J. exp. Biol. (1982), **97**, 411–420
Printed in Great Britain

RELATIONSHIP BETWEEN MUSCLE FORCE AND MUSCLE AREA SHOWING GLYCOGEN LOSS DURING LOCOMOTION

BY R. B. ARMSTRONG AND C. R. TAYLOR

Department of Physiology, Oral Roberts University, Tulsa, Oklahoma 74171, *and Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts* 02138

(*Received* 20 *August* 1981)

SUMMARY

This experiment was designed to study the relationship between the cross-sectional area of rat skeletal muscle showing glycogen loss and the muscle forces exerted during exercise. Muscular force exerted by the extensors of the elbows and ankle was increased by 24 % by loading rats with 24 % of their body mass while running them on a treadmill at 30 m.min^{-1}. $\dot{V}_{O_2}$ increased by 24 % and stride frequency was unchanged when the rats ran with loads. Cross-sectional areas of the elbow and ankle extensor muscles showing glycogen loss were compared from rats running with and without the load. We found a nearly direct proportionality between the changes in force and the changes in muscle area showing glycogen loss, i.e. when the force of the extensors was increased by 24 %, the cross-sectional area of the elbow extensors showing glycogen loss increased by 28 %, and that of the ankle extensor group increased by 24 %. The more peripheral muscles in each group accounted for a greater proportion of the increase in cross-sectional area of the group showing glycogen loss (i.e. lateral and long heads of triceps brachii muscle accounted for 91 % of the increase in the elbow extensor group, and gastrocnemius muscle accounted for 84 % of the increase in the ankle extensor group). Most of the increases in muscle area showing glycogen loss occurred in fast-twitch-glycolytic fibres (84 % in the elbow and 88 % in the ankle). The data suggest that increasing muscle force requirements by 24 % by loading resulted in proportional increases in cross-sectional area of muscles recruited to produce the force, i.e. that spatial recruitment primarily accounted for the elevation in force. The relatively greater increases in cross-sectional area showing glycogen loss of peripheral muscles within a group indicate the importance of studying whole groups of muscles when considering muscular recruitment patterns during exercise.

INTRODUCTION

Forces exerted by skeletal muscles increase with increasing locomotory speed (Alexander, 1977; Cavagna, Heglund, & Taylor, 1977; Walmsley, Hodgson, & Burke, 1978). Recruitment of additional muscle fibres serves as one important mechanism for providing these increases in muscular force (Stein, 1974). Evidence

from electromyography (Smith *et al.* 1977; Walmsley *et al.* 1978), determination of forces exerted by muscles using strain guages attached to tendons (Walmsley *et al.* 1978), and estimations of loss of glycogen from muscles using histochemical techniques (Sullivan & Armstrong, 1978) all indicate that animals first recruit the deeper muscles and deeper muscle fibres within those muscles, but as they increase in speed more peripheral muscles and muscle fibres start to be recruited. During quiet standing, muscular force is provided almost entirely by deep, slow-twitch extensor muscles (Smith *et al.* 1977; Walmsley *et al.* 1978). When animals walk, the deep slow muscles continue to provide most of the force (Walmsley *et al.* 1978), although slow and fast oxidative fibres are also recruited in the more superficial muscles (Armstrong *et al.* 1977). As animals increase their speed during running and galloping, this peripheral recruitment of fibres and muscles continues (Armstrong *et al*).1977; Smith *et al.* 1977; Sullivan & Armstrong, 1978; Walmsely *et al.* 1978).

A technique was described recently for quantitatively increasing the forces exerted by locomotory muscles of running animals by having them carry loads (Taylor *et al.* 1980). In the present study we used this technique to investigate whether animals would utilize the same peripheral pattern of fibre recruitment when force is increased by carrying a load as has been observed when it is increased by increasing speed.

METHODS

Animals and training

Male Sprague-Dawley rats ($n = 26$) weighing 277 ± 43 (S.D.) g were used in the experiment. They were housed in groups of 13 animals per cage and were provided food (commercial rat chow) and water *ad libitum*. Temperature was maintained at 24 ± 2 °C. Days were divided into 12 h each of light and darkness. The rats were trained to run on a motor-driven treadmill with a packload. The pack was fashioned from a lightweight elastic tape and the appropriate mass was obtained by adding lead shot. Running speed and added mass were gradually increased over a 3-week period (10 min per day, 5 days per week) until the rats could run for 10 min at 30 $m.min^{-1}$ with a load of 24 % of body mass.

Procedures

The forces exerted by the extensor muscles of rats were increased by 24 % as the animals ran on a treadmill by having them carry a load equal to 24 % of their body mass. Newton's second law states that force (F) is a function of mass (m) and acceleration (a):

$$F = ma. \tag{1}$$

If the average accelerations of the centre of mass during the stride of an animal running at the same velocity are constant, force may be varied by changing the mass supported by the animal's muscles:

$$a = k, \quad \Delta F = k\Delta m. \tag{2}$$

It has been demonstrated that average accelerations of the centre of mass of animals do not change while carrying loads between 7 and 27 % of their body mass (Tay[illegible]

al. 1980). Therefore, increasing the mass of a rat running at a constant velocity by 24% should increase the required muscular force of the muscles supporting the animal (i.e. extensors) by 24%.

$\dot{V}_{O_2}$ during running at 30 m.min^{-1} was determined for each rat with and without the load. The rats ran in a plexiglass box that slid on the surface of the tread. Air was drawn through the box at a rate of 3–10 l.min^{-1} (STP). Part of the exhaust air was dried and metered through an oxygen analyser (Beckman F-3). $\dot{V}_{O_2}$ was calculated assuming an R value of 0·80 using the following equation as described by Tucker (Tucker, 1968).

$$\dot{V}_{O_2} = \frac{\text{Flow}_{air}(\Delta_{O_2})}{0{\cdot}9581} \tag{3}$$

The box was checked for exhaled air leaks by decreasing the flow rate 30%. This would increase the magnitude of exhaled air that was lost. No differences were found between the two measurements. Flow meters were calibrated daily by drawing N_2 through the box at a known rate and monitoring changes in O_2 concentration using the equation:

$$\text{Flow}_{air} = \frac{\text{Flow}_{N_2}(0{\cdot}2094)}{\Delta_{O_2}} \tag{4}$$

Accuracy of the system was better than ±3%.

Stride frequency was measured simultaneously with $\dot{V}_{O_2}$ to indicate whether accelerations of the centre of mass of the rats changed with loading. The interval for 25 strides was timed with a stopwatch.

Rats were then randomly divided into three groups for the histochemical studies: (1) controls, (2) loaded runners, and (3) unloaded runners. At least 48 h following the previous bout of exercise, the latter two groups were run for 10 min at 30 m.min^{-1} with a load equalling 24% of their body mass (group 2), or without a load (group 3). $\dot{V}_{O_2}$ was determined during the run. Runners were discarded if they did not run steadily at the front of the treadmill box or if their $\dot{V}_{O_2}$ showed fluctuations during the run. Immediately following exercise, the rats were decapitated and the following muscles rapidly excised: the lateral (L), long (Ln), and medial (M) heads of triceps brachii; the soleus (S); the plantaris (P); and the gastrocnemius (G). One cross-section of each muscle was mounted on a specimen holder and frozen in 2-methylbutane cooled in liquid N_2 for histochemistry. An adjacent cross-section of each muscle was frozen in liquid N_2 for subsequent determination of glycogen concentration using the anthrone procedure (Seifter *et al.* 1950). All muscles were frozen within 6 min. Glycogen loss in the muscles during this time frame is insignificant (Armstrong & Peterson, 1981). The sequence in which the muscles were removed was the same for all rats.

Muscle analysis

Serial sections cut from the muscle samples prepared for histochemistry were stained for diphosphopyridine nucleotide-diaphorase (Novikoff, Shin, & Drucker, 1961) and myofibrillar adenosine triphosphatase (Padykula & Herman, 1955) so fibres in the sections could be classified as fast-twitch-oxidative-glycolytic (FOG),

fast-twitch-glycolytic (FG), or slow-twitch-oxidative (SO) (Peter *et al.* 1972). A third serial section from each sample was stained for glycogen with the periodic acid-Schiff's reagent (Pearse, 1961). Glycogen contents of at least 100 fibres of each fibre type in each cross-section were subjectively estimated under the light microscope by assigning the fibres a staining intensity of 4, 3, 2, or 1 for those with dark, moderate, light, or negative stains, respectively (Armstrong *et al.* 1977). Fibre populations were estimated by classifying 100–200 fibres in fascicles distributed throughout the muscle cross-sections, and fibre diameters were measured using a micrometer eyepiece on the microscope. Percentages of a given fibre type per cross-sectional area of each muscle, average PAS staining intensity of each fibre type in each sample, and maximal and minimal percentages of muscle cross-sectional areas showing glycogen loss were calculated as previously described (Armstrong *et al.* 1977; Sullivan & Armstrong, 1978). The average of the maximum and minimum values was then calculated and used to evaluate the changes in area showing glycogen loss. It was necessary to calculate limits and use the averages of the limits for the percentage of fibres showing glycogen loss after exercise, because there were four categories of glycogen staining intensity. For example, if after running there were fibres with no glycogen remaining (rating no. 1), there was no way of knowing if these fibres were darkly (no. 4), moderately (no. 3), or lightly (no. 2) stained before exercise. If they were darkly stained (no. 4), then a relatively low proportion of fibres would have been active, which would be indicated by the minimal value. On the other hand, if the no. 1 fibres after exercise were no. 2 fibres before, the no. 2 fibres after exercise were no. 3 fibres before, etc. a relatively high proportion of fibres would have been active during the running. The maximal value would reflect this situation. Determination of cross-sectional area of muscles in absolute terms is extremely difficult, particularly for complex pennate muscles like plantaris, gastrocnemius, and triceps brachii, long head. Therefore, to estimate the proportional contribution of individual muscles to total muscle group cross-sectional area, we used the mass of the muscles, recognizing the shortcoming of the approach.

Statistical analysis

Differences among means for glycogen concentration (biochemistry) in each muscle from the three groups were tested with a one-way analysis of variance and Tukey's *w*. Differences between means for area showing glycogen loss for fibre types within muscles and muscles from loaded and unloaded runners were tested with an independent t test.

RESULTS

When rats ran with a load equal to 24% of their body mass, stride frequency was the same and $\dot{V}_{O_2}$ was increased by 24% over that observed when they ran without the load at the same speed (Table 1). From these two measurements, we concluded that the loading increased the force exerted by the extensors by about 24%. The assumptions involved in this conclusion have been discussed previously (Taylor *et al.* 1980).

There was nearly a direct proportionality between the increase in force and the

Table 1. *Oxygen consumption and stride frequency of rats running on a treadmill with a load equalling 24 % of body mass and without a load at a speed of 30 m.min^{-1}*

	$\dot{V}_{O_2}$ (ml $O_2.g^{-1}.h^{-1}$)	Stride frequency (strides.min^{-1})
Unloaded	4·13 ± 0·07	160 ± 0·29
Loaded	5·14 ± 0·06[a]	158 ± 0·37
Change (%)	+24·0 ± 0·46	−1·3 ± 0·21

Values are the means of 74 values on 26 rats ± S.E.M.
[a] Mean for loaded rats significantly different from that for unloaded ($P < 0·01$).

Table 2. *Percentages of cross-sectional areas of the elbow and the ankle extensor muscle groups showing a loss of glycogen in rats after running at 30 m.min^{-1} with a load equal to 24 % of body mass or without a load*

(A mean value calculated by averaging the upper and lower limits is given. The limits ± S.E.M. are also given in parentheses (see Materials and Methods for discussion of limits).)

	Elbow extensors	*n*	Ankle extensors	*n*
Unloaded	40 % (36 ± 3 to 44 ± 4)	5	46 % (42 ± 5 to 50 ± 4)	7
Loaded	51 % (43 ± 6 to 59 ± 8)	6	57 % (52 ± 5 to 62 ± 7)	7
Change	+28 %		+24 %	

increase in total extensor muscle area showing loss of glycogen (Table 2). The cross-sectional area showing glycogen loss in the elbow extensor group increased by 28 %, and in the ankle extensor group by 24 %. Forty per cent of the cross-sectional area of the elbow extensor group showed a loss of glycogen when the rats ran without a load, which increased to 51 % when they carried the load. Forty-six per cent of the ankle extensor group showed a loss when the animals ran without a load. This increased to 57 % in the loaded animals.

Most of the increase in cross-sectional area showing glycogen loss occurred in the peripheral muscles of each group (Table 3). In the elbow extensor group, 91 % of the increase in cross-sectional area occurred in the lateral and long heads, and only 9 % in the deep medial head (triceps brachii muscle). FG fibres contributed 84 % of the increase, FOG fibres 10 %, and SO fibres 6 % (Table 4). In the ankle extensor group, 84 % of the increase in cross-sectional area occurred in the more peripheral gastrocnemius muscle. The more medial plantaris and soleus muscles accounted for 9 and 7 % of the increase. FG fibres contributed 88 % of the increase in cross-sectional area in the ankle extensor group, FOG fibres 6 %, and SO fibres 6 %.

Muscle glycogen concentrations of the control and exercised rats are presented in Table 5. Significant decreases in muscle glycogen in the loaded over the unloaded condition occurred in L and Ln in the elbow extensor muscle group, and in G in the ankle extensor group. These are the same muscles that had large increases in the area of FG fibres showing glycogen loss in the loaded rats.

Table 3. *Percentages of cross-sectional areas of the individual muscles comprising the elbow and the ankle extensor groups showing a loss of glycogen in rats after running at 30 m.min^{-1} with a load equal to 24 % of their body mass or without a load*

(A mean value calculated by averaging the upper and lower limits is given. The limits ± S.E.M. are also given in parentheses (see Materials & Methods for discussion of limits).)

Elbow extensor group (triceps brachii muscle)

	Medial head (mass = 8 % of group)	*n*	Lateral head (mass = 18 % of group)	*n*	Long head (mass = 74 % of group)	*n*
Unloaded	65·5 % (60 ± 10 to 71 ± 9)	5	47·5 % (42 ± 7 to 53 ± 9)	6	35·5 % (32 ± 4 to 39 ± 4)	5
Loaded	83·0 % (80 ± 9* to 86 ± 9)	6	70·5 % (62 ± 10* to 79 ± 7*)	7	43·0 % (35 ± 6 to 51 ± 10)	7
Change	+27 %		+48 %		+21 %	
Relative contribution of each muscle to increase in cross-sectional area of total muscle group	9 %		33 %		58 %	

Ankle extensor group

	Soleus (mass = 6 % of group)	*n*	Plantaris (mass = 16 % of group)	*n*	Gastrocnemius (mass = 78 % of group)	*n*
Unloaded	63·5 % (60 ± 8 to 67 ± 8)	7	52·5 % (47 ± 8 to 58 ± 9)	7	43·5 % (40 ± 5 to 47 ± 4)	7
Loaded	76·0 % (73 ± 6 to 79 ± 6)	7	59·0 % (54 ± 9 to 64 ± 11)	7	55·0 % (50 ± 6* to 60 ± 8*)	7
Change	+20 %		+12 %		+26 %	
Relative contribution of each muscle to increase in cross-sectional area of total muscle group	7 %		9 %		84 %	

* Means of loaded rats significantly larger than those from unloaded rats ($P < 0{\cdot}05$).

Table 4. *Relative contributions of FOG, FG, and SO fibres to the increase in cross-sectional area of muscle groups and individual muscles within groups showing a loss of glycogen after rats ran with a load equal to 24 % of body mass compared to rats running without a load at a speed of 30 m.min^{-1}*

	Fibre type		
	FG	FOG	SO
Elbow extensor group	84 %	10 %	6 %
Medial head of triceps	14 %	22 %	64 %
Lateral head of triceps	93 %	7 %	0 %
Long head of triceps	89 %	10 %	1 %
Ankle extensor group	88 %	6 %	6 %
Soleus	0 %	4 %	96 %
Plantaris	98 %	2 %	0 %
Gastrocnemius	88 %	6 %	6 %

Table 5. *Muscle glycogen concentrations of control rats and rats after running with and without loads equalling 24 % of body mass*

Condition	Muscle M	L	Ln	S	P	RG‡	WG‡
Control	20·4	26·5	25·2	28·1	26·6	28·8	30·9
	±4·4	±3·2	±3·4	±3·9	±2·5	±3·0	±1·9
	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Unloaded	4·3*	13·4*	20·6	8·0*	16·5*	8·9*	28·2
	±1·3	±1·3	±2·8	±1·7	±1·6	±2·7	±1·2
	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Δ %	−79	−49	−18	−72	−38	−69	−9
Loaded	4·0*	8·9*†	12·7*†	8·8*	12·6*	5·4*	23·6*†
	±1·8	±2·0	±1·9	±1·5	±1·8	±2·0	±0·8
	(9)	(9)	(9)	(9)	(9)	(9)	(9)
Δ %	−80	−66	−50	−69	−53	−81	−24

Values are given in mmoles glucose units × kg^{-1} wet weight and represent means ± S.E.M. Numbers of animals are included in parentheses.

* Mean for exercised animals significantly lower than for control rats ($P < 0.05$).

† Mean for loaded animals significantly lower than for unloaded rats ($P < 0.05$).

‡ For biochemistry gastrocnemius muscle was divided into a deep red portion composed primarily of FOG and SO fibres (RG) and a peripheral white portion composed of FG fibres (WG).

DISCUSSION

Patterns of recruitment of muscles and muscle fibres

The additional cross-sectional area of muscle recruited to provide the higher forces in the loaded rats came primarily from the more peripheral muscles of the extensor group. This pattern of peripheral recruitment is similar to that observed when animals increase force as they increase locomotory speed (Smith *et al.* 1977; Sullivan & Armstrong, 1978; Walmsley *et al.* 1978) or jump (Smith *et al.* 1977). Thus, it appears to be a general pattern. Most of the increase in cross-sectional area in the elbow extensor group of the loaded rats was in the more peripheral long and lateral heads of the triceps brachii muscle and there was only a small contribution made by the medial head. The situation in the ankle extensor group was the same, with the peripheral gastrocnemius muscle being primarily responsible for the increase in cross-sectional area.

There is a deep to peripheral organization of fibres within the extensor muscles (Sullivan & Armstrong, 1978). Most deep fibres of these muscles are SO or FOG, and the more peripheral, FG. Thus, the addition of primarily FG fibres when the rats were loaded indicates that fibres within muscles, like muscles within groups, are also recruited peripherally with increasing force.

The results of these experiments emphasize the importance of including whole muscle groups rather than individual muscles, or small samples (e.g. biopsy samples) from individual muscles, when studying either muscular recruitment patterns during exercise or the acute or chronic effects of exercise on muscles. If we had utilized muscle samples taken by biopsy instead of sectioning the entire cross-sectional area of a group, our findings would have varied enormously depending on which muscles the biopsy came from, or whether it was obtained from a deep or peripheral area within the muscle.

Significance of the direct proportionality between force and cross-sectional area of muscle showing a loss of glycogen

The direct proportionality observed between the increase in muscular force and the increase in the cross-sectional area of muscles showing a loss of glycogen is intriguing. The simplest explanation is that the cross-sectional area showing glycogen loss is a direct measure of the cross-sectional area of muscle fibres that were active. Area of active muscle then increased in direct proportion to the force exerted by the muscle. This would require that muscle lengths, shortening and lengthening velocities, and motor unit discharge frequencies were approximately the same in the loaded and unloaded rats. In our experiments the animals ran the same velocity and had the same stride frequency (indicating they also had the same accelerations of their centre of mass) when they ran with the load and without the load. The extensor muscular forces in the loaded condition should have increased proportionately in all phases of the contractile cycle, i.e. during the active lengthening, shortening, and static phases. In other words, the forces required for positive and negative accelerations of the centre of mass, as well as for isometric support, should have increased by 24 % in the loaded rats. Also, it seems reasonably certain that muscle lengths and velocities of shortening and lengthening were the same in the loaded and unloaded animals (Taylor *et al.* 1980).

A second, more complex interpretation of our results could be that the direct proportionality between force and cross-sectional area showing glycogen loss was fortuitous and does not represent a direct proportionality between force and cross-sectional area of active fibres. This would be the case if the muscular force in the loaded rats was increased by increasing discharge frequencies of the same muscle motor units that were active when rats ran without loads. Stein (1974) pointed out in his review of peripheral control of locomotion that the relative importance of motor unit recruitment and increased frequency of discharge (rate coding) remains controversial. If the increase in force was accomplished solely by rate coding, it would be possible to explain our results by postulating that cycling among motor units increased in direct proportion to force. Cycling could be due either to fatigue of active motor units or a neural control mechanism.

Several indirect lines of evidence argue against fatigue-induced cycling of units under the conditions that were employed in these experiments. Since the treadmill exercise was of relatively low intensity (Shepherd & Gollnick, 1976) and only lasted for 10 min, it is doubtful if significant fatigue of motor units occurred. This would be particularly true in the unloaded condition when primarily high-oxidative fibres were recruited to produce the required muscular forces (Armstrong *et al.* 1977; Armstrong *et al.* 1974; Baldwin *et al.* 1973; Sullivan & Armstrong, 1978). Also, in rats running at low to moderate speeds, most of the glycogen is lost during the first minutes of exercise, with little further loss occurring (Armstrong & Ianuzzo, 1977; Baldwin *et al.* 1973). For example, in rats running at 23 $m.min^{-1}$ up a 5° incline, significant glycogen loss occurred in the muscles in the first 10 min, but no further loss was observed over the next 20 min (Armstrong & Ianuzzo, 1977). If significant cycling of units continued because of fatigue, one would expect a steady decline in muscle glycogen stores as new units were recruited.

Evidence against motor unit cycling as a normal neural mechanism is not as clear. However, the common observation (Armstrong *et al.* 1977; Armstrong *et al.* 1974; Gollnick *et al.* 1973; Sullivan & Armstrong, 1978) that some fibres of a given type are devoid of glycogen after exercise, whereas others of the same type apparently have lost no glycogen, would suggest that a certain number of motor units within types are recruited during rhythmic exercise and the others are not. Also, the concept of the 'size principle' (Henneman & Olsen, 1965) would support the supposition that specific motor units are recruited for generation of force as long as fatigue or other limiting factors do not interfere.

One of the most obvious ways of experimentally deciding between these two explanations of the direct proportionality between force and cross-sectional area showing depletion would be to investigate the relationship over a greater range of forces. This could be accomplished either by having rats run while varying the load or by varying the speed while the rat ran with the same load. While theoretically appealing, these experiments are not feasible because of the variability inherent in the glycogen loss technique. We selected our experimental conditions so that the load and speed were maximal. Still we were barely able to move out of the experimental noise. Lower loads and/or speeds accentuate the problems of noise in the technique because less glycogen is lost by the fibres.

In the absence of any evidence to the contrary, we prefer the simple explanation of our results.

The authors wish to thank Ms Pat Marum and Ms Carolyn Johnson for their assistance in the collection and analysis of the data. The study was supported by NIH Grants AM25472 and AM18140.

REFERENCES

ALEXANDER, R. McN. (1977). Terrestrial locomotion. In *Mechanics and Energetics of Terrestrial Locomotion* (ed. R. McN. Alexander and G. Goldspink). pp. 168–203. London: Chapman and Hall.

ARMSTRONG, R. B. & IANUZZO, C. D. (1977). Exercise-induced muscle glycogen depletion and repletion in diabetic rats. *Life Sci.* **20**, 301–308.

ARMSTRONG, R. B., MARUM, P., SAUBERT IV, C. W., SEEHERMAN, H. J. & TAYLOR, C. R. (1977). Muscle fiber activity as a function of speed and gait. *J. appl. Physiol.: Respirat. Environ. Exercise Physiol.* **43**, 672–677.

ARMSTRONG, R. B. & PETERSON, D. F. (1981). Patterns of glycogen loss in muscle fibers: response to arterial occlusion during exercise. *J. appl. Physiol.: Respirat. Environ. Exercise Physiol.* **51**, 552–556.

ARMSTRONG, R. B., SAUBERT IV, C. W., SEMBROWICH, W. L., SHEPHERD, R. E. & GOLLNICK, P. D. (1974). Glycogen depletion in rat skeletal muscle fibers at different intensities and durations of exercise. *Pflügers Arch. ges. Physiol.* **352**, 243–256.

BALDWIN, K., REITMAN, J. S., TERJUNG, R. L., WINDER, W. W. & HOLLOSZY, J. O. (1973). Substrate depletion in different types of muscle and in liver during prolonged running. *Am. J. Physiol.* **225**, 1040–1050.

CAVAGNA, G. A., HEGLUND, N. C. & TAYLOR, C. R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243–R261.

GOLLNICK, P., ARMSTRONG, R. B., SAUBERT IV, C. W., SEMBROWICH, W. L., SHEPHERD, R. E. & SALTIN, B. (1973). Glycogen depletion patterns in human skeletal muscle fibers during prolonged work. *Pflügers Arch. ges. Physiol.* **244**, 1–12.

HENNEMAN, E. & OLSEN, C. B. (1965). Relations between structure and function in the design of skeletal muscles. *J. Neurol.* **28**, 581–598.

NOVIKOFF, A. B., SHIN, W. & DRUCKER, J. (1961). Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. *J. biophys. biochem. Cytol.* **9**, 47–61.

PADYKULA, H. A. & HERMAN, E. (1955). The specificity of the histochemical method of adenosine triphosphatase. *J. Histochem. Cytochem.* **3**, 170–195.

PEARSE, A. G. E. (1961). Histochemistry, Theoretical and Applied, Boston, Mass.: Little, Brown, p. 832.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1972). Metabolic profiles of three types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11**, 2627–2633.

SEIFTER, S., DAYTON, S., NOVIC, B. & MUNTWYLER, E. (1950). The estimation of glycogen with the anthrone reagent. *Archs Biochem.* **25**, 191–200.

SHEPHERD, R. E. & GOLLNICK, P. D. (1976). Oxygen uptake of rats at different work intensities. *Pflügers Arch. ges. Physiol.* **362**, 219–222.

SMITH, J. L., EDGERTON, V. R., BETTS, B. & COLLATOS, T. C. (1977). EMG of slow and fast ankle extensors of cat during posture, locomotion, and jumping. *J. Neurophysiol.* **40**, 503–513.

STEIN, R. B. (1974). Peripheral control of movement. *Physiol. Rev.* **54**, 215–243.

SULLIVAN, T. E. & ARMSTRONG, R. B. (1978). Rat locomotory muscle fiber activity during trotting and galloping. *J. appl. Physiol.: Respirat. Environ. Exercise Physiol.* **44**, 358–363.

TAYLOR, C. R., HEGLUND, N. C., MCMAHON, T. A. & LOONEY, T. R. (1980). Energetic cost of generating muscular force during running: a comparison of large and small animals. *J. exp. Biol.* **86**, 9–18.

TUCKER, V. A. (1968). Respiratory exchange and evaporative water loss in the flying budgerigar. *J. exp. Biol.* **48**, 67–87.

WALMSLEY, B., HODGSON, J. A. & BURKE, R. E. (1978). Force produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J. Neurophysiol.* **41**, 1203–1216.