Effect of Lysine and Glycine Upon Arginine Requirement of Guinea Pigs.* (28036)

B. L. O'DELL AND W. O. REGAN
Department of Agricultural Chemistry, University of Missouri, Columbia

Arginine is recognized as an essential amino acid for young growing rats, the requirement being about 0.2% of the diet(1). This is low compared to the chick which has a nearly 10-fold higher requirement(2). Adult rat and man require none in the diet. In contrast to the rat, the arginine requirement of the growing guinea pig is not met by 25% of casein (3).

The concept of physiological antagonism between natural occurring and structurally related amino acids is generally accepted and has been demonstrated in higher animals for a few amino acids such as leucine and isoleucine(4). A lysine-arginine antagonism has been observed in a lysine-less mutant of neurospora(5) and has recently been suggested to occur in the growing chick(6,7,8). Under certain conditions chicks can tolerate high levels of glycine(9) but rats cannot(10). This communication deals with the toxicity of dietary lysine and glycine for the guinea pig and its counteraction by arginine.

Materials and methods. Weanling guinea pigs of selected sex, reared in our colony and weighing from 160 to 200 g, were caged in groups of 4 on raised wire floors. Feed and water were supplied *ad libitum* and weights were recorded weekly for a 4-week period.

The basal diet contained: Washed casein 30; sucrose 43.3; cellulose (Solka Floc) 15; salts(11) 4; potassium acetate 2.7; magnesium oxide 0.5; soybean oil 4; DL-methionine 0.2; ascorbic acid 0.2; choline Cl 0.1 and the vitamin and chlortetracycline supplements previously described(11,12).† Analysis of the casein indicated a dietary arginine level of about 1.2%.

Supplementary amino acids were added at the expense of sucrose. Sufficient water was added to the diet at time of feeding to make a thick paste, and the feed was supplied fresh twice daily. Wetting the feed improved texture and acceptability so that the rate of gain was increased about 1 g per day. When extra ascorbic acid was given, 25 mg per animal was supplied in a small quantity of drinking water at the morning feeding.

Results. Female guinea pigs were used in the first trial to determine whether or not the arginine requirement was met by 30% casein. Addition of 0.5% arginine HCl (B) to the basal diet increased the growth rate about 20% (Table I). Extra ascorbic acid supplied in the drinking water (C) also increased growth rate and under these conditions arginine did not give a statistically significant additional stimulation. Although the basal diet contained 200 mg % of ascorbic acid, moistening the feed resulted in destruction of sufficient ascorbic acid as to render the diet inadequate in this respect. When adequate ascorbic acid was supplied in the present study 30% casein approached adequacy for females. An explanation for the difference in response to arginine in the presence and absence of extra ascorbic acid is not obvious. Reid(13) found that 30% casein supported maximum growth rate in

TABLE I. Growth Stimulation of Female Guinea Pigs by Arginine.

Diet No.	Supplements: Arginine HCl, %	Supplements: Ascorbic acid in H_2O, mg/day	No. animals	Avg daily gain, 4 wk, g
A	0	0	24	5.5 ±.13*
B	.5	0	18	6.6 ±.23
C	0	25	8	6.2 ±.25
D	.5	25	8	6.5 ±.24

* Standard error of mean. Statistically B>A, $p<0.01$; C>A, $p<0.02$.

* Contribution from Missouri Agri. Exp. Station. Appoved by Director as Journal Series No. 2509. Supported in part by National Science Foundation grant.

† The authors gratefully acknowledge gifts of vitamins and amino acids from Merck and Co., Rahway, N. J.; Hoffmann-LaRoche, Nutley, N. J., and General Mills, Minneapolis, Minn.

TABLE II. Lysine-Arginine and Glycine-Arginine Antagonism in Male Guinea Pigs.

Diet No.	Supplements* Arg HCl	Lys HCl	Gly	No. of animals	Avg daily gain, 4 wk, g
	———	%	———		
A	0	0	0	15	7.2 ±.15†
B	.5	0	0	15	7.8 ±.27
C	0	2.0	0	12	6.5 ±.17
D	.5	2.0	0	14	7.7 ±.23
E	0	0	2	11	5.6 ±.39
F	.5	0	2	12	7.4 ±.33

* Extra ascorbic acid (25 mg/day) offered in water.

† Standard error of mean. Statistically, B>A, $p<0.06$; C<A, $p<0.01$; D>C, $p<0.01$; E<A, $p<0.01$; F>E, $p<0.01$.

guinea pigs of mixed sex whereas a 20% casein diet required an arginine supplement.

Males were used in the second trial and extra ascorbic acid was supplied throughout. The results obtained when excess lysine and excess glycine were added with and without supplementary arginine are shown in Table II. As observed in other species, excess lysine depressed growth rate significantly, but the depression was completely reversed by supplementary arginine. Addition of 2% glycine also depressed growth and the effect was overcome by arginine.

So far as the authors are aware this is the first report of lysine-arginine antagonism in a growing mammal, but a similar antagonistic effect has been demonstrated for the enzyme arginase(14). Casein contains a relatively high concentration of lysine and a low concentration of arginine. Its high lysine content and the resulting unfavorable ratio of lysine to arginine probably accounts for the suggestion that the arginine of casein is not as available as that in plant proteins(3). The fact that the guinea pig has a higher arginine requirement than the other commonly used mammals makes it amenable to a study of an antagonism involving this amino acid. The chick can well tolerate as much as 4% supplementary glycine(9) and excess glycine appears to decrease its arginine requirement(2). The fact that arginine ameliorates the toxicity of excess glycine as well as excess glycine in the guinea pig suggests that these are not specific antagonisms and that a more general mechanism may be involved.

Summary. The growing guinea pig has an unusually high arginine requirement as compared to other mammals. A diet based on 30% casein (1.2% arginine) was slightly deficient. Addition of 2% lysine HCl or of 2% glycine depressed growth rate but the depression was alleviated by 0.5% arginine HCl. The results suggest lysine-arginine and glycine-arginine antagonism but the function of arginine may be more general than specific antagonism.

1. Borman, A., Wood, T. R., Black, H. C., Anderson, L. G., Oesterling, M. J., Womack, M., Rose, W. C., *J. Biol. Chem.*, 1946, v166, 585.

2. Savage, J. E., O'Dell, B. L., *J. Nutrition*, 1960, v70, 129.

3. Heinicke, H. R., Harper, A. E., Elvehjem, C. A., *ibid.*, 1955, v57, 483.

4. Harper, A. E., Benton, D. A., Elvehjem, C. A., *Arch. Biochem. Biophys.*, 1955, v57, 1.

5. Doermann, A. H., *Arch. Biochem.*, 1944, v5, 373.

6. Snetsinger, D. C., Scott, H. M., *Poultry Sci.*, 1961, v40, 1675.

7. Jones, J. D., *Fed. Proc.*, 1962, v21, 1.

8. O'Dell, B. L., Limbaugh, C. L., Savage, J. E., *ibid.*, 1962, v21, 8.

9. Fisher, H., Scott, H. M., Johnson, B. C., *J. Nutrition*, 1955, v55, 415.

10. Hier, S. W., Graham, C. E., Klein, D., PROC. SOC. EXP. BIOL. AND MED., 1944, v56, 187.

11. O'Dell, B. L., Vandepopuliere, J. M., Morris, E. R., Hogan, A. G., *ibid.*, 1956, v91, 220.

12. O'Dell, B. L., Regan, W. O., Hogan, A. G., *ibid.*, 1957, v96, 553.

13. Reid, M. E., *Fed. Proc.*, 1956, v15, 570.

14. Hunter, A., Downs, C. E., *J. Biol. Chem.*, 1945, v157, 427.

Received October 16, 1962. P.S.E.B.M., 1963, v112.