J. Physiol. (1974), **241**, *pp.* 59–67
With 3 plates
Printed in Great Britain

SELECTIVE GLYCOGEN DEPLETION IN SKELETAL MUSCLE FIBRES OF MAN FOLLOWING SUSTAINED CONTRACTIONS

By P. D. GOLLNICK,* J. KARLSSON, KARIN PIEHL AND B. SALTIN*

From the Department of Physiology, Gymnastik-och idrottshögskolan, Stockholm, Sweden

(*Received 12 November 1973*)

SUMMARY

1. Six healthy males performed sustained contractions with different tensions related to their maximal voluntary contraction (MVC). The isometric exercise consisted of efforts to extend the knee when flexed at an angle of 90°.

2. Biopsy samples were taken from the lateral portion of M. quadriceps femoris before and after different periods (6–45 min) during a series of sustained contractions. Total glycogen content was determined on each muscle sample. In order to evaluate whether the glycogen depletion occurred preferentially in slow twitch (ST) or fast twitch (FT) fibres, serial sections of the muscle samples were stained for myofibrillar ATPase and glycogen (PAS reaction).

3. In all experiments a selective glycogen depletion was observed. At low tensions, the ST fibres and at higher tensions the FT fibres became glycogen depleted. The critical tension at which this conversion in glycogen depletion from ST to FT fibres took place was 20 % MVC.

4. It is concluded that at sustained contractions of less than 20 % MVC there is a major reliance upon ST fibres and above that level a primary dependence upon FT fibres. It is further suggested that restriction of blood flow and thus availability of oxygen at forces higher than 20 % MVC may be the explanation for the present findings.

INTRODUCTION

Static muscular contractions representing small (5–10) percentages of maximal voluntary contractile strength (MVC) can be maintained for

* Present addresses: P. D. Gollnick, Department of Physical Education for Men, Washington State University, Pullman, Washington 99163, U.S.A. B. Saltin, August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark.

long periods of time. However, there is a disproportionate decline in isometric endurance time as tension increases (Monod & Scherrer, 1957; Rohmert, 1960). This decline in endurance time appears to occur before the point of complete circulatory occlusion (Barcroft & Millen, 1939; Edwards, Hill & McDonnell, 1972). Such an effect could result from a difference in the contractile and/or metabolic characteristics of the motor units activated to produce increases in isometric tension. Glycogen depletion patterns in human muscle fibres during bicycle exercise point to differences in the characteristics of the motor units activated in submaximal as opposed to supramaximal exercise (Gollnick, Piehl & Saltin, 1974). Changes in muscle lactate levels during isometric exercise at different forces may also indicate a shifting pattern of motor unit recruitment.

The present study was undertaken to examine the glycogen depletion pattern in human skeletal muscle fibres following varying isometric exercise regimens in an attempt to obtain additional information about the contractile and metabolic events associated with this type of muscular activity.

METHODS

Subject and protocol

Six male physical education students served as subjects for the experiments. Before the experiments, MVC was determined for knee extension at a 90° angle using the strain gauge technique (Karlsson & Ollander, 1972). In the weeks before the experiment, subjects were also given an opportunity to practise maintaining different isometric tensions for varying periods of time. The subjects were fully informed about the procedures involved in the experiments and they had all given an oral consent before any biopsies were taken.

At glycogen concentrations less than 80–90 m-mole glucose units.kg^{-1}, changes in the glycogen content of muscle fibres are discernible using histochemical techniques (Gollnick, Piehl, Saubert IV, Armstrong & Saltin, 1972*b*; Piehl, 1974). The following procedure was employed in order to ensure that the present subjects had muscle glycogen stores below this critical level. On the day before the isometric exercise experiments, subjects exercised on the bicycle ergometer for 1 hr at a workload corresponding to 60–70 % of their maximal oxygen uptakes. Subjects were then asked to limit their intake of carbohydrate-rich foods so as to maintain relatively low muscle glycogen levels until the following day.

On the day of the experiment, the MVC was determined. The variation in MVC from day to day was within 5 %. Samples were thereafter taken from the lateral portion of the quadriceps muscle using the needle biopsy technique (Bergström, 1962). Subjects then performed the isometric contractions for that particular day. The experiment included repeated contractions at forces of 10, 15 and 20 % MVC maintained to exhaustion; 25, 30, 35 and 40 % MVC (up to 30 times) maintained to exhaustion (45 sec to several minutes) with 1–2 min rest periods between sessions; 15 and 20 % MVC performed intermittently for 10 sec with 10 sec rest periods for up to 90 min; and 60 contractions at 40 and 50 % MVC with tensions maintained for 20 sec with 40 sec rest periods. The exercise was interrupted for only 5–10 sec in order to take the biopsies during sustained contractions. All exercise programmes led

to exhaustion. No subject participated in more than three experiments with at least 1 week elapsing between each experiment.

The muscle samples were divided into two parts. One part of the sample was quickly frozen in liquid nitrogen and stored at $-80°$ C until subsequently analysed for glycogen content (Karlsson, Diamant & Saltin, 1970). The rest of the sample was prepared for histochemical analysis. Sections were cut in a cryostat at $-20°$ C. Staining intensity for myofibrillar ATPase activity was determined using 10 μm thick sections at a pH of 9·4 after pre-incubation at a pH of 10·3 (Padykula & Herman, 1955). Serial sections 16 μm thick were stained for glycogen employing the periodic acid-Schiff (PAS) reaction (Pearse, 1961). The relative glycogen content in individual muscle fibres was estimated on the basis of PAS staining intensity, as described by Gollnick *et al.* (1972*b*). The muscle fibres were identified as fast twitch (FT) and slow twitch (ST) on the basis of heavy and light myofibrillar ATPase staining respectively. The rationale for this nomenclature has been discussed elsewhere (Gollnick, Armstrong, Saubert IV, Piehl & Saltin, 1972*a*).

RESULTS

Muscle lactate at rest averaged 2·3 m-mole.kg^{-1} wet wt. Only a small increase in muscle lactate occurred during intermittent exercise at all the tensions studied. This was also true of the lowest tensions (10 or 15% MVC) when continuously sustained to exhaustion. Major increases in muscle lactate were observed at isometric tensions calling for 20% of MVC or more when continuously maintained to exhaustion. The peak value of 18·7 m-mole.kg^{-1} wet wt. was observed at a tension of 25% MVC. Muscle lactate was lower at the highest tension studied and averaged 15·0 m-mole.kg^{-1}.

At rest, the glycogen content of the muscle samples averaged 48 m-mole glucose units.kg^{-1} wet wt. This value was 20–30 m-mole.kg^{-1} less than values found in a similar population of healthy young men (Karlsson *et al.* 1970; Gollnick *et al.* 1972*b*). The difference is attributable to the previous day's exercise and the limited intake of carbohydrate in the 18–20 hr preceding the experiment. The rather low glycogen content was also histochemically discernible on the basis of over-all lighter PAS staining of the sections. However, all fibres were stained, even though it was evident in these conditions that FT fibres stained more darkly than ST fibres (Pl. 1). A 10–30 m-mole glycogen depletion was observed after isometric exercise. Values for several individual experiments are presented in the legends for Pls. 1–3. A final glycogen concentration less than 19 m-mole glucose units.kg^{-1} wet muscle was not found in any case.

At isometric tensions of less than 20% MVC, there was preferential glycogen depletion in ST fibres (Pl. 1). This pattern was constant, irrespective of the exercise pattern or whether or not exercise was maintained to exhaustion. There was no apparent reduction in PAS staining in FT fibres under these conditions (Pl. 1). At a tension of 20% MVC or more,

FT fibres became PAS negative with no change in the staining intensity of ST fibres (Pl. 2). This was observed after only 10 min exercise at a tension of 20 % MVC and after 6 min of exercise at 25 % MVC when exercise was performed intermittently (10 sec of contraction with 10 sec of rest). At a tension of 40 % MVC and performed with several exhaustive contractions (40–60 sec duration with 1 min rest intervals), some FT fibres became PAS negative after fifteen contractions (about 10 min of continuous contraction). After thirty such exhaustive contractions at 40 % MVC, all FT fibres were PAS negative, whereas the staining intensity of ST fibres did not differ significantly from its pre-exercise appearance (Pl. 3).

DISCUSSION

The general pattern in the endurance sessions examined in the present study was similar to the pattern previously reported by several laboratories (Monod & Scherrer, 1957; Rohmert, 1960; Karlsson & Ollander, 1972). In absolute terms, however, the low tensions were maintained for shorter periods of time than is commonly reported. This was most probably due to sub-normal, initial muscle glycogen levels as a result of the previous day's bicycle exercise and the reduced carbohydrate consumption. Thus, a relationship appears to exist between the initial glycogen content of muscle and performance in static work.

The principal question arising from the present results is: how is the glycogen depletion pattern in muscle fibres at different isometric tensions to be interpreted? Even though the present experiment provided no direct answer, some insight may be derived from existing knowledge on the physiological properties of the different fibre types and the mode of motor unit recruitment. Thus, it is known that peak tensions are not significantly different per unit of muscle, even though slow-contracting muscles develop tension more slowly than fast-contracting muscles (Close, 1967). While developing and maintaining tension, fast-contracting muscles also consume more ATP than slow-contracting muscles (Goldspink, Larson & Davies, 1970). In muscles composed of different fibre types, i.e. most mammalian muscles, motor units are composed of the same fibre type (Edström & Kugelberg, 1968). Control of the motor units is based on differences in the activation thresholds of the fibres' afferent motor nerves (Henneman & Olson, 1965). As a general rule, fibres with a high oxidative capacity are contained in motor units with low activation thresholds (Close, 1972). However, a continuum of thresholds for motor unit activation undoubtedly exists within each fibre type (Burke, 1968; Engel, 1970). Differential rates of glycogen depletion within a given fibre type, both during isometric (see Pls. 1–3) and dynamic exercise (Gollnick *et al.* 1974),

also support the concept of motor units with different activation thresholds. Although this general information about the metabolic and contractile properties of skeletal muscle is primarily derived from studies using animal muscle, it appears reasonable to assume that the general findings are also applicable to human skeletal muscle. An attempt will be made to interpret current findings within this general context.

At low isometric tensions (less than 20 % MVC) only ST fibres were depleted of their glycogen. It seems reasonable to assume that the glycogen-depleted fibres had been active in the exercise, since there is no other major metabolic pathway for the elimination of glycogen in skeletal muscle. Thus, low isometric tensions were most probably maintained via contraction of ST fibres. Since FT fibres were more darkly glycogen-stained at rest, it may be argued that FT fibres had also been active but that they retained PAS staining due to their higher initial glycogen content. It should be emphasized, however, that there was no change in the PAS staining of FT fibres, even after nearly 30 min of sustained isometric tension. Moreover, the chemical determination of total glycogen content only demonstrated a slight (18 m-mole.kg^{-1}) decline. Thus, the concept of continuous FT fibre activation seems unlikely, as FT fibres became PAS negative at higher tensions after a much shorter exercise period, even though they initially displayed PAS staining which was darker than ST fibre staining. FT fibres may have been activated at the onset of the contraction when tension-development speed was high. FT fibres may have been inactivated when the tension produced by ST fibres became sufficient to sustain the contraction. Some evidence has been published indicating a shift in the recruitment pattern in human skeletal muscle early in contraction (Grimby & Hannerz, 1968; Milner-Brown, Stein & Yemn, 1973).

FT fibres were the only fibres to become PAS negative at isometric tensions calling for 20 % of MVC or more. This points to an activation of motor units containing these fibres. At a tension of 20 % MVC, only a few FT fibres became PAS negative, suggesting that only a small number of motor units with these fibres may have been recruited at this tension. Since these fibres probably rely on anaerobic metabolism during prolonged contraction and also consume ATP more quickly than ST fibres at similar tensions, they probably consumed glycogen at an accelerated rate. In this context, it may be worth while noting that there is a significant increase in both muscle and blood lactate at an isometric tension of 25 % of MVC. This would be consistent with the activation of FT fibres.

Nearly all FT fibres were found to PAS negative in muscle samples taken after 30 contractions lasting 30–45 sec at 40 % MVC. By contrast, ST fibres retained PAS staining similar to the staining found in samples taken at rest. Thus, it would appear that all FT fibres had been active.

Since the tension developed by contracting muscle is proportional to the number of motor units contracting, one wonders why FT fibres are recruited at such low relative tensions if they really are the most difficult fibres to activate. Thus, if 50 % of a muscle consists of ST fibres (the percentage indicates the area occupied by a fibre in a muscle (Gollnick *et al.* 1972*a*)), why are these fibres alone unable to sustain tensions of about 50 % of MVC? The answer may be related to the speed of tension development. Thus, multiple contractions at relative high tensions may require the recruitment of a large number of fast motor units during the early stage of each contraction. Part of the answer may also lie in the effect of the different tensions on the muscle's afferent blood flow. For example, Barcroft & Millen (1939) reported that blood flow to the calf muscle might be restricted at isometric tensions corresponding to, and greater than, 20 % MVC, and this conclusion was also reached, after studies of the quadriceps muscle, by Edwards *et al.* (1972). Regional differences may exist within a muscle or between different muscle groups as there are results indicating that some blood flow may continue with tensions of 60 % MVC or more in the forearm (Humphreys & Lind, 1963). It is uncertain whether changes in blood flow and oxygen transport contribute to the current results on glycogen depletion patterns in fibres at different tensions. However, if they do, static and dynamic exercise display a similar response.

As mentioned above, the continuous recruitment of FT fibres from the onset of dynamic exercise appears to be related to whether or not the workload requires an energy expenditure exceeding the individual's maximal aerobic power. Thus, an important factor in the recruitment of FT fibres may be the relative hypoxia present in the muscle in supra-maximal dynamic exercise or in static efforts when blood flow is either restricted or occluded. It should be noted that there appears to be a difference when static and dynamic exercise are compared. In very intense bicycle exercise, ST fibres are depleted of glycogen as expected. This does not occur in isometric contractions greater than about 20 % of MVC. This suggests that ST fibres are not recruited to the same extent in the two experimental circumstances. The regulatory mechanisms involved in recruitment of ST fibres are unknown. Thus, large amounts of oxygen are consumed during high-intensity dynamic exercise. This probably takes place primarily in ST fibres through the oxidation of glycogen. Conversely, blood flow may be greatly restricted or completely absent during high-tension static exercise. In these circumstances, the low anaerobic capacity of ST fibres should result in slow glycogen depletion. The contribution made by ST fibres to exercise in such conditions cannot be assessed in the present experiments.

In summary, a selective glycogen depletion pattern indicative of a

differential recruitment of muscle fibres can be said to occur in isometric exercise of varying intensity (% MVC). The finding of a major reliance on ST fibres up to exercise intensities of 20 % MVC, followed by apparent dependence on FT fibres, was of special note. The present results also provide a basis for greater understanding of the reasons why muscle lactate accumulation is more prominent at isometric tensions exceeding 20 % MVC.

This study was supported by grants from the Swedish Medical Research Council (40X–2203) and the Research Council of the Swedish Sports Federation. P. D. Gollnick was the recipient of Visiting Professorship supported by the Swedish Medical Research Council (14V–4014).

REFERENCES

BARCROFT, H. & MILLEN, J. L. E. (1939). The blood flow through muscle during sustained contractions. *J. Physiol.* **97**, 17–31.

BERGSTRÖM, J. (1962). Muscle electrolytes in man. *Scand. J. clin. Lab. Invest.* suppl. 68.

BURKE, R. E. (1968). Firing patterns of gastrocnemius motor units in the decerebrate cat. *J. Physiol.* **196**, 631–654.

CLOSE, R. (1967). Properties of motor units in fast and slow skeletal muscles of the rat. *J. Physiol.* **193**, 45–55.

CLOSE, R. (1972). Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* **52**, 129–197.

EDSTRÖM, L. & KUGELBERG, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. *J. Neurol. Neurosurg. Psychiat.* **31**, 424–433.

EDWARDS, R. H. T., HILL, D. K. & MCDONNELL, M. (1972). Myothermal and intramuscular pressure measurements during isometric contractions of the human quadriceps muscle. *J. Physiol.* **224**, 58–59*P*.

ENGEL, W. K. (1970). Selective and nonselective susceptibility of muscle fiber types. *Archs Neurol. Psychiat., Chicago* **22**, 97–117.

GOLDSPINK, G., LARSON, R. E. & DAVIES, R. E. (1970). The immediate energy supply and the cost of maintenance of isometric tension for different muscles in the hamster. *Z. vergl. Physiol.* **66**, 389–397.

GOLLNICK, P. D., ARMSTRONG, R. B., SAUBERT IV, C. W., PIEHL, K. & SALTIN, B. (1972*a*). Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *J. appl. Physiol.* **33**, 312–319.

GOLLNICK, P. D., PIEHL, K. & SALTIN, B. (1974). Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J. Physiol.* **241**, 45–57.

GOLLNICK, P. D., PIEHL, K., SAUBERT IV, C. W., ARMSTRONG, R. B. & SALTIN, B. (1972*b*). Diet, exercise, and glycogen changes in human muscle fibers. *J. appl. Physiol.* **33**, 421–425.

GRIMBY, L. & HANNERZ, J. (1968). Recruitment order of motor units on voluntary contraction: changes induced by proprioceptive afferent activity. *J. Neurol. Psychiat., Lond.* **31**, 565–573.

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

HUMPHREYS, P. W. & LIND, R. A. (1963). The blood flow through active and inactive muscles of the forearm during sustained hand-grip contractions. *J. Physiol.* **166**, 120–135.

KARLSSON, J., DIAMANT, B. & SALTIN, B. (1970). Muscle metabolites during submaximal and maximal exercise in man. *Scand. J. clin. Lab. Invest.* **26**, 385–394.

KARLSSON, J. & OLLANDER, B. (1972). Muscle metabolites with exhaustive static exercise of different duration. *Acta physiol. scand.* **86**, 309–314.

MILNER-BROWN, H. S., STEIN, R. & YEMN, R. (1973). The orderly recruitment of human motor units during voluntary isometric contractions. *J. Physiol.* **230**, 359–370.

MONOD, H. & SCHERRER, J. (1957). Capacité de travail statique d'un groupe musculaire synergique chez l'Homme. *C. r. Seanc. Soc. Biol.* **151**, 1358–1361.

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*, Appendix 9, p. 832. Boston, Mass: Little Brown.

PIEHL, K. (1974). Time course for refilling of glycogen stores in human muscle fibres following exercise-induced glycogen depletion. *Acta physiol. scand.* **90**, 297–302.

ROHMERT, W. (1960). Determination for the recovery pause for static work of man. *Int. Z. angew. Physiol.* **18**, 123–164.

EXPLANATION OF PLATES

PLATE 1

All micrographs are serial sections (× 120) of the vastus lateralis muscle stained for myofibrillar ATPase (*A*) and glycogen (PAS) (*B*); sections stained for ATPase activity show ST and FT fibres as light or dark respectively.

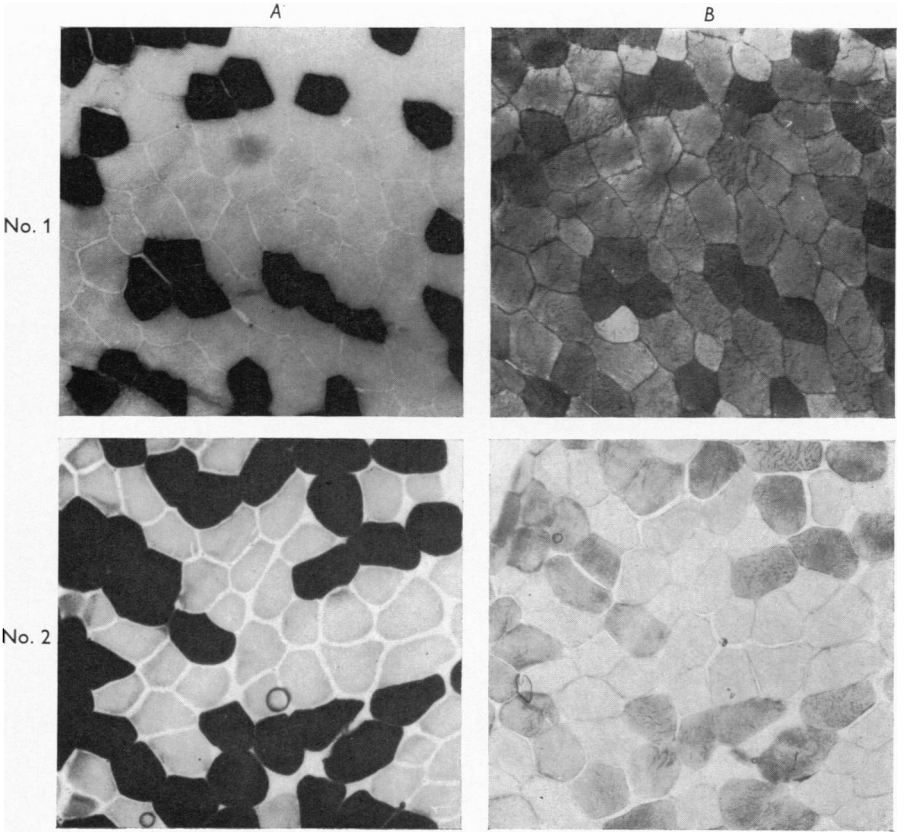
Section 1 (*A* and *B*) illustrates the general appearance of PAS staining at rest. Muscle glycogen content was 63 m-mole glucose units.kg^{-1} wet wt. FT fibres are clearly more darkly stained than ST fibres indicating a higher glycogen content.

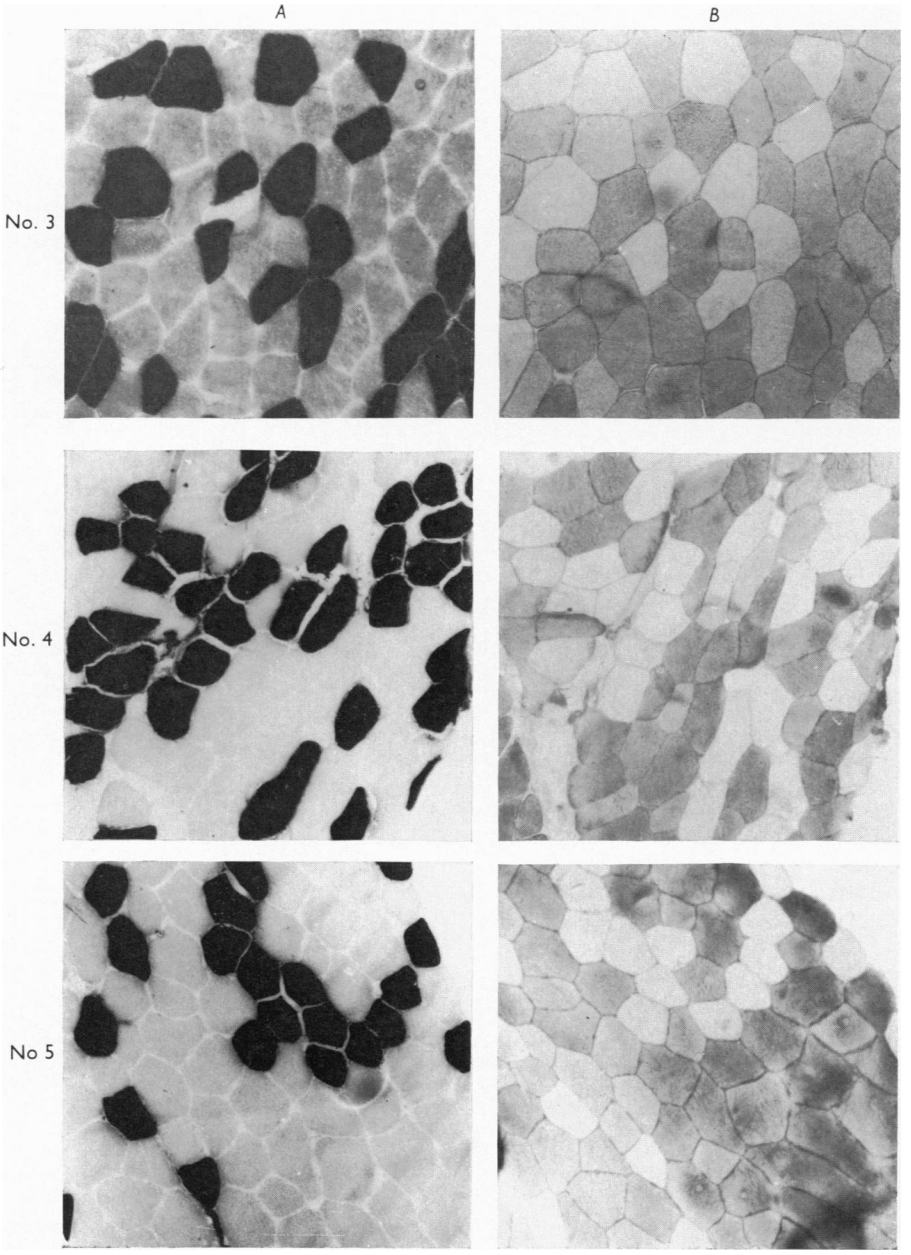
Section 2 shows the PAS staining pattern (2*B*) in muscle fibres having maintained continuous isometric tension amounting to 15 % MVC for 26 min. At this point the subjects were unable to sustain the contraction. These sections demonstrate glycogen depletion only in ST fibres with no apparent changes in the PAS staining intensity of FT fibres. Glycogen was reduced by 19 m-mole glucose units.kg^{-1} wet wt. (from 54 to 33) during exercise.

PLATE 2

Micrographs 3 (*A* and *B*) demonstrate the PAS staining pattern (3*B*) in the fibres of a muscle having maintained continuous isometric tension amounting to 20 % MVC for 15 min. Glycogen content declined by 10 m-mole glucose units.kg^{-1} in the exercise. This depletion apparently occurred in FT fibres only.

Micrographs 4 and 5 (*A* and *B*) are sections of muscle samples obtained after successive isometric exercise sessions at a tension of 35 % MVC maintained until exhaustion with 2 min inter-session rest periods. Section 4 is from a sample taken after ten sessions. These micrographs show preferential glycogen depletion from FT fibres. However, some FT fibres are still dark, perhaps indicating differential recruitment of motor units of FT fibres. Section 5 shows that all FT fibres were PAS negative after twenty exhaustive sessions of contractions at 35 % MVC, whereas there were few discernible changes in the PAS staining intensity of ST fibres. These changes are particularly dramatic in view of the fact that FT fibres were initially far more darkly stained than ST fibres. The glycogen content of the final muscle sample amounted to 25 m-mole glucose units.kg^{-1}.


A
B
No. 1
No. 2


A
B
No. 3
No. 4
No 5

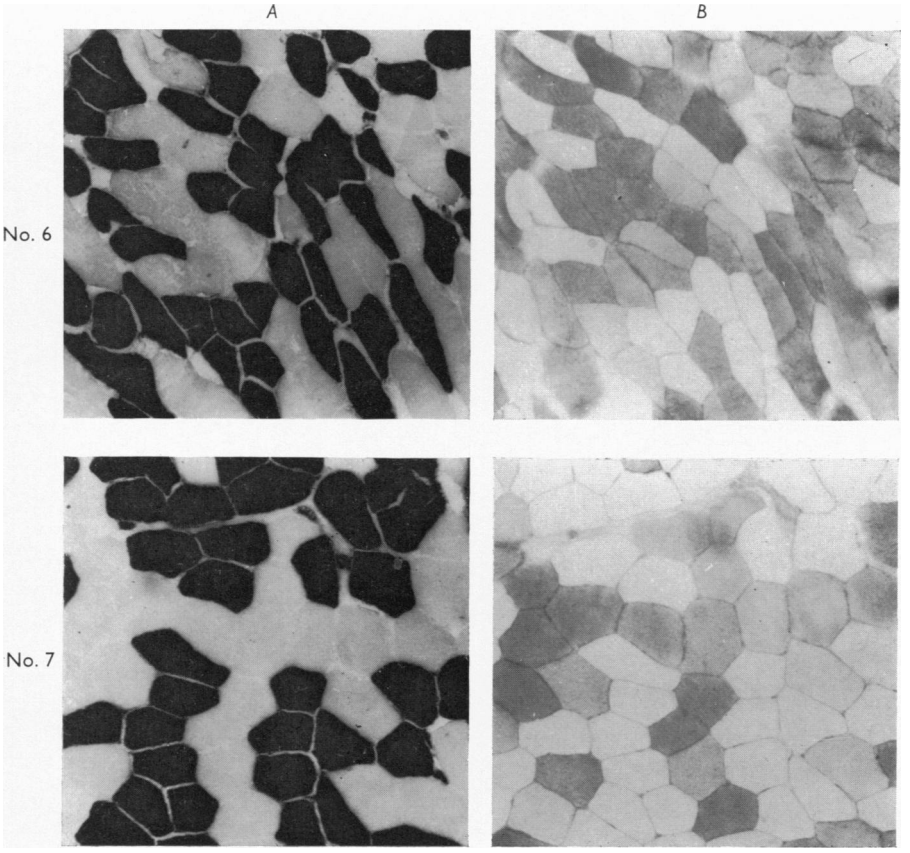

A
B
No. 6
No. 7

PLATE 3

Micrographs 6 and 7 (*A* and *B*) are a series of samples taken after repeated isometric contractions at a tension of 40 % MVC. One min rest periods were allowed between the exhaustive sessions. These micrographs also show differential rate of glycogen depletion in FT fibres after fifteen exercise sessions (6). However, all FT fibres were PAS negative after thirty sessions. As was the case at 35 % MVC, no change in PAS staining occurred in ST fibres. The glycogen content after the last exercise session amounted to 19 m-mole glucose units.kg^{-1} wet muscle.