Effect of Dietary Putrescine on Whole Body Growth and Polyamine Metabolism (43100)

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Abstract. **Putrescine (1,4-diaminobutane) is the simplest of the mammalian polyamines. These are small, positively charged molecules which are essential for cell growth and are thought to play a role in regulation of anabolic events such as synthesis of DNA, RNA, and protein. Recent reports have indicated the potential for dietary precursor amino acids of putrescine to alter tissue putrescine concentrations. The current study was conducted to determine the physiologic significance of these effects by feeding up to flooding doses of putrescine to determine any influence on whole body growth and polyamine metabolism. A total of 96 chicks were fed purified crystalline amino acid diets containing 0.0, 0.2, 0.4, 0.6, 0.8, or 1.0% purified putrescine (four birds per pen, four pens per diet) for 14 days. The feeding of 0.2% putrescine increased growth rate beyond that of controls while further supplements reduced growth and were toxic when 0.8 and 1.0% putrescine were fed. Hepatic and muscle concentrations of ornithine increased with dietary putrescine while the effect in kidney was much less. Putrescine concentrations in liver, kidney, and muscle rose when 0.4% putrescine or more was fed. This effect was particularly obvious in muscle in which there were also increases in the concentrations of spermidine and spermine. In a subsequent similar experiment, putrescine was fed at 0.0, 0.1, 0.2, 0.3, 0.4, or 0.5% to determine the effect on the activities of the key enzymes regulating polyamine synthesis. The feeding of putrescine at even 0.1% caused a rapid reduction in hepatic ornithine decarboxylase activity while S-adenosylmethionine decarboxylase and arginase activities were not influenced by diet. It was concluded that excess tissue putrescine can be toxic to whole organisms but small, orally administered doses of this metabolite can promote growth.**

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The polyamines (putrescine, spermidine, and spermine) are essential for cell growth and it has been suggested that these molecules, perhaps because of their positive electrical charge, promote anabolic events such as synthesis of DNA, RNA and protein, stabilization of ribosomes, and increased amino acid uptake by cells (1). The first reaction in the synthesis of the polyamines is conversion of arginine to ornithine catalyzed by arginase (EC 3.5.3.1). The subsequent synthesis of putrescine from ornithine is catalyzed by the key regulatory enzyme ornithine decarboxylase (EC 4.2.2.17; ODC). Spermidine and spermine are formed by the sequential addition of aminopropyl groups derived from decarboxylated *S*-adenosylmethionine. This decarboxylation is catalyzed by *S*-adenosylmethionine decarboxylase (EC 4.1.1.50; AdoMetDC), a second key regulatory enzyme in the synthesis of the polyamines.

Although it has been demonstrated that the feeding of lysine to chicks to induce renal arginase will lead to an increase in renal ornithine concentration and subsequently to an increase in renal putrescine concentration (2) and that the feeding of inducers of ODC coupled with ornithine will have a similar effect (3), it is not clear if these tissue-specific events are adequate to influence whole body metabolism. Cellular polyamine concentrations are tightly regulated in a manner that has been described as intricate and exquisite (4). It has also been demonstrated that the feeding of potent ODC inhibitors such as 2-difluoromethylornithine can greatly reduce tissue polyamine concentrations without causing severe growth depression in rats (5). An experiment was therefore conducted to examine the potential for dietary supplements of putrescine to flood tissues

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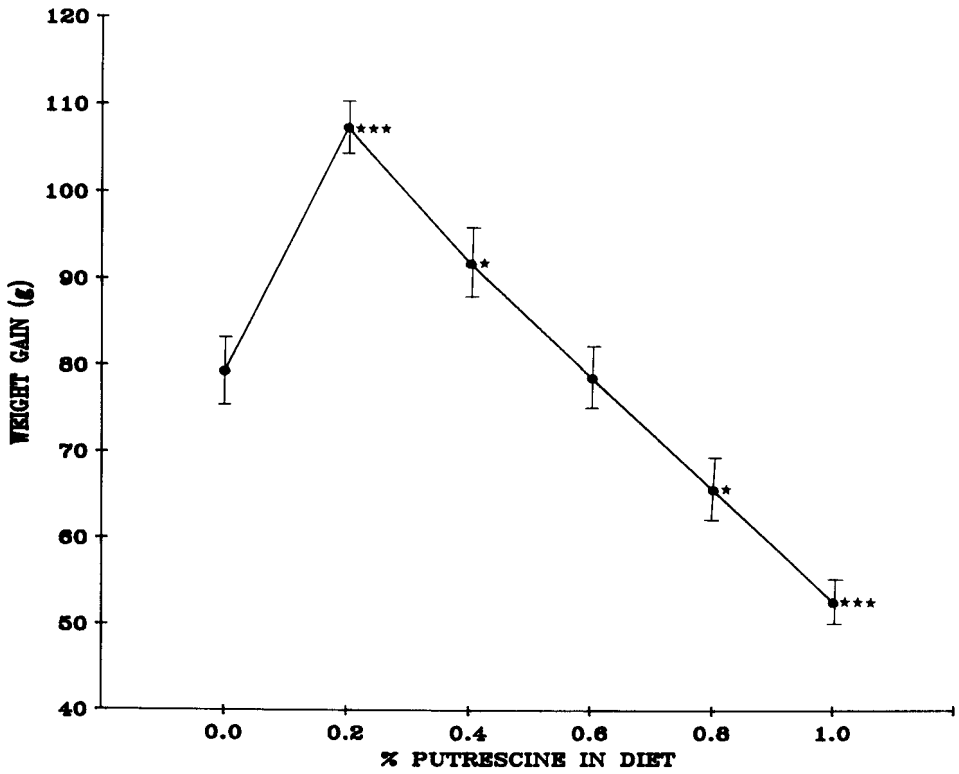

Figure 1. Weight gain of chicks fed varying dietary concentrations of putrescine. Values are mean (n = 16) ± SE and were compared with controls (0% putrescine) using Student's t test (*$P < 0.05$, ***$P < 0.001$).

with this molecule and so override regulation of polyamine metabolism and alter whole body growth.

Materials and Methods

Animals and Diets. In the first experiment, a total of 96 one-week-old Leghorn cockerel chicks (Shaver Poultry Breeding Farms Ltd., Cambridge, ON) were fed purified crystalline amino acid diets (6) containing 0.0, 0.2, 0.4, 0.6, 0.8, or 1.0% putrescine (Ames Laboratories, Inc., Milford, CN) (four birds per pen, four pens per diet) for 14 days. Putrescine was added to diets at the expense of cellulose to maintain all diets as isoenergetic and isoproteinaceous as possible. Birds were housed in electrically heated, thermostatically controlled cages with raised wire floors and were exposed to continuous lighting with feed and water supplied *ad libitum*. The second experiment was of similar design except that putrescine was provided at 0.0, 0.1, 0.2, 0.3, 0.4, or 0.5%. Weight gain and feed consumption were monitored during the growing period, after which time all birds were killed by instantaneous cervical dislocation and livers, kidneys, and a sample of breast muscle were excised, immediately frozen in liquid nitrogen, and stored at −80°C until analyzed.

Enzyme Assays. Tissue enzyme activities were determined in the second experiment only. ODC and AdoMetDC activities were measured in liver and kidney according to Eloranta *et al.* (7) while renal arginase activity was assayed according to the method of Smith and Lewis (8).

Analysis of Amino Acids and Polyamines. Liver, kidney, and muscle samples were homogenized for 30 sec (Ultra-Turrax, Tekmar Co., Cincinnati, OH) (1 part tissue to 8 parts water) and centrifuged at 20,000g for 30 min (Sorval RC5C centrifuge with SW-24 rotor; Dupont Inc., Newtown, CT). Supernatant was then deproteinized using the Amicon Micropartition System (YMT membrane, centrifuged for 60 min at 3000 g). Arginine, ornithine, putrescine, spermidine, and spermine in the deproteinized supernatant were then simultaneously analyzed in liver, kidney, and muscle as their *N*-heptafluorobutyrylisobutyl derivatives (9) using the capillary gas-liquid chromatographic technique of Bedford *et al.* (2) with electron capture detection.

Statistical Analyses. Statistical comparisons of treatment means with controls (0.0% dietary putrescine) were made using Student's t test (10).

Results

Animal Growth. The effect of dietary putrescine on chick growth is illustrated in Figure 1. The feeding of putrescine at 0.2 and 0.4% of the diet increased the growth rate of chicks compared with controls (0.0% putrescine). Growth rate returned to control levels when 0.6% putrescine was fed while chicks fed higher

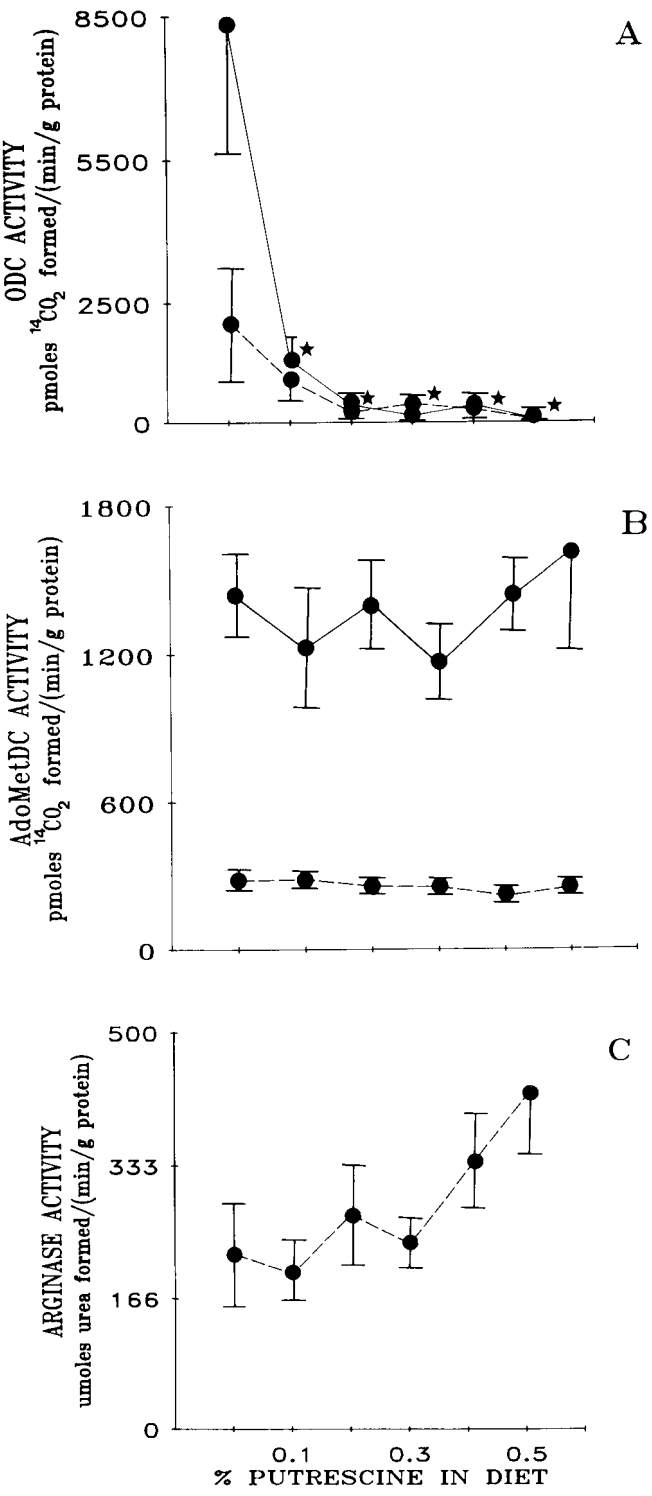

Figure 2. Effect of dietary putrescine concentration on chick liver (●——●) and kidney (●- -●) activities of ODC (A), AdoMetDC (B), and arginase (C). Values are mean (n = 14) ± SE and were compared with controls (0% putrescine) using Student's t test (*$P < 0.05$).

concentrations of putrescine grew more slowly than controls. The slowly growing birds did not exhibit any specific lesions. The feeding of toxic concentrations of putrescine depressed food intake and the ratio of weight gained to food consumed (data not shown).

Enzyme Activities. The effects of dietary putrescine on hepatic and renal ODC and AdoMetDC and renal arginase are given in Figure 2. Hepatic ODC activity was sharply and significantly reduced when 0.1% or more putrescine was fed. The trend in renal ODC activity was similar but less marked and was not significant ($P > 0.05$). Hepatic and renal AdoMetDC and renal arginase activities were not affected by diet ($P > 0.05$).

Tissue Concentrations of Amino Acids and Polyamines. The effect of dietary putrescine on the concentrations of ornithine, putrescine, spermidine, and spermine is depicted in Figure 3. The feeding of putrescine greatly increased concentrations of ornithine in muscle and liver while there was little effect in kidney. Tissue arginine concentrations were unaffected by diet.

There was a significant accumulation of putrescine in liver, kidney, and muscle when 0.4% putrescine or higher concentrations were fed. The accumulation was particularly obvious in muscle as was the accumulation of spermidine and spermine. The addition of putrescine to the diet decreased hepatic concentrations of spermidine and renal concentrations of spermine while renal concentrations of spermidine were increased.

Discussion

These experiments show that the feeding of putrescine at moderate intakes promotes growth of chicks, although a toxicity develops at high intakes. The polyamines are not known to have any nutritive value and it is not clear how such a growth promotion might be brought about. The purified diet used in these studies meets all of the nutritional requirements for chick growth while minimizing the presence of naturally occurring polyamines.

It has recently been reported that oral administration of putrescine to calves fed soybean-based milk replacer can promote the development of the gastrointestinal tract epithelium and perhaps promote nutrient absorption (11). This was associated with increased intracellular concentrations of putrescine in the intestinal cells. Similar observations have been made in young swine (12). It is not clear, however, whether putrescine is subsequently transported to other tissues and what effect this might have on metabolism of polyamines in peripheral tissues. Some of the anabolic properties of polyamines have been attributed not to putrescine but to spermidine and spermine (1). It is also possible that putrescine could be promoting food intake due to organoleptic properties not related to tissue metabolism.

Increased tissue putrescine concentrations coincided with the onset of diet toxicity, perhaps indicating that growth depression is due to the energetic costs of putrescine excretion. These data demonstrate that pu-

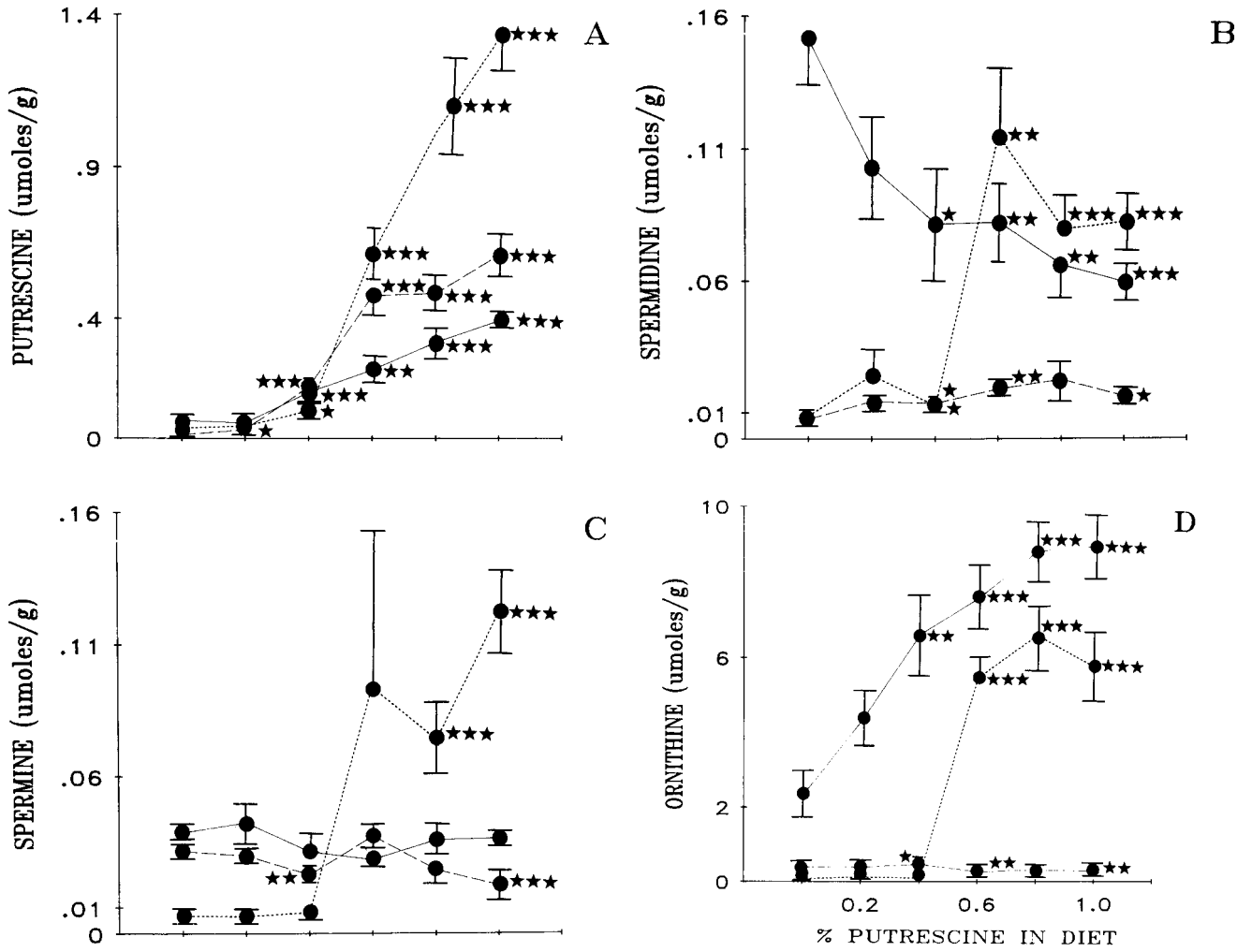

Figure 3. Effect of dietary putrescine concentration on chick liver (●——●), kidney (●– –●) and muscle (●· · · · ·●) concentrations of putrescine (A), spermidine (B), spermine (C), and ornithine (D). Values are mean (n = 8) ± SE and were compared with controls (0.0% putrescine) using Student's t test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

trescine was absorbed from the gastrointestinal tract and transported to peripheral tissues. The large accumulation of putrescine in muscle confirms the earlier observation that this tissue may be atypical with respect to putrescine metabolism (3). The relatively low AdoMetDC activity in muscle (3) suggests that the increased concentrations of muscle spermidine and spermine might result from synthesis in other tissues and subsequent uptake by muscle from the blood. Active transport of polyamines across membranes has been described (13). Increased polyamine concentrations in muscle may be desirable, moreover, as this tissue has a lower overall rate of protein turnover than liver or kidney and might be particularly responsive to any putrescine-induced promotion of cell growth.

Ornithine also accumulated in muscle and liver with the feeding of putrescine while the effect on kidney was much less (Fig. 3). It has been proposed that endogenously synthesized ornithine is more effective as a precursor for putrescine synthesis than exogenous ornithine (14) but it is unlikely that this would result in putrescine synthesis in muscle because of the relatively low ODC activity (2).

The effect of dietary putrescine on hepatic ornithine concentration was undoubtedly due to the rapid reduction in hepatic ODC activity (Fig. 2). Renal and hepatic AdoMetDC activity and renal arginase activity were largely unaffected by diet. The chick lacks a functional urea cycle (15) and the kidney is the major site of arginase activity.

It can be concluded that the feeding of putrescine to chicks results in increased tissue putrescine concentrations and an increase in overall growth rate, although the latter occurs only with moderate concentrations of dietary putrescine. The dietary levels of putrescine must be chosen with care, however, to avoid potential putrescine toxicity.

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