Respiration Physiology, 78 (1989) 265–280
Elsevier

RESP 01606

Interaction of allometry and development in the mouse *Mus musculus*: heart rate and hematology

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(Accepted for publication 30 August 1989)

Abstract. The contribution of body mass changes to developmental adjustments in heart rate and hematology has been investigated in the mouse *Mus musculus*. Both resting heart rate (f_H) and hematological variables including erythrocyte concentration, hemoglobin concentration, blood oxygen capacity, hematocrit, mean corpuscular hemoglobin and mean corpuscular volume, changed considerably during the increase in body mass from birth (1 g) to adulthood (maximum of 50 g). There were two phases of change, one characteristic of preweaned mice (approximately < 10 g) and the other of postweaned mice (approximately 10–50 g). In preweaned mice resting f_H was about 1/2 of the value predicted on the basis of interspecific allometric data from mammals. f_H increased steadily until body mass reached 10 g, then began to decrease with further mass increase at the same rate as predicted from interspecific allometric data. Erythrocyte concentration, hematocrit, hemoglobin concentration and blood oxygen capacity were all significantly lower in preweaned mice compared with postweaned mice. It is suggested that the progressive heart rate increase in very young mice may be to increase cardiac output to compensate for the neonatal anemia. After weaning, hematological variables showed little or no further change with increasing body mass. Collectively, these data indicate that during the early phases of postnatal growth, developmental factors other than body mass have the greatest influence on heart rate and hematology, and allometric data derived from interspecific studies on adults have little predictive value in neonates. After weaning, however, body mass is the major influence on these variables, and allometric data derived from interspecific studies on adults are reasonably accurate as predictors. We conclude that interspecific allometric studies must be properly regarded as the study of *adult* animals of different body sizes, and that untested assumptions about the applicability of these data to intraspecific studies of immature specimens should be made with extreme caution.

Allometry; Body mass; Growth; Heart frequency; Hematocrit; Hemoglobin concentration; Red blood cell

The scaling of physiological characters to body mass has been extensively documented (for review see Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984). This knowledge of physiological allometry has been based on combined data from a wide phylogenetic range, frequently including different orders or even different classes. Yet, there are a few indications that interspecific allometric data may not accurately predict changes intra-

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specifically. For example, Heusner (1982) and Wieser (1984) have emphasized the difference in allometry of basal metabolism when comparing intraspecifically (mass exponent, $b = 0.66$) and interspecifically ($b = 0.75$). They further indicated that there can be several phases in the developmental change of basal metabolism, each described by its own allometric equation.

While the same physical and biological considerations that have been suggested to account for the effects of body mass on physiological function in interspecific comparisons should also apply intraspecifically, this hypothesis has been but rarely tested. The present study focuses on the intraspecific allometry of cardiac performance and hematology in a mammal. These two factors are highly interdependent, since an increase in heart rate and cardiac output may act to offset a reduced hemoglobin concentration and blood oxygen capacity, for example. It is known from interspecific studies that resting heart rate (Stahl, 1967) and cardiac output (Günther, 1975) in adult mammals of different species decreases with body mass to the power of -0.25, generally paralleling the associated fall in metabolic rate with increasing body mass. Stroke volume (Günther, 1975) and heart mass (Prothero, 1979), however, are directly proportional to body mass across a wide range of mammalian species, indicating that adjustments in cardiac output related to changes in body mass are largely the result of adjustments in heart rate. Allometric studies on single mammalian species show qualitatively similar changes in cardiac performance, with a decrease in resting heart rate with increasing body size during development being reported for humans (Wong and Cassels, 1960) and white-tailed deer (Jacobsen, 1978).

Hematological allometry has also been investigated, though again almost all studies have been interspecific in nature. Interestingly, there appear to be differences when comparing inter- and intra-specific studies. Blood hemoglobin concentration (and presumably blood oxygen capacity) is nearly independent of body mass when examined interspecifically in mammals (see Peters, 1983), yet hemoglobin concentration and hematocrit of a newborn human infant are about $4.5\ g \cdot dl^{-1}$ and 8% higher, respectively, than that of the much heavier normal adult (Wintrobe *et al.*, 1981). In contrast, in urodele amphibians these hematological variables actually scale positively with body mass (Burggren *et al.*, 1987).

One complicating factor with intraspecific allometric studies of cardiac performance or hematology (or indeed any physiological or anatomical variable) involves possible effects of development *per se*. Interspecific studies have typically compared mature adults of species exhibiting widely different body masses. In order to generate meaningful allometric relationships in intraspecific studies, it is necessary to examine individuals over a very wide range of body mass. Usually, this also means that data from animals of different developmental stages are being lumped together, although some investigations have capitalized upon lower vertebrate species that show wide variation in body mass in single developmental stages (*e. g.* Burggren *et al.*, 1987). For most vertebrate species, however, changes in development and changes in body mass occur simultaneously. Yet, the interactions between body mass and development on physiological factors remains largely unexplored for any vertebrate.

The intent of the present study was to quantify selected cardiac and hematological variables within a single mammalian species, the mouse *Mus musculus*, to determine if changes due to development were superimposed upon those predicted strictly on the basis of body mass changes. We hypothesize that postnatal adjustments of cardiac and hematological variables in the mice are due solely to changes in body mass. Therefore, the intraspecific allometric relationship developed for cardiac and hematological variables in the developing mice should be predictable from interspecific allometric studies. Deviation from the interspecific relationship will indicate that developmental factors independent of body mass are influencing the measured variables.

Materials and methods

A total of 131 mice (*Mus musculus*, inbred D-L strain) were used for cardiac and hematological measurements. Body mass ranged from 1 g (newborn) to 50 g (two years of age). Separate groups of animals were used for measurements of heart rate (62 animals) and hematology (69 animals). Prior to measurements, mice were kept at 22 °C in 12L/12D photoperiod and given food and water *ad libitum* prior to use. Heart mass measurements were made on 48 of the mice used for hematological measurements.

Mice were categorized as 'preweaned' or 'postweaned'. At weaning, mice general have fully developed pelage and open eyes. Body mass at weaning is somewhat variable, but weaning usually occurs at approximately 9–11 g. Thus, preweaned mice included newborns (about 1–2 g) up to a body mass of approximately 10 g. Postweaned mice included the body mass range of approximately 10–50 g.

Surgical preparations. Mice were anesthetized with 2% halothane in air during all surgical procedures. Resting heart rate (f_H) was recorded using chronically implanted electrodes constructed from 45 gauge insulated copper wires. A pair of electrode wires, bared 1 mm at their tips, penetrated the dorsal skin of the neck and ran to both sides of the chest. A third electrode wire serving as ground was implanted subcutaneously in the lumbar region. Fine sutures secured all the wires to the skin at their point of emergence, and the wires were braided together to form a single cable. Electrode implantation required approximately 20 min. ECG electrodes were connected to a biopotential preamplifier of a Narco MK-IV chart recording system. An instantaneous heart rate signal was generated using a Narco biotachometer and was recorded on a second channel.

Body temperatures were measured chronically along with f_H in 32 mice using a 0.127 mm diameter thermocouple connected to a Sensortek BAT-12 meter. For the relatively nonambulatory newborn and preweaned mice, the thermocouple was inserted into the rectum for 0.5–1.5 cm, depending on the size of the animal. For larger mice that were more active and showed a tendency to chew a rectally-implanted thermocouple, the tip of the thermocouple was implanted under anesthesia into the abdominal cavity via a lateral skin and body wall penetration, and was secured by sutures to the skin.

The thermocouple wire was led up over the shoulder, and braided together with the ECG electrodes.

Immediately after electrodes and thermocouple implantation, mice were transferred to an experimental chamber in a thermostatted water bath. The chamber consisted of a 1-L open Plexiglas jar containing a 5 cm thick layer of ground corn cobs for bedding.

Experimental protocol. For postweaned mice, temperature of the water bath was kept between 31 to 33 °C, which is their metabolic thermoneutral zone at a body temperature of 38 °C (Pearson, 1947). Preweaned mice are either complete or partial poikilotherms (Lagerspetz, 1962; Bryant and Hails, 1975; confirmed by present experiments). Given that any developmental differences in f_H could have been offset by small differences in body temperature among preweaned mice, the temperature of the water bath was elevated to between 35 and 38.5 °C to maintain body temperature (Tb) close to 38 °C. However, in many preweaned mice Tb was still 1–2 °C above or below 38 °C at the time of f_H measurement. Therefore, a series of preliminary experiments were performed to determine the temperature sensitivity of f_H in preweaned mice. Resting heart rate and body temperature were measured simultaneously over a range of ambient temperature from 30 to 40 °C during heating and cooling. Q_{10}s for f_H were calculated from the slope of the linear regression line on a semilogarithmic plot of heart rate against body temperature. The relationship between Q_{10} and the slope is $\log(Q_{10}) = 10 \cdot S$, where S is the slope of the regression line. Using these data, any f_H sampled at a body temperature (Tb) other than 38 °C was corrected to heart rate at 38 °C as follows: f_H at 38 °C = f_H at Tb $\cdot Q_{10}^{(38 - Tb)/10}$, where f_H is heart rate at body temperature of T °C.

After surgery, all mice were allowed 20 min in the experimental chamber to recover from anesthesia. Instantaneous heart rates were sampled regularly at 5 min intervals from resting ECG's between 30–240 min after electrode implantation. A representative recording of the ECG and instantaneous heart rate is indicated in fig. 1. Even completely

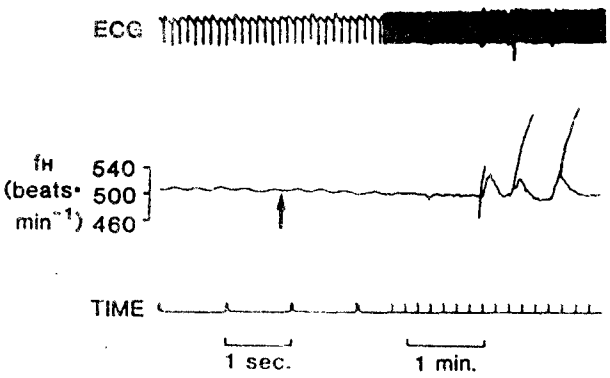

Fig. 1. A representative recording of the electrocardiogram and heart rate in an unrestrained, unanesthetized mouse, *Mus musculus* (body mass = 11.8 g). The arrow indicates a typical sample point contributing to mean heart rate for this animal. Note also periods of higher heart rate usually associated with movement, as indicated by the transient artifact in the ECG signal.

inactive mice that appeared to be sleeping showed brief spontaneous increases in heart rate of 20–40 beats · min^{-1}. Thus, f_H for each individual was calculated using the average of the ten lowest instantaneous heart rates in the measurement period.

Heart mass and ventricular mass. Hearts were dissected from 48 deeply anesthetized mice. All attached blood vessels were removed and the heart was immersed and rinsed in mammalian Ringer's solution to flush blood from the cardiac chambers. The heart was then gently blotted with absorbant paper to remove all Ringer's solution from the interior of the heart. The weight of the heart was determined, and both atria were then removed and the ventricular mass measured.

Hematology. The chest of deeply anesthetized mice was opened to expose the heart. A 100 μl blood sample was collected by cardiac puncture into a heparinized Hamilton syringe. Total elapsed time from first disturbance of the mouse to completed sampling never exceeded 3 min. Hematocrit (Hct), hemoglobin concentration ([Hb]), oxygen carrying capacity ($C_{O_2}^{max}$), and red blood cell concentration ([RBC]) were determined on individual whole blood samples from all but the smallest mice. In several neonates insufficient blood could be collected for all four measurements. Thus, after determination of Hct, [Hb] and [RBC] on whole blood, the remaining blood was diluted by 50% for measurement of oxygen capacity.

Hematocrit was determined on 30 μl blood samples in heparinized capillary tubes after centrifugation at 10,400 rpm for 3 min. Hemoglobin concentration was measured on 35 μl blood samples by a Radiometer OSM-2 spectrophotometric hemoximeter. Blood oxygen capacity was measured on 10 μl of blood as described in Burggren *et al.* (1987). Erythrocyte concentration was determined on a 20 μl blood sample initially diluted 1 : 500 with mammalian saline (Coulter Isoton II) and was further diluted to 1 : 50000 and measured in a Coulter Counter model ZF. Three counts were made on each sample and the mean of the three counts calculated. Mean corpuscular hemoglobin was calculated by dividing hemoglobin concentration by erythrocyte concentration. Mean corpuscular volume was calculated by dividing hematocrit by erythrocyte concentration.

Data analyses. Heart rate and hematological data were plotted against body mass. Logarithmic scales were used for certain variables. Linear regression lines were generated by the least squares method. For some variables the data were divided into two groups representing different developmental stages. Regression lines of the two groups were compared by analysis of covariance for a single variable. In the group where regressions were not significant, the mean was calculated. A Student *t*-test was performed to determine the difference between means. Observed resting heart rate and heart mass of individual mice were also compared with the predicted values for mammals (Stahl, 1967; Prothero, 1979) by paired *t*-tests.

Results

Temperature effects on heart rate. As expected, fH increased as Tb increased in preweaned mice (fig. 2). Preliminary analysis of the data for preweaned mice indicated mice from 1–5 g showed different responses than mice from 5–10 g; consequently, the data were analyzed separately according to body mass group. The increase in fH with increasing Tb was significant in both groups ($P < 0.01$). The slope of the regression line for fH *vs* Tb for mice of 1–5 g was significantly lower than that for mice of 5–10 g ($P < 0.001$). The mean Q_{10} calculated for heart rate was 1.56 and 1.69 for animals of

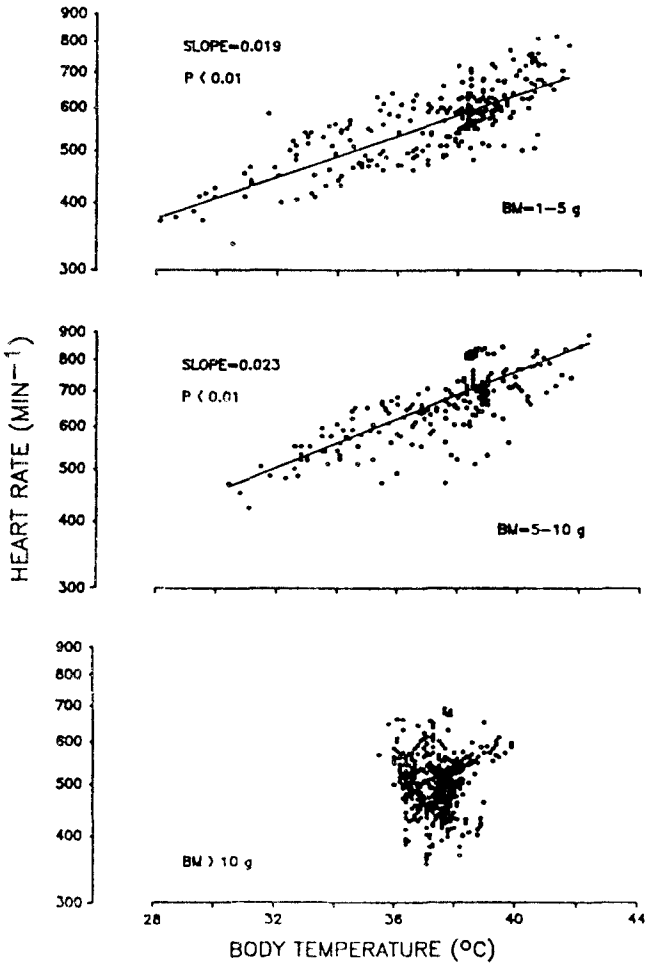

Fig. 2. Relationship between heart rate and body temperature in 33 *Mus musculus*. Data points include multiple measurements on each animal. Mice were placed in one of three body mass classes, as indicated. Also indicated for each class is the slope of the linear regression, as well as the significance level of the relationship.

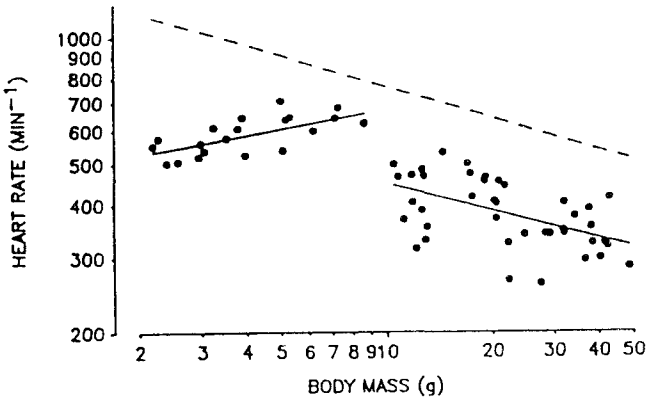

Fig. 3. Relationship between heart rate and body mass in 62 *Mus musculus*. Each point represents the mean heart rate for a single animal ($N = 10$). Note that both scales are logarithmic. Separate regression lines were calculated for preweaned and postweaned mice. The dashed line is the value predicted from interspecific studies on mammals (Stahl, 1967). Statistics pertaining to the linear regressions are given in table 1.

1–5 g and 5–10 g, respectively. Slight variations in Tb above and below 38 °C were recorded in postweaned mice. However, the range of body temperature was very small and there was no apparent effect of temperature on f_H ($P > 0.1$) (fig. 2)

Allometry of heart rate. As stated above, data for f_H were measured at a Tb of 38 °C (postweaned mice) or, if Tb varied from this in preweaned mice, were corrected for the temperature difference using the calculated Q_{10} for f_H appropriate for the body mass class. Resting heart rate in 62 *Mus musculus* between 2 g and 50 g varied from 260–706 min^{-1}. Plotting these data on a logarithmic scale reveals distinct differences between preweaned mice and postweaned mice (fig. 3). Unexpectedly, heart rate in preweaned mice showed a positive correlation with body mass (slope = + 0.15, table 1), rather than a negative correlation predicted from interspecific analysis of mammalian heart rate (*e. g.* Stahl, 1967). Heart rate increased from 527 beats · min^{-1} at a birth

TABLE 1

Exponents, constants and statistical significance describing the relationship between the measured cardiac variables and body masses presented in figures 3 and 4. The regression lines are expressed as $\log(Y) = \log(a) + b \cdot \log M$, where Y is the variable and M is body mass in grams. N = number of animals, P = significant level of regression, and r^2 = coefficient of determination.

Variable	M	N	$\log(a)$	b + SE	P	r^2
Heart rate	1–10	21	2.676	0.152 ± 0.039	<0.001	0.448
	10–50	41	2.869	− 0.217 ± 0.053	<0.001	0.300
Heart mass	1–43	48	− 2.311	0.969 ± 0.030	<0.0001	0.958
Ventricles mass	1–43	47	− 2.395	1.001 ± 0.032	<0.0001	0.957

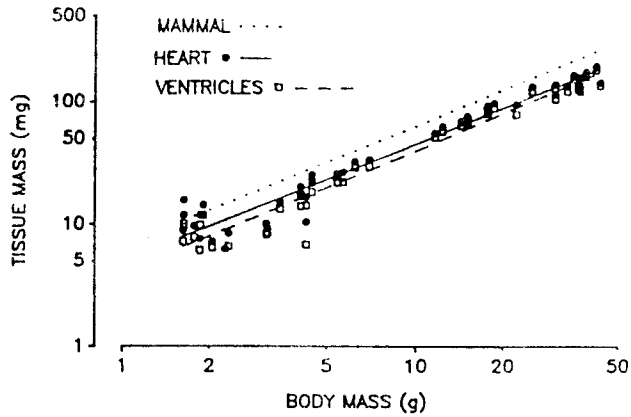

Fig. 4. Relationship between total heart mass (atria plus ventricles) and ventricular mass determined in 48 *Mus musculus*. The dotted line is the value predicted from interspecific studies on mammals (Prothero, 1979). Statistics pertaining to the linear regressions are given in table 1.

weight of approximately 2 g to 673 beats · min^{-1} at 10 g. Resting f_H in postweaned mice decreased with increasing body mass, with a regression line slope of −0.22 (table 1).

Heart mass and ventricular mass. Total heart mass (atria and ventricles) and ventricle mass increased with increasing body mass (fig. 4) as described by the following allometric equations:

$$\text{Heart mass} = 0.0049 \cdot M^{0.97} \quad (1)$$

$$\text{Ventricle mass} = 0.0040 \cdot M^{1.00} \quad (2)$$

where heart, ventricle, and body mass are in grams. Neither exponent differs significantly from 1.0 (table 1). Generally, the heart of mice accounts for 0.49% of the total body mass, with the two ventricles accounting for 0.40% of body mass.

Hematology. Significant differences in every measured hematological variable occurred with increase in body mass during development. Preliminary examination of these data revealed that, as for Tb and f_H, all measured and calculated variables showed large differences between preweaned mice and postweaned mice. Thus, the hematological data were separated into 'preweaned' and 'postweaned' groups for statistical analyses.

Hematological variables could be grouped into one of two categories on the basis of their changes with body mass, as evident in figs. 5 and 6. In the first category, represented by [Hb], $C_{O_2}^{max}$, and Hct (fig. 5), separate linear regressions on data from preweaned and postweaned mice showed these variables to be independent of body mass *within* the two body mass classes ($r^2 < 0.03$, $P > 0.1$). Yet, for each variable the mean values for preweaned mice were significantly lower than for postweaned mice ($P < 0.0001$).

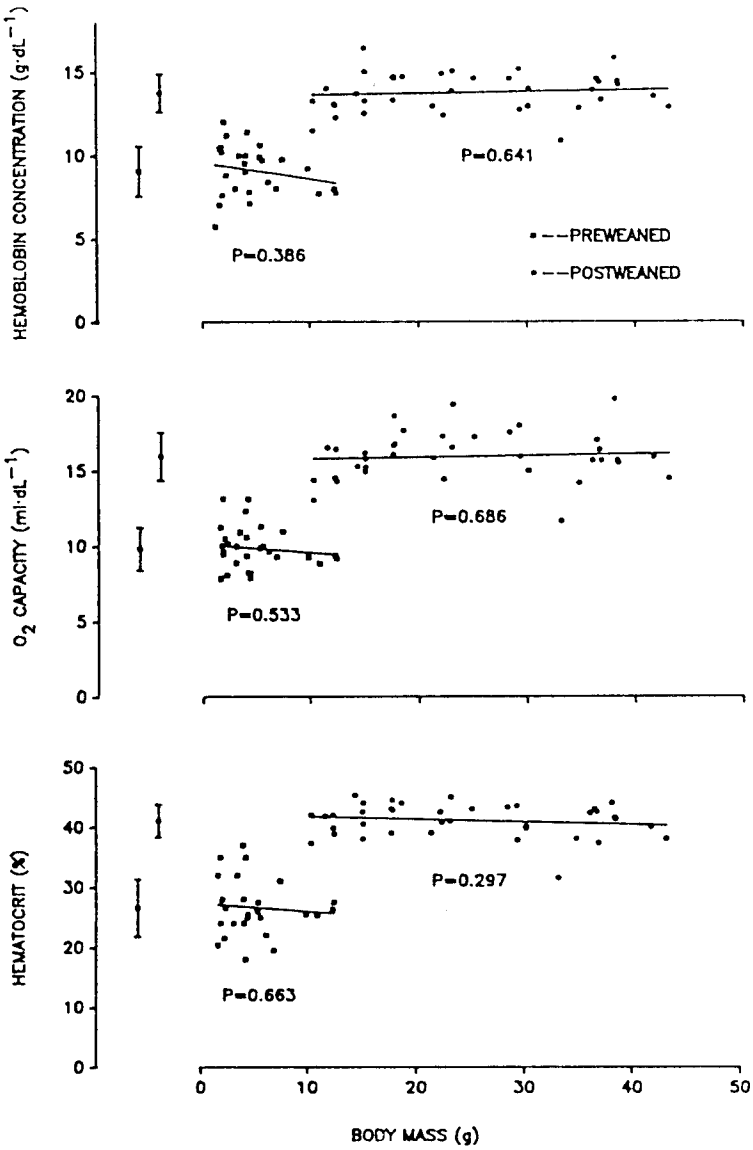

Fig. 5. Relationship between hemoglobin concentration, blood oxygen capacity, hematocrit and body mass in 68 *Mus musculus*. Data were divided into two groups, preweaned (approximately < 10 g) and postweaned (approximately > 10 g) as described in the text. Linear regressions were calculated for each class and each variable, but in every case the relationships were not significant – *i. e.* the variables were independent of body mass within body mass classes. Also indicated to the left of the diagram are the mean values ± 1 standard error for each of the two body mass classes. Mean values for preweaned mice are significantly different from those of postweaned mice at $P = 0.0001$ level. See text for further details.

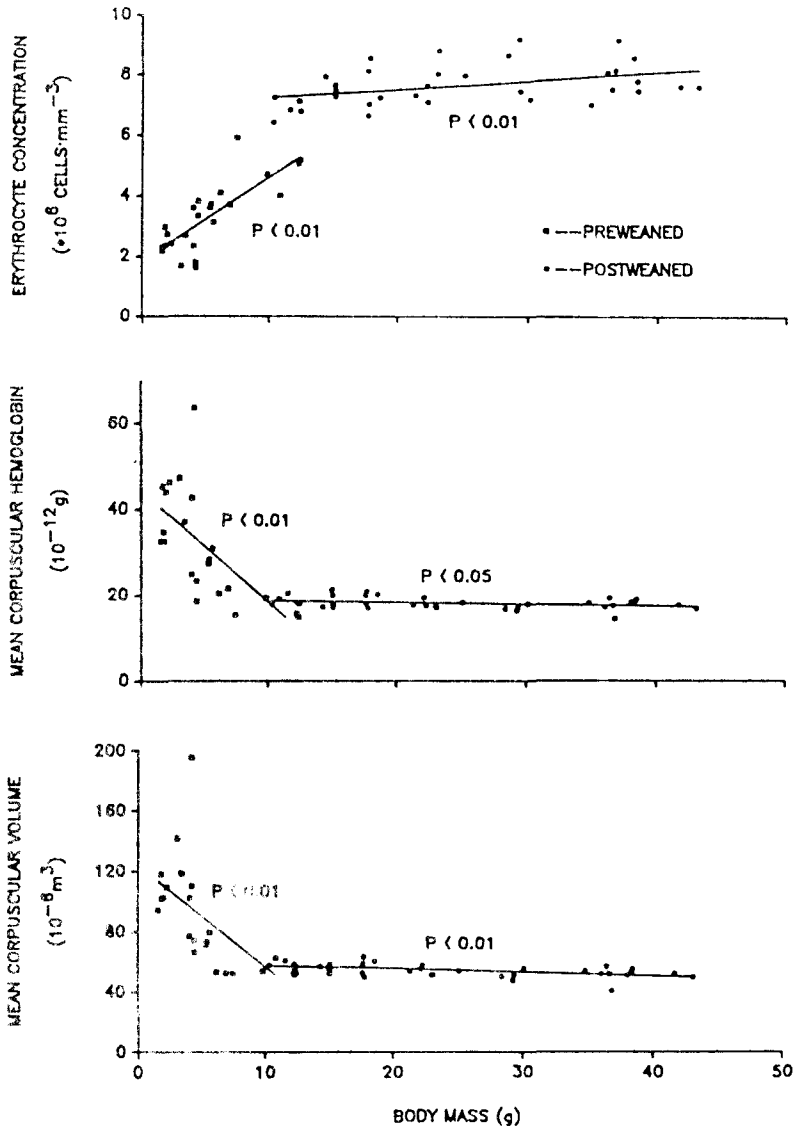

Fig. 6. Relationship between erythrocyte concentration, mean corpuscular hemoglobin, mean corpuscular volume and body mass in 61 *Mus musculus*. Data were divided into two preweaned and postweaned groups as described in the text. Linear regressions were calculated for each class and each variable. In every case, the values were significantly affected by body mass both within and between groups. See text for further details.

The second category of hematological variables consisted of [RBC], MCH, and MCV (fig. 6). Each of these variables changed significantly and sharply with body mass in preweaned mice, but thereafter changed only slightly with increasing body mass after weaning. [RBC] increased with body mass in preweaned mice, while MCH and MCV both decreased with body mass in preweaned mice.

TABLE 2

Exponents, constants and statistical significance describing the relationship between the measured and calculated hematological variables and body masses presented in fig. 6. The regression lines are expressed as $Y = a + b \cdot M$, where Y is the variable and M is body mass in grams. N, P, and r^2 are the same as in table 1.

Variable	M	N	a	b + SE	P	r^2
[RBC]	1–13	25	1.817E6	0.281E6 ± 0.45E6	<0.0001	0.632
	10–43	36	6.974E6	0.028E6 ± 0.01E6	<0.001	0.185
MCH	1–13	24	44.281	−0.258 ± 0.604	<0.001	0.453
	10–43	36	19.226	−0.045 ± 0.022	<0.05	0.113
MCV	1–13	23	124.037	−6.688 ± 1.867	<0.01	0.379
	10–43	36	59.817	−0.230 ± 0.060	<0.001	0.304

Finally, the ratio of $C_{O_2}^{max}$ and [Hb] was independent of body mass both within and between body mass groups, and had a mean value of 1.14 ± 0.11 ml·g^{-1} (N = 65).

Discussion

Hematology. The present study has revealed a significant neonatal anemia in *Mus musculus*, with [Hb], $C_{O_2}^{max}$, Hct, and [RBC] all being considerably depressed below adult levels. While [RBC], MCH, and MCV begin to change towards adult levels immediately after birth, [Hb], $C_{O_2}^{max}$ and Hct did not change significantly with body mass from birth until weaning. However, these three variables all showed a rather marked change coincident with weaning (data were not collected serially during each mouse's development, so we are unable to determine if the transition is actually this abrupt within individuals). An increase in [RBC] during early development in immature mice was also observed by Kienle and Strong (1959) and Grewal (1962), though in both of these studies (RBC) at birth was nearly twice that found in the present study.

Previous studies on the ontogeny of hematology in mice have shown that [Hb] and Hct fall after birth, reaching their lowest levels at about 10–14 days of age (approximate body mass = 7–9 g), then rise again to reach adult levels following weaning at 21 days (body mass = 9–11 g) (Grewal, 1962; Kingston *et al.*, 1978). In the present study the initial decrease in these variables immediately after birth was not apparent, but the subsequent development period leading to maturity showed a similar pattern of rise in [Hb] and Hct. The initial fall in postnatal MCH and MCV values in the present study has been reported earlier (Grewal, 1962). Mean corpuscular volume and MCH are quite constant in adult mice over their entire body mass range. The rapid decrease of the MCV in the neonatal mice is partly due to the disappearance of the hepatic generation of fetal erythrocytes, and partly to a decrease in size of newly produced erythrocytes (Bannerman, 1983).

Differences in hematological variables such as [RBC] between previous studies and the present one may be attributable to genetic differences in mouse strains used (Russell *et al.*, 1951). Alternatively, the newborn mouse receives its iron supply by placental transfer, and thus erythropoiesis in the newborn may be strongly influenced by maternal iron status (Kingston *et al.*, 1978), which was an uncontrolled variable in both previous and present experiments. As well, mean [Hb] is higher in neonates of first litter than in later litters in normal mice (Kingston *et al.*, 1978), which also was not controlled for.

In spite of differences in [RBC] at birth and in patterns of early change in some hematological parameters between studies, the picture that emerges from combination of both previous studies and the present one is that (1) at some time early in development immature mice show an anemia when compared with adults, (2) the changes in hematology leading up to weaning are in a direction counter to that predicted on the basis of simple body mass change, and (3) once weaned, there appears to be no influence of either changes in body mass or development. Interspecific hematological studies of adult mammals similarly indicate an almost total independence of body mass (Adolph, 1949; Prothero, 1980; Schmidt-Nielsen, 1984)

Heart mass. Heart mass is directly proportional to adult body size in a wide range of mammalian species (Prothero, 1979, discussed in Schmidt-Nielsen, 1984). The present study shows that this relationship also holds intraspecifically, at least for *Mus musculus*. Heart mass scales to body mass with the exponent 1.00 from 1 g newborns to very large 50 g adults.

The observed heart mass (0.49% total body mass) in mice was very consistent, but was slightly lower than predicted heart mass (0.58%) from the equation based on interspecific comparison in a wide range of adult mammals (Prothero, 1979). Differences between studies in the extent to which the veins and arteries were trimmed from the atria and ventricles may account for some of this small difference in ratio of heart to body mass.

Heart rate. 1. Adult rates: comparison with previous studies. The values for resting f_H in postweaned mice recorded in the present study are lower than the previously published values. Resting f_Hs calculated from the regression line for postweaned mice are 445 beats · min^{-1} at 10 g, falling to 313 beats · min^{-1} at 50 g. In contrast, resting f_H predicted from interspecific allometry of small adult mammals (Stahl, 1967) are 762 beats · min^{-1} down to 510 beats · min^{-1} for the same body size range. Richards *et al.* (1953) also recorded a mean heart rate of 632 min^{-1} in adult mice, although body mass was not reported. Furthermore, mean heart rates for adults of seven different mouse strains with a mean body mass from 16.9 and 23.5 g varied from 533 beats · min^{-1} to 699 beats · min^{-1} (Blizard and Welty, 1971). Despite these discrepancies, it is important to emphasize that in postweaned mice the rate of decline of heart rate with increasing body mass is similar to that reported for mature mice in previous studies.

Several factors could account for the lower heart rates of postweaned mice reported

in this study. First, previous studies on resting f_H in adult mice have been performed at temperatures of 20–25 °C, which are considerably below the reported thermoneutral zone of 31 to 33 °C for *Mus musculus* (Pearson, 1947), which was the temperature used for postweaned mice in this study. Oxygen consumption in homeotherms increases when ambient temperature is outside the thermoneutral zone. Thus, the higher mean heart rate for *Mus musculus* in previous studies may reflect a higher metabolic rate due to ambient temperatures outside the thermoneutral zone. Second, the present study measured heart rate from unrestrained, undisturbed and even sleeping animals, thus providing a better approximation of resting, undisturbed rates, in contrast to the use of restrained animals (*e. g.* Richards *et al.*, 1953). Third, heart rate is affected by genetic factors. Blizard and Welty (1971) measured heart rate in seven different strains of mice, and found that differences between strains of mice of similar age accounted for approximately 42% of the total heart rate variance.

2. *Effect of temperature.* The Q_{10} for heart rate in poikilothermic, preweaned mice over a body temperature range of 30–42 °C ranged from 1.5–1.7. This is lower than the Q_{10} of 2 to 3 observed for most biological processes. However, Richards *et al.* (1953) reported that heart rate was 240 min^{-1} and 615 min^{-1} in newborn mice at body temperatures of 23 °C and 37 °C, respectively. Calculation from the above data results in a Q_{10} for heart rate of 1.96, which is slightly higher than those found in this study for mice of 1–5 g (1.56) and 5–10 g (1.69), but still lower than many other physiological processes. Q_{10}s for f_H of 1.5–2 have also been reported for anuran amphibians (W. Burggren, R. Infantino and D. Townsend, unpublished data) and a similar range (1.4–1.9) is found in avian embryo at a body temperature range of 35–40 °C (see review in Bennett and Dawson, 1979).

Presumably, the isolation of preweaned mice from their brooding mothers under the experimental condition would have little impact on the measured f_Hs. Brooding decreases oxygen consumption at 25 °C in developing mice (Bryant and Hails, 1975). However, this effect was attributed to preventing heat loss and therefore disappeared as newborn mice attained pelage. In this study, the isolated neonates were maintained at 35–38.5 °C to eliminate artifacts due to heat loss. f_H in this study was sampled within 4 h of measurement (see Materials and methods for details). For most preweaned mice, f_H was collected within 3 hours and this reduced any effects of fasting.

3. *Effect of body mass.* In postweaned mice resting f_H scaled to body mass with exponent −0.22. This value is not significantly different from −0.25 ($P = 0.995$), which is the value of the exponent in the allometry equation relating resting heart rate and body mass in interspecific studies of f_H in mammals (Stahl, 1967). Thus, it would appear that in postweaned mice, the changes in resting heart rate can be related strictly to changes in body mass.

Resting f_H in newborn mice is only about 1/2 of that predicted from interspecific allometric studies on adult mammals. However, this should not be seen as a severe deficiency in neonatal cardiac function because resting f_H in postweaned mice is also considerably lower than that predicted from interspecific allometry. In fact, extending the regression line for postweaned mice back to the preweaned range (fig. 3) would

indicate that fH in newborn mice is actually close to the line extrapolated back from adults, while it is fH in older preweanlings that deviates further from the extended line as body mass increases. Therefore, fH both at birth and as adults seems consistent with a simple scaling function, while older preweanlings appear exceptional. Body temperature was carefully controlled for in both newborn and older preweaned mice, and was not a contributing factor to these differences in resting fH. The distinctive pattern of fH change in preweaned mice indicates a large influence of developmental changes that override the effects predicted by a 'simple' change of body mass. Interestingly, in the rat, the increase in fH during fetal development continues after birth until 40 days, thereafter dropping with further development (Adolph, 1967). While these data were not examined in the context of body mass changes, together with the present experiments they do suggest that this complex pattern of heart rate change in neonates may occur in other rodent species, at least.

Relationship between heart rate, hematology and development. This study has documented two distinct phases of change related to growth and development in *Mus musculus*. In postweaned mice of all sizes, changes in heart rate, heart mass, and the six measured hematological variables scale with body mass as predicted by interspecific allometric analysis of adult mammals. Thus, after weaning, the measured physiological and morphological features change strictly in response to the constraints and demands of changing body mass. Prior to weaning, however, measured changes in cardiac and hematological variables show sharp discrepancies with values predicted from interspecific allometric equations. We propose that *developmental factors* other than changes in body mass are primarily responsible for the observed patterns of ontogenetic change.

What is the mechanism behind the increase in fH in preweaned mice, and what is its physiological purpose? The former question is largely beyond the scope of this study, but evidence for newborn rats suggest the increase of resting fH in the neonatal mice may be due to an increase in sympathetic stimulation of the heart. Wekstein (1965) studied fH in the preweaning rat and found that in the first 3 days after birth resting fH was unresponsive to adrenergic blockers. From 10 days of age on, however, the progressive increase of fH with increasing age was completely prevented by adrenergic blockade.

The physiological purpose behind the increase in resting fH in preweaned mice has not been identified explicitly, but our hematological measurements showing a neonatal anemia are highly relevant. The oxygen capacity of the blood of preweaned mice is only 2/3 of that evident after weaning. If oxygen consumption of preweaned mice is to be maintained at similar levels to that evident in smaller postweaned mice (there are no data to indicate otherwise), then either cardiac output or tissue oxygen extraction must increase considerably for adequate delivery of oxygen to the tissues. Stroke volume is generally assumed to be closely related to ventricle mass (Holt *et al.*, 1968). The constancy of ventricular mass, and by implication constancy of stroke volume, would indicate that disproportionate increases in stroke volume are not occurring to increase cardiac output in newborn mice. Thus, the increase in fH during growth prior to weaning

may be to produce an increase in cardiac output to compensate for the low oxygen capacity of blood during early period of growth. Once erythropoietic events generate the blood oxygen capacity typical of postweaned mice, f_H then begins to decrease according to increase in body mass.

Summary: comparison of intraspecific vs interspecific allometry. The findings of the present study on *Mus musculus* indicate that, at least for heart rate and many aspects of hematology, interspecific allometric data are generally poor predictors of intraspecific relationships. Two distinct phases were seen in the intraspecific analysis of change in both heart rate and hematology. The first phase, occurring from birth up to weaning at approximately 10 g body mass, is characterized by patterns of change which are the antitheses of those predicted by interspecific allometric studies. The second phase, occurring after weaning, is qualitatively different from the first phase in that all findings can be predicted from allometric relationships determined from as interspecific studies. Thus, the first phase is characterized by changes that include developmental aspects in addition to body mass changes, while the second phase is correlated strictly with further increases in body mass. Together with previous studies of basal metabolism (Heusner, 1982; Wieser, 1984), it would appear that interspecific allometric studies must be properly regarded as the study of *adult* animals of different body sizes, and that untested assumptions about the applicability of these data to intraspecific studies of immature specimens should be made with extreme caution.

Acknowledgements. This study was supported by U.S. NSF operating grant DCB8608658 to WWB.

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