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Original Article

The influence of thyroid hormones on the growth of the lungs in perinatal rats

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ABSTRACT The growth and rates of protein turnover in the perinatal lung have been studied in the rat during normal development between late gestation and weaning, and after altering their thyroid status. The aim was to establish what influence thyroid hormones have on the early stages of growth in the lungs. Perinatal hypothyroidism was induced by administering propylthiouracil (PTU) via the mothers' drinking water from late gestation and throughout lactation. A precocious and elevated surge of thyroid hormones was induced by daily injections of T_4 from day 3 *postpartum* onwards. Hypothyroidism in the neonate, but not the fetus, significantly retarded the growth of the animal and its lungs. This was attributable to a decrease in both the pulmonary rates of protein synthesis and protein degradation; the effect on the former rate exceeding that on the latter. Neonatal hyperthyroidism did not significantly alter protein turnover or the growth of the lungs, compared with euthyroid control tissues. This contrasts with the accelerated growth of some other body tissues in the presence of excess thyroid hormones.

KEY WORDS: *perinatal lung growth, protein synthesis, thyroid status, RNA, DNA*

Introduction

In the fetus the lung emerges from a small dense vascular structure to become a compliant tissue with a large surface area ideally suited for gaseous exchange (Thurlbeck, 1975). The timely growth and development of the airways, respiratory musculature and extra thoracic components, such as chemo-receptors and medullary central control, are all crucial for active respiration at birth.

The temporal maturation of the lungs in late gestation, in terms of their ability to produce surfactant and anti-oxidant enzymes, appears to be largely endocrine driven and is vital for survival in pre- and full-term offspring. Thyroid hormones, independently or in conjunction with adrenal cortical steroids, have been implicated in the growth and maturation of the perinatal lung (Ayromlooi *et al.*, 1983; Ballard, 1990). For example, respiratory distress syndrome has been described in high proportions of congenitally-hypothyroid infants (Smith *et al.*, 1975), while thyroidectomy of fetal lambs leads to decreased body and lung weights (Blakthavathsalan *et al.*, 1981). In the adult rat, thyroidectomy reduces the diameter of the alveolar type II cells by one-third, but all other parenchymal cells were unaffected (Redding and Douglas, 1972).

Surprisingly, the growth and rates of protein turnover in the whole lung have been little studied; this being particularly true of the perinatal period. Protein turnover in the lung of adult animals has been measured in various normal and pathological conditions, but generally using *in vitro* methods which leave doubts concerning the accuracy of the absolute rates so measured (Chiang *et al.*, 1979; Rannels *et al.*, 1979; Clark *et al.*, 1980; Watkins and Rannels, 1980). Although a study from this laboratory (Goldspink, 1987) covered most of the lifespan of the rat, the suckling period was not investigated. Hence, in the absence of reliable information concerning the perinatal period we have examined the normal developmental changes in lung growth, its rates of protein turnover and the effects induced by either lowering or increasing the endogenous levels of thyroid hormones.

Results

During the development of the perinatal rat there is an approximate 6- and 4-fold increase in the circulating concentrations of T_3 (Fig. 1) and T_4 (Fig. 2), respectively between late gestation and weaning. These levels subsequently decline to lower values in the adult (Figs. 1 and 2). Although the oral administration of PTU to the mothers did not produce hypothyroidism in the fetus, it did effec-

Abbreviations used in this paper: PTU, propylthiouracil; T_3, tri-iodothyronine; T_4, thyroxine.

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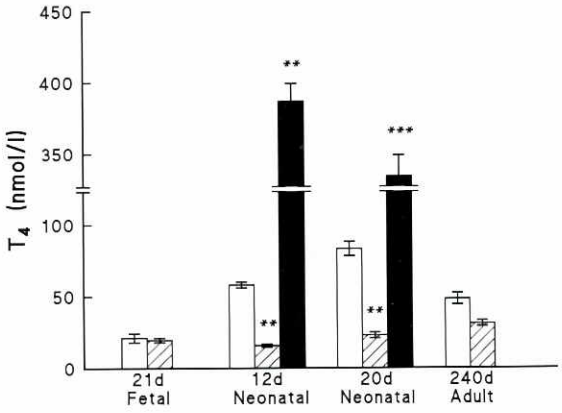

Fig. 1. Age- and treatment-related changes in T_3 plasma concentrations. *Total T_3 concentrations were measured in euthyroid controls (❑), PTU-treated (▨) and T_4 injected (■) fetuses or neonates using a double antibody ^{125}I radioimmunoassay. Results are the means±SEM of 3-12 plasma samples, derived from the pooled blood of 12-13 fetuses, 2-3 neonates or individual adult animals. *P<0.05; **P<0.01; ***P<0.001.*

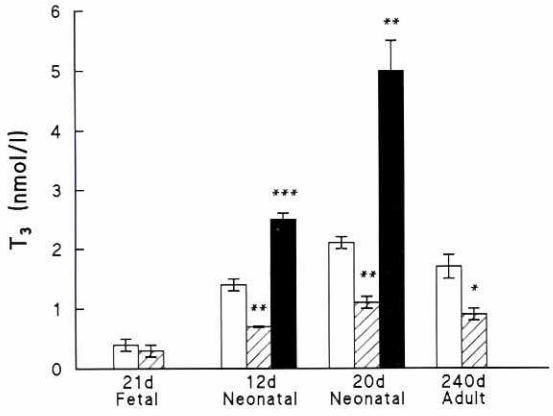

Fig. 2. Age- and treatment-related changes in T_4 plasma concentrations. *Total T_4 concentrations were measured in the same plasma samples as for T_3 in control (❑), PTU-treated (▨) and T_4 injected (■) animals, as in Fig.1. *P<0.05; **P<0.01 or ***P<0.001, compared with control values.*

tively block T_4 and substantially reduce T_3 levels in the neonates between birth and 20 days *postpartum*. In contrast, the postnatal administration of exogenous T_4 both advanced and increased circulating levels of the thyroid hormones. These experimental manipulations of the thyroid status could then be studied alongside the normal developmental changes helping to establish the impact of thyroid hormones on the growth of the neonatal lungs.

Two groups of control neonates were initially used to allow for possible differences arising from the different routes of administering PTU (i.e. orally) or exogenous T_4 (i.e. by injection). The latter could conceivably have stressed the animals. However, no significant differences were found between these two groups, nor the male and female pups within them (data not shown). Hence, these data have been pooled and presented as one group of euthyroid controls.

In the euthyroid perinate the lungs undergo rapid growth, as reflected by 4-fold increases in their wet weight, protein and DNA contents and a 2.5-fold increase in their RNA (Figs. 3-7). The increase in DNA (Fig. 6) and protein:DNA (Fig. 7) can provide useful, if rather crude, indications of whether the enlargement of the tissue is occurring through changes in cell number and/or in cell size. From these measurements it is apparent that most of the growth of the lungs during the 21 days studied arose from massive increases in cell number rather than changes in cellular hypertrophy (Figs. 6 and 7). The types of cells undergoing an increase in number were not however identified.

Despite the 4-fold increase in the weight and protein mass of these perinatal lungs, the growth rates approximately halved, i.e. they declined from 7.3%/day in the fetal tissue to 3.6%/day at 20 days *postpartum*. During this period the total rate of protein synthesis increased by 40%. This was, however, largely due to the large increase in the protein mass since the fractional rate of protein synthesis actually declined from 60 to 22%/day (Tables 1 and 2). This age-related fall in the fractional synthetic rate was paralleled by a similar 38% reduction in both the ribosomal capacity and the ribosomal activity.

None of the measurements of growth in the fetal lung were significantly affected by maternal PTU treatment (Figs. 3-7; Table 1). This is perhaps not surprising since the slightly lowered T_3 and T_4 levels (Figs. 1 and 2) in these fetuses were not significantly different from those of the euthyroid controls. Hence, we cannot be certain

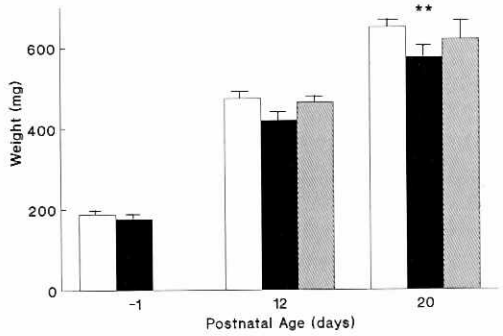

Fig. 3. Changes in the wet weights of the lungs in relation to age and thyroid status. *The wet weights of the whole lungs were determined and expressed as means SEM for a minimum of 6 control (❑), hypo- (■) or hyper- (▨) thyroid perinates. **P<0.01.*

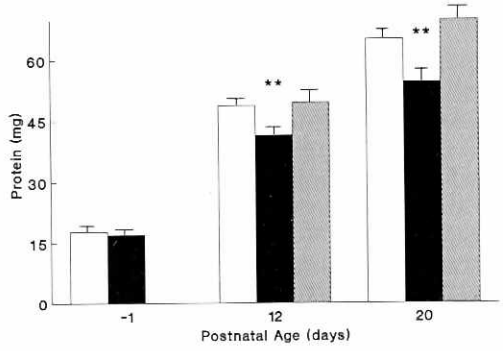

Fig. 4. Protein contents of the perinated lungs of eu-, hypo- and hyperthyroid rats. *The protein contents of the same lungs as in Fig.3 were measured (Lowry et al., 1951) for controls (❑), hypo- (■) and hyper- (▨) thyroid rats. **P<0.01.*

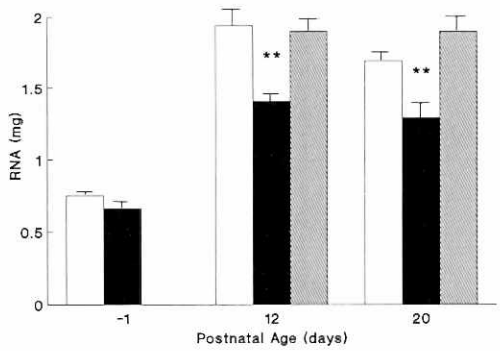

Fig. 5. Effects of thyroid status on the RNA content of the perinatal lungs. *Total RNA was extracted and measured spectrophotometrically (Ashford and Pain, 1986) in the same lungs as described in Fig. 3. Control (□), hypo- (■) and hyper- (▨) thyroid animals. **P<0.01.*

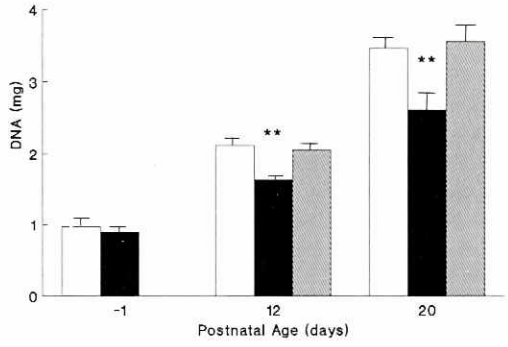

Fig. 6. DNA content of the lungs from eu-, hypo- and hyper-thyroid perinates. *DNA was extracted and measured (Goldberg and Goldspink, 1975) for the same tissues as in Fig. 3. **P<0.01. In tissues possessing mononucleate cells this can give an indication of cell number.*

that a true state of hypothyroidism existed in the fetuses.

In contrast, by 12 and 20 days *postpartum* the thyroid hormones were significantly lowered and the lungs of the hypothyroid neonates consistently growth retarded (Figs. 3-7). This suppression of growth (i.e. protein accumulation) was a consequence of a 40-60% inhibition of protein synthesis, which in turn was linked to decreases in both the ribosomal activity and the ribosomal capacity (Table 2). As the rates of protein synthesis were suppressed to a greater extent than that required to explain the 16% reduction in the protein mass, it follows that protein degradation must also have decreased in response to the low levels of thyroid hormones. Meaningful rates of protein degradation cannot be readily measured *in vivo* (Waterlow *et al.*, 1978). However, indirect rates can be calculated by measuring the rates of protein synthesis and tissue growth (i.e. accumulation of protein) between 12 and 20 days and subtracting the latter value from the former (Garlick *et al.*, 1979). When these calculations are made, the control lungs possess a rate of protein degradation equal to 18.7%/day with this falling to 10%/day in the PTU-treated animals.

No attempts were made to examine the effect of hyperthyroidism on fetal lung growth because unlike hypothyroidism this could not be accomplished without affecting maternal metabolism, making it difficult to interpret any possible changes in fetal lung growth. It had been anticipated that hyperthyroidism would produce opposite changes in lung growth to those observed with thyroid insufficiency. This was not, however, the case. No significant changes in the rates of protein turnover or lung growth were observed with an excess of thyroid hormones (Figs. 3-7; Table 2).

It may, therefore, be concluded that thyroid hormone insufficiency but not excess is capable of modifying the growth of the neonatal lung and the whole animal (Fig. 8).

Discussion

The information available concerning the rates of protein turnover and the normal developmental growth of the lungs is of a very limited nature. This is particularly true for the perinatal period. Although a report from this laboratory did measure protein turnover in the lungs over most of the lifespan of the rat (Goldspink, 1987), no measurements were made during the period of suckling. This has been rectified in the present study, whilst also investigating the role of thyroid hormones during perinatal development.

Between birth and weaning the lungs' fractional rate of protein synthesis fell from 60-22%/day and was accompanied by a 40% decrease in both the ribosomal capacity and ribosomal activity. Over larger age ranges the developmental fall in protein synthesis in the lung, and other body tissues, is primarily attributable to an age-related fall in the ribosomal capacity (Waterlow *et al.*, 1978; Kelly and Goldspink, 1984; Lewis *et al.*, 1984; Goldspink, 1987). These age-related changes in synthesis rates represent average changes within the whole lung, and do not identify changes within the many individual cell types present.

When the normal neonatal surge of thyroid hormones (Figs. 1 and 2) was suppressed by treatment with PTU, the rates of protein synthesis, protein degradation and growth of the lungs were decreased. This inhibition of protein synthesis was due to decreases in both the ribosomal capacity and ribosomal activity (Table 2). Although lung growth and its rate of synthesis in the fetus were consistently reduced after PTU treatment, these changes were slight and non-significant (Figs. 3-7 and Table 1). This is perhaps not surprising since the thyroid hormones in the fetus were only marginally lowered (Figs. 1 and 2). PTU is, however, known to cross both the placental and mammary gland membranes (Low *et al.*, 1979) and to block T_4 synthesis in the thyroid gland and its

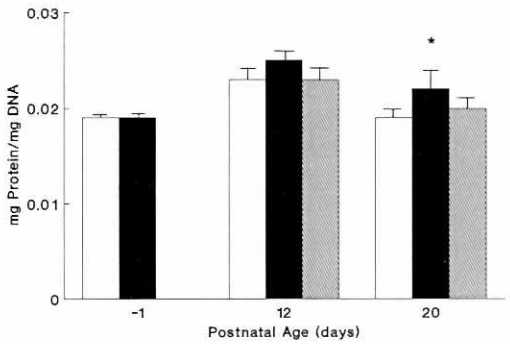

Fig. 7. Pulmonary cell size in relation to age and thyroid status. *Cell size can be determined from the protein:DNA ratios using data from Figs. 4 and 6 for control (□), hypo- (■) and hyper- (▨) thyroid animals. *P<0.05, compared to control values.*

TABLE 1

PROTEIN SYNTHETIC RATES IN CONTROL AND PTU-TREATED FETAL LUNGS

	Protein synthesized			Ribosomal activity
	(%/day)	(mg/day)	(mg/day/ mg of RNA)	(mg RNA/ g protein)
Control	60.5	10.6	14.2	42.3
	±4.0	±0.4	±0.4	±1.4
PTU-treated	50.7	8.7	12.9	39.3
	±3.5	±1.0	±0.8	±1.2

The fractional rates of protein synthesis (i.e. %/day) in the fetal lungs were measured *in vivo* (Garlick *et al.*, 1980) after administering ^{3}H-phenylalanine via the mothers' tail vein (Lewis *et al.*, 1984). Total rates of synthesis are the products of the fractional rates and the respective protein masses (Fig. 4), and the ribosomal capacities calculated from the appropriate RNA (Fig. 5) and protein (Fig. 4) contents. The results are from 7 lungs per group and are presented as means±SEM for control and PTU-treated fetuses.

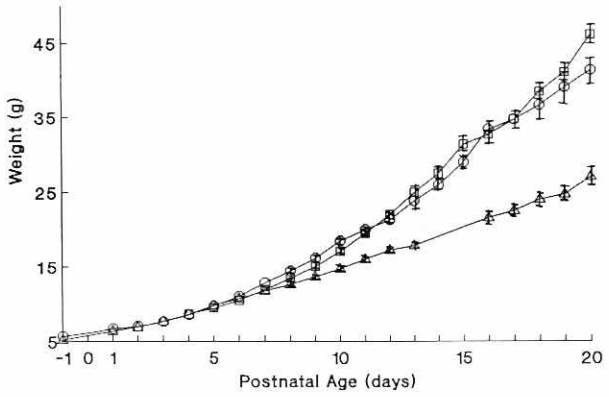

Fig. 8. Body weights were recorded in euthyroid control (○), PTU-treated (Δ) and T_4-injected (□) perinates between late gestation and weaning. *Values are means± SEM, ranging from 90 animals per group at birth to 8 per group by 20 days. From day 9 onwards the hypothyroid animals were significantly (P<0.001) lighter than their controls.*

subsequent peripheral conversion to T_3. PTU should, therefore, have been delivered successfully to the fetal circulation. However, the rat is known to be relatively immature at birth and only starts to synthesize and store thyroid hormones around 18 days of gestation. Furthermore, the rat's hypothalamic-pituitary axis is only fully developed after day 4 *postpartum* (Walker *et al.*, 1980). Hence, there may have been insufficient time to induce fetal hypothyroidism in this particular animal model. PTU was, however, clearly effective during the suckling period. Treatment with PTU was continued throughout the study period to prevent the transfer of T_3 and T_4 within the mother's milk (Koldov, 1980) sky as well as to block the activity of the neonate's thyroid gland. PTU has no known side effects and is used in human pregnancies to treat Graves' disease (Cheron *et al.*, 1981). It is, therefore, reasonable to conclude that the low levels of thyroid hormones *per se* and not chemical side effects due to PTU treatment are responsible for retarding the cell proliferation (Fig. 6) and the growth of the lungs. In related studies we have found that other body tissues in the same hypothyroid neonates were much more severely growth retarded, e.g. the diaphragm (48%), the ventricles (45%) and the atria of the heart (24%) and the liver (28%). As in the lung the rates of protein synthesis were inhibited. The lungs do therefore appear to be one of the better protected tissues within these neonates, which in whole body terms are 34% growth retarded, relative to their controls, by 20 days *postpartum* (Fig. 8).

We are not aware of any previous studies on the perinatal lung so these results cannot be readily compared with the findings of earlier investigations. However, in adult rats thyroidectomy reduces the diameter of the alveolar type II cells by approximately one-third, while other parenchymal cells are unaffected (Ruel *et al.*, 1982). The reverse was also true, with the type II cells of the adult lungs undergoing hypertrophy in response to 6 days of T_4 injections

TABLE 2

RATES OF PROTEIN SYNTHESIS IN THE LUNGS OF EU-, HYPO-, AND HYPER-THYROID NEONATES

	12 days				20 days			
	%/day	Protein synthesis mg/day	mg/day /mg of RNA	Ribosomal activity mg RNA/g protein	%/day	Protein synthesis mg/day	mg/day /mg of RNA	Ribosomal activity mg RNA/g protein
Control	24.5	12.2	6.1	39.7	22.3	14.8	8.8	26.2
	±1.7	±1.7	±0.3	±1.0	±1.3	±0.9	± 0.4	±0.9
PTU-treated	13.3	5.5	3.9	34.3	13.4	7.3	5.7	23.7
	±1.2	±0.3	±0.3	±1.2	±0.8	±0.7	±0.2	±1.2
% decrease	51***	59***	44***	14**	40***	51***	34***	9*
T_4 injected	27.1	13.3	7.0	38.8	25.5	18.5	9.4	27.1
	±1.8	±1.1	±0.4	±1.3	±1.4	±1.3	±0.3	±0.7
% increase	11	9	15	-2	14	25*	9	4

Rates of protein synthesis were measured in the lungs of neonates after they had each received on injection (I.P.) of ^{3}H-phenylalanine (Garlick *et al.*, 1980). These values are means±SEM for a minimum of 12 animals per group and were derived in a similar manner to those described in Table 1. *P<0.05; **P<0.01; ***P<0.001.

(Redding and Douglas, 1972). In the present study we did not examine the responsiveness of different individual alveolar cell types, either with respect to their numbers or their rates of protein turnover. Likewise, we are assuming that it is the low levels of T_3 and T_4 which produce the growth retardation of the lungs. Nonetheless, other indirect effects cannot be ruled out. For example, the mother's milk production could be compromised by the treatment with PTU and subsequent hypothyroidism. Also the impaired development of the CNS might restrict the suckling ability of the hypothyroid pups. There does, however, appear to be no diminution of appetite in the hypothyroid pups, since, like euthyroid neonates, they are found to possess stomachs engorged with milk. Furthermore, hypothyroid pups fostered by lactating euthyroid mothers remain growth retarded (Tamasy *et al.*, 1984), suggesting that it is the low thyroid hormone levels *per se* that exert the main effect on the neonatal lung growth.

Although the T_3 and T_4 levels were substantially increased above normal after the administration of T_4, this did not produce any thyrotoxic effects since the body growth of these animals was normal (Fig. 8). Surprisingly, advancing and elevating these hormone levels did not produce any enhancement of growth in the neonatal lungs. This contrasts with significant increases in the size of striated muscles, such as the atria and ventricles of the heart and the diaphragm in the same animals. The failure to induce any hypertrophy of the lungs does not however exclude the possibility that the hyperthyroid state might have enhanced the maturation of the lungs. Certainly these T_4-injected pups experienced earlier opening of their eyes and unfolding of their ear pinnae, compared with the controls.

It would, therefore, appear that certain levels of thyroid hormones are required for normal lung growth and maturation. However, once this minimal level has been reached excessive thyroid hormones do not appear to further enhance the growth of the lungs. In other body tissues, e.g. the heart and diaphragm, this threshold level may be higher than it is for the lungs.

Materials and Methods

Female Wistar rats weighing 310±15 g were mated with proven males; pregnancy being determined by palpation of the uterus from day 11 onwards. The pregnant dams were then subdivided into 3 groups; one destined to produce control (i.e. euthyroid) fetuses or neonates, the second hypothyroid and the third hyperthyroid neonates. All animals had free access to water and were fed *ad libitum*. Animals were either killed before full term or allowed to give birth. In the latter cases all litter sizes were reduced to 12 pups. Changes in thyroid status were achieved as follows.

Perinatal hypothyroidism was induced by adding propylthiouracil (PTU) to the drinking water (200 mg/l) of both pregnant (from day 12 of gestation) and lactating dams, through to weaning. The effects of hypothyroidism were determined on fetal lungs at 21 days of gestation and in the neonates at either 12 or 20 days *postpartum*. Appropriate euthyroid controls were used for comparison.

Precocious hyperthyroidism was induced in the neonates by administering T_4 (S.C., maximum vol. of 200 µl) from day 3 through to either day 12 or day 20 *postpartum*, at a dose of 0.1 µg/g of body weight. Appropriate controls received a similar volume of the 0.9% saline vehicle to allow for any stress caused by the daily injections.

All experimental procedures were standardized and performed between 09.00 and 12.00 h to minimize possible diurnal variations. Protein synthesis was measured *in vivo* after an injection of a large dose of phenylalanine to flood the precursor pools (Garlick *et al.*, 1980). This injection was administered (I.P.) to each neonatal rat or to each mother (I.V.) for the fetal lungs (Goldspink, 1987), and contained 150 µmol of the amino acid, including 65 µCi of L-(4-^{3}H) phenylalanine (specific radioactivity 24 Ci/mmol; from The Radiochemical Center, Amersham, Bucks., UK), in 1 ml of 0.9% NaCl per 100 g body wt. All animals, i.e. mothers, fetuses and neonates, were decapitated 10 min after commencing the injection. Abdominal and thoracic cavities were immediately opened and the whole animal submerged in ice-cold NaCl (0.9%). The perinatal lungs were rapidly dissected out and within a few seconds frozen in liquid nitrogen.

The frozen tissues were then pulverized between chilled metal plates and homogenized in ice-cold 0.3 M $HClO_4$ in a ground-glass homogenizer. The specific radioactivities of the phenylalanine both in the 'flooded' tissue pools and covalently bound in protein were measured in the homogenates of the lungs by the method described by Garlick *et al.* (1980). This involved the prior hydrolysis of the washed protein pellets in 6 M HCl at 110°C for 24 h and the conversion of phenylalanine into ß-phenethylamine (Garlick *et al.*, 1980). All measurements of radioactivity were made in a LKB scintillation counter (efficiency for ^{3}H of 25%) in Optiphase (Pharmacia) scintillant with the use of an external standard.

The fractional rates of synthesis (i.e. k_s, the percentage of protein mass synthesized per day) were calculated from

$$k_s = \frac{S_B}{S_A t} \times 100$$

where S_A and S_B are the specific radioactivities of phenylalanine in the free tissue pools (i.e. intracellular and extracellular) and protein respectively, and t is the time in days.

Protein was measured in the same preparations by the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma, Kingston upon Thames, Surrey, UK) as standard. RNA and DNA were sequentially extracted and measured as previously described (Ashford and Pain, 1986; Goldberg and Goldspink, 1975).

Radioimmunoassays for thyroid hormones

Samples (50 µl) of mixed whole blood were collected at the time of killing and assayed for T_3 and T_4 using in-house radioimmunoassays modified from the method of Luxton *et al.* (1977).

Statistical analysis

The various parameters associated with the growth of the lungs are presented as means±SEM. Student's unpaired *t* test was used to establish the level of significance between the values of the control and thyroid manipulated groups of animals.

Acknowledgments

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