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REVIEW ARTICLE

STEREOLOGICAL ANALYSIS OF SKELETAL MUSCLE TISSUE

Ida Eržen , Miroslav Kališnik

Institute of Anatomy, Institute of Histology and Embryology, Medical Faculty, University Edvard Kardelj, 61105 Ljubljana

ABSTRACT

The components of the skeletal muscle tissue are more or less oriented in space - they show characteristics of spatial anisotropy. This property must be obligatory taken into account, when evaluating length density (L_V) and surface density (S_V), when the latter is not estimated on vertical sections. Nevertheless volume (V_V) and numerical density (N_V) are independent of the degree of anisotropy. Several methods and examples of their application for the stereological analysis of anisotropic structures are demonstrated, as are also the most frequently used variables, characteristic of the analysis of the skeletal muscle tissue.

1. INTRODUCTION

The aim of this review article is a clear explanation of basic principles to be taken into account during stereological analysis of the skeletal muscle tissue. It is not its purpose to give a sophisticated theoretical explanation of the above principles or to develop the pertaining stereological theory. The interested reader will find such data quoted in this article in the literature, too. The authors wish to close the gap existing between the mathematically trained theoreticians and research workers who only want to apply stereological methods in order to solve their scientific problems.

Skeletal muscle tissue is mixed, consisting of specific components - skeletal muscle fibres and unspecific interstitial tissue - connective tissue with blood and lymph vessels and nerves (Fig. 1). Some components of skeletal muscle tissue are more or less oriented in space, they show characteristics of spatial anisotropy. This anisotropy must be taken into account when planning and performing some stereological measurements (boundary density, length density, and according to some models surface density), but other variables such as volume density and numerical density are not influenced by anisotropy. The major part of this article will be devoted to the problems of anisotropy. Stereological analysis of anisotropic structures requires isotropic uniform random sections - in such a case basic formulae, valid for isotropic objects can be applied (Mattfeld and Mall, 1984). The equations for anisotropy are optional and supplement the average values obtained with the basic stereologic equations. The special equations for anisotropy may supply additional information, or they may require less labour (two measurements vs. measurements at all angles). However the basic stereological equations are valid for any kind of structure (whether isotropic, partially- or completely oriented. If the structure is not isotropic then randomness is supplied by random measurements.

Two basic approaches to the stereological analysis of anisotropic structures will be described in the present paper,

i) methods, based on the Saltykov orientation equations (Underwood, 1970), which assume simply that there are two subsets in the microstructure - one oriented and one random. The two directed measurements are all that is needed.

ii) methods, based on the Dimroth-Watson distribution ($-\infty < K < \infty$) as well as on the approximation of it called the Marriott distribution (Weibel, 1980), which is valid for moderate degrees of anisotropy ($-1 < K < 1$). The model supposes that line elements cluster about the axis of anisotropy

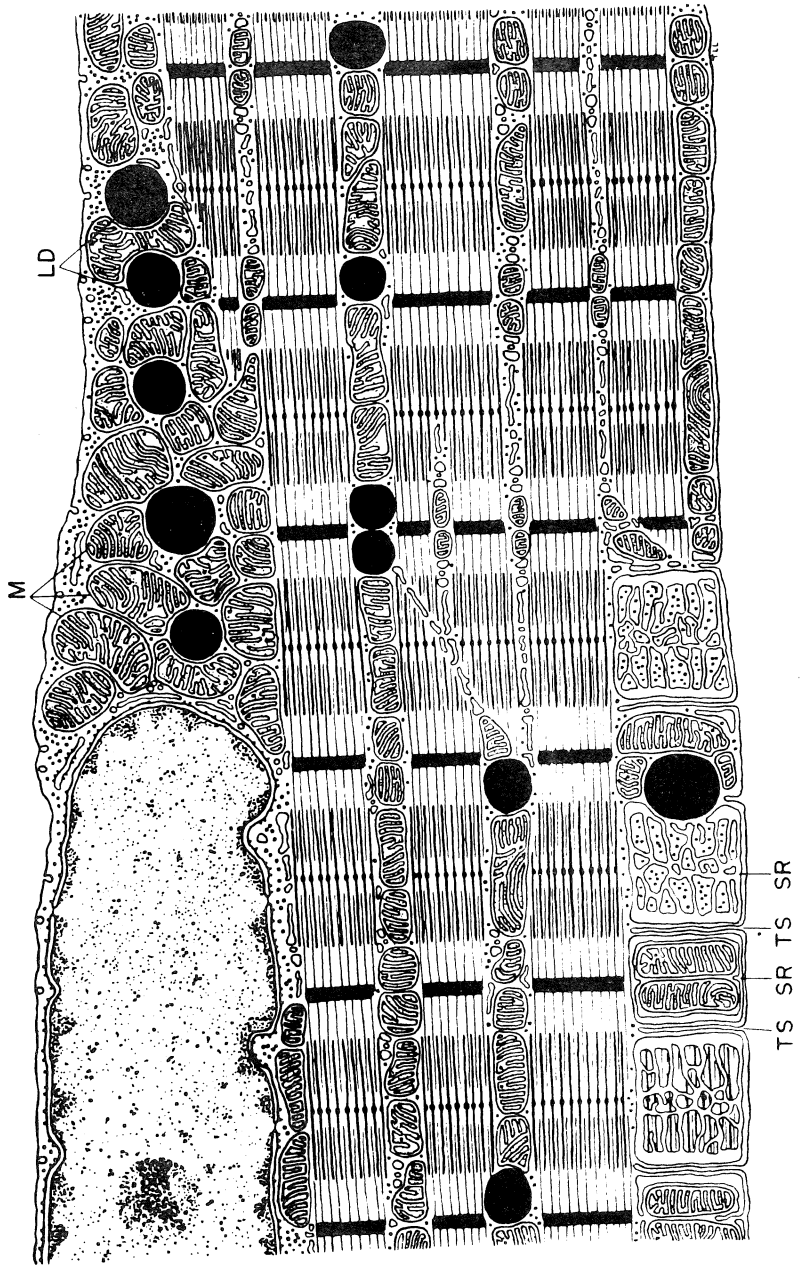

Fig.1. Scheme of skeletal muscle fibre (LD- lipid droplets, M mitochondria, SR sarcoplasmic reticulum, TS transversal tubules). (T. Lentz, 1971, p. 89)
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or surface elements constitute a plate-like structure ($K > 0$). On the other hand, line elements cluster with their directions about a fixed plane, or surface elements constitute a tube-like structure ($K < 0$).

2. SAMPLING

Sampling, as the key phase of the stereological analysis, must on the one hand be adjusted to the fact that skeletal muscle tissue is anisotropic. This proves true above all for the direction of sectioning, where the preferential axis of the structure arrangement in space must be taken into consideration (skeletal muscle fibres, myofibrils, sarcoplasmic reticulum, capillaries and thelike) (See Fig.2).

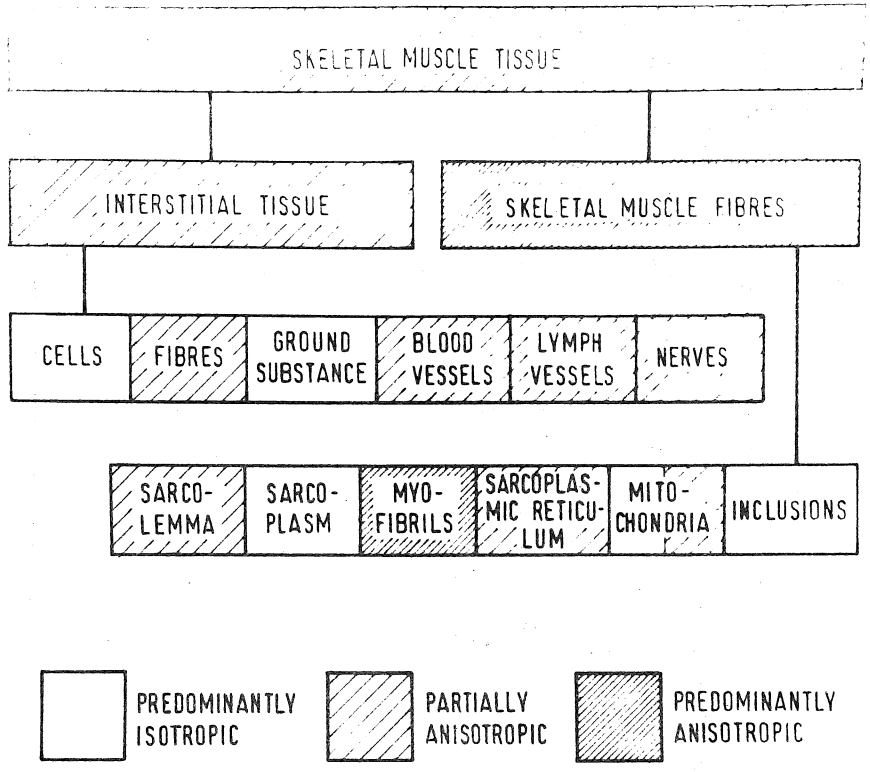

Fig.2. The hierarchical model of skeletal muscle tissue

On the other hand, sampling must be performed according to the rules valid for stereological analysis in general. So the sample size depends e.g. on the variability between animals and, at a lower level of the structure investigated, as well as on the desired accuracy and permitted deviation of our estimations from the true values. It is often desirable to use a multi-level or cascade sampling design at different magnifications, whereby the object phase at one level becomes the reference phase in the next level.

Each level can be regarded as an independent sampling design (Cruz-Orive, Weibel, 1981). When sampling on the electron microscopical level the periodicity of skeletal muscle fibre structures, i.e. exchanging of A-band with I-band, must additionally be considered.

3. SPATIAL ANISOTROPY OF SKELETAL MUSCLE TISSUE

The elements of skeletal muscle tissue are more or less oriented in space (see the hierarchical model). The preferential axis is determined by the miofibrils, which are considered to be totally anisotropic, other structures more or less follow the preferential axis, they lie either parallel (nuclei, longitudinal sarcoplasmic reticulum) or perpendicular to it (t tubules, lateral cisternae).

The shape of the traces, being dependent on the angle of sectioning (Θ), reflects the anisotropic structure of the skeletal muscle tissue. The angle of sectioning is determined by the angle between the preferential axis and the normal to the sectioning plane: $\Theta=0^{o}$ for transverse-sections and $\Theta = 90^{o}$ or $\pi/2$ for longitudinal sections respectively.
Volume density (V_V) and numerical density (N_V) are independent of anisotropy, which, nevertheless, must be taken into consideration with surface density (S_V) and length density (L_V). The stereological analysis of anisotropic structures is based on several mathematical models (Underwood, 1970; Weibel, 1979; Weibel, 1980; Mathieu et al., 1982).
Anisotropy in space is often also reflected by anisotropy in the plane.

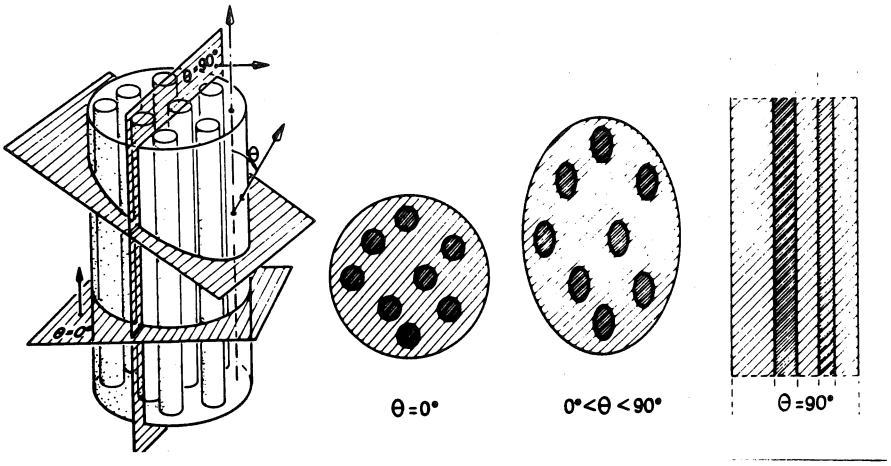

Fig. 3. Dependence of the shape of traces on the angle of sectioning (Weibel, 1979, p. 216)
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3.1. Anisotropy in the plane

The analysis requires the proper choice and application of the test system used. The system recommended should be capable of destroying anisotropy (e.g. the semicircular Merz system). The usual lattice system, randomly placed on structures, could be used as well; in this case, intersections with vertical and horizontal test lines respectively, should be counted separately. The mean of the intersections obtained should be taken for further analysis (Weibel, 1980). Directing the test lines at an angle of 19^{o} and 71^{o} to the axis of anisotropy respectively gives precise results only with objects where the particles are aligned in a strictly parallel fashion (total anisotropy). Such precision is in practice impossible to achieve because the results of this method may be wrong, especially for partially anisotropic structures (Weibel,1980). The test triangle or test lines rotating in the plane can also be used for sampling. The basic formula, used for calculating boundary density (B_A) of any structure is as follows:

$$B_A = \frac{\pi}{2} I_L \qquad (1)$$

According to Underwood (1970) approximation to the total value of B_A for oriented or partially oriented lines in a plane is

$$B_A = (I_L)_\perp + \left(\frac{\pi}{2} - 1\right)(I_L)_\parallel = (I_L)_\perp + 0.571\ (I_L)_\parallel \qquad (2)$$

Directed measurements are required, i.e. $(I_L)_\perp$ are intersections perpendicular and $(I_L)_\parallel$ intersections parallel to the axis of anisotropy.

3.2. Anisotropy in space

As already mentioned, anisotropy must be considered in determining length density (L_V) and also surface density (S_V), when it is not estimated on vertical sections (cf. Baddeley et al. 1985). If the degree of anisotropy is known, analysis in one sectioning plane is sufficient. If the degree of anisotropy is unknown, however, reliable results will be obtained only if the anisotropic object is cut in two or three planes, perpendicular to each other. The greater the quotient between the results obtained in the different planes, the higher the degree of anisotropy. Classical formulas, valid for isotropic objects, must be adjusted for anisotropy (see Underwood, 1970; Weibel, 1979; Weibel, 1980; Mathieu et al., 1982). Several approaches to the determination of length and surface density, as well as the degree of anisotropy will be described; for easier understanding we have taken the liberty of unifying the symbols.

Length density will be considered in detail in the section "Capillaries".

Surface density can be determined in several ways.
The easiest way is to use "vertical" sections, e.g. plane sections parallel to a predetermined "vertical axis". In muscle tissue this means longitudinal sections. The recommended test system should consist of a grid of cycloid curves. Care must be taken that the vertical axis of the test system is aligned with the vertical direction on the section. Surface density is estimated according to the equation (3).

$$\text{est } S_V = 2 \ (p/l \ ) \sum_{i=1}^{n} I_i \ / \sum_{i=1}^{n} P_i \qquad (3)$$

I - number of intersection points between the test lines and the surface
P - number of test points which hit the reference space
p/l - ratio of test points to test curve length (in real units)
n - number of micrographs

For details see Baddeley et al. (1985)!

When surface density is determined according to other models, which will be described later in the text , it is necessary to know the shape of the particles arranged anisotropically. Two basic types of anisotropic systems can be distinguished: the linear system, in which single elements (tubules) are parallel to the preferential axis (e.g. skeletal muscle fibres, myofibrils, longitudinal sarcoplasmic reticulum) and the planar system, in which single elements (lamellas) are perpendicular to the preferential axis (e.g. terminal cisternae of the sarcoplasmic reticulum) (Fig.4).

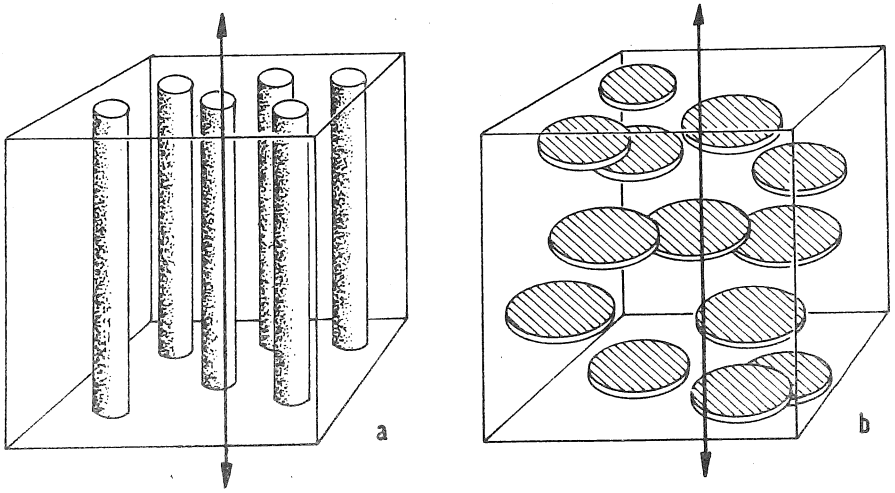

Fig. 4. Tubular (a) and lamellar (b) systems

3.3. Basic stereological approaches for estimating surface density

3.3.1. Underwood's method (1970)

Though developed for materials science the method was applied in biological literature as well (cf. Crowe and Baskin, 1977 and 1979), but nowadays other methods, explained in the text later, are more recommended.

a) Linearly oriented systems (tubular structures) are analysed on longitudinal sections. A test system of parallel lines, which is placed parallel ($\parallel$) and then perpendicular ($\perp$) to the preferential axis, is used.

$$S_{V_{lin}} = \frac{\pi}{2} \cdot (I_L)_{\perp} + (2 - \frac{\pi}{2}) (I_L)_{\parallel} = \tag{4}$$

$$= 1,571\ (I_L)_{\perp} + 0,429\ (I_L)_{\parallel}$$

The degree of orientation (anisotropy) is

$$\Omega_{lin} = \frac{(I_L)_{\perp} - (I_L)_{\parallel}}{(I_L)_{\perp} + (\frac{4}{\pi} - 1)(I_L)_{\parallel}} = \frac{(I_L)_{\perp} - (I_L)_{\parallel}}{(I_L)_{\perp} + 0,273\ (I_L)_{\parallel}} \tag{5}$$

b) Planar (lamellar) systems are analysed on sections which are parallel to the orientation axis, but perpendicular to the lamellas.

$$S_{V_{pl}} = (I_L)_{\perp} + (I_L)_{\parallel} \tag{6}$$

The degree of orientation is

$$\Omega_{pl} = \frac{(I_L)_{\perp} - (I_L)_{\parallel}}{(I_L)_{\perp} + (I_L)_{\parallel}} \tag{7}$$

3.3.2. Weibel's method (1980)

The stereological analysis of anisotropic structures is based on Dimroth-Watson orientation distribution.
(Theoretical considerations of the method are explained in detail elsewhere (Cruz-Orive et al., 1985).)

$$S_V = c_1 \cdot I_L \qquad (8)$$

$$S_V = c_2 \cdot B_A \qquad (9)$$

Factors c_1 and c_2 are dependent on the degree of anisotropy, which is expressed by the concentration parameter K and by the angle of sectioning (designated as Θ or α), determined by the angle between the preferential axis and the normal to the sectioning plane. Dimroth-Watson distribution is normally very difficult to handle, except in special cases of longitudinal and transverse sections. The coefficients c_1 and c_2 can be calculated following Weibel (1980).

For partially anisotropic structures it is possible to calculate the factors c_1 and c_2, if the preferential axis and the degree of anisotropy is known. Otherwise the following approach is recommended in practice: one should determine the boundary density on a few transverse and longitudinal sections. From the ratio of boundary densities obtained on transverse and longitudinal sections, the ratio of factors c_2 on transverse and longitudinal sections is determined; on the basis of this ratio it is possible to read the values for K and c_2 from table 2 (Meznarič, Eržen, 1984).
Note some special cases:

For isotropic structures

$$K = 0,\ c_1 = 2\ ,\ c_2 = \frac{4}{\pi}$$

It follows that

$$S_V = 2\, I_L \qquad \text{and} \qquad (10)$$

$$S_V = \frac{4}{\pi}\, B_A \qquad (11)$$

If normals to the particle surface are parallel to the preferential axis, K is positive, but when normals are perpendicular to the preferential axis, K is negative. For structures, equivalent to parallel straight tubules

$K \to -\infty$

$c_2 = 1$ for transverse sections

$c_2 = \pi/2$ for longitudinal sections

On the other hand for lamellar structures the following holds true:

$K \to \infty$

$c_2 = 1$ for sections parallel to the anisotropy axis, i.e. perpendicular to the lamelle

$c_2 = \infty$ for sections parallel to the lamelle

Per agreement the preferential axis lies parallel to the tubular structures (e.g. skeletal muscle fibres, myofibrils, longitudinal sarcoplasmic reticulum) and perpendicular to the lamellar structures (e.g. terminal cisternae of the sarcoplasmic reticulum). The application of the method will be described in detail in the section "Sarcoplasmic reticulum".

3.3.3. Case of moderate anisotropy (in Weibel, 1980)

For structures with a moderate degree of anisotropy which can be modelled by the Marriott distribution , S_V can be determined without knowing the degree of anisotropy, if structures are analysed on two section planes perpendicular to each other.

$$S_V = \frac{4}{3\pi} (B_{A_1} + 2 B_{A_2}) \quad (12)$$

B_{A_1} - boundary density obtained on transverse sections

B_{A_2} - boundary density obtained on longitudinal sections

(Definitions for B_{A_1} and B_{A_2} /Weibel, 1980, p.303/ have been reversed. Unless the potential users are aware of this they will obtain the wrong results.)

The method is valid for the Marriott distribution with $-1 \leqslant K \leqslant 1$

The degree of anisotropy is estimated by

$$K = \frac{4 \left[(B_{A_2} / B_{A_1}) - 1 \right]}{2 (B_{A_2} / B_{A_1}) - 1} \quad (13)$$

Before starting the stereological analysis the following limitations of the method must be tested:

For elongated structures ($0 < K \leqslant 1$) the ratio of measurements should be:

$$B_{A_2} / B_{A_1} \leqslant 3/2$$

Whereas for flattened structures ($-1 \leqslant K < 0$) the ratio should be:

$$B_{A_1} / B_{A_2} \leqslant 6/5$$

The method is not suitable for structures showing high degree of anisotropy.

4. STEREOLOGICAL ANALYSIS OF SKELETAL MUSCLE TISSUE ON THE LEVEL OF OPTICAL MICROSCOPY

Transverse-sections are the most suitable for stereological analysis. When estimating the surface density of muscle fibres, it must be taken into account that surface density nearly equals boundary density ($S_V \doteq B_A$ and not $S_V = \frac{4}{\pi} B_A$ as holds true for isotropic structures). Some of the most applicable cases of the stereological analysis will be described.

4.1. Estimating the volume density of connective tissue in muscle tissue is a good measure of the quality of meat and meat products (Vuković et al., 1981).

4.2. Estimating the volume and numerical density of fibre types determined by histochemical reactions (e.g. myofibrillar adenosinetriphosphatase) serves to evaluate changed muscle condition, either after a change in activity (training, immobilisation) or in nerve-muscle diseases. The latter frequently cause (selective) atrophy of the muscle fibres,

4.3. so the determination of muscle fibre diameter is a variable frequently used. In the stereological analysis the similarity between muscle fibres and cylinders helps to derive some variables, such as the average diameter ($\bar{D}$). If the diameter variability is small, the following equation can be used:

$$\bar{D} = 2 \frac{P_P}{I_L} \qquad (14)$$

where P_P means the proportion of points that fall on the transverse sections of the cylinders. I_L, however, means the intersection density between the cylinder surface and the test lines. If the diameters differ a great deal, estimations of volume density (V_V) and surface density (S_V) must be made for each size category of diameters separately. The ratio between V_V and S_V gives average diameters for every category separately. The formula (14) is valid

for isotropic cylinders (Kališnik, 1977). Because the skeletal muscle fibres are highly anisotropic cylinders the formula (14) should be modified for the transverse sections ($\Theta=0^{o}$) as follows:

$$\bar{D} = \frac{8}{\pi} \frac{P_P}{I_L} \qquad (15)$$

4.4. Stereological analysis of nuclei

Nuclei are highly anisotropic structures lying with their longitudinal axis parallel to the preferential axis of the muscle fibre. On transverse-sections it is possible to determine the surface areal and numerical areal density of nuclei in the muscle fibre (Muller, 1976).

The method applied by Atherton and James (1980) recommends estimating the numerical density of nuclei on serial semi-thin sections, according to the De Hoff's formula:

$$N_V = \frac{N_A}{\bar{L}} \qquad (16)$$

N_A = number of nuclear profiles seen per unit area of transverse section

$\bar{L}$ = average caliper diameter of the nuclei in a direction perpendicular to the section.

The mean volume of nuclei is given by the relation:

$$\bar{v} = V_V / N_V \qquad (17)$$

On the electron microscopical level the evaluating of the proportion of euchromatin and heterochromatin reflects the nuclear activity (Wakayama et al., 1979, James, Čabrić, 1982; Čabrić, James, 1983).

For the method of measuring the volume fraction of heterochromatin within myonuclei and the mean intercept length across heterochromatin and euchromatin see James et al. (1982). The method consists of placing a series of transparent screens bearing randomly oriented lines of known length on micrographs and recording whether heterochromatin or euchromatin lies under the end of each test line.

4.5. Capillaries

Capillaries are partially anisotropic structures. Whether anisotropy in the specimen will be preserved or not is determined by the fixation method: only fixation with perfusion preserves anisotropy, but simply immersing fresh muscle tissue in a fixative causes anisotropy to be lost (Mathieu et al., 1982). The stereological variable most frequently determined is the length density of the capillaries (L_V). Additional information can be obtained from anisotropic linear structures. The tissue must be cut in two planes, one being parallel ($\parallel$) and the other perpendicular ($\perp$) to the preferential axis. Several evaluation methods are possible : some authors (Atherton, James, 1980, James, 1981, James, 1982) recommend the first method (according to Underwood), because in vivo capillaries have two directions of orientation both longitudinal and transverse, whereas Weibel's methods are based on the assumption that the capillaries are oriented within an unimodal distribution.

4.5.1. The method according to Underwood (1970):

$$L_V = (Q_A)_{\perp} + (Q_A)_{\parallel} \qquad (18)$$

$Q_{A_{\perp}}$ = transection density on transverse-sections

$Q_{A_{\parallel}}$ = transection density on longitudinal sections

The degree of anisotropy (Ω) i.e. fractional length of oriented (anisotropic) capillaries, is:

$$\Omega_{13} = \frac{(Q_A)_{\perp} - (Q_A)_{\parallel}}{(Q_A)_{\perp} + (Q_A)_{\parallel}} \qquad (19)$$

Ω_{13} (the degree of anisotropy of a one-dimensional structure - a capillary- in a three dimensional structure - space, in actual fact, in muscle) takes values from 0 (the structure is isotropic) to 1 (the structure is totally anisotropic).

4.5.2. The method according to Weibel (1980)(formulae are only approximations based on Marriott's distribution and hold true for structures with a moderate degree of anisotropy).

$$L_V = \frac{2}{3} (Q_{A_1} + 2Q_{A_2}) \qquad (20)$$

Q_{A_1} = transection density on transverse-sections

Q_{A_2} = transection density on longitudinal sections

From the ratio between both transection densities (Q_{A_1}) and (Q_{A_2}) it is possible to determine the concentration parameter K.

$$K = 2 \left[1 - \left(\frac{Q_{A_2}}{Q_{A_1}} \right) \right] \qquad (21)$$

Both formulas may be used only for partially anisotropic structures with a low degree of anisotropy. K takes values from -1 to +1. (K=0 when the structure is isotropic; K takes the absolute value 1 when the structure is totally anisotropic. K is positive when the curve lies more or less parallel to the preferential axis, and negative when the curve is more or less perpendicular to the preferential axis.)

4.5.3. A high degree of anisotropy (where anisotropy can be satisfactorily modelled by the Dimroth-Watson distribution and K takes values from $-\infty$ to $+\infty$) is evaluated in the following way (Weibel, 1980, Mathieu et al., 1982):

$$L_V = c_1 \cdot Q_A \qquad (22)$$

Factor c_1 depends on the angle between the preferential axis and the normal to the plane of sectioning, and on the concentration parameter K (K is a measure for the concentration of structures round the preferential axis, and determines the degree of anisotropy). If K = 0, the structure is isotropic, so

$$L_V = 2\, Q_A \qquad (23)$$

For structures arranged with a high degree of anisotropy, table 1 shows the values for c_1 and K. Mathieu et al. (1982) recommend calculating transection densities (Q_A) on cross ($\Theta=0$) and longitudinal ($\Theta=\frac{\pi}{2}$) sections. The method again assumes that the Dimroth-Watson model holds. From the ratio between both transection densities the quotient between factors c_1 obtained on transverse and longitudinal sections is obtained , and from this values for K and c_1 can be determined from the table 1. (linear interpolation should be used, if necessary).

$$Q_{A(0)} \,/\, Q_{A(\pi/2)} = \qquad (24)$$

$$= c_1\ (K, \pi/2) \,/\, c_1\ (K,0)$$

See table 1!

The sample sizes should be calculated according to the equations (25) and (26)

$$n(\pi/2) / n(0) = \left\{ Q_A(0) / Q_A(\pi/2) \right\}^{1/2} \qquad (25)$$

$n(\pi/2)$ is the number of sections used to estimate $Q_A(\pi/2)$

$n(0)$ is the number of sections used to estimate $Q_A(0)$

$$n(0) = \frac{1}{A\varepsilon^2} \frac{1}{Q_A(0)} \left\{ 1 + \left(\frac{Q_A(0)}{Q_A(\pi/2)} \right) \right\}^{1/2} \qquad (26)$$

A – the area of a section

ε – relative standard error of R

R – $\hat{Q}_A(0)/\hat{Q}_A(\pi/2)$

$\hat{Q}_A(0)$ and $\hat{Q}_A(\pi/2)$ are measured on two independent sets of perpendicular ("transverse") and longitudinal sections respectively (Mathieu et al., 1982).

For an example see Mathieu et al. (1982)!

5. STEREOLOGICAL ANALYSIS AT THE ELECTRON MICROSCOPICAL LEVEL

5.1. Sarcoplasmic reticulum

This is a partially anisotropic structure in the skeletal muscle fibre. The longitudinal sarcoplasmic reticulum as well as transversal tubules can be analysed according to principles valid for tubular structures (or elements arranged linearly). Terminal cisternae, on the other hand, can be analysed according to principles valid for lamellar structures (or elements arranged planarly) (Crowe, Baskin, 1979). Volume and surface densities are determined, the containing space being either muscle fibre or A-band or I-band. For the electrical properties of a cell, the ratio between the sum of the outer surfaces of the transversal tubules and the myofibril surface to the muscle fibre surface is important (Eisenberg, Kuda, 1976).

Several methods for the estimation of surface density are available:

5.1.1. Evaluation on oblique sections (the angle between the preferential axis and normal to the sectioning plane should be greater than 75^{o}) (Weibel, 1972). In this case the relationship between S_V and B_A is

$$S_V \approx B_A \qquad (27)$$

Another advantage of sampling on oblique sections is that all the elements of the skeletal muscle fibre, which otherwise is a periodic structure, are sampled equally (for the application of the method see Hoppeler et al., 1973).

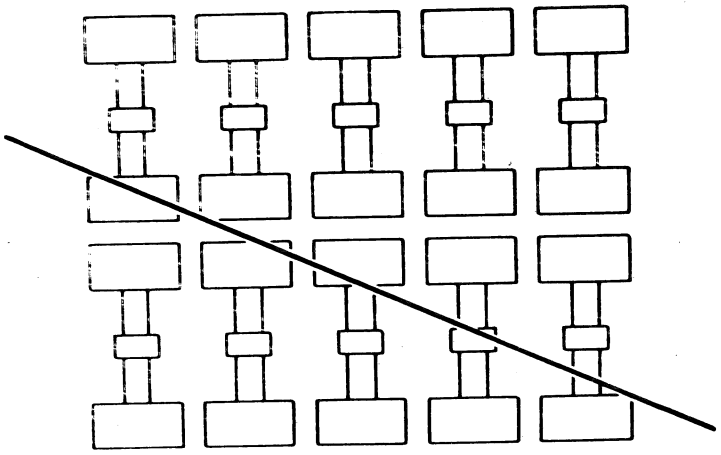

Fig.5. Sampling of periodic structures on oblique sections. (Weibel, 1972, p. 237.) (Reproduced by permission of Blackwell Scientific Publications Ltd.)

5.1.2. The method according to Underwood (1970) requires analysis on longitudinal sections; the test system of parallel lines, on position parallel (ıı) and the second

position perpendicular ($\perp$) to the orientation axis, is used. The surface density of the sarcoplasmic reticulum, except the terminal cisternae, is analysed according to the method used for linearly arranged structures, according to eq. (4) and the degree of orientation according to eq. (5). The surface density of terminal cisternae is analysed according to the formula for planarly arranged systems (6), and the percentage of planarly arranged systems according to eq. (7) respectively.

5.1.3. The method according to Weibel (1980), based on Dimroth-Watson orientation distribution according to eq. (8) and (9) respectively.

$$B_{A(K,0)} / B_{A(K,\frac{\pi}{2})} = \tag{28}$$

$$= c_2 (K, \tfrac{\pi}{2}) / c_2 (K,0)$$

See table 2.

Example: (Meznarič, Eržen, 1984)

The boundary density of the sarcoplasmic reticulum was determined on transverse- ($\Theta=0^{\circ}$) and longitudinal sections ($\Theta=90^{\circ}$ or $\pi/2$).

$B_{A(K,0)} = 5.52\ \mu m^{-1}$

$B_{A(K,\frac{\pi}{2})} = 3.85\ \mu m^{-1}$

The ratio between both boundary densities should be calculated

$$B_{A(K,0)} \; / \; B_{A(K,\frac{\pi}{2})} = 5.52 : 3.85 = 1.4337$$

According to eq. (28) this ratio equals the ratio between c_2 on longitudinal and c_2 on transverse-sections

$$c_2 \; (K,\tfrac{\pi}{2}) \; / \; c_2 \; (K,0) = 1.43$$

The sarcoplasmic reticulum is a tubular system, so the concentration parameter K should be negative. The quotient value 1.43 should be looked for in the lower right column of table 2. The value 1.43 approximately equals the following values:

$K = -4$

$c_2 \; (K,0) = 1.0341$

$c_2 \; (K,\tfrac{\pi}{2}) = 1.4789$

On the basis of the values obtained it is possible to calculate surface density according to eq. (9).

$$S_V = c_2 \cdot B_A = 1.0341 \times 5.52 = 5.70 \; \mu m^{-1}$$

(calculated from the boundary density determined on transverse-sections)

or

$$S_V = 1.4789 \times 3.85 = 5.69 \; \mu m^{-1}$$

(calculated from the boundary density obtained on longitudinal sections), which again shows that the results obtained in both ways correlate well.
Thus the degree of anisotropy and factor c were determined from several longitudinal and transverse-sections of the same material. Stereological analysis

can now proceed in only one sectioning plane, which partly simplifies our work.

5.1.4. The method of Weibel (1980) according to eq. (12) and (13).
For the application of stereological methods for the analysis of the sarcoplasmic reticulum see also Hoppeler et al., 1973; Mobley, Eisenberg, 1975; Van Winkle, Schwartz, 1978; Salmons, 1981; De Coster et al., 1981 !

5.2. Mitochondria
Three groups of mitochondria, differing in their localisation and orientation in skeletal muscle fibres as well as in their function can be distinguished:
- groups of mitochondria, situated under the sarcolemma, specially developed in highly oxidative fibres (isotropic structures),
- mitochondria, situated between miofibrils (lying parallel to the preferential axis,
- mitochondria, situated at the Z-line (lying perpendicular to the preferential axis) (Eisenberg, Kuda, 1975).

The most frequently determined stereological variables in the analysis of mitochondria:

5.2.1. On semi-thin sections, stained with cresyl violet, the surface areal density of subsarcolemmal mitochondria in the muscle fibre ($A_{A_{sm,mf}}$) can be determined.

$$A_{A_{sm}} = A_{sm} / A$$

The boundary length of the subsarcolemmal mitochondrial zone per boundary length of muscle fibre

$$B_{B_{sm}} = B_{sm} / B$$

From the ratio between A_{sm} and B_{sm} the average width of the mitochondrial zone is calculated

$$D_{sm} = A_{sm} / B_{sm}$$

Such data are especially valuable when estimating fibre type transformation (e.g. training) (Müller, 1976).

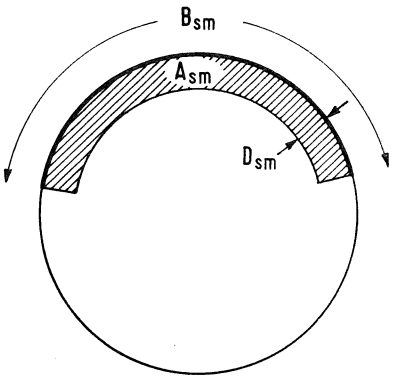

Fig.6. Scheme for the analysis of the subsarcolemmal mitochondrial zone (A_{sm} - area, B_{sm} - boundary, D_{sm} - average width).

5.2.2. On the electron microscopical level estimation of the following stereological variables is reasonable: volume density of mitochondria in the muscle fibre ($V_{V_{mit,mf}}$), namely in the subsarcolemmal outer zone (o) ($V_{V_{mit,o}}$) and in the central core of the muscle fibre (c) ($V_{V_{mit,c}}$) separately. For the latter variable the containing space can be either A- or I-band (Eisenberg, Kuda, 1975).
Instead of numerical volume density it is better to determine numerical areal density, N_{mit} / A.

For the functional condition of a mitochondrion itself it is possible to get useful data from the following stereological variables (Eisenberg in Weibel, 1979): volume density of the mitochondrial matrix per mitochondrion, surface density of cristae and inner mitochondrial membrane per mitochondrion (mitochondrial cristae must be considered as anisotropic structures).

5.3. Other variables, frequently determined in skeletal muscle fibre:
sarcomere length and Z-band width are measured directly, but the angle between the preferential direction of the miofibrils and the sectioning plane must be considered.

6. CONCLUSIONS

For every structure where the degree of anisotropy is not known, or else we do not know whether the structure is anisotropic or not, at least an orientational analysis in two sectioning planes, exactly determined as regards the preferential direction, is recommended. The more the results obtained in both planes differ, the higher the degree of anisotropy is only on the basis of these data it is possible to decide on the proper approach to the stereological analysis.

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Table 1.
Multiplicative correlation coefficients $c_1(K,\Theta)$ for transversal ($\Theta=0$) and longitudinal ($\Theta=\frac{\pi}{2}$) sections, and their ratio, for different values of K, based on the Dimroth - Watson distribution (Mathieu et al., 1982, p. 135)
(Reproduced by permission of Blackwell Scientific Publications Ltd.)

K	$c_1(K,0)$	$c_1(K,\frac{\pi}{2})$	$\frac{c_1(K,\frac{\pi}{2})}{c_1(K,0)}$
0.00	2.0000	2.0000	1.000
0.10	1.9347	2.0347	1.052
0.20	1.8722	2.0722	1.107
0.30	1.8126	2.1126	1.165
0.40	1.7560	2.1560	1.228
0.50	1.7025	2.2025	1.294
0.60	1.6520	2.2521	1.363
0.70	1.6046	2.3049	1.436
0.80	1.5602	2.3609	1.513
0.90	1.5188	2.4199	1.593
1.00	1.4803	2.4820	1.677
1.50	1.3274	2.8332	2.134
2.00	1.2279	3.2319	2.632
2.50	1.1649	3.6464	3.130
3.00	1.1251	4.0509	3.601
3.50	1.0993	4.4318	4.032
4.00	1.0819	4.7856	4.423
4.50	1.0698	5.1140	4.781
5.00	1.0608	5.4207	5.110
6.00	1.0485	5.9823	5.705
7.00	1.0405	6.4918	6.239
8.00	1.0348	6.9622	6.728
9.00	1.0305	7.4019	7.183
10.00	1.0272	7.8163	7.610
12.00	1.0223	8.5845	8.397
14.00	1.0189	9.2888	9.116
16.00	1.0164	9.9430	9.782
18.00	1.0145	10.5566	10.406
20.00	1.0130	11.1363	10.993
22.00	1.0118	11.6872	11.551
24.00	1.0108	12.2133	12.083
26.00	1.0099	12.7175	12.593
28.00	1.0092	13.2025	13.082
30.00	1.0086	13.6703	13.554
∞	1	∞	∞

Table 2.
Multiplicative coefficients for stereological formulae applicable to anisotropic structures, based on Dimroth-Watson distribution, as a function of the concentration parameter (modified after Weibel, 1980 , table 10.1 , p. 298)
(Reproduced by permission of Academic Press.)

$\kappa > 0$	$c_1(\kappa,0)$	$c_1(\kappa,\frac{\pi}{2})$	$c_2(\kappa,0)$	$c_2(\kappa,\frac{\pi}{2})$	$\frac{c_2(\kappa,\frac{\pi}{2})}{c_2(\kappa,0)}$
0.0	2.0000	2.0000	1.2732	1.2732	1.0000
0.25	1.8420	2.0920	1.3318	1.2463	0.9358
0.5	1.7025	2.2025	1.4021	1.2193	0.8696
0.75	1.5820	2.3325	1.4849	1.1930	0.8034
1.0	1.4803	2.4820	1.5801	1.1682	0.7393
1.25	1.3961	2.6498	1.6869	1.1456	0.6791
1.5	1.3274	2.8331	1.8036	1.1256	0.6241
1.75	1.2720	3.0286	1.9280	1.1083	0.5748
2.0	1.2278	3.2319	2.0575	1.0937	0.5316
3.0	1.1251	4.0509	2.5789	1.0560	0.4095
4.0	1.0819	4.7856	3.0466	1.0382	0.3408
5.0	1.0608	5.4207	3.4509	1.0289	0.2982
6.0	1.0485	5.9823	3.8085	1.0234	0.2687
7.0	1.0405	6.4918	4.1328	1.0196	0.2420
8.0	1.0348	6.9622	4.4323	1.0169	0.2294
9.0	1.0305	7.4019	4.7122	1.0149	0.2154
10.0	1.0272	7.8163	4.9760	1.0133	0.2036
12.0	1.0223	8.5845	5.4651	1.0110	0.1850
16.0	1.0164	9.9430	6.3299	1.0081	0.1593
20.0	1.0130	11.1363	7.0896	1.0064	0.1420
∞	1	∞	∞	1	
$\kappa < 0$					
0.0	2.0000	2.0000	1.2732	1.2732	1.0000
-0.25	2.1746	1.9246	1.2253	1.2992	1.0603
-0.5	2.3629	1.8637	1.1864	1.3234	1.1474
-0.75	2.5616	1.8148	1.1553	1.3456	1.1647
-1.0	2.7671	1.7758	1.1305	1.3656	1.2080
-1.25	2.9757	1.7447	1.1107	1.3833	1.2454
-1.5	3.1847	1.7199	1.0949	1.3988	1.2776
-1.75	3.3914	1.7001	1.0823	1.4123	1.3049
-2.0	3.5941	1.6840	1.0721	1.4240	1.3282
-3.0	4.3502	1.6444	1.0468	1.4581	1.3929
-4.0	5.0147	1.6244	1.0341	1.4789	1.4301
-5.0	5.6051	1.6128	1.0268	1.4928	1.4538
-6.0	6.1401	1.6055	1.0221	1.5028	1.4703
-7.0	6.6320	1.6002	1.0187	1.5103	1.4826
-8.0	7.0897	1.5964	1.0163	1.5162	1.4919
-9.0	7.5201	1.5935	1.0144	1.5209	1.4993
-10.0	7.9265	1.5910	1.0129	1.5248	1.5054
$-\infty$	∞	$\frac{\pi}{2}$	1	$\frac{\pi}{2}$	

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