of LH-NIH-B1. The response was unchanged by pentobarbital or atropine administration and occurred in androgenized females.

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## Relation of Chick Kidney Arginase to Growth Rate and Dietary Arginine.* (29768)

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Chicks cannot synthesize arginine and when fed a semi-purified diet based on casein as source of protein have a high requirement for this amino acid(1,2,3). Furthermore, chicks fed a sub-optimal amount of arginine show an unusually wide variation in growth rate(4). A study of arginine metabolism showed a considerably greater excretion of urea by chicks fed free arginine, and the authors suggested that the high requirement was due in part to the cleavage of arginine by kidney arginase(5). If this postulate is correct, the high variability in growth rate could result from differences in kidney arginase activity and one would expect an inverse relationship since high arginase activity would be detrimental when arginine is limiting.

Arginase is found in moderate amounts in chick kidney but only in trace amounts in the liver of this species(6,7). Rat liver is a rich source of arginase and the activity can be increased or the enzyme induced in this tissue by high protein diets(8). There is no evidence that this enzyme, or any other, can be induced in non-hepatic tissues of postnatal animals(9). However, arginase has been induced in a variety of animal cells cultured in the presence of ribonucleic acid (10). If kidney arginase is induced by dietary arginine, this phenomenon would accentuate the requirement for dietary arginine when the diet is supplemented with the free amino acid. This paper is concerned with the relationship between growth rate and kidney arginase levels in chicks fed a sub-optimal level of arginine and with the induction of arginase in chick kidney by dietary arginine.

*Materials and methods.* Male chicks of a crossbred broiler strain were reared from hatching to 4 weeks of age in battery brooders. Feed and water were supplied *ad libitum* and weights were recorded weekly. Two types of diets were fed. One was a semi-purified diet (S) of the following percentage composition: Casein 35, DL-methionine 0.5, L-arginine HCl 0.72, glycine 1.5, glucose hydrate 43.3, salts(11) 6.0, K acetate 2.7, soybean oil 10.0, choline Cl 0.2 and the vitamin supplement previously described(3). This diet contained about 30% of protein and 1.8% of arginine. Supplements were added

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in lieu of glucose. The other diet was a practical type diet (P) which contained: Soybean oil meal 36, corn 59.5, $CaHPO_4$ 2.0, Ca $CO_3$ 1.3%, NaCl 0.5, DL-methionine 0.1, and the usual vitamin and trace mineral supplements. This diet contained about 22% protein and 1.4% arginine. Supplements were added at the expense of total diet.

At the end of the 4-week period kidneys were removed, weighed and kept frozen until assayed. The enzyme assay was based on the method of Van Slyke and Archibald(12). A 10% homogenate of tissue was prepared in cold $MnCl_2$ solution ($5 \times 10^{-4}$M). Appropriate dilutions were made and preincubated at 37°C for 15 minutes before the substrate was added. Preliminary results showed $5 \times 10^{-4}$M to be the optimal manganese concentration for activation of arginase in this tissue. At least 3 different tissue levels (0.25 to 2.0 mg per tube) were used for each kidney sample and one ml of homogenate was incubated with 0.5 ml of 0.85 M arginine at pH 9.5 for 30 minutes. The protein was precipitated with metaphosphoric acid and urea determined with alpha-isonitrosopropiophenone. To obtain a suitable standard curve, the sulfuric-phosphoric acid mixture should contain a small quantity of ferric ion. In this study the solution was made approximately $10^{-2}$M with respect to $Fe^{+++}$. Arginine activity was expressed as micromoles of urea released per hour per gram of wet tissue. Values for 3 or more determinations usually varied not more than ± 10% of the mean.

*Results and discussion.* Arginase was determined in kidney tissue from 36 chicks fed the semi-purified diet (S) which contained a level of arginine that supported a submaximal growth rate. The ratio of individual weight to group average weight was plotted against the kidney arginase activity as shown in Fig. 1. There was an inverse relationship between arginase activity and the individual body weight. The equation of the line fitted by the method of least squares is Y = 27,340-17,380 X and the coefficient of correlation is −0.61 ($P<.001$).

The growth rate and kidney arginase activities of chicks fed the 2 basal diets with and

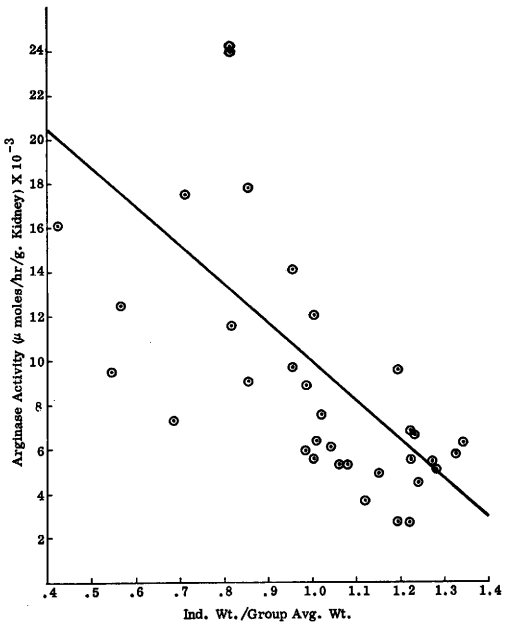


FIG. 1. Inverse correlation of kidney arginase concentration with relative growth rate. The curve was calculated from 36 values, but for convenience 2 extreme values were omitted from the plot.

without amino acid supplementation are shown in Table I. As noted by other investigators a high variability in growth rate occurred among chicks fed the semi-purified diet (S). The kidney arginase activity was also higher on the semi-purified than on the practical diet ($P<0.001$). Diet S contained more protein than diet P but since the ornithine cycle is not operative in the chick, one would not expect the protein level to affect the arginase level as it does in the rat. It is more likely that the difference is due to the higher arginine content of the purified diet and that free arginine has a greater effect than arginine in protein. When the purified diet was supplemented with a total of 2.4% of arginine HCl, kidney arginase was increased significantly above that of the basal group. This level of arginine supported a maximum rate of growth and the coefficient of variation was low. Furthermore, there was no difference between the kidney arginase activity of those in the upper and those in the lower 25% body weight groups. Presumably, the maximum induction of arginase had occurred in all chicks but the excess of dietary arginine was sufficient to overcome any depressing

TABLE I. Elevation of Chick Kidney Arginase Concentration by Dietary Arginine.

| Ration description | Chicks analyzed: No. | Chicks analyzed: Wt 4 wk | Chicks analyzed: Coef of variation | Kidney: Wt | Kidney: Arginase* |
|---|---|---|---|---|---|
| | | (g) | (%) | (% B.W.) | (μmoles/g/hr) |
| S (basal) | 18 | 546 ± 30† | 23.1 | .48 | 7,560 ± 940[a] |
| S + 1.7% (2.4% total) Arg HCl | 14 | 613 ± 18 | 11.0 | .51 | 12,000 ± 830[a] |
| P (practical) | 10 | 529 ± 18 | 11.0 | .42 | 3,540 ± 410[b] |
| P ± 1.45% Arg HCl | 10 | 542 ± 27 | 15.7 | .44 | 13,620 ± 1530[b] |
| P + 2.0% Gly HCl | 8 | 539 ± 15 | 7.7 | .46 | 2,690 ± 260 |

* Arginase values labeled with the same letter differ statistically ($P < 0.005$) as determined by the "t" test.
† Standard error of mean.

effect on growth. In chicks fed the practical type diet, supplementary arginine also increased the kidney arginase and to a level slightly higher than that observed among chicks fed the purified diet with a high level of arginine. Supplementary glycine had no effect on kidney arginase and at least to this extent arginase adaptation is specific for dietary arginine.

Nesheim and Hutt(13) have observed strain differences in the arginine requirement of chicks and Fisher and Griminger(14) found genetic differences in growth potential of chicks fed an arginine deficient diet. The latter workers reported that preliminary investigation did not reveal any correlation between arginase activity and growth potential. Smith and Lewis(7) fed 2 practical type diets which contained 0.8 and 1.2% of arginine and were not able to demonstrate a difference in kidney arginase in relation to arginine content of diet. In the present study the higher level of arginine was supplied as the free amino acid rather than as a component of protein. It is possible that only the free amino acid will cause elevation of kidney arginase but on the other hand the contradictory results may stem from differences in the magnitude of arginine supplementation. It is not clear from our results whether the differences in kidney arginase that correlate inversely with growth rate are due to genetically determined levels at hatching or to inherent ability for arginase adaptation. The fact that there is no apparent correlation between body weight and kidney arginase among chicks fed the practical diet or among those fed the purified diet with excess arginine supports the concept that the difference among individuals is related to their capacity for arginase induction. In any case the induction of arginase in an animal which has a dietary requirement for the substrate may partially explain the chick's high requirement for arginine and the fact that there does not seem to be a sharply defined level that supports maximum growth rate.

*Summary.* Chicks reared to 4 weeks of age on a purified diet supplemented with a suboptimal level of arginine showed a highly variable growth rate within the group which was inversely correlated with the concentration of arginase in their kidneys. Supplementation of this diet, as well as a practical type diet, with excess arginine caused a highly significant elevation of kidney arginase levels. This induction of kidney arginase activity was apparently specific inasmuch as glycine supplementation was without effect.

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## Effect of Egg Yolk and Phosphatides on Anthrax Infection of Rats and Guinea Pigs. (29769)

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The treatment of spores of *Bacillus anthracis* with whole chicken egg yolk has been shown to reduce the number of spores required to induce lethal infection in mice(1) rats(2,3) and guinea pigs(3). Neither the component of egg yolk nor the mechanism responsible for this effect on anthrax infection have been described. Among the constituents of egg yolk are lecithin and phosphatidyl ethanolamine, phosphatides which are quite active in diverse biological systems. Their effect on anthrax infection of rats and guinea pigs has been compared with that of whole chicken egg yolk and the results indicate that the activity of phosphatidyl ethanolamine present in yolk could account for the effect of yolk on anthrax infection.

*Materials and methods.* *B. anthracis* employed in these studies was the highly virulent Vollum V1b strain. All inocula were 1 ml. Aerosolization of spores was accomplished with a modified Henderson apparatus (4). The spore suspension, containing $4 \times 10^{10}$ spores/ml, was prepared in 1957 and stored as a phenolated suspension at 4°C (Fort Detrick Lot No. 189). Although the guinea pig subcutaneous lethal dose 50% ($LD_{50}$) was $< 10$ spores, the intraperitoneal $LD_{50}$ ($IPLD_{50}$) was 190 spores. Spores were "heat-shocked" (60°C for 30 minutes) 48 hours prior to use. Vegetative cells were harvested as chains from nutrient agar plates incubated at 37°C for 18 to 20 hours following seeding with spores. Spore and chain concentrations were determined by the conventional colony count method.

Egg yolk was freshly prepared for each use and unless otherwise noted was employed in a final concentration of 80%. Phosphatides, lecithin, phosphatidyl ethanolamine (PE), phosphatidyl serine (PS) and phosphatidyl inositol (PI), were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Aqueous suspensions were utilized. Guinea pigs of the Hartley strain weighing 350 to 450 g were obtained from the Animal Farm, Fort Detrick, Md. Rats, 28-day-old Sprague-Dawley strain weanling female, were obtained from Charles River Breeding Laboratories, Brookline, Mass.

*Results.* As shown in Table I i.p. inoculation of guinea pigs with *B. anthracis* spores suspended in 80% fresh chicken yolk resulted in 19-fold greater infectivity than spores suspended in water. $LD_{50}$'s were 10 and 190 respectively, a difference significant at the

TABLE I. Effect of Chicken Egg Yolk and PE as Suspending Fluid upon the $IPLD_{50}$ of Anthrax Spores for Guinea Pigs and Rats.

| Spore suspending fluid | Guinea pig $IPLD_{50}$ spores | Rat $IPLD_{50}$ spores |
|---|---|---|
| Water | 190 | $1.4 \times 10^3$ |
| 80% Yolk | 10 | $1.4 \times 10^3$ |
| PE (15 mg/ml) | 26 | $1.3 \times 10^3$ |

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