

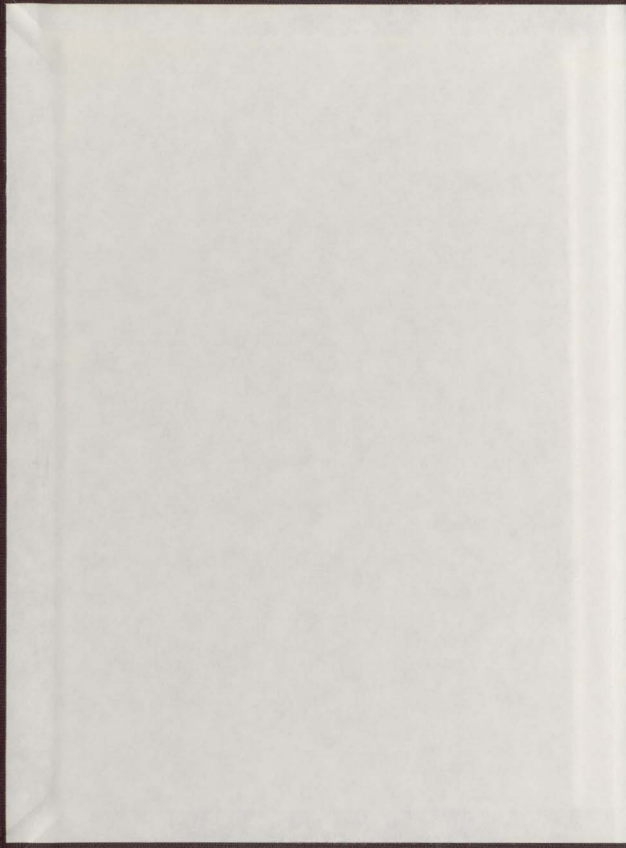
THE MECHANICAL AND HISTOCHEMICAL
CHARACTERISTICS OF AN IN VIVO
NERVE-MUSCLE PREPARATION OF NORMAL
AND DYSTROPHIC AVIAN MUSCLE:
EFFECTS OF SEX, DISEASE AND AGE

CENTRE FOR NEWFOUNDLAND STUDIES

**TOTAL OF 10 PAGES ONLY
MAY BE XEROXED**

(Without Author's Permission)

SUSAN ELLEN HOWLETT



141039



THE MECHANICAL AND HISTOCHEMICAL
CHARACTERISTICS OF AN IN VIVO NERVE-MUSCLE PREPARATION
OF NORMAL AND DYSTROPHIC AVIAN MUSCLE:
EFFECTS OF SEX, DISEASE AND AGE

by



Susan E. Howlett, B.Sc.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Medicine
Memorial University of Newfoundland

October, 1982

St. John's, Newfoundland

Canada

Abstract

An in vivo nerve-muscle preparation of the posterior latissimus dorsi muscle (PLD) has been used extensively to study contractile, pharmacological and electrophysiological characteristics of avian muscles affected by hereditary muscular dystrophy. The use of the preparation is limited since there is no convenient artery for cannulation to assess the acute effects of substances of potential therapeutic value. A preparation that appears to meet this requirement, the extensor digitorum communis (EDC) muscle preparation, is developed in the present study. The mechanical and histochemical characteristics of the EDC muscle compare favorably to those reported for normal and dystrophic avian muscle in the PLD preparation.

The gene for muscular dystrophy in chickens is thought to be autosomal recessive but personal observations and communications with some workers in the area suggest that the gene is expressed differently in males and females. The mechanical characteristics of the EDC muscle have been examined in old and young groups (old - greater than 6 months; young - 6-9 weeks) of normal and dystrophic chickens with respect to sex. Results showed that age-related sex differences were apparent for mechanical parameters known to distinguish normal and dystrophic birds, that is ability to rise from the supine position.

wing apposition score, post-tetanic potentiation, twitch-tetanus ratio and post-tetanic contracture. The sex differences observed in young birds indicate that the females were more severely affected by the disease than were the males. In the older birds the contractile responses of the EDC in males showed classic signs of dystrophy while females were often not significantly different from normal. If the inheritance pattern is truly autosomal recessive then perhaps there is an endogenous "protective" factor in the mature female. It is suggested that the lowered plasma cholesterol in the egg laying hen could account for the observed sex differences. Defects have been reported in a variety of membranes in human and animal muscular dystrophies. Since cholesterol is known to decrease membrane fluidity and since increases in plasma cholesterol have been reported in dystrophic chickens, a dramatic decrease in plasma cholesterol could perhaps improve the dystrophic condition.

ACKNOWLEDGEMENTS

To my supervisor, Dr. Ted Hoekman, for his understanding and patient teaching, I extend my heartfelt thanks. I would also like to thank the members of my supervisory committee, Dr. Detlef Beiger and especially Dr. Sue White who gave me encouragement when I needed it the most. Conversations with Dr. Paul Redfern and statistical advice from Dr. Dave Bryant and Dr. Jack Strawbridge proved both informative and invaluable.

I greatly appreciate the technical help I received, from Janet Robinson and Jim Ross in our lab, from the audiovisual department in the Faculty of Medicine and from the secretaries on the fifth floor.

I would like to acknowledge the financial support I received in the form of a University Fellowship from the office of the Dean of Graduate Studies.

Finally, I would like to thank my family and my friends; they understood when I was discouraged and always urged me to go on.

Table of Contents

	Page
List of Tables	ix.
List of Figures	xx
Chapter 1	
Introduction	1
1.1 The Definition of Muscular Dystrophy	1
1.2 Hereditary Animal Models of Muscular Dystrophy	1
1.3 The Genetically Dystrophic Chicken	5
1.3.1 Clinical features of avian dystrophy	5
1.3.2 Inheritance pattern of avian dystrophy	5
1.3.3 The pedigree of the dystrophic chicken	8
1.3.4 Gross pathological changes	9
1.3.5 Changes in interstitial tissues	10
1.3.6 Microscopic pathology of the cell organelles	11
1.3.7 Microscopic pathology of the sarcotubular system	12
1.3.8 Histochemical profile of the dystrophic avian muscle fibre	15
1.3.9 Muscle enzyme abnormalities	18
1.3.10 Acetylcholinesterase distribution in dystrophic muscle	22
1.3.11 Myotonia in dystrophic chicken muscle	26
1.3.12 Contractile characteristics of dystrophic chicken muscle	27
1.3.13 Electrophysiology of dystrophic chicken muscle	29
1.4 The Rationale for the Present Study	31
1.4.1 Problems with existing <u>in vivo</u> avian nerve-muscle preparations	31
1.4.2 Identification of a suitable <u>in vivo</u> preparation	32
1.4.3 The <u>in vivo</u> EDC nerve-muscle preparation	33
1.4.4 The aims of this research	33
Chapter 2	
Materials and Methods	
2.1 Birds	37

2.2	Clinical Assessment	38
2.2.1	General	38
2.2.2	Exhaustion Score	38
2.2.3	Wing Apposition Score	38
2.3	Surgery	43
2.3.1	Preparatory surgery	43
2.3.2	Exposure of the EDC and the radial nerve	43
2.3.3	Immobilization of the preparation	48
2.3.4	Stimulating and recording electrodes	48
2.3.5	Core temperature and vital signs	51
2.4	Mechanical and Electrical Circuits	51
2.4.1	Recording circuits	51
2.4.2	Stimulus control circuits	52
2.5	Experimental Protocol	52
2.6	Measurement Techniques	54
2.6.1	Post-tetanic potentiation and twitch/tetanus ratio	54
2.6.2	Twitch contraction time and half-relaxation time	54
2.6.3	Latent period, duration of the active state plateau, tetanus half-relaxation time and post-tetanic contracture	57
2.6.4	Maximal rate of rise of tension	64
2.6.5	Grams tension/muscle wet weight	64
2.6.6	Statistical analysis	67
2.7	Histology	67
2.7.1	SDH and myosin ATPase reactions	67
2.7.2	AChE stain	68

Chapter 3

Results

3.1	Clinical Signs of Dystrophy	69
3.1.1	General	69
3.1.2	Exhaustion score	69
3.1.3	Wing apposition	72
3.2	Mechanical Characteristics of Dystrophy	77
3.2.1	Post-tetanic potentiation	77
3.2.2	Twitch/tetanus ratio	84
3.2.3	Twitch contraction time	95
3.2.4	Twitch time to half-relaxation	95
3.2.5	Latent period	100
3.2.6	Duration of the active state plateau	100

3.2.7	Tetanus time to half-relaxation	105
3.2.8	Post-tetanic contracture	110
3.2.9	Maximal rate of rise of tension	110
3.2.10	Twitch-grams tension/muscle wet wt.	121
3.2.11	Tetanus-grams tension/muscle wet wt.	124

3.3 Histology

3.3.1	Succinate dehydrogenase stain	124
3.3.2	Myosin ATPase stain	129
3.3.3	Acetylcholinesterase stain	134

Chapter 4

Discussion

4.1	Histochemical Characterization of the <u>In Vivo</u> EDC Nerve-Muscle Preparation	137
4.1.1	SDH and myosin ATPase muscle histochemistry	137
4.1.2	Acetylcholinesterase histochemistry	138
4.2	Mechanical Characteristics of the <u>In Vivo</u> EDC Nerve-Muscle Preparation	139
4.2.1	Twitch contraction time and half-relaxation time	139
4.2.2	Twitch and tetanus-grams tension/muscle wet wt.	143
4.2.3	Twitch/tetanus ratio	144
4.2.4	Latent period	144
4.2.5	Duration of the active state plateau and tetanus half-relaxation time	145
4.2.6	Rate of rise of tetanic tension	149
4.2.7	Post-tetanic potentiation	150
4.2.8	Post-tetanic contracture	152
4.3	Sex Differences in Hereditary Avian Muscular Dystrophy	154
4.3.1	Exhaustion score	154
4.3.2	Wing apposition score	156
4.3.3	Mechanical characteristics	156
4.4	The Effects of Age on the Mechanical Characteristics of Normal and Dystrophic Muscle	159
4.5	A Possible Explanation for Sex Differences Observed in the Phenotypic Expression of Hereditary Avian Muscular Dystrophy	160
4.5.1	A generalized membrane defect in muscular dystrophy?	160
4.5.2	Why does dystrophy fail to progress in female dystrophic chickens?	162

4.5.3	Altered membrane cholesterol as a primary defect in muscular dystrophy	163
4.5.4	Exercise and cholesterol	166
4.5.5	Conclusions.	167
	Summary	169
	References	171
	Appendix 1	183
	Appendix 1.1	183
	Appendix 1.2	186

List of Tables

Table		Page
1.	Classification of the Muscular Dystrophies	3
2.	The Weights of Normal vs. Dystrophic Birds for the Young Group	71
3.	The Mechanical Responses of the PLD and EDC Muscles in Normal (line 412) and Dystrophic (line 413) Chickens	141

x.

List of Figures

Figure	Page
1. Pedigree of the Dystrophic Chicken	7
2. Muscles of the Distal Avian Wing	35
3. The Exhaustion Score	40
4. The Wing Apposition Score	42
5. Tracheotomy and Jugular Cannulation	45
6. The EDC Nerve-Muscle Preparation	47
7. Plexiglass Chamber	50
8. Sample Twitch and Tetanic Responses	56
9. Measurement of Twitch Contraction and Half-Relaxation Times	59
10. Measurement of Active State Plateau, Latent Period and Tetanus Half-Relaxation Time	61
11. Measurement of Post-Tetanic Contracture	63
12. Calibration of Force Transducer	66
13. Exhaustion Score: Young and Old Groups	74
14. Wing Apposition Score: Young and Old Groups	76
15. Interaction Effects for Wing Apposition Score	79
16. Post-Tetanic Potentiation vs. Frequency Young Group	81
17. Post-Tetanic Potentiation vs. Frequency Old Group	83

18.	Interaction Effects for Post-Tetanic Potentiation: Young Group	86
19.	Interaction Effects for Post-Tetanic Potentiation: Old Group	88
20.	Twitch-Tetanus Ratio vs. Frequency: Young Group	90
21.	Twitch-Tetanus Ratio vs. Frequency: Old Group	92
22.	Interaction Effects for Twitch-Tetanus Ratio: Young Group	94
23.	Twitch Contraction Time: Young and Old Groups	97
24.	Interaction Effects for Twitch Contraction Time	99
25.	Twitch Half-Relaxation Time: Young and Old Groups	102
26.	Latent Period: Young and Old Groups	104
27.	Tetanus Half-Relaxation Time: Young and Old Groups	107
28.	Duration of the Active State Plateau: Young and Old Groups	109
29.	Post-Tetanic Contracture vs. Frequency: Young Group	112
30.	Post-Tetanic Contracture vs. Frequency: Old Group	114
31.	Interaction Effects for Post-Tetanic Contracture: Young Group	116

32.	Rate of Rise vs. Frequency: Young Group	118
33.	Rate of Rise vs. Frequency: Old Group	120
34.	Twitch Tension per Gram of Muscle: Young and Old Groups	123
35.	Tetanic Tension per Gram of Muscle: Young and Old Group	126
36.	Interaction Effects for Tetanic Tension per Gram of Muscle	128
37.	Succinate Dehydrogenase Stain	131
38.	Myosin ATPase Stain	133
39.	Acetylcholinesterase Stain	136
40.	Active State Plateau Duration and Concomitant Muscle Twitch	147

Chapter 1

Introduction

1.1 The Definition of Muscular Dystrophy

The muscular dystrophies are a group of genetically determined disorders characterized by progressive necrosis of the muscle fibres accompanied by weakness. On the basis of clinical presentation, inheritance pattern, age of onset, distribution of affected muscles and the presence or absence of myotonia, cases of muscular dystrophy can be divided into a number of categories (see Table 1); the distinction between disorders based on pathological material is much less clear. The nature of the primary defect in muscular dystrophy has yet to be unequivocally demonstrated. There is no known cure for any of these conditions; in the worst cases (and in all cases of Duchenne muscular dystrophy) the disease is eventually fatal.

1.2 Hereditary Animal Models of Muscular Dystrophy

Animal models of the disease which satisfy the

Table 1. Classification of the muscular dystrophies
(adapted from Gardner-Medwin, 1980).

CLASSIFICATION OF THE MUSCULAR DYSTROPHIES

- 1) X-Linked Recessive Muscular Dystrophies
 - Severe (Duchenne)
 - Benign (Becker)
 - Benign with early contractures (Dreiffuss)
 - Scapulo-peroneal ?
- 2) X-Linked Dominant Muscular Dystrophies
 - Muscular dystrophy confined to females
- 3) Autosomal Recessive Muscular Dystrophies
 - Scapulohumeral (? limb girdle)
 - Quadriceps myopathy
 - Autosomal recessive muscular dystrophy of childhood
 - Congenital
- 4) Autosomal Dominant Muscular Dystrophies
 - Fascioscapulohumeral
 - Scapulo-peroneal ?
 - Proximal (late onset)
 - Distal (infantile onset)
 - Distal (adult onset)
 - Ocular
 - Oculopharyngeal
 - Myotonic

4

criteria used to define human dystrophies, notably the hamster, the mouse and the chicken, have been extensively studied. Although no animal myopathy is a "perfect" model of any human dystrophy, considering the difficulty encountered in distinguishing the various human disorders it is perhaps unreasonable to expect that one exists. Certain features of the animal models are consistent with many aspects of the human dystrophies (Mendell et al., 1979). The morphological changes in the muscle fibres of the dystrophic hamster are not marked but this animal appears to be a superior model for studying the cardiomyopathy that figures prominently, particularly in the terminal states, in patients with muscular dystrophy (Hunter, 1980). Murine dystrophy is characterized by extensive necrosis of the muscle fibres closely resembling that in the human disorders. The profound neural involvement in the dystrophic mouse model, as evidenced by myelination of the spinal roots (Bradley and Jenkinson, 1973; Jaros and Bradley, 1978) and impaired impulse transmission (Rasminsky, 1978; Rasminsky, 1980), is perhaps a fundamental difference between murine muscular dystrophy and human muscular dystrophy. On the other hand, the presence of defects in systems other than the neuromuscular system indicates there is a multisystem defect in this model; multisystem defects have also been reported in several of the human dystrophies.

1.3 The Genetically Dystrophic Chicken

1.3.1 Clinical features of avian dystrophy.

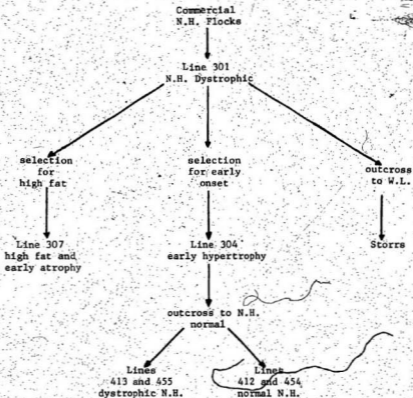
Muscular dystrophy of the domestic chicken was first reported by Asmundson and Julian in 1956. The mutation originated in commercial lines of New Hampshire chickens, selectively bred to produce birds with large pectoral muscles. These birds were unable to right themselves when placed on their backs and there was excessive resistance to passive apposition of the wings behind the back at the elbow joints. The inability of dystrophic birds to rise from the supine position is a reliable assay of dystrophy and can statistically discriminate normal and dystrophic birds in preclinical stages as early as 10 days posthatch (Wilson et al., 1979). In addition, the dystrophic birds have a broad body, short thick limb bones and manifest an initial muscle hypertrophy followed, in some birds, by severe atrophy (Julian and Asmundson, 1963). The disease is progressive in chickens but it does not substantially shorten the life span of affected birds.

1.3.2 Inheritance pattern of avian dystrophy.

Traditionally, muscular dystrophy of the domestic fowl has been attributed to a simple autosomal recessive gene, however, it may be codominant since there is some penetrance of the gene in heterozygotes (Julian and

Figure 1. The pedigree of the dystrophic chicken (adapted from Wilson et al., 1979).

Pedigree of the Dystrophic Chicken



Asmundson, 1963). The gene is not sex linked but is possibly expressed differently in males and females (Julian and Asmundson, 1963; Wilson et al., 1979). This distinction has not been systematically evaluated.

1.3.3 The pedigree of the dystrophic chicken. The original mutant, line 301, was a somewhat heterogeneous mixture of birds, some birds undergoing early muscle hypertrophy and some birds undergoing early muscle atrophy (Julian and Asmundson, 1963). Subsequent selection for birds with high fat content and early atrophy of the pectoral muscles produced the line 307 birds and selection for early onset of symptoms accompanied by pectoral hypertrophy gave rise to the line 304 birds (see Figure 1). Birds from line 304 were outcrossed to normal New Hampshire birds and progeny from the F2 generation were segregated to produce lines of homozygous dystrophic birds (lines 413 and 455) and lines of genetically matched controls (lines 412 and 454) (see Figure 1). It was hoped that in addition to creating lines of dystrophic birds with genetically matched controls, the out-crossing of the original line would diminish or eliminate those characteristics potentially due to "background" genes and limit the phenotypic expression of the gene to those characteristics determined by the dystrophic gene. The recent emergence of lines 412 and 413 has prompted a plethora of studies re-evaluating the nature of the phenotypic expression of the gene. Lines 412 and

413 are now extensively used in research; lines 454 and 455 are less frequently employed (Wilson et al., 1979). The so-called "Storrs" strain was produced by selection of birds which were homozygous dystrophic from among the offspring of the original line 301 New Hampshire birds outcrossed to normal White Leghorn birds (see Figure 1). Normal White Leghorn chickens are generally used as controls for the Storrs birds. Randall and Wilson (1980) have recently shown that certain "classical" biochemical and physiological manifestations of the dystrophic phenotype differ in the various lines of dystrophic chickens (Randall and Wilson, 1980).

1.3.4 Gross pathological changes. The physical characteristics of whole dystrophic muscle differ markedly from normal, even early in the disease. Affected muscles are firm, tough, opaque (normal avian muscles are somewhat translucent) and relatively resistant to the blade of a scalpel. When removed from the body, normal muscle is pliable and, if folded, retains its shape. By contrast, dystrophic muscles are "rubbery" and fail to retain a folded shape (Julian and Asmundson, 1963).

Although the majority of the skeletal muscle mass is involved in advanced stages of the disease, initially there is selective involvement of the "white" muscles (Julian and Asmundson, 1963).

In the older lines of dystrophic chickens a wide

variation in fibre size was apparent; muscles exhibited both hypertrophic fibres and atrophic fibres in cross-section but few fibres of normal size (Julian and Asmundson, 1963; McMurtry et al., 1972). Crowe and Baskin (1979) reported a large percentage of hypertrophied fibres in line 413 birds; few atrophied fibres were evident. Splitting of hypertrophied fibres (thought to be a compensatory response to increase the surface area and improve the oxygen perfusion of the hypertrophied fibres thus facilitating aerobic metabolism) and large regions of actively degenerating fibres are features of the disease common to all major lines of dystrophic chickens (Julian and Asmundson, 1963; McMurtry et al., 1972; Libelius et al., 1979).

1.3.5 Changes in the interstitial tissues. Normal muscle fibres are tightly packed and adopt irregular polygonal shapes in cross-section. The loose packing and elliptical shape of dystrophic fibres in cross-section results from the loss of contractile material and the marked increase in the connective tissues surrounding individual dystrophic fibres in line 301 (Julian and Asmundson, 1963), line's 304 and 307 (McMurtry et al., 1972) and line 413 (Crowe and Baskin, 1979) chickens. The origin of the increase in connective tissues has not yet been elucidated. Fat infiltration and replacement of degenerating dystrophic muscle fibres by adipose tissue has

been demonstrated in all major lines of dystrophic chickens (Julian and Asmundson, 1963; Cosmos, 1970; McMurtry et al., 1972; Crowe and Baskin, 1979). The origin of these adipocytes in regions of muscle vacated by necrotic fibres is unknown. It has been suggested that either these are not true adipocytes but phagocytes capable of storing fat or there is a failure of fat metabolism in necrotic cells (Cosmos, 1970).

1.3.6 Microscopic pathology of the cell organelles. Crowe and Baskin (1979) reported a decrease in the total volume of cell organelles and an increase in the amount of empty cytoplasm in line 413 chickens. The total volume of myofibrillar material was shown to decrease in the same birds (Crowe and Baskin, 1979). The loss of myofibrillar protein and cell organelles appears to be a result of active lysosomal destruction of materials in degenerating fibres.

Central nucleation, a prominent abnormality of the muscle fibre reported in a variety of human and animal dystrophies, is a common feature of normal chicken muscle and, as such, is not a reliable marker of avian dystrophy (Julian and Asmundson, 1963). Other aberrations in the nuclei of dystrophic chickens are apparent; a slight increase in the volume fraction of nuclei and a large increase in the cross-sectional area of individual nuclei have been demonstrated in line 413 chickens (Crowe and

Baskin, 1979). McMurtry et al. (1972) found an increase in the total number of nuclei in several of the older lines of dystrophic chickens using stereological techniques. This increase was primarily evident in the peripheral nuclei (defined as those nuclei which touch the sarcolemma). Recently, Yorita et al. (1980) showed an increase in the total number of nuclei in line 413 birds, and in particular, an increase in the number of satellite cell (dormant myoblasts) nuclei; satellite cells are situated at the periphery of muscle cells.

Increases in the number and decreases in the size of mitochondria resulting in an overall increase in the surface to volume (S/V) ratio have been reported in the pectoralis (Crowe and Baskin, 1979) and the PLD (Beringer, 1978) muscles of line 413 chickens. This may be a another compensatory response to muscle fibre hypertrophy; as the fibre diameter increases the conditions for aerobic metabolism are progressively less favorable.

1.3.7 Microscopic pathology of the sarcotubular system. Earliest reports on the ultrastructure of dystrophic chicken muscles did not examine the sarcotubular systems, probably because of technological limitations. Shafiq et al. (1972) reported dilation and proliferation of the sarcoplasmic reticulum (SR) of line 304 birds, however, a subsequent study (Malouf and Sommer, 1976) used an extracellular marker to show that SR vesicle preparations

were often contaminated with vesicles from the transverse tubules (t-tubules). That enquiry demonstrated that it was dilation and proliferation of the t-tubules and not of the SR which caused the vesiculations seen in the dystrophic muscles of line 304 birds. Using stereologic analysis of the pectoralis in line 413 birds, Crowe and Baskin (1979) observed an increase in the S/V ratio of the t-tubules which seemed to be caused in part by dilation and vacuolization in the longitudinal direction. A decrease in the S/V ratio of the SR was accompanied by a loss of orientation at the triad structure. Beringer (1978) used a similar set of techniques on the PLD of line 413 birds; he found increases in the S/V ratio of the t-tubules and decreases in the S/V ratio of the SR. The changes were less marked in the PLD than in the pectoralis.

A decrease in the number of intramembrane particles, thought to represent a decrease in the number of ion channels or functional enzymes, has been reported in the membranes of both the t-tubules (Crowe and Baskin, 1978) and the SR (Beringer, 1978; Crowe and Baskin, 1978) of dystrophic (line 413) chickens. In the case of the t-tubules this does not necessarily represent reduction of the functional capacity of a unit area of membrane since the increased surface density of the t-tubule membranes results in a 125 % increase in the number of particles per unit volume of muscle (Crowe and Baskin, 1979). The t-tubule proliferation may be a compensatory response to

muscle fibre hypertrophy, to preserve the relationship between extracellular space and the volume of muscle tissue. Unequivocal determination of the number of intramembrane particles in the SR proved difficult. The number of intramembrane particles per unit volume of muscle appears dramatically reduced in the free SR; however, in the junctional SR, the presumed site of active calcium release and reaccumulation, the number of particles per unit volume of muscle is unchanged (Beringer, 1978; Crowe and Baskin, 1979). If calcium release and calcium sequestration are accomplished to the same extent by all the SR, these functions may be compromised in dystrophic muscle. On the other hand, if junctional SR is primarily responsible for release and/or reaccumulation of calcium, these functions are probably not dramatically altered in dystrophic chicken muscle.

Injection of the extracellular marker, horseradish peroxidase, has demonstrated that the the marked vacuolization (the formation of regions of active fibre degeneration within the myofibril) of dystrophic muscle is a result of t-tubule endocytosis (Libelius, et al., 1979). They proposed that lysosomal enzymes, which have been reported to be increased in dystrophic muscle (Bird, 1975), are incorporated into the fibre by t-tubule endocytosis; subsequent activation of lysosomes within the muscle fibre caused the degeneration.

1.3.8 Histochemical profile of the dystrophic avian muscle fibre. Since the earliest reports that the so-called "white" muscles of the dystrophic chicken were severely affected by the disease while the "dark" muscles were seemingly spared (Julian and Asmundson, 1963), the phenomenon has been extensively investigated. Attempts to quantify these rather subjective observations have been hampered by the inability of researchers to agree upon a scheme of classification for the different muscle fibre types (Burke and Tsaiiris, 1974).

In the adult avian and amphibian skeletal muscles, two physiologically distinct muscle fibre types exist, the tonic (red, slow, beta, dark) and the phasic (white, fast, alpha, twitch) fibres. They have been characterized on the basis of many differences of which the following are of primary importance. (1) Tonic muscle fibres are multiply innervated, they have more than one end plate per fibre whereas those in phasic muscle are focally innervated, having a single end plate per fibre; (2) phasic muscle fibres respond to stimulation with an action potential while mature tonic muscle fibres produce a graded potential; (3) the time course of the phasic contraction is short relative to that of the tonic contraction; (4) tonic muscle fibres are small in diameter, contain many mitochondria and possess a profuse blood supply while phasic fibres are large diameter, rich in glycolytic but not oxidative enzymes and are less

extensively vascularized. Mammalian muscles, with rare exceptions, contain no tonic fibres. Muscles referred to as fast and slow in mammals are focally innervated and all produce an action potential. Fast muscle fibres are large in diameter, fast contracting and rich in glycolytic enzymes, while slow muscle fibres are small in diameter, slow contracting and have abundant supplies of the enzymes involved in oxidative metabolism. Different authors have reported varying numbers of intermediate fibre types possessing characteristics particular to both fast and slow fibres (Burke and Tsairis, 1974). Intermediate fibres have also been reported in avian and amphibian muscles.

In the chick embryo, alignment of the myoblasts and subsequent fusion to form myotubes can occur without the influence of the nerve but further development (i.e. differentiation into specific fibre types) depends upon the fibre receiving successful innervation. Contact with the muscle cell is made in ovo by the growing nerve terminal between days 6-12. One explanation of how this occurs is that if enough acetylcholine (ACh) is released onto the muscle membrane (which is sensitive to ACh all over) by the first nerve to make contact, an action potential will be generated. This initial action potential is thought to cause the release of lysosomal enzymes which stop redundant contacts from being formed and decrease extrajunctional ACh sensitivity. This process results in a phasic fibre. Conversely if little ACh is released then only an epp is generated; the

decrease in extrajunctional ACh sensitivity and release of lysosomes occurs over the area of passive decay of the epp. Several contacts may be formed on one fibre, producing a multiply innervated tonic fibre. This hypothesis on the formation of nerve-muscle contacts has been suggested by Vrbova, Gordon and Jones (1978).

It has been suggested that this process is impaired in some way in dystrophy and thus dystrophic muscle retains some fetal muscle characteristics. Cosmos (1966) and Cosmos and Butler (1967) showed that fetal avian muscle had high levels of the enzymes associated with oxidative metabolism and low levels of the enzymes associated with glycolytic metabolism. They maintained that the neural signal to differentiate, that is to "switch" from fetal aerobic to adult anaerobic enzyme profiles, was required by the prospective phasic fibres only. In dystrophic muscle ontogenic analysis revealed that the ability of phasic muscles to complete the transition from fetal to adult characteristics was impaired; since tonic muscles were never asked by the nerve to differentiate fast fibre types, phenotypic signs of the disease were never expressed. Studies in which minced phasic muscle was transplanted between neonatal normal and dystrophic hosts showed that each muscle transplant expressed its own inherent genotype (that of the donor), regardless of the genotype of the host (Cosmos, 1974). Evidently, peripheral motor nerves of the

dystrophic genotype were capable of differentiating, regulating and maintaining normal muscle; dystrophic muscle was unable to respond to the neural dictate to differentiate fast fibre types. Cross-reinnervation of the phasic posterior latissimus dorsi (PLD) muscle with the nerve of the tonic anterior latissimus dorsi (ALD) muscle showed that a majority of the fibres in the cross-reinnervated PLD had adopted the characteristics of a tonic muscle and failed to express the phenotypic characteristics of dystrophy (Cosmos et al., 1979).

Recently, Kikuchi et al. (1980) mixed equal volumes of minced normal and dystrophic muscle and transplanted the minces into normal and dystrophic hosts. Regeneration of these tissues produced a mosaic fibre pattern with nuclei from each genotype expressing their inherent phenotype. Some fibres appeared to vary greatly along their length in the intensity of stain for an oxidative enzyme; it was concluded that these were hybrid fibres resulting from the fusion of myoblasts arising from both normal and dystrophic nuclei.

1.3.9. Muscle enzyme abnormalities. Plasma creatine phosphokinase (CPK) levels are usually dramatically increased in human muscular dystrophies and can often be used to distinguish pre-clinical cases of the disease. Elevated levels of CPK, presumably due to "leakage" from damaged muscle fibres, have been reported in

line 304 dystrophic chickens (Holliday et al., 1965) and in the newer line 413 birds (Wilson et al., 1979). In contrast, CPK levels were reduced in the muscles of dystrophic chickens (Mahler., 1979; Stewart et al., 1981). Examination of CPK isoenzymes in dystrophic chicken muscle has shown that the fetal muscle profile predominates; the MB and BB isoenzymes which were normally not present in adult muscle, were found in appreciable quantities in adult dystrophic (line 455) chicken muscle (Mahler, 1979). In addition, levels of the MM isoenzyme characteristically found in normal adult muscle were reduced in the dystrophic chicken (Mahler, 1979). Stewart et al. (1981) reported decreased muscle CPK and concomitant increases in serum CPK for the Starrs line of dystrophic chickens. Prior to hatching and at one week ex ovo, CPK activity was similar in normal and dystrophic birds; muscle levels of MB isoenzyme increased with age while MM levels decreased with age. Although Cosmos et al. (1979) have suggested that affected muscles in the dystrophic chicken never leave the fetal stage, Stewart et al. (1981) proposed that CPK activity regresses to the fetal stage in older birds.

Serum pyruvate kinase (PK) levels increased in parallel with the rate of appearance in plasma of CPK in line 413 birds (Liu et al., 1980). The observed isoenzyme in the plasma was not the embryonic form of PK (M2) but the isoenzyme normally found in mature muscle (M1). This study suggests that the muscle does indeed mature from

the fetal stage. It is interesting to note that regenerating muscle has been reported to give multiple CPK isoenzymes (MM, MB and BB) and the single isoenzyme (MI) of PK (Liu et al., 1980).

The relative amount of lactate dehydrogenase (LDH) in the muscles of dystrophic (Storrs) chickens was found to be reduced (Kaplan and Cahn, 1962). This was due to the failure of levels of the "M" isoenzyme, which normally predominates in adult fast of these findings is that the alterations in muscle isoenzyme profiles and the apparent shift from glycolytic to oxidative metabolism in dystrophic muscle may be interpreted as characteristic of regenerating muscle rather than as the retention of the muscle in the fetal state. This suggestion is consistent with the reported proliferation of satellite cells in dystrophic muscle which is actively undergoing degeneration twitch muscle, to increase during muscle maturation. Kaplan and Cahn (1962) demonstrated that the isoenzyme found both in dystrophic muscle and in normal slow tonic muscle is the "H" or fetal variety. A similar reduction of LDH in denervated muscle suggested that the effect may not be specific to the disease process but rather is due to generalized muscle damage (Dawson and Kaplan, 1965).

The relative proportions of the myosin isoenzymes, designated M1, M2 and M3 in order of electrophoretic mobility, were similar in normal (line 412) and dystrophic (line 413) chickens up to 3 weeks ex

ovo (Obinata et al., 1980). At three weeks M1 levels, which were initially low in newly hatched chicks, continued to increase in normal birds but began to fall dramatically in the dystrophic birds. By comparison, the M2 and M3 isoenzymes found in abundance in the neonate, declined in both groups until 3 weeks post hatch; levels continued to decline in the normal chickens and to increase in the dystrophic birds. Similar reports of fetal myosin predominance in human dystrophy have been recently cited in the literature (Fitzsimons and Hoh, 1981). Nevertheless, the biochemical evidence that the primary effect of avian dystrophy is to "fixate" the muscles in the undifferentiated embryonic state is not conclusive.

Cardinet et al. (1972) showed that glycolytic enzyme activities, specifically phosphorylase, phosphoglyceromutase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, LDH and glycerophosphate dehydrogenases with the notable exception of hexokinase, were decreased in dystrophic (line 301) chicken pectoralis muscles. Enzyme activity levels associated with oxidative metabolism, such as isocitrate dehydrogenase, malate dehydrogenase and glutamic oxaloacetate transaminase, were reported to be increased in the dystrophic pectoralis muscles (Cardinet et al., 1972). Consistent differences between glycolytic enzyme levels of normal and dystrophic lateral adductor muscles were not reported. One explanation of these findings is that the alterations in muscle isoenzyme

profiles and the apparent shift from glycolytic to oxidative metabolism in dystrophic muscle may be interpreted as characteristic of regenerating muscle rather than as the retention of the muscle in the fetal state. This suggestion is consistent with the reported proliferation of satellite cells in dystrophic muscle which is actively undergoing degeneration and regeneration.

Recently, Woodward et al. (1979) found increases in hexokinase activity in both the pectoralis (fast-twitch) and the thigh (slow-tonic) muscles in Storrs birds. Increases in glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase have been shown in the pectoralis muscles of line 413 chickens (King et al., 1981) and in the pectoralis and to a limited extent in the thigh muscles of Storrs birds (Park et al., 1979). These enzymes (hexokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) are associated with the pentose phosphate pathway, a pathway which produces pentose phosphates for nucleic acid synthesis and NADPH for reductive biosynthesis (i.e. fatty acid synthesis). Increased activity of these enzymes may reflect increased biosynthetic activity in hypertrophied muscle (King et al., 1981).

1.3.10 Acetylcholinesterase distribution in dystrophic muscle. Wilson et al. (1968) first reported overproduction of the enzyme, acetylcholinesterase (AChE),

in the fast twitch muscles of chickens with hereditary muscular dystrophy. Subsequent studies revealed that the pattern of AChE seen in dystrophic muscle was similar to that found in embryonic muscle. Specifically, Wilson et al. (1970) noted high levels of AChE, the presence of low molecular weight AChE isoenzymes and extrajunctional localization of AChE. In older birds the levels of this enzyme were dramatically increased in the plasma (Wilson et al., 1973).

Denervation experiments have shown that nerve-muscle interaction reversibly regulates AChE levels in the muscle. Severing the nerve causes a marked increase in extrajunctional AChE activity over the entire muscle fibre; in normal muscle the AChE is restricted to within a 50 μ m radius of the end plate whereas in dystrophic muscle the AChE radiates roughly 250 μ m from the end plates (Patterson and Wilson, 1976a; Patterson and Wilson, 1976b). Jedrzejczyk et al. (1973) found that the end plate levels of AChE (the bound synaptic fraction) were reduced in dystrophic chicken muscle whereas levels of the soluble sarcoplasmic fraction were increased. Recent characterization of the specific isoenzymes of AChE that constitute the soluble and insoluble fractions support these findings (Lyles et al., 1980).

It is apparent that AChE activity is under neural control. Wilson et al. (1975) have suggested that in dystrophic muscle some defect, possibly in the muscle fibre

membrane, interrupts the nerve "trophic" influence that represses the presence of embryonic AChE distribution. A recent report, however, showed that AChE levels were initially lower than normal in dystrophic embryonic muscle; the enzyme levels remained low until 2 weeks ex ovo at which point the levels rose dramatically to peak at roughly 4 months (Lyles et al., 1980).

Limb bud transplantation experiments performed prior to muscle differentiation and outgrowth support the idea that the primary lesion in muscular dystrophy is myogenic in origin. The nerves of dystrophic chickens were shown to be capable of regulating and maintaining normal muscles; conversely dystrophic muscles (limb buds) transplanted into normal hosts exhibit an AChE profile identical to that found in the muscles of genotypically dystrophic birds (Linkhart et al., 1975; Linkhart et al., 1976).

Recently, Johnson et al., (1981) demonstrated that cultured normal and dystrophic muscles were equally able to develop normal AChE activity implying that dystrophic muscle has the basic capacity to synthesize AChE. Subsequent addition of a neuronal fraction to the culture medium caused an increase in the AChE activity of the normal muscle but had no effect on the dystrophic muscle; the dystrophic muscle either failed to respond to the trophic influence of the nerve or failed to relay the appropriate "metabolic message".

Indirect evidence that the primary lesion is a failure of the muscle to respond to the regulatory influences of the nerve comes from the study of axonal transport, a process thought to play an important role in trophic function. DeSantis et al. (1976) found that retrograde axonal transport of the label, horseradish peroxidase, was similar in normal and dystrophic birds. Another study looked at the fast and slow components of anterograde and retrograde axonal transport of AChE isoenzymes and could find no difference in the flow rates of normal and dystrophic nerves (DiGiamberdino et al., 1979). A recent report compared the rate of axoplasmic transport of substances in the radial nerve in normal and dystrophic birds and concurred with earlier work, no difference in flow rate was noted (Strosska et al., 1981). It is important to note that this does not preclude qualitative differences in the nature of the substances transported in normal and dystrophic nerves.

Wilson et al. (1973) first found that plasma levels of AChE were dramatically elevated in dystrophic chickens, particularly in the older birds. Lyles et al. (1980) concur with these results and suggest that excess muscle AChE is actively eliminated into plasma; since AChE is normally bound to the post-synaptic membrane it probably does not leak out of "holes" in the membrane, a mechanism that has been proposed to explain the elevated plasma CPK and PK seen in avian dystrophy. The levels of

pseudocholinesterase or butyrylcholinesterase (BChE) isoenzymes are increased in the muscle but not in the plasma (Wilson et al., 1973; Lyles et al., 1980). Since AChE and BChE levels are controlled by similar mechanisms, it is surprising that plasma BChE is not increased in avian dystrophy. Lyles et al. (1980) suggest that excess muscle BChE is either not eliminated into plasma in the same way as AChE or that the addition of excess muscle BChE has no net effect on the circulating endogenous levels of AChE.

1.3.11 Myotonia in dystrophic chicken muscle.

Holliday et al. (1965) first described abnormalities in muscle electrical activity, detectable by EMG, in dystrophic (line 301) chicken muscles. They reported that impaling fast-twitch dystrophic muscle fibres with a recording electrode produced a spontaneous "myotonic" discharge, 5-15 seconds in duration, in birds as young as 16 days ex ovo; the severity of these abnormalities increased with age. Slow-tonic muscles exhibited nearly normal EMG characteristics, even in advanced stages of the disease. The heightened mechanical excitability and the failure of neuromuscular blockade with d-tubocurarine to antagonize this activity suggests that the dystrophic chicken fibre is myotonic. Repetitive myotonic volleys in response to mechanical stimulation have also been demonstrated in the biceps muscle at 17 days ex ovo (Linkhart et al., 1976) and in the PLD muscle at 25-38 days

ex ovo (Enrikin and Bryant, 1977) in line 413 birds.

1.3.12 Contractile characteristics of dystrophic chicken muscle. The in vivo mechanical responses of a muscle affected by avian muscular dystrophy, the PLD muscle of line 304 birds, have been determined (Hoekman, 1974; Hoekman, 1976). Similar results have recently been obtained for the PLD muscle in the newer line 413 birds (Hoekman, unpublished results).

The indirectly elicited muscle twitch generated significantly less tension per gram of muscle in the dystrophic PLD when compared to the normal PLD; the tetanic tension generated per gram of muscle was not reduced in dystrophic birds. The twitch-tetanus ratio was significantly depressed in line 304 and 413 birds reflecting the pronounced attenuation of the twitch response in dystrophic muscle. Twitch contraction time and time to half-relaxation were similar in normal and dystrophic muscle. Large increases in the post-tetanic potentiation observed in dystrophic muscle were reported. This increase was attributed to the propensity of the muscle membrane to hyperpolarize after repetitive stimulation (Albuquerque and Warnick, 1971).

Hyperpolarization would tend to increase the duration and amplitude of the action potential and result in a post-tetanic prolongation of the active state and an increase in twitch tension. The rate of rise of tension was markedly

reduced during the twitch and during the tetanus, probably due to an increase in the series elastic compliance. While the reduced rate of rise of the twitch in conjunction with a normal contraction time for that response is seemingly paradoxical, Hoekman (1976) suggested that if the active state plateau duration were reduced in dystrophic muscle a slower contracting muscle would generate less tension. Since Hoekman (1976) reported a significant increase in the frequency at which fusion occurred in dystrophic muscle, indirect evidence exists that the active state plateau is in fact, shortened in dystrophic muscle.

1.3.13 Electrophysiology of dystrophic chicken muscle. The electrophysiological characteristics of dystrophic chicken muscle were first examined by Albuquerque and Warnick (1971) in an in vitro preparation of the PLD muscle. They demonstrated pre- and post-synaptic alterations at the neuromuscular junction of muscle fibres of both Storrs and line 304 dystrophic birds. Specifically they found a decrease in the frequency and amplitude of miniature end plate potentials (mepps) and a concomitant increase in the mcpp time course. Batrachotoxin caused a dramatic increase in mcpp frequency, an effect blocked by tetrodotoxin, indicating that transmitter was available for use at the dystrophic neuromuscular junction. In a subsequent study (Warnick et al., 1979) it was shown that these changes in mcpp

frequency, amplitude and time course were mirrored in the newer line 413 birds. In addition, the mean quantal size was decreased, the mean quantal content increased and the facilitation of transmitter release severely attenuated in line 413 birds. By 12 weeks of age the mep frequency was reduced effectively to zero for both lines. Warnick et al. (1979) suggested that the depression of transmitter release could be the result of either decreased sodium conductance or defects in the mechanism for calcium translocation or both.

Values obtained for the muscle membrane cable properties such as the input resistance, the membrane resistance, the time constant and the membrane capacitance were attenuated in line 304 birds (Albuquerque and Warnick, 1971), however, examination of the cable properties in the newer line 413 showed that they were not significantly different from normal (Warnick et al., 1979). By definition, increased fibre diameter increases selected membrane cable properties. The presence of many fibres undergoing compensatory hypertrophy and thus unwittingly being "selected" for impalement could account for the observed increase in cable properties in the line 304 birds (Warnick et al., 1979). Muscle fibre hypertrophy cannot totally account for the observed altered cable properties since hypertrophied muscle fibres have also been demonstrated in line 413 birds (see section 1.3.4). It has been suggested that reduced ionic conductances (in

particular the sodium conductance) also contribute to the altered cable properties (Warnick et al., 1979). In addition, the outcrossing of the line 304 birds to produce line 412 and line 413 may have changed the nature of the expression of the disease.

Although indirect stimulation of dystrophic muscle initially produced an action potential, repetitive stimulation elicited a decrementing response eventually resulting in an end-plate potential (epp). The rate of rise of the action potential was significantly decreased. These findings are consistent with the idea that sodium conductance and/or calcium translocation mechanisms have been in some way compromised as a function of the disease process.

Warnick et al. (1979) compared the electrophysiological characteristics of the old lines 200 and 304 with the newer lines 412 and 413 using an in vitro preparation of the PLD muscle. The decay of the action potential to an epp with repetitive stimulation was noted in both lines 304 and 413 but the time course of the action potential was altered only for line 413. The resting membrane potential (RMP), overshoot and the threshold for generating an action potential were increased in the newer line.

It has been suggested that the dystrophic fibres may be "functionally denervated"; denervation of muscle may result in the following, a decrease in RMP, an increase in

extrajunctional acetylcholine sensitivity, the development of tetrodotoxin-resistant action potentials and an increase in membrane resistance. Although extrajunctional acetylcholine sensitivity (see section 1.3.10) and increased membrane resistance (Lebeda and Albuquerque, 1975) and impaired synaptic transmission have been reported in dystrophic chicken muscle, there is little evidence to support the view that dystrophic chicken muscles are even functionally denervated. Directly elicited action potentials and RMP's were not qualitatively different in line 200 and line 304 birds; tetrodotoxin resistant action potentials were never recorded in either normal or dystrophic denervated tissue. The long increase in membrane resistance may be explained as due to the combined effects of increased fibre diameter and alteration in ionic conductances (Lebeda and Albuquerque, 1975).

1.4 The Rationale for the Present Study

1.4.1 Problems with existing in vivo avian nerve-muscle preparations. Previous work done on in vivo dystrophic avian muscle has used the PLD preparation (Hoekman, 1976). Although this preparation has contributed substantially to our knowledge of the mechanical characteristics of dystrophic chicken muscle, there are

limitations to its use as a research tool. Primarily, there is no convenient artery to cannulate for close intra-arterial (IA) injection of microlitre quantities of drugs to assess the pharmacological properties of the preparation and to evaluate the acute effects of substances of potential therapeutic value. In addition, surgical access to the PLD involves major trauma to several overlying muscles, often resulting in bleeding and a general decrease in the viability of the preparation.

1.4.2 Identification of a suitable in vivo preparation. In an effort to eliminate these constraints, a new nerve-muscle preparation was sought. Bryan and Letinsky (1979) reported that alterations in the histochemical and electrophysiological properties of the dystrophic digit extensor muscle, the extensor digitorum II, conformed to those previously reported for other preparations. The superficial anatomical location, easy surgical access to muscle tendons and the relatively discrete blood supply of the digit extensor muscles made them suitable for study. Unequivocal identification of the extensor digitorum II muscle based upon available avian anatomy books proved impossible. Due to discrepancies in the nomenclature of avian wing muscles it is not possible to assert that the muscle reported in the present study is the extensor digitorum II muscle. The muscle examined in this study has been named the extensor digitorum communis

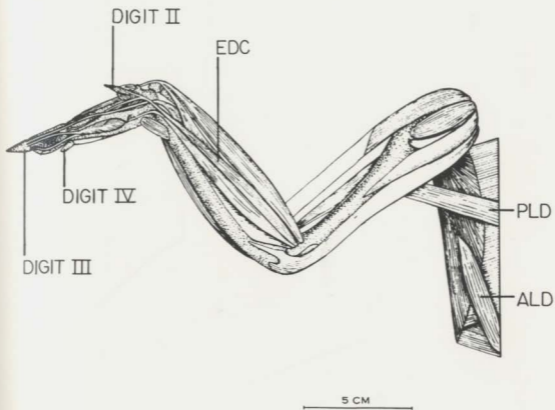
(EDC) muscle (see Figure 2) according to the naming system devised by Sullivan (1962). Preliminary examination of the EDC muscle indicated that it appeared to conform to the specifications outlined in the previous section.

1.4.3 The in vivo EDC nerve-muscle preparation.

The EDC muscle (see Figure 2) originates in the elbow joint and inserts on the middle digit (III) sending a collateral tendon to the first digit (II). It is innervated by the radial nerve. The blood supply is via the radial artery which arises from the bifurcation of the brachial artery into the ulnar and radial branches. There are five major advantages to this preparation. (1) insertion of a cannula into the ulnar artery and advancement of the tip to the junction of the brachial and radial arteries allows injection of microlitre quantities of drugs directly to the EDC with the minimum washout time; (2) the superficial location of the muscle allows relatively non-traumatic surgical access; (3) long free tendons at both ends of the muscle permit easy removal of the muscle for in vitro comparison studies; (4) the peripheral location of the EDC makes it suitable to set up bilateral preparations; (5) the accessible radial nerve can be readily cut to examine the long-term effects of denervation.

1.4.4 The aims of this research. The aims of this research were threefold. (1) to describe the new

Figure 2. The extensor digitorum communis (EDC), posterior latissimus dorsi (PLD) and anterior latissimus dorsi (ALD) muscles; also shown, the EDC tendons to the second and third digits (adapted from Sullivan, 1962).



preparation with respect to histology and contractile physiology and to compare the results with similar results obtained in the PLD; (2) to perform quantitative tests for the presence of sex differences using clinical and physiological measures of dystrophy; (3) to examine the effects of age using two groups of birds, a young, sexually immature group and an older, sexually mature group.

Chapter 2

Materials and Methods

2.1 Birds

Dystrophic New Hampshire chickens (Line 413) and their genetically matched controls (Line 412) from lines developed by the Dept. of Avian Sciences, University of California at Davis, were reared locally at the Memorial University Vivarium. Breeding flocks of both normal and dystrophic birds were found to produce fertile eggs; the fertility rate was slightly lower for the dystrophic flock. Despite reports advocating artificial insemination as the only way to insure a reliable source of dystrophic eggs, it was found that the reduced hatch could be compensated for by incubating more dystrophic eggs than needed.

The birds were housed "on the floor" in large enclosed pens. Food (Masterfeeds Chick Starter #16533 for the younger birds and Masterfeeds 15% Layer #23846 for the laying flock) and water were provided ad libitum.

2.2 Clinical Assessment

2.2.1 General. Birds were transported from the animal care facility to the laboratory where they were weighed and their sex determined. If the sex was not readily apparent, as was the case for some of the younger birds, verification was made by autopsy at the end of the experiment.

2.2.2 Exhaustion score. The birds were assayed for dystrophy using the exhaustion score test (Entrikin et al., 1978). Using both hands the bird was placed on its back, released and allowed to right itself as many times in succession as possible to a maximum of 20 (see Figure 3).

2.2.3 Wing apposition. A second clinical assay of dystrophy, the wing apposition test, was performed in this study (Chou et al., 1975). Grasping the bird by both forearms, the experimenter attempted to appose the elbow joints behind the back. The degree to which this maneuver was opposed by passive resistance was measured in centimeters separation between the "elbows" providing a second semi-quantitative index of dystrophy (see Figure 4).

Figure 3. The exhaustion score. (a) The normal chicken is able to right itself when placed on its back in the supine position; (b) the dystrophic chicken is unable to right itself when placed on its back in the supine position.



Figure 4. The wing apposition score. (a) There is no resistance to passive apposition of the wings behind the back in the normal bird; (b) there is excessive resistance to passive apposition of the wings behind the back in the dystrophic bird.



2.3 Surgery

2.3.1 Preparatory surgery. Young (6-12 weeks) and old (greater than 6 months) normal and dystrophic chickens were assayed for dystrophy using standard exhaustion score and wing apposition tests, weighed and anesthetized by intra-venous (IV) injection of urethane (1.2 g/kg). A tracheotomy was performed (see Figure 5) and respiration was supported by a conventional small animal respirator. The jugular vein was cannulated with PE-50 tubing (see Figure 5) to establish a route for later infusion of fluids and anesthetic supplements. The neck skin was then sutured together.

2.3.2 Exposure of the EDC and the radial nerve. The feathers were removed from the dorsal aspect of the right wing and an incision made in the skin overlying the EDC muscle (see Figure 6). Approximately 1 cm of the deep fascia covering the muscle was dissected away from the distal end to provide a point of contact for the surface EMG electrode. The distal tendon was exposed and severed from its insertion on the middle digit (III) (see Figure 2). The muscle was fixed to the transducer hook with a square knot; the free end of the tendon was used to form half the knot with a length of 1-0 silk serving as the other half (see Figure 6). Steel pins were driven through

Figure 5. (a) Tracheotomy; (b) cannulation of the jugular vein.

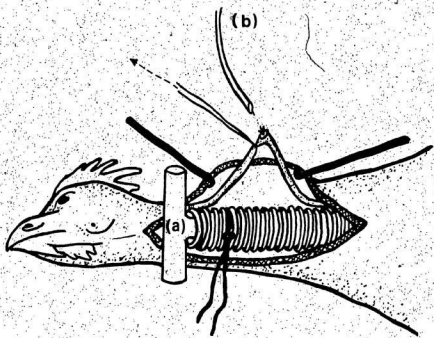
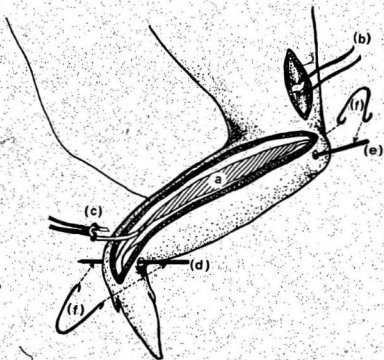


Figure 6. (a) The EDC muscle; (b) snaring the radial nerve; (c) formation of a square knot with 1-0 silk and the EDC tendon; (d) steel pin through the wrist joint; (e) steel pin through the elbow joint; (f) "horseshoe" brackets for attachment to chamber.

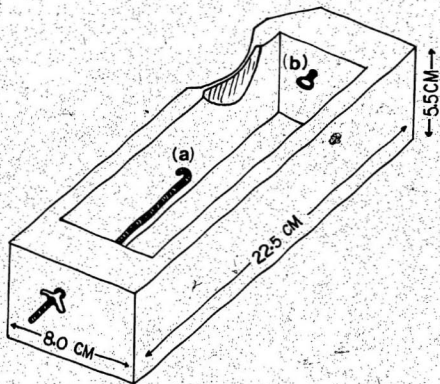


the elbow and wrist joints to secure the wing in the muscle bath (see Figure 6). The portion of the radial nerve overlying the humerus was exposed and snared with a loose ligature (see Figure 6). A 10-gauge biopsy needle was inserted into the abdominal air sacs to provide unidirectional ventilation with humidified air (Burger and Lorenz, 1960).

2.3.3 Immobilization of the preparation. The feathers were removed from the region overlying the thoracic vertebrae and two incisions, just long enough to accommodate a vertebral clamp, were made on either side of the vertebral crests. The bird was suspended by this clamp in a modified stereotaxic apparatus. The wing was immobilized by hooking the curved ends of two "horseshoe-shaped" brackets over the ends of the steel pins protruding from the wrist and elbow joints (see Figure 6) and clamping these brackets to the proximal and distal ends of a plexiglass chamber (see Figure 7). To insure immobility tension was exerted on the forearm by tightening an externally mounted wing nut on the threaded rod which also served as the proximal site of attachment of the wing to the chamber (see Figure 7).

2.3.4 Stimulating and recording electrodes. Bipolar platinum iridium stimulating electrodes were positioned beneath the radial nerve and were shielded from

Figure 7. Scale drawing of plexiglass chamber for immobilization of the wing. "Horseshoe" brackets attach at point (a) for wrist and point (b) for elbow.



the underlying tissues by a thin strip of parafilm. A monopolar platinum iridium recording electrode was placed in contact with the surface of the muscle to record the muscle EMG. The vertebral clamp served as a reference electrode for the electrical responses.

2.3.5 Core temperature and vital signs. The nerve was covered with gauze soaked in mineral oil and the entire wing was immersed in mineral oil warmed to 34°C with a thermoregulated heat lamp. The bird was placed on a heating pad and covered with a "space blanket" to reduce heat loss; core temperature was monitored with an oral temperature probe. Throughout the experiment the EKG was monitored.

2.4 Mechanical and Electrical Circuits

2.4.1 Recording circuits. The isometric contractile responses of the EDC were recorded using a Grass model FTO3C force-displacement transducer modified with a right angle attachment lever to accommodate the hook on the EDC tendon. The output of the transducer was amplified by a DC preamplifier with an integral transducer bridge input circuit (gain = 1-10K) and displayed simultaneously on a Gould Brush 220 chart recorder and on a

Tektronix 5111 storage oscilloscope. The EMG was recorded between the surface electrode and ground (at the vertebral clamp); this signal was directed through an AC coupled preamplifier (gain = 1-10 K) and monitored on the oscilloscope. The mechanical and electrical responses of the muscle were recorded intermittently on magnetic tape at 3 3/4 inches per second for later analyses using a Hewlett-Packard Model 3964-A four channel FM tape recorder.

2.4.1.2 Stimulus control circuits. The radial nerve was stimulated by the output of two Tektronix PG-505 pulse generators controlled by a custom made digital pulse counter. The pulse counter enabled the delivery of a designated number of stimuli at two independent rates; automatically alternating between the two pulse generators. The output of both pulse generators was coupled to the muscle through a W-P Instruments NP-1 stimulus isolation unit.

2.5 Experimental Protocol

At the start of the experiment the muscle length was adjusted with a rack-and-pinion drive mounted to the force-transducer to determine the length required to generate peak twitch tension, the resting length (l_0) in

the intact bird. The optimal conditions for stimulation were also determined (the preferred polarity for the stimulus pulse and the voltage which was 25% supramaximal for the twitch response). Finally, the baseline resting tension was set to zero using the balance bridge and a 300 Hz, 50 pulse tetanus was delivered to take the "slack" out of the mechanical connections. If any deviation in the baseline was observed after the tetanus the baseline was reset to zero and the procedure repeated until no further shifts were evident.

Throughout the experiment the muscle was stimulated by one pulse generator at a frequency of 0.2 Hz with a pulse duration of 0.2 msec. At 5 minute intervals (after delivering 60 pulses) the pulse counter switched the stimulus control to the second pulse generator and the muscle was stimulated to contract tetanically by a 50 pulse train at a frequency of 100, 200, 300, 400, 500 or 600 Hz with a pulse duration of 0.2 msec; frequencies were delivered in a random sequence for each experiment. When the 50 pulse train was completed, each muscle being tested at all frequencies, the stimulus control switched back to the first pulse generator and the cycle was reinitiated.

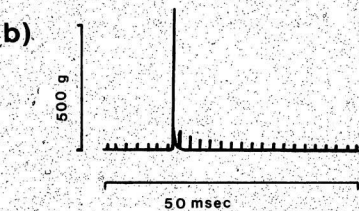
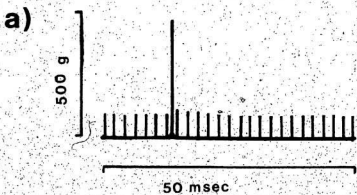
2.6 Measurement Techniques

Stimulation of the muscle following the protocol described above generated a series of twitches and tetani for each of the different frequencies used (see Figure 8). From these data several other parameters can be measured or computed and analysis performed with respect to age, sex and stimulus frequency.

2.6.1 Post-tetanic potentiation and twitch/tetanus ratio. From the data recorded directly on the chart recorder at slow speed (25 mm/min) during the experiment (see Figure 8) the following measurements were made. The ratio of the amplitude of the first twitch after a tetanus to the amplitude of the last twitch before the tetanus is an index of the degree of post-tetanic potentiation (PTP); this parameter was examined across frequencies for all muscles. The amplitude of the twitch (P) and the tetanus (P_0), were measured at each frequency; twitch/tetanus ratios (P/P_0) were calculated for each frequency of stimulation.

2.6.2 Twitch contraction time and half-relaxation time. Records of the twitch data on magnetic tape were replayed on the chart recorder at normal tape speed and fast chart speed (125 mm/sec) allowing precise measurement

Figure 8. Sample series of twitch and tetanic responses recorded directly on the chart recorder during the experiment for normal (a) and dystrophic (b) birds.



of the time to peak contraction (T_c) and time to half-relaxation ($1/2 T_r$) in milliseconds (see Figure 9).

2.6.3 Latent period, duration of the active state plateau, tetanus half-relaxation time and post-tetanic contracture. These measurements were made using simultaneous expansion of the tetanic time course and the concomitant EMG activity (see Figure 10) which facilitated correlation of the electrical and mechanical events in the muscle. This was usually done by playing back taped records at 15/32 inches per second and recording the response on the chart recorder at fast speed (125 mm/sec). The latent period was defined as the time from the first stimulus artifact to the onset of tension development in the muscle. The duration of the active state plateau was measured using the method of Ritchie (1954); the latent period was subtracted from the time from the last stimulus artifact and the onset of the initial decay of tension in the muscle following termination of tetanic stimulation at 100 Hz. Using the same record, the time to half-relaxation ($1/2 T_r$) for the tetanus was calculated. Finally, the expanded time scale of the tetani in dystrophic preparations illustrated the phenomenon of post-tetanic contracture (PTC) (see Figure 11); a significant isometric tension persisted in dystrophic muscle long after the tetanic stimulation had stopped. To normalize this response for muscles of different sizes the PTC's were

Figure 9. Measurement of the muscle twitch contraction time (a) and twitch half-relaxation time (b).

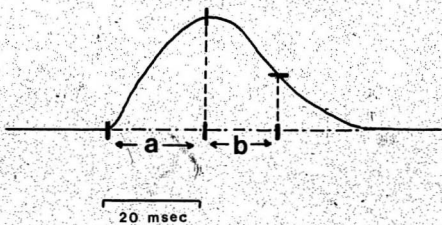


Figure 10. Simultaneous expansion of tetanus time course and muscle EMG activity for measurement of (a) the latent period; (b) duration of the active state plateau (note that the time measured for the latent period, "a", must be subtracted from "b" to obtain an accurate estimate of the active state plateau); (c) tetanus half-relaxation time.

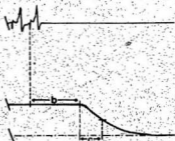
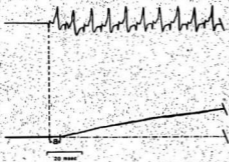
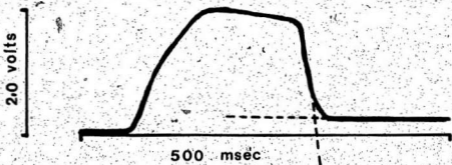


Figure 11. The measurement of the post-tetanic contracture was obtained in volts from the point of intersection of the dashed lines. This value was ultimately expressed as a percentage of the twitch amplitude for a given experiment.



first measured in millivolts at each frequency and were expressed as percentages of the maximum twitch amplitude (in millivolts) for each experiment .

2.6.4 Maximal rate of rise of tetanic tension.

The maximal rate of rise of tension for each tetanus in a series was determined with a passive RC differentiator and expressed as a percentage of the height, in millivolts, of the largest tetanus in the series ($\% P_0/\text{msec.}$)

2.6.5 Grams tension/muscle wet weight. To test the accuracy of the transducer and to obtain a conversion factor to convert millivolts deflection (recorded directly on the chart recorder) into grams-tension (a more conventional means of evaluating muscle strength), a calibration curve (mV vs. g) was calculated for known weights across the entire range of gain settings used on the amplifier in these experiments (see Figure 12). The linearity of these curves was evident in that in no case was the correlation coefficient for regression analysis, r , less than 0.99. Since the correlation between theoretical and observed values was so high and since all the lines passed through the origin the regression equation reduced to the form $y = mx$. Thus, the value m was applied as a conversion factor enabling values of x in millivolts to be directly converted to values of y in grams. In this manner, the amplitude of the maximum twitch and the maximum


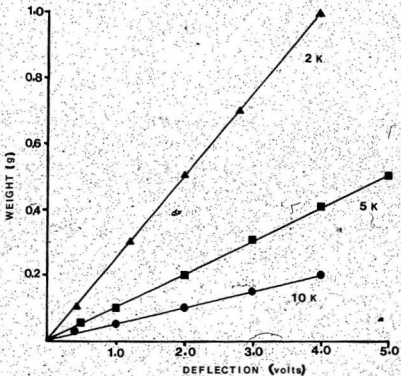


Figure 12. Calibration curve for the transducer at the three gain settings (2K, 5K and 10K) used in the present experiment.



tetanus for each experiment were expressed in grams-tension. These absolute values in grams were normalized for different size muscles by dividing the tension (in grams) by the wet weight of the muscle (in grams) producing values for the grams tension generated per gram of muscle.

2.6.6 Statistical analysis. All graphs and histograms are presented as the mean plus or minus the standard error of the mean. The effects of dystrophy, sex and any possible interaction effects were examined using a standard two-way analysis of variance computer program for a 2 X 2 factorial design; the results of this analysis are summarized in Appendix 1. The level of significance selected for this study was $p < 0.05$. Significant effects related to disease are denoted by a plus (+) sign and significant effects of sex are denoted by two plus (++) signs.

2.7 Histology

2.7.1 SDH and myosin ATPase reactions. Normal and dystrophic chickens of both sexes were exsanguinated by decapitation, the EDC and PLB muscles quickly excised, cut into fifths, mounted on cork and rapidly frozen in isopentane cooled in liquid nitrogen. The tissue was

allowed to equilibrate in the cryostat for 1 hour, cut in 14 micron sections and mounted on slides. The slides were air dried for 1-2 hours for the succinate dehydrogenase (SDH) reaction; alternate sections were stored overnight in the refrigerator for the myosin ATPase (ATPase) reaction. All SDH reactions were run according to the methods of Barks and Anderson (1968) as modified by Cosmos and Butler (1967) for avian muscle and myosin ATPase reactions followed the procedures of Guth and Samaha (1971) as modified by Cosmos et al. (1979).

2.7.2 AChE stain. For the demonstration of motor end plates on whole avian muscle tissue the author modified the stain for acetylcholinesterase (AChE) in the following manner (Silver, 1974). Both EDC and PLD muscles were removed as described above. Whole muscle preparations were tied by the tendons with 4-0 silk to plexiglass frames and slightly stretched. The tissue was rinsed in cold saline, fixed in buffered formalin for 5-10 minutes, rinsed well in phosphate buffer and incubated in the staining medium at 37° C for approximately 2 hours. Then the tissue was rinsed several times in water and the reaction developed in 1% ammonium sulphide for less than 1 minute. Finally, the muscles were dehydrated in alcohol, cleared in xylene and examined under a dissecting microscope.

Chapter 3

Results

3.1 Clinical Signs of Dystrophy

3.1.1 General. Subjective observation indicated that sexual development was retarded in the dystrophic chicken; the sex of normal birds was relatively simple to determine from external characteristics at six weeks of age whereas in young dystrophics it was always necessary to make this distinction post-mortem. In addition, there was a tendency for the young dystrophic birds to be smaller than the normals of comparable age but this was not statistically significant (see Table 2).

3.1.2 Exhaustion score. Data for the exhaustion scores of the young group (see Figure 13a) showed a highly significant decrease ($p < 0.01$) in the ability of the dystrophic birds to rise from the supine position. This was true of both sexes; normal males were able to rise an average of 19.3 ± 0.7 times while dystrophic males were only able to rise 1.2 ± 0.6 times and normal females were able to rise 19.2 ± 0.8 times while dystrophic females were able to rise 1.2 ± 1.2 times. The effects of sex and the

Table 2. The weights of normal birds vs. dystrophic birds for the young group. These differences were not significant at $p < 0.05$.

WEIGHT OF NORMAL VS. DYSTROPHIC BIRDS
FOR THE YOUNG GROUP

	MALE	FEMALE
NORMAL	597.5 ± 98.0 g	601.0 ± 83.4 g
DYSTROPHIC	464.8 ± 55.2 g	508.0 ± 46.1 g

interaction of sex and disease were not significant.

Findings for the old birds (see Figure 13b) indicated that while the results for the females were similar to those obtained for the younger birds (normals were able to rise 18.3 ± 1.6 times and dystrophics were able to rise 1.7 ± 0.6 times), the dystrophic males were never able to rise (normal males flipped an average of 19.5 ± 0.3 times). The effects of both sex and disease were significant ($p < 0.01$) but the test for an interaction was not significant. The age related decline in the ability of dystrophic chickens to right themselves was not observed in dystrophic females.

3.1.3 Wing apposition. The passive ability to appose the the elbow joints behind the back was severely attenuated ($p < 0.01$) in dystrophic chickens of either sex for both age groups examined in this study. Results for the young group showed that there was never any passive resistance to this movement in normal chickens (wing apposition equaled 0 cm regardless of the sex of the bird); the degree of apposition in dystrophic males was 7.9 ± 0.7 cm and in dystrophic females was 7.9 ± 1.2 cm (see Figure 14a). Effects due to sex or interaction were not noted.

Results for the old group showed that age did not affect the performance of normal chickens on the wing apposition test; for both males and females wing apposition again equaled 0 cm. The wing apposition scores of the

Figure 13. Exhaustion score data for the young group (N=6 for males and N=5 for females) (a) and the old group (N=10 for males and N=7 for females) (b). + = the effect of disease was significant ($p < 0.05$); ++ = the effect of sex was significant ($p < 0.05$). NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female. Note: the exhaustion score data do not represent true means since a score of 20 means 20 or more and hence does not conform to a normal distribution.

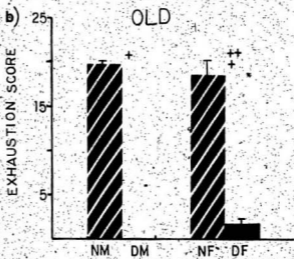
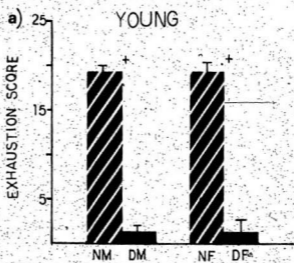
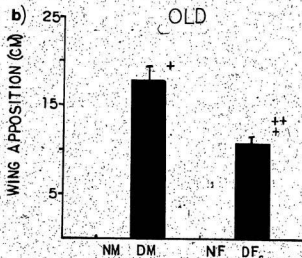
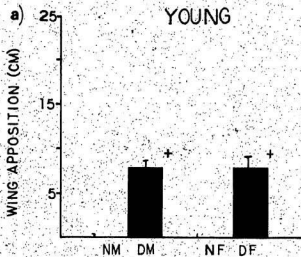


Figure 14. Wing apposition score for the young group (N = 6 for males and N = 5 for females) (a) and the old group (N = 10 for males and N = 7 for females) (b). + = the effect of disease was significant ($p < 0.05$); ++ = the effect of sex was significant ($p < 0.05$). NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.



female dystrophics were slightly increased (wing apposition equaled 10.9 ± 0.8) while those of the male dystrophics were substantially increased (wing apposition equaled 18.1 ± 1.5 cm) as shown in Figure 14b). In this case the main effects of sex and disease and their interaction were significant ($p < 0.01$). When the interaction effect was considered (see Figure 15) it became apparent that the expression of this characteristic of the disease was significantly attenuated in older dystrophic females.

3.2 Mechanical Characteristics of Dystrophy

3.2.1 Post-tetanic potentiation. The magnitude of PTP has been plotted as a function of frequency for young (Figure 16) and old (Figure 17) normal and dystrophic birds.

In the young group the effect of sex was not significant at any frequency examined although the effect of disease, enhancement of the PTP, was significant at frequencies of 100 Hz, 200 Hz and 300 Hz ($p < 0.05$); there was a significant interaction at 300 Hz ($p < 0.05$).

Results from the old birds also failed to demonstrate any sex-related effect on the PTP; increases in the magnitude of the PTP correlated with the disease process were significant at all frequencies examined.

Figure 15. The effect of the interaction of sex and disease on the wing apposition score for normal (N) and dystrophic (D) birds. The effect was not significant for the young birds (Y) and was significant ($p < 0.01$) for the old birds (O). ● = male; ○ = female.

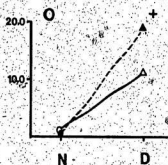
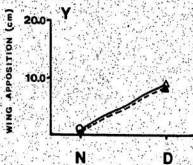


Figure 16. Magnitude of the post-tetanic potentiation vs. the frequency of stimulation for the young birds (N = 6 for males and N = 5 for females). + = the effect of disease was significant ($p < 0.05$). ● = normal males; ▲ = dystrophic males; ○ = normal females; △ = dystrophic females.

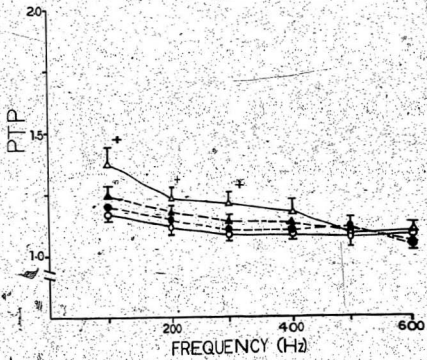
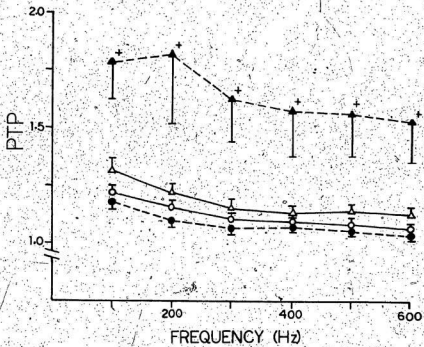


Figure 17. Magnitude of the post-tetanic potentiation vs. the frequency of stimulation for the old birds (N = 10 for males, and N = 7 for females). + = the effect of disease was significant ($p < 0.05$). ● = normal males; ▲ = dystrophic males; ○ = normal females; △ = dystrophic females.



($p < 0.05$). The interaction effect of sex and disease was significant at 100 Hz ($p < 0.05$).

When both interaction effects were examined individually it was apparent that in the young group the females exhibited a larger degree of PTP; linked to the effect of disease, than did the males (see Figure 18) whereas in the old group the males showed a larger disease-related PTP than did the females (see Figure 19).

3.2.2 Twitch/tetanus ratio. The mean P/Po's have been plotted against frequency of stimulation for both the young group (see Figure 20) and the old group (see Figure 21).

In the younger group the effect of disease was depression of the value of the P/Po; this effect was significant at a frequency of 100 Hz ($p < 0.05$). Sex did not have a significant influence on the P/Po at any frequency for the younger birds.

The sex of the bird had no significant effect on the older group, however, the effect of dystrophy was significantly related to a reduced P/Po at 100 Hz, 200 Hz, 300 Hz and 400 Hz ($p < 0.05$).

The only interaction effect apparent in the P/Po data was observed for the young group at 400 Hz ($p < 0.05$). Considered separately (see Figure 22), the interaction shows that dystrophy depressed the P/Po in young females while enhancing it in males. This trend is apparent.

Figure 18. The effect of the interaction of sex and disease on the post-tetanic potentiation for normal (N) and dystrophic (D) birds of the young group. + - the effect was significant at $p < 0.05$. ● - male; ○ - female.

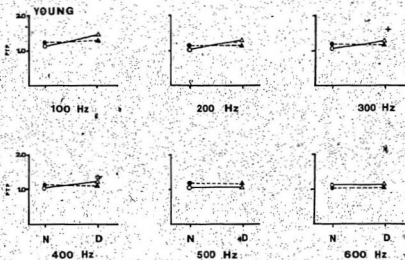
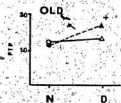
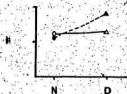


Figure 19. The effect of the interaction of sex and disease on the post-tetanic potentiation for normal (N) and dystrophic (D) birds of the old group. + - the effect was significant at $p < 0.05$. ● - male; ○ - female.



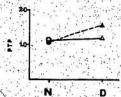
100 Hz



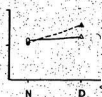
200 Hz



300 Hz



400 Hz



500 Hz



600 Hz

Figure 20. The twitch/tetanus ratio vs. the frequency of stimulation for the young group (N = 6 for males and N = 5 for females). + = the effect of disease was significant ($p < 0.05$). ● = normal males; ▲ = dystrophic males; ○ = normal females; △ = dystrophic females.

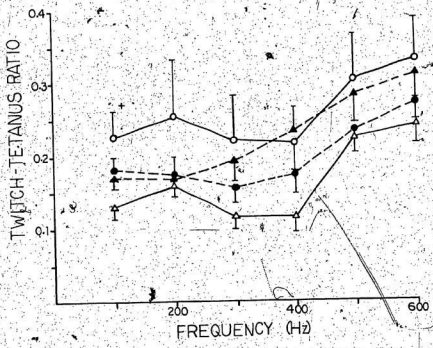


Figure 21. The twitch/tetanus ratio vs. the frequency of stimulation for the old group (N = 10 for males and N = 7 for females). + - the effect of disease was significant ($p < 0.05$). ● - normal males; ▲ - dystrophic males; ○ - normal females; △ - dystrophic females.

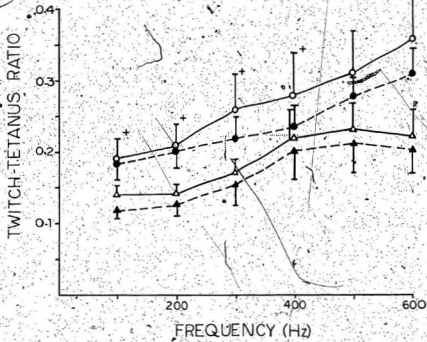
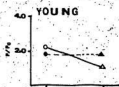


Figure 22. The effect of the interaction of sex and disease on the twitch/tetanus ratio for normal (N) and dystrophic (D) birds of the young group. * - the effect was significant at $p < 0.05$. ● - male; ○ - female.



100 Hz



200 Hz



300 Hz



400 Hz



500 Hz



600 Hz

although not significant, at all frequencies (see Figure 22).

3.2.3 Twitch contraction time. Data for the T_c for the young group are illustrated in figure 23a. The observed T_c for normal males was 21.0 ± 0.4 msec and for dystrophic males was 21.8 ± 0.9 msec. The value for normal females was 22.0 ± 1.7 msec whereas that observed for dystrophic females was 16.0 ± 0.7 msec. There was a significant effect of sex ($p < 0.05$), a significant effect of disease ($p < 0.05$) and a significant interaction (see Figure 24) effect ($p < 0.01$) due to the shortened contraction time observed for dystrophic females.

The old group was relatively homogeneous across both sex and disease (see Figure 23b); the contraction times for normal and dystrophic males were 24.3 ± 1.3 msec and 21.5 ± 1.6 msec respectively while the values for the normal and dystrophic females were 22.6 ± 2.0 msec and 22.1 ± 1.6 msec respectively. The effects of either disease or sex or the interaction of the two were not significant.

3.2.4 Twitch time to half-relaxation. The twitch $1/2 T_r$ was not shown to be affected by dystrophy, sex or the interaction of these variables for two age groups examined in this study. The value obtained for young normal males was 15.7 ± 1.2 msec, for young dystrophic males was 15.8 ± 0.7 msec, for young normal females was

Figure 23. The twitch contraction times for the young group (N = 6 for males and N = 5 for females) (a) and the old group (b) (N = 10 for males and N = 7 for females). + = the effect of disease was significant ($p < 0.05$); ++ = the effect of sex was significant ($p < 0.05$). NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.

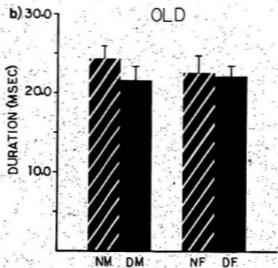
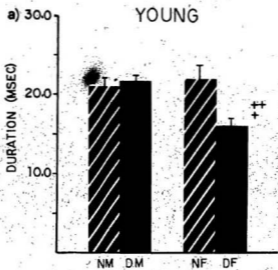
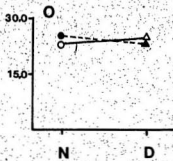
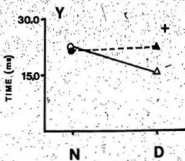


Figure 24. The effect of the interaction of sex and disease on the twitch contraction time for normal (N) and dystrophic (D) birds. The effect was significant ($p < 0.01$) for the young birds (Y) and not significant for the old birds (O). ● - male; ○ - female.



18.2 \pm 2.5 msec and for young dystrophic females was 16.0 \pm 0.7 msec (see Figure 25a). Older birds exhibited 1/2 T₁ times slightly longer than those observed for the young group; the times for old normal and dystrophic males were 20.6 \pm 1.4 msec and 19.3 \pm 1.4 msec respectively and for old normal and dystrophic females were 18.7 \pm 2.7 msec and 18.3 \pm 1.0 msec (see Figure 25b).

3.2.5 Latent period. There were no effects attributable to sex, disease or their interaction on the latent period of the muscle. The latent periods for young males were found to be 5.8 \pm 0.5 msec for the normals and 5.5 \pm 0.3 msec for the dystrophics; findings for young females showed a mean latent period of 6.0 \pm 0.6 msec for normals and 5.6 \pm 0.2 msec for dystrophics (see Figure 26a). Corresponding values in the old group were recorded as follows: 6.9 \pm 0.5 msec for normal males, 6.7 \pm 0.4 msec for dystrophic males, 6.4 \pm 0.8 msec for normal females and 6.5 \pm 0.3 msec for dystrophic females (see Figure 26b).

3.2.6 Duration of the active state plateau. The duration of the plateau of active state in young birds was not affected by either disease, sex or the interaction of these factors. The mean value observed for the normal males was 15.3 \pm 0.7 msec and that for the dystrophic males was 16.4 \pm 1.5 msec; results for the normal and dystrophic

Figure 25. The twitch half-relaxation times for the young group (N = 6 for males and N = 5 for females) (a) and the old group (N = 10 for males and N = 7 for females) (b). NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.

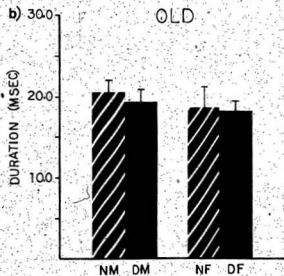
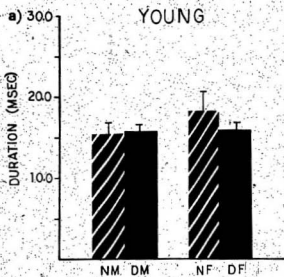
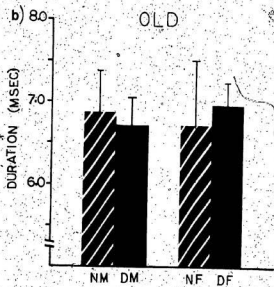
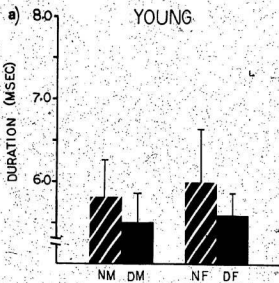


Figure 26. The duration of the latent periods for the young group (N = 6 for males and N = 5 for females) (a) and the old group (N = 10 for males and N = 7 for females) (b).
NM - normal male; DM - dystrophic male; NF - normal female;
DF - dystrophic female.



females were 18.4 ± 2.4 msec and 14.2 ± 1.8 msec respectively (see Figure 27a).

In the old group disease was shown to significantly shorten the duration of the active state plateau ($p < 0.05$); tests for effects due to sex and the interaction of sex and disease were not significant. The effect of disease was more pronounced in the older males (24.6 ± 2.9 msec for the normals versus 16.2 ± 1.4 msec for the dystrophics) than in the older females (20.4 ± 3.2 msec for the normals versus 17.8 ± 1.0 msec for the dystrophics). These results are shown in Figure 27b.

3.2.7 Tetanus time to half-relaxation. In the young group the tetanus $1/2$ Tr for a 100 Hz tetanus was found to be 13.5 ± 1.2 msec for the normal males, 14.7 ± 1.4 for the dystrophic males, 16.6 ± 3.2 for the normal females and 14.8 ± 1.6 for the dystrophic females (see Figure 28a).

Although not significant, a notable trend towards depression of the time to $1/2$ Tr was evident in the older males; the mean time for the normals was 23.1 ± 2.9 msec while the time for the dystrophics was 15.8 ± 1.4 msec (see Figure 28b). Such a trend was not apparent in the data recorded for the older females. A $1/2$ Tr time of 20.4 ± 3.2 msec was recorded for the normal females while 22.6 ± 3.1 msec was the corresponding mean for the dystrophics (see Figure 28b).

Figure 27. . The duration of the active state plateau for the young (N = 10 for males and N = 7 for females) (a) and the old (N = 10 for males and N = 7 for females) (b) groups. + = the effect of disease was significant ($p < 0.05$). , NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.

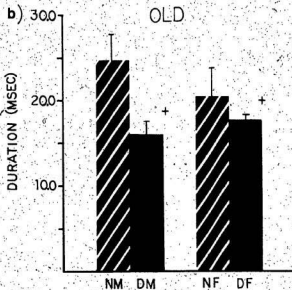
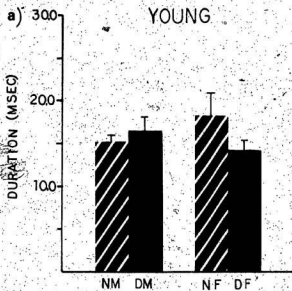
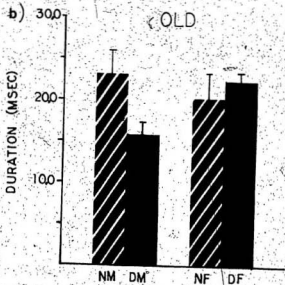
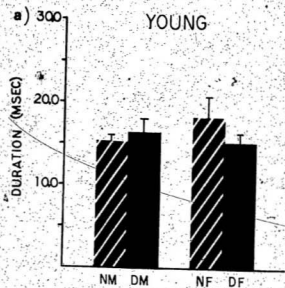


Figure 28. The tetanus half-relaxation times for the young (N = 6 for males and N = 5 for females) (a) and the old (N = 10 for males and N = 7 for females) (b) groups. NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.



3.2.8 Post-tetanic contracture. The PTC has been plotted against frequency as the percentage of the maximum twitch observed in each experiment for the young (see Figure 29) and the old (see Figure 30) groups.

The effect of dystrophy, increased magnitude of the PTC, was found to be significant in the younger birds at all frequencies. The main effects of sex and the interaction of sex and dystrophy were significant at all frequencies except at 300 Hz. When these findings were examined in another way (see Figure 31), young females were shown to be substantially more affected by the disease process than the males; the PTC was much greater in the female dystrophics than in either the male dystrophics or the normals.

Data for the older birds showed a highly significant ($p < 0.01$) disease-related enhancement of the PTC at all frequencies. The effects of sex and the interaction of sex and dystrophy were not significant at any frequency, despite a trend in the data at the lower frequencies for males to show a greater disease-related effect than the females (see Figure 30).

3.2.9 Maximal rate of rise of tetanic tension. The percentage of the maximum tetanus occurring per msec or the rate of rise of tension has been plotted against frequency of stimulation for the young (see Figure 32) and the old (see Figure 33) birds.

Figure 29. The magnitude of the post-tetanic contracture vs. the frequency of stimulation for the young birds (N = 6 for males and N = 5 for females). + = the effect of disease was significant ($p < 0.05$); ++ = the effect of sex was significant ($p < 0.05$). ● = normal males; ▲ = dystrophic males; ○ = normal females; △ = dystrophic females.

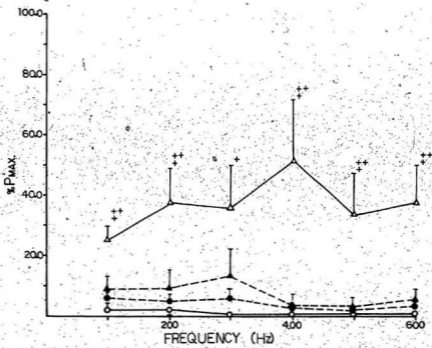


Figure 30. The magnitude of the post-tetanic contracture vs. frequency of stimulation for the old birds (N = 10 for males and N = 7 for females). + - the effect of disease was significant ($p < 0.05$). ● - normal males; ▲ - dystrophic males; ○ - normal females; △ - dystrophic females.

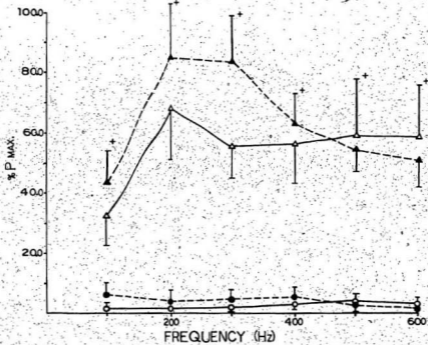


Figure 31. The effect of the interaction of sex and disease on the post-tetanic contracture for normal (N) and dystrophic (D) birds of the young group. + = the effect was significant at $p < 0.05$. ● = male; ○ = female.

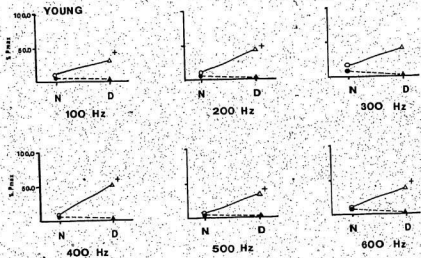


Figure 32. The maximal rate of rise of tension vs. frequency of stimulation for the young group (N = 6 for males and N = 5 for females). + - the effect of disease was significant ($p < 0.05$). ● - normal males; ▲ - dystrophic males; ○ - normal females; △ - dystrophic females.

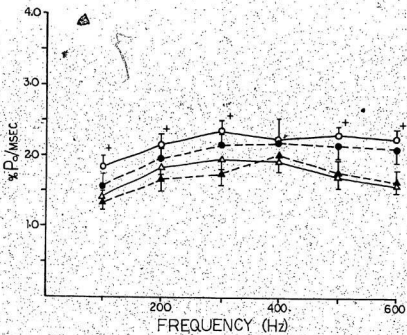
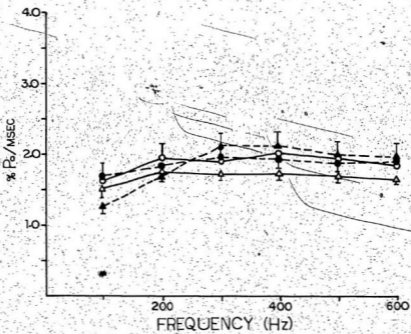


Figure 33. The maximal rate of rise of tension vs. frequency of stimulation for the old group (N = 10 for males and N = 7 for females). ● = normal males; ▲ = dystrophic males; ○ = normal females; △ = dystrophic females.



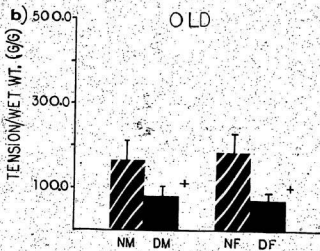
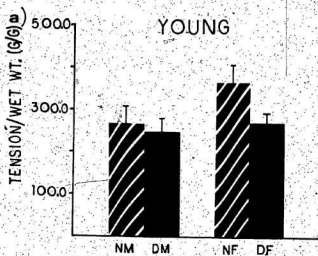
In the young group the effect of disease, depression of the rate of rise of tension, was significant at all frequencies ($p < 0.05$) except 400 Hz (see Figure 32). Quantitative differences related to either sex or the interaction of sex and disease were not significant.

The effects of sex, disease or their interaction were not significant at any frequency for the older group (see Figure 33).

3.2.10 Twitch-grains tension/muscle wet weight. Figure 34a illustrates that the effects of disease, sex or the interaction of sex and disease on the tension generated per gram of muscle wet weight for a twitch were not significant for the young group. Values obtained were 268.9 ± 33.1 g/g for normal males, 251.3 ± 44.2 for dystrophic males, 363.9 ± 47.5 g/g for normal females and 273.7 ± 17.5 g/g for dystrophic females.

For the old group there was a significant ($p < 0.05$) attenuation of the ability of the muscle to generate tension (see Figure 34b); the amount of tension generated per gram of muscle wet weight was 161.9 ± 43.6 g/g for the normal males, 79.4 ± 20.1 g/g for dystrophic males, 182.3 ± 56.0 g/g for normal females and 73.9 ± 12.4 g/g for dystrophic females. Tests for the effects of sex and the interaction of sex and disease were not significant.

Figure 34. The twitch tension per gram of muscle for the (N = 6 for males and N = 5 for females) (a) and the old (N = 10 for males and N = 7 for females) (b) groups. + = the effect of disease was significant at $p < 0.05$. NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.



3.2.11 Tetanus-grams tension/muscle wet weight.

For the young birds the effects of both sex and disease were not significant (see Figure 35a); muscles from normal and dystrophic males generated 2.02 ± 0.3 kg/g and 1.59 ± 0.19 kg/g in response to a tetanus respectively and muscles from normal and dystrophic females generated 1.92 ± 0.21 kg/g and 2.47 ± 0.22 kg/g tension in response to a tetanus respectively. There was, however, a significant interaction effect. When considered alone, the interaction effect showed that the young dystrophic females generated more tension per gram of muscle than did the young dystrophic males whereas the situation was reversed in the normals (see Figure 36).

As indicated by Figure 35b, the amount of tension generated per gram of muscle in birds from the old group was significantly reduced from 1.09 ± 0.21 kg/g in normals to 0.71 ± 0.07 kg/g in dystrophics whereas the corresponding values for females were 0.98 ± 0.18 kg/g for normals and 0.56 ± 0.07 kg/g for dystrophics. The effects of sex and the interaction of sex and disease were not significant.

3.3 Histology

3.3.1 Succinate dehydrogenase stain. Photographic

Figure 35. The tetanic tension per gram of muscle for the young (N = 6 for males and N = 5 for females) (a) and the old (N = 10 for males and N = 7 for females) (b) groups. + - the effect of disease was significant at $p < 0.05$. NM = normal male; DM = dystrophic male; NF = normal female; DF = dystrophic female.

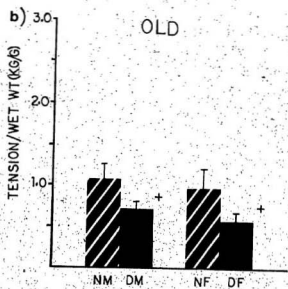
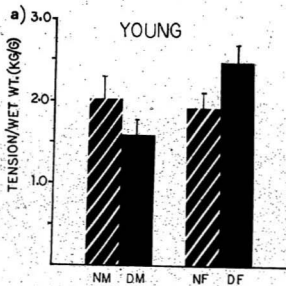
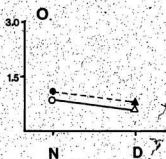
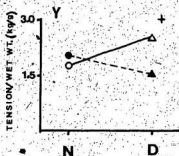


Figure 36. The effect of the interaction of sex and disease on the tetanic tension generated per gram of muscle for normal (N) and dystrophic (D) birds. The effect was significant for the young (Y) birds ($p < 0.05$) and was not significant for the old (O) birds. ● - male; ○ - female.



representation of the results of the stain for succinate dehydrogenase (SDH) are presented in Figure 37a for normal EDC, Figure 37b for dystrophic EDC, Figure 37c for normal PLD and Figure 37d for dystrophic PLD. No attempt was made to quantitate the histochemical analysis, rather examination of the EDC muscle and subsequent comparison with similar material from the PLD was performed to determine the relative proportions of fast and slow fibres on a purely subjective level with respect to prior work done in other muscles. While the normal PLD had very little SDH activity, the normal EDC had a notable number of fibres with some SDH activity. The overall level of SDH activity increased in both muscles in dystrophic birds. Variation in fibre size was a prominent feature of dystrophic tissue.

3.3.2 Myosin ATPase stain. The results of the myosin ATPase stain are presented in Figure 38. Figure 38a illustrates the acid pre-incubation for the normal EDC muscle and Figure 38b displays the alkali pre-incubation of the normal EDC muscle. Again, statistical analysis of the fibre types was not done; attempts were made to correlate these data with similar findings in other muscles. Since virtually no fibres stable in both acid and alkali preincubations were found in the EDC muscle no tonic fibres were present in the muscle. Apparently the SDH staining fibres in the EDC muscle were of the slow twitch variety.

Figure 37. Succinate dehydrogenase stain for (a) normal EDC; (b) dystrophic EDC; (c) normal PLD; (d) dystrophic PLD.

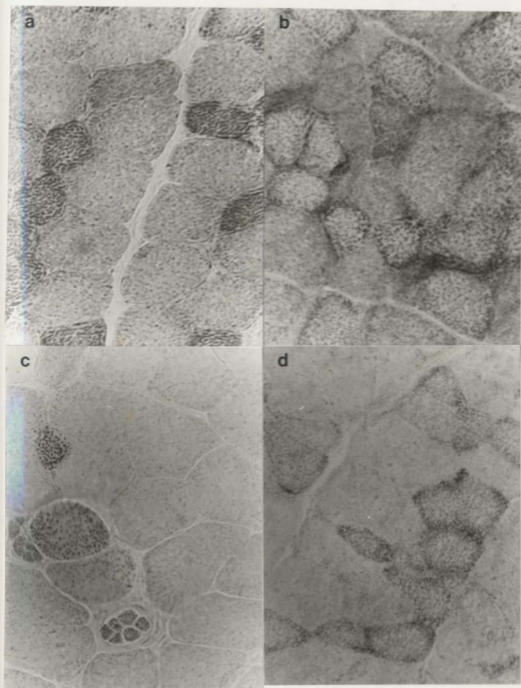
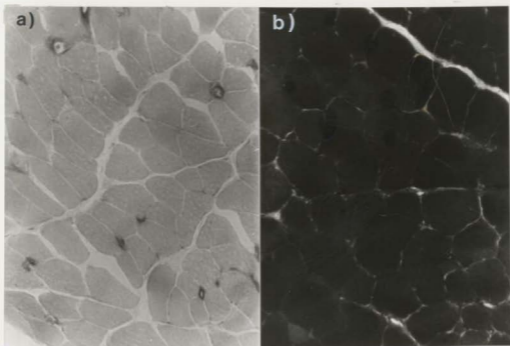
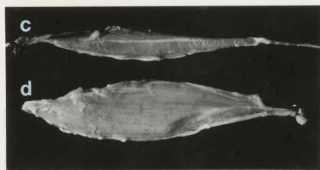


Figure 38. Myosin ATPase stain for normal EDC; (a) acid pre-incubation; (b) alkali pre-incubation.



3.3.3 Acetylcholinesterase stain. Photographs of the whole muscle stain for AChE are shown for normal EDC (Figure 39a) and dystrophic EDC (Figure 39b), and for normal PLD (Figure 39c) and dystrophic PLD (Figure 39d). In addition, fibres from several of these preparations were teased apart and examined under a dissecting microscope to verify the apparent "en plaque" nature of the motor end plates. The focal banding pattern characteristic of avian twitch muscle was readily apparent in the normal PLD and EDC muscles. A notable proliferation of end plates was observed in dystrophic EDC and PLD muscles.

Figure 39. Acetylcholinesterase stain for (a) normal EDC; (b) normal PLD; (c) dystrophic EDC; (d) dystrophic PLD.



Chapter 4

Discussion

As stated in the introduction, the aims of this research were threefold; (1) to describe the new EDC preparation with respect to histology and contractile physiology and compare these results with similar data reported for the PLD; (2) to perform quantitative tests for the presence of sex differences using clinical and pathophysiological measures of dystrophy; (3) to examine the effects of age using two groups of birds, a young sexually immature group and an older sexually mature group.

4.1 Histochemical Characterization of the In Vivo EDC Nerve-Muscle Preparation

4.1.1 Succinate dehydrogenase and myosin ATPase muscle histochemistry. Histochemical examination of the EDC muscle using stains for SDH and myosin ATPase indicates that although the muscle clearly contains mostly fast twitch fibres, it appears to contain a larger component of slow twitch fibres than does the PLD. While the muscles are not equivalent in this regard, they are comparable.

There are certain advantages to studying a muscle preparation with a heterogeneous fibre population. It is notable that muscles affected by dystrophy in the human are heterogeneous composites of slow and fast twitch fibres. Furthermore, Randall et al. (1980) have shown that the expression of certain characteristics of the avian disease (i.e. lactate dehydrogenase activity, EMG activity, muscle fibre diameter and total lipid content) may be expressed to varying degrees in the same muscles from different lines of birds and, indeed, within different fast twitch muscles in the same bird. Hoekman et al. (1982) have evaluated the mechanical properties of dystrophic muscle in three different lines of birds using two different muscles and shown that the expression of dystrophy in the mechanical properties of the muscle differs both between the various lines of birds and between different muscles within the same line. Since some of these changes may be related to the composition of the population of fibres present in particular muscles, the use of the EDC preparation may allow the investigation of relative changes occurring in fast and slow twitch fibres within the same muscle.

4.1.2 Acetylcholinesterase histochemistry. The stain for AChE on whole muscle mounts made it possible to distinguish patterns of innervation common to either twitch or tonic muscle with the use of a dissecting microscope. The focal banding arrangement of the endplates seen in the

PLD is also evident in the EDC; this distinction is particularly striking when compared to the appearance of the endplates using the same stain on whole mounts of the ALD.

The primary reason for attempting this stain was to determine the anatomical location of the endplates in the EDC muscle in vivo and thus facilitate the recording of miniature endplate potentials (mepps). One limitation of this preparation is that the regions containing motor endplates in the EDC are located on the relatively inaccessible ventral aspect of the muscle near the periphery. Recording of mepps is very difficult in such a preparation.

4.2 Mechanical Characteristics of the In Vivo EDC Nerve-Muscle Preparation.

Although the mechanical responses of the EDC muscles recorded in the present study were grouped according to sex, certain of these data were pooled (see Table 3) to facilitate comparison with data previously reported for the PLD (Hoekman et al., 1982).

4.2.1 Twitch contraction time and half-relaxation time. Both the isometric twitch contraction times and the

Table 3. The mechanical responses of the PLD and EDC muscles in normal (line 412) and dystrophic (line 413) chickens. (Data for the PLD muscle from Hoekman et al., 1982).

COMPARISON OF THE MECHANICAL PROPERTIES OF NORMAL
AND DYSTROPHIC EDC AND PLD MUSCLES

	NORMAL		DYSTROPHIC	
	PLD	EDC	PLD	EDC
twitch contraction time (msec)	22.3 ± 1.0	21.5 ± 0.8	22.8 ± 0.9	18.3 ± 1.6
twitch half-relaxation time (msec)	19.3 ± 1.1	16.8 ± 1.3	19.5 ± 0.9	15.9 ± 0.5
twitch tension/muscle wet wt. (g/g)	171.6 ± 10.5	312.2 ± 30.6	93.1 ± 13.0	261.5 ± 24.5
tetanic tension/muscle wet wt. (g/g)	0.57 ± 0.03	1.97 ± 0.17	0.58 ± 0.08	1.99 ± 0.19
twitch-tetanus ratio	0.21 ± 0.02	0.32 ± 0.13	0.17 ± 0.02	0.16 ± 0.02
latent period (msec)	6.9 ± 0.2	5.9 ± 0.4	7.0 ± 0.2	5.6 ± 0.2
active state plateau duration (msec)	18.3 ± 1.0	16.7 ± 1.2	11.8 ± 0.6	15.3 ± 0.9
tetanus half-relaxation time (msec)	37.4 ± 4.1	14.9 ± 1.6	22.2 ± 1.3	14.7 ± 1.0

Table 3 continued

DYSTROPHIC

NORMAL

	PLD	EDC	PLD	EDC
rate of rise of tension (% Po/msec)	3.06 ± 0.28	2.26 ± 0.12	2.63 ± 0.22	1.83 ± 0.13
post-tetanic potentiation	1.12 ± 0.03	1.09 ± 0.01	1.36 ± 0.05	1.16 ± 0.02
post-tetanic contracture (% maximum twitch)	0	3.4 ± 1.9	100.2 ± 36.6	23.7 ± 8.6
number of birds	6	11	6	11

* = Significantly different from normal at $p < 0.05$.

twitch half-relaxation times were reported to be of similar magnitude in the PLD muscles of normal (line 200) and dystrophic (line 304) chickens (Hoekman, 1976). Table 3 indicates that similar results were obtained for the PLD and EDC muscles of line 412 and 413 birds.

4.2.2 Twitch and tetanus - grams tension/muscle wet weight. Hoekman (1976) reported that the twitch tension per gram of muscle wet weight generated by the dystrophic (line 304) PLD was significantly less than that generated by the normal PLD (line 200). The tetanic tension generated per gram of muscle wet weight by the dystrophic (line 200) PLD was not significantly different from that generated by the normal (line 200) PLD, in fact the dystrophic PLD generated more tension per gram of muscle than did the normal PLD. Similar results were observed for the PLD of line 412 (normal) and line 413 (dystrophic) birds (see Table 3), although these results were not statistically significant at $p < 0.05$. In the EDC muscle of the normal (line 412) and dystrophic (line 413) birds this trend in the data was preserved but the magnitude of the difference was reduced even further (Table 3).

One notable difference between the PLD and the EDC is that the tension generated per gram of muscle wet weight is greater in the EDC for both twitch and tetanic stimulation. This suggests that the EDC is a stronger

muscle having a greater effective cross-sectional area than the PLD thus forming more cross bridges per unit area.

4.2.3 Twitch/tetanus ratio. The twitch to tetanus ratio was previously shown to be significantly reduced in the dystrophic (line 304) PLD muscle (Hoekman, 1976); the change was due to a decreased twitch tension, augmented by a slightly increased tetanic tension. The twitch-tetanus ratio in the PLD and the EDC muscles of line 412 and 413 birds are also reduced, but not significantly (see Table 3). This is predictable since the reduction in twitch tension is not as severe in the line 413 birds as it is in the line 304 birds.

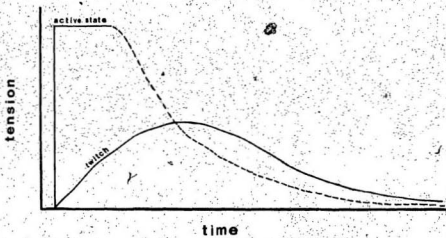
4.2.4 Latent period. The latent period of the muscle is the time from stimulation to the onset of muscle shortening. Since the stimulus was delivered indirectly in the present study, the latent period includes both the nerve conduction and synaptic transmission time from the point of stimulation in addition to the time to stretch out the series elastic elements of the muscle. This parameter was not examined in earlier studies using birds from the normal line 200 and the dystrophic line 304. As illustrated in Table 3, there is no significant difference between the latent periods recorded in normal and dystrophic birds for either the PLD or the EDC muscles. The only notable difference is that the latent period for

the EDC is longer than that for the PLD. The effect of the long tendons on both ends of the EDC muscle increasing the series elastic compliance, could account in part for the increased latent period observed in that muscle, despite the fact that the stimulating electrodes were located at a point on the nerve further from the muscle in the EDC preparation than in the PLD preparation.

4.2.5 Duration of the active state plateau and tetanus half relaxation time. The duration of the active state plateau reflects the true time course of the maximal contractile activation process and is thought to represent the amount of time a saturating concentration of calcium is available to interact with the myofilaments (Sandow & Brust, 1966). The onset of active state is very rapid; this is followed by a plateau phase and a slower decay phase (Casser & Hill, 1924; Hill, 1950; Hill, 1951). An estimate of the rate of decay can be obtained by measuring the time to half relaxation for a tetanus; the decay phase probably reflects the rate of calcium reaccumulation by the sarcoplasmic reticulum. The time course of the active state and concomitant muscle twitch are illustrated in Figure 40. Although the onset of the ability of the muscle to develop maximal tension is rapid, a single twitch never attains maximal tension. This is because the muscle must first shorten against its series elastic elements before it can do external work. When this internal shortening has

Figure 40. The time course of both the active state and concomitant muscle twitch.





been accomplished, the active state has already begun to decay and the muscle relaxes (see Figure 40).

Table 3 indicates that the duration of the active state plateau is significantly reduced in the PLD of line 413 birds when compared to line 412 birds; a trend in the same direction is seen in the EDC of line 413 birds although this difference is not statistically significant. The tetanus time to half-relaxation was also significantly reduced in the PLD muscles of line 413 chickens. This was not true of the EDC muscle where virtually no change was apparent in the half-relaxation time for a tetanus. The duration of the plateau of active state and the time to half-relaxation for a tetanus were not measured in line 200 and line 304 birds.

The observed decrease in the active state plateau duration could result from either more active calcium reaccumulation or a decrease in the amount of calcium released from the sarcoplasmic reticulum. The idea that calcium is more rapidly reaccumulated by the sarcoplasmic reticulum is consistent with the observation that half-relaxation time is decreased in the same muscle (the PLD) that exhibits the largest decrease in the active state plateau duration. On the other hand, there is evidence that intracellular calcium levels are decreased in some dystrophic muscles (Cosmos, 1964).

It is important to note that the time to half-relaxation in the EDC muscle is much shorter in the normal

EDC than in either the normal or the dystrophic PLD (see Table 3). Possibly the system for calcium sequestration is already operating at its maximum level in the EDC.

4.2.6 Rate of rise of tetanic tension. The rate of rise of tetanic tension has been reported to be reduced in dystrophic PLD (line 304) muscle when compared to normal PLD (line 200) muscle (Hoskman, 1976). This trend is apparent in the data for the 412 and 413 birds (see Table 3) but it is only significantly reduced in the EDC muscle. Note that values for the normal EDC were much lower than those observed for the normal PLD.

It has been demonstrated that the rate of rise of tension is directly related to the velocity of shortening and inversely related to the series elastic compliance of the muscle (Katz, 1939; Hill, 1951). A reduced velocity of shortening, perhaps reflecting a slowing of the myosin ATPase could account for the decreased rate of rise of tension seen in dystrophic muscle. The observation that the rate of rise of tension in the normal EDC is slower than that in the normal PLD was predicted, since the long tendons at either end of the EDC should increase the series elastic compliance of that muscle. Furthermore, the EDC is probably a slightly slower muscle than the PLD since it does have a larger component of slow twitch fibres as defined by histochemistry.

When confronted with a reduced rate of rise of

tension, the observation that the twitch time course is of normal duration seems inconsistent. Hockman (1976) suggested that if the active state plateau duration were reduced, the peak twitch tension would occur at the same time in normal muscle as it would in dystrophic muscle, albeit at a reduced tension. Since a reduction in the duration of the active state plateau has now been demonstrated in dystrophic muscle, this hypothesis appears to be supported.

4.2.7 Post-tetanic potentiation. The application of a tetanic stimulus to a muscle via a peripheral nerve causes potentiation of subsequent twitch stimuli (Hughes, 1958). While PTP has been demonstrated in any muscle in which it has been sought, the origin of this phenomenon is a subject of current investigation. It has been demonstrated that the PTP observed in slow muscle (i.e. soleus) and the PTP observed in fast muscle (i.e. gastrocnemius) are similar in appearance but arise from many different mechanisms (Standaert, 1963; Standaert, 1964). In the soleus the underlying basis of the PTP is a short train of action potentials which has been coined the post-tetanic repetition (PTR) and is primarily neural in origin. Standaert (1963) hypothesizes that this activity is produced by a generator potential which develops between the unmyelinated nerve terminal and the last node of Ranvier; presumably the hyperpolarization of the small,

unmyelinated nerve terminals in response to tetanic stimulation is much greater than that in the large myelinated fibres. If the potential difference between the last node and the nerve terminal is of sufficient magnitude, repetitive activity will ensue (Standaert, 1963). In the gastrocnemius, PTP is primarily a muscular phenomenon; it occurs in curarized muscle. PTP is seen too infrequently in fast muscle to account for the PTP (Standaert, 1964). There is evidence (Macfarlane, 1953; Shanarina, 1961) that the muscle membrane also hyperpolarizes in response to repetitive stimulation. This hyperpolarization results in muscle action potentials of longer duration and consequently the duration of the active state plateau is increased; this potentiates the twitch (Standaert, 1964). On the other hand, Winegrad (1968) suggests that since the rate of calcium uptake by the sarcoplasmic reticulum decreases as saturation is approached, tetanic stimulation results in prolongation of the active state due to a direct effect on calcium transport processes. Either mechanism will account for the PTP and probably both are occurring to some extent.

The magnitude of post-tetanic potentiation in dystrophic (line 304) muscle has been shown to be increased when compared to that observed in normal (line 200) muscle (Hoekman, 1976). In the line 412 and 413 birds the PTP is significantly enhanced in both the PLD and the EDC muscles (present study).

Since pronounced hyperpolarization of the dystrophic muscle membrane in response to repetitive stimulation has been reported in vitro (Albuquerque and Warnick, 1971), it is likely that the PTP observed in dystrophic muscle reflects a post-tetanic hyperpolarization of the muscle membrane causing increased action potential duration and potentiation of subsequent twitches. Decreased intracellular calcium sequestration, as suggested by Winegrad (1968), could play a role in these processes. Another possibility is that the increased proportion of slow twitch muscle fibres in dystrophic muscle results in an increase in the PTR; since indirect muscle stimulation was used in the present experiment, involvement of the motor nerve and neuromuscular junction cannot be excluded.

4.2.8 Post-tetanic contracture. The post-tetanic contracture (PTC) is the amount of tension remaining in a muscle after tetanic stimulation if relaxation is assumed to be a logarithmic function of time and the early part of the response is extrapolated to zero tension. While the phenomenon has been reported previously (Hoekman, 1976), it has not been systematically described in dystrophic muscle. As indicated in Table 3, the PTC for the dystrophic (line 413) birds is much greater than that for the normals (line 412) in both the EDC and PLD; values for the normals were negligible for both muscles. In addition, the magnitude of the PTC for dystrophic muscle was greater in the PLD than

in the EDC.

The origin of the PTC is not known. The most probable explanation for the phenomenon is that it results from the myotonia of dystrophic chicken muscle. Repetitive (myotonic) action potentials in dystrophic chicken muscle have been demonstrated as a result of muscle stimulation (Holliday et al., 1965), and are often noted on the EMG trace during experimentation (Howlett, unpublished results). It is also possible that the increased number of slow twitch fibres reported in dystrophic muscle contribute to the PTC.

In summary, the EDC and the PLD appear to be similar muscles. There are differences between them but most of these can be explained in terms of the fibre type composition and the gross morphology of the two muscles. Since the EDC contains a larger proportion of slow twitch fibres than does the PLD, progression of the disease proceeds at a slower rate in this muscle. The comparisons in Table 3 are based on six week old birds; note that most of the "differences" between the PLD and the EDC are discrepancies between the magnitude, not the direction, of the effect. These become insignificant when one examines the EDC in older birds. Clearly the disease progresses faster in the PLD than in the EDC.

4.3 Sex Differences in Hereditary Avian Muscular Dystrophy

Although the gene for muscular dystrophy of the domestic fowl is autosomal recessive (Asmundson and Julian, 1956; Julian and Asmundson, 1963; Wilson et al., 1979), it has generally been noted that the expression of the gene may differ in males and females. Many researchers routinely use only male birds for experimentation while others pool the data obtained from male and female birds. If, indeed, a difference does exist in the phenotypic expression of the disease in males and females, pooling data for both sexes may increase the chances of making a Type II error on tests of significance.

A systematic investigation of these sex differences has never been reported, so the existence of such differences remains speculative. Few studies have even attempted to separate birds on the basis of sex. One study that did showed that on several clinical assays of dystrophy males were more severely affected by the disease than females; however the authors failed to discuss either these results or the implications of such findings for the avian model of muscular dystrophy (Chou et al., 1975).

4.3.1 Exhaustion score. Chou et. al. (1975) reported that male dystrophics lost the ability to rise

from the supine position earlier and at a faster rate than did females. The righting ability of males dropped to zero between 40-50 days ex ovo, while at day 100 the females still retained some ability to right themselves. These findings correlate well with those reported in the present study. The young dystrophic group (see Figure 13a) showed a marked decrease in the ability to rise from the supine position for both sexes. The disability increased progressively with age in male birds but not in females (see Figure 13b).

The inability of dystrophic birds to rise from the supine position appears to be a stable indicator of avian dystrophy which has been reported in all lines of genetically dystrophic chickens (Wilson et al., 1979).

It is not likely that the impaired righting ability is related to relative muscle strength, since dramatic declines in this parameter are apparent before concomitant decreases in muscle strength are seen (see Figures 34a, 34b, 35a & 35b). Although the specific defect responsible for impaired righting ability is not known it has been suggested that the myotonia of the dystrophic chicken muscle could produce muscle rigidity (Entrikin et al., 1977). It is also possible that a decrease in wing flexibility and/or selective hypertrophy of muscles used in abduction or adduction of the wing produces the observed effect.

4.3.2 Wing apposition score. The degree to which the wings can be elevated beyond the horizontal plane, the so-called wing apposition score, has been shown to be compromised in dystrophic chickens (Chou et al., 1975). When these effects were separated on the basis of sex, the data of Chou et al. (1975) are again in agreement with the findings reported in the present study; dystrophic males were more severely affected by the disease than the dystrophic females. While the ability to appose the wings deteriorated with age in the males, this manifestation of the disease did not appear to have progressed further with age in the females (see Figures 14a & 14b). The origin of the decreased flexibility of the wings in dystrophic chickens is unknown; possibly increased connective tissue decreases the elasticity of the muscles or hypertrophy of certain muscle groups hinders passive muscle movement. The myotonia may also play a role since the contractures are sensitive to percussion and other mechanical manipulation.

4.3.3 Mechanical characteristics. The presence of sex differences on mechanical assays of dystrophy has never been documented.

In the present study, for those mechanical assays of dystrophy that most reliably separated normal and dystrophic birds, the young females were affected by the disease to the same or to a greater extent than were the young males. The general effect of age was to dramatically

increase the severity of the effects on disease indices in the older males whereas their values were not appreciably affected by age in the females. Specifically data showed marked age-related increases in the magnitude of the PTP for the dystrophic males that clearly were not observed for the dystrophic females (see Figures 16 & 17). The twitch to tetanus ratios recorded for the young dystrophics were decreased in magnitude for the females but not for the males (see Figure 20); in the older birds the twitch to tetanus ratios were depressed for the male dystrophic group but did not appear to be changed as a result of age for the females (see Figure 21). The disease-related enhancement of the PTC was substantial for the young females and absent for the young males (see Figure 29). In the older group there was a marked enhancement of the PTC for the males whereas the PTC did not "worsen" in the dystrophic females (see Figure 30).

There were significant interaction effects of sex and disease for both the twitch contraction time and the tetanic tension generated per gram of muscle for the young group. There was a significant decrease in the contraction time (see Figure 23a) for young dystrophic females when compared to age-matched males. This finding was rather unexpected since contraction times have been reported to be within the normal range for dystrophic avian muscle. This is not consistent with the observations that both the twitch tension per gram of muscle (see Figure 34a) and the

duration of the active state plateau (see Figure 27a) were similar in both the normal and dystrophic females of the young group. At present no simple explanation for the decrease in the contraction times can be offered. One possibility is that the duration of the active state plateau is actually reduced for the young dystrophic females. Figure 27a indicates an obvious trend in the data in that direction whereas the active state plateau duration actually appears increased for the young dystrophic males. Although a decreased duration of the active state plateau is consistent with a decreased twitch contraction time for the young females, this is contingent upon little or no decrease in the rate of rise of tension. Since the maximal rate of rise of tension is reduced in the young group for both sexes (see Figure 32), it is not clear whether the reduction in the active state plateau duration for the young females is sufficient to account for the shortened twitch contraction time.

The sex of the bird had no apparent bearing upon the twitch tension generated per gram of muscle (see Figures 34a & 34b), the duration of the active state plateau (see Figures 27a & 27b) or the rate of rise of tension (see Figures 32 & 33) although these parameters significantly decreased with age as a function of disease. Finally, there were no effects attributable to sex or disease on the muscle latent period (see Figures 26a & 26b), the twitch half-relaxation time (see Figures 25a &

25b) or the tetanus half-relaxation time (see Figures 28a & 28b).

4.4 The Effects of Age on the Mechanical Characteristics of Normal and Dystrophic Muscle

A qualitative assessment of the effects of age on the mechanical properties of normal and dystrophic muscle will be briefly considered in this section.

The following effects were noted. The duration of the latent period was increased in old muscles from normal and dystrophic birds. This was probably a result of increased connective tissue; increased connective tissue has been reported in ageing human muscle (Tomlinson et al., 1969). This tends to increase the series elastic compliance of the muscle and thus increase the latent period. In addition, the nerve would be longer in older birds so the conduction time would be prolonged. The duration of the active state plateau increased with age, particularly for normal muscle; this suggests either that there is more calcium available in adult muscle, that the calcium reaccumulation system is less efficient in ageing muscle or that there was an age-dependent increase in the duration of the action potential. There was a general age-related slowing of both the twitch and the tetanic half-relaxation times in normal and dystrophic muscle. Since the half-

relaxation times for twitch and tetanic stimuli were also increased it seems possible that the increase in duration of the active state results from a decrease in the rate of calcium reaccumulation by the sarcoplasmic reticulum. The tension generated per gram of muscle for either a twitch or a tetanus was reduced in older muscle in both normal and dystrophic birds. Reduction in muscle strength has also been reported for elderly humans (Campbell et al., 1973) and probably reflects the loss of muscle fibres, particularly those of the fast twitch variety.

4.5 A Possible Explanation for the Sex Differences Observed in the Phenotypic Expression of Hereditary Avian Muscular Dystrophy

4.5.1 A generalized membrane defect in muscular dystrophy? Recent developments in the field of muscular dystrophy research suggest that the primary defect is a "generalized membrane defect" essentially affecting all (or most) of the membranes of the body to some extent. The theory holds that the genetic fault in the muscular dystrophies affects an enzyme, structural protein or other membrane constituent that results in a membrane abnormality; this abnormality causes the degeneration of the muscle fibres. Controversy in the literature no longer

centres around whether muscular dystrophy is a primary neuropathy or a primary myopathy, rather whether the existence of a non-specific membrane defect in dystrophic tissues could account for most of the data.

Clinical abnormalities in such diverse tissues as erythrocytes, nervous tissue, liver and ectodermal derivatives have been reported for some of the human dystrophies, in particular for Duchenne dystrophy (Rowland, 1980). In addition, defects in the activity of membrane-bound enzymes (i.e. sodium-potassium ATPase, adenylyl cyclase, AChE, protein kinase and calcium ATPase) have been demonstrated (see Rowland, 1980). Similar findings have been reported in the genetically dystrophic chicken. Alterations in the activity of membrane bound enzymes in the muscle (adenylyl cyclase, sodium-potassium ATPase), liver (adenylyl cyclase, sodium-potassium ATPase) and erythrocyte (adenylyl cyclase) membranes of dystrophic birds have been demonstrated (Rodan et al., 1974; Rodan et al., 1979). In addition, several sarcolemmal enzymes (Owens, 1979) have been shown to be altered in dystrophic chicken muscle. That muscular dystrophy principally manifests itself as a muscle disorder is reasonable; alterations in the delicate microenvironment of the muscle membrane or membrane of the sarcoplasmic reticulum could readily interfere with the functioning of the muscle whereas similar alterations in the membrane structure of static systems such as myelin would fail to cause such notable effects.

4.5.2 Why does dystrophy fail to progress in female dystrophic chickens? There are a number of sex-related physiological differences that are predictable based on previous work. The hypothesis advanced in the present study is that the failure of the disease to progress in mature female chickens may be due to the massive loss of cholesterol associated with egg-laying in the hen.

Evidence for sex differences in cholesterol levels in avian species originates in the atherosclerosis literature. Chickens are a good model of experimental atherosclerosis; they develop hypercholesterolemia and arterial plaques readily when maintained on a high-cholesterol diet. As a consequence, variations in the cholesterol levels of male and female chickens have been well documented. Mature females on a normal diet appeared to have lower serum-cholesterol levels than age-matched males (Stamler et al., 1954). The egg-laying hens regularly "cleared" large quantities of cholesterol from their bodies via egg-laying. Oviduct ligation of mature hens resulted in serum cholesterol levels similar to those seen in roosters of the same age; this was probably caused by reabsorption and recirculation of the yolk cholesterol (Stamler et al., 1954).

That the females initially appeared "worse" than the males on the assays of dystrophy used in the present experiment is not an unexpected result since levels of

cholesterol gradually rise in females to peak when egg-laying commences at approximately 4 months of age (Lorenz et al., 1938).

4.5.3 Altered membrane cholesterol as a primary defect in muscular dystrophy. There are a number of means whereby cholesterol can alter the properties of membranes in ways that are consistent with the symptoms of avian muscular dystrophy. Cholesterol is known to be a primary determinant of membrane fluidity (Oldfield and Chapman, 1972). It has been established that changes in membrane cholesterol can alter the activity of various membrane-bound enzymes and transport proteins (Farias et al., 1975) in addition to reducing the permeability of membranes to some molecules and monovalent cations (Papahadjopoulos, 1973). Recently, Madden et al. (1979) demonstrated that the enzyme activity of calcium ATPase in the sarcoplasmic reticulum decreased as the cholesterol:phospholipid ratio of the membrane increased. Furthermore, this effect was reversible upon extraction of the cholesterol.

There are several theories which attempt to explain how these effects of cholesterol, reduction of membrane fluidity and inhibition of the activity of intrinsic membrane enzymes, produce the muscle fibre necrosis associated with dystrophy. One such theory holds that inhibition of membrane pumps, in particular the sodium-potassium ATPase, produces osmotic imbalance within

the cell and subsequent cell lysis (Papahadjopoulos, 1973). Cullen and Fulthorpe (1975) suggest that the altered permeability of the sarcolemma allows calcium influx; the calcium causes a locally hypercontracted sarcomere that "rips" the adjacent sarcomeres initiating necrosis. Another calcium theory suggests that excessive calcium influx causes increased calcium uptake by the mitochondria, a process that steals energy from essential cell functions (Wrogemann and Pena, 1976).

Hsu and Kaldor (1971) examined the lipid components of fragmented sarcoplasmic reticulum from dystrophic chickens and noted abnormalities in the composition of these membranes. Specifically, they found elevated membrane cholesterol and triglyceride levels; the composition of triglycerides comprising the membranes was similar in fractions from normal and dystrophic birds. The total phospholipid levels remained the same but the relative contributions of individual phospholipids to that total was altered. In addition, Hsu and Kaldor (1971) reported poor uptake of calcium by fragmented dystrophic sarcoplasmic reticulum. The cholesterol:phospholipid ratio was increased in dystrophic sarcoplasmic reticulum fragments and the amount of lipid per mg protein was increased. The suggestion was made that reduced calcium uptake by dystrophic sarcoplasmic reticulum resulted from a decreased number of carrier proteins per unit area, for that ion. Since it has been shown that the incorporation

of cholesterol into membranes can alter the permeability characteristics of the membrane, it is also possible that the cholesterol is responsible for the alterations in sarcoplasmic reticulum function (Rothfield and Finkelstein, 1968).

Sha'afi et al. (1975) reported higher membrane microviscosity in the membranes of muscle, liver, and erythrocytes in genetically dystrophic chickens. This increase was particularly marked in muscle. Since cholesterol is known to stabilize membranes (i.e. decrease the fluidity of membranes) by binding within the fluid phase of the membrane between the hydrophobic tails of the phospholipids (Oldfield and Chapman, 1972), the reported increase in the viscosity of dystrophic membranes is hardly surprising. Eckstein et al. (1979), however, reported no decrease in the fluidity of erythrocyte membranes in the chicken. This may be a subtle difference between the Storrs and the Line 413 dystrophic birds; Sha'afi et al. (1975) used Storrs birds whereas Eckstein et al. (1979) examined the Line 413 birds.

This change in the cholesterol content of dystrophic tissues appears to be an early manifestation of the disease. Stewart et al. (1977) reported that serum, liver and muscle cholesterol levels were significantly elevated in dystrophic birds when compared to their normal counterparts as early as 9 days in ovo. It is also worthy to note that the cholesterol content was elevated in the

dystrophic pectoralis, a fast twitch muscle, but not in the slower thigh muscle; the fast twitch muscles are preferentially destroyed by the disease process.

It is not likely that the increased membrane cholesterol in dystrophic tissues is a direct effect of increased serum cholesterol. Sources of cholesterol are the diet and synthesis in the liver and the small bowel. The body appears to carefully maintain the serum cholesterol level at a predetermined value under normal conditions despite variations in both diet and demand, presumably by regulation of synthesis. There are doubtless many mechanisms responsible for the regulation of cholesterol but these are largely unknown. The anomalous gene responsible for avian dystrophy could exert its primary effect on factors responsible for the regulation of synthesis, metabolism or uptake of cholesterol.

4.5.4 Exercise and cholesterol. Endurance exercise in man has been shown to decrease total serum cholesterol levels (Martin et al., 1977; Wood et al., 1977). Hardy et al. (1962) showed that differences in serum cholesterol were observed between chickens raised "on the floor" and chickens raised in a battery. In both cases the normals and dystrophics were fed on similar diets, thus it seems likely that the alterations in serum cholesterol reflect the reduced activity levels of chickens raised in a battery. Transient improvements have been reported in some

of the human dystrophies (Duchenne, fascioscapulohumeral and limb-girdle) upon institution of an exercise regimen (Vignos and Watkins, 1966). In addition, it has been shown that exercise alone can improve the righting ability and lower the serum CPK levels in chickens with hereditary muscular dystrophy (Entrikin et al., 1977; Hudecki et al., 1978).

Although it is reasonable to assume that exercise-related improvements in the human and animal dystrophies are related to hypertrophy of surviving muscle fibres, exercise effects on the plasma-cholesterol levels cannot be ruled out. Recall that Madden et al. (1979) showed that the decreased calcium ATPase activity observed in sarcoplasmic reticulum vesicles that were incubated in high cholesterol was an effect that was reversible upon removal of the cholesterol from the medium.

4.5.5 Conclusions. In summary, the hypothesis proposed is that the failure of dystrophy to progress in female chickens is directly related to the dramatic decrease in serum cholesterol at maturity that is associated with egg-laying. A certain amount of support for this theory exists. Several workers have reported membrane abnormalities associated with the dystrophic condition in the chicken. Specifically, they noted that membrane cholesterol content and serum cholesterol levels are decreased in avian muscular dystrophy. Exercise, which

is known to transiently improve motor skills in both patients and birds with muscular dystrophy, has been shown to decrease serum cholesterol levels. Increased serum cholesterol is capable of altering membrane fluidity, membrane permeability and the activity of intrinsically bound membrane proteins. Several theories have been advanced to explain how such membrane defects could produce the symptoms of muscular dystrophy.

Summary

From the results obtained in the present study the following points can be made.

- 1) The new in vivo nerve-muscle preparation of the EDC muscle is similar to the PLD muscle preparation with respect to histochemistry and physiologic parameters of contractility.
- 2) The EDC preparation has several advantages over the PLD preparation; it is readily accessible with minimal surgical trauma and a convenient intra-arterial injection route exists which facilitates the assessment of acute pharmacological responses.
- 3) Quantitative sex differences as measured by clinical and physiological assays of dystrophy do exist in genetically dystrophic chickens despite the fact that the gene is autosomal.
- 4) In the younger, sexually immature group the females are as severely affected by the disease as the males. In some cases the females appear to be "worse" than the males. When these results are compared to those in the older, sexually mature group, the disease progresses substantially

in the males and fails to progress in the females.

5) A body of evidence exists that supports the idea that the failure of the disease to progress in females is due to the dramatic decrease in serum cholesterol associated with egg-laying in the hen.

References

- Albuquerque, E.X. and Warnick, J.E. 1971. Electrophysiological observations in normal and dystrophic chicken muscles. *Science*. 172: 1260-1261.
- Asmundson, V.S. and Julian, L.M. 1956. Inherited muscle abnormality in the domestic fowl. *Jour. Hered.* 47: 248-252.
- Barak, T. and Anderson, P.J. 1968. Oxidative enzymes. pages 312-313 in *Histochemistry Theory, Practice and Bibliography*. Holber Medical Division, Harper and Row Publishers Inc., New York.
- Beringer, T. 1978. Stereologic analysis of normal and dystrophic avian W myofibres. *Exp. Neurol.* 61: 380-394.
- Bird, J.W. 1975. Skeletal muscle lysosomes. pages 75-109 in J.R. Dingle and R.T. Dean Eds., *Lysosomes in biology and pathology*. North Holland, Amsterdam.
- Bradley, W.G. and Jenkinson, M. 1973. Abnormalities of peripheral nerves in murine muscular dystrophy. *J. Neurol. Sci.* 18: 227-247.
- Bryan, J.S. and Letinsky, M.S. 1979. Morphology and electrophysiology of dystrophic chicken muscle. *Soc. Neurosci. Abs.* 5: 477.
- Burger, R.E. and Lorenz, F.W. 1960. Artificial respiration in birds by unidirectional air flow. *Poultry Sci.* 39: 236-237.
- Burke, R.E. and Tsairis, P. 1974. The correlation of physiological properties with histochemical characteristics in single motor units. *Ann. N.Y. Acad. Sci.* 228: 145-159.

- Campbell, M.J., McComas, A.J. & Petito, F. 1973. Physiological changes in aging muscles. *J. Neurol. Neurosurg. & Psychia.* 36: 174-182.
- Cardinet, G.H., Freedland, R.A., Tyler, W.S. and Julian, L.M. 1972. Morphologic, histochemical and quantitative enzyme study of hereditary avian muscular dystrophy. *Am. J. of Vet. Res.* 33: 1671-1684.
- Chou, T., Hill, E.J., Bartle, E., Wooley, K., LeQuire, V., Olson, W., Roelofs, R. and Park, J.H. 1975. Beneficial effects of penicillamine treatment on hereditary avian muscular dystrophy. *J. Clin. Invest.* 56: 842-849.
- Cosmos, E. 1964. Intracellular distribution of calcium in developing breast muscle of normal and dystrophic chickens. *J. Cell. Biol.* 23: 241-252.
- Cosmos, E. 1966. Enzymatic activity of differentiating muscle fibres. *Dev. Biol.* 13: 163-181.
- Cosmos, E. 1970. Ontogeny of red and white muscles: the enzymic profile and lipid distribution of immature and mature muscles of normal and dystrophic chickens. pages 193-207 in A.T. Milhorat Ed., *Exploratory Concepts in Muscular Dystrophy and Related Disorders.* Excerpta Medica, Amsterdam.
- Cosmos, E. 1974. Muscle transplants: role in the etiology of hereditary muscular dystrophy. pages 368-373 in A.T. Milhorat Ed., *Exploratory Concepts in Muscular Dystrophy II.* Excerpta Medica, Amsterdam.
- Cosmos, E. and Butler, J. 1967. Differentiation of fibre types in muscle of normal and dystrophic chickens: A quantitative and histochemical study of the ontogeny of muscle enzymes, pages 197-204 in A.T. Milhorat Ed., *Exploratory Concepts in Muscular Dystrophy and Related Disorders.* Excerpta Medica, Amsterdam.

- Cosmos, E. Butler, J. Allard, E.P. and Mazlish, J. 1979. Factors that influence the phenotypic expression of genetically normal and dystrophic muscles. *Ann. N.Y. Acad. Sci.* 317: 571-592.
- Crowe, L.M. and Baskin, R.J. 1978. Freeze fracture of intact membranes. *J. Ultrastruc. Res.* 62: 147-154.
- Crowe, L.M. and Baskin, R.J. 1979. Stereologic analysis of dystrophic chicken muscle. *Am. J. Path.* 95: 295-316.
- Cullen, M.J. and Fulthorpe, J.J. 1975. Stages in fibre breakdown in Duchenne muscular dystrophy. *J. Neurol. Sci.* 24: 179-200.
- Dawson, D.M. and Kaplan, N.O. 1965. Factors influencing the concentration of enzymes in various muscles. *J. Biol. Chem.* 240: 3215-3221.
- DeSantis, M., Hoekman, T. and Limwongse, V. 1976. Retrograde transport of peroxidase in motor neurons innervating slow and fast muscles: absence of a difference between normal and dystrophic chickens. *Brain Res.* 119: 454-458.
- DiGiamberardino, L., Couraud, J.Y. and Barnard, E.A. 1979. Normal axonal transport of acetylcholinesterase forms in peripheral nerves of dystrophic chickens. *Brain Res.* 160: 196-202.
- Eckstein, T.B., Randall, W.R. and McNamee, M.G. 1979. Erythrocyte plasma membrane fluidity in avian muscular dystrophy. *Exp. Neurol.* 64: 315-326.
- Entrikin, R.K. and Bryant, S.H. 1977. Membrane electrical effects of phenytoin on skeletal muscle of dystrophic chickens. *Fed. Proc.* 36: 499.

- Entrikin, R.K., Swanson, K.L., Weidoff, P.M., Patterson, G.T. and Wilson, B.W. 1977. Avian muscular dystrophy: functional and biochemical improvement with diphenylhydantoin, *Science*, 195: 873-875.
- Farias, R.N., Bloj, E., Morero, R.D., Sineriz, F. and Trucco, R.E. 1975. Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. *Bioch. Biophys. Acta*. 415: 231-251.
- Fitzsimons, R.B. and Hoh, J.E.Y. 1981. Embryonic and fetal myosins in human skeletal muscle. The presence of fetal myosins in Duchenne muscular dystrophy and infantile spinal muscular atrophy. *J. Neurol. Sci.* 52: 367-384.
- Gardner-Medwin, D. 1980. Clinical features and classification of the muscular dystrophies. *Brit. Med. Bull.* 36: 109-115.
- Gasser, H.S. and Hill, A.V. 1924. Dynamics of muscular contraction. *Proc. Roy. Soc. B.* 96: 398.
- Guth, L. and Samaha, F.J. 1971. Procedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurol.* 28: 365-367.
- Hardy, L.B., Auger, H.V. and Wilcox, F.H. 1962. Genetic differences in serum cholesterol in chickens. *Am. J. Physiol.* 202: 997-1001.
- Harris, J.B. and Slater, C.R. 1980. Animal models: what is their relevance to the pathogenesis of human. *Brit. Med. Bull.*, 36: 193-197.
- Hill, A.V. 1950. Development of the active state of a muscle during the latent period. *Proc. Roy. Soc. B.* 137: 320.
- Hill, A.V. 1951. The effect of series compliance on the tension development in a muscle twitch. *Proc. Roy. Soc. B.* 138: 325-328.

- Hoekman, T.B. 1974. An in vivo nerve-muscle preparation in the study of avian muscular dystrophy. Pharmacologist. 36: 233.
- Hoekman, T.B. 1976. Isometric contractile properties of the posterior latissimus dorsi muscle in normal and dystrophic chickens. Exp. Neurol. 53: 729-743.
- Hoekman, T.B. 1977. Fatigability of normal and dystrophic chicken muscle in vivo. Exp. Neurol. 54: 565-578.
- Hoekman, T.B., Howlett, S.E., Umanee, V. and Redfern, P. 1982. Comparison of in vivo contractile and pharmacologic responses of a fast twitch skeletal muscle in normal (line 412 New Hampshire and White Leghorn) and dystrophic (line 413 New Hampshire and Storrs White Leghorn backcross) chickens. Sixth Nat. Sci. Workshop of the Musc. Dyst. Assoc. of Canada Abs.
- Hoekman, T.B. unpublished results.
- Holliday, T.A., Van Meter, J.R., Julian, L.M. and Assundson, V.S. 1965. Electromyography of chickens with inherited muscular dystrophy. Am. J. Physiol. 209: 871-876.
- Howlett, S.E. unpublished results.
- Hsu, Q. and Kaldor, G. 1971. Studies on the lipid composition of fragmented sarcoplasmic reticulum of normal and dystrophic chickens. Proc. Soc. Exp. Biol. Med. 138: 733-737.
- Hudecki, M.S., Pollina, C., Bhargava, J.E., Fitzpatrick, J.E., Privitera, C.A. and Schmidt, D. 1978. Effect of exercise on chickens with hereditary muscular dystrophy. Exp. Neurol. 61: 65-73.
- Hughes, J.R. 1958. Post-tetanic potentiation. Physiol. Rev. 38: 91-113.

- Hunter, S. 1980. The heart in muscular dystrophy. *Brit. Med. Bull.* 36: 133-134.
- Jaros, E. and Bradley, W.G. 1978. Development of the myelinated lesion in the ventral root of the dystrophic mouse. *J. Neurol. Sci.* 36: 317-339.
- Jedrzejczyk, J., Wieckowski, J., Rymaszewska, T. and Barnard, E.A. 1973. Dystrophic chicken muscle: altered synaptic acetylcholinesterase. *Science*. 180: 404-408.
- Johnson, D.D., Bailey, S. and Wenger, B.S. 1981. Trophic action of sheep sciatic nerve extracts from normal and dystrophic chick embryos: failure of dystrophic muscle to respond. *Exp. Neurol.* 73: 421-429.
- Julian, L.M. and Asmundson, V.S. 1963. Muscular dystrophy of the chicken. pages 458-497 in G.H. Bourne and N.S. Golarz Eds., Karger, N.Y.
- Kaplan, N.O. and Cahn, R.D. 1962. Lactic dehydrogenases and muscular dystrophy in the chicken. *Proc. Natl. Acad. Sci.* 48: 2123-2130.
- Katz, B. 1939. The relation between force and speed in muscular contraction. *J. Physiol.* 96: 45-64.
- Kikuchi, T., Doerr, L. and Ashmore, C.R. 1980. A possible mechanism of phenotypic expression of normal and dystrophic genomes on succinate dehydrogenase activity and fibre size within a single myofibre of muscle transplant. *J. Neurol. Sci.* 45: 273-286.
- King, D.B., King, C.R. and Jacaruso, R.B. 1981. Avian muscular dystrophy: thyroidal influence on pectoralis muscle growth and glucose-6-phosphate dehydrogenase activity. *Life Sci.* 28: 577-585.
- Lebeda, F.J. and Albuquerque, E.X. 1975. Membrane cable properties of normal and dystrophic chicken muscle fibres. 47: 544-557.

- Libelius, R., Jirmanova, I., Lundquist, L., Teskeff, S. and Barnard, E.A. 1979. T-tubule endocytosis in dystrophic chicken muscle and its relation to muscle fibre degeneration. *Acta Neuropath.* 48: 31-38.
- Linkhart, T.A., Yee, G.W. and Wilson, B.W. 1975. Myogenic defect in acetylcholinesterase regulation in muscular dystrophy of the chicken. *Science.* 187: 549-557.
- Linkhart, T.A., Yee, G.W., Wieberg, P.S. and Wilson, B.W. 1976. Myogenic defect in muscular dystrophy of the chicken. *Dev. Biol.* 48: 447-457.
- Liu, P.K.S., Barnard, E.A. and Barnard, P.J. 1980. Blood plasma pyruvate kinase as a marker of muscular dystrophy: Properties in dystrophic chickens and hamsters. *Exp. Neurol.* 67: 581-600.
- Lorenz, F.W., Chaikoff, I.L. and Entenman, C. 1938. The endocrine control of lipid metabolism in the bird II. The effects of estrin on the blood lipids of the immature domestic fowl. *J. Biol. Chem.* 126: 763-769.
- Lyles, J.M., Barnard, E.A. and Silman, I. 1980. Changes in the levels and forms of cholinesterases in the blood plasma of normal and dystrophic chickens. *J. Neurochem.* 34: 978-987.
- MacFarlane, W.V. 1953. Intracellular records of repetitive activity at the end plate of skeletal muscle fibres. *J. Physiol.* 119: 42P.
- Madden, T.D., Chapman, D. and Quinn, P.J. 1979. Cholesterol modulates the activity of calcium-dependant ATPase of the sarcoplasmic reticulum. *Nature.* 279: 538-541.
- Mahler, M. 1979. Progressive loss of mitochondrial creatine phosphokinase activity in muscular dystrophy. *Bioch. Biophys. Res. Comm.* 88: 895-906.

- Malouf, N.N. and Sommer, J.R. 1976. Chicken dystrophy - the geometry of the t-tubules. *Amer. J. Path.* 84: 299-318.
- Martin, R.P., Haskell, W.L. and Wood, P.D. 1977. Blood chemistry and lipid profiles of elite distance runners. *Ann. N.Y. Acad. Sci.* 301: 346-360.
- McMurtry, S.L., Julian, L.M. and Asmundson, V.S. 1972. Hereditary muscular dystrophy of the chicken. *Arch. Path.* 94: 217-224.
- Mendell, J.R., Higgins, R., Sahenk, Z. and Cosnos, E. 1979. relevance of the genetic animal models of muscular dystrophy to human dystrophy. *Ann. N.Y. Acad. Sci.* 317: 409-430.
- Obinata, T., Takano-Ohmuro, H. and Matsuda, R. 1980. Changes in troponin-T and myosin isoenzymes during development of normal and dystrophic chicken muscles. *FEBS Letters.* 120: 195-198.
- Oldfield, E. and Chapman, D. 1972. Dynamics of lipids in membranes: heterogeneity and the role of cholesterol. *FEBS Letters.* 23: 285-297.
- Owens, K. 1979. Biochemical studies of dystrophy in the young chicken: lysosomal and sarcolemmal enzymes. *Ann. N.Y. Acad. Sci.* 317: 247-262.
- Papahadjopoulos, D., Cowden, M. and Kimelberg, R. 1973. Role of cholesterol in membranes: effect on phospholipid-protein interaction, membrane permeability and enzymatic activity. *Bioch. Biophys. Acta.* 330: 8-26.
- Park, J.H., Hill, E.J., Chou, T.H., LeQuire, V., Roelofs, R. and Park, C.R. 1979. Mechanism of action of penicillamine in the treatment of avian muscular dystrophy. *Ann. N.Y. Acad. Sci.* 317: 356-369.
- Patterson, G.T. and Wilson, B.W. 1976a. Distribution of extrajunctional acetylcholinesterase in muscle from normal and dystrophic chickens. *Exp. Neurol.* 50: 214-225.

- Patterson, G.T. and Wilson, B.W. 1976b. Distribution of acetylcholinesterase activity in normal, dystrophic and denervated muscles of the chicken. *Exptl. Neurol.* 52: 250-262.
- Randall, W.R. and Wilson, B.W. 1980. Properties of muscles from chickens with inherited muscular dystrophy. *J. Neurol. Sci.*, 46: 145-155.
- Rasminsky, M. 1978. Ectopic generation of impulses and cross talk in spinal nerve roots of "dystrophic" mice. *Ann. Neurol.* 3: 351-357.
- Rasminsky, M. 1980. Ephaptic transmission between single nerve fibres in the spinal nerve roots of dystrophic mice. *J. Physiol.* 305: 151-169.
- Ritchie, J.M. 1954. The duration of the plateau of full activity in frog muscle. *J. Physiol.* 124: 605-612.
- Rodan, S.B., Hintz, R.L., Sha'afi, R.I. and Rodan, G.A. 1974. The activity of membrane bound enzymes in muscular dystrophic chickens. *Nature.* 252: 589-591.
- Rodan, G.I., Rodan, S.B., Raible, D.G., Cutler, L.S., Wacholtz, M. and Sha'afi, R.I. 1979. Adenylate cyclase in muscular dystrophy. *Ann. N.Y. Acad. Sci.* 317: 670-689.
- Rothfield, L. and Finkelstein, A. 1968. Membrane Biochemistry. *Ann. Rev. Bioch.* 37: 463-491.
- Rowland, L.P. 1980. Biochemistry of muscle membranes in Duchenne muscular dystrophy. *Muscle and Nerve.* 3: 3-20.
- Sandow, A. and Brust, M. 1966. Contractility of dystrophic mouse muscle. *Amer. J. Physiol.* 194: 557-563.

- Sha'afi, R.I., Rodan, S.B., Hintz, R.L., Fernandez, S.M. and Rodan, G.A. 1975. Abnormalities in membrane microviscosity and ion transport in genetic muscular dystrophy. *Nature*. 254: 525-526.
- Shafiq, S.A., Askansas, V., Asiedu, A. and Milhorat, A.T. 1972. Structural changes in human and chicken muscular dystrophy. *Muscle Biol.* 1: 255-272.
- Shanarina, N.M. 1961. Pessimal response of a single muscle fibre to indirect stimulation. *Secenov Physiol. J. USSR. (Eng)*. 47: 91.
- Silver, A. 1974. pages 67-98 in *The Biology of Cholinesterases*. Elsevier. Amsterdam.
- Stanler, J., Pick, R. and Katz, L.N. 1954. Inhibition of cholesterol-induced coronary atherogenesis in the egg producing hen. *Circulation*. 10: 251-254.
- Standaert, F.G. 1963. Post-tetanic repetitive activity in the cat soleus nerve; its origin, course and mechanism of action. *J. Gen. Physiol.* 47: 53-70.
- Standaert, F.G. 1964. Mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. *J. Gen. Physiol.* 47: 987-1001.
- Stewart, P.A., Werstfluk, E.S., Vickers, J.D. and Rathbone, M.P. 1977. Elevated cholesterol in tissues of chicken embryos with hereditary myotonic muscular dystrophy. *Exp. Neurol.* 57: 475-485.
- Stewart, P.A., Percy, M.E., Chang, L.S. and Thompson, M.W. 1981. Creatine kinase isozyme transition in chicks with hereditary muscular dystrophy. *Muscle and Nerve*. 4: 165-173.
- Stromska, D., Ochs, S. and Muller, J. 1981. Rate of axoplasmic transport in the dystrophic chicken. *Exp. Neurol.* 74: 530-547.

- Sullivan, G.E. 1962. Anatomy and embryology of the wing musculature of the domestic fowl (gallus). Aust. J. Zool. 10: 458-518.
- Tonlinson, B.E., Walton, J.N. and Rebeiz, J.J. 1969. The effects of ageing and of cachexia upon skeletal muscle. A histopathological study. J. Neurol. Sci. 9: 321-346.
- Vignos, P.J. and Watkins, M.P. 1966. The effects of exercise in muscular dystrophy. JAMA. 197: 121-126.
- Vrbova, G., Gordon, T. and Jones, R. 1978. Nerve-Muscle Interaction. John Wiley and Sons. New York.
- Wernick, J.E., Lebeda, F.J. and Albuquerque, E.X. 1979. Junctional and extrajunctional aspects of inherited muscular dystrophy in chickens: development and pharmacology. Ann. N.Y. Acad. Sci. 317: 263-282.
- Wilson, B.W., Montgomery, M.A. and Asmundson, R.V. 1968. Cholinesterase activity and inherited muscular dystrophy of the chicken. Proc. Soc. Exp. Biol. Med. 129: 199-206.
- Wilson, B.W., Kaplan, M.A., Merhoff, W.C. and Mpr, S.S. 1970. Innervation and the regulation of acetylcholinesterase activity during the development of normal and dystrophic chick muscle. J. Exp. Zool. 174: 39-54.
- Wilson, B.W., Linkhart, T.A., Walker, C.R. and Nieberg, P.S. 1973. Tissue acetylcholinesterase in plasma of chick embryos and dystrophic chickens. J. Neurol. Sci. 18: 333-350.
- Wilson, B.W., Taylor, R.G., Fowler, W.M., Patterson, G.T., Nieberg, P.S., Linkhart, S.G., Linkhart, T.A. and Fry, D.M. 1975. Incidence of acetylcholinesterase in the sarcoplasm of human and chicken muscles. J. Neurol. Sci. 26: 133-146.

Wilson, B.W., Randall, W.R., Patterson, G.T. and Entrikin, R.K. 1979. Major physiologic and histochemical characteristics of inherited dystrophy of the chicken. *Ann. N.Y. Acad. Sci.* 317: 224-246.

Winegrad, S. 1968. Intracellular calcium movements of frog skeletal muscle during recovery from a tetanus. *J. Gen. Physiol.* 51: 65-83.

Wood, P.D., Haskell, W.L., Stern, M.P., Lewis, S. and Perry, C. 1977. Lipoprotein distribution in male and female runners. *Ann. N.Y. Acad. Sci.* 301: 748-763.

Woodward, J., Park, J.H. and Colwick, S.P. 1979. The increase in hexokinase activity in hereditary avian-muscular dystrophy. 586: 641-644.

Wrogemann, K. and Pena, S.D.J. 1976. Mitochondrial calcium overload: a general mechanism for cell necrosis in muscle disease. *Lancet.* 1: 672-673.

Yorita, T., Nakamura, H. and Nonaka, I. 1980. Satellite cells and muscle regeneration in the developing dystrophic chicken. *Exp. Neurol.* 70: 567-575.

Appendix 1

Appendix 1.1 Summary of the Results of the Analysis of Variance for the Young Group

(see End of table for abbreviations)

Dependent Variable	Factor	Significance
1) Exhaustion Score	S	ns
	D	p<0.01
	SxD	ns
2) Wing Apposition Score	S	ns
	D	p<0.01
	SxD	ns
3) Twitch grams tension per muscle wet wt.	S	ns
	D	ns
	SxD	ns
4) Tetanus grams tension per muscle wet wt.	S	ns
	D	ns
	SxD	p<0.01
5) Twitch contraction time	S	p<0.05
	D	p<0.05
	SxD	ns
6) Twitch half-relaxation time	S	ns
	D	ns
	SxD	ns
7) Tetanus half-relaxation time	S	ns
	D	ns
	SxD	ns
8) Latent period	S	ns
	D	ns
	SxD	ns
9) Duration of the active state plateau	S	ns
	D	ns
	SxD	ns
10) PTP 100Hz	S	ns
	D	p<0.05
	SxD	ns

11) PTP 200 Hz	S D SxD	ns p<0.05 ns
12) PTP 300 Hz	S D SxD	ns p<0.01 p<0.05
13) PTP 400 Hz	S D SxD	ns ns ns
14) PTP 500 Hz	S D SxD	ns ns ns
15) PTP 600 Hz	S D SxD	ns ns ns
16) Twitch:tetanus 100 Hz	S D SxD	ns p<0.05 ns
17) Twitch:tetanus 200 Hz	S D SxD	ns ns ns
18) Twitch:tetanus 300 Hz	S D SxD	ns ns ns
19) Twitch:tetanus 400 Hz	S D SxD	ns ns p<0.05
20) Twitch:tetanus 500 Hz	S D SxD	ns ns ns
21) Twitch:tetanus 600 Hz	S D SxD	ns ns ns
22) % Po/msec 100 Hz	S D SxD	ns p<0.05 ns
23) % Po/msec 200 Hz	S D SxD	ns p<0.05 ns

24) \dot{X} Po/msec 300 Hz	S D SxD	ns p<0.05 ns
25) \dot{X} Po/msec 400 Hz	S D SxD	ns ns ns
26) \dot{X} Po/msec 500 Hz	S D SxD	ns p<0.05 ns
27) \dot{X} Po/msec 600 Hz	S D SxD	ns p<0.01 ns
28) PTC 100 Hz	S D SxD	ns p<0.01 p<0.01
29) PTC 200 Hz	S D SxD	p<0.05 p<0.01 p<0.05
30) PTC 300 Hz	S D SxD	ns p<0.05 ns
31) PTC 400 Hz	S D SxD	p<0.05 p<0.05 p<0.05
32) PTC 500 Hz	S D SxD	p<0.05 p<0.05 p<0.05
33) PTC 600 Hz	S D SxD	p<0.05 p<0.01 p<0.01

Abbreviations:

S - sex

D - disease

SxD - interaction of S and D

ns - not significant

PTP - post-tetanic potentiation

 \dot{X} Po/msec - maximal rate of rise of tetanic tension

PTC - post-tetanic contracture

Appendix 1.2. Summary of the Results of the Analysis of Variance for the Old Group

(see end of table for abbreviations)

Dependent Variable	Factor	Significance
1) Exhaustion Score	S	p<0.01
	D	p<0.01
	SxD	ns
2) Wing Apposition Score	S	p<0.01
	D	p<0.01
	SxD	p<0.01
3) Twitch grams tension per muscle wet wt.	S	ns
	D	p<0.05
	SxD	ns
4) Tetanus grams tension per muscle wet wt.	S	ns
	D	p<0.05
	SxD	ns
5) Twitch contraction time	S	ns
	D	ns
	SxD	ns
6) Twitch half-relaxation time	S	ns
	D	ns
	SxD	ns
7) Tetanus half-relaxation time	S	ns
	D	ns
	SxD	ns
8) Latent period	S	ns
	D	ns
	SxD	ns
9) Duration of the active state plateau	S	ns
	D	p<0.05
	SxD	ns
10) PTP 100Hz	S	ns
	D	p<0.01
	SxD	p<0.05
11) PTP 200 Hz	S	ns
	D	p<0.05
	SxD	ns

12) PTP 300 Hz	S	ns
	D	p<0.01
	SxD	ns
13) PTP 400 Hz	S	ns
	D	p<0.05
	SxD	ns
14) PTP 500 Hz	S	ns
	D	p<0.01
	SxD	ns
15) PTP 600 Hz	S	ns
	D	p<0.01
	SxD	ns
16) Twitch:tetanus 100 Hz	S	ns
	D	p<0.01
	SxD	ns
17) Twitch:tetanus 200 Hz	S	ns
	D	p<0.01
	SxD	ns
18) Twitch:tetanus 300 Hz	S	ns
	D	p<0.05
	SxD	ns
19) Twitch:tetanus 400 Hz	S	ns
	D	ns
	SxD	ns
20) Twitch:tetanus 500 Hz	S	ns
	D	ns
	SxD	ns
21) Twitch:tetanus 600 Hz	S	ns
	D	p<0.01
	SxD	ns
22) I Po/msec 100 Hz	S	ns
	D	ns
	SxD	ns
23) I Po/msec 200 Hz	S	ns
	D	ns
	SxD	ns
24) I Po/msec 300 Hz	S	ns
	D	ns
	SxD	ns

25) \dot{X} Po/msec 400-Hz	S	ns
	D	ns
	SxD	ns
26) \dot{X} Po/msec 500 Hz	S	ns
	D	ns
	SxD	ns
27) \dot{X} Po/msec 600 Hz	S	ns
	D	ns
	SxD	ns
28) PTC 100 Hz	S	ns
	D	p<0.01
	SxD	ns
29) PTC 200 Hz	S	ns
	D	p<0.01
	SxD	ns
30) PTC 300 Hz	S	ns
	D	p<0.01
	SxD	ns
31) PTC 400 Hz	S	ns
	D	p<0.01
	SxD	ns
32) PTC 500 Hz	S	ns
	D	p<0.01
	SxD	ns
33) PTC 600 Hz	S	ns
	D	p<0.01
	SxD	ns

Abbreviations:

S = sex

D = disease

SxD = interaction of S and D

ns = not significant

PTP = post-tetanic potentiation

 \dot{X} Po/msec = maximal rate of rise of tetanic tension

PTC = post-tetanic contracture

