# Thyroid morphology and function and its role in thermoregulation in the newborn southern elephant seal (Mirounga leonina) at Macquarie Island

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(Accepted 9 November 1990)

#### INTRODUCTION

The southern elephant seal pup (*Mirounga leonina*) experiences a dramatic drop in its environmental temperature at birth, from the intrauterine environment (approximately 37 °C) to an average ambient temperature of 4·1 °C. With an average wind speed of 16 knots (8·2 m s<sup>-1</sup>), the wind chill factor may generate temperatures as low as -32 °C (calculated from Consolazio, Johnson & Pecora, 1963). During the breeding season there is a 73 % likelihood of rain and a 29 % possibility of snow occurring.

The newborn pup receives no thermal protection from the cow, or from physical features of the environment, as the birth site is on exposed beaches. The pup has negligible physical insulation (such as blubber) and has a wettable fur; the natal pelage is wet from amniotic fluid for approximately six hours after birth. Behavioural means of maintaining body temperature are not employed.

These factors create the potential for the pup to be thermally stressed at birth or during the first week of life until it acquires an insulating blubber layer. The newborn harp seal pup (*Phoca groenlandica*) shivers, using glycogen stores in its muscles until its fur is dry (Blix, Grav & Ronald, 1979). In addition to shivering the pup uses brown adipose tissue (BAT) to maintain body temperature (Blix *et al.* 1979). BAT has not been reported in the newborn southern elephant seal.

The thyroid gland has been shown to be particularly active immediately after birth and assists in maintaining body temperature through increased production of thyroid hormones (Cabello & Levieux, 1980; Haidmayer & Hagmuller, 1981; Ślebodziński, Nowak & Zamyslowska, 1981; Polk, Wu & Fisher, 1986). Morphological techniques have been used to observe thyroid activity in newborn pups of two land-breeding phocid species, the harbour seal (*Phoca vitulina*) (Harrison, Rowlands, Whitting & Young, 1962) and grey seal (*Halichoerus grypus*) (Amoroso et al. 1965) together with the ice-breeding harp seal (*Phoca groenlandica*) (Leatherland & Ronald, 1979). Although the thyroid glands were found to show 'wide differences in their histological appearance' (Amoroso et al. 1965, p. 430) in newborn harbour and grey seals, both Harrison et al. (1962) and Amoroso et al. (1965) believed the thyroid gland is 'strikingly active immediately after birth' (Harrison et al. 1962, p. 7). Thyroid follicular epithelial cells were cuboidal to columnar, the size of the follicles was reduced and there was vacuolation of the colloid (Harrison et al. 1962; Amoroso et al. 1965). However, in the harp seal only one animal of ten showed any

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morphological change in the thyroid (Leatherland & Ronald, 1979). These conflicting results may be due to the small numbers of animals used, whose precise age was unknown.

Recent studies have shown that it is possible to determine ultrastructurally whether thyroglobulin is being produced for storage in the lumen of the follicle, or if it is being broken down with resultant secretion of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) (Seljelid, 1967; Fujita, 1975, 1981, 1988; Ericson & Engstrom, 1978; Engstrom & Ericson, 1981; Ericson, 1981; Uchiyama, Murakami & Igarashi, 1986). Thus it is possible to assess thyroid gland activity more accurately than by light microscopical means.

The thyroid hormones  $T_4$  and  $T_3$  are known to increase the metabolic rate of mammals and consequently to increase body temperature (Kaciuba-Uscilko, Legge & Mount, 1970; Moore, Moore & Moore, 1978; Andrews, Lynch & Moore, 1979). Leatherland & Ronald (1979) and Engelhardt & Ferguson (1980) reported extremely high  $T_4$  and  $T_3$  levels in harp and grey seals on the first day *postpartum*, decreasing to adult levels by the third day.

Newborn elephant seal pups are able to maintain body temperature within the range 36.5 °C to 39.1 °C (G. Little, unpublished data). The aim of this paper is to investigate the role of the thyroid in thermoregulation in the newborn southern elephant seal pup. Morphological and endocrinological techniques were used to estimate thyroid gland secretory activity.

#### MATERIALS AND METHODS

# Study site, Groups

The study was conducted during the 1985 pupping season at Macquarie Island (159 °E, 54 °S). Thirty-seven pups of known age, representing eight groups, were sampled at random during the pupping season from early September to early November. The groups were at 0 hours (4 pups), 2 hours (3 pups), 6 hours (5 pups), 24 hours (6 pups), 2 days (5 pups), 5 days (5 pups), 10 days (5 pups) and 20 days (4 pups). Serial blood samples were collected from an additional six pups followed for varying times and included in the  $T_4$  and  $T_3$  analysis.

### Collection of blood and tissue samples

Each pup was removed from the harem on a sling or stretcher. Blood samples were collected from the extradural vertebral venous sinus (Hubbard, 1968, p. 346) using an 18 gauge needle. Twenty millilitres of blood were collected into screw-topped plastic vials containing 100 mg EDTA. The samples were left at ambient temperature (4–7°) until they were centrifuged in the laboratory. The plasma was stored in 5 ml plastic bottles at -20 °C until assayed in Australia. Plasma  $T_4$  and  $T_3$  concentrations were measured by radioimmunoassay (Wynn, Wallace, Kirby & Annison, 1988).

Thyroid glands were collected from animals killed under anaesthetic with sodium pentobarbitone injected into the extradural vertebral venous sinus (Hubbard, 1968). A midline ventral incision was made in the cervical region, the thyroid was exposed, then removed, and a thin transverse section from the middle of each lobe was placed in 3% glutaraldehyde/4% paraformaldehyde in 0·1 m cacodylate buffer, pH 7·2. Left and right lobes were subsequently weighed in the laboratory. After 24 hours fixation, the tissue was stored in 0·1 m cacodylate buffer until the samples were returned to Australia for further processing.

Five millimetres sections of left and right lobes were postfixed in 1% osmium tetroxide, processed through a graded series of alcohols and embedded in LR White

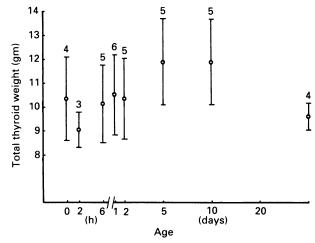


Fig. 1. Total thyroid weight of elephant seal pups aged 0 hours to 20 days of age. The number of animals for each group is shown.

(London Resin Company). Two micrometre sections were cut and stained with 1% toluidine blue. Ultrathin sections were cut, mounted on uncoated 300 mesh grids and stained with uranyl acetate and lead citrate. They were examined using a Zeiss 10 electron microscope at 60 kV.

For scanning electron microscopy pieces of thyroid gland, fixed as above, were dehydrated in ethanol and transferred to amyl acetate before critical point drying. The dried tissue blocks were placed in a vacuum evaporator, gold coated and viewed with a Cambridge 600 stereoscan microscope.

## Morphometry

Measurement of thyroid epithelial cell height was made at a magnification of  $\times$  1200 using 2  $\mu$ m thick, toluidine blue stained sections. An Olympus microscope with a camera lucida attachment was linked to an Apple IIe computer and digitising tablet utilising the Magellan stereological programme to measure directly from the section, the apparent epithelial cell height (Halasz & Martin, 1985).

For each animal, four sections from different areas of the thyroid gland were measured. A Weibel graticule in the eyepiece of the microscope was used to select cells randomly for measurement in 10 different fields on each slide.

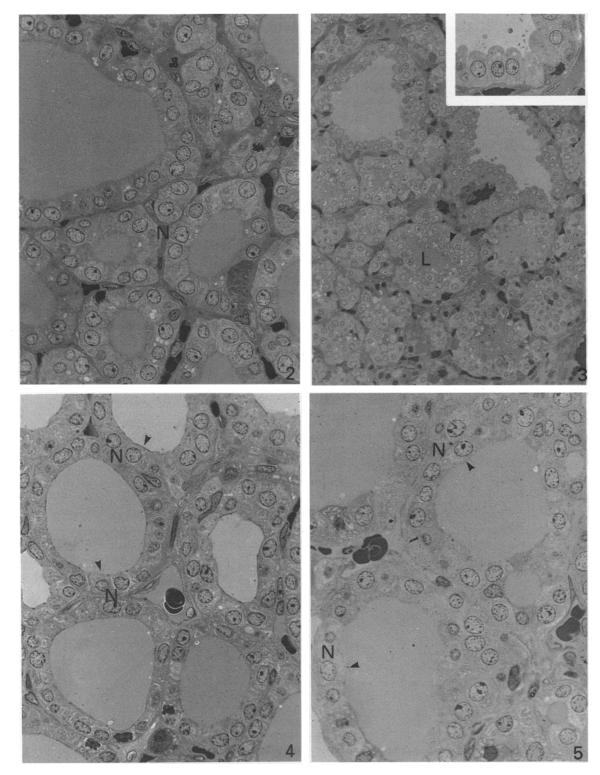
## Statistical analysis

Tests for statistical significance between age groups of thyroid weight, thyroid epithelial cell height and plasma  $T_4$  and  $T_3$  concentrations were carried out using one way analysis of variance (ANOVA) and the multiple comparison Student–Newman–Keuls (SNK) test (Q value, Zar, 1974). Paired t tests were used to test for statistical significance between left and right thyroid gland weight and between male and female total gland weight.

#### RESULTS

#### Thyroid weight

Total thyroid weight did not vary with age up to 20 days (Fig. 1). No difference in weight between left and right lobes was found (left lobe,  $5.46 \pm 1.8$  g; right lobe,



Hours postpartum	No. pups	TE (μm)	SE	
0	4	11.82	1.17	
2	3	12-18	0.37	
6	5	10-99	1.86	
24	6	11.75	1.58	
48	5	11.62	2.54	
120	5	8.49	1.6	
240	5	8.87	1.49	
480	4	8.93	0.68	

Table 1. Mean thyroid epithelial cell height (TE) and standard error (SE) for southern elephant seal pups during the first 480 hours (20 days) after birth

 $5.15\pm2.1$  g; t=0.9517, NS). Mean total thyroid weight was  $10.6\pm2.7$  g (n = 37). No significant difference in total thyroid weight was observed between male and female pups (t=1.17, NS).

# Thyroid epithelial cell height

Table 1 shows the thyroid epithelial cell heights for the eight age groups. A one way ANOVA revealed significant differences among ages in thyroid epithelial cell height (F = 5.19, P < 0.001). Thyroid epithelial cells are cuboidal to columnar during the first 48 hours *postpartum*, but decrease in height between 48 hours and five days of age (Q = 4.28, P < 0.05).

# Light microscopy

The position and relative size of the nuclei within the thyroid epithelial cells differ between the age groups. At birth the nuclei occupy a basal position, but by 6 hours the nuclei occupy a subapical position (Figs. 2, 3). At 24 hours and 2 days the nuclei are located centrally within the cell. The nucleus at 5 days occupies the majority of the cell, while at 10 and 20 days, nuclear position and relative size is similar to that in 24 hours and 2 days old animals.

The number of large vacuoles within the cells appears to increase dramatically at 6 hours of age (Fig. 3). Fewer large vacuoles are present in 24 hours and 2 days old pups and 5 days old pups show very little vacuolation in the apical cytoplasm (Figs. 4, 5). At 10 and 20 days there is a tendency for the number of large vacuoles to increase, but they are less frequent than during the first 2 days *postpartum*.

### Electron microscopy

Ultrastructurally the large vacuoles in the apical cytoplasm are seen to be colloid droplets delimited by a single membrane. At 0 hours the majority of the droplets have

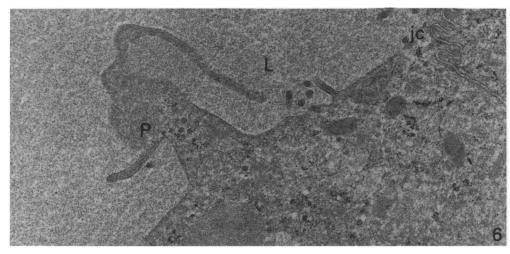
Fig. 2. High power photomicrograph of thyroid from 0 hours pup. Note the cuboidal to columnar epithelium with nuclei (N) occupying the basal regions of the cell. ×677.

Fig. 3. Light micrographs of thyroid from a 6 hours pup. Note the presence of papillary ingrowths (arrowhead) protruding into the lumen (L).  $\times 434$ . Inset. Epithelial cells are columnar with vacuoles present in the apical cytoplasm.  $\times 655$ .

Fig. 4. Thyroid epithelial cells (arrowheads) from a 2 days old pup. The extensive vacuolation of the epithelial cells as observed at 6 hours is not present.  $\times$  685.

Fig. 5. Thyroid epithelial cells from a 5 days old pup. Cells are low cuboidal (arrowheads) with the nucleus (N) occupying the majority of the cell.  $\times$  726.

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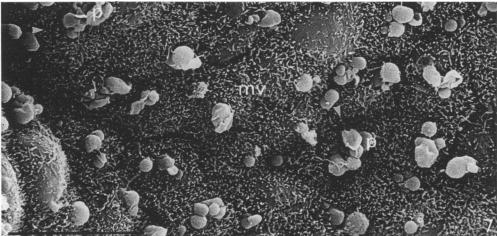
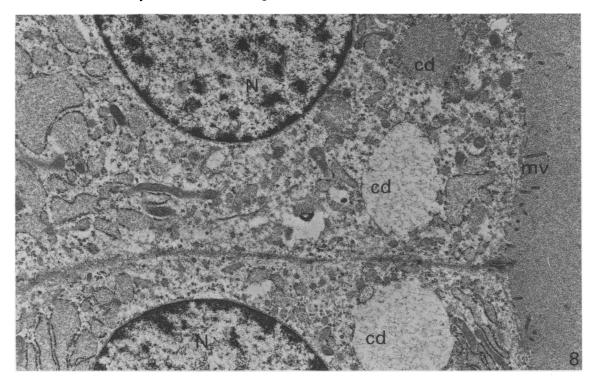


Fig. 6. High power electron micrograph of the apical surface of a thyroid epithelial cell from a 0 hours pup. A pseudopodium (p) protrudes into the lumen (L) of the follicle adjacent to a junctional complex (jc).  $\times 22916$ .

Fig. 7. Scanning electron micrograph of apical surface of thyroid epithelial cells from a 0 hours pup. Many pseudopodia (p) extend into the follicle lumen adjacent to cell junctions (arrowhead). Covering the apical surface are many microvilli (mv). Bar,  $10~\mu m$ .

a similar density to the colloid in the follicular lumen and are restricted to the apical cytoplasm. Pseudopodia can be observed protruding into the lumen and apparently engulfing colloid in the majority of follicles (Figs. 6, 7). At 2 hours the colloid droplets exhibit varying densities and are confined to the apical portion of the cell (Fig. 8). Many extensive pseudopodia protrude into the lumen in the majority of follicles (Fig. 9).

Colloid droplets are not confined to the apical cytoplasm at 6 hours as they were at 0 hours and 2 hours but are distributed throughout the cytoplasm (compare Figs. 8 and 10). Pseudopodia are abundant and protrude into the lumen in the majority of follicles (Fig. 11). Cisternae of the rough endoplasmic reticulum (RER) tend to be enlarged at 6 hours (Fig. 10). Numbers of pseudopodia appear to decrease by 24 hours, after which they are rarely observed in follicles from two to 20 days. Cisternae of the RER at 24 hours, 2 days and 5 days tend to be dilated (Fig. 12). Few colloid



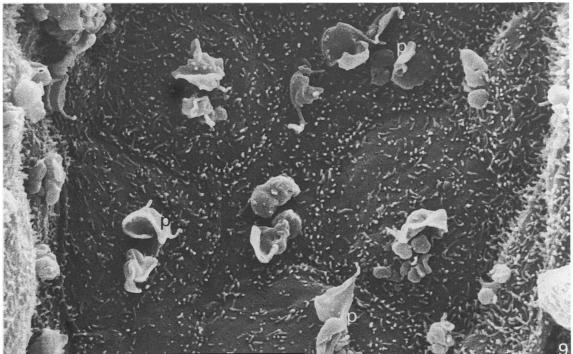
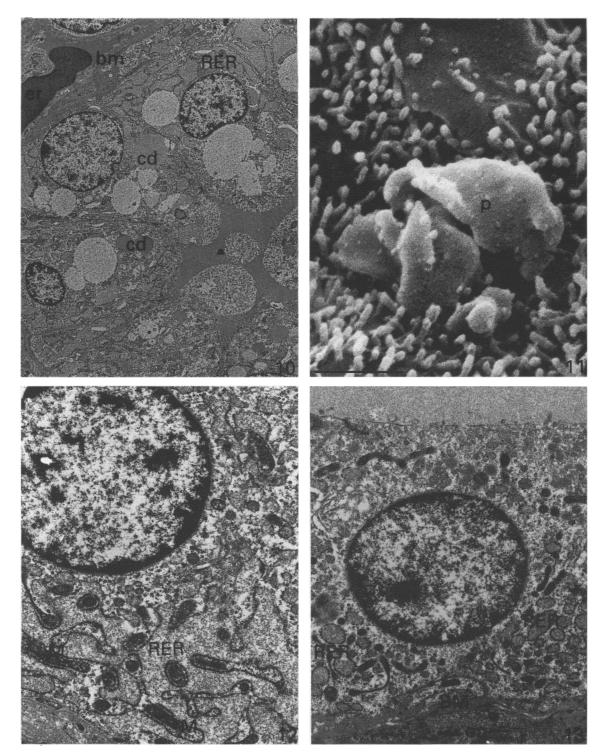


Fig. 8. Thyroid epithelial cells from a 2 hours pup. Colloid droplets (cd) of varying densities are present in the apical cytoplasm. Large ovoid nuclei (N) are situated in the middle of the cell. Microvilli (mv) protrude into the lumen of the follicle. ×8410.

Fig. 9. High power scanning electron micrograph of apical surface of thyroid epithelial cells from a 2 hours pup. Large extensive pseudopodia (p) extend into the lumen. Bar,  $10~\mu m$ .



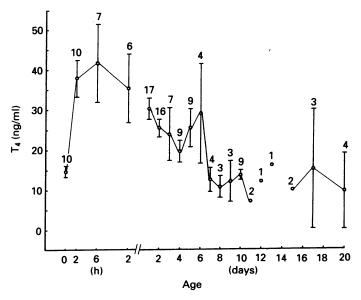


Fig. 14. Graph of plasma T<sub>4</sub> values for elephant seal pups from 0 hours to 20 days of age. The number of animals at each age group is shown.

droplets and minimal numbers of lysosomes are present in the apical cytoplasm from five to 20 days. The RER tends to decrease in size at 10 and 20 days (Fig. 13).

# Plasma concentrations of thyroid hormones

Variation in the plasma concentration of  $T_4$  from birth to 20 days is shown in Figure 14. A one way ANOVA indicated significant variation in  $T_4$  concentration with age (F = 3.09, P < 0.001).  $T_4$  levels increased approximately three-fold from birth  $(14.6 \pm 1.4 \text{ ng/ml})$ , to peak at 6 hours  $(41.7 \pm 9.5 \text{ ng/ml})$ , Q = 5.864, P < 0.005) postpartum after which it steadily declined. Between 7 and 15 days  $T_4$  concentration ranged from 7 to 13.5 ng/ml, that is less than at birth, after which it increased to 20 days  $(16.5 \pm 9.5 \text{ ng/ml})$ .

Plasma concentration of  $T_3$  from birth to 20 days is shown in Figure 15. A one way ANOVA showed significant variation in  $T_3$  concentration with age (F = 1.67, P < 0.05). It increased eight-fold between birth and 24 hours (Q = 4.474, P < 0.05) postpartum, remained high until 120 to 168 hours, then decreased to 20 days.

Fig. 10. Low power electron micrograph of thyroid follicle from a 6 hours pup. Many colloid droplets (cd) of varying densities are present throughout the cytoplasm. Cisternae of the rough endoplasmic reticulum (RER) in the basal portion of the epithelial cells are dilated. An erythrocyte (er) is present in the capillary adjacent to the basement membrane (bm) of the follicle.  $\times$  3203.

Fig. 11. Large pseudopodia (p) extending into the lumen from the apical surface of a thyroid epithelial cell of a 6 hours pup. Bar,  $1 \mu m$ .

Fig. 12. Dilated cisternae of the rough endoplasmic reticulum (RER) are present in the thyroid epithelial cells of 5 days old pups. Mitochondria (M) are present among the rough endoplasmic reticulum.  $\times$  7777.

Fig. 13. Thyroid epithelial cells of a 20 days pup exhibiting greatly reduced rough endoplasmic reticulum (RER) in the basal portion of the cell. The nucleus of an endothelial cell (en) is observed adjacent to the basement membrane (BM) of the epithelial cells.  $\times$  6600.

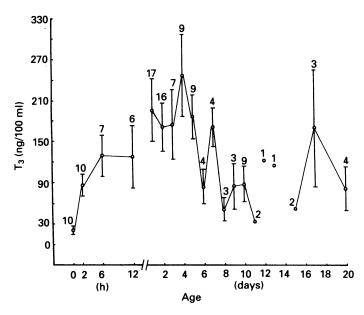


Fig. 15. Plasma T<sub>3</sub> values of elephant seal pups aged 0 hours to 20 days. The number of animals at each age group is shown.

#### DISCUSSION

During the first 48 hours, and particularly within the first 24 hours, after birth the thyroid gland of the newborn southern elephant seal pup exhibits markedly increased secretory activity.

No significant change in total thyroid weight with increasing age was demonstrable in the pups during the first 20 days of life. The major component of growth during this period is blubber (Bryden, 1968 a) evidenced by a significant increase in girth (Little, Bryden & Barnes, 1987). The ratio of total thyroid weight to body weight in the elephant seal,  $0.175 \pm 0.018$  g/kg is similar to that found in the grey seal and harbour seal (Harrison et al. 1962; Amoroso et al. 1965). As in the grey and harbour seals, no significant difference in thyroid weight between sexes and no significant difference between right and left lobes of the thyroid was demonstrated in the elephant seal.

The change in cell height of the thyroid epithelium in the southern elephant seal pup suggests that thyroid hormone secretion is high during the first 48 hours *postpartum*, but declines after that. This pattern of thyroid activity is similar to that in the newborn lamb, pig and infant, where there is an initial peak followed by a decrease in secretion of thyroid hormones (Erenberg, Phelps, Lam & Fisher, 1974; Cabello & Levieux, 1980; Parker, Williams, Aherne & Young, 1980a; Cabello, 1983; Wrutniak & Cabello, 1987).

The ultrastructure of the thyroid epithelial cells is strongly suggestive of increased secretion of thyroid hormones during the first 6 hours of postnatal life. In glands acutely stimulated by thyroid stimulating hormone (TSH) engulfment of colloid by pseudopodia is observed within 30 minutes of administration (Ketelbant-Balasse, Rodesch, Nève & Pasteels, 1973; Ketelbant-Balasse, VanSande, Nève & Dumont, 1976; Ericson, 1981). The presence of colloid droplets of varying density distributed throughout the cytoplasm and not confined to the apical cytoplasm indicates that absorbed thyroglobulin is being hydrolysed and that  $T_4$  and  $T_3$  are being released (van

den Hove-Vandenbroucke, 1980; Ericson, 1981; Uchiyama et al. 1986). These morphological characteristics have been well documented in rats and pigs under varying experimental conditions of thyroid stimulation, such as injection of TSH (Nève, Authelet & Golstein, 1981), thyroxine (Engstrom & Ericson, 1981; Ericson, 1981), or after subtotal thyroidectomy (Krupp & Lee, 1986). Corresponding morphological changes occur in the thyroid gland of animals exposed to cold (Del Conte & Stux, 1954; Seibel & Knigge, 1972; Dobrowolska, Rewkiewicz-Dziarska & Szarska, 1976; Frankel & Lange, 1980).

Few reports describe the morphology of the thyroid gland during the early neonatal period. With the exception of one (Leatherland & Ronald, 1979, which gives equivocal results), all reports demonstrate histological features which indicate increased secretory activity, (Harrison et al. 1962; Amoroso et al. 1965; Slebodzinski & Srebro, 1968; Slebodzinski, 1972; Etling & Larroche, 1975; Larroche, 1976; Roy, Saigal, Nandad & Nagpal, 1978; Parker et al. 1980a). Significant changes in the organelles of thyroid epithelial cells occur over a 24 hours period in conjunction with variations in TSH concentrations in the rat (Murakami & Uchiyama, 1986; Uchiyama et al. 1986). The most dramatic changes in thyroid morphology occur during the first 6 hours after birth in the newborn southern elephant seal.

However, in the study by Leatherland & Ronald (1979), harp seals were sampled at one and five days of age. Only one five days old animal indicated thyroid hyperactivity with columnar epithelial cells. The lack of morphological changes observed in the thyroid gland of harp seals may be due to the inability to determine age precisely in that species, as the dramatic morphological changes occur within 24 hours after birth in the Southern elephant seal.

Leatherland & Ronald (1979) suggest that the elevated plasma  $T_4$  and  $T_3$  levels in neonatal harp seals may be derived from  $T_4$  or  $T_3$  in the milk (Štrabák, Macho, Škultétyová & Michaličková, 1978). Southern elephant seal pups first suckle between three and seven hours after birth at Macquarie Island (Bryden, 1968 b), whereas harp seals suckle within one hour after birth (Blix et al. 1979). The pups sampled between zero and 6 hours in the present study had not suckled, so the dramatic increases in  $T_4$  and  $T_3$  before 6 hours could not have resulted from transmission of these hormones in the milk.

Fifty per cent of circulating  $T_3$  is secreted by the thyroid gland in sheep (Fisher, Chopra & Dussault, 1972) whereas only 15% of circulating  $T_3$  in man is secreted by the thyroid gland, the remaining 85% produced through peripheral deiodination of  $T_4$  (Braverman, Ingbar & Sterling, 1970). These differing mechanisms for producing  $T_3$  may be utilised by the harp and southern elephant seals. The peripheral deiodinating system may be more mature in the harp seal and therefore responsible for the increased  $T_3$  levels (Leatherland & Ronald, 1979), thus explaining the lack of morphological indications of increased secretory activity by the thyroid gland.

Plasma T<sub>4</sub> levels are lower in newborn elephant seals than in newborn lambs (Cabello & Levieux, 1980; Cabello, 1983; Wrutniak & Cabello, 1987), pig (Parker, Williams, Aherne & Young, 1980b), human infants (Abuid, Stinson & Larsen, 1973; Erenberg et al. 1974) and captive grey and harp seals (Engelhardt & Ferguson, 1980). However, they are similar to the serum T<sub>4</sub> levels recorded for free-living newborn harp seals (Leatherland & Ronald, 1979). John, Ronald & George, (1987) suggest that the decreased serum T<sub>4</sub> levels obtained in the study by Leatherland & Ronald (1979) result from the blood samples being collected from harp seals in their natural environment and using a different assay method. Leatherland & Ronald (1979) and John et al. (1987) used serum samples whereas plasma samples were used in the present study and

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a different radioimmunoassay method was used. The elephant seal samples were stored for one year after returning from Macquarie Island before being assayed. Two other possibilities may explain the low  $T_4$  values in the elephant seal. The thyroid gland may be selectively secreting  $T_3$  in preference to  $T_4$  and, either in association or as a separate factor, rapid peripheral 5'-monodeiodination of  $T_4$  to  $T_3$  may be taking place.

The newborn southern elephant seal pup shows an eight-fold increase in  $T_3$  concentration at 24 hours after birth the most dramatic increase of any newborn mammal studied to date, and the concentration remains elevated until 168 hours (see Fig. 15). Newborn harp and harbour seals have raised  $T_3$  levels during the first day after birth (Leatherland & Ronald, 1979; Engelhardt & Ferguson, 1979, 1980).

It is suggested that this large increase in T<sub>3</sub> concentration in the first day postpartum plays an important role in maintaining body temperature by increasing the metabolic rate of the newborn elephant seal. The metabolic rate has been shown to be elevated soon after birth in newborn Weddell seals, Leptonychotes weddellii (Elsner, Hammond, Davison & Wyburn, 1977), harp seal (Davydov & Makarova, 1964; Blix et al. 1979) and harbour seals (Miller, Rosenmann, Irving & Morrison, 1973; Miller & Irving, 1975). Metabolic rate of newborn seals is 1.5 to 3 times that of animals of similar size (Lavigne et al. 1986; Worthy, 1987). A recent study of California sea lion pups, Zalophus californianus (Thompson, Ono, Oftedal & Boness, 1987) indicates they have a metabolic rate 2.38 times that predicted for adult mammals of similar size. Thompson et al. (1987) suggest that the high metabolic rate at birth is needed to maintain body temperature, as sea lion pups may have a thin subcutaneous fat layer and a relatively high lower critical temperature compared with other pinnipeds. As the blubber layer which provides physical insulation in the elephant seal increases, the need for physiological mechanisms to maintain body temperature decreases, which may explain the decrease in  $T_3$  levels by 10 days of age.

The pituitary-thyroid axis is functional at birth in the southern elephant seal and the thyroid gland of the pup is responsible for the production of circulating  $T_4$  and  $T_3$ . From the evidence presented, the thyroid gland of the southern elephant seal is markedly active at birth and is responsible for the elevated levels of circulating  $T_4$  and  $T_3$ . The thyroid gland appears to play a vital role in maintaining the body temperature of the poorly equipped newborn seal when it enters the harsh environment of the sub-Antarctic.

#### SUMMARY

The thyroid gland of the newborn southern elephant seal pup exhibits markedly increased secretory activity during the first 24 hours after birth. Thyroid epithelial cell height is cuboidal to columnar for pups from birth to 48 hours postpartum after which it decreases by five days of age. Ultrastructurally the thyroid epithelial cells show pseudopodia protruding into the lumen at zero, two and six hours after birth. After 24 hours postpartum pseudopodia are rarely observed in thyroid follicles from two to 20 days old pups. The number of colloid droplets increases by six hours after birth and they are distributed throughout the cytoplasm. At 24 hours and two days, few colloid droplets are observed. Plasma T<sub>4</sub> concentration increases three-fold from birth, to peak at six hours postpartum after which it steadily declines. Plasma concentration of T<sub>3</sub> increases eight-fold between birth and 24 hours postpartum. T<sub>3</sub> levels remain high until five days to seven days, then decrease to 20 days. The observed changes in thyroid epithelial cell height and ultrastructure is strongly suggestive of increased secretion of thyroid hormones during the first six hours of postnatal life. This pattern of thyroid

activity is similar to that in other newborn mammals which have been examined. The thyroid gland of the southern elephant seal is markedly active at birth and is responsible for the elevated levels of  $T_4$  and  $T_3$ , thus playing a vital role in maintaining the body temperature of the newborn seal when it enters the harsh environment of the sub-Antarctic.

I would like to thank Mr Ron Newman (CSIRO Prospect) for his invaluable assistance in completing the T<sub>4</sub> and T<sub>3</sub> assays. Many thanks are due to Professor M. M. Bryden for constructive criticism of this paper. I thank all members of the 1985 Australian National Antarctic Research Expedition at Macquarie Island who assisted in the field. The support given to me by the Director of the Antarctic Division, Department of Science and the Director of the Tasmanian National Parks and Wildlife Service is gratefully acknowledged.

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