Thyroid Hormone Influence upon Lung Surfactant Metabolism

Abstract. *Thyroid administration and thyroidectomy in the rat profoundly affect the morphological characteristics of the type II pneumonocyte and the quantitative harvest of lung surfactant obtained from alveolar washings. The correlation of the ultrastructural changes with quantitative alterations in lung surfactant is evidence that the lamellar bodies within this cell are the source of the surface-active phospholipids utilized at the air-liquid interphase of lung alveoli. Our findings suggest that in the rat, L-thyroxine may be a potent regulator of lung surfactant metabolism.*

Little is known about the controlling mechanisms that govern the production and turnover of lung surfactant within alveoli. This vital lining material, primarily phospholipids (*1*), lowers surface tension at the alveolar fluid-air interphase, thereby facilitating inflation of air sacs with relatively small changes in air pressure. Inadequate surfactant production, increased degradation, or both, are considered to be responsible for the respiratory distress syndrome of the human newborn (*2*), a worldwide disease which usually results from, and frequently accompanies, premature birth.

L-Thyroxine (T_4), an important in vivo regulator of lipid metabolism, was investigated as a possible regulator of surfactant metabolism. The ultrastructure of potential source cells for this surface-active agent was examined in lungs from rats that were thyroidectomized, treated with L-thyroxine, or normal. The influence of varying amounts of thyroxine upon lung surfactant production was studied by the use of a new method for quantitatively harvesting surfactant from rat lungs.

Eighty-one adult female rats (CDBS-Charles River) maintained free of respiratory disease were divided into five groups. Fifteen rats were made hyperthyroid by daily subcutaneous injections of 1 mg of L-thyroxine per kilogram for 6 days. On the seventh day they were killed. These animals had serum protein-bound iodine concentrations above 20 μg/100 ml and a 10 to 15 percent fall in weight. An additional 12 rats injected for 15 days with 1 mg of L-thyroxine per kilogram displayed similar alterations in weight and protein-bound iodine. These two groups of rats were compared with 30 littermates that were injected daily with 0.15*M* saline; and killed at the same times as test groups. A progressive weight gain was observed in the control animals and their mean protein-bound iodine was 2.61 μg/100 ml.

In another experiment, a group of 12 rats were killed 6 to 8 weeks following thyroidectomy and compared with 12 littermates in whom a similar operation without thyroidectomy was performed. Hypothyroidism was verified in the thyroidectomized group by a lack of weight gain in 11 of 12 animals and five random protein-bound iodine determinations which were substantially lower than normal.

Each rat from the above five groups was killed by an overdose of pentobarbital administered intraperitoneally. The lungs were removed and weighed. Our quantitative method was derived from a qualitative assay technique (*3*). Each pair of lungs was washed via the tracheobronchial tree with 0.15*M* saline approximately ten times or until the lavage fluid became clear. The lungs were then dried in an oven at 75°C for 24 hours and reweighed. The combined washings were differentially centrifuged at 600*g* to separate cellular macrophages, then at 16,000*g* to deposit an acellular crude pellet of lung surfactant. The crude surfactant pellet, after dialysis to remove electrolytes, was separated into three bands by ultracentrifugation within a linear sucrose density gradient. At equilibrium only the center band ($\rho = 1.035$) was highly surface active, which distinguished it from the top layer of cholesterol and triglycerides (*3*) and a pellet of mucoproteins. This central band was isolated, freed of sucrose by dialysis, lyophilized, and weighed. The quantity of surfactant was expressed in milligrams per gram of wet weight of fresh lung. A wet weight to dry weight ratio for each pair of lungs was also calculated.

Blocks of lung tissue (1 mm^3) from six pairs of lungs from each of the

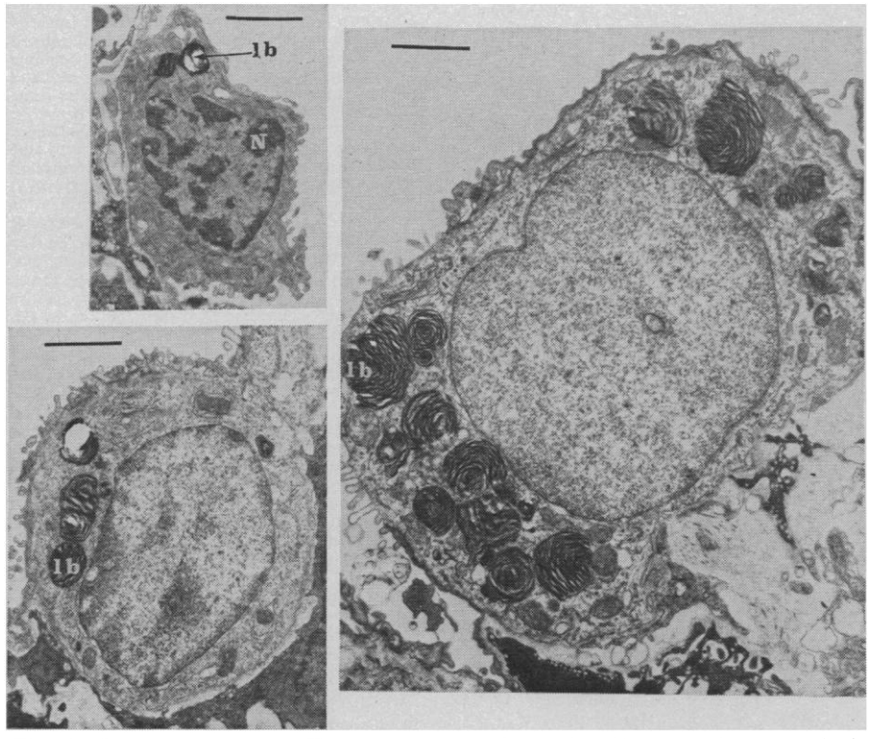

Fig. 1. Representative type II pneumonocytes from thyroidectomized (upper left), euthyroid (lower left), and thyroxine-treated (right) rats are compared. The type II cell obtained from a thyroidectomized rat is smaller in size. The nuclear chromatin (*N*) is clumped and there are fewer and smaller lamellar bodies (*lb*) present in the cytoplasm. The euthyroid rat's pneumonocyte contains several normal-appearing osmiophilic lamellar bodies. A type II cell from a thyroid-treated animal (right), has more and much larger lamellar bodies. Both the nucleus and cytoplasm are hypertrophied. The line marker on each electron micrograph denotes 2 μm.

above groups were coded to eliminate bias and submitted to W.H.J.D. for evaluation by electron microscopy. The specimens were fixed for 2 hours in 4.0 percent formaldehyde containing 0.25*M* sucrose buffered to *p*H 7.4. Tissue blocks were then rinsed for 15 minutes in 0.1*M* cacodylate buffer (*p*H 7.4) containing 0.25*M* sucrose, and placed in glass vials in a vacuum to remove all tissue air. The blocks were postfixed in 1 percent osmium tetroxide in cacodylate buffer for 30 minutes, then stained for 2 hours with 0.5 percent magnesium uranyl acetate in 0.9 percent sodium chloride. Dehydration was carried out in a graded series (70; 90; 100 percent) of hydroxypropylmethacrylate before the tissue blocks were embedded in Epon-Araldite. This procedure offered the advantage of preserving the fine structure of the alveolar type I and type II pneumonocytes, as well as the endothelial, interstitial, and terminal nonrespiratory bronchiolar Clara cells. Since both the alveolar type II pneumonocyte (*4*) and Clara cells (*5*) have been suggested as sites of surfactant production, their fine structural responses to varying amounts of thyroxine were compared in euthyroid, thyroid-treated, and thyroidectomized rats. All statistical comparisons of morphological and quantitative data were done by Student's *t*-test.

Only the type II alveolar cells exhibited marked cellular changes that correlated with varying thyroxine concentrations. Figure 1 shows representative pneumonocytes obtained from rats that were thyroidectomized, normal, or treated with L-thyroxine for 6 days. The nuclear chromatin was frequently clumped in the smaller type II cells from hypothyroid rats, suggesting inactivity. The significant morphological alterations are shown in Table 1. With increased amounts of thyroid hormone, there is a distinct stepwise enlargement of alveolar type II cells combined with increases in size and number of their lamellar inclusion bodies (Table 1 and Fig. 1).

The quantity of lung surfactant obtained from the five groups of rats is shown in Fig. 2. Compared with the control rats' mean surfactant pool of 1.55 mg, the thyroxine-treated rats showed statistically significant increases to 2.20 mg (T_4-treated, 6 days; $P < .03$) and 2.63 mg (T_4-treated, 15 days; $P < .004$) per gram of wet weight of lung. In contrast, the thyroidectomized rats showed lower mean recoverable surfactant pools (1.07 mg) compared with their sham-operated littermates (1.55 mg) ($P < .07$). In all five groups, the ratio of dry to wet weight of lung was similar, indicating that variation in water content of the lung did not account for the wide differences in recoverable surfactant pools. In addition, the chemical nature of the purified surfactant did not appear to change. Mean phosphorous determinations (microgram of phosphorous per milligram of surfactant) (*6*) were similar in all five groups, indicating that the proportion of phospholipids present was unchanged.

Table 1. Mean cell diameter, number of lamellar bodies, and lamellar body diameters of type II pneumonocytes from rats that were treated with thyroxine for 6 days (T_4), thyroidectomized (T_x), or euthyroid (controls). Standard deviations are given in parentheses after each value.

Treatment	Cell diameter (μm)	Lamellar bodies/cell (No.)	Lamellar body diameter (μm)
T_4	12.2 (2.4)*	8.8 (5.2)†	1.8 (0.30)*
Controls	9.3 (1.9)	5.4 (3.3)	1.1 (0.25)
T_x	6.7 (1.2)*	3.4 (2.5)	0.8 (0.19)*

* Statistically significant compared to controls ($P < .001$). † Statistically significant compared to controls ($P < .05$).

The hormone induced morphological alterations in the type II pneumonocyte suggests augmented storage and production of lung surfactant. Our quantitative and biochemical analysis of the recoverable surfactant from alveolar washings of rats implies that increased secretion occurs as well. Although the physiological role of altering the surfactant pool in terms of utilization and the kinetics of biosynthesis and degradation are not fully understood, we have shown evidence that increased thyroid hormone concentrations are associated with increased available lung surfactant on the alveolar surface.

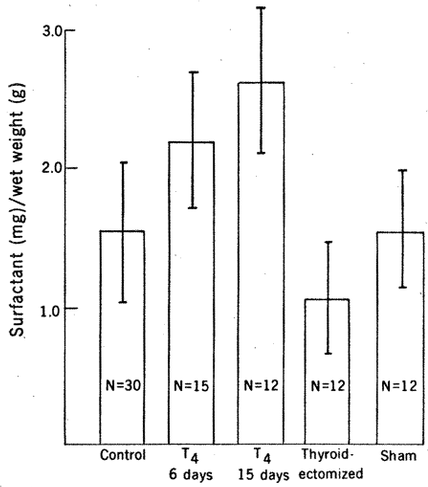

Fig. 2. The quantity of purified surfactant harvested from lung washings is expressed on the ordinate. Compared to their littermate controls, rats treated with thyroxine for 6 (T_4, 6 days) and 15 days (T_4, 15 days) have increased quantities of recoverable surfactant. Sham-thyroidectomized rats had a similar mean quantity of surfactant as the controls, but more than the thyroidectomized animals. Brackets indicate 1 standard deviation. The numbers of rats in each group are also given within the bars.

In striking contrast to the type II pneumonocyte, other cells within the lung parenchyma were relatively unchanged. Clara cells were not altered by varying hormone levels, suggesting that this cell type has little or no participation in the phospholipid metabolism related to lung surfactant. The Clara cells from euthyroid rats contained ample quantities of smooth endoplasmic reticulum in addition to mitochondria and electron-opaque granules. In both thyroid-treated and thyroidectomized rats these cells displayed similar fine structure with no detectable alteration in amount or arrangement of organelles. The fine structure of adjacent ciliated epithelial cells of terminal bronchioles and capillary endothelial cells was also unaffected by varying thyroxine.

These new observations lead to speculation that thyroid hormone is important to the maturing lung of the human fetus. The human fetal thyroid is known to begin trapping iodine at 10 to 12 weeks (*7*), and by 20 to 24 weeks, the age of viability, low concentrations of thyroxine-like compounds are found in blood (*8*). Free and total thyroxine continue to rise in response to rapidly rising thyroid-stimulating hormone (*9*). At full term and shortly after delivery these values are well above those of an adult (*10*). It has also been observed that osmiophilic inclusions and surface activity do not appear until late in gestational development (*11*). Recently, however, Wu *et al.* have reported that thyroxine administration to rabbit fetuses accelerates maturation of the lung, with earlier appearance of surface activity and osmiophilic lamellar inclusions within type II pneumonocytes (*12*). No information is available about thyroid function in human infants with respiratory distress syndrome. Conversely, the

incidence of this syndrome has not been reported to be increased in cretins. Nevertheless, our observations suggest that a possible relationship between thyroid function and respiratory distress syndrome requires further investigation.

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Tolerance to Sheep Red Cells: Breakage with Thymocytes and Horse Red Cells

Abstract. *Mice rendered tolerant to sheep red cells and then given normal thymocytes, made no antibody when immunized with these cells. When immunized with horse red blood cells, however, they made significant amounts of noncross-reacting antibody to sheep red blood cells. This suggests that antibody-making precursor cells (B cells) which are nontolerant but nonactivatable by specific antigen, may exist in tolerant hosts.*

The discovery that two types of lymphocytes cooperate in the immune response to certain antigens (*1*) raised the question of where the cellular basis of immunological tolerance lay. It appears that cells in the thymus can easily be rendered tolerant (*2–4*). There is also evidence that cells which have been educated in the thymus and have subsequently migrated to peripheral lymphoid tissues (T cells) can also be rendered tolerant (*5, 6*). The situation with antibody-making precursor cells (B cells), both those in the bone marrow and emigrés in peripheral tissues, is less clear. While several workers have evidence that bone marrow cells of tolerant animals are tolerant (*4, 7*) others have been unable to confirm this (*3, 6*). Even when tolerance was demonstrated in cells in the bone marrow, it was much shorter lived than the tolerant state of the intact animal (*8*). There is no information available that bears directly on the question of whether peripheralized B cells can be rendered tolerant.

Thus it is possible that the tolerant state is a property mediated largely if not exclusively by T cells. This hypothesis has been discussed at length (*9*). Supporting evidence is as follows.

1) Precursor cells competent to make antibodies against "tolerated" antigens have been shown to exist in unresponsive mice; they can only be stimulated, however, by cross-reacting antigens (*10*). Administration of the tolerated antigen with the cross-reacting antigen may prevent the B cell stimulation (*11*).

2) Allogeneic T cells, which can replace the contribution of carrier-specific syngeneic cells (also T cells) in antibody responses to haptens (*12*) allow rats tolerant to sheep red blood cells (SRBC) to make antibodies to SRBC, while syngeneic T cells do not (*13*).

These results are consistent with the notion that B cells of tolerant mice may be normal, that tolerant T cells actively prevent antibody formation, and that some types of nonspecific T cell stimulation can activate the otherwise unresponsive B cells. Our report supports this concept.

Male CBA mice were made tolerant to SRBC as described (*14*). Seven-week-old mice were thymectomized, lethally irradiated (850 roentgens), and then given 5×10^6 syngeneic bone marrow cells and 15×10^6 syngeneic thymocytes. After reconstitution they were given a total of 2.5×10^{10} SRBC intraperitoneally in nine doses, divided over 30 days. Four days after the last injection, serums were titrated, and those mice that had made antibody were eliminated from these experiments. Half of the remaining mice were then given a second inoculation of 15×10^6 syngeneic thymocytes; half of these mice, as well as half of the mice that

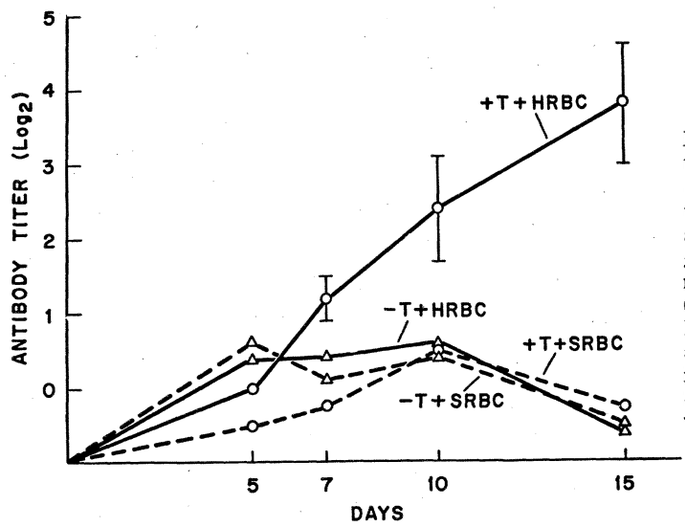

Fig. 1. Antibody to SRBC response of mice rendered tolerant to SRBC and then either given 15×10^6 normal thymocytes (+T) or not (−T). Half of each resultant group was immunized with SRBC and half with HRBC.