FIRST BREATH

BY SHANNON SIMPSON

Some marsupials are born so small they don't use their lungs for their first few days of life. What can this tell us about lung structure and function in the immature lung?

Gas exchange across the newborn dunnart's skin enables it to respire without functional lungs.

The newborn marsupial is the only example of a mammal that does not rely on its lungs to exchange oxygen and carbon dioxide. Instead, a number of marsupial newborns use their skin as the major organ of gas exchange. For example, dunnarts are born with lungs so structurally and functionally immature that they do not take their first breath until 3–4 days after birth.

The idea that a newborn mammal could survive without functioning lungs has challenged some longstanding ideas about respiration in mammals. How could a newborn marsupial survive without lung function? Did its apparent prematurity provide clues that could help explain lung development in other mammals – and possibly help in understanding problems associated with breathing disorders in pre-term babies?

The comparative short gestation and premature birth of marsupials is often attributed to the lack of nutrients and oxygen reaching the foetus as the placenta is poorly interfaced with the uterine wall. Marsupials are therefore characterised by development of the young in the pouch and a prolonged reliance on lactation to raise their young.

This reproductive strategy does, however, make marsupials a great model to study organ development, as the young are born far less developed than their placental counterparts and are easily accessible in the pouch through many more stages of organ development.

Adding to the interest of the marsupial as a model for lung development is the fact that all mammalian species, marsupials included, undergo the same developmental stages of structural lung development. However, the timing of each developmental stage and the degree of lung maturation at birth varies widely between species, with marsupials being born particularly less mature.

The fat-tailed dunnart (*Sminthopsis crassicaudata*) is a small nocturnal insect-eating marsupial that is born no bigger than a grain of rice after just 13.5 days gestation. Because most marsupials are born so small – a mere 15 mg in the case of the dunnart – they have a large surface area of skin with respect to the volume of their body, and this ratio permits exchange of gases through their skin. They also have a very low metabolic rate and hence a low rate of oxygen consumption. If the skin is a sufficient membrane for oxygen uptake and carbon dioxide removal, then the newborn dunnart does not need to have functioning lungs.

To quantify the amount of gas exchange through the skin and lungs, researchers in Prof Peter Frappell's laboratory at La Tobe University used a technique called closed-system respirometry, which works on the principle of what goes in must come out. The rate at which the animal consumes oxygen and produces carbon dioxide is dependent on the metabolic demands of the animal at that time. Therefore, if an animal is placed in a sealed chamber for a period of time, the amount of oxygen in that chamber will decrease and the amount of carbon dioxide will increase from atmospheric values.

In order to characterise how much gas exchange was occurring across both the skin and the lungs, newborn dunnarts were fitted with a minute face-mask (Fig. 1). The mask opening passed through a wall that separated a temperature-controlled chamber into two discrete compartments: one in which the exchange of oxygen and carbon dioxide across the skin could be measured, and another measuring gas exchange across the lungs via the face-mask.

At the same time breathing (if it occurred) could be detected with a very sensitive transducer connected to the compartment in which the face-mask opened. This transducer sensed the small changes in pressure that occurred when microlitre volumes were inspired and expired.

This set-up allowed us to determine that the dunnart was not breathing at birth, and that the newborn dunnart relies almost completely on its skin to breathe (Fig. 2). A regular breathing pattern was not established until about 4 days of age.

After the commencement of ventilation, the young fat-tailed dunnart's breathing pattern was characterised by a post-inspiratory pause, which is typical of newborn marsupials. The percentage of gas exchange across the skin decreased with the onset of breathing but continued to play a role until the young reached approximately 1 gram, which is equivalent to a 40–45-day-old dunnart. Interestingly, this is also the age where the animal ceases to rely on the pouch to maintain body temperature and generates its own heat by raising its metabolic rate.

Other research in our laboratory has shown that larger newborn marsupials, such as the tammar wallaby (*Macropus eugenii*), which is born weighing ~380 mg, do breathe at birth but still rely on their skin for approximately one-third of their oxygen requirements. As the lungs in the tammar wallaby are more developed at birth, the joey becomes totally reliant upon its lungs within 4–5 days.

Dunnarts are born with lungs so structurally and functionally immature that they do not take their first breath until 3–4 days after birth.

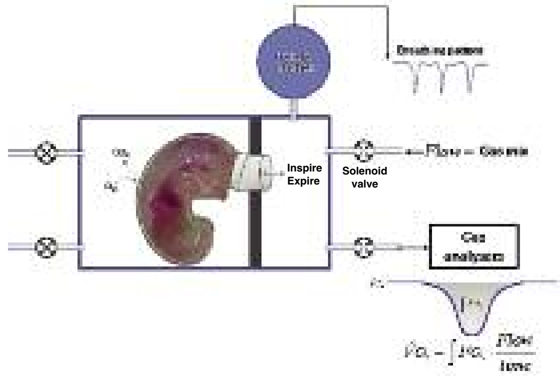

Figure 1. A minute facemask is fitted to a newborn dunnart to measure the exchange of gas by the skin and/or lungs.

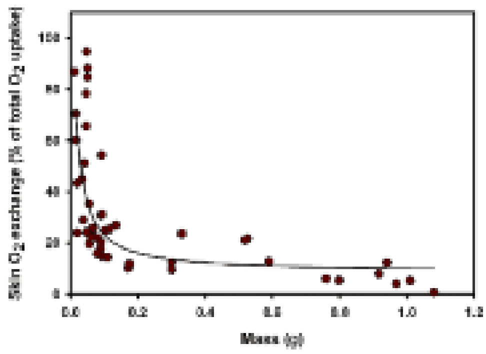

Figure 2. Exchange of oxygen across the skin in dunnart pouch young as a percentage of the total rate of oxygen consumption. While almost 100% at birth, oxygen consumption across the skin decreases until the young weighs approximately 1 gram, which is equivalent to about 45 days after birth.

Establishing that the newborn dunnart had no ventilation or pulmonary gas exchange raised the question: why? It seems feasible that the use of gas exchange across the skin, and a delay in the onset of breathing, is an indication that the lung is struc-

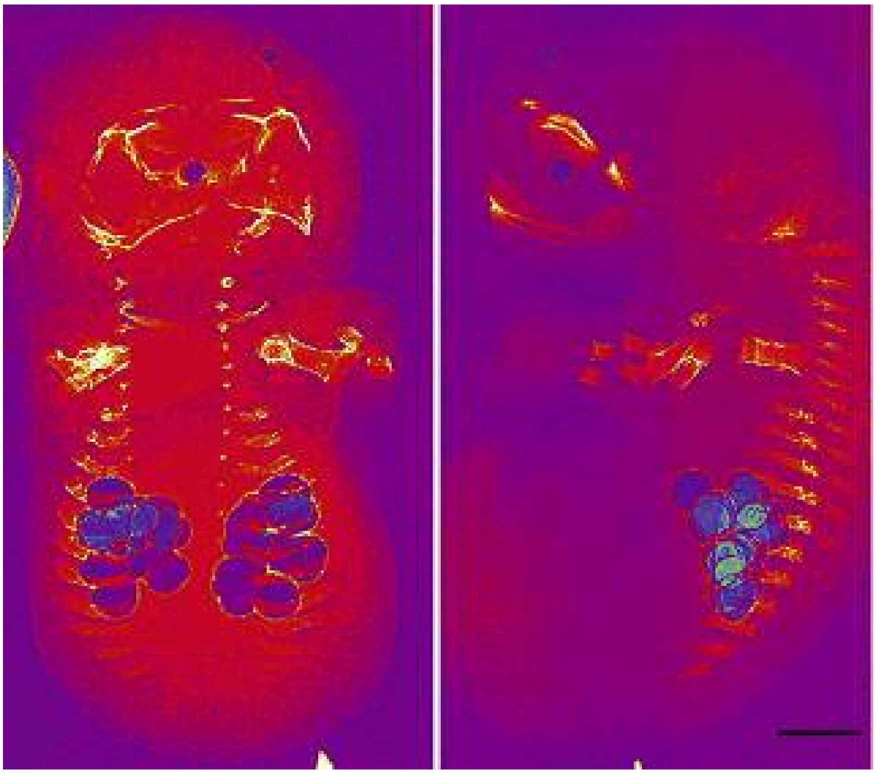

Figure 3. Image of a fat-tailed dunnart 72 hours after birth (left is a ventral view, right is a lateral view). The air within the lungs is visible within little more than a few primitive air sacs. The skeletal formation of the jaws, forelimbs and ribs are also clearly visible. Images are from frozen specimens and are shown here with a false colour overlay. The scale bar represents 350 μm.

turally and/or functionally underdeveloped at the time of birth. To have functioning lungs newborns must:

- replace the fetal fluid in the lungs with air;
- have appropriate lung structure for gas exchange (e.g. alveoli, a thin air/blood barrier);
- have the correct cells to produce surfactant so that the alveoli will not collapse;
- have the capacity to generate a respiratory rhythm;
- have an innervated and muscularised diaphragm (the main muscle of inspiration); and
- have a chest wall that is rigid enough to prevent distortion when the diaphragm contracts.

Answering these questions requires a multidisciplinary approach. We are integrating the physiology with structural morphology studies in a project involving physiologists from Dr Stuart Hooper's laboratory at Monash University and synchrotron physicists from Dr Rob Lewis' department at Monash University. We are also collaborating with the Victorian Department of Primary Industries to determine which genes are expressed in the early stages when the immature lung is no more than a collection of simple air-sacs.

The most structurally underdeveloped lungs encountered at birth to date have been described in small, low birth-weight marsupials such as the eastern and northern native cats, the Tasmanian devil, the American opossum and the quokka wallaby. In these low birth-weight marsupials, the lung is composed of a few highly vascularised sacs. A human infant born with this lack of structural lung development would be grossly premature and most likely not survive.

It has been shown that the tammar wallaby's lung contains surfactant at birth and should be able to function. We have also demonstrated that the chest wall in the newborn tammar wallaby is so compliant that the rib cage moves inwards during contraction of the diaphragm. This leads to inefficient diaphragmatic functioning and potential fatigue, and thus limits the efficiency of breathing. Collapse of the rib cage and increased abdominal distortion leads to a situation in human infants known as paradoxical breathing, which can be problematic for pre-term infants.

We have also imaged newborn dunnarts to investigate the development of the three-dimensional structure of the lung using a synchrotron radiation source in collaboration with our colleagues from Monash University and Japan's SPring-8 synchrotron facility. The radiographs reveal that just two air-filled sacs are present within the first hour of the newborn dunnarts' life, with the number of air sacs increasing over the first 4 days (Fig. 3). This is presumably due to the actual number of air sacs increasing and becoming filled with air as a result of body movements.

Upon the commencement of ventilation, at about 4 days, we see a marked proliferation of the lung from a few large air sacs to a complex alveolar structure at around 40 days. Three-dimensional reconstructions from about 1500 individual images enable calculations of the lung surface area and volume, which is useful for determining the gas exchange capacity of the lung, with the aim of improving the understanding of early lung development in mammals.

Through our integrative approach we have been able to characterise the structural and functional development of the respiratory system in the neonatal marsupial. Our findings reveal the incredibly early stages of development that the lungs and other aspects of the respiratory system are at birth in the marsupial. This, and the ease of access to newborn marsupials, reveals the exciting potential that newborn marsupials have to offer medical research in furthering our understanding of lung development.

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