each group. However, when intact liver slices were used in the same type of uric acid oxidizing system, the slice of non-Dalmatian liver was greatly superior in oxidizing uric acid than the slice of Dalmatian liver. The inference from these studies was that the intact hepatic cellular membrane prevented the association between uric acid in the blood and the uricase present in the hepatic cell.

In recent studies we have shown that although a constant load of uric acid is delivered to the Dalmatian liver, both by hepatic artery and portal vein, it fails to oxidize the uric acid as efficiently as the mongrel dog.

**Observations:** Eight Dalmatians and six mongrel dogs were anesthetized and small cannulae were placed in each of the following sites: (1) abdominal aorta; (2) renal vein; (3) portal vein; (4) hepatic vein; (5) jugular vein. After baseline blood samples were taken from these sites, a rapid injection of uric acid (40/mg/kg) was given into the jugular vein. At intervals of five minutes after this bolus injection and for a period of 40 minutes, samples were drawn simultaneously from each site cannulated and uric acid levels were determined on each sample (Table I). The arteriovenous urate differences for liver, kidney and gastrointestinal tract were calculated.

From these studies two important points became evident: (1) The hepatic “clearance” of uric acid in the mongrel dog was greater than the Dalmatian for, in the latter, the arteriovenous difference was similar for all organs excepting the kidney. (2) There was no measurable loss of uric acid into the gastrointestinal tract in either group. (This fact is rather significant when it is remembered that in humans some 200-400 mg of uric acid per 24 hours is secreted into the gastrointestinal tract and is oxidized.) From this, we deduced that the circulating uric acid in the Dalmatian is prevented from being oxidized presumably by some block in the membrane permeability to uric acid in the Dalmatian hepatic structure.

If such a problem exists in one region of the purebred dog, does this account for the differences in handling uric acid in the renal tubule and might it not be present also in some other more accessible areas such as the red
Uric Acid Transport in Man, the Dalmatian and the non-Dalmatian Dog

TABLE I

Mean Values* of Serum Uric Acid (mg/100ml) Determined at Intervals Following I.V. Injection of 40 mg/kg of Uric Acid

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>NON-DALMATIAN</th>
<th>DALMATIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Renal Vein</td>
<td>Hepatic Vein</td>
</tr>
<tr>
<td>0</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>5.8</td>
</tr>
<tr>
<td>10</td>
<td>4.1</td>
<td>3.1</td>
</tr>
<tr>
<td>15</td>
<td>3.8</td>
<td>2.4</td>
</tr>
<tr>
<td>20</td>
<td>3.6</td>
<td>1.8</td>
</tr>
<tr>
<td>30</td>
<td>2.9</td>
<td>1.6</td>
</tr>
<tr>
<td>40</td>
<td>1.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Eight Dalmatians and six mongrel dogs were studied

blood cell or the white blood cell? In humans the polymorphonuclear leucocyte is capable of concentrating uric acid—a situation which is occasionally seen in gouty patients whose peripheral circulating polymorphonuclear cells contain one or more large crystals of uric acid.

Uric acid is also present within human red blood cells and Danish workers have studied the transport of uric acid across the red cell membrane. They have shown that although physical diffusion of uric acid occurs, an active transport system—"mediated transport"—accounted for the majority of transfer of uric acid from the suspending medium to the red blood cell. This urate transport was altered by pH changes, temperature changes, and changes in concentrations to which the Michaelis equation for enzyme reaction rates could be applied. This enzyme mediated transport is blocked by the presence of other purines, i.e., hypoxanthine, and accelerated in the presence of estrogens. We have conducted such studies on 40 normal adult and gouty patients in an endeavor to determine the presence of urate transport abnormalities. No such discrepancy has been noted.

By using this system on two Dalmatians and "a number" of mongrel dogs, Harvey and Christensen suggested that a red cell membrane transport block existed in the Dalmatian's erythrocytes and not in those of the mongrel dog. Such a circumstance would help explain the problem at the hepatic cell membrane in the Dalmatian but, unfortunately, we have not been able to confirm these studies even though performed identically and repeatedly on erythrocytes of 10 purebred Dalmatians and 14 mongrel dogs.
Duncan and Curtiss

Method

The details of the method have been previously delineated.\textsuperscript{13} Basically the movement of uric acid across the red cell membrane is monitored by using radioactive uric acid (labelled $^{14}$C in the "2"-position). In the steady state the levels of uric acid within the cell and in the suspending medium do not change but a steady influx and efflux of uric acid molecules is constantly occurring. The influx is measured by the rate of decline of concentration of labelled uric acid in the suspending medium during 60 and 120 minutes of incubation. If hypoxanthine has previously been added to the medium, the active transport of uric acid is inhibited.

Fresh samples of blood were centrifuged and the red cells separated, washed, and suspended in a physiological buffer containing a uric acid concentration equal to the previous plasma level. These cells (5 ml) were incubated with 5 ml of buffer at 24°C for 45 minutes to permit a steady state to be established and then at zero time 0.4 microcuries of uric acid in 0.11 mg of stable uric acid contained in a 0.2 ml volume was added. Aliquots were taken immediately after mixing at zero time, and at 60 minutes and 120 minutes and at intervals between. The cells were rapidly centrifuged at 0°C in a two-minute period and samples 0.2 ml of the supernatant were transferred to scintillation-counting vials. The transfer across the red blood cell membrane and into the red cell was represented by the fall of radioactivity of the supernatant fluid and can be cross-checked subsequently by separation of the red blood cells then promoting their hemolysis and evaluating the concentration of intracellular radioactivity separately. While normal physical diffusion occurs, evidence of the presence of a mediated transport system is appreciated by the more rapid fall in the radioactivity of the supernatant fluid. If hypoxanthine is added to the incubating medium, mediated transport is stopped and the slow decline in the radioactivity of the supernatant represents physical diffusion factor alone.

Results

Table II shows the percentage of the radioactive uric acid present at zero time, 60 minutes, and 120 minutes following its introduction to the system. Human, Dalmatian and non-Dalmatian red cells have been studied. Included also in the study on the supernatant are figures available for these changes when hypoxanthine is present in the suspending medium. In the human the concentration of the labelled uric acid fell from 100% at zero time to 81.9% at 60 minutes and 75.3% at 120 minutes. When hypoxanthine was added, the mediated transport system was blocked and the fall of radioactivity at 60 minutes was 89.3% and at 120 minutes was 84.3% which represented the slow physical diffusion factor. In the studies involving the sets of dogs no evidence of a mediated transport of uric acid was found for the studies in the presence and absence of hypoxanthine were the same and also the same for each breed. Whereas a difference is known to exist between human and canine red cell transport, Harvey and Christensen stated that a 13% difference between the mediated and the physical diffusion transport systems was evident in mongrel dogs but was not present in Dalmatians. In
TABLE II

Uric Acid Transfer into Red Blood Cells

Percentage Radioactivity Present in Supernatant at Intervals Following Zero Time. (± Standard Deviation)

<table>
<thead>
<tr>
<th>Medium</th>
<th>0 Minutes</th>
<th>60 Minutes</th>
<th>120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human RBC's</td>
<td>Buffer only. 100</td>
<td>81.9 ± 3.4</td>
<td>75.3 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>With hypoxanthine 100</td>
<td>89.3 ± 4.0</td>
<td>84.3 ± 4.4</td>
</tr>
<tr>
<td>Mongrel RBC's</td>
<td>Buffer only. 100</td>
<td>92.0 ± 2.2</td>
<td>85.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>With hypoxanthine 100</td>
<td>92.9 ± 2.0</td>
<td>86.4 ± 2.4</td>
</tr>
<tr>
<td>Dalmatian RBC's</td>
<td>Buffer. 100</td>
<td>92.7 ± 2.3</td>
<td>85.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>With hypoxanthine 100</td>
<td>93.4 ± 1.6</td>
<td>87.4 ± 2.1</td>
</tr>
</tbody>
</table>

our own studies, no such difference was demonstrable.

This method of observing the concentrating of labelled purines, by suspended red blood cells, forms the basis of the present tests used in confirming the diagnosis of the Lesch-Nyhan syndrome. In this syndrome, a partial or complete deficiency of the enzyme hypoxanthin-guanine phosphoribosyl transferase presents itself clinically in children with mental deficiency, neurological abnormality, self-mutilating tendencies, hyperuricemia, and often uric acid lithiasis. In this group of patients allopurinol has proven partially effective in reducing the hazard of uric acid stone formation.

In the gouty patients and idiopathic uric acid stone formers, allopurinol has proven eminently effective. Here, again, it would naturally follow that the uric acid stone formation and hematuria of Dalmatians should be susceptible to the beneficial effects of allopurinol since nucleoprotein degradation in both man and dog are similar. Using seven Dalmatians and three mongrels which received 100 and 200 mg/day of allopurinol, our studies showed the average uric acid output of the Dalmatian could be reduced from 580 to 420 mg per 24-hour period—a 27% decrease. We have not conducted any long term studies on the frequency of stones in the dog population but one can deduce that if uric acid load should be decreased, its associated problems also should be reduced.

The uric acid problems in man are beginning to be understood and while some uric acid studies on the chimpanzee have been recently described which show a very close similarity to man, the Dalmatians' abnormalities are much more readily accessible and per-
mit the understanding of uric acid metabolism and membrane transport problems in man as well as the other aberrations found in this dog and other purebreds. A closer attention to this breed with respect to the study of biochemistry, physiology, pathology, and genetics could be productive at a faster and more economical rate with mutual benefit to both man and dog.

Acknowledgement

Considerable technical help is acknowledged from the Surgical Research personnel and particularly Mary Jo Lassilla, Jerry Mackenzie, Vladimir Sadler, and W. M. Konde, D.V.M.

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