cium ions have been shown to be specifically required when *Azotobacter vinelandii* O is fixing atmospheric nitrogen. Ammonium ion eliminates the need for molybdenum and calcium and has a sparing action on the requirement for iron. High levels of iron stimulate the knallgas reaction of *A. vinelandii* O fixing N_2 in the presence of H_2, but molybdenum in the range tested had no effect on this reaction. Low concentrations of calcium ions in a nitrogen-fixing culture result in an extended lag phase of growth. Decrease in the concentration of phosphate in the presence of low levels of calcium brings about a further increase in the lag, but this increase is not observed if the calcium supply is adequate.

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Uricolysis in Normal and Leukemic Individuals. (22821)

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Hyperuricemia occurs with moderate frequency in leukemia and other blood dyscrasias. The hyperuricemia has been ascribed to increased nucleo-protein catabolism. In studies on the uric acid pool, Benedict(1) and her associates consistently found more urate produced than excreted. Using larger amounts of labeled uric acid, Wyngaarden (2) demonstrated that uricolysis occurs in significant amounts in humans. We attempted (3) to demonstrate the site of this uricolysis. The rate of urate disappearing from the formed elements of blood incubated with plasma was of a significant amount and could account for a large part of the degraded urate. Among our non-gouty hospitalized controls were several leukemics. These patients demonstrated a markedly lower rate of uricolysis than did the normals or even the gouty patients, the gouty patients being considerably lower than the normals. It is quite possible therefore, that elevated plasma urate levels in leukemics may be secondary, at least in part, to an impaired mechanism for uricolysis.

Methods. 50 ml of heparinized blood were obtained. A 5 ml sample was removed to serve as a whole blood specimen and the rest centrifuged. The plasma was removed and the buffy coat pipetted off. The buffy coat was centrifuged in a narrow tube in either the patient's own plasma or isotonic saline. After 3-4 separations, high concentrations of white blood cells relatively free of red blood cells were obtained. The red blood cell preparations were obtained by pipetting the red cells from the bottom of the tube and freed of white blood cells in the same manner as above. Each patient had the following samples incubated at 37° under 5% CO_2–95% O_2: 1. Whole blood. 2. Plasma. 3.White blood cells (0.5 ml) and the same patient's plasma (3. ml). 4. Red blood cells (0.5 ml) and the same patient's plasma (3. ml). Blood counts were done on preparations 3 and 4. On white cell preparations, the red counts

TABLE I. Mean Percentage Decrease in Plasma Urate after Incubation.

Subjects	No. of determinations	Mean plasma level	Whole blood	Plasma	White cells	Red cells
Normals	25	5.1 ± .1*	34.4 ± 2.3	2.3 ± 2.1	39.0 ± 2.2	29.8 ± 2.5
Leukemics	11	9.5 ± .2	22.4 ± 1.0	4.2 ± .3	21.4 ± .7	13.0 ± .7

* ± stand. error.

were less than 0.5 million, and in the red cell preparation, the white counts were less than 1000. For technical reasons, the patients chosen had increased leukocyte counts ranging from 18,000 to 542,000. Samples of plasma were withdrawn at 0, 4 and 24 hours of incubation and the plasma urate determined by the Yü modification of the Buchanan, Block and Christman method.

The results of 25 determinations on normal hospitalized patients as compared with 11 determinations on 7 leukemic patients are given in Table I.

To prove that the diminished rate of uricolysis was not related to the serum urate level, samples of normal sera were fortified by the addition of urate to hyperuricemic levels with no significant change in the rate of uricolysis. Of the leukemic patients, 3 had myelogenous leukemia, and 4 had lymphatic leukemia. There was no significant difference between the two types of leukemias we studied so far as diminished rates of uricolysis nor could differentiation be made as to the type of leukemia from the pattern of uricolysis. Aside from uricase, which has never been demonstrated in humans, 2 known enzyme systems are capable of oxidizing urate at a physiological pH. The first of these is peroxidase, which will oxidize urate in the presence of peroxide or a source of peroxide (as glucose and glucose oxidase) and the second is cytochrome-cytochrome oxidase(4). The end products of the peroxidase degradation were studied by Agner and by Tuttle and Cohen. The end products of cytochrome-cytochrome oxidase degradation have not been extensively studied.

Peroxidase is present to 1-2% of the dry weight of leukocytes of the myeloid series, while erythrocytes have both cytochrome and peroxidative activity(5). Agner states that leukocytes isolated from the blood in myeloid leukemia were very rich in peroxidase. The enzymatic activity of leukocytes in infections and in chronic myelogenous leukemia has been studied in regard to phosphatase content by Valentine(6). During a leukocytosis, the phosphatase activity may be as high as 5 times the normal and was found to be lower than normal in chronic myelogenous leukemia. Variable amounts of other enzymes are therefore also probable in disease states.

All urate determinations were total chromogen determinations and were not subjected to uricase degradation. Serious errors may still arise using a specific enzyme like uricase because of oxidation of other chromogenic substances or binding by the buffer of still other chromogenic substances(7). It is conceivable that uricolysis may proceed at a normal or even accelerated rate in leukemias but that substances reducing arsenophosphotungstic acid may be liberated into the plasma with the result that a defect in uricolysis is being demonstrated in the sample taken.

Summary. 1. Uricolysis occurs in whole blood as well as in plasma incubated with white or red blood cells. The rate of uricolysis is not dependent on the hyperuricemia in normals. There is marked defect in uricolysis in leukemics under the same experimental conditions. 2. Hyperuricemia in leukemia, may be in part due to diminution in the rate of uricolysis.

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