SELECTIVE GLYCOGEN DEPLETION
PATTERN IN HUMAN MUSCLE FIBRES AFTER EXERCISE
OF VARYING INTENSITY AND AT VARYING
PEDALLING RATES

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SUMMARY

1. Glycogen depletion pattern in human skeletal muscle fibres was studied after bicycle exercise of varying intensity performed at different pedalling rates. Work intensities studied were equivalent to 30–150 % of $\dot{V}_{O_2 \, max}$ with pedalling rates of 30–120 rev/min.

2. Glycogen depletion increased dramatically with increasing exercise intensity; depletion was 2·7 and 7·4 times greater respectively at workloads demanding 64 and 84 % $\dot{V}_{O_2 \, max}$ than at workloads calling for 31 % $\dot{V}_{O_2 \, max}$. Even greater rates of glycogen utilization occurred at supramaximal loads.

3. Slow twitch, high oxidative (ST) fibres were the first to lose glycogen (reduced PAS staining) at all workloads below $\dot{V}_{O_2 \, max}$. Progressive glycogen depletion occurred in fast twitch (FT) fibres as work continued. Large quantities of glycogen remained in the muscle after 3 hr of exercise at low exercise intensity. This was almost exclusively found in FT fibres. At workloads exceeding maximal aerobic power, there was an initial depletion of glycogen in both fibre types. Varying the pedalling rate and, thus, the total force exerted in each pedal thrust had no effect on the pattern of glycogen depletion in the fibres.

4. Results point to primary reliance upon ST fibres during submaximal endurance exercise, FT fibres being recruited after ST fibres are depleted of glycogen. During exertion requiring energy expenditure greater than the maximal aerobic power, both fibre types appeared to be continuously involved in carrying out the exercise.

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INTRODUCTION

Human skeletal muscle contains two major fibre types which are histochemically distinguishable on the basis of differences in myofibrillar ATPase activity (Dubowitz & Pearse, 1960; Edström & Nyström, 1969; Gollnick, Armstrong, Saubert IV, Piehl & Saltin, 1972a). We designate fibres with low and high levels of myofibrillar ATPase activity as slow twitch (ST) and fast twitch (FT) respectively (Gollnick *et al.* 1972a), since contractile speed is closely related to myosin ATPase activity (Barnard, Edgerton, Furukawa & Peter, 1971). ST fibres are also histochemically characterized by heavy staining for oxidative capacity and slight staining for glycolytic capacity. By contrast, FT fibres possess low oxidative and high glycolytic capacity (Gollnick *et al.* 1972a).

The physiological characteristics of the muscle fibres suggest different patterns of use and perhaps different metabolic modes for each major type. Some evidence for such a view was obtained in experiments in which the glycogen depletion pattern was examined in exercised muscle. In these experiments, ST fibres were the first to become depleted of glycogen during bicycle exercise at loads requiring 60–80 % of maximal oxygen uptake ($\dot{V}_{O_2 \text{ max.}}$), even though there was a constant decline in total muscle glycogen (Gollnick, Piehl, Saubert IV, Armstrong & Saltin, 1972b; Gollnick, Armstrong, Saubert IV, Sembrowich, Shepherd & Saltin, 1973a). As exercise continued, FT fibres also became depleted of glycogen. A similar glycogen depletion pattern was observed during prolonged running (Costill, Gollnick, Jansson, Saltin & Stein, 1973). By contrast a workload of 150 % $\dot{V}_{O_2 \text{ max.}}$ performed at a pedalling rate of 90–104 rev/min resulted in initial glycogen depletion in FT fibres (Gollnick, Armstrong, Sembrowich, Shepherd & Saltin, 1973b). One possible interpretation of these findings is that there is primary reliance on motor units containing ST fibres during light and moderate exercise, motor units with FT fibres being involved only in the later stages of such exercise. Both types of motor units are probably activated during heavy exercise. In the latter circumstance, the differential rate of oxidative metabolism, produced either by differences in oxidative capacity or availability of oxygen, coupled with a higher energy consumption per unit of tension developed (Goldspink, Larson & Davies, 1970), probably produce initial glycogen depletion in FT fibres.

Data presently available concerning glycogen depletion pattern in human skeletal muscle fibres during exercise encompass limited exercise intensities (% $\dot{V}_{O_2 \text{ max.}}$) and speeds. It is possible that glycogen depletion patterns might be significantly altered if exercise was performed at extreme speeds or intensities. The present investigation was therefore

undertaken in order to study the glycogen depletion pattern in human skeletal muscle fibres following exercise comprising cycling at pedalling rates ranging from 30 to 120 rev/min and with workloads sustained for from 1 min to several hours. The results of such a study may enhance the understanding of factors responsible for preferential glycogen loss in one fibre type or the other during exercise, and thus give an indication of neuromuscular control mechanisms during exercise in man.

METHODS

Subjects

Thirteen male physical education students whose mean age was 24·5 (range 20–30) years participated in the experiment. The subjects average weight and height were 72·8 (64–84) kg and 182 (169–193) cm respectively. The average $\dot{V}_{O_2\,max.}$ amounted to 4·46 (3·76–5·29) l..min^{-1}. All subjects were well informed about the procedures to be used and they had participated in similar type experiments prior to giving their consent for the participation in the present investigation.

Experimental

Oxygen uptake ($\dot{V}_{O_2}$) measurements were made using the open circuit method. Expired air was collected in Douglas bags and its volume determined with a Tissot spirometer. Gas analysis was performed using either the Haldane or micro-Scholander methods. $\dot{V}_{O_2\,max.}$ was determined during bicycle exercise using the 'levelling-off' criterion, i.e. several submaximal and maximal workloads were used at separate occasions in order to determine each subject's workload–$\dot{V}_{O_2}$ relationship and thereby clearly define the point where no further increase in $\dot{V}_{O_2}$ occurred in spite of an increase in workload.

Blood lactate concentrations were determined with an enzymatic method (Scholz, Schmitz, Bücher & Lampen, 1959).

Samples were obtained from the quadriceps femoris (vastus lateralis) muscle using the needle biopsy technique (Bergström, 1962). Resting samples were taken with subjects in a supine position and exercise samples were taken with subjects seated on the bicycle ergometer, exercise being interrupted only long enough to take the biopsy (5–10 sec). A portion of the sample was immediately frozen in liquid nitrogen and stored at −80° C for subsequent glycogen and lactate assay (Karlsson, Diamant & Saltin, 1970). The rest of the muscle sample was mounted on a specimen holder in O.C.T. embedding medium (Ames Tissue-Tek) and frozen in isopenthane cooled in liquid nitrogen. Serial cross-sections (10 μm thick) were cut in a cryostat at −20° C and mounted on cover glasses for histochemical analysis. Myofibrillar ATPase activity was estimated at a pH of 9·4 after pre-incubation at a pH of 10·3, as described by Padykula & Herman (1955). Serial sections (16 μm thick) were stained for glycogen using the periodic acid-Schiff (PAS) reaction (Pearse, 1961).

Comments

The relative glycogen content of individual muscle fibres was estimated from PAS staining intensity. In this method, muscle sections were examined under the light microscope and the staining intensity of fibres was rated as dark, moderate, light or negative. In our experience, PAS staining in muscle samples from non-exercised

subjects is fairly uniform, and all fibres are stained dark when the glycogen content exceeds approximately 80 m-mole glucose units/kg wet wt. (Gollnick *et al.* 1972*b*; Piehl, 1974). The average glycogen content of samples taken at rest was 80 m-mole. kg^{-1}. Thus, all fibres were darkly stained with only minor exceptions. This method of rating glycogen content in muscle fibres is an adaptation of the method described by Kugelberg & Edström (1968), who also demonstrated that the method can be useful in identifying fibres which have been contracting. However, its application entails some inherent disadvantages. One consideration is the objectivity and reliability of the rating procedure. Thus, the arbitrary choice of only four categories makes it necessary to include fibres with some differences in the same category. It may also be difficult to apply the rating system objectively under such circumstances. In order to assess this disadvantage, a random selection of slides were rated by different individuals who obtained essentially the same results. Multiple sections of the same sample also produced similar staining intensities and ratings.

Rapid resynthesis of glycogen within muscle fibres might also obscure the glycogen depletion pattern. This does not appear to be a major limitation, since glycogen resynthesis rates are known to be relatively slow in comparison to glycogenolysis (Hultman, Bergström & Roch-Norlund, 1971) and a reciprocal relationship is also known to exist between the activation of the enzymes glycogen phosphorylase and synthetase (Staneloni & Piras, 1969). A major limitation involved in using PAS staining for identifying fibres which were active during exercise is that a considerable decline in glycogen levels may be necessary before any change in staining becomes apparent. This was not really a major problem in the present study, since the muscle glycogen content was not especially high and the exercise sessions were long enough to produce a clear differentiation between the fibres.
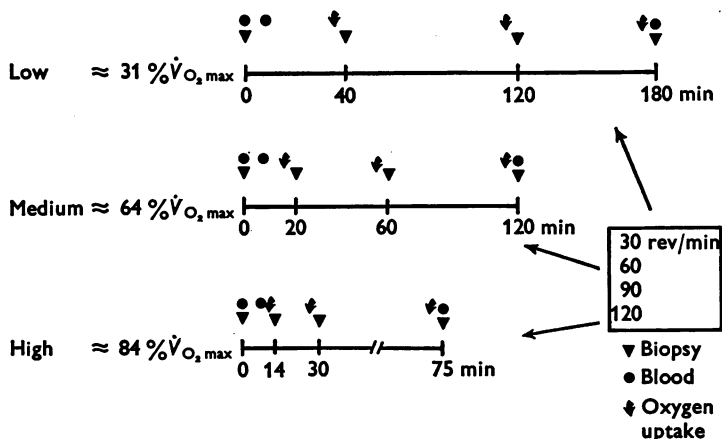
Text-fig. 1. Protocol for experiments at low, medium and high exercise intensities indicating the points at which blood and biopsy samples were taken and heart rate and $\dot{V}_{O_2}$ measurements were made.

Procedure

The subjects were assigned to groups which exercised at loads requiring an energy expenditure representing approximately 30, 60 or 90 % $\dot{V}_{O_2 \text{ max.}}$ at a pedalling rate of 60 rev/min in bicycle exercise. These workloads were referred to as low, medium and high respectively. Subjects then followed the protocol illustrated in Text-fig 1.

They arrived at the laboratory at various times throughout the day. Diet was uncontrolled, but subjects were asked not to engage in any prolonged exercise on the day of an experiment. Muscle biopsy samples were taken at rest and at intervals shown in Text-fig. 1. During the $\dot{V}_{O_2}$ measurements, a sample of blood was taken from the fingertip for lactate assay. Subjects then pedalled on the bicycle at the pedalling rate to be used during the day with no load on the bicycle, and $\dot{V}_{O_2}$ determinations were made. The load for the day was then added and the experiment begun. Subjects exercised for 3 hr, 2 hr or 1 hr (or until exhausted) at the low, medium and high loads respectively. Some subjects also exercised at loads with energy requirements of approximately 120 or 150 % $\dot{V}_{O_2 \, max.}$ at 40, 60 or 90 rev/min after completion of the preliminary part of the experiment. The initial exercise test for each subject was performed at a pedalling rate of 60 rev/min. The pedalling rate in the other exercise sessions was selected at random with an interval of at least 1 week between each experiment. Workloads were adjusted so that the total external work output was constant for each subject. Under these conditions there was some variation in $\dot{V}_{O_2}$ from test to test.

RESULTS

Mean oxygen uptake for the three workloads (low, medium and high) were 1·39, 2·86 and 3·91 l. min^{-1} respectively when the pedalling rate was 60 rev/min (Text-fig. 2). These $\dot{V}_{O_2}$ values represented 31, 64 and 83 % respectively of the subjects maximal aerobic power. The R values varied from 0·93 at the heaviest workload to 0·81 at the lightest workload. Energy exchange for the three workloads, estimated from the R values and $\dot{V}_{O_2}$ values, averaged 6·4, 14·4 and 17·1 kcal.min^{-1} respectively. Based on R and $\dot{V}_{O_2}$ observations during the exercises the mean rate of glucose oxidation was estimated to 0·6 g.min^{-1} at the low workload as compared to 2·1 and 3·3 g.min^{-1} at the medium and high exercise intensities. Since the total duration of exercise varied, the total glucose oxidized averaged 112, 257 and 183 g respectively at the low, medium and high levels of exercise intensity. The rate of glycogen depletion in the thigh and the changes in blood lactate concentration were related to work intensity (Text-fig. 2).

The two fibre types displayed no differential PAS staining pattern before exercise (Text-fig. 3). After 40 min of exercise at the lightest workload at a pedal rate of 60 rev/min, muscle glycogen declined from 84 to 71 m-mole glucose units.kg^{-1} wet wt. At this point, a distinct differentiation in PAS fibre staining was evident, 75 % of the ST fibres and only 5 % of the FT fibres then being rated as moderately stained. All other fibres were still darkly stained. No further change in the PAS staining of FT fibres was noted after 2 hr of work, but at this point no ST fibres were PAS dark and 2 % were PAS negative. After 3 hr of exercise, 33 % of the ST fibres were PAS negative and only 8 % were moderately stained. Only a minor change in the intensity of PAS staining of FT

fibres was observed following the third hour of exercise. This differentiation in the intensity of PAS staining of the fibre types during prolonged, low-intensity exercise is illustrated in Pl. 1.
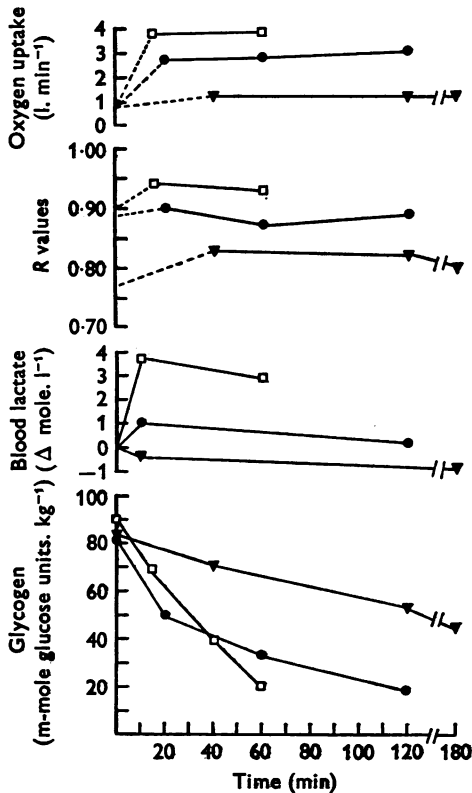

Text-fig. 2. Changes in mean value for $\dot{V}_{O_2}$, R value, blood lactate and muscle glycogen at low (▼), medium (●) and high (□) exercise intensities performed at 60 rev/min.

ST fibres were also the first to display reduced PAS staining during prolonged, moderately severe (medium) exercise. This is in accordance with our previous observations (Gollnick *et al.* 1973*a*). Total carbohydrate utilization was greatest at this workload and an average of only 18 m-mole glucose units . kg^{-1} wet muscle remained in the muscle after 2 hr of exercise. Histochemical staining for glycogen revealed that 95 % of the ST fibres were PAS negative, whereas only 53 % of the FT fibres were negative.

It is noteworthy that when subjects exercised to exhaustion at the high workload, the change in PAS staining was similar to, but faster than, the change observed at the lighter workloads. Thus, after only 14 min of

exercise, 37 % of the ST fibres were rated as moderately stained as compared to 14 % of the FT fibres. The remaining fibres of both types remained PAS dark. After 40 min of exercise some ST fibres were PAS negative but none was still rated as PAS dark. By contrast, 17 % of the FT fibres were
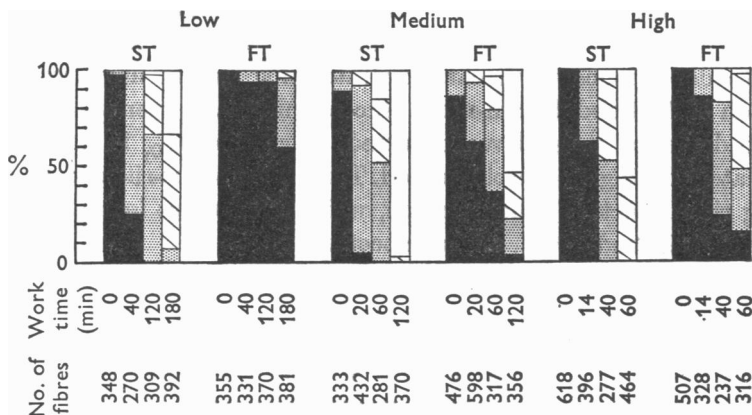
Text-fig. 3. Summary of the PAS staining pattern in ST and FT fibres as a function of time during exercise at 60 rev/min at low, medium and high intensities.
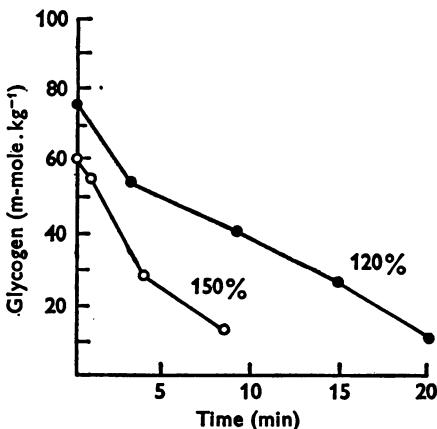

Text-fig. 4. Mean values for the glycogen depletion (in glucose units) from the lateral portion of the quadriceps femoris muscle having performed the two supramaximal workloads.

lightly stained, 24 % darkly stained and the remaining 41 % were moderately stained. At exhaustion (60 min of exercise) 56 % of the ST fibres were PAS negative and the rest were lightly stained. By contrast, 15 % of the FT fibres were rated as PAS dark and only 3 % were negative.

Micrographs illustrating this selective change in PAS staining are shown in Pl. 1.

Total work duration averaged 21 and 8 min respectively for the test demanding an energy exchange equivalent to 120 and 150 % of $\dot{V}_{O_2 max}$. This exercise was performed in 3 min (120 %) and 1 min (150 %) sessions with 10 min rest pauses between sessions. The estimated rates of energy exchange were 28·3 and 35·6 kcal.min^{-1} for the two loads. Assuming complete dependence upon carbohydrate, this should have resulted in oxidation of 38·3 and 48·2 m-mole.glucose min^{-1}. An even greater carbohydrate depletion must have occurred, since max. $\dot{V}_{O_2}$ is not attained immediately during an exercise period. This circumstance is reflected in the striking rise in lactate concentration, which reached at least 14 m-mole. kg^{-1} at the 120 and 150 % loads respectively.
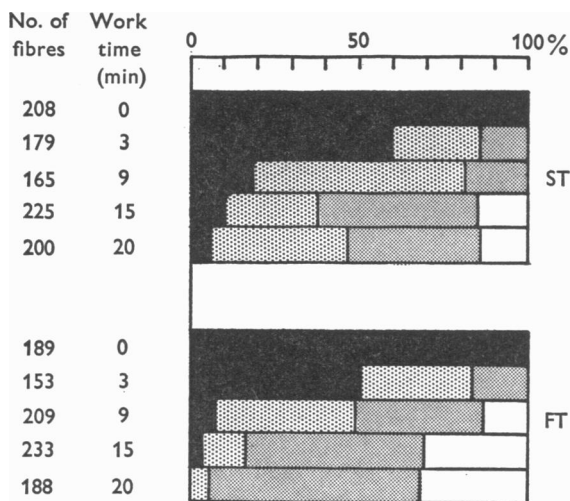
Text-fig. 5. Summary of changes in PAS staining in the fibres of the lateral portion of the quadriceps femoris muscle during bicycle exercise at 60 rev/ min requiring 120 % of $\dot{V}_{O_2 max}$. This tabulation demonstrates the rapid decline in PAS staining in both fibre types, resulting in the early appearance of PAS negative FT fibres.

The rapid glycogen depletion, which averaged 3·1 and 9·5 m-mole glucose units.kg^{-1}.min^{-1} at the two intensities (Text-fig. 4), provided further evidence. The pattern of the change in PAS staining was similar for both supramaximal loads. However, it differed from the submaximal loads in that a decline in PAS staining intensity occurred in both fibre types from the start of exercise but was most pronounced in FT fibres

(Text-fig. 5). Micrographs demonstrating this pattern are shown in Pl. 1. Changing the pedalling rate at a given exercise level had no effect on glycogen depletion or the pattern of fibre depletion as indicated by a decline in PAS staining.

<div align="center">DISCUSSION</div>

The present results clearly demonstrate that differential rates of glycogen depletion occur in human skeletal muscle fibres during exercise and with workloads requiring less than 100 % of $V_{O_2 \, max}$. ST fibres are the first to lose glycogen. FT fibres only become PAS negative before ST fibres at supramaximal workloads. The question arises as to the interpretation of these data in terms of fibre activation. On the basis of the above, it seems reasonable to assume that the loss in glycogen from fibres is a direct result of fibre contraction during exercise.

At the lightest workload, irrespective of pedalling rate, total glycogen depletion was rather modest. At this workload, however, selective glycogen depletion occurred in ST fibres. This glycogen loss in ST fibres was observed at a rather early stage after the onset of exercise. This suggests that the fibres were heavily engaged in this rather light exercise and consumed glycogen at a rapid rate. From this standpoint it is apparent that the true rate of glycogen utilization by contracting fibres cannot be accurately assessed by measuring changes in the total glycogen of muscle samples. As exercise continued, additional ST fibres became PAS negative or became more lightly stained than average ST fibres in initial samples. This could be interpreted as pointing to subsequent recruitment of additional motor units. The reason for such recruitment is unknown, since the total force exerted on the pedals at this light workload was slight (Gollnick, Karlsson, Piehl & Saltin, 1974). It is also known that other energy sources, such as blood glucose, plasma FFA and intramuscular lipids, are utilized during prolonged, low-intensity exercise. The rate of oxidation of such substrates or, in the case of blood glucose and FFA, their rate of entry into the muscle may be insufficient to satisfy exercise energy requirements.

In the Methods section, it was suggested that the magnitude of glycogen synthesis is negligible when there is concomitant glycogenolysis in the muscle cell. On this basis it would appear that most of the glucose taken up by skeletal muscle during exercise is used to support exercise metabolism.

At the high workload producing exhaustion within 60 min, ST fibres were the first to be depleted of glycogen. Moreover, a substantial amount of glycogen was still available after termination of exercise but almost all of it was in FT fibres. At this level of exercise, both muscle lactate accumulation (Karlsson & Saltin, 1970) and depleted muscle glycogen stores

(Saltin & Karlsson, 1971) were ruled out as possible factors causing or related to subject exhaustion. The present data provide further support for the hypothesis that muscle lactate is not limiting as its concentration only amounted to 5·7 m-mole.kg^{-1} and a rather high lactate turnover also may be assumed (Jorfeldt, 1970). Can reduced glycogen stores be an explanation? Earlier statements may now require reassessment. The number of glycogen-depleted muscle fibres is very high, and even though peak tension developed with each pedal thrust only amounted to 10 % (120 rev/min) to 35 % (30 rev/min) of maximal voluntary contraction (MVC), that tension may still have been too high to be produced by the remaining glycogen-filled fibres (Gollnick *et al.* 1974). Thus, glycogen depletion (in individual fibres) may be the cause of muscle fatigue. This explanation of fatigue is only valid, as has been discussed above, if other available substrates cannot be utilized at a rate fast enough to cover requisite energy demands by glycogen-depleted fibres.

It is tempting to suggest that the shift in glycogen depletion pattern, featuring an initial decline in PAS staining intensity, from ST to FT fibres at the supramaximal loads was due to the increased tension developed by the muscles. This is obviously a possibility, since the highest tensions were developed at the loads equivalent to 120–150 % $\dot{V}_{O_2 max.}$ ranging from 15 up to 35 % MVC at high and low pedalling rates respectively. However, as demonstrated in the same paper (Gollnick *et al.* 1974) the force exerted on the pedals in some submaximal exercise tests performed at slow pedal rates, e.g. 60 and 90 % $\dot{V}_{O_2 max.}$ at 30 rev/min, was above the force required for supramaximal loads utilizing fast pedalling rates. As indicated above, the force exerted on the pedals during dynamic exercise only amounted to approximately 1/3 MVC. Thus, the results indicate that the factors producing the shift in the initial glycogen depletion from ST to FT fibres are more closely related to relative workload, i.e. availability of oxygen, than to tension developed or speed of contraction.

Muscular strength and the relative force exerted on the pedals may not be completely discounted as factors contributing to the manner in which glycogen is depleted from the fibres. Two of the subjects, who exercised at 90 % of $\dot{V}_{O_2 max.}$, may illustrate this point. These subjects had values for $\dot{V}_{O_2 max.}$ of 4·9 and 5·3 l.min^{-1} respectively. The subject with the lower aerobic power possessed the highest, voluntary, contractile strength in his knee extensors. Thus, the subject with the higher $\dot{V}_{O_2 max.}$ exerted a greater proportion of his maximal contractile force during exercise. There was more rapid glycogen depletion from FT fibres in this subject during exercise, and nearly all his FT fibres were PAS negative at exhaustion. By contrast the other subject, who exercised for a comparable length

of time at the same relative workload, retained PAS staining in FT fibres, even at exhaustion.

Even though these circumstances were observed in two subjects, the present data may imply that the availability of oxygen is a critical factor. Thus, when the supply of oxygen becomes insufficient, the activation of FT fibres with their high anaerobic potential would result in a rapid reduction in their glycogen stores. Under these conditions, continued use of ST fibres is obvious. This is reasonable, since there is still considerable energy production in ST fibres via aerobic pathways under these conditions.

In conclusion, the present results are in accordance with the view that human skeletal muscle is composed of fibres in motor units with varying activation thresholds. Even though there appear to be significant differences in the activation of the two major fibre types found in the muscle we studied, differences apparently exist even within a given fibre type. Results indicate that there is preferential use of ST fibres when a prolonged period of dynamic exercise is performed calling for energy expenditure less than 100 % of $\dot{V}_{O_2 \text{ max}}$. This use is independent of the speed at which the contraction is performed. At very light loads, e.g. one requiring 30 % of $\dot{V}_{O_2 \text{ max}}$, only a few fibres appear to be activated, resulting in local depletion of glycogen in the ST fibres. The results point to two ways of activating FT fibres, viz. (1) by increasing workload to more than 100 % of $\dot{V}_{O_2 \text{ max}}$ or (2) by continuing exercise at lighter loads until a large number of ST fibres become depleted of their glycogen stores.

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P. D. GOLLNICK, KARIN PIEHL AND B. SALTIN
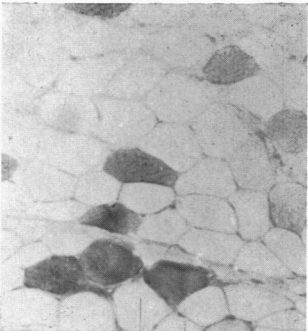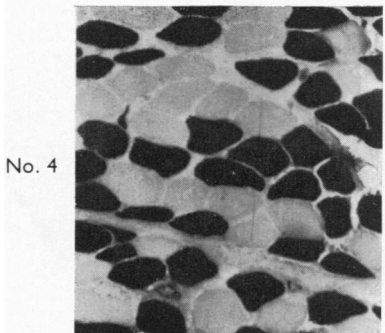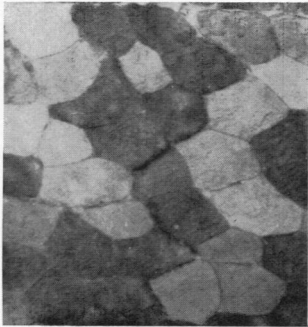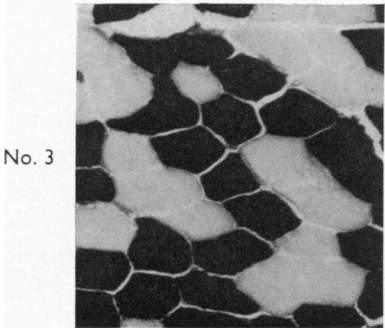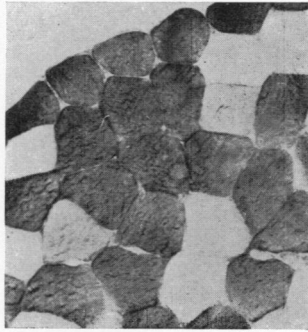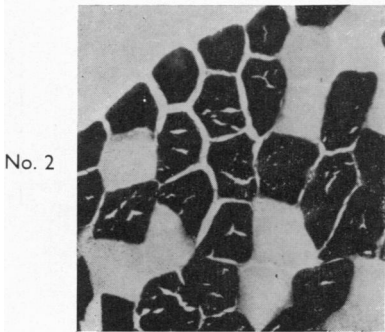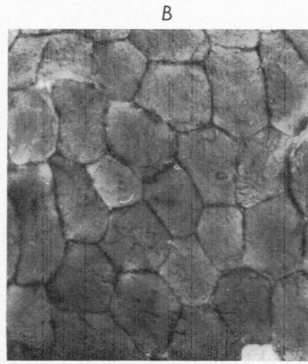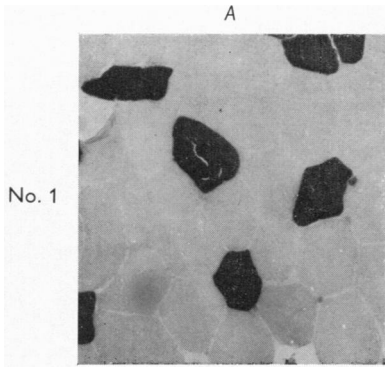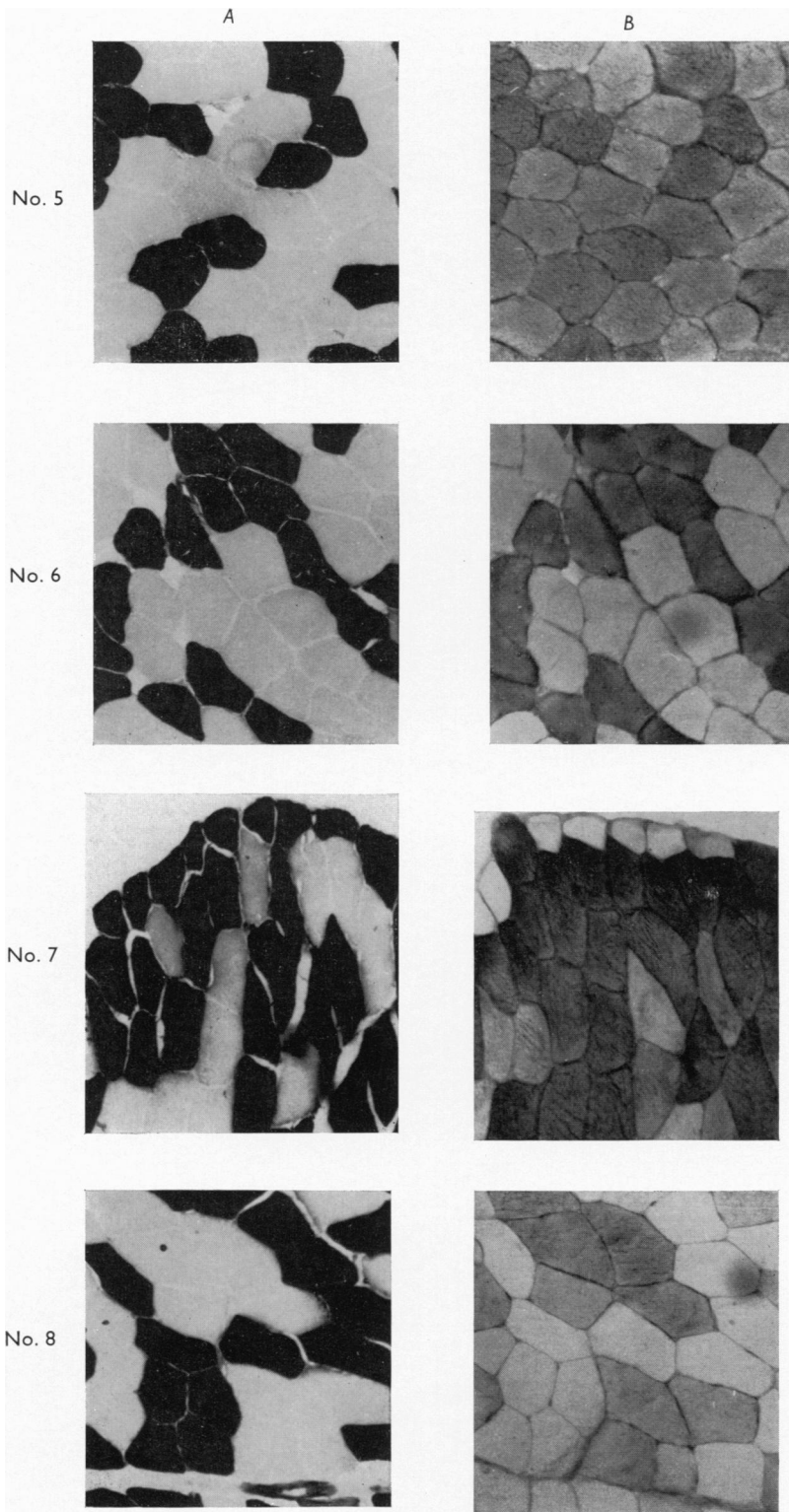
EXPLANATION OF PLATES

Serial sections of muscle samples displaying changes in PAS staining following various exercise programmes. Samples A were stained for myofibrillar ATPase showing the ST (light) and FT (dark) fibres. Sections labelled B were stained for glycogen with the PAS reaction. Section 1 ($A + B$) is from one subject at rest. It illustrates the generally homogeneous PAS stain seen for all subjects. Section 2 was taken after 180 min of work at 30 % of $\dot{V}_{O_2\,max.}$ with a pedalling rate of 60 rev/min. It illustrates the striking difference in PAS staining in ST and FT fibres. Section 3 is taken after 40 min of work on the medium workload and shows the selective loss of PAS stain in the ST fibres. At the end of this work after 2 hr (section 4) it is evident that also some FT fibres have lost glycogen while some still stain dark. Sections 5 and 6 are from one subject working on the high workload. No. 5 is taken after 14 min and no. 6 at the end of exercise. Note that selective depletion in the ST fibres is already present after 14 min of work. Sections 7 and 8 show the glycogen staining pattern after the first and seventh exercise sessions on one of the supra-maximal (120 % of $\dot{V}_{O_2\,max.}$) workloads. Note the early appearance of PAS negative FT fibres followed by gradual decline in both fibre types.