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CONTRACTILE AND ANABOLIC RESPONSES OF RAT SKELETAL
MUSCLE TO DIANABOL AND EXERCISE

BY

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A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

Doctor of Philosophy
(Physiology)

TO

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Barry Martin Lubek, Ottawa, Canada, 1982.

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Abstract

The purpose of this study was to investigate the ability of Dianabol and exercise to increase skeletal muscle contractile strength. It was hypothesized that if the contractile strength was to be affected by this treatment, then the increased strength was a manifestation of an enhanced concentration of respiratory enzymes and/or contractile protein.

In order to examine this problem, a technique was developed to permit the determination of total protein, PFK and SDH concentrations from a single tissue homogenate. Creatine and actin concentrations were also determined in separate samples from the same tissues. An *in situ* lateral gastrocnemius preparation for nerve stimulated isometric contractions was developed to compliment an *in situ* soleus preparation for the contractility studies.

Animals received treatment with Dianabol ($0.075 \text{ mg } 2 \times \text{week}^{-1}$) and a progressive exercise programme for either four weeks (series 1) or eight weeks (series 2 and 3). At the end of the treatment period, series 1 animals were sacrificed, and the soleus, plantaris, medial and lateral gastrocnemius muscles were removed, blotted and frozen for biochemical assay. Series 2 and 3 animals were anaesthetized following the treatment period permitting the monitoring of twitch and tetanic contractions as well as fatigue. These animals were then sacrificed and the muscle tissues treated as those of series 1.

Following four weeks of treatment, the concentration of total protein, PFK, SDH, creatine and actin was significantly greater in

the lateral gastrocnemius of animals receiving Dianabol plus exercise treatment when compared to control tissues. These parameters were unaffected by any of the treatments employed in the other muscles studied.

In series 2 and 3, the lateral gastrocnemius isometric twitch and tetanic tension was significantly greater in the Dianabol-exercise treated animals than the controls. The other parameters of contractility studied were not affected. The contractility of the soleus was not altered by any of the treatments employed.

Biochemical analysis of the muscles used for series 2 and 3 contractility studies again revealed that the concentrations of total protein, PFK, SDH, creatine and actin were significantly elevated in the Dianabol-exercise treated lateral gastrocnemius only.

Withdrawal of steroid injections midway through the series 3 treatment period while maintaining exercise administration negated the effect on the lateral gastrocnemius creatine concentration observed in the same tissues receiving Dianabol plus exercise for the entire treatment period.

It is concluded from the results of this study that concomitant administration of Dianabol and exercise for eight weeks can have a marked effect on isometric force characteristics of skeletal muscle in young adult male rats. The changes in contractile tension in this tissue are accompanied by increases in enzymatic and

contractile protein concentrations. The changes in enzyme concentrations however, appear to promote an enhanced metabolic capacity which may not be utilized as there was no effect on the fatigue profile of this muscle, as studied in these experiments.

Introduction

Since the early work of Kochakian, it has generally been assumed that testosterone, its metabolites, and more recently certain synthetic derivatives can have a marked effect on muscle growth and metabolism. The majority of these studies have employed castrated animals to which hormone supplements have been administered.

Whereas in many target tissues dihydrotestosterone has been established as the active androgen/anabolic, it has been demonstrated that in skeletal muscle testosterone acts directly to promote DNA replication and protein synthesis (Powers and Florini, 1975), acting through specific testosterone receptors (Michel and Baulieu, 1976; Powers and Florini, 1975).

The development of orally active testosterone derivatives has enabled new uses for anabolic hormones. In the cattle industry, anabolics have been administered with the intention of yielding increased weight gains over shorter periods of time (Heitzman, 1976; Heitzman et al., 1977). In humans, although anabolic steroids are indicated for use in deficiency states resulting in protein loss (Liddle and Burke, 1960) or abnormalities (Sanchez-Medal et al., 1969), the market of athletics has become a major consumer.

Numerous reports are available in the literature demonstrating increased mass due to steroid treatment alone, or in conjunction with exercise (Freed et al., 1975; Johnson et al., 1972; Johnson and O'Shea, 1969). Concomitant with increased weight, reports of increased

strength and stamina can also be found (Casner and Early, 1971). However, equally as numerous are publications refuting these findings (Stone et al., 1978; O'Shea, 1971, 1970; Fowler et al., 1965). In fact, Ariel and Seville (1972) reported that humans receiving placebos while believing they were consuming anabolic steroids also demonstrated increased strength.

Anabolic steroid induced hypertrophy

Justification in animal research for the belief that "megadosing" with anabolic steroids results in increased mass or strength is scant in the literature. Both macroscopic (Kochakian and Tillotson, 1957; Kochakian, Tillotson and Endahl, 1956; Papanicalou and Falk, 1938) and combined light and electron microscopic studies have been used to assess structural alterations in skeletal muscle. The results of these reports suggest that anabolic steroids induce skeletal muscle hypertrophy in sedentary animals. In a more detailed study, Wydra (1972) reported that mice treated with methandrostenolone ($1.65 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) alone or in combination with exercise, developed muscular hypertrophy, and that the hypertrophy induced by the steroid administered in conjunction with exercise was not different from that observed with steroid alone.

In fact, however, the involvement of androgens in body weight gains in the adult has been questioned. Hervey and Hutchinson (1973) reported that castrated adult male rats did not have significantly altered growth curves from control animals. In the same study

Hervey and Hutchinson (1973) observed that doses of testosterone of $1 \text{ mg} \cdot \text{day}^{-1}$ given to adult male rats that were either normal or castrated, or even ovariectomized adult females, had little effect on body weight and composition of lean tissue. Doses higher than $1 \text{ mg} \cdot \text{day}^{-1}$ given to adult males resulted in substantial losses of weight.

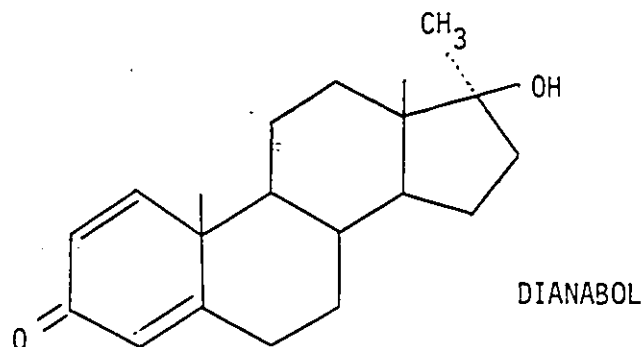
Effect of synthetic androgens on sexual tissues

If the evidence presented above suggests the literature to be contradictory with regard to the ability of these compounds to cause muscular hypertrophy or increased body weight, there is much more agreement amongst the few existing articles about the effect on sexual tissues. The dependence of these tissues on testosterone or dihydrotestosterone for growth and function was definitively established by Kenyon et al. in 1940. Since then, several authors have reported various anabolic androgens incapable of supporting growth and function of these tissues (Arnold, Potts and Baylor, 1963a, b; Dorfman and Kincl, 1963; Edgren, 1963). Boris et al. (1970) reported on a most comprehensive study of this topic. In all, 12 androgens were injected subcutaneously in immature male rats for 10 days, after which organs were excised and removed. Under these conditions, they observed a dose dependent depression of testicular weight in animals injected with any of the steroids. They also observed that few of the synthetic steroids affected seminal vesicle or ventral prostate weights at doses up to $200 \text{ } \mu\text{g} \cdot \text{rat}^{-1} \cdot \text{day}^{-1}$. Doses above this significantly increased the weights of

these organs. The apparent contradiction in these results accentuates the importance of the age and endocrine status of the experimental animal. The animals employed in the study by Boris et al. were maximally 35 days old at sacrifice, which would suggest they were both sexually and endocrinologically immature. At that point in time the exogenously applied steroids may have had a direct effect at the higher doses, but might not have exerted significant negative feedback on the hypothalamo-hypophyseal-gonadal axis, which has been the suggested mechanism (Stone, Rush and Lipner, 1978; Holm and Aldercruetz, 1976; Harkness, Kilshaw and Hobson, 1975). This is supported by the reports of testicular atrophy in intact young adult guinea pigs and atrophy of the ventral prostate in intact rats following methandrostenolone administration (Stone, Rush and Lipner, 1978; Brooks, 1979a, b).

Structure of the synthetic anabolic steroids

It is generally accepted that both testosterone and estrogen exert anabolic characteristics. Indeed, the synthetic anabolics produced to date are derivatives of these compounds. The most commonly used (anecdotal) of the synthetic anabolic steroids is Dianabol (methandrostenolone, methandienone, 1,4-androstadien-17 α -methyl-17 β -ol-3-one). This compound, being a testosterone



derivative, retains the cyclopentanoperhydrophenanthrene nucleus, but differs from its parent compound in that it is methylated in the 17 α position and double bonds exist in both the 1 and 4 positions of ring A. The 17 α -methylation reportedly enhances oral absorption and the alteration of the A ring structure prolongs the biological half life by interfering with metabolism within the liver (Murad and Haynes, 1980). Dianabol is absorbed rapidly following oral administration. However, like testosterone, absorption is more constant and controlled if injected subcutaneously in an oil vehicle.

Effect of Dianabol and exercise on muscle strength

Very few reports in the literature address this subject. There do exist several papers as reviewed by Ryan (1976), which provide subjective evidence both supporting and opposing the hypothesis that Dianabol administration alone or combined with exercise can increase muscular strength. However, following an exhaustive literature search, only one paper reported the results of a study of the effect of Dianabol administration combined with high intensity exercise on muscle contractility (Stone and Lipner, 1978). These authors observed that administration of Dianabol with exercise did not enhance further the increased contractility of the gastrocnemius-plantaris muscle group (*in situ*) attained by exercise alone.

Fitts and Holloszy (1977) also reported the ability of exercise to alter several contractile parameters of the soleus muscle in rats. These authors observed a decreased soleus time to peak

tension and $\frac{1}{2}$ relaxation time of isometric twitch contractions in animals that had received 19-27 weeks of an endurance (prolonged running) treadmill running programme. This programme also, however, resulted in a decreased maximal tetanic tension. High intensity (sprint) exercise for a three week period has been reported to result in increased soleus maximal isometric tetanic contraction tension while the twitch contraction time of the same muscle decreased (Staudte, Exner and Pette, 1973).

The inconsistencies in the results of the reports above emphasize the fact that the intensity of the exercise programme employed may result in a specificity of muscles responding to that exercise. This specificity of response is governed by probably two factors: 1) for exercise to affect a muscle, the muscle must be recruited in that movement, and 2) the intensity of the exercise will differentially select fibres recruited in a particular movement, depending on the fibre type profile of the muscles (Salmons and Sreter, 1976; Lomo et al., 1974).

The only study known to this author with respect to the effect of Dianabol administration on muscle contractility was performed by Richardson and Smith (1981). These authors observed that administration of this anabolic androgen for 30 days did not result in any "strength" or "stamina" changes of the whole gastrocnemius. Any comment on their results is severely restricted by the fact that these terms were not defined and there was no discussion of the methodology employed or data in the report.

If indeed, as would appear to be generally accepted, administration of Dianabol with exercise will improve strength to a greater degree than exercise alone, then two mechanisms can be proposed by which this result may be manifest, both involving protein synthesis. Muscular strength may be increased by enhancing the metabolic capacity of the muscle tissue or by increasing the concentration of contractile element within that tissue.

Effect of anabolic androgens on skeletal muscle protein synthesis

The fact that the steroid employed in the present study is classed as anabolic suggests that it is a member of a family of compounds which have the ability to affect skeletal muscle protein synthesis. The activity of many metabolic enzymes has been shown to be affected by castration and testosterone replacement therapy (Apostolakis, Matzelt and Voigt, 1963; Loring, Spencer and Villee, 1961). Some of the above results have been contradicted by the observations of Eckstein et al. (1960) who reported that succinate dehydrogenase activity in the rat levator ani was not affected by castration or administration of androgen supplements following castration.

However, the suggestion that androgens increase skeletal muscle protein synthesis is further supported by the reports of several authors who observed an androgen induced increased RNA and DNA synthesis (Galavazi and Szirmai, 1971a, b; Arvill, 1967; Saunders, Steciw and Kline, 1962) as well as enhanced uptake of

various radioactive amino acids (Breuer and Florini, 1965; Kochakian, Hill and Harrison, 1964; Costa, Kochakian and Hill, 1962). Similar observations have been reported in tissues of animals treated specifically with Dianabol (Bullock, Peters and White, 1969; Yudaev and Pokrovskii, 1966).

Effect of exercise on skeletal muscle protein synthesis

In light of the specificity of muscular response to a particular exercise suggested above, it is interesting that several publications in the literature report of investigations of exercise induced enzymatic changes in muscle tissue. Endurance exercise has been shown to result in an enhanced concentration of aerobic enzymes in humans (Holloszy, 1975; Gollnick et al., 1973; Morgan et al., 1971). This finding is supported by data from investigations employing rodents (Baldwin et al., 1972; Gollnick and Ianuzzo, 1972). The high intensity sprint running programme utilized by Staudte, Exner and Pette (1973) induced hexokinase and citrate synthetase activities in both slow and fast muscle. The activity of several other enzymes of the soleus was also increased. Holloszy (1975) employed a programme of endurance training adjusted to include short bouts of sprinting and observed alterations of enzymes involved in aerobic and anaerobic respiration in the gastrocnemius. Several review papers are available addressing this topic (Poortmans, 1978; Holloszy, 1975).

Following a review of the literature, it becomes obvious that there must be a common denominator determining the ability of any

particular muscle to respond to a particular exercise. This common entity is generally accepted to be the profile of the respiratory and contractile characteristics of the individual fibres found within a given muscle. This system, as described by Peter et al. (1972) classifies fibres into slow oxidative and fast fibres, with the fast fibres being subcategorized into fast glycolytic and fast oxidative glycolytic, the classification being with reference to the predominating respiratory enzymes and contractile speed. In light of this information, it would appear that one could predict, at least to some degree, the participation of a muscle in a given movement, and whether repetition of this movement will induce enzyme synthesis. Indeed, several authors have demonstrated that exercise, under certain conditions, can actually alter the metabolic profile of a muscle (Barnard, Edgerton and Peter, 1970a, b; Pattengale and Holloszy, 1967).

This overview of the literature is of necessity somewhat contradictory; it is an accurate reflection of the state of knowledge in the field. Investigations have been undertaken to study the effect of exercise on muscle and others to observe the effects of anabolic androgens. Amongst the many questions remaining unanswered, three would appear to be of paramount importance:

- 1) Can the administration of synthetic androgens and exercise enhance the synthesis of specific proteins in skeletal muscle above that observed with exercise treatment alone?, 2) If the answer to question 1) is affirmative, do these changes affect the contractile

strength of the muscle?, and 3) What are the side effects attributable to administration of synthetic androgens?

The present investigation was designed to generate data which should provide some insights into the above questions, with emphasis on questions 1 and 2.

Statement of the Problem

Dianabol is a synthetic androgen derivative which is used extensively for non medical purposes by amateur and professional athletes. However, the hypothesis that this compound is capable of increasing muscle size and strength is based on extrapolations made from results of studies on the effects of the parent compound testosterone on specific androgen responsive skeletal muscles. The importance of questioning the use of Dianabol for these purposes is amplified because of the possible side effects which are associated with the administration of large doses of this compound. The present study was designed to investigate the ability of Dianabol to increase muscle size and strength, and the reversibility of these effects.

The Aims of the Project

It was hypothesized that protein synthesis could be enhanced in an exercising rat by concomitant administration of the synthetic androgen Dianabol. This anabolic activity could result in an increase in metabolic and/or contractile protein, or even a non-specific increase in protein generally. Increases in contractile protein concentration could be manifest as an enhanced contractile strength whereas alterations in metabolic enzyme concentrations may affect the rate or degree of fatigue of a muscle, or its recovery from fatigue. By monitoring the twitch and tetanic contractions, the fatigue profile, as well as the total protein, phosphofructokinase, succinate dehydrogenase, creatine and actin concentrations of tissues from normal, exercised and Dianabol injected animals, any relationship(s) between these parameters could be observed.

Specifically, this study was designed to investigate
a) whether or not administration of Dianabol and exercise can enhance protein synthesis in exercising tissue and b) if a) is positive is the contractility of that particular muscle affected?

Effect of Dianabol and Exercise on Rat Growth

Methods

Male Sprague Dawley rats obtained from Charles River Laboratories (Montreal, Quebec) were used for this study. They were purchased weighing 125-150 g and allowed to acclimate to the new housing for 1 week. Accommodation during this period was in plastic cages, 4 animals per cage, with food and water provided *ad libitum*. The room environment was controlled at 21.5 ± 1 and 55 per cent humidity, with a 10 hour lights on cycle. The plastic cages were changed twice weekly, and the water freshened daily. Standard Purina Rat Chow in pellet form was the food provided.

Experimental groups and treatment administration

After the 1 week acclimatization period, the animals were randomly divided into groups and placed in individual plastic cages (Table 1). Animals in any of the Dianabol treatment groups, which included Dianabol (D), Dianabol withdrawal (DW), Dianabol plus exercise (DE), and Dianabol withdrawal plus exercise (DWE), received semi-weekly subcutaneous (Monday evening, Friday morning) injections of 0.075 mg Dianabol in 0.2 ml of a 15% ethanol in corn oil vehicle. Dianabol (methandrostenolone, or 1,4-androstandien-17 α -methyl-17 β -ol-3-one) was obtained courtesy of Ciba-Geigy Canada Ltd., Montreal, or purchased from Steroid Plus Research Laboratories Inc., Denville, New Jersey. The corn oil used was a standard household brand (Mazola). Injections were given with a 23 gauge 2.54 cm. needle. One third of the control (C) and control plus exercise (CE) animals

Table 1. Rat groups.

<u>NUMBER OF RATS</u>														<u>DURATION OF TREATMENT.</u>	<u>SACRIFICE DATE</u>
<u>SERIES</u>	<u>STARTED</u>					<u>SURVIVED</u>									
	<u>C</u>	<u>D</u>	<u>DW</u>	<u>CE</u>	<u>DE</u>	<u>DVE</u>	<u>C</u>	<u>D</u>	<u>DW</u>	<u>CE</u>	<u>DE</u>	<u>DVE</u>			
1	10	10		10	10		10	10		10	10		4 weeks	October 1980	
2	10	10		10	10		8	7		7	6		8 weeks	August-September 1980	
3	10	5	5	10	5	5	10	5	5	8	5	5	8 weeks	November 1981	

Rats were randomly divided into groups 1 week after arrival. All were housed in individual cages in a controlled environment.

Figure 1. Experimental protocol. Following a 1 week acclimatization period, rats were randomly divided into groups. Animals in exercise groups participated in a progressive treadmill running programme, as described in Appendix A. Steroid treated groups received 0.075 mg Dianabol in 0.2 ml of a 15% ethanol in oil vehicle, injected subcutaneously semi-weekly. Number of animals per group as described in Table 1.

Groups

C - control

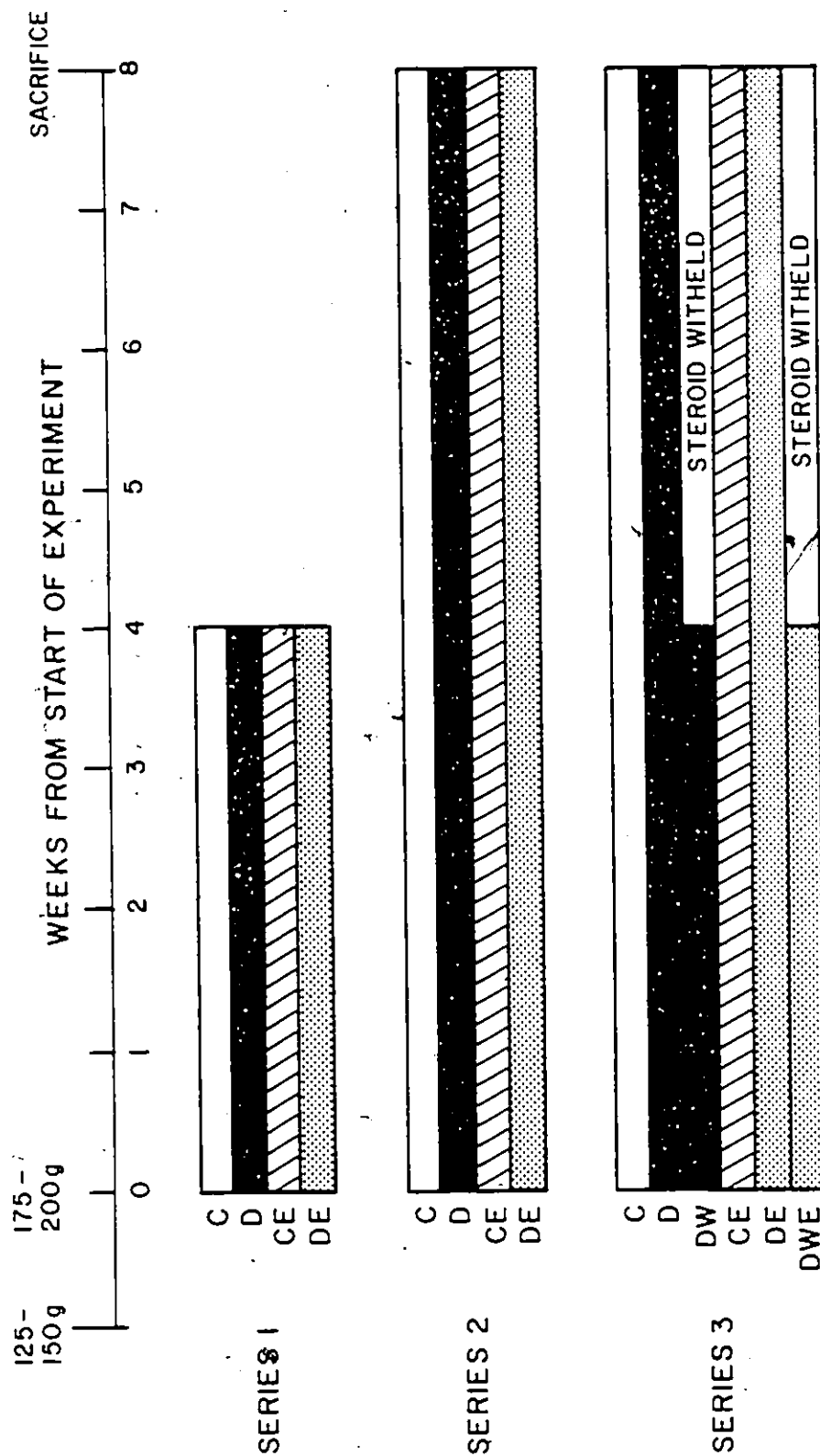
D - Dianabol

DW - Dianabol withdrawn

CE - Control-exercise

DE - Dianabol-exercise

DWE - Dianabol-withdrawn-exercise



C - CONTROL
 D - DIANABOL
 DW - DIANABOL WITHDRAWN
 CE - CONTROL - EXERCISE
 DE - DIANABOL - EXERCISE
 DWE - DIANABOL - WITHDRAWN - EXERCISE

formed a sub-group of controls, receiving 0.2 ml vehicle only injections.

Animals undergoing exercise treatment (CE, DE and DWE groups) were given a programme of progressive medium duration, moderate intensity treadmill running, as described by Reed (1972, appendix A). The running exercise was performed on a Quinton Instruments model rodent treadmill (Quinton, Mass.) with a 10° incline. Exercise was always performed midway through the off part of the light cycle.

Tissue sampling

Three series of animals were employed for this investigation (Table 1 and Figure 1). Series 1 involved only 4 groups (C, D, CE and DE) for a period of 4 weeks. At the end of this period, the animals were sacrificed by cervical dislocation after which the adrenals, ventral prostate, the heart (trimmed to ventricles) as well as the soleus, plantaris, medial and lateral gastrocnemius of both legs were removed, blotted, weighed and rapidly frozen in liquid nitrogen for subsequent biochemical analysis.

The series 2 and 3 animals were maintained for a period of 8 weeks. One half of series 3 groups D and DE animals received the steroid injections for only the first 4 weeks of the investigation, after which no further injections were given, but exercise was continued. These animals comprise groups DW (Dianabol withdrawal) and DWE (Dianabol withdrawal plus exercise), the 4 week Dianabol free period denoted as withdrawal.

Starting times for series 2 and 3 were staggered in order to facilitate the sacrifice of 2 animals per day (1 steroid treated and 1 respective control) at the end of the 8 week period. For the contractility studies the animals were anaesthetized (sodium pentobarbital, 70 mg/kg) and the surgery performed (appendix D, and chapter 4). Only 2 animals per day could be studied because of the difficulty and time required for these procedures. Immediately following completion of the contractility studies, the animals were treated precisely as those of series 1 (4 week study). It was felt that performing both the biochemical and contractility determinations on the same muscle tissue would provide a more accurate appraisal of any muscle response to the various treatments. Accordingly, in the series 2 and 3 rats, muscles of the right hind limb were used for both the biochemical analysis and contractility experiments.

Data analysis

Standard statistical methods were used in evaluating the results of the experiments. Quantitative results are expressed throughout as mean $\pm$ standard error of the mean (SEM) for each group. In order to compare the effects of the various treatments on each parameter studied, data were analyzed using one-way analysis of variance. For comparisons between two means, Tukey's test was used. P values of 0.05 or less were considered to be significant.

Results

Effect of Dianabol and/or exercise on body weight gain

All animals involved in series 1 of this investigation continued to gain weight throughout the 4 week treatment period (Figure 2). The mean starting weight for group C was 190.6 ± 2.6 g which increased at an almost constant rate to a mean weight at sacrifice of 378.5 ± 7.2 g. Administration of Dianabol (group D) did not have any effect on animal growth, as the mean weights of these animals over the 4 week period followed the same trend as group C.

Both groups of animals maintained on the exercise programme (groups CE and DE) had body weights which were less than the C and D groups. This trend was apparent 1 week following the beginning of treatment. The group CE weights were significantly less than those of groups C and D at week 2, the mean weights being 268.7 ± 4.1 g, 289.4 ± 4.7 g and 290.9 ± 5.0 g, respectively. The mean body weight of group DE (291.8 ± 9.0 g) became significantly less than that of group C and D beginning at week 3 (327.0 ± 5.6 g and 327.5 ± 5.3 g, respectively), and as was observed in group CE, this significant difference in body weights was maintained for the duration of the experiment. Although the mean body weights at sacrifice of groups CE and DE (322.2 ± 6.7 g and 339.7 ± 6.8 g, respectively) were both significantly less than the mean body weight of group C (378.5 ± 7.2 g) and D (375.4 ± 6.3 g), the confidence intervals were different, that for CE being $p < 0.01$ and for DE at $p < 0.05$.

· Figure 2. Effect of 4 weeks treatment with Dianabol and exercise on male rat body weight gain. Rats were housed in individual cages in controlled ambient conditions. Experimental protocol was as described in Figure 1 and the text. Points represent group means. Standard errors of less than 15 g are omitted.

Symbols denote significant difference from: *Control, †Dianabol treatment by Tukey's test ($p < 0.05$).

Groups

- Δ - Control (10)
- ▲ - Dianabol (10)
- - Control-exercise (10)
- - Dianabol-exercise (10)

Number of animals per group indicated in parentheses

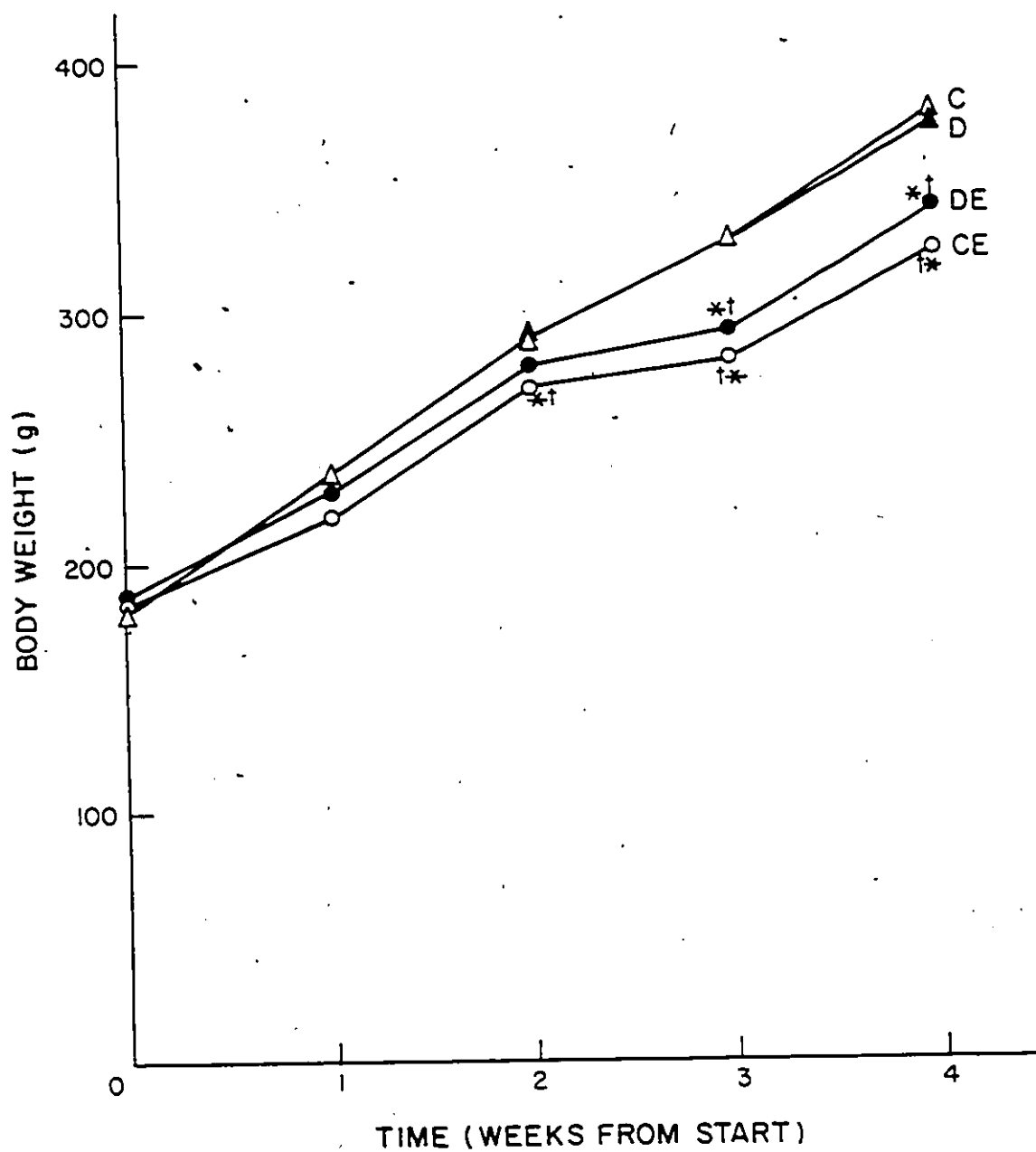


Figure 3, a composite of mean weights of animals in series 2 and 3, illustrates that similar observations were made of these animals over the first 4 weeks of the 8 week treatment period. Although animals of group C and D continued to grow after 4 weeks, the rate of body weight gain plateaued beginning at week 5, the mean body weights of groups C being 384.8 ± 8.2 g and 415.4 ± 8.1 g following week 5 and 8, respectively. Again, as noted above with series 1, animals of the CE group had significantly lesser mean body weights than group C, beginning at week 2. This difference persisted throughout the 8 week period, the difference between the 2 groups increasing until approximately week 5. From week 5 to week 8, the CE animals continued to gain weight whereas, as noted above, the group C animals' weights had plateaued, and thus the difference between the 2 groups decreased.

Interestingly, the observation at 4 weeks that the DE animals mean weights appeared to be somewhat greater than the CE group mean was continued such that by week 5 the mean DE group weight was significantly greater than that of group CE, the means being 356.3 ± 9.4 g and 317.7 ± 10.5 g, respectively, as well as remaining significantly less than the mean of group C or D (384.8 ± 8.2 g and 380.0 ± 11.4 g, respectively). The mean body weight of group DE continued to increase throughout the 8 week period, attaining a level which by week 6 was no longer significantly different from the controls. However, animals of group DWE, which had the steroid injections withheld after week 4, did not maintain

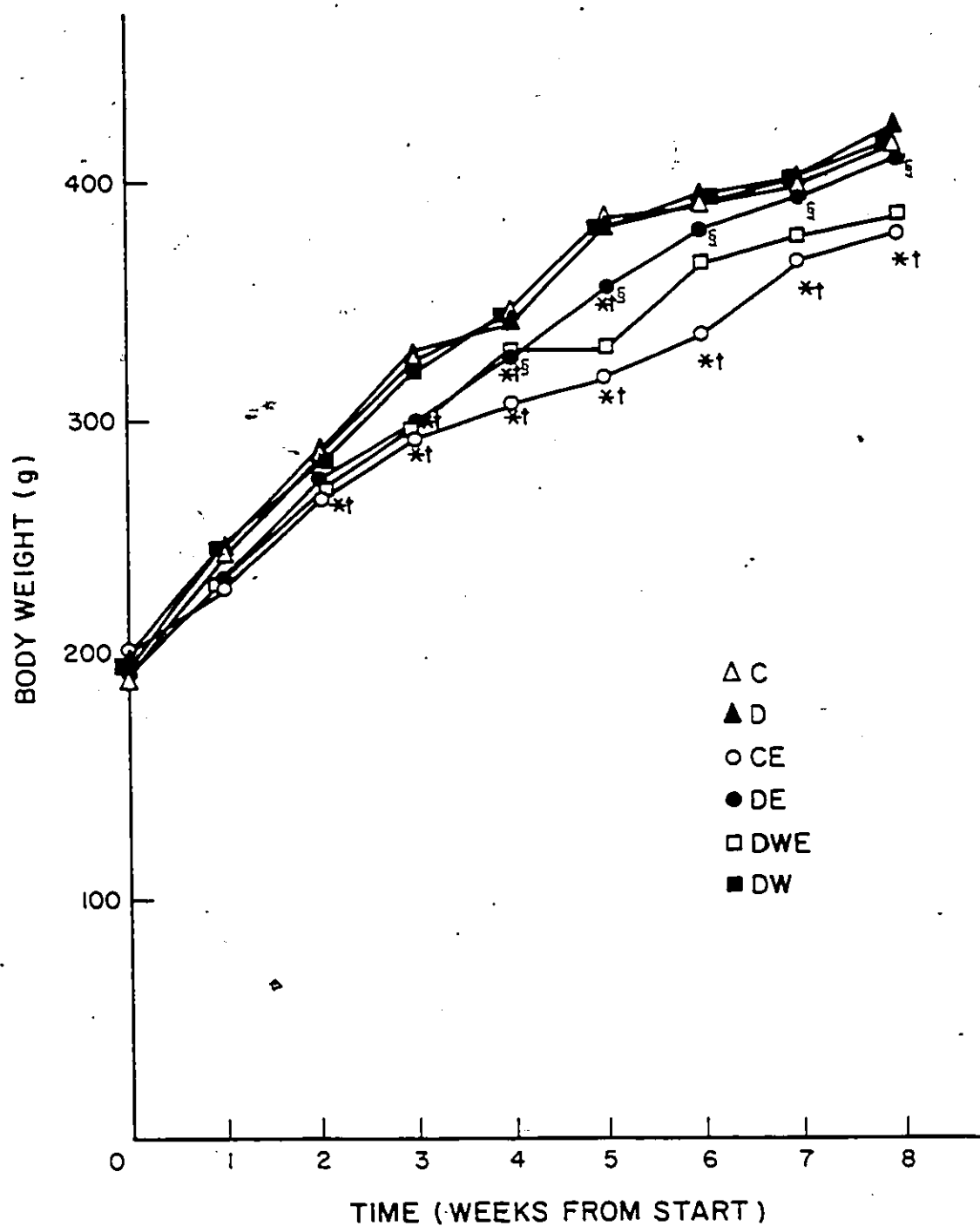
Figure 3. Effect of 8 weeks treatment with Dianabol and exercise (composite of series 2 and 3) on male rat body weight gain. Rats were housed in individual cages in controlled conditions. Experimental protocol was as described in Figure 1 and the text. Points represent group means. Standard errors of less than 15 g are omitted.

Symbols denote significant difference from: *Control, +Dianabol, §Control-exercise treatment, by Tukey's test ($p < 0.05$).

Groups

- Δ - Control (18)
- ▲ - Dianabol (12)
- - Dianabol withdrawn (5)
- - Control-exercise (15)
- - Dianabol-exercise (11)
- - Dianabol-withdrawn-exercise (5)

Number of animals per group indicated in parentheses



the steroid-exercise induced weight gain. Indeed, only 1 week following the cessation of steroid injections, the DWE mean body weight declined to 330.0 ± 9.3 g, which was no longer significantly different from the mean of group CE. This trend continued for the duration of the experiment. Whereas steroid withdrawal had a marked effect on mean body weight of the DWE group, there was no effect of withdrawal on group DW. This was to be expected however, because the steroid treatment alone (group D) did not result in any changes in body weight over the experimental period when compared to group C.

The fact that the group DE body weight was elevated with respect to groups DWE and CE suggests that injections of Dianabol may reduce the negative effect of exercise on body weight gain.

Effect of Dianabol on normalized organ weights

In light of the previous discussion regarding the reported decreased androgenicity of Dianabol relative to testosterone, the weight of both the testes and ventral prostate were recorded.

The recording of primary and accessory sex organ weights in this study was not for the purpose of quantitative analysis but to verify the absorption of Dianabol from the oil vehicle via subcutaneous injection.

The adrenal glands were also weighed to gain some insight into the stressfulness of the treatments, as well as the cardiac ventricles for an indication of the treatment effects on a second muscle type.

The ventral prostate was the only sex organ observed to respond to the administration of the synthetic androgen. Following the 4 week study (Table 2), as well as both 8 week series (Tables 3 and 4) the mean normalized ventral prostate weight of the Dianabol treated groups was significantly less than that of the respective control group. There was no apparent effect of steroid treatment on normalized testicular, adrenal or ventricular weight. Withdrawal of steroid treatment (Groups DW and DWE, Table 4) after 4 weeks did not diminish the steroid induced atrophy of the ventral prostate.

The diminished ventral prostate weight observed in the steroid injected animals presumably reflects the effect of Dianabol on endogenous androgen production and illustrates that Dianabol was absorbed via the chosen route of administration.

The apparent exercise induced increase of normalized organ weights relative to the respective non exercised group is an artifact of the lesser body weight of the leaner exercising animals; use of this body weight in normalization exaggerates relative organ weight. Whereas this trend was also observed in comparing the series 1 ventral prostate weights, it was not in series 2 and 3 animals. The mean normalized weights of this organ from groups DE and DWE of series 3 animals were not different from that of the D or DW respective control (Table 4), even though the group DE mean body weights were equivalent to that of groups C and D. These data suggest a larger mean absolute ventral prostate weight in the DE animals which was indeed the situation as the mean absolute ventral

Table 2. Effect of 4 weeks treatment with Dianabol and exercise on rat organ weights.

ABSOLUTE WEIGHT AT SACRIFICE [NORMALIZED TO mg/100 g BODY WEIGHT]				
	<u>ADRENALS</u>	<u>VENTRAL PROSTATE</u>	<u>TESTIS</u>	<u>VENTRICLES</u>
CONTROL (10)	24.48 ± 1.2 [11.9 ± 0.7]	247.5 ± 14.3 [118.9 ± 6.8]	1653.7 ± 41.0 [811.6 ± 20.1]	511.8 ± 9.3 [251.2 ± 4.4]
DIANABOL (10)	24.27 ± 1.0 [11.5 ± 0.7]	207.0 ± 14.1* [100.2 ± 6.1]*	1678.6 ± 42.5 [789.7 ± 18.9]	512.0 ± 15.7 [241.7 ± 18.5]
CONTROL- EXERCISE (10)	29.61 ± 1.3 [15.3 ± 0.8]*	263.2 ± 9.2 [133.1 ± 6.1]	1646.2 ± 45.9 [918.9 ± 34.1]*	512.6 ± 14.3 [276.3 ± 7.8]*
DIANABOL- EXERCISE (10)	29.29 ± 1.1 [15.8 ± 0.6]*+	210.4 ± 11.9§ [114.2 ± 6.3]§	1662.7 ± 49.8 [902.2 ± 26.6]†	517.2 ± 11.9 [275.4 ± 6.2]

1) Values represent mean ± S.E.M.

2) Symbols represent significant difference by Tukey's test ($p < 0.05$)
From: *Control, +Dianabol, § Control-Exercise treatment.

3) Number of animals per group indicated in parentheses.

Table 3. Effect of 8 weeks treatment (series 2) with Dianabol and exercise on rat organ weights.

ABSOLUTE WEIGHT AT SACRIFICE [NORMALIZED TO mg/100 g BODY WEIGHT]				
	<u>ADRENALS</u>	<u>VENTRAL PROSTATE</u>	<u>TESTIS</u>	<u>VENTRICLES</u>
CONTROL (8)	56.77 ± 3.95 [12.85 ± 0.81]	574.06 ± 26.99 [130.26 ± 6.45]	3240 ± 120 [737.86 ± 36.39]	1231 ± 60 [279.08 ± 14.2]
DIANABOL (7)	64.07 ± 2.31 [12.80 ± 0.50]	520.80 ± 16.62* [118.98 ± 8.64]*	3332 ± 80 [755.77 ± 16.52]	1213 ± 60 [274.46 ± 9.9]
CONTROL- EXERCISE (7)	54.32 ± 1.32 [17.8 ± 1.26]*	573.59 ± 22.21 [170.20 ± 16.27]*	3134 ± 140 [925.93 ± 26.62]*	1174 ± 50 [331.13 ± 14.6]
DIANABOL- EXERCISE (6)	65.00 ± 5.30 [17.5 ± 0.85]*+	493.85 ± 13.56 [114.40 ± 7.32]*s	3167 ± 80 [861.70 ± 42.62]+	1152 ± 50 [311.03 ± 6.5]±

1) Values represent mean ± S.E.M.

2) Symbols represent significant difference from: *Control, +Dianabol, sControl-Exercise treatment, by Tukey's test (p < 0.05).

3) Number of animals per group indicated in parentheses.

Table 4. Effect of 8 weeks treatment with Dianabol and exercise (series 3) on rat organ weights.

ABSOLUTE WEIGHT AT SACRIFICE [NORMALIZED TO mg/100 g BODY WEIGHTS]				
	<u>ADRENALS</u>	<u>VENTRAL PROSTATE</u>	<u>TESTIS</u>	<u>VENTRICLES</u>
CONTROL (10)	46.9 ± 2.1 [11.3 ± 0.6]	572.3 ± 21.9 [140.2 ± 6.0]	3189 ± 99.8 [744.6 ± 17.6]	1205.4 ± 130.7 [265.1 ± 3.8]
DIANABOL (5)	43.7 ± 2.7 [11.4 ± 1.2]	536.8 ± 14.9 [117.4 ± 4.2]*	3290 ± 162.0 [755.9 ± 50.1]	1100.0 ± 54.2 [268.4 ± 7.0]
DIANABOL- WITHDRAWAL (5)	48.2 ± 1.1 [11.7 ± 0.8]	578.9 ± 35.9 [124.0 ± 7.9]*	3347 ± 56.4 [719.8 ± 28.5]	1145.9 ± 73.6 [268.9 ± 2.3]
CONTROL- EXERCISE (8)	49.0 ± 3.0 [14.3 ± 0.9]*	661.5 ± 15.4 [180.4 ± 7.9]*	2877 ± 121.6 [826.3 ± 20.7]	1065.8 ± 48.4 [285.0 ± 8.6]
DIANABOL- EXERCISE (5)	51.9 ± 3.4 [14.3 ± 0.7]*+	516.8 ± 10.9 [126.8 ± 6.1]§	2978 ± 83.1 [873.2 ± 27.8]	1039.0 ± 61.9 [286.2 ± 8.2]
DIANABOL- WITHDRAWAL- EXERCISE (5)	50.1 ± 1.2 [14.8 ± 0.7]*+	490.2 ± 26.3 [126.6 ± 2.8]*§	3070 ± 43.1 [878.1 ± 39.8]*Δ	977.8 ± 60.7 [278.2 ± 4.2]

1) Numbers represent mean ± S.E.M.

2) Symbols represent significant difference from: *Control, +Dianabol, § Control-Exercise, ΔDianabol-withdrawal, treatment by Tukey's test ($p < 0.05$).

3) Number of animals per group indicated in parentheses.

prostate weight for group D and DE, respectively, were 536.8 ± 10.9 mg and 512.7 ± 17.1 mg.

Effect of Dianabol and exercise on skeletal muscle weights

The mean weights of the soleus, plantaris, medial and lateral gastrocnemius muscles of the hind limb are summarized in Table 5 (series 1), Table 6 (series 2) and Table 7 (series 3). From these results it is apparent that treatment with either Dianabol or exercise alone or the two combined had no effect on mean muscle weight in any of the series of animals. It is interesting to note that the muscles of the right leg of animals involved in series 2 and 3 are approximately 20 per cent heavier than the same muscles of the contralateral limb. The right hind limb soleus and lateral gastrocnemius were utilized for the contractility experiments of chapter 4. As discussed in conjunction with the muscle water content data in chapter 3, this increased muscle weight may be accounted for in terms of an increased water content (Appendix D).

Table 5. Effect of 4 weeks treatment with Dianabol and Exercise on rat muscle weights at sacrifice (series 1).

	<u>Soleus (mg)</u>	<u>Plantaris (mg)</u>	<u>Medial Gastroc (mg)</u>	<u>Lateral Gastroc (g)</u>
CONTROL (10)	158.7 ± 7.2	367.3 ±10.9	918.7 ±28.2	1.00 ±0.02
DIANABOL (10)	168.5 ± 5.8	368.5 ±10.4	922.1 ±25.0	1.02 ±0.03
CONTROL- EXERCISE (10)	147.0 ± 3.3	331.0 ± 8.9	808.0 ±25.2	0.88 ±0.03
DIANABOL- EXERCISE (10)	152.0 ± 6.5	353.7 ±12.5	834.1 ±23.1	0.94 ±0.02

1) Numbers represent ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 6. Effect of 8 weeks treatment (series 2) with Dianabol and exercise on rat muscle weights at sacrifice.

	LEFT				RIGHT			
	SOLEUS (mg)	PLANTARIS (mg)	MEDIAL GASTROC. (g)	LATERAL GASTROC. (g)	SOLEUS (mg)	PLANTARIS (mg)	MEDIAL GASTROC. (g)	LATERAL GASTROC. (g)
CONTROL (8)	169.7 ±11.3	427.6 ±20.0	1.02 ±0.05	1.13 ±0.04	207.1 ± 9.4	470.3 ±15.3	1.10 ±0.03	1.36 ±0.06
DIANABOL (7)	175.2 ±13.1	426.3 ±18.1	1.02 ±0.06	1.08 ±0.04	222.1 ±12.7	478.9 ±21.9	1.09 ±0.08	1.38 ±0.04
CONTROL- EXERCISE (7)	181.2 ±11.3	436.2 ± 7.7	0.93 ±0.03	1.11 ±0.04	228.0 ±12.2	497.8 ±12.2	1.21 ±0.06	1.35 ±0.05
DIANABOL- EXERCISE (6)	162.6 ± 9.6	431.2 ± 9.7	0.94 ±0.06	1.08 ±0.05	199.7 ±12.3	483.7 ±19.2	1.04 ±0.07	1.27 ±0.06

1) Number represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 7. Effect of 8 weeks (series 3) treatment with Dianabol and exercise on rat muscle weights at sacrifice.

	LEFT				RIGHT			
	SOLEUS (mg)	PLANTARIS (mg)	MEDIAL GASTROC (g)	LATERAL GASTROC (g)	SOLEUS (mg)	PLANTARIS (mg)	MEDIAL GASTROC (g)	LATERAL GASTROC (g)
CONTROL (10)	165.0 ± 8.0	425.1 ±12.2	1.03 ±0.04	1.11 ±0.05	204.2 ± 8.3	482.4 ± 3.8	1.23 ±0.04	1.34 ±0.07
DIANABOL (5)	168.8 ±18.7	422.9 ±22.8	0.82 ±0.04	1.09 ±0.06	200.2 ±10.0	499.5 ±24.1	1.22 ±0.08	1.34 ±0.06
DIANABOL- WITHDRAWAL (5)	166.6 ± 6.7	416.3 ±15.5	1.03 ±0.04	1.09 ±0.05	203.2 ± 2.7	506.9 ±15.9	1.21 ±0.06	1.39 ±0.02
CONTROL- EXERCISE (8)	150.5 ± 9.8	400.1 ±18.1	0.91 ±0.02	1.15 ±0.05	187.3 ±10.2	499.7 ± 7.5	1.12 ±0.04	1.25 ±0.05
DIANABOL- EXERCISE (5)	151.3 ± 8.3	373.7 ±10.8	0.95 ±0.05	1.07 ±0.06	209.6 ± 9.2	497.3 ± 9.6	1.22 ±0.07	1.35 ±0.07
DIANABOL- WITHDRAWAL- EXERCISE (5)	158.2 ±12.3	396.9 ±11.1	0.94 ±0.05	1.01 ±0.05	192.2 ± 2.3	500.5 ± 8.1	1.16 ±0.05	1.28 ±0.04

1) Numbers represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Discussion

Drug dose and administration

The choice of dosage and administration of Dianabol for this investigation became an extremely difficult task. A survey of the literature unveiled a definite lack of consistency in this area, which is magnified by the fact that various anabolic steroids also have quite different potencies (Boris, Stevenson and Trimal, 1970a, b). Doses of anabolic agents ranging from $0.35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for methandrostenolone (Stone, Rush and Lipner, 1978) to $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of methylandrostanediol (Hickson et al., 1976) or even $25 \text{ mg} \cdot \text{day}^{-1}$ for testosterone propionate (Kochakian and Cockrell, 1958) have been used in previous work. The dose chosen for this investigation was based on a pilot study in the rat in which overall body weight was monitored for a 4 week period, with groups of rats being administered doses of Dianabol ranging from a high of 2.0 mg to 0.075 mg semi-weekly.

It is recognized that Kochakian (1938) reported administration of Dianabol semi-weekly greatly reduces its efficacy relative to lesser daily doses. However, to minimize any suffering that might be caused to the animals by the injections, and the fact that one half would be required to perform exercise 5 times per week, semi-weekly injections appeared to be the most adequate compromise.

Only the lowest dose of Dianabol used (0.075 mg or $0.375 \text{ mg} \cdot \text{kg}^{-1}$ starting weight) resulted in any tendency toward greater weight gains, thus this dose was chosen for future studies.

Suitability of the rat as a model for humans

The inconsistency of the literature data required that much consideration be given to the choice of an animal model. This being primarily a study of muscle, first consideration was given to the guinea pig which has androgen responsive masseter and several other skeletal muscles (Papanicolaou and Falk, 1938; Kochakian and Tillotson, 1957). The docility of this animal enhanced its appeal as a model. However, the lack of availability of both a reliable exercise programme and a consistent healthy supply of animals made this model less attractive. Governed by the size of animal which could be run on the treadmill, the necessity of large enough muscle tissue quantities to perform all the biochemical analysis, and cost, the rat was chosen by process of elimination.

Effect of Dianabol and exercise on rat growth

Observation of the growth pattern of all 3 series of animals can only be interpreted to suggest that administration of this dose of Dianabol to young adult male rats does not cause marked changes in body weight. This corroborates the results of other studies in rats (Stone and Lipner, 1978; Kochakian and Endahl, 1959) and larger animals (Heitzman, 1976; Heitzman et al., 1977; Young and Pluskal, 1977). Bavetta et al. (1962) also observed similar results using 0.04 or 0.14 mg·day⁻¹ 17 α -methyltestosterone. It has been suggested that an increased growth rate is not observed in rats of this size (about 200 g) when anabolic agents are administered because they

already have a positive nitrogen balance and because the doses of steroid used are very low (Kochakian, 1952; Arnold et al., 1963).

The effect of Dianabol on weight gain may also be somewhat species specific as Kruze et al. (1966) using a much larger dose of $1.5 \text{ mg} \cdot \text{kg}^{-1}$ every second day in rabbits reported a significant increase in both male and female body weight. The observations in rats also contrast with those of human studies where unequivocally an increased mass is observed following steroid administration alone or in conjunction with exercise (Johnson and O'Shea, 1969; Johnson et al., 1972; Freed et al., 1975; Ryan, 1976). Indeed, the steroid plus exercise combination in the present study did result in larger body weights over the 8 week period than did exercise alone.

A major difference between the growth pattern of the rat and human is the fact that humans tend to stop growing, i.e. gaining weight, towards the end of adolescence (Sinclair, 1969), whereas the rat continues to gain weight throughout most of its lifespan. This may be one reason that a steroid induced weight gain was not observed in the rat, because it may in fact have been preempted by the variation in normal growth patterns. As discussed above, the young adult rat gains weight in an almost linear fashion until 5 weeks of the experiment (or 13-14 weeks of age). The weight gain was approximately 50 g per week throughout this period. It may be difficult to induce an even more rapid growth pattern with any anabolic steroid. The effectiveness on body weight of Dianabol in combination with exercise may be a function of the fat content of the animal. Several authors

(Kochakian, 1935; Kochakian and Murlin, 1935; Funk and Harrow, 1930) have shown testosterone and many of its synthetic derivatives to be highly lipid soluble. Thus the exercised animals which are, of course, much leaner, would not sequester as much of the steroid, providing larger circulating plasma concentrations to act at target tissues. As early as 1950, Kochakian, Robertson and Bartlett and later Laron and Kawadlo (1963) and Fisum and Sharkevich (1971) suggested that increased weight gain in rats administered steroids may be a function of the fat content of the animal, particularly if low doses are used.

The effect of exercise was to reduce body weight gain with age. The particular exercise programme chosen has been used extensively by several investigators (Reed, 1972; Wilkinson, 1978) and was designed to recruit all known fibre types in rat skeletal muscle tissue. However fast fibre types of muscle tend to be affected more with this programme (Reed, 1972). Similar effects of exercise on animal (Stone, Rush and Lipner, 1978) and human (Parizkova, 1974) body weight have been reported in the literature. The fact that animals receiving both steroid and exercise had significantly larger body weights relative to exercising animals is an interesting observation. Combined with the fact that the muscles of these animals were not heavier than the same tissues from any other group, this result suggests that this weight change may be due to water retention induced by the steroid. In 1939, Sandberg et al., reported that testosterone in castrated young rats caused a retention of

sodium and chloride as well as other salts. Other authors made similar reports in man (Kenyon, 1938; Kenyon et al., 1938 1940, 1944) and dogs (Thorn and Harop, 1937). Some time later Kim (1961) in a well controlled study reported that adult rats administered several different anabolic androgens including Dianabol, had a decreased urinary sodium and potassium content. A similar effect was observed following estrogen administration (Knowlton et al., 1942; Dignam, Voskian and Assali, 1956; Landau et al., 1957).

The mechanism by which androgens may have this effect remains unknown, but the result in mice has been an increased kidney weight due to edema (Pfeiffer, Emmel and Gardner, 1940; Kochakian, 1941; Wrete, 1943; Feyel, 1943). In rats implanted with testosterone propionate pellets, Korenchevsky et al. (1941) observed a 16 per cent increased kidney weight with enlargement of the convoluted tubule cells. Other reports of increased kidney weights following steroid administration are numerous (Selye, 1940a; Ludden et al., 1941). This effect on the kidney resulting in edema appears to be a direct action, as it can be blocked with the anti-androgen cyproterone acetate (Ohno and Lyon, 1970; Broulik, Starka and Ston, 1975). In contrast to this Rieger et al. (1972) reported that administration of estrogen resulted in increased plasma renin concentration, which suggests an involvement of aldosterone. However, since plasma aldosterone was not elevated this is not likely.

The involvement of Dianabol administration on water retention due to a direct action on the kidney resulting in sodium retention is further supported by the fact that the difference in body weight of animals of series 3 of the present study which continued to exercise while steroid treatment was withheld and the control exercise group, began to decrease immediately following the cessation of steroid injections. As discussed in chapter 3, any observed protein synthesis which may have been induced by the synthetic steroid is most certainly not reversed in this short period of time, and thus a decrease in sodium uptake with the ensuing water retention can not be explained by a reduced carrier mechanism.

Effect of Dianabol and exercise on organ weights

The supportive role that androgens play in regard to sex organ function has been known for over half a century. Several authors have reported atrophy of the primary or secondary sex organs following administration of methandrostenolone (Stone, Rush and Lipner, 1978; Boris, Taylor, Secord and Murray, 1978). In the present study, a significantly lesser ventral prostate weight was observed in all Dianabol treated groups relative to their respective control. This atrophy due to exogenous steroid may be explained in 2 ways. Firstly, the synthetic androgen could competitively inhibit testosterone action at the target organ by occupying receptor binding sites, or perhaps more likely, it may interfere with the hypothalamo-hypophyseal-gonadal axis decreasing testosterone secretion and thus circulating plasma levels. In either situation, the lesser androgenic capabilities of Dianabol would result in a

lack of support for growth of the ventral prostate.

Whereas the latter reasoning might be more generally accepted, reports in the literature are contradictory on the effects of anabolic steroid administration on circulating levels of anterior pituitary hormones. A markedly decreased circulating plasma luteinizing hormone (LH) concentration was reported following 6 weeks Dianabol administration to rats (Stone, Rush and Lipner, 1978; Stone, 1978), but follicle stimulating hormone (FSH) levels were not affected. This would explain why ventral prostate atrophy occurred in the present study, while the primary sex organ weights were unaffected. However, studies in humans report the reverse effect on anterior pituitary hormones in that Dianabol administration to male athletes results in a depression of plasma FSH levels whereas LH is not affected (Harkness, Kilshaw and Hobson, 1975; Holma and Aldercruetz, 1976).

The obvious answer to this problem would be to concomitantly determine plasma testosterone and Dianabol concentrations following Dianabol administration. A review of the literature does not provide any insight into this question, but some unpublished data of Dugal and Masse (1982) confirms testosterone levels in the male human to decline during Dianabol administration, which would appear to contradict studies discussed above. A severe decrease in spermatogenesis has also been observed in humans following synthetic androgen consumption (Hudson, 1981).

Samples of blood were taken weekly from the series 2 animals (see chapter 3) in an attempt to quantify plasma levels of testosterone and Dianabol during Dianabol administration in the rat, as no reports are available in the literature on this subject. However, the low concentrations of both of these compounds in the rat, combined with the small samples which could be taken, given that the animals had to survive 8 weeks, have made this determination technically very difficult. Thus, the data are still unavailable.

It was very interesting to find that neither an atrophy nor hypertrophy of the adrenal gland occurred in any of the treated groups. Hypertrophy of this organ might have been caused by the stress of either exercise or steroid injections. Although this response to stress is a widely reported phenomenon in rats (Yuwiler, 1976), the animals used in the present study appeared to acclimate to any of the stressful factors, as the adrenal weights were unaffected by any treatment. This suggestion is further supported by the fact that plasma corticosterone levels from animals of any treated group were within the normal range but the control group, which was handled much less frequently, had very high circulating levels of this steroid. This lack of effect on adrenocortical secretion was also reported by Kinson and Lubek (1981). For a more elaborate discussion of this subject, chapter 3 should be consulted.

Adrenal atrophy would be indicated if Dianabol interfered with the hypothalamo-hypophyseal-adrenal axis to cause a reduction or inhibition of anterior pituitary secretion of adrenocorticotrophic hormone (ACTH). Again, however, this did not appear to be the situation.

One characteristic response of the heart to exercise is left ventricular hypertrophy (Van Lier et al., 1964; Van Lier and Northup, 1957; Bloor, Leon and Pasyk, 1968), which is considered an important adaptive mechanism to sustain function with increased circulatory load (Norman, 1962). The fact that there was no increase of ventricular weight observed in any of the exercised animals involved in this investigation suggests that the specific exercise programme used did not result in a significant increase in circulatory load. This suggestion is indirectly supported by the fact that the adrenals also showed no characteristic stress symptoms, which would normally be associated with an increased circulatory load.

Effect of Dianabol and exercise on skeletal muscle weight

Similarly, observations of skeletal muscle hypertrophy have been recorded following Dianabol administration with intensive training in humans (O'shea and Winkler, 1970; Ward, 1973), or even Dianabol alone (Ward, 1973; Winnay and Mya-Tu, 1975). In the rat, at least one investigator has observed skeletal muscle hypertrophy following Dianabol administration (Pongratz and Mittlebach, 1969). However, Stone, Rush and Lipner (1978) reported no change in the weight of this tissue. The results of the present study did not indicate any muscular hypertrophy following treatments with steroid and/or exercise, which may suggest the changes reported by others were a result of the muscle specificity of the particular exercise programme employed, which differed from that of this study.

Biochemical Response of Skeletal Muscle to Dianabol and Exercise

Methods

Determination of total protein, succinate dehydrogenase, and phosphofructokinase concentration

A piece of frozen muscle tissue was weighed in a glass homogenizing tube containing 1 ml of ice cold homogenizing medium (0.15 M KCl, pH 7.4), as described by Paterson, Layberry and Nadkarni (1972). The tube was then placed in an ice water bath and a further quantity of medium added to yield a 10 per cent homogenate. If less than 100 mg of tissue were available, as was often the situation encountered with soleus muscle tissue, 1 ml of homogenizing medium was still used as this was the minimum volume required for the total procedure. The tissue was then homogenized by hand with a glass pestel, while the tube was kept immersed in the ice water bath.

Succinate dehydrogenase content was determined by a modification of the method of Cooperstein, Lazarow and Kurfess (1950, Appendix B). Immediately following homogenization, a 0.10 ml aliquot of homogenate was transferred to a precooled glass tube and 0.3 ml of 0.044 M potassium phosphate buffer pH 7.4 added, resulting in a 0.033 M buffer concentration as described in the original paper. The determination measures spectrophotometrically the rate of reduction of cytochrome C at 550 nm.

As soon as the aliquot for determination of SDH was buffered, a second 0.5 ml aliquot of homogenate was transferred to a glass tube and 2.5 ml of 0.36 N potassium hydroxide was added. This provided a means of digesting all of the tissue protein for accurate quantification. The tubes were covered and placed in a 37°C water bath until digestion was complete, usually about 4 hours. During this period the tubes were vortex mixed each half hour. When digestion was complete, a 0.5 ml aliquot of digest was diluted to 10 ml with distilled water and this sample then used for protein determination by the method of Lowry et al. (1951). This is a colorimetric measurement of the reduction of phosphomolybdic-tungstic mixed acid at 640 nm, room temperature.

The remainder of the original tissue homogenate was transferred to a precooled centrifuge tube and spun at 100,000 x g, 4°C for 60 minutes. Following centrifugation, the supernatant was transferred to a glass tube. Immediately before assay, an aliquot of the supernatant was diluted with ice cold distilled water to yield a 2 per cent preparation (1:4). Phosphofructokinase activity was determined by an adaptation of the methods of Mansour (1966) and Lea and Walker (1965) as described by Paterson, Layberry, and Nadkarni (1972). This method involves the spectrophotometric measurement of the oxidation of NADH at 340 nm, 37°C. The supernatant is added to a mixture containing ATP, aldolase glyceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase buffered with tris-magnesium chloride, pH 8.2. All

ingredients were provided in excess, making the quantity of PFK the rate limiting factor. The rate of change of absorption by NADH is ultimately proportional to the quantity of PFK added to the cuvette, as this governs the amount of substrate for the other enzymes.

The enzymatic analyses were monitored with a Unicam SP800 UV spectrophotometer (Unicam Instruments Ltd., England), fitted with a Pye AR55 linear recorder.

Protein concentrations were determined using a Coleman III spectrophotometer (Perkin Elmer Corp., Illinois).

Determination of creatine concentration

A second piece of tissue from the same muscle was homogenized in 2.0 ml of 6 per cent perchloric acid (PCA) for determination of tissue total creatine content, as described by Ennor and Stocken (1948). In this method, all creatine phosphate is hydrolyzed following protein precipitation, providing an accurate determination of total tissue creatine. Creatine is incubated with butane 2,3-dione in the presence of α -naphthol (which intensifies the colour) at basic pH and room temperature for 30 minutes. The absorbance is then monitored at 510 nm, 25°C on the Unicam SP800 UV spectrophotometer described above.

Determination of actin concentration

The amount of actin in acetone powders prepared from muscle samples was determined by the method of Anderson (1976). This method uses double radioisotope labelling and the isolation of actin

specific peptides to estimate the *per cent* (w/w) of actin in complex protein mixtures. The protein contents of the muscle samples were determined as previously described except 20 μ l aliquots of muscle acetone powders which had been dissolved in 70 *per cent* formic acid were used.

Due to the extreme cost of the actin determination, and the availability of equipment, the number of samples which could be analyzed was severely limited. Of the series 1 tissues available, 5 from each treatment group were analyzed for this compound (20 in total) because of the implications on the choice of tissues for the contractility experiments of series 2 and 3. In series 2, only 2 group C and 5 group DE tissues could be analyzed, the 2 controls to give an indication that the mean control concentration had not changed significantly. In series 3, 2 group C, 2 group DE, and 5 group DWE tissues were analyzed for actin.

Determination of tissue water content

Water content of the tissues, expressed as a *per cent* of the tissue wet weight was determined by drying a weighed sample of tissue in an oven (Despatch) at 200°C for 48 hours and reweighing. A third weighing was done following a further 24 hours of oven drying. If the dry tissue weight did not change over the final 24 hour period, the tissues were assumed to be dehydrated.

Determination of plasma corticosterone concentration

Plasma corticosterone levels were measured by a micro-modification (Cowan et al., 1974) of the method of Silber, Busch

and Oslapas (1958). Blood samples were taken by ventricular puncture at weekly intervals with a 26 gauge 5/8 inch needle from the animals anaesthetized with Metofane (methoxyflurane, Pitman Moore Ltd., Scarborough). The blood was immediately transferred to a heparinized tube and centrifuged for 3 minutes. The plasma was then separated, frozen, and stored at -70°C for subsequent analysis.

The method of Silber, Busch and Oslapas is a fluorometric analysis based on the measurement of the fluorescence of corticosterone in sulphuric acid at 530 nm, room temperature.

Results

Effect of Dianabol and exercise on skeletal muscle total protein concentration

Following 4 weeks of treatment with Dianabol and exercise, the total protein concentration of the lateral gastrocnemius was significantly elevated in only the DE group relative to group C (Table 8). Both group D and CE appeared to have elevated mean protein concentrations relative to group C, but these differences were not significant.

The same observations were made when treatment was extended to 8 weeks duration (Tables 10 and 12). Withdrawal of Dianabol injections (groups DW and DWE, Table 12) produced no effect on the tissue total protein content when compared to group D and DE, respectively.

Total protein concentrations of the soleus, plantaris and medial gastrocnemius samples from the series 1 animals discussed above, were the same for any muscle, irrespective of the treatment group (Table 9). This result was sustained in the data obtained from analysis of series 2 and 3 tissues, illustrated in Tables 11 and 13, respectively.

Perhaps of greater importance than the increased protein concentration in the lateral gastrocnemius of the DE group as noted, is the fact that the protein content of these tissues also markedly increased. The mean protein content of the group C and DE tissues for each series are illustrated in Table 14. The fact

Table 8. The effect of 4 weeks treatment with Dianabol and exercise on lateral gastrocnemius total protein, PFK, SDH, actin and creatine concentrations (series 1).

	<u>LATERAL GASTROCNEMIUS</u>				
	<u>TOTAL PROTEIN</u>	<u>PFK</u>	<u>SDH</u>	<u>ACTIN</u>	<u>CREATINE</u>
CONTROL (10)	160.0 ± 9.6	0.20 ±0.01	13.5 ±0.9	7.98 ±0.6	27.1 ±0.9
DIANABOL (10)	183.5 ±12.2	0.19 ±0.02	16.2 ±1.7	12.2* ±0.2	31.5 ±2.1
CONTROL- EXERCISE (10)	177.8 ± 8.3	0.19 ±0.02	17.4 ±1.4	9.60 ±0.5	32.3 ±0.4
DIANABOL- EXERCISE (10)	212.2* ±14.5	0.28* _§ ± ±0.03	20.3* ±2.2	12.2* ±0.7	37.1* ±2.6

Total Protein mg·g tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Actin per cent of Total Protein

Creatine μ moles·g tissue wet wt⁻¹

- 1) Values represent mean ± S.E.M.
- 2) Symbols indicate significant difference from: *Control
+Dianabol; §Control-exercise treatment, test by Tukey's ($p < 0.05$).
- 3) Number of animals per group indicated in parentheses except
actin analysis where N = 5 in all groups.

Table 9. The effect of 4 weeks treatment with Dianabol and exercise on total protein, PFK, SDH and creatine concentrations of the soleus, plantaris and medial gastrocnemius muscles (series 1).

	SOLEUS				PLANTARIS				MEDIAL GASTROCNEMIUS			
	TP	PFK	SDH	CREATINE	TP	PFK	SDH	CREATINE	TP	PFK	SDH	CREATINE
CONTROL (10)	145.5 ± 8.2	0.08 ± 0.00	18.8 ± 1.4	19.6 ± 0.6	175.8 ± 6.4	0.13 ± 0.01	15.7 ± 1.3	26.2 ± 0.8	180.7 ± 6.6	0.14 ± 0.01	14.20 ± 1.7	30.1 ± 1.5
DIANABOL (10)	146.3 ± 7.7	0.08 ± 0.07	21.1 ± 1.5	17.6 ± 0.5	175.2 ± 7.1	0.17 ± 0.01	12.2 ± 1.4	30.5 ± 3.0	172.2 ± 7.7	0.14 ± 0.02	16.7 ± 1.6	34.8 ± 1.3
CONTROL- EXERCISE (10)	154.4 ± 5.7	0.08 ± 0.01	16.3 ± 1.3	17.5 ± 1.0	124.5 ± 7.2	0.22* ± 0.03	22.0+ ± 2.2	26.5 ± 0.4	177.7 ± 7.9	0.20 ± 0.02	21.8 ± 1.8	31.7 ± 0.6
DIANABOL- EXERCISE (10)	150.5 ± 6.6	0.07 ± 0.00	22.7 ± 1.5	18.3 ± 0.3	176.1 ± 5.2	0.20* ± 0.02	22.2*+ ± 1.9	29.0 ± 0.7	179.7 ± 4.7	0.20 ± 0.02	15.6 ± 1.1	29.9 ± 1.4

Total Protein mg·g tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Creatine μmoles·g tissue wet wt⁻¹

1) Values represent mean ± S.E.M.

2) Symbols indicate significant difference from: *Control, +Dianabol treatment, by Tukey's test (p < 0.05).

3) Number of animals per group indicated in parentheses.

Table 10. The effect of 8 weeks treatment (series 2) with Dianabol and exercise on the lateral gastrocnemius concentration of total protein, PFK, SDH, creatine and actin.

	<u>LATERAL GASTROCNEMIUS</u>				
	<u>TOTAL PROTEIN</u>	<u>PFK</u>	<u>SDH</u>	<u>CREATINE</u>	<u>ACTIN</u>
CONTROL (8)	132.7 ± 5.0	0.18 ±0.03	15.6 ±0.7	23.3 ±1.9	7.5
DIANABOL (7)	147.2 ± 3.6	0.24 ±0.03	21.0 ±1.0	24.8 ±1.9	
CONTROL- EXERCISE (7)	141.8 ± 4.0	0.16 ±0.02	17.8 ±0.5	25.3 ±1.3	
DIANABOL- EXERCISE (6)	156.7* ± 1.6	0.34*§ ±0.05	27.2*§ ±1.0	30.0*§ ±1.7	11.9* ±0.7

Total Protein mg·g Tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Creatine μmoles·g tissue wet wt⁻¹

Actin per cent of Total Protein

- 1) Values represent mean ± S.E.M.
- 2) Symbols indicate significant difference from: *Control treatment, §Control-Exercise treatment, by Tukey's test ($p < 0.05$).
- 3) Number of animals per group indicated in parentheses, except actin where N for Control = 2 and Dianabol-Exercise = 5

Table 11. The effect of 8 weeks treatment (series 2) with Dianabol and exercise on the soleus concentration of total protein, PFK, SDH and creatine.

	<u>SOLEUS</u>			
	<u>TOTAL PROTEIN</u>	<u>PFK</u>	<u>SDH</u>	<u>CREATINE</u>
CONTROL (8)	130.2 ± 3.0	0.16 ±0.03	14.9 ±0.6	21.6 ±1.5
DIANABOL (7)	123.1 ± 2.8	0.19 ±0.03	12.3 ±0.5	24.0 ±1.0
CONTROL- EXERCISE (7)	136.4 ± 4.4	0.16 ±0.03	16.3 ±0.8	22.5 ±1.9
DIANABOL- EXERCISE (6)	136.0 ± 3.7	0.20 ±0.02	12.5 ±1.0	23.9 ±1.7

Total Protein mg·g tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Creatine μmoles·g tissue wet wt⁻¹

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 12. The effect of 8 weeks treatment (series 3) with Dianabol and exercise on lateral gastrocnemius total protein, PFK, SDH, creatine and actin concentrations.

LATERAL GASTROCNEMIUS

	<u>TOTAL PROTEIN</u>	<u>PFK</u>	<u>SDH</u>	<u>CREATINE</u>	<u>ACTIN</u>
CONTROL (7)	133.8 ± 5.5	0.18 ±0.01	12.22 ±0.94	20.17 ±0.97	10.8
DIANABOL (5)	144.9 ±10.2	0.25 ±0.03	21.54 ±0.88	25.61 ±1.73	
DIANABOL- WITHDRAWAL (5)	146.4 ± 5.5	0.20 ±0.05	13.78 ±0.75	19.96 ±1.47	
CONTROL- EXERCISE (7)	145.5 ± 2.2	0.19 ±0.02	14.08 ±0.50	23.12 ±1.49	
DIANABOL- EXERCISE (5)	161.2*§ ± 2.9	0.34*§ ±0.01	29.06*§ ±1.43	30.45*§ ±1.88	13.5*
DIANABOL- WITHDRAWAL- EXERCISE (5)	162.1*§ ± 4.6	0.36*§ ±0.06	27.96*§ ±1.31	22.67 ±0.87	13.9* ±0.2

Total Protein mg·g tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Creatine μmoles·g tissue wet wt⁻¹

Actin per cent of Total Protein

- 1) Values represent mean ± S.E.M.
- 2) Symbols indicate significant difference from: *Control, §Control-Exercise treatment, by Tukey's test ($p < 0.05$).
- 3) Number of animals per group indicated in parentheses, except actin where N for Control = 2, Dianabol-Exercise = 3, Dianabol-Exercise = 5

Table 13. The effect of 8 weeks treatment (series 3) with Dianabol and exercise on soleus total protein, PFK, SDH and creatine concentrations

	<u>SOLEUS</u>			
	<u>TOTAL PROTEIN</u>	<u>PFK</u>	<u>SDH</u>	<u>CREATINE</u>
CONTROL (7)	137.3 ± 6.4	0.12 ±0.01	14.3 ±1.0	15.79 ±0.32
DIANABOL (5)	125.4 ± 5.9	0.14 ±0.01	16.2 ±0.8	13.87 ±0.51
DIANABOL- WITHDRAWAL (5)	135.4 ± 6.1	0.13 ±0.01	16.7 ±0.5	14.80 ±0.99
CONTROL- EXERCISE (2)	128.4 ± 7.4	0.15 ±0.02	19.0 ±0.8	13.10 ±0.92
DIANABOL- EXERCISE (5)	138.0 ± 7.6	0.13 ±0.02	17.4 ±0.6	14.30 ±0.76
DIANABOL- WITHDRAWAL- EXERCISE (5)	134.9 ± 3.8	0.13 ±0.01	14.9 ±1.0	14.48 ±1.01

Total Protein mg·g tissue wet wt⁻¹

PFK I.U.·mg Protein⁻¹

SDH mI.U.·mg Protein⁻¹

Creatine μmoles·g tissue wet wt⁻¹

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses

Table 14. Effect of Dianabol-Exercise treatment on lateral gastrocnemius protein content.

	<u>SERIES 1</u>	<u>SERIES 2</u>	<u>SERIES 3</u>
CONTROL	160.3 ± 6.3 (10)	174.4 ± 2.4 (8)	179.9 ± 4.9 (7)
DIANABOL- EXERCISE	199.8* ± 8.1 (10)	209.9* ± 8.5 (6)	234.3* ± 13.2 (5)

Protein Content mg

- 1) Values represent mean ± S.E.M.
- 2) Symbol indicates significant difference from control, by Tukey's test ($p < 0.05$).
- 3) Number of animals per group indicated in parentheses

that the protein content of the DE lateral gastrocnemius tissues was significantly increased above control levels suggests that protein synthesis in this muscle was stimulated by Dianabol-exercise treatment.

Effect of Dianabol and exercise on skeletal muscle PFK and SDH concentration

Tissue PFK and SDH concentrations of series 1 muscles are also illustrated in Tables 8 and 9. While a significant elevation of SDH concentration was observed in the group DE lateral gastrocnemius relative to only group C, PFK was significantly elevated in relation to all other treatment groups. In light of the fact that the enzyme concentrations are expressed per mg protein, and the protein and enzyme concentrations are elevated in the same tissue, the magnitude of this increase is even larger than would first be apparent.

Similar observations were derived from the analysis of series 2 and 3 tissues (Tables 10 and 12). Unlike the 4 week treated tissues, both the SDH and PFK concentrations of group DE were significantly greater than groups C and CE when treatment was continued for 8 weeks. However, the concentrations of these enzymes were not further enhanced by the prolongation of treatment.

Withdrawal of steroid injections (Table 12) had no effect on the increased lateral gastrocnemius concentration of these 2 enzymes induced by DE treatment.

Whereas the PFK and SDH concentrations of the soleus and medial gastrocnemius were not altered by any treatment employed (Tables 9, 11 and 13), the concentration of PFK and SDH in the plantaris muscle of series 1 animals (Table 9) was significantly enhanced in both the CE and DE treatment groups relative to group C. The SDH concentration of these 2 groups was also significantly elevated relative to group D. These results suggest that although an exercise induced alteration of enzyme concentrations was observed in the plantaris, concomitant steroid administration did not result in further enhancement. Only the DE combination treatment resulted in elevated enzyme levels in the lateral gastrocnemius.

Effect of Dianabol and exercise on skeletal muscle creatine concentration

Analysis of skeletal muscle tissue creatine concentration produced results which would provide for a similar interpretation to that given to the tissue biochemical analysis discussed to this point. Creatine concentration was significantly elevated in the series 1 lateral gastrocnemius group DE tissues relative to group C, as illustrated in Table 8. Again, the mean creatine concentration in both group D and CE tissues was greater than group C, but the difference was not significant. The creatine concentration in the soleus, plantaris and medial gastrocnemius tissues was not altered by any of the various treatments.

Increasing the duration of treatment to 8 weeks produced similar results (Tables 10 and 12) i.e., the creatine concentration of the lateral gastrocnemius DE group was significantly elevated above control levels, and the soleus concentration of this compound was unaffected by any treatment. While withdrawal of Dianabol injections did not affect the enhancement of protein or enzyme concentrations induced by the DE treatment, the group DWE lateral gastrocnemius creatine concentration was the same as all other treatment groups.

This suggests that the effect of the DE treatment on creatine concentration can only be maintained if both components of the treatment are present.

Although continuation of treatment for 8 weeks sustained the elevation of creatine levels observed at 4 weeks, no further enhancement was seen.

Effect of Dianabol and exercise on skeletal muscle actin concentration

The lateral gastrocnemius actin concentration following 4 weeks of treatment is illustrated in Table 8. In light of the results discussed above, and the extreme cost of the actin analysis, only lateral gastrocnemius tissue samples were analyzed for this contractile protein. As observed with all other tissue proteins analyzed, the group DE tissues had a significantly greater concentration of actin, than did group C. The actin concentration was also significantly greater in group D relative to group C.

The elevation of tissue actin concentration was maintained in group DE when treatment was prolonged to 8 weeks (Tables 10 and 12), but was not enhanced further. Tissues of the 8 week group D animals were not analyzed for this compound because of the extreme cost.

Once again (Table 12) withdrawal of the steroid following 4 weeks of treatment did not significantly alter the actin concentration of group DWE relative to group DE (Table 12).

These results suggest the actin content of the lateral gastrocnemius is significantly enhanced by D treatment, but the possibility that this may be due to steroid administration can not be dismissed.

Effect of Dianabol and exercise on skeletal muscle water content

The water content of the 4 muscles studied in series 1, expressed as a *per cent* of the tissue wet weight, are illustrated in Table 15. There were no significant differences between group means of any one muscle type. However, it is interesting that in both series 2 (Table 16) and series 3 (Table 17) the water content of the muscles used for the contractility studies (right hindlimb) was greater than the same muscle from the contralateral limb which did not undergo contraction (Table 25).

This observation can account for the difference in absolute weights between the muscles used for contractility studies and those of the contralateral limb, the muscles of the right hindlimb being about 20 *per cent* heavier, as described in chapter 1 (Tables 6 and 7). This concept is discussed in further detail in Appendix C.

Table 15. Effect of 4 weeks treatment with Dianabol and exercise on skeletal muscle water content (series 1).

(per cent of tissue wet weight)

	<u>SOLEUS</u>	<u>PLANTARIS</u>	<u>MEDIAL GASTROCNEMIUS</u>	<u>LATERAL GASTROCNEMIUS</u>
CONTROL (10)	70.3 ±0.3	72.1 ±0.8	73.0 ±0.6	72.9 ±0.3
DIANABOL (10)	71.7 ±0.5	73.0 ±0.3	74.2 ±0.9	73.2 ±0.5
CONTROL- EXERCISE (10)	70.0 ±0.5	72.5 ±0.7	72.7 ±0.4	73.4 ±0.5
DIANABOL- EXERCISE (10)	69.2 ±0.6	72.0 ±0.5	72.1 ±0.4	72.7 ±0.8

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 16. Effect of 8 weeks treatment with Dianabol and exercise (series 2) on skeletal muscle water content.

(per cent of tissue wet weight)

	<u>SOLEUS</u>	<u>PLANTARIS</u>	<u>MEDIAL GASTROCNEMIUS</u>	<u>LATERAL GASTROCNEMIUS</u>
CONTROL (8)	78.4 ±1.3	78.7 ±0.8	78.3 ±0.5	78.7 ±0.6
DIANABOL (7)	77.8 ±0.8	79.4 ±1.0	78.0 ±0.3	77.5 ±0.8
CONTROL- EXERCISE (7)	78.8 ±0.4	78.6 ±0.6	77.8 ±0.6	78.4 ±0.9
DIANABOL- EXERCISE (6)	78.1 ±0.8	78.8 ±0.4	78.4 ±0.8	78.3 ±0.3

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 17. Effect of 8 weeks treatment with Dianabol and exercise (series 3) on skeletal muscle water content.

(per cent of tissue wet weight)

	<u>SOLEUS</u>	<u>PLANTARIS</u>	<u>MEDIAL GASTROCNEMIUS</u>	<u>LATERAL GASTROCNEMIUS</u>
CONTROL (7)	78.5 ±0.5	78.6 ±0.4	78.6 ±0.9	78.9 ±0.4
DIANABOL (5)	78.0 ±0.8	78.2 ±0.8	79.4 ±0.6	79.2 ±0.6
DIANABOL- WITHDRAWAL (5)	78.6 ±0.3	78.7 ±0.3	79.4 ±0.5	79.6 ±0.6
CONTROL- EXERCISE (7)	78.3 ±0.3	78.3 ±0.8	78.5 ±0.7	79.0 ±0.5
DIANABOL- EXERCISE (5)	78.6 ±0.5	78.0 ±0.5	78.2 ±0.3	78.8 ±0.9
DIANABOL- WITHDRAWAL- EXERCISE	78.8 ±0.6	78.6 ±0.4	78.9 ±0.8	79.5 ±0.6

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses

Again, in series 2 and 3, the water content of any one muscle was not affected by any of the various treatments.

Effect of Dianabol and exercise on plasma corticosterone concentration

The results of the plasma corticosterone analysis of weekly blood samples from the series 2 animals are plotted in Figure 4. The group C plasma corticosterone levels were consistently significantly greater than each of the treated groups throughout the 8 week period. Whereas there was no consistent trend in differences between means of the 3 treatment groups, the group DE plasma corticosterone concentration was elevated significantly above that of group D and CE at week 3. Furthermore, the plasma concentration of corticosterone was significantly higher in group D with respect to groups CE and DE in weeks 6, 7 and 8. From these data there does not appear to be any effect of any of the treatments on plasma corticosterone levels.

Figure 4. Effect of 8 weeks Dianabol and exercise treatment (series 2) on plasma corticosterone levels. Blood samples were obtained weekly by ventricular puncture from rats under Metofane anaesthesia. Experimental protocol was as described in Figure 1, series 2.

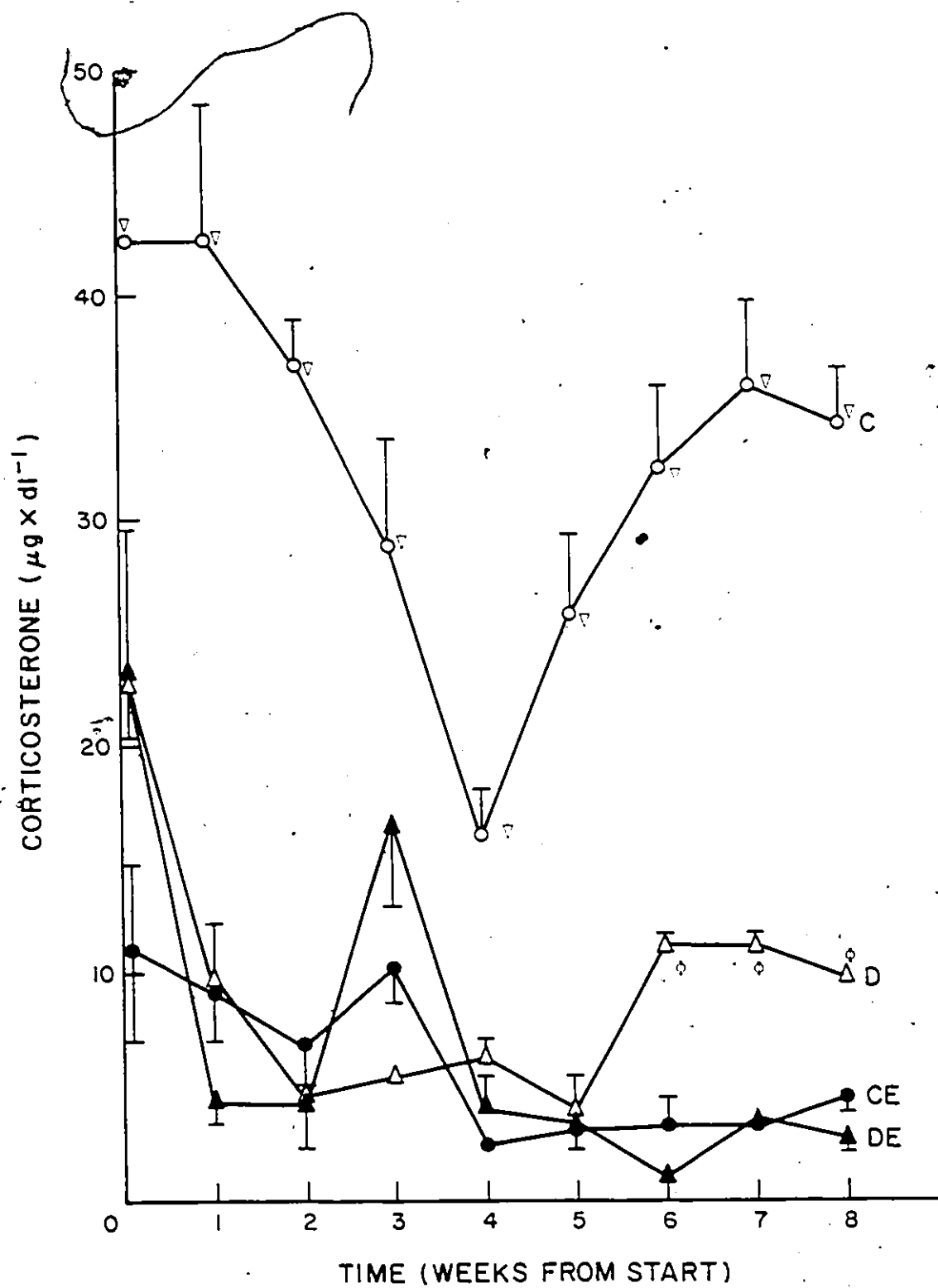
Values represent group mean $\pm$ S.E.M.

Groups

- - Control (6)
- △ - Dianabol (7)
- - Control-exercise (7)
- ▲ - Dianabol-exercise (6)

Symbols denote significant difference from: ▽ D, CE and DE, ◇ groups CE and DE treatment by Tukey's test ($p < 0.05$).

Number of animals per group indicated in parentheses



Discussion

Effect of Dianabol and exercise on skeletal muscle total and enzymatic proteins

In the present study the total protein concentration and content were significantly elevated in the lateral gastrocnemius of animals receiving the combination of Dianabol and treadmill running exercise, relative to the control tissues. There was a trend toward elevated protein concentrations in the animals receiving Dianabol or exercise alone, but the difference between means of these groups and the controls was not significant. In fact, the DE total protein concentration appears to be a summation of the individual increases observed with the Dianabol and exercise treatments individually. This suggests prolongation of the administration period may result in a significant increase in protein concentration in these treatment groups.

Although this phenomenon is interesting, perhaps of even greater importance is the fact that both the PFK and SDH concentrations of the lateral gastrocnemius group DE tissues were also significantly elevated above those observed in control tissues.

Selection of a method of assaying phosphofructokinase and succinate dehydrogenase concentrations as markers of anaerobic and aerobic metabolism, respectively, was based on practicality and the importance of these enzymes. Practically, an assay was developed to determine the concentration of both of these enzymes as well as total protein from the same tissue homogenate. This

was thought to produce a more accurate determination of these parameters by eliminating variability due to the choice of tissue sample. PFK has been shown to be the rate limiting enzyme of glycolysis (Ui, 1966) and can thus govern the rate of skeletal muscle production of metabolic energy from carbohydrates. Succinate dehydrogenase was chosen as a marker of aerobic metabolism, as it is an enzyme of the tricarboxylic acid cycle. Ironically, SDH is the only enzyme in the citric acid cycle which transfers the hydrogen from a substrate to a flavoprotein without the participation of nicotinic adenine dinucleotide (NAD) (Harper and Mayes, 1973). The assay uses oxidized cytochrome C which is then reduced by the action of SDH. Thus by monitoring the concentrations of PFK and SDH, one can gain some insight into the relative metabolic capacity of a particular tissue.

Effect of androgens on skeletal muscle enzymes

Reports in the literature on the effects of androgens on skeletal muscle enzymes are contradictory. Several authors have observed skeletal muscle oxidative and glycolytic enzymes to be responsive to androgen treatment. Loring, Spencer and Villee (1961) at first reported that whereas the SDH activity in the rat levator ani muscle was not altered by castration or subsequent injections of 5 mg testosterone for 3 weeks, both malate and citrate dehydrogenase concentrations decreased in the castrates and were restricted to normal in the animals receiving testosterone supplements. Repetition of the experiment with more controlled

conditions resulted in SDH activity responding in the same manner as the other enzymes. Apostolakis, Matzelt and Voigt (1963) did their experiment correctly the first time and observed that testosterone propionate was able to reverse the effects of castration on the levator ani malate dehydrogenase, glyceraldehyde phosphate dehydrogenase, and diphosphofructose aldolase activities, but they reported the activity of these enzymes to be increased by castration. These authors also reported a decrease in activity of α -glyceraldehyde dehydrogenase and a disappearance of tryptophan pyrolase activity. The testosterone propionate supplements in castrates resulted in a normal α -glyceraldehyde dehydrogenase activity, and a 4-fold enhancement of tryptophan pyrolase activity. The activities of glyceraldehyde-3-phosphate dehydrogenase, creatine phosphokinase and lactate dehydrogenase were not altered by either treatment. These results are contrary to those of Eckstein et al. (1960) who reported that the SDH activity in the rat levator ani was the same in the tissues from controls, 30 day castrates, and those receiving testosterone propionate following castration.

It was suggested earlier in the discussion of this chapter that the increased quantity of enzymes measured in the DE lateral gastrocnemius tissues may indicate an increased metabolic capacity, but not necessarily a chronically increased activity. The reports discussed above illustrate that androgenic steroids increased the activity of a number of enzymes. However, several authors have

reported that the overall metabolic activity of the levator ani (Eisenberg, Gordan and Elliott, 1949; Leonard, 1950) and the grouped dorsal thigh muscles (Leonard, 1950) was not affected by castration or androgen supplement following castration. If the enzyme activity is increased, i.e., in a tissue homogenate, more substrate is converted per minute, but the overall metabolic activity is not increased in the intact muscle, then one may conclude that the quantity of enzyme has increased, but that the increased potential metabolic capacity can only be realized if the quantity of substrate available is also enhanced.

Three lines of evidence support the hypothesis that androgens enhance *de novo* synthesis of protein. Many investigators have reported an increased uptake of radioactive amino acids in the presence of testosterone or its propionate derivative (Breuen and Florini, 1965) and in castrates supplemented with testosterone (Breuen and Florini, 1965; Kochakian, 1969; Kochakian, 1966; Kochakian, Hill and Harrison, 1964) into the levator ani, as well as the diaphragm (Costa, Kochakian and Hill, 1962). Further support was provided by Bresloff et al. (1974), who observed that animals whose muscular protein content had been reduced by glucocorticoid administration had normal concentrations following testosterone administration.

Similar observations have been reported with Dianabol. Yudaev and Pokrovskii (1966) measured an increased uptake of (2-¹⁴C) glycine into both the tibial muscles of adult rats and tibial and

cardiac muscle of immature rats following 7 days administration of 1 mg/100 g body weight⁻¹ of the synthetic steroid. Bullock et al. (1968) observed similar changes in the psoas muscle of rats following Dianabol administration. The ability of Dianabol to reverse a glucocorticoid induced 50 per cent decrease in (U-¹⁴C)-L-leucine uptake in the rat psoas muscle was reported by White (1967).

The question as to whether the extra proteins synthesized in response to testosterone or its derivatives were the same, was a matter of concern. Florini (1970) incubated the soleus muscle from normal and castrated rats receiving testosterone propionate supplements with (³H)-leucine or (U-¹⁴C)-L-leucine and isolated the protein fraction by disc electrophoresis. The fractions exhibited identical patterns of ³H- and ¹⁴C-radioactivity, suggesting the proteins synthesized under the influence of testosterone and its propionate ester were indeed the same.

It is interesting to note that the uptake of radioactive amino acids induced by testosterone or its derivatives can be blocked by actinomycin D (Breuen and Florini, 1965; Kochakian et al., 1971; Frieden et al., 1964) a compound which has been shown to block *de novo* protein synthesis (Reich et al., 1961).

Watson (1963) reported that protein synthesis requires at least 3 forms of RNA. Several investigators have observed increases in skeletal muscle tissue RNA concentration following androgen administration (Galavazi and Szenai, 1971a, b; Saunders, Steciw and

Kline, 1962; Sheppard et al., 1964, 1965; Arvill, 1967; Heim et al., 1969; Kochakian, Hill and Harrison, 1964; Marcais and Kochakian, 1971):

Thus, androgens can influence not only the availability of precursor substrates for protein synthesis in muscle tissue, but also the quantity of RNA available to direct the process.

Effects of exercise on skeletal muscle enzymes

A survey of the literature on the responsiveness of skeletal muscle enzymes to exercise presents one with a vast quantity of data. It is relatively easy to determine the specificity of an exercise for a particular muscle group in the human, because of ease of communication and the reintroduction of the Duchenne muscle biopsy technique by Bergstrom (1962) which enables rapid determination of the concentrations of various compounds in muscle samples. It has thus become apparent that an exercise may be specific not only to a muscle group, but also to the type of metabolic processes of that group. These changes have been well documented in several review articles (Holloszy, 1975; Poortmans, 1978).

In light of this specificity skeletal muscle fibres are generally accepted to be of 3 types based on the predominant metabolic pathway and contractile speed. In 1958, Eccles, Eccles, and Lundberg proposed that muscle fibres were innervated by either "phasic" high frequency or "tonic" low frequency motor neurons. This was confirmed nearly 20 years later by Grimby and Hannerz (1976).

At about the same time, it was reported that the predominant contractile characteristics of a particular muscle fibre were determined by the nerve impulse pattern i.e. frequency and duration (Salmons and Sreter, 1976; Lomo et al., 1974; Salmons and Vrbova, 1969). Through the studies of Kugelberg and Edstrom (1963), Burke (1968), Burke et al. (1971) and Barnard et al. (1971) on single motor units, it was confirmed that the fast or slow contractile twitch characteristics of a muscle fibre did indeed correlate with its myosin ATPase profile. As a result much research effort has been focused on the identification of the predominant biochemical characteristics of different skeletal muscles through staining techniques with particular enzyme specificity.

In the classification system proposed by Peter et al. (1972) muscle fibres may be classified as slow oxidative (SO), which have a predominance of oxidative enzymes and are usually recruited for slow tonic movement (Burke, 1975; Hannerz, 1973) or fast. The fast fibres are subdivided into 2 groups, those with a high capacity for both glycolytic and oxidative metabolism (termed fast oxidative glycolytic, Fog) and those with high glycolytic but low oxidative capacity (termed fast glycolytic, FG). A muscle is classified or "typed" according to the predominant fibre quantity and contractile speed, such as 85 per cent slow oxidative.

Using the above classification system, the muscles studied in the present investigation are typed as follows: soleus, 84 per cent

SO, and 16 per cent FOG, plantaris, 6 per cent SO, 53 per cent FOG, and 41 per cent FG, and the medial and lateral gastrocnemius 5 per cent SO, 37 per cent FOG, and 58 per cent FG (Ariano, Armstrong and Edgerton, 1973).

The importance of classifying tissues as proposed by Peter et al. (1972) becomes relevant when discussing exercise related changes in muscle enzymes. If the predominant fibre characteristic of a particular muscle or group of muscles is known, as well as the type of movement the muscle contributes to, then by altering the frequency of this movement (exercise), the effects of the exercise on the muscle metabolism can be studied.

Holloszy (1975) reported that endurance exercise induces approximately 2-fold increases in the levels of tricarboxylic acid cycle enzymes in the leg muscles of humans. This has been supported by several other investigators working with rodents (Baldwin et al., 1972; Gollnick and Ianuzzo, 1972), as well as humans (Gollnick et al., 1973; Morgan et al., 1971; Varnauskas et al., 1970). Modification of the endurance training programme described by Holloszy (1975) to include short bouts of sprinting, produced increased hexokinase, phosphorylase and PFK activities, as well as all enzymes of the citric acid cycle in the gastrocnemius of rats (Huston et al., 1976; Dohm et al., 1973). Staudte, Exner and Pette (1973) subjected rats to a totally short term, high intensity sprint exercise programme. They reported increases in the activity of hexokinase and citrate synthetase in both the slow soleus and fast rectus femoris muscles.

The soleus also had significantly elevated glycogen phosphorylase, triosephosphate dehydrogenase and creatine kinase activities.

Indeed, it has been shown that the biochemical characteristics of specific muscles can be altered to such a degree by various types of exercise that the predominant fibre type is altered (Edgerton, 1969; Barnard, Edgerton and Peter, 1970a, b; Pattengale and Holloszy, 1967).

The exercise programme utilized in the present study has been shown to be somewhat specific to fast oxidative glycolytic fibres (Reed, 1972) and thus might affect the enzymes of the plantaris, medial and/or lateral gastrocnemius, whichever of these muscles are utilized by the rat in treadmill running. The relative degree of utilization of each of these muscles could only be determined by electromyogram studies during exercise, which is beyond the scope of this investigation.

Exercise treatment alone did not result in concentration changes of any of the enzymes quantified in the present investigation excepting the 4 week treated plantaris. This may be a result of the exercise period being too short, as 8 weeks training has been suggested to be a minimal time period to induce observable enzyme changes in rat skeletal muscle (Hickson, Heusner and Van Huss, 1976).

In light of the above information, the significance of the results of this study is further enhanced because marked elevations of the lateral gastrocnemius protein content as well as protein and enzyme concentrations were recorded after only 4 weeks treatment in the DE group, and were maintained when the treatment was extended

to 8 weeks. That the lateral gastrocnemius was responsive to the treatment was somewhat surprising, as various enzymes of this muscle have been reported non-responsive to androgen treatment by other authors (Kochakian, Hill and Harrison, 1964; Bresloff et al., 1974; Kochakian and Cockrell, 1958; Scow and Roe, 1953), and this muscle is rarely studied in relation to exercise.

In the present study the lateral gastrocnemius SDH concentration from control animals was 13.5, 15.6 and 12.2 mIU-mg protein⁻¹ for series 1, 2 and 3. Unfortunately, many authors illustrate enzyme concentrations as *per cent* of controls without reporting any actual values, making comparisons to the results of the present study difficult. Ianuzzo and Chen (1979), also using the methodology of Cooperstein, Lazarow and Kurfess (1950), obtained values of 3.8-4.0 I.U.-g tissue⁻¹ in rat plantaris muscle, and PFK concentrations between 48 and 51 I.U.-g tissue⁻¹. Although these values are from the plantaris muscle, the fibre composition of this muscle and the gastrocnemius are similar, as discussed earlier in this chapter. Thus the values may be used for comparative purposes. Dohm et al. (1973) reported slightly larger concentrations of these enzymes in the gastrocnemius muscle. The difference may be due to methodology, as they used a semi-purified mitochondrial preparation for quantification of SDH, as opposed to the tissue homogenate of this study. As well, they assayed the enzyme at 37°C which Cooperstein, Lazarow and Kurfess (1950) demonstrated gives

falsely elevated concentration. The values reported by Huston et al. (1976) for PFK in the whole gastrocnemius were 54.9 and 87.0 I.U.·g tissue⁻¹ in untrained and trained animals, respectively, which when converted to units used in the present study are very similar. Patterson, Layberry and Nadkarni (1972) observed cardiac muscle PFK concentrations that when converted are also very similar to those of the lateral gastrocnemius in this study.

These comparisons suggest that the assay conditions employed in the current investigation for the particular proteins studied do produce results from control tissues which are comparable to published values. Following a review of current literature, the observations of the present study appear to be the first report of specific enzymatic changes produced by chronic administration of Dianabol and exercise.

Effect of Dianabol and exercise on skeletal muscle creatine and actin concentrations

The concentration of both creatine and actin was significantly elevated in the lateral gastrocnemius following Dianabol-exercise treatment, relative to control tissues. The elevations were of the same *per centage* (150-200) as the increases observed in protein and enzyme concentrations discussed above.

The purpose of quantifying the concentration of these 2 compounds was to correlate any increases in contractility observed in chapter 4 with alterations in tissue contractile proteins contents. Actin was measured directly and myosin may be inferred from the increased protein content and actin concentration of the tissue.

Whereas the creatine concentration was significantly elevated in only the DE group lateral gastrocnemius, actin concentration was also significantly elevated in the same tissue of group D, suggesting the effect on this contractile protein may be due to Dianabol administration alone. However, the lateral gastrocnemius twitch and tetanic tensions were not changed in this latter group (chapter 4), and the content of protein as well as the concentration of total protein, SDH and PFK were also not altered by this treatment. Thus, even if the elevated level of this contractile protein was maintained in the 8 week studies, there would be questionable physiological significance.

Creatine is primarily synthesized in the liver and kidney in the rat (Baker and Miller, 1940). The synthesis is a process involving 2 enzymes, amidinotransferase and methyltransferase, and uses arginine, glycine and S-adenosylmethionine as precursors (du Vigneaud et al., 1940; Borsook and Dubnoff, 1941; Walker, 1957). Krisko and Walker (1966) demonstrated that creatine synthesis can be influenced by androgens. In their study, kidney homogenates from castrated male rats had a lesser amidinotransferase activity than homogenates of control tissues. As well, whereas normal male rat kidney homogenates had amidinotransferase activities markedly greater than the female counterpart, the activity of this enzyme was significantly enhanced in kidney tissues from females treated with testosterone propionate.

The influence of creatine on muscle contractile proteins appears to be to enhance *de novo* synthesis. Ingwall, Morales

and Stockdale (1972) reported that skeletal muscle cells synthesized myosin at a faster rate when incubated in the presence of creatine, although the fact that this has subsequently been disputed by Fry and Morales (1980) detracts from the significance of this observation. The response was dose dependent in the range of 10-100 mM creatine. That the myosin formed was synthesized *de novo* was further substantiated by their observation that (³H)-leucine was incorporated at a greater rate into the cells incubated in the presence of creatine.

Thus myosin synthesis may be enhanced *in vivo* if the muscle tissue creatine levels can be increased. The methodology used in the present study was conducive to this action. Sutton (1978) and Sutton et al. (1969), have demonstrated that circulating levels of growth hormone are significantly increased by exercise. Growth hormone accelerates the uptake of creatine by skeletal muscle (Tan and Ungar, 1979), a process which Fitch and Shields (1966) demonstrated to be active.

It is of great interest to note that the above discussion may be the first identifiable process which can be used to explain why any change noted occurred in DE group tissues in the present study. Only the combination of androgens which stimulate creatine synthesis, and exercise which promote growth hormone secretion, enhance creatine concentrations in muscle tissue which may result in myosin synthesis.

Although the suggestion that myosin as well as actin is enhanced in the DE group lateral gastrocnemius may be considered somewhat speculative, this idea is supported by the report of Rogozkin (1979a, b) that methandrostenolone in a dose of $0.5 \text{ mg} \cdot \text{kg}^{-1}$ stimulated myosin synthesis in rat gastrocnemius muscle, and by the observations of Loble and Lovie (1979), Potter (1974), Tregear and Squire (1973) and Hanson and Huxley (1957) that the muscle tissue actin:myosin ratio remains constant at all times.

The large increase in the concentration of the contractile protein relative to the increase in total protein concentration was of some concern, because it suggested that the concentration of other proteins must decrease. In series 3, for example, the contractile protein concentration of group DE was 25 per cent greater than that of group C. Assuming that in normal conditions, 70 per cent of muscle protein is associated with the contractile elements, then this would increase to 88 per cent in the group DE tissues. Thus the enzymatic and other proteins could only comprise 12 per cent of the total tissue proteins.

The observations of the present study that the total protein content, as well as the actin concentration increased in the DE group lateral gastrocnemius which was also capable of generating greater contractile tensions than the control tissues substantiate the suggestion that myosin as well as actin content of the DE lateral gastrocnemius increased. Madena-Pyrgaki et al. (1978) also reported that both exercise and the anabolic steroid nandrolone decanoate individually significantly elevated the total contractile protein

concentration in atrophied rat gastrocnemius muscle. Unfortunately, these investigators did not study the effect of combining the treatments.

Effect of Dianabol and exercise on skeletal muscle water content

Although no muscle hypertrophy due to any of the treatments employed was indicated on the basis of tissue wet weights (chapter 2), the tissue water content was determined to confirm that the changes observed in this chapter were not due to altered water contents. As discussed earlier, the water content of any particular muscle was also not affected by any of the treatments employed. However, the tissues of the right hindlimb used for contractility studies in series 2 and 3 were composed of 6-8 per cent more water than were the same tissues of the contralateral limb, based on wet and dry weights. The wet weights of these tissues were also approximately 20 per cent more than the left hindlimb tissues. This difference in weight may be accounted for by the change in water content, probably due to an osmolarity difference between the Tyrode's solution and the intracellular muscle fluid.

The osmolarity of the Tyrode's solution used was 290 mosmoles. Several authors, as reviewed by Conway (1957), have suggested an intracellular muscle osmolarity to be 340 mosmoles or greater. If such an osmotic gradient did exist, all muscles exposed to the bathing medium would be expected to have an increased water content, as did indeed occur.

Effect of Dianabol and exercise on plasma corticosterone concentration

None of the treatments appeared to have any effect on plasma corticosterone levels, as the concentrations observed for all but the control samples were within the normal range reported by other authors (Mazurkiewicz-Kwilecki and Bielkiewicz, 1982; Verdiere, Rose and Schwartz, 1977). The purpose of determining plasma concentration of this steroid was to provide an indication of the amount of stress to the animal induced by Dianabol injections and/or exercise. The elevation of plasma corticosterone levels in rats due to stress has been reviewed in great detail (Yuwiler, 1976). Interestingly, in the present study the only animals to have significantly elevated plasma corticosterone levels were the controls. This was probably due to the degree of handling of the animals. Whereas those receiving the various treatments were handled minimally 4 times per week (group D) and usually more often (Groups CE and DE), the control animals were handled only once or twice per week, and therefore these latter animals had not accommodated to being handled. These results suggest that the control group utilized was not adequate for studying this parameter as an indication of stress.

General

A consistent trend was observed within the results of this chapter. Only the lateral gastrocnemius responded to treatment with Dianabol and exercise, and neither of these treatments individually had significant effects on the parameters studied. The specificity of the treatment was confirmed by the fact that the soleus muscle

continued to show no consistent changes to any of the treatments employed either for 4 weeks or 8 weeks. The Dianabol-exercise treatment resulted in a non-specific increase in *de novo* protein synthesis, as not only was the total protein concentration and content significantly elevated in this tissue relative to the control animals, but so were the protein fractions of PFK, SDH and actin. The changes in the protein content and actin concentration also indirectly suggest an elevated myosin concentration in this tissue.

The implications to the contractility studies of chapter 4 are obvious; the increased contractile protein concentrations correlate with the increased strength of the lateral gastrocnemius. However, the increased metabolic capacity did not appear to make a significant contribution as the fatigue profiles were the same for all treatment groups and the controls.

Withdrawal of Dianabol injections after 4 weeks (series 3) did not reverse the effects of the Dianabol-exercise combination excepting the tissue creatine concentration. Indeed, this result was predictable in light of the fact that the biological half life of the synthetic androgens is considerably longer than testosterone as these compounds are still detectable in the urine for 2 weeks following cessation of administration (Dugal and Masse, 1982), and because the mechanism of action is to induce *de novo* protein synthesis, a process which is probably not reversed immediately. These results may also suggest that even though the urine and therefore plasma concentrations of steroid are not detectable 2 weeks after the

cessation of administration, the effects of the Dianabol on the lateral gastrocnemius cells may persist for considerably longer.

The fact that the lateral gastrocnemius creatine concentration reverted to control levels within 1 week of the cessation of Dianabol injections (group DWE), whereas significantly greater concentrations were maintained in the DE group tissues, suggests that the effect of this particular anabolic steroid on creatine synthesis is not via *de novo* synthesis of amidinotransferase in the kidney, but it may, instead, increase the specific activity of the enzyme.

Effect of Dianabol and exercise on some contractile characteristics of the lateral gastrocnemius and soleus

Methods

Following 8 weeks of treatment, as described in Table 2, the rats were anaesthetized with Somnotol (sodium pentobarbital $70 \text{ mg} \cdot \text{kg}^{-1}$, I.P.). When complete anaesthesia had been achieved, as judged by the loss of the righting reflex and tail pinch response, the soleus and lateral gastrocnemius muscles of the right hindlimb were prepared for study as described in Appendix D. The first muscle studied was the soleus.

The muscle having been secured with a rigid clamp at the tendon origin, the foot was also immobilized in a plexiglass bath (Figure 5a). A piece of stainless steel wire attached to the distal tendon was secured to the force displacement transducer, which fed into an amplifier. The output of the amplifier was connected simultaneously to a Grass model 5D polygraph (Grass Instruments Inc., Quincy, Mass.) and Tektronix (Portland, Oregon) D12 (with a 601 display unit) and 502A oscilloscopes. Photographs of the image on the second oscilloscope were taken with a Polaroid (Cambridge, Mass.) CR-9 camera using type 657 film. This procedure provided immediate confirmation of successful recording of the contractions.

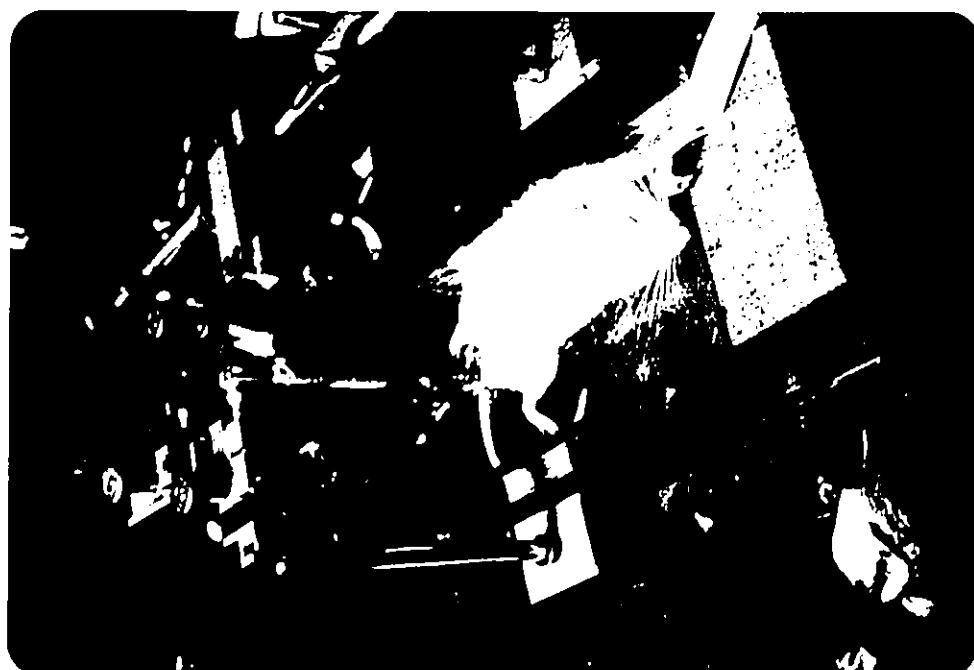
Different transducers were used in series 2 and 3 contractility studies. The one employed for series 2 was a home made transducer which had an output of 6 mV per gram tension, a compliance of less than $1 \text{ } \mu\text{m} \cdot \text{g}^{-1}$ and a resonant frequency greater than 1 Hz. Series 3

Figure 5. *In situ* contractility studies of the soleus and lateral gastrocnemius. Animals were anaesthetized with sodium pentobarbital and prepared surgically as outlined in Appendix D. Treatment protocol was as described in Figure 1, series 2 and 3. Studies of isometric twitch and tetanic contractions were performed *in situ* with the soleus and lateral gastrocnemius of the right hind limb. The exposed tissues were kept moist with Tyrode's solution.

- a) illustrates the animal secured in the plexiglass bath with all equipment in place for the soleus studies.
- b) the *in situ* soleus preparation. The tendon of the origin is secured with a stationary clamp, and the distal tendon attached to the transducer with a stainless steel wire. A 4 to 11 volt stimulus was applied via the soleus branch of the tibial nerve.
- c) illustrates the positioning of the soleus muscle during the contractility studies. It was separated slightly from surrounding tissues minimizing frictional interference during contraction.
- d) the lateral gastrocnemius *in situ* preparation. A 6 to 18 volt stimulus was applied via the lateral gastrocnemius branch of the tibial nerve.



(b)



(a)



(c)



(d)

contractility studies were performed with a Grass model FT03C transducer. The output of the transducer with the springs employed was linear to a load of 2 kg tension. In this mode, the grass transducer had an output of $2 \text{ mV} \cdot \text{g}^{-1}$ tension, and a compliance of $0.5 \text{ } \mu\text{m} \cdot \text{g}^{-1}$. Both transducers had a 6V supply.

The transducer was replaced at the completion of the series 2 study for 2 reasons. First, the original transducer developed an unrepairable crack in one bridge lead. However, more importantly, it became obvious at the end of this series that the transducer output was not linear over the required range of tensions. Although the linearity of the transducer output had been confirmed to a load of 900 g tension, the tension generated by the lateral gastrocnemius in certain instances was greater than this, and appeared to have exceeded the linear range of transducer. For this reason, the author accepts the fact that the values of the lateral gastrocnemius tetanic tensions illustrated for series 2 in this chapter may not be accurate in absolute terms, but are still valid on a comparative basis. If anything, the significant differences observed in the series 2 lateral gastrocnemius tetanic tensions would be even greater, because the output of the transducer would have been in a region of lesser slope at the greater tension. The identification and analysis of this problem is confirmed by the fact that the mean twitch tensions of both the soleus and lateral gastrocnemius, as well as the mean tetanic tensions of the former are similar in a series 2 and 3.

tissues, these tensions being definitely within the linear range of both transducers. However, the lateral gastrocnemius mean tetanic tensions are markedly different for the series 2 and 3 animals, and the difference increases proportionately to the tension generated.

Once the lower limb was clamped in place, the animal was further immobilized by securing the forelimbs fully extended. A bipolar electrode was then placed under the tibial nerve using a micromanipulator, and the nerve elevated slightly by the electrode to ensure good contact. In this fashion, the muscle was slightly elevated from the limb to minimize friction against other tissues (Figure 5b, c). The stimulus voltage and length of the muscle were optimized for maximal twitch tension and the voltage was then doubled for the remainder of the experiment. Stimulation of the soleus varied between 4 and 11 volts, with the pulse duration standardized to 50μ seconds. Maximal tetanic contractions were elicited by varying the pulse frequency from 70-110 pulses per second with a train duration of 500 msec. Fatigue was then induced by a train frequency of 0.6-sec^{-1} for 5 minutes. Tetanic tension during recovery was studied by reducing the train frequency to $.05\text{-sec}^{-1}$.

Typical twitch and tetanic contractions of both the soleus and lateral gastrocnemius are illustrated in Figure 6. Fatigue and recovery tracings from both muscles are reproduced in Figure 7.

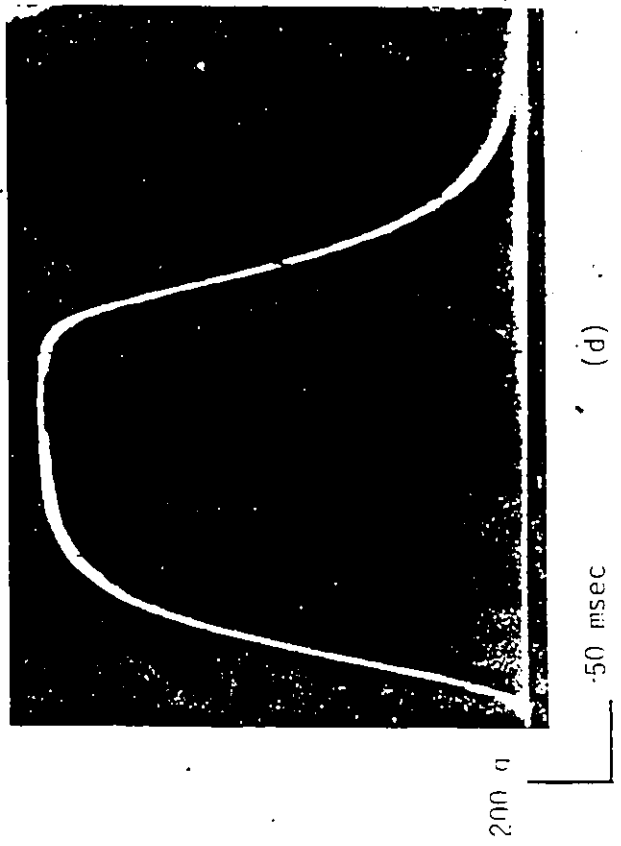
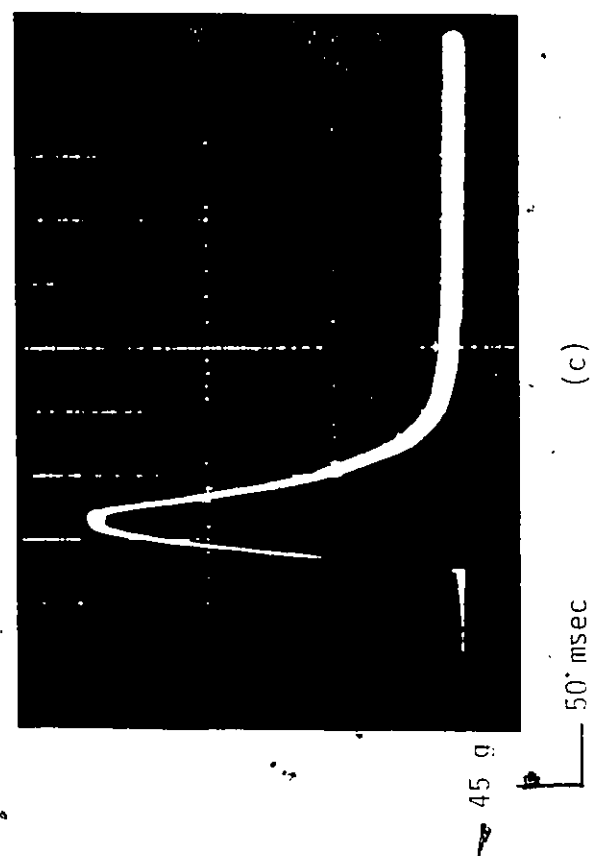
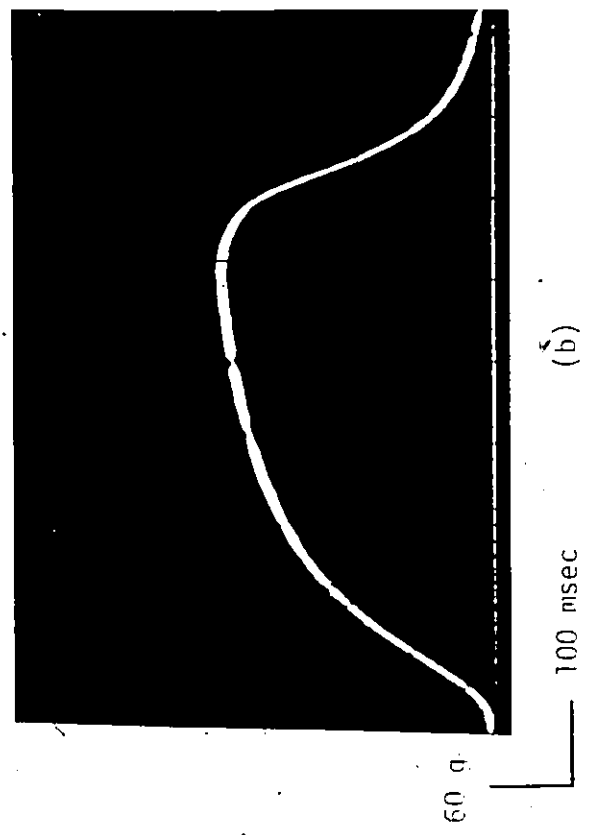
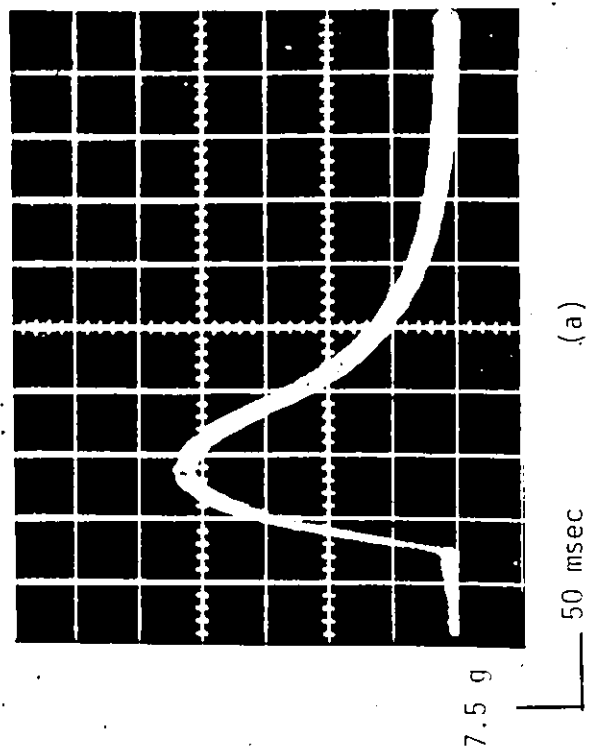
The lateral gastrocnemius studies were performed in the same fashion as described for the soleus (Figure 5d) except that the nerve

stimulated was the branch of the tibial innervating the lateral gastrocnemius, the stimulus voltage ranged between 6 and 18, the tetanic stimulation frequency was between 130 and 170 pulses·sec⁻¹, and the train frequency used to induce fatigue was 1.0·sec⁻¹.

The limb was irrigated with Tyrode's solution at room temperature throughout the studies (Appendix D).

Figure 6. Photographic record of oscilloscope tracings of soleus and lateral gastrocnemius twitch and tetanic contractions. Animals were anaesthetized with sodium pentobarbitol and prepared surgically as outlined in Appendix D. Treatment protocol was as described in Figure 1, series 2 and 3. Studies of isometric twitch and tetanic contractions were performed *in situ* with the soleus and lateral gastrocnemius of the right hind limb.

- a) soleus twitch. Stimulation procedure as described in the text.
Time to peak measured from point of departure from baseline. Time to $\frac{1}{2}$ relaxation measured from the twitch peak.
- b) soleus tetanus. Stimulation procedure as described in the text.
Time to $\frac{1}{2}$ relaxation measured from point where tension decline begins.
- c) lateral gastrocnemius twitch. Stimulation procedure as described in the text. Time to peak measured from point of departure from baseline. Time to $\frac{1}{2}$ relaxation measured from the twitch peak.
- d) lateral gastrocnemius tetanus. Stimulation procedure as described in the text. Time to $\frac{1}{2}$ relaxation measured from point where tension decline begins.



Results

The effect of Dianabol and exercise on skeletal muscle twitch contractions

Series 2 and 3 twitch contraction parameters are illustrated in Tables 18, 19 and 20, 21, respectively. As in the biochemical data discussed in the previous chapter, the total twitch tension generated was significantly greater in the DE group lateral gastrocnemius than that of all other groups. The twitch tensions normalized to $g \cdot 100 g^{-1}$ muscle weight followed the same trend, as one might have expected because the various treatments employed had no effect on mean muscle weights. Group D and CE mean tensions were not significantly different from group C. Interestingly, the mean twitch tension of group DWE (Table 20) remained significantly greater than that of group C and was not different from the mean of group DE.

The mean twitch tension of the soleus muscle was not altered by any of the treatments utilized, when expressed in terms of absolute tension generated or on a normalized basis.

The mean time to peak tension and $\frac{1}{2}$ relaxation times of the lateral gastrocnemius and soleus twitch contraction were not altered by any of the treatments employed in either series 2 (Tables 18 and 19) or series 3 (Tables 20 and 21).

The effect of Dianabol and exercise on skeletal muscle tetanic contractions

The mean tetanic tension for series 2 and 3 lateral gastrocnemius and soleus muscles are also found in Tables 18, 19 and 20, 21,

Table 18. Effect of 8 weeks treatment with Dianabol and exercise on lateral gastrocnemius twitch and tetanic contractions *in situ* (series 2).

	<u>LATERAL GASTROCNEMIUS</u>						
	<u>TWITCH</u>				<u>TETANUS</u>		
	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO PEAK (MSEC)</u>	<u>TIME TO ½ RELAX (MSEC)</u>	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO ½ RELAX (MSEC)</u>
CONTROL (8)	19.8 ±1.8	265.2 ±20.4	34.1 ±1.9	21.5 ±2.0	53.4 ±5.2	726.8 ±73.0	52.9 ±2.6
DIANABOL (7)	19.2 ±2.0	257.8 ±28.9	37.2 ±1.6	19.5 ±1.6	53.8 ±7.6	728.8 ±62.6	54.2 ±4.4
CONTROL EXERCISE (7)	19.4 ±1.4	263.2 ±19.0	31.7 ±2.5	21.7 ±2.5	50.4 ±8.8	710.4 ±81.6	52.0 ±2.0
DIANABOL EXERCISE (6)	30.3*±5 ±2.2	383.4*±5 ±27.0	30.0 ±1.8	20.0 ±1.8	89.6*±5 ±5.6	1138.0*±5 ±82.4	43.0 ±2.5

Normalized to 100 mg tissue wet weight

- 1) Values represent mean ± S.E.M.
- 2) Symbols indicate significant difference from: *controls, +Dianabol, §Control-exercise treatment, by Tukey's test ($p < 0.05$).
- 3) Number of animals per group indicated in parentheses

Table 19. Effect of 8 weeks treatment with Dianabol and exercise on soleus twitch and tetanic contractions *in situ* (series 2).

	<u>SOLEUS</u>							
	<u>TWITCH</u>				<u>TETANUS</u>			
	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO PEAK</u>	<u>TIME TO ½ RELAX (MSEC)</u>	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO ½ RELAX (MSEC)</u>	
CONTROL (8)	12.6 ±1.0	26.2 ±3.4	72.5 ±4.8	86.9 ±7.4	85.2 ±6.8	176.4 ±14.2	135.0 ± 5.0	
DIANABOL (7)	13.1 ±1.6	29.6 ±3.0	72.4 ±6.2	87.9 ±5.9	85.7 ±10.6	190.2 ±22.2	133.6 ± 9.4	
CONTROL - EXERCISE (7)	18.0 ±2.0	31.0 ±3.2	67.2 ±6.6	67.6 ±3.1	108.4 ±3.2	186.4 ±16.4	130.8 ±10.1	
DIANABOL - EXERCISE (6)	16.6 ±1.0	33.2 ±2.6	64.2 ±6.2	64.2 ±5.2	117.1 ±8.2	234.2 ±25.4	120.8 ± 5.5	

Normalized to 100 mg Tissue wet weight.

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses

Table 20. Effect of 8 weeks treatment with Dianabol and exercise on lateral gastrocnemius twitch and tetanic contractions *in situ* (series 3).

	<u>LATERAL GASTROCNEMIUS</u>						
	<u>TWITCH</u>				<u>TETANUS</u>		
	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO PEAK (MSEC)</u>	<u>TIME TO ½ RELAX (MSEC)</u>	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO ½ RELAX (MSEC)</u>
CONTROL (10)	20.9 ±2.5	281.1 ±17.6	30.5 ±4.1	17.6 ±1.8	93.4 ±5.9	1252.7 ±120.5	58.1 ±2.4
DIANABOL (5)	21.0 ±2.2	281.8 ±12.6	29.5 ±4.8	18.2 ±2.2	79.9 ±7.9	1068.5 ±101.5	58.7 ±5.3
DIANABOL - WITHDRAWAL (5)	21.9 ±1.2	286.6 ±13.5	30.9 ±3.0	17.0 ±5.6	83.0 ±2.4	1153.5 ± 57.8	66.7 ±7.6
CONTROL - EXERCISE (8)	23.1 ±1.3	288.9 ±19.1	36.7 ±2.7	21.1 ±2.5	94.4 ± 6.7	1134.9 ± 92.5	54.5 ±2.4
DIANABOL - EXERCISE (5)	31.6*+§Δ ±2.6	420.8*+§Δ ±37.6	34.3 ±3.7	20.5 ±2.1	126.9*+§Δ ±18.5	1707.6*+§Δ ±119.4	48.7 ±6.1
DIANABOL - WITHDRAWAL - EXERCISE (5)	30.5*+§Δ ±2.0	390.4*+§Δ ±19.5	35.5 ±2.6	20.8 ±2.0	140.4*+§Δ ± 8.1	1748.0*+§Δ ±130.8	47.9 ±3.7

Normalized to 100 mg tissue wet weight

1) Values represent mean ± S.E.M.

2) Symbols indicate significant difference from: *Controls, +Dianabol, §Control-exercise
ΔDianabol treatment, Tukey's test ($p < 0.05$).

3) Number of animals per group indicated in parentheses.

Table 21. Effect of 8 weeks treatment with Dianabol and exercise on soleus twitch and tetanic contractions *in situ* (series 3).

	<u>SOLEUS</u>						
	<u>TWITCH</u>				<u>TETANUS</u>		
	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO PEAK (MSEC)</u>	<u>TIME TO ½ RELAX (MSEC)</u>	<u>NORM. TENSION (g)</u>	<u>TOTAL TENSION (g)</u>	<u>TIME TO ½ RELAX (MSEC)</u>
CONTROL (10)	17.7 ±1.9	35.6 ±3.3	71.2 ±6.6	89.2 ±6.7	92.1 ±9.3	184.2 ±12.5	128.6 ± 1.5
DIANABOL (5)	14.4 ±1.4	28.9 ±1.6	57.8 ±3.2	67.8 ±5.0	93.9 ±7.6	188.7 ±14.2	124.7 ± 5.1
DIANABOL - WITHDRAWAL (5)	14.7 ±1.3	29.9 ±5.6	69.0 ±9.4	76.3 ±1.3	101.0 ±9.4	205.0 ±16.0	108.2 ± 8.2
CONTROL - EXERCISE (8)	14.0 ±0.9	26.2 ±2.3	52.4 ±4.6	77.2 ±5.9	105.7 ±6.1	197.7 ±11.0	84.1 ± 4.2
DIANABOL - EXERCISE (5)	14.8 ±1.2	31.0 ±2.4	62.2 ±4.8	80.4 ±7.7	99.4 ±6.7	207.9 ± 8.9	88.0 ± 6.2
DIANABOL - WITHDRAWAL - EXERCISE (5)	15.1 ±1.7	29.0 ±3.4	57.8 ±6.8	97.9 ±10.5	108.5 ±4.2	208.4 ±13.4	96.7 ±15.0

Normalized to 100 mg tissue wet weight

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses

respectively. The effect of the various treatments on tetanic tension followed the same trend as observed with twitch tension in that there was a significant increase in the mean tension generated by the group DE lateral gastrocnemius, when compared to any other group except group DWE. Again, withdrawal of the synthetic steroid (Table 20) did not reverse this effect, the mean tetanic tension of group DWE tissues being significantly greater than all other groups except that of group DE. Administration of D, DW, and CE treatments did not affect lateral gastrocnemius mean tetanic tension.

Tetanic tension of the soleus muscle was not altered by any of the treatments employed in the present study (Tables 19 and 21).

As observed with the parameters of the twitch contraction, the mean $\frac{1}{2}$ relaxation time of the tetanic contraction was not changed in either the lateral gastrocnemius or soleus by any of the treatments employed.

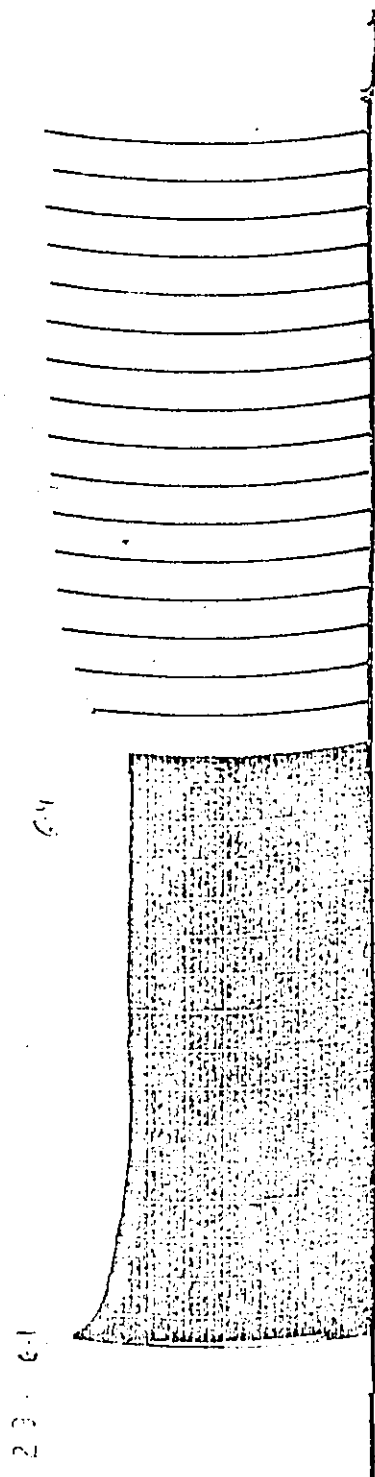
The effect of Dianabol and exercise on skeletal muscle fatigue

Sample fatigue and recovery tracings for the soleus and lateral gastrocnemius are illustrated in Figures 7a and 7b, respectively. Tensions in the fatigue studies were calculated as a *per cent* of the initial tension.

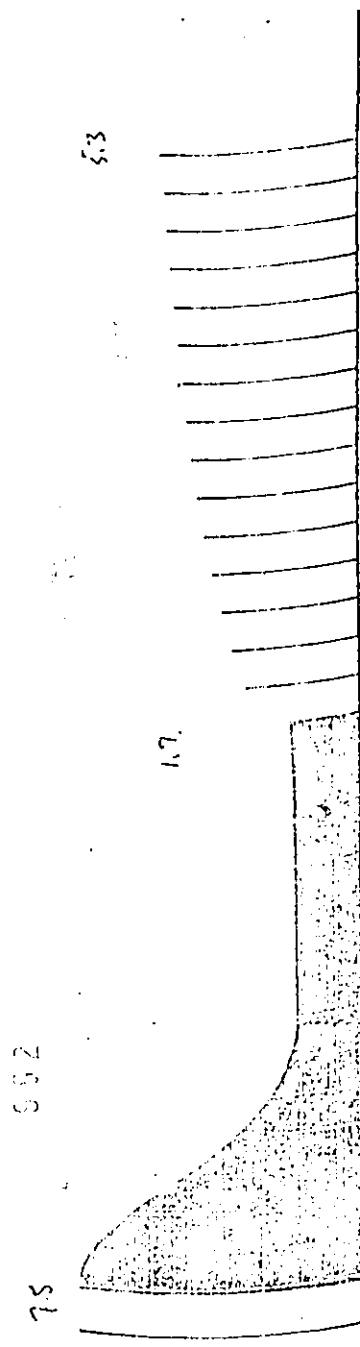
The fatigue characteristics of the soleus muscle were not affected by any of the treatments employed (Tables 22 and 23). In both series 2 and 3 studies, the degree of fatigue varied from 64-76 *per cent* of the initial tetanic tension, and recovered to 99-104 *per cent* of the initial tension. The time to $\frac{1}{2}$ recovery was rapid

Figure 7. Polygraph recordings of fatigue and recovery of the soleus and lateral gastrocnemius. Animals were anaesthetized with sodium pentobarbitol and prepared surgically as outlined in Appendix D. Treatment protocol was as described in Figure 1, series 2 and 3. Fatigue studies were performed following the contractility study of each muscle. Muscles were fatigued for 5 minutes, and recovery followed for 5 minutes.

- a) a typical recording of soleus fatigue and recovery.
- b) a typical recording of lateral gastrocnemius fatigue and recovery.



(a)



(b)

Table 22. Effect of 8 weeks treatment with Dianabol and exercise on lateral gastrocnemius and soleus fatigue characteristics *in situ* (series 2).

	<u>LATERAL GASTROCNEMIUS</u>			<u>SOLEUS</u>		
	<u>FATIGUE</u> <u>(% INITIAL)</u>	<u>TIME TO</u> <u>½ RECOVERY</u> <u>(MIN)</u>	<u>RECOVERY</u> <u>(% INITIAL)</u>	<u>FATIGUE</u> <u>(% INITIAL)</u>	<u>TIME TO</u> <u>½ RECOVERY</u> <u>(MIN)</u>	<u>RECOVERY</u> <u>(% INITIAL)</u>
CONTROL (8)	29.1 ±2.3	<2.0	79.1 ±4.8	67.7 ±2.6	<0.67	99.3 ±1.5
DIANABOL (7)	24.2 ±2.5	<1.33	84.9 ±7.1	64.2 ±4.1	<0.67	98.7 ±1.3
CONTROL - EXERCISE (7)	28.3 ±3.0	<1.67	87.7 ±3.9	76.2 ±2.3	<0.67	104.4 ±2.0
DIANABOL - EXERCISE (6)	30.0 ±3.3	<1.67	88.1 ±1.5	76.9 ±1.2	<0.67	99.1 ±4.0

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

Table 23. Effect of 8 weeks treatment with Dianabol and exercise on lateral gastrocnemius and soleus fatigue characteristics *in situ* (series 3).

FATIGUE CHARACTERISTICS - GROUP 3						
	LATERAL GASTROCNEMIUS			SOLEUS		
	FATIGUE (% INITIAL)	TIME TO ½ RECOVERY (MIN)	RECOVERY (% INITIAL)	FATIGUE (% INITIAL)	TIME TO ½ RECOVERY (MIN)	RECOVERY (% INITIAL)
CONTROL (10)	32.4 ±3.4	<2.0	79.4 ±8.4	72.1 ±4.0	<0.67	102.6 ± 2.1
DIANABOL (5)	31.0 ±5.8	<1.33	84.8 ±1.3	67.8 ±9.4	<0.67	98.7 ± 3.8
DIANABOL - WITHDRAWAL (5)	34.1 ±1.2	<1.67	80.6 ±4.4	69.0 ±7.8	<0.67	102.7 ± 3.7
CONTROL - EXERCISE (8)	26.1 ±3.4	<1.67	82.3 ±4.8	66.1 ±5.2	<0.67	99.1 ± 3.6
DIANABOL - EXERCISE (5)	34.3 ±3.1	<1.67	84.0 ±2.3	73.4 ±4.3	<0.67	101.9 ± 3.1
DIANABOL - WITHDRAWAL - EXERCISE (5)	31.5 ±2.9	≈1.33	82.5 ±7.1	75.4 ±1.3	<0.67	99.6 ± 3.2

1) Values represent mean ± S.E.M.

2) Number of animals per group indicated in parentheses.

in all cases, and reached essentially 100 *per cent* of initial tension at the end of the recovery period.

The lateral gastrocnemius tensions during fatigue decreased to a much larger degree than did the soleus. The tetanic tension of this muscle following 5 minutes stimulation varied from 24-34 *per cent* of initial tension, with no differences between group means. Following 5 minutes recovery, the tetanic tension generated increased to 70-84 *per cent* of the initial tension. Whereas the soleus time to $\frac{1}{2}$ recovery following fatigue was short, the lateral gastrocnemius time to $\frac{1}{2}$ recovery varied from 1.33 to 2.0 minutes. There were no differences between means of the various groups.

Discussion

Effect of Dianabol and exercise on lateral gastrocnemius contractility

In the present study both the twitch and tetanic tensions of the lateral gastrocnemius were increased significantly above control values by Dianabol-exercise treatment. Other contractile parameters were unaffected. As well, neither Dianabol nor exercise treatments individually had an effect on any of the contractile parameters studied.

Very few studies have dealt with gastrocnemius contractility in the past. In fact, only one study known to this author utilized the whole gastrocnemius muscle of rats in contractility study (Richardson and Smith, 1981). Unfortunately, the methodology employed and the terminology used were not explained and therefore prohibit any effective comparison to data obtained in the present study. The authors did claim that Dianabol administered in a dose of $0.35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 30 days was ineffective in increasing the "strength" or "stamina" of the gastrocnemius muscle. The route of Dianabol administration was not stipulated.

It is interesting that the De group lateral gastrocnemius of the present study had a significantly greater contraction tension than the other treatments, as this was the only tissue studied to have an increased concentration of enzymatic and contractile proteins, as well as total protein. It was somewhat surprising to observe that the fatigue characteristics of this tissue were not altered. If muscle fatigue is a result of intracellular biochemical changes (Dawson et al., 1977) then, one might have expected that the degree of fatigue or recovery from fatigue may have been affected by the enhanced

metabolic enzyme concentrations observed in the same tissues as described in chapter 3. The fact that this did not occur supports the hypothesis that even though the metabolic capacity of these tissues may have been increased by the enhanced PFK and SDH concentrations, this capacity may not necessarily be utilized unless increased amounts of substrate are also provided in some way.

Stone and Lipner (1978) studied the effects of high intensity exercise and Dianabol ($0.35 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ s.c., for 6 weeks) on the contractile parameters of an *in situ* gastrocnemius-plantaris preparation. They observed that the administration of the steroid provided no further enhancement of tension generation by this muscle group than did the exercise alone. The high intensity short duration training employed in their study resulted in significant increases in isometric twitch and tetanic tension following 3 and 6 weeks treatment.

Effect of Dianabol and exercise on soleus contractility

A greater number of reports in the literature deal with the contractile parameters of the soleus muscle. In the present study, the soleus muscle contraction and fatigue parameters were unaffected by any of the treatments employed.

Using a similar *in situ* preparation, Close (1967) reported that soleus muscles of 12 week old rats developed a mean twitch tension of $29.09 \text{ g} \cdot \text{g tissue}^{-1}$ with a mean time to peak of 35.5 msec. and a mean $\frac{1}{2}$ relaxation time of 51.0 msec. The total tension observed by Close was somewhat greater than tensions recorded in the present study. As well, Close observed that time to peak tension development

and $\frac{1}{2}$ relaxation times were considerably shorter.

The difference between the 2 works may be accounted for by the fact that the present study was executed at room temperature, and that by Close at 35-36°C. Close himself, in 1968, demonstrated that twitch tension development and several timed parameters of the soleus twitch and tetanic contraction, including the 2 mentioned above, are affected by temperature i.e. increasing the bathing medium temperature from 20°C to 37°C decreases the value of the time parameters. In fact, the soleus tetanic tension at 20°C was approximately 75 per cent of that observed at 35°C. Adjusting the tension observed by Close for room temperature produces values very similar to those recorded in the present study.

Similarly, the mean tetanic tension generated by the group C soleus was 184.2 g with a $\frac{1}{2}$ relaxation time of 128.6 msec as compared to 145.0 g total tension with a $\frac{1}{2}$ relaxation time of 51.0 msec by Close. These data are further supported by the observations of Fitts and Holoszy (1977) if converted to similar units. It is interesting to note that these latter authors reported that soleus muscles from rats undergoing an exercise programme of endurance treadmill running for 19-27 weeks had significantly shorter time to peak twitch tension and $\frac{1}{2}$ relaxation time than did control animals. However, the same exercised tissues developed less tetanic tension than did the controls. Staudte, Exner and Pette (1973) also reported a decrease in rat soleus isometric twitch contraction time following only 3 weeks of high intensity treadmill running.

They, however, observed an increased maximum tetanic tension. This apparent contradiction suggests that the type of exercise programme employed may differentially affect various contractile parameters of a particular muscle. The difference may also be due, in part, to temperature as the study of Staudte, Exner and Pette was executed at 30°C.

Thus, it appears that high intensity exercise can change some contractile characteristics of the soleus, but the moderate programme employed in the present investigation does not affect the basic parameters studied. It is possible that prolongation of the training period may result in some alterations of contractile parameters of either muscle studied. However, there does not appear to be any trend in the data to support this. Indeed, the fact that all soleus contractile parameters measured were unaffected by any treatment employed, combined with the results discussed in chapter 3 that biochemical parameters were also not changed, suggests that prolongation of the treatment period will not result in significant differences, i.e. those muscles that did not respond to any treatments at 4 weeks, still did not respond at 8 weeks.

Concluding Remarks

In this study, Dianabol-exercise treatment resulted in several significant changes when compared to control young adult male rats. This treatment increased the weight gained over the eight week period when compared to Control-exercise animals.

The extra weight appeared to be due to water retention, presumably in the extracellular compartment, as no hypertrophy was observed in the muscles or organs studied.

Significant biochemical and contractile changes were induced in the lateral gastrocnemius by Dianabol-exercise treatment. Marked elevations in the concentration and content of total protein, succinate dehydrogenase, phosphofructokinase, creatine and actin were observed. *In situ* isometric twitch and tetanic tensions were also significantly increased. However, because fatigue parameters were not altered, the data have been interpreted to suggest that the increased protein concentrations observed provided an enhanced metabolic capacity which was not utilized. This may have occurred either because larger amounts of substrate were not concomitantly made available, or that there is a superabundance of enzymes needed for the metabolic requirements of the particular muscle and therefore increasing the concentration further had no effect.

In light of these data, the first question one might ask refers to the reasons for the response being specific to the lateral gastrocnemius of animals receiving Dianabol-exercise treatment.

The mechanism of the synergistic response to the Dianabol-exercise treatment has been addressed to some extent in chapter 3, with regard to creatine. In that discussion an hypothesis was formulated to explain how the individual components of the Dianabol-exercise treatment might contribute to enhance lateral gastrocnemius myosin concentration. It was proposed that Dianabol induces creatine synthesis in the liver, and exercise increases muscle creatine concentration by elevating plasma growth hormone levels. Muscle creatine concentration has been suggested as a modulating factor of the rate of myosin synthesis. One would predict this response to be non-specific however, as there is no evidence to suggest a skeletal muscle tissue target organ specificity for growth hormone.

Therein may be the primary role of Dianabol, to induce increased protein synthesis, which has been discussed in detail in chapter 3. Thus, in this hypothesis, exercise, by increasing circulating growth hormone levels (in the case of myosin), acts in a permissive role to regulate the synthesis of various proteins.

This hypothesis relies on the presence of androgen receptors in muscle tissue. It is known that testosterone enters the muscle cell to combine with receptors directly, resulting in the induction of protein synthesis, unlike other target tissues where the dihydro derivative is the most potent androgen. This then suggests that the lateral gastrocnemius contains a higher concentration of androgen receptors than the other muscles studied which have a similar fibre type profile, permitting enhancement of protein synthesis to a greater degree. This suggestion is not without precedent as

this is presumably the reason for the responsiveness to androgens of the masseter and temporal muscles of the guinea pig and the levator ani of the rat.

With the publication recently of techniques for the isolation of androgen receptors (Michel and Baulieu, 1980; Krieg, Smith and Bartsch, 1978; Krieg, 1976; Dube, Lesage and Tremblay, 1976) it may be possible to quantify the androgen receptor population in individual tissues and test this hypothesis.

A second, and equally plausible hypothesis, is that Dianabol can enhance protein synthesis to the same degree in all of the muscles studied, but the muscle remains in a prepared state until protein is needed, and that exercise initiates this requirement. Because the potential of the protein synthesis mechanism has been enhanced under these conditions, when protein is required, a markedly increased production occurs. This is to suggest that Dianabol enters all cells to the same degree, and binds to receptors. However, the muscle cells which are used to a greater degree on a relative basis in a particular exercise i.e., the lateral gastrocnemius in the present study, would require a greater amount of protein to be synthesized in order to maintain normal function. The protein requirement of these tissues would be elevated not only because of the increased metabolic requirement, but also because exercise itself results in increased protein catabolism. If this second hypothesis is correct, then the anabolic action of Dianabol would indeed be one of "priming" the protein synthesizing mechanism of muscle cells, but the full potential

of this anabolic action is only manifest when the cells have a markedly increased requirement.

Some insight into the validity of this hypothesis may be gained by performing 2 experiments, which could be done concomitantly. These would involve comparing electromyogram recordings from the individual muscles of animals participating in the same exercise programme employed in the present study, as well as analyzing the glycogen content of these same muscles following exercise. These experiments would provide for the determination of which muscles were being used during the treadmill running exercise.

Finally, a third hypothesis also equally possible, would be that exercise increases the androgen receptor bed in skeletal muscle and thus amplifies the effect of the synthetic anabolic steroid. This suggestion reverses the role of exercise and Dianabol discussed in the second hypothesis, in that exercise would be priming the muscle by inducing an increase in the concentration of androgen receptors in muscle(s). If the exercise were specific to a muscle, then the androgen receptor bed of only that muscle would be expanded. The steroid, entering the cells of all muscles would induce protein synthesis to a greater degree in this muscle because of the increased binding to receptors.

When the present study was designed, the question asked was: Can the administration of Dianabol alone or combined with exercise increase muscle size or strength? The results definitively suggest that Dianabol, in the dose employed in these experiments, does not have any effect on the parameters studied. However, the combination

of Dianabol administration and the particular exercise employed did result in marked changes in several biochemical and contractile parameters in at least one muscle in young adult male rats. The major source of androgens in the female being the adrenal cortex administration of exogenous synthetic androgens would probably result in an accentuation of the changes observed in the male.

Bearing in mind the dangers of extrapolating from animal studies to humans, the results of this study have direct implications on athletes. This segment of the populace has been using (read abusing) these compounds with the intention of improving muscle size and strength. However, the idea that anabolic steroids could do so in the young or mature adult is not based on scientific evidence, and in fact, equal numbers of reports can be found in the literature to support or contradict this point. The results of the present study suggest that muscle strength can be improved by the administration of Dianabol combined with exercise. However, the effects on sexual tissue maintenance and function can not be overlooked.

Appendix A

Exercise Programme

The exercise programme employed is illustrated in Table 24.

The programme employed was as described by Reed (1974), an intermittent moderate intensity, medium duration progressive exercise programme. Animals were run 5 days week⁻¹ during the off part of the light cycle, with controlled temperature and humidity. The treadmill was kept in the same room that the animals were housed in.

Table 24. Exercise Programme.

wk.	Day of wk.	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Run Speed (m. min ⁻¹)	Total Time of Prog. (min: sec)	Total Work Time (sec)
C	4=T	40:00	10	1	1	5.0	27.4	40:00	---
	5=F	40:00	10	1	1	5.0	27.4	40:00	---
1	1=M	00:10	10	40	3	5.0	22.4	49:30	1200
	2=T	00:10	10	40	3	5.0	27.4	49:30	1200
	3=W	00:10	10	40	3	5.0	27.4	49:30	1200
	4=T	00:15	15	19	4	5.0	36.6	52:00	1140
	5=F	00:15	15	19	4	5.0	36.6	52:00	1140
2	1=M	00:15	15	19	4	5.0	36.6	52:00	1140
	2=T	00:15	15	19	4	5.0	45.7	52:00	1140
	3=W	00:15	15	19	4	5.0	45.7	52:00	1140
	4=T	00:15	15	19	4	5.0	45.7	52:00	1140
	5=F	00:15	15	19	4	5.0	45.7	52:00	1140
3	1=M	00:15	15	19	4	5.0	45.7	52:00	1140
	2=T	00:15	15	18	4	5.0	54.9	50:00	1080
	3=W	00:15	15	18	4	5.0	54.9	50:00	1080
	4=T	00:15	15	18	4	5.0	54.9	50:00	1080
	5=T	00:15	15	18	4	5.0	54.9	50:00	1080
4	1=M	00:15	15	18	4	5.0	54.9	50:00	1080
	2=T	00:15	15	18	4	5.0	64.0	50:00	1080
	3=W	00:15	15	18	4	5.0	64.0	50:00	1080
	4=T	00:15	15	18	4	5.0	64.0	50:00	1080
	5=F	00:15	15	18	4	5.0	64.0	50:00	1080
5	1=M	00:15	15	18	4	5.0	64.0	50:00	1080
	2=T	00:15	15	14	5	5.0	73.2	53:45	1050
	3=W	00:15	15	14	5	5.0	73.2	53:45	1050
	4=T	00:15	15	14	5	5.0	73.2	53:45	1050
	5=F	00:15	15	14	5	5.0	73.2	53:45	1050
6	1=M	00:15	15	14	5	5.0	73.2	53:45	1050
	2=T	00:20	20	11	5	5.0	73.2	55:00	1100
	3=W	00:20	20	11	5	5.0	73.2	55:00	1100
	4=T	00:20	20	11	5	5.0	73.2	55:00	1100
	5=F	00:20	20	11	5	5.0	73.2	55:00	1100
7	1=M	00:20	20	11	5	5.0	73.2	55:00	1100
	2=T	00:25	25	9	5	5.0	73.2	55:25	1125
	3=W	00:25	25	9	5	5.0	73.2	55:25	1125
	4=T	00:25	25	9	5	5.0	73.2	55:25	1125
	5=F	00:25	25	9	5	5.0	73.2	55:25	1125
8	1=M	00:25	25	9	5	5.0	73.2	55:25	1125
	2=T	00:30	30	8	5	5.0	73.2	57:30	1200
	3=W	00:30	30	8	5	5.0	73.2	57:30	1200
	4=T	00:30	30	8	5	5.0	73.2	57:30	1200
	5=F	00:30	30	8	5	5.0	73.2	57:30	1200

Appendix B

Determination of succinate dehydrogenase concentration

Succinate dehydrogenase concentration was determined by an adaptation of the method of Cooperstein, Lazarow and Kurfess (1950). Two alterations to the methodology were made. The first was the homogenization medium. To facilitate determination of total protein, SDH, and PFK concentrations from the same tissue sample, the tissues were homogenized by hand in 0.15 M KCl, pH 7.4, with a glass pestle and homogenizing tube partially submersed in an ice water bath. Immediately following homogenization, an aliquot was transferred to a glass 12 x 75 mm tube and 0.3 ml of a 0.044 M phosphate buffer solution added. This concentration of phosphate buffer was used in order that the phosphate concentration in the diluted homogenate was 0.033 M, as in the original method.

Figure 8 is a copy of an original spectrophotometer tracing of samples homogenized in 0.15 M KCl, pH 7.4 (cuvettes 1 and 3) as in the present study, or in 0.033 M PO_4^- , pH 7.4 (cuvettes 2 and 4) as in the original method. The parallelism of the rate of change in optical density of the solutions illustrates that the change in homogenization procedure did not affect the assay results.

The second modification adapted was in the calculation of the amount of cytochrome C being reduced per minute. The original method suggested the amount of succinate used was proportional to the rate of change of the log of the concentration of oxidized cytochrome C ($\log[\text{C}_{\text{ox}}] \cdot \text{min}^{-1}$). However, in the range of enzyme

concentrations in the tissues in the present study, plotting the rate of change of the reduced cytochrome C concentration vs time ($[C_{red}] \cdot \text{min}^{-1}$) also demonstrated a linear relationship. Figure 9 illustrates this method of plotting the sample. Using a linear function simplified the calculation of SDH concentration, as the rate of change in optical density of the cytochrome C solution could be monitored directly without further mathematical manipulations.

Figure 8. Spectrophotometer recording of SDH assay. A sample lateral gastrocnemius tissue was frozen in liquid nitrogen and homogenized in either a) 0.15 M KCl, pH 7.4, or b) 0.033 M PO_4^- buffer, pH 7.4. All other assay protocol was as described in the text. Sample aliquots from a) were added to cuvettes 1 and 3 and from b) to cuvettes 2 and 4. The rate of reduction of cytochrome C was monitored for approximately 7 minutes.

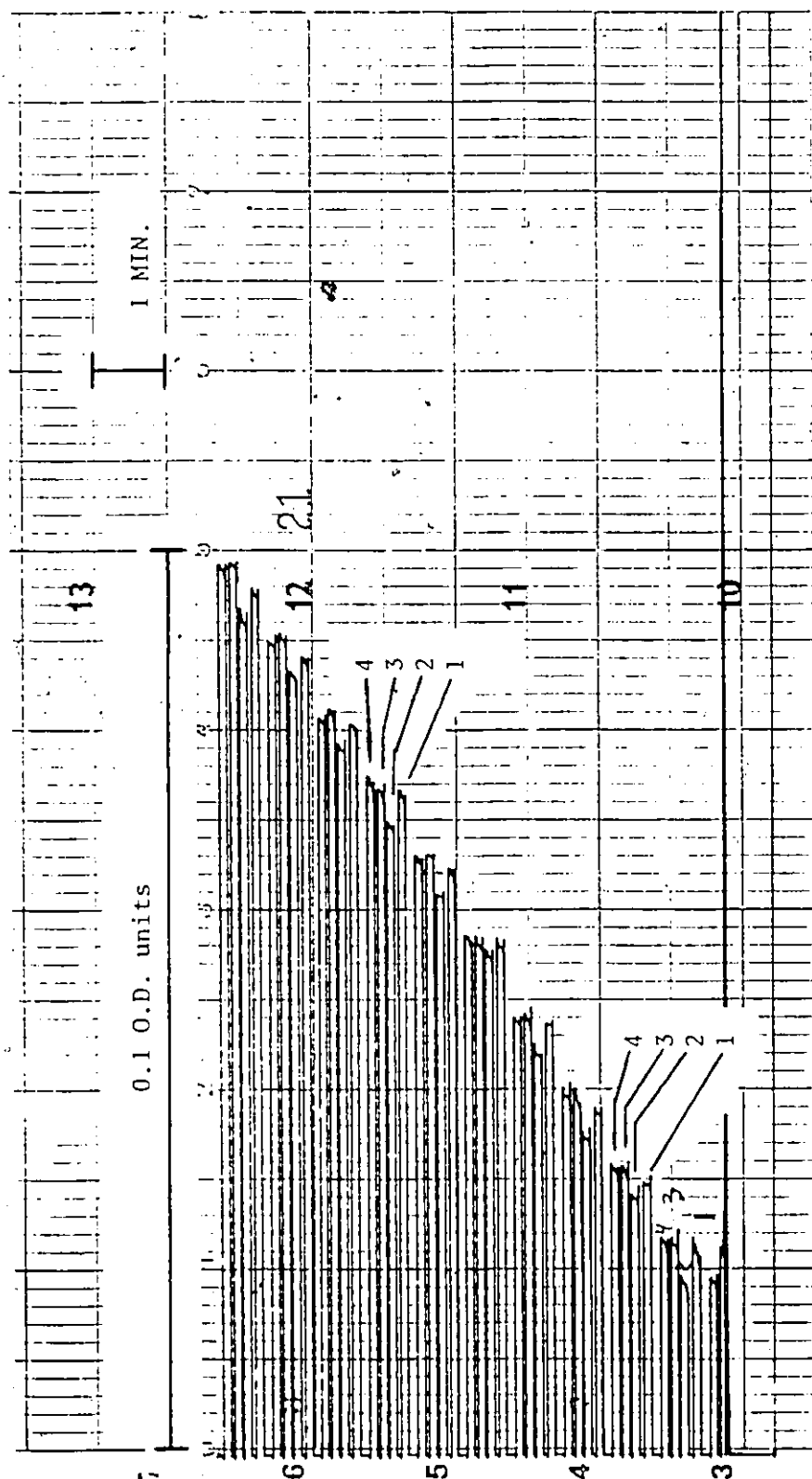
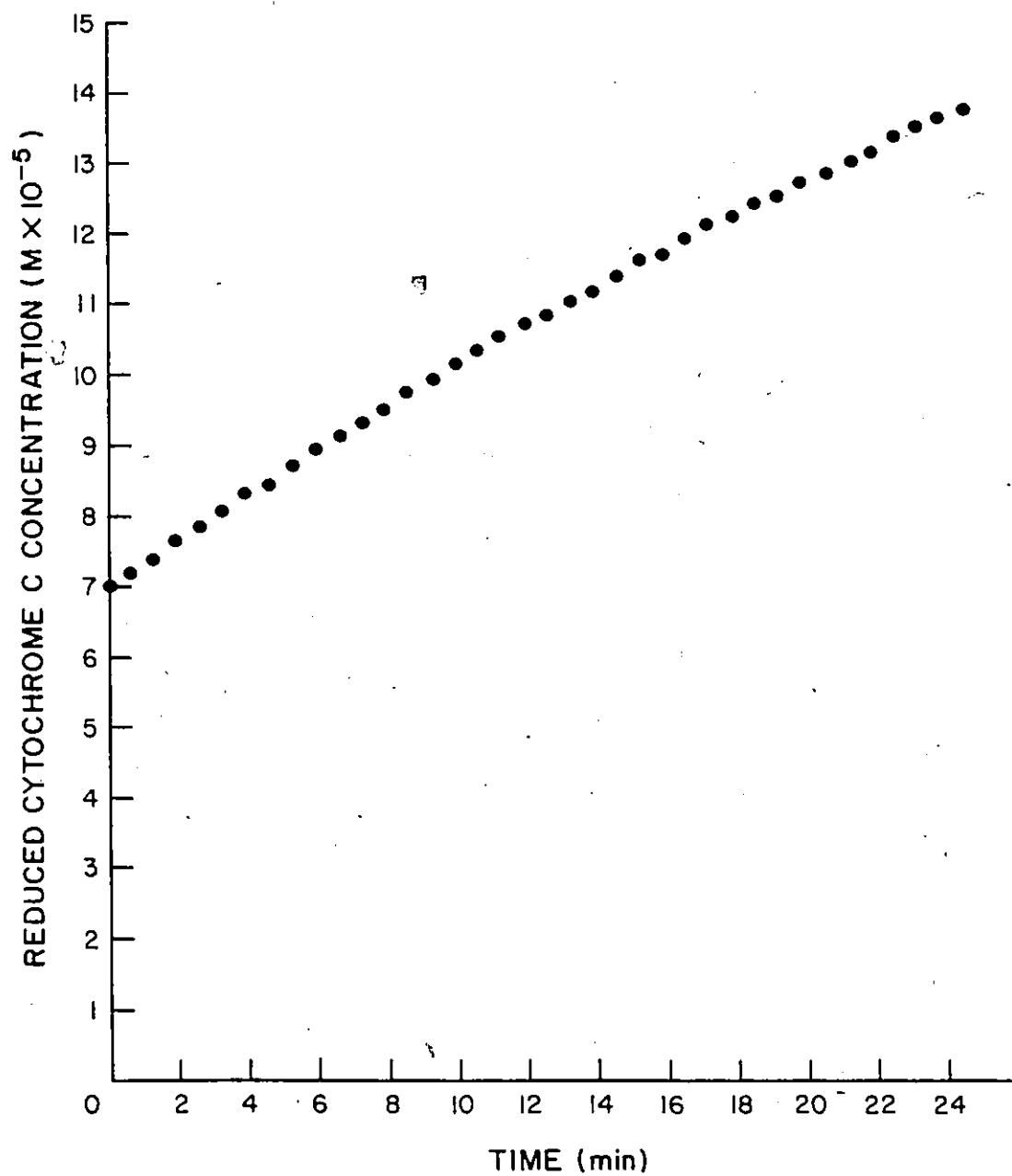


Figure 9. Rate of change of reduced cytochrome C concentration. Assay protocol was as described in the text. The concentration of reduced cytochrome C plotted against time illustrates the rate of change to be linear for approximately 15 minutes.



Appendix C

Relationship between tissue weight and *per cent* water content

The question being addressed is whether the seven *per cent* increase in tissue water content observed in the tissues used for contractility studies when compared to the same tissues of the contralateral limb (Table 25) can account for a 20 *per cent* increase in tissue weight.

If we assume a) that the protein and lipid concentration of any given muscle is the same in both hind limbs of the animal, and b) for the sake of simplicity that the tissue is made up only of protein, lipid and water, then the observation that the water content (as a fraction of tissue wet weight) was seven *per cent* greater in the tissues used for contractility experiments means that the contribution of protein to the weight of the tissue has declined by seven *per cent*.

If one were to perform the appropriate calculations, a five *per cent* increase in water content could account for a 20 *per cent* increase of tissue weight. Similarly, a seven *per cent* increase in water content could account for a 33 *per cent* muscle weight increase. The difference between the calculated values and the seven *per cent* water increase and 20 *per cent* tissue weight increase observed in this study are presumably due to the inherent error involved in the determination of the tissue water content.

Using a five *per cent* change to simplify numbers for an example, and starting with the same 72 *per cent* water, the right hind limb muscles would be composed of 77 *per cent* water by weight. If the left hind limb muscle weighed 1000 mg, 720 mg would be water, and 280 mg protein and lipid. The right hind limb muscle would weigh 20 *per cent* more, or 1200 mg, of which 77 *per cent* by weight is water, or 924 mg. Subtracting the water weight from the total tissue weight, leaves 276 mg of protein and lipid in the right hind limb.

Table 25. Water content of contralateral soleus and lateral gastrocnemius muscles of the hind limb following contractility studies (series 3).

	<u>(per cent of tissue wet weight)</u>			
	<u>SOLEUS</u>		<u>LATERAL GASTROCNEMIUS</u>	
	<u>LEFT</u>	<u>RIGHT</u>	<u>LEFT</u>	<u>RIGHT</u>
CONTROL	71.8 ±0.9	78.5 ±0.5	72.3 ±0.4	78.9 ±0.4
DIANABOL	72.4 ±0.6	78.0 ±0.8	71.9 ±0.8	79.2 ±0.6
DIANABOL WITHDRAWAL	72.6 ±0.5	78.6 ±0.3	72.4 ±0.3	79.6 ±0.6
CONTROL EXERCISE	71.7 ±0.7	78.3 ±0.3	72.0 ±0.8	79.0 ±0.5
DIANABOL EXERCISE	71.2 ±0.3	78.6 ±0.5	71.7 ±0.5	78.8 ±0.9
DIANABOL- WITHDRAWAL EXERCISE	72.1 ±0.8	78.8 ±0.6	72.3 ±0.4	79.5 ±0.6

Appendix D

Surgical procedures for contractility studies

Following 8 weeks of treatment, the rats were anaesthetized with Somnotol (sodium pentobarbitol $70 \text{ mg} \cdot \text{kg}^{-1}$, I.P.). When complete anaesthesia had been achieved, as judged by the loss of the righting and tail pinch responses, the right hind limb was shaved and a lateral longitudinal incision made from the midsection of the paw to the knee joint. All surgical techniques were performed using a Carl Zeiss dissecting microscope, and the anatomical nomenclature used is as described by Greene (1959). The surgery took approximately 90 minutes from the beginning to the point when the soleus studies began.

Supplements of anaesthetic were given as required, in amounts of one quarter the original dose.

The underlying fascia was then cut through and using forceps, the soleus muscle was separated along its anterior aspect from the tibia. The fibular branch of the tibial nerve was cut, care being taken to preserve the posterior tibial artery and vein. Fatty tissue was trimmed from the common distal ligament of the posterior compartment muscles at the lateral tibial condyle, leaving the saphenous circulation to the foot intact. The heads of the gastrocnemius muscle were then exposed by reflecting back the skin, beginning at the knee, until the muscles of the posterior cavity of the lower leg were completely exposed. Great care was taken not to damage the large saphenous vessels.

At the bifurcation of the tibial nerve the fibular branch was cut and completely removed from the surface of the lateral gastrocnemius. A cotton swab soaked with Tyrode's solution aerated with 5 per cent CO_2 in O_2 was used to irrigate the tissues while the soleus muscle, its innervation and blood supply were more freely exposed.

The Tyrode's solution was prepared with a final ionic concentration of Na, 145.5 mM; Ca, 2.5 mM; K, 4.7 mM; Mg, 3.1 mM; SO_4^- , 3.1 mM; Cl, 129.0 mM; HCO_3^- , 25 mM; HPO_4^- , 1.2 mM; and glucose, 5.5 mM.

The soleus was teased away from the lateral gastrocnemius and plantaris muscles, care being taken to grasp only connective tissue and not muscle whenever possible. The isolation of the soleus in this fashion exposed the innervation (a branch of the tibial nerve) and the blood supply (external sural artery). The extraneous tissue was cleaned away from the nerve and it was electrically stimulated to confirm its integrity. The stimulation threshold was determined, and usually found to be 1-2 volts. A bipolar electrode was used to deliver the output of a Grass S-4 stimulator, adjusted to deliver a 50 μsec DC pulse. This aspect of the leg was then covered with cotton batting soaked in Tyrode solution and the upper sector of the limb re-exposed.

The sural nerve was severed and removed from the surface of the lateral gastrocnemius for a length of about 1 cm, care being taken not to damage the superficial sural artery and small

saphenous vein. The tibial, common peroneal and sural nerves, as well as the saphenous artery and great saphenous vein, and the popliteal artery and vein were exposed and freed of fatty and connective tissue. The tibial nerve trunk was followed until the quadruple division was exposed, and the branch innervating the lateral head of the gastrocnemius was separated from other tissue. Functional integrity of the lateral gastrocnemius innervation was confirmed using the same stimulation procedure described for the soleus.

At this point the upper portion of the lower limb was well irrigated with cotton batting soaked in Tyrode's solution, and the distal tendon (Achilles) re-exposed and isolated. The soleus tendon was separated from that of the plantaris and gastrocnemius, leaving as much tendon tissue as possible on the soleus. It was then severed and a short wire attached with 000 silk suture. The plantaris tendon was then separated from that of the gastrocnemius and a second wire attached to the latter tendon. Because of the tension generated during a tetanic contraction of the lateral gastrocnemius, the suture of this tendon had to be very strong. Adequated holding strength was achieved by using three sutures, two around the circumference of the tendon, sandwiching a third which went through the centre of the tendon. The plantaris was then teased away from the gastrocnemius.

The soleus having been prepared for stimulation, the deep and superficial peroneal nerves and the peroneus tendons were cut.

The muscle was then carefully lifted and connective tissue cut to free it from surrounding structures. The animal was then placed in the apparatus for study of the contractile characteristics of the soleus.

At the end of the above procedure, the animal was removed from the apparatus and prepared for study of the lateral gastrocnemius. For this the tibial nerve innervation of the soleus was cut as close to the muscle as possible. The nerve was then isolated near its quadruple division and the branch to the lateral gastrocnemius reidentified and tested for function with the S-4 stimulator. The viability of the lateral gastrocnemius preparation having been confirmed, the 3 remaining branches of the tibial nerve were severed. The medial and lateral heads of the gastrocnemius were then teased apart toward the insertion as much as possible, care being taken not to damage any lateral gastrocnemius fibres. The lateral gastrocnemius, as with the soleus, was elevated by the distal tendon, and as much remaining connective tissue as possible cut. The animal was then replaced in the apparatus and the contractile characteristics of the lateral gastrocnemius studied.

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