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Serine Utilization in the Chick as Influenced by Dietary Pyridoxine (40802)

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Almquist and Mecchi (1) and Almquist and Grau (2) demonstrated that glycine is required by chicks for maximum rate of growth in diets containing casein or an amino acid mixture. Akrabawi and Kratzer (3) and Baker *et al.* (4) as well as later studies by Rabbani *et al.* (5) and Featherston (6) showed that serine could replace dietary glycine. Sugahara *et al.* (7) found that 8% of serine depressed the growth of chicks when used as the source of nonessential amino acids in a chick diet. Shen *et al.* (8) found that the growth depression of chicks caused by feeding 2.86% of L-serine could be alleviated by the addition of extra pyridoxine and folic acid to the diet.

Naber *et al.* (9) and Kratzer and Lantz (10) demonstrated that glycine, but not serine, depressed the growth of chicks and poults when added to a diet marginal in folic acid. This was presumed to be due to the fact that folic acid is required for the interconversion of glycine and serine so that glycine toxicity was spared by the failure of serine to be converted to glycine in a deficiency of this vitamin. Deodhar and Sakami (11) demonstrated that pyridoxine is also required for the interconversion of glycine and serine.

The present study was undertaken to determine whether a deficiency of pyridoxine would act similar to folic acid in reducing the toxicity of glycine for young chicks. When it was found that excess serine rather than glycine was growth depressing in a marginal deficiency of pyridoxine, the nutritional relationship between these two amino acids was studied.

The experimental methods. Four experiments were carried out using a commercially available broiler strain of chicken. Various supplements of glycine, serine or pyridoxine were made to the basal diet (Table I) at the expense of glucose. The chicks were housed in electrically heated batteries and feed and water were provided *ad libitum*. The first experiment was conducted for 21 days and the second one for 15 days. In the third experiment, the chicks were fed for only 7 days before blood samples were taken for plasma amino acid analysis and liver slices were taken for [^{14}C]serine incubation studies. In Experiment 4, the chicks were fed between ages of 4 and 13 days and [^{14}C]serine was injected 2 hr before the birds were killed and tissue samples taken.

Blood was collected by heart puncture with heparin and centrifuged at 480*g*. Aspartate aminotransferase (EC 2.6.1.1.) activity in the plasma was determined by the method of Reitman and Frankel (16). Plasma which had been stored at −20°C was deproteinized with sulfosalicylic acid and the free amino acids determined by a Beckman Spinco Model 121 amino acid analyzer.

In the liver slice experiment (Experiment 3), the chicks were killed by cervical dislocation and the livers were removed immediately. Liver slices (0.4 mm) were prepared with a Stadie–Riggs microtome and 0.5 g were placed with 3 μCi L-[*U*-^{14}C]serine (specific activity 159 μCi per mmole) in 5 ml of Krebs–Ringers phosphate buffer (pH 7.4). The mixture was incubated for 2 hr at 37°C with shaking in a water bath while oxygen was swept through at 30 ml per minute. The reaction was stopped by adding 1 ml of 30% trichloroacetic acid and the mixture was homogenized in a Virtis "45" homogenizer.

In the *in vivo* study (Experiment 4) chicks were injected (three chicks per treatment) intraperitoneally with 27.3 μCi L-[*U*-

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TABLE I. COMPOSITION OF A PYRIDOXINE-DEFICIENT BASAL DIET

Ingredients	Percentage
Isolated soybean protein[1]	27.00
Glucose	60.02
Cellulose (Solka Floc)	2.50
Soybean oil	3.50
DL-Methionine	0.20
Glycine	0.18
Vitamin mixture[2]	0.59
Mineral mixture[3]	5.61
Choline chloride (50%)	0.40

[1] Ralston–Purina, RP-100, basal diet provides 1.3 mg of pyridoxine per kilogram by calculation.

[2] Vitamin mixture (per kg diet): stabilized vitamin A palimitate, 4485 IU; cholecalciferol, 4500 ICU.; *dl*-α-tocopheryl acetate, 50 IU; (in mg) menadione sodium bisulfite, 1.5; riboflavin 15; thiamine hydrochloride, 15; niacin, 50; calcium pantothenate, 20; folic acid, 6; biotin, 0.6; vitamin B_{12}, 0.02; ethoxyquin, 100.

[3] Mineral mixture contributed (g/kg diet): CaHPO4·H_2O, 17.94; $CaCO_3$ 15.35; KH_2PO_4, 8.67; NaCl, 5.2; MnSO4·H_2O, 0.30; FeSO4·$7H_2O$, 0.43; $MgSO_4 \cdot 7H_2O$, 2.6; $NaMoO_4 \cdot 5H_2O$, 0.0072; Na_2SeO_3, 0.00175; KIO_3, 0.009; $CuSO_4 \cdot 5H_2O$, 0.026; $ZnCO_3$, 0.13; $CoCl_2$, 0.0015, KCl, 0.867.

^{14}C]serine (specific activity 159 μCi per mmole) per kilogram body weight. The chicks were killed 2 hr later by cervical dislocation and the livers were removed rapidly and homogenized with 4 vol of chilled phosphate buffer (pH 7.5, 0.025 *M*). An aliquot of the homogenate was deproteinized with 10% trichloroacetic acid. The homogenates from both the *in vivo* and *in vitro* experiments were treated similarly. The precipitate was heated in a water bath at 60°C or 30 min with 70% ethanol and then washed twice more with the same solution. The liver protein obtained was then dissolved in 88% formic acid; 10 ml of scintillation solution (PCS, Amersham Corporation, Arlington Heights, Ill.) was added to the solute and radioactivity was determined in a liquid scintillation spectrometer (Packard Tri-Carb liquid scintillation counter). Liver protein content was determined by the Lowry method (12). Another part of the homogenate was deproteinized with 15% sulfosalicylic acid. The free amino acids in the supernatant were separated on silica gel plates using phenol–water and *n*-butanol–acetic acid–water (80:20:20). The gels containing glycine and serine were scraped off and counted for radioactivity. Free plasma serine was determined according to the method of Yemm and Cocking (13). A third part of the homogenate was used for phospholipid extraction. The phospholipids were extracted as described by Vohra *et al.* (14). The extracted phospholipids were dissolved in chloroform–methanol (3:1) and an aliquot was taken for the determination of radioactivity. The individual phosphatides were separated on silica gel plates using chloroform–methanol–water (70:30:4). The plates were left for an hour, dried, and then exposed to iodine vapor. The gels containing phospholipids were scraped off and the radioactivity was counted. After the extraction of phospholipids, the phospholipid content of the livers was determined as total acid soluble phosphorous according to the method of Fiske and Subbarow (15).

Results and discussion. In Experiment 1, there was no difference in body weight, mortality, or aspartate aminotransferase activity with various levels of pyridoxine without the addition of glycine and serine (Table II). However, when 4% glycine was added, aspartate aminotransferase activity was significantly reduced and when 5.6% DL-serine was added there was a significant reduction in body weight gain, high mortality, and reduced plasma aspartate aminotransferase activity with low levels of pyridoxine. These conditions were corrected by the addition of 5 mg of pyridoxine·HCl per kilogram. The fact that aspartate aminotransferase activity was reduced in chicks with reduced body weight gain is consistent with the observations of Sifri *et al.* (17) and Daghir *et al.* (18) that aspartate aminotransferase activity is more sensitive to pyridoxine levels than is body weight gain.

In the second experiment, DL-serine again depressed growth very severely and caused high mortality when fed along with 0.5 mg of pyridoxine·HCl per kilogram of diet but there was no growth reduction with 5 mg of pyridoxine·HCl per kilogram of diet (Table III). Two chicks in Group 4 which survived being fed the diet containing 5.6% DL-serine and 0.5 mg of pyridoxine·HCl per kilogram of diet for 10

TABLE II. BODY WEIGHT GAIN, FEED CONSUMED, MORTALITY RATE AND ASPARTATE AMINOTRANSFERASE ACTIVITY OF CHICKS FED THE DIETS FROM 3–21 DAYS OF AGE (EXPERIMENT 1)

Supplement						
Glycine (%)	DL-Serine (%)	Pyridoxine HCl (mg/kg diet)	Body weight gain (g)	Feed consumed (g/chick)	Mortality[1]	Aspartate aminotransferase activity (units/ml)[2]
0.0	0.0	0.5	240 ± 4.0^{ab3}	278 ± 51.0^{a}	0/14	140.6 ± 2.51^{a}
0.0	0.0	1.0	256 ± 14.0^{a}	338 ± 26.0^{ab}	0/14	123.5 ± 3.92^{ab}
0.0	0.0	5.0	269 ± 11.5^{a}	339 ± 10.5^{a}	0/14	144.3 ± 20.76^{a}
4.0	0.0	0.5	170 ± 40.0^{b}	250 ± 54.0^{ab}	0/14	97.6 ± 4.21^{b}
4.0	0.0	1.0	213 ± 2.0^{ab}	253 ± 13.5^{a}	0/14	150.8 ± 3.82^{a}
4.0	0.0	5.0	205 ± 18.5^{ab}	257 ± 22.0^{a}	0/14	144.5 ± 4.60^{a}
0.0	5.6	0.5	58 ± 12.0^{c}	124 ± 17.5^{b}	12/14	63.9 ± 1.46^{c}
0.0	5.6	1.0	98 ± 7.5^{c}	173 ± 27.5^{ab}	2/14	103.0 ± 5.23^{abc}
0.0	5.6	5.0	228 ± 6.5^{ab}	311 ± 7.5^{ab}	0/14	148.0 ± 7.63^{a}

[1] Number of dead chicks/total number of chicks per treatment.
[2] Sigma-Frankel Units and the values represent three samples from three different chicks per treatment.
[3] Mean ± SEM and those with different superscripts are significantly different ($P < 0.05$).

days showed remarkable recovery after being injected with pyridoxine and fed the high pyridoxine diet. Three comparable chicks maintained on the low pyridoxine diet died in the next 4-day period. The addition of DL-glycine at 2% to the 5.6% serine diet did not significantly improve growth or reduce the high mortality.

Plasma-free serine (Table IV) was increased markedly with the addition of serine to the diet. Glycine was also increased and showed a marked rise when the pyridoxine level was reduced. Cystathionine, although at a much lower level, showed an increase with a reduction in pyridoxine. Taurine showed a reduction with a deficiency of pyridoxine. These changes are consistent with the work described by Aboaysha and Kratzer (19). These results indicate that there is no decrease in plasma glycine in a deficiency of pyridoxine and actually, the level is increased so that the ratio of glycine and serine is about similar to the control birds

TABLE III. BODY WEIGHT GAIN AND MORTALITY OF CHICKENS FED VARIOUS LEVELS OF GLYCINE, DL-SERINE AND VITAMIN B_6 (EXPERIMENT 2)

	Supplements			Body weight gain (g)		Mortality	
Group	Glycine (%)	DL-Serine (%)	Pyridoxine HCl (mg/kg)	3–10 days	11–15 days	3–10 days	11–15 days
1	0.0	0.0	0.5	77.0^{a2}	60.0^{a}	0/14	0/10
2	0.0	1.0	0.5	72.0^{a}	14.0^{b}	0/14	5/10
3	0.0	2.0	0.5	62.0^{ab}	—	1/14	6/6
4a	0.0	5.6	0.5	28.5^{b}	—	9/14	3/3
4b[3]	0.0	5.6	0.5	—	107.0^{c}	0/14	0/2
5	2.0	5.6	0.5	39.5^{b}	−2.0^{b}	0/14	7/10
6	0.0	0.0	5.0	91.5^{ac}	102.0^{c}	0/14	0/10
7	0.0	1.0	5.0	104.0^{c}	97.0^{ac}	0/14	0/10
8	0.0	2.0	5.0	101.5^{ac}	112.0^{c}	0/14	0/10
9	0.0	5.6	5.0	83.0^{ac}	106.0^{c}	0/14	0/10
10	2.0	5.6	5.0	90.5^{ac}	119.0^{c}	0/14	0/10

[1] Number of dead chicks/total number of chicks/treatment.
[2] Means with different superscripts differ statistically at $P < 0.05$.
[3] The chicks of this group were fed the diet with Group 4a from 3 to 10 days of age and on Days 11 and 12 were injected intraperitoneally with 80 mg pyridoxine·HCl and switched to the diet of Group 9 on Day 11.

TABLE IV. PLASMA-FREE AMINO ACIDS IN CHICKS (EXPERIMENT 3)

Dietary Supplement			
DL-Serine, %	0.0	4.0	4.0
Pyridoxine·HCl mg/kg	5.0	0.5	5.0
Plasma Amino Acids, u moles/100 ml			
Serine	108	533	522
Glycine	77	423	148
Cystathionine	1.2	2.8	1.0
Taurine	16.7	6.5	15.0
Total essential amino acids	395	818	314
Total nonessential amino acids	492	900	829

without the addition of serine. These results would indicate that the growth-depressing effect of serine at a low level of pyridoxine is not related to a failure to convert serine to glycine. Total nonessential amino acids were elevated by the addition of serine and were not appreciably changed with a deficiency of pyridoxine. On the other hand, total essential amino acids were influenced little by the addition of serine but were greatly increased by the deficiency of pyridoxine.

In the *in vitro* experiment (Experiment 3) in which liver slices were incubated with [*U*-^{14}C]serine there was a significant reduction in the incorporation of ^{14}C into protein when the chicks were deficient in pyridoxine (Table V). This probably indicates a reduction in protein synthesis in the deficiency state. There was a significant increase in ^{14}C in the total phospholipids when 4% of serine was added to the diet. Reducing the pyridoxine supplementation greatly reduced the incorporation of ^{14}C serine into phospholipids. There was considerably greater radioactivity in the serine from the serine-supplemented pyridoxine-deficient chicks than from the other two groups while there was little difference in acitivity in the glycine fraction between any of the groups.

In Experiment 4 (Table VI) the chicks supplemented with 0.5 mg of pyridoxine·HCl per kilogram grew very slowly in comparison with the other groups. The deficiency of pyridoxine also resulted in a reduced level of protein in the liver. The total phospholipid content of the liver, however, was similar for the two groups receiving the 4% DL-serine regardless of the pyridoxine supplementation and was significantly greater than the group receiving adequate pyridoxine but no added serine. In the ^{14}C study *in vivo* there was no difference in the incorporation of ^{14}C into protein. There was, however, an increase in the incorporation of ^{14}C into total phospholipids with an increase in serine content of the diet and a significant reduction when pyridoxine was reduced. Phosphatidyl serine showed no difference in any of the groups but phosphatidyl choline showed an increase with the addition of serine to the diet and a decrease when pyridoxine·HCl was reduced from 5 to 0.5 mg per kilogram diet in the presence of 4% serine. This suggests that pyridoxine has some function in the conversion of phosphatidyl serine to phosphatidyl choline.

The results of this study show that the addition of higher levels of serine can have an adverse effect if pyridoxine is not ade-

TABLE V. *In vitro* INCORPORATION OF [*U*-^{14}C]SERINE[1] INTO LIVER PROTEIN, PHOSPHOLIPIDS, AND FREE GLYCINE AND SERINE (EXPERIMENT 3).

Supplement[2]		Body weight gain (g)	cpm/Flask/2 hr			
DL-Serine (%)	Pyridoxine·HCl (mg/kg diet)		Protein	Phospholipids	Serine[2] (free)	Glycine[2] (free)
0	5.0	25.5 ± 3.6	15,214 ± 1,061[a3]	1,154 ± 228[a]	10,100	11,700
4	0.5	9.5 ± 1.5	7,735 ± 308[b]	617 ± 180[a]	71,600	9,200
4	5.0	18.2 ± 1.5	14,244 ± 1,179[a]	7,122 ± 193[b]	11,200	8,100

[1] 1.1×10^5 dpm of L-[*U*-^{14}C]serine was added to each flask and values are expressed as cpm/flask/2 hr.
[2] Serine and glycine values represent pooled samples from three different chicks in each treatment.
[3] Mean ± SEM for three chicks and those with different superscripts are significantly different at ($P < 0.05$).

TABLE VI. BODY WEIGHT GAIN, WEIGHT, PHOSPHOLIPID AND PROTEIN CONTENT OF THE LIVER OF CHICKS, AND INCORPORATION OF [^{14}C]SERINE INTO PROTEIN AND LIVER PHOSPHOLIPIDS (EXPERIMENT 4).

Parameter	DL-Serine (%) / Pyridoxine·HCl (mg/kg)	0 / 5.0	4.0 / 0.5	4.0 / 5.0
Body weight gain (g)		263 ± 6.4[2]	86 ± 8.2[a]	262 ± 34.7[b]
Liver weight (g)		14.46 ± 2.5	7.50 ± 0.3	14.87 ± 0.56
Percentage body weight		4.16 ± 0.8	3.98 ± 0.4	4.05 ± 0.2
Protein (mg)/liver g		104.0 ± 5.4[a]	54.0 ± 2.2[b]	98.0 ± 2.6[a]
Phospholipids (μmole)/g liver		29.17 ± 4.19[a]	38.36 ± 1.37[b]	39.65 ± 1.47[b]
Phospholipids (μmole)/mg Protein		0.28 ± 0.02	0.71 ± 0.1	0.46 ± 0.06
L-[*U*-^{14}C]Serine incorporation into				
Protein (cpm/mg)		210 ± 100	332 ± 30	352 ± 158
Phospholipids (cpm/μmole)[3]		156 ± 39[a]	239 ± 49[a]	543 ± 157[b]
Phosphatidyl serine (cpm/μmole P-lipid)		83 ± 41	103 ± 29	89 ± 34
Phosphatidyl choline (cpm/μmole P-lipid)		114 ± 24[a]	151 ± 41[a]	310 ± 28[b]

[1] Ten chicks/group initially for growth data and three samples from three chicks, treatment for chemical analysis.

[2] Mean ± SEM and those with different superscripts are significantly different at ($P < 0.05$).

[3] Molecular weight assumed to be 787.

quate. Pyridoxine supplementation can completely reverse the adverse effects of added serine. This effect appears to be unrelated to the conversion of serine to glycine since growth was not improved by the addition of glycine and plasma glycine levels appeared to be normal. By the use of [*U*-^{14}C]serine it appears that there is a failure to convert phosphatidyl serine into phosphatidylcholine although the phosphatidylserine level in the liver appeared to be adequate.

Summary. In experiments with young chicks the addition of serine to a diet containing isolated soybean protein caused growth depression with marginal levels of pyridoxine. The growth depression can be overcome by the addition of extra dietary pyridoxine. Serine appeared to be readily converted to glycine and there is a suggestion that the metabolic defect is in the conversion of phosphatidylserine to phosphatidylcholine.

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