

Respiratory function in a newborn marsupial with skin gas exchange

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Abstract

The Julia Creek dunnart (*Sminthopsis douglasi*) is a marsupial born after ≈ 12 days of gestation. At birth, the newborn is ≈ 4 mm long and weighs ≈ 15 mg. Gaseous metabolism (oxygen consumption rate, \dot{V}_{O_2} , rate of carbon dioxide production, \dot{V}_{CO_2}) was measured separately across the airways (lungs) and the rest of the body (skin). At pouch temperature (36°C) total \dot{V}_{O_2} (i.e. skin + lungs) averaged 15 ± 2 S.E.M. $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. At birth the skin contributed almost the total gaseous metabolism, and at 3 weeks $\approx 1/3$ of the total. The compliance of the respiratory system, per unit of body weight, was similar to that of other newborn mammals. During the first postnatal days breathing was an occasional event determined by gross body movements. Artificial expansion of the lungs temporarily stopped breathing, presumably a manifestation of the Hering–Breuer reflex. By the 2nd–3rd week breathing was regular, pulmonary ventilation (\dot{V}_E) averaged $263 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, tidal volume (V_T) $3.4 \text{ ml}\cdot\text{kg}^{-1}$, breathing frequency (f) $87 \text{ breaths}\cdot\text{min}^{-1}$. Lowering ambient temperature in steps from 36 to 20°C reduced both lung and skin gaseous metabolism. \dot{V}_E and f , at first, were little affected but eventually they dropped in approximate proportion to metabolism, whereas V_T remained unchanged. In conclusion, for the newborn dunnart gas exchange through the skin is a requirement because of the inefficient \dot{V}_E . To what extent the \dot{V}_E adjustments to changes in metabolic rate reflect mechanisms of regulation remains unresolved. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The dunnart at birth it is one of the smallest newborn mammals known (Tyndale-Biscoe and

Renfree, 1987). After a gestation of ≈ 12 days, the newborn is ≈ 4 mm long and weighs ≈ 15 mg (Selwood and Woolley, 1991; Frigo and Woolley, 1997). The skeleton is entirely cartilaginous (Frigo and Woolley, 1996) and the internal organs are visible through the transparent skin. The lungs are represented by a small number of air sacs of spherical shape on either side of the heart. In one

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species, the Julia Creek dunnart (*Sminthopsis douglasi*, Fig. 1), we measured the exchange of oxygen and carbon dioxide and found that, during the first postnatal days, it occurred almost entirely through the skin, the only example so far demonstrated in a mammal of any age (Mortola et al., 1999). In the present study we aimed to quantify some aspects of the ventilatory function of this unusual newborn with respect to its mechanical characteristics and its response to a decrease in metabolic demands. Specifically we aimed to measure (1) the compliance of the respiratory system, to estimate whether skin-breathing was an unavoidable requirement of an animal born with immature lungs, and (2) the ventilatory changes at different temperatures, to assess to what extent pulmonary ventilation was tracking metabolic rate in an animal that in the early neonatal period largely bypasses ventilation for gas exchange.

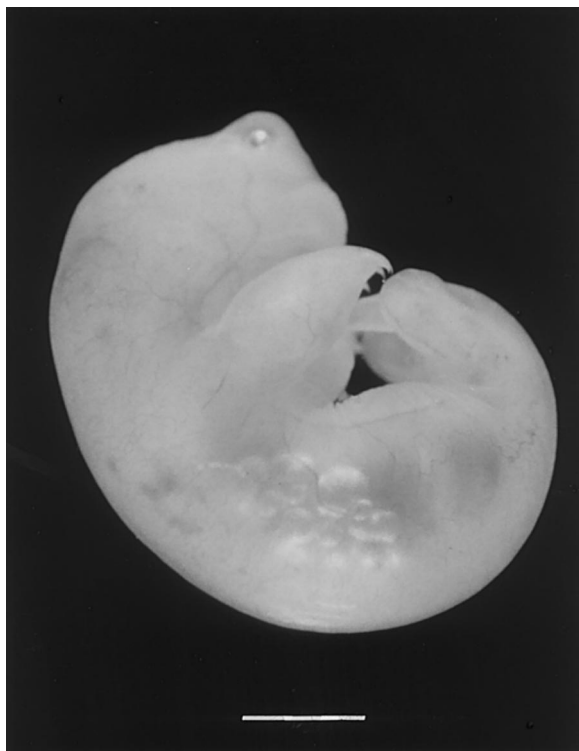


Fig. 1. The Julia Creek dunnart (*S. douglasi*) at the day of birth (day 0). It is possible to see the spherical air sacs of the lungs through the transparent skin. Scale bar, 1 mm.

Data of the relative contribution of skin and lung to total gas exchange at 36°C (Fig. 7, top panel) were presented in an earlier communication (Mortola et al., 1999).

2. Methods

Experiments were conducted on the pouch-young of the Julia Creek dunnart (*S. douglasi*), following approval of the Ethics Committee of La Trobe University. After ≈ 20 years from the last report of a specimen, the Julia Creek dunnart, a small dasyurid marsupial, was found alive in the north-west area of Queensland, Australia (Woolley, 1992) and a colony established in the laboratory¹.

Close to term (Woolley, 1990) the pouch of the pregnant female was inspected frequently to ascertain the day of birth (day 0). On the experimental day, the pouch was opened and, with the help of a magnifying lens, one of the pups (litter size 6–8 young) was gently removed from a teat. This was done for a total of 22 pouch-young from five litters, at various ages between postnatal day 0 and 21.

A minute mask made from a short length of polyethylene tube was sealed to the face of the joey, covering both mouth and nostrils, using drops of a removable dental polyether material. The low weight and minimal movement of the neonates ensured that the seal was maintained. The tube passed through a thin rubber stopper which was placed in the centre of a moist cylindrical chamber of 0.5–2 ml in size. The stopper, therefore, completely separated the chamber into two compartments; one which housed the body of the animal (to measure skin gas exchange) and another which communicated with the airways (to measure lung gas exchange, Fig. 2). Injection of a small quantity of air into one compartment and the absence of pressure transmission to the other indicated complete separation of the compartments. Each compartment had ingoing and out-

¹ A successful breeding colony has been established for a number of years by Dr P. A. Woolley, Department of Zoology, La Trobe University, Melbourne, Vic. 3083, Australia.

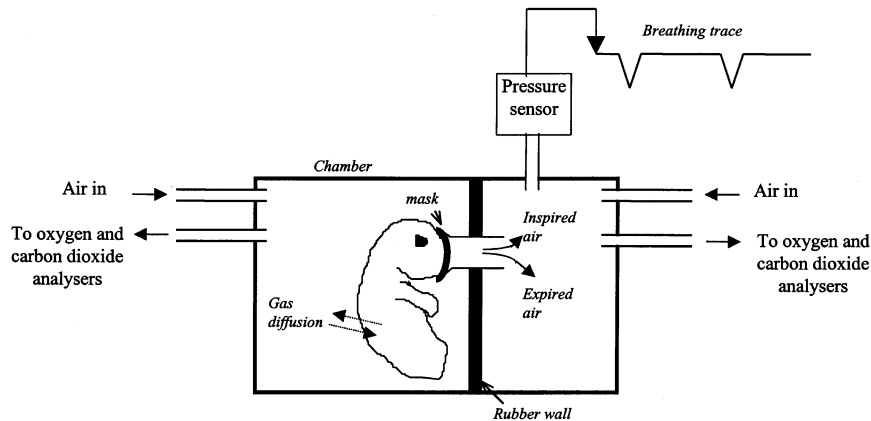


Fig. 2. Schematic of the set-up used to measure the exchange of respiratory gases and ventilation in the newborn joey. A small mask is attached to the face of the joey and the joey positioned in the chamber in such a way that the opening of the facemask protrudes through a rubber stopper that acts as a wall, dividing the chamber into two compartments. Air can flow through both compartments. The compartment that communicates with the facemask measures pulmonary gas exchange, while the compartment that encloses the body of the joey measures skin gas exchange. A pressure transducer was connected to the compartment that communicated with the facemask to record pressure fluctuations associated with breathing.

going tubes for circulation of air by roller pumps, and the whole chamber was maintained at pouch temperature (36°C) by a water bath (Fig. 2).

2.1. Gaseous metabolism

After temperature and humidity equilibration the individual compartments were sealed for 5–15 min, depending upon the animal's age. At the end, the compartments were individually flushed with a constant airflow of $20 \text{ ml} \cdot \text{min}^{-1}$, and the gas forced through a drying column before being analysed for O_2 and CO_2 concentration by appropriate gas analyzers. The output of these analyzers was recorded on paper and their digital signal acquired by computer for later analysis. The rates of oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) were calculated from the time integral of the gas concentration curves (Frappell et al., 1989), multiplied by the flow, and the reciprocal of the time during which the compartment was maintained sealed. In most animals measurements were performed at ambient temperatures (T_a) of 36 and 28°C. In four animals measurements were also collected at T_a of 32, 24, and 20°C. In all cases the change in T_a was obtained by adjusting the temperature of the water bath.

Temperature stability was usually achieved within 10–15 min.

2.2. Ventilation

The compartment which communicated with the animal's airways was connected to a sensitive pressure transducer (Model PT5, Grass Inst., 6 cmH_2O). The oscillations in pressure, calibrated for volume by injecting 2 μl of gas, were recorded on paper and acquired on computer for a period of 1–3 min. Pulmonary ventilation (\dot{V}_E) was calculated as the product of the average tidal volume (V_T) and breathing frequency (f). Measurements of \dot{V}_E were performed immediately after those of gaseous metabolism.

2.3. Pressure–volume curve

The compartment that communicated with the airways was opened and a glass capillary tube containing a soap bubble was connected to the open end of the polyethylene tube that formed the mask attached to the face of the animal. The movement of the soap bubble due to breathing was magnified by a microscope and was recorded on tape by a video camera (Hyper HAD digital,

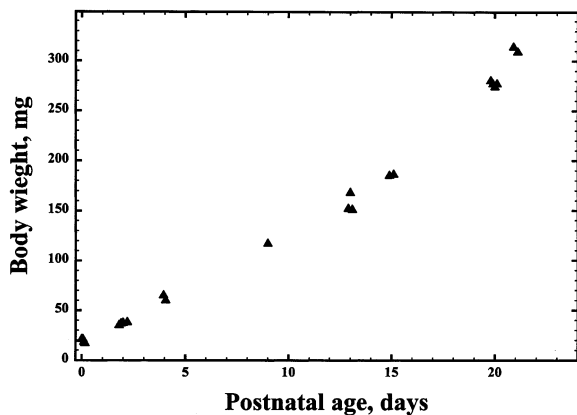


Fig. 3. Body weight (mg) of the pouch-young dunnarts, as a function of postnatal age (days). At birth, the newborn weighs < 20 mg.

Sony) connected to a video monitor (Sony) via a video cassette recorder (SVHS, Panasonic). Negative pressures were generated in the compartment that housed the body by applying a gentle vacuum through a connecting tube. These negative body surface pressures, measured by a water manometer, corresponded to positive trans-respiratory system pressures and inflated the lungs by a volume which was calculated from the in-

spiratory movement of the soap bubble and the dimensions of the glass capillary tube. Pressures were applied from 0 to 20 cmH₂O, in steps of 5 cmH₂O during inflation and deflation. Before the next pressure step was applied, we waited for termination of volume 'creeping', a reflection of the visco-elastic properties of the respiratory system, as indicated by a stable position of the soap bubble. In each animal the pressure–volume (P–V) curve was repeated twice and the results averaged.

2.4. Data presentation

Data are presented as group means \pm 1 SEM. The age and number of animals used for each measurement is presented in the pertinent Section 3 and in the figure legends. Values of gaseous metabolism and \dot{V}_E are expressed normalised by the weight of the animal in kilograms, the former at standard temperature, pressure and humidity (stpd; 1 ml O₂ stpd = 0.0446 mmol O₂), the latter at body temperature, pressure and humidity (BTPS).

3. Results

3.1. Body growth

The body weights of the 22 pouch-young studied are represented in Fig. 3. On the day of birth the weight was 17–20 mg, and it increased \approx 15-fold by the end of the 3rd week. The air sacs were visible at all ages through the transparent skin. In some animals at 2 weeks of age the diameter of the air sacs was measured from calibrated video images, and averaged 395 μ m. The trachea, measured in same-age specimen post-mortem, had a diameter of 250 μ m, and a length of 1420 μ m.

3.2. Respiratory system compliance

The average P–V curves of the respiratory system of various age groups are represented in Fig. 4, with the volume normalised by the weight of the animal in kilogram. At day 2–4 the relation-

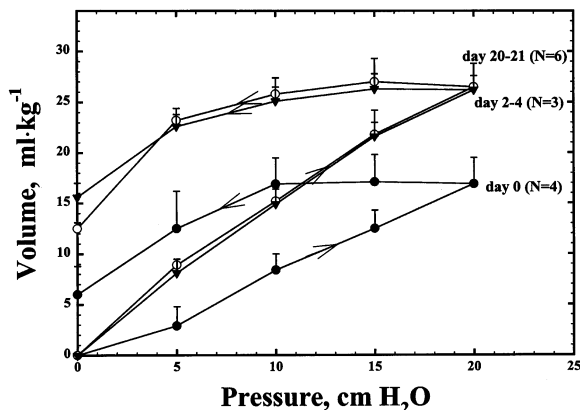


Fig. 4. P–V curves of the respiratory system at the day of birth (day 0), after a few days (day 2–4) and at 3 weeks (day 20–21). V represents the increase in lung volume above the resting volume at P = 0, and is normalised by the weight of the animal in kilogram. Symbols are mean values, bars represent 1 S.E.M. Arrows indicate the direction of inflation and deflation. N, number of animals

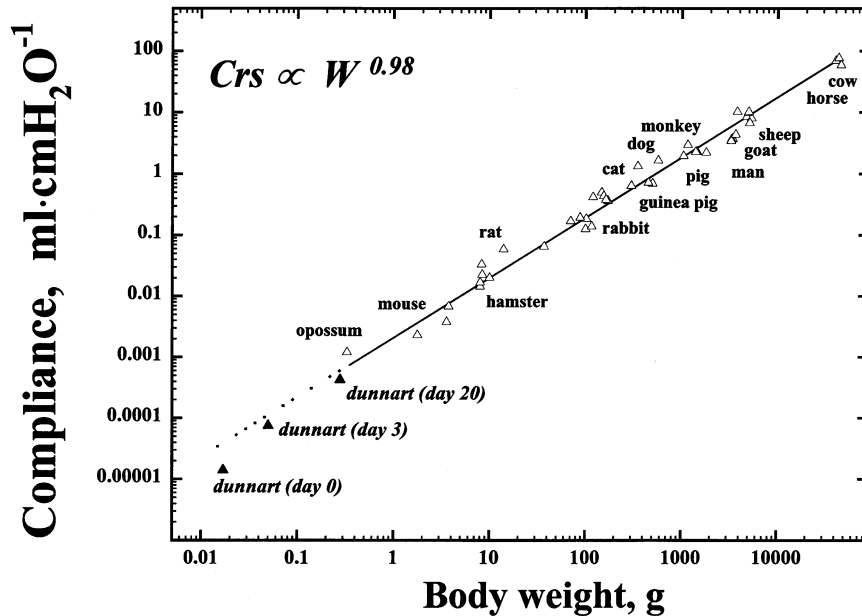


Fig. 5. Allometric relationship of the compliance of the respiratory system (C_{rs}) in newborn mammals. Open symbols are literature data, and C_{rs} was found to be proportional to the first power of body mass, $C_{rs} \propto W^{0.98}$ (from Mortola, 1987, and additional data cited in text). Filled symbols refer to the dunnarts at the ages indicated.

ship was essentially similar to that at 3 weeks of age, and the volume per kilogram was about 70% greater than at the day of birth. At all ages the P–V curve presented a substantial hysteresis. The compliance of the respiratory system (C_{rs}), measured on the inflation limb over 10 cmH₂O inflation, averaged 0.8 ± 0.2 ml·kg^{−1}·cmH₂O^{−1} at day 0, and 1.5 ± 0.1 and 1.5 ± 0.2 ml·kg^{−1}·cmH₂O^{−1} at day 2–4 and 3 weeks, respectively. These values are slightly lower (day 0) or similar (older ages) to those expected from the allometric relationship of newborn mammals (Fig. 5).

3.3. Breathing pattern response to lung inflation

As lung volume was increased by the application of negative body surface pressure breathing activity, measured from the movement of the soap bubble in the capillary tube, ceased for variable periods of time. This interruption was more marked the larger the inflation (i.e. with more negative body surface pressures). We interpreted this response as a manifestation of the

Hering–Breuer inflation reflex, although no attempts were made to verify its vagal origin.

3.4. Gaseous metabolism

At 36°C, the total (lungs + skin) \dot{V}_{O_2} of 15 pouch-young between 20 and 320 mg (day 0–21) averaged 15.1 ± 1.7 ml·kg^{−1}·min^{−1}. The values of \dot{V}_{CO_2} were very similar and the respiratory exchange ratio (RER) averaged 1.16 ± 0.09 . Neither total \dot{V}_{O_2} ·kg^{−1} nor total \dot{V}_{CO_2} ·kg^{−1} changed significantly with age. Indeed, \dot{V}_{O_2} had an approximately linear relationship with body weight (W), $\propto W^{1.16}$ at 36°C and $\propto W^{1.02}$ at 28°C (Fig. 6). The skin contributed almost the total gas exchange in the smallest animals, and $\approx 1/3$ of the total in the 3-week old (Fig. 7, upper panel). The RER for the skin displayed no apparent age relationship, averaging 1.48 ± 0.18 .

At 28°C total \dot{V}_{O_2} averaged 6.9 ± 0.6 ml·kg^{−1}·min^{−1} and, as at 36°C, it was independent of body mass (Fig. 6). The relative contributions of skin and lungs to total gas exchange were similar to that at 36°C (Fig. 7, lower panel).

3.5. Pulmonary ventilation

In the youngest animals pulmonary ventilation was a casual event. The animals were either immobile or moved continuously, as the result of generalised or local skeletal muscle twitches. Occasionally, this resulted in some air moving into the lungs. First observations at day 0 revealed that the air sacs were clearly expanded with air in all animals. In the youngest animals, however, it was not uncommon for the air sacs to gradually deflate over a period of many minutes, only to re-expand following some vigorous generalised motor activity. The youngest pouch-young in which we could clearly recognise respiratory-like movements was a 4-day old; at $T_a = 36^\circ\text{C}$, its breathing acts were at a rate of 46 min^{-1} , with an average V_T of $0.14 \mu\text{l}$, or $\approx 2 \text{ ml}\cdot\text{kg}^{-1}$. In older animals the breathing pattern was regular and at 3 weeks $V_T \text{ kg}^{-1}$ was $\approx 3\text{--}4 \text{ ml}\cdot\text{kg}^{-1}$, with f reaching values of $106 \pm 8 \text{ breaths}\cdot\text{min}^{-1}$.

Average values of V_T , f and \dot{V}_E of a group of ten animals of the 2nd–3rd week of life are presented in Fig. 8, as function of the corresponding values of total (skin + lungs) \dot{V}_{O_2} and \dot{V}_{CO_2} , at $T_a = 36$ and 28°C . On the assumption that the total dead space (V_D) was 3 times the tracheal volume, alveolar ventilation (\dot{V}_A) was also calculated, as $\dot{V}_A = (V_T - V_D) \times f$. With the drop in T_a

from 36 to 28°C and the consequent decrease in metabolism both \dot{V}_E and \dot{V}_A decreased. This was due to a decrease in f , whereas V_T remained unaltered. Neither \dot{V}_E/\dot{V}_{O_2} nor \dot{V}_E/\dot{V}_{CO_2} differed significantly between the two temperatures.

3.6. Gradual decrease in temperature

The relationship between gaseous metabolism and \dot{V}_E at T_a of 36 , 32 , 28 , 24 and 20°C was studied in four 2-week old animals (Fig. 9). The overall pattern of the changes in \dot{V}_E indicated an approximate proportionality between \dot{V}_E and \dot{V}_{O_2} or \dot{V}_{CO_2} (dashed lines). However, \dot{V}_E decreased little between 36 and 32°C , and much more as T_a was lowered further. The decrease in \dot{V}_E was almost exclusively due to the decrease in f .

4. Discussion

At 36°C the total $\dot{V}_{O_2}\cdot\text{kg}^{-1}$ of the dunnart pouch-young averaged $\approx 15 \text{ ml} \times \text{kg}^{-1} \times \text{min}^{-1}$. This value is lower than in small eutherian newborns but similar to that of other newborn marsupials (Farber et al., 1972; Baudinette et al., 1988). During postnatal development \dot{V}_{O_2} increased approximately in proportion to body mass. This pattern is also quite different from that of eutherian newborns, but it has been observed in another marsupial, the tammar wallaby (Baudinette et al., 1988). Presumably, the low \dot{V}_{O_2} and its constancy throughout postnatal development may be related to development in the warm environment of the pouch, which frees the newborn marsupial from the energetic cost of thermogenesis. Hence, the O_2 requirements of the newborn dunnart and their postnatal changes are not particularly different from those of other larger marsupials. The important difference is that a major portion of gas exchange is through the skin.

In adult mammals cutaneous gas exchange cannot be an important contributor to the total gaseous metabolism because of the high mass-specific metabolic rate and the poor gas diffusion properties of the skin tissues. Even in the shrew, one of the smallest mammal's with large body

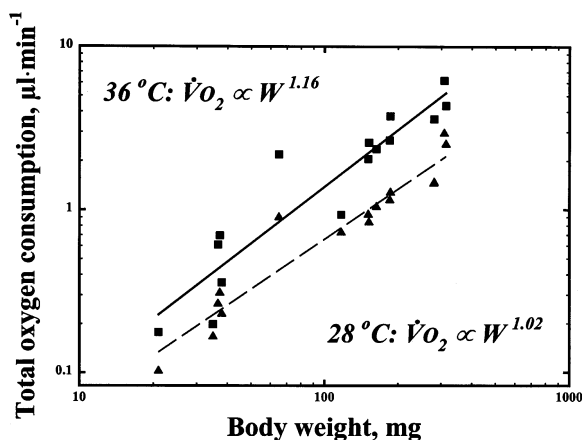


Fig. 6. Oxygen consumption (\dot{V}_{O_2} , $\mu\text{l} \times \text{min}^{-1}$) as function of body weight (W , mg) during the first 3 weeks of postnatal life in dunnarts at the ambient temperatures of 36 (squares) and 28°C (triangles).

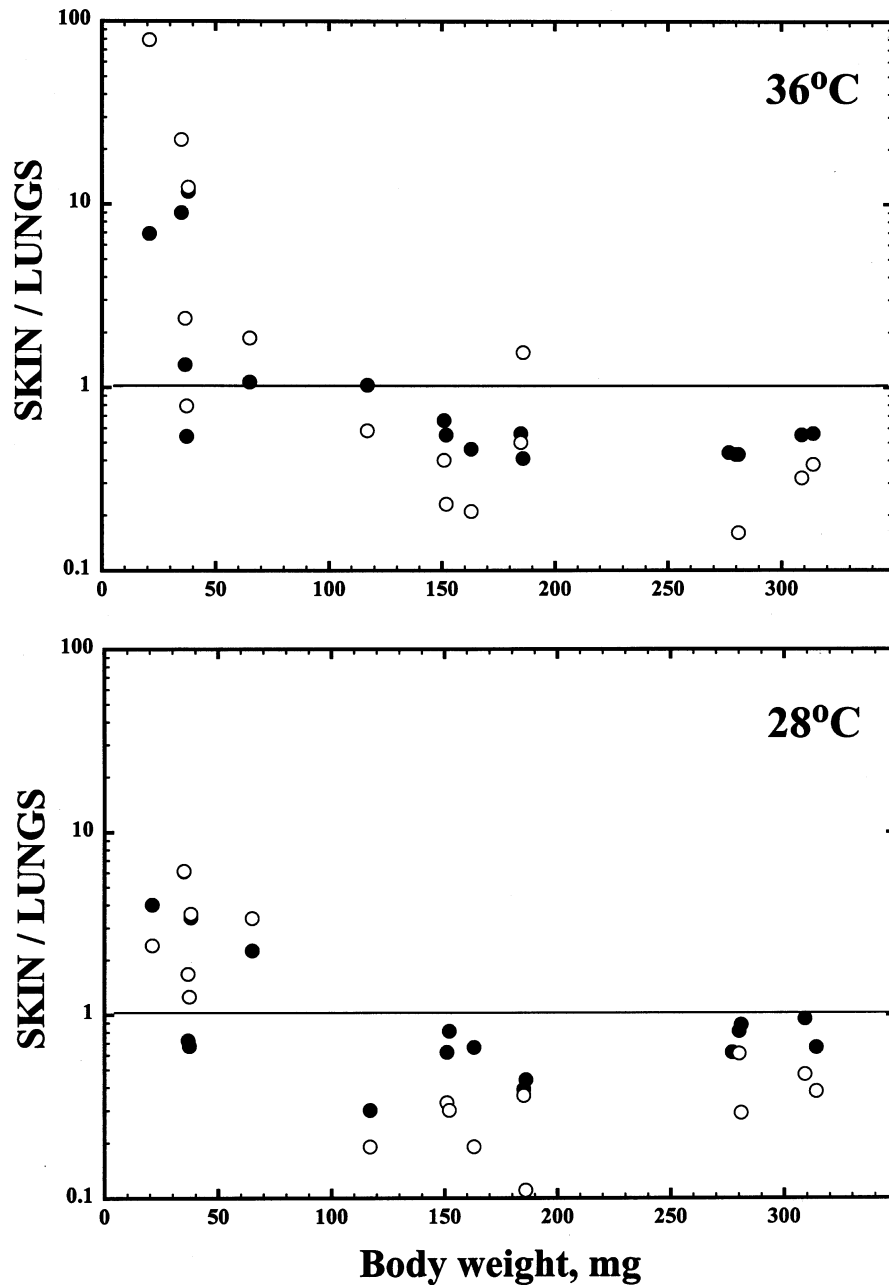


Fig. 7. Ratio between pulmonary and skin gas exchange in animals of different body weight, at pouch temperature (36°C, top panel), and at 28°C (bottom panel). The Y-axis is a logarithmic scale. The horizontal line (at unity) indicates an equal contribution of skin and lungs to total gas exchange. Open symbols, oxygen consumption; filled symbols, carbon dioxide production.

surface-to-mass ratio, the skin was found to contribute not more than 3% of total gaseous metabolism (Mover-Lev et al., 1998). The possi-

bility of the skin contributing a sizeable fraction of gas exchange could be more realistic in small newborn marsupials, because they are born very

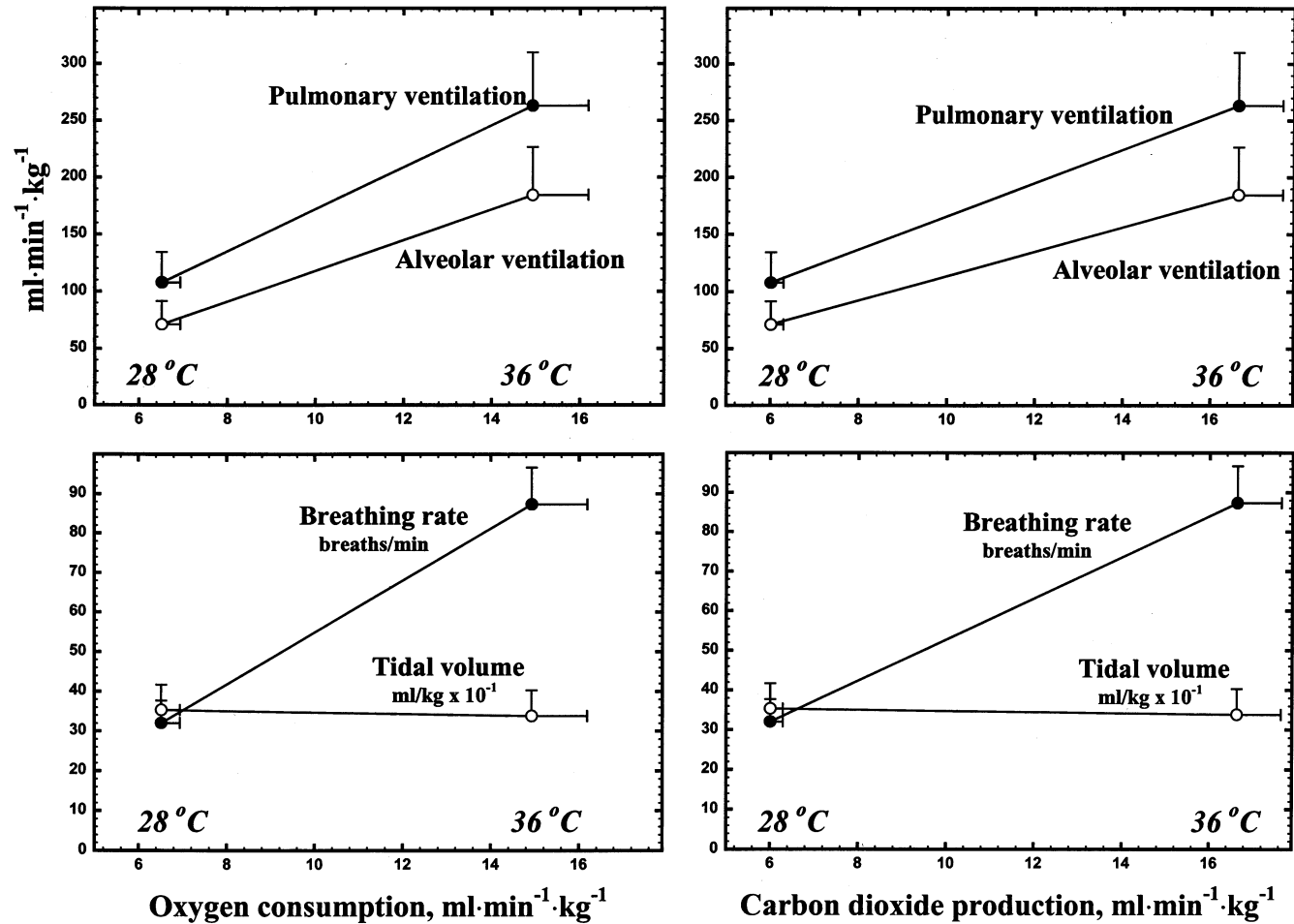


Fig. 8. Average values of pulmonary and alveolar ventilation, tidal volume and breathing rate in pouch-young dunnarts of 2–3 weeks of age at 36 and 28°C, represented as functions of the corresponding values of total oxygen consumption (panels at left) and carbon dioxide production (panels at right). Alveolar ventilation was calculated assuming a dead space equivalent to three times the tracheal volume. Symbols are mean values of a group of ten animals, bars 1 S.E.M.

small following a short gestation and have negligible thermogenic needs. However, in the 1-g tammar wallaby joey Baudinette et al. (1988) have shown that cutaneous gas exchange contributed only $\approx 4\%$ of the total \dot{V}_{O_2} . Given the metabolic requirements of a newborn mammal of this size (1 g), even if the cutaneous tissue layers were capable of high gas diffusion rates, the surface-to-mass ratio would be too low for the skin to provide an important contribution to the \dot{V}_{O_2} (Mortola et al., 1999). The newborn dunnart at birth is almost two orders of magnitude smaller and, to our knowledge, this is the only mammal, whether newborn or adult, in which cutaneous gas exchange has been demonstrated to be very important (Mortola et al., 1999).

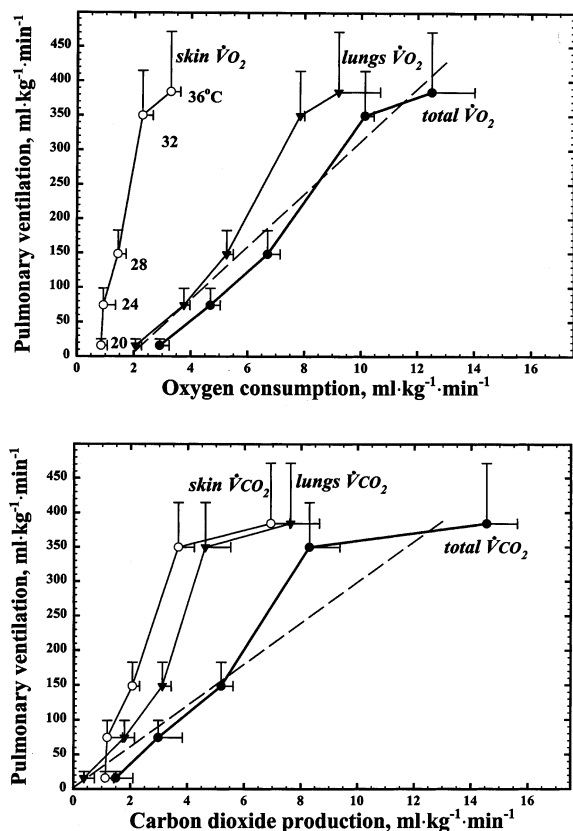


Fig. 9. Pulmonary ventilation as a function of gaseous metabolism through the skin, lungs, and total (skin + lungs) at various ambient temperatures between 36 and 20°C. Values are means of four animals at 2–3 weeks of age, bars represent 1 S.E.M. Dashed line represents the linear fit through all the data points of total gas exchange.

After normalization by body weight, the value of C_{rs} of the 3-day old is comparable to that of the newborn opossum (Frappell and Mortola, 1989) and close to the value predicted from the allometric relationship. Only at day 0, C_{rs} kg⁻¹ was low, but even at this age the difference from other species was not very large. Hence, it seems probable that at birth the dunnart lung, as is the case for other newborn marsupials (Krause et al., 1976), has a functional complement of surfactant material lining the most peripheral airway. With a surface tension (T) of 5 mN m⁻¹ (Possmayer, 1998), the large radius of the air sacs in the newborn dunnart (r , ≈ 200 μ m) implies that the recoil pressure $P = 2T/r$ is only ≈ 50 N m⁻², or ≈ 0.5 cmH₂O. This very low recoil pressure favours the maintenance of air inside the lungs. Normalised by body mass, the linear dimensions of the dunnart trachea were large in comparison to other newborn species (Mortola and Fisher, 1980). Hence, from the viewpoint of passive mechanics, it would seem that neither the compliance of the respiratory system nor the resistance to flow in the large airways should pose a problem to inspiration. Rather, the major factors hindering \dot{V}_E in the early postnatal days appears to be in the active properties of the system, namely the lack of coordination of the respiratory muscles. Videotape observations indicated that entrance of air occurred at random during the wriggling, presumably when the combinations of muscle contractions favoured the generation of some negative airways pressure. Hence, in the early postnatal period pulmonary ventilation was a casual, almost accidental event determined by gross body movements. The high viscosity of the neonatal respiratory system apparent from the long time required to reach volume stability at each pressure step and the large hysteresis area is a characteristic of other small newborns (Sullivan and Mortola, 1987). This characteristic delays the transmission of muscle force to the airways further reducing the efficiency of the breathing act. Occasionally, the air sacs were observed to gradually empty. This was unlikely to be due to deflation through the airways because of the very low recoil pressure. It is more probable that it

indicated the occurrence of airways closure. In such a closed system, gradual deflation of the air sacs is expected when O_2 uptake by the pulmonary capillaries exceeds CO_2 elimination. In conclusion, it seems that gas exchange through the skin for the newborn dunnart is not simply an alternative mechanism to pulmonary gas exchange but rather it is the only possibility for survival in a mammal born with inefficient pulmonary ventilation.

Because of their very small size it is reasonable to assume that the values of body temperature were equal to those of T_a . Over the temperature range 36–20°C the Q_{10} values for \dot{V}_{O_2} and \dot{V}_{CO_2} were between 1.6 and 2.5. These values are close to the Q_{10} of enzymatic reactions, and therefore in agreement with the ectothermic behaviour of these newborns. With the drop in temperature and metabolism \dot{V}_E decreased in approximate proportion. Because the drop in \dot{V}_E was entirely determined by the decrease in f , this should imply that alveolar ventilation also changed in proportion with gaseous metabolism. The constancy of the ventilation–metabolism ratio as temperature drops is a characteristic of homeothermic mammals with thermogenic responses to cold (Mortola and Gautier, 1995), but its occurrence in this ectothermic newborn is intriguing and difficult to interpret in absence of blood gas data. As temperature decreased, the similarity in ventilation–metabolism could imply that the partial pressure of CO_2 in the arterial blood (Pa_{CO_2}) remained around the same value. This would be different from the temperature responses of most ectothermic vertebrates in which Pa_{CO_2} has a positive slope with temperature (Jackson, 1989). If Pa_{CO_2} remained constant the drop in skin gas exchange with temperature might indicate a progressive lowering in skin gas conductance, its perfusion, or both. The results presented in Fig. 7 also suggest that for a small drop in temperature, between 36 and 32°C, \dot{V}_E changed little, and \dot{V}_E/\dot{V}_{CO_2} increased by $\approx 74\%$ (from 27 to 47). This should lower Pa_{CO_2} at least as much as expected from the effect of temperature on the physical characteristics of body fluids. This pattern, therefore, would be in agreement with a ventilatory regulation aiming to protect the constant net charge of

proteins and specifically of the imidazole group of histidine (alaphastat regulation; Reeves, 1977). If this interpretation of the data was correct, it would imply the existence of some mechanisms controlling \dot{V}_E in the dunnart pouch-young. We have no indications about what they could be, or about their efficacy. However, the finding of a response to lung inflation so reminiscent of the classic Hering–Breuer reflex suggests that at least the most basic regulatory mechanisms of \dot{V}_E may be operative even in a newborn born after 12 days of gestation.

In conclusion, for the newborn dunnart gas exchange through the skin is not simply an option, but a requirement because of the inefficient \dot{V}_E . The latter is attributable to the immature neuro-muscular development rather than the passive mechanical properties of the respiratory system. After a couple of weeks the lungs contribute $\approx 2/3$ of total gas exchange. At this time \dot{V}_E tracks temperature induced changes in metabolic rate, but whether the \dot{V}_E adjustments reflect mechanisms of regulation remains unresolved.

Acknowledgements

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