THE MORPHOLOGY OF ERYTHROCYTES AND THE
FINE STRUCTURE OF MYOCARDIUM IN DYSTROPHIC
MICE FOLLOWING EXHAUSTIVE EXERCISE

BY

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Bryan A Mist 1977
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Abstract

The purpose of this study was to resolve conflicting literature on the question of whether exhaustive exercise is detrimental to animals with muscular dystrophy. Normal and genetically dystrophic mice were obtained and assigned to various experimental groups. After swimming to exhaustion on day 1, 11 and 17, mice were either sacrificed immediately or 24 hours post-exercise and compared with control groups of non-exercising normal and dystrophic animals. Assessment of the effects of exercise were achieved by examining myocardial fine-structure, erythrocyte morphology and changes in body weight and heart weight.

There was a noticeable increase in the percentage of deformed erythrocytes following exercise. This increase was greatest immediately following exercise and more so on day 1. There was a tendency for dystrophic mice to exhibit more deformed erythrocytes than non-dystrophic mice but the contribution of a dystrophic factor to surface alterations in the erythrocyte was not as great as the contribution of exercise. No quantifiable differences in myocardial fine-structure were observed between groups. Supercontracted myofibrils were occasionally found in dystrophic mice that had been immediately sacrificed following exhaustive exercise.
but this feature did not appear frequently enough to distinguish this group clearly from the others. Dilated T-tubules, isolated mitochondrial alterations and increased lipid were common findings but were not specific to any one group. The gains in body weight of the exercising dystrophic mice were markedly lower than for non-exercised dystrophic mice but their heart weight / body weight ratios were increased.

It was concluded that while exhaustive exercise appeared to have no detectible adverse effects on the myocardium of 6-8 week old dystrophic mice, further work is needed to determine the implications of a concomitant increased erythrocytic deformation in exercised dystrophic animals.
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Chapter 1

Introduction

Walton (1974) has attempted to define the term 'muscular dystrophy' by suggesting that the term be reserved for cases of progressive, genetically determined, primary, degenerative myopathy. While this definition tends to be very general, it is perhaps the best attempt so far to define a disease about which so little is precisely known.

The disease origin is uncertain, but the actual study of dystrophy dates back only to the mid-19th century. Edward Meryon (1852) has been credited with giving the first clear account of progressive muscular paralysis in young boys and he was able to demonstrate that it was due to 'granular degeneration' of the muscles. Later Duchenne (1868) gave a vivid description of this disorder and laid the foundation from which the study of this disease began.

The British neurologist, Sir William Gowers presented a very comprehensive account of the disease process in 1879 and prefaced a paper to 'Lancet' in that year with the
following paragraph:

'The disease is one of the most interesting, and at the same time most sad, of all those with which we have to deal: interesting on account of its peculiar features and mysterious nature; sad on account of our powerlessness to influence its course, except in a very slight degree, and on account of the conditions in which it occurs. It is a disease of early life and early growth. Manifesting itself commonly at the transition from infancy to childhood, it develops with the child's development, grows with his growth, so that every increase in stature means an increase in weakness, and each year takes him a step further on the road to a helpless infirmity, and in most cases, to an early and inevitable death.'

Despite the passage of almost 100 years, research efforts of many individuals and groups and an accumulating volume of literature on the dystrophic process, the message of the above paragraph is as meaningful today as it was in 1879.

Duchenne's or childhood muscular dystrophy is accepted by all to be of a genetic origin but its etiology is
unknown. Likewise, it is generally accepted that no treatment is at present known which has any definite influence upon muscular dystrophy. Some of the many remedies which have been advocated for muscular dystrophy include glycine, vitamin E, corticosteroids, multiple amino-acid and vitamin therapy, adenosine triphosphate, high energy anabolic steroids and physical methods of treatment.

Fowler (1973) concluded by stating that after careful evaluation, all treatment programmes have been found eventually to be ineffective, and that the discovery of any proper therapeutic agent or procedure must await a fuller understanding of the dystrophic defect.

Much new knowledge of the dystrophic process, emerging over the last 10 years or so, has resulted from experimentation on various animal mutants which have been shown to exhibit a primary hereditary myopathy very similar to the human myopathy. In particular the disease in the mouse (Michelson et al 1955), chicken (Asmundson and Julian 1956) and the hamster (Homburger et al 1962) has been shown to parallel very closely the human disorder (West and Murphy 1960, Pearce and Walton 1963, Homburger et al 1966, Meier and Southard 1970). These animals have been used extensively as models for experiments not possible on humans.
While it is, at the present time, a little premature to equate directly these animal diseases with human dystrophy, their study has advanced understanding of the disorder in man. With this in mind, it seems relevant both to discuss findings from, and to use, animal studies, in the hope that they may shed some light on possible beneficial research into human muscular dystrophy.

**Purpose of Study.**

It is the purpose of this thesis to investigate the effects of an exercise programme on muscular dystrophy in genetically dystrophic mice. A review of the literature on muscular dystrophy suggests that the evaluation of exercise treatment in muscular dystrophy has not received the degree of investigation it deserves. There appear in the literature, many statements with respect to exercise and dystrophy which seem to have been made from scant scientific evidence. Walton (1974) for example has stated:

'Physical activity in all cases of muscular dystrophy is beneficial'.

'Patients should be encouraged to exercise as much as possible'.
'Inactivity is detrimental'.

'Over vigorous exercise may be harmful'.

These statements suggest that the level and extent of physical activity that is optimally beneficial for people with progressive, degenerative muscular dystrophy has already been well established. Indeed, it is now common clinical practice to keep these people active for as long as possible and there appears to be general agreement that mild forms of exercise are beneficial. If it is the case that mild exercise is helpful and, as the above statement suggests, over vigorous exercise may be harmful, (the word 'may' implying that nobody really knows for certain), then it is important to determine definitely whether exercise is harmful and to quantitate the intensity, frequency and duration at which it becomes so? It is primarily to this question that this thesis is directed.

A thorough review of the literature shows that the statement, 'over vigorous exercise may be harmful', is lacking solid supportive evidence and has been made despite conflicting interpretations and findings from research studies. Most of the evidence in favour of the statement is unconvincing and has resulted from limited and narrow investigative experiments on inadequately exercised,
previously untrained subjects. For example, Fowler et al (1968) measured serum enzymes in untrained normal and dystrophic subjects after exercise to exhaustion and found enzyme elevations (particularly creatine phosphokinase) to be considerably greater in dystrophic subjects. This finding along with the fact that serum enzyme levels were shown to decrease during bed rest has prompted Fowler to caution against over vigorous exercise on the basis that elevated serum enzyme levels result from tissue breakdown and cellular damage. In another study, Homburger et al (1966) found that severe exercise of swimming to exhaustion of from one to four hours in untrained dystrophic hamsters aggravated cardiac necrosis. However, similar results have also been obtained from untrained normal animals following exhaustive exercise (Laguens and Gomez-Dumm 1967, Pelosi and Agliati 1968, King and Gollnick 1970, Banister et al 1971) but not in trained animals (Banister et al 1971).

The claim that carefully controlled exercise training can reduce or eliminate harmful effects of over vigorous exercise in dystrophics has recently gained considerable support in the literature (Wilson et al 1971, Ho et al 1975, Knudson et al 1976, Carrow et al 1976), and further questions the validity of the argument against this form of exercise. However, while results from these studies are certainly
promising they also are not totally convincing. For example, Wilson et al (1971) could find no adverse effects on dystrophic mice forced to swim to exhaustion on 56 consecutive days. But in their experiment the mice swam for an average of only 2-8 minutes per day. Pilot studies preliminary to this thesis indicated that dystrophic mice can swim for much longer periods (up to 50 minutes) which questions whether the mice in Wilson's study (1971) were really exhausted.

Past studies of exercise effects on muscular dystrophy may be criticised for neglecting to consider other aspects of the disease. A considerable volume of investigation in the past has concentrated on skeletal muscle. This is perhaps not surprising since the myopathy was originally studied from its manifestation in skeletal muscle. It is only recently that attention has focused on the very important myocardial aspect of dystrophic myopathy. (The reason for this focus of attention will be emphasised in section two of the review of literature). Cardio-respiratory failure is the major cause of death in dystrophy and vigorous exercise appears to improve cardio-respiratory function in normal animals. Thus it seems that an examination of a similar possible mechanism deserves further study in dystrophic animals.

No study to date has attempted an electron
microscopic evaluation of cardiac muscle following an exhaustive exercise programme in dystrophic animals. This approach has previously been used for normal animals (Banister et al. 1971), when it was found that cardiac muscle fine structure adapted to regular exercise stress and showed less focal damage and mitochondrial disruption after exhaustive exercise following a period of training. Thus it seems relevant to determine if a similar adaptation is possible in cardiac muscle of dystrophic animals.

A final aspect of the dystrophic myopathy which has yet to be examined with respect to vigorous exercise, or exercise of any type, is that concerning abnormal erythrocytes in dystrophy. Recent studies have opened this new approach to muscular dystrophy research, and as no study has yet been reported that has examined the effects of exercise on erythrocyte morphology in dystrophic mice it is included as a major portion of this thesis. The rationale for its inclusion is outlined in section three of the review of literature.

As both myocardium and erythrocytes have important roles in exercise it is the purpose of this thesis to examine the effects of exhaustive exercise on these aspects of physiological function in dystrophic mice.
without this evaluation the level and extent of physical activity that is optimally beneficial to muscular dystrophics seems to have been incompletely assessed and prematurely determined.

OBJECTIVE OF STUDY.

The present study has, as its principal objective, the evaluation of daily, exhaustive, swimming exercise of 30-50 minutes duration on the course of the dystrophic process in myocardium and erythrocytes of Bar Harbor 129/Re-J genetically dystrophic mice. This investigation is not aimed specifically at finding a treatment for muscular dystrophy by exercise therapy although the results may provide useful information to this end. The study is simply an attempt to determine the effect of exhaustive exercise, on the myocardium and erythrocytes of dystrophic mice.

The methods of evaluation to be used include:

1. Study of fine structural changes of the myocardium accompanying acute and chronic exhaustive exercise by transmission electron microscopic (TEM) techniques.
2. Study of changes in the surface features (morphology) of erythrocytes accompanying acute and chronic exhaustive exercise by scanning electron microscopic (SEM) techniques.

3. Observation of any differences in body weight, heart weight and endurance swimming time in exhaustive exercise resulting from the exercise programme.

The rationale for these approaches is outlined in the following summary of the literature.
Chapter 2

Review of Literature.

This review of related literature is classified under four major sub-headings:
2. Cardiac muscle and muscular dystrophy.
3. Erythrocytes and muscular dystrophy.
4. Swimming exercise for dystrophic mice.


While it is not the major objective of the proposed investigation to study skeletal muscle, a brief review of the exercise - skeletal muscle relationship in dystrophy helps to provide a rationale and an appreciation of why this aspect is more deserving of evaluation.

In 1963, Bonsett performed an anatomical study, uncovering the entire muscular system of a pseudo-hypertrophic muscular dystrophy victim. He was able to demonstrate that not only were all skeletal muscles
abnormal but that also there was a distinct pattern of wasting with certain muscles degenerating to a greater extent than others. Bonsett concluded from his work that the pattern of wasting bore a relationship to the function of the muscle. This in turn was determined by the muscle's location in the body. The rate of degeneration was also related to, or affected by the work destiny of the muscle. For example, postural muscles were more affected than the muscles engaged in a non-postural role. Further, he suggested that the difference in rate and degree of degeneration of a muscle was an expression of the difference in imposed loading. It is well established however, that different muscles have different proportions of fiber types and Bonsett's observation may have merely indicated a process of selective degeneration.

If these findings can be applied generally to all skeletal muscle, it would seem that a task that demands increased or prolonged activation of any given muscle would increase its degree of degeneration. Exercise would then appear to be a contra-indicated activity.

This conclusion does not seem to be supported by the few studies that have considered this problem. Dystrophic mice also exhibit a distinct pattern of muscular atrophy
which proceeds from the hind limb quarters to the forelimb musculature (Michelson et al 1955). One would expect both hind limbs and forelimbs of dystrophic mice to atrophy equally if the problem was simply one of the work load being imposed upon the muscle. Thus there appears to be some other factor or factors which determine the pattern of wasting in dystrophy besides the postural theory suggested by Bonsett.

Among the first to investigate the question of dystrophy and exercise were Abramson and Rogoff (1952) and Hoberman (1954). They subjected patients with advanced Duchenne (pseudo- hypertrophic) dystrophy to active and assisted, resistive weight lifting exercises designed to increase muscle strength. Hoberman found no increase in muscle strength with exercise while Abramson and Rogoff reported small improvements in some (approximately half) of the exercised patients. However, while Hoberman used the same criterion as Abramson and Rogoff, he applied it more stringently, so what he considered insignificant improvement was significant in the work of Abramson and Rogoff. This probably explains the major difference in the results between the two studies.

Vignos et al (1963) found in a study of 27 patients with progressive muscular dystrophy of childhood, that
duration of ambulation from onset of symptoms could be increased from an average of 4.4 years to 8.7 years by a comprehensive physical therapy programme with emphasis on passive stretching of flexion contractures about joints and by bracing for ambulation when independent walking was no longer possible.

As an extension of this work, Vignos and Watkins (1966), evaluated a maximum resistance exercise programme carried out over a period of 12 months in 24 patients with muscular dystrophy. They found an improvement in muscle strength in all patients throughout the first 4 months of exercise and this was followed by a plateau which was maintained throughout the period of observation. Abramson and Rogoff (1952) also noticed that improvement was most rapid during the initial 8-10 weeks of treatment and that this was followed by a leveling off. However, Vignos and Watkins (1966) could not demonstrate an improvement in functional abilities proportional to the increase in muscle strength. This point suggests that perhaps the exercise programme used by the above workers was not an optimal one for dystrophic patients and that other forms of exercise could possibly contribute more to the maintenance of functional muscle mass. While it appears that Vignos and
Watkins were referring to their maximum resistance exercise programme, their concluding statement,

'The opinion that active forms of exercise are deleterious in muscular dystrophy is not supported by our results. The suggestion that exercise programs should consist of only a small number of repetitions because of rapid, easy fatigue which might contribute to further deterioration of muscle strength is also not supported.'

offers the possibility that more active forms of exercise may be helpful. Further evidence that a more active, endurance type exercise program may contribute greater gains to improvement in functional ability is provided by the results of several other investigations.

Adams and Chandler (1974) found that a reduced vital capacity in children with pseudo-hypertrophic muscular dystrophy could be alleviated, up to 11% of predicted normal values, following a program of active swimming and breathing exercise. They were also able to show that during periods without regular therapy there were marked decreases in vital capacity. The significance of this improvement in respiratory muscle function, indicated by improved vital capacity with exercise, is enhanced considerably if one
considers that respiratory problems are a major complication and cause of death in this disease.

A second investigation by Wilson, Carrow and Walker (1971) is one of the very few studies which has attempted to evaluate long term, exhaustive exercise effects on the dystrophic process. They forced dystrophic mice to swim until exhaustion on 56 consecutive days and could find no evidence of a decreased ability to swim by the end of the programme. This fact suggests not only maintenance of functional ability but also disputes the fact that increased activity leads inevitably to an increased degeneration of the muscle. Further evidence is provided in their finding that dystrophic exercised mice had a significant increase in the number of fibers with large diameters compared with non-exercising dystrophic controls. They concluded that forced swimming exercise not only induced muscle fiber hypertrophy but that it also appeared not to have any adverse effects on the mice.

A recent report by Carrow et al (1976) examined histochemical features of chronically exercised dystrophic hamster skeletal muscle. Treated over an 8 week period, the hamsters were subjected to a daily progressive forced swimming program (30-60 min per day). While muscles from
dystrophic sedentary animals exhibited general myopathic features; i.e. wide variation in fiber sizes, central nuclei, necrosis, phagocytosis and inflammation, muscles from dystrophic exercised animals showed an absence of both necrosis and overall fiber destruction as well as generalised muscle integrity comparable to that of normal animals.

It seems from this general review that active forms of exercise are beneficial, or at worst harmless, to dystrophic skeletal muscle. There appears little doubt that maintainence of muscular strength during the course of a progressing weakness is possible to some extent in dystrophy with exercise. This is important in that retention of ambulatory status in the dystrophic individual for as long as possible offsets severe joint contractures, scoliosis, obesity and social retardation. However, to generalise findings, as some investigators have done, and to conclude that because exercise does not appear to effect skeletal muscle adversely it is acceptable to encourage physical activity in dystrophics, is failing to consider other important aspects of the disease. The need exists then to assess other types of exercise programmes and other aspects of this disease. Two such aspects - cardiac myopathy and abnormal erythrocytes - are the subjects of review in the following sections.
2. CARDIAC MUSCLE.

Reports of myocardial lesions in cases of progressive muscular dystrophy have appeared sporadically in earlier literature (Northacker and Netsky 1950), but up to 1951 only some 30 cases had been published (Storstein and Austarheim 1954).

However, over the course of the last 20 years a developing awareness of the myocardial involvement in dystrophy has emerged and has been confirmed by recent autopsy studies.

Leth and Wulff (1975), investigated retrospectively the causes of death in progressive muscular dystrophy of 46 patients who died in Denmark during the period 1960-1973. Microscopic examination of the myocardium showed that dystrophic involvement of the heart occurred in all of the patients with the childhood type muscular dystrophy, (in 16 out of 20 (80%) patients with Duchenne type muscular dystrophy and in 4 out of 6 patients with limb girdle type muscular dystrophy). Latent or manifest cardiac insufficiency was encountered in more than 95% of patients with definite dystrophic involvement of the heart. Further,
autopsy findings indicated that in 41% of cases the main cause of death was of cardiac origin and another 43% of pulmonary origin.

In another study of cardiac pathology in Duchenne type progressive muscular dystrophy, Hotchi (1975), presented autopsy findings from 60 cases. He showed that the causes of death were congestive heart failure (14 cases), cardio-pulmonary insufficiency (19 cases) and infectious diseases (27, including 22 cases of pneumonia). Gross and microscopic myocardial fibrosis was observed in 51 out of 57 cases (90%).

Thus patients with progressive muscular dystrophy frequently develop cardio-pulmonary complications. That the involvement of the heart muscle does play an important role in the terminal course of the disease suggests that early and intensive treatment is of decisive significance.

Several other studies have investigated the cardiac involvement in animals with muscular dystrophy (Homburger et al 1962, Jasmin and Bajusz 1962, Bajusz et al 1966 and 1969, Demany and Zimmerman 1969, Paterson et al 1972). Forbes and Sperelakis (1972) studied the fine structure of cardiac muscle from dystrophic mice and found widespread degenerative changes particularly in the ventricular muscle. Changes such
as increased lipid, swollen mitochondria, dilated sarcoplasmic reticulum, super contracted myofibrils and partially separated inter-calated discs were observed.

With cardiomyopathy in dystrophy now so widely reported in the literature it is perhaps pertinent to question the common medical recommendation of keeping patients with muscular dystrophy active, on the grounds that physical activity stresses cardiac function and may aggravate the existing disease condition already present.

Storstein and Austarheim (1954) query the wisdom of active exercise training and warn against exertion when subjects show signs of heart disease. They state:

'when the heart is affected by the disease, the patients activities must be limited, although otherwise the patient would be encouraged to keep on the move'.

In 1966 Homburger et al, described cardiac myopathy in the Syrian hamster and found that severe exercise in the form of swimming to exhaustion of from one to four hours accelerated the disease process.

However, it has been well documented in exercise studies on normal animals that chronic, sub-maximal,
continuous work appears beneficial in protecting against cardiac damage if performed regularly over a period of time. King and Gollnick (1970) and Banister et al (1971), were able to show extensive myocardial damage following exhaustive exercise in untrained rats. The myocardial changes were almost completely reversed 24 hours post-exercise and Banister et al (1971) demonstrated less damage immediately following exercise after 3-4 weeks of training, suggesting a mechanism of adaptation to the exercise stress which allows the cardiac muscle to resist degenerative structural changes.

The question arises from this of whether cardiac muscle undergoing dystrophic degeneration is also capable of reversible changes 24 hours post-exhaustive exercise. It may be that, because of the degenerative nature of dystrophic muscle, exhaustive exercise may greatly magnify the myocardial damage to the extent that reversible changes are not possible within a 24 hour period. If this were so, daily exhaustive exercise would appear to be contra-indicated. As mentioned earlier, Wilson et al (1971) could find no adverse effects with dystrophic mice exercised exhaustively for 56 consecutive days. But they did not study the cardiac myopathy in these animals and the extent to which the mice in their study were exhausted is questionable.
Another question which arises, is whether eventual adaptation to the exercise stress is possible in dystrophic muscle following a lengthy period of training, similar to that adaptation Banister et al (1977) found in normal rats. In other words, is an endurance type exercise program of a chronic, regular nature capable of providing myocardial protection against the dystrophic degeneration?

An important consideration with both the above questions is the ability of dystrophic muscle to repair itself following damage. It is well established that dystrophic skeletal muscle retains (at least initially) its ability to regenerate. Hudgson et al (1967), were able to demonstrate widespread regenerative activity in the pre-clinical phase of the disease e.g. coarse myofibrils, large central nuclei containing prominent nucleoli and large quantities of RNA (ribo-nucleic acid).

Pearson (1962), has also reported evidence of regeneration in skeletal muscle biopsies taken from mildly affected dystrophics and Desmedt and Borenstein (1976), have provided electromyographic evidence of regeneration in Duchenne muscular dystrophy.
But the fact that the myopathy is progressively degenerative suggests that the regenerative activity must be abortive and ineffective. Hudgson et al (1967) suggests that either there is a defect in the mechanism of regeneration or that the fiber necrosis simply outstrips the rate of regeneration.

It is thought possible, that if the exercise treatment is provided early enough in the course of the disease, at a time when the regenerative mechanisms appear to be most active, then a protective function may be afforded to the myocardium which would significantly delay the degenerative process. Findings in this direction have been presented by Howells and Goldspink (1974), in looking at the effect of exercise on the progress of the myopathy in dystrophic hamster skeletal muscle fibers. They subjected male dystrophic hamsters of different ages to a weight lifting exercise regime of 5 weeks duration and found that the effect of the exercise depended on the age of the animal. The most beneficial effect, in terms of reduced fiber degeneration, appeared to be in the very young animals (4 weeks), whereas in the older dystrophic muscles (40 weeks), exercise seemed to aggravate the condition rather than alleviate it. However, it seems that hamster myopathy is not nearly so severe as that found in murine dystrophy and it is
conceivable that other forms of exercise may have a different
effect on the progress of the myopathy. Further work is
required to clarify this, particularly with respect to
cardiac myopathy.

Several very recent investigations have offered
convincing evidence of the need to assess the influence of
chronic endurance type exercise on dystrophic myocardium.
Sockolov and Irwin (1976) examined the exercise performance
in 6-11 yr. old boys with Duchenne muscular dystrophy and
compared their cardio-respiratory responses during a bicycle
ergometer test with those from normal boys. At rest, the
dystrophic group had significantly higher heart rates (HR)
102 vs. 89 (mean values), and lower stroke volume (SV) 31
vs. 39 ml. than normal subjects. During maximal work, the
dystrophic group had lower peak values for work rate, 400 vs.
600 kgm per min; endurance 7.2 vs. 12.5 min; oxygen uptake
0.37 vs. 1.35 l/min; HR 136 vs. 189 beats/min; SV 41 vs.
60 ml; and cardiac output 5.20 vs. 11 l/min. Thus, low
cardiorespiratory capacity is a characteristic feature of
muscular dystrophy.

That myocardial function can possibly be improved
with training in dystrophic hamsters has been shown by
Knudson et al (1976). They evaluated cardiac function as
contractility measured from left ventricular pressure curves and expressed as \( \frac{dP}{dT}/kp \). Their results indicated that cardiac contractility was not as severely depressed in the trained hamsters as in the nontrained controls. It seems possible then, that training may have a positive effect in forestalling cardiac failure.

Until the present time the relationship between exercise and cardiac fine structure in muscular dystrophy has been largely ignored. Investigators have chosen to concentrate their efforts on skeletal muscle and findings from these studies, have, to some extent, offered the possibility that the cardiac muscle could benefit from regular exercise stress. Certainly the findings from studies on cardiac function in muscular dystrophy, warrants a much deeper examination of this question. In particular, the need exists to assess in greater detail, chronic endurance type exercise of an exhaustive nature which is known greatly to stress cardiac function and to evaluate the extent of any changes that this programme will have on cardiac fine structure.
3. Erythrocytes and Muscular Dystrophy.

While the fundamental defect in genetic muscular dystrophy remains obscure, there has emerged in recent years a hypothesis that the muscular defects in dystrophic patients may represent specific manifestations of a generalised membrane disorder. The discovery that this possible membrane defect exists in tissues other than muscle has directed attention towards a more widespread approach to this problem. In particular, the readily accessible red blood cell has become a focus of interest to many investigators in this field and there is ample evidence now available in the literature suggesting that erythrocyte membranes in dystrophy are abnormal in enzymatic activity, lipid composition and morphological characteristics. A brief summary of some of these findings will help to illustrate the nature of this membrane defect and will at the same time provide a sound rationale for the study of erythrocyte morphology in this thesis.

Morse and Howland (1973) reported surface alterations of erythrocytes from dystrophic mice as revealed by scanning electron microscopy. 50% of the erythrocytes were distorted in dystrophic mice compared with 4% for normal mice.
Matheson and Howland (1974) confirmed this finding in human muscular dystrophy. The percentage range of distorted cells in Duchenne dystrophy was 20% to 98% compared with a range of only 3% to 7% for normal donors.

Lumb and Emery (1975) also found an increased percentage of echinocytes (crenated discs) in boys with Duchenne dystrophy - 8% compared with 3% for controls while Miller et al (1976) reported a large increase in the number of stomatocytes (cupped shaped cells) in dystrophic patients compared with normal controls.

However, a study of five Duchenne dystrophy patients by Miale, Frias and Lawson (1975) could find only one of the patients with a percentage of deformed erythrocytes falling just outside the normal range.

Reduced deformability of erythrocyte membranes from patients with Duchenne dystrophy has been demonstrated by Percy and Miller (1975). Using a method of cell elastimetry, they determined the amount of negative pressure required to aspirate erythrocytes into micro-pipettes. They found that of the Duchenne dystrophy erythrocytes, 25% were aspirated at negative pressures greater than 100mmHg compared with only 6% of control erythrocytes.
Further evidence that there does exist some abnormality of erythrocytes in muscular dystrophy is reported by Brown et al (1967), Peter et al (1969), Araki et al (1971), and Mawatari et al (1976). Their evidence indicates that a red blood cell membrane ATPase obtained from patients with Duchenne muscular dystrophy is abnormal in that it is stimulated by ouabain. This is in marked contrast to inhibition by the same method from normal patients. This finding has also been disputed (Probstfield et al 1972).

A final observation of abnormal erythrocyte features in dystrophy is presented by Toffelmire and Boegman (1976). They determined the average RBC lifespan from normal hamsters to be 30 days while in the dystrophic animals the lifespan was 57 days.

Thus, the bulk of the literature tends to be in favour of some membrane defect in erythrocytes of muscular dystrophic patients and animals. Whether this is a direct effect of the disease process or an indirect effect is not known but it is interesting to speculate as to its significance. Perhaps the most obvious question that arises is whether the oxygen transport function of the RBC is affected. It has been reported that the degenerative changes occurring in dystrophic muscle are not too dissimilar to the
changes that occur following hypoxia. That hypoxia could be a contributing cause of muscle fiber necrosis in dystrophy does have some possible supportive evidence.

Demos (1961), advanced a vascular hypothesis of the cause of muscular dystrophy, suggesting that the muscle degeneration was due to ischemia and abnormal muscle blood flow. This hypothesis has recently been propounded more strongly by Hathaway et al (1970). Bradley et al (1975) were unable, however, to confirm a vascular cause of muscular dystrophy in both animal and human experiments. Thus, if there does exist a contributing hypoxic condition in dystrophy it may result from either abnormal blood flow or abnormal erythrocyte function.

The relevance of this argument attains greater significance if an attempt is being made to evaluate exhaustive exercise stress on the dystrophic process because of the increased oxygen requirement. Working with normal rats, Gollnick et al (1965) have suggested that the higher circulatory rate, increased temperature and increased compression of the red blood cells by the muscles during exercise may produce a weakening of the cell wall. His evidence to support this suggestion that the structural integrity of the red cell is weakened by the regular exercise
of training is a decreased resistance of red cells from trained rats to osmotic pressure.

Davis (1939), also found a lowered osmotic resistance of erythrocytes during training although he reported higher osmotic resistance of erythrocytes (ORE) immediately after exercise. Further evidence is available from an investigation by Halicka et al (1969). They reported a lowered ORE in sportsmen and were able to demonstrate that morphological features of the RBC's showed certain departures from the normal.

Lieberman and Acel (1923) experimented with human and animal material and reported that ORE declined over the first few minutes of strenuous physical exercise with subsequent disintegration of the most fragile cells.

Other workers (Barbaszowa et al 1929, Thorner 1929, 1932) have shown increased ORE in rats, dogs and man following training, while McAtee and Grollman (1967) could find no change in ORE following a 52 week exposure to daily swims by rats.

The above findings suggest that exercise stress and training does have some effect on the cell membrane of the
erythrocyte. The question then is what affect does exercise have on the structural integrity of the erythrocyte membrane from dystrophic blood, which has been reported by a number of investigators to be defective and abnormal?

Miale et al (1975), has reported normal ORE from non exercised Duchenne muscular dystrophic patients but they also found normal erythrocyte morphology which disputed the findings of several other workers.

There is clearly a need to clarify the above question if the effects of exercise and training on dystrophy are to be fully assessed. As the first stage in this assessment it is proposed to examine morphological features of dystrophic erythrocytes in order to determine if they are excessively susceptible to altered morphology following exercise stress.

4. Swimming Exercise For Dystrophic Mice.

An extensive review of swimming in small laboratory animals has been presented by Dawson and Horvath (1970). A perusal of this paper offers a full appreciation of the many factors affecting swimming and also of the effects resulting from this form of exercise stress. While there is no
The effects of daily forced swimming exercise on dystrophic mice has been examined by Wilson, Carrow and Walker (1971). Dystrophic mice could swim for only an average of 2 to 8 minutes per day until exhaustion forced

Scltan (1962) was the first to investigate the swimming capabilities of dystrophic mice. He found that dystrophic mice swim much more slowly than normal littermates and that they interrupt the strong kicking movements of the hind legs with occasional periods where one leg is extended in a passive condition directly behind the body while the other continues to kick vigorously.

Gey and Kennard (1964) measured the work and power capacity of dystrophic mice during stationary swimming. They were able to show that dystrophic mice performed less work and developed less power (approximately one-sixth) than the normal mice of the same litter. Also, fatigue was more evident in the dystrophic mice and appeared earlier than in the normal.

intention of reproducing any of their material in this summary, there have been several other papers which have looked specifically at the effects of swimming by dystrophic mice.
them to stop. However, the length of time each mouse swam was quite variable and the range in swimming time was 0.5 to 23.5 min. Fatigue of the hind limbs occurred frequently (swimming initially involving all four limbs) and terminated with motion in the hind limbs being limited to twitching.

These above findings, perhaps, question the suitability of dystrophic mice as subjects for exercise studies and Wilson et al (1971) raise the question as to whether swimming constitutes a sufficient exertion to test the effects of severe exercise. However, if one considers the severity of the disease in dystrophic mice and the degree of exhaustion after swimming it would seem that only a very limited swimming time would be required to produce a marked effect. Further support for this view is provided by the work of Wilson et al (1971) that only 2 to 8 minutes of swimming time per day for 56 days was sufficient to produce a significant increase in fiber diameters in exercised dystrophic mice compared with non exercised dystrophic mice.

The extreme weakness of the hind limbs of these animals, characterised by a dragging of the hind limbs when in motion, rules out the other common form of animal exercise, treadmill running. The support given the mice by
the water during swimming exercise largely eliminates this problem while at the same time allowing a sufficient period of sustained activity to ensure adequate stress to be placed on the cardiovascular system.
Male mice of strains 129/ReJ (normal) and 129/ReJ-dy (dystrophic) from the Jackson Laboratories, Bar Harbor, Maine were used in this investigation. All animals were housed in individual woven wire pens 24cm in length and 18cm in width, kept in a thermally regulated room with hours of dark and light regulated by a fixed time clock and provided with purina mouse chow and water ad libitum. 31 pairs of mice (31 dystrophic and 31 normal) aged 5-7 weeks and weighing 10-20 grams were obtained and divided into the groups described in table I.

The non-exercised animals (groups 1 and 5) were not physically stressed in any way and were kept in their cages until the time of sacrifice.

The experimental animals (groups 2, 3 and 4) were exercised exhaustively by swimming, each day for the required period of time. Swimming was performed in a circular glass tank, 29cm in diameter and 30cm in depth. Warm water, maintained at a temperature of 35 degrees celsius plus or
Table I. A description of the experimental groups outlining the time of sacrifice and the number of mice per group.
Table I

<table>
<thead>
<tr>
<th>Group</th>
<th>Group Description</th>
<th>Sub-group Description</th>
<th>Time of Sacrifice</th>
<th>Number</th>
<th>Abbreviated Group Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>non-exercised</td>
<td>dystrophic</td>
<td>rest</td>
<td>4</td>
<td>1NEC</td>
</tr>
<tr>
<td>2</td>
<td>exercised</td>
<td>dystrophic</td>
<td>immed.*</td>
<td>4</td>
<td>2ECI</td>
</tr>
<tr>
<td></td>
<td>1 day.</td>
<td>dystrophic</td>
<td>24 hours+</td>
<td>4</td>
<td>2EC</td>
</tr>
<tr>
<td>3</td>
<td>exercised</td>
<td>dystrophic</td>
<td>immed.</td>
<td>3</td>
<td>3EDI</td>
</tr>
<tr>
<td></td>
<td>11 days.</td>
<td>dystrophic</td>
<td>24 hours</td>
<td>4</td>
<td>3ED</td>
</tr>
<tr>
<td>4</td>
<td>exercised</td>
<td>dystrophic</td>
<td>immed.</td>
<td>8</td>
<td>4EDI</td>
</tr>
<tr>
<td>5</td>
<td>non-exercised</td>
<td>dystrophic</td>
<td>rest</td>
<td>4</td>
<td>5NEC</td>
</tr>
<tr>
<td></td>
<td>exercised</td>
<td>dystrophic</td>
<td>rest</td>
<td>4</td>
<td>5NED</td>
</tr>
</tbody>
</table>

* sacrificed immediately following exhaustive exercise.

+ sacrificed 24 hours post-exhaustive exercise.
minus 2 degrees, was added to a level 6cm below the top of the tank.

Mice were placed into the tank either individually or two at a time and the water stirred to prevent effortless floating. The mice were kept active by prodding if necessary and were exercised until they sank beneath the surface of the water and made no effort to resurface. They were then removed from the water and rested for approximately 1 minute. Following this rest interval, the mice were placed back into the tank for a further period of swimming. This procedure was repeated until the mice were either not capable of swimming following a rest interval or until a total swimming time of 40-50 minutes had been achieved.

The total time of swimming was recorded for each animal on each occasion that it swam. At exhaustion the animals were promptly removed from the tank and placed into a drying cage for 30 minutes before being replaced into their holding pens. Drying was achieved utilising an electric fan heater kept at a distance of several feet from the animals.

All animals from groups 1 and 5 (sedentary) were sacrificed at rest. These two groups provided non-exercising control animals both before and at the end of the training
period. Animals from each of the groups 2 and 3 (exercised) were further divided into two equal sub-groups. Those mice from the first sub-group were sacrificed immediately following exhaustive exercise while those from the second were sacrificed 24 hours post exhaustive swimming. All animals from group 4 were sacrificed immediately following exhaustive exercise.

This procedure (Banister et al 1971) was followed for three reasons:

1. It has previously been able to demonstrate the extent to which dystrophic myocardium was able to resist degenerative structural changes resulting from the exercise stress.

2. It has previously been able to show the extent to which any changes in 1. above were reversible following 24 hours of rest i.e. the reparable ability of dystrophic myocardium.

3. It provided a suitable approach for the investigation of possible changes in erythrocyte morphology following exhaustive exercise stress.

All mice were sacrificed under light ether anesthesia. An extended abdominal incision was made and a blood sample taken from the abdominal aorta and placed into a
heparinised test tube in readiness for scanning electron microscopic preparation. Subsequently, a thoracotomy was performed and the entire heart quickly excised, trimmed of fatty and connective tissue, sliced in half and placed into a previously weighed beaker containing a 3% glutaraldehyde solution, buffered with 0.1M sodium cacodylate, pH 7.4 (Sabatini et al 1963, Carson 1973). The beaker, solution and heart were weighed together and the difference from the previously weighed beaker and solution was taken as the heart weight.

Transmission Electron Microscopic Preparation of Ventricular Myocardium

Immediately after the heart weight had been determined, the ventricular tissue sample was dissected into very small portions, to allow adequate penetration of the fixative, and placed into small vials containing approximately 1ml of the buffered fixative medium (the same solution that the heart was weighed in) and left for 1.5 - 2 hours at 4 degrees celsius.

The tissues were then thoroughly washed three times with 0.1M sodium cacodylate buffer for 15 minutes each time.
After the third wash the tissues were post fixed in 2% osmium tetroxide buffered with 0.1M sodium cacodylate for a further 1.5 - 2 hours at 4 degrees C. (Palade 1952, Carson 1973). They were again thoroughly washed 3 times with buffer for 15 minutes each time.

Tissues were dehydrated in an ethanol series - 30%, 50%, 70%, 85%, 95% and 3 changes of 100% ethanol - for 15 minutes with each step. They were then transferred to a propylene oxide : 100% ethanol mixture (1 : 1) and finally 3 changes of 100% propylene oxide - each change being 15 minutes.

Epoxy resin embedding was based upon the method of Luft (1961). Equal amounts of resin mixture and propylene oxide (1 : 1) were added to the tissues and left overnight to allow the propylene oxide to evaporate. Tissues were then placed into pure resin mixture for 24 hours to allow complete penetration. Following this period, samples were flat embedded in pure resin mixture and placed into an oven at 65 degrees C. for 3 - 4 days to allow polymerization to occur.

The specimens were trimmed from the resin and mounted on epoxy resin 'blocks' and placed back into the oven for 2 - 3 days at 65 degrees C. After trimming excess resin
from the 'blocks' to expose the tissue they were sectioned on a Reichert Om U2 ultramicrotome, stained with 2% uranyl acetate (10 minutes) and lead citrate (5 minutes) (Carson 1973), and viewed under a Zeiss EM - 9A electron microscope (Fig. 1).

Scanning Electron Microscopic Preparation of Erythrocytes

Preparations of erythrocytes for viewing under the scanning electron microscope have been outlined by several authors (Bessis and Weed 1972, Morse and Howland 1973, Matheson and Howland 1974). The method used in this investigation was very similar to that described by Morse and Howland 1973.

0.05ml (approximately one drop) of a blood sample taken from the abdominal aorta was diluted 10 - 20 fold in isotonic saline (0.9% NaCl) containing 0.5% glutaraldehyde. After incubation for 1 hour at 25 degrees C. the cells were sedimented at 1,000g for 10 minutes, washed and centrifuged 2 times with isotonic saline, and finally diluted to 3.0ml with saline. A small drop of cell suspension was placed on a 'Millipore' filter of 0.22 micrometre pore diameter. Pieces
Figure 1. The Zeiss EM - 9A Transmission Electron Microscope.
of filter were fixed to aluminium stubs with silver conductive paint, coated with approximately 200 Angstroms of gold and examined with an Etec Autoscan scanning electron microscope (Fig. 2) at 20kV.
Figure 2. The Etec Autoscan Scanning Electron Microscope.
Chapter 4

Treatment of Data

1. Body weights, heart weights and swimming times.

All animals were weighed at the commencement of the experimental period. (The animals were approximately 6 weeks of age at this time). At the time of sacrifice, body weights and heart weights were measured and the heart weight/body weight ratios calculated.

Swimming time was recorded for each exercising animal on each occasion that it swam and a mean swimming time per animal determined.

From the above collected data, mean values per group of mice were obtained and statistically analysed by an analysis of variance (least significant difference procedure, \( P = .01 \)) to determine if any significant differences existed between group means.

2. Erythrocytes.

It was not felt necessary to perform the scanning electron microscopic study blindly. The method of preparation ensured
a completely random distribution of red blood cells. In addition to this, the microscope was de-focused until the site on the specimen stub to be photographed had been chosen. Thus, although it was known from which animal the sample had been taken the red blood cells that were photographed were not in any way selected.

The data for this portion of the study was obtained directly from the electron micrographs of the erythrocytes. For each animal 6-10 photographs (at magnifications ranging from 800X up to 4,000X) were taken from different locations on the specimen stub and the negatives developed and printed. Each photograph contained, in most cases, between 50 and 300 cells which provided a total cell count per animal of between 500 and 1000. The number of abnormal, distorted cells were counted as were the number of normal cells. The percentage of distorted cells was then calculated for each animal and a mean percentage value for each group of mice obtained. These values were then subjected to an analysis of variance as outlined above.

The criterion that was used for determining the abnormality of erythrocyte morphology were the following:

Erythrocyte morphology was considered to be abnormal only if the cells were clearly distorted and,
1. were not recognisable as biconcave discocytes i.e. if viewed alone the erythrocytes could not be recognised as such.

2. exhibited surface irregularities involving projections from the cell surface.

3. Myocardium.

All transmission electron microscopic examinations were performed blindly. The tissue blocks were mixed and coded by a person not involved in the study, prior to sectioning and viewing under the microscope. It was not until after the photographs had been taken and viewed that the code was deciphered for those involved in the investigation. This procedure prevented any bias in the selection of material to be photographed from the different groups of animals.

The final analysis of the fine structure of the myocardium was made from the electron micrographs. Up to 20 representative micrographs were taken for each animal at magnifications ranging from 1,800X up to 21,000X. This range was utilised in order to obtain a good coverage of cellular information. Higher magnifications provided information from too specific an area to provide generally useful data.

An effort was made to quantify transmission electron
microscopic work to obtain the best possible representation of information. This was achieved by various methods as follows:

1. Each of the 11 groups consisted of 3-4 animals.
2. More than one myocardial specimen (tissue) was prepared for each animal. From these tissues 4 blocks were prepared for sectioning, although in some cases not all were used.
3. Several grids per animal were obtained for viewing under the microscope. This allowed more than one site of the myocardium to be examined.
4. A large number of photographs (up to 20) were taken for each animal. Many of these were at low to medium magnifications in order to achieve adequate cellular representation.
5. Animals in each group were considered together. This allowed only those features common to a group of animals to be reported and it eliminated unrepresentative findings.
6. Representation was further ensured by performing this portion of the thesis blindly. This allowed the myocardial information to be assessed from approximately 500 unbiased photographs.
7. The analysis of the micrographs was performed by two investigators in discussion format. This reduced any bias in the interpretation of the information.
Chapter 5

RESULTS

For ease of presentation of results the abbreviated group names as outlined in table I of the material and methods section will be used. Also, in keeping with previously laid down format, and to aid clarity of presentation, results are classified under appropriate sub-sections.

1. Swimming Times, Body Weights and Heart Weights.

A. Swimming times.

Mean swimming times for each animal and for each group of animals are listed in table II.

The animals in groups three and four were not exhaustively exercised on the first 3 days but were introduced gradually to the exercise stress with swimming times of 12 min, 15 min and 20 min. On the fourth and subsequent days they were exercised for as long as they could continue to swim. This procedure may account for the slightly shorter swimming times for the 11 and 17 day
Table II. The mean swimming times for each animal and for each group of animals.
<table>
<thead>
<tr>
<th>Group</th>
<th>Days of Exercise</th>
<th>Mean Swimming Time For Each Individual Animal (minutes)</th>
<th>Mean Swimming Time per Group (minutes)</th>
<th>Mean S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2EDI</td>
<td>1</td>
<td>40 35 35 50</td>
<td>40</td>
<td>7.00</td>
</tr>
<tr>
<td>2ED</td>
<td>1</td>
<td>40 30 35 20</td>
<td>31</td>
<td>8.50</td>
</tr>
<tr>
<td>2ECI</td>
<td>1</td>
<td>30 45 60 40</td>
<td>44*</td>
<td>12.50</td>
</tr>
<tr>
<td>2EC</td>
<td>1</td>
<td>30 30 50 30</td>
<td>35</td>
<td>10.00</td>
</tr>
<tr>
<td>3EDI</td>
<td>11</td>
<td>21 31 34 -</td>
<td>29</td>
<td>6.81</td>
</tr>
<tr>
<td>3ED</td>
<td>11</td>
<td>22 25 29 -</td>
<td>25*</td>
<td>3.51</td>
</tr>
<tr>
<td>4EDI</td>
<td>17</td>
<td>36 34 32 -</td>
<td>34</td>
<td>2.00</td>
</tr>
</tbody>
</table>

* Significantly different from each other (P < 0.01) by Least Significant Difference Procedure (L.S.D.)
dystrophic exercised groups. There was no significant difference in swimming times between any of the dystrophic groups (L.S.D. procedure).

The similarity of swimming times between groups can be explained by stating that the animals were exercised at a level which induced exhaustion in 30 - 50 minutes. Thus, while the non-dystrophic exercised groups (2ECI and 2EC) were swum to exhaustion in approximately the same time period these animals were capable of swimming faster and much more strongly than the dystrophic mice. Some of the dystrophic animals could not attain this period of time due to early onset of fatigue. Evidence of exhaustion in all animals was seen in the way in which they lay on their sides, breathing in a forced manner when removed from the water.

Subjective observations indicated no noticeable improvement in swimming ability with increasing days of exercise. If any trend did exist it was towards a greater difficulty in swimming in the dystrophic animals. This was particularly evident in the time taken by the mice to begin swimming once placed into the water. Initially this was only 2-3 min. but in the latter stages of the exercise programme this duration of time extended to 15 min. This was a general finding and did not apply to all animals. This may
have been due to a learning factor i.e. the mice had learned that to swim meant exhaustion and fatigue and that floating was a better alternative. It may also have been due to accumulating after effects of previous days of exercise.

Fatigue of the hind limbs appeared to be the most common cause for the termination of exercise. This was observed in the mice as a rigid extension of the hind limbs, characterised in some cases with rapid twitching. If in this state, the mice were removed from the water and rested for about one minute they could then be replaced back into the tank for a further period of swimming. Thus exhaustion of dystrophic mice may result either from a temporary inability to activate skeletal muscles of the hind limb, or an inability of the muscles to relax from the contracted state in readiness for the next stimulus.

B. Body Weights and Heart Weights.

The data on body weight, heart weight and the heart weight / body weight ratio is summarised in table III.

The purpose of this section of the study was to assess differences in the body weights and the heart weights of the dystrophic exercised (groups 3 and 4) vs. the
Table III. Mean values for each group of animals for body weight, heart weight and the heart weight / body weight ratio.
<table>
<thead>
<tr>
<th>Group No. of Animals</th>
<th>Days of Exercise (N=40)</th>
<th>Body Wt. at Beginning (grams)</th>
<th>Body Wt. at Sacrifice (grams)</th>
<th>Heart Wt. at Sacrifice (grams)</th>
<th>Heart Wt. / Body Wt. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1NED</td>
<td>3</td>
<td>-</td>
<td>9.75</td>
<td>10.80</td>
<td>0.021*</td>
</tr>
<tr>
<td>1NEC</td>
<td>4</td>
<td>-</td>
<td>18.42</td>
<td>18.42</td>
<td>0.086</td>
</tr>
<tr>
<td>2EDI</td>
<td>4</td>
<td>1</td>
<td>10.40</td>
<td>11.47</td>
<td>0.042</td>
</tr>
<tr>
<td>2ED</td>
<td>4</td>
<td>1</td>
<td>12.00</td>
<td>11.97</td>
<td>0.061</td>
</tr>
<tr>
<td>2ECI</td>
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<td>1</td>
<td>20.12</td>
<td>20.62</td>
<td>0.074</td>
</tr>
<tr>
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<td>1</td>
<td>18.50</td>
<td>19.23</td>
<td>0.081</td>
</tr>
<tr>
<td>3EDI</td>
<td>3</td>
<td>11</td>
<td>11.30</td>
<td>12.30</td>
<td>0.049</td>
</tr>
<tr>
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<td>3</td>
<td>11</td>
<td>11.40</td>
<td>11.70</td>
<td>0.049</td>
</tr>
<tr>
<td>4EDI</td>
<td>3</td>
<td>17</td>
<td>10.00</td>
<td>10.10</td>
<td>0.040</td>
</tr>
<tr>
<td>5NED</td>
<td>4</td>
<td>-</td>
<td>10.67</td>
<td>13.05</td>
<td>0.040</td>
</tr>
<tr>
<td>5NEC</td>
<td>4</td>
<td>-</td>
<td>16.75</td>
<td>20.12</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Mean values for all animals:
- Dystrophic = 10.78
- Non-dystrophic = 11.62+ 0.043 0.00370

Mean values for all animals:
- Dystrophic = 18.44 19.59+ 0.080 0.00400

* only one animal.
+ Significantly different P < .01 (L.S.D.)
dystrophic non-exercised (group 5) mice.

Because groups 1 and 2 were sacrificed within three days of beginning the experiment and also because these animals had either no exercise or only one bout of exercise, any change in body weight or heart weight was considered meaningless in respect of the assessment sought. For this reason they will be discussed but not examined in detail.

Figure 3 shows the changes in body weight for groups 3, 4 and 5 over the duration of the exercise period. It can be seen that weight gain was least in the exercised dystrophic animals and greatest in the non-exercised mice. The weight gain differences between exercised dystrophic and non-exercised dystrophic mice was not significant by the L.S.D. procedure (P < .01).

However, that this difference in weight gain was so noticeable after only 11 and 17 days of exercise is perhaps good evidence that the exhaustive swimming exercise was having a marked effect on these animals. This, along with the fact that dystrophic mice are already very much underweight when compared with non-dystrophic mice (11.62g vs. 19.59g respectively = mean values for all dystrophic and all non-dystrophic animals - P < .01) is perhaps sufficient
Figure 3. Changes in body weight (grams) for groups 3, 4 and 5 over the duration of the exercise period. The values given are the means and the standard errors for each group of animals.
Figure 3.

- **O** = 5NEC = non exercised, non dystrophic
  - = 3.37

- **•** = 5NED = non exercised, dystrophic
  - = 2.38
  - = 0.65

- **Δ** = 3EDI and 3ED
  - = 11 days of exercise, dystrophic
  - = 0.10

- **♦** = 4EDI = 17 days of exercise, dystrophic

**Y-axis:** BODY WEIGHT (GRAMS)

**X-axis:** TIME (DAYS)

6 Weeks of age
reason alone to question this type of exercise programme.

Strong evidence that exhaustive swimming was detrimental can be seen in the results from group 4. Of 8 dystrophic mice that were started on the programme of daily swims only 3 were surviving at the end of 15 days - the other 5 animals had died during the 2 week period. One animal from the 11 day exercise group also died. Thus, 6 exercising dystrophic mice died during the experimental period compared with 0 animals from the non-exercising control groups. Subjective observations verified that swimming mice appeared to be weaker and more sickly with time compared with controls.

Figure 4. shows the heart weight / body weight ratios for groups 3, 4 and 5. It can be seen from this that the exercised dystrophic animals had much higher ratios than the non-exercised dystrophic group although this difference was not significant at the 1% confidence level. Groups 3 and 4 (exercised dystrophic mice) had a heart wt. / body wt. ratio similar to that of the non-exercised, non-dystrophic group (SNEC). The most probable explanation for this increased ratio for exercised dystrophic animals is cardiac hypertrophy resulting from swimming exercise.
Figure 4. The means and standard errors of the heart weight / body weight ratios (10) for groups 3, 4 and 5 at the end of the experimental period.
Figure 4.

[Graph showing heart weight/body weight for different groups: 5NED, 5NEC, 3EDI, 3ED, 4EDI for No Exercise, 11 Days Exercise, and 17 Days Exercise.]
However, the heart weight / body weight ratios for groups 1 and 2 dystrophic animals are more variable and do not exhibit such a clearly defined pattern. Group 1NED had a very low ratio (0.0019) but only one animal's heart weight was measured for this group. Group 2ED had a ratio at the other extreme (0.0050) which was the highest ratio of any group. With only one exposure to swimming stress this high ratio cannot be explained as cardiac hypertrophy resulting from the exercise. No obvious explanation can be provided of why this one group does not adhere to the general pattern set by the other groups.

2. Erythrocyte Morphology.

The results of the scanning electron microscopic investigation of erythrocyte morphology are summarised in table IV and presented in figures 5 to 27.

Table V presents a comparison of results obtained from this study with results reported in the literature. Only data from groups 1 and 5 (non-exercised animals) are included from the present results. No results in table V are from exercised dystrophics.
Table IV. The mean percentages of deformed erythrocytes for each animal and for the different groups of animals with standard deviations.
### Table IV

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of Exercise</th>
<th>% of Deformed R.B.C's. For Each Animal</th>
<th>Total No. of Cells Counted / Group</th>
<th>Mean % of Deformed Cells / Group</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1NED</td>
<td>-</td>
<td>60 3 1</td>
<td>3,156</td>
<td>21.3</td>
<td>33.5</td>
<td></td>
</tr>
<tr>
<td>1NEC</td>
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<td>4 10 20</td>
<td>3,145</td>
<td>11.3*</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>2EDI</td>
<td>1</td>
<td>61 74 18 41</td>
<td>3,963</td>
<td>48.5</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td>2ED</td>
<td>1</td>
<td>22 8 8 32</td>
<td>3,623</td>
<td>17.5</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>2ECI</td>
<td>1</td>
<td>19 54 4 41</td>
<td>2,447</td>
<td>29.5</td>
<td>22.3</td>
<td></td>
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<tr>
<td>2EC</td>
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<td>19 5 7</td>
<td>2,318</td>
<td>10.3*</td>
<td>7.5</td>
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</tr>
<tr>
<td>3EDI</td>
<td>11</td>
<td>35 26 7</td>
<td>1,851</td>
<td>22.6</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>3ED</td>
<td>11</td>
<td>12 22 6</td>
<td>2,356</td>
<td>13.3*</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>4EDI</td>
<td>17</td>
<td>32 26 14</td>
<td>2,516</td>
<td>24.0</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>5NED</td>
<td>-</td>
<td>4 8 1 5</td>
<td>1,925</td>
<td>4.5*</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>5NEC</td>
<td>-</td>
<td>5 4 3 3</td>
<td>3,840</td>
<td>3.7*</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

* Groups that were found to be significantly different (P < .01) from group 2EDI (48.5% of deformed cells).

Mean for all dystrophic animals \( (N = 24) \) = 21.4%
Mean for all non-dystrophic animals \( (N = 14) \) = 13.7%
Mean for all dystrophic non-exercising animals = 12.5%
Mean for all non-dystrophic non-exercising animals = 7.5%
Mean for all dystrophic exercising animals = 25.0%
Mean for all non-dystrophic exercising animals = 20.0%
Mean for all exercising animals = 22.5%
Mean for all non-exercising animals = 10.0%
Table V. A comparison of studies from the literature which report various percentages of deformed erythrocytes from dystrophic mice and human dystrophic patients.
Table V

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>No. of Subjects</th>
<th>Cells Counted</th>
<th>% of Cells Distorted</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/ReJ normal *</td>
<td>7</td>
<td>6,985</td>
<td>7.5</td>
<td>Present Study</td>
</tr>
<tr>
<td>129/ReJ-dy dyst.</td>
<td>7</td>
<td>5,081</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>129ReJ normal</td>
<td>10</td>
<td>294</td>
<td>4.1</td>
<td>Morse and Howland (1973).</td>
</tr>
<tr>
<td>129/ReJ-dy dyst.</td>
<td>8</td>
<td>187</td>
<td>51.6</td>
<td></td>
</tr>
<tr>
<td>Human normal</td>
<td>5</td>
<td>3,314</td>
<td>4.2</td>
<td>Matheson</td>
</tr>
<tr>
<td>Human normal</td>
<td>2</td>
<td>-</td>
<td>0.5</td>
<td>Miale et al (1975)</td>
</tr>
<tr>
<td>Human D.M.D.</td>
<td>5</td>
<td>-</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>Human normal</td>
<td>17</td>
<td>-</td>
<td>3.8</td>
<td>Lumb and Emery (1975).</td>
</tr>
<tr>
<td>Human D.M.D.</td>
<td>9</td>
<td>-</td>
<td>8.6</td>
<td></td>
</tr>
</tbody>
</table>

* 129/ReJ = strain of mice.
+ D.M.D. = Duchenne Muscular Dystrophy.
Figure 5. The means and standard errors for each of the 11 groups showing the percentages of deformed erythrocytes.
Figure 6. Normal, smooth surfaced erythrocytes with characteristic biconcave features.

Magnification = x 7,800

Group = 5NEC
Figure 7. An example of deformed erythrocytes showing surface irregularities and loss of the characteristic biconcave features.

Magnification = X 12,000

Group = 2EDI
Figure 8. Close-up view of a normal erythrocyte.

Magnification = $X \times 20,000$

Group = 5NEC

Figure 9. Close-up view of a crenated (spiculed) red blood cell showing a complete loss of discocyte form.

Magnification = $X \times 20,000$

Group = 5NED

Figure 10. Normal and crenated cells existing together.

Magnification = $X \times 7,000$

Group = 1NED

Figure 11. Both normal and deformed cells showing intermixed arrangement and distorted cells at various stages of development.

Magnification = $X \times 3,500$

Group = 4EDI
Figure 12 and Figure 13. An example of the intra-group variation showing normal cell morphology for one animal and a high number of deformed cells for another animal of the same group. Both micrographs are from non-exercised dystrophic animals.

Magnification = X 2,800

Group = 1NED
Figure 14. A high number of deformed cells from a 1 day exercised dystrophic animal (immediately sacrificed).

Magnification = X 2,800

Group = 2EDI

Figure 15. An enlargement from the same animal as in figure 14 showing severely distorted cells.

Magnification = X 7,700

Group = 2EDI
Figure 16. A high number of deformed red cells in a one day dystrophic exercised animal that was immediately sacrificed following exercise. (Compare with figure 17).

Magnification = X 3,850

Group = 2EDI

Figure 17. Fewer deformed cells from a one day exercised dystrophic animal, sacrificed 24 hours post exercise.

Magnification = X 3,850

Group = 2ED
Figure 18. 1 day exercised, non-dystrophic animal, immediately sacrificed, showing a number of misshapen cells but not the number or the severity of deformed cells seen in a similarly treated dystrophic animal. (Compare with figure 14 and figure 16).

Magnification = X 5,250

Group = 2ECI

Figure 19. 1 day exercised, non-dystrophic animal, sacrificed 24 hours post exercise and exhibiting fewer misshapen cells than the immediately sacrificed animal in figure 18.

Magnification = X 4,550

Group = 2EC
Figure 20. A 1 day exercised, non-dystrophic animal, immediately sacrificed displaying an increased number of deformed cells. (Compare with figure 21).

Magnification = X 3,500

Group = 2BECI

Figure 21. From a non-exercised, non-dystrophic animal showing very few distorted cells.

Magnification = X 3,850

Group = 5NEC

These two figures illustrate the effect of exercise on erythrocyte morphology.
Figure 22. A high number of deformed red cells from a one day exercised, dystrophic animal, immediately sacrificed. (Compare with figure 23).

Magnification = X 5,250

Group = 2EDI

Figure 23. A fewer number of deformed cells from a 17 day exercised, dystrophic animal, immediately sacrificed.

Magnification = X 5,250

Group = 4EDI
Figure 24. Deformed cells from a 17 day exercised, dystrophic animal, immediately sacrificed exhibiting more misshapen cells than the non-exercised dystrophic animal (figure 25) but fewer than in the 1 day exercised dystrophic mice (figure 22).

Magnification = X 7,000

Group = 4EDI

Figure 25. Fewer deformed erythrocytes from a non-exercised dystrophic animal.

Magnification = X 7,000

Group = 5NED
Figure 26. A large number of morphologically normal cells, from a non-exercised non-dystrophic animal.

Magnification = X 4,200

Group = 5NEC

Figure 27. A large number of normal cells from a non-exercised dystrophic animal.

Magnification = X 4,200

Group = 5NED

These two figures illustrate normal surface features of erythrocytes from the non-exercised dystrophic and non-dystrophic animals.
Perhaps the most striking observation from table IV is the tremendous amount of intra-group variability. An example of this is group 1NED which has an unusually high percentage of deformed erythrocytes for one animal (60%), yielding a misleading mean percentage value for that group (21%) and a very large standard deviation (33.5). That this percentage is unusually high is verified by a similar control group (5NED) which has a mean percentage figure of only 4%. Thus, of 7 non-exercising dystrophic mice only one has a percentage value greater than 8%, that being the 60% value.

In an attempt to reduce the intra-group variability as much as possible the percentage values will be examined collectively rather than individually with an emphasis being placed on inter-group comparisons. If this procedure is followed then definite patterns emerge. The most obvious of these is the high percentage of deformed red blood cells found in the exercised groups. In particular, the 4 groups of mice that were exercised to exhaustion and immediately sacrificed displayed the highest mean percentages of all groups. The greatest number of deformed erythrocytes were shown to be present in the blood of dystrophic mice sacrificed following one bout of exhaustive exercise (2EDI = 48%). After 11 and 17 days of exercise fewer deformed cells were observed in dystrophic animals sacrificed immediately
succeeding swimming. There was also a noticeable reduction in the percentage of deformed cells in those dystrophic animals sacrificed 24 hours post exhaustive exercise although this value was still generally higher than that for the control non-exercising groups.

The non-dystrophic exercised mice (2EC1 and 2EC) displayed a similar trend to the dystrophic animals with only the magnitude of this trend being different. If sacrificed immediately following exhaustive exercise on day 1, the non-dystrophic exercised mice showed 30% of erythrocytes to be deformed compared with 48% for the corresponding dystrophic group. If sacrificed 24 hours post exhaustive exercise then the mean percentage values were 10% vs. 17% respectively.

In summary, it would seem from these results that exercise stress does have a definite effect on the morphological features of erythrocytes as viewed by the scanning electron microscope. This effect appears to be more prevalent in exercised mice sacrificed immediately following exhaustive swimming and also in mice sacrificed after 1 day of exercise compared with those animals sacrificed following 11 and 17 days of swimming. A higher occurrence of deformed cells appear in group 2 dystrophic mice compared with group 2 non-dystrophic animals.
The nature of the erythrocyte deformation does not appear to differ between any of the groups. The characteristic morphological feature of the deformed cells was crenations projecting out from the cell surface and the loss of the normal biconcave shape towards a more spherical form. Cells could be seen at various stages of the deformation process with some cells very densely crenated and others retaining their biconcave features but showing early development of surface protrusions.

In all samples the distorted erythrocytes seemed to be evenly distributed and interspersed with normal, smooth surfaced discocytes. Occasionally, a stomatocytic (cup shaped) cell was observed but they were not found in excessive numbers.


Prior to uncoding the transmission electron microscopic results, they were viewed in an attempt to determine whether they could be categorised into exercised vs. non-exercised or dystrophic vs. non-dystrophic by general observation of fine structural features. While it
was noted at this time that many of the electron micrographs showed increased lipid, contracted and supercontracted myofibrils, dilated T-tubules, increased numbers of ribosomes and isolated mitochondrial disruption, no animal could be categorised with certainty to have been exercised or to be dystrophic. Indeed, the overall features of each animal mirrored those features common to a normal myocardium. The conclusion from this prior observation was that no gross or obvious pathologic or abnormal feature occurred frequently enough to enable groups to be clearly distinguished from one another. Thus, it was realised that any differences that would exist on decoding the information would be of a minute nature and would not be widespread but confined to localised regions of the myocardium.

Following this former procedure, the code was deciphered and the electron micrographs re-evaluated in more detail. While most of the fine structural features remained common to all groups, one feature was found that could be confined to a single category of animals. This observation was myofibrillar supercontraction, appearing only in dystrophic mice that were sacrificed immediately following exercise (Fig. 30 - 33). However, supercontraction was not widespread and occurred only in isolated fibers in close
Figure 28. A longitudinal section of ventricular myocardium displaying normal cell features:

ID = Intercalated disc.
IS = Intercellular space.
L = Lipid.
M = Relaxed myofibrils.
MI = Mitochondria.
N = Nucleus.
T = T-tubules.

Magnification = X 5,400

Group = 2EDI
Figure 29. A longitudinal section of relaxed ventricular myocardium showing normal sarcomere arrangements:

\[ A = A - \text{Band.} \]
\[ I = I - \text{Band.} \]
\[ Z = Z - \text{Line.} \]
\[ \text{MI} = \text{Mitochondria.} \]

Magnification = X 21,900

Group = 3EDI
Figure 30. A 17 day exercised dystrophic animal, sacrificed immediately following exercise, showing a region of supercontracted myofibrils (SM) separated by an intercalated disc from a region of stretched myofibrils (SF).

Magnification = X 5,400  
Group = 4EDI

Figure 31. A 1 day exercised dystrophic animal, sacrificed immediately, displaying supercontraction (SM), and a clumping together of Z-lines. 5-6 Z-lines appear to be in the space normally occupied by a single sarcomere.

Magnification = X 5,400  
Group = 2EDI

Figure 32. A 1 day exercised dystrophic animal, sacrificed immediately, showing fibers in a high state of contraction.

Magnification = X 5,400  
Group = 2EDI

Figure 33. A magnified region from figure 32 showing a loss of normal sarcomere features (banding).

Magnification = X 21,900  
Group = 2EDI
proximity to an intercalated disc. Very often, these focal sites of supercontraction in which 5 or 6 Z-lines could be observed in the space normally occupied by a single sarcomere, was surrounded by and adjacent to normal myofibrillar arrangements. With this occasional exception, the myocardium of dystrophic mice had an appearance which could not be distinguished from non-dystrophic myocardium.

Mitochondrial alterations (Fig. 34 - 35) were observed in only a small number of animals with the most severe changes being observed in a non-dystrophic animal sacrificed 24 hours post exercise on day 1. In most cases the mitochondria were not morphologically affected by the exercise stress and retained their normal features i.e. dense matrix, orderly arrangement of cristae and a continuous outer membrane. Occasionally, very large mitochondria were seen (Fig. 36 - Fig. 38) that had lost all evidence of cristae formation but had retained their shape and electron dense matrix. An attempt to isolate these mitochondria to a particular category of mice was not successful.

Increased lipid was evident in many animals and was distributed widely throughout the ventricular muscle, usually in close association with mitochondria. There existed no tendency for this lipid deposition to occur in excess only in
Figure 34. From a non-dystrophic animal, sacrificed 24 hours post exercise on day 1, showing a region of mitochondrial damage (MD).

Magnification = x 21,900

Group = 2EC

Figure 35. From a dystrophic animal sacrificed immediately following exercise on day 1 displaying large mitochondria, mitochondrial division (DM) and the loss of mitochondrial cristae (LC) and matrix.

Magnification = x 21,900

Group = 2EDI
Figure 36

and

Figure 37. A non-exercised dystrophic animal showing a region of many large mitochondria and great variation in mitochondrial size.

Magnification = X 5,400

Group = 5NED

Figure 38. High magnification from the same group, exhibiting very large mitochondria (MI) separating narrow myofibrils (M).

Magnification = X 21,900

Group = 5NED
dystrophic animals (Fig. 39 - 40).

The transverse tubules were seen in several electron micrographs to be disrupted and swollen into isolated vacuoles (Fig. 41 - 44). More widespread dilation of the T-tubules was evident in some animals, occurring over a much larger area of the ventricular myocardium. Once again however, no group pattern was evident.

Structural integrity of the intercalated discs appeared to be maintained in all animals with no discernable disruption or separation of the discs.

In summary, almost all fine structural myocardial features that have been observed and considered abnormal in other work with dystrophic mice and exercising animals have been seen in the present results. However, the infrequency of observation and the lack of consistency with which these findings could be associated with a particular category of mice has prevented any clear distinction between groups.

This suggests perhaps that:

1. for some reason the cardiac myopathy had not expressed itself in these animals and

2. the exercise was not sufficiently stressful to induce visible alterations.

Both of these points will be taken up in the discussion.
Figure 39. A region of high lipid deposition from a non-dystrophic animal.

L = Lipid.

Magnification = $x\ 21,900$

Group = 5NEC

Figure 40. Non-dystrophic animal showing excess lipid (L).

Magnification = $x\ 5,400$

Group = 2EC
Figure 41. A 17 day exercised dystrophic animal showing dilated T-tubules but otherwise normal appearing myocardium.

Magnification = X 5,400

Group = 4EDI

Figure 42. A non-exercised dystrophic animal displaying several large mitochondria (MI) and marked dilation of the T-tubules which have disrupted into vacuoles (V).

Magnification = X 21,900

Group = 5NED
Figure 43. A 1 day exercised, non-dystrophic animal, sacrificed immediately, showing a slight separation of an intercalated disc (ID) and disrupted T-tubules (V).

Magnification = X 21,900

Group = 2ECI

Figure 44. A low magnification of the same animal showing a marked dilation of the T-tubules (T).

Magnification = X 5,400

Group = 2ECI
Figure 45. From an 11 day exercised dystrophic animal, sacrificed immediately, showing normal cellular features and normal arrangement of myofibrils, mitochondria and intercalated discs.

Magnification = X 5,400

Group = 3EDI

Figure 46. An enlarged micrograph of figure 45.

Magnification = X 21,900

Group = 3EDI
Figure 47
and
Figure 48. A 17 day exercised dystrophic animal, immediately sacrificed, showing no obvious myocardial damage or disruption.

Magnification = x 21,900 = Figure 47.

= x 5,400 = Figure 48.

Group = 4EDI
Chapter 6

Discussion

Most studies which have investigated the effects of exercise on dystrophy in animals have used swimming as the stress of choice (Soltan 1962, Gey and Kennard 1964, Homburger et al 1966, Wilson et al 1971, Howells and Goldspink 1974, Ho et al 1975 and Carrow et al 1976). However, swimming has often been questioned (Wilson et al 1971) as suitable exercise for dystrophic mice on the grounds that they fatigue early and are unable to swim fast enough and/or long enough to achieve the effects of severe exercise. Gey and Kennard (1964) reported that dystrophic mice could swim against resistance for only about 1.5 minutes while Wilson et al (1971) found that dystrophic mice swam for only an average of 2 - 8 minutes before exhaustion. In light of these findings it would appear necessary to make some attempt to assess the stressfulness of the swimming exercise for the mice in this study and to determine from the results that have been obtained, if the swimming did provide an adequate test of exhaustive exercise. A number of points can be offered to support the claim that it did and as evidence of exhaustive exercise is a crucial consideration in this thesis they will be discussed at some length.
The first point that should be raised is that concerning the duration of swimming time, particularly in view of the above reports that dystrophic mice can swim for only 1 - 8 minutes before exhaustion. The findings from this study dispute the fact that dystrophic mice can only swim for this length of time. Results from table II show mean swimming times for mice in this investigation ranging from 25 to 40 minutes per day which is much longer than previously reported. The reason for this large discrepancy is almost certainly due to the stringency with which the criteria was applied to determine the point of exhaustion, plus the short rest intervals (30 - 60 seconds) approximately every 10 - 15 minutes, which allowed the mice to recover sufficiently from what appeared to be a temporary exhaustive state, to continue swimming for a much longer total duration. It should be noted, however, that swimming times equal to and in excess of times recorded in this thesis have been reported for dystrophic Syrian hamsters (Ho et al 1975, Carrow et al 1976). But the myopathy in hamsters is not nearly so severe as that found in murine dystrophy (Howell and Goldspink 1974). The severity of the disease was nowhere more evident than in the uncoordinated, often desperate action of these mice attempting to swim. That swimming was so difficult for them should have ensured that even minimal activity was stressful. Thus, the combination of the duration of swimming
time for mice in this study and the severity of disease should have been adequate to produce marked effects of the exercise stress.

However, it may be argued that 40 minutes of swimming does not alone provide convincing evidence of stressful activity and that the intensity of exercise is also a necessary consideration. It may well be that cessation of swimming was due entirely to a localised exhaustion of the skeletal muscles of the hind limbs and that stress on the cardio-vascular system was negligible. The question which emerges in light of this reasoning, then, is whether the 40 minutes of swimming in this study was of a sufficient intensity to impose an 'overload' on the cardiovascular functions. In an attempt to resolve this uncertainty, attention will be focussed on discussion of the body weight and heart weight results.

Body Weight.

It is well accepted that dystrophic animals weigh less than non-dystrophic animals. It is also well established (Gollnick et al 1967, van Huss et al 1969 and Ho et al 1975) that normal, chronically exercised animals weigh less than normal sedentary animals that have been equally
matched and treated otherwise identically. A similar trend is seen in the results of the present study in which the dystrophic exercised mice have much lower weight gains than dystrophic non-exercised mice. However, this finding lacks support from the literature. Several studies (Soltan 1962, Wilson et al 1971) have demonstrated that changes in body weight of stressed dystrophic mice do not differ significantly from those of the non-stressed mice. But, while in these studies mice swam for 12 and 56 days respectively, the duration of swimming time was only 1-8 minutes per day compared with 25-40 minutes in the present investigation. It is doubtful that such a short duration (1-8 min. per day) of swimming would produce marked changes in the body weight. In contrast to the present results, and also to the normal trend for non-dystrophic animals, Ho et al (1975) reported that dystrophic hamsters, exercised by swimming, weighed more than dystrophic sedentary hamsters following 8 weeks of training (30-60 min. per day, 5 days per week). As mentioned earlier in the discussion, the severity of disease for hamsters and mice is not the same however, and it maybe that the reduced weight gain of the mice exercised by swimming in this study was only an initial response to the exercise which may have been reversed if the period of swimming had been longer. In fact Ho et al (1975), could find no difference in body weight between swimmers and
non-swimmers after 4 weeks of training which endorses the findings for dystrophic mice (Soltan 1962, Wilson et al 1971). Only at 8 weeks was there a measurable difference. One other study (Howell and Goldspink 1974) reported that weight loss due to exercise stimulus was most noticeable in old dystrophic animals but did not appear to affect the young animals.

Thus, the finding in this study that dystrophic exercised mice have a much smaller weight gain than dystrophic sedentary mice is contrary to the bulk of the literature for these animals but supports the literature for non-dystrophic exercised animals. It also appears that the nature of the exercise programme in the present research was much more demanding than others have reported. In support of this claim is the fact that, whereas the 11 and 17 day dystrophic exercised animals gained only 0.65g and 0.10g respectively (5% and 1% of initial total body weight), dystrophic sedentary mice gained 2.38g (18% of initial total body weight) over the same 17 day period. That this difference was so noticeable after only 11 and 17 days of exercise is perhaps sound evidence that the swimming exercise was indeed stressful. A point that is worthy of mention is that this greatly reduced body weight gain may not be beneficial in exercised dystrophic mice that are already very much underweight.
Cardiac Hypertrophy.

Further information is provided by the heart weight/body weight ratios which are often used as indicators of cardiovascular stress imposed by endurance training. A number of investigators (see review by Dawson and Horvath 1970) have shown that various chronic swimming regimes produce cardiac hypertrophy and a corresponding increase in the heart weight/body weight ratio of normal animals. However, very few investigators have examined this cardiac response to physical activity in dystrophic animals. One such study (Ho et al 1975) reported that normal swimming hamsters had higher heart weight/body weight ratios than did the dystrophic swimming hamsters but there were no differences between normal sedentary hamsters and the dystrophic sedentary hamsters. They also point out a delayed response of exercise induced cardiac hypertrophy in myopathic hamsters, noticing a difference in the heart weight/body weight ratio between exercised and non-exercised animals at 8 weeks but not at 4 weeks. This has been explained by a retarded growth rate which appears to be a characteristic feature of dystrophic hamsters during the first half of their lives (Bajusz et al 1966).
In the present study, non-exercised, non-dystrophic animals had a higher ratio (heart weight / body weight), than did non-exercised dystrophic animals, which disputes the findings of Ho et al (1975). It was also demonstrated that the 11 and 17 day exercised dystrophic mice had a higher ratio than the non-exercising dystrophic mice that acted as the control group. Once again, this challenges the observations of Ho et al (1975), in that the cardiac hypertrophy noticed by them was less rapid.

In light of these differences and also in light of the low body weight gains for the exercised dystrophic mice in this study, it is perhaps pertinent to question the rapidity with which the cardiac hypertrophy developed. However, it seems that the adaptive response of the heart to endurance exercise occurs rapidly and is evident following a single training session (Hickson et al 1976). The same authors report the half time (T½) for cardiac hypertrophy to be 4.9 days. It would seem then, that 11-17 days of an exhaustive swimming programme is sufficient to allow significant changes to occur.

Another point which can be challenged with respect to exercise induced cardiac hypertrophy, is the variability seen in the heart weight / body weight ratios of the mice in this
study. Examination of table III shows that the highest and the lowest ratio were both recorded in dystrophic groups. The range for dystrophic animals was 0.0019 - 0.0050 compared with a range of 0.0035 - 0.0046 for non-dystrophic animals. Marked variation in heart weights from human dystrophic patients have also been reported (Hotchi 1975). This variation may well depend on the extent to which the myocardium is involved in the myopathy. This involvement itself varies tremendously from one dystrophic animal to another (this point will be discussed further on), and may result in differing degrees of pathological hypertrophy. Thus, it may be argued that the hypertrophy is not exercise induced at all but is pathological in nature. The general trend however, was towards an increased heart weight / body weight ratio for the exercised dystrophic mice. While it cannot be definitely concluded that this increased ratio was exclusively the product of the exercise stimulus, the consideration discussed above would support the view that swimming was sufficiently stressful to enable cardiac hypertrophy to develop. If it may be assumed that any exercise induced cardiac hypertrophy that did occur is related to an increased functional capacity of the myocardium (Crews and Aldinger 1967), then a reduction in a depressed cardiac function (Knudson et al 1976, Sockolov and Irwin 1976) which result from exercise training may have a positive effect on forestalling cardiac failure in dystrophy.
One final point that should be discussed with respect to the exercise programme, concerns the high percentage (40%) of exercising dystrophic animals that died during the course of the experimental period. One of seven animals (14%) died from the 11 day exercise group while 5 of 8 (62%) died in the 17 day exercise group. No animals from the non-exercising group died during the same time period. These facts strongly suggest that exercise was in some way implicated with the deaths and was exerting a detrimental affect on the mice. It has been well established (Hotchi 1975, Leth and Wulff 1975, Leth and Wulff 1976) that cardiorespiratory complications are the major cause of death in dystrophics. The question of cardiac failure will be discussed at some length in the next section. It is sufficient to say at this point that no evidence was apparent from the myocardial fine structure investigation that implicated cardiac failure as a cause of death.

Respiratory failure (with possible resulting cardiac complications) appeared to be the most likely cause of death. The animals that did die were observed to grow gradually weaker over a 2-3 day period with a noticeable decrease in their swimming time. Difficulties with respiration were clearly visible on the day prior to death. It seems that either pneumonia or pulmonary emphysema would adequately fit
this description. Chronic swimming has been reported to produce pulmonary emphysema in rats (Tura 1960). By forcing rats to swim to exhaustion daily for 90 days, Tura (1960) found that only 29 of an original 60 rats survived such a 3 month training period. It was suggested that the rigorous exhaustion criterion used by Tura, which caused the animals to aspirate a good deal of water before being removed from the swim tank, was responsible for this low survival rate. This may well explain the primary cause of death in the present study, particularly if the high susceptibility of dystrophic mice to respiratory failure and infection is considered. There is no doubt that the difficulty these mice had in swimming plus the stringent criterion for exhaustion did force them to aspirate a good deal of water before removal. An attempt was made to try to reduce deaths in this way by placing the mice into heated drying cages for 20-30 minutes immediately following swimming. But this obviously was not a sufficient answer to the problem.

The susceptibility of mice to respiratory failure and the latter apparent enhancement during the course of the swimming programme, perhaps poses a need for increased care with human dystrophic patients. In recent years many rehabilitation centers for dystrophic children have started to advocate the swimming training during the initial stages
of the myopathy. While swimming does appear to be beneficial (Harris and Cherry 1974, Adams and Chandler 1974), its application and use should be carefully controlled.

Myocardial Fine Structure.

The lack of consistent fine structural changes of the myocardium in this investigation is surprising in that cardiac myopathy in dystrophy has been frequently reported in the literature (see review). There may be several reasons why, in the present study, it was not possible to differentiate between the dystrophic and the non-dystrophic animals on the basis of myocardial fine structural differences.

First, it seems that cardiac involvement in muscular dystrophy is not always apparent. Opinions differ concerning the incidence of myocardial involvement, but it has been estimated that the heart is affected in about 50 - 85% of advanced cases (Bajusz et al 1966). Leth and Wulff (1976) found the incidence of cardiac involvement in 19 male subjects with the Duchenne type of progressive muscular dystrophy to be 84% while in the dystrophic mouse, cardiomyopathy presents a wide spectrum of morphological abnormalities, many of which are not manifest in every animal
(Forbes and Sperelakis 1972). This latter finding, has been shown to be the case in the present investigation. Almost all abnormal features which have been reported to exist in dystrophic myocardium have been presently observed. Unfortunately they have neither occurred frequently enough, nor been sufficiently confined to the dystrophic group clearly to distinguish dystrophic from non-dystrophic animals.

Secondly, it seems that cardiac myopathy in dystrophy is more focal compared with the more diffuse changes that occur in skeletal muscle. This would tend to increase the difficulty of detecting changes, particularly in fine structure. However, the diverse procedures used in this study should have located any pathological changes that were present had they existed. In addition to viewing several sites of the myocardium under the electron microscope, thick (1 micron) sections, stained with toluidine blue, were prepared from several dystrophic animals and viewed under the light microscope. This allowed a much greater area of the myocardium to be examined, and although it was not done for all animals, it did confirm the electron microscopic findings that no abnormal features existed.
The age of the animals (6 - 8 weeks) is a third, and perhaps the most likely reason why the dystrophic mice did not exhibit widespread cardiac myopathy. Forbes and Sperelakis (1972), observed abnormalities in the myocardium of dystrophic mice at 7 - 12 weeks of age, but their findings were not consistent amongst all animals. Also, they state that there appeared to be a progression of degenerative events, implying that the incidence of diseased cells would be higher in hearts from older dystrophic mice. Gilroy et al (1963), Bajusz et al (1969) and Hotchi (1975) are also of the opinion that the dystrophic changes of the myocardium do not appear until late in the course of the disease. Thus, it maybe, that the dystrophic mice in this present study had not reached the age at which the cardiac myopathy expresses itself in a clearly visible form. Perhaps the rare sites of structural alterations that were observed may have been an indication of the initial development of the cardiac myopathy.

That no widespread fine structural changes were seen even following exhaustive swimming exercise is perhaps further evidence that no major abnormality of the myocardium existed in the dystrophic mice in this study. Focal sites of mitochondrial disruption and rare myofibrillar supercontractility were the only signs of myocardial changes
following exhaustive swimming but they were observed in only a small number of the exercised animals. It was not possible, by examination of myocardial fine structure, to distinguish exercised mice from non-exercised mice.

However, this finding appears to be contrary to the bulk of evidence in the literature which reports that myocardial alterations, particularly mitochondrial disruptions, do occur following exhaustive swimming in both normal (Laguens et al 1966, Laguens and Gomez-Dumm 1967, Arcos et al 1968, King and Gollnick 1970) and dystrophic (Homburger et al 1966) animals. It seems that the only possible explanation that can be offered for this contradiction of findings is that the swimming exercise in the present study was not imposing a sufficient stress on the myocardium to induce the structural alterations that have been observed in other research. In light of what has been discussed previously, this seems highly unlikely (but nevertheless possible).

It is perhaps worth speculating at this point on the significance of the brief 30 - 60 second rest intervals approximately every 10 - 15 minutes that were required by the mice in order for them to continue swimming. The increase in total swimming time that this procedure achieved is
sufficient reason to question the nature of the exhaustive state in the dystrophic mice of this study. It may be that the 2 - 8 minutes of swimming per day by mice in Wilson's et al (1971) study, represented only a temporary exhaustive state, which was found in the present study to be reversed following a 30 - 60 second rest interval. If this view could be endorsed, then the meaningfulness of Wilson's et al (1971) conclusion that 56 consecutive days of 'exhaustive' swimming does not have an adverse effect on the mice is, to some extent, lost.

To pursue this point further, it has been speculated (Cullen and Fulthorpe 1975), that the central process in the breakdown of the fibre structure in dystrophy, is the formation of contraction clumps and the loss of a very fine balance between the tendency to contract and the tendency to relax. Cullen and Fulthorpe (1975) suggest, that a destructive sequence of events could occur. Arrival of an action potential would trigger the normal excitation-contraction cycle resulting in a shortening of the sarcomeres. An inability to relax would then result in a further shortening of the sarcomeres on arrival of the next action potential. Continuing excitation (as would exist under exercise conditions) would lead to excessive contraction, the piling up of Z-lines and disorganisation of the normal spatial relationship of the sarcomeres.
If this sequence of events could be applied to the present findings, then the 30 - 60 second rest intervals which allowed the mice to greatly improve their total swimming time may attain the utmost importance. It was certainly found that a rigid extension of the hind limbs and an apparent inability to activate the hind limb muscles was a characteristic feature of the exhaustive state. It would appear reasonable then, if indeed excessive contraction was responsible for the temporary inability to swim, to expect a 30 -60 second rest interval to have some influence on the release of the contraction that would enable a stimulus (action potential) to excite the relaxed muscle and hence to allow swimming to recommence. If this is so, then the duration, frequency and intensity of exercise become important considerations. Fowler et al (1968), found that both duration and intensity of exercise were critical factors in inducing serum enzyme elevations. In human dystrophic patients elevations were greater after relatively mild but cumulative exercise than after short duration, maximal work on the bicycle. Thus, duration of exercise appears to be a critical factor in muscular dystrophy. Perhaps these findings suggest that the ideal exercise programme for dystrophics is one which utilises short duration, intense activity with frequent rest intervals which would allow the muscles to re-establish a contractable state.
If the above considerations can also be applied to cardiac function, then exercise of long duration without rest intervals may induce sufficient myofibrillar supercontractility that sudden cardiac failure could result. Evidence that supercontracted myofibrils exist in dystrophic myocardium has been observed in the present study and supports findings from other work (Forbes and Sperelakis 1972). However, while Forbes and Sperelakis (1972), report supercontractility in sedentary dystrophic mice, the only evidence found in this study of myocardial supercontraction existed in dystrophic mice that were sacrificed immediately following exhaustive exercise. In retrospect, several of the dystrophic mice that were removed from the water after exhaustion did not have a visible heart beat and lay motionless on their sides for several seconds. It was thought at the time that this was due to the fact that the mice had been submerged beneath the surface of the water for 3 - 4 seconds before removal. But the possibility exists that it may have been a temporary heart stoppage resulting from supercontracted myofibrils and loss of cardiac contractility. Depressed cardiac contractility in dystrophic animals has been reported in the literature (Knudson et al 1976), and perhaps provides further support for the claim that prolonged continuous activity, may be detrimental to dystrophic myocardium.
The local sites of supercontraction in the dystrophic mice were not found frequently and certainly not in all animals. A possible reason for this is that the 2 - 3 minutes required to sacrifice the animal and to dissect out the heart may have been sufficient time for the myofibrils to release excessive contraction. That the mice could recommence swimming after a one minute rest adds weight to this argument, if it is assumed that over contracted myofibrils was the reason for the stoppage. Other possible reasons of why myofibrillar supercontraction was not observed in many of the dystrophic myocardiums were discussed earlier with respect to the absence of visible cardiac myopathy in these animals. It is sufficient to say at this stage, that the fact that myofibrillar supercontraction existed at all in exercised dystrophic myocardium when almost no other abnormal features were displayed may emphasis the importance of this structural alteration.

Thus, it seems that the design of an exercise programme for dystrophics may have to take into account supercontractility of myofibrils. From the present findings it appears that the termination of exercise results primarily from inactivation of skeletal muscles of the hind limbs. That this occurs first may be an important factor in preventing failure of the myocardium which appears to exhibit
similar features but of a much less severe nature (Hotchi 1975). It is suggested that regular rest intervals during prolonged exercise may prevent the formation of contraction clumps and hence slow down the rate of atrophy which appears to stem from these sites (Cullen and Fulthorpe 1975). If this could be achieved, while at the same time beneficial stress is applied to the cardio-vascular system, then exhaustive prolonged exercise may not be detrimental. That supercontracted myofibrils were seen in dystrophic mice sacrificed following 17 days of exercise perhaps suggests that training is not capable of reducing or preventing this supercontraction from occurring.

To conclude the discussion of the electron microscopic investigation of myocardial fine structure it can only be stated that,

A. widespread visible signs of cardiac myopathy were absent from the dystrophic animals and that,

B. no consistent structural changes of the myocardium were visible following exhaustive exercise.

As the age of the animals was the most likely reason for the absence of cardiomyopathic features, it may well be that strenuous exhaustive exercise has no adverse effects on the myocardium of young animals. In support of this claim,
Howell and Goldspink (1974), conclude that the effects of exercise on skeletal muscle fibres is beneficial in the very young animals but in older dystrophic muscles, exercise seems to aggravate the condition rather than alleviate it.

Thus, early and intensive treatment therapy of the cardiac symptoms appears to be of great importance. If beneficial cardiovascular stress can be applied by way of an exercise programme during the initial stages when the cardiac myopathy has not developed, then it may significantly delay the onset of cardiac complications which are a major problem in the later stages of dystrophy.

Erythrocyte Morphology.

The finding in this investigation of increased morphological alterations in erythrocytes from exercised mice is, perhaps, further evidence that the swimming exercise was exerting an influence on the cardiovascular system. This claim could be disputed on the grounds that similar deformities of the erythrocyte surface have been frequently described in non-exercised dystrophics (Morse and Howland 1973, Matheson and Howland 1974 and Miller et al 1976). Also, these surface changes do not appear to be specific for the muscular dystrophies as such changes have been noted
clinically in association with severe liver disease, uraemia, splenectomy, haemoglobinopathies and beta-lipoprotein deficiency (Percy and Miller 1975). Further, it has been argued (Miller et al 1975), that the erythrocyte deformities observed by the scanning electron microscope (SEM) are in vitro artifacts produced by the fixation procedure. In support of this claim, Miller et al (1976) could find no evidence that erythrocytes of dystrophic patients were misshapen in vivo. They suggest that the shape changes that they observed were probably the result of intrinsic biochemical membrane differences that respond to fixation in an abnormal way.

While it is conceded that the methods of SEM preparation for erythrocytes have yet to be firmly standardised and may, in part, be responsible for the changes that have been reported, it would seem from the results of this study that exercise stress was in some way implicated in the morphological changes that were observed. Several reasons can be offered to support this view:

1. The non-exercising mice (groups 1 and 5) displayed generally lower percentages of deformed cells when compared with the exercising mice (10% and 22.5% respectively).

2. There was a higher percentage of deformed erythrocytes in those animals that were sacrificed
immediately following exhaustive exercise when compared with those sacrificed 24 hours post exhaustive exercise.

3. The untrained mice that were swum to exhaustion and then sacrificed exhibited a higher percentage of deformed cells when compared with the trained mice that were sacrificed following 11 and 17 days of swimming.

4. Non-dystrophic exercised mice also exhibit a high percentage of deformed erythrocytes which suggests that the deformities observed in this study are not due entirely to a dystrophic 'factor' and are, at least in part, due to exercise.

These reasons do not however, eliminate the possibility that fixation procedures were responsible for the morphological changes. The low percentages of deformed erythrocytes from non-exercising mice to some extent dispels this possibility. But it may have been that the exercise stress increased the susceptibility of the erythrocytes to in vitro alterations. While it has not been examined in this thesis, it has been reported elsewhere (Matheson and Howland 1974), that distortions of dystrophic erythrocytes are visible under a light microscope when unfixed cells are diluted with saline solution and viewed at a magnification of X450. This information would suggest that alterations in the surface features do not reflect damage from fixation and do exist in vivo in that condition.
It is perhaps necessary at this point to make an attempt to assess the relative contributions of exercise and dystrophy to the percentages of distorted erythrocytes. If all dystrophic mice in this experiment \((N = 24)\) are compared with all non-dystrophic mice \((N = 14)\), then the respective percentages of deformed cells are 21.4% and 13.7%. This data would seem to imply that a dystrophic 'factor' is at least partly responsible for the altered surface features. However, it can be countered that this difference is due to the greater number of exercised dystrophic animals and that exercise, not dystrophy, is the critical factor responsible for the changes.

Examination of results in table IV offers convincing evidence that this is so. The non-exercised mice (group 5) display almost identical percentage values for the dystrophic and the non-dystrophic animals. Likewise, the dystrophic non-exercised mice in group 1 show very normal values if the obviously non-typical value of 60% is overlooked. Thus, it seems with the non-exercised animals that the difference between dystrophic and non-dystrophic mice with respect to erythrocyte deformations is minimal. This aspect of the present research both disputes (Morse and Howland 1973, Matheson and Howland 1974, Miller et al 1976) and endorses (Lumb and Emery 1975, Miale et al 1975) findings from the literature (see table V).
In contrast with groups 1 and 5, all exercising groups had an increased percentage of deformed cells with the dystrophic mice from group 2 having a higher percentage than the group 2 non-dystrophic animals. This would seem to suggest that exercise is the key factor and also that dystrophic erythrocytes are possibly more susceptible to exercise induced changes. It is interesting to note that the 11 and 17 day exercised mice have a lower percentage of deformed cells compared with the 1 day exercised mice which perhaps indicates that either the exercise stress has less effect with training or that the erythrocytes adapt in some way to the stress.

While the nature of this investigation has not lent itself to answer the question as to what is causing the abnormal surface features of the erythrocytes some attempt has been made in the literature to do this. Gollnick et al (1965), suggests that the higher circulatory rate, increased temperature and increased compression of the cells by the muscles during exercise contribute to a weakening of the cell wall. Lieberman and Acel (1923), reported that the osmotic resistance to erythrocytes declined over the first few minutes of strenuous physical exercise with subsequent disintegration of the most fragile cells. Removal of the
most fragile cells in this way may be a factor in the reduced percentage of deformed cells in the 11 and 17 day exercised mice.

A number of theories have been proposed for the high incidence of deformed erythrocytes in dystrophy. It has been reported that the ability of red blood cells to deform and change shape is an important determinant of the life span of circulating red cells (Williamson et al 1975). Also, it has been demonstrated (Toffelmire and Boegman 1976), that the life span of erythrocytes from dystrophic hamsters is twice that of normal hamsters (58 days and 30 days respectively). Thus, the high percentage of deformed erythrocytes that have been reported in dystrophy may be a reflection of the age of the red cell. These findings may explain the results of Percy and Miller (1975), that while dystrophic erythrocytes are more deformed they are less deformable.

A second theory is that the morphological changes of dystrophic erythrocytes may reflect alterations in the lipid component of the membrane (Matheson and Howland 1975), as red cell membranes from patients with muscular dystrophy have been shown to differ from normal ones with respect to fatty acid and phospholipid composition (Kunze et al 1973).
Increased phosphorylation of red cell spectrin in patients afflicted with Duchenne muscular dystrophy has also been reported (Roses et al. 1975). It has been suggested that phosphorylation of spectrin may induce some type of conformational change in a protein network throughout the membrane that adjusts the shape of the cell (Shchet and Layzer 1976).

Still another theory is that myosin and actin-like proteins exist on the cytoplasmic (inner) surface of the red cell membrane. These proteins exhibit ATPase activity which is similar to that of myofibrillar ATPase. It has been demonstrated (Brown et al. 1967, Peter et al. 1969, Araki et al. 1971 and Mawatari et al. 1976), that the red cell membrane ATPase obtained from Duchenne dystrophy patients is abnormal. Thus, the association of an abnormal ATPase activity with the fibrillar proteins of the cell membrane may be responsible for the process of deformability (Percy and Miller 1975). It would seem also, if this were correct, that the increased ATPase requirement during exercise may increase the susceptibility of the cell to even greater change.

At the present time much of the erythrocyte investigation in dystrophy is controversial. Enough studies have shown that abnormal features do exist in the red cell
but the literature on erythrocyte morphology is contradictory and confusing. The finding from this study that erythrocytes of non-exercised dystrophic mice have a normal morphological appearance perhaps disputes (together with other studies in the literature) that they exist in dystrophy. There is little doubt however, that they exist following exercise. But as non-dystrophic mice also have an increased number of distorted erythrocytes following exercise this may be a normal physiological response.

**Until such a time as the significance and implications of the deformed red cells are known it is not possible to conclude whether exhaustive exercise is beneficial or detrimental to the erythrocytes. But as the morphologically altered cells also appear in normal exercised animals, there appearance in dystrophic animals would not seem to be an argument, at this stage, in favour of limiting the extent of physical activity in dystrophics.**
Chapter 7

Summary and Conclusions

The purpose of this study has been to evaluate the effect of exhaustive swimming on the myocardium and the erythrocytes of dystrophic mice. It would seem from the findings that no serious adverse effects resulted from the nature of the exercise programme. Although many of the mice did die during the course of the experimental period, this was thought to be associated more with the conditions of exercise rather than with any direct physiological effect of the exercise itself.

No consistent structural changes of the myocardium was observed following exercise in the dystrophic mice which suggests that the myocardiums of 6-8 week old dystrophic mice are no more susceptible to exercise induced alterations than are the myocardiums of normal mice. However, this point cannot be generalised to include older animals as the susceptibility to myocardial damage following exercise may increase as the disease process advances. Evidence that young dystrophic mice may actually benefit from an exhaustive exercise programme is seen with the heart weight / body weight ratios which increased for the exercising dystrophic
animals. It is normally assumed that any cardiac hypertrophy resulting from exercise training is related to an improvement in cardiac function.

The reduced body weight and the increased percentages of deformed erythrocytes following exercise in dystrophic mice cannot at the present time be considered detrimental as both of these features also appear in non-dystrophic animals. However, as the significance of these findings in dystrophic animals has yet to be adequately determined, they cannot be considered to be beneficial either.

It is perhaps a worthwhile exercise at this stage to attempt to relate the findings from this study to possible applications in human muscular dystrophy. It should be emphasized however, that to directly equate murine dystrophy with human dystrophy is at this stage premature but the similarities between the two myopathies does at least provide the opportunity for speculation.

One of the important points to emerge is that early intensive treatment appears to be a necessity. If in human dystrophy, as seems to occur in murine dystrophy, no cardiomyopathy appears until the later stages of the disease, then this early period would seem to be the ideal time to
develop a cardiac protection against the disease process. As cardiac failure is one of the two major causes of death in human dystrophy it would seem that the sooner this protection is developed and the longer that it is maintained the greater will be its value for the patient.

However, a problem which arises is how best can this cardiovascular 'reserve' be developed. It was found in the present study with mice, and is probably equally applicable to humans, that skeletal muscle fatigue is a limiting factor in exercise. Hence, it is possible that skeletal muscle fatigue develops before an adequate stress can be placed on the cardiovascular system to induce the beneficial changes. It would seem imperative then, to distribute the exercise over as many muscle groups of the body as possible so as to prevent localised muscle fatigue. In this respect, swimming would appear to be a better form of exercise than riding a bicycle. But while the swimming exercise itself may be ideal it appears from the present study that the conditions under which swimming takes place are not entirely suitable to dystrophics who are very susceptible to respiratory infections. Thus, while it is the cardiovascular system which is of primary concern, the skeletal muscle weakness and the respiratory problems cannot be neglected.
The finding that deformed erythrocytes exist in both murine and human dystrophy has provided a further link for comparative purposes. The results from the present study possibly suggest that deformed red cells, like the cardiomyopathy, do not appear until late in the course of the disease. The appearance of the deformed erythrocytes following exercise however, may mean that the abnormality exists in the red cells of young animals but in a latent form. The finding of erythrocyte surface alterations has prompted many investigators to propose a systemic membrane defect theory as the primary cause of muscular dystrophy. While the erythrocyte provides an excellent membrane 'model' for investigation purposes, there is at the present time no uniformity of findings. Until such time as the confusion is sorted out and the significance of the red cell surface alterations determined, very little comment can be made as to the importance of the surface changes following exercise.

Questions For Future Considerations

1. There is an urgent need to settle the controversy surrounding abnormal erythrocytes in dystrophy. Do the distorted erythrocytes really exist and if they do what is their significance?
2. What are the long term effects of an endurance exercise programme on the cardiovascular responses of human dystrophic patients?

3. What is the optimal exercise programme for a dystrophic subject with respect to intensity, frequency and duration. e.g. should dystrophics exercise continuously for prolonged periods or should their activity periods be interspersed with frequent rests. What should the intensity of this exercise be?

4. To what extent is age a factor in determining the effect of the exercise. Is exercise only beneficial to the very young dystrophics and if it is, at what stage does it become detrimental?

5. If exercise studies are to be carried out with animals, then if possible, the 129/ReJ-dy strain of dystrophic mice should be avoided and the C57B1/6J-dy strain of mice or the dystrophic Syrian hamster used. The severity of the disease in the 129/ReJ-dy strain makes exercise for these animals very difficult and because of their short life span (4-7 months) and their susceptibility to respiratory infection they are not suited to long term studies.
Bibliography


