ALLANTOIN AND URIC ACID METABOLISM
IN LEUKEMIC CATTLE

by

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requirements for the degree

MASTER OF SCIENCE

Pathology

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1969

Approved by:

[Signature]
Major Professor
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ALLANTOIN AND URIC ACID METABOLISM IN LEUKEMIC CATTLE

Neoplasia of the hematopoietic tissues in cattle is almost exclusively of lymphocytic origin\textsuperscript{13} and is one of the most common neoplasm affecting this species\textsuperscript{17}. The condition has been referred to by various authors as lymphosarcoma, lymphoblastoma, lymphocytoma, lymphadenosis, leucosis and leukemia\textsuperscript{3,7,13,16,17}.

Because it is hemopoietic in origin, hematological and histopathological studies have been reported\textsuperscript{1,7,16,17}.

The acute form of the disease is sufficiently characteristic that diagnosis may be based on signs\textsuperscript{17}. However, in the chronic form, the disease is not characteristic and physical examination and laboratory findings may not substantiate a diagnosis\textsuperscript{3,17}.

Although the Bendixen scale\textsuperscript{1} has been an accepted criterion for diagnosis of chronic leukemia on a herd basis, quantitative estimation of lymphocytes is seldom adequate to confirm a diagnosis.

Diagnosis of lymphosarcoma in cattle with normal or slightly elevated lymphocyte counts is difficult as animals with normal hematology may subsequently found to be affected by lymphosarcoma, although the primary reason for presentation was one of a number of unrelated conditions such as digestive disturbances, abortion or lameness\textsuperscript{17}.

Increased urinary excretion of uric acid has been reported in leukemia of man\textsuperscript{4,18}. Unlike man, the bovine liver contains uricase\textsuperscript{14} which converts uric acid to allantoin and carbon dioxide.
Because of the presence of this biochemical abnormality in man and the difficulty of ante-mortem diagnosis of chronic leukemia, nucleic acid metabolism in leukemic cattle was investigated. As lymphocytic infiltration of the kidneys has been reported as a common sequel to chronic leukemia, renal function studies were also performed.

Materials and Methods

In 1964 a closed herd of Holstein cows having a high incidence of leukemia was established. Since that time the herd has been maintained in isolation, fed on an adequate maintenance ration and allowed to breed and reproduce at random. Complete management, clinical, hematological and necropsy records have been maintained. Eight mature Holstein cows having normal to greatly elevated lymphocyte counts were selected as subjects in this investigation. Three comparable mature Holstein cows from another herd fed a similar diet were selected as controls.

Prior to the start of the experiment the following procedures were performed on all animals: total and differential leukocyte counts, hemoglobin, packed cell volume, sulfobromophthalein (BSP) half-time clearance (T1/2) and complete physical examination.

Two experiments were performed. One in December, 1968 and a second in February, 1969, each a replica of the other.

The cows were placed in stanchions and the urinary bladder cannulated with a No. 26F* retention catheter. The 24-hour urine

* C. R. Bard, Inc., Murray Hill, N.J.
volume was measured and a 250 ml. aliquot from each animal identified and refrigerated. Blood samples were collected at the start of the experiment and 20 hours later. Serum was removed and stored at -20 C.

Urine and serum were examined by previously described methods for urea nitrogen, uric acid using a modification of the Caraway method and creatinine by the Folin-Wu method. Creatinine clearance was calculated in the normal manner. The \( T_{1/2} \) for BSP was measured according to the method described by Cornelius. Urine solutes were measured using a Fiske osmometer. Allantoin was determined by a modification of the Young-Conway method (See Appendix).

Results.

On physical examination no superficial lymph nodes were enlarged. No abnormalities were detected by rectal palpation, except for bi-lateral enlargement of the external iliac lymph nodes in cow 2.

Hemoglobin and packed cell volumes (PCV) were within normal limits in all cows. Leukocyte counts ranged from 7,000/cu.mm. to 105,400/cu.mm. while lymphocyte counts ranged from 4,340/cu.mm. to 103,800/cu.mm. (Table 1)

Values for BSP clearance (\( T_{1/2} \)) were within normal limits for all experimental cows.

Total urine uric acid in the leukemic cows (Fig. 1) was slightly higher than that of the control group (Fig. 2). Increased urine volume (Table 3) probably accounted for the total increase as the uric acid concentration was not appreciably higher in leukemic cows. Uric acid values obtained from serum of the leukemic cows were slightly higher than those of the non-leukemic group.

Total urine allantoin was markedly elevated in the leukemic cattle (Figs. 1 & 2). Although concentrations of urine allantoin were slightly higher in the leukemic group, the marked increase in urine output accounted for the greatly elevated total values.

There was a greater allantoin to uric acid ratio in the urine and serum of leukemic cows. The average serum allantoin to uric acid ratio in leukemic cows was .52 (range of .30 to .73) as compared to an average of .097 (range of .06 to .16). Urine allantoin to uric acid ratios were also higher in leukemic cattle (ave. 6.1, range 5.4 to 7.7) than in the controls (ave. 4.7, range 3.2 to 6.2).

Normal creatinine clearance for healthy cows is given by Poulsen\(^15\) as 846± 132 ml/min/500 kg body weight. Cows 2, 11, 13, and 14 had reduced creatinine clearance as all were less than 500 ml/min (Table 3).

Urine osmolarity ranged from 959 to 1440 mOs/liter in the leukemic cattle and 740 to 800 mOs/liter in the controls. (Table 3)

With the exception of cows 13 and 19, serum urea and creatinine were within normal limits for healthy animals. The
urea nitrogen concentration in these cows was slightly elevated on both occasions. The urine volume of leukemic cows was higher than that of the control animals and greater than reported for normal animals.

All animals had a variable daily urine urea output that was unrelated to urine volume. As animals were on a maintenance diet, urine urea output was considered an index of protein intake.

Total urine creatinine of leukemic cattle was variable with cows 2, 13, 14 and 19 having the lowest output. (Table 2) One control cow, 72D had a reduced urinary creatinine output and a slightly lower urine urea output.

Discussion

Uric acid is formed partly from purines of exogenous sources (food) and in part from endogenous purines which are the result of nucleic acid metabolism\(^2,10,12\). Although the site of uric acid formation has not been precisely determined, the liver has been established as the site of uric acid destruction\(^14\). Species and breed differences exist in the handling of uric acid excretion. In man filtered uric acid is more or less completely reabsorbed in the proximal tubules and uric acid secretion is added to the tubular urine by a secretory mechanism located more distally in the nephron that the reabsorption mechanism\(^11\). The action of uricase contained in the liver of non-primate mammals converts uric acid to allantoin and carbon dioxide\(^12,14\).
leukemic cattle probably were producing and destroying nucleic acid-rich lymphocytes at a much greater rate than normal. Thus the primary reason for high uric acid and allantoin values would seem to be over-production rather than excretion failure. However, not all uric acid is eliminated in urine as there is evidence to suggest a small amount of biliary excretion of uric acid which is in turn degraded by intestinal bacteria. Uric acid is also secreted in small amounts by the kidney tubules.

Cattle with leukemia had elevated serum and urine allantoin and uric acid. As these alterations in uric acid and allantoin were consistent, the examination of urine and serum for allantoin and uric acid may aid in making a definitive diagnosis in animals that do not present a clinical and hematologic profile typical of leukemia.

As shown by the data obtained, the increase in urine and serum uric acid was not as great as that of allantoin. The increased urine volume probably accounted for the total increased output of uric acid as the concentration was not significantly higher in the leukemic group.

Allantoin levels in the urine of leukemic cattle were markedly increased when compared to the controls. In addition, the allantoin to uric acid ratio in serum and urine of leukemic cows was greater. These data suggested that increased uric acid production in leukemic cattle provided more substrate for the action of uricase.

Although the creatinine clearance was diminished and the blood urea nitrogen slightly elevated in some leukemic cattle,
there was little other evidence of decreased renal function. Urine osmolarity in leukemic cattle was comparable to or greater than urine osmolarity in the controls.
Table 1

Homograms and sulfobromophthalein clearance of experimental animals

<table>
<thead>
<tr>
<th>Leukemic Cows&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Hb gms%</th>
<th>PCV %</th>
<th>Leukocytes mm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Lymphocytes mm&lt;sup&gt;3&lt;/sup&gt;</th>
<th>ESP T&lt;sub&gt;1/2&lt;/sub&gt; min</th>
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<td>2</td>
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<td>24.5</td>
<td>104,600</td>
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<tr>
<td>6</td>
<td>10.7</td>
<td>35.0</td>
<td>18,450</td>
<td>12,350</td>
<td>4.75</td>
</tr>
<tr>
<td>8</td>
<td>12.2</td>
<td>33.5</td>
<td>30,800</td>
<td>27,450</td>
<td>4.37</td>
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<td>11</td>
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<td>41.3</td>
<td>13,750</td>
<td>15,300</td>
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<tr>
<td>13</td>
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<td>33.0</td>
<td>8,800</td>
<td>6,450</td>
<td>4.50</td>
</tr>
<tr>
<td>14</td>
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<td>37.8</td>
<td>12,800</td>
<td>15,450</td>
<td>4.87</td>
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<tr>
<td>15</td>
<td>11.1</td>
<td>32.5</td>
<td>23,650</td>
<td>33,900</td>
<td>4.12</td>
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Control Cows

<p>| | | | | | |</p>
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<td>9,700</td>
<td>6,305</td>
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<td>72D</td>
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<td>37.0</td>
<td>7,500</td>
<td>5,325</td>
<td>5.50</td>
</tr>
<tr>
<td>75D</td>
<td>13.0</td>
<td>38.5</td>
<td>9,800</td>
<td>6,174</td>
<td>5.90</td>
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<sup>1</sup> - Average of two determinations at eight week intervals
Table 2

Serum and urine allantoin and uric acid values of leukemic and normal cows

<table>
<thead>
<tr>
<th>Leukemic Cows¹</th>
<th>Serum U.A. mg/100ml</th>
<th>Urine U.A. mg/100ml</th>
<th>Total Urine U.A. G/24 hrs</th>
<th>Serum Allantoin mg/100ml</th>
<th>Urine Allantoin mg/100ml</th>
<th>Total Urine Allantoin G/24 hr</th>
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<td>.83</td>
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<td>.66</td>
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<td>.31</td>
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<td>59.9</td>
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<td>.86</td>
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<td>.62</td>
<td>342</td>
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<td>48.8</td>
<td>5.70</td>
<td>.58</td>
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<td></td>
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<tr>
<td>B127</td>
<td>.69</td>
<td>45.8</td>
<td>2.84</td>
<td>.11</td>
<td>215</td>
<td>13.33</td>
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<tr>
<td>72D</td>
<td>.69</td>
<td>76.5</td>
<td>3.83</td>
<td>.05</td>
<td>241</td>
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<tr>
<td>75D</td>
<td>.79</td>
<td>56.0</td>
<td>3.23</td>
<td>.05</td>
<td>345</td>
<td>19.87</td>
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</table>

¹ - Average of two determinations at eight week intervals
Table 3

Renal function studies in leukemic and normal cows

<table>
<thead>
<tr>
<th>Leukemic Cows</th>
<th>Urine Vol. (^1) L/24 hrs</th>
<th>Urine Solutes (^1) mOs x 10(^3)</th>
<th>Serum Urea</th>
<th>Total Urine (^1) G/24 hrs</th>
<th>Serum Creatinine (^1) mg/100ml</th>
<th>Total Urine Creatinine (^1) G/24 hrs</th>
<th>Creatinine Clearance ml/min</th>
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<tr>
<td>2</td>
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<td>9.95</td>
<td>20.9</td>
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<td>4.0</td>
<td>275</td>
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<td>6</td>
<td>14.5</td>
<td>14.80</td>
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<td>1.73</td>
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<td>663</td>
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<td>18.2</td>
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<td>7.1</td>
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<td>19</td>
<td>11.7</td>
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<td>23.2</td>
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<td>16.7</td>
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<td>75D</td>
<td>5.7</td>
<td>8.55</td>
<td>12.5</td>
<td>17.9</td>
<td>1.28</td>
<td>16.9</td>
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\(^1\) - Averages of two determinations at eight week intervals
Total uric acid, allantoin and solutes in 24 hour urine collections from cows with lymphoma

1st and 2nd Collections Parallel

Figure 1
Total uric acid, allantoin and solutes in 24 hour urine collections from normal cows

<table>
<thead>
<tr>
<th>Uric Acid - Gms</th>
<th>Allantoin - Gms</th>
<th>Solutes - mOs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grams</td>
<td></td>
<td>mOs x 10^3</td>
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Figure 2
LITERATURE CITED


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<tr>
<td>15</td>
<td>Poulsen, E.</td>
<td>Renal Clearance in the Cow</td>
<td>Yearbook</td>
<td>Royal Veterinary and Agricultural College, Copenhagen</td>
<td>(1957): 97-126.</td>
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APPENDIX
Allantoin Determinations

Estimation of allantoin in serum and urine was made by a modification of the Young-Conway method (31).

Five ml. of serum was added to 10 ml. of triple-distilled water, and the mixture warmed to 40°C in a water bath. While stirring mechanically, 8 grams of sodium sulfate was slowly added. This mixture was filtered with light vacuum and the filtrate immediately chilled to freezing in an ice-salt bath. After chilling the tube was filled with a crystalline-like mass which was centrifuged at 2000 r.p.m. for 10 minutes. This yielded 2.5 to 3.0 ml. of supernatant fluid.

Two ml. of supernatant fluid was placed in a chemically clean test tube and 0.2 ml. of 1N sodium hydroxide added. This mixture was placed in a boiling water bath for 10 minutes then immediately chilled to freezing after which 0.3 ml. 1N hydrochloric acid was added and again placed in boiling water for 2 minutes. Tube and contents were immersed in an ice-salt bath and chilled.

In very dim light, 0.4 ml. of phenylhydrazine hydrochloride solution (0.05 gm in 15 ml. of dist. HOH) was added after which the mixture was placed in a dark 30°C water bath for 15 minutes. Tube and contents were placed in the ice-salt bath and chilled. To this was added 1.2 ml. concentrated HCl and 0.4 ml. potassium ferricyanate solution (0.25 gm in 15 ml. dist. HOH). The tubes were kept cool and color allowed to develop in the dark for 7
minutes. Transmittance was read spectrophotometrically in a 12 x 75 mm. cuvette at 530 μm.

Urine allantoin was determined in a like manner using a 1:500 or 1:1,000 dilution. At these dilutions deproteinizing with sodium sulfate was unnecessary.

Each series of allantoin determinations were read against a freshly prepared allantoin standard containing 0.5 mg/100 ml. Phenylhydrazine and potassium ferricyanate were prepared daily for each series of determinations.
<table>
<thead>
<tr>
<th>Leukemic Cows</th>
<th>24 hr. Urine Volume (ml)</th>
<th>24 hr. Urine Allantoicin (mg/100ml)</th>
<th>Total Urine Allantoicin (mg/24hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>13,640</td>
<td>24.68</td>
</tr>
<tr>
<td>6</td>
<td>b</td>
<td>14,320</td>
<td>33.96</td>
</tr>
<tr>
<td>8</td>
<td>a</td>
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<td>b</td>
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<tr>
<td>24 hr. Urine</td>
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<td>Serum Allantoicin (mg/100ml)</td>
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<td>Serum Allantoicin (mg/100ml)</td>
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## BASE LINE STUDIES

Hemograms and sulfobromophalein clearance of experimental animals.

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<th>Band</th>
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<th>Lymphocytes</th>
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a 1st Collection

b 2nd Collection
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<th>Basophils</th>
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# RENAL FUNCTION STUDIES

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<th>Urine Urea Nitrogen (total mg)</th>
<th>Urine Solutes (mOs x 10³)</th>
<th>Creatinine Clearance (ml/min)</th>
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a  1st Collection  

b  2nd Collection
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<th>Urine Solutes (mOs x 10^3)</th>
<th>Creatinine Clearance (ml/min)</th>
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### URINE AND SERUM CREATININE

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- **a** 1st Collection
- **b** 2nd Collection
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<td>Urine Uric Acid mg/100 ml</td>
<td>Total Urine U.A. (Gms)</td>
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a 1st Collection
b 2nd Collection
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<th>Control Cows</th>
<th>Serum U.A. (mg/100ml)</th>
<th>Urine 24 hr. Vol. (ml)</th>
<th>Urine Uric Acid mg/100 ml</th>
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I wish to extend my sincere appreciation to my Major Professor, Dr. E. H. Coles and the other committee members, Drs. G. W. Osbaldiston and A. C. Strafuss. Thanks also to Dr. W. E. Moore who assisted in some of the calculations. A special thanks to Kristi Wilson, M. T. (ASCP) whose good humor was inversely proportional to my own while performing some of the chemical analyses.
ALLANTOIN AND URIC ACID METABOLISM
IN LEUKEMIC CATTLE

by

WILLIAM J. BRACKEN

B. S., Kansas State University, 1955
D. V. M., Kansas State University, 1955

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969
ABSTRACT

As the result of an extensive study on a closed herd of cattle having a high incidence of lymphosarcoma, it was appreciated that ante-mortem confirmation of this malignancy in the individual cow was often difficult.

In the non-primate mammals uric acid is the primary precursor of allantoin. Derived from the break-down of nuclei of all cells as well as nucleic acid-rich leukocytes, it is subjected to the action of uricase and catabolized into allantoin and carbon dioxide. Thus, it was considered possible that serum and urine concentrations of allantoin might prove to be of diagnostic value in the chronic form of bovine leukemia. If such is true, elevated lymphocyte counts and serum and urine allantoin might be confirmatory.

In an attempt to study nucleic acid metabolism, allantoin and uric acid in serum and urine were evaluated in eight cows from this herd. Serum and urine allantoin concentrations were elevated when the values in leukemic animals were compared to those of unaffected controls.

As lymphocytic infiltration of the kidneys is a frequent sequel to this condition, renal function studies were also performed. Creatinine clearance values in four of the eight leukemic cows were near normal range, the other four had reduced clearance rates.