Effect of Different Levels of Hexahomoserine on Growth and Hematopoiesis in Rats. (17580)

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In a previous report(1) it was shown that young pigs stop growing and develop anemia when small amounts of hexahomoserine (α-amino-ϵ-hydroxy caproic acid) are added to an otherwise normal mixed-grain ration. The complete inhibition of growth of the pigs after ingestion of the compound for only one week was unexpected in view of the low level fed in the diet (3 g DL-hexahomoserine daily). To obtain more information on the properties of this compound, groups of white rats have been maintained for 28 days on diets containing different levels of hexahomoserine. In these experiments a separation of the growth-retarding and anemia-producing effects was observed.

Experimental. DL-hexahomoserine was synthesized from dihydropyran‡ by the method of Gaudry.(2) DL-hexahomoserine and DL-lysine monohydrochloride replaced equivalent weights of dextrin in the lysine-deficient basal zein ration shown in Table I. Young white rats from the stock colony were divided into groups of 6. Each litter was distributed as evenly as possible among the experimental groups, one rat to each group, and each group contained 3 males and 3 females. The rats were housed in individual cages with wire screen floors, and were fed *ad libitum*. The animals were weighed every fourth day. On the 28th day red blood cell counts(3) were made on blood from the tip of the tail, and the animals were then autopsied.

Results. The effect of the different levels of hexahomoserine on weight gain and red blood cell count is summarized in Table II. The average growth curves for animals in Groups I-VII inclusive, made by plotting the average total weight gains of each group at 4 day intervals, all have a positive slope characteristic for the level of lysine, or lysine and hexahomoserine, fed. In Group VIII, the curve reached a maximum (3 g) at the end of 8 days and then flattened out. The growth curves are not reproduced here.

The data in Table II show that the lowest level of DL-hexahomoserine fed (0.083%-Group IV) produced an average total weight gain (53 g) which is 80% of that of the control animals (Group I) receiving the same level of lysine without hexahomoserine. Increasing increments of hexahomoserine produced further reductions in average total gain. Statistical analysis§ of the average total gains in Groups IV-VII inclusive was made by plotting a regression curve using the percents of DL-hexahomoserine as the abscissae and the average total gains as ordinates. The regression of gain on amount of hexahomoserine was negative and highly significant, and appeared to be linear from 0 to 0.33% DL-hexahomoserine. Extrapolation of the curve to zero gain intersected the X axis at 0.6% DL-hexahomoserine. This level is about half way between the levels of DL-hexahomoserine fed to the animals in Groups VII and VIII.

To determine the effect of low levels of lysine alone on the growth rate and red blood cell count, the rats in Groups II and III were fed the basal zein diet supplemented with 1.5% and 1.0% of DL-lysine · HCl, respectively. At the 1.5% level (see Table II) the

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† Journal paper No. 435, Purdue University Agric. Exp. Sta.

1. Mertz, E. T., Beeson, W. M., Waltz, R. H., Jr., and Gaudry, R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 609.

‡ Supplied through the courtesy of E. I. du Pont de Nemours and Co.

2. Gaudry, R., *Can. J. Research*, 1948, B, v26, 387.

3. Todd, J. C., and Sanford, A. H., *Clinical Diagnosis by Laboratory Methods*, p. 223, W. B. Saunders Co., Philadelphia, 1940.

§ We are indebted to Professor S. R. Miles for the statistical analyses.

TABLE I.
Composition of Basal Ration.

Component	%	Vitamin mixture: Component	Vitamin mixture: Mg per 100 g basal ration
Zein	26.1	Thiamin hydrochloride	.3
Dextrose	27.6	Riboflavin	.3
Dextrin	26.49	Calcium pantothenate	.5
Lard	11.0	Nicotinic acid	3.0
Mineral mixture(5)	5.2	Pyridoxine hydrochloride	.5
Celluflour	2.0	Choline chloride	100
Liver extr. (Lilly)	.5	Inositol	20
DL-tryptophan	.4	Para-amino benzoic acid	5
Glycine	.13	Biotin	.01
L-histidine · HCl	.20	Folic acid	.01
Vit. mixture	.38	Vit. K (Menadione)	.50
		Nopco XX*	250
	100		380.12

* Contains 3,000 I.U. of Vit. A and 400 I.U. of Vit. D_2 per g.

TABLE II.
Effect of DL-Hexahomoserine on Growth and Red Blood Cell Count.

Group	Diet,* %	Avg total gain (28 days) g	Avg RBC count (28th day) cells × 10^6/cmm
I	2 DL-lysine · HCl	66	6.1
II	1.5 " "	52	6.1
III	1.0 " "	21	5.4
IV	2.0 " " + 0.08 DL-hexahomoserine	53	5.9
V	2.0 DL-lysine · HCl + 0.11 DL-hexahomoserine	51	6.0
VI	2.0 DL-lysine · HCl + 0.17 DL-hexahomoserine	49	5.7
VII	2.0 DL-lysine · HCl + 0.33 DL-hexahomoserine	32	5.7
VIII	2.0 DL-lysine · HCl + 1.0 DL-hexahomoserine	3†	3.9†

* The lysine and hexahomoserine each replaced an equivalent weight of dextrin in the basal zein ration (see Experimental Section).
† One rat died on the 24th day and is not included in the average.

rats made an average total gain that is approximately the same as that made by rats receiving 2% DL-lysine · HCl and 0.08-0.17% DL-hexahomoserine. At the 1.0% level (Group III) the rats made an average total gain that is intermediate between that made by rats on the diets containing 2% DL-lysine · HCl and either 0.33% or 1.0% of DL-hexahomoserine. The average red blood cell count of the animals receiving inadequate amounts of lysine (Group III) was lower than that of the animals receiving 0.08-0.33% DL-hexahomoserine in the diet.

At autopsy, gross examination of the internal organs of all of the animals was made, and sections of the bone, liver, spleen, testes and kidneys were taken from two or more animals in each group for microscopic examination. No gross abnormalities aside from emaciation were observed among the rats in Groups I-VII inclusive. Five of the rats in Group VIII (including the rat which died on the 24th day) had enlarged spleens. The one animal in Group VIII with a normal spleen had a red blood cell count of 4,900,000 per

5. Heinemann, W. M., Ensminger, M. E., Cunha, T. J., and McCulloch, E. C., *J. Nutrition*, 1946, v31, 107.

cmm (28th day), was very emaciated (showed no weight gain in 28 days), and apparently was only slightly anemic.

The microscopic sections from the rats selected from Groups I-VI inclusive were negative. The spleen and bone marrow sections from the animals selected from Group VII showed evidence of a small increase in hematopoietic activity. The sections of the spleen and bone marrow from 4 animals with enlarged spleens in Group VIII showed marked hematopoiesis. These animals in Group VIII were either manufacturing new red blood cells at an increased rate, or the new cells were piling up because of the lack of maturation factor(s). The red cell counts made on the blood of the 4 animals with enlarged spleens on the 28th day varied from 3.4-3.9 million per cmm, indicating a definite state of anemia.

The data presented above show that low levels of hexahomoserine can inhibit growth without producing a significant drop in the level of the red blood cells, or a marked change in the blood-forming organs. If the feeding of DL-hexahomoserine at the lower levels had been continued for a longer time than 28 days, it is possible that definite symptoms of anemia may have appeared. Pagé and Gingras(4) have found that the oral administration of DL-hexahomoserine to rats causes a rise in the level of blood plasma amino nitrogen which is not reduced by the addition of lysine to the ration. If the increased plasma amino nitrogen is actually DL-hexahomoserine nitrogen, then the effect of daily ingestion of the compound may be cumulative, and all animals may eventually show growth-retardation even when fed extremely low levels of the compound. Anemia might also occur under these conditions. Studies on the effect of feeding very low levels of hexahomoserine for long periods of time are in progress.

Summary. Young white rats were fed diets containing 2% DL-lysine·HCl and different levels of DL-hexahomoserine for 28 days. When the diet contained 0.08-0.33% DL-hexahomoserine, the growth rate was reduced without simultaneous development of anemia. When the diet contained 1% DL-hexahomoserine, growth was almost completely inhibited and the majority of the animals showed definite symptoms of anemia.

4. Pagé, E., and Gingras, R., *Fed. Proc.*, 1949, v8, 122.

Received November 28, 1949. P.S.E.B.M., 1950, v73.

Cultivation of Mengo Encephalomyelitis Virus in the Embryonated Hen Egg.* (17581)

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Several reports have appeared in the literature on the cultivation of the Columbia SK (1), MM(2,3) and encephalomyocarditis (EMC)(4) viruses. It has been adequately demonstrated(5,6,7) that these 3 agents are closely related to the virus of Mengo encephalomyelitis (ME). It might be expected, therefore, that the pathogenicity of ME virus for the embryonated egg would be similar to that of other agents of this group.

Material and Methods. The strain of ME virus isolated from the central nervous system of a paralyzed rhesus monkey(8) was employed. It had been passed 3 times intracerebrally in mice prior to being used for the inoculation of eggs. The diluent employed in all experiments was a solution of 0.2% bovine albumin in buffered saline(9), con-

* This study was made at the Laboratories of the International Health Division of The Rockefeller Foundation, New York, during tenure of a Rockefeller Foundation fellowship, while on leave of absence from the Yellow Fever Research Institute, Entebbe, Uganda.