control, the average time for the initial ovulation in 23 nonlactating puerpera was found to be 10.2 weeks postpartum. There is considerable variation in the appearance time of ovulation in the absence of lactation. This may be attributed to varying degrees of gonadotrophic stimulation, sufficient in some cases to culminate in ovulation as early as the sixth postpartum week.

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Growth Retardation and Corneal Vascularization with Tyrosine and Phenylalanine in a Purified Diet.

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(Introduced by J. M. Sherman.)
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In a previous study dealing with the antagonistic action of certain amino acids toward the growth of *Streptococcus bovis* in a synthetic medium, it was found that a combination of *dl*-phenylalanine and *l*(-)tyrosine showed a marked inhibitory effect.[1] This inhibition of growth could be counteracted by the addition of *l*(-)tryptophane to the medium. Increased amounts of nicotinic acid added to the medium showed no effect. Krehl, Teply, Sarma, and Elvehjem[2] have shown that growth retardation in rats produced by the addition of corn grits to the diet could be counteracted by adding a small amount of tryptophane or nicotinic acid to the diet. In view of this fact the work with amino acid antagonisms was extended in order to determine whether the same phenomenon could be demonstrated in rats.

The following basal ration, similar to that used by Krehl *et al.*[2] was used: Labco vitamin-free casein 10, sucrose 83, corn oil 3, U.S.P. salt I 4, and cystine 0.15 parts. Vitamins were incorporated in the basal ration in the following amounts: thiamine 0.2, riboflavine 0.3, pyridoxine 0.25, calcium pantothenate 2.0, choline chloride 100, inositol 10, 2-methylnaphthaquinone 0.1, biotin 0.01, and folic acid 0.0115 mg per 100 g. Halibut liver oil (diluted 1:2 with corn oil) was fed at a level of 2 drops per week, with α-tocopherol included at 0.5 mg per drop. Additions to this diet were made by replacing an equal weight of sucrose. Substances added to the basal diet were thoroughly mixed with it using a mortar and pestle. Weanling, male rats averaging from 45 to 55 g were used throughout these experiments. Five rats were placed on each diet except in the case of a few control diets in which 3 were used.

The addition of 1% *dl*-phenylalanine and 1% *l*(-)tyrosine to the basal ration produced a substantial growth retardation (Table I) which was accompanied in about 50% of the cases by vascularization of the cornea and marked edematous swelling of the feet. With the addition of levels of phenylalanine and tyrosine as high as 2% each, growth was almost completely inhibited over a 3-week period. At this higher level more severe external lesions were noted and they occurred in 100% of the cases. Spontaneous healing of the external lesions was noted with all of the levels used in these experiments. When 2% phenylalanine and 2% tyrosine were used, the lesions began to appear on the 6th day and healing became apparent on the 15th to 18th day.

Contrary to the results obtained with *Streptococcus bovis*, it was found that the addition of tyrosine or phenylalanine alone to the basal ration produced growth retardation and external lesions to an extent which

[1] Niven, C. F., Jr., and Washburn, Mary R., unpublished.

[2] Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A., *Science*, 1945, **101**, 489.

TABLE I.
Effect of Nicotinic Acid and Tryptophane on Growth Retardation in Rats on Diets with Added Phenylalanine and Tyrosine.

Ration used	G gained per week* and range
Basal	19 (18-20)
" 1% *dl*-phenylalanine + 1% *l*(−)tyrosine	13 (8-19)
" 1% " + 1% " + 2 mg % nicotinic acid	11 (8-13)
" 1% " + 1% " + 100 " *l*(−)tryptophane	14 (11-18)
" 1% " + 1% " + 500 " nicotinic acid	16 (11-22)
" 1% " + 1% " + 100 " " " + 1% *l*(−)tryptophane	15 (13-17)
" 2% " + 2% "	1 (0.7-2)
" 2% " + 2% " + 100 mg % nicotinic acid	4 (3 -6)
" 2% " + 2% " + 1% *l*(−)tryptophane	4 (2.6-7)
" 2% " + 2% " + 500 mg % nicotinic acid	6 (4.6-7)
" 2% " + 2% " + 2% *l*(−)tryptophane	7 (5 -8)
" 1.5% *l*(−)tyrosine	12 (9-14)
" 4% "	2.4 (2- 3)
" 4% *dl*-phenylalanine	6 (4- 9)
" 100 mg % nicotinic acid	18 (16-19)
" 1% *l*(−)tryptophane	17 (16.6-17.6)

* Average of three weeks.

suggests that the effect produced by the addition of phenylalanine to a diet containing tyrosine may merely add to the effect of the tyrosine. Using deuterium-labeled phenylalanine, Moss and Schoenheimer[3] have shown that when a diet is supplemented with 2% phenylalanine, 20 to 30% of this is converted to tyrosine in the growing rat. The rate of conversion was not affected by the addition of an equal amount of tyrosine to the diet.

Lesions similar to those observed in the present study were previously described by Hueper and Martin[4] as a result of the addition of 10 parts of tyrosine to a diet containing 18 parts of casein and 100 mg % of nicotinic acid.

Corneal vascularization has been seen to occur in a variety of conditions including tryptophane deficiency,[5] lysine and methionine deficiency, and on diets low in protein.[6]

The growth retardation produced by the addition of 1% of phenylalanine and 1% tyrosine could not be counteracted by adding 2 mg % of nicotinic acid or 100 mg % of tryptophane to the diet (Table I). However, a very large amount of nicotinic acid (500 mg %) did appreciably decrease the growth retardation, as well as the appearance of external lesions. There seemed to be no advantage to the simultaneous addition of nicotinic acid and tryptophane.

The almost complete inhibition of growth by the addition of 2% phenylalanine and 2% tyrosine could be alleviated to some extent by the incorporation of 500 mg % of nicotinic acid or 2% of tryptophane to the diet. On the other hand, the addition of nicotinic acid or tryptophane to the basal diet had no growth stimulatory effects.

Two per cent phenylalanine and 2% tyrosine produced no deleterious effects when incorporated in the following diet: Argentine casein 25, Brewer's yeast 6, dried liver 4, sucrose 10, U.S.P. salt I 4, cooked starch 47.9, cellulose 3, and choline 0.1 parts per 100. Mixed tocopherols in corn oil were fed at a level of 11.6 mg per day.

The reason for the rather striking response of the rat toward a relatively low protein diet supplemented with phenylalanine and tyrosine is not known at the present time. It is hoped that experiments now in progress will aid in clarifying some unanswered questions. These experiments, however, serve to demonstrate possible complications arising

[3] Moss, A. R., and Schoenheimer, R., *J. Biol. Chem.*, 1940, **135**, 415.

[4] Hueper, W. C., and Martin, G. J., *Arch. Path.*, 1943, **35**, 685.

[5] Totter, J. R., and Day, P. L., *J. Nutrition*, 1942, **24**, 159.

[6] Sydenstricker, V. P., Hall, W. K., Hock, C. W., and Pund, E. R., *Science*, 1946, **103**, 194.

from diets containing certain amino acids in quantities which are out of proportion to that found in most natural proteins.

Summary. The addition of 1% *dl*-phenylalanine and 1% *l*(-)tyrosine to a purified diet containing 10% casein produced growth retardation and external lesions. Phenylalanine is converted to tyrosine in the anima and so may add to the effect of the tyrosine. The addition of relatively large amounts o nicotinic acid or *l*(-)tryptophane will appre ciably alleviate the deleterious effects of thes amino acids.

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Utilization of Intramuscularly Injected Carotene.

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Carotene administered parenterally has been shown to be poorly utilized.[1,2] Subcutaneous injection of carotene in oil solution[1] or intravenous, intraperitoneal or intrasplenic injections[2] of aqueous colloidal suspensions of carotene have failed to increase stores of vitamin A in the liver of rats. Deficiency symptoms and death have occurred while considerable amounts of the injected carotene were still present in the body.[1,2] Studies in this laboratory of intramuscularly-injected carotene in oil solution have yielded similar results. Carotene in sesame oil, with and without added tocopherols, when injected intramuscularly, has been found to restore growth to depleted rats for a short time only. Upon ensuing death, a considerable fraction of the carotene was found at the site of injection. After being solubilized in water by means of a suitable agent, intramuscularly-injected carotene was as efficiently utilized as carotene administered orally.

Experimental Methods and Results. When carotene is dissolved in Tween 80, (polyoxyalkylene derivative of sorbitan monoleate†) the solution may be diluted indefinitely with water without precipitation of the carotene. A comparative study of the absorption o carotene in Tween 80 solution and in sesam oil solution was made. 0.5 mg of carotene and 0.2 mg of α-tocopherol in 0.1 cc of solven was injected into the thigh muscles of normal adult rats and the rate at which the carotene passed from the muscle determined. The data are presented in Table I.

The curative effect of intramuscularly-injected carotene as compared with that administered orally was measured by a study o the growth and duration of cure of vitami A-depleted rats. Seventy male weanling rats were fed the U.S.P. XII vitamin A free diet. After 6 weeks on this diet, when cessation o growth and the appearance of ocular symptoms had marked the depletion of body store of vitamin A, 40 of the most typically de ficient rats were divided by weight into groups. Rats dying within 3 days after re ceiving the supplement were not included in the data. From a comparison of the growth curves and the data on duration of cure. (Fig. 1) it may be seen that an intramuscular injection of carotene in sesame oil (Group 3) is poorly utilized as compared with the utilization of the same supplement given orally (Group 4). When the carotene was injecte in Tween 80 (Group 1) the curative effec was as good as, if not better than, that obtained in the groups receiving carotene orally either in Tween 80 or oil (Groups 2 and 4).

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1 Lease, V. G., Lease, E. J., Steenbock, H., and Baumann, C. A., *J. Lab. and Clin. Med.*, 1942, **27**, 502.

2 Sexton, E. L., Mehl, J. W., and Deuel, H. J., *J. Nutrition*, 1946, **31**, 299.

† Atlas Powder Co.