

## Muramiduria and Hyperkalemia in the Chloroleukemic Rat<sup>1</sup> (36807)

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The presence of high levels of serum muramidase (lysozyme) in patients with acute granulocytic and acute monomyelocytic leukemia is well known (1, 2). In some of these patients, severe hypokalemia with kauresis has been noted (3, 4). The loss of potassium has been attributed to tubular damage due to renal deposition of muramidase. However, the mechanism for handling muramidase and the nature of the renal damage caused by the small molecular weight enzyme remains obscure.

The transplanted chloroleukemia in the rat is cytologically similar to human acute granulocytic leukemia; it is also associated with markedly elevated serum and urinary muramidase levels (5). Preliminary studies are presented which demonstrate excessive potassium loss in chloroleukemic rats and is related to the degree of muramiduria. It is apparent that the chloroleukemic rat furnishes an excellent model for the investigation of the metabolism of muramidase and the effect of this enzyme on the kidney.

**Methods and Materials.** Chloromas (Shay) were transplanted by subcutaneous passage of 0.5 to 1.0 ml suspension of chloroma cells into 3-wk-old Sprague-Dawley pups. Chloroleukemia was induced by intravenous injection of a similar suspension cephalad into the jugular vein. Blood samples were obtained from the tail and on occasion, from the inferior vena cava for determination of muramidase activity by the lysoplate method of Osserman and Lawlor (2). Urine samples

were collected for 24 hr from rats in metabolic cages and analyzed for muramidase. Serum and urine potassium and sodium determinations were carried out by standard methods. Potassium intake was measured on the basis of the amount of diet (containing 0.093% K) consumed. Serum and urine muramidase and electrolytes were measured before and after chloromatous tumors or chloroleukemia developed.

**Results.** Rats developing chloroma and chloroleukemia following transplantation show significant increase in serum muramidase after 10–14 days and subsequently develop muramiduria. Chloroma-bearing animals had a mean serum muramidase level significantly above the normal range (see Table I). No urinary muramidase was detected in control animals, but urine levels in the leukemic rats ranged from 3.3–20,250  $\mu\text{g}/24$  hr. Although mean serum K in 17 rats with chloromas was not significantly different than control levels, the mean urinary K excretion in 16 leukemic rats was significant compared to control animals at the level  $p < .005$  (see Table I). Although there was no direct correlation between the amounts of muramidase and K excreted in the urine over a 24-hr period ( $r = .3997, < p < .1$ ), 3 of 4 rats followed serially showed simultaneous elevations in urine muramidase and potassium (see Fig. 1).

**Discussion.** Studies on patients with acute myelomonocytic leukemia have demonstrated the association of hypermuramiduria and hypokalemia (3, 4). Two major theories exist concerning the renal clearance of muramidase and the effect on the kidney of high serum muramidase levels. Muramiduria results either from an overloading of the renal threshold for lysozyme or from elevated serum enzyme levels directly causing tubular damage. In human subjects with leukemia and sus-

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TABLE I. Experimental Results (Mean  $\pm$  1 SD).

	Serum Mur. ( $\mu\text{g/ml}$ )	Urine Mur. ( $\mu\text{g}/24\text{ hr}$ )	Urine K (mEq/24 hr)
Chloromatous rats	75.1 $\pm$ 8.22 (11) <sup>a</sup>	1762.4 (23)	0.8615 $\pm$ 0.0572 (16)
Controls	11.6 $\pm$ 0.71 (7)	0 (14)	0.5013 $\pm$ 0.0590 (11)

<sup>a</sup> No. of rats is given in parentheses.

pected renal damage due to muramidase, studies have been retrospective; prospective metabolic studies in acutely ill leukemic patients are impractical and would require prolonged periods of observation to achieve sufficient information. The chloroleukemia in the rat is cytologically similar to human acute granulocytic leukemia. Clearance studies by Greenberger, Rosenthal and Moloney (6) have demonstrated rapid disappearance of the enzyme from the serum in normal rats while in chloromatous rats with elevated serum muramidase, clearance is significantly delayed and muramiduria occurred. Our studies demonstrating the appearance of hyperkalemia with increased enzyme in the urine favors the mechanism of renal tubular damage. Although there was no histologic abnormality evidenced by light microscopy, Osserman and Azar's (7) findings of abnormal "droplets" in human kidneys by electronmi-

croscopy favors the mechanism of direct tubular damage. In this laboratory, further studies are now being carried out employing fluorescent antimuramidase antibody for localization of the enzyme in the chloroleukemic rat kidney.

*Summary.* The transplanted rat chloroleukemia cytologically similar to human acute myelocytic leukemia is associated with markedly elevated serum and urinary muramidase levels. Current metabolic studies demonstrate excessive potassium loss in the urine and appears to be related to the degree of muramiduria. The experimental myelogenous leukemia furnishes an excellent model for the investigation of the enzyme effect on the kidney and present studies suggest that muramidase causes a direct tubular damage.

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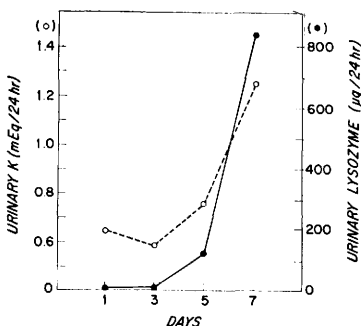


FIG. 1. Relationship of urinary potassium and muramidase (lysozyme) in the chloroleukemic rat.

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