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Body size allometry of mammalian blood heat output as assessed by microcalorimetry[1]

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Abstract

The heat output of heparinized blood samples from seven mammalian species (hamster, rat, hedgehog, dog, swine, human and sheep) was determined by ampoule microcalorimetry and related to body mass. Typical microcalorimetric records consist of an initial plateau and a terminal decline of metabolic rate. A decrease in the plateau heat output with increasing body mass was found, corresponding to the allometric relationship between specific basal metabolic rate and body mass. Moreover, the duration of the plateau was nearly reciprocal to the level of heat output, indicating a relatively constant "energy content" of blood samples which in smaller species, due to their higher thermal power, is utilized faster than in larger ones. The presence of scaling effects in a physiological cell suspension may elucidate the still unknown regulatory mechanisms of metabolic size allometry.

SHORT INTRODUCTION TO METABOLIC ALLOMETRY

Contrary to what one might expect, the metabolic rate of mammals is not proportional to body mass. This well-known, though little understood phenomenon is illustrated by the famous mouse-to-elephant curve, presented by Benedict [1] in 1938, which is reproduced in schematic form in Fig. 1. In this figure, the basal metabolic rates (on the y axis) of mouse and elephant are plotted against body mass (on the x axis). The mouse weighs 30 g, the elephant 3000 kg; thus the elephant has 100 000 times the mass of the mouse. If the turnover rate were directly proportional to body mass,

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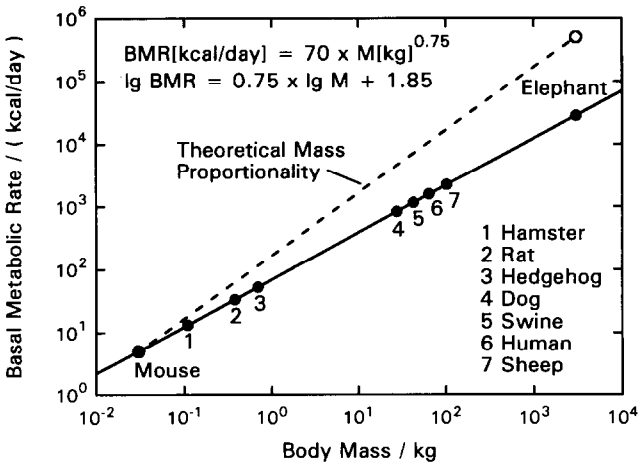

Fig. 1. Metabolic size allometry in mammals. The basal metabolic rate (BMR) of mammals increases with body mass (M) to the power of 0.75. Thus, the BMR of the elephant as compared with the mouse is lower than would be expected in the case of direct mass proportionality. When plotted on log scales, the exponent of the power function becomes the slope of the line. The underlying formulae are given in the traditional units used by Kleiber [3].

the basal metabolic rate of the elephant would also be 100 000 times that of the mouse (broken line). Remarkably, however, it is more than one order of magnitude lower, suggesting a reduction in metabolism with increasing body mass.

The body size dependences of physiological parameters can be expressed, as first proposed by Huxley [2], by a mathematical expression relating, in this case, the basal metabolic rate (BMR) and body mass (M) in the form of a power function. Given a direct proportionality between BMR and M, the exponent of this equation would be 1.0, indicating isometry. Because, however, the exponent differs from 1.0, the equation is called allometric. The allometric body size relationship of mammalian BMR in its widely accepted form was formulated by Kleiber [3] in 1961, the exponent of this relationship being 0·75 (for traditional reasons, no SI units are used in this case)

$$\mathrm{BMR\ (kcal\ day^{-1})} = 70\ M\ (\mathrm{kg})^{0.75}$$

If this equation is expressed in logarithmic terms or, as already shown in Fig. 1, plotted on log coordinates, a linear equation results, transforming the exponent of the power function into the slope of the line

$$\log \mathrm{BMR} = 0.75 \log M + \log 70$$

Another slightly different representation of the allometric relationship is achieved if the specific, i.e. weight-related, basal metabolic rate (SBMR) is

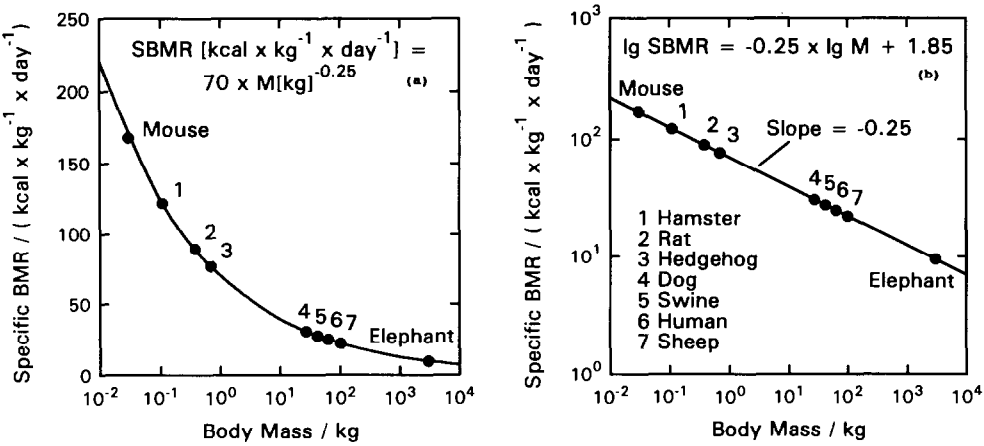

Fig. 2. Metabolic size allometry in mammals. (a) If the specific, i.e. the weight-corrected, basal metabolic rate is plotted instead of the absolute turnover values, the reduction of metabolism with increasing body mass is evident. (b) Logarithmic transformation again leads to a linear relationship with a corresponding negative slope. The numbers refer to the species investigated in this study which in reality, of course, would not exactly lie on the mouse-to-elephant regression curve.

taken instead of the absolute turnover values (Fig. 2(a))

$$\text{SBMR (kcal kg}^{-1}\text{ day}^{-1}) = 70M\text{ (kg)}^{-0.25}$$

This kind of graph is especially illustrative of the metabolic reduction occurring with increasing body mass. As described above, logarithmic transformation leads to a linear relationship, in this case with negative slope, which again corresponds to the exponent of the underlying power function (Fig. 2(b)).

$$\log \text{SBMR} = -0.25 \log M + \log 70$$

The allometric relationship will be applied in this form below.

It may now be asked why the turnover rate is not proportional to body mass. Following a traditional explanation going back to the 19th century German physiologist Rubner [4], smaller mammals, due to their greater surface-to-volume ratio, lose more heat to the surroundings and therefore need a higher endogenous heat production per unit of mass than larger species in order to maintain a constant body temperature.

However, there are two main objections to this so-called "surface rule". Firstly, metabolic size allometry also applies (at a lower absolute turnover level) to poikilothermic organisms which do not have a constant body temperature [5]. Secondly, Kleiber's exponent of 0.75 is significantly different from the 0.67 which would be expected, for mathematical reasons, in the case of "true" surface proportionality of metabolic rate.

Thus, the surface theory, though quite plausible, is probably not correct.

However, no alternative convincing explanation of metabolic size allometry exists, and even advanced theories on "biological similarity" omit the physiological mechanisms which could be regulating in this intriguing relationship (for reviews see refs. 6–11).

In the search for these fundamental mechanisms, it is interesting to know whether Kleiber's rule only applies to the intact organism, or whether it also holds true at the tissue and/or cellular level.

With respect to isolated tissues, the validity of metabolic size allometry was demonstrated by Krebs [12] in 1950 although his results have never been reproduced on a broader scale. We have made similar observations in a microcalorimetric study on ischemic tissue samples in which the metabolic difference between a large and a small species seemed to be transformed into a time factor of metabolic decline, suggesting that the size influence is still operating under anaerobic conditions [13].

As far as the cellular metabolism is concerned, very few experimental efforts seem to have been directed to size effects. However, in an interesting paper by Sernetz et al. [14], dealing with the "fractal structure" of metabolism, the assumption was made (and reinforced by a single observation on mononuclear leucocytes) that isolated cells do not exhibit a metabolic dependence on body mass.

AIM OF THE STUDY AND EXPERIMENTAL PROCEDURE

We decided to perform microcalorimetric measurements on blood samples from various mammals of different body mass. From these experiments, information on the presence or absence of metabolic size allometry in a "natural cell suspension" was expected. Moreover, because the main cellular component of blood samples consists of erythrocytes, and because their metabolism is mainly anaerobic, this investigation was expected to provide further insight into the body size dependence of anaerobic metabolism.

Thus far, seven mammalian species have been investigated which, in order to demonstrate the size scale considered, are included in Figs. 1 and 2. In general, the animals were being subjected to other types of experiments and would have been sampled anyway. Hence, most of them were under narcosis and some influence of the anesthetic pretreatment on blood heat output might be assumed. Nevertheless, whenever a comparison between various anesthetic procedures in one species was possible, no differences in the microcalorimetric results were noted.

Samples were collected in heparinized cups or syringes and transferred into 5 ml stainless steel ampoules for microcalorimetric measurement. In most cases, two specimens of different volumes, e.g. 0.5 and 1.0 ml or 1.0 and 2.0 ml, were prepared from the same blood sample so that the result of one experiment represents a mean value of two calorimetric curves at

different absolute heat output levels. Incidentally, no significant influence of the sample volume on the shape of the thermal power curve was observed.

In addition, the hematocrit value from each sample was determined to allow correction of the thermal power values not only to the blood, but also to the cell volume (for details see below).

Measurements were performed on a 2277 Thermal Activity Monitor (TAM), assembled by ThermoMetric AB, Järfälla, Sweden, and distributed in Germany by C3 Analysentechnik, Baldham [15]. The incubation temperature was 37°C. Preparation of the specimens normally took 10 min so that, with an additional 20 min of thermal equilibration, the microcalorimetric measurement normally started 30 min after the sampling procedure. In cases where somewhat longer distances between the veterinary and the calorimetric laboratories were involved, the samples were ice-chilled during transport. Thus, assuming typical temperature influence on metabolic rate, the net "normothermic" latency time between sampling and onset of measurement never exceeded 45 min.

Thermal power curves were recorded over six or ten hours, in smaller or larger species, respectively. In view of the relatively slow time course of blood heat output, no attempts were made to correct dynamically the resulting microcalorimetric curves.

The shape of the calorimetric curve regularly obtained on measurement of heparinized whole blood samples in the static ampoule mode is demonstrated, for human blood, in Fig. 3(a). Two main phases may be

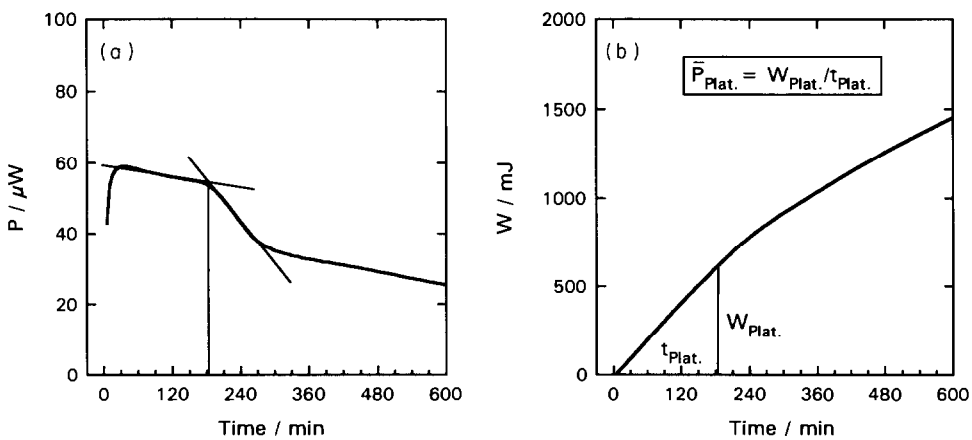

Fig. 3. Blood microcalorimetry, demonstrated by a representative human sample (the author's own). (a) If measured in the static ampoule mode, blood samples give a limited, more or less steady plateau of heat output, followed by a terminal decline in metabolic rate. (b) When the total amount of heat (energy) liberated during the plateau phase is divided by the duration of the plateau, a mean power value results which is largely independent of slight instabilities occurring in the thermal power curve.

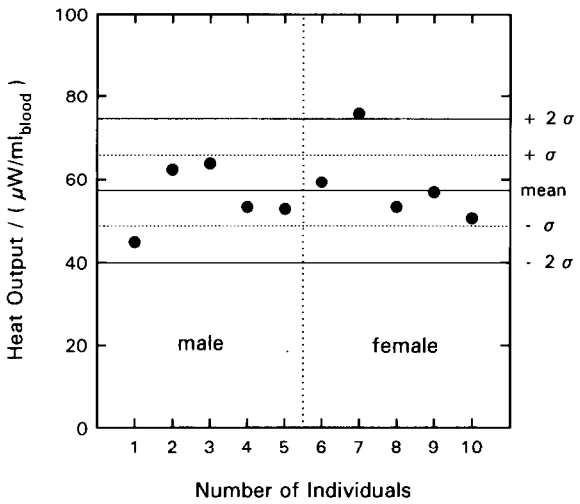

Fig. 4. Standard heat output of adult human blood. Samples were taken from ten volunteers of both sexes. Determination of thermal power by the method described in Fig. 3 led to a standard range which is in good accordance with data reported in the literature [18].

distinguished in the course of thermal power, namely an initial "plateau" of metabolism which, probably due to sedimentation phenonema in the sealed ampoule [16], can sometimes be a little more uneven than in the case presented, and a terminal decline in metabolic rate which is apparently due to the exhaustion of glucose reserves and the accumulation of acid metabolites in the measuring ampoule [17].

If this curve is integrated and the total amount of energy liberated during the plateau period is divided by the duration of the plateau (Fig. 3(b)), an average thermal power value results which is largely independent of slight instabilities in the curve and leads to good reproducible results.

This is demonstrated in Fig. 4 where a standard range for the heat production of blood samples from healthy human adults (all laboratory personnel) is given, in perfect agreement with an earlier report in the literature [18].

Thus, ampoule microcalorimetry may be regarded as a simple, but reliable method of heat output determination in blood samples (for reviews see refs. 19–21).

RESULTS AND DISCUSSION

If the mean blood heat output values from the seven species investigated, i.e. golden hamster, rat, hedgehog, dog, swine, human, and sheep, are compared (see Table 1) a striking dependence on body size is clear: the blood samples of the smaller species have a significantly higher thermal power than those of larger species. If these values are plotted on log scales

TABLE 1

Results of the comparative study on blood heat output in seven mammalian species of differing body mass. Data are tabulated as mean values and standard errors of the mean. The decrease in blood heat output with increasing body mass is evident

Species	n	Body mass/kg	Blood heat output/ μW per ml of blood
Hamster	8	0.107 ± 0.005	121.56 ± 1.75
Rat	5	0.378 ± 0.028	132.28 ± 10.41
Hedgehog	9	0.696 ± 0.048	97.24 ± 6.48
Dog	5	27.8 ± 0.8	57.40 ± 2.25
Swine	7	42.9 ± 2.1	44.74 ± 1.83
Human	10	65.0 ± 1.7	57.26 ± 2.73
Sheep	7	103.1 ± 5.0	24.66 ± 1.60

(Fig. 5), a surprisingly good linear regression is obtained

$$\log P = -0.19 \log M + 1.97 \; (r = 0.91)$$

which corresponds to the power function

$$P(\mu\text{W per ml of blood}) = 93.3\, M(\text{kg})^{-0.19}$$

and, hence, indicates an allometric relationship between metabolic rate and body size.

With these results it must be remembered that different species have different hematocrit values. The hematocrit indicates the contribution of

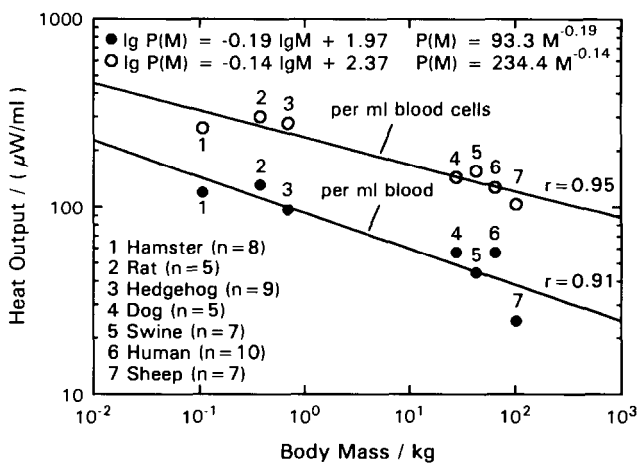

Fig. 5. Body size allometry of mammalian blood heat output. If the blood heat output values of various mammals are plotted against body mass, an allometric relationship results, similar to that seen for specific basal metabolic rates of the whole body (see Fig. 2(b)). If the data are corrected for the differing hematocrit values and given in μW per ml of cells instead of per ml of blood, an even better regression coefficient is obtained.

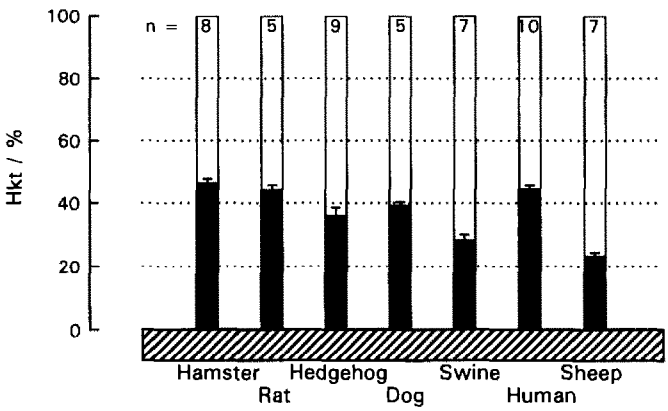

Fig. 6. Hematocrit values of the mammalian species investigated in this study (mean ± SEM). The black areas indicate the relative blood cell volume which, due to their dominant number, mainly consists of red cells (erythrocytes). As shown by the direct comparison, the hematocrit value is a non-scaleable parameter with some species-specific peculiarities.

"corpuscular elements" to the blood volume or, briefly, the "relative blood cell volume". This is mainly composed, due to their dominant number, of red cells or erythrocytes. As shown in the inter-specific comparison (Fig. 6), the hematocrit is a "non-scaleable" parameter, i.e. the red cell content of a given blood volume is relatively independent of body size. However, there are some species-specific features. Thus, for instance, in sheep, the hematocrit is somewhat lower than in other mammals, and therefore it is not surprising that the heat output per ml of blood in this species is below the overall regression line. If such deviations are corrected mathematically and the heat output is given in microWatts per ml of cells instead of per ml of blood, a somewhat modified regression line results

$$\log P = -0.14 \log \mathrm{M} + 2.37$$

corresponding to the power function

$$P(\mu\mathrm{W \text{ per ml of cells}}) = 234.4\, M(\mathrm{kg})^{-0.14}$$

which has an even better regression coefficient ($r = 0.95$) than the original curve in Fig. 5.

The slope of the line or the exponent of the corresponding power function differs from Kleiber's exponent of 0.75. However, this is not unusual in such experiments, and many more species will have to be studied before a final statement on the allometric parameters can be made. Moreover, two out of the three small mammals studied (golden hamster and hedgehog) are hibernators which are known to have, even in the waking state, somewhat lower basal metabolic rates than comparable species [22, 23]. If this also applies to blood samples, it would explain why

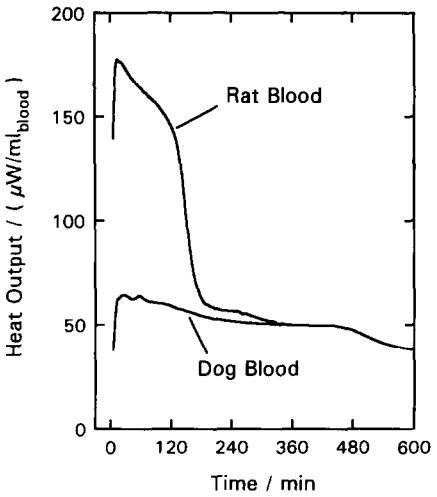

Fig. 7. Typical thermal power curves from rat and dog blood samples. In the smaller species where the heat output is higher, the metabolic plateau is shorter. Moreover, it has a slightly negative slope and is terminated by a metabolic fall-off which is much steeper than in the larger species.

the heat output values in these small species are somewhat lower than expected, leading to too low a negative slope of the regression line. Lastly, the so-called plateaus of blood heat output are not as flat in small mammals as in larger ones (see Fig. 7), and if the metabolic rate had been determined by back-extrapolation to the sampling time rather than by averaging the plateau metabolism, somewhat higher heat output values would have been obtained, especially in the smaller species. Because, however, this procedure would be influenced by additional elements of uncertainty, the more unambiguous method of plateau averaging was preferred in this case.

Apart from the overall relationship between blood heat output and body mass, there is one additional observation to be made in comparing blood heat output from small and large mammalian species. This is demonstrated in Fig. 7 in which two representative microcalorimetric curves from rat and dog blood are plotted together. It is obvious that the plateau of the smaller species is much higher, but also shorter, than in the larger species in which the heat output is lower and the final metabolic decline occurs later. If, starting from this observation, the parameters in question are related in all the species investigated, a nearly reciprocal relationship is found between the height of heat output and the length of the plateau (see Fig. 8, for this purpose the latency time between the sampling procedure and the onset of measurement was added to the measured plateau time in order to obtain the overall plateau duration). If this is true, the product of the two parameters, i.e. the total amount of heat or energy liberated during the

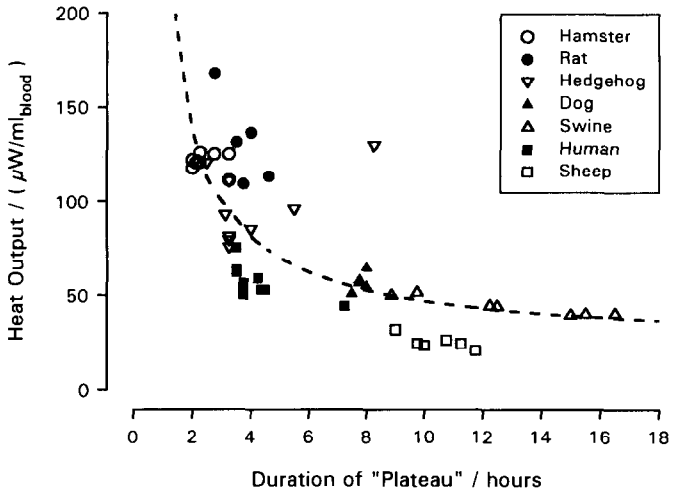

Fig. 8. Thermal power vs. duration of plateau in blood samples from different mammalian species; the duration of the plateau, in this case, includes the latency period between blood sampling and onset of microcalorimetric measurement. A roughly reciprocal relationship exists between the two parameters, indicating that the earlier the "energy content" of the blood samples is exhausted, the higher the species-specific thermal power.

plateau phase, should be a relatively species-independent constant which, in smaller species, because of their higher metabolic rate is utilized faster than in larger ones. If this parameter is calculated from the underlying data, it is remarkably uniform among the species (Fig. 9). Thus, the "energy content" of blood samples appears to be a non-scaleable parameter, similar

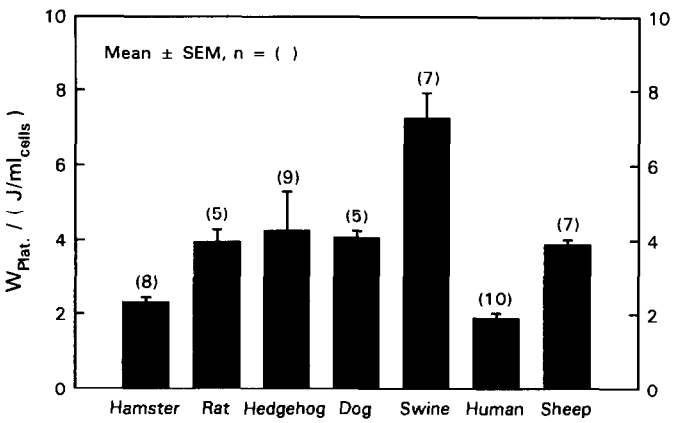

Fig. 9. Total calculated plateau heat of mammalian blood samples. Multiplication of the total duration of the plateau (including the latency period between sampling and onset of measurement) by the mean thermal power (corrected to ml of blood cells) yields the "energy content" of the blood samples which, with some exceptions, is remarkably uniform among the species.

to the hematocrit values. As in the latter, however, there are some species-specific peculiarities including, for unknown reasons, a remarkably low value in humans.

The relatively high value in swine, however, is probably due to methodological rather than biological reasons because the animals from which the samples were taken underwent surgical procedure with blood loss and volume substitution. This led to a hemodilution with the hematocrit values being somewhat lower in this series than would have been expected in untreated animals [24, 25]. An analysis of the data shows that the hematocrit value, if properly corrected for, does not influence the level but the duration of the metabolic plateau, in that the longest plateaus are generally found in the samples with the lowest hematocrit values. Equally, a low hematocrit, be it physiological or artificial, tends to prolong the plateau duration in comparable cellular heat output levels (Fig. 10). This is obviously due to the fact, that the substrate reserves per number of cells and the distribution space for the metabolic end-products are higher in the more "diluted" samples.

With respect to the substrates involved, it must be remembered that although the mainly anaerobic turnover rate of red cells is lower than the aerobic white cell metabolism, it accounts, again due to their dominant number, for at least 50% of the total heat produced by blood samples [18]. Because, moreover, half of the erythrocyte thermogenesis comes directly

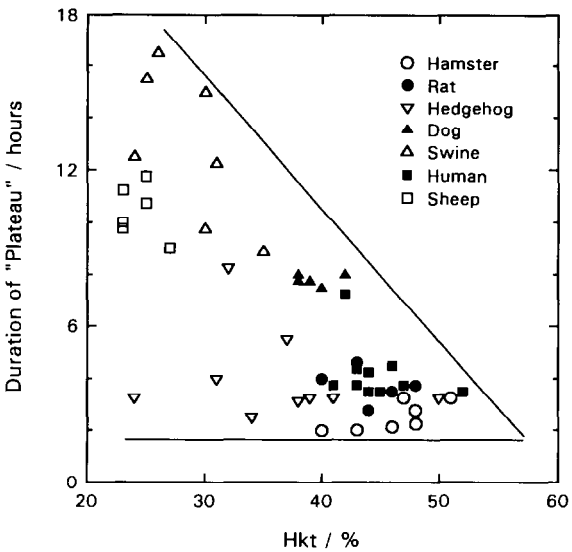

Fig. 10. Influence of sample hematocrit on the plateau duration in mammalian blood calorimetry. Whereas the heat output level per ml of cells is largely independent of hematocrit, the duration of the plateau at comparable heat output levels tends to be longer in species, or samples, with lower hematocrit values.

from the glycolytic pathway, the availability of glucose is probably the main determining factor of plateau duration in blood calorimetry [17, 20]. Thus, it may well be that random variations in the blood sugar level, e.g. from sampling on an empty stomach or after glucose infusion, are partly responsible for the size-independent peculiarities of blood "energy content" described above. Apart from this, the results seem to confirm what has already been observed in ischemic tissue samples, namely the presence of scaling effects even under mainly anaerobic conditions.

CONCLUSIONS AND METHODOLOGICAL PROSPECTS

In summary, it is concluded that, contrary to current assumptions, size effects on metabolic rate are also found in natural cell suspensions. Although this observation provides no new explanation of the allometric relationship per se, it contributes to the invalidation of predictions derived from other scaling theories. Moreover, blood, as compared to the whole organism or tissue samples, is easier to analyse with respect to both the appropriate measuring conditions and the "intrinsic" physiological parameters which are the chief candidates for exercising influence on cellular metabolism, e.g. hormone levels, membrane composition, enzyme content. In this way, the results presented may offer a new approach to the yet unsolved regulatory mechanisms of metabolic size allometry.

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