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THE ROLE OF THYROID HORMONES IN MATURATION OF THE ADRENALINE-SENSITIVE LUNG LIQUID REABSORPTIVE MECHANISM IN FETAL SHEEP

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SUMMARY

1. Following thyroidectomy at 106–118 days fetal sheep were infused continuously with triiodothyronine (T_3) from 110, 118, 125 or 131 days ($n = 12$) or with thyroxine (T_4) from 118 days ($n = 4$) until the fetuses were delivered. Lung liquid secretion or absorption rates, heart rate, blood pressure and arterial blood gases were measured before and during 45 min periods of fetal infusions of adrenaline ($n = 60$) at 3–8 day intervals. The effects of T_3 or T_4 replacement on the response to adrenaline were compared with data previously obtained in groups of euthyroid (control) and thyroidectomized (Tx) fetuses.

2. Fetuses infused with T_4 (50 μg/day) following thyroidectomy had plasma T_4 and T_3 concentrations in the normal fetal range. Fetal plasma T_3 levels in fetuses infused with T_3 (60 μg/day) were at or above the high end of the normal range for full-term fetuses. Those receiving 120 μg of T_3 per day had levels equivalent to those normally seen in the postnatal T_3 surge.

3. Normal maturation in the lung of the reabsorptive response of fetal lung liquid to adrenaline was seen in the fetuses infused with T_3 or T_4 from 118 days. A marginal advance in maturation was seen in fetuses infused with T_3 from 110 days and a delay in maturation in those infused with T_3 from 125 and 131 days.

INTRODUCTION

Throughout fetal life the alveolar epithelium secretes lung liquid, driven by active Cl^- transport, into the alveolar spaces (Olver & Strang, 1974). Around the time of birth the direction of flow of liquid across this epithelium is reversed so that the liquid is reabsorbed into the fetal circulation (Brown, Olver, Ramsden, Strang & Walters, 1983). This switch from secretion to absorption is induced by an acute rise in fetal adrenaline concentration brought about by the stress of labour and delivery (Brown *et al.* 1983) and is mediated by amiloride-blockable entry of Na^+ across the apical membrane of the alveolar cell (Olver, Ramsden, Strang & Walters, 1986). The pulmonary epithelium of the fetal sheep becomes increasingly sensitive to adrenaline in the last few weeks of gestation (term = 147 days). Whereas a given rise in fetal adrenaline levels (0·1–1·0 ng/ml) produces only a small fall in the resting secretion

rate in fetuses of less than 130 days gestation, it brings the secretory process to a halt around 130 days, and results in reabsorption of increasing magnitude thereafter (Brown *et al.* 1983). The reabsorptive response can also be induced by the addition of dibutyryl cyclic AMP to fetal lung liquid and the magnitude of this response also shows a striking increase with gestation which closely parallels that to adrenaline (Olver, Ramsden & Walters, 1987).

We have recently shown that maturation of the reabsorptive response to both these substances is severely inhibited by removing the fetal thyroid gland at 118 days (Barker, Brown, Ramsden, Strang & Walters, 1988). Thus fetal thyroid hormones are crucial to maturation of components of the adrenaline-activated Na^+ transport mechanisms which lie beyond the generation of intracellular cyclic AMP.

In order to confirm the role of thyroid hormones in this maturational process and to assess the relative importance of thyroxine (T_4) and its active metabolite triiodothyronine (T_3), we have infused these hormones in previously thyroidectomized fetuses, commencing infusion at 110, 118, 125 or 131 days gestation and continuing until the fetuses were delivered. We have compared the maturation of the responsiveness to adrenaline in these fetuses with that taking place in euthyroid (Brown *et al.* 1983) and hypothyroid fetuses (Barker *et al.* 1988). A preliminary account of some of these experiments has been published (Barker, Strang & Walters, 1989*a*).

METHODS

Surgical procedure

Surgery was performed on the fetuses of sixteen pregnant ewes (Clun Forest) of known tupping dates. The gestational age at surgery was determined by the gestation at which T_3 or T_4 infusion commenced, i.e. surgery at 106–107 days in three fetuses, infusion with T_3 from 110 days; surgery at 114–115 days in three fetuses, infusion with T_3 from 118 days; and surgery at 118 days in six fetuses, infusion with T_3 either from 125 days (three fetuses) or 131 days (three fetuses). In the four fetuses infused with T_4 from 118 days, surgery was performed at 114–117 days. Anaesthesia was induced with thiopentone (12–18 ml) and maintained with inhaled fluothane (1–5%). Fetal thyroidectomy and catheter insertion into the fetal trachea, carotid artery and jugular vein were as described in Barker *et al.* (1988). Benzylpenicillin (300 mg/day I.M.) and streptomycin (500 mg/day I.M.) were given to the ewe during the first two postoperative days during which time no experiments were performed.

Experimental procedure

Experiments were conducted at 3–8 day intervals following the commencement of thyroid hormone infusion ($n = 60$). In each case T_3 or T_4 was infused continuously until the fetus was delivered spontaneously or died. At the start of each experiment the exteriorized loop of catheter containing fetal lung liquid was interrupted, and the distal (lung) end of the catheter connected to a glass burette, under sterile conditions. By lowering the burette about half of the liquid in the fetal lung could be syphoned off into the burette. At the start of the experiment an impermeant tracer (^{125}I-labelled albumin, 1–2 μCi) was added to the liquid and by repeated elevation and lowering of the burette, even mixing of this tracer in lung liquid was maintained throughout the experiment. The use of a glass burette was a modification of our previous method in which a 50 ml syringe was used to mix the lung liquid (Walters & Olver, 1978). Lung liquid secretion or absorption rate was calculated from the changing concentration of the tracer over a 30 min period (Brown *et al.* 1983). Secretion or absorption rates were measured during two periods of observation in each experiment. After 30 min of mixing, lung liquid was sampled at intervals of 5 min until seven samples had been collected, allowing calculation of the resting secretion rate. Adrenaline (0·5 μg/min) was then infused through the fetal jugular catheter; 15 min after the commencement of this infusion a further seven samples were taken at 5 min intervals to allow calculation of the new secretion or

absorption rate. The condition of the fetus was monitored during the experiments by measurement of arterial blood gas tension, heart rate and blood pressure. The blood pressure transducer was connected to the fetal arterial catheter and fixed at a level which was judged to be approximately the height of the fetal heart. This did not allow comparison of the absolute systolic and diastolic blood pressure in the different groups but permitted measurement of changes in mean blood pressure during adrenaline infusion. In some fetuses there was evidence of partial or complete blockage of the arterial catheter and in these instances the tracing of heart rate and blood pressure were not used for analysis. At the end of each experiment sulphamethazine (165 mg) was both added to lung liquid and infused into the fetal circulation to prevent infection. The loop of tracheal catheter was then re-established to allow free passage of lung liquid to the larynx.

The daily dose of T_3 or T_4 (Henning, Berlin, FRG and Glaxo, London) was diluted to a 10 ml volume with sterile distilled water and infused continuously (Graseby Syringe Driver, type MS16a, Graseby Medical plc, Watford). T_3 was infused at 60 μg/day in the experiments started at 110 and 118 days gestation and at 120 μg/day from 125 and 131 days gestation. High rates of infusion were used to ensure relatively high plasma concentrations in the face of the rapid clearance of T_3 which has been reported in the fetal sheep (Fraser & Liggins, 1988). Although we did not precisely determine the half-life in our experiments, we have shown that following an intravenous bolus to the fetal sheep, the concentration of T_3 falls to its resting level within 24 h which indicates a much shorter half-life than the 24–36 h quoted for the adult by De Groot, Larsen, Refetoff & Stanbury (1984). T_4 was infused at 50 μg/day in experiments started at 118 days.

Thyroid hormone and adrenaline concentrations in fetal plasma

Thyroxine (T_4) and triiodothyronine (T_3) concentrations were measured using a commercial radioimmunoassay kit (Amerlex-M, Amersham). The lower limit of detection for T_4 was 3·1 ng/ml and for T_3 was 0·09 ng/ml. Samples of fetal blood taken for adrenaline estimation during the control period and adrenaline infusion were centrifuged at 4 °C and immediately stored at −70 °C. Plasma catecholamine levels were measured by a double-isotope modification (Brown & Jenner, 1981) of the radioenzymatic method of Da Prada & Zurcher (1976). There was a significant rise in resting fetal plasma adrenaline levels during adrenaline infusion at 0·5 μg/min (0·05 ± 0·01 to 0·93 ± 0·04 ng/ml, $n = 38$), similar to that previously reported in experiments on both euthyroid (Brown *et al.* 1983) and thyroidectomized fetuses (Barker *et al.* 1988).

Control data

Experiments on thyroidectomized (Barker *et al.* 1988) and non-thyroidectomized (Brown *et al.* 1983) fetuses carried out previously in our laboratory using an identical experimental protocol to that outlined above provided control data for adrenaline responsiveness in this study. Control thyroid hormone concentrations were measured in samples from chronically catheterized non-thyroidectomized fetuses undergoing an investigation of glucose transport in the fetal lung (Barker, Boyd, Ramsden, Strang & Walters, 1989*b*).

RESULTS

Thyroid hormone concentrations: infusion of T_4

Continuous intravenous infusion of T_4 (50 μg/day) was started 2 or 3 days after thyroidectomy at 114–117 days gestation. At the time of starting the infusions the mean fetal plasma T_4 levels were 19·3 ng/ml (range 3·9–42·7 ng/ml, $n = 4$) and plasma T_3 levels were at or below the lower limit of detection of the assay (0·09 ng/ml). Following hormone infusion mean plasma T_4 concentration rose to 77·7 ng/ml (S.E.M. 7·7 ng/ml, $n = 13$) which is similar to the mean value for euthyroid controls in Barker *et al.* 1988 (99 ng/ml, S.E.M. 10·6). The rise in plasma T_3 concentrations in these T_4-infused fetuses during the infusion period was similar to that seen in non-thyroidectomized fetuses (Fig. 1). T_3 data from only three of the four T_4-infused fetuses is shown, as the arterial sampling catheter in one of these fetuses became blocked at 131 days. Since these fetuses had been thyroidectomized

prior to infusion, the increasing level of T_3 in fetal plasma were presumed to reflect the changing peripheral metabolism of T_3 in the fetus (see Discussion). The effect of these changes can be expressed as the ratio of T_3 to T_4 concentration at difference gestational ages (T_3/T_4) which showed a progressive increase with gestation as in the euthyroid fetuses of similar gestations (Fig. 1). The small fall in T_3 levels in one of the T_4-infused fetuses (fetus a in Fig. 1) was attributed to a large fall in T_4 concentration possibly due to an undetected technical failure in the T_4 infusion; the T_3/T_4 ratio increased in this experiment in the same way as in the others (see Fig. 1*A*).

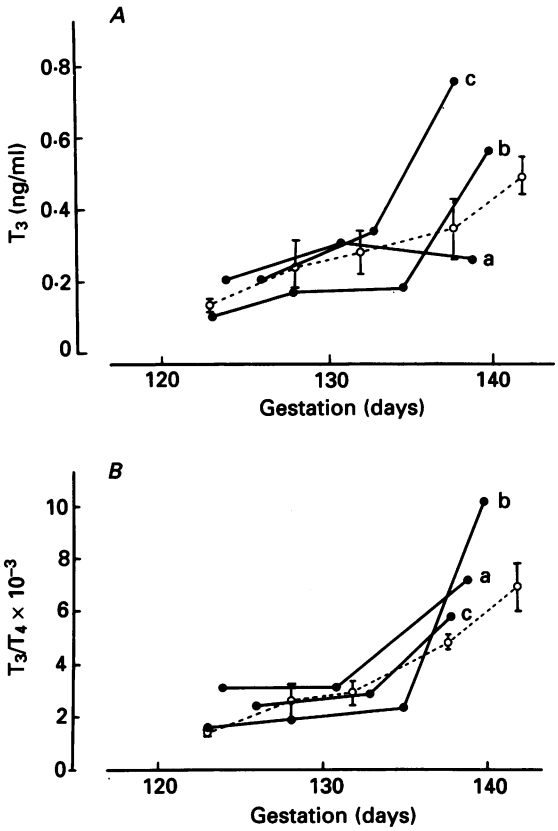

Fig. 1. T_3 levels in fetal plasma and T_3/T_4 ratio during T_4 infusion to thyroidectomized fetal sheep. ●, values from three fetuses (a, b, c) thyroidectomized at 114–117 days and infused with T_4 (50 μg/day), starting either 2 or 3 days later and continuing until delivery or death of the fetus. ○, mean values (±S.E.M.) from eight non-thyroidectomized fetuses in 5 day gestational age groups.

Thyroid hormone concentrations: infusion of T_3

In all fetuses in this group, fetal plasma T_3 concentrations had fallen below the limits of detection of the assay by the time T_3 infusion was started, but rose to a stable level by the third day of infusion. In fetuses where the T_3 infusion rate was 60 μg/day, plasma T_3 concentration rose to a mean of 0·87 ng/ml (S.E.M. 0·10 ng/ml. $n = 15$), while those infused at the higher rate of 120 μg/day had mean T_3 levels of

2·78 ng/ml (S.E.M. 0·31 ng/ml, $n = 27$). Concentrations of T_3 in the normal fetal sheep (ng/ml) are: 0·17 at 122 days, 0·21 at 127 days, 0·28 at 132 days, 0·31 at 137 days and 0·63 at 142 days (see Fig. 1*A*). The values obtained at the higher infusion rate compare with the peak of the postnatal surge which reaches values between 2 and 3 ng/ml at 2 h (Nathanielz, Silver & Comline, 1973*a*; Fisher, Dussault, Sack & Chopra, 1977).

Effect on heart rate, blood pressure and arterial blood gas tensions

During adrenaline infusion there was a significant rise both in heart rate (T_3-infused group: 153±5 to 169±4 beats/min, $n = 14$; T_4-infused group: 158±5 to 177±10 beats/min, $n = 10$) and mean blood pressure (T_3 group: a rise of 5·9±0·8 mmHg, $n = 22$; T_4 group: a rise of 4·2±1·6 mmHg, $n = 9$). These changes were very similar to those reported for control (Brown *et al.* 1983) and thyroidectomized fetuses (Barker *et al.* 1988). There were no significant differences in the responses of fetuses infused with 60 or 120 μg T_3 per day. Arterial blood gas tensions before and during adrenaline infusion were unchanged in all groups.

Maturation of the lung liquid reabsorptive response in T_4-infused fetuses

Infusion of T_4 at 50 μg/day in four fetuses from 118 days following thyroidectomy at 114–117 days restored the maturation of the reabsorptive response to adrenaline which had been inhibited in the hypothyroid fetuses (Fig. 2). The gestation at which the reabsorptive responses was first seen may have been delayed slightly when compared to the control data but the magnitude of the response was the same in both groups within all the gestational age groups (Table 1). There was no apparent dependence of reabsorptive capacity on the absolute concentration of T_4 or T_3 in these fetuses. Thus fetus 'a' in Fig. 1 had the largest reabsorptive response to adrenaline (−21·3 ml/h, Fig. 2) of the mature T_4-infused fetuses whilst its plasma T_3 and T_4 levels were the lowest in the group.

Maturation of the lung liquid reabsorptive response in T_3-infused fetuses

In the fetuses in which T_3 was infused from 110 and 118 days, an infusion rate of 60 μg/day was used and the infusion was started within 4 days of thyroidectomy. A higher rate of infusion (120 μg/day) was used when fetuses were infused from 125 and 131 days. In these latter groups there was a longer period of hypothyroidism between the time of thyroidectomy (115 to 118 days) and the start of the T_3 infusion (125 or 131 days) than in fetuses infused from 110 and 118 days in which T_3 infusion was started within 4 days of thyroidectomy.

When the T_3 infusion was started at 110 days, the reabsorptive response to adrenaline was first seen after 130 days gestation, some 3 weeks after the infusion had commenced. The magnitude of the response to adrenaline infusion before 135 days was significantly greater than in the control group (Table 1) and the earliest gestation at which the reabsorptive response appeared may have been brought forward by a few days as a result of the T_3 infusion (Fig. 3). T_3 supplementation from 118 days resulted in a pattern of maturation very similar to the T_4-infused and control groups. In these fetuses the reabsorptive response to adrenaline infusion was seen from 130 days onwards (Fig. 3) with no significant differences noted from the control fetuses

in secretion or absorption rates during adrenaline infusion within any of the gestational age groups (Table 1).

In the three fetuses infused with T_3 (120 μg/day) from 125 days, the capacity to reabsorb lung liquid was delayed until 135 days gestation – some 10 days after the T_3 infusion had commenced and about 5 days later than in the control group (Fig. 3

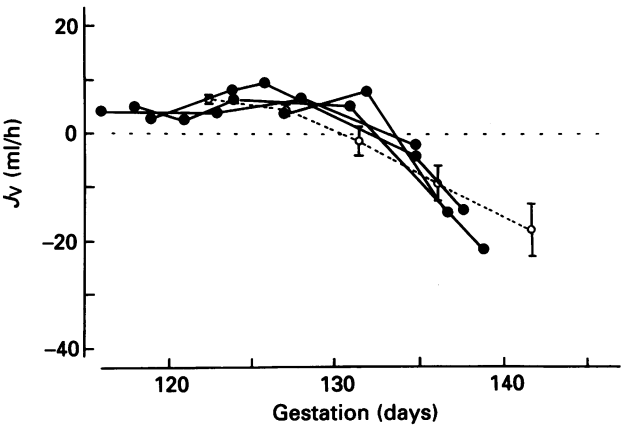

Fig. 2. Lung liquid secretion and absorption rates during 45 min periods of adrenaline infusion (0·5 μg/min) to thyroidectomized fetal sheep infused with T_4. Thyroidectomy was performed at 114–117 days and infusion with T_4 (50 μg/day) started 2 or 3 days later. ●, values from four thyroidectomized, T_4-infused fetuses. ○, mean values (± S.E.M.) from twenty-five euthyroid, non-thyroidectomized fetuses (Brown *et al.* 1983) in 5 day gestational age groups. The dashed line at zero delineates the secretion rate (values above zero, $+J_V$) or absorption rate (values below zero, $-J_V$) during adrenaline infusion.

and Table 1). After this gestation the magnitude of the response to adrenaline infusion was not significantly different from that of the control group. The three fetuses infused with T_3 (120 μg/day) from 131 days experienced a similar delay in the appearance of the reabsorptive response (Fig. 3) with reabsorption of lung liquid being seen in this group only after 138–141 days gestation. The magnitude of the response after this gestation was similar to that seen in the control group.

Resting secretion rates in control, thyroidectomized and T_3-treated fetuses

Over the period of study (120–147 days gestation) resting secretion rates in control fetuses showed a small rise between 130 and 140 days gestation before falling back to the levels seen at around 120 days (Brown *et al.* 1983). In the thyroidectomized fetuses resting secretion rates were significantly lower than in the control group below 130 days gestation, but these were in the restored in the T_3-infused fetuses whose secretion rates were similar to control values in all gestational age groups. The T_4-infused fetuses had resting secretion rates which were not different from control values except in the 120–124 day age group which had lower values (Table 2).

TABLE 1. Secretion ($+J_V$) or absorption ($-J_V$) rates of fetal lung liquid measured during a 45 min period of stimulation by an I.V. adrenaline infusion (0·5 μg/min) in thyroidectomized fetal sheep following T_3 or T_4 supplementation

	J_V (ml/h)					
	T_3 (from day below)					
Gestional group (days)	110	118	125	131	T_4 (from day 118)	Control (non-Tx)
115–119	6·3±0·7	—	—	—	—	4·0±0·9
n	3	—	—	—	—	3
120–124	4·6±0·4	5·9±1·6	—	—	4·5±1·2	6·5±0·6
n	3	3	—	—	3	9
125–129	2·3±1·0	6·7±2·7	11·5±3·9*	—	6·8±2·4	4·5±1·0
n	3	3	3	—	3	12
130–134	−13·3±1·5*	−5·4±3·6	10·5±0·9*	11·1±3·8*	6·9	1·3±2·4
n	3	3	3	3	2	10
135–139	−13·3	—	−15·7±6·8	5·9±2·0	−11·0±4·1	−9·2±3·3
n	2	—	4	6	5	11
140–144	—	—	—	−10·7±3·5	—	−17·9±4·8
n	—	—	—	5	—	4

* Significantly different from control value on *t* test ($P < 0·05$).

T_3 was given continuously at 60 μg/day in fetuses whose infusions started at 110 or 118 days and at 120 μg/day in fetuses whose infusion began at 125 or 131 days. T_4 was infused continuously in three fetuses at a rate of 50 μg/day. Control data for non-thyroidectomized (non-Tx) fetuses taken from Brown *et al.* (1983). Values are means±S.E.M.

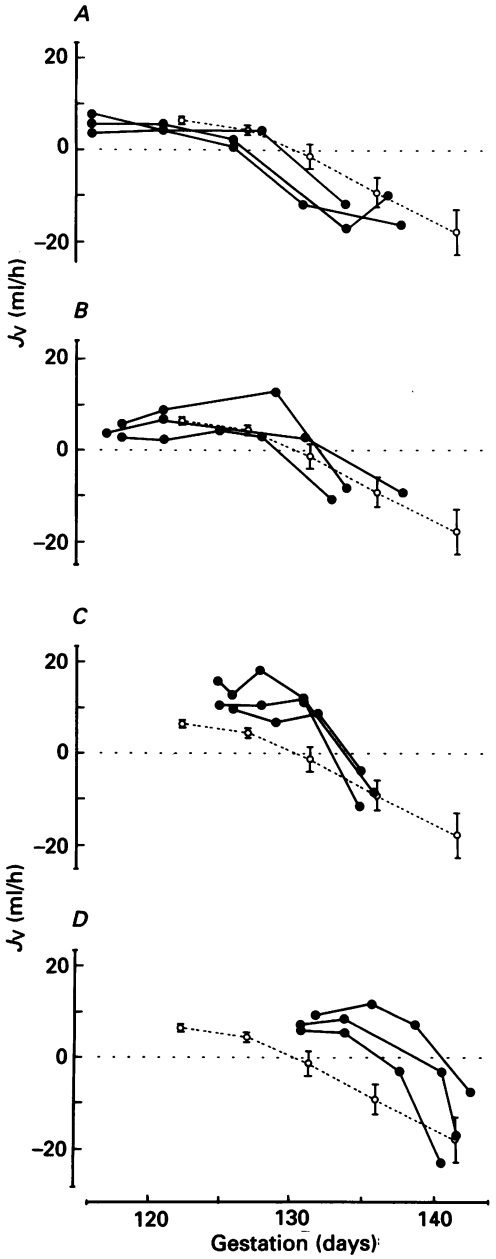

Fig. 3. Lung liquid secretion and absorption rates during 45 min periods of adrenaline infusion at 0·5 μg/min to thyroidectomized fetal sheep infused continuously with T_3. In each panel the dashed line at zero delineates the secretion rate (values above zero, $+J_V$) or absorption rate (values below zero, $-J_V$) during adrenaline infusion. ●, secretion or absorption rates during adrenaline infusion in T_3-infused fetuses. ○, mean values (±S.E.M.) for non-thyroidectomized fetuses (from Brown *et al.* 1983) in 5 day gestational age groups. *A*, fetal thyroidectomy at 106–107 days, T_3 infusion (60 μg/day) from 110 days. *B*, fetal thyroidectomy at 114–115 days, T_3 infusion (60 μg/day) from 118 days. *C*, fetal thyroidectomy at 118 days, T_3 infusion (120 μg/day from 125 days. *D*, fetal thyroidectomy at 118 days, T_3 infusion (120 μg/day) from 131 days.

TABLE 2. Comparison of resting (i.e. unstimulated by adrenaline) lung liquid secretion rates in fetal sheep in control, thyroidectomized (Tx), T_3 infused (60 μg/day) from 110–118 days (T_3), or T_4 infused (50 μg/day) from 118 days (T_4) preparations

Gestional group (days)	Lung liquid secretion rate (ml/h)			
	Control	Tx	T_3	T_4
120–124	11·7 ± 1·0	5·9 ± 1·4*	9·1 ± 1·2	7·4 ± 0·8
n	9	5	7	3
125–129	13·2 ± 1·3	8·3 ± 1·0*	15·0 ± 3·2	13·3 ± 2·6
n	12	6	6	3
130–134	17·3 ± 2·0	16·0 ± 3·9	13·1 ± 3·4	15·3 ± 2·6
n	10	6	6	3
135–139	17·0 ± 1·5	10·5 ± 2·5*	—	18·2 ± 3·2
n	11	5	—	4
140+	10·3 ± 2·8	14·3 ± 1·4	—	—
n	4	5	—	—

* Significantly different from control value on unpaired *t* test ($P < 0·05$).

Control data are taken from Brown *et al.* (1983) and thyroidectomized data from Barker *et al.* (1988). Values are means ± S.E.M.

DISCUSSION

Thyroid hormone levels in the fetus

The relative impermeability of the sheep placenta to maternal thyroid hormones (Comline, Nathanielsz & Silver, 1970; Hopkins & Thorburn, 1972) allows unhindered manipulation of fetal thyroid hormone levels by fetal thyroidectomy with or without hormone replacement. It was shown by Barker *et al.* (1988) that within a few days of fetal thyroidectomy the T_4 in fetal plasma falls to very low levels and that fetal levels of T_3 and rT_3 were at or below the limit of detection of the assay. Only a small proportion of the deiodination products of T_4 (the active hormone T_3 and its inactive counterpart rT_3) are secreted by the thyroid gland, the major fraction of these two hormones being derived from peripheral deiodination (Chopra, 1976). Throughout most of fetal life in the sheep and in other mammalian species, T_4 is deiodinated predominantly to rT_3 so that levels of this inactive hormone are relatively high compared to T_3 (Chopra, Solomon, Chopra, Wu, Fisher & Nakamura, 1978). During the last 3 weeks of gestation in the fetal sheep, rT_3 levels decrease and T_3 levels increase (Fisher *et al.* 1977). These changes are thought to result from an increase in T_3 production by outer ring deiodination of T_4 (Wu, Klein, Chopra & Fisher, 1978) and a simultaneous decrease in T_3 catabolism to T_2 due to decreasing activity of placental α-monoiodinase (Roti, Braverman, Fang, Alex & Emerson, 1982). The high metabolic clearance rate of T_3 reported by Fraser & Liggins (1988) probably reflects the activity of this placental enzyme. This also explains why we had to use such high rates of T_3 infusion to achieve the required plasma levels. Our thyroidectomized fetuses infused with T_4 showed the normal progressive increase both in the levels of T_4 and in the T_3/T_4 ratio during the period of study (118–140 days) confirming that the increase in T_3 levels usually seen in the fetus at this time results from changes in peripheral metabolism and not glandular secretion. The small fall in T_3 concentration seen in one fetus was closely related to a relatively larger fall in T_4 concentration.

However, the T_3/T_4 ratio in this fetus was similar to that in other infused and normal fetuses of the same gestation.

Infusion of T_4 at 50 μg/day resulted in fetal plasma T_4 levels which were similar to those in full-term euthyroid controls (Nathanielsz, Comline, Silver & Thomas, 1973*b*; Fisher *et al.*1977; Fraser & Liggins, 1988; Barker *et al.* 1989*a*). In fetuses where T_3 was infused at 60 μg/day, fetal plasma T_3 concentrations were about 60–100% higher than seen in euthyroid fetuses at full term (Fisher *et al.* 1977; Barker *et al.* 1988) while those infused at the higher rate of 120 μg/day had mean T_3 levels some 5-fold higher than seen at full term. The T_3 levels seen in this high infusion rate group were similar to those reported for newborn lambs during the postnatal T_3 surge (Nathanielsz *et al.* 1973*a*; Sack, Beaudry, DeLamater, Oh & Fisher, 1976).

The role of thyroid hormones in the responsiveness of fetal tissues to adrenaline

Thyroid hormones are known to affect the sensitivity of peripheral tissues to catecholamines (for review see Landsberg, 1977). The present results demonstrate the influence of thyroid hormones on maturation of the lung liquid reabsorptive capacity of the fetal pulmonary epithelium.

Barker *et al.* (1988) reported that fetal thyroidectomy at 118 days prevented the appearance of a reabsorptive response to infused adrenaline or to dibutyryl cyclic AMP added to lung liquid during the last 3 weeks of gestation (Barker *et al.* 1988). The present results demonstrate that following thyroidectomy, infusion of T_4 from 118 days at a rate equivalent to mature fetal T_4 production (Nathanielsz *et al.* 1973*b*) leads to maturation of adrenaline responsiveness in the normal way. This confirms the dependence of the reabsorptive mechanism in fetal alveolar epithelium on normal fetal thyroid hormone production during the final weeks of gestation.

A similar pattern of maturation was seen in fetuses infused with the active hormone T_3 from 118 days. Again, lung liquid reabsorption during adrenaline infusion was seen only after 130 days, while fetuses infused with T_3 from 110 days showed only a small advance in the timing of adrenaline responsiveness. Our inability to induce an earlier appearance of this response, despite exposure to high levels of T_3 in relatively immature fetuses, suggests that the maturation of T_3 metabolism which allows the normal rise in T_3 during the last 3 weeks of gestation cannot be the only factor controlling the appearance of this reabsorptive capacity. The fetuses given the higher rate of T_3 infusion from 125 and 131 days (following thyroidectomy at 118 days) were refractory to adrenaline for up to 10 days from the start of T_3 infusion. These later starting dates for infusion were chosen to assess the possible importance of other fetal factors, e.g. corticosteroid hormones, which appear around the time of the onset of the reabsorptive response. The delay in the appearance of the reabsorptive response suggests that 7–10 days exposure to adequate levels of T_3 is required to effect the changes in the epithelial Na^+ transport system which allow absorption to occur. Alternatively, the pulmonary epithelium may have been less responsive to T_3 since these fetuses experienced 1 or 2 weeks of profound hypothyroidism following thyroidectomy before T_3 replacement was started, whereas replacement had commenced shortly after thyroidectomy in fetuses infused from 110–118 days. Our inability, in the T_3-infused fetuses, to bring forward

the gestation at which reabsorption was first seen (in spite of T_3 concentrations similar to those at term) and the variability of the rise in T_3 seen after 130 days in the normal fetus suggest that T_3 has a permissive effect on development of the reabsorptive mechanism. The timing of the appearance of the reabsorptive response may indicate that a threshold T_3 concentration is required for the development of this response which, at 130 days gestation, would be about 0·3 ng/ml (Fig. 1). It is also clear that the plasma concentration of T_3 above a threshold value is not critically important in determining the magnitude of the response since there was no relationship between the relatively high T_3 levels seen in the T_3-infused fetuses and reabsorption rates after 130 days. Thus it is likely that there are additional fetal influences determining the maturation of the response to adrenaline.

The cardiovascular responsiveness to catecholamines does not appear to have been affected by the thyroid status of these fetuses. The resting heart rate and the rise in both heart rate and mean blood pressure during adrenaline infusion were very similar in the control, thyroidectomized, T_4- and T_3-infused fetuses, suggesting that thyroid hormones at the concentrations experienced by these fetuses do not have a direct or indirect influence on cardiac rate. This contrasts with the stimulatory effect of hyperthyroidism on cardiac rate reported in studies on the atria of adult rats (Thier, Gravenstein & Hoffmann, 1962) and the reported enhancement of β-adrenergic receptor activity on the myocardium in the hyperthyroid fetal rabbit (Das, Bandyopadhyay, Bandyopadhyay, & Neogi, 1984).

Mechanisms of action of thyroid hormones

Our current observations that adrenergic effects on the fetal lung are affected by fetal thyroid activity could reflect the action of thyroid hormones at one or more of several intracellular sites, including the β-adrenoreceptor, the intracellular second messenger system or part of the effector mechanism responsible for Na^+ reabsorption.

Previous studies (Olver *et al.* 1987) have shown unresponsiveness of the immature fetal pulmonary epithelium to dibutyryl cyclic AMP and maturation of the reabsorptive response similar to that seen with adrenaline infusion. This indicated that maturation of β-receptors, known to occur during late gestation in the fetal lung (Whitsett, Darovek-Beckerman, Manton & Adams, 1981), is not critical to the development of this particular adrenergic response. It implicated the gestational maturation of intracellular components further along the intracellular signalling system than the generation of cyclic AMP or of some component of the sodium transport system. Failure of mature thyroidectomized sheep fetuses to respond to dibutyryl cyclic AMP established that thyroid hormones influenced these intracellular events (Barker *et al.* 1988) and this conclusion was supported by the observation that the development of β-adrenoreceptors in the lung is not influenced by thyroid hormones (Giannopoulos & Smith, 1982).

The small decrease in resting secretion rate in the thyroidectomized fetuses and its restoration by T_3 infusion suggests that the underlying Cl^--driven secretory process may itself be influenced by thyroid hormones. Thyroid hormones are known to have a general influence on the activity of the basolateral Na^+–K^+-ATPase pump (Lo, August & Liberman, 1976) thought to be present in type II alveolar cells (Goodman, Fleischer & Crandall, 1983). According to the model of sodium-linked secondary

active chloride transport (Frizzell, Field & Schultz, 1979) proposed for fetal lung liquid secretion (Olver *et al.* 1986), any dysfunction of this pump due to hypothyroidism could influence both resting secretion and adrenaline-induced reabsorption of lung liquid. Our present results and those of Barker *et al.* (1988) suggests that while thyroid hormones may have a minor influence on resting secretion mechanisms, they are crucial to the maturation of mechanisms responsible for the reabsorption of lung liquid. This latter effect is likely to be due to the influence of these hormones on a component of the mechanism responsible for the reversible opening of sodium channels on the apical membrane of the alveolar epithelium (Olver *et al.* 1986).

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