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THE EFFECT OF THYROIDECTOMY IN THE FETAL SHEEP ON LUNG LIQUID REABSORPTION INDUCED BY ADRENALINE OR CYCLIC AMP

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SUMMARY

1. In fetal sheep at 113–120 days' gestation, thyroidectomy was performed and tracheal, arterial and venous catheters inserted. Following a recovery period experiments were performed from 120–145 days to measure changes in lung liquid secretion or its absorption in response to I.V. adrenaline infusion or to introduction of dibuteryl cyclic AMP into lung liquid. The results were compared with those previously obtained in non-thyroidectomized fetuses.

2. Plasma levels of thyroid hormones in non-thyroidectomized fetuses confirmed the pattern found by previous workers. In thyroidectomized fetuses the levels of thyroxine (T_4), tri-iodothyronine (T_3) and reverse T_3 (rT_3) were very low except in one fetus which showed biochemical evidence of thyroid regeneration towards the end of gestation.

3. In thyroidectomized fetuses the normal response to adrenaline infusion (diminution of reversal of lung liquid secretion) was profoundly suppressed and very little gestational maturation in this response took place, except in the one fetus with evidence of thyroid regeneration in which a normal reabsorptive response developed in late gestation.

4. In thyroidectomized fetuses, the normal response to dibuteryl cyclic AMP was greatly reduced and its increase with gestation which normally parallels that seen during adrenaline infusion did not take place.

INTRODUCTION

The reabsorption of fetal lung liquid which normally takes place during labour in response to a rise in plasma adrenaline has been shown to depend on the initiation of active sodium transport from the luminal to the abluminal side of the pulmonary epithelium (Brown, Olver, Ramsden, Strang & Walters, 1983; Olver, Ramsden, Strang & Walters, 1986). It has also been argued that changes in pulmonary blood flow and vascular pressure are unlikely to play an important role either in the formation of lung liquid (Olver & Strang, 1974) or in the reabsorption induced by

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adrenaline (Olver *et al.* 1986). It is a striking characteristic of fetal lung development – in the sheep at least – that the sensitivity of this reabsorptive mechanism to a given adrenaline concentration increases by more than an order of magnitude during the last 1–2 weeks of gestation (Brown *et al.* 1983). A parallel maturation of the reabsorptive response is also seen when the adrenergic stimulus is replaced by direct exposure of the pulmonary epithelium to cyclic AMP introduced into lung liquid (Olver, Ramsden & Walters, 1987). Hence maturation of the reabsorptive response is thought not to depend exclusively on development of β-adrenergic receptors but to involve one or more components beyond cyclic AMP in the chain of intracellular reactions initiated by β-adrenergic stimulation.

It seemed likely that the gestational development of this reabsorptive mechanism was under some kind of endocrine control; and, of possible agents which might play a role in this process, thyroid hormones were considered particularly likely in view of their known effect in augmenting several types of β-adrenergic response (reviewed by Sterling, 1979). It is also known that thyroid hormones promote maturation of structural aspects of lung development and of the surfactant system (for review see Ballard, 1986).

In order to determine whether thyroid hormones do indeed play a role in development of the reabsorptive response, we performed thyroidectomy in fetal sheep of less than 120 days gestation – some 2 weeks before the reabsorptive response to adrenaline infusion is regularly observed (Brown *et al.* 1983). This allowed us to compare maturation of the reabsorptive response to adrenaline and to cyclic AMP in a group of hypothyroid fetal sheep with that taking place in the euthyroid fetus. Preliminary accounts containing details of some of these experiments have been published (Barker, Brown, Ramsden, Strang & Walters, 1987; Barker, Ramsden, Strang & Walters, 1987).

METHODS

Surgical procedure. Operations were performed under sterile conditions on fifteen pregnant ewes (Clun Forest) of known tupping dates at 113–120 days gestation (mean, 118 days). Anaesthesia was induced with intravenous sodium thiopentone and followed by inhaled fluothane 1·5–2·5%, in a closed circuit. The fetal head was delivered through a hysterotomy and fetal thyroidectomy performed taking care not to damage the recurrent laryngeal nerves. Catheters inserted into the trachea, carotid artery and jugular vein were exteriorized for use during experiments as described by Walters & Olver, (1978). The ewes were given a 3 day post-operative course of intramuscular benzylpenicillin (300 mg/day) and streptomycin (500 mg/day) during which time no experiments were performed.

Experimental procedure. At the start of each experiment, the tracheal loop was interrupted under sterile conditions and a 60 ml syringe connected via a 3-way tap to the distal limb of the tracheal catheter. The fetal lung, trachea and tubing thus formed an isolated lung liquid compartment. Gentle withdrawal of the plunger allowed aspiration of about half the total lung liquid into the syringe. Repeated aspiration and re-injection allowed substances introduced during the experiment to be mixed evenly in lung liquid. Lung liquid secretion rate was calculated from the changing concentration of an impermeant tracer (^{125}I-albumin) added to lung liquid, exactly as described by Brown *et al.* (1983). The calculated changes in volume showed either a linear increase, reflecting secretion or a linear decrease, reflecting absorption.

In twenty-seven experiments on nine fetuses, the effects of intravenous adrenaline infusion on lung liquid secretion rate were measured. After a 30 min mixing period, lung liquid was sampled at intervals of about 5 min until seven samples had been collected. Adrenaline was then infused at

0·5 μg/min. Fifteen minutes after commencing this infusion, further samples of mixed lung liquid were taken to calculate the new secretion rate. In five thyroidectomized fetuses, seventeen experiments were performed in which dibutyryl cyclic AMP (db-cAMP, 5 mg) was added to lung liquid to give a final concentration of 10^{-4} M. In these experiments secretion rate before and 90–120 min after addition of db-cAMP was measured. The condition of the fetus was monitored during experiments by measurement of arterial blood gas tension, heart rate and blood pressure. Adrenaline infusion caused a rise in mean heart rate from 151 to 172 beats/min in the non-thyroidectomized (non-Tx) and from 153 to 158 beats/min in the thyroidectomized (Tx) fetuses. The increase in mean arterial pressure (2–3 mmHg) was similar in the two groups. No changes in arterial P_{O_2} or P_{CO_2} were observed. A small decrease in mean arterial blood pH from 7·31 to 7·29 during infusion was similar to that previously seen in non-Tx fetuses. Additions of cyclic AMP to lung liquid produced no changes in any of these measurements. At the end of each experiment 165 mg of sulphamethazine was both added to lung liquid and infused into plasma to prevent infection. The loop of tracheal catheter was then re-established to allow free passage of lung liquid towards the larynx. A minimum interval of 2 days between experiments was allowed.

Thyroid hormone and adrenaline concentrations in fetal plasma. Thyroxine (T_4) and tri-iodothyronine (T_3) concentrations were measured using a commercially available (Amerlex-M, Amersham) radioimmunoassay kit. The lower limit of detection for T_4 was 3.10 ng/ml and for T_3 was 0·09 ng/ml. Reverse T_3 (rT_3) was measured with a commercial kit (Serono); the lower limit of detection was 0·02 ng/ml. Samples from non-thyroidectomized fetuses were diluted four times to increase accuracy at the upper limits of detection of this assay (2 ng/ml) as relatively high levels of rT_3 are normally seen in the sheep fetus. Samples of fetal arterial blood taken for adrenaline estimation during the control period and adrenaline infusion were centrifuged at 4 °C. Samples were stored at −70 °C and the measurements made within six months of collection. Plasma catecholamines were measured by a double-isotope modification (Brown & Jenner, 1981) of the radioenzymatic method of Da Prada & Zurcher (1976).

Calcium concentrations. Samples for plasma calcium estimation were taken from the arterial catheter in fetal sheep. Measurements were made on a Technicon Smac computer-controlled biochemical autoanalyser utilizing the method of Gitelman (1967). These levels were 'corrected' for differences in plasma albumin between Tx and non-Tx fetuses to a standard albumin concentration of 27 g/l by adding or subtracting 0·02 mM-calcium per gram of difference between the observed albumin concentration and 27 g/l (see Editorial: Correcting the calcium, 1977). Lung liquid calcium was measured on a Roche-Cobas-Bio centrifugal analyser using a methylthymol blue-chelating reaction (Gindler & King, 1972).

Statistics. Unless otherwise stated, all values given are mean (±standard error of the mean). Paired data and non-paired data were compared using paired and non-paired Student's t tests. Regressions were calculated using the least-squares method. Statistically significant results assume a P value of $< 0·05$.

Control data. Experiments on non-thyroidectomized fetuses carried out in our laboratories (Brown *et al.* 1983; Olver *et al.* 1987) formed the control group for this study and provided the protocol for the adrenaline infusion and db-cAMP experiments on the thyroidectomized fetuses described in this paper.

RESULTS

Thyroid hormone concentrations

Table 1 gives values of thyroid hormone concentration in thyroidectomized (Tx) and control (non-Tx) fetuses. Control hormone concentrations were measured in samples taken from non-Tx fetuses which had been subjected to the same surgical procedures as the Tx fetuses, except for thyroidectomy, and which were undergoing similar experiments in another project. In fourteen out of fifteen of the Tx fetuses levels of thyroxine (T_4), tri-iodothyronine (T_3) and reverse T_3 (rT_3) were near or below the lower limits of detection for the respective assays in clear distinction to levels measured in the control group which were in the range expected for normal

TABLE 1. Mean values (±S.E.M.) for plasma concentrations of thyroid hormones

	T_4 (ng/ml)		T_3 (ng/ml)		rT_3 (ng/ml)	
	Non-Tx	Tx	Non-Tx	Tx	Non-Tx	Tx
120–129 days gestation	98·8 (±10·6)	7·2 (±0·8)	0·21 (±0·03)	0·09 —	3·20 (±0·33)	0·09 (±0·03)
n (samples)	5	29	10	18	5	16
n (below detection)*	0	6	0	18	0	0
130+ days gestation	70·6 (±21·4)	5·6 (±0·7)	0·36 (±0·05)	0·09 —	3·94 (±0·29)	0·04 (±0·02)
n (samples)	8	27	17	11	5	15
n (below detection)*	0	5	0	11	0	3
Number of fetuses	4	14	8	7	5	9

* Lower limits of detection (ng/ml) were: $T_4 = 3{\cdot}10$, $T_3 = 0{\cdot}09$, $rT_3 = 0{\cdot}02$. These levels were used in calculating the means when the concentration was below the limit. Hence the means for the Tx fetuses are overestimated.

sheep fetuses at the gestations studied (Nathanielsz, Comline, Silver & Thomas, 1973; Fisher, Dussault, Sack & Chopra, 1977; Nwosu, Kaplan, Utiger & Delivoria-Papadopoulos, 1979; Mathur, Brown, Krane, Thomas & Nathanielsz, 1980). In one thyroidectomized fetus there was biochemical evidence of regeneration of thyroid function in late gestation. In this fetus T_4 levels rose from 5 ng/ml at 127 days to 26 ng/ml at 145 days. At this gestation T_4 levels in the other Tx fetuses were negligible, and in the non-Tx fetuses were about 55 ng/ml. The results from this fetus are not included in Table 1 and the effects of adrenaline infusion were analysed separately from the others in the thyroidectomized groups.

Calcium concentrations

To determine effects of possible inadvertent parathyroidectomy in the Tx fetuses, we measured total calcium levels in plasma and lung liquid of both Tx and non-Tx fetuses. Plasma albumin (g/l) was significantly lower in the Tx fetuses (mean in Tx fetuses, $22{\cdot}2 \pm 0{\cdot}4$, $n = 25$; mean in non-Tx fetus = $27{\cdot}2 \pm 0{\cdot}6$, $n = 8$). When allowance was made for the difference in albumin concentrations, the mean plasma calcium value in the Tx fetuses ($2{\cdot}92 \pm 0{\cdot}09$ mM, $n = 25$) was not significantly different to that in the control group ($3{\cdot}11 \pm 0{\cdot}06$ mM, $n = 8$). There were likewise no significant differences in the mean calcium concentrations in lung liquid (Tx = $0{\cdot}35 \pm 0{\cdot}02$ mM, $n = 40$; non-Tx = $0{\cdot}36 \pm 0{\cdot}03$ mM, $n = 25$).

The effect of adrenaline infusion

Fetal lung liquid secretion rate was measured before and during intravenous infusion of adrenaline (0·5 μg/min) at various gestational ages. Table 2 gives the results of these experiments in Tx fetuses (excluding the fetus showing evidence of thyroid regeneration) and of experiments on non-Tx fetuses from Brown *et al.* (1983).

At gestations below 130 days, the mean resting secretion rate (J_v^c) in the Tx fetuses was significantly lower than in non-Tx fetuses but in the mature groups these rates were not significantly different. The most striking difference between the two groups however was in their response to adrenaline. In non-Tx fetuses of less than 130 days gestation, the usual response to infusion was a slight slowing of secretion rate, whereas fetuses over this gestation responded to the same stimulus with reabsorption of lung liquid. This reabsorptive response increased considerably with advancing gestational age. In contrast, in the Tx fetuses at gestations under 130 days, there was negligible slowing of secretion rate during adrenaline infusion and beyond this gestation, although significant slowing of secretion did occur in some of the fetuses, reabsorption was observed in only three out of seventeen (18%). In contrast reabsorption was seen in eighteen of twenty-four (72%) non-Tx fetuses with gestations over 130 days. The reabsorption rate in the three Tx fetuses referred to above was considerably less than expected for euthyroid fetuses of this gestation (Fig. 1).

In the one Tx fetus with evidence of thyroid regeneration the responsiveness to adrenaline resembled that of the euthyroid rather than the hypothyroid group. Thus, at 144 days, the secretion rate in this fetus changed from 17 ml/h during the control period to reabsorption at 21 ml/h during the adrenaline infusion.

Relationship of secretion and absorption rates to plasma adrenaline concentrations

The mean plasma concentrations of adrenaline in carotid arterial blood before and during adrenaline infusions were not significantly different in the Tx and non-Tx fetuses. Before infusion, the adrenaline concentration (ng/ml) in Tx fetuses was

TABLE 2. Mean (±S.E.M.) secretion rates before (J_v^c) and after (J_v^a) intravenous infusion of adrenaline at 0·5 μg/min in euthyroid (non-Tx) and thyroidectomized (Tx) fetuses

Gestation (days)	n		J_v^c (ml/h)		J_v^a (ml/h)	
	Non-Tx	Tx	Non-Tx	Tx	Non-Tx	Tx
120–124	9	5	11·7 (±1·0)	5·9 (±1·4)	6·5 (±0·6)	4·7 (±1·3)
125–129	12	6	13·2 (±1·3)	8·3 (±1·0)	4·5 (±1·0)	4·9 (±0·4)
130–134	10	6	17·3 (±2·0)	16·0 (±3·9)	−1·3 (±2·4)	9·3 (±1·4)
135–139	11	5	17·0 (±1·5)	10·5 (±2·5)	−9·2 (±3·3)	1·9 (±2·4)
140+	4	5	10·3 (±2·8)	14·3 (±1·4)	−17·9 (±4·8)	3·7 (±4·0)

Negative values of J_v indicate absorption. Euthyroid data from twenty-five fetuses, Tx data from nine fetuses. Data for euthyroid fetuses from Brown *et al.* (1983).

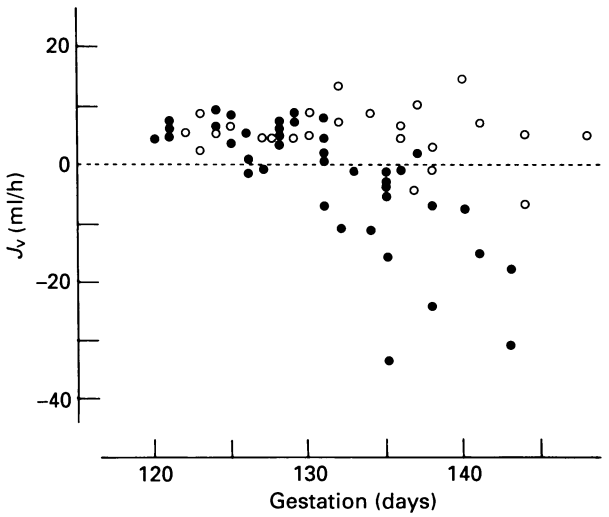

Fig. 1. Ordinate: J_v, lung liquid secretion rate during I.V. adrenaline infusion at 0·5 μg/min. Negative values indicate absorption. Abscissa: gestational age at time of experiment. ●, euthyroid (non-Tx) fetuses; ○, thyroidectomized (Tx) fetuses.

0·09 (±0·03, $n = 23$) and in the non-Tx fetuses was 0·10 (±0·01, $n = 32$). During adrenaline infusion the concentration rose to 1·61 (±0·28, $n = 25$) in the Tx fetuses and to 1·01 (±0·14, $n = 18$) in the non-Tx fetuses.

In non-Tx fetuses of over 130 days gestation, Brown *et al.* (1983) demonstrated dependence of secretion rate (J_v) on plasma adrenaline concentration [A], and as

gestation advanced this dependence increased strikingly, as shown by gestational changes in the intercept and slope of the regressions of J_v on ln[A] (Fig. 2). In the thyroidectomized fetuses the dependence of J_v on [A] was much less and showed little or no change with gestational age (Fig. 2). In the euthyroid (non-Tx) fetuses an

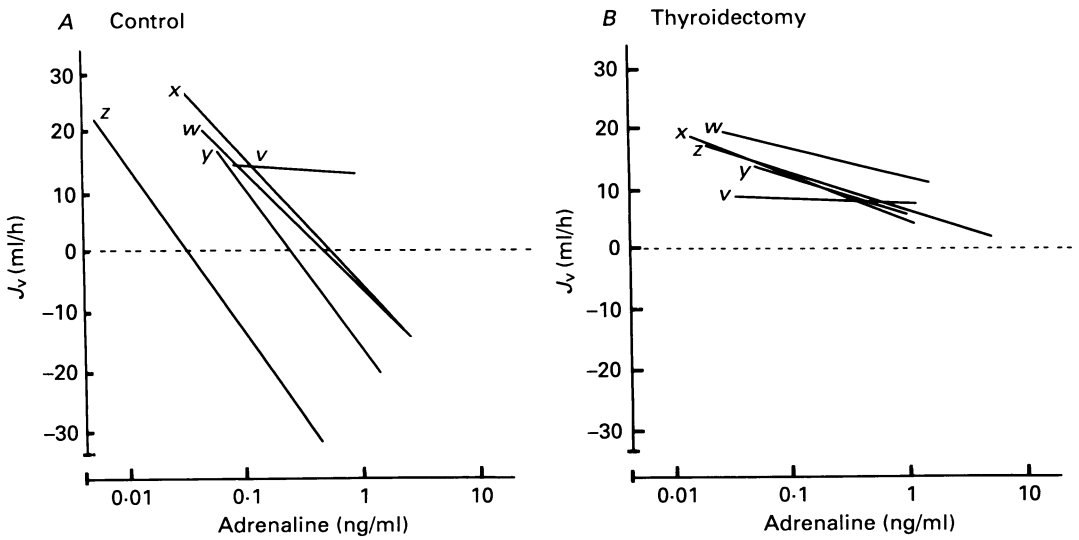

Fig. 2. Regressions of J_v on ln[A] at five gestations. v = 129–131 days, w = 132–134 days, x = 135–137 days, y = 138–140 days, z = 141+days. The range of data points used to calculate the regressions is indicated by the length of the lines illustrated. *A*, regressions for non-Tx fetuses calculated from data of Brown *et al.* (1983). *B*, regressions for Tx fetuses. Parameters of the regression $J_v = a + b$ ln[A] are given below. $[A_I]$ (ng/ml) is the intercept of regression at $J_v = 0$. For the Tx fetus this value was obtained by extrapolation of regressions x, y and z.

Gestation (days)		n	a	b	$[A_I]$(ng/ml)
v	Tx	6	−10·4 (±5·3)	0·6 (±0·9)	—
(129–131)	Non-Tx	8	−17·6 (±10·8)	0·8 (±2·0)	—
w	Tx	6	−11·8 (±2·6)	2·0 (±1·1)*	—
(132–134)	Non-Tx	11	7·0 (±2·6)	8·4 (±1·1)	0·43
x	Tx	7	−4·6 (±2·3)	3·3 (±1·1)*	4·03
(135–137)	Non-Tx	11	6·5 (±3·6)	9·1 (±1·9)	0·49
y	Tx	4	−5·7 (±5·7)	2·8 (±3·2)	7·66
(138–140)	Non-Tx	10	17·5 (±5·1)	11·5 (±2·7)	0·22
z	Tx	6	−6·3 (±2·6)	2·7 (±1·2)*	10·31
(141+)	Non-Tx	4	42·5 (±9·3)	11·14 (±2·4)	0·03

* Significantly different from non-Tx value, $P < 0·05$.

estimate of the minimum concentration required to induce reabsorption $[A_I]$ was obtainable from the intercept of the regression of J_v on ln[A] at $J_v = 0$ (see Brown *et al.* 1983) and this decreased from 0·43 to 0·029 ng/ml during the gestation period between 132 and 144 days. In the Tx fetuses, estimates of $[A_I]$ could be made only by extrapolation of the regressions. When this was carried out on the regressions

shown as x, y and z in Fig. 2, $[A_I]$ values between one and two orders of magnitude greater in the thyroidectomized than in the non-thyroidectomized fetuses were obtained (see Fig. 2 legend).

The effect of dibuteryl cyclic AMP (db-cAMP) added to lung liquid

It has been shown that reabsorption of lung liquid can be induced directly by db-cAMP, added to lung liquid and that this effect increases with gestation in close

TABLE 3. Mean (±S.E.M.) secretion rates before (J_v^c) and 90–120 min after adding db-cAMP (10^{-4} M) to lung liquid (J_v^{AMP}) in euthyroid (non-Tx) and thyroidectomized (Tx) fetuses

Gestation (days)	n		J_v^c (ml/h)		J_v^{AMP} (ml/h)	
	Non-Tx	Tx	Non-Tx	Tx	Non-Tx	Tx
120–130	7	8	11·0 (±1·3)	6·9 (±0·4)	4·8 (±1·5)	6·9 (±0·7)
130–134	6	3	16·2 (±1·6)	5·2 (±0·5)	3·3 (±2·1)	6·2 (±1·7)
135–139	6	3	14·1 (±2·3)	9·2 (±0·5)	−15·6 (±6·5)	6·4 (±1·1)
140+	3	3	14·4 (±4·6)	10·9 (±1·0)	−41·2 (±7·6)	9·3 (±1·4)

Negative values indicate absorption. Non-Tx data from eight fetuses, Tx data from five fetuses. (Data for euthyroid fetuses from Olver *et al.* 1987.)

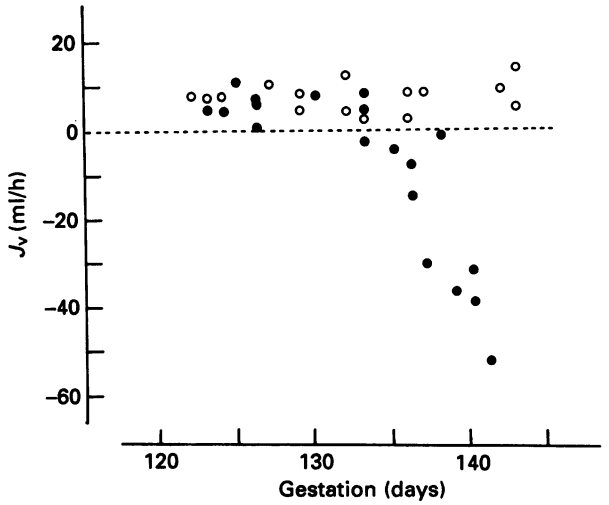

Fig. 3. Ordinate: J_v, 90–120 min after adding dibuteryl cyclic AMP (10^{-4} M) to lung liquid. Abscissa: gestational age at time of experiment. ●, euthyroid (non-Tx) fetuses; ○, thyroidectomized (Tx) fetuses.

parallel to the maturation of the response to adrenaline (Olver *et al.* 1987). To test this response after thyroidectomy, we carried out eighteen experiments on five thyroidectomized fetal sheep to measure the effect of adding 5 mg db-cAMP to lung liquid to give an estimated initial concentration of 10^{-4} M. It had been shown by Olver *et al.* (1987) that the maximal effect of db-cAMP took place in the second hour

after putting this substance into lung liquid. For this reason the effect on J_v was measured at 90–120 min after adding db-cAMP. As shown in Table 3 and Fig. 3, which compare results in the Tx fetuses with those in the non-Tx fetuses reported by Olver *et al.* (1987), no increase with gestation in the response to db-cAMP was seen in the Tx fetuses.

DISCUSSION

The pattern of T_4, T_3 and rT_3 concentrations in the non-thyroidectomized control fetuses as well as the very low levels observed after thyroidectomy were similar to those found by previous investigators (Nathanielsz *et al.* 1973; Fisher *et al.* 1977; Nwosu *et al.* 1979; Mathur *et al.* 1980). The data from the thyroidectomized fetuses confirm that materno-fetal transfer of thyroid hormones in late gestation is slight although not necessarily absent. It is noteworthy that the small rise in T_3, normally taking place from 130 days (Nwosu *et al.* 1980), coincides with development of the reabsorptive response to adrenaline, suggesting that T_3 could be an effective agent determining this development (Tables 1 and 2).

Thyroidectomy prevented most of the gestational maturation in the response to adrenaline except in the one fetus showing biochemical evidence of thyroid regeneration. The lack of maturation in the remaining fetuses is most evident when the response is related to the adrenaline level in carotid arterial plasma. Since both pulmonary arterial and carotid flows in the fetus are largely derived from a common pool, most of which has not passed through the lungs, the carotid arterial concentration of adrenaline can be taken as a good approximation to the strength of the adrenergic stimulus delivered to the abluminal surface of the pulmonary epithelium. The residual maturation observed could have been due to minimal feto-maternal transfer of thyroid hormones. Thyroidectomy also impaired the reabsorptive response to cyclic AMP and virtually abolished its gestational maturation (Fig. 3). It follows that the effect of thyroid hormones cannot be attributed to their action on β-adrenergic receptors and this conclusion is in accord with the observation of Giannopolous & Smith (1982) that the development of β-adrenoreceptors in the fetal lung is uninfluenced by T_3. It must be concluded therefore that thyroid hormones are required for the maturation of a component or components beyond cyclic AMP in the intracellular signalling system normally activated by β-adrenergic stimulation. Olver *et al.* (1986) proposed a model for transepithelial ion transport in the fetal lung to explain the switch from net chloride secretion in the fetus (Olver & Strang, 1974) to net sodium reabsorption in response to adrenaline. In this model a key role is played by apical membrane sodium channels, capable of reversible opening in response to a rise in cyclic AMP. If the model is correct, thyroid hormones could be required either for synthesis of a protein or proteins controlling channel opening, or for synthesis of the channels themselves. These mechanisms imply mediation of hormone action by nuclear T_3 or T_4 receptors, but at present an alternative mechanism for thyroid hormone action via mitochondrial receptors cannot be excluded (for review, see Sterling, 1979).

Although the thyroidectomized fetuses were not studied in labour or after birth our results suggest that their reabsorptive response to endogenous adrenaline would

not have been sufficient to ensure adequate clearance of lung liquid allowing normal independent ventilation. Hopkins & Thorburn (1972) found that most thyroidectomized sheep fetuses survived for less than an hour after natural delivery. There were probably several reasons, including hypothermia, for this limited survival and there appear to have been no detailed studies of post-natal respiratory function in the hypothyroid animal. Fetal thyroid status clearly affects the incidence of respiratory problems in the human newborn since low cord levels of both T_3 and T_4 are associated with an increased risk of Respiratory Distress Syndrome (Redding & Pereira, 1974; Cuertas, Findall & Engel, 1976; Abassi, Merchant & Abramson, 1977). Similarly in infants found to be hypothyroid in a neonatal screening programme, the incidence of respiratory distress was some ten times commoner than expected (Fernhoff, Brown & Elsas, 1987). Since thyroid hormones are known to influence lung growth and maturation of the surfactant system, it must remain uncertain what contribution any deficiency in the lung liquid transport system might make to these clinically observed respiratory difficulties. In this connection it is important that infants with congenital hypothyroidism generally have T_4 levels of 20–40 ng/ml (DeGroot, Larsen, Refetoff & Stanbury, 1984) whereas our thyroidectomized sheep fetuses all had levels below 10 ng/ml. Presumably there is either a more efficient transfer of material hormone to the human than to the sheep fetus or there is a significant residual production of thyroid hormones in most cases of human congenital hypothyroidism.

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