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Purine Metabolism in High and Low Uric Acid Lines of Chickens: Phosphoribosylpyrophosphate (PRPP) Synthetase Activities and PRPP Pool Sizes[1] (41755)

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Abstract. Phosphoribosylpyrophosphate synthetase activities were examined in liver and kidney tissues of two genetic lines of chickens selected for their plasma uric acid levels. Previous work demonstrated that the high uric acid line (HUA) has significantly greater *de novo* uric acid synthesis rates in kidney tissue compared to the low uric acid line (LUA). In addition, xanthine dehydrogenase activity in liver and kidney tissues was significantly higher in the HUA compared to the LUA line. The activity of PRPP synthetase, which provides a key substrate for the rate limiting step in *de novo* purine biosynthesis, was found to be significantly elevated ($P < 0.05$) in liver and kidney tissues of the HUA line. The mean value of kidney PRPP synthetase activity was 30.2 ± 0.7 nmole PRPP and 19.1 ± 1.6 nmol PRPP produced/mg protein/hr, respectively, for the HUA and LUA lines. The PRPP pool size in kidney tissue was also significantly higher in the HUA line. The mean level of PRPP was 319.8 ± 37.2 pmole/g of wet tissue compared to 176.7 ± 12.4 pmole PRPP/g of wet tissue in the LUA line. The mean values of liver PRPP synthetase activities were 11.4 ± 0.6 nmole PRPP and 9.0 ± 0.4 nmole PRPP produced/mg protein/hr, respectively, for the HUA and LUA lines. The PRPP pool size in liver tissues was significantly higher in the HUA line as well. The mean level of PRPP was 550.7 ± 72.5 pmole/g of wet tissue compared to 313.6 ± 31.4 pmole PRPP/g of wet tissue in the LUA line. The enzyme from the HUA birds does not differ from controls with respect to Michaelis constants, inhibition phenomena, and phosphate activation. The increased PRPP synthetase activity found in HUA tissues may be due to structural alterations of the molecule resulting in an enzyme with a greater V_{max}, an increase in the amount of enzyme protein, or a lowered level of a nondialyzable inhibitor.

Phosphoribosylpyrophosphate (PRPP) synthetase (E.C. 2.7.6.1) catalyzes the conversion of ribose-5-phosphate and adenosine-5′-triphosphate (ATP) to PRPP and adenosine-5′-monophosphate (AMP). The reaction was first described by Kornberg and his co-workers in 1954 during pioneering work on the incorporation of orotic acid into pyrimidine nucleotides (1). Since this initial discovery, the end product (PRPP) has been demonstrated to participate in over 17 different enzyme reactions (2).

Human PRPP synthetase is inhibited by a wide variety of end products of purine, pyrimidine, and pyridine metabolism (3). Inhibition appears to occur at three different sites on the enzyme. Adenosine-5′-diphosphate (ADP) inhibits activity competitively with respect to Mg-ATP. PRPP and 2,3-diphosphoglyceric acid (2,3-DPG) are both competitive inhibitors with respect to ribose-5-phosphate. Most of the remaining inhibitors act in a noncompetitive manner by a mechanism termed "heterogeneous nucleotide pool inhibition" (3).

PRPP amidotransferase (E.C. 2.4.2.14) catalyzes the first committed step in *de novo* purine biosynthesis. In the rat liver (4) and in other cells and tissues (5) PRPP is not only the rate limiting substrate for this reaction, but for the entire *de novo* biosynthetic pathway. Lipstein and his co-workers demonstrated that PRPP levels in the liver of the uricotelic chicken were 200-fold greater than in the ureotelic rat (6). The increased availability of PRPP was thought to be due in part to the relatively low amounts of competing

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purine salvage activity found in avian liver tissue. These workers found that the activity of PRPP synthetase in rat and chicken liver homogenates were 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 (7).

A number of cases of elevated PRPP synthetase activity and concomitant hyperuricemia have been reported in man in recent years. Sperling and his co-workers (8) reported increased erythrocyte PRPP synthesis in two brothers suffering from gouty arthritis and uric acid lithiasis. Further work revealed that the mutant PRPP synthetase, when measured in crude hemolysates, was more active at physiological phosphate concentrations than the normal enzyme and activity was less sensitive to inhibition by guanosine-5′-diphosphate (GDP), ADP, 2,3-DPG, and AMP (9). Phosphate activation curves and substrate saturation curves for ribose-5-phosphate and ATP were similar for both enzymes when measured using partially purified enzyme preparations. The abnormal protein appears to be altered in its regulatory capacity while the catalytic properties remain unchanged.

Increased PRPP synthetase activity and pool sizes were reported in cultured lymphocytes from patients with the Lesch–Nyhan Syndrome (10), a disease characterized by a deficiency of hypoxanthine/guanine phosphoribosyltransferase (HGPRT) and accompanying behavioral and neurological disorders (11, 12).

Becker (13) reported increased PRPP synthetase activity in hemolyzates from two brothers suffering from purine overproduction and gout. Using antibody to purified human PRPP synthetase it was demonstrated that these individuals had normal amounts of immunoreactive protein, but 2.5–3.0-fold higher activity per enzyme molecule (14). This abnormality differs from that described by Sperling (9) in that activity was increased over a wide range of inorganic phosphate concentrations while there was no altered response to inhibitors (15). Neither mutant differed from the normal enzyme with respect to substrate affinity. In 1974 a mutant enzyme was reported which possessed an increased affinity for ribose-5-phosphate. Fibroblasts cultured from this patient had both increased PRPP and decreased ribose-5-phosphate concentrations (16).

In 1980 Becker and his co-workers described a superactive PRPP synthetase in cultured fibroblasts from a hyperuricemic 14-year-old male (17). The mutant enzyme was resistant to ADP and GDP feedback inhibition and demonstrated hyperbolic rather than sigmoidal inorganic phosphate activation in "incompletely dialyzed" extracts. While the enzyme showed normal affinity constants for substrates and for Mg^{2+}, there was twice the normal activity per immunoreactive enzyme molecule. The enzyme therefore possessed both abnormal regulatory and catalytic properties.

Our investigations center on the etiology of uric acid overproduction in the high uric acid (HUA) line of chickens (18). Previous work in our laboratory demonstrated that in addition to significantly greater *de novo* uric acid synthesis rates in kidney tissues of the HUA line, xanthine dehydrogenase levels in both liver and kidney tissues are also elevated (19). The activity of the purine salvage enzyme HGPRT was also elevated in the liver tissues of the HUA line (20).

This study was initiated to examine the activity of PRPP synthetase in tissue extracts of the HUA line for a possible abnormality which might be linked to the hyperuricemia and articular gout commonly found in these birds. PRPP synthetase activity was also examined in tissues of birds fed varying levels of dietary protein.

Materials and Methods. *Experimental chickens and husbandry.* The original stocks of HUA and low uric acid (LUA) birds were obtained from R. E. Austic, Cornell University in 1975 and have been continually selected according to plasma uric acid levels. The LUA chickens served as control birds throughout the study since they were derived from a similar ancestry as the HUA line. Male broiler chickens (Hubbard × Hubbard) were obtained from Fors Farms, Puyallup, Washington.

All HUA and LUA birds were maintained in electrically heated wire brooders until approximately 4 weeks of age, then reared in floor pens for the remainder of the study. The birds were fed a chick starter ration (23% crude protein (CP)) until 14 weeks of age and then

fed chicken developer (13% CP) until the 22nd week. After the 22nd week, the birds were maintained on layer mash (17.5% CP).

Male broiler chicks were maintained in electrically heated wire brooders. Birds were fed a chick starter ration for the first 8 days then divided into four groups of 15 each (5 birds/pen) and fed purified diets containing 29, 44, 66, or 82% crude protein (Table I) for 2 weeks.

Determination of plasma uric acid. Blood samples were taken via cardiac puncture using heparinized syringes. Plasma uric acid levels were determined by a micromethod modification (21) of the uricase spectrophotometric procedure of Feichtmeir and Wrenn (22).

Acetone powder preparation. Tissue samples were taken from HUA and LUA birds at 13 months of age and from the broiler chicks at 3 weeks of age. Immediately after collection of blood samples, livers and kidneys from each bird were removed, weighed, and a 1- to 2-g portion of each tissue was excised for PRPP pool analysis. The balance of the tissues were frozen in a dry ice–acetone mixture and stored at −20°C. The fresh tissue samples were mixed with a 1-ml volume of 0.05 *M* Tris–HCl buffer, pH 7.4 (4°C), equal to 10-fold the weight in grams and the mixture was blended in a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.). The homogenate was clarified by centrifugation at 1500*g* and the supernatant frozen for later analysis. Frozen tissues were pulverized with a mallet and suspended in a 1-ml volume of acetone (−20°C) equal to 10-fold the weight in grams. The tissues were blended thoroughly in a Sorval Omnimixer. The acetone solution was filtered off through a Büchner funnel and the resulting cake was resuspended in half the original volume of acetone and filtered. After a second resuspension and filtering, the cakes were placed *in vacuo* for 2 days at 4°C over concentrated sulfuric acid and 2 days over Drierite (W. A. Hammond Drierite Co., Xenia, Ohio). The acetone powder preparations were stored dessicated at −20°C prior to use.

Reagents. [*carboxy*-^{14}C]Orotic acid, omnifluor, and hyamine hydroxide were purchased from New England Nuclear, Boston, Massachusetts. Ethylene diamine tetraacetic acid (EDTA), β-mercaptoethanol, ribose-5-phosphate, adenosine-5′-triphosphate (ATP), guanosine-5′-monophosphate (GMP), 2,3-diphosphoglyceric acid (2,3-DPG), adenosine-5′-diphosphate (ADP), and the mixed enzymes: orotidine 5′-phosphate pyrophosphorylase and orotidine 5′-phosphate decarboxylase were purchased from Sigma Chemical Company, St. Louis, Missouri. Reagents were of analytical grade.

Assay of PRPP synthetase activity. The assay for PRPP synthetase was a modification of the assay of Kornberg (7). PRPP synthetase

TABLE I. COMPOSITION OF PURIFIED DIETS (PERCENTAGE)

Ingredient	Dietary protein level (%)			
	29	44	66	82
Isolated soybean protein[a]	28.74	45.98	68.97	86.21
Glucose monohydrate[b]	62.24	45.25	22.26	5.02
Vitamin mix[c]	0.05	0.05	0.05	0.05
Mineral mix[d]	7.44	7.44	7.44	7.44
Linoleic acid	1.00	1.00	1.00	1.00
Choline chloride (50%)	0.28	0.28	0.28	0.28
D-L-methionine	0.10	—	—	—
Glycine	0.20	—	—	—

[a] Soybean protein: grade II, U.S. Biochemical Corporation, Cleveland, Ohio.

[b] Cerelose, Corn Products Co., Argo, Ill.

[c] The vitamin mixture contributed the following to the diet (mg/kg diet): thiamine HCl, 100; niacin, 100; riboflavin, 16; Ca-pantothenate, 20; Cyanocobalamin, 0.02; pyridoxine HCl, 6; biotin, 0.6; folic acid, 4; inositol, 100; menadione sodium bisulfite, 5; *d*-α-tocopherol acetate, 20; cholecalciferol, 1200 ICU; retinyl palmitate, 10,000 IU.

[d] The mineral mixture contributed the following to the diet (g/kg diet): $NaMoO_4 \cdot 2H_2O$, 0.01; $CuSO_4$, 0.02; KI, 0.05; $ZnCO_3$, 0.02; $FeC_6H_5O_7$, 0.40; $MnSO_4 \cdot 2H_2O$, 0.42; KCl, 3.00; $MgSO_4 \cdot 7H_2O$, 5.00; NaCl, 6.00; Na_2HPO_4, 7.20; KH_2PO_4, 12.00; $Ca_3(PO_4)_2$, 13.95; and $CaCO_3$, 15.00.

activity is linear with respect to protein concentration and time. Twelve milligrams of each acetone powder preparation was extracted gently in 4 ml of 1 m*M* sodium phosphate buffer, pH 7.2, containing 1 m*M* EDTA and 1 m*M* β-mercaptoethanol for 30 min at room temperature in an orbital mixer. The extract was dialyzed for 2 hr at 4°C against 50 vol of extraction buffer with one change. The dialyzed extract was centrifuged at 1500*g* for 15 min at 4°C and the supernatant withdrawn for protein determination and enzyme assay. Protein determination was done by the Coomassie blue dye-binding method of Bradford (23) using bovine γ globulin as the standard. After making appropriate dilutions with dialysis buffer, 200-μl aliquots of the supernatant were added to culture tubes (15 × 85 mm) containing 200 μl of 135 m*M* Tris–NaOH buffer, pH 7.4, with 81 m*M* inorganic phosphate, 10.8 m*M* $MgCl_2$, 2.7 m*M* EDTA, 0.946 m*M* ribose-5-phosphate, and 1.35 m*M* ATP. In the inhibition studies inorganic phosphate was adjusted to a final concentration of 1 m*M*. The reaction was initiated by the addition of 100 μl of 0.05 *M* Tris–HCl buffer, pH 7.4, containing 0.1 μCi [*carboxy*-^{14}C]orotic acid and 1 mg of the mixed enzymes (orotidine 5′-phosphate pyrophosphorylase and orotidine 5′-phosphate decarboxylase: 0.32 units/mg). The pH of this reaction mixture was 7.33. The culture tubes were stoppered with a rubber septum (14 × 18 mm) fitted with a plastic disposable center well (Kontes, San Leandro, Calif.). Each center well contained a 11 × 60-mm folded Whatman No. 1 filter paper saturated with 125 μl of hyamine hydroxide. The tubes were incubated at 37°C for 2 hr and the reactions were terminated by the injection of 100 μl of 30% w/v trichloroacetic acid through the septum into the reaction mixture. The tubes were equilibrated at room temperature for 90 min to trap the remaining [^{14}C]CO_2 evolved in the reaction. The papers in the center wells were removed and placed in 10 ml of scintillation counting fluid (4 g/liter omnifluor in 70% toluene and 30% absolute ethanol).

PRPP pool size determination. PRPP pool size determinations are linear within the range used here. One-milliliter aliquots of the thawed supernatants were placed in 12 × 75-mm culture tubes and incubated in a boiling water bath for 30 sec. The tubes were then immediately chilled in an ice-water bath and centrifuged at 1500*g* for 15 min. The clear supernatants (devoid of detectable PRPP synthetase activity) were used in the determination of PRPP pool sizes. This heat treatment removes interfering enzymes without a detectable loss of PRPP (24). Two hundred-microliter aliquots of each supernatant were added to 15 × 85-cm culture tubes. Assay conditions for pool size determinations were the same as for PRPP synthetase except the buffer contained no ribose-5-phosphate or ATP.

Statistical analysis of the data was done with Duncan's new multiple-range test (25).

Results. The mean plasma urate level in HUA birds was greater than that of the LUA line ($P < 0.05$) (Table II). There were no significant differences between plasma urate levels of males and females in the LUA line. HUA males, however, had nearly twice the levels of the corresponding females.

Within lines, the liver PRPP synthetase activities were significantly greater in males than females. Between lines, the HUA males and females had greater PRPP synthetase activity in liver tissue than the corresponding birds of the LUA line. The specific actvity in kidney tissues was about double that of the livers. There was no significant difference in activity between sexes within genetic lines, however, both males and females of the HUA breed had approximately 50% greater activity in kidneys than the controls in the LUA line.

Liver PRPP pool sizes were greater in females than in males. Between lines, both males and females of the HUA line had significantly larger pools than the corresponding controls in the LUA line. In kidneys, the males of the HUA line had greater PRPP pool sizes than females, but there was no statistical difference between the sexes of the LUA line. HUA males had larger pool sizes than their controls, but there was no statistical difference between the females of the two lines. When compared as a group, the birds of the HUA line had significantly greater kidney PRPP pool sizes than those of the LUA line.

In broiler chicks daily protein consumption and plasma uric acid levels increased with increasing dietary protein except in those birds fed the 82% CP diet where it was equivalent

TABLE II. PRPP SYNTHETASE ACTIVITIES AND PRPP POOL SIZES IN HUA AND LUA TISSUES

Breed-Sex	Plasma UA ± SEM†	PRPP synthetase activity ± SEM‡		PRPP pool sizes ± SEM§	
		Liver	Kidney	Liver	Kidney
LUA-females	5.83^{a} ± 0.56	7.7^{a} ± 0.6	19.6^{a} ± 2.8	416.1^{a} ± 32.3	183.2^{a} ± 19.1
LUA-males	6.46^{a} ± 0.27	10.1^{b} ± 0.4	18.6^{a} ± 1.7	221.5^{b} ± 30.5	169.6^{a} ± 16.2
LUA mean	6.16* ± 0.38	9.0* ± 0.4	19.1* ± 1.6	313.6* ± 31.4	176.7* ± 12.4
HUA-females	13.65^{b} ± 1.86	9.7^{b} ± 0.5	29.1^{b} ± 1.2	744.8^{c} ± 130.5	252.6^{a} ± 37.0
HUA-males	25.48^{c} ± 2.46	13.0^{c} ± 0.7	31.3^{b} ± 0.6	395.3^{a} ± 35.1	387.0^{b} ± 59.0
HUA mean	19.25* ± 2.03	11.4* ± 0.6	30.2* ± 0.7	550.7* ± 72.5	319.8* ± 37.2

Note. All values represent the mean of 10 observations.
† mg uric acid/dl plasma.
‡ nmole PRPP synthesized/mg protein/hr.
§ pmole PRPP/g wet tissue.
^{a,b,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 ($P < 0.05$).

to that of birds fed the 66% CP diet (Table III). Liver PRPP synthetase activity decreased with increasing levels of dietary protein, whereas the kidney activity did not change. Liver PRPP pool sizes did not differ between birds fed the 29, 44, and 66% diets. Birds fed the 82% diet, however, had significantly greater PRPP pool sizes. Kidney pool sizes ranged from between 5 and 21% that found in livers. PRPP levels in kidneys of birds fed the higher protein diets (66 and 82%) were significantly lower than those fed the 29 and 44% CP diets.

Michaelis constants (apparent) for ATP and ribose-5-phosphate were not detectably different between the HUA and LUA PRPP synthetase preparations (Table IV, Fig. 1). ADP, GDP, and 2,3-DPG inhibition curves for the kidney enzyme from both breeds were virtually identical (Fig. 2). In addition, phosphate activation curves for the enzyme from both

TABLE III. THE DIETARY CONSUMPTION, PLASMA URIC ACID LEVELS, PRPP SYNTHETASE ACTIVITIES, AND PRPP POOL SIZES IN BROILER CHICKS

	Dietary protein level (%)			
	29	44	66	82
Feed consumption (g/chick/day)	16.07^{a} ± 1.13†	13.54^{b} ± 0.86	$11.60^{b,c}$ ± 0.35	9.25^{c} ± 0.32
Energy consumption (kcal ME/chick/day)	51.33^{a} ± 3.62	$43.89^{a,b}$ ± 2.80	$38.15^{b,c}$ ± 1.15	30.76^{c} ± 1.07
Protein consumption (g/chick/day)	4.59^{a} ± 0.32	5.98^{b} ± 0.38	7.66^{c} ± 0.23	7.56^{c} ± 0.26
Plasma uric acid (mgs/dl)	11.43^{a} ± 0.59	15.74^{b} ± 0.61	26.32^{c} ± 2.30	25.63^{c} ± 1.77
Liver PRPP synthetase activity‡	13.8^{a} ± 0.50	$12.5^{a,b}$ ± 0.7	11.2^{b} ± 0.5	9.0^{c} ± 0.4
Kidney PRPP synthetase activity‡	56.0^{a} ± 1.1	55.4^{a} ± 1.3	55.1^{a} ± 0.9	53.4^{a} ± 1.0
Liver PRPP pool size§	594.1^{a} ± 67.0	590.3^{a} ± 111.4	649.5^{a} ± 108.2	1090.3^{b} ± 130.9
Kidney PRPP pool size§	106.8^{a} ± 10.2	123.8^{a} ± 17.3	62.6^{b} ± 10.6	57.4^{b} ± 9.9

Note. Nutrient consumption values represent the mean of three groups of five chicks each. Uric acid levels, tissue PRPP pool sizes, and PRPP synthetase activities represent the mean of 10 observations.
† Values are means ± SEM.
‡ nmole PRPP synthesized/mg protein/hr.
§ pmole PRPP/g wet tissue.
^{a,b,c} Values within rows with the same letter in superscript are not significantly different ($P > 0.05$).

TABLE IV. CHARACTERISTICS OF PRPP SYNTHETASE FROM KIDNEY TISSUES OF HUA AND LUA BIRDS

	Apparent K_m ATP (μM)	Apparent K_m ribose-5-P (μM)	I_{50} ADP (μM)	I_{50} GDP (μM)	I_{50} 2,3-DPG (μM)
HUA	13.25	40.00	26	23	700
LUA	11.43	35.21	26	20	800

Note. K_m values were obtained from the Lineweaver–Burk plots shown in Fig. 3. Inhibition constants (I_{50}) were obtained from activity curves shown in Fig. 4.

lines were sigmoidal and superimposable when plotted as a percent of maximal activity (Fig. 3).

Discussion. Our results indicate that males and females of the HUA line had significantly greater PRPP synthetase activity in liver and kidney tissues compared to the LUA line (Table II). In addition, plasma uric acid levels correlated significantly ($P < 0.05$) with liver ($r = +0.52$) and kidney ($r = +0.59$) PRPP synthetase activities. PRPP pool sizes in HUA livers and kidneys were 70 to 80% larger than the corresponding controls. The large variation in sample values within groups probably reflects the labile nature of this compound. PRPP concentrations in erythrocytes and cultured cells from humans possessing elevated PRPP synthetase activites also have been generally higher than normal (8, 10, 13, 15, 17). Since PRPP is the rate limiting substrate for *de novo* purine biosynthesis in the chick liver (6), the elevated PRPP synthetase activities and PRPP pool sizes in the HUA bird may play an important role in the hyperuricemia and articular gout found in this breed. The relatively large PRPP pools in liver tissue compared to the kidney may also be due in part to the low levels of the PRPP utilizing purine salvage enzyme HGPRT in the avian liver (20). Lipstein and his co-workers reported that the level of adenine phosphoribosyltransferase (APRT) was 95% lower in the livers of chicks compared with that in rats (6). In addition, activity of HGPRT in the chick liver was 90% lower than the activity found in the rat. These workers suggest that the relatively low levels of purine salvage activity found in the avian liver tissue may be important in maintaining the higher rates of *de novo* purine synthesis. The PRPP sparing effect of lowered HGPRT and APRT levels may be especially important when considering the relatively higher K_m for this substrate of glutamine phosphoribosylpyrophosphate amidotransferase compared with the salvage enzymes (26, 27).

Broiler chicks forced to convert large amounts of dietary nitrogen to uric acid did not respond by increasing renal or hepatic PRPP synthetase activities (Table III). An increased flux of nitrogen through the *de novo* purine biosynthetic pathway is therefore un-

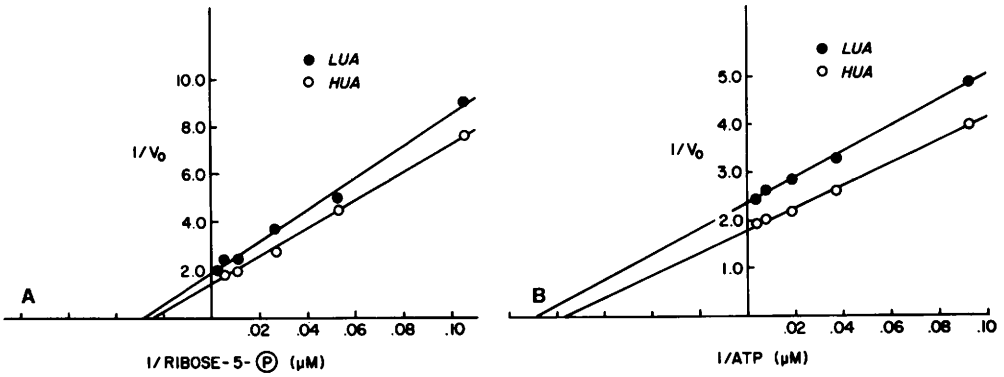

FIG. 1. Kinetics of PRPP synthetase in dialyzed kidney acetone powder preparations. (A) Lineweaver–Burk plot of initial reaction velocities at variable ribose-5-phosphate levels. (B) Lineweaver–Burk plot of initial reaction velocities at variable ATP levels.

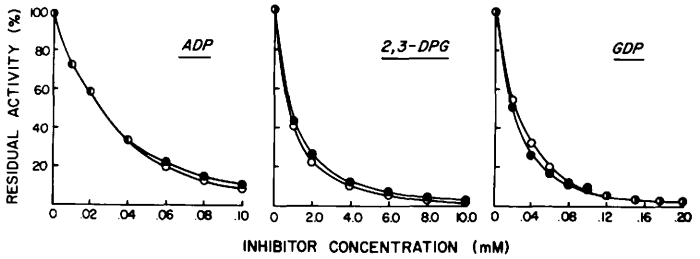

FIG. 2. Inhibition of LUA (●) and HUA (○) PRPP synthetase from dialyzed kidney acetone powder preparations of ADP, 2,3-DPG, and GDP at 1 mM inorganic phosphate.

likely to be responsible for elevated hepatic and renal PRPP synthetase activity found in the HUA birds. The feed consumption and ME intake of birds fed the high-protein diets were substantially less than optimum for broilers of this age (28). The decrease in broiler hepatic PRPP synthetase activity with elevated dietary protein levels may be due to increased degradation of the enzyme in response to an energy deficient diet. This type of behavior has been documented with other proteins (29). Increased dietary protein intake only affected liver PRPP pool sizes in chicks fed the 82% CP diets. The increased liver pool size seen in these birds may in part be due to significantly lowered levels of competing hepatic HGPRT (20). Renal PRPP pool sizes appear depleted in birds fed the higher protein diets (66 and 82%). The lowered pool sizes in these tissues may simply reflect a depletion of this substrate by increased rates of *de novo* uric acid production.

The apparent Michaelis constants for ATP and ribose-5-phosphate were not detectably different between the HUA and LUA breeds (Table IV). The values were remarkably similar to those found for the purified human PRPP synthetase (14 μM for ATP and 33 μM for ribose-5-phosphate) (30). The I_{50} of ADP, GDP, and 2,3-DPG were also virtually identical in both breeds. At 1 mM phosphate the avian enzyme was slightly more sensitive to feedback inhibition than the human enzyme by the competitive inhibitor ADP and noncompetitive inhibitor GDP (9). The avian PRPP synthetase was about 10-fold more sensitive than the human enzyme to inhibition by the competitive inhibitor 2,3-DPG. Phosphate activation curves for both HUA and LUA lines are superimposable (Fig. 3). The values were expressed as a percentage of the activity measured at 32.8 mM phosphate (the level used in routine assays) in order to compensate for differences in enzyme quantity.

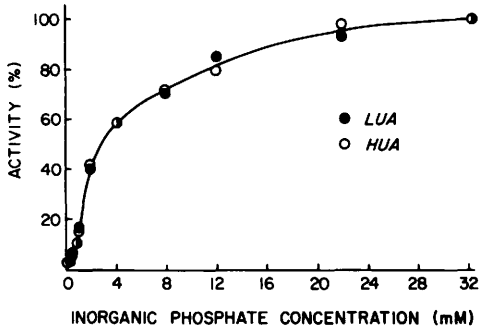

FIG. 3. Activation of LUA (●) and HUA (○) PRPP synthetase by increasing levels of inorganic phosphate.

The elevated hepatic and renal PRPP synthetase activities and PRPP pool sizes may be important factors in the hyperuricemia and articular gout common to the HUA line. The increased activity does not appear to result from abnormal substrate affinities or regulatory properties of the enzyme, but may be due to structural alterations of the molecule resulting in an enzyme with a greater V_{max}, an increase in the amount of enzyme protein, or a lowered level of a nondialyzable inhibitor.

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