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Comment: Polyamines and protein degradation

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Wajnberg and Fagan [1] have recently shown that polyamines can inhibit the ATP-dependent ubiquitin-linked proteolytic pathway in rabbit reticulocyte lysate. The association of increased polyamine levels and growth is a well established phenomenon in which inhibition of protein breakdown could be one facet. The principal difficulties of assessing the significance of effects of polyamines, however, are first how to determine the specificity, since polyamines influence many processes, and second, in the present instance, to question to what extent changes in the rates of protein synthesis and degradation in vivo correlate in general with changes in measured polyamine concentrations.

Recently there has been considerable interest [2–7] in the ability of polyamines to inhibit translation of ornithine decarboxylase (ODC) mRNA and in several cases [3–9] it has also been observed that polyamines *accelerate* ODC breakdown. The feedback regulation of the synthesis and degradation of the enzymes of polyamine synthesis constitutes a special case, but it is worth noting that neither the changing concentrations of the polyamines in cells nor the activities of ODC and *S*-adenosylmethionine decarboxylase are always moving in concert – for the polyamines, for example, the rise in concentration of putrescine, followed by its decline, usually precedes movements in spermidine concentration, which are earlier than those of spermine.

The effects of spermidine and spermine on synthesis of the enzymes of polyamine metabolism in reticulocyte lysate are in a submillimolar and micromolar range, respectively. Persson et al. [5] observed that at spermidine concentrations above 0.6 mM, synthesis of all proteins was strongly inhibited. Kameji and Pegg [4] likewise observed inhibition of total protein synthesis in lysates with mixtures of spermidine and spermine in similar concentration ranges. With isolated reticulocyte ribosomes optimal stimulation of protein synthesis is also in the submillimolar range [10,11]. These numbers are less than the concentration of polyamines in the reticulocyte [10], though probably similar to those for the cytosol [12], probably because much of the polyamine content is likely to be bound in the nucleus [13]. Effective concentrations of polyamines in these studies are thus markedly below those used by Wajnberg and Fagan [1] which were mM.

An additional problem of correlation arises when we look at polyamine concentrations and rates of protein synthesis and degradation in muscle. Red muscle has a higher polyamine content than white [14] but a higher rate of protein turnover [15]. The denervated

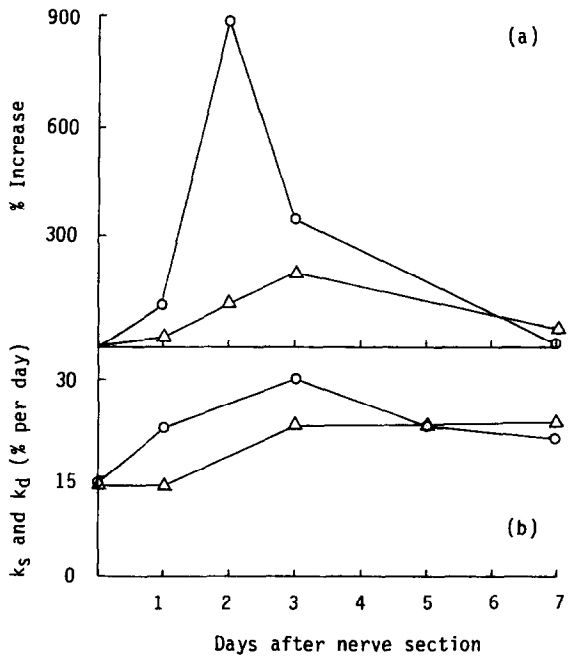

Fig.1. Effect of unilateral denervation of the rat hemidiaphragm (a) on the relative changes in concentrations of putrescine (○) and spermidine (△), and (b) on the fractional rates of protein synthesis (k_s) and degradation (k_d) in the days following nerve section [17,18]. The increase in spermine concentration in a was very slight until 7–10 days following nerve section.

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diaphragm also provides an inverse correlation between polyamine concentrations and rates of degradation. Following unilateral denervation the rat hemidiaphragm undergoes a transient hypertrophy in which there is considerable increase in both RNA and protein content [16]. There is also an increase in the concentration of polyamines [17], that of putrescine being most rapid and marked, followed closely by that of spermidine, whereas change in spermine concentration is rather sluggish (fig.1a). The increased protein mass arises from an enhanced rate of protein synthesis [18], but is more or less coincident with an increased rate of protein degradation (fig.1b). The increased rate of protein degradation is not consistent with a possible suppression of breakdown by polyamines. Moreover the concentrations of polyamines in the muscle do not reach values in excess of 150–200 nmol/g [14,17]. The hypertrophy phenomenon lasts for a period of days. Kremzner et al. [14] observed enhanced levels of polyamines in dystrophic biceps femoris muscle and denervated gastrocnemius muscle in the weeks following nerve section where there is marked tissue wastage.

These last observations may mean no more than that the ATP-dependent ubiquitin-linked pathway of protein degradation has little importance in muscle, but it does also beg the question of whether inhibition of the pathway observable in reticulocyte lysate by polyamines is of any physiological importance or relevance.

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Reply: Polyamines and ATP-dependent protein breakdown

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Wajnberg and Fagan [1] recently showed that the readdition of polyamines to dialyzed rabbit reticulocyte lysate inhibits the ATP + ubiquitin-dependent proteolytic system. To determine whether polyamines inhibit proteolysis in vivo is more difficult. If it were possible to measure rates of proteolysis in cells and tissues depleted of polyamines without affecting other biological processes, then one might be able to examine the role of polyamines in regulating rates of protein breakdown. At present, there are no compounds that rapidly, specifically and completely deplete the intracellular pool of polyamines. Alternatively, one could correlate rates of protein synthesis and degradation in vivo with changes in measured polyamine concentration as Manchester has done [2]. It is unlikely, however, that using this approach will enable one to delineate the role that polyamines play in regulating rates of protein turnover. Following unilateral denervation of rat hemidiaphragm, Manchester reports a rise in polyamine concentration and an increase in the fractional rates of *overall* protein degradation [2,3]. Since the higher concentrations of polyamines in certain tissue preparations were not associated with a decrease in protein breakdown, Manchester [2] was led to ques-

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