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Effect of Adenosine 3′,5′-Monophosphate on Serine Metabolism in Chick Tissues (39354)

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Previous research suggest control mechanisms for glycine and serine metabolic enzymes differ for rats and chicks (1). An explanation for this species difference in cell regulation of serine enzymes is the large amount of glycine or serine needed for uric acid production in chicks. Seventy-five percent protein diets fed to chicks produced high levels of serum uric acid and also increased activities of serine biosynthetic enzymes, 3-phosphoglycerate dehydrogenase, and phosphoserine phosphatase, in chick liver, whereas activity for the catabolic enzyme, serine dehydratase, remained the same compared to control chicks (1). This same high protein diet fed to rats has been shown to increase serine dehydratase activity in rat liver and simultaneously decrease 3-phosphoglycerate dehydrogenase and phosphoserine phosphatase activities (2, 3).

The *de novo* synthesis of serine dehydratase also occurs after intraperitoneal injections of cyclic AMP in rats (4). The increased enzyme activity is completely blocked if Actinomycin D is administered 30 min prior to the injection of the cyclic nucleotide. High protein carbohydrate free diets have been shown to increase cyclic AMP levels in perfused rat liver (5), hence correlating the two experimental parameters.

Serine biosynthetic enzyme activities are increased in hepatic tissue of chicks fed crystalline amino acid diets supplemented with 2% glycine or serine compared to enzyme activities from chicks fed no glycine or serine (6). Oral intubation studies with rats suggest glycine or serine play an important role increasing the transport of cyclic AMP in liver tissue (7).

The present investigation was initiated to determine the *de novo* synthesis of serine metabolic enzymes in chicks fed crystalline amino acid diets with supplemental glycine and in chicks given high doses of cyclic 3′,5′-AMP by intraperitoneal injections.

Materials and methods. One-hundred-day-old broiler chicks were distributed at random into five groups of 20 chicks each. The chicks were placed in electrically heated wire batteries and fed *ad libitum* an amino acid diet deficient in glycine and L-serine (Table I) for 3 days. Two groups of chicks were then fed the basal diet with 2% supplemented glycine, and the remaining three groups were fed the basal diet deficient in glycine and L-serine. Chicks in group 1 were fed the diet with 2% supplemented glycine and were intraperitoneally injected daily with 1 ml of 0.9% NaCl. The chicks in group 2 were fed the 2% glycine supplemented diet and were injected daily with 0.5 ml of Actinomycin D (10 μg/ml). The chicks of groups 3, 4, and 5 were fed the basal diet and were intraperitoneally injected with 1 ml of 0.9% NaCl, 0.5 ml of cyclic 3′,5′-AMP (5 mg/ml), and 0.5 ml of Actinomycin D (10 μg/ml) + 0.5 ml of cyclic 3′,5′-AMP (5 mg/ml), respectively, for a 5-day period. The chicks in group 5 fed the basal diet were injected intraperitoneally with Actinomycin D, 30 min prior to the injection of cyclic 3′,5′-AMP. The birds were sacrificed on Day 8 and livers were removed for enzyme determination.

Enzyme preparation and assay. Chicks

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TABLE I. Composition of Amino Acid Diets.

Basal diet	
Ingredient	g/kg
L-Valine	10.00
L-Leucine	14.00
L-Isoleucine	8.00
L-Threonine	7.00
L-Glutamic acid	100.00
L-Tryptophan	2.25
DL-Methionine	7.00
L-Cystine	3.50
L-Phenylalanine	7.00
L-Tyrosine	7.00
L-Lysine · HCl	14.00
L-Arginine · HCl	14.00
L-Histidine · HCl	4.50
L-Proline	10.00
Corn oil	90.00
Antiacid[a]	5.00
Salts mixture[b]	60.00
Cellulose[c]	30.00
$NaHCO_3$	10.00
Choline chloride	2.00
Vitamin mix[d]	5.00
Vitamin E supplement (10 mg/g)	1.00
Glucose monohydrate[e]	588.75

[a] Antiacid: 3 parts $Mg_2Si_3O_8$ and 1 part $Al(OH)_3$.

[b] The salt mixture supplied the following reagent minerals (g/kg diet): $NaMoO_4 \cdot 2H_2O$, 0.01; $CuSO_4$, 0.02; KI, 0.05; $ZnCO_3$, 0.20; $FeC_6H_5O_7$, 0.40; $MnSO_4 \cdot 2H_2O$, 0.42; KCl, 3.0; $MgSO_4 \cdot 7H_2O$, 5.0; NaCl, 6.00; Na_2HPO_4, 7.20; KH_2PO_4, 12.0; $Ca_3(PO_4)$, 13.95; and $CaCO_3$, 15.00.

[c] Solka Floc, Brown Company, Berlin, New Hampshire.

[d] The vitamin mixture supplied the following vitamins (mg/kg diet): thiamine · HCl, 100.0; niacin, 100.0; riboflavin, 16.0; D-calcium pantothenate, 20.0; cyanocobalamin (mg/g), 20.0; pyridoxine · HCl, 6.0; biotin, 0.6; folic acid, 4.0; inositol, 100.0; menadione sodium bisulfite, 5.0; retinyl palmitate, 10,000 IU; and cholecalciferol, 1200 ICU.

[e] Cerelose, Corn Products Company, Argo, Illinois.

were sacrificed by cervical dislocation and livers were removed and weighed. Liver tissues were prepared and assayed for D-3-phosphoglycerate dehydrogenase (EC 1.1.1.s), phosphoserine phosphatase (E.C. 3.1.3.3) and L-serine dehydratase as described by Fallon *et al.* (8). Glycerate dehydrogenase (EC 1.1.1.29) was determined by the method of Willis and Sallach (9). The enzyme assay procedures were each adjusted for chick tissue to allow for linear initial velocity studies.

Protein determinations were made according to the method of Lowry *et al.* (10) using crystalline bovine serum albumin as a standard.

Results and discussion. The specific activity of D-3-phosphoglycerate dehydrogenase and phosphoserine phosphatase was increased in liver tissue from chicks injected daily with 3′,5′-AMP above that of those fed the same basal diet and injected with physiological saline (Table II). The specific activity of these two enzymes was increased in liver tissues from chicks fed the basal diet supplemented with 2% glycine, and injected with 0.9% NaCl above that of liver tissue from chicks fed the basal diet and injected with saline. Actinomycin D completely inhibited the increase in enzymatic activity of D-3-phosphoglycerate dehydrogenase in liver tissue from chicks injected with cyclic 3′,5′-AMP or fed the basal diet supplemented with 2% glycine. These data suggest a *de novo* synthesis of D-3-phosphoglycerate dehydrogenase and phosphoserine phosphatase in liver tissue of chicks injected with cyclic 3′,5′%-AMP or fed a basal amino acid diet supplemented with 2% glycine.

L-Serine dehydratase and glycerate dehydrogenase content of liver tissue were not significantly affected by the injection of cyclic 3′,5′-AMP or the addition of 2% glycine to the amino acid basal diet (Table II).

The regulation of serine biosynthetic enzymes in chick liver tissue by cyclic 3′5′-AMP is a reversal of conditions observed in mammals (3).

It was previously mentioned that the metabolic enzyme, L-serine dehydratase is induced by the intraperitoneal injections of cyclic 3′,5′-AMP in rats. It is possible that cyclic 3′,5′-AMP is the regulator of serine catabolism in rats and serine synthesis in chicks. The possible difference in enzyme regulation in liver tissue by cyclic 3′,5′-AMP may be due to the essential nature of glycine or L-serine for the chick. The *de novo* synthesis of serine biosynthetic enzymes in liver tissue from chicks fed a basal amino acid diet supplemented with 2% glycine may be due to the availability of cyclic 3′,5′-AMP to the hepatic tissue.

Summary. The effect of cyclic 3′,5′-AMP and supplemental dietary glycine upon *de novo* synthesis of serine metabolic enzymes in chick livers were examined. Chicks fed crystalline amino acid diets containing 2% glycine had approximately twofold the activ-

TABLE II. THE EFFECT OF CYCLIC 3′,5′-AMP AND ACTINOMYCIN D ON D-3-PHOSPHOGLYCERATE DEHYDROGENASE, PHOSPHOSERINE PHOSPHATASE, GLYCERATE DEHYDROGENASE, AND L-SERINE DEHYDRATASE ACTIVITY IN LIVER OF CHICKS FED VARIOUS PROPORTIONS OF GLYCINE FOR 5 DAYS.

		Specific activity[a]			
		D-3-PGD[b]	PP[c]	PGD[d]	S-D[e]
Diet	Daily treatment	mμ Moles NAD/mg protein/hr × 10^{-3}	mμ Moles Pi/mg protein/hour	mμ Moles NADH/mg protein/hour	mμ Moles NAD/mg protein/hour
1. Basal	1 ml 0.9% NaCl	13.8 ± 0.3c	67 ± 7b	304 ± 23a	70 ± 4a
2. Basal + 2% Glycine	1 ml 0.9% NaCl	25.4 ± 0.4a	130 ± 9a	326 ± 21a	69 ± 5a
3. Basal + 2% Glycine	5 μg Actinomycin D	14.3 ± 0.3c	75 ± 5b	312 ± 17a	69 ± 6a
4. Basal	2.5 mg of cyclic AMP	23.5 ± 0.5b	123 ± 10a	309 ± 18a	71 ± 6a
5. Basal	5 μg Actinomycin D + 2.5 mg cyclic AMP	13.3 ± 0.4c	63 ± 6b	291 ± 14a	68 ± 4a

[a] Means of three observations/group ± SEM. Each observation consisted of tissue samples from five chicks. Means with a common lettered subscript in a vertical row are not statistically different ($P < 0.05$).
[b] D-3-Phodphoglycerate dehydrogenase (EC 1.1.1.s).
[c] Phosphoserine phosphatase (EC 3.1.3.3).
[d] D-Glycerate dehydrogenase (EC 1.1.1.29).
[e] Serine dehydratase (EC 4.2.1.13).

ity in liver for 3-phosphoglycerate dehydrogenase and phosphoserine phosphatase compared to liver tissue from chicks fed diets lacking in dietary glycine. Chicks subjected to daily intraperitoneal injections of cyclic 3′,5′-AMP and fed diets containing no dietary glycine contained biosynthetic enzyme activity similar to glycine-fed chicks suggesting a correlation between glycine and cyclic AMP for serine enzyme induction. The elevated enzyme activity in liver of chicks fed dietary glycine or injected with cyclic AMP was inhibited when chicks were also injected with Actinomycin D indicating *de novo* synthesis of 3-phosphoglycerate dehydrogenase and phosphoserine phosphatase. Dietary glycine or cyclic AMP, however, did not change serine dehydratase and glycerate dehydrogenase activities in chick liver.

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