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Hepatic Purine Enzyme Profiles and Uric Acid Overproduction in Muscular Dystrophy and in Inherited Tophaceous Gout (40199)

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Many diseases which lead to continued tissue synthesis and breakdown are associated with hyperuricemia and clinical gout (1). Muscular dystrophy, for example, includes a group of disorders characterized by progressive atrophy of skeletal muscle (2). We have previously reported that amino acid-dependent alterations in polysome aggregation determine the rate of RNA breakdown and that an unidentified product of RNA breakdown regulated purine biosynthesis (3). Although the biochemical mechanism of the relationship among hyperuricemia, gout, and tissue synthesis and breakdown is usually thought to be due to the increased turnover of purine nucleic acids, a detailed evaluation of purine metabolism under these conditions has not been reported. A unique opportunity to study the mechanisms of the above relationships occurred with the recent discovery (4) of a genetically selected line of chickens which overproduced uric acid but did not develop clinical gout (dystrophic) and another line of the same strain which overproduced uric acid and developed severe tophaceous gout (dystrophic gouty). Consequently, the hepatic activities of 5′-nucleotidase (total IMP phosphohydrolases EC 3.1.3.1 + EC 3.1.3.5), adenosine deaminase (EC 2.5.4.4), adenosine kinase (EC 2.7.1.20), hypoxanthine phosphoribosyltransferase (EC 2.4.2.8), adenine phosphoribosyltransferase (EC 2.4.2.7), adenylate deaminase (EC 3.5.4.6) and purine nucleoside phosphorylase (EC 2.4.2.1), and uric acid production in control and genetically selected lines of dystrophic and dystrophic gouty chickens are reported.

Materials and methods. One day-old chicks from a line of New Hampshire chickens with inherited muscular dystrophy and a normal control line of New Hampshire chickens (4) were given free access to a 22% protein commercial chick starter ration for 3 weeks. During the last 5 days of this period, food intake was monitored and total excreta was collected daily for individual birds. Uric acid production was determined as previously reported (5).

At the end of the 3-week period, all birds were fed an 80% protein diet (5) for the next 2 weeks. Since dystrophic birds ate less than controls, the amount of food offered to some control birds was restricted to that eaten by the dystrophic birds. Control birds fed restricted amounts of food were necessary in order to evaluate possible differences in enzyme activities due to different food intake levels. When the data were evaluated the level of food intake did not affect the enzyme activity levels and consequently, the data from *ad libitum*-fed and restricted-fed control chicks were pooled.

After two weeks on the 80% protein feeding, the birds were weighed, examined for gout (4) and approximately 3 ml of blood were collected in EDTA by cardiac puncture. The birds were killed, the livers quickly removed and clamped in liquid nitrogen. Blood plasma was isolated by centrifugation (1000*g* for 5 min, 20°) and uric acid concentration measured (6). Five representative carcasses from each group were assayed for water, nitrogen, inorganic material (7) and lipid (8).

Each frozen liver was weighed and powdered in a mortar containing liquid nitrogen. One and a half grams of the powdered frozen liver were homogenized with 4 ml of 0.05 *M* Tris containing 0.15 *M* KCl at 0°, pH 7.2, and the homogenate was centrifuged at 12,000*g* for 10 min at 4°. The supernatant was dialyzed against 10 vol of the homogenizing buffer changed 3 times. The protein concentration (9, 10) of the dialyzed extract was approximately 40 mg/ml. All enzyme assays were completed within 6 weeks during which time the extracts were stored at −70°.

Adenylate deaminase activity was measured from the radioactivity in IMP, derived

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from [8-^{14}C]AMP. The reaction mixture contained 5 m*M* [8-^{14}C]AMP (specific activity 0.2 Ci/mole), 50 m*M* Tris–succinate buffer (pH 7.4), 300 m*M* KCl, 10 μl of liver tissue extract and deionized water in a final volume of 200 μl. The incubation was conducted and the reaction terminated as described previously (11, 12). Products and reactants were isolated by thin layer chromatography using a mixture of butyric acid, 1.5 *M* NaOH, and 0.15 *M* EDTA (14:3:3, v/v) to develop the cellulose plates.

Purine nucleoside phosphorylase was determined essentially as previously described (13). The reaction mixture contained 5 m*M* inosine, 50 m*M* potassium phosphate buffer (pH 7.4) and commercial xanthine oxidase (250 mg protein). Before use, the xanthine oxidase preparation was dialyzed (14). In order to convert absorbance to uric acid concentration, a uric acid standard was used. The standard contained all the reagents present in the enzyme assay, except the liver preparation which was replaced with bovine serum albumin.

The activities of 5′-nucleotidase, adenosine kinase, hypoxanthine phosphoribosyltransferase, adenine phosphoribosyltransferase and adenosine deaminase were measured by the methods we have previously reported (11, 12) but modified for chicken liver to obtain maximum activity and linear product formation for at least 15 min with up to 1 mg of tissue extract protein in each reaction. The data were analyzed by analysis of variance (15) and mean values compared using Duncan's multiple range test (16) at the 5% probability level.

Results. The results are summarized in Table I. The dystrophic gouty birds were smaller than dystrophics or controls. Both dystrophic lines ate less and had higher plasma uric acid levels than the controls. Uric acid production (output), corrected for differences in body weight and dietary protein intake, was 1.7 and 3.8 times greater in the dystrophic and dystrophic gouty birds, respectively, compared to controls. Uric acid output in dystrophic gouty birds was 2.2 times greater than that in dystrophic birds. The body composition of all groups of birds was similar.

Adenosine deaminase, adenylate deaminase and purine nucleoside phosphorylase activities were greater in dystrophic and dys-

TABLE I. URIC ACID PRODUCTION AND HEPATIC PURINE ENZYME LEVELS IN CONTROL, DYSTROPHIC AND DYSTROPHIC GOUTY CHICKENS.

	Control	Dystrophic	Dystrophic gouty
Food intake (g/day)	32.3 ± 1.4^a (13)	24.1 ± 0.9^b (7)	19.2 ± 0.8^b (3)
Protein intake (g/day)	7.0 ± 0.3^a (13)	5.3 ± 0.2^b (7)	4.2 ± 0.2^b (3)
Daily uric acid output (mMoles per prot. intake per BW$^{.75}$)	1.69 ± 0.4^a (13)	2.91 ± 0.2^b (7)	6.42 ± 0.2^c (3)
Body weight (Kg$^{.75}$)	0.38 ± 0.06^a (20)	0.36 ± 0.03^a (9)	0.27 ± 0.05^b (12)
Liver weight (g)	6.2 ± 1.3^{a,b} (20)	6.7 ± 0.8^b (9)	5.3 ± 1.3^a (12)
Plasma uric acid (m*M*)	0.39 ± 0.03^a (20)	0.49 ± 0.08^b (9)	0.74 ± 0.12^b (12)
Body composition (% wet basis)			
Protein	21.2 ± 0.6^a (5)	20.6 ± 0.6^a (5)	20.3 ± 0.5^a (5)
Fat	5.6 ± 0.2^a (5)	5.6 ± 0.3^a (5)	4.8 ± 0.4^a (5)
Inorganic material	3.4 ± 0.1^a (5)	3.6 ± 0.2^a (5)	3.8 ± 0.2^a (5)
Water	70.4 ± 0.4^a (5)	69.8 ± 0.9^a (5)	70.5 ± 0.2^a (5)
Enzyme activities (μMoles product per liver per hr/BW$^{.75}$)			
Adenine phosphoribosyltransferase	16.3 ± 1.3^a (20)	20.2 ± 1.7^{a,b} (9)	22.9 ± 1.1^b (12)
Adenosine deaminase	147 ± 6^a (20)	188 ± 7^b (9)	168 ± 9^b (12)
Adenosine kinase	1871 ± 75^a (20)	1919 ± 65^a (9)	2195 ± 103^b (12)
AMP deaminase	652 ± 25^a (20)	802 ± 49^b (9)	846 ± 56^b (12)
Hypoxanthine phosphoribosyltransferase	26.5 ± 1.4^a (20)	32.1 ± 2.1^b (9)	39.4 ± 2.3^c (12)
5′-Nucleotidase (IMP)	4114 ± 225^a (20)	4106 ± 437^a (9)	5143 ± 273^b (12)
Purine nucleoside phosphorylase	8148 ± 598^a (20)	10811 ± 329^b (9)	10469 ± 472^b (12)

* Entries are means ± SE. The number of birds are shown in parentheses. Data on food intake, protein intake and daily uric acid output were obtained from individual birds during the last 5 days of the third week on the commercial chick starter diet (22% protein). The rest of the data were obtained from birds consuming the 80% protein diet (4) during the last 2 weeks of the experiment.

$^{a-c}$ Horizontal values not sharing a common superscript are different at the 5% level of probability.

trophic gouty chickens than in the controls. The activities of these enzymes did not differ between the dystrophic and dystrophic gouty groups.

The activities of 5′-nucleotidase and adenosine kinase were greater in the dystrophic gouty group than in the control or dystrophic groups. The activities of these two enzymes were the same in the control and dystrophic groups.

Hypoxanthine phosphoribosyltransferase activity was greater in dystrophic gouty chickens than in dystrophic chickens. Dystrophic chickens also had a higher activity level of this enzyme than did the controls. Adenine phosphoribosyltransferase, in general, showed a pattern of activity similar to that of hypoxanthine phosphoribosyltransferase.

Discussion. A scheme outlining purine, metabolism is shown in Fig. 1. The purpose of the present study was to evaluate uric acid production and purine metabolism by measuring the levels of purine degrading and reutilizing enzymes in two selected lines of genetically dystrophic chickens known to overproduce uric acid. One of the lines was known to develop severe tophaceous gout while the other line was known not to develop gout (4). Dystrophic birds have a progressive atrophy of skeletal muscle and since conditions of tissue breakdown are frequently associated with the first episodes of clinical gout (1, 2), the present study sought to describe alterations in hepatic purine enzyme levels of dystrophic and gouty chickens.

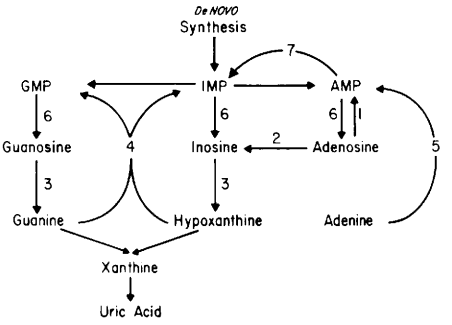

FIG. 1. Diagrammatic representation of purine metabolism. The reactions catalyzed by 1, adenosine kinase; 2, adenosine deaminase; 3, nucleoside phosphorylase; 4, hypoxanthine-guanine phosphoribosyltransferase; 5, adenine phosphoribosyltransferase; 6, 5′-nucleotidase; and 7, adenylate deaminase are outlined.

Since the activity of 5′-nucleotidase was much greater than that of the other purine enzymes, it was important to ensure that such high activity did not interfere with the other enzyme assays. A 5′-nucleotidase inhibitor (TTP) was included in the incubation mixture of the adenosine kinase assay. The concentrations of TTP used inhibited 5′-nucleotidase by 61%. Adenosine kinase was not inhibited by TTP.

On the other hand, TTP inhibited the activity of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase by approximately 50% and consequently was not included in these assays. All the products of the adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase assays were isolated by thin layer chromatography (17) in the absence of TTP and the product nucleotides from adenine and hypoxanthine accounted for at least 90% of the total product activity.

To ensure that all products of the adenylate deaminase reaction were accounted for, the reaction products were also subjected to two dimensional thin layer chromatography (17). The only labelled nucleotide product was IMP.

Measurement of adenylate deaminase in the presence of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride (18) showed that of the total AMP used by the liver preparation, approximately 4% was found in inosine, hypoxanthine, xanthine and uric acid. Therefore, determination of adenylate deaminase by recovering only IMP accounted for more than 90% of the total activity.

Dystrophic gouty chickens produced 2.2 times more uric acid than the dystrophic birds. Dystrophic birds produced 1.7 times more uric acid than the controls. This basis for expressing uric acid production was selected because uric acid output is a function of protein intake (5, 19–21) and of metabolic body size (19). Thus, it appears that there are two levels of excess production of uric acid, one associated with the dystrophic condition and the other with the dystrophic gouty condition.

Enzyme activities were expressed as total liver activity per unit metabolic body size because of the role of the liver in uric acid synthesis (22, 23) and because the carcasses

were of similar composition.

Overproduction of uric acid in the dystrophic relative to control birds was associated with increased activities of adenosine deaminase (28%), hypoxanthine phosphoribosyltransferase (21%), adenylate deaminase (23%), and purine nucleoside phosphorylase (33%). The increased activities of adenosine and adenylate deaminases would increase the flow of adenine nucleotide and nucleoside to IMP and GMP, thus increasing uric acid production (24). The increased activity of purine nucleoside phosphorylase found in dystrophic birds in the present study agreed with a previous report (25) which showed increased activity of this enzyme was associated with enhanced uric acid production. Xanthine dehydrogenase levels were also enhanced when uric acid production was increased (20, 25) and this enzyme was previously reported (4) to be elevated in these genetically selected lines of birds.

The overproduction of uric acid in the dystrophic gouty birds, above the level in the dystrophic birds, was associated with increased activities of 5′-nucleotidase (25%), hypoxanthine phosphoribosyltransferase (23%) and adenosine kinase (15%).

The increased 5′-nucleotidase activity in the dystrophic gouty chickens may be an important factor in uric acid overproduction and gout in these birds, since this enzyme is the first step in purine degradation and is very active in chicken liver. Also, a cytosolic form of 5′-nucleotidase has been suggested as a key enzyme in nitrogen elimination in uricotelic animals (25–28).

Although the increased activities of 5′-nucleotidase and adenosine kinase were associated with the gouty condition in the dystrophic birds, the ratio of 5′-nucleotidase/adenosine kinase was remarkably constant for all groups of birds. In view of the very high activity of 5′-nucleotidase compared with adenosine kinase, it is very likely that the deposition of adenosine in chicken liver was determined by the relative activities of 5′-nucleotidase and adenosine kinase, rather than adenosine kinase and adenosine deaminase as appears to be the case in the erythrocyte (29). Consequently, increased levels of the salvaging enzymes, adenine and hypoxanthine phosphoribosyltransferases, and adenosine kinase, associated with uric acid overproduction, may represent an attempt to overcome the enhanced purine catabolism associated with increased activities of adenosine and AMP deaminases and 5′-nucleotidase.

Although the present data suggest that uric acid overproduction concomitant with muscular dystrophy has a different biochemical basis than the uric acid overproduction concomitant with inherited tophaceous gout, the small increments (15–25%) in enzyme activities compared with the large increment (220%) in uric acid production suggest the involvement of additional factors. Such factors might include other purine enzymes and key purine intermediates. For further studies, the present genetically selected lines of chickens offer unique possibilities for identifying key purine enzymes and substrates which may be involved in regulating purine synthesis.

Summary. Although uric acid overproduction frequently, but not always, leads to gout in humans, it is not known if different biochemical aberrations in purine metabolism might be responsible for that uric acid overproduction which culminates in gout and that which does not. To investigate this possibility hepatic purine enzymes were measured in a line of chickens which overproduced uric acid but did not develop clinical gout (dystrophic) and in another line of the same strain which overproduced uric acid and developed severe tophaceous gout (dystrophic gouty). Adenosine and adenylate deaminases and nucleoside phosphorylase were elevated in both the dystrophic and dystrophic gouty chickens compared with controls. The activities of these three enzymes were not different between the dystrophic and dystrophic gouty groups. 5′-nucleotidase and adenosine kinase were elevated only in dystrophic gouty chickens. Hypoxanthine phosphoribosyltransferase was elevated in dystrophic chickens and was further elevated in dystrophic gouty chickens. To the extent that the hepatic purine enzyme activities reflect events at the molecular level, the present study demonstrates that different biochemical mechanisms may be involved in the uric acid overproduction associated with tissue atrophy (dystrophy) and inherited tophaceous gout.

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