ingly accurate numerical solutions to be obtained over hundreds of cycles or more. If parameters similar to those of the actual circuit are used, these computations show the phenomena of synchronization and periodicity described above.

States with intrinsic noise can be produced by a second coupling scheme which incorporates a finite value of R_c. In this case, a change in one of the diode voltages will immediately change the other diode current, rather than simply affecting its rate of change, as in the circuit with coupling provided by the resistor R. We now find that the trajectories are no longer periodic for certain broad ranges of applied voltage. The spectra contain a great deal of broadband noise (Fig. 2B). Although several peaks are still visible, high-resolution spectra show even the sharpest one to have a full width at half maximum of about 100 Hz. (In the periodic states the spectral lines have widths of a few hertz at most, and perhaps much less.) The corresponding phase space projection (Fig. 2C) uniformly fills an irregularly shaped region of the I_1–I_2 plane. This behavior is clearly nonperiodic, and is qualitatively different from the states described above. Although it is not difficult to find physical parameters that yield this type of chaos, only periodic states are observed if the system is scaled down to much lower frequencies (by increasing the inductances). In this physically realizable dissipative dynamical system, we have shown experimentally that the nature of the coupling is much more important than the number of degrees of freedom in generating chaos.

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Muscular Dystrophy: Inhibition of Degeneration in vivo with Protease Inhibitors

Abstract. *The protease inhibitors leupeptin and pepstatin were used in vivo in genetically dystrophic chickens to determine their effects on the histological and biochemical changes observed in this disease. These compounds appear to delay the degeneration of muscle tissue which is characteristic of this disorder and thus may have potential therapeutic value in the treatment of muscular dystrophy.*

A striking feature of muscular dystrophy is the extensive loss of sarcoplasmic and contractile proteins and their replacement by fat and connective tissue. The mechanism involved in this degradation is unknown but a persistent observation in neuromuscular degenerative disorders has been a marked elevation in the activity of acidic and neutral proteases and other lysosomal hydrolases (*1*).

We recently reported that the use of the protease inhibitors pepstatin and leupeptin (*2*, *3*) could delay the degeneration of both normal and dystrophic muscle cell cultures. We thought that because of their low toxicity and nonimmunogenic nature these inhibitors might have potential therapeutic value for the treatment of muscular dystrophy and other degenerative muscle diseases. Therefore, we studied the effects of protease inhibitors in vivo in genetically dystrophic chickens.

The chickens, both normal and dystrophic, were hatched in our facilities from eggs supplied by L. Pierro, University of Connecticut, Storrs. The chicks

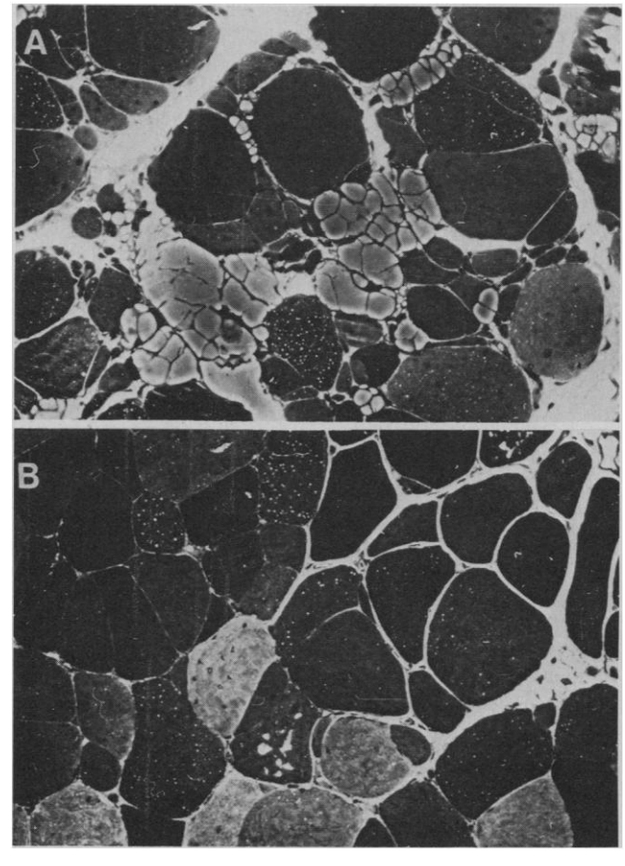

Fig. 1. Light micrographs of transverse sections of dystrophic pectoralis major muscle from an untreated 4-month-old chick (A) and from a chick injected with pepstatin and leupeptin for 4 months (B) (× 280).

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received twice-weekly injections (direct into the pectoralis major muscle) of a combination of leupeptin and pepstatin (8 mg of each per kilogram of body weight) dissolved in 10 percent dimethyl sulfoxide. Injections were started 1 day after hatching. Controls were either injected with solvent or were not injected at all; no differences were detected between these two types of controls. Creatine phosphokinase (E.C. 2.7.3.2) was assayed in blood obtained at the time the chicks were killed, according to the method of Oliver (*4*). The data reported are from seven different experiments conducted on animals aged 3 and 4 months. Figure 1 shows the histology of muscle from untreated dystrophic (DU) chickens and from treated dystrophic (DT) chickens. The photomicrographs shown are representative of results obtained after 4 months of treatment. A slight hypertrophy of the DT muscle was noted when compared to DU muscle after 1 month of treatment (not shown). In DU chickens aged 3 and 4 months there was much variation of fiber diameters with a progressive increase of fatty tissue and fiber degeneration (Fig. 1A). In contrast, DT muscle showed little fatty infiltration and the general architecture of the muscle was better preserved (Fig. 1B). These qualitative observations were corroborated by histological determination of fiber diameters and fiber numbers from four sets of photomicrographs of DT and DU muscle. The area occupied by myofibers per unit area of cross-sectioned muscle was calculated from these values; in DU muscle there was a decrease in myofiber area of 45 to 49 percent in 4 months. In DT muscle, however, a decrease of only 14 to 21 percent was found. These figures substantiate the greater replacement of myofibers by fat and connective tissue in the DU sample (Fig. 1A).

The DT animals at 4 months of age (three animals) had a ratio of pectoralis muscle mass to body weight that was 1.85 ± 0.3 times that of the 4-month-old DU animals. This suggests that leupeptin or pepstatin, or both, inhibits some enzyme, or enzymes, involved in the turnover of dystrophic muscle protein and thereby reduces muscle tissue atrophy. Studies in vitro have shown that leupeptin is a potent inhibitor of both cathepsin B (*5*) and the muscle Ca^{2+}-activated protease (*6*). These results are of special significance because of the reports that higher concentrations of both cathepsin B (*7*) and Ca^{2+}-activated protease (*8*) appear to be present in dystrophic muscle and that higher concentrations of Ca^{2+} are present in dystrophic muscle as compared to normal (*9*). The Ca^{2+}-activated protease is known to act on the Z line of the myofibril (*10*) and it is possible that leupeptin exerts its influence by inhibiting this enzyme, in vivo, which may be the initial step in muscle protein turnover and degradation. We recently reported (*11*) that both pepstatin and leupeptin enter muscle cells in tissue culture and inhibit, in situ, 85 percent of the cathepsin D activity and 50 percent of the neutral protease activity. If protein turnover can be considered to be a "cascade" process involving several enzymes, inhibition of any one enzyme in the sequence could be responsible for turning off protein catabolism. Pepstatin, therefore, may act by inhibiting an enzyme further down in the process; this may explain its effectiveness found in previous studies (*2*).

Data on creatine phosphokinase activity in the serum of these animals at 3 and 4 months of age were as follows: normal, 2,100 ± 300 U/liter; DU, 17,650 ± 1,500 U/liter; and DT, 4,200 ± 280 U/liter. The activity of this enzyme, which is known to increase in human and avian muscular dystrophy, has been used in diagnosis of the human disease. In the present experiments the creatine phosphokinase activity in the DT animals was about double that in the normal animals but approximately one-fourth that in the DU animals. This is a biochemical indication that the degeneration of muscle tissue was diminished in the DT animals.

In several laboratories the symptoms of dystrophy have been partially alleviated by means of penicillamine (*12*), methysergide (*13*), and Dilantin (*14*). Although improvements in the values for creatine phosphokinase activity and righting ability were demonstrated, no histological evidence was presented in these studies and it is, therefore, difficult to interpret the mechanism by which these drugs are effective.

Although the direct role of specific proteases in muscle protein turnover in normal and diseased states has not been well established, there is little doubt that these proteases are somehow involved when one considers the large quantity of protein which must be catabolized. Recent evidence (*15*) suggests that in normal protein turnover, myofibrillar proteins are initially released into the cytoplasm with the aid of specific proteases and that the terminal degradation may involve lysosomal cathepsins. A persistent observation by numerous workers has been the increased proteolytic activity, at both acidic and neutral *p*H values, observed in a variety of wasting disorders of both animal and human muscle. This increase in proteolytic activity is probably secondary to the unknown primary disorder of Duchenne muscular dystrophy (*1*). The mechanism of protein catabolism, unlike protein synthesis, is little understood. However, there is no doubt that it will be of extreme importance for us to understand this process in order to treat degenerative disorders, particularly those of neuromuscular origin. The studies presented here do not suggest that protease inhibitors completely inhibit muscle degeneration. The success in delaying muscle atrophy, however, suggests that these specific protease inhibitors may be useful as potential therapeutic agents in neuromuscular degenerative disorders, as well as providing us with a better insight into the pathogenesis of these diseases.

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