On the Heterogeneity of Skeletal Muscle Fibers: The Intermediate Fibers

In recent years, it has been possible to demonstrate 2 major types of muscle fibers in most animal species through enzyme histochemical reactions[1–3] and their selective staining by lipid and basophilic dyes under appropriate conditions of tissue processing[4–6]. The so-called red muscle fibers (type I) are known to be rich in mitochondria and react strongly to succinic dehydrogenase[1]. The white muscle fibers (type II) are less reactive to oxidative enzymes, larger in size, and contain more glycogen[6,7] as well as glycolytic enzymes[2,8].

In view of the fact that between these 2 extreme and somewhat opposite types of muscle fibers there exist all possible intermediate gradations (Figure 1), the question arises whether one category of intermediate muscle fibers exists as such, as has been suggested by some workers[2,3,9], or if their occurrence simply reflects variations in their degree of functional and metabolic activity. In an attempt to individualize an intermediate muscle fiber in morphological terms, experiments were undertaken to evaluate by semiquantitative means the enzymatic activity of the whole spectrum of muscle fibers encountered in different muscle specimens, with regard to the size of the fibers and the relative proportion of mitochondria.

Material and methods. The muscle specimens originated from a series of 20 female Wistar rats weighing 100–130 g. The animals were killed by esanguination and various muscles from the hind limbs were dissected and rapidly frozen in liquid air. Cryostat frozen sections were stained for the demonstration of succinic dehydrogenase (SDH)[10]. After several trials, the quadriceps on the one hand and the soleus on the other were selected as being the most appropriate muscles in that they provided the full range of evaluation criteria: while the former muscle is highly heterogenous, type I muscle fibers predominate in the latter. Final readings were made on 2 or more tangential sections in 3 delimited areas in order to ensure an adequate distribution of (type I) and (type II) muscle fibers.

The sections were projected on a ground glass transparent screen with an optical system providing a total magnification of 645 X, using a light source of 500 W. The intensity of the reaction in each individual fiber was measured with a photovolt 520-M photometer and the surface of the same fibers was determined with a Keuffel & Esser planimeter. Readings of the intensity of light were made after calculating the correction index in unstained preparations. The values are expressed on a scale proportional to the log of the optical density. All measurements were made on 12 fibers in 10 different serial sections, for calculations of the standard error.

The relative proportion of mitochondria in individual muscle fibers was studied in light microscopy in sections stained for SDH at 1000 X magnification. The glycogen content in the respective fibers was measured by densimetry in fresh frozen sections stained with PAS after lipid extraction with pyridine.

Results. The staining patterns of the muscle fibers in the quadriceps are shown in Figure 2. The numerical values on the abscissa correspond to the log of the optical density of formazan precipitates. They run between 5 and 70, with a standard error of ± 1, and the equivalent number of fibers for each reading is indicated on the ordinate. It can be seen that the distribution frequency delineates 2 separate peaks, permitting differentiation between the 2 groups of fibers. The first peak corres-

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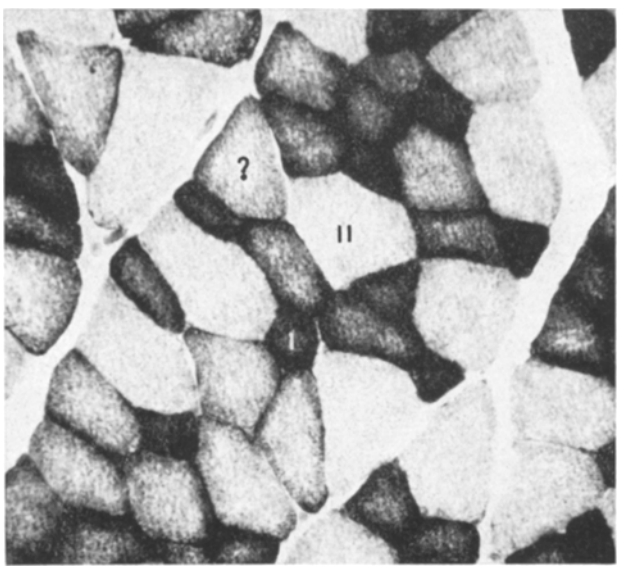

Fig. 1. SDH activity in the normal quadriceps of the rat. Note the differences in the staining reaction and the size of type I and type II muscle fibers. The ? refers to an intermediate category of fiber.

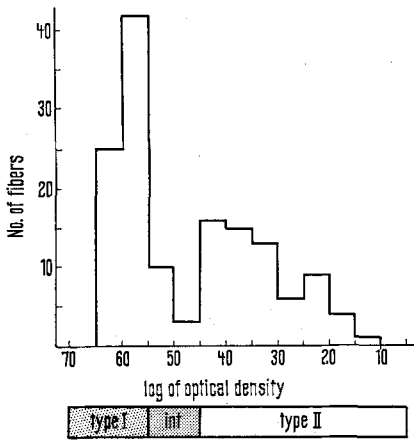

Fig. 2. Histogram of SDH reactivity in the quadriceps. The height of the columns represents the number of fibers within the same range of reactivity to SDH, as determined by photometric measurements. 2 groups are discernible, one corresponding to type I and intermediate fibers (int), and the second to type II.

ponds to the maximum reacting fibers (type I), with sharp progression and regression of neighbouring values; but with the second peak, the transition is less abrupt, because of a wider range of the corresponding type II white fibers. It should be noted, however, that an intermediate category of fibers is hardly discernible in the quadriceps. On the other hand, if the readings of the optical density of the soleus muscle fibers are applied to the same scale, it can be seen that the distribution of frequency falls into a much narrower range; 2 separate peaks are outlined with the presence of intermediate reacting muscle fibers (Figure 3). In fact, typical type II muscle fibers are not demonstrable in the soleus with this position of reference.

Data pertaining to the distribution of frequency in relation to the size of fibers are given in Figure 4. Three separate groups within a range of 3–23 cm^2 are distinguishable, with the respective values lying between 3 and 9, 9 and 13, 13 and 25. This distribution pattern, however, is demonstrable only in those muscles that comprise a relatively high proportion of type I fibers. Figure 5 shows the intensity of the reaction to succinic dehydrogenase in relation to the size of the fibers, each dot representing 1 fiber. There is a close relationship between the intensity of the staining reaction and the size of the muscle fibers.

A survey of the mitochondrial population in each individual fiber enables us to observe that the occurrence of larger mitochondria, whether subsarcolemmal or intermyofibrillar, was characteristic of the small- and the medium-size fibers, although in lesser amount in the latter. In those fibers that exhibited a weak staining reaction to succinic dehydrogenase, very few large mitochondria could be evidenced. These fibers were almost exclusively equipped with small paired mitochondria near the Z band.

Figure 6 illustrates the intensity of the staining reaction to PAS in relation to the size of muscle fibers. Here, 3 groups can be individualized, with a greater frequency for the intermediate values. It is seen that, in the first group, the staining reaction tends to increase in relation to the size of the fibers, whereas the reverse is true as far as the third group of fibers is concerned. However, this does not apply to those regions formed almost exclusively by white muscle fibers.

Discussion. Photometric readings afford a more accurate and objective evaluation of the SDH activity when appraisal is made on tissue sections. This mode of evaluation is particularly useful when related to planimetric measurements, which were absent in previous studies dealing with the identity of muscle fibers[9]. The intermediate group of muscle fibers is more apparent in the soleus, which however, does not comprise the entire

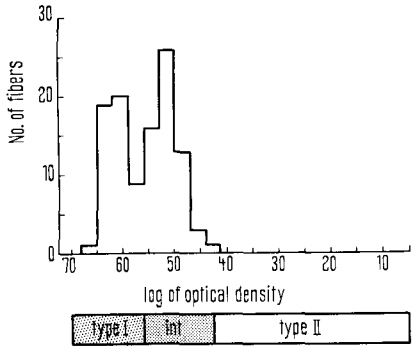

Fig. 3. Histogram of SDH reactivity in the soleus. In this particular muscle, the reactivity pattern of the fibers to SDH corresponds to type I and intermediate fibers (int). None of the type II fibers are demonstrable.

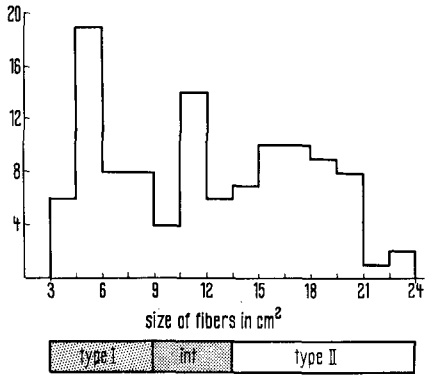

Fig. 4. Histogram of the size of fibers in quadriceps. Each column represents the number of muscle fibers of the same size, as determined by planimetric measurements. 3 groups are distinguishable: type I, intermediate (int), and type II fibers.

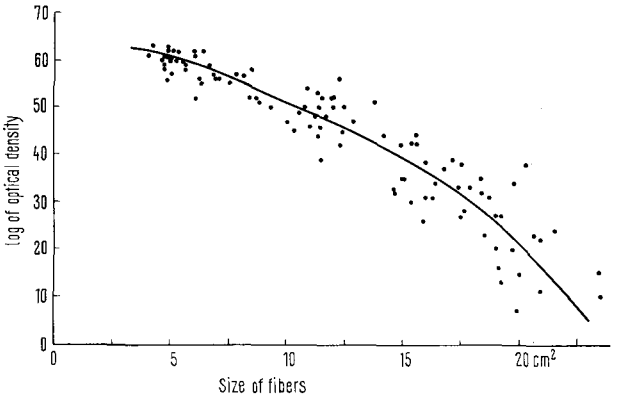

Fig. 5. SDH reactivity in relation to the size of the muscle fibers. Each fiber is indicated by a dot that coordinates the size and the density. Note the close relationship between these 2 parameters.

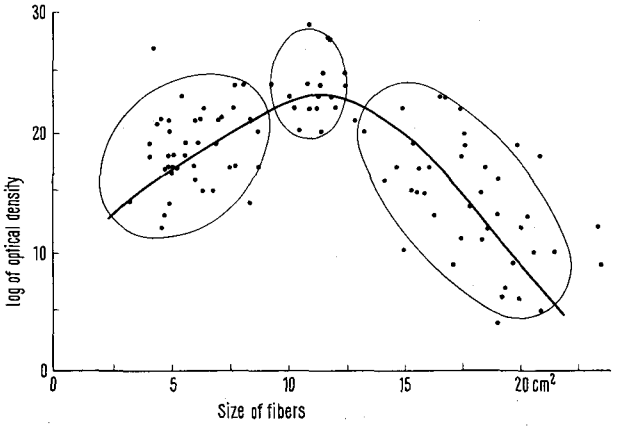

Fig. 6. Intensity of the PAS-staining reaction in relation to the size of fibers. Each dot represents 1 fiber. Note that the staining reaction is higher for the fibers that are intermediate in size (central area).

spectrum of the different type of fibers. The significance of these observations is strengthened by the fact that the 2 categories of fibers (type I and intermediate) are demonstrable as much by planimetric means as by the optical density of the tetrazolium-staining reaction.

Photometric determinations are more critical when dealing with the PAS reaction because of the smaller scale between the 2 extremes of the readings. However, it is apparent that the intermediate size fibers contain more glycogen than the other 2 categories. The functional significance of the intermediate fibers is unknown. It is believed from embryogenesis studies of the chicken muscle that they are precursors of white-muscle fibers[11]. But even if their exact role remains undetermined, the existence of a third category of fibers in well-differentiated muscle has significant morphologic bearings. Such fibers are distinctive in their size, their weaker reaction to succinic dehydrogenase and a smaller proportion of the large type of mitochondria, and their relatively high glycogen content[12].

Résumé. Les mesures d'intensité de la réaction à la succino-déshydrogénase et de la surface des fibres des muscles quadriceps et soléaire chez le rat nous ont permis d'individualiser un groupe de fibres intermédiaires qui se situe entre les fibres rouges (type I) et blanches (type II). Les fibres intermédiaires ont une activité enzymatique et une population mitochondriale distinctes, une taille moyenne et une teneur élevée en glycogène.

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Einbau von ^{3}H-Uridin in Follikelepithelzellen degenerierender Oozyten beim Zebrafisch, *Brachydanio rerio* (Ham. Buch.)

In Ovarien vieler Tierarten treten regelmässig degenerierende Eizellen auf[1-3]. An der Resorption dieser Oozyten ist das Follikelepithel aktiv beteiligt, wobei es durch Einfaltung zu mehrschichtigen Zelleisten kommt, die in die Dottermassen hineinragen[1], oder zur Einwanderung von Follikelepithelzellen[4]. Letzteres ist auch bei *Brachydanio rerio* der Fall. Schon bei Beginn der Degeneration sind im Zytoplasma dieser Zellen histologisch Vakuolen[1] und Kohlenhydrateinschlüsse[5] und elektronenmikroskopisch abnorme Lamellenstrukturen[4] nachweisbar, was auf einen grundlegenden Funktionswandel hindeutet. Am Beispiel der RNS-Synthese wurde autoradiographisch untersucht, inwieweit mit der veränderten Funktion der Follikelepithelzellen eine Stoffwechselumstellung einhergeht.

Ausgewachsenen weiblichen Zebrafischen wurde i.p. ^{3}H-Uridin (0,025 ml wässrige Tracerlösung = 12,5 µC pro Versuchstier) verabreicht. Nach Inkubationszeiten von 1–12 h wurden die Tiere getötet und von den Ovarien nach histologischer Aufbereitung Autoradiogramme hergestellt (Kodak-Strippingfilm AR 10). Die Expositionszeit betrug 28 Tage.

Nach kurzen Inkubationszeiten (1–4 h) waren in den Autoradiogrammen degenerierender Eizellen vorwiegend die Zellkerne des Follikelepithels markiert, während nach längerer Inkubation zunehmend auch ausserhalb der Kerne Silberkörner lagen. Die Markierung war in allen Fällen um ein Vielfaches höher als in normal sich entwickelnden Follikeln. Figur 1 zeigt das Autoradiogramm einer fast ausgewachsenen Eizelle (Stadium IV[6]) zu Beginn der Degeneration. Die intensive Schwärzung über dem Follikelepithel steht in starkem Kontrast zur geringen Markierung bei normal sich entwickelnden Eizellen. In der Zona radiata sind mehrere Aussparungen deutlich markiert, in denen im histologischen Bild nach feulgen-positiv erscheinen, findet sich eine starke Markierung, in der Dotterschollenbruchstücke ausgespart sind. Nach zweistündiger RNase-Behandlung bei 37 °C (0,5%ige RNase-Lösung, 40 Kunitz-Einheiten/mg) war in den Autoradiogrammen nur noch 10–12% der ursprünglichen Radioaktivität nachweisbar.

Die hohe RNase-empfindliche ^{3}H-Uridin-Einbaurate ist Ausdruck einer gesteigerten RNS-Synthese in den Follikelepithelzellen degenerierender Oozyten. Es erfolgt also neben histologisch und elektronenmikroskopisch

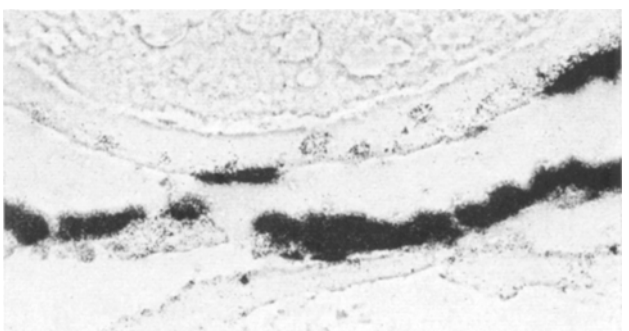

Fig. 1. Autoradiogramm einer degenerierenden Eizelle (Stadium IV[6]) nach zwölfstündiger Inkubation mit ^{3}H-Uridin. Das stark markierte Follikelepithel ist teilweise durch die Präparation von der Zona radiata gelöst. Dadurch wird die Markierung über einwandernden Zellen auffälliger. × 560.

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