sistant, one sensitive, and one lethal product; and (c) two sensitive and two lethal products. Type a is scored as parental ditype. Types b and c are scored as tetratype and nonparental ditype, respectively, with the lethal products taken as recombinants carrying both resistance markers. No evidence for linkage between this mutant and the other Mendelian markers has been found.

This genetic analysis indicates that at least three loci in *C. reinhardi* can mutate to yield erythromycin resistance expressed at the level of the chloroplast ribosome. If the suggestion of Sager and Ramanis (*6*) that uniparental genes are carried on chloroplast DNA is proved correct, our results are similar to those of studies on erythromycin resistance in yeast (*4*). Mendelian and "cytoplasmic" resistance mutations have now been found in both organisms. A major difference is that, in the *Chlamydomonas* mutants described here, it has been possible to trace both classes of mutations to expression as altered ribosomes. In *E. coli* many independently isolated resistant strains all gained resistance by alteration of one and the same ribosomal protein (*1*); in contrast, our results show that in *Chlamydomonas* several loci may be involved.

Since a combination of mutant 12S3 with any of the other Mendelian genes in the same cell is lethal, it appears that there is a strong interaction between their gene products. A reasonable interpretation is that the ribosomal component which is altered in each case, whether protein or RNA, leads to resistance without seriously affecting the function of the ribosome. However, when the gene products are combined in the same cell, the result is either no ribosome or one that does not function in protein synthesis. An implication of this analysis is that a functional chloroplast ribosome is required for the growth and division of the cell under the zygote germination conditions used. A search for conditions which might "save" these cells is necessary. A contrasting situation is found in cells containing both gene 2L1 and either of the two types of Mendelian genes; these cells grow normally. It will be valuable to identify the nature of the alterations in the ribosomes for each of these three different types of mutations.

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Mammalian Motor Units: Physiological-Histochemical Correlation in Three Types in Cat Gastrocnemius

Abstract. *The correlation among a variety of physiological properties and the histochemical characteristics of muscle fibers belonging to single motor units in a mixed mammalian muscle is directly demonstrated. The population of motor units making up the cat gastrocnemius was classified into three nonoverlapping groups on the basis of a combination of physiological parameters. The muscle fibers belonging to motor units of each physiological type exhibited a distinctive histochemical profile, such that the three basic histochemical "fiber types" exactly matched the three physiologically defined groups. Within each individual motor unit, the muscle fibers were histochemically uniform.*

Many mammalian muscles are composed of muscle fibers that differ from one another in a variety of morphological and histochemical characteristics (*1*). Most attempts to relate the histochemical characteristics of muscle fibers to their physiological properties have depended on indirect evidence based on the properties of whole muscle (*1*, *2*), a process sometimes leading to conflicting conclusions (*3*). The muscle fibers making up a given skeletal muscle are organized into functional entities, the motor units, each consisting of a group of muscle fibers and the single motoneuron innervating them (*4*). More direct evidence of a correlation between one physiological parameter, resistance to fatigue, and oxidative enzyme activity of the active muscle fibers has been obtained by stimulating individual motoneuron axons and examining both physiological responses and muscle fiber histochemistry in the same motor unit (*5*). Utilizing a similar technique, we have now obtained direct correlation of a variety of physiological parameters of single motor units with the reactivity of their muscle fibers in a number of histochemical stains. The motor units studied both physiologically and histochemically were representative of the entire popu-

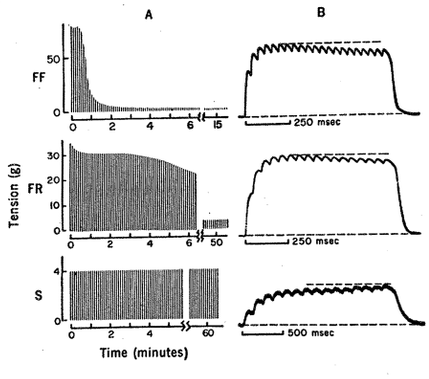

Fig. 1. (A) Graphs of the tension (ordinates) produced by three different motor units during 40 per second tetani, each lasting 330 msec and repeated every 1 second throughout the durations shown on the abscissas. Note rapid decline in tension within 2 minutes in the type FF motor unit (row 1) and greater resistance to fatigue in the FR (row 2) and S (row 3) units. (B) Records of unfused tetanus responses in the same three motor units, showing the slight decline in tension ("sag") in late portions of the tetani in FF and FR units, and the absence of "sag" in the S unit. Tension scales same in A and B.

lation of units found within the cat gastrocnemius. We have shown that motor units in the cat medial (MG) and lateral (LG) gastrocnemius can be classified into three distinct types on the basis of a combination of physiological parameters, and that each type so defined has a characteristic histochemical profile.

In adult cats anesthetized with pentobarbital, short depolarizing current pulses delivered through an intracellular micropipette penetrating a single motoneuron activated only one motor unit (*6*, *7*). Mechanical responses of each unit studied were recorded with the MG or LG tendon connected to a strain gauge through a low compliance link, with muscle length constant and passive tension held at 80 to 100 g. The animals were maintained with normal blood pressure and body temperature, and muscle temperature was kept between 35° and 37°C.

To characterize the population of gastrocnemius motor units as completely as possible, we recorded a variety of mechanical responses from a large number of such units and then evaluated the patterns of distribution of the different properties found within the population sampled. Two of the physiological parameters studied, (i) susceptibility to fatigue during prolonged repetitive stimulation and (ii) the shape of the tension output during unfused tetani, permitted the most distinct separation of units into three nonoverlapping groups.

Assessment of fatigue susceptibility was based on the tension output of units during trains of stimuli at 40 pulses per second, each train lasting 330 msec and repeated every 1 second. After 2 minutes of such repetitive stimulation, the tension output during the trains declined in some units to less than 25 percent of the tension produced by the first train in the series ("fatigue index" < 0.25; Fig. 1A, row 1) (*8*). In other units, tension output after 2 minutes remained greater than 75 percent of the initial level (fatigue index > 0.75; Fig. 1A, rows 2 and 3).

When a gastrocnemius motor unit was stimulated with pulses recurring at regular intervals which were about 125 percent of the contraction time, the shape of the unfused tetanus produced had one of two configurations. With some units, the envelope of tension rose to maximum within four to eight contractions and then fell slightly, or "sagged" (Fig. 1B, rows 1 and 2) (*9*). In other units, no such "sag" developed (Fig. 1B, row 3).

With the two physiological parameters, three types of gastrocnemius motor units were defined (Table 1). Only three motor units out of the total sample of 117 units studied could not definitely be classified into one of the three types. Twitch contraction times (*10*) and tension outputs, which were also measured, were highly correlated with the unit types (Table 1), but these parameters in themselves were less useful in establishing a scheme for motor

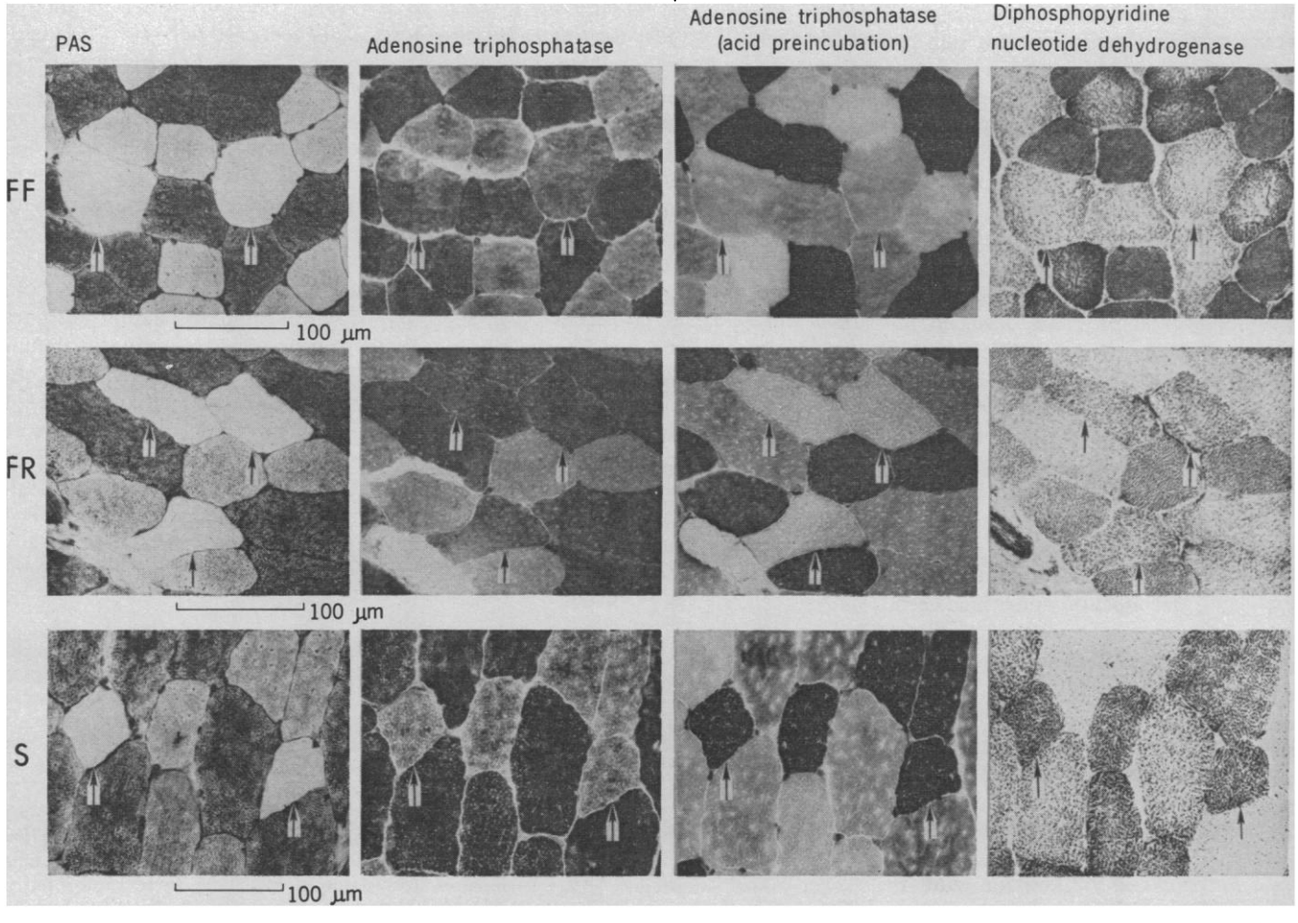

Fig. 2. Four histochemical stains of serial sections containing muscle fibers belonging to the same motor units for which physiological data are shown in Fig. 1. Fibers of the studied units identified as the unstained muscle fibers in PAS sections (column 1, arrows). The same fibers are indicated in serial sections stained with other histochemical reactions (arrows). Acid preincubation before adenosine triphosphatase staining (column 3) was in acetate buffer at *p*H 4.65.

unit classification. The nomenclature used for this classification denotes certain physiological features of each motor unit type: type FF, fast contracting, fast fatigue; type FR, fast contracting, fatigue resistant; type S, slowly contracting. The type names designate fast or slow contraction speed because this has been a key parameter used in previous work on motor unit physiology (*2*, *6*). When all the parameters were available for analysis, it was evident that the two types of fast contracting units (FF and FR) had twitch contraction times of 55 msec or less, while the slowly contracting type S units had contraction times > 55 msec. However, with contraction times alone we would not have been able to discern that dividing point precisely, and we therefore preferred to use the "sag" property to separate the FR and S groups distinctly. Although both FR and S units had fatigue indices > 0.75 after 2 minutes of stimulation, the FR units eventually fatigued during more prolonged repetitive stimulation while most S units showed very little fatigue with stimulation continued up to 1 hour (compare graphs in Fig. 1A, rows 2 and 3).

In experiments designed to determine the histochemical profile of motor units characterized physiologically, only one unit was studied in each gastrocnemius head per animal. After the recording of unit mechanical responses and the determination of the fatigue index, the repetitive stimulus trains were continued for variable lengths of time (15 minutes for FF units, 30 to 60 minutes for FR units, and 45 to 60 minutes for S units) to deplete the muscle fibers belonging to the studied unit of their glycogen content (*5*, *11*). The whole muscle containing the unit studied was then quickly removed from the animal, cut into cross-section blocks and rapidly frozen (*12*). Serial cross sections (10 to 15 μm), cut in a cryostat, were stained with the following histochemical reactions: periodic acid–Schiff (PAS) for glycogen (*13*); reduced diphosphopyridine nucleotide dehydrogenase (*14*), an oxidative enzyme in mitochondria and probably in some elements of the sacroplasmic reticulum; succinic dehydrogenase (*15*), an oxidative enzyme in mitochondria; and adenosine triphosphatase at *p*H 9.4 (*16*), for myofibrillar adenosine triphosphatase activity. The same adenosine triphosphatase reaction was also carried out after prior incubation (preincubation) of serial sections in acetate buffer at *p*H 4.65 (*17*), and also after preincubation in edetic acid buffer at *p*H 4.35 (*18*). These preincubation procedures have been useful in differentiating muscle fiber types (*17*, *18*). Glycogen-depleted fibers were not found in unstimulated control muscles.

Table 1. Comparison of several physiological parameters of gastrocnemius motor units with histochemical profiles of muscle fibers belonging to physiologically characterized motor units. Units were assigned to different physiological types on the basis of fatigue index and presence or absence of "sag" in unfused tetani. Diphosphopyridine nucleotide dehydrogenase, DPNH dehydrogenase; succinic dehydrogenase, SDH.

Unit characteristics	Motor unit types		
	FF	FR	S
Physiological properties			
Fatigue index	< 0.25	> 0.75	> 0.75
"Sag" in unfused tetanus	Present	Present	Absent
Twitch contraction time			
Range (msec)	20 to 47	30 to 55	58 to 110
Median (msec)	34	40	73
Tetanic tension (maximum)			
Range (g)	30 to 130	4.5 to 55	1.2 to 12.6
Median (g)	60	20	5
Number of units	51	32	31
Histochemical profiles			
Myofibrillar adenosine triphosphatase staining	High	High	Low
Adenosine triphosphatase staining*	Low	Low	High
Adenosine triphosphatase staining†	Moderate	Low	High
DPNH dehydrogenase and SDH staining	Low	Moderate to high	High
Number of units	17	7	5

* Preincubation at *p*H 4.35. † Preincubation at *p*H 4.65.

After prolonged repetitive stimulation of a single motoneuron, the muscle fibers innervated by it were depleted of glycogen and were thereby identifiable in PAS-stained sections as unstained fibers outlined against the red staining fibers of surrounding unstimulated motor units (*5*, *19*) (Fig. 2, column 1). Oxidative enzyme and adenosine triphosphatase staining reactions were unaltered by such stimulation.

The histochemical profiles of 27 physiologically characterized gastrocnemius motor units have been studied (see Fig. 2 and Table 1). The distribution of the reaction product of the oxidative enzyme within fibers was different for each motor unit type (*20*). Muscle fibers of FF units were usually large in diameter and sparsely supplied with capillaries. Fibers of FR units were variable in diameter and those of S units were generally small. Fibers of both FR and S motor units were liberally supplied with capillaries.

Our study of the present material indicates that all of the muscle fibers in a given motor unit have the same histochemical profile. No exceptional fibers have been found in the units systematically examined on this point. This is essentially in accord with previous results (*5*, *19*), although in the rat occasional exceptional fibers have been described (*5*).

In addition to showing that the fatigue susceptibility of motor units of cat gastrocnemius can be correlated with the oxidative enzyme activity of their muscle fibers, as noted earlier in the rat (*5*), we have now demonstrated that contraction speed and several other physiological parameters are, in a tripartite classification scheme, highly correlated with a number of morphological and histochemical characteristics of the muscle fibers in the same motor units. Unstimulated muscle fibers with histochemical profiles and morphology like those of FF and FR unit fibers were rich in glycogen whereas those similar to S unit fibers were glycogen-poor. The implied abundance of glycogen in FF unit fibers, considered together with the low oxidative enzyme activity and sparse capillary supply, suggest that they depend primarily on anaerobic glycolysis for energy [see (*1*) and (*21*)]. This could explain their rapid fatigue, since glycogen is quickly depleted during repetitive activity (*11*). In contrast, the fatigue-resistant S unit fibers have less glycogen [that is, not readily depleted, see (*11*)], high oxidative enzyme activity, and a rich capillary supply, which suggests primary utilization of aerobic energy pathways. Fibers of FR units appear to have both aerobic and anaerobic capabilities. These interrelated

morphological, histochemical, and physiological characteristics are very likely connected with the ways in which the different motor unit groups, even within a single muscle, are utilized by the central nervous system in various types of movement [see (*2*) and (*22*)].

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8. The decline in tension during repetitive stimulus trains over the 2-minute period used to assess the fatigue index did not appear to be due to fatigue of the neuromuscular junction. Muscle fiber action potentials (EMG responses) recorded from the muscle surface over the stimulated units usually changed little over this period. This suggests that the number of muscle fibers participating in the mechanical response was essentially constant and indicates that the diminution of mechanical tension output was due primarily to fatigue in the excitation-contraction coupling mechanism or in the contractile mechanism itself.
9. EMG responses were of constant shape and amplitude throughout unfused tetani showing "sag," which suggests that the tension decline was not due to muscle fibers dropping out of response. Other evidence suggests that the "sag" is not due to true fatigue but rather results from subtle changes in the kinetics of excitation-contraction coupling or in the contractile mechanism.
10. As used here, contraction time denotes the interval between initiation of the EMG response and the peak of the mechanical twitch response. Maximally potentiated twitches, recorded after a series of repeated short tetani (posttetanic potentiation), were used for these measurements in order to have all of the units in the series in an approximately comparable state. This represents a difference in technique from an earlier study (*6*) and appears to account in part for differences from the earlier results.
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20. The reaction products of the DPHND and SDH stains in fibers of type FF units were distributed in a fine, very interrupted intermyofibrillar network that sometimes showed a tendency to increase in density toward the fiber periphery. In FR unit fibers, the particles were distributed in a coarser, interrupted intermyofibrillar network that usually showed a tendency to increase in density toward the periphery, with subsarcolemmal clumps. In fibers of type S units, the reaction products were distributed uniformly in a dense uninterrupted network without peripheral accumulation, and subsarcolemmal clumps were not prominent. The pattern of distribution of these reaction products has been used in the past as an important criterion in the classification of muscle fiber types [J. M. Stein and H. A. Padykula, *Amer. J. Anat.* **110**, 103 (1962)].
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Creation of "Amyloid" Fibrils from Bence Jones Proteins in vitro

Abstract. *"Amyloid" fibrils have been created from some human Bence Jones proteins by proteolytic digestion under physiologic conditions. These fibrils with an antiparallel, β-pleated sheet conformation consist of only a portion of the variable region of the immunoglobulin light polypeptide chain and share the physical properties of amyloid fibrils. The relation between amyloidosis and immunoglobulins is thus more firmly established and a pathogenetic mechanism for amyloid fibril formation is suggested.*

Amyloid fibrils, the structures that are generally recognized as the characteristic and lethal component deposited in tissues in the disease amyloidosis (*1*), have certain well-defined properties. These include a green polarization birefringence after being stained with Congo red (*2*), a protein consisting of polypeptide chains in an antiparallel conformation and β-pleated sheet structure, as judged by x-ray crystallography (*3*), a distinguishing (*4*) but occasionally variable appearance (*5*) when viewed by electron microscopy, and a relative resistance to enzymic degradation (*6*). Evidence obtained by amino acid sequence and immunochemical studies of purified amyloid fibril proteins (*7*) suggests that amyloid fibrils consist primarily of the amino-terminal variable segment of the light polypeptide chain of homogeneous immunoglobulins in those cases studied. If indeed this is their source, then it should be possible to produce a variable region fragment having the characteristics of amyloid

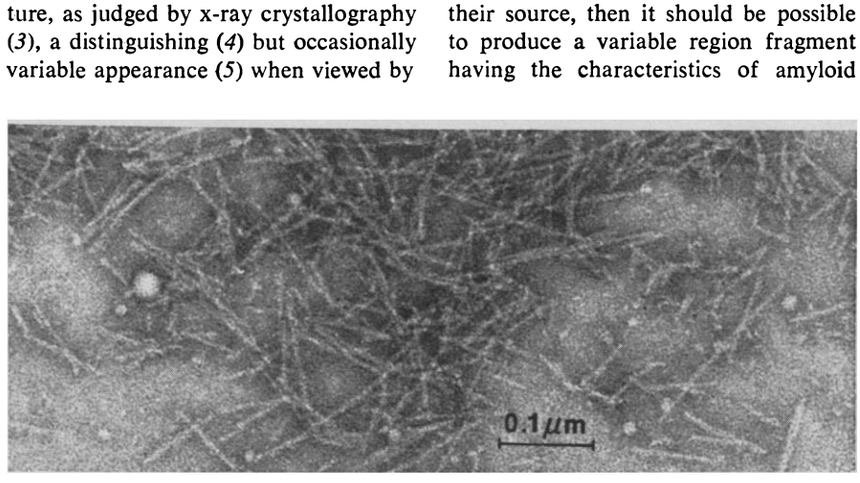

Fig. 1. Electron micrograph of fibrils formed by peptic digestion of a λ Bence Jones protein, Nic, after incubation at *p*H 3.5 for 2 hours at 37°C.