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Three-dimensional morphometry of mammalian cells. I. Diameters

Morfometría tridimensional de células de mamíferos. I. Diámetros

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Three-dimensional measurements of eleven kinds of cells, obtained from serial sections of five different organs, excised from eleven adult mammals of different body sizes –from a 40 g mouse to a 450 kg cow– were made. In order to minimize technical errors all organs were submitted to standardized fixation and staining procedures. Twenty cell diameters (at the nuclear level) were measured in each of the 7 μm serial tissue section which were made in two planes, after a 90° rotation of the fixed and embedded organ specimens. The mean values of the cell diameter measurements were submitted to a cluster analysis by means of a computer program, to establish the cell type groups with similar morphometric characteristics. The dendrograms of the cell-type groupings were then compared with the results obtained by applying the traditional statistical analysis of the cell sizes (in micrometers) in the three dimensions of space, and also with the principal component analysis. With the three statistical methods we came to analogous conclusions. The empirical allometric exponents for the three cell diameters, when expressed independently as functions of body mass, are not significantly different from zero, and in consequence cell sizes are independent of body mass. The physiological meaning of the body-size-independence of the mean three cell diameters is discussed.

A characteristic feature of all organisms is its cellular structure. Small and large animals have cells of roughly the same size (around 10 μm) as has been stated by Teissier (1939); this is also valid for the red cell size which is body-size-independent (Altman and Dittmer, 1964; Calder, 1984; Schmidt-Nielsen, 1984). No adequate and rational explanation has been given up to present for the cell size constancy. It is obvious that a reduced cell diameter may decrease the diffusion distance from the plasma membrane to the center of each cell and also that an increase of the ratio of cell surface area (A) to cell volume (V) should increase the rates of diffusion in tissues of smaller mammals (Peters, 1983). The consequence of the invariance of cell size in mammals of different body mass (M) is that larger animals have "more cells, nor larger cells", as stated by Munro (quoted by Calder, 1984).

It is interesting to note that Teissier's (1939) study on cell sizes in vertebrates is based on the measurements made by numerous authors which, unfortunately, have used different fixation and staining methods, and in consequence may have led to quantitative results which are not strictly comparable. To avoid the latter objection, Maldonado *et al.* (1973) performed one-dimensional measurements on different cell types obtained from homeotherms (from 20 g to 600 kg body mass) as well as from poikilotherms (from 2.6 to 624 g body mass) by using standardized fixation and staining procedures.

All previously mentioned comparative studies were mainly concerned with one-dimensional measurements of cell diameters, and this kind of information is sufficient, from a quantitative point of view, only for spherical or cubical cells, two geometric forms which are rather scarce among mammalian cells.

In the present study three cell dimensions (lenght, width and depth) were determined under strict constant fixation and staining conditions. These three main diameters corresponding to eleven kinds of cells and

obtained from five different organs of eleven adult mammals of different sizes (from a 40 g mouse to a 450 kg cow) were measured. Furthermore, instead of the commonly used qualitative method of cell size comparison we have applied quantitative criteria by means of the so called "cluster analysis". This numerical procedure allows to establish the similarities or dissimilarities among different cell types by taking into account the corresponding three diameters (D_1, D_2 and D_3).

The constancy of cell diameters, which are in the micrometer realm, is analyzed in relation to the diffusion *distances* inside each cell and in consequence of the *time* requirements as a function of these distances (cell radius). The invariance of cell sizes which we found in the present study confirms the idea that body-size-independence is due to diffusion-limited processes inside all cells.

MATERIAL AND METHODS

Cells from the liver, large intestine, kidney, cerebellum, and skin, obtained from eleven adult mammals, were studied. In case of the hamster and the rat, both sexes were investigated separately. The corresponding body masses (g) are indicated in Table I.

The three diameters (D_1, D_2 and D_3) of the following cell types were measured:

1) glomerular epithelium (kidney); 2) proximal convoluted tubule (kidney); 3) Henle loop (kidney); 4) fibrocyte (kidney); 5) fibroblast (kidney); 6) adipocyte (skin); 7) goblet cell (large intestine); 8) Purkinje cell (cerebellum); 9) granular cells (cerebellum); 10) sebaceous gland cell (skin), and 11) hepatocyte (liver).

TABLE I

Body weights of nine species of mammals (11 animals)

Species	Name	Body weight (g)
1. Rockefeller mouse	*Mus musculus*	40
2. Hamster (m)	*Mesocricetus auratus*	135
3. Hamster (f)	*Mesocricetus auratus*	168
4. Rat (f)	*Rattus norvegicus*	189
5. Rat (m)	*Rattus norvegicus*	200
6. Cat	*Felis catus*	2,700
7. Dog	*Canis familiaris*	5,300
8. Sheep	*Ovies aries*	12,000
9. Pig	*Sus scrofa*	120,000
10. Horse	*Equus caballus*	270,000
11. Cow	*Bos taurus*	450,000

In order to decide which fixation and staining techniques should be preferred in the present investigation, several fixation methods were compared (formalin 10%; Bouin-Hollande; Duboscq-Brazil), the latter being the procedure which yielded the best results (the least cell retraction). On the other hand, among the staining techniques (Van Gieson; ferric hematoxylin of Heidenhain; acetic-thionine; hematoxylin-eosin) the best cell boundary contrasts (plasma membranes) and optimal nuclear stainings were obtained by means of the Van Gieson's method.

Fixation. Organ fixation is small animals (Rockefeller mouse, rat, and hamster) was initiated by perfusing a 5% formaldehyde solution through the aorta of the anesthetized animal. This fixation process was continued by submerging a fragment of the corresponding organ (5x5x5 mm) into the Duboscq-Brazil fixation solution during 24 hours (McManus, 1968). In all other instances the organs were excised within twenty minutes *postmortem* and maintained for 24 hours in the Duboscq-Brazil fixative.

Embedding and Sections. Organ fragments were embedded in paraffin, and 30 serial sections of 7 μm thickness were made from each block. The third dimension (depth) was obtained in a similar fashion after rotating the paraffin blocks in 90°.

Staining. All sections were stained in accordance with Van Giesson's technique (McManus, 1968).

Measurement of cell diameters. Cell body (soma) diameter measurements were performed at the nuclear level (with the exception of the adipocytes) by means of an ocular micrometer attached to a Leitz microscope (amplification 400x).

Statistical analysis. The numerical data (mean of 20 cell diameter measurements) corresponding to the three dimensions of space (D_1, D_2, D_3) were arranged in a decreasing order (major, medium, and minor diameters), and submitted to three independent statistical procedures: a) descriptive statistics; b) cluster; and c) principal component analysis. Within the possible strategies of hierarchical grouping we have chosen the simple means method and cell dissimilarities were established by the Euclidean distances (Everitt, 1980; Chatfield and Collins, 1980).

In order to obtain the corresponding allometric equations (Eq. 1) a regression analysis (least squares method) was employed between the mean cell diameters (D_1, D_2 and D_3) and body weight.

The principal component analysis was applied to the 121 three-dimensional data, corresponding to the major, the medium, and the minor cell diameters.

RESULTS

The cell diameter (D_1, D_2 and D_3) mean values, expressed in micrometers, were obtained from 20 individual measurements, performed on eleven different kinds of

TABLE II

Each number corresponds to the mean value of 20 individual measurements of the diameters of eleven different types of cells, which were obtained from eleven mammals, from the mouse (1) to the cow (11).
The mean cell diameters were arranged in accordance to their sizes: D_1 = major cell diameter; D_2 = medium cell diameter; and D_3 = minor cell diameter. In each column, the mean values (X) and the standard errors (SE) are indicated

		Cell types										
	N	1	2	3	4	5	6	7	8	9	10	11
		Major diameter (D_1)										
	1.	12.05	14.00	10.60	3.60	9.80	31.00	15.00	16.70	4.95	20.20	18.90
	2.	21.25	16.10	11.90	6.00	14.25	47.50	13.30	19.20	5.90	16.00	15.90
	3.	25.75	17.00	12.15	6.30	14.30	45.25	14.80	17.50	5.75	17.15	16.65
S	4.	24.00	16.90	14.00	6.70	12.65	33.55	14.45	17.35	6.00	15.85	17.70
	5.	18.00	15.65	13.20	6.00	14.00	56.50	14.45	18.80	6.60	15.40	16.65
	6.	23.25	16.45	14.75	6.40	14.75	50.50	14.20	27.70	6.65	16.00	15.75
	7.	21.75	16.00	12.95	6.40	14.10	52.50	16.05	18.45	5.40	16.10	17.20
	8	20.05	16.85	13.50	4.50	10.90	46.75	16.05	42.75	7.45	23.80	15.90
	9.	17.20	16.00	12.30	6.90	11.55	71.75	19.20	37.00	7.60	18.85	14.40
L	10.	34.00	18.95	13.50	4.90	12.70	42.50	17.30	41.50	7.35	18.35	18.55
	11.	18.30	16.85	14.60	7.80	10.60	85.50	18.95	46.60	7.40	18.75	16.40
	$\bar{X}$	21.42	16.43	13.04	5.95	12.69	51.21	15.80	27.60	6.46	17.86	16.73
	SE	1.70	0.36	0.37	0.36	0.52	4.76	0.59	3.61	0.28	0.76	0.39
		Medium diameter (D_2)										
	1.	10.30	12.80	10.40	3.45	5.90	27.75	14.10	13.00	4.45	15.50	15.70
	2.	13.85	15.35	10.60	4.00	6.20	35.50	12.55	16.35	5.60	13.85	15.30
	3.	14.50	15.30	11.15	5.25	5.45	37.75	12.30	16.00	4.70	15.15	16.10
A	4.	14.85	15.80	12.10	5.60	6.55	30.45	14.10	15.90	5.20	15.30	16.60
	5.	11.85	14.05	7.90	4.30	5.05	36.25	14.40	15.65	5.90	15.15	15.70
M	6.	13.45	16.40	10.00	4.95	6.35	39.50	14.00	21.55	6.25	14.90	15.50
	7.	11.80	15.70	12.25	4.70	6.05	40.75	13.95	17.95	5.00	16.06	15.70
	8.	14.75	15.20	10.55	4.50	8.80	35.50	14.30	28.70	7.10	19.75	15.50
	9.	16.20	15.25	10.70	5.55	5.65	51.25	16.60	30.20	7.00	17.15	13.20
	10.	12.20	15.40	11.30	3.90	7.00	38.25	14.70	30.80	7.34	17.60	16.30
I	11.	14.40	15.05	10.60	5.50	5.55	67.50	17.55	25.55	6.90	17.20	16.40
	$\bar{X}$	13.47	15.12	10.69	4.70	6.23	40.04	14.41	21.06	5.95	16.15	15.64
	SE	0.52	0.29	0.35	0.22	0.31	3.29	0.46	1.99	0.31	0.50	0.27
		Minor diameter (D_3)										
	1.	2.40	11.95	9.80	3.00	2.75	24.35	13.70	12.85	3.80	15.25	15.60
N	2.	3.40	13.80	7.55	3.10	3.40	22.20	11.35	16.20	5.30	11.35	14.70
	3.	5.00	13.35	10.00	4.65	3.95	23.75	11.00	15.00	4.55	14.10	15.80
	4.	5.70	13.70	9.90	4.15	3.00	26.15	13.60	15.90	4.40	13.85	15.80
	5.	3.05	13.55	7.75	3.80	3.45	23.95	12.05	15.35	5.30	14.35	14.85
	6.	5.20	14.80	9.80	4.55	3.40	30.00	10.90	20.50	5.80	14.50	14.65
	7.	4.75	12.50	10.50	3.75	3.25	31.50	13.20	15.55	4.55	12.70	14.70
A	8.	3.10	14.10	7.85	3.90	2.75	35.00	14.00	23.55	7.10	17.05	14.65
	9.	3.20	14.70	7.65	3.60	2.85	35.50	11.75	17.00	6.85	14.20	12.85
	10.	3.80	13.80	8.20	3.55	3.35	33.55	14.45	17.75	6.95	16.40	16.20
	11.	3.60	14.85	8.90	3.65	3.10	39.25	12.45	18.50	6.15	17.00	14.95
	$\bar{X}$	3.93	13.74	8.90	3.79	3.20	29.56	12.59	17.10	5.52	14.61	14.98
	SE	0.32	0.28	0.34	0.16	0.11	1.75	0.38	0.88	0.34	0.53	0.27

cells from 11 different mammals (total number of measurements = 7260) which are summarized in Table II.

The similarities of the eleven mammalian cell types can be established by means of the cluster analysis, which leads to the dendrogram illustrated in Fig. 1. As shown in this dendrogram, the greatest similarities exist between cell types 10 and 11, then between the types 2 and 7, followed by types 4 and 9. An equivalent conclusion is obtained by means of the traditional statistic analysis (Fig. 2), in which again the greatest similarities occur between cell types 10 and 11, 2 and 7, and finally between 4 and 9. These three groups of cells not only have similar shapes (D_1, D_2 and D_3) but also the absolute sizes (expressed in μm) were taken into account in this analysis. It is interesting to note, that in accordance with the dendrogram (Fig. 1) and the traditional statistical analysis (Fig. 2), the greatest dissimilarities occur with cell types 1 (glomerular epithelium cells), 6 (adipocytes), and 8 (Purkinje cells). With regard to the principal component analysis, it is worth mentioning that the first and second principal component explain 99.3% of the cell diameter variability. From the two principal component scores we obtained four clusters, as shown in Fig. 3, which confirm our previous conclusions (see Fig. 1 and 2).

With regard to the original hypothesis, concerning the eventual invariance of cell diameters, we have submitted the numerical data summarized in Table II to a log-log regression analysis, in order to obtain the corresponding empirical allometric equation for each cell diameter (D_1, D_2 and D_3), whose results are given in Table III. Since the 95% confidence limits for the exponents (b) include zero, we can conclude that no scale effect is present. The above mentioned equation is:

$$Y = aM^b \qquad (1)$$

where

Y is any physiological, morphological or ecological variable, which appears to be

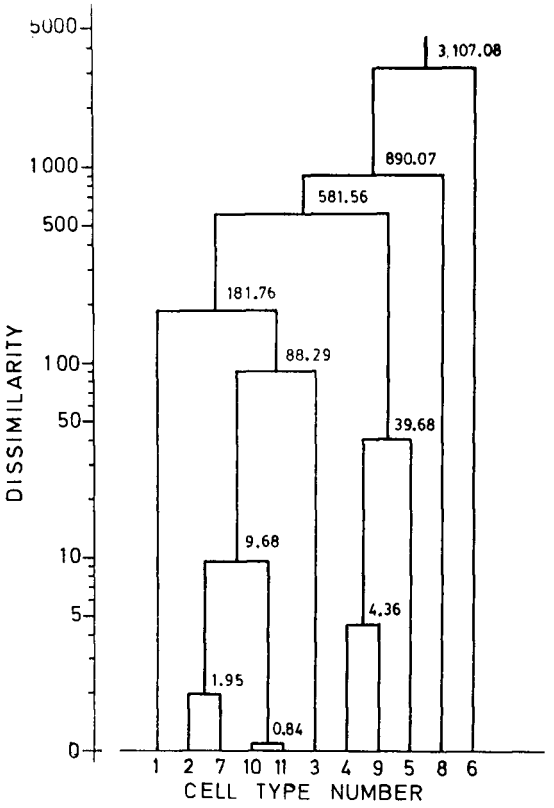

Fig. 1: Dendrogram of the eleven cell types. Ordinate: logarithmic scale for the cell dissimilarities. Abscissa: cell type numbers.

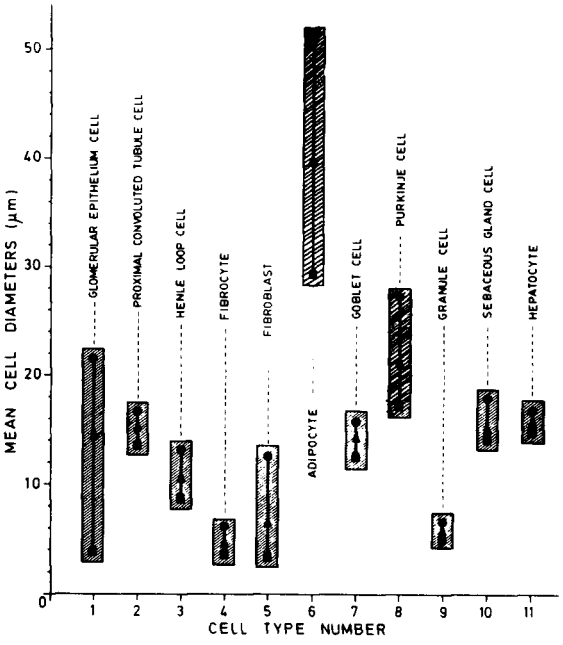

Fig. 2: Mean cell diameters (μm) of eleven types of mammalian cells, with indication of the mean ($\bar{X}$) of the major diameters (full circle), the medium diameters (triangle), and minor diameters (square).

TABLE III

Allometric equations of the relationship between the three cell diameters (μm) and body mass (g), in eleven mammals and for the eleven kinds of cells (N = 121)

Cell diameter	Coefficient (a)	Exponent (b)	95% confidence limits of exponent (b)
Major (D_1)	15.03	0.027	−0.067, 0.060
Medium (D_2)	12.02	0.025	−0.092, 0.059
Minor (D_3)	9.07	0.013	−0.028, 0.054

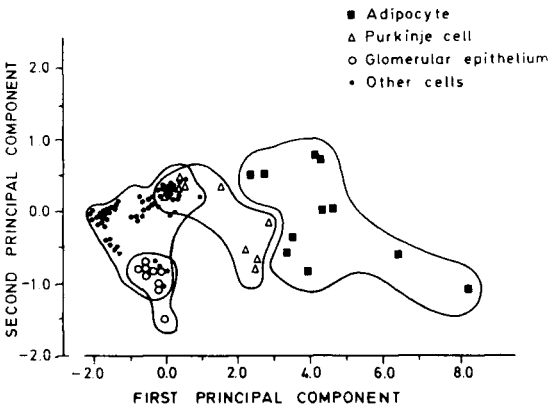

Fig. 3: Cell-type grouping in accordance to the first and the second principal component analysis.

correlated with size; *a* is an empirical parameter; *M* is body mass or organ mass (in g or kg); *b* is the scaling factor, or the allometric exponent.

With regard to the eventual sex differences, only two species were compared: hamster (animals Nº 2 and 3) and rats (animals Nº 4 and 5), as shown in Table I and II. From the unpaired t-test of these two species we may conclude, provisionally, that the mean values of all cell diameters (D_1, D_2 and D_3) are not significantly different ($p > 0.2$) between both sexes.

DISCUSSION

Cell diameters of different sized mammals have been studied in the past by Teissier (1939) and Szarsky (1976). Nevertheless, in most of these works only one cell diameter was measured. In addition, the comparisons among cells from various species were based on the numerical data obtained from several authors, which utilized different fixation and staining techniques. Only Maldonado *et al.* (1973) employed a standardized fixation and staining method throughout their work. On the other hand, the great majority of authors have calculated cell areas (A) and cell volumes (V) based on a single cell diameter, which is valid only if one assumes that the cell geometry is either a sphere or a cube. In consequence, the calculated figures may be erroneous, since the three cell diameters were not taken into account. In order to avoid these possible sorces of error, in the present study we have measured the three diameters from eleven kinds of mammalian cells independently. The calculated areas (A) and volumes (V) of these cells, as well as their A/V ratios, will be analyzed in a forthcoming publication.

Nevertheless, it should be emphasized that the present cell morphometry deals only with the three dimensions of the soma of eleven kinds of cells, and does not include the cellular processes. With regard to the range of the mean cell diameters, they vary from 4 μm of fibrocytes (type 4) and 5 μm of granule cells (type 9), to the large Purkinje cell (type 8) with diameters between 17 μm and 27 μm (Fig. 1 and Table I). A completely different kind of cells is represented by the adipocyte (type 6), whose diameters vary between 30 μm and 60 μm; but in this case we are dealing with a fat-reservoir-cell, whose protoplasma is located exclusively under the cell surface.

For cell size comparisons in animals of different sizes we have utilized Huxley's (1932) allometric equation which represents a convenient scaling procedure (Calder, 1984; McMahon & Bonner, 1983; Peters, 1983; Schmidt-Nielsen, 1984), due to its simplicity and general applicability.

In a previous morphometric analysis, Maldonado *et al.* (1973) found that the mean cell diameter of seven different types of cell was 13.82 μm for a 20 g mouse, 19.45 μm for a 15 kg dog, and 17.93 μm for a 600 kg cow. If the logarithms of these mean cell diameters (D) are plotted againts the logarithms of body mass (M), expressed in grams, one can obtain the following allometric equation in its logarithmic form:

$$\text{Log } D = 0.028 \log M + 1.123 \qquad (2)$$

The slope (b = 0.028) of Eq. 2 indicates, that the cell diameter (D) is almost invariant in *homeotherms*. When the same methodology was applied to the cell sizes of *poikilotherms* (2.6 g and 50 g frogs, and a 624 g toad), the mean cell diameter values were 18.32, 18.71 and 24.17 μm, respectively. The corresponding allometric equations is:

$$\text{Log } D = 0.049 \log M + 1.225 \qquad (3)$$

in which the slope of the logarithm of body mass (M) is only slightly larger than that of Eq. 2.

In contradistinction to the above mentioned results (Eq. 2 and 3), we found (Table III) that the three cell diameters (D_1, D_2 and D_3), expressed as allometric equations, have exponents (b) whose 95% confidence limits include the zero value, and consequently the cell diameters in mammals do not obey to the so called "scale effect".

The constancy of cell dimensions can be related with the exchange processes between the cell protoplasm and the surrounding medium. The exchange of matter between the interior of each cell and its environment is governed primarily by Ficks's law of diffusion (Florey, 1966).

$$dm/dt = -SK(dc/dx) \qquad (4)$$

whereby the transport of matter (m) depends upon the cell surface area (S), the difussion constant of a specific substance across membranes and protoplasm (K) and the concentration gradient (dc/dx) among the distance (x) that the substance (m) has to be transported. Assuming that three of these parameters are kept constant (S, K, c) then the transport of matter is inversely proportional to the distance (x) between the cell surface (plasma membrane) and the center of each cell (nucleus). In order to illustrate the paramount importance of this distance (x), with regard to the time (t) required for the diffusion process (assuming that K $\sim 10^{-5}$ cm^2 sec^{-1}) let us examine the following numerical data (Berne & Levy, 1983):

Diffusion distances (μm) (x)		Times required: (t)
1		0.5 millisecond
10		50 milliseconds
100	(0.1 mm)	5 seconds
1000	(1 mm)	8.3 minutes
10000	(1 cm)	14 hours

From these data we may conclude, that the diffusion time in the millisecond range is only possible in the microscopic realm (μm). When the diffusion distance (x) is within the millimeter, or centimeter range, the diffusion time (t) increases very markedly. In consequence, all cell diameters must be restricted to the micrometer scale (μm).

Finally, we would like to discuss briefly the biological meaning of the constancy of cell diameters in multicellular organisms. The metabolic rate or oxygen consumption (V_{O_2}) of multicellular organisms should be proportional to the sum of the metabolisms of all cell masses, i.e., $\dot{V}_{O_2} \propto M$. Alternatively, the metabolic rate could also be related to the exchange surface of all cells, and in the latter case $\dot{V}_{O_2} \propto M^{2/3}$, a relationship which would agree with a geometric similarity (Günther, 1975). Nevertheless, extensive metabolic measurements reviewed by Kleiber (1947) have yielded a relationship of $\dot{V}_{O_2} \propto M^{3/4}$ for both homeotherms and poikilotherms, and also for unicellular organisms (Hemmingsen, 1960). Notwithstanding, the latter conclusions may not be valid for the cells which conform the tissues of multicellular organisms, since in this case, the cells cannot be taken as isolated units, because they pertain to organized structures.

Krebs (1950) found that the *in vitro* metabolism, expressed per gram and per hour, of tissue sections obtained from homeotherms of increasing body mass (M), decreased as the animal size got larger. The allometric exponents (b) for the metabolism of liver and kidney tissue slices were −0.115 and 0.064, respectively. It is likely, that the latter values for the allometric exponent (b) are not significantly different from zero. Besides the above mentioned *in vitro* studies, recently Langer (1985) has determined the oxygen consumption ($\dot{V}_{O_2}$) of mononuclear leucocytes obtained from the blood of six mammals of different sizes. He found that the oxygen consumption (10^{-12} l_{O_2}/cell/hour) was independent of body weight (kg), since the calculated allometric equation for $\dot{V}_{O_2}$ per cell and per unit time, was:

$$\log \dot{V}_{O_2} = -0.005 \log M + 0.059 \quad (5)$$

where *M* is the body mass (kg). Since the 95% confidence limits of the exponent (b) were −0.048 and 0.038, the author concluded that the exponent (b) is probably not different from zero.

In summary, the allometric exponents (b) for cell metabolism ($\dot{V}_{O_2}$) obtained *in vitro*, both for tissue slices (Krebs, 1950) and for isolated leucocytes (Langer, 1985), are essentially equal to zero. In contrast, the standard metabolic rate for the whole organisms, when expressed per unit mass (M^{-1}), has an allometric exponent of b = −0.25. This figure is based on the fact, that the metabolic rate for all organisms (Hemmingsen, 1960) is proportional to $M^{3/4}$, and when this standard metabolism is expressed per unit body mass ($M^{3/4}/M$) this yields $M^{-1/4}$ or b = −0.25. The different exponents, b = 0.0 for the metabolism *in vitro*, and b = −0.25 for the metabolism *in vivo*, may be related to the organization of the whole organism (Günther & Morgado, 1984), in contradistinction with the conditions which prevail in isolated cells or in tissue slices *in vitro*.

In sum, the present morphometric study emphasizes the paramount importance of the constancy of cell sizes, which is directly related to the exchange of matter in all organisms (unicellular and multicellular), provided that the cell diameters are within the micrometer range.

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