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Purine Metabolism in High and Low Uric Acid Lines of Chickens: *De Novo* Uric Acid Synthesis in Isolated Hepatocytes and Phosphoribosylpyrophosphate Amidotransferase Activities[1] (41966)

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Abstract. The *de novo* biosynthesis of uric acid was examined in isolated hepatocytes from the high and low uric acid lines of chickens. Rates of incorporation of radiolabeled glycine into uric acid by hepatocytes from the high uric acid (HUA) line were approximately 3.6-fold greater than found in low uric acid (LUA) control hepatocytes. Uric acid synthesis rates in these cells were positively correlated with plasma uric acid levels ($r = +0.77$; $P < 0.01$). The activity of phosphoribosylpyrophosphate (PRPP) amidotransferase was measured in acetone powder preparations from liver and kidney tissues of the HUA and LUA lines. Activities in kidney tissues were about 21% lower than those found in livers. PRPP amidotransferase activities in liver and kidney tissues did not correlate significantly with plasma uric acid levels. The increased synthesis of uric acid in the HUA line may be the result of the increased PRPP synthetase activities and PRPP pool sizes previously reported for these tissues.

De novo biosynthesis of uric acid in birds does not appear to be restricted to hepatic tissues, since incorporation of labeled glycine into uric acid has also been reported to occur in isolated cells from the chicken kidney, spleen, thymus, and bursal tissues (1, 2). *De novo* purine biosynthesis in avian tissues has also been demonstrated using acetone powder preparations from chicken livers and kidneys (3) and using cell-free enzyme preparations from pigeon livers (4). Isolated cell suspensions provide a convenient method for studying purine metabolic pathways *in vivo*. Jones and Buttery (5) reported that substrate and intermediate stimulation of uric acid production in isolated cells was similar to that found in perfused livers. The ability to make numerous observations on a given sample while maintaining viable cells over several hours is a distinct advantage in conducting metabolic research. This communication examines *de novo* uric acid synthesis in isolated hepatocytes prepared from tissues of the high and low uric acid lines of chickens (6). The high uric acid line (HUA) is characterized by hereditary hyperuricemia and articular gout. HUA birds have been previously shown to synthesize more uric acid in acetone powder preparations from kidney tissues than low uric acid (LUA) control birds (3).

The activity of phosphoribosylpyrophosphate (PRPP) amidotransferase (EC 2.4.2.14) was measured in acetone powder preparations from HUA and LUA birds. This enzyme catalyzes the first committed step in *de novo* purine biosynthesis and is subject to feedback inhibition by numerous purine nucleotides (7–9). The avian hepatic PRPP amidotransferase appears to be induced during the feeding of high-protein diets (10), but not during estradiol-induced liver growth (11). Since PRPP amidotransferase exhibits major control over *de novo* purine nucleotide and uric acid synthesis, determination of the relative activities of this enzyme in HUA and LUA tissues may be of value in explaining the overproduction of urates in the hyperuricemic line of chickens.

Materials and Methods. *Experimental chickens and husbandry.* The original stocks of HUA and LUA birds were obtained from R. E. Austic, Cornell University, in 1975 and have been selected according to plasma uric acid levels. The LUA chickens served as control birds throughout the study since they

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were derived from a similar ancestry as the HUA line.

All HUA and LUA birds were maintained in electrically heated wire-floored brooders throughout the study. The birds were fed a chick starter ration [containing 23% crude protein (CP)] until 14 weeks of age and then fed a chicken developer diet (13% CP) throughout the study.

Reagents. [1-^{14}C]Glycine was purchased from New England Nuclear, Boston, Massachusetts, and L-[U-^{14}C]glutamine was purchased from Amersham, Arlington Heights, Illinois. Phosphoribosylpyrophosphate, β-mercaptoethanol, bovine pancreatic insulin, Hepes, amino acids, purine bases, Dowex 50W-X4 cation-exchange resin, Sigmacell Type 100, hyaluronidase (Type I-S) 300 units/mg, and collagenase (Type II) 260 units/mg were purchased from Sigma Chemical Company, St. Louis, Missouri. Reagents were of analytical grade.

Isolation of chicken hepatocytes and de novo uric acid synthesis. Hepatocytes were isolated by a modification of the procedure of Badenoch-Jones and Buttery (5). Eight-month-old roosters from the HUA and LUA lines were anaesthetized with ether and fitted with a cannula extending from the right atrium into the posterior vena cava. The cannula was tied in place and the liver was perfused with oxygenated 0.019 *M* Hepes-buffered Ca^{2+}-free Hanks' solution (12) at 4°C. The hepatic portal vein was cut to allow the medium to flow through the liver and perfusion continued until the effluent was clear and essentially free of blood cells (generally about 300 ml). The livers were then finely minced with a razor blade and rinsed extensively over cheesecloth with the perfusion solution. Five grams of each sample was placed in a 50-ml Erlenmeyer flask containing 20 ml of hyaluronidase–collagenase digestion medium (1 mg each/ml in Ca^{2+}-free Hanks' solution). The samples were incubated at 37°C for 60 min in an orbital water bath. A stream of oxygen was gently blown over the surface of the suspension and the contents were pipeted frequently to enhance the dispersal of cells. After the digestion period, the suspensions were cooled on ice before centrifuging at 500*g* for 15 min at 4°C and the supernatant was discarded. The pellet was resuspended in 40 ml of 0.026 *M* Hepes-buffered Eagle's medium containing 0.32 m*M* glycine (GIBCO, Grand Island, New York) and briefly centrifuged until the samples reached 50*g*. This latter step pelleted tissue particles while individual cells and small clumps remained in suspension. The supernatant was withdrawn and centrifuged at 500*g* for 15 min and the resulting supernatant discarded. The pellet was washed twice with the Hepes-buffered Eagle's medium and resuspended in 10 ml of this solution. The cell suspensions (approximately 7 million cells/ml) were transferred to a 50-ml Erlenmeyer flask containing 1 ml of Hepes-buffered Eagle's medium with 2.25 μCi [1-^{14}C]glycine and 1 μg of bovine insulin. The flasks were incubated at 40°C in an orbital water bath at 150 rpm and 1-ml samples were taken at 0.5-hr intervals and added to 1 ml of 2 *N* perchloric acid in glass conical centrifuge tubes. One microgram of bovine insulin was added to the cell suspensions at 1-hr intervals. Viability of the incubating cells was assessed by trypan blue exclusion at 0, 1, and 2 hr. Initial viability averaged 92.5 ± 0.95% and after 2 hr of incubation viability decreased to 84.5 ± 1.67%. The samples were capped and placed in a boiling water bath for 60 min to yield free purine bases. After centrifugation, the clear hydrolysates were applied to Dowex 50W-X4 cation exchanges (40 × 8 mm) previously washed with 1 *N* HCl. The pellets were washed with 1 ml of 1 *N* HCl and the supernatants were applied to the columns. The columns were then washed with 8 ml of 1 *N* HCl and the effluent was collected in glass conical centrifuge tubes and evaporated to dryness in a boiling water bath under a stream of air. To this residue was added 6 ml of 8.5 m*M* Li_2CO_3 and the sample was mixed vigorously with several milligrams of acid washed sand (Ottawa) to promote solubilization of the uric acid and urate salts. The procedure for the isolation of the mercuric salt of uric acid is a modification of the method of Bergmann and Dikstein (13). The tubes were placed in a boiling water bath and 1.5 ml of 0.1 *M* mercuric acetate in 5% (v/v) acetic acid was added dropwise. The mixture was incubated in the boiling water bath for 10 min and then placed in an ice-water bath for 30 min. The

precipitate was pelleted by centrifugation at 500*g* for 15 min at 4°C and the supernatant was discarded. The pellet was washed by centrifugation with two 4-ml aliquots of 0.01 *M* mercuric acetate in 1% (v/v) acetic acid saturated with uric acid. The pellet was dried and extracted with 400 μl of 0.5 *M* NaCl in 1% (v/v) acetic acid. An aliquot of this extract was chromatographed on cellulose TLC (Sigmacell Type 100) with 19 μg of nonlabeled uric acid which served as a marker. The solvent system consisted of 79% H_2O:15% methanol:6% concentrated HCl. The ultraviolet absorbing areas corresponding to uric acid were removed and added to 10 ml of Biocount (Research Products International Corp., Grove Village, Ill.) and counted in a Packard Tri-Carb 460 liquid Scintilation counter. This chromatographic system gives clear separation between uric acid and the following compounds: glycine, serine (formed from glycine), xanthine, hypoxanthine, adenine, and guanine. Recovery of exogenously added uric acid from the cell preparations was 85.9 ± 3.1%.

Assay of PRPP amidotransferase activity. The assay for PRPP amidotransferase was based on the measurement of the PRPP-dependent deamidation of [U-^{14}C]glutamine. PRPP amidotransferase activity was linear with respect to protein concentration and time. Acetone powders of both livers and kidneys were prepared as previously described (14, 15). Thirty milligrams of each acetone powder preparation was extracted in 4 ml of 25 m*M* Tris–HCl buffer, pH 7.0, containing 3 m*M* $MgCl_2$ and 1 m*M* β-mercaptoethanol using a glass-teflon homogenizer (Arthur H. Thomas Co., Philadelphia, Pa.). The extracts were centrifuged at 1500*g* for 15 min at 4°C and the supernatant was withdrawn for protein determination and enzyme assay. Protein determination was done by the Coomassie blue dye-binding method of Bradford (16) using bovine γ-globulin as the standard. Aliquots of liver preparations (150 μl) and of kidney preparations (100 μl) were adjusted to 200 μl total volume with the extraction buffer and added to 200 μl of the reaction buffer containing 25 m*M* Tris–HCl buffer, pH 7.0, containing 3 m*M* $MgCl_2$, 3 m*M* PRPP, 1 m*M* β-mercaptoethanol, and 0.1 μCi L-[U-^{14}C]glutamine (40 Ci/mole) in 12 × 75 mm culture tubes. To determine the amount of interfering glutaminase present in the preparations, control reactions were run in parallel without PRPP. The tubes were incubated at 37°C for 2 hr and the reactions were terminated by placing them into an ice-water bath. Separation of the glutamic acid product from glutamine was accomplished by anion-exchange descending chromatography using Whatman DE52 paper. Forty microliters of each reaction mixture was spotted along with 50 μg of glutamine and 50 μg of glutamic acid to serve as markers. After developing with distilled water, the chromatographs were dried and the amino acid spots visualized using ninhydrin spray (0.5% w/v in acetone). The ninhydrin reactive spots corresponding to glutamic acid were excised and extracted with 0.75 ml of 0.01 *N* HCl in glass scintillation vials. Ten milliliters of BioCount (Research Products International Corp.) was added to the mixture and the vials were counted in a Packard Tri-Carb 460 liquid scintillation counter.

Determination of plasma uric acid. Blood samples were taken via cardiac puncture using heparinized syringes. Plasma uric acid levels (14, 15) were determined by a micromethod modification (17) of the uricase spectrophotometric procedure of Feichtmeir and Wrenn (18).

Statistical analysis of the PRPP amidotransferase data was done with Duncan's new multiple range test (19). The effect of breed difference on *de novo* uric acid synthesis was determined by the Student *t* test (20). The model included sources of variation due to time of sampling.

Results. Isolated hepatocytes from HUA tissues synthesized uric acid at a rate 3.6-fold greater than LUA hepatocytes (Table I). Uric acid synthesis rates in these cells correlated favorably with plasma uric acid levels ($r = +0.77$; $P < 0.01$).

The activities of PRPP amidotransferase in acetone powder preparations from HUA and LUA tissues are shown in Table II. Activities in kidney tissues were about 21% lower than those found in livers. Within the LUA line females showed greater activity in liver samples than males while there were no significant differences between sexes in kidney tissues. In the HUA line females showed

TABLE I. *De Novo* Biosynthesis of Uric Acid from [1-^{14}C]Glycine in Isolated Hepatocytes from HUA and LUA Lines

Breed	Plasma UA (mg uric acid/dl plasma)	Incorporation rate (per hr)	
		dpm/10^6 cells	dpm/mg protein
HUA	36.32 ± 2.27[a]	642 ± 114[a]	579 ± 59[a]
LUA	8.06 ± 1.01[b]	179 ± 46[b]	127 ± 33[b]

Note. All values represent the means ± SEM of five observations.

[a,b] Means within a column with the same letter in superscript are not significantly different ($P > 0.01$).

greater levels of activity in their kidneys compared to males, while no differences were seen in the livers. Between the breeds, females of the low line had significantly higher amounts of activity in their liver preparations and lower amounts of activity in their kidneys. Male birds showed no differences in PRPP amidotransferase activities in either liver or kidney tissues. When lines were compared over both sexes, the birds of the HUA line had greater activity in kidney tissues compared to the LUA line. PRPP amidotransferase activities in liver and kidney tissues did not correlate significantly with plasma uric acid levels.

Discussion. The results shown in Table I clearly demonstrate an increased rate of *de novo* uric acid synthesis in hepatocytes from the HUA birds compared to controls. Our data suggest that the increased synthesis does not appear to be caused by an increase in the specific activity of PRPP amidotransferase (Table II). Previous work in our laboratory (15) showed that livers and kidneys of HUA birds possess significantly greater PRPP pool sizes. In rat liver (21), chicken liver (22), and other cells and tissues (23) PRPP is the rate limiting substrate not only for PRPP amidotransferase, but also for the entire *de novo* biosynthetic pathway. Lipstein and his coworkers demonstrated that PRPP levels in the liver of the uricotelic chicken were 200-fold greater than in the ureotelic rat (22). The increased availability of PRPP was thought to be due in part to the relatively low amounts of competing purine salvage activity found in the avian liver tissue. These workers found that the activity of PRPP synthetase in rat and chicken liver homogenates was comparable. Earlier studies, however, using liver acetone powder preparations demonstrated a 29-fold greater activity of PRPP synthetase in the chicken tissue compared to the rat. Data from our laboratory showed increased activity of PRPP synthetase in HUA kidneys and livers compared to controls (15). The importance of PRPP availability for *de novo* purine synthesis in the chick liver was also demonstrated by Welch and Rudolph using a liver growth-promoting

TABLE II. PRPP Amidotransferase Activities in HUA and LUA Tissues

Breed/sex	Plasma UA (mg uric acid/dl plasma)	PRPP amidotransferase activity (pmole glutamic acid produced/mg protein/hr)	
		Liver	Kidney
LUA			
Females	5.83 ± 0.56[a]	138.70 ± 5.68[a]	85.14 ± 5.41[a]
Males	6.46 ± 0.27[a]	110.25 ± 13.19[b]	85.12 ± 5.38[a]
LUA-mean	6.16 ± 0.38*	123.64 ± 8.08	85.13 ± 3.70*
HUA			
Females	13.65 ± 1.86[b]	111.95 ± 1.31[b]	112.24 ± 4.15[b]
Males	25.48 ± 2.46[c]	122.59 ± 4.57[a,b]	95.77 ± 5.21[a]
HUA-mean	19.25 ± 2.03*	117.27 ± 3.19	103.57 ± 3.81*

Note. All values represent the means ± SEM of 10 individual tissue samples. Plasma UA levels were previously reported (14, 15).

[a–c] Means within a column with the same letter in superscript are not significantly different ($P > 0.05$).

* Mean values of breeds within a column are significantly different ($P < 0.05$).

hormone: β-estradiol (11). While the activity of PRPP amidotransferase and several other key purine metabolic enzymes remained unchanged in treated birds, the intracellular PRPP pool sizes doubled and the rates of *de novo* purine biosynthesis tripled. The increased rates of *de novo* uric acid synthesis in hepatocytes from the HUA line may simply be due to increased rates of synthesis and availability of PRPP in these birds.

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