16804

Effect of the Amino Acid Hexahomoserine on Growth and Hematopoiesis in Swine.

E. T. MERTZ, W. M. BEESON, R. H. WALTZ, JR., AND R. GAUDRY.
(Introduced by W. A. Hiestand.)

From the Departments of Agricultural Chemistry and Animal Husbandry, Purdue University, Lafayette, Ind., and the Department of Biochemistry, Medical School, Laval University, Quebec, Canada.*

The synthesis of the amino acid, α-amino-ε-hydroxycaproic acid, was accomplished recently by one of the authors,[1] and the name hexahomoserine, was proposed for the new compound. It was shown that hexahomoserine could not replace lysine in the diet of rats, and that it was probably toxic.[2] Further studies[3] showed that the incorporation of hexahomoserine in the diet of the rat caused a drop in the hemoglobin level, red cell count, and red cell volume of the blood. Evidence was cited[3] indicating that hexahomoserine may be identical with the anemia-producing factor of deaminized casein.[4]

Inasmuch as feeding experiments with hexahomoserine have been limited to the rat, it appeared desirable to determine whether this compound would produce anemia in an unrelated species. The data presented below show that young pigs develop anemia and in addition, stop growing, when small amounts of hexahomoserine are added to an otherwise normal diet.

Experimental. Two male and 2 female purebred Duroc weanling pigs, whose weights ranged from 53-60 lb, were paired for the feeding experiments. The 4 pigs were kept in individual pens and were fed twice daily with amounts to satisfy the individual appetite of each animal. All animals received, throughout the course of the experiment, a ration consisting of ground corn 58.75%, ground wheat 20%, Purdue supplement V 20% (this supplement consists of meat and bone scraps 20%, fish meal 20%, soybean oil meal 40%, cottonseed meal 10%, and alfalfa leaf meal 10%), mineral mixture 1% (this consists of equal parts of limestone, steamed bone meal, and salt), and concentrated cod liver oil (NOPCO XX) 0.25%.

For a period of 27 days, male 72 and female 70 each received, in addition to the above diet, 3 g of DL-hexahomoserine daily. The hexahomoserine was mixed with the first half of the daily ration. The other pigs, male 73 and female 71, received only the basal ration.

A record was kept of daily food consumption and weekly weight gains. Twice a week red blood cell,[5] white blood cell,[5] platelet,† and reticulocyte[5] counts were made, and red blood cell diameters[5] were measured, in blood samples taken from a lancet wound in the ear tip. Once a week, red blood cell, hemoglobin,[6] cell volume,[7], icteric index,[5] prothrombin,[8] non-protein nitrogen,[9] total plasma pro-

* Journal paper No. 347, Purdue University Agricultural Experiment Station.

1 Gaudry, R., *Can. J. Research*, 1948, B, **26**, 387.

2 Gingras, R., Pagé, E., and Gaudry, R., *Science*, 1947, **105**, 621.

3 Pagé, E., Gaudry, R., and Gingras, R., *J. Biol. Chem.*, 1947, **171**, 831.

4 Hogan, A. G., Powell, E. L., and Guerrant, R. E., *J. Biol. Chem.*, 1945, **137**, 41.

5 Todd, J. C., and Sanford, A. H., *Clinical Diagnosis by Laboratory Methods*, pp. 238, 249, 294, and 386, W. B. Saunders Co., Philadelphia, 1936.

† Determined by measuring the ratio of platelets to red blood cells in blood diluted with 12% $MgSO_4$ and stained with Wright's stain.

6 Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

7 Wintrobe, M. M., *J. Lab. Clin. Med.*, 1929, **15**, 287.

8 Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 595.

9 Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, pp. 420 and 453, P. Blakiston Co., Philadelphia, 1937.

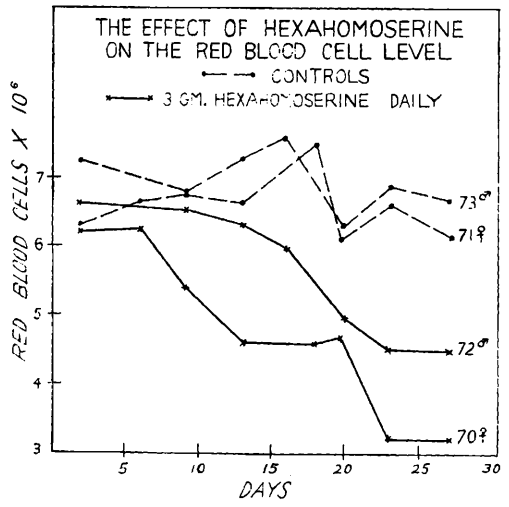

FIG. 1.

tein,[9] albumin,[9] globulin,[9] and fibrinogen[9] determinations were made on blood samples drawn from the anterior vena cava.

Results. The effect of hexahomoserine on the red blood cell level of the swine is shown in Fig. 1. The red blood cell level of pig 70 began to fall about 9 days after hexahomoserine had been added to the diet. By the 27th day, the red cell count had dropped to 3.2 million. At this point, the feeding of hexahomoserine was discontinued. One week after hexahomoserine was removed from the diet, the red cell count of this pig was still 3.2 million. The following day the pig died. Autopsy‡ showed marked enlargement of the spleen, lesions of swine pox, and a myocarditis characterized by accumulations of mononuclear cells. Microscopic examination of the bone marrow revealed no abnormal increase or reduction in the content of immature red blood cells. The bone marrow appeared to be functioning normally in the anemic animal.

The curve in Fig. 1 shows that pig 72 did not have a lowered erythrocyte count until about the 20th day after hexahomoserine had been added to the diet. On the 27th day, the last day on which the compound was fed, the erythrocyte count was 4.4 million, as compared with 6.6 million for the control pig 73. One week after the feeding of hexahomoserine had been discontinued, the erythrocyte count of pig 72 had risen to 5.1 million, and the hemoglobin level and cell volume were back to normal (15.3 g of Hb and 35.7 ml of total cells per 100 ml of blood compared with 15.2 g of Hb and 36.2 ml of total cells for pig 73). Ten days after the removal of hexahomoserine from the diet, the erythrocyte count of pig 72 had risen to 6.1 million. The animal was maintained on the basal ration for a total of 5 weeks after the hexahomoserine was dropped from the diet, and was then autopsied. Macroscopic and microscopic examination of the various tissues, including the bone marrow, showed no abnormalities; the ingestion of hexahomoserine had apparently caused no permanent damage.

The hemoglobin and cell volume values obtained on the blood of the four pigs are summarized in Table I. The blood of pig 70 shows a marked drop and the blood of pig 72 a slight drop in hemoglobin and cell volume.

The white blood cell, platelet and reticulocyte counts, the red blood cell diameter, and the icteric index, prothrombin, non-protein nitrogen, total plasma protein, albumin, globulin and fibrinogen values obtained on the blood of the two pigs receiving hexahomoserine showed no significant deviations from the values obtained simultaneously on the 2 normal control pigs. The experimental data are not reproduced here. It should be pointed out that the level of hexahomoserine fed in these studies may have been too low to produce changes in any blood constituent except the red blood cell.

In addition to depressing the level of red blood cells, hexahomoserine affected the growth rate of the pigs (Fig. 2). The compound appeared to have a delayed action on growth, for the two animals consumed normal quantities of feed and gained weight at a normal rate for approximately one week after the compound was added to the diet. After this initial period, however, both animals consumed less feed and stopped growing. During the last 18 days of the experiment, the animals

‡ We are indebted to Dr. L. P. Doyle, Department of Veterinary Science, Purdue University, for performing the autopsies on pigs 70 and 72.

TABLE I.
Effect of Hexahomoserine on Hemoglobin Level and Blood Cell Volume of Swine.

No. of days on diet	Hemoglobin (g Hb/100 ml blood)				Cell volume (ml cells/100 ml blood)			
	70 ♀ *	71 ♀ †	72 ♂ *	73 ♂ †	70 ♀	71 ♀	72 ♂	73 ♂
0	12.8	15.1	14.9	12.4	31.0	36.5	36.0	31.0
6	14.4	14.3	—	—	29.5	33.0	—	—
12	9.9	14.8	13.2	15.2	29.7	35.9	32.8	39.4
18	10.3	15.0	12.0	15.6	25.0	34.6	29.6	35.6
27	6.4	15.0	12.5	14.7	22.5	39.5	32.0	39.0

* Animal received 3 g of hexahomoserine daily.
† Control animal. Received no hexahomoserine.

receiving hexahomoserine each consumed an average of 2.2 lb of feed per day, and gained little or no weight; the control animals each consumed an average of 3.5 lb of feed per day and gained weight in a normal manner.

Removing hexahomoserine from the diet of pigs 70 and 72 did not cause an immediate resumption of growth. Pig 70, when returned to the basal ration, lost 6 pounds during the first week and succumbed on the eighth day. Pig 72 recovered from the anemia in about 10 days but did not show normal weight gains for one month.

Discussion. The data presented above indicate that no major constituent of the blood except the red blood cell is affected when 3 g of DL-hexahomoserine are fed daily for 27 days to young (50-60 lb) pigs. If the decrease in circulating red cells in the blood of these pigs were due to a destruction of the red cells by hexahomoserine, one would expect an increase in the icteric index of the blood. No increase was observed. It is possible that hexahomoserine slows down or prevents the release of mature red cells to the blood stream. The finding of a normal supply of immature cells in the bone marrow of the anemic pig 70, and the presence of a normal icteric index and reticulocyte count in both of the anemic animals would support this hypothesis.

The inhibition of growth by hexahomoserine may be due to the development of a lysine deficiency in the animals. No data are available on the lysine requirements of swine, but lysine has been found essential for growth in all species tested to date. Hexahomoserine is closely related structurally to lysine, and may be a metabolic antagonist of the latter. Further studies on the mechanism of the action of hexahomoserine are in progress.*

Summary. Two Duroc pigs were fed hexahomoserine (α-amino, ε-hydroxy caproic acid) for a period of 27 days. A marked lowering of the red blood cell count was observed in both animals. No major constituent of the blood, except the red blood cell, was affected. In addition to developing anemia, the pigs made no significant weight gains after one week on the diet containing hexahomoserine, and it is concluded that the amino acid inhibits growth.

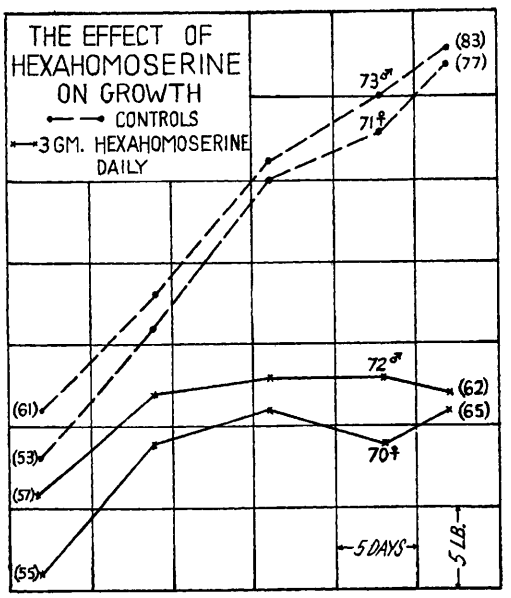

FIG. 2.
The numbers in parentheses denote the initial and final weights of the pigs.

* Since this paper was written, it has been shown conclusively that lysine is essential for the growth of pigs (Mertz, E. T., Shelton, D. C., and Beeson, W. M., *J. Animal Sci.*, 1948, **7**, 530).