Morphometric Analysis and Oxygen Consumption of Developing Mouse Diaphragm Muscle

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Abstract

The composition of muscle fibres in the male mouse diaphragm was investigated at four periods ranging in ages from 3 days prior to birth, 3 and 14 days postpartum and in the adult. Standard morphometry was used to estimate the mitochondrial, sarcoplasmic reticulum, sarcoplasm and myofibrils content. The respiratory activity of isolated mitochondria was measured biochemically, using cytochrome c as a substrate. The maximal aerobic capacity (VO_2 max) was estimated for cold exposed animals using the Kalabukhov - Skvortzov method. The volume of mitochomdria, sarcoplasmic reticulum and myofibrils increases during animal development. The oxidative capacity at each of the four stages was in direct proportion to the total volume of their mitochondria. Maximal aerobic capacity (VO_2 max) per gram body mass after cold exposure, was 1.2 times greater in juvenile mice (+14 days) than in suckling (+3 days) mice and 1.5 times greater in juvenile mice than in adult animals. This greater oxidative potential in juvenile mice is achieved by a greater ratio between the total volume of mitochondria and volume of diaphragm muscle.

Key words: diaphragm, morphometry, development, oxygen consumption.

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The metabolic rate in a mammal depends largely on two main factors: body size and ambient temperature. But there are also other determinants in an homeotherm animal, e.g. physical exercise. When an animal is involved in physical stress, or is exposed to cold beyond its normal ambient temperature range, the metabolic rate is affected, and the need for oxygen increases. The diaphragm, which is the most important inspiratory muscle, responds immediately to an oxygen demand. Therefore, the diaphragm and the other respiratory muscles have to be trained relatively early, during foetal life of an animal to be a match for its adult life metabolic rate. The respiratory movements in foetuses have been recorded many times in various species [4, 9, 10, 13, 16, 18]. In the early stages of lung development, the air passages and saccules are filled with fluid, which is in communication with the amniotic fluid. During the last third of gestation when spontaneous foetal breathing occurs, the lung fluid is pumped in and out. These pseudo -respiratory movements, which occur long before the delivery of an animal, may initiate and facilitate the process of muscle fibre differentiation in all respiratory muscles.

The present experiments were designed to elucidate the correlation betweem the morphological observations made and the physiological and biochemical responses of selected muscle fibre compartments: mitochondria, sarcoplasmic reticulum, myofibrils and sarcoplasm of the diaphragm to variable environmental factors which occur during animal development.

Materials and Methods

Physiology

Before the experiment started all the animals were kept at room temperature (20°C). and the average body temperature was 35.8°C for adults, 30.7°C for juveniles and 30.5°C for sucklings. The resting metabolic rate (RMR) was measured at low temperatures (1°C or 2.5°C) and at room temperature, (20°C), in 13 juvenile animals (14 day old) and 7 adult mice. In 12 sucklings (3 day old) mice the measurements were limited only to the 2O°C series. This series of 30-minute long measurements was carried out in a closed circuit manometric respirometer of the Kalabukhov-Skvortzov system in chambers of 500 or 1000 cm^3 capacity [7]: The mice were placed in wire net cages (8.5 x 4 x 4 cm) that prevented the animals' movements.

After removing the animals from the respiratory chambers, rectal (or oral in suckling mice) temperatures were taken within 20 s with an electronic probe Etron TL-2000 [7], accurate to the nearest 0.2°C. The rectal temperatures were taken in 8, 9, 10, 12 and 13-day old mice and each age group consisted of 15 animals. The temperature was taken again after each separate animal was exposed for 30 min to 20°C ambient temperature. This procedure was followed to determine the age at which a mouse acquires the ability to maintain a stable body temperature when outside the nest.

Biochemistry

Twenty four animals, males KP outbred mice were used in the biochemical study. Groups of 3 to 6 animals were anesthetized with ether and killed at developmental periods representing ages of -3 days before delivery (foetus), +3 days postpartum (suckling), +14 days postpartum (juvenile) and adults (Tab. 1). The whole diaphragm was immediately excised, washed in phosphate buffered solution (PBS), weighed and finally minced and suspended in 4 volumes of ice cold phosphate buffer (0. 05M), pH 7.0. Subsequently, the suspension was homogenized in a glass homogenizer and then centrifuged (10min, 600xg). The supernatant, containing mitochondria, was used to measure the cytochrome c oxidase activity [8]. Cytochrome c oxidase activity was measured spectrophotometrically at 550nm. The supernatant was diluted 2-5 times with the 0.1M phosphate buffer, pH 7.6, containing 0.6 ml of 0.1% cytochrome c reduced with sodium hydrosulfite ($Na_2S_2O_4$). The enzyme activity was expressed in μM 0_2.min^{-1}.mg proteins. Protein concentration was estimated by means of the Bradford method [5].

Animals for morphology

Twenty male KP outbreed mice were used in the morphological study. Groups of 5 mice were anesthetized with ether and killed at developmental periods representing approximate ages of -3, +3, +14 days and adults. These ages correspond to weight (means, SD) of 0.99 ±0.033; 1.41 ±0.062; 4.95 ±0.043; 20.86 ±2.41g respectively (Tab. 1).

Electron microscopy

After death, the diaphragm muscles were rapidly removed from animals and weighed. The muscles were completely cut into thin longitudinal strips and fixed in a 6.26% solution of glutaraldehyde in 0.1M sodium cacodylate buffer adjusted to 430 mOsm with NaCl (total osmolarity of the fixative 1100 mOsm, pH 7.4). Samples from arbitrary locations of the muscle strips were postfixed in 1.0% OsO_4 and embedded im Epon 812. The thin sections, of a pale gold color, were cut with an LKB Ultratome, model 1802A. The sections were stained in uranyl acetate and then with lead citrate. Thin sections cut transversely to the long axis of the muscle fibres were photographed using a Philips 300, Tesla BS 500 and JEM - 100-SX electromicroscopes at a magnification of 20,000 x. The microscopes were calibrated for each series of pictures taken with carbon grating, which had 21600 lines/cm (E.F. Fullam, Schenectady, N.Y.- No 321).

Morphometry

Five tissues blocks from each of the five animals were sectioned for a stereological analysis of each individual diaphragm. Five micrographs from each section (block) were taken in consecutive frames, yielding approximately 50 muscle fibre profiles for analysis from each 14 day old animal and adult. Up to 20 muscle fibre profiles for each foetus (-3 days) and suckling animal (+3 days) were analyzed. Contact prints of 35 mm films and 6x9 cm negatives were projected onto a screen fitted with a double square lattice test system C-64 (576 test points) and B-36 (144 test points). Point counting was done at a final magnification 97,200 x [24]. The volume density of all structures was expressed in $cm^3.cm^{-3}$ in a reference volume of muscle fibre. The subsarcolemmal mitochondria [$V_{v(ms,\ f)}$] were defined as any that were localized at the fibre border close to the sarcolemma. The interfibrillar mitoehondria [$V_{v(mi,\ f)}$] were defined as any that were intermingled with myofibrils. The total mitochondria [$V_{v(\ mt,\ f)}$] were the sum of subsarcolemmal and interfibrillar mitochondrial volume densities. The sarcoplasmic reticulum volume densities [$V_{v(sr,\ f)}$], were defined as all the smooth vesicles, tubules and cisternae associated with the myofibrils. The sarcoplasmic volume densities [$V_{v(spl,\ f}$] were defined as all the residual structures i.e. lipid droplets, endoplasmic reticulum profiles, glycogen and fibre nuclei. The mean values of all structures analyzed were compared between stages of development by an unpaired Student's t-test. Also, the coefficient of variation (CV in %) was calculated within the limits of each stage. The errors were calculated as standard errors of ratios [6].

Results

Maximal oxygen uptake rate

The results of RMR measurements are given in Table 2. The highest metabolic rates were noted in 14 day old mice which also showed the highest drop in body temperature (Tb) after exposure to 1.3°C. Following a warmimg-up period, which lasted 15 min, T_b in all animal studied returned to their normal temperature of 30.7°C. In the temperature range applied (0.2°C), the decrease in metabolic rate in adult mice amounts to 4.12% while in 14 day old mice the metabolic rate decreases only insignificantly (3.28%). The oxygen consumption during the 5-minute period of measurement in each animal decreases in both the 3 day and 14 day old mice. The most remarkable drop in oxygen consumption, however, was recorded during exposure of the animals to the low temperature. In the 3 day old mice, oxygen consumption dropped in the range of 1.0 - 0.3 ccm/g x 5 min compared with 14 day old mice in the range of 1.7 - 0.6 ccm/g x 5 min. In both cases the decrease in the oxygen consumption was associated with lowering body temperatures (T_b) of the animals (Tab. 2). The measurements of T_b in juvenile animals whose ages ranged from 8 to 13 days, separately exposed to 20°C of ambient temperature, revealed an evident increase in their body temperatures. In 13-day old mice T_b attained the level normally recorded in the animals remaining in the nest with their mother. This last experiment was designed to show that the basic thermoregulatory abilities are acquired in about two week old mice.

Mitochondrial oxygen consumption

The specific activity of oxygen consumption was measured with cytochrome c as the substratum, which covered the last step of the respiratory chain. The values for foetus and suckling diaphragm muscle increase respectively; however, the values for juvenile diaphragm muscle were lower than that of suckling and reached statistical significance ($p < 0.01$). The highest values were consistently found in fully developed diaphragm muscle (adult). Biochemical data on isolated mitochondria were expressed in μM O_2 per min^{-1} per mg of protein and per g of intact diaphragm muscle (Tab. 3).

Morphometry of muscle fibres components

The adult mice have over 24, 18 and 5 times more of their total body mass, compared to foetuses, suckling and juveniles respectively. The mass of diaphragm of adult mice is 15 times, 12 times and 4 times greater than those of foetuses, suckling

Table 1. Body mass and diaphragm mass as percentage of body mass [Means, Mb; Md; units, g; (%); SD; SEM; CV in %]

	Foetus (-3 days)	Suckling (+3 days)	Juvenile (+14 days)	Adult
Mb	0.996	1.39	4.95	24.71
n	7	13	5	5
SD	0.033	0.076	0.044	2.43
SEM	0.013	0.044	0.020	1.08
CV	3.3	5.4	0.88	9.84
Md	0.0056	0.007	0.02	0.085
SD	0.0018	0.001	0.004	0.002
SEM	0.0004	0.0004	0.002	0.001
CV	32	19.2	18.2	2.6
%	0.5	0.5	0.4	0.3

*Table 2. Maximal oxygen consumption rate (VO_{2max}) in mice of different age, exposed to low ambient temperature. (n), number of animals; SD, standard deviation; cv, coefficent of variation in %. Statistical level of significance, * $p < 0.05$.*

	Body weight in g		Resting Metabolism Rate	
Age	Body weight in g	Ambient temperature	Oxygen consumption $ccmO_2/g \times h$	Body temperature after experiment
Suckling	2.68	18.3	6.39*	22.0
n	12			
sd	0.69		2.06	1.1
cv	25.7		32.2	5.0
Juvenile	6.29	19.6	7.61	31.6
n	13			
sd	1.18		2.42	3.2
cv	10.7		31.8	10.1
	6.33		12.63*	16.0
n	13	1.3		
sd	1.25		7.04	2.9
cv	19.7		55.7	18.1
Adult	25.6	17.5	5.12	36.5
n	7			
sd	4.7		1.28	0.9
cv	18.3		25.0	2.4
	25.6		8.28*	29.8
n	7	2.5		
sd	4.7		1.53	3.0
cv	18.3		18.5	10.1

and juvenile respectively (Tab. 1) and is similar to its total volume (Tab. 5).

Mitochondria

In the random muscle samples, mitochondrial volume increases significantly in each of the following stages of development from foetuses to adults. The total volume of mitochondria, (V_{mt}), was calculated as the product of volume density of total mitochondria, [$V_{v(mt, f)}$] multiplied by the muscle volume, in turn obtained by multiplying the muscle mass by its specific weight (1.06g x cm^{-3}, [17]). The volume density of total mitochondria, [$V_{v(mt, f)}$], increases with animal maturation. The volume density of interfibrillar mitochondria, [$V_{v(mi,f)}$] is usually higher in the three initial stages of maturation, but in the adult mice it is lower, when the subsarcolemmal mitochondria predominate (Fig. 1). In the juvenile stage, however, all the values of mitochondrial density [($V_{v(mi, f)}$; $V_{v(ms, f)}$; $V_{v(mt,f)}$], are lower from those of previous stages of development (suckling animals) (Tab. 4).

Myofibrils.

The volume density of myofibrils, [$V_{v(fi, f)}$] increases by about 66% from the foetuses to the next three stages of development. The most significant increase of the $V_{v(fi, f)}$ occurs when adult mice and foetuses are compared ($p < 0.01$) but it is not significant between suckling and juveniles. In the adult stage the $V_{v(fi, f)}$ is significantly higher from those of suckling and juvenile stages (Tab. 5).

Sarcoplasmic reticulum

The total volume of sarcoplasmic reticulum (sr) increases slowly from the foetuses to suckling by a factor of 1.7 and then more rapidly in the juvenile stage by a factor of 3.09 to reach its highest value in the adult stages (factor 5.7). The values of the volume density, however, of sr per muscle fibre volume increases by an average factor of 1.26 from the first to the last stage of development (Tab.5). The total volume of sr was calculated as the product of volume density of sr multiplied by the total volume of muscle fibres, [$V_{(f)}$].

Sarcoplasm

The volume density of sarcoplasm decreases by about the same amount that the myofibrils increase. In the juvenile stages the $V_{v(spl, f)}$, is again lower than in the suckling stages (Tab. 5).

Discussion

Although the diaphragm has been subjected to numerous anatomical studies, a clear understanding of its morphological organization at the quantitative ultrastructural level during an animal's development is still lacking. In this study we have examined some of the physiological, biochemical and morphological characteristics in the development of the mouse diaphragm. Particular note has been made in the connection with their ontogeny, during which the animals gain the ability to maintain stable body temperature. The analysis of the individual diaphragm muscle fibres at the four levels of mouse development, reveals distinct ultrastructural differences between the foetus (-3 days), suckling (+3days), juvenile (+14 days) and adult forms. Their diaphragm muscles were found to differ, first of all in mass and then, consequently, in total volume of mitochondria as well as in volume density of these organelles. The mass of the diaphragm muscle tissue increases linearly ($r = 0.9999$) during mouse development and in the adult it is 25 times greater than that in the foetus. A similar correlation ($r = 0.9691$), was found between the estimated mitochondrial content and their volume density ($r = 0.9496$). The muscle tissue of adult mice diaphragm contained 20 times higher volume of mitochondria than the muscle samples of foetuses and suckling mice, but only 2.5 times higher than that of juvenile animals (Tab. 4). It is not surprising however, that such a dramatic increase in mitochondrial amount is achieved by juvenile animals in only two weeks. It is absolutely necessary to maintain the high breathing activity, since the 14 days old mice showed the highest metabolic rates. When exposed to the low temperatures (Table 3.), the juvenile mice decrease their metabolic rate to only 3.29% and differ marginally from that of adult amimals (4.12%). It is therefore evident that juvenile animals have already acquired the basic thermoregulatory abilities. The maximal metabolism measured in this study is represented by a maximum oxygen consumption utilized for the generation of heat in thermogenesis [23] in contrast to the maximum oxygen consumption attained during a physical effort [22]. The first of these two alternative ways of forcing the maximum metabolism can be followed only after the animals have attained the ability to thermoregulate i.e. in the various species of homeotherms between several to 12 or more days of life.

The other way of measuring maximum metabolism can be applied after 2 - 3 weeks of life. In our experiment we have determined the age of young mice in which they attained the ability to maintan stable body temperature. We exposed the animals to 20°C ambient temperature at 12 to 13 days of life i.e. with the body weight slightly above 6 g. The mice at the age of 2 weeks can maintain a stable body temperature; the same they had in the nest, with their mother.

Special attention was paid to the mitochondria, their distributions in the muscle fibres, and to their size and fine structure. Mitochondria in the young muscle fibres are small, more or less evenly dispersed and their interior is characterized by a relatively low density of cristae (Fig. 1). We did not count the number of mitochondria, since these structures lack well defined shape, especially in the more advanced stages of muscle fibres development (Fig. 1). The mitochondrial number is a poor morphometric parameter to be related to a muscle fibres aerobic capacity. It has neither a functional nor morphometric meaning. The mitochondria are often very long, complicated structures whose size and shape are difficult to estimate from thin sections and very difficult to interpret with respect to their spatial and functional meaning [14]. These features allow no reliable calculation of their diameter and numerical density. However the increase of cytochrome c oxidase activity goes hand in hand with increases of mitochondrial volume ($r = 0.9719$) and correlates well with the stage of the animals developmemt as well as with their muscle fibre maturation. At the level of individual muscle fibres of the diaphragm the greater mass coupled with the volume densities of mitochondria ($r = 0.9496$). The increase of cytochrome c activity observed during muscle fibres differentiation appears to be determinated by the increase of the mitochondrial inner membranes surface [19] but to a lesser degree by mitochondrial volume density. In the muscle fibres mitochondria are situated in two configurations: close to the myofibrils arranged as interfibrillar lattices and in a form of peripheral rim or clusters (Fig.

*Table 3. Maximal respiratory rates of isolated mitochondria from diaphragm muscle fibres of mice at four stages of development, with cytochrom c as a substrate. Rates, expressed as cytochrome c oxidase activity per mg of protein and g of diaphragm muscle were obtained on five preparations of diaphragm from each stage of development. Statistical level of significance, * $p < 0.05$, ** $p < 0.01$, for unpaired Student's test was used to compare the means.*

Age of mice	Body mass in g	Diaphragm mass in g	Cytochrome c oxidase activity, μM O_2 x min^{-1}	
			per mg of protein	per g of muscle
Foetus	1.2	0.004	60.6**	265.7**
sd	0.6	0.0018	28	112.0
cv (%)	50	45	46	40
Suckling	2.4	0.008	120.7**	399.8
sd	0.3	0.001	26	71
cv	24	13	21	20
Juvenile	5.3	0.012	192.5**	320.0*
sd	0.5	0.006	36	116
cv	20	50	36	40
Adult	25.7	0.100	297*	101.1**
sd	4.4	0.016	69	58
cv	25	16	23	20

*Table 4. Quantitative morphological data of mitochondrial content for diaphragm muscle fibres of mouse during development. Mean volume of mitochondria [$V_{(mt)}$]; volume density of total, interfibrillar and subsarcolemmal mitochondria, [$Vv_{(mt, f)}$, $Vv_{(mi, f)}$, $Vv_{(ms, f)}$]. n = 5 each stadium; SEM = standard error of mean; SD = standard deviation; CV = coefficient of variation in %; statistical level of significance * $p < 0.05$, ** $p < 0.01$, for unpaired Student's t-test was used to compare the means.*

Age	$V_{(mt)}$	$Vv_{(mi,f)}$	$Vv_{(ms, f)}$	$Vv_{(mt, f)}$
	($cm^3 . 10^{-4}$)	($cm^3 . cm^{-3}$)	($cm^3 . cm^{-3}$)	($cm^3 . cm^{-3}$)
Foetus (-3 days)	6.0	6.6*	5.1	11.1
SEM		0.01	0.01	0.01
sd		0.04	0.04	0.04
cv		60	78	36
Suckling (+3 days)	9.2	8.5*	5.4	13.9
SEM		0.01	0.01	0.01
sd		0.04	0.04	0.06
cv		47	74	43
Juvenile (+14 days)	85.5	6.8*	4.5	11.3
SEM		0.01	0.01	0.02
sd		0.02	0.04	0.04
cv		29.4	88	35
Adult	190.0	10.1**	13.6**	23.7**
SEM		0.02	0.03	0.03
sd		0.07	0.10	0.12
cv		70	73	52

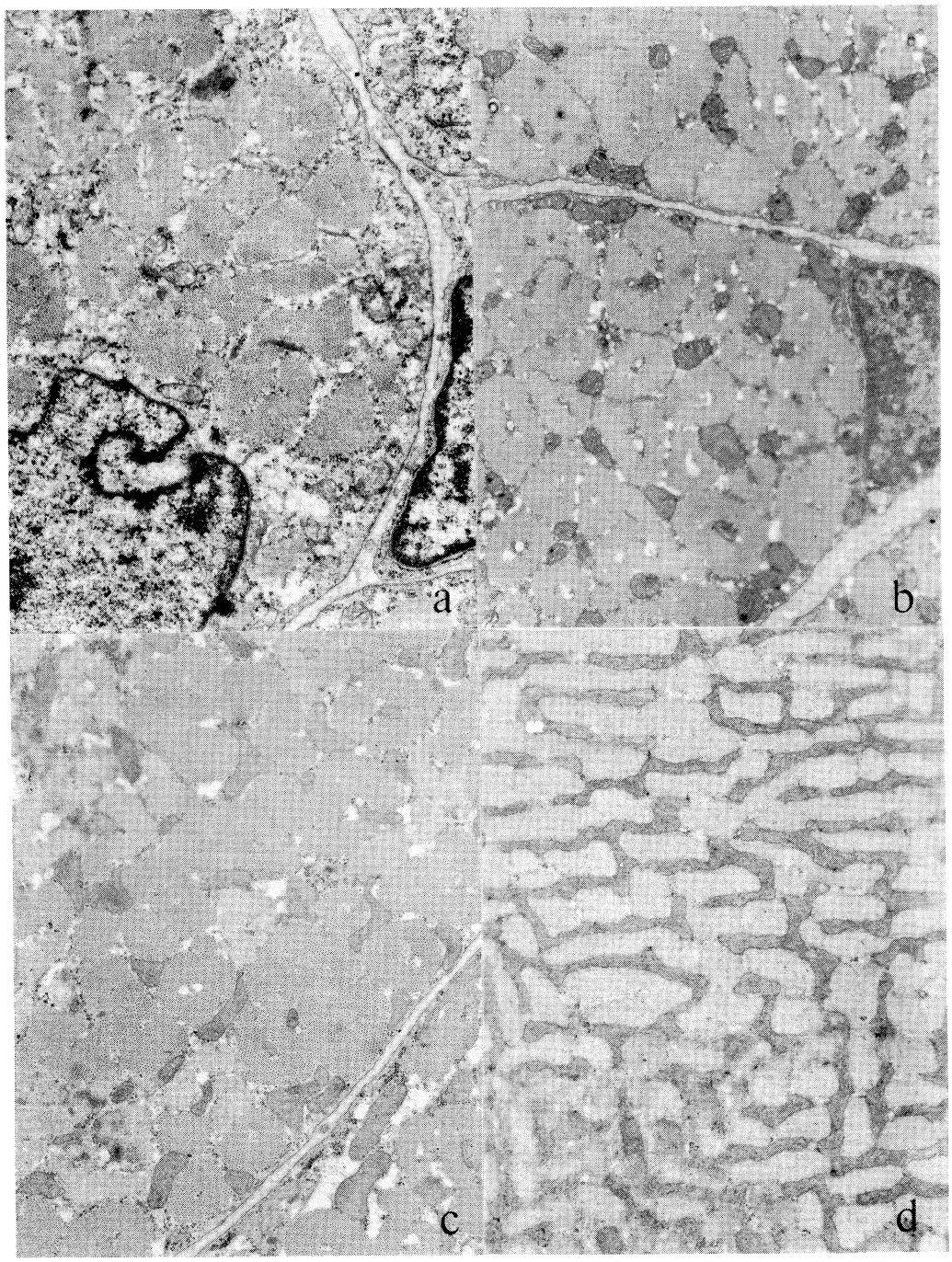

Figure 1 a-d. Comparison of cross-sections of samples of diaphragm muscle fibres from four stages of mouse development. (a), from -3 days ;(b) +3 days ; (c) 14 days and (d), adult mouse. Note the variability of the mitochondria distribution, differentiation in their size and shape in consequent stages of animals development. Magnification x 10,260.

1). Both mitochondrial groups are characterized by an uneven distribution. The functional role of the subsarcolemmal mitochondria has not been fully elucidated. The mitochondria are situated close to both sarcolemma and capillaries and therefore to the O_2 supply. On the other hand they are not in close contact with the internal myofibrils. The subsarcolemmal mitochondria could then possibly serve as the main reservoir for O_2 and ATP which diffuses into the interior of the muscle via the inner mitochondrial lattice system The mitochondrial distribution in the last two stages of the animal's maturation is

*Table 5. Quantitative morphological data for diaphragm muscle fibres of mouse during development. Mean volume of diaphragm muscle [$V_{(md)}$], volume density of myofibrils [$Vv_{(fi, f)}$]; volume of sarcoplasmic reticulum [$V_{(sr)}$]; volume density of sarcoplasmic reticulum [$Vv_{(sr, f)}$]; volume density of sarcoplasm [$Vv_{(spl, f)}$]. n = 5 each stadium; SEM = standard error of mean; SD = standard deviation; CV = coefficient of variation in %. Statistical level of significance, * p < 0.05, ** p < 0.01 for unpaired Student's t-test was used to compare the means*

Age	$V_{(md)}$ ($cm^3 . 10^{-3}$)	$Vv_{(fi,f)}$ ($cm^3 . cm^{-3}$)	$V_{(sr)}$ ($cm^3 . 10^{-5}$)	$Vv_{(sr, f)}$ ($cm^3 . cm^{-3}$)	$Vv_{(spl, f)}$ ($cm^3 . cm^{-3}$)
Foetus (-3 days)	5.2	49.7**	4.2	0.8*	30.4**
SEM		0.02		0.001	0.03
sd		0.13		0.006	0.13
cv		26		75	42
Suckling (+3 days)	6.6	74.8**	7.26	1.1*	11.5**
SEM		0.02		0.001	0.02
sd		0.09		0.005	0.15
cv		12		45	20
Juvenile (+14 days)	18.8	74.5	22.5	1.2	14.7*
SEM		0.03		0.002	0.02
sd		0.01		0.007	0.07
cv		13		58	47
Adult	80.1	76.0*	128.1	1.6*	2.8*
SEM		0.03		0.008	0.008
sd		0.12		0.02	0.02
cv		15		12	71

characterized by their zonal orientation. The peripheral mitochondria are big and more or less oval with densely packed cristae, while the inner mitochondria are slender and very long. The later form a very characteristic lattice at the level of I/A junctions of the sarcomere, when the cross-sections of the fibres are viewed (Fig. 1). The development of the inner mitochondrial lattice system begins very early but it is difficult to accept a suggestion by Bakeeva and her colleagues [2] that process of lattice formation begins immediately after birth and that this process is directly connected with the contractile activity of the diaphragm which, according to these authors [2], does not function in embryos. However the diaphragm does not remain inactive in foetuses, during their final last days of gestation. The diaphragmatic activity usually appeared in conjunction with the general tonic activity of eye, neck, and hindlimb muscles which starts several weeks before delivery. The respiratory movements of the term foetuses in different species are well known facts and have been recorded many times in different species [4, 9, 10, 13, 16, 18]. The internal mitochondria form a homogeneous population and they are absolutely distinct from the subsarcolemmal mitochondria. They have a very dense and fibrous matrix and are poorly equipped with cristae. The characteristic structure, distribution and organization in the form of a continuum at the two levels of each sarcomere implies their particular function (Fig. 1). A mitochondrial lattice described in this study has also been recorded in cardiomyocytes in culture [1] as well as in rat diaphragm and limb [18, 2] and was interpreted as an electrically united system carrying the mitochondrial electric potential from the peripheral to the internal mitochondrial system [1]. This form of lattice may also create an interconnected canal system, which may efficiently conduct the excess of oxygen from the peripheral part of muscle fibres to their interior, to be used there by deeply localized mitochondria. Oxygen dissolves in lipids approximately 10 times better than in water, thus the mitochondrial membrane system may provide a suitable medium for it. There are several effects that may favor the use of the inner mitochondrial membrane as a route for the intra cellular oxygen transport [2, 3, 11, 12, 15, 20, 21, 25]. The function of a lattice mitochondrial system was postulated recently by Amchenkova et al., [1], who discussed the possibility that an extended mitochondrial system might be the route for any substance that was concentrated in a mitochondrial compartment or in their membrane system.

It was not surprising however, to find a positive correlation between muscle fibres volume and the total volume of sarcoplasmic reticulum (r = 0.9993) as well as between the volume densities of sr, [$Vv_{(sr, f)}$], and total muscle fibres volume during mice development, (r = 0. 7326). However, the ratio between the volume densities of sarcoplasm stays in a reverse correlation, (r = - 0.7414), to the total volume of the muscle fibres during their differentiation during mice ontogeny {[$Vv_{(spl, f)}$], V_f}.

To interpret these events we conclude that the sarcoplasmic reticulum establishes the reservoir for Ca^{2+} in the state of calsequestrin. The functional significance of this reservoir is a

well known fact. Therefore the increase in volume of this reservoir in any subsequent stages of the myofibres maturation during animals development seems to be obvious. It also reflects well the muscle fibres ability of faster and sustained contraction, which they acquire during development.

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