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Muscle fibre type populations of human leg muscles

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Synopsis. Four selected leg muscles (gastrocnemius, soleus, vastus lateralis and intermedius) from thirty-two humans were autopsied within 25 hr of death and examined histochemically. The results of histochemical myofibrillar adenosine triphosphatase activity demonstrated that the soleus and vastus intermedius muscles have a higher proportion of slow twitch fibres (70%, 47%) than their synergists, gastrocnemius and vastus lateralis, respectively. The gastrocnemius contains about 50% slow twitch fibres and the vastus lateralis about 32%. Similar proportions of slow and fast twitch fibres have been reported for these hindlimb muscles in other mammals. Human muscles, however, differ from other mammalian muscles in that the proportion of slow and fast twitch fibres were similar in the superficial and deep regions of the muscles examined. Fast twitch oxidative glycolytic fibres in sedentary humans were observed less frequently, and they are less prominent in terms of oxidative enzymatic activity when compared to similar fibres of several laboratory mammals studied previously.

Introduction

The relationship of histochemical to physiological properties of mammalian hindlimb muscles has been firmly established in whole muscles consisting of a homogeneous population (Barnard *et al.*, 1971; Peter *et al.*, 1972) and in heterogeneous muscles by testing single motor units (Burke & Tsairis, 1973; Stephens *et al.*, 1973). It has been demonstrated that analogous muscles of five different mammals (guinea-pig, rat, cat, lesser bushbaby and slow loris) generally have similar proportions of fast twitch oxidative glycolytic (FOG), fast twitch glycolytic (FG) and slow twitch oxidative (SO) fibres. For example, the soleus and vastus intermedius muscles contain a higher percentage of SO fibres than any of the other major hindlimb extensors (Ariano *et al.*, 1973). Both of these muscles are situated deep within a muscle group and are in an anatomical position to resist gravitation. The data in this paper demonstrate that a similar relationship exists in the leg muscles of humans.

Materials and methods

Autopsied muscle samples were taken from the gastrocnemius (lateral and medial portions), soleus, vastus lateralis and vastus intermedius within 25 hr of death. The mean time from death to collection of the sample was 12 ± 1 (SEM) hr (range = 4–25). The mean age of the subjects was 59 ± 3 years and ranged from 21 to 83 years. Samples were taken from twenty-two males and ten females. Carcinoma was the cause of death in 42% of the cases, myocardial infarction in 24%, coronary artery disease in 9%, and leukemia, brain aneurysm and pulmonary embolism making up most of the other cases.

Muscles were frozen in isopentane cooled with liquid nitrogen as described by Edgerton & Simpson (1969). Sections, 10 μm thick, were assayed for myofibrillar adenosine triphosphatase (myo-ATPase) according to the method of Padykula & Herman (1955) as modified by Guth & Samaha (1969), reduced nitocinamide adenine dinucleotide diaphorase (NADH-D) according to the procedure of Novikoff *et al.* (1961) and alpha-glycerophosphate dehydrogenase (α-GPD) activity (Wattenberg & Leong, 1960). Muscle fibres were typed as FOG, FG, and SO according to the profiles described by Edgerton & Simpson (1969) and Peter *et al.* (1972).

Results

The proportions of myo-ATPase dark fibres, or fast twitch (type II according to Engel, 1962) and light fibres, or slow twitch fibres (type I) of the soleus, medial and lateral gastrocnemius and total gastrocnemius muscles are illustrated in Fig. 1. It is clear that the soleus contains a higher proportion of slow twitch fibres (70%) than the gastrocnemius (50%). It is also apparent that the medial and lateral gastrocnemius are similar with respect to fast and slow twitch fibre populations. Data from the vastus intermedius (VI) and deep and superficial vastus lateralis (VL) are presented in Fig. 2. The ratio of the percentage of slow fibres in the soleus and gastrocnemius is very similar to that of the vastus intermedius and vastus lateralis. For example, the soleus has 40% more slow fibres than the gastrocnemius and the vastus intermedius has 47% more than the lateralis (Figs. 1, 2). Note, however, the large range in percentage of fast twitch fibres in each muscle (Figs. 1, 2).

Analysis of NADH-D, α-GPD as well as myo-ATPase activities of each fibre revealed FOG, FG, and SO fibres in the proportions shown in Fig. 3. Note the consistently low proportion of FOG compared to SO and FG fibres in all four muscles.

There are three particular features of human skeletal muscle which make them histochemically distinct from other mammals. First, the proportion of slow to fast fibres is more homogeneous throughout the depth of the muscle. This is evident from the similar proportions of slow muscle fibres in deep and superficial region of the vastus lateralis (Fig. 3). Secondly, within a single muscle fibre, NADH-D staining is more evenly dispersed throughout its cross-sectional area than is true of other mammals (Ariano *et al.*, 1973; Burke & Tsairis, 1973; Edgerton & Simpson, 1969). Thirdly, fast fibres of a muscle have a range of NADH-D activities as is true of other mammals, but the level of NADH-D activity as shown histochemically rarely surpasses the NADH-D activity of slow twitch fibres (Fig. 4).

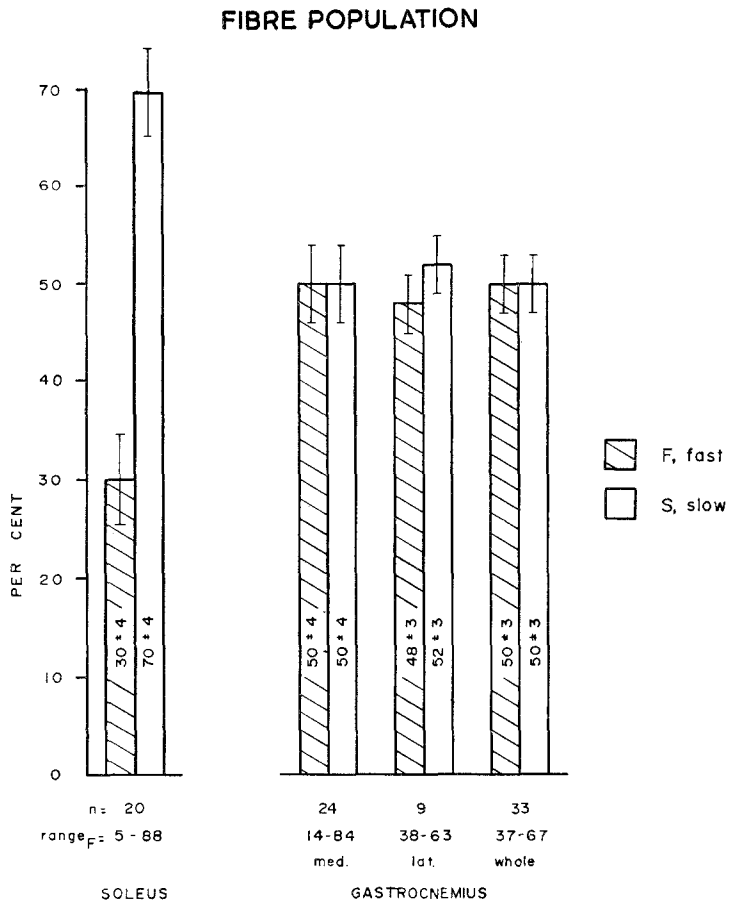

Figure 1. Mean percentage ± SEM of slow and fast twitch fibres of the soleus, medial and lateral gastrocnemius and gastrocnemius muscles without regard to medial or lateral head identified. The range of fast twitch (F) fibre is shown below each set of bars. The number of individual autopsies from which the samples were taken is shown as *n*. Fast and slow twitch fibres were identified on the basis of myo-ATPase activity.

Discussion

In the light of numerous studies that involve muscle biopsies of humans, these data are particularly relevant in that they demonstrate a homogeneous dispersion of fibre types from superficial to deep regions of the VL. This even distribution is also evident in the data of Johnson *et al.* (1973), and from the multiple biopsies taken from the same subject by Edgerton *et al.* (in press). This tends not to be the case in the laboratory mammals studied previously (Ariano *et al.*, 1973). Johnson *et al.* (1973) found no difference between deep and superficial samples in the human gastrocnemius. We have also found that the medial and lateral gastrocnemius have similar proportions of slow and fast twitch fibres. The results of Johnson *et al.* (1973) were similar in this regard.

With respect to the proportion of slow and fast twitch populations in the human vastus intermedius (47% slow twitch), almost identical percentages have been reported

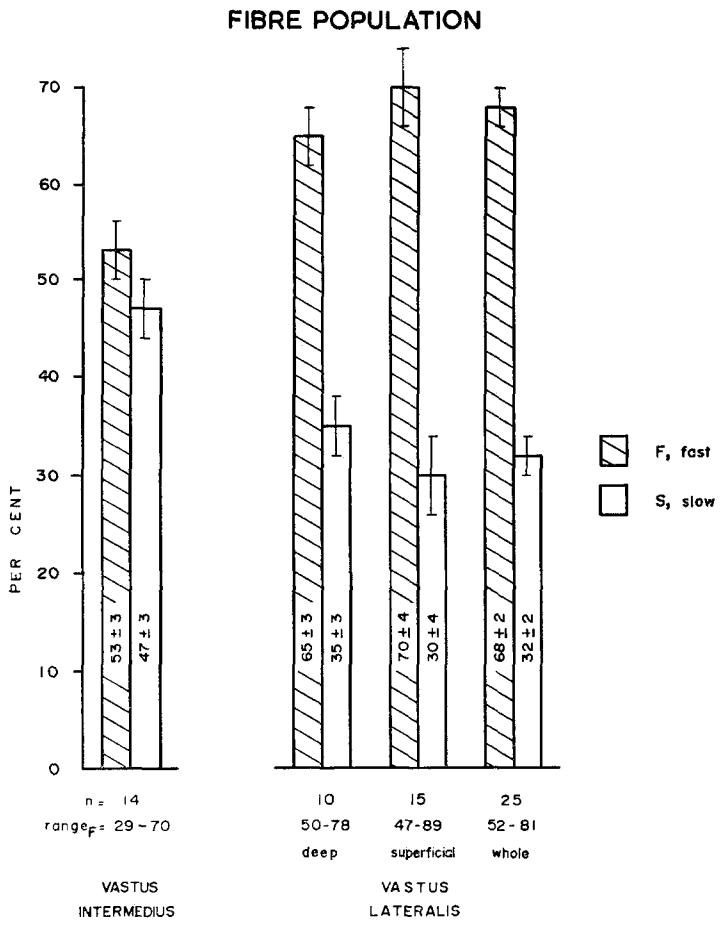

Figure 2. Mean percentage ± SEM of slow and fast twitch fibres of the vastus intermedius and vastus lateralis are shown. The range of fast twitch (F) is also shown. Deep and superficial sections of the vastus lateralis were analysed. The number of individual autopsies from which the samples were taken is shown as *n*. Fast and slow twitch fibres were identified on the basis of myo-ATPase activity.

for the *Galago senegalensis* (44% slow twitch). In the soleus a mean of about 70% slow twitch fibres was found in human, while the *Galago* had about 87% slow twitch fibres (Ariano *et al.*, 1973).

A higher proportion of slow twitch fibres in the human soleus compared to other muscles has been reported previously by Edström & Nystrom (1969). In the two samples examined by these authors, 78 and 65% of the fibres were slow twitch (staining procedures included myo-ATPase). Johnson *et al.* (1973) reported from 77 to 100% type I (slow twitch) fibres for the soleus in six subjects, and Buchthal & Schmalbruch (1970) reported that 89% of the fibres in the soleus from six subjects had high Sudan Black B staining (called C fibres). These authors did not find a marked difference in the contraction time of fibre bundles between the soleus and gastrocnemius muscle. However, we have recently found a more rapid loss of integrated (frequency and amplitude) electro-

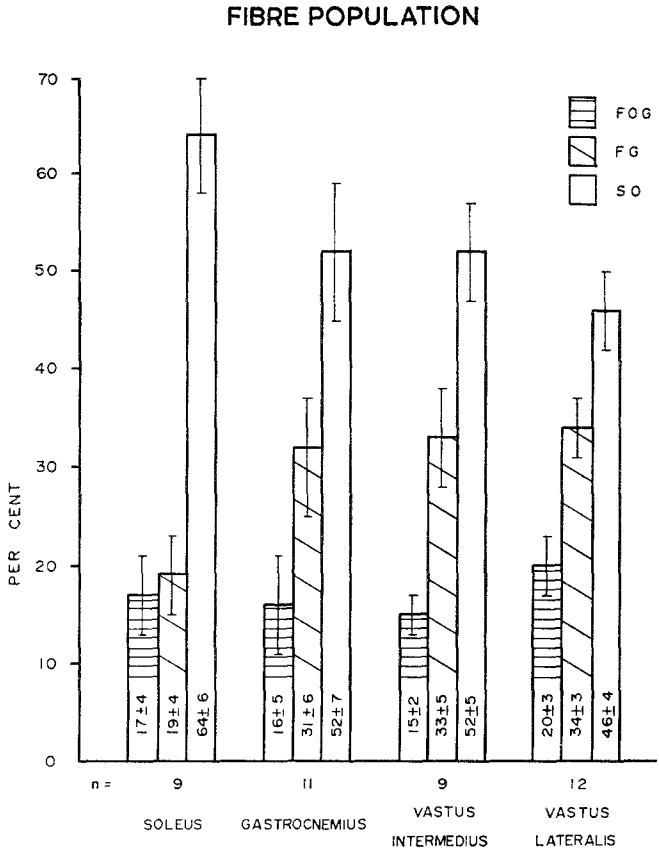

Figure 3. The mean percentage ± SEM of fast twitch oxidative glycolytic (FOG), fast twitch glycolytic (FG) and slow twitch oxidative (SO) fibres of the soleus, gastrocnemius, vastus intermedius and vastus lateralis are shown. The number of samples analysed on this basis is indicated by *n*. These categories were based on myo-ATPase, NADH-D, and α-GPD activity as described by Edgerton & Simpson (1969).

myographic activity in the gastrocnemius than soleus with the progressive loss of muscle tension yielding capacity by the calf of the leg (unpublished observations). This is consistent with the greater resistance to fatigue of SO than FG fibres (Burke & Tsairis, 1973) and the marked drop in EMG amplitude of FG but not SO units when stimulated repeatedly (Stephens *et al.*, 1973).

Human muscle fibres differ from other mammals in most cases in that FOG fibres are less prominent. In our samples, less than 20% of the fibres had high myo-ATPase, oxidative and glycolytic enzymatic activity, whereas percentages greater than 24 and up to 78 are found in the guinea-pig and rat. It has been demonstrated that endurance-trained athletes generally have a greater proportion of fibres having a high oxidative capacity than is true of non-trained controls (Gollnick *et al.*, 1972). Similar results have been reported in trained and stimulated animals (Baldwin *et al.*, 1972; Edgerton *et al.*, 1969; Pette *et al.*, 1973). From our autopsy samples of presumably sedentary subjects, prac-

tically all fast twitch fibres had an oxidative capacity less than the slow twitch fibres (Fig. 4).

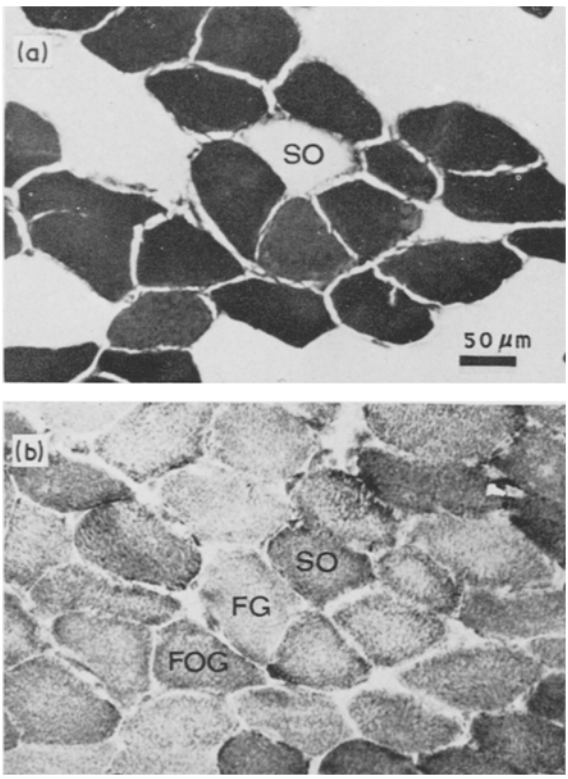

Figure 4. An example of cross-sections of human soleus muscle stained for myo-ATPase (*a*) and NADH-D activity (*b*). Note that the NADH-D activity for the myo-ATPase darkly stained fibres does not appear to exceed the NADH-D activity of any of the low myo-ATPase fibres. SO = slow twitch oxidative; FG = fast twitch glycolytic; FOG = fast twitch oxidative glycolytic.

The justification of calling a muscle fibre fast or slow twitch on the basis of myofibrillar ATPase has been challenged. It should be noted that the previous reports by Samaha & Yunis (1973) and Guth (1973), suggesting that adult mammalian muscle cannot be accurately classified fast or slow twitch by myosin ATPase, are erroneous. The single exception in adult mammalian hindlimb muscle (rat soleus) cited by these authors is, in fact, supportive rather than contradictory to the realization that dark staining myo-ATPase fibres have a relatively shorter twitch contraction time than those fibres which stain lightly. Close (1967) found 4 of 38, or about 11%, of single units in the rat soleus to be significantly faster than the remainder of the slower soleus motor units. This is well within the range that can be expected on the basis of histochemical observations (Ariano *et al.*, 1973), and if one calculates the percentage from the data in Fig. 4 of Ariano *et al.*'s paper, 24% of the motor units of the soleus made up the significantly faster population. This percentage is almost identical to the histochemical slow–fast distinction (Ariano *et al.*, 1973). A similar conclusion regarding the relationship of the percentage of high myo-ATPase fibres and contraction time is valid with the EDL of Close's paper (Ariano *et al.*, 1973). This point has been made earlier by Edgerton & Simpson (1971) and by Close (1972). However, caution is warranted with respect to myo-ATPase staining as an indication of twitch speed in neonatal and adult extraocular muscles. Also discrediting

the supposition of Samaha & Yunis (1973) and Guth (1973) that the myo-ATPase is not a reliable indication of actual myofibrillar ATPase, but of mitochondrial ATPase, is the simple observation that the red and white vastus lateralis stain similarly with myo-ATPase (dark) even though the red vastus has almost ten times more mitochondria (Peter *et al.*, 1972).

One can more validly take issue with the nomenclature used. Clinicians traditionally use type I and II as labels for low and high myo-ATPase fibres respectively. The specificity of the histochemical staining for myo-ATPase has been verified in single muscle fibres assayed quantitatively (Taylor *et al.*, 1974). Our view is that one should not use abstract terms when data exist that support a physiologically sensible terminology. We further feel that there are other properties of skeletal muscle fibres besides its twitch speed that characterizes the fibre's functional capacity. Thus, we have chosen to describe its metabolic properties which reflect the fatigue properties. It seems reasonable that clinicians, particularly, would employ a system which includes metabolic properties. It appears that many investigators use the dichotomy of fibres with respect to contraction time or myo-ATPase because it is simple and reliable. However, it should be recognized that such a nomenclature ignores other potentially important information, namely, metabolism.

Conclusion

The histochemical results of this paper show that the relationship of the relatively slow soleus and fast gastrocnemius in the calf and slow vastus intermedius and fast vastus lateralis in the thigh is similar in humans as it is in other mammals studied previously. Therefore, these two pairs of muscles provide a useful model for studying how slow and fast muscles are used to perform specific types of movements in humans.

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References

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973). Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* **21**, 51–5.

BALDWIN, K., KLINKERFUSS, G. H., TERJUNG, R. L., MOLE, P. A. & HOLLOSZY, J. O. (1972). Respiratory capacity of white, red and intermediate muscle: Adaptive response to exercise. *Am. J. Physiol.* **222**, 373–8.

BARNARD, R. J., EDGERTON, V. R., FURAKAWA, T. & PETER, J. B. (1971). Histochemical, biochemical and contractile properties of red, white and intermediate fibers. *Am. J. Physiol.* **220**, 410–14.

BUCHTHAL, F. & SCHMALBRUCH, H. (1970). Contraction times and fiber types in intact human muscle. *Acta physiol. scand.* **79**, 435–52.

BURKE, R. E. & TSAIRIS, P. (1973). Anatomy and innervation ratios in motor units of cat gastrocnemius. *J. Physiol. (Lond.)* **234**, 749–65.

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

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* **52,** 129–97.

EDGERTON, V. R. & SIMPSON, D. R. (1969). The intermediate fiber of rats and guinea pigs. *J. Histochem. Cytochem.* **17,** 828–38.

EDGERTON, V. R. & SIMPSON, D. R. (1971). Dynamic and metabolic relationships in the rat extensor digitorium longus muscle. *Exp. Neurol.* **30,** 374–6.

EDGERTON, V. R., SALTIN, B., ESSÉN, B. & SIMPSON, D. R. (1975). Glycogen depletion in specific types of human skeletal muscle fibers in intermittent and continuous exercise. In: *Metabolic Adaptation to Prolonged Physical Exercise* (eds. H. Howard & J. R. Poortmans), in press.

EDGERTON, V. R., GERCHMAN, L. & CARROW, R. (1969). Histochemical changes in rat skeletal muscle after exercise. *Exp. Neurol.* **24,** 110–23.

EDSTRÖM, L. & NYSTROM, B. (1969). Histochemical types and sizes of fibres in normal human muscles. A biopsy study. *Acta neurol. scand.* **45,** 257–69.

ENGEL, W. K. (1962). The essentiality of histochemical and cytochemical studies of skeletal muscles in the investigation of neuromuscular disease. *Neurology* **12,** 778–84.

GOLLNICK, P., ARMSTRONG, R. B., SAUBERT, C. W. IV, PIEHL, K. & SALTIN, B. (1972). Enzyme activity and fiber composition in skeletal muscle of trained and untrained men. *J. appl. Physiol.* **33,** 312–19.

GUTH, L. (1973). Fact and artifact in the histochemical procedure for myofibrillar ATPase. *Exp. Neurol.* **41,** 440–50.

GUTH, L. & SAMAHA, F. J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscles. *Exp. Neurol.* **25,** 138–52.

JOHNSON, M. A., POLGAR, J., WEIGHTMAN, D. & APPLETON, D. (1973). Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J. neurol. Sci.* **18,** 111–29.

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 for adenosine triphosphatase. *J. Histochem. Cytochem.* **3,** 170–95.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11,** 2627–33.

PETTE, D., SMITH, M. E., STAUDTE, H. W. & VRBOVÁ, G. (1973). Effects of long term electrical stimulation on some contractile and metabolic characteristics of fast rabbit muscles. *Pflügers Arch.* **338,** 257–72.

SAMAHA, F. J. & YUNIS, E. J. (1973). Quantitative and histochemical demonstration of calcium activated mitochondrial ATPase in skeletal muscle. *Exp. Neurol.* **41,** 431–9.

STEPHENS, J. A., GERLACH, R. L., REINKING, R. M. & STUART, D. G. (1973). Fatigueability of medial gastrocnemius motor units in the cat. In: *Control of Posture and Locomotion* (ed. R. B. Stein, K. G. Pearson, R. S. Smith & J. B. Redford), p. 179–85. New York: Plenum Press.

TAYLOR, A. W., ESSÉN, B. & SALTIN, B. (1974). Myosin ATPase in skeletal muscle of healthy men. *Acta physiol. scand.* **91,** 568–70.

WATTENBERG, L. W. & LEONG, J. L. (1960). Effects of coenzyme Q_{10} and menadione on succinate dehydrogenase activity as measured by tetrazolium salt reaction. *J. Histochem. Cytochem.* **8,** 296–303.