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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tion whether (i) the ATP + ubiquitin-dependent proteolytic pathway demonstrated in reticulocytes [4], skeletal muscle and liver [5] has any importance and (ii) the physiological relevance of the finding that polyamines inhibit this pathway in reticulocyte lysates [1]. Little is known about the relative importance or contribution of the ATP-dependent system to overall rates of proteolysis in mammalian cells or its modulation by regulatory molecules such as polyamines. Our observations, based on direct biochemical evidence, merely open up this possibility. However, we do not believe that a lack of correlation between polyamine levels and *overall* rates of protein breakdown in specific models can be used as evidence against polyamines affecting rates of ATP + ubiquitin-dependent protein breakdown.

In this regard, we have recently found that skeletal muscle contains another soluble ATP-dependent proteolytic pathway that does not require ubiquitin or the enzymes which conjugate ubiquitin to proteins [6]. Like the ATP-dependent, ubiquitin-dependent proteolytic system [4,5], this energy-dependent ubiquitin-independent protease is inactivated by the serine protease inhibitors diisofluorophosphate [6] and dichloroisocoumarin [6] (table 1). The ATP-dependent, ubiquitin-independent enzyme was not affected by pepstatin, an inhibitor of aspartic proteases [7], by leupeptin or E64, inhibitors of thiol proteases [8,9], or the metal chelator dipicolinic acid (table 1). To determine whether polyamines inhibited this new enzyme, spermine at high concentration (10 mM) was incubated with partially purified enzyme in the presence or absence of ATP. Spermine inhibited the ATP-dependent, ubiquitin-independent degradation of [^{3}H]globin by 25% (table 1). When spermine was incubated with the ATP-dependent ubiquitin-conjugate degrading enzyme the degradation of ubiquitin-lysozyme conjugates was inhibited by 100% [1]. These data suggest that physiological concentrations of polyamines may inhibit more specifically the ATP + ubiquitin-dependent proteolytic activity and may not play a significant role in regulating other proteolytic pathways requiring ATP.

Table 1

Effect of inhibitors on the ATP-dependent, ubiquitin-independent proteolytic activity isolated from chicken skeletal muscle

Addition	ATP-dependent [^{3}H]globin degradation (% inhibition)
Pepstatin (50 μM)	0
Leupeptin (100 μM)	0
E-64 (100 μM)	0
Dipicolinic acid (5 mM)	0
Spermine (10 mM)	25
Dichloroisocoumarin (0.3 mM)	100

Partially purified enzyme (10 μg) was preincubated at 37°C for 15 min in the presence of inhibitor at the concentration indicated. [^{3}H]Globin (20 μg) was then added in the presence or absence of ATP (5 mM) and the mixtures incubated for 1 h at 37°C. The % inhibition of the ATP-dependent degradation of globin was calculated by determining the release of acid-soluble radiactivity.

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