TECHNICAL PAPERS

The Amino Acid Requirements of Swine: I. Tryptophan[1]

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It is common knowledge that swine require proteins of high biological value for maximum growth and efficiency of food utilization. However, no studies have as yet been reported on the amino acids that are essential for the growing pig. Research on amino acid requirements has been confined largely to the rat (*10*), chicken (*1, 3*) dog (*14*), mouse (*2*), and man (*11–13*). Studies on the amino acid requirements of swine have been delayed until purified rations could be developed that would support normal growth in pigs. The research of Hughes (*5, 6*), Hughes and Ittner (*7*), McRoberts and Hogan (*9*), Wintrobe, *et al.* (*15, 16*), and Heinemann, *et al.* (*4*) has contributed substantially to perfecting purified diets that will meet the nutritional needs of the pig. Our modification of these diets has resulted in a purified ration which will produce a growth rate superior to a balanced natural feedstuff diet, thus permitting the study of amino acid requirements.

Recently, Luecke, McMillen, Thorp, and Tull (*8*) have demonstrated a relationship between nicotinic acid and tryptophan for the growing pig. Their results indicate that when corn constitutes a major part of the ration, nicotinic acid deficiency occurs. The addition of either 30 mg of nicotinic acid daily/pig or 200 mg of DL-tryptophan alleviated the deficiency and gave a growth response. It is not clear whether tryptophan promoted this response by serving as a precursor for nicotinic acid or by some other mechanism. Our studies show conclusively that tryptophan is an essential amino acid for the growing pig, even in the presence of adequate nicotinic acid.

To identify tryptophan as an essential amino acid, it was necessary to design a purified diet adequate in all the known nutrients with the exception of tryptophan. This was accomplished by modifying the diet previously reported by Heinemann, *et al.* (*4*). The tryptophan-low diet was composed of hydrolyzed fish protein (26.1%), dextrose (30.2%), dextrin (25.0%), lard (11.0%), mineral mixture (5.2%), celluflour (2.0%), and liver extract (0.5%). The water-soluble vitamins were added to the diet in the same quantities as listed by Heinemann, *et al.* (*4*), except that thiamin chloride was fed at a level of 0.40 mg/kg of live weight/pig daily. Each pig was fed 1,500 I.U. of vitamin A, 200 I.U. of vitamin D, 50 mg of α-tocopherol, and 2 mg of vitamin K daily. The hydrolyzed fish protein was chemically treated by a special process to remove most of the tryptophan. DL-tryptophan was added to the control diet (group A) at a level of 0.4% of the total ration. This level was apparently adequate to meet the tryptophan requirements of the growing pig.

Four male and four female purebred Duroc weanling pigs averaging 50 lbs were paired for this experimental study. All the pigs were closely related and had the same treatment prior to the starting of the experiment. The pigs were kept in individual pens equipped with raised wire-screen floors and were fed twice daily in amounts to satisfy the individual appetites of each animal. An adjustment period of 7 days was allowed for changing the pigs gradually from a natural grain ration to the experimental diets.

The control animals (group A—four pigs) receiving the purified ration plus 0.4% of DL-tryptophan made an average daily gain of 1.42 lbs for a period of 28 days after the adjustment period. These pigs had glossy, smooth hair coats and showed no outward signs of ill health or unthriftiness. Twenty-eight days after the adjustment period the pigs averaged 96 lbs in weight and were removed from the experiment. In a previous experiment, similar pigs on a balanced ration of natural feedstuffs gained 1.25 lbs/pig daily, which indicated that our purified diet plus 0.4% DL-tryptophan supported growth better than the natural grain ration.

The pigs (group B—four pigs) receiving the tryptophan-deficient diet showed a weight loss averaging 2.13 lbs/pig at the end of the first week on the purified diet, while those receiving 0.4% DL-tryptophan gained an average of 8.9 lbs in the first week. These data definitely show that the lack of tryptophan in the diet of the pigs has an immediate retarding effect on growth rate.

Two pigs kept on the tryptophan-deficient diet for 21 days lost an average of 5.5 lbs during that period and showed considerable reduction in appetite, rough hair coat, and severe symptoms of inanition. After 21 days, 0.4% DL-tryptophan was added to the diet. This caused an immediate increase in food consumption and resulted in a growth rate of 1.47 lbs/pig daily for the remaining 19 days of the experiment. The animals took on a more thrifty, healthy appearance, and gradually the gloss returned to the hair coat.

The other two pigs were kept on the tryptophan-deficient diet for 14 days, after which 0.4% DL-tryptophan was added. Prior to the addition of tryptophan the pigs lost 3.5 lbs in 14 days; after tryptophan was added there

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was an average gain of 1.06 lbs daily for 26 days. The improvement in appearance was similar to that previously described.

The above data show that tryptophan is an indispensable amino acid for growing pigs. In addition, a purified ration has been formulated which is capable of producing a rate of growth equal to that obtained on a well-balanced mixture of natural feedstuffs.

The complete details of this experiment will be published in a later paper.

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Trypanosoma cruzi Endotoxin (KR) in the Treatment of Malignant Mouse Tumors[1]

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Verification of the results of Roskin and Klyueva (*7* and several earlier papers) in producing the cancerolytic toxin, KR, from the lysed cells of *T. cruzi* was recently claimed by Malisoff (*8*). Since this mode of "biotherapy" is still looked upon by some as one of the promising leads in the treatment of cancer, our own largely negative findings are outlined here for the sake of critical comparison.

Since March 1945, 8 different strains of *T. cruzi* have been tested by us against 5 varieties of malignant tumors in over 1,300 experimental mice. The trypanosomes covered a wide range of virulence and were originally derived from various mammalian and insect hosts (man, monkey, armadillo, triatome). Included among the 8 *T. cruzi* strains were the "Soule" strain ("S" strain), on which Malisoff based his positive data, and the "Wellcome" strain ("W–BH" strain, inadvertently referred to as the "R" strain in an earlier publication, *4*). The latter material was the source of Roskin and Klyueva's allegedly effective KR preparations. The tumors were: transplantable sarcomas 37 and 180 in Swiss and A-mice; transplantable squamous cell carcinoma 119 in A-mice; transplantable and spontaneous mammary adenocarcinoma in C3H- and dba-mice.

Infections of mice with the various *T. cruzi* strains, *i.e.* active Chagas' disease, resulted in consistent inhibition of tumor growth, but produced very few tumor regressions and generally did not lengthen the life of infected cancerous mice beyond that of controls (*5, 6*). Tumor inhibition was sometimes accompanied by loss in body weight. These weight losses were closely correlated with the graded virulence of the several trypanosome strains.

The parasites were not "positively tumorotropic," as stated by the Russian investigators who found leishmanial stages in malignant growths to the exclusion of normal host tissues. Under our experimental conditions, cancer cells proper were only rarely parasitized. The infection was lightly present in the stroma of some tumors and was heavily concentrated in heart, liver, spleen, kidneys, small intestine, and skeletal muscle.

Cancerous mice infected with the always lethal W–BH strain died within 8–13 days of inoculation. Weight loss in these animals was considerable, and tumor growth was almost completely suppressed. When the infection in such mice was checked by drugging with the quinoline derivative, Bayer 7602, the previously inhibited tumors resumed their usual growth rate, and the hosts eventually died of cancer. From the available evidence, tumor inhibition by living *T. cruzi* does not appear to be a specific phenomenon caused by response to specific toxins, but can more adequately be attributed to competition for essential dietary factors and to general depletion of the host-system by the infection. Active Chagas' disease has, therefore, no clinical value in the treatment of malignant growths.

The killed trypanosome preparations of Roskin and Klyueva contained the "cancerolytic endotoxin," KR, which was claimed to have caused the complete regression of a variety of neoplasms in experimental rodents and in 13 out of 60 cancer patients. Follow-ups on the human cases treated are too brief for final appraisal, and evaluation is further confused by X-ray treatment in several instances. Duplicating as closely as possible the Russian techniques, we followed two general types of procedure:

(1) Cultures of *T. cruzi* (Brazil strain) at the height of growth were heat-killed by 30-min exposures to a temperature of 49° C ± 1°. This material was injected intraperitoneally into cancerous mice, or the tumor pieces were soaked in the preparation for 8 hrs prior to implanting. Injections were without effect, and the latter pretreatment did not reduce the number of "takes" or alter subsequent growth, as compared with an equal num-

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