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Dietary Effects on the Need for Glycine by the Chick (33770)

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While the need for glycine by the growing chick was shown by Almquist *et al.* (1), Akrabawi and Kratzer (2) showed that the requirement depends on the level of arginine in 24% casein diets and the presence of L-serine in amino acid mixture diets. Since serine hydroxymethyltransferase (E.C. 2.1. 2.1, L-serine tetrahydrofolate 5,-10-hydroxymethyltransferase) catalyzes the interconversion of glycine–serine in the liver (3–5), we wished to determine whether dietary arginine changes the glycine requirement by influencing the activity of this enzyme. This reaction was also studied by observing the effect of 2-levels of L-serine in the amino acid mixture diet on the plasma level of glycine and other amino acids of 2-week-old chicks.

Because L-serine was shown (2) to support growth of chicks equal to that of glycine when an amino acid mixture diet was fed, but not with a casein diet, there is a question of whether adequate L-serine was used. This was studied by comparing the response of chicks fed added glycine to those fed a higher level of L-serine in a 24% casein diet.

Materials and Methods. Day-old Arbor Acre chicks were weighed individually and put into groups of comparable weight and weight distribution. They were housed in electrically heated batteries with raised wire floors and were supplied feed and water *ad libitum*. The experimental diets were based on corn starch and 24% casein or 26.2% amino acid mixture (2). Both diets contained soybean oil, glycerol, cellulose, vitamin mixture, and chromic bread at levels of 5, 5, 3, 1.1, and 1% respectively. They also contained enought calcium, phosphorous, and other minerals to satisfy the dietary requirements. In Expts. 1 and 2, two groups of chicks were raised on a 24% casein diet with 0.4 or 1.2% L-arginine·HCl. After 2 weeks, 3 chicks from each group were killed by neck dislocation. The liver from each chick was quickly removed, weighed, and frozen on dry ice. The livers, each wrapped individually were stored at —10° for not longer than 1 week before assay. The activity of serine hydroxymethyltransferase was determined on a crude homogenate according to the method of Scrimgeour and Huennekens (6). A 1-g liver sample was homogenized in 3 ml of 0.1 *M* phosphate buffer (pH 7.5). Two-tenths ml of the crude homogenate was added to the reaction mixture. A 0.3-ml aliquot of the supernatant was used in formaldehyde assay according to the method of Nash (7).

In Expts. 3 and 5, different levels of glycine and L-serine were used. Nigrogen retention was determined according to the methods of Hill *et al.* (8) and Hill and Anderson (9) on excreta collected during days 11 through 13 from the onset of feeding. Body weight gains and feed efficiency were analyzed statistically according to Snedecor (10). In Expt. 4, an amino acid mixture diet containing 2.2% L-arginine·HCl was fed with different levels of glycine or L-serine. A sample of 0.5 ml of blood from each of three 2-week-old chicks of average weight was ob-

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TABLE I. Effect of L-Arginine • HCl on the Specific Activity of Serine Hydroxymethyltransferase in the Livers of 2-Week-Old Chicks Fed a 24% Casein Diet (Expts. 1 and 2).

Suppl. L-arginine • HCl (%)	Expt. no.	Chick wt.[a] (g)	Liver wt.[a] (g)	Specific activity[b] (μmoles of formaldehyde/10 min/g of wet liver)
0.4	1	133	5.5	11.9 (10.6–13.9)
1.2		194	7.7	6.0 (4.0– 9.2)
0.4	2	137	7.0	10.3 (8.8–12.8)
1.2		202	6.9	7.3 (5.1– 9.8)

[a] Average of 3 chicks.

[b] Average of 3 chicks, 2 determinations/chick, and (range).

tained by heart puncture and centrifuged. The plasma was deproteinized with the addition of 3 vol of 5% sulfosalicylic acid. The sample was centrifuged and the supernatant was transferred to a beaker and dried at room temperature in a forced draft oven. The sample was then extracted with diethyl ether to remove excess sulfosalicylic acid and the amino acids were brought into solution with an equal mixture of 0.5 *M* citrate buffer (pH 2.2) and 20% sucrose solution. The preparation was then frozen until it was analyzed with a modified Beckman model 120B amino acid analyzer.

Results and Discussion. The results of Expts. 1 and 2 are shown in Table I. In both experiments, the differences in the average specific activity of serine hydroxymethyltransferase were not statistically significant ($p > 0.05$). If the need for glycine in the low arginine-fed chicks were due to limited synthesis of glycine, one would expect these chicks to have reduced enzyme activity in their livers. The lack of differences in enzymatic activity indicates that this is not the reason for the glycine requirement under these conditions.

If the level of arginine does not influence glycine synthesis, one would also expect that the response of chicks fed a casein diet with supplementary L-serine would be similar to that of chicks fed amino acid mixture diets. Experiment 3 was designed to test such a hypothesis. The results shown in Table II indicate clearly that a level of 1.9% L-serine in 24% casein diets was not sufficient to support growth, feed efficiency, or nitrogen retention equal to that of 1.2% glycine. This is surprising since a level of 1.9% L-serine was sufficient to promote growth equal to that with 1.2% glycine when chicks were fed an amino acid mixture diet (2). The amino acids provided by the 24% casein diet, however, were similar to those of the amino acid mixture except for cystine which was 0.60% in the amino acid mixture and 0.07% in the casein diet. Serine is needed for the *in vivo* synthesis of cystine from methionine (11,

TABLE II. Effect of Glycine or L-Serine on Average 2-Week Body Weight Gain, 7–14-Day Daily Gain, Feed Consumption, Feed Efficiency, and Percentage Nitrogen Retention of Chicks Fed 24% Casein Diets Containing 0.4% L-Arginine • HCl (Expt. 3).

Suppl. (%)						
L-Serine	Glycine	Wt. gain[c] (g)	7–14-day daily gain (g)	Feed consumed (g)	Feed/gain	Nitrogen retained (%)
—	—	100[a]	7.7 ± 3.5[d]	174	1.76[a]	63[a]
—	1.2	150[b]	13.1 ± 1.9	212	1.42[a]	67[a]
1.9	—	114[a]	9.3 ± 3.5	183	1.60[a]	64[a]
1.9	1.2	140[b]	11.7 ± 2.2	205	1.44[a]	68[a]

[c] Average of triplicate groups, 5 chicks/group. Figures with different superscripts (*a* or *b*) are significantly different ($p < .05$).

[d] ± Standard deviation.

TABLE III. Effect of Glycine or L-Serine on the Plasma Serine, Glycine, Cystine, Methionine, and Arginine Levels of Chicks Fed Amino Acid Mixture Diets Containing 2.2% L-Arginine • HCl (Expt. 4).

Suppl. (%)		Plasma (μmole/ml)^{a}				
L-Serine	Glycine	Ser	Gly	Cys	Meth	Arg
0	0	0.390	0.279	0.109	0.223	0.244
0	1.2	0.537	1.130	0.133	0.273	0.268
1.9	0	1.127	0.307	0.164	0.211	0.233
1.9	1.2	1.211	1.073	0.128	0.240	0.256

^{a} Average of three determinations, 1 determination/chick for each treatment.

TABLE IV. Effect of Glycine or L-Serine on Average 2-Week Body Weight Gain, 7–14-Day Daily Gain, Feed Consumption, Feed Efficiency, and Percentage Nitrogen Retention of Chicks Fed 24% Casein Diets Containing 0.4% L-Arginine • HCl (Expt. 5).

Suppl. (%)						
L-Serine	Glycine	Wt. gain^{c} (g)	7–14-day daily gain (g)	Feed consumed (g)	Feed/gain	Nitrogen retained (%)
0	0	145^{a}	12.2 ± 2.3^{d}	210	1.51^{a}	53^{a}
0	1.2	167^{b}	14.7 ± 2.9	211	1.34^{b}	57^{a}
3.36^{e}	0	170^{b}	14.9 ± 2.5	218	1.34^{b}	58^{a}

^{c} Average of triplicate groups, 5 chicks/group. Figures with different superscripts (*a* or *b*) are significantly different ($p < .05$).

^{d} ± Standard deviation.

^{e} L-Serine (3.36%) = 2 × 1.2% glycine on molecular basis.

12) in the rat and presumbaly also in the chick. When 1.9% L-serine was added to the amino acid mixture diet in Expt. 4, the ratio of plasma cystine to methionine increased from 0.49 to 0.78 (Table III). Adding 1.2% glycine to the same diet did not alter the plasma cystine to methionine ratio. Also, the addition of either L-serine or glycine to diet increased plasma glycine or serine levels respectively.

In Expt. 5 a level of 3.36% L-serine was compared to 1.2% glycine for its effect on the growth of chicks fed 24% casein diets. The results shown in Table IV indicate clearly that such a level of L-serine in the diet was sufficient to support growth, feed efficiency and nitrogen retention equal to that of 1.2% glycine.

The results of Expt. 5 and those of Akrabawi and Kratzer (2) show that chicks fed either amino acid mixture or casein diets are able, in the presence of sufficient amounts of L-serine in the diet, to synthesize glycine at a rate sufficient to satisfy their need and hence to support their maximum growth. The fact that chicks raised on a casein diet require a higher level of L-serine compared to those raised on amino acid mixture diets may be due to the extremely low level of cystine in casein protein and its possible effect in imposing a higher physiological need for serine. Dean and Scott (13), using amino acid mixture diets with 0.45% DL-methionine and no L-serine, observed a dietary requirement of 0.35% cystine for the maximum growth of 7-day-old chicks. The question is then raised whether a cystine requirement, even at a higher level of methionine, could have persisted because of the lack of serine in their diets.

Summary. There was no difference in the activity of serine hydroxymethyltransferase in the liver of chicks fed a 24% casein diet and 1.2% L-arginine compared with 0.4% L-arginine·HCl. The presence of L-serine or glycine in an amino acid mixture diet increased the plasma glycine or serine level respectively and the presence of L-serine in the same diet increased the ratio of plasma cystine to methionine. A level of 1.9% L-serine failed to improve the growth of chicks fed a 24% casein diet while the level of 3.36% L-serine supported growth of chicks equal to that of 1.2% glycine.

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Correlation Between Mitotic Activity and Transplantability of Pituitary Tumors Following Radiothyroidectomy* (33771)

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Profound cellular changes occur in the anterior pituitary of mice following the injection of a thyroid-destructive dose of ^{131}I (1, 2). Thyrotropic tumors invariably appear several months after the radiothyroidectomy (3) and these may be successfully transplanted to isologous hosts.

In spite of the early and rapid increase in the size of the thyrotrope population after radiothyroidectomy (4), transplantability is not attained before several months. It seemed worthwhile to investigate if a parallelism existed between cell proliferation and acquisition of transplantability.

Materials and Methods. Male and female LAF_1 mice were radiothyroidectomized by intraperitoneal injection of 200 μCi of ^{131}I to each animal. Four, 8 and 12 months later, a group of 8 radiothyroidectomized mice of each sex was treated as follows. One hr prior to sacrifice, each animal received an intraperitoneal injection of thymidine-^3H (1 μCi/g of body wt.). After death, the pituitary was divided approximately in two equal parts. One part was placed in Bouin-Hollande solution to which 5% chrome alum was added just prior to fixation. The other half was again cut in two parts. One part was implanted subcutaneously in the lower back of an isologous female recipient and the other part was similarly implanted in a male recipient. The recipient animals were about 3 months of age and had been radiothyroidectomized 1 month previously.

The pituitary halves fixed in the modified Bouin-Hollande solution were processed for radioautography (5). The frequency of radioactive cells was established on a large size population on account of their scarcity in some group of animals. The following counting procedure was adopted. For each animal, a mean number of all types of anterior pituitary parenchymal cells was established in 50 random fields delineated by a ruled square on an ocular disc. With this value on hand, several hundred fields could be scanned for labeled cells without the tedious task of counting the total number of cells in each field. This procedure allowed us to survey complete sections, representing a population of several thousand anterior pituitary cells per animal. The frequency of labeled cells was then uniformly expressed per 10,000 pituitary cells.

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