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CAPILLARITY, OXIDATIVE CAPACITY AND FIBRE COMPOSITION OF THE SOLEUS AND GASTROCNEMIUS MUSCLES OF RATS IN HYPOTHYROIDISM

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SUMMARY

1. Muscle capillarity, mean and maximal diffusion distances and muscle fibre composition were evaluated in frozen sections stained for myosin ATPase of the soleus and the white area of the gastrocnemius medial head (gastrocnemius) of rats made hypothyroid by the injection of propylthiouracil (PTU) (50 mg kg^{-1}) every day for 21 or 42 days.

2. Oxygen consumption in the presence of excess ADP and P_i with pyruvate plus malate as substrates and the activity of cytochrome *c* oxidase were measured in muscle homogenates.

3. Treatment with PTU decreased body oxygen consumption and the concentration of triiodothyronine in plasma. The capacity of the soleus and gastrocnemius muscles' homogenates to oxidize pyruvate plus malate and their cytochrome *c* oxidase activity were reduced after 21 or 42 days of treatment with PTU.

4. Fibre composition in the soleus muscle was changed by treatment with PTU. There was a decrease in the proportion of type IIa or fast glycolytic oxidative fibres and an increase in type I or slow oxidative fibres. After 21 days of PTU administration there was also an increase in the proportion of fibres classified as IIc. The changes in fibre composition are believed to be the result of changes in the types of myosin synthesized by the fibres. Therefore, the fibres classified as IIc are, most probably, IIa fibres in the process of changing their myosin to that of the type I fibres. No changes in fibre composition were evident in the white area of the gastrocnemius medial head, an area made up of IIb or fast glycolytic fibres.

5. The indices of capillarity: capillary density and capillary to fibre ratio, as well as mean and maximal diffusion distances from the capillaries, were not changed by the treatment with PTU in the muscles studied.

6. The lack of changes in capillarity in spite of significant changes in oxidative capacity indicates that in skeletal muscle capillarity is not necessarily related to the oxidative capacity of the fibres.

INTRODUCTION

Administration of excess thyroid hormone has a marked effect on the anatomy and physiology of skeletal muscles. Changes in fibre composition and in oxidative capacity have been found in the soleus and some other skeletal muscles of hyperthyroid rats (Ianuzzo, Patel, Chen, O'Brien & Williams, 1977; Winder & Holloszy, 1977; Wiles, Young, Jones & Edwards, 1979; Fitts, Winder, Brooke, Kaiser & Holloszy, 1980; Johnson, Olmo & Mastaglia, 1983). The muscles that seem to be more readily affected by hyperthyroidism are those mostly made up of slow oxidative or type I fibres, like the soleus, and muscles mostly made up of fast oxidative glycolytic or type II a fibres, like the red portion of the quadriceps. On the other hand, muscles mostly made up of fast glycolytic or type II b fibres do not change their fibre composition or oxidative capacity even after prolonged treatment with excess thyroid hormones (Winder & Holloszy, 1977; Capo & Sillau, 1983).

Hypothyroidism has been reported to decrease the activities of the oxidative enzymes succinate dehydrogenase, citrate synthase, and cytochrome *c* oxidase in the soleus, the extensor digitorum longus and the quadriceps muscles in rats (Gollnick & Ianuzzo, 1972; Baldwin, Hooker, Campbell & Lewis, 1978; Baldwin, Hooker, Herrick & Schrader, 1980; Nicol & Johnston, 1981). Also hypothyroidism is known to increase the proportion of type I fibres in the soleus and in the respiratory muscles (Ianuzzo *et al.* 1977; Johnson *et al.* 1983; Ianuzzo, Chen, O'Brien & Keens, 1984). These studies, with the exception of that of Baldwin *et al.* (1980), involved muscles mostly made up of type I fibres (like the soleus) or muscles with an heterogenous fibre composition (like the quadriceps). This makes it difficult to appreciate the possible effects of hypothyroidism on type II a and type II b fibres.

Tissue capillarity is generally assumed to be directly related to the oxidative capacity of the tissues. In skeletal muscle, fibres that show a high oxidative potential are surrounded by a greater number of capillaries than fibres that depend mostly on their anaerobic capacity for energy production (Romanul, 1965). Further, conditions that increase the oxidative capacity of the muscles like chronic electrical stimulation, endurance exercise or hyperthyroidism are accompanied by increases in the density of the capillary network (Brown, Cotter, Hudlicka & Vrbova, 1976; Brodal, Ingjer & Hermansen, 1977; Capo & Sillau, 1983). Others, however, have failed to find a correlation between oxidative capacity and capillarity (Maxwell, White & Faulkner, 1980). Similarly in hyperthyroid rats the capillarity of the white area of the gastrocnemius medial head was considerably higher than that of euthyroid rats, however, the oxidative capacity was not different (Capo & Sillau, 1983). Other factors such as the cross-sectional area of the fibres (f.c.s.a.) (Brodal *et al.* 1977; Ripoll, Sillau & Banchero, 1979) and, probably, capillary blood flow (Hudlicka, 1982) also seem to be important in determining tissue capillarity.

The present report deals with the effects of hypothyroidism on the oxidative capacity and capillarity of the soleus and the white area of the gastrocnemius medial head of the rat.

METHODS

Female Wistar rats with a body weight of 306 ± 19 (mean $\pm$ S.D.) g were housed in pairs matched for body weight and injected I.P. every day with propylthiouracil (PTU) (50 mg kg^{-1} body weight) or with saline for 21 or 42 days. This dose of PTU is in excess of what is required to prevent synthesis of thyroid hormones by the thyroid gland (Iino, Yamada & Greer, 1961).

On the day of the last injection the animals were lightly anaesthetized with sodium pentobarbitone (20 mg kg^{-1} I.P.) and body oxygen consumption ($B\dot{V}_{O_2}$) was measured in an oxygen consumption chamber (Harvard Apparatus) kept at $23 \pm 0{\cdot}5$ °C with the aid of a water circulator. The average of at least three similar measurements was considered the $B\dot{V}_{O_2}$ of the animal. The values obtained in ml O_2 min^{-1} kg^{-1} were corrected to s.t.p.d. conditions and expressed as mmol O_2 min^{-1} kg^{-1}.

One day after the last injection the animals were killed by decapitation and a small sample of blood collected to measure the levels of triiodothyronine (T3) in plasma using commercially available radioimmunoassay kits (Amersham Co.). The solei and the gastrocnemius–plantaris muscles were rapidly removed, cleaned of excess fat and connective tissue, blotted dry and weighed to the nearest milligram. The soleus and the gastrocnemius medial head from the right leg were cut transversely at the widest point in the belly, and frozen in isopentane cooled to -140 °C with liquid N_2. These frozen specimens were stored at -70 °C for later study. The remainder of the right soleus and the left soleus and the white area of the gastrocnemius, located in the medial part of the gastrocnemius medial head (from now on referred to as gastrocnemius), were minced with scissors and known amounts were homogenized in ice-cold buffers to measure the capacity of the homogenates to oxidize pyruvate plus malate and the activities of cytochrome *c* oxidase.

To measure the capacity to oxidize pyruvate plus malate the tissues were homogenized in a buffer containing 300 mM-sucrose, 10 mM-Tris HCl and 2 mM-EDTA (pH 7·4). Oxygen uptake by the homogenates in the presence of excess ADP and P_i was followed at 37 °C using a Clark electrode and an oxygen monitoring system (Winder, Baldwin, Terjung & Holloszy, 1975). Cytochrome *c* oxidase activity of muscle homogenates prepared in 10 mM-phosphate buffer (pH 7·4) was determined at 37 °C following the procedure of Ferguson-Miller, Brautigan & Margoliash (1976).

The heart was removed, cleaned of fat, vessels and atria and the ventricles were blotted dry and weighed to the nearest milligram.

Cross-sections of approximately 12 μm thickness from the frozen specimens were obtained in a cryostat. These sections were processed by the ATPase technique after pre-incubation for 5 min at pH 4·5 (ATP 4·5). This technique permits the identification of at least three fibre types (Dubowitz & Brooke, 1973). To stain microvessels the same ATPase technique after pre-incubation for 5 min at pH 4·0 (ATP 4·0) was used (Sillau & Banchero, 1977).

Photomicrographs of the ATP 4·5 and ATP 4·0 preparations of the soleus and gastrocnemius were taken and information on fibre density, fibre cross-sectional area (f.c.s.a.), capillary density (c.d.) and capillary to fibre ratio were obtained as described earlier (Capo & Sillau, 1983). The average number of fibres counted was 489 in the soleus and 423 in the gastrocnemius. The area used to count capillaries was approximately 1·45 mm^2.

Photomicrographs of the ATP 4·0 preparations of the soleus muscle were also used to measure diffusion distances by the 'closest individual' method (Kayar, Archer, Lechner & Banchero, 1982*a*; Kayar, Lechner & Banchero, 1982*b*). A grid of nine points arranged in uniform rows and columns was laid at random over the projected photomicrographs and the distance from each point to the nearest capillary was recorded. This procedure was repeated until a total of 200 distances was obtained for each animal. Mean diffusion distance ($\bar{R}$) was taken as the arithmetic mean of the 200 distances measured. The distances were then ordered in ascending value and the 190th distance was taken as the maximal diffusion distance (R_{95}) (Kayar *et al.* 1982*a*, *b*).

Expected values of $\bar{R}$ and R_{95} for the range of values of c.d. obtained in the present investigation were calculated for two models of distribution of capillaries in the cross-section. One model assumes that the microvessels are distributed in an hexagonal array while the other assumes a random distribution. For the model that assumes an hexagonal array

$$\bar{R} = 0{\cdot}4037\ (\text{c.d.}^{-1})^{\frac{1}{2}} \quad \text{and} \quad R_{95} = 0{\cdot}7110\ (\text{c.d.}^{-1})^{\frac{1}{2}}.$$

For the model that assumes a random distribution

$$\bar{R} = 0{\cdot}5000\ (\text{c.d.}^{-1})^{\frac{1}{2}} \quad \text{and} \quad R_{95} = 0{\cdot}9765\ (\text{c.d.}^{-1})^{\frac{1}{2}}.$$

These equations predicting the values of $\bar{R}$ and R_{95} were developed by Kayar and co-workers (1982*a*, *b*). The equation to calculate R when the capillaries are distributed in an hexagonal array is not the same that appears in the original publication since this equation was found to be in error (Homer, 1984).

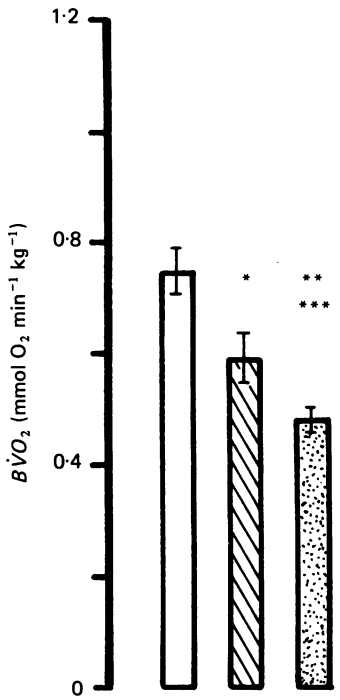

Fig. 1. Oxygen consumption of control rats (open column) and of rats treated with PTU for 21 days (hatched column) or 42 days (stippled column). Vertical lines indicate the S.E. of the mean. * Different from control ($P < 0.05$). ** Different from control ($P < 0.01$). *** Different from rats treated for 21 days ($P < 0.05$).

RESULTS

Thyroid state of animals

Body oxygen consumption was not different between the controls after 21 or 42 days of saline administration, therefore, these results were pooled. PTU treatment for 21 or 42 days reduced $B\dot{V}_{O_2}$ by 21 and 30% respectively (Fig. 1). The weight of the ventricles normalized for body weight (mg g^{-1}) was not different in the two groups of controls or in the two groups of experimental animals. The mean of the pooled values for the control animals (2.55 ± 0.07) (mean $\pm$ S.E. of mean) was significantly different ($P < 0.01$) from the mean for the pooled values of the experimental animals (2.29 ± 0.04). T3 concentration (ng ml^{-1}) was significantly lower in the animals receiving PTU for 21 days (0.46 ± 0.02) or 42 days (0.16 ± 0.02) than in the controls (0.74 ± 0.07).

Body weight and muscle weight

Control animals showed a small gain in body weight. Some of the experimental animals also showed an increase in body weight but the majority, especially those

that received PTU for 6 weeks, lost weight. These weight losses were small and after 21 or 42 days of treatment, control and PTU-treated animals had similar body weight. The weights of the soleus and gastrocnemius–plantaris muscles were related linearly to body weight and the treatment did not seem to affect these relationships (Fig. 2).

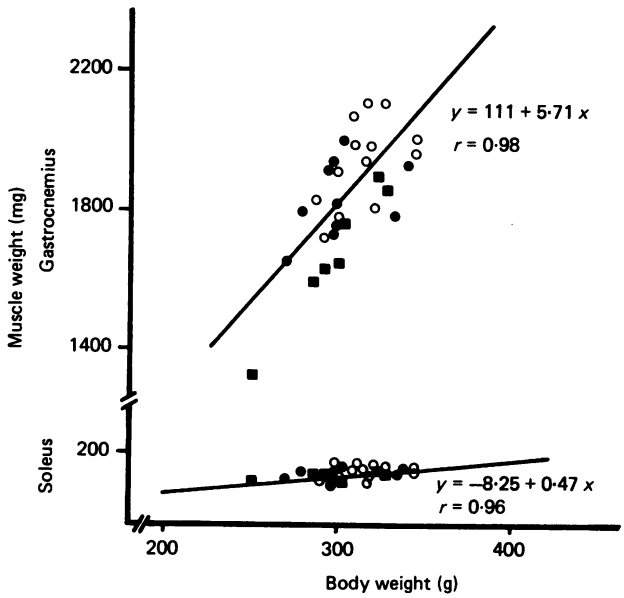

Fig. 2. Relationships between gastrocnemius–plantaris and soleus muscle weights with body weight in control rats (○) and rats treated with PTU for 21 days (●) and 42 days (■).

Muscle biochemistry

In control animals the capacity to oxidize pyruvate plus malate and the activity of cytochrome *c* oxidase of soleus homogenates were higher than those of the gastrocnemius (Figs. 3 and 4). This reflects the higher oxidative capacity of the soleus muscle. PTU treatment resulted in a drop in the oxidative capacity in both the soleus and the gastrocnemius. The capacity to oxidize pyruvate plus malate decreased by approximately 50 % in both muscles after 21 days of PTU administration. 42 days of treatment did not result in lower values than those found after 21 days of PTU administration (Fig. 3). Cytochrome *c* oxidase activity was also significantly decreased by the treatment. After 21 days the activities in the soleus and gastrocnemius were reduced by 19 and 38 % respectively. Treatment for 42 days resulted in even lower values for the activity of cytochrome *c* oxidase in the soleus (to 49 % of the control values) but not in the gastrocnemius (Fig. 4).

Muscle fibre area and fibre composition

Mean f.c.s.a. was related to muscle weight. In the soleus the values of muscle weight *vs.* f.c.s.a. of the control animals fell around the regression line reported previously by Ripoll *et al.* (1979). Treatment with PTU did not seem to affect the relationship between f.c.s.a. and muscle weight. In the gastrocnemius, f.c.s.a. was also related to

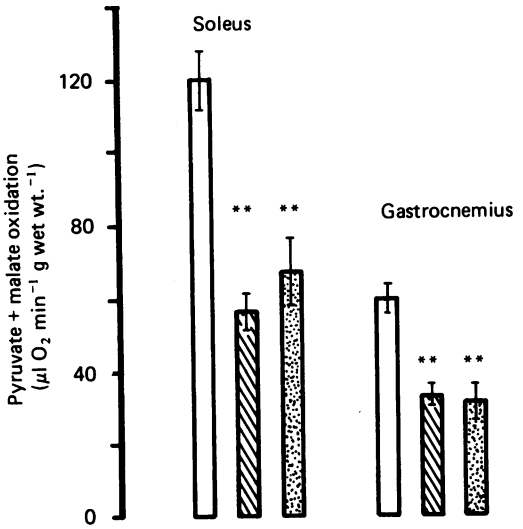

Fig. 3. Pyruvate plus malate oxidation in the presence of excess ADP by soleus and gastrocnemius muscle homogenates of control rats (open columns) and of rats treated with PTU for 21 days (hatched columns) or 42 days (stippled columns). ** Different from controls ($P < 0.01$). Vertical lines indicate the S.E. of the mean.

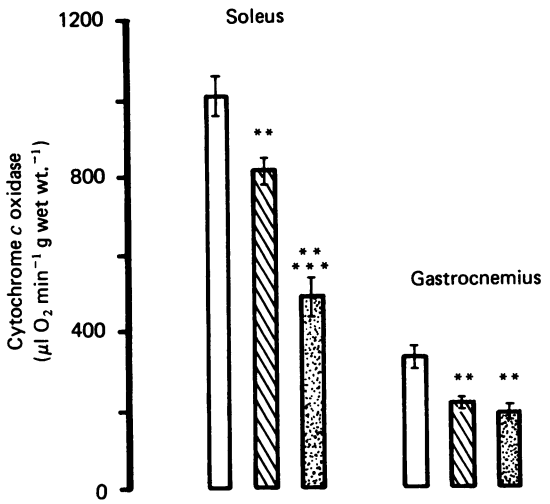

Fig. 4. Cytochrome *c* oxidase activity of soleus and gastrocnemius muscle homogenates of control rats (open columns) and of rats treated with PTU for 21 days (hatched columns) or 42 days (stippled columns). Vertical lines indicate the S.E. of the mean. ** Different from controls ($P < 0.01$). *** Different from rats treated for 21 days ($P < 0.01$).

the weight of the gastrocnemius–plantaris muscle and PTU treatment did not seem to alter this relationship.

Three types of fibres were evident in the soleus of control animals: type I, type II a and type II c. In the gastrocnemius of control animals only type II b was present. The histochemical characteristics of these fibre types have already been described

TABLE 1. Soleus muscle fibre composition (%) of control (saline) rats and of rats injected with propylthiouracil (PTU) for 21 or 42 days

Treatment	IIa	I	IIc
Saline (21 or 42 days) ($n = 12$)	13·4±2·9†	84·5±2·8	1·8±0·7
PTU (21 days) ($n = 9$)	2·3±1·3**	90·6±2·1	7·1±1·8**
PTU (42 days) ($n = 7$)	1·9±1·7*	96·5±2·2**	1·6±0·7

† = values are means±S.E. of means.
n = number of observations.
* = different from controls ($P < 0.05$).
** = different from controls ($P < 0.01$).

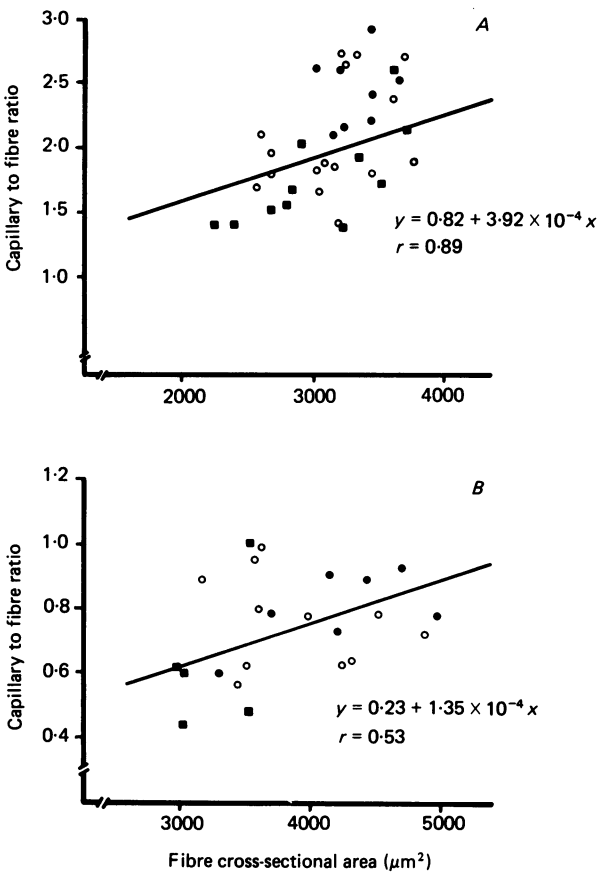

Fig. 5. Capillary to fibre ratio *vs.* fibre cross-sectional area in the soleus (*A*) and gastrocnemius (*B*) muscles of control rats (○) and rats treated with PTU for 21 days (●) or 42 days (■). Regression line in *A* is from data by Ripoll *et al.* (1979).

(Dubowitz & Brooke, 1973). Treatment with PTU did not change the fibre composition of the gastrocnemius. On the other hand soleus muscle fibre composition was changed by treatment with PTU. The proportion of type I fibres increased and there was a decrease in the proportion of type IIa fibres (Table 1).

Muscle capillarity and diffusion distances

In the soleus, capillary to fibre ratio values increased linearly with increasing f.c.s.a. (Fig. 5*A*). Similar results have been reported previously in a variety of muscles from different mammals (Brodal *et al.* 1977; Ripoll *et al.* 1979; Aquin, Sillau, Lechner & Banchero, 1980). An analysis of covariance demonstrated that the line obtained with the data of the present experiment was not significantly different from the line

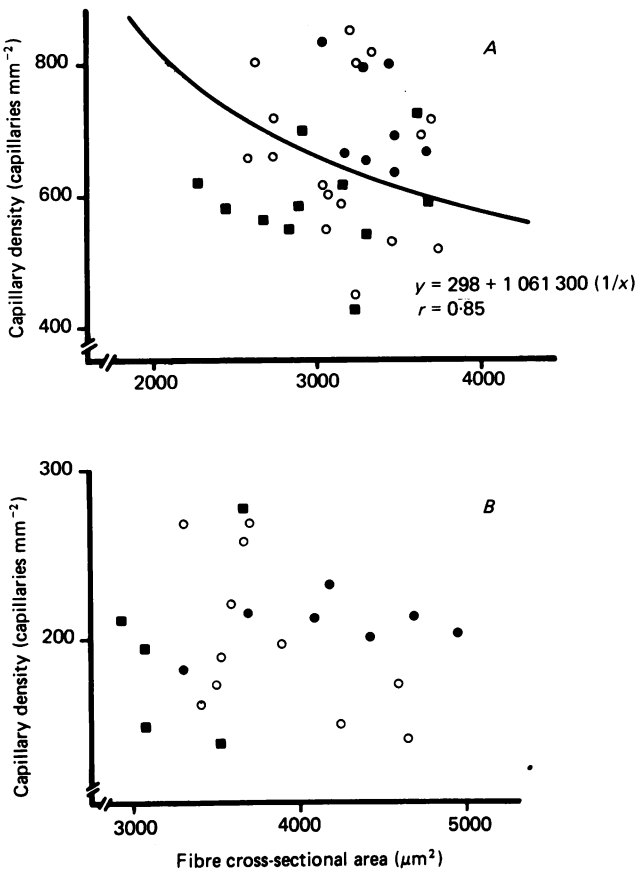

Fig. 6. Capillary density *vs.* fibre cross-sectional area in the soleus (*A*) and gastrocnemius (*B*) muscles of control rats (○) and rats treated with PTU for 21 days (●) or 42 days (■). Regression line in *A* is from data by Ripoll *et al.* (1979).

calculated by Ripoll *et al.* (1979) that included a large number of observations over a wide range of f.c.s.a. In the gastrocnemius, also, our values of capillary to fibre ratio increased linearly with increasing values of f.c.s.a. (Fig. 5*B*). The regression line in Fig. 5*B* is the best linear fit of the values obtained in the present experiment. No previously reported line seems to be available.

Values of c.d. are known to decrease hyperbolically with increasing f.c.s.a. (Ripoll *et al.* 1979; Aquin *et al.* 1980). No such correlations were evident from our results in

the soleus (Fig. 6*A*) and gastrocnemius (Fig. 6*B*). This is most probably due to the limited range of values of f.c.s.a. Nevertheless, in the case of the soleus our values fell around a regression line previously reported (Ripoll *et al.* 1979). No similar regression line is available for the gastrocnemius to the best of our knowledge.

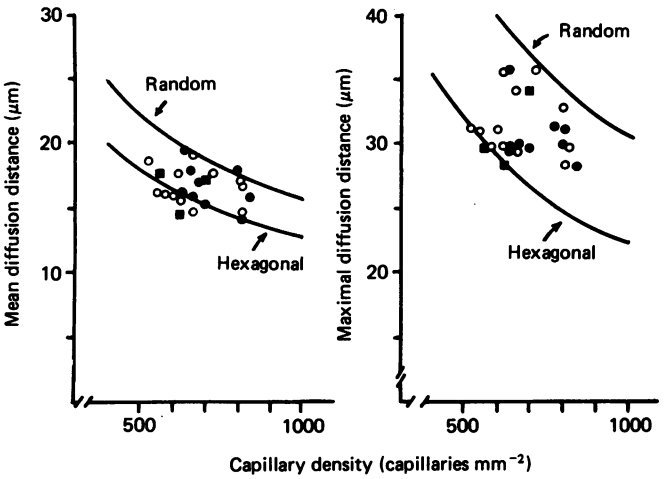

Fig. 7. Mean ($\bar{R}$) and maximal (R_{95}) diffusion distances in the soleus muscles of control rats (○) and in the soleus of rats treated with PTU for 21 days (●) or 42 days (■). The curves represent theoretical values of $\bar{R}$ and R_{95} when the capillaries in the cross-section are distributed in a random array (upper curves) or in an ordered (hexagonal) array (lower curves).

At a given f.c.s.a. the values of capillary to fibre ratio and c.d. of the hypothyroid animals do not seem to be different from those of the controls. This indicates that hypothyroidism did not evoke growth or attrition of capillaries in the skeletal muscles studied.

Mean ($\bar{R}$) and maximal (R_{95}) diffusion distances were not different between control and experimental animals (Fig. 7). The majority of the values of $\bar{R}$ and R_{95} fell between predicted values for different c.d.s assuming an hexagonal or a random array of the capillaries in the cross-section (Fig. 7).

DISCUSSION

Values of $B\dot{V}_{O_2}$ and T3 plasma concentration in the control animals were within values reported previously for the rat (Nicol & Johnston, 1981; Capo & Sillau, 1983; Threatte, Barney, Baker & Fregly, 1983). Rats treated with PTU for 21 days were hypothyroid as judged by their values of $B\dot{V}_{O_2}$ and T3 concentration in plasma which were significantly lower than those of the controls. The animals that received PTU for 42 days also had lower values of $B\dot{V}_{O_2}$ and T3 plasma concentration than controls.

At the end of the treatment the hypothyroid and control rats had similar body weights. Some investigators have found that hypothyroid rats have lower body weights than controls, due to the slow body growth that usually accompanies the

condition (Ianuzzo, Patel, Chen & O'Brien, 1980; Johnson *et al.* 1983). Others (Nicol & Johnston, 1981; Matoba, Sugiura & Murakami, 1982), however, found no changes in the growth rates of rats made hypothyroid. These discrepancies seem to be related to differences in the procedures used to induce a decrease in the production of thyroid hormones that results, many times, in different degrees of hypothyroidism.

Changes in fibre composition in muscles of hypothyroid animals have been previously reported (Ianuzzo *et al.* 1977; Nicol & Johnston, 1981; Matoba *et al.* 1982; Johnson *et al.* 1983). Ianuzzo *et al.* (1977) used the ATPase technique after alkaline pre-incubation and found an increase in the proportion of alkaline labile fibres in the soleus and plantaris muscles of hypothyroid rats. This was interpreted as an interconversion of type IIa and/or type IIb to type I fibres. Others (Nicol & Johnston, 1981) using the ATPase technique after acid pre-incubation (pH = 4·3) found a decrease in the proportion of IIa fibres and an increase in the proportion of type I fibres in the soleus of hypothyroid rats. For our studies of fibre types we have pre-incubated the muscle sections at a slightly higher pH (pH = 4·5) than Nicol & Johnston (1981). This permits a better way to analyse fibre composition in the rat because it allows for the visualization of fibre types not clearly revealed by pre-incubation at an alkaline or at a too acid pH.

In the present investigation the soleus of the hypothyroid rats after 42 days of PTU administration showed a higher proportion of type I fibres and a lower proportion of type IIa fibres than the soleus of the control rats but no change in the proportion of IIc fibres. However, after 21 days of PTU administration and associated with an increase in the proportion of type I fibres, there was a significant increase in fibres that were classified as type IIc based on the intensity of their ATPase reaction. It is probable that they represent IIa fibres in the process of changing their myosin ATPase to the one that histochemically resembles that of the type I fibres. These changes in fibre population most probably represent changes in the myosin ATPase synthesized by the fibres (Ianuzzo *et al.* 1980). It is not clear, however, whether the fibres that undergo changes in their histochemical characteristics given by their myosin ATPases, also change their oxidative capacity and their physiological properties. There is evidence that suggests that changes in the myosin ATPase characteristics of the fibres are not necessarily related to changes in other biochemical or physiological characteristics (Fitts *et al.* 1980; Capo & Sillau, 1983).

Gustafsson, Tata, Lindberg & Ernster (1965) found that the oxidative capacity is reduced in the muscles of hypothyroid animals. Baldwin *et al.* (1978) reported that thyroid deficiency resulted in a decreased activity of citrate synthase, and a lower concentration of cytochrome *c* in the quadriceps of neonatal rats. Also the activity of succinate dehydrogenase has been reported to be lower in the gastrocnemius muscle of thyroidectomized rats (Gollnick & Ianuzzo, 1972). More recently Nicol & Johnston (1981) induced mild hypothyroidism in rats and found that the activity of cytochrome *c* oxidase was decreased in the soleus but not in the extensor digitorum longus. This difference in the response between the soleus and the extensor digitorum longus may be related to the different fibre composition of these two muscles. It is known that the response of the oxidative enzymes of the muscles to thyroid hormones is affected by the fibre composition of the muscle. Winder & Holloszy (1977) and Capo & Sillau (1983) have shown that muscles made up of mostly type I or slow oxidative fibres

increased their oxidative capacity in response to hyperthyroidism while muscles mostly made up of type IIb or fast glycolytic fibres are unresponsive even after prolonged treatments with relatively high doses of thyroid hormones. Muscles made up of mostly IIa or fast oxidative glycolytic fibres have a response to thyroid hormones that is intermediate between that of muscles made up of I or IIb fibres (Winder & Holloszy, 1977). As expected, the oxidative capacity of the soleus was reduced by hypothyroidism. However, contrary to the lack of response found in hyperthyroidism, our results also showed that 21 or 42 days of hypothyroidism resulted in a decrease in the oxidative capacity of the white area of the gastrocnemius medial head (type IIb fibres). This behaviour could be explained if we assume that during euthyroid conditions the aerobic capacity of the type IIb fibres is already maximally stimulated by the existing levels of thyroid hormones. Thus, administration of excess hormone does not result in changes in oxidative capacity. On the other hand if the concentration of thyroid hormones goes below normal limits this results in understimulation and lower values of oxidative capacity. In turn, this explanation could have its base on differences in the number and/or affinity of nuclear receptors for thyroid hormones (Winder, Fitts, Holloszy, Kaiser & Brooke, 1980). Further studies are needed to clarify this interesting phenomenon.

The degree of capillarization of the skeletal muscle has been shown to change with changes in the oxidative capacity of the fibres. Chronic electrical stimulation of a fast muscle, like the tibialis anterior, with a frequency pattern resembling that of a slow motoneurone results in an increase in the oxidative capacity of the fibres. Also, animals trained for endurance exercise, exposed to chronic cold or made hyperthyroid by administration of thyroid hormones have muscles with higher than normal oxidative capacities. In all these experimental conditions the higher oxidative capacity is accompanied by an increased capillarity of the muscles (Brodal *et al.* 1977; Hudlicka, Tyler & Aitman, 1980; Sillau, Aquin, Lechner, Bui & Banchero, 1980*a*; Capo & Sillau, 1983). The increased capillarity present in the experimental conditions mentioned is the result of a proliferation of new microvessels. At a given f.c.s.a. the c.d. or capillary to fibre ratio of the experimental animals were higher than those of the controls (Brodal *et al.* 1977; Hudlicka *et al.* 1980; Sillau *et al.* 1980*a*; Hudlicka, 1982; Capo & Sillau, 1983). Capillary proliferation also occurs during normal growth. As muscle fibres increase in girth the number of capillaries around each fibre increases (Ripoll *et al.* 1979; Aquin *et al.* 1980) but at the same time c.d. decreases and diffusion distances from the capillaries increase (Kayar *et al.* 1982*b*).

It is not clear what mechanisms are involved in the elicitation of capillary proliferation associated with increased oxidative capacity or normal growth. Some researchers believe that tissue hypoxia is the principal stimulus for capillary proliferation (Valdivia, 1958; Banchero, 1975). This belief is partially based on results of increased capillarity in animals exposed to chronic hypoxia. Although more recent data have seriously questioned these findings (Sillau, Aquin, Bui & Banchero, 1980*b*) there is other evidence indicating that severe tissue hypoxia induces capillary proliferation (Knighton, Hunt, Scheuenstuhl, Halliday, Werb & Banda, 1983). Further, increased oxygen utilization of the muscle induced by chronic electrical stimulation or hyperthyroidism could result in the development of hypoxic areas in the tissue. It is also conceivable that hypoxic areas would also develop during normal

growth due to the longer diffusion distances associated with the increase in the cross-sectional areas of the fibres (Kayar *et al.* 1982*b*). The presence of hypoxic areas under these conditions may trigger the growth of capillaries.

Although hypoxia may be a stimulus for the development of capillaries some evidence indicates that it may not be the only one. Drug-induced chronic vasodilation has been shown to result in higher capillarity in the skeletal and cardiac muscles (Hudlicka, 1982). Recently we found an increased capillarity in the white area of the gastrocnemius' medial head of hyperthyroid rats in the absence of an increased oxidative capacity (Capo & Sillau, 1983). However, blood flow to the muscles has been reported to be increased in hyperthyroidism (Kontos, Shapiro, Mauck, Richardson, Patterson & Sharpe, 1965; Frey, 1967*a*, *b*) and this was suggested as an explanation for the capillary growth observed in the gastrocnemius of the hyperthyroid rats.

In the animals treated with PTU for 3 or 6 weeks the values of capillarity were not different from the values of c.d. and capillary to fibre ratio found in the controls in spite of their significantly lower values of oxidative capacity (Figs. 5–6). The lack of a clear association between the changes in oxidative capacity and capillarity of the muscles in the present experiment, together with previous findings (Maxwell *et al.* 1980), raises doubts about the generally accepted idea that muscle capillarity is mostly determined by its oxidative capacity. Capillary proliferation is evident in muscles after 4 days of electrical stimulation and before 14 days in animals given excess thyroid hormones (Brown *et al.* 1976; Capo & Sillau, 1983). We have also found that 21 days after cessation of excess thyroid hormone administration capillarity of the soleus muscle returns to control values (Sillau, 1985). It therefore appears that 6 weeks should have been enough time to see clear changes in muscle capillarity in the muscle of the hypothyroid animals.

In terms of tissue oxygenation, more relevant than capillary density are the actual distances that the oxygen molecules have to travel. The 'closest individual' method provides a way to measure mean ($\bar{R}$) and maximal (R_{95}) diffusion distances. The actual measurement is independent of c.d. but the values obtained depend on c.d. and on the way in which the capillaries are distributed in the tissue (Kayar *et al.* 1982*a*, *b*). This dependence of $\bar{R}$ and R_{95} on c.d. and also on the way in which the capillaries are arranged in the muscle cross-section is evident from the theoretical lines of Fig. 7. Thus, it is possible to have similar values of capillarity but longer diffusion distances if the capillary distribution changes from a hexagonal to a random array. However, our results do not show any indication that such a change took place in the PTU-treated animals.

As previously stated, two main factors have been suggested as stimulus for capillary proliferation in the muscle of mammals: tissue hypoxia and increased capillary blood flow (Hudlicka, 1982). By analogy it could be assumed that higher than normal P_{O_2} and/or lower than normal capillary blood flow would result in rarefaction of capillaries. Tissue oxygen levels are the result of a balance between oxygen supply and oxygen consumption by the tissues. The lower oxygen capacity present in the muscles of the hypothyroid rats reflects, most probably, a lower oxygen consumption. This would result, if tissue blood flow does not change, in a higher tissue P_{O_2}. In this case the lack of change in capillarity would mean that higher than normal levels of tissue P_{O_2} do not result in attrition of microvessels. However, there is

evidence indicating that tissue perfusion is reduced in hypothyroidism. Cardiac output is considerably reduced and peripheral resistance is increased in hypothyroid animals (Freedberg & Hamolsky, 1974). The increased peripheral resistance is, at least in part, the consequence of an increased catecholamine discharge by adrenergic neurones and of a shift from β-adrenergic-mediated vasodilation to α-adrenergic-mediated vasoconstriction (Zoster, Tom & Chappel, 1964; Stoeffer, Jiang, Gorman & Pikler, 1973; Spaulding & Noth, 1975). Reduced tissue perfusion could compensate for the reduced oxygen utilization of the hypothyroid rats, resulting in normal values of tissue P_{O_2}. In this case the lack of change in tissue capillarity would mean that low tissue perfusion does not result in an attrition of microvessels. Direct measurements of blood flow and tissue oxygenation are necessary to reach solid conclusions regarding the possible effects of tissue P_{O_2} and blood flow in the regulation of tissue capillarity.

In summary we have shown that hypothyroidism induced by PTU administration results in a decrease in oxidative capacity of 'red' and 'white' muscle fibres without a concomitant change in the values of capillarity or diffusion distances from the capillaries to the muscle tissue.

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