

On the pulmonary toxicity of oxygen. 4. The thyroid arena.

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Abstract

Normally developed thyroid function is critical to the transition from fetal to neonatal life with the onset of independent thermoregulation, the most conspicuous of the many ways in which thyroid secretions act throughout the body. A role for thyroid secretions in growth and maturation of the lungs as part of the preparation for the onset of breathing has been recognized for some time but how this contributes to tissue and cell processes and defenses under the duress of respiratory distress has not been well examined. Extensive archival autopsy material was searched for thyroid and adrenal weights, first by gestational age, and then for changes during the first hours after birth as ratios to body weight. After a gestational age of 22 weeks the fetal thyroid and adrenal glands at autopsy in those with hyaline membrane disease are persistently half the size of those in "normal" infants dying with other disorders. When the thyroid is examined shortly after birth it reveals a post natal loss of mass per body weight of similar orders of magnitude which does not occur in the control group. A clinical sample of premature infants with (12) and without (14) hyaline membrane disease was tested for T_4 , TSH, TBG, and total serum protein. The results also demonstrate a special subset with lower birth weights at the same gestational age, and lower serum T_4 and total serum protein. Ventilatory distress in newborn rabbits was induced by bilateral cervical vagotomy at 24 hours post natal following earlier injection of thyroxine (T_4) or thyroid stimulating hormone (TSH) and comparisons were made with untreated animals and by dose. Early life thyroidectomy was performed followed by exposure to either air or 100% oxygen. A final experiment in air was vagotomy after thyroidectomy. Composite analysis of these methods indicates that thyroid factors are both operative and important in the newborn animal with ventilatory distress. This work and the archival data indicate those infants destined to develop hyaline membrane disease through respiratory distress are a distinct developmental and clinical subset with the point of departure from otherwise normal development and maturation in the second or early third trimester. This interval is known to be a period of marked variation in the overview indicators of fetal progress through gestational time. The initiating factor or circumstance which then separates this special subset from normal future development is placed by these observations firmly into the period when human fetal TSH dramatically rises 7-fold (17.5-25.5 weeks) followed by a lesser 3 to 4 fold increase in T_4 which is extended into the early third trimester. The earlier part of this interval is characterized by the thyrotrophic action of chorionic gonadotropin (hCG). The possibility that abnormalities in the intrauterine environment secondary to maternal infection play a role within this time frame is indicated by the demonstration that interleukin-2 (IL-2) induces an anterior pituitary release of TSH. Since IL-2 has this property and is not an acute phase cytokine, some form of chronic infection or an immunopathic process seems more likely as a possible active factor in pathogenesis.

Introduction

Earlier work from this laboratory demonstrated a complex but systematic relationship between the amount of oxygen to which the lung was exposed and the extent and severity of lung injury in a neonatal animal model for hyaline membrane disease (Shanklin, 1969; Shanklin and Lester, 1972). The model is based on the effects of bilateral cervical vagotomy (BCV) on the lung (Farber, 1937). When Farber's model using adult rabbits is transferred to the newborn rabbit the resulting lesion is that of human neonatal hyaline membrane disease (Shanklin and Berman, 1964). The frequent occurrence of intermediate severity of the lesion in the model offered a way to ascertain certain pathogenetic factors of importance.

Firstly, the physiological concept of the effects of the partial pressure of gases was shown to be a consequence of normobaric environments at one atmosphere absolute (1.0 ATA) at or near sea level. These experiments used oxygen at 7, 21, 40, and 60 percent against nitrogen, and at 100% oxygen in hypobaric (0.2 and 0.6 ATA), normobaric, and hyperbaric (3.0 ATA) total pressure (Shanklin, 1969).

Secondly, the oxygen effect is dependent on which second gas was used as diluent in the inhaled mixture. In these circumstances, oxygen at 20 and 60 percent was studied with argon, helium, neon, and nitrogen, and also at 20, 40, 60, and 80 percent against sulfur hexafluoride. The results were then compared to those from 100% oxygen (Shanklin and Lester, 1972).

Extensions of the work using the same gases, and hydrogen, at oxygen percentages lower than the atmospheric 20-21% will be reported elsewhere.

The discussion of the diverse but systematic effects by diluent gas considered five sites and the categories of physical and physicochemical attributes of each gas likely to be operant there, seeking thereby to identify one or more factors in the process of injury to the lung observed in this model. These were: (a) the external body surface and thermal conductivity with metabolic effects, (b) the conducting airways, mediated by flow dynamics including turbulence, (c) the gas-tissue interface, at which a stochastic or coordinated competition from the specific mix of the inhaled gas mixtures was the controlling factor, (d) within the pulmonary tissue itself, without specifying at what level, and (e) systemically, mediated through metabolic consequences with secondary injury to the lung (Shanklin and Lester, 1972, pp. 149-150).

Two of these sites, *external body surface* and *systemic effects*, readily imply participation of animal metabolism as a feature, whether by a direct pathogenetic role, as an adjunct factor, or as a supplement to the more obvious effect of the percentage of oxygen in any given inhalant mix (Shanklin and Lester, 1972). A third, prospect (d), *within the pulmonary tissue itself*, would relate to specific aspects of pulmonary metabolism. The data presented in that report did not allow further definition of the matter (Shanklin and Lester, 1972).

The question arises whether such metabolic effects are due to activation or participation of ordinary metabolic reactants, such as thyroxine (T_4), or triiodothyronine (T_3), or the size of the animal subject, or both, or some other feature. The animal model used in these studies introduces a metabolic factor in that no nutritional or fluid support is provided between birth and vagotomy, or after the procedure. Little is known about the effects of immediate postnatal nutrient deprivation, especially in premature infants, a state once very common in clinical practice (Stone, 1945), and, from their important distinctions from adults, even pregnant adult women, the lessons from short term fluid and caloric deprivation in adults may not be directly applicable (Felig, et al., 1969; Felig, et al., 1972; Kim & Felig, 1972).

Maternal starvation in rabbits for 48 hours prior to delivery has profound effects on the newborn rabbits, with higher blood concentrations of free fatty acids and triglycerides and a greater maximal rate of heat production over the first three days after birth (Edson and Hull, 1977). Normalization

of glycogen reserves in man, monkeys, sheep, pigs, dogs, rats, guinea pigs, and rabbits reveals that the rabbit has the lowest hepatic storage of these eight species, circa 35 mg/gram wet weight liver, and that this falls dramatically the first day after birth even when the newborn has suckled (Shelley, 1961). Total carbohydrate reserves in infants dying with respiratory distress syndrome/hyaline membrane disease were consistently lower in heart, liver, diaphragm, and leg muscles, very roughly about half that found in deaths from non-respiratory causes (Shelley, 1964).

Thyroxine has decided effects on the fetal lung when applied in certain ways in rats (Redding, et al, 1972; Sosenko and Frank, 1987), rabbits (Wu, et al., 1973), and from indirect evidence, in humans (Mashiach, et al, 1978; Redding and Pereira, 1974). A principal result is enhanced histologic maturation when given directly to the late developing fetal rat (Redding, et al., 1972) or rabbit (Wu, et al., 1973), but not when given to the dam or doe (Wu, et al., 1973). Another effect pertains to maturation of lung permeability (Barker, et al., 1990; Hemberger and Schanker, 1978). It should be noted that most such experiments consider only the effect of additional T_4 , T_3 , or thyrotropin (TSH) which may override the usual physiologic homeostatic mechanisms. The result may be considered as an induced dysthyroidal state roughly comparable to the dysthyroidism of progressive fetal failure of cellular development with coordinated function of the thyroid axis. The relation of thyroid factors to oxygen challenge has been explored with thyrotropin releasing hormone (TRH) in rats, but without the added factor of induced ventilatory distress (Rodriguez, et al., 1991; Rodriguez-Pierce, et al, 1992). Described here are the effects of T_4 , TSH, and thyroidectomy in the newborn rabbit under various circumstances. T_4 was chosen instead of T_3 to invoke the normal processes of conversion *in vivo*.

These aspects have to be placed in the context of normal but dramatic changes in the regulatory matrix of thyroid function upon birth. First, there is an acute release of TSH in the human newborn, from a mean value of 9.5 μ U/ml in cord blood to 86 μ U/ml in the neonatal circulation within 30 minutes after birth (Fisher and Odell, 1969). Second, cord blood has low T_3 and high T_4 concentrations with a sharp rise in T_3 from 51 ± 3 ng/100 ml in cord blood to 191 ± 16 ng/100 ml in neonatal blood at 90 minutes after birth (Abuid, et al., 1973). The change in T_4 is minimal at 90 minutes, from 12.2 ± 2.0 μ g/100 ml cord blood to 14.1 ± 1.9 μ g/100 ml in the circulation (Abuid, et al., 1973). The timing and slope of these plots indicates the surge in TSH precedes and promotes the increase in T_3 . Reported here are the results of 26 premature newborns, 14 controls that did not show signs of respiratory distress and, 12 that did. The birth values of serum T_4 , TSH, TBG, FTI, and total serum protein, are examined against gestational age, birth weight, and Apgar Scores.

The evolutionary aspects of thyroid factors has been examined in part. Changes occur in both structure and function of the gill of the teleost fish *Anabas testudineus* (Bloch) depending on thyroid status (Sreejith, et al., 2007).

This paper considers the role of thyroid factors in the pathogenesis of hyaline membrane disease in the premature infant. Specifically, data are presented on these five subjects:

- [1] the growth of the human fetal thyroid and immediate postnatal loss of glandular mass,
- [2] the parallel growth of the human fetal adrenal glands as a surrogate marker for fetal glucocorticoid resources,
- [3] the influence of weight loss between birth and surgery on the further weight loss after BCV or other experimental interventions as a marker for metabolic drain,
- [4] the effect of T_4 and TSH on outcome following bilateral cervical vagotomy to induce respiratory distress,
- [5] the effect of thyroidectomy on survival and lung injury with and without added oxygen and when followed by vagotomy.

Materials and methods

Archival studies:

During twelve years as pathologist-in-chief in the Laboratory of Pathology, Chicago Lying-In Hospital (CLIH) (1967-1978), the opportunity arose to review in detail the cumulative records compiled by my predecessor, Edith L. Potter, M.D., Ph.D., (1901-1993) through the modality of the City of Chicago Perinatal Mortality Study initiated in the mid-1930s by Herman N. Bundesen, M.D., Sc.D. (1882-1960), long time Commissioner of Health of Chicago. Approximately 7,500 autopsy records were on file in this archive.

Further review was enhanced by having the records converted to microfiche by the University of Chicago Joseph Regenstein Library Department of Photoduplication, after a first reading of the archive. Whilst all records were not complete for data pertinent to this work, most were useful in many ways. In numerous cases, stillborn and newborn, there were descriptions of microscopic slides, complete autopsy reports, and a few details from the clinical course. Intrapartum stillbirths were very common in the earlier years of the Potter archive. These latter were excluded from this review when weights were either absent or only of the major organs. Since this was not an epidemiological study no attempt was made to ascertain the proportion of cases excluded while seeking to fulfill certain quotas as generally representative of the whole.

The records on microfiche were thoroughly reviewed four times. The first review was retrograde, comparing newborn adrenal and thyroid organ weights between infants with hyaline membrane disease (HMD) and those dying from other causes on a *gestational age* basis, but excluding major malformation syndromes. This review obtained 450 premature newborns, 154 with hyaline membrane disease at autopsy and 296 without HMD. This information was placed in a spread sheet (ProStat v4.51 for Windows, Poly Software International, Pearl River, N.Y., 10965) [Table 1 and Figure 1]. The second review revisited the database from its origin to ascertain whether the thyroid to body weight ratio changed with survival time after birth. This time period was chosen to minimize any possible effect from immediate postnatal treatment. The third and fourth reviews were dedicated to verification and to the capture of any overlooked pertinent material.

Thence, phase one of the second review was to create a similar spread spreadsheet with four subsets of information: [1] nonmacerated late stillborns, to ascertain the baseline at the end of intrauterine life, [2] newborns with conditions other than HMD, [3] newborns with histological diagnoses of or findings consistent with pulmonary edema, a precursor to HMD (Shanklin, 1959; Shanklin, 1964), and [4] newborns with a histological diagnosis or findings consistent with hyaline membrane disease *per se*.

After subsets [1] and [2] obtained one hundred cases each, preliminary analysis showed newborns dying in the first 30 minutes could not be easily distinguished by time analysis from the stillborn subset (data not shown); most represented the singular failure of onset of respiration. These cases differed enough from those surviving over 30 minutes that this early subset was set aside and the newborn analysis was extended to obtain 100 cases each. This required reviewing 906 cases overall.

A slower rate of accession occurred in cases with pulmonary edema (PE) and HMD from the initial four year archive 1934-1937. The quota of 50 cases of PE and HMD required extending the first phase through 1942. The number of records over these time frames which were reviewed in order to obtain the data is shown in Table 2.

Weight loss in experimental animals:

Newborn rabbits were weighed at birth, at the time of vagotomy, and at autopsy. This generated data for determining the rate of loss of weight for each interval and each experiment. The results are reported as the slope of loss regressions for comparison.

Laboratory assessment of premature newborns:

Following approval by the Institutional Review Board under an expedited protocol for collection of blood samples, 30 premature newborns were sampled at birth for total serum protein (Lowry, et al, 1957) and for T₄, TSH, thyroid binding globulin (TBG), and free thyroxine index (FTI) in the hospital clinical laboratory by methods in common use at the time with internal standards and controls (Cassidy, et al., 1968; Henry, 1984; Man, et al., 1969). Four of the control cases were in a much higher weight range beyond those with hyaline membrane disease and these were omitted from analysis for a better comparison.

The basic animal model (BCV) and preparation:

Pregnant New Zealand and Dutch rabbits at term were induced by oxytocin (Shanklin, 1966). Immediately after delivery the newborns were placed in air in incubator boxes at 34°C with 50% relative humidity, separate from the doe. They were maintained there until 24 hours of age without caloric supplementation. At 24 hours all animals were subjected to bilateral cervical vagotomy as previously described (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967). Almost all vagotomies took less than one minute to complete, including positioning and restraining the subject for the procedure. A few drops of dilute local anesthetic were used in all cases (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967).

Close observation of all animals was maintained. Animals were removed after death and were autopsied according to the methods and procedures previously reported (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969). In brief, the extent of lesion was determined by a grid approximation formula on the pleural surface of the lungs, with each of the four principal lobes considered separately. These values were combined by a weighted formula in which each upper lobe was one-sixth and each lower lobe was one-third of the whole. Estimates were enabled in part because the grossly apparent reaction discretely follows lobular lines. The result is a percentage of total pleural surface. The determination was performed under a dissecting microscope at 15-30X to verify airlessness and fluid collection of each lobular zone. Lungs were fixed in Bouin's fluid and the lesions were then confirmed microscopically.

Thyroidectomy:

This was performed at two hours after birth. The external surgical approach was the same. After the trachea was identified and isolated bilaterally, careful dissection of the thyroid gland was commenced from the dorsal edge of each lobe and final detachment of the gland from the trachea was at the ventral interlobar isthmus. The minimal blood loss was compressed briefly by small sterile gauze pads. Generally, the procedure was completed within three minutes.

Thyroxine:

Injectable L-thyroxine (T₄) Flint was obtained from the hospital pharmacy and was administered by dorsal subcutaneous injection between the scapulae in doses of 1.0, 10.0, or 100.0 mg/kg body mass. The injections were at three hours post natal.

Thyrotropin:

Injectable thyrotropin (TSH) Armour was also obtained from the hospital pharmacy and was administered by dorsal subcutaneous injection between the scapulae in doses of 0.1, 1.0, 10.0, or 100.0 mU/kg body mass. The injections were at three hours post natal.

A total of 245 newborn rabbits was used in the specific experiments reported here, including 126 BCV air only controls for comparison.

Assignment of animals from given litters to a group was as before, with special attention to large litters and by weight (Shanklin, 1969). The sex ratio of individual groups was left to random sort by the minimum test group size as noted above (Shanklin and Berman, 1964; Shanklin and Sotelo-Avila, 1967; Shanklin, 1969) since the sex of the newborn animal is readily determined only at autopsy.

Analysis by litter, weight, and sex showed no inherent bias to the distribution, with random, or nonsystematic placement between comparable, adjacent, and sequential groups.

Graphic and statistical analysis:

The time intervals postvagotomy as used in this text were taken as a whole and as particular segments of the survival curves, A, B, and C, in time sequence order. Extensive prior experience had shown a triphasic response when the per cent surviving as \log_{10} (on the ordinate) is plotted against the survival in hours (on the abscissa) (Shanklin, 2010). Mean gross long change (GLC), the extent of the lesion which results from the experimental intervention, was considered as the mean \pm the standard error of the mean (SEM) for the whole set as well as each subset, when appropriate..

The slopes of phases A, B, and C were calculated from the log scale conversions. Where useful, Student's *t test* and Chi-square testing was applied for small sample comparisons. Values of *p* are reported as one-tailed tests of significance since the origin of all such scales is theoretically zero with a unidirectional time vector. Areas under the survival curves was determined by printing the graph on quadrille paper and measuring the result in centimeters with square counts at uneven plot lines (Shanklin, 2010). The result is reported in arbitrary units of area.

Global analysis by statistical means (\pm s.e.m) has little meaning in light of the differences in the slopes and intercepts of the three phases and was not used. Rather, the comparisons are by survival against time and by extent of pulmonary lesion for each time frame.

Formal analysis and graphs were done with the aid of ProStat 4.51 and 5.5 for Windows (Poly Software International, Inc., P.O. Box 80, Pearl River, NY 10965), as noted for archival studies; preliminary graphs and slopes of regression equations were calculated using Abstat 5.0 for DOS and 1.96 for Windows (Anderson-Bell Corporation, P.O. Box 745160, Arvada, CO 80006); .

The experimental protocols were approved by the relevant animal research committee of the University of Chicago, the recipient of the grants-in-aid as noted.

Results

Archival studies by gestational age:

The first phase of review sought the size of the thyroid and adrenal glands on a gestational age basis. As noted, a total of 450 cases were enrolled starting in the mid-1960s and working backward in time. The following table and graph shows progressive divergence of the thyroid:body weight ratio and similar changes in adrenal weight. [Table 1, Figure 1]. This total included 154 autopsy proven cases of hyaline membrane disease and 296 control autopsies, excluding known syndromes and gross or global anomalies.

The data show close similarities for birth weights before 31 weeks and progressive disparity afterwards. The crown-heel and crown-rump lengths are much closer numerically. There is also a consistent higher proportion of males in the HMD cases which is typical of the disorder (Shanklin, 1963), a factor relevant to the role of sex hormones, especially estrogens, in the pathogenesis and progression of the respiratory distress syndrome (Shanklin and Wolfson, 1970). The most striking feature is the lower absolute weight of the thyroids for the infants with hyaline membrane disease. The difference in thyroid weight at 22-27 weeks is statistically significant at $p < 0.02$ and the overall trend is highly significant, $p = 0.001$. The difference in adrenal weights shows divergence later at

28-30 weeks gestational age. The p values for differences in adrenal weights are in Table 3.

When the absolute weights are normalized against the top mean weights for thyroid and adrenals the striking persistence of the differential is shown graphically [Figure 1].

In the second phase of the Potter archive a total of 4,588 perinatal autopsies from 1934 through 1942 was reviewed [Table 2]. As accessions grew (the busiest year was 1938 with 830 perinatal autopsies) more and more hospitals in Cook County, Illinois, and the City of Chicago, participated in the Bundesen program. Eventually 53 hospitals other than the three CLIH services (the CLIH in house obstetrical service, the CLIH Home Service, and the Chicago Maternity Center) were registered. Of the 53 "outside services" the most important was Provident Hospital which had an informal arrangement with CLIH staff. The outside hospitals contributed 2,827 cases of which 2,154 (76.19%) had sufficient data for assessment. The three main sequence services contributed 1,751 cases of which 1,717 (98.06%) had the appropriate particularities. Table 4 has further details of the cases by stillborns and newborns, the latter with and without hyaline membrane disease.

This table might seem to suggest a paradox, that the university obstetrical services had a higher stillbirth experience but this is an artefact from the higher frequency of complete reports which also displayed a higher rate of respiratory distress syndrome/hyaline membrane disease [Table 5].

When presented in graphical form an interesting pattern and differences in the prevalence of the lesions between the CLIH services and the outside hospitals emerge [Figure 2].

Thyroid gland weight loss shortly after birth:

The thyroid:body weight ratios had very different profiles between infants with hyaline membrane disease and those without. The latter had a flat mean line from birth through 230 hours post natal, with a regression confirmed ratio of 0.005200 (plot not shown). By contrast infants with hyaline membrane disease displayed a biphasic curve, with a change point at 12 hours. The normalized ratio change from 0 to 12 hours was minus 30.9% with a total decline by 66 hours of 68.2%. The ratio at 66 hours, by regression analysis, was 0.0025291. The equation is part of the legend for Figure 3. Thus, the immediate post natal change brought the relationship into the range of the overall distinction over gestational time as shown in Figure 1, offering as one possibility the difference is due to immediate post natal effects after the first 30-60 minutes.

Circulating thyroid factors and serum protein in premature newborns:

Preliminary study revealed several controls with birth weights well above the highest of the respiratory distress (RDS) subset (N = 4); these were set aside for direct comparison of the others, a final total of 26 cases, 14 normal controls and 12 infants with respiratory distress syndrome by clinical and radiological criteria. This adjustment actually reduced the significance of the two group comparison of birth weights. Remarkably, a highly significant difference was observed in the Apgar Scores of these premature infants [Table 6].

When the Apgar scores are plotted against birth weight (data not shown) there is a flat plot for the control scores but a steady increase by birth weight for the hyaline membrane cases. This might be construed to suggest the maturational vectors for immediate post natal adaptability are normally well developed early in gestation but are much retarded in those fetuses which manifest respiratory distress at and soon after birth.

This subset of premature newborns with respiratory distress syndrome are also a distinct thyroid arena with respect to birth weight, serum thyroxine, and total serum protein [Tables 7a and 7b].

The difference in birth weight is noteworthy, even by inspection, and the difference in T_4 , 1.18 $\mu\text{g}/\text{dl}$, is a decrease of 15.57% from the control. This is significant at $p = 0.0455$. By contrast,

the difference for TSH is smaller at 9.53% (1.04 µg/dl), and is not significant, $t = 0.82$, $p = 0.2125$.

The total serum protein had a very different range and a significant mean difference [Table 7b]. The range of gestational ages for RDS/HMD cases began a month earlier but the mean gestational age is not statistically different. The controls had a range of 28-38 weeks against 24-

37 weeks for RDS/HMD cases. The means were 33.71 ± 0.822 and 31.58 ± 1.311 weeks respectively. The difference, 2.13 weeks, is not significant ($p = 0.844$). The median gestational ages were 33.5 and 32.0 weeks, respectively, a difference of 1.5 weeks.

Although a limited study, in comparison to the archival data, important differences are seen in infants with respiratory distress syndrome, viz, lower birth weight, lower total serum protein, and lower T_4 values, and in clinical manifestations of birth adaptability as is summarized by the Apgar score. By contrast, although TSH, FTI, and thyroid binding globulin were all a little lower in HMD/RDS cases, they were not significantly so ($p = 0.2125$, 0.1760 , and 0.0698 , respectively). Finally, 10 of the 12 HMD/RDS cases were managed in part by enhanced oxygen environment at 30-60%, against none of the 14 non-HMD/RDS infants.

Experimental results following prevagotomy thyroxine:

One hundred twenty six newborn rabbits in a fasting state were subjected to bilateral cervical vagotomy at age 24 hours with air as the inhalant gas, the control group. As previously reported, animals from birth to BCV and after surgery were maintained in an incubator set at 34°C and 50% relative humidity (Shanklin and Sotelo-Avila, 1967). Each batch of four to six animals was followed to their demise and the lungs were examined and the autopsy weight determined. Survival analysis showed this particular control subset to have followed the same pattern of demise as every prior air only subset studied over the years (Shanklin and Berman, 1964, Shanklin and Lester, 1972, Shanklin, 2010). This control experience is the baseline in Figure 4. Table 8 displays the mean and median survival of the control group, values which are very similar to those previously observed in air only treatments following BCV (Shanklin and Berman, 1964; Shanklin, 1969; Shanklin and Lester, 1972; Shanklin, 2010) as well as post thyroxine and post thyrotropin at 3 hours of age. The group control mean lung change was 7.70 ± 1.56 per cent of lung surface. Subset means were: Phase A, 4.23 ± 1.05 , Phase B, $33.6.62 \pm 10.68$, and Phase C, 43.00 ± 7.93 per cent. Despite the large standard error for Phase B (the result of only 6 animals in the group), the difference between Phases A and B was significant, $t = 6.11593$, $p = < 0.0001$. The further difference between Phases B and C was not significant, $p = 0.2519$.

Thirty six newborn rabbits in a fasting state were injected at age 3 hours with thyroxine, 12 at 1 mg/kg, 13 at 10 mg/kg, and 11 at 100 mg/kg. These were then compared to the air only post BCV control set of 126 similar animals. After subset analysis of the survival curves, which revealed little difference by dose as to survival, the data were then combined into a single data set [Figure 4]. Phase A after pre-BCV thyroxine was dramatically shortened, giving the curve a much higher profile prior to a sharp decline in Phase C. The mean survivals were very different. The control mean was 6.87 ± 1.14 hours with a median of 3.00 hours. The combined thyroxine mean survival was 19.92 ± 3.07 hours, with a median of 14.1 hours. Table 9 compares just the mean values (no SEM) for all of the subsets by dose and phase following thyroxine to the whole thyroxine and control observations. When viewed graphically (not shown) several points found in the tabular data deserve attention. Mean lung change in Phase C at 1.0 mg/kg thyroxine appears low for late survivors but can also be viewed as beyond the influence of the least dose utilized. Although there is little difference in the mean survival in Phase A, they bear a precise linear relationship in the semi-log plot. The same is true for both survival and lung injury in Phase B. The smallest group in all experimental runs is Phase C and although the basic change

in late survival is always abrupt over a relatively short interval, comparisons of lung change and other features is sometimes less assured than at earlier times in the experiments.

The negative or harmful effects of middle range (10 mg/kg) and high dose (100 mg/kg) thyroxine in this system are well shown in Phase A. The gross lung change at 10 mg/kg in Phase A is 20.77%, 2.49 times the result at 1.0 mg/kg, and 25.90% at 100 mg/kg, 3.1 times the lowest value. As noted above, this is an exponential correlation against the log dose of thyroxine [Table 9]. By contrast, the mean lung injury are elevated in the 25-33% range during Phase B, the differences having a linear relationship to \log_{10} dose. These values combine in the entire thyroxine result shown in Figure 5.

Experimental results following prevagotomy thyrotropin:

As seen in Figure 4, thyrotropin elicits a survival profile different in important ways from either controls or thyroxine effects. First, the proportion of animals dying in Phase A is intermediate between the other two subsets and conversion to Phase B occurs slightly later in time. Second, Phase B is greatly prolonged such that conversion to Phase C occurs past 65 hours post-BCV. Moreover, the pattern of lung change has a distinctive profile, with the result at 10 mU/kg markedly lower than the other three dose levels [Figure 6]. The figure offers the suggestion this is a presumptively optimal protective dose level since both lower doses and the maximum, 100 mU/kg, all have mean gross lung injuries in the uppermost percentage ranges of the entire study following vagotomy as the only surgical intervention, viz, over 20%

The high variation prompted several recalculations dropping one or more subsets from consideration but these had only minor effects on the profile of the survival curve so all four were left intact as a composite group for presentation [Figure 4].

Table 9 shows the composite mean survival to be 15.09 hours, less than that for thyroxine which is explicable with a longer Phase A but mean gross lung change just 1.05 times higher than the thyroxine effect (22.46% vs. 21.38%). These are, in effect, the same value. Of much interest is the ratio between the areas under the curve [Table 8], 1.02, again, in effect, the same value. It appears both pre-BCV treatments have the same appreciable effect on survival and lung injury, the differing modality and position in the operational scheme of their physiologic actions notwithstanding. When taken together in comparison to the controls, a striking pattern does emerge. The areas under the survival curve are 1.47 (thyroxine) and 1.50 (TSH) of the control value [Table 8] but the extent of lung injury is 2.78 (thyroxine) and 2.92 (TSH) time that of the control result [Table 10].

From this aspect alone one is entitled to conclude, both thyroxine and thyrotropin in pharmacological doses promote lung injury well beyond the deleterious effects of induced respiratory distress. The probing exception is 1.0 mg/kg thyroxine in Phase C, a lung change of 7.0%, no different than the overall control mean, about one sixth of that in Phase C in the controls. It is tempting to conclude the effect of added thyroxine at this level has worn off, the lung reverting to a Phase A status, were it not for the equally demonstrable fact that the actual Phase A injury after 1.0 mg/kg thyroxine was 8.33%, not significantly different from the control value. Exhaustion or downregulation of the pertinent receptors or the feedback loops is a more likely arena for explanation of the result in the final phase of response to this thyroxine dose, if they are involved at all. Thyroxine in this system appears to be working as a drug; the added thyrotropin seems to be mixed in with the thyroid stimulating and modulating events that normally follow birth.

Experimental results following thyroidectomy:

Thyroidectomy two hours post natal was performed to approximate total acute thyroid failure but necessarily leaves intact, at least transiently, the residual actions of circulating and already

receptor and protein bound T_4 , T_3 , and TSH. Eighteen newborn rabbits were thyroidectomized and placed into chambers with either air (10) or 100% oxygen (8). A vastly different survival pattern emerged [Figure 7], which, by visual inspection, lacks Phase A characteristics. The two runs followed closely similar survival curves which could not be distinguished except by duration with a distinctive distribution [Table 10]. There is a displacement in what resembles Phase B at 46 hours post thyroidectomy (at age 48 hours) which may be explained by the distribution difference between air and oxygen exposures. The distribution is highly significant by χ^2 , $p = 0.0073$. The mean survival of the thyroidectomy-air subset (Figure 7) contains the comparison of these 18 animals to two then subjected to BCV after 22 hours in air (the timing of vagotomy was the same as in the animals depicted in Figure 4). Both survived BCV by about one hour and revealed advanced gross lung change, remarkably both at 52% but with different lobar distribution. This dramatic result prompted no further dual surgeries since the main interest was in a comparison between oxygen and air effects on the thyroid ablated animals. However, when placed in a weight loss graphic, they fit exactly due to the very short survival. In the full series of 20 animals, overall weight loss was 26% on a reasonably steady linear slope (-0.00244806) with two outliers, both late air survivors (data not shown). The characteristic previously demonstrated of life prolongation due to oxygen (Shanklin, 2010) is suggested also by this distribution. Although the mean gross lung injury is highly variable it shows strong trends in this small sample. That in oxygen was twice that in air, 2.04 times [Table 11] but with $p = 0.0713$. When the survival time frame is limited to those beyond 48 hours, the ratio rises to 2.33, but with $p = 0.1180$, showing the effect of the smaller sample. The lesser difference in survival (ratio = 1.288) between both groups taken as a whole, is closer to significance, as a unidirectional variable, with $p = 0.0603$. The difference in mean survival after 48 hours is not significant, as expected ($p = 0.2948$).

Discussion

The foregoing experiments, archival review, and clinical study well establish four conclusions:

- [1] Thyroid factors are involved in the respiratory distress of newborn humans and rabbits;
- [2] Thyroid factors are operative within the context of a background metabolic deprivation state which is intrinsic to the animal experiments as performed and which may be present in the underweight fetuses illustrated in Table 1 and Figure 1;
- [3] There is a distinct subset of human premature newborns whose development and functional status eventuates in respiratory distress clinically and in hyaline membrane disease pathologically which is reflected in archival autopsy studies and in concurrent clinical observation; and
- [4] Although there is an overall aspect shown in experimental studies of the thyroid arena, there is variability of a sort which might be best understood by factors not under investigation.

There is also and appropriately, variation in the results in hospitalized premature newborns, and in the archival autopsy database. These variations often emphasize aspects of the pathogenetic network which, if not the cause of the disease, are clearly important parts of the framework. The fetal thyroid develops and matures along certain general lines across gestational time but is subject to variations of pattern, size, and function; it is also influenced by other tissues and organs and pathological states of the fetus [Figure 11]. Thyrotoxicosis has been described *in utero*, apparently the same lesion shown in Figures 11B and 11D (Serup and Petersen, 1979). And, intrauterine ^{131}I treatment has been implicated in neonatal thyroid agenesis (Exss and Graewe, 1974). Maternal thyroid status does influence the growth and development of the fetus (Mestman, et al., 1969). Finally, abnormalities of brain development which interrupt the formation and maturation of the hypothalamic-pituitary axis often result in atypical thyroids (Shanklin, 1991).

Overview of the results:

The onset of breathing and balanced regulation of ventilation are the imperative challenge at birth in newborn animals and human infants (Jansen and Chernick, 1988). As to these experiments and archival studies, the results in general validate the investigation of the whole animal once it became apparent both thyroxine and TSH, although given on a pharmacological challenge basis, had their effects through disturbance of thyroid homeostasis without putting it beyond all aspects of normal function. Perhaps near the head of a list of necessary functions is the question of the duration of effects of T_4 and TSH. This has not been expressly studied in newborn rabbits or infants, more especially those in respiratory distress but the question has been investigated in adult rabbits (Brown-Grant, et al, 1954), adult sheep (De Pablo-Dávila, et al, 1980; Miralles-García, et al, 1981); and in adult rats (Hintze, et al., 1991; Kaplan and Yaskoski, 1980; Larsen and Frumess, 1977; Nathanielsz, 1971).

Systemic disease alters the peripheral conversion of thyroxine to triiodothyronine (De Pablo-Dávila, et al, 1980; Miralles-García, et al, 1981). The kidney is especially important in thyroid metabolism through: (a) excretion of the hormones and metabolites in the urine and as a major site for extrathyroidal conversion of T_4 to T_3 (De Pablo-Dávila, et al, 1980) The normal elimination stage half-life for T_4 was reported as 33.4 ± 4.4 hours. The comparable half life of T_4 in induced acute liver insufficiency studies from the same laboratory (Miralles-García, et al., 1981) was 26.9 ± 3.5 hours. Physical and emotional stress was applied to adult rabbits by Brown-Grant, et al (1954) including laparotomy, physical restraint, intraperitoneal turpentine, and induced hemorrhage. They primed rabbits with ^{131}I and then followed the thyroid release of radiolabeled T_4 under various conditions. The release periods ranged from 20 to 40 hours. Nathanielsz (1971) approached this from the standpoint of the thyroxine distribution space in the adult rabbit, a statement of the circulating pool of [^{125}I] T_4 , not the rate of secretion. The half life of T_4 in this compartment was 11 hours. Significantly, the rate of conversion of T_4 to T_3 was significantly reduced in rats fasted for 48 hours (Hintze, et al, 1991). More recently, Nagao, et al. (2011), demonstrated an effect of thyroidectomy on thyroxine metabolism and turnover in seven week old Sprague-Dawley rats. Injection of radiocarbon labeled thyroxine ($^{13}\text{C}_9$) T_4 was followed for 17 days. In normal rats the labeled T_4 had cleared by the fifth day whereas in the post thyroidectomy animals it took nine days to clear and after two days peripheral T_4 stabilized from the release of thyroxine from peripheral tissues (Nagao, et al., 2011). Their results translated into a half life in the controls of 14.16 hours and 27.84 hours following thyroidectomy.

Whilst not precise in terms of time frame nor from newborn rabbits as a direct comparison model, these several time intervals fit reasonably well to those which resulted from the experimental side of this report.

The Potter Archive:

There are no studies of comparable nature, size, or by historic period to the Potter Archive. Changes in the clinical care of newborns since World War II, and declines in both the mortality rate and the frequency of informed autopsy study, militate against there being another opportunity for similar data to be obtained. It is noteworthy, accordingly, to see several presumed or intuitively considered aspects to be confirmed by the archive. The role of thyroid factors was well assessed by Redding and Pereira (1974) whose earlier work was in part a stimulus for these studies (Redding, et al., 1969; Redding, et al, 1972). Those reports considered thyroid factors as a feature of maturation of the pulmonary surfactant system but did not infer a pathogenetic role of the thyroid in hyaline membrane disease. However, with a considerable number of cases in the Archive, viewed in retrograde fashion, 1965 back to 1955, a period prior to “modern” measures of clinical support and treatment beyond those of the 1930s and 1940s,

of the developing thyroid gland, as more colloid accumulates, there will be fewer cells in each milligram of tissue. But to take this to a deeper level, it should be noted the number of specific nuclear T_3 receptors in the lung, as the peripheral action site for thyroid effects on lung, varies in the rabbit. Lindenberg, et al., (1978) found 2400 specific binding sites per pulmonary cell in the fetus contrasted to 1120 sites per adult lung cell. Thus, size or size ratios have to be considered as an indirect marker for functional capacity as a rule, but when the data base is large and the trend is consistent in one direction, then greater weight may be placed on the result.

The archival collection of 450 newborns, 154 with hyaline membrane disease, has an utterly consistent pattern of small thyroids from midgestation to term. These cases had highly varied survival times but subgroup coherence is shown in the mean crown-heel and crown-rump lengths. Fetal body length is a better index of gestational age and maturity than birth weight and it seemed well advised to determine whether an immediate post natal loss of thyroid mass had occurred as it seemed unlikely such a definitive ratio could be maintained in the awareness that average post natal survival differs considerably over gestational time, those near midgestation surviving just a few hours as an upper limit (Shanklin and Zhang, 2007). A return to the Potter Archive in early 2011 resulted in the information found in Tables 2, 4, and 5, and Figures 2 and 3 on the average. This study yielded information on the thyroid:body mass ratio in the first hours after birth.

There is virtually no information on this point with a singular exception. Little (1991) considered the effect on newborn pups of the southern elephant seal of being born into the almost freezing environment of Macquarie Island in far South Pacific ocean. He concluded there was little effect overall up to 50 hours after birth but when the results were plotted by time after birth there appeared a distinctive 12% drop in thyroid mass in the first two hours and a 3% increase in thyroid epithelial height which later displayed a 24.5% loss of vertical dimension (data not shown). Thyroid mass increased after about 120 hours post natal. There are important differences, of course, between newborn rabbits in an experimental setting in a heated incubator and elephant seal pups in their native habitat with considerable reserves of adipose tissue. In the seal pup T_4 peaked at two hours and T_3 at four days (Little, 1991).

The second phase of archival review is also a unique dataset, for the same reasons noted above. Figure 3 shows the biphasic decline in the thyroid:body mass ratio. The question arises whether the 30.9% decline from birth to 12 hours plus a subsequent decline to a final reduction of 68.2% at 66 hours after birth accounts for the remarkable difference seen in Table 1 and Figure 1. The numbers themselves bracket the range of ratios found in the first phase review. The data was drawn from the two reviews under a different rubric and, although the body weight is progressive over gestational time, it is a secondary variable (Fig. 8). Some insight into this comes from Figure 10. This takes the paired mean body weights by gestational age range and converts them to a weight ratio. The earliest gestational period, 22-27 weeks, has a ratio of 1.104 which rapidly declines with the passage of time. The same calculation of the thyroid weights as an HMD/control ratio yields an irregular series: 0.736, 0.750, 0.723, 0.603, 0.695, for the intervals 22-27, 28-30, 31-33, 34-36, and 37-39 weeks respectively (data not shown graphically). These non-linear relationships, all being less than the body weight ratios across the same time frame, are evidence against mass loss of the thyroid post natal over gestational time as the sole or a major controlling factor operant in Figure 3. The inference is while there is a tendency toward smaller fetuses the thyroids become disproportionately even smaller. The critical message of the analysis which was drawn from 4,588 autopsy records is that the control cases *did not show this effect at all*. Accordingly, there is some intrinsic aspect of the thyroid gland or of the thyroid arena in the pathophysiological or developmental sense which gives rise to these evident abnormalities of time resultant thyroid size.

Progressive weight loss of the experimental animal from birth to surgery and after vagotomy or other procedures, is evidence of a metabolic effect at work in the model. Table 1 provides mean birth weights by gestational age ranges for hyaline membrane cases and control newborns. The

ratio of these mean weights is plotted in Figure 10, the weight of HMD cases as the antecedent value and the control cases as the consequent variable. Only in the first time set does the ratio exceed 1.00 and afterwards it declines to around 85% (15% loss) at 32 weeks, remaining there for the remainder of gestational time.

The clinical study:

There have been many reports on studies of thyroid function in both healthy and distressed newborns and premature newborns (Carrascosa, et al., 2004; Clemente, et al., 2007; Cuestas and Engel, 1979; Delange, et al., 1984; LaFranchi, 1999; Redding and Pereira, 1974; van Wassenaer, et al., 1997; Williams, et al., 2005). The earliest of these is that of Redding and Pereira (1974). In some respects this is similar to the study report here. It is larger but TSH was not determined, but included normal full term infants as a collateral control, a feature absent from the present study. Redding and Pereira reported 79 premature infants, 40 with RDS and 39 without. The mean cord blood T_4 in RDS infants was $7.2 \pm 1.4 \mu\text{g/dl}$ against the control value of $8.5 \pm 1.3 \mu\text{g/dl}$, the former 15.3% less than the latter. Remarkably, although the results here are slightly lower, the difference [Table 7a] is almost exactly the same, 15.6%. Moreover, they reported progressive values. Premature infants with RDS at less than 29 weeks = 5.6 ± 1.2 , 30-33 weeks = 7.2 ± 1.4 (the same as whole group mean), and at 34-37 weeks = $7.9 \pm 1.1 \mu\text{g/dl}$. The controls were all above 29 weeks but also showed progression: 8.5 ± 1.3 at 30-33 weeks, and $9.0 \pm 1.9 \mu\text{g/dl}$ at 34-37 weeks. The differences were all significant at either $p < 0.05$ or < 0.01 .

Cuestas and Engel (1979) reported a lower mean TSH peak in the RDS group ($32.8 \pm 9.6 \mu\text{U/ml}$) than the controls ($60.9 \pm 21.8 \mu\text{g/dl}$), with $p < 0.005$. The RDS value was 53% of normal. Williams, et al. (2005) recruited 780 infants at a gestational age of 23-24 weeks and followed them for 28 days post natal. They correlated thyroid factor values with most of the ills which afflict extremely premature infants. There was a positive correlation between low TSH and oxygen dependence at 28 days.

Alone of the eight papers cited here, Redding and Pereira (1974) reported the Apgar Scores of both subsets. They reported the scores in a different format, the frequency of those less than 6: 26/40 infants with RDS (65.0%) and 12/39 controls (30.8%). Moreover, only three other than Redding and Pereira reported cord blood values as point of birth determinations (Carrascosa, et al., 2004; Clemente, et al., 2007; van Wassenaer, et al., 1997). These are confirmatory and appear to establish low thyroid function amongst the causal factors on a time sequence basis if for no other reason.

Animal experiments: prevagotomy thyroxine:

The effect of prevagotomy thyroxine (T_4) is shown in Figures 4, 5, 9, and 12, and in Tables 8 and 9. Figure 4 demonstrates an early positive effect on survival, considerable shortening of Phase A and about a 20% extension of Phase B (42.85 hours versus 34.29). However, final Phase C has a sharper onset and a steep decline to zero survival for a maximum just 75% of the air only controls. This qualifies as a hormetic effect, essentially, albeit spread over a time dynamic rather than as a dose response curve (Calabrese and Baldwin, 2001; Robertson and Grutsch, 1987). That thyroxine is having a specific pulmonary effect, as well as the probable influence on metabolism, is shown in Figures 5, 9, and 12. Figure 5 is a 2-dimensional plot of mean gross lung injury against the \log_{10} of the dose applied at 3 hours of age, 21 hours before vagotomy which clearly shows the exponential relationship, implying a possible primary forcing role to T_4 when added as a pharmacological substance over a three point grid beyond the physiological range of newborn rabbits. Figure 12 is a 3-dimensional plot which combines the \log_{10} dose, the mean gross lung change, and mean survival for each dose level. The third factor smooths out the very small difference in values found from the initial linear regression seen in Figure 5 and provides an even clearer picture of the forcing effect of thyroxine on the lung experiencing respiratory distress, although paradoxically there is a reduced worsening as the dose is increased.

This is not a contradiction of the positive influence of thyroid factors on surfactant function in the neonatal lung since that is an effect of nuclear triiodothyronine (T_3) receptors in the lung (Luo, et al., 1989). The result implies an excess of T_4 which might have been circulating as free thyroxine (the index, FTI) thus being available for a specific dynamic effect which in this experimental model is enhanced lung injury. Against this explanation is a lower FTI in RDS/HMD cases [Table 11] which was also found by Redding and Pereira (1974): infants with RDS, 1.99 ± 0.30 ; controls, 2.54 ± 0.18 , a significant difference with $p < 0.01$. Since serum protein [Table 7b], including thyroid binding globulin, was significantly lower in the RDS/HMD subset (4.05 ± 0.21 g/dl) compared to the controls (4.69 ± 0.18 g/dl) there might have been less binding capacity. Not reported in Tables 7a or 7b were determinations of thyroid binding globulin specifically. The RDS/HMD subset had 17.408 ± 1.490 μ g/dl and the controls 19.857 ± 0.761 μ g/dl; this small difference approached significance with $p = 0.0698$ [Table 11].

The other side of the equation is whether a reduction in carrying capacity results in a higher free thyroxine index (FTI). Table 11 shows it did not clinically. The difference between RDS/HMD cases and the controls, 0.612, is even less significant, with $p = 0.1760$. The lower FTI in the larger series reported by Redding and Pereira (1974) was significant and had a difference of a similar order of magnitude of 0.55. Just why their range of FTI values is so different is not known but may be related to the method of calculation of the index. Redding and Pereira (1974) used the formula: $FTI = T_4/\%T_3$. Brien, et al. (1974), who reported values in the same range (7.5-18.0) as in the current study, used the formula: $FTI = T_4/T_3$, as was done here.

Nevertheless, the lung injury in these experimental animals cannot be explained on the basis of the binding capacity of serum proteins, whether specific or nonspecific. The difference in thyroid binding globulin is insufficient to account for the pulmonary effect which has an ascending if complex quantitative response to dose applied. Although their paper does not provide the number of animals per determination, Myant and Osorio (1959) reported total serum protein in rabbit fetus and on the day of birth as 4.446 g/dl (30th day of gestation), 4.598 g/dl (31st day), and 4.598 g/dl for the first postnatal day, values well within the range found in the clinical arm of this study.

The present report contains no information on thyroxine receptors (TR). Keijzer, et al. (2007), using mRNA and protein analysis found TR α 2 to occur first in developing rat lung, followed by TR β 1, but no TR β 2 in either form. TR α 1 protein was identified in lung but its mRNA was not. Keijzer, et al. (2007) found the mRNA for TR α alpha was ten times as high as in the liver but in brain it was 100 times the hepatic concentration. They concluded there was extensive post-transcriptional regulation which varies according to the organ or tissue. This is congruent with the current finding that lung injury is produced by thyroxine across a pharmacological range in a reverse exponential fashion (i.e., higher doses result in more injury but on a progressively diminishing scale). Thyroxine thus has no potential role in the clinical management of respiratory distress syndrome. This same conclusion was reached by Stahnke, et al. (1986), but on very different grounds from a study of clinical laboratory data on RDS newborns.

Animal experiments: prevagotomy thyrotropin:

The effect of thyrotropin is shown in Tables 8 and 9 and in Figures 4 and 6. Figure 4 conveys what might have been anticipated, a delay in the trophic effect, a doubling of the length of the curve in Phase A, a substantial increase in Phase B, 1.45 times that following thyroxine and 1.8 times that of the air only controls, and a terminal slope in Phase C intermediate to both of the others. Despite these aspects, the fact they occur after considerable mortality has passed is the explanation for the overall shorter mean survival shown in Table 8, just over 15 hours against almost 20 for thyroxine, actually 75.7% of the latter. Both mean survivals greatly exceed that for the air only controls at 6.87%. The median survival for TSH treated animals is 40% higher than the controls but both are under five hours. The area under the survival curve is essentially the same for post thyroxine and post thyrotropin animals. This can be taken as evidence of a net

common effect on the thyroid arena, by thyroxine directly, and by thyrotropin through its usual channels, viz, the trophic process. These comparative distinctions are summarized as regression slopes in the far right column of Table 9.

From this point the data develop a compelling complexity. Figure 6 has a very different aspect of the thyrotropin effect. When gross lung change is plotted against the \log_{10} of the dose of thyrotropin the U-shape of hormesis (Calabrese and Baldwin, 2001; Robertson and Grutsch, 1987), with a low dose range shoulder, appears. Remarkably, it appears the system of thyroid stimulations and peripheral actions, possibly including nonthyroidal conversion of T_4 to T_3 , is least disturbed by a dose of 10 mU/kg thyrotropin. Perhaps even more remarkable is the close approximation of quantitative gross lung change at all three of the other doses, 0.1, 1.0, and 100 mU/kg. When independently plotted, linear regression yields a mean which fully brackets all three data points within one standard error of the mean (SEM). This seems to violate biochemically one known attribute of thyrotropin effect, the stimulation of cAMP phosphoinositide signaling (Boutin, et al, 2011). The current data set does not provide information on the presence of or the relative activity of low or high affinity thyrotropin binding sites, either in the thyroid (Lefort, et al., 1984) or elsewhere (Ishigaki, et al., 1989). Lefort, et al. (1984) concluded that low affinity sites do not participate in the stimulation of adenylate cyclase.

Nor is TSH likely to be active in peripheral organs which convert T_4 to T_3 . Hepatic and renal deiodinases are very active, accounting for perhaps 80% of the peripheral conversion (Hillgartner and Romsos, 1987), although it has been suggested the renal activity is directed at least in part toward the excretion of iodides by further deiodination of T_3 (Shimoda and Greer, 1972). The population of TSH receptors outside of the thyroid gland is limited and liver appears to have few if any (Endo, et al., 1993) while kidney does (Dutton, et al., 1997). TSH receptors have been identified in the anterior pituitary (Theodoropoulou, et al, 2000) offering the possibility of a feedback loop through thyrotropin-releasing hormone. Rates of conversion have been reported in slices of human tissue, kidney, liver, heart, and skeletal muscle (Albright and Larson, 1959). Only kidney of these four showed measurable conversion, averaging 22.30 ± 2.84 per cent ($N = 10$).

Animal experiments: early neonatal thyroidectomy:

The experimental results are entirely summarized in Figure 7 and Table 10. There is a continuum along the survival curve for animals whether treated after surgery to an environment of air or 100% oxygen. The principal finding related to the distribution of survival along the continuum. Twice as many oxygen treated animals survived past 48 hours, and no oxygen treated animal died before 48 hours [Table 10]. This is a classic χ^2 scenario with a highly significant result, $p = 0.0073$. There was a strong trend in the results of gross lung injury as noted above, a mean in oxygen twice that in air, but the small samples did not yield the standard p threshold of 0.05.

Antenatal thyroidectomy in the lamb followed immediately by delivery failed to change the T_4 levels at 60 minutes after birth but there was a much lower value T_3 , after fetal thyroidectomy, 39.0 ± 4.8 ng/dl, versus 153.0 ± 20.1 in sham operated fetal lambs (Polk, et al., 1986). Follow up study revealed no change in 5'-monodeiodinase for any tissue from either sham or late fetal thyroidectomized subjects. Polk, et al (1986) concluded the post natal surge in T_3 came from the thyroid and not from peripheral sources. In the present work thyroidectomy was timed to get past the surge at two hours post natal, allowing for that immediate effect to have occurred prior to environmental challenge. One cannot assess how much of a factor this might have been relative to the late fetal procedure used by Polk, et al. (1986) as it was not part of the current protocol. The previously mentioned report of Nagao, et al (2011) invoked the reinfusion of T_4 from storage status elsewhere, from possible enterohepatic recirculation following excretion in bile, or from extrathyroidal production. Either of the first two might be applicable given the similarity of time frames in the current study and theirs. Nagao, et al. (2011) offered no data to support the third suggestion.

Concluding comments:

The difficulties in sorting out the effects of thyroid arena hormones, from thyrotropin-release hormone, through TSH, and in the peripheral conversion of T_4 , have been long known. In one sense this is the profound dilemma of intact animal investigation, those forces regularly applied to maintain homeostasis, whilst disturbed by the experiments, may play a necessary role in the process. By combining historical data, from what might advisedly be called *near to natural conditions*, but no longer available concurrently or prospectively, with more up to date clinical assessments and animal experiments, some idea of the pathogenesis has been drawn together if for no other reason than to rule out certain aspects, such as a possible beneficial role for thyroxine in lung injury, leaving a field for future consideration less cluttered than presently. The more modern cellular techniques, however, would need to be applied to an animal model and although much has been learned from neonatal rabbits a larger research animal with a longer gestation would enable study of the rather early gestational period suggested here as the time when the pathogenesis gets underway.

This period has been the subject of increasing interest. The importance of TSH to the early pathogenesis of hyaline membrane disease is highlighted by two aspects. First, the α -subunit of TSH and hCG are identical (Garcia-Campayo, et al., 2002; Norman, et al., 1987) and hCG has been acknowledged as a weak thyrotropin despite the differences in the β -subunit (Hershman, 1999). Although weaker in molar effect, the considerable increase in levels of hCG in the four week periods, 9-12 and 13-16, to maximum levels of 288,000 and 254,000 mIU/mL respectively (McPherson and Pincus, 2006) offers the possibility of a thyrotrophic effect secondary to hCG as an additive stimulus to the expected changes in formulation and maturation of the hypophyseal-pituitary-thyroid stimulating processes in the first half of the second trimester. Second, there is evidence of a relationship between the level of hCG and intrauterine growth retardation (Haddad, et al., 1999; Ilagan, et al., 2004; Liu, et al., 1999; Onderglu and Kabukcu, 1997; Vaillant, et al., 1996) although there is some question whether very early low values (Haddad, et al., 1999) or elevated values around the end of the first trimester or in the early second trimester is the important finding (Ilagan, et al., 2004; Liu, et al., 1999; Onderglu and Kabukcu, 1997; Vaillant, et al., 1996). There is no other reported link similar to the data in this report. It may be the factor which distinguishes those fetuses which later will be underweight relative to their gestation [Table 1, Figure 1] and have hyaline membrane disease is some form of integrated interaction between placental hCG and fetal TSH-releasing hormone, TSH, and the modulating effects of fetal thyroid activity.

This paper concerns major aspects of the metabolic adaptations of newborns. The basic metabolic transformation which follows birth is a reversal of the dominant principal fetal energy food source. Prenatally, the nutrient supply is rich in carbohydrates and low in lipids with both gluconeogenesis and ketogenesis absent to low. Postnatally these factors are reversed and gluconeogenesis increases toward adult levels soon after birth and a rate limiting enzyme, phosphoenolpyruvate carboxykinase (PEPCK) emerges and becomes significant (Girard, 1990).

However, in this vagotomy model, the transition is delayed in part by the non-provision of the programmed postnatal nutrients. Although the metabolic factors discussed herein, especially thyroxine, are integral to the maturation, production, and release of pulmonary surfactant, that aspect of the scheme will be reported elsewhere.

Of interest quantitatively, with the more or less consistent half size of the thyroid and adrenal across gestational time (Table 1 and Figure 1), is the observation that total carbohydrate reserves in infants dying from hyaline membrane disease are about half those found in other neonatal deaths (Shelley, 1964). The juxtaposition of these findings is evidence of a deeper metabolic factor, likely progressing through gestation and marking the special subset also seen here in the clinical data. The latency of the absolute and relative weights for the adrenals can be taken as evidence of a more primary or necessary role for the thyroid arena.

Detailed examination of the role of the adrenal gland and cortical hormones were not undertaken here but some aspects of the interrelation between thyroid factors and adrenal factors with the pulmonary surfactant system have been reported (Ohashi, et al., 1991; Rooney, et al., 1986). Other factors, some with neurovascular actions, such as aminophyllin, have been found to enhance surfactant production (Sevanian, et al., 1979).

But there is another aspect. Genetic mutations in the TSH-receptor gene have been implicated in both congenital hypothyroidism (Bretones, et al., 2001) and in congenital hyperthyroidism (Chester, et al., 2008). The genetic factors presumably involved in alleles of receptors can be addressed indirectly by deeper analysis of maternal, paternal, and litter effects in the research database from which these specific experiments were drawn. This will be reported elsewhere in the context of an overall assessment of the neonatal rabbit model.

A new and unique perspective on this is that of Pei, et al. (2011). Knock-in mutations of the nuclear co-repressor SMRT (silencing mediator of retinoid and thyroid hormone receptors) in C57BL/6 mice produced a previously identified respiratory distress syndrome caused by prematurity of type I pneumocytes, the principal peripheral lung epithelium most responsible for oxygen, carbon dioxide, and water exchange between the peripheral air spaces and the intrinsic tissue of the lung.

The demonstration of a stimulatory effect of IL-2 on pituitary cells, including thyrotropes, has not been well developed following the original report (Artz, et al., 1995); theirs is not an isolated observation. Collateral evidence comes from instances of hyperthyroidism (Koukkou, et al., 1995) and the observation that therapeutic use of recombinant IL-2 in renal cell carcinoma leads to thyroid dysfunction including transient hyperthyroidism (Jacobs, et al., 1991). Thus, another trophic layer is added to the thyroid arena when TSH-releasing hormone is taken into account (Guillaume, et al., 1985). There are no comparable studies as yet during pregnancy in either humans or experimental animals. Whilst these and potentially other considerations may be found to operate within the developing thyroid arena, one fact remains very clear from the archival analysis: the control cases *do not show these several effects at all*. Therefore there is an intrinsic aspect of thyroid gland development behind the pathophysiological abnormalities and of thyroid size which causes or promotes the respiratory distress syndrome and hyaline membrane disease. The evidence across gestational time strongly suggests the inference the point of origin lies in the early to mid-second trimester when chorionic gonadotropin is still ascendant and TSH rises dramatically, pushing the fetal thyroid gland to its maximal relative weight.

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References

- Abuid, J., Stinson, A., Larsen, P.R., 1973. Serum triiodothyronine and thyroxine in the neonate and the acute increases in these hormones following delivery. *J.Clin.Invest.* 52, 1195-1199.
- Albright, E.C., Larson, F.C., 1959. Metabolism of L-thyroxine by human tissue slices. *J.Clin. Invest.* 38, 1899-1903.
- Artz, E., Sauer, J., Buric, R., Stalla, J., Renner, U., Stalla, G.K., 1995. Characterization of interleukin-2 (IL-2) receptor expression and action of IL-2 and IL-6 on normal anterior pituitary cell growth. *Endocrine.* 3, 113-119.
- Barker, P.M., Strang, L.B., Walters, D.V., 1990. The role of thyroid hormones in maturation of the adrenaline-sensitive lung liquid reabsorptive mechanism in fetal sheep. *J.Physiol.* 424, 473-485.
- Boutin, A., Allen, M.D., Geras-Raaka, Huang, W., Neumann, S., Gershengorn, 2011. Thyrotropin receptor stimulates internalization-independent persistent phosphoinositide signaling. *Mol.Pharmacol.* 80, 240-246.
- Bretones, P., Duprez, L., Parma, J., David, M., Vassart, G., Rodien, P., 2001. A familial case of congenital hypothyroidism caused by homozygous mutations of the thyrotropin receptor gene. *Thyroid* 11, 977-980.
- Brien, T.G., Fay, J.A., Griffin, E.A., 1974. Thyroid status in the newborn infant: effective thyroxine ratio and free thyroxine index. *Arch.Dis.Childh.* 49, 225-227.
- Brown-Grant, K., Harris, G.W., Reichlin, S., 1954. The effect of emotional and physical stress on thyroid activity in the rabbit. *J.Physiol.* 126, 29-40.
- Calabrese, E.J., Baldwin, L.A., 2001. The frequency of U-shaped dose responses in the toxicological literature. *Toxicol.Sci.* 62, 330-338.
- Carrascosa, A., Ruiz-Cuevas, P., Potau, N., Almar, J., Salcedo, S., Clemente, M., Yeste, D., 2004. Thyroid function in seventy-five healthy preterm infants thirty to thirty-five weeks of gestational age: a prospective and longitudinal study during the first year of life. *Thyroid* 14, 435-442.
- Cassidy, C.E., Benotti, J., Peno, S., 1968. Clinical evaluation of the determination of thyroxine iodine. *J.Clin.Endocrinol.Metab.* 28, 420-421.
- Clemente, M., Ruiz-Cuevas, P., Carrascosa, A., Potau, N., Almar, J., Salcedo, S., Yeste, D., 2007. Thyroid function in preterm infants 27-29 weeks of gestational age during the first four months of life: results from a prospective study comprising 80 preterm infants. *J.Pediatr.Endocrinol.Metab.* 20, 1269-1280.
- Chester, J., Rotenstein, D., Ringkanaanont, U., Steuer, G., Carlin, B., Stewart, L., Grasberger, H., Refetoff, S., 2008. Congenital neonatal hyperthyroidism caused by germline mutations in the TSH receptor gene. *J.Pediatr.Endocrinol.Metab.* 21, 479-486.
- Cuestas, R.A., Engel, R.R., 1979. Thyroid function in preterm infants with respiratory distress syndrome. *J.Pediatr.* 94, 643-646.

- Delange, F., Dalhem, A., Bourdoux, P., Lagasse, R., Glinoe, D., Fisher, D.A., Walfish, P.G., Ermans, A.M., 1984. Increased risk of primary hypothyroidism in preterm infants. *J.Pediatr.* 105, 462-469.
- De Pablo-Davilá, F., Miralles-García, J.M., Hernandez-Miguel, E., García-Iglesias, C., Corrales-Hernández, J.J., del Pozo, S.C., 1980. Study of the kinetics of the thyroid hormones and the peripheral conversion rate of thyroxine to triiodothyronine in acute renal insufficiency under experimental conditions. *Horm.Metab.Res.* 12, 529-536.
- Dutton, C.M., Joba, W., Spitzweg, C., Heufelder, A.E., Bahn, R.S., 1997. Thyrotropin receptor expression in adrenal, kidney, and thymus. *Thyroid* 7, 879-884.
- Edson, J.L., Hull, D., 1977. The effect of maternal starvation on the metabolic response to cold of the newborn rabbit. *Pediatr.Res.* 11, 793-795.
- Endo, T., Ohno, M., Kotani, S., Gunji, K., Onaya, T., 1993. Thyrotropin receptor in non-thyroid tissues. *Biochem.Biophys.Res.Comm.* 190, 774-779.
- Exss, R., Graewe, B., 1974. Congenital athyroidism in the newborn infant from intra-uterine radioiodine action. *Biol.Neonate* 24, 289-291.
- Farber, S., 1937. Studies on pulmonary edema. I. The consequences of bilateral cervical vagotomy in the rabbit. *Am.J.Exp.Med.* 66, 397-404.
- Felig, P., Marliss, E., Cahill, G.F., Jr., 1969. Plasma amino acids and insulin secretion in obesity. *N.Eng.J.Med.* 281, 811-816,
- Felig, P., Kim, Y.J., Lynch, V., Hendler, R., 1972. Amino acid metabolism during starvation in human pregnancy. *J.Clin.Invest.* 51, 1195-1202
- Fisher, D.A., Odell, W.D., 1969. Acute release of thyrotropin in the newborn. *J.Clin.Invest.* 48, 1670-1677.
- Garcia-Campayo, V., Kumar, T.R., Boime, I, 2002. Thyrotropin, follitropin, and chorionic gonadotropin expressed as a single multifunctional unit reveal remarkable permissiveness in receptor-ligand interactions. *Endocrinology* 143, 3773-3778.
- Girard, J., 1990. Metabolic adaptation to change of nutrition at birth. *Biol.Neonat.* 58, Supp. 1, 3-15.
- Guillaume, J., Schussler, G.C., Goldman, J., 1985. Components of the total serum thyroid hormone concentrations during pregnancy: high free thyroxine and blunted thyrotropin (TSH) response to TSH-releasing hormone in the first trimester. *J.Clin.Endocrin.* 60, 678-684.
- Haddad, B., Abirached, F., Louis-Sylvestre, C., Le Blond, J., Paniel, B.J., Zorn, J.R., 1999. Predictive value of early human chorionic gonadotrophin serum profiles for fetal growth retardation. *Hum.Reprod.* 14, 2872-2875.
- Hemberger, J.A., Schanker, L.S., 1978. Effect of thyroxine on permeability of the neonatal rat lung to drugs. *Biol.Neonate* 34, 299-303.
- Henry, J.B., *Clinical diagnosis and management by laboratory methods*, 17th ed., 1984, W.B. Saunders Co., Philadelphia, pp. 307-312.
- Hershman, J.M., 1999. Human chorionic gonadotropin and the thyroid: hyperemesis gravidarum and trophoblastic tumors. *Thyroid* 9, 653-657.

- Hillgartner, F.B., Romsos, D.R., 1987. Iodothyronine 5'-deiodination in rats fed low protein diets: lack of correlation with energy balance. *J.Nutr.* 117, 368-375.
- Hintze, G., Braverman, L.E., Ingbar, S.H., 1991. The effect of surgical stress on the in vitro metabolism of thyroxine by rat liver, kidney, and brain. *Endocrinology* 128, 146-152.
- Ilagan, J.G., Stamilio, D.M., Ural, S.H., Macones, G.A., Odibo, A.O., 2004. Abnormal multiple marker screens are associated with adverse perinatal outcomes in cases of intrauterine growth restriction. *Am.J.Obstet.Gynecol.* 191, 1465-1469.
- Ishigaki, S., Abramovitz, M., Listowsky, I., 1989. Glutathione-S-transferases are major cytosolic thyroid hormone binding proteins. *Arch.Biochem.Biophys.* 273, 265-272.
- Jacobs, E.L., Clare-Salzler, M.J., Chopra, I.J., Figlin, R.A., 1991. Thyroid function abnormalities associated with the chronic outpatient administration of recombinant interleukin-2 and recombinant interferon-alpha. *J.Immunother.* 10, 448-455.
- Jansen, A.H., Chernick, V., 1988. Onset of breathing and control of respiration. *Sem.Perinatol.* 12, 104-112.
- Kaplan, M.M., Yaskoski, K.A., 1980. Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. *J.Clin.Invest.* 66, 551-562.
- Keijze, R., Blommaart, P-J.E., Labruyère, W.T., Vermeulen, J.L.M., Doublabi, B.Z., Bakker, O., Tibboel, D., Lamers, W.H., 2007. Expression of thyroid hormone receptors A and B in developing rat tissues: evidence for extensive posttranscriptional regulation. *J.Mol.Endocrinol.* 38, 523-535.
- Kim, Y.J., Felig, P., 1972. Maternal and amniotic fluid substrate levels during calorie deprivation in human pregnancy. *Metabolism* 21, 507-512.
- Koukkou, E., Panayiotidis, P., Thalassinou, N., 1995. Serum soluble interleukin-2 receptors as an index of the biological activity of thyroid hormones in hyperthyroidism. *J.Endocrinol.Invest.* 18, 253-257.
- LaFranchi, S., 1999. Thyroid function in the preterm infant. *Thyroid* 9, 71-78.
- Larsen, P.R., Frumess, R.D., 1977. Comparison of the biological effects of thyroxine and triiodothyronine in the rat. *Endocrinology* 100, 980-988.
- Lefort, G.P., Amr, S., Carayon, P., Nisula, B.C., 1984. Relevance of the low and high affinity thyrotropin-binding sites of human thyroid membranes to the stimulation of adenylate cyclase. *Endocrinology* 114, 1005-1011.
- Lindenberg, J.A., Brehier, A., Ballard, P.L., 1978. Triiodothyronine nuclear binding in fetal and adult rabbit lung and cultured lung cells. *Endocrinology* 103, 1725-1731.
- Little, G.L., 1991. Thyroid morphology and function and its role in thermoregulation in newborn elephant seal (*Mirounga leonina*) at Macquarie Island. *J.Anat.* 176, 55-69.
- Liu, D.F., Dickerman, L.H., Redline, R.W., 1999. Pathologic findings in pregnancies with unexplained increases in midtrimester maternal serum human chorionic gonadotropin levels. *Am.J.Clin.Pathol.* 111, 209-215.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J.Biol.Chem.* 193, 265-275.

- Luo, M., Faure, R., Tong, Y.A., Dussault, J.H., 1989. Immunocytochemical localization of the nuclear 3,5,3'-triiodothyronine receptor in the adult rat: liver, kidney, heart, lung and spleen. *Acta Endocrinol (Copenh)*. 120, 451-458.
- Man, E.B., Reid, W.A., Hellegers, A.E., Jones, W.S., 1969. Thyroid function in human pregnancy. II. Serum butanil-extractable iodine values of pregnant women 14 through 44 years. *Am.J.Obstet.Gynecol.* 103, 328-337.
- Mashiach, S., Barkai, G., Sack, J., Stern, E., Goldman, B., Brish, M., Serr, D.M., 1978. Enhancement of fetal lung maturity by intra-amniotic administration of thyroid hormone. *Am.J.Obstet.Gynecol.* 130, 289-293.
- McPherson, R.A., Pincus, M.R., 2006. *Henry's clinical diagnosis and management by laboratory methods*, 21st ed., Saunders, Philadelphia, ISBN 1-4160-0287-1.
- Mestman, J.H., Niswonger, J.W., Anderson, G.V., Manning, P.R., 1969. Evaluation of thyroid function during and after pregnancy. *Am.J.Obstet.Gynecol.* 103, 322-327.
- Miralles-García, J.M., García-Igesias, C., García-Díez, L.C., De Pablo-Dávila. F., 1981. The kinetics of the thyroid hormones and the peripheral conversion rate of thyroxine to triiodothyronine in acute liver insufficiency under experimental conditions. *Horm.Metab.Res.* 13, 158-164.
- Myant, N.B., Osorio, C., 1959. Serum proteins, including thyroxine-binding proteins, in maternal and foetal rabbits. *J.Physiol.* 146, 344-357.
- Nagao, H., Imazu, T., Hayashi, H., Takahashi, K., Minato, K., 2011. Influence of thyroidectomy on thyroxine metabolism and turnover rate in rats. *J.Endocrin.* 210, 117-123.
- Nathanielsz, P.W., 1970. The effect of diet and acute starvation on the deiodination of thyroxine and triiodothyronine in the thyroidectomized rat. *J.Physiol.* 206, 701-710.
- Norman, R.J., Haneef, R., Buck, R.H., Joubert, S.M., 1987. Measurement of the free alpha subunit of human glycoprotein hormones by a monoclonal antibody-based immunoradiometric assay, and further exploration of antigenic sites on the choriogonadotropin molecule. *Clin.Chem.* 33, 1147-1151.
- Ohashi, T., Takada, S., Motoike, T., Tsuneishi, S., Matsuo, M., Sano, K., Makamura, H., 1991. Effect of dexamethasone on pulmonary surfactant metabolism in hyperoxia-treated rat lungs. *Ped.Res.* 29, 173-177.
- Onderglu, L.S., Kabukçu, A., 1997. Elevated second trimester human chorionic gonadotropin level associated with adverse pregnancy outcome. *Int.J.Gynaecol.Obstet.* 56, 245-249.
- Pei, L., Leblanc, M., Barish, G., et al.(15 authors!), 2011. Thyroid hormone receptor repression is linked to type I pneumocytes-associated respiratory distress syndrome. *Nat.Med.* 17, 1466-1472.
- Polk, D.H., Wu, S.Y., Fisher, D.A., 1986. Serum thyroid hormones and tissue 5'-monodeiodinase activity in acutely thyroidectomized newborn lambs. *Am.J.Physiol.* 251, E151-E155.
- Redding, R.A., Douglas, W.H.J., Stein, M., 1972. Thyroid hormone influence upon lung surfactant metabolism. *Science* 175, 994-996.
- Redding, R.A., Hauck, C.T., Steim, J.M., Stein, M., 1969. New activity on lung surfactant. *New Eng.J.Med.* 280, 1298 -1299.

- Redding, R.A., Pereira, C., 1974. Thyroid function in respiratory distress syndrome (RDS) of the newborn. *Pediatrics* 54, 423-428.
- Robertson, A.D., Grutsch, J.F., Jr., 1987. Biphasic responses, quantal signals and cellular behavior. *J.Theor.Biol.* 125, 41-60.
- Rodriguez, M.P., Sosenko, I.R.S., Antigua, M.C., Frank, L., 1991. Prenatal hormone treatment with thyrotropin releasing hormone and with thyrotropin releasing hormone plus dexamethasone delays antioxidant enzyme maturation but does not inhibit a protective antioxidant enzyme response to hyperoxia in newborn rat lung. *Pediat.Res.* 30, 522-527.
- Rodriguez-Pierce, M.P., Sosenko, I.R.S., Frank, L., 1992. Prenatal thyroid releasing hormone (sic) and thyroid releasing hormone (sic) plus dexamethasone lessen the survival of newborn rats during prolonged high O₂ exposure. *Pediat.Res.* 32, 407-411.
- Rooney, S.A., Gobran, L.I., Chu, A.J., 1986. Thyroid hormone opposes some glucocorticoid effects on glycogen content and lipid synthesis in developing fetal rat lung. *Pediat.Res.* 20, 545-550.
- Serup, J., Petersen, S., 1979. Fetal thyrotoxicosis *in utero*. *Biol.Neonate* 35, 175-179.
- Sevanian, A., Gilden, C., Kaplan, S.A., Barrett, C.T., 1979. Enhancement of fetal lung surfactant production by aminophylline. *Pediat.Res.* 13, 1336-1340.
- Shanklin, D.R., 1959. Cardiovascular factors in the development of pulmonary hyaline membrane. *Arch.Pathol.* 68, 49-57.
- Shanklin, D.R., 1963. The sex of premature infants with hyaline membrane disease. *South.Med.J.* 56, 1018-1022.
- Shanklin, D.R., 1964. The influence of fixation on the histologic features of hyaline membrane disease. *Am.J.Pathol.* 44, 823-837.
- Shanklin, D.E., 1966. Oxytocin induction of pregnant rabbits; with special reference to the stillbirth rate. *AmJ.Obstet.Gynecol.* 94, 242-248.
- Shanklin, D.R., 1969. On the pulmonary toxicity of oxygen. I. The relationship of oxygen content to the effects of oxygen on the lung. *Lab.Invest.* 21, 439-448.
- Shanklin, D.R., 1991. Pathologic studies of fetal thyroid development. *Adv.Perinatal Thyroidology*, 299, 27-46.
- Shanklin, D.R., 2010. On the pulmonary toxicity of oxygen. III. The induction of oxygen dependency by oxygen use. *Exp.Mol.Pathol.* 89, 36-45.
- Shanklin, D.R., Berman, P.A., 1964. An experimental model for hyaline membrane disease. *Biol.Neonate* 6, 340-360.
- Shanklin, D.R., Lester, E.P., 1972. On the pulmonary toxicity of oxygen. II. The effect of the second gas. *Biol.Neonate* 20, 140-158.
- Shanklin, D.R., Sotelo-Avila, C., 1967. The effects of components of vagotomy on the lung and the effects of anesthesia on vagotomy induced lung change. *Biol.Neonate* 11, 61-86.
- Shanklin, D.R., Wolfson, S.L., 1970. Aqueous estrogens in the management of respiratory distress syndrome. *J.Reprod.Med.* 5, 53-71.

- Shanklin, D.R, Zhang, J., 2007. Neonate with a single umbilical vessel. *J.Reprod.Med.* 52, 529-532.
- Shelley, H.J., 1961. Glycogen reserves and their changes at birth and in anoxia. *Br.Med.Bull.* 17, 137-143.
- Shelley, H.J., 1964. Carbohydrate reserves in the newborn infant. *Br.Med.J.* 1, 273-275.
- Shimoda, S-I., Greer, M.A., 1972. Iodine metabolism: preferential renal excretion of iodide derived from triiodothyronine deiodination.
- Sosenko, I.R.S., Frank, L., 1987. Thyroid hormone depresses antioxidant enzyme maturation in fetal rat lung. *Am.J.Physiol.* 253, R592-R598.
- Sreejith, P., Beyo, R.S., Prasad, G., Sunny, F., Oommen, O.V., 2007. Thyroid status alters gill ionic metabolism and chloride cell morphology as evidence by scanning electron microscopy in a teleost *Anabas testudineus* (Bloch): short and long term *in vivo* study. *Indian J.Exp.Biol.* 45, 1015-1021.
- Stahnke, N., Stenzel, E., Hellwege, H., 1986. Thyroid function in prematures with respiratory distress syndrome. *Acta Endocrin.Suppl(Copenh).* 279, 354-360.
- Stone, e.l., 1945. *The new-born infant; a manual of obstetrical pediatrics.*, 3rd ed., Lea & Febiger, Philadelphia, pp. 289-290.
- Theodoropoulou, M., Arzberger, T., Gruebler, Y., Korali, Z., Mortinin, P., Joba, W., Heufelder, A.E., Stalla, G.K., Schaaf, L., 2000. Thyrotrophin receptor protein expression in normal and adenomatous human pituitary. *J.Endocrin.* 167, 7-13.
- Vaillant, P., David, E., Constant, I., et al., 1996. Validity in nulliparas of increased beta-human chorionic gonadotrophin at mid-term for predicting pregnancy-induced hypertension complicated with proteinuria and intrauterine growth retardation. *Nephron* 72,557-563. (10 authors)
- Van Wassenaer, A.G., Kok, J.H., Dekker, F.W., de Vijlder, J.J., 1997. Thyroid function in very preterm infants: influences of gestational age and disease. *Pediatr.Res.* 42, 604-609.
- Williams, F.L., Ogston, S.A., van Toor, H., Visser, T.J., Hume, R., 2005. Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. *J.Clin. Endocrinol.Metab.* 90, 5954-5963.
- Wu, B., Kikkawa, Y., Orzalesi, M.M., Motoyama, E.K., Kaibara, M., Zigas, C.J., Cook, C.D., 1973. The effect of thyroxine on the maturation of fetal rabbit lungs. *Biol.Neonate* 22, 161-168.

Legends

Table 1:

Archive tally of 450 newborns from the decade 1955-1965, with size of subsets, proportion of males, mean usual measurements (body weight, crown-heel length, and crown rump length) with mean weights of thyroid and both adrenals from 154 neonatal autopsies with hyaline membrane disease and 296 others, excluding gross malformations and known syndromes.

Table 2:

Year by year inventory of 4,588 microfiche autopsy records in the Potter Archive with data breakdown by classification categories as to source, stillbirth or livebirth, and net cases fit for review.

Table 3:

The pattern of divergence from normal of the adrenal glands is delayed or latent, shown here as the difference in their weight from normal and the significance by t test. A significant difference appears after 28 weeks, whilst that for the thyroid occurs 4-5 weeks earlier.

Table 4:

Ascertainment of the prevalence of hyaline membrane disease, 1934-1942, in the three Chicago Lying-In Hospital services and from the 53 outside cases in the Bundesen-Potter study. The CLIH services had twice the prevalence overall in their premature newborns.

Table 5:

The progression of the prevalence of hyaline membrane disease 1934-1942 differed between the CLIH services and outside hospitals from year to year, approximating a three-fold difference in 1940-1942.

Table 6:

Apgar Scores at birth in the clinical study of 1972, 24-28 weeks gestational age.

Tables 7a and 7b:

Laboratory data obtained from the clinical study of 26 premature newborns.

Table 8:

Characteristics of newborn rabbit survival after bilateral cervical vagotomy either in air only controls or with thyroxine or thyrotropin at age 3 hours.

Table 9:

Comparative means and regression slopes of phases A, B, and C, with especial details referent the thyroxine series.

Table 10:

The significance of survival after thyroidectomy by exposure to oxygen or air. $\chi^2 = 7.204$, $p = 0.0073$.

Table 11:

Thyroxine carrying capacity of serum proteins.

Legends

Figure 1.

Normalized weights of thyroid and both adrenals from 450 neonatal autopsies, 154 with hyaline membrane disease and 296 others, excluding gross malformations and known syndromes, plotted by midpoints of three week gestational ranges. The values for both glands in all subsets track each other closely throughout the third trimester, with HMD values approximately half those of the control cases. From the Potter Archive 1950-1965. Of possible interest is the observation that normalized HMD adrenal weights are slightly higher than the thyroid which is the reverse of the control group after 30 weeks.

Figure 2.

Annual prevalence of hyaline membrane disease is the earliest part of the Archive which shows the beginning of diagnosed HMD and a sharp rise in the prevalence at Chicago Lying-In Hospital with its two affiliated services contrasted to the other 53 institutions under the Bundesen Plan. The change coordinates closely with the introduction into premature neonatal care of the Hess Oxygen Bed.

Figure 3.

Thyroid:body weight ratios by duration of post natal survival in 50 cases from the earliest years of the Archive, 1934-1942, a period of typically minimalist treatment of the premature newborn. The ratio is thyroid weight in milligrams divided by autopsy weight in grams. The secondary decline from 12 to 66 hours post natal has a regression equation: Ratio = $-.00000650629$ (survival in hours) + 0.000683954 (by least squares analysis). By contrast, thyroids of 100 contemporaneous control cases showed no decline in the ratio for up to ten days post natal (data not shown).

Figure 4.

Semi-log₁₀ plot of survival of 225 newborn rabbits after bilateral cervical vagotomy: 36 after pre-BCV thyroxine at age 3 hours, 63 after pre-BCV thyrotropin at 3 hours, and 126 air controls after BCV only. The control curve shows the typical result of such experiments, a sharp loss of animals by 10 hours post-BCV (Phase A), an extended Phase B to about 45 hours, and an accelerated final Phase C. By contrast thyroxine pretreatment greatly shortened Phase A with a similar length of Phase B and a sharper final Phase C. The thyrotropin effect on Phase A was along the control plot until about 4 hours when an extremely protracted Phase B set in, about twice as long as that of either of the two other subsets, and then a moderately sharp final Phase C. These survival plots are congruent with the interpretation of Shanklin and Sotelo-Avila (1967) which concluded Phase A was likely the result of partial laryngeal constriction because bilateral cervical vagotomy also interrupts the recurrent laryngeal nerve. From this, then, thyroxine and thyrotropin may act against the loss of that motor nerve action but the mode or manner of this remains unknown.

Figure 5.

The extent of pulmonary injury in the thyroxine subset was exponentially proportionate to the \log_{10} dose of thyroxine. The same relationship held true for the degree of change in Phases A and B (data not shown) and this fact drives the result for the whole series shown here. The regression equation is: Mean gross lung change = $6.05 (\log_{10} \text{dose}) + 15.6439$.

Figure 6.

The extent of pulmonary injury in the thyrotropin subset differed considerably from the thyroxine subset. This plot of mean gross lung change against the \log_{10} dose of thyrotropin shows no difference when the dose was 0.1, 1.0 or 100 mU/kg and a regression analysis of these three subsets resulted in a flat line with a slope of zero. By contrast the dose of 10 mU/kg significantly reduced the extent of lung injury. Thyrotropin has systemic effects beyond its influence over thyroxine secretion by the thyroid but whether its prime function or some aspects of its secondary roles results in what gives the appearance of a "protective" role or is simply that dose least disruptive of TSH homeostasis which is not shown in the data as derived by this experiment. Note that all three other mean results fall within one standard error the subset mean.

Figure 7.

The effect of thyroidectomy at two hours post natal required a different mode of graphic analysis. This plot gives the survival post thyroidectomy. Eight of 20 newborn rabbits were placed in 100% oxygen and these were followed until demise along with ten left in air. Remarkably, 60% of those in air died before 48 hours post surgery but all eight in oxygen survived beyond 48 hours. Two animals were subjected to BCV at the usual age of 24 hours (22 hours post thyroidectomy) and left in air for their entire survival interval. Remarkably, one died at 0.95 hour post-BCV and the other at 1.02 hours post-BCV, and both had gross lung change at the 52% level, remarkably quick lesion formation compared to BCV controls. The survival difference without BCV between oxygen and air runs is very highly significant by χ^2 , with a p value of >0.0001 despite a Student's t test = 1.63938, $p = 0.0603$.

Figure 8.

Ratio of thyroid weight to body weight by gestational age. The ratio rose at a slightly increased rate from 12-13 weeks, at which time the ability of thyroid to concentrate iodine begins. There is then a progressive rise in the ratio to midgestation under the influence of rising fetal TSH which peaks around 25 weeks, about one month after the ratio becomes maximal. There is little change during the later part of the second trimester to 30 weeks, after which somatic growth becomes more marked, resulting in a decline in the ratio (Adapted from Shanklin, 1991).

Figure 9.

Effect of pre-BCV thyroxine on body weight loss following vagotomy. The control animals lost about 25% of body mass by 75 hours post-BCV and the thyroxine group tracked the same rate up to 35 hours after which there was marked acceleration of weight loss, 28% by 50 hours. The light lines around the heavy mean regression line are the individual subset weight losses, a fairly tight and consistent result. There was no evident effect on the ratio of surgical weight to birth weight in any subgroup, including the controls (data not shown).

Figure 10.

When the birth weights of the HMD and the non-HMD subsets (Table 1) are directly compared as a ratio there is a dramatic fall in the ratio from 24.5 to 32 weeks gestational age and an effectively constant relationship thereafter. Thus, premature newborns lose 15% of their relative thyroid weight by 32 weeks.

Figure 11.

Samples of the variation in thyroid histopathology during gestational ages 18.5 to 36 weeks.

- A. Image obtained by scanning original H&E cross section of the cervical tissues. Estimated gestation 31-33 weeks, birth weight = 2010 g, Nonmacerated antepartum fetal death from massive abruption of the placenta. Monochorionic, diamniotic twin #1, parabiologic recipient with enlarged thyroid gland, about twice normal volume, mildly hyperactive. Mild maternal gestosis. Lung showed extensive, deep aspiration of amniotic content.
- B. Image obtained by scanning original H&E cross section of the cervical tissues. Gestational age 33 weeks, birth weight 2249 g. Liveborn survived 9 hours, poor color and muscle tone, tachycardia and tachypnea. Clinical diagnosis of pneumonia treated with ampicillin/gentamicin. Failed to respond to these and general supportive measures. Autopsy revealed congestive heart failure with diffuse myocardial necrosis. Thyroid 15X normal size with diffuse hypersecretory state (toxic congenital goiter), much more active than Figure 11A. The clarity of the thyroid epithelium is apparent even at this magnification.
- C. H&E. Original magnification, 100X. 18.5 weeks, birth weight 223 g, survived 4 hours, despite no active resuscitation. Intrauterine diagnosis of Klinefelter syndrome confirmed with chromosome analysis, XXY and numerous Barr bodies. Autopsy revealed extreme somatic and pulmonary immaturity, the latter in the organoid phase. Scattered thyroid follicles with scant colloid accumulation against a generally an immature prefollicular gland.
- D. H&E. Original magnification, 100X. 36 weeks, birth weight 2170 g. Survived 55 minutes; no air could be put into the lung due to extensive tracheal atresia, a 5.2 cm missing segment; some air possible by needle placement in lower trachea. Autopsy also revealed cleft common upper trachea and esophagus, post ampullary duodenal atresia, and lower facial bony anomalies on a square head. The thyroid was moderately enlarged with disseminated hyperactivity as shown by the circumferential vacuoles at the colloid:lining cell interfaces.
- E. H&E. Original magnification 100X. 33 weeks, birth weight 1450 g. Survived 30 hours. Osteogenesis imperfecta congenita. Obvious blue sclerae. Partial premature closure of foramen ovale, bilateral cardiac hypertrophy. Abundant indolent colloid in thyroid follicles, many of which are over distended.

Figure 12:

Three-dimensional plot of thyroxine dose, survival in hours, and extent of gross lung injury for each of the three dose levels. The connecting line is precisely linear, strongly indicative of a forcing effect of thyroxine.

Tables

Table 1: Differential thyroid and adrenal weights: newborns both with and without hyaline membrane disease.

Gestation (weeks)	Number of cases	Males (%)	Mean body weight (g)	Mean CH length (cm)	Mean CR length (cm)	Mean thyroid weight (g)	Mean adrenal weight (g)
22-27	71 non-HMD 28 HMD	40.6 60.7	886 ± 47 978 ± 13	34.0 ± 0.4 36.0 ± 0.6	23.0 ± 0.4 24.0 ± 0.4	0.76 ± 0.04 0.56 ± 0.05	3.7 ± 0.33 3.6 ± 0.19
28-30	50 non-HMD 32 HMD	40.0 50.0	1192 ± 70 1183 ± 75	38.0 ± 0.5 38.0 ± 0.7	26.0 ± 0.4 26.0 ± 0.7	0.84 ± 0.05 0.63 ± 0.04	5.0 ± 0.32 4.0 ± 0.24
31-33	50 non-HMD 48 HMD	42.0 60.2	1884 ± 123 1586 ± 45	42.0 ± 0.8 42.0 ± 0.5	29.0 ± 0.7 29.0 ± 0.4	1.01 ± 0.09 0.73 ± 0.03	5.7 ± 0.25 4.5 ± 0.18
34-36	44 non-HMD 18 HMD	46.0 55.5	2267 ± 88 1954 ± 103	45.0 ± 0.8 44.0 ± 0.7	31.0 ± 0.4 31.0 ± 0.5	1.36 ± 0.11 0.82 ± 0.06	7.5 ± 0.55 5.4 ± 0.44
37-39	81 non-HMD 28 HMD	55.5 67.8	2706 ± 96 2248 ± 91	49.0 ± 0.4 47.0 ± 0.6	34.0 ± 0.3 32.0 ± 0.8	1.41 ± 0.08 0.98 ± 0.07	8.4 ± 0.60 5.8 ± 0.33

Data as means ± SEM

Table 2: Record by record inventory of CLIH perinatal autopsy archive:

Year	1934	1935	1936	1937	1938	1939	1940	1941	1942	Total
Total	46	162	262	602	830	630	731	667	658	4588
Outside cases	0	6	91	405	589	425	451	436	424	2827
No record	0	6	3	6	6	9	20	25	19	94
No lung slide	0	0	0	54	7	8	7	12	12	100
Pediatric age+	0	0	7	57	96	92	72	71	82	477
Placenta only	0	0	0	0	0	0	2	0	0	2
Net for review	0	0	81	288	480	316	350	328	311	2154
Stillborn	0	0	61	170	278	209	237	232	201	1388
Newborn	0	0	20	118	202	107	113	96	110	766
HMD/VC	0	0	1	11	8	9	10	12	8	59
Percentage	0.00	0.00	5.00	9.32	3.96	8.41	8.85	12.5	7.27	7.70
Main sequence	46	155	171	197	235	205	280	231	231	1751
No record	1	0	1	1	0	0	0	0	4	7
No lung slide	6	3	3	3	0	1	2	3	6	27
Net for review	39	152	167	193	235	204	278	228	221	1717
Stillborn	29	107	125	137	153	143	199	168	145	1206
CLIH	9	84	79	88	96	80	123	104	99	762
Home Service	4	8	12	7	8	5	18	6	6	74
CMC	16	15	34	42	49	58	58	58	40	370
Newborns	10	45	40	56	82	61	79	60	76	509
CLIH	8	36	26	43	44	49	64	45	53	368
Home Service	1	4	10	5	5	2	4	1	5	37

Table 3. Delayed divergence of adrenal weights in hyaline membrane disease.

Gestational range	Difference in weight	Degrees of freedom	t	p
22-27 weeks	0.1 g	97	0.2632	0.3965
28-30	1.0	80	2.5000	0.0072
31-33	1.2	96	3.895	0.0001
34-36	2.1	60	2.982	0.0021
37-39	2.6	107	3.797	0.0001

Table 4. Overview of autopsy cases in the Potter Archive.

Category	Outside services	CLIH services
Total autopsies	2827	1751
Net for review	2154 (76.17%)	1717 (98.06%)
Stillborn	1388 (64.44%)	1206 (70.24%)
Newborn	766 (35.6%)	509 (29.76%)
Hyaline membrane disease	59	77
Per cent HMD	7.70	15.13

Table 5. Year by year accession of cases of RDS/HMD, 1934-1942.

Source	1934	1935	1936	1937	1938	1939	1940	1941	1942	Total
Outside RDS/HMD	0	0	1	11	8	9	10	12	8	59
Per cent	0.0	0.0	5.0	9.32	3.96	8.41	8.85	12.5	7.27	7.70
CLIH RDS/HMD	0	2	2	8	1	6	19	21	18	77
Percent	0.0	4.45	5.0	14.3	1.22	9.84	24.0	35.0	23.7	15.13

Table 6: Apgar scores at birth, 24-38 weeks gestational age.

Subset	Number	Apgar range	Mean Apgar Score
Control-normal			
Control-normal	14	3-10	8.21 ± 0.48
RDS/HMD	12	2-9	5.33 ± 0.58
			t = 3.845, p = 0.0004

Table 7a: Mean birth weight, serum T₄, and serum TSH.

Subset	Number	Gestational Range (weeks)	Mean Birth Weight (grams)	Mean T ₄ µg/dl	Mean TSH µIU/ml
Normal Controls	14	28-38	2020.79 ± 93.54	7.58 ± 0.45	10.91 ± 0.76
RDS/HMD	12	24-37	1693.83 ± 143.64	6.40 ± 0.49	9.87 ± 1.03
Difference			p = 0.0309	p = 0.0455	p = 0.2125

Table 7b: Total serum protein values.

Subset	Number	Range of protein as g/dl	Mean serum protein as g/dl
Normal controls	14	3.70 – 5.95	4.69 ± 0.18
RDS/HMD	12	2.45 – 4.90	4.05 ± 0.21
			t = 2.325, p = 0.0144

Table 8: Characteristics of survival after pre-BCV thyroxine and TSH.

Experiment	Number	Mean survival (± sem)	Median survival	Area under survival curve (ratio referent control)
Air controls	126	6.87 ± 1.14 hr	3.0 hr	78.38 (1.00)
Thyroxine at 3 hours	36	19.92 ± 3.07	14.1	115.41 (1.47)
Thyrotropin at 3 hours	63	15.09 ± 2.91	4.2	117.62 (1.50)

Table 9: Comparative means and regression slopes for Phases A, B, and C, thyroxine and thyrotropin pretreatment at age three hours before bilateral cervical vagotomy at 24 hours compared to air only controls.

Dose level	Survival phase	Mean survival	Mean lung change	Regression slope
Thyroxine	Entire run	19.92 (hours)	21.38 %	
	A			-0.0377719
	B			-0.0186038
	C			-0.1748650
1 mg/kg	A	4.05	8.33	
	B	28.32	24.93	
	C	50.80	7.00	
10. mg/kg	A	3.75	20.77	
	B	32.38	29.00	
	C	43.64	30.00	
100 mg/kg	A	3.50	25.90	
	B	36.23	33.33	
	C	49.06	29.67	
Air controls	Entire run	6.87	7.70	
	A	3.30	4.23	-0.139982
	B	24.96	33.63	-0.00926989
	C	55.26	43.00	-0.0243192
TSH series	Entire run	15.09	22.46	
	A			-0.05339
	B			-0.00863854
	C			-0.0747942

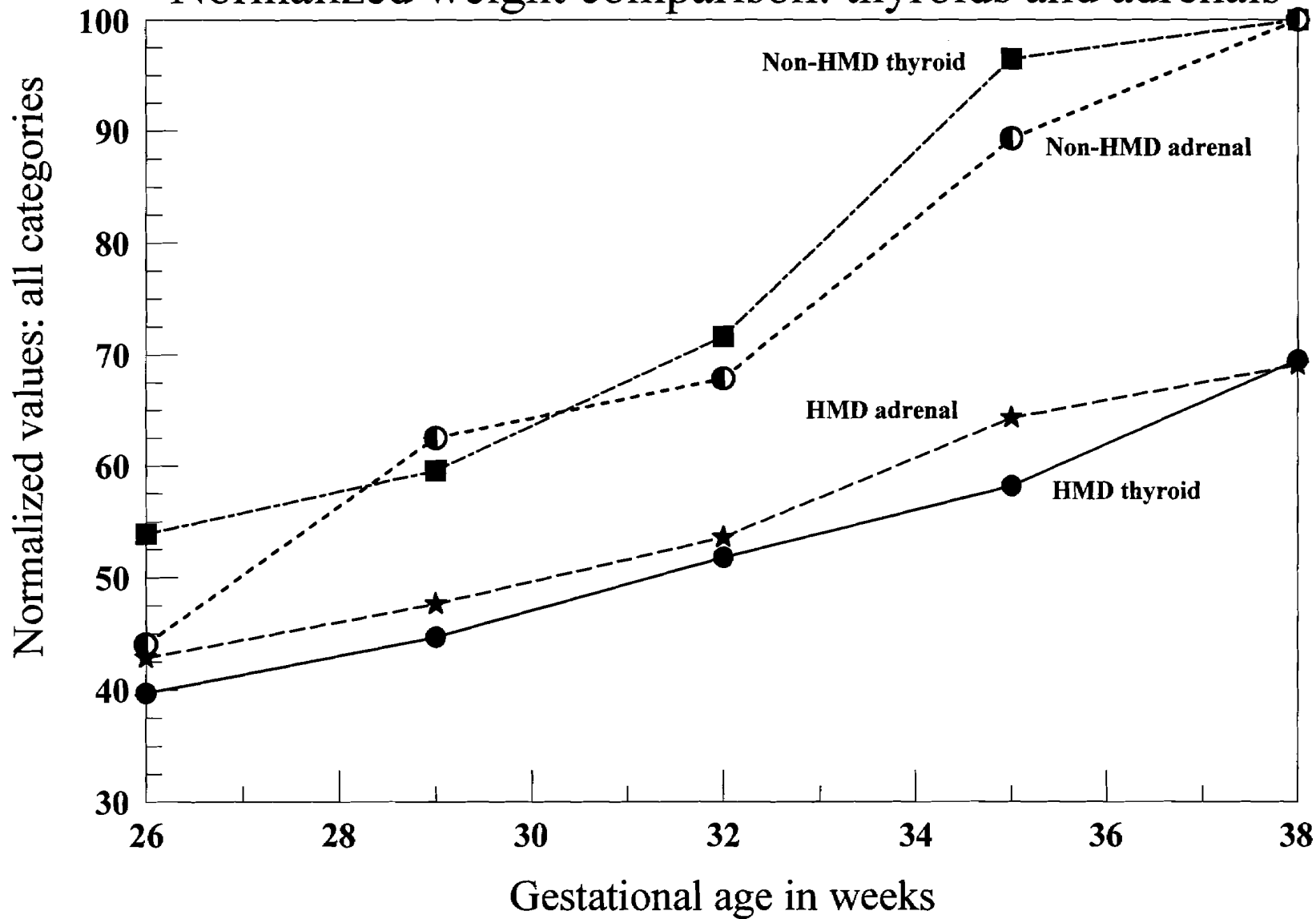
Table 10: Survival distribution following thyroidectomy of newborn rabbits by atmospheric environment.

Environment	Up to 48 hours	Beyond 48 hours	Total
Air	Found 6 Expected 3.333	Found 4 Expected 6.667	10
Oxygen	Found 0 Expected 2.667	Found 8 Expected 5.333	8
Total	6	12	18

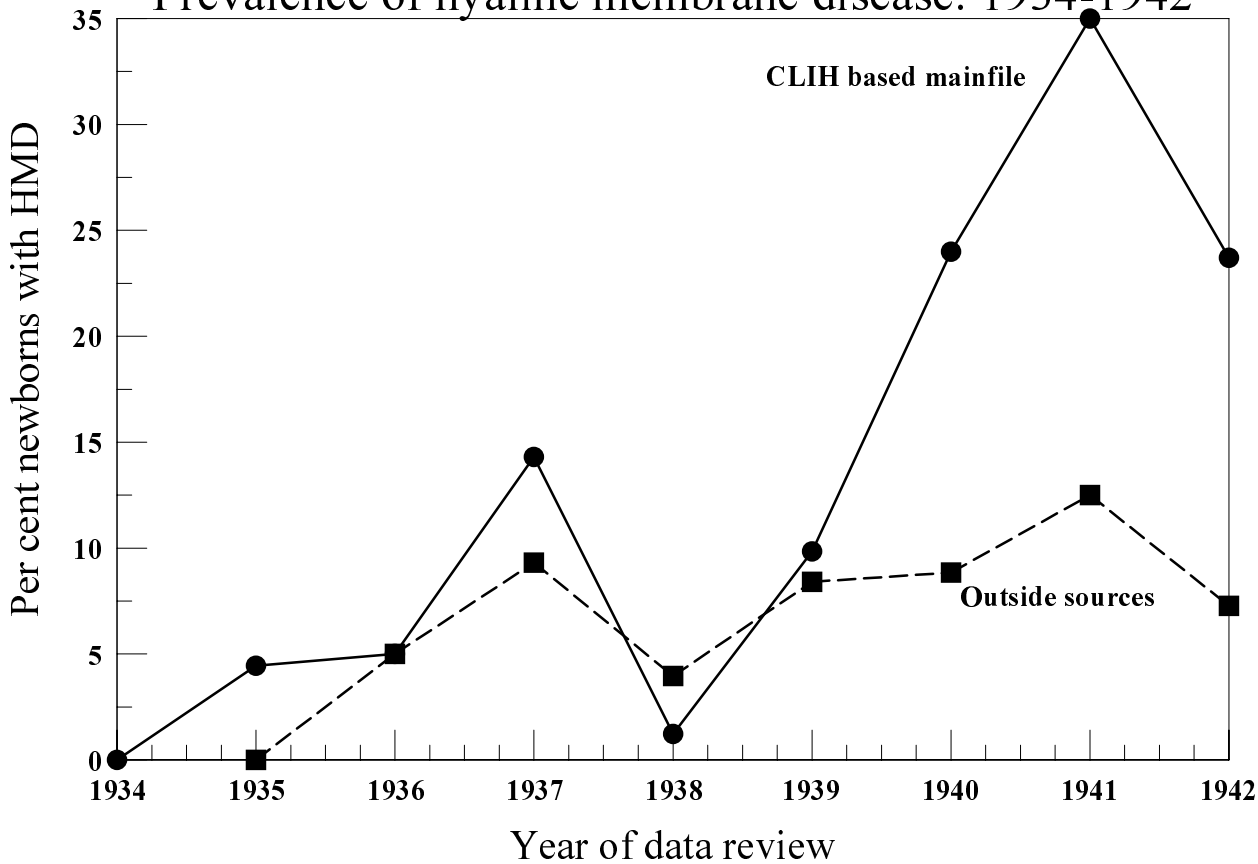
Table 11: Thyroxine carrying capacity of serum proteins, clinical study.

Modality	Controls	RDS/HMD cases	p value
Thyroid binding globulin	19.857 ± 0.761 mcg/dl	17.408 ± 1.490 mcg/dl	0.0698
Free thyroxine index	8.029 ± 0.507	7.417 ± 0.365	0.1760

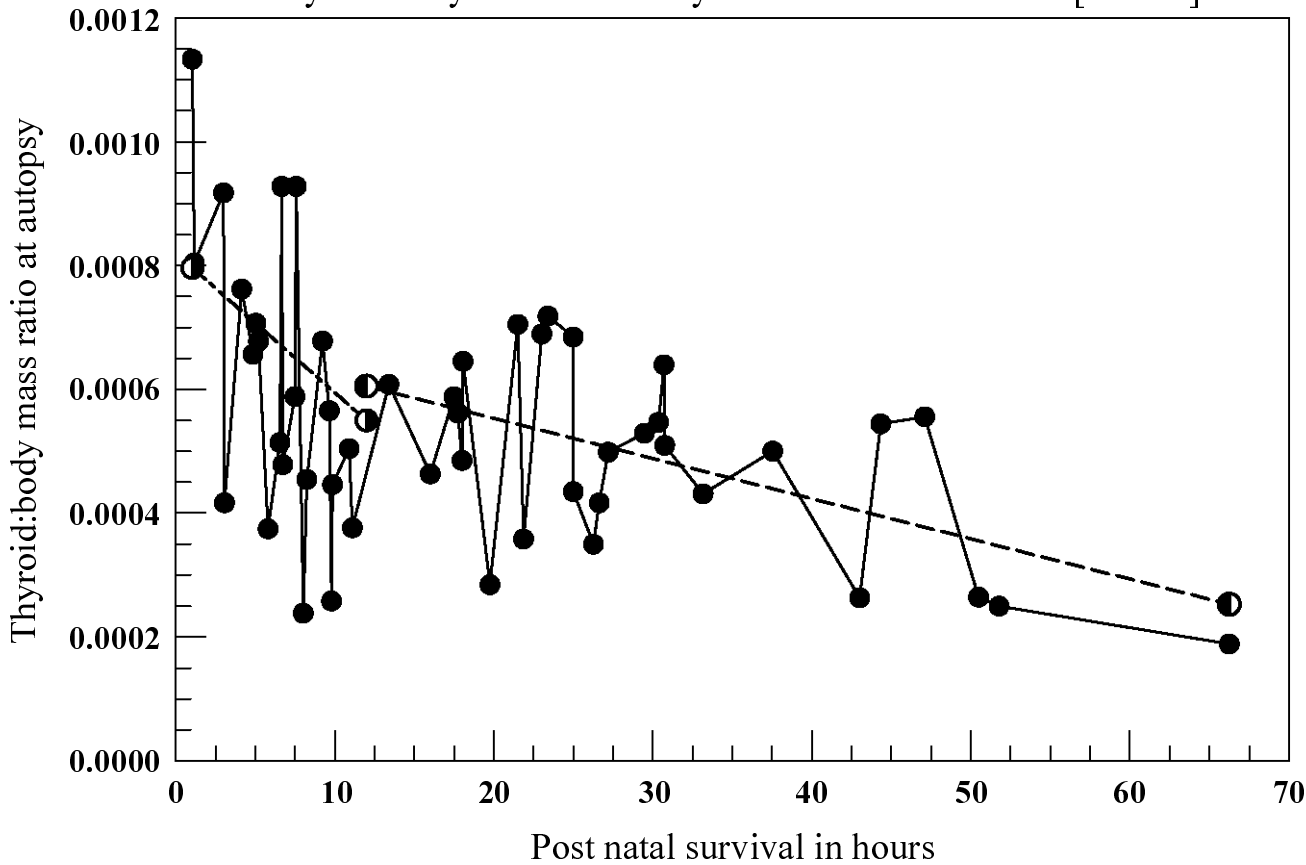
Normalized weight comparison: thyroids and adrenals



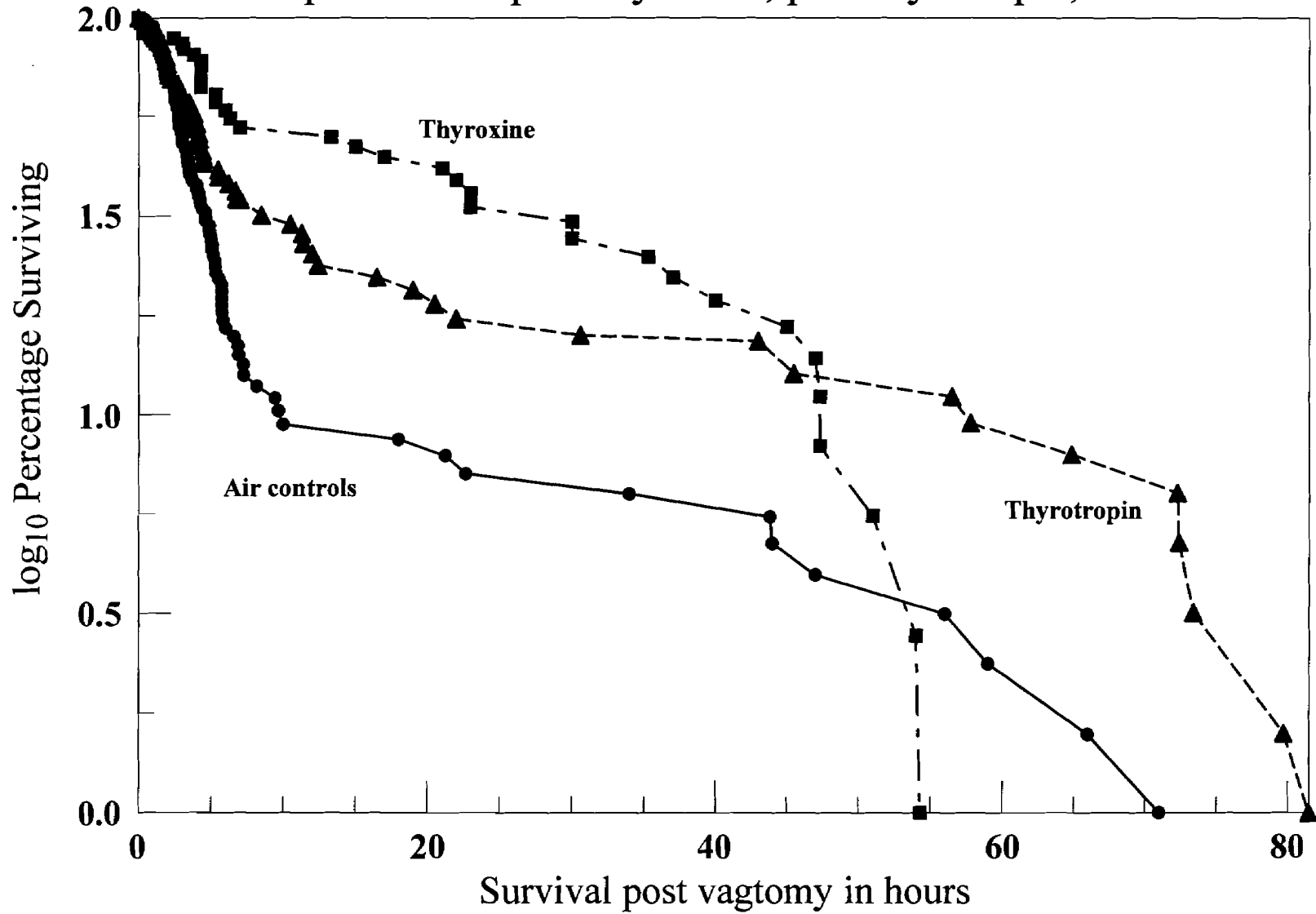
Prevalence of hyaline membrane disease: 1934-1942



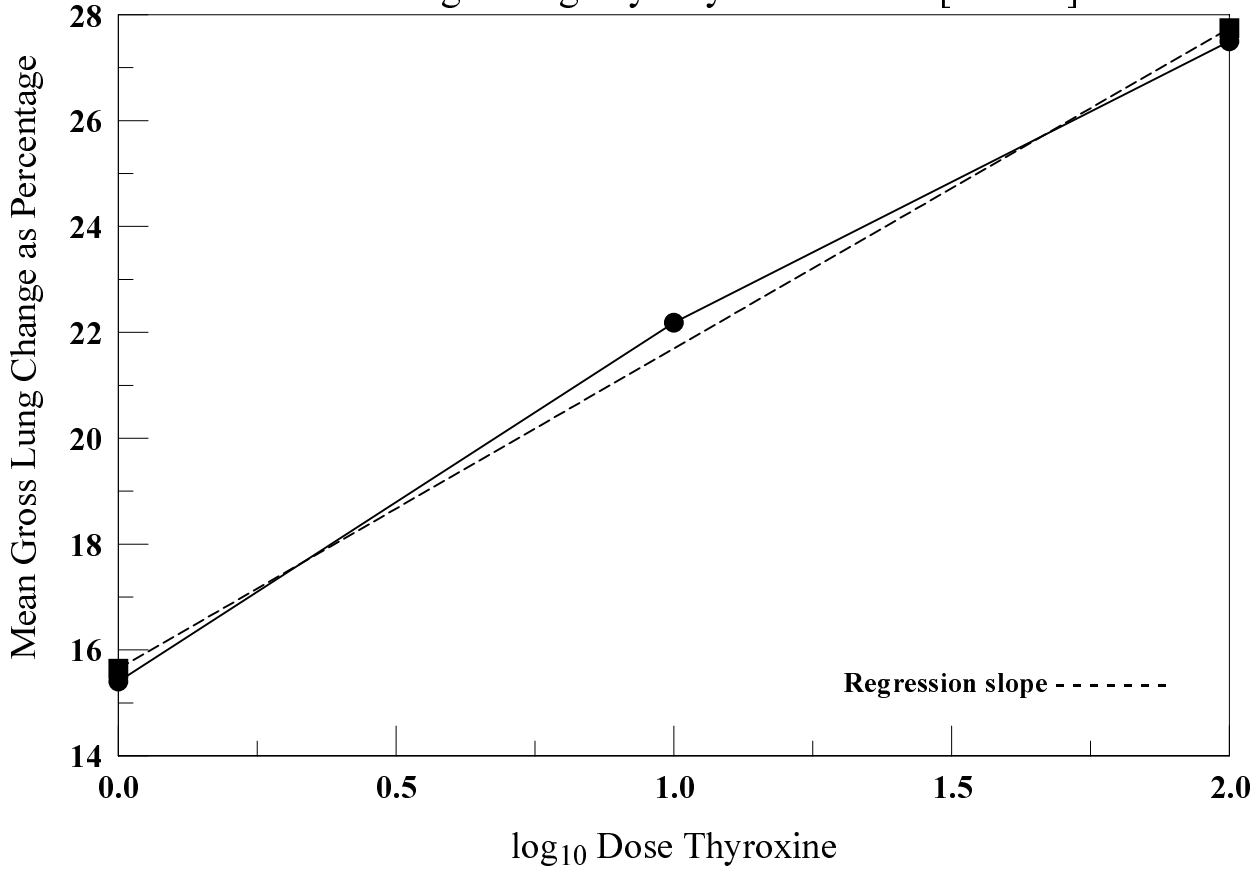
Thyroid:body mass ratios - hyaline membrane disease [N = 50]



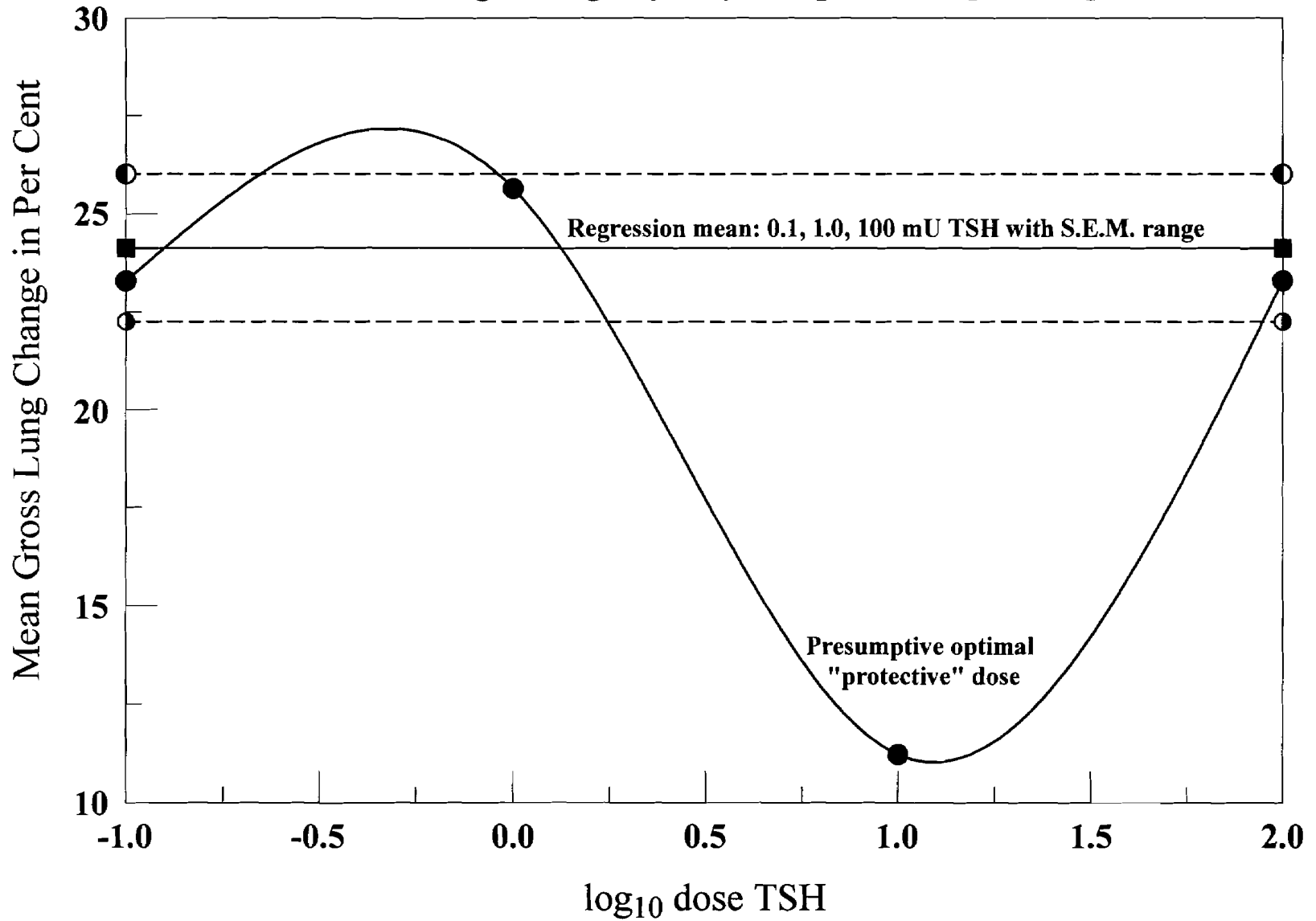
Survival profiles for post-thyroxine, post-thyrotropin, and controls



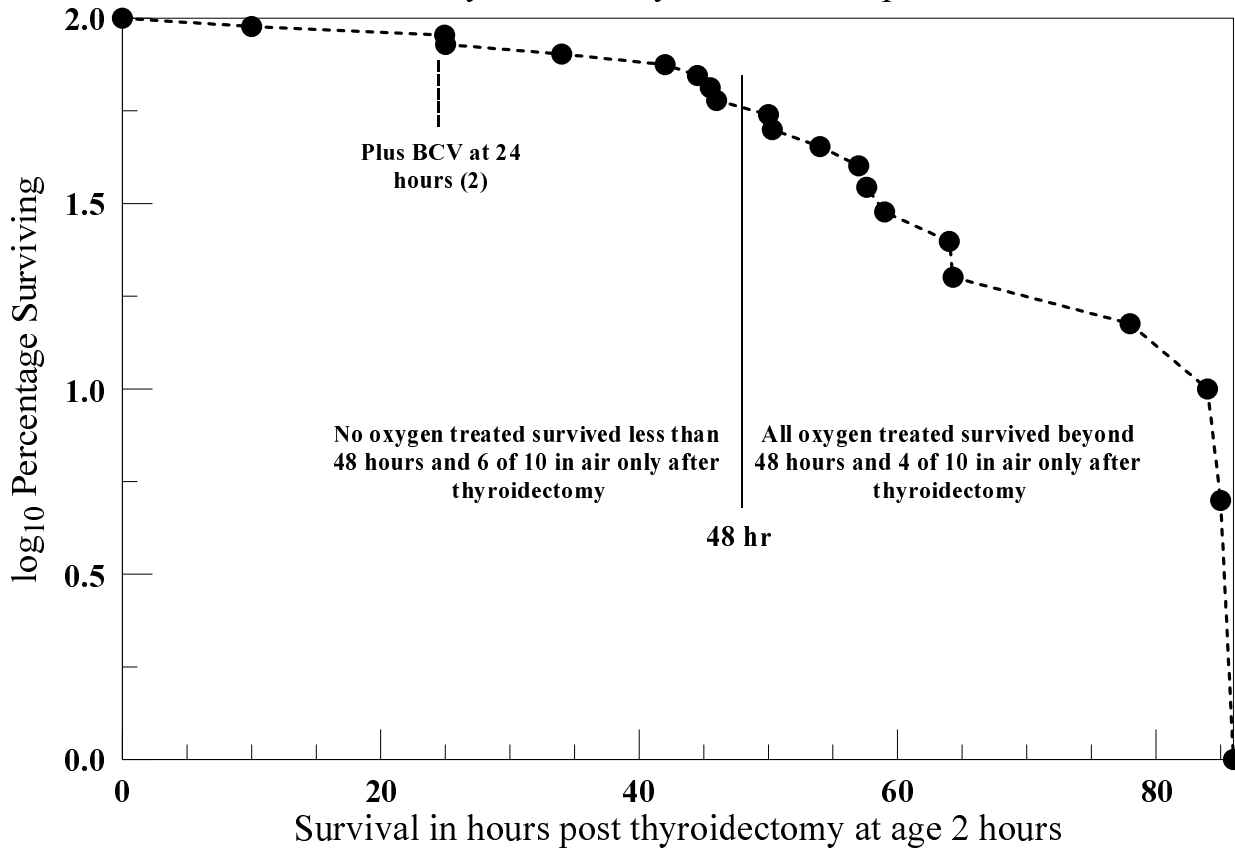
Gross Lung Change by Thyroxine Dose [N = 36]



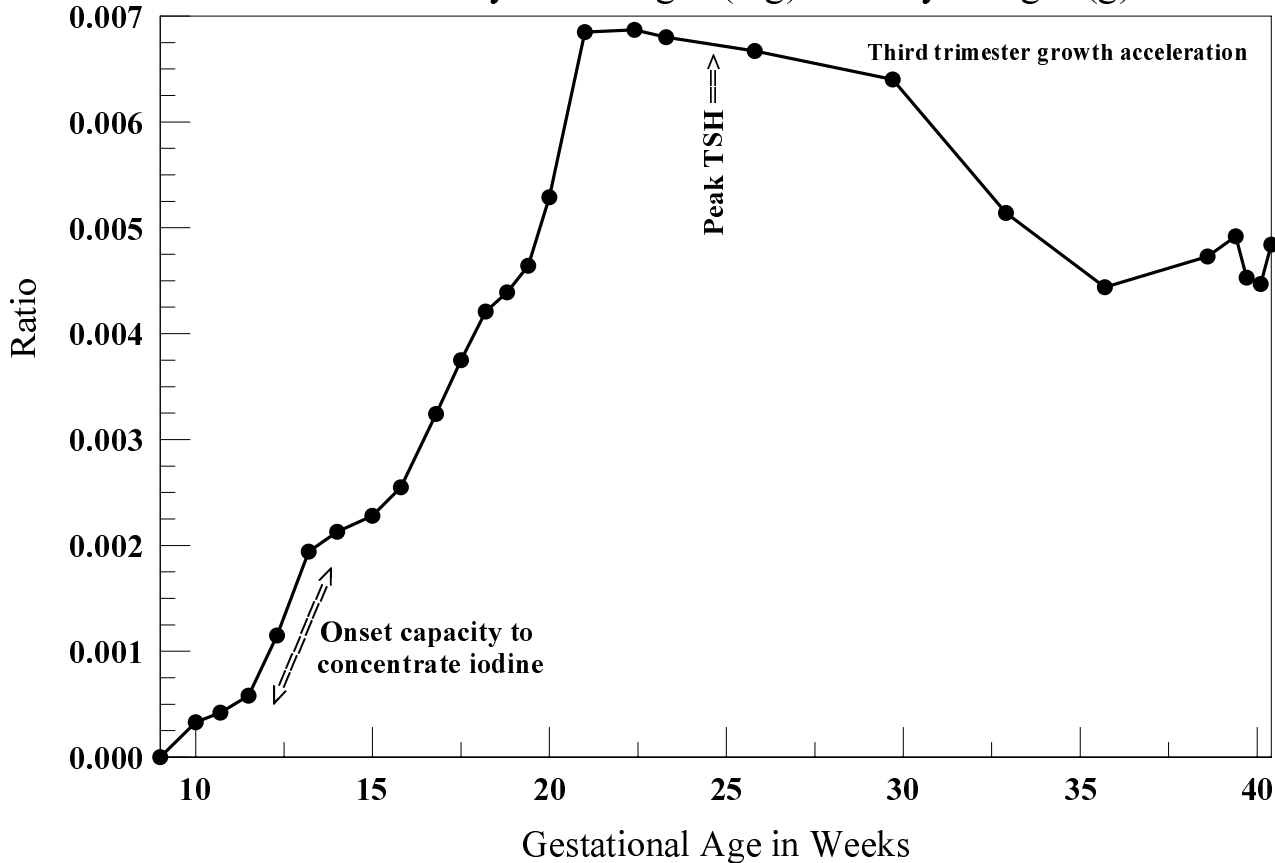
Gross Lung Change by Thyrotropin Dose [N = 63]



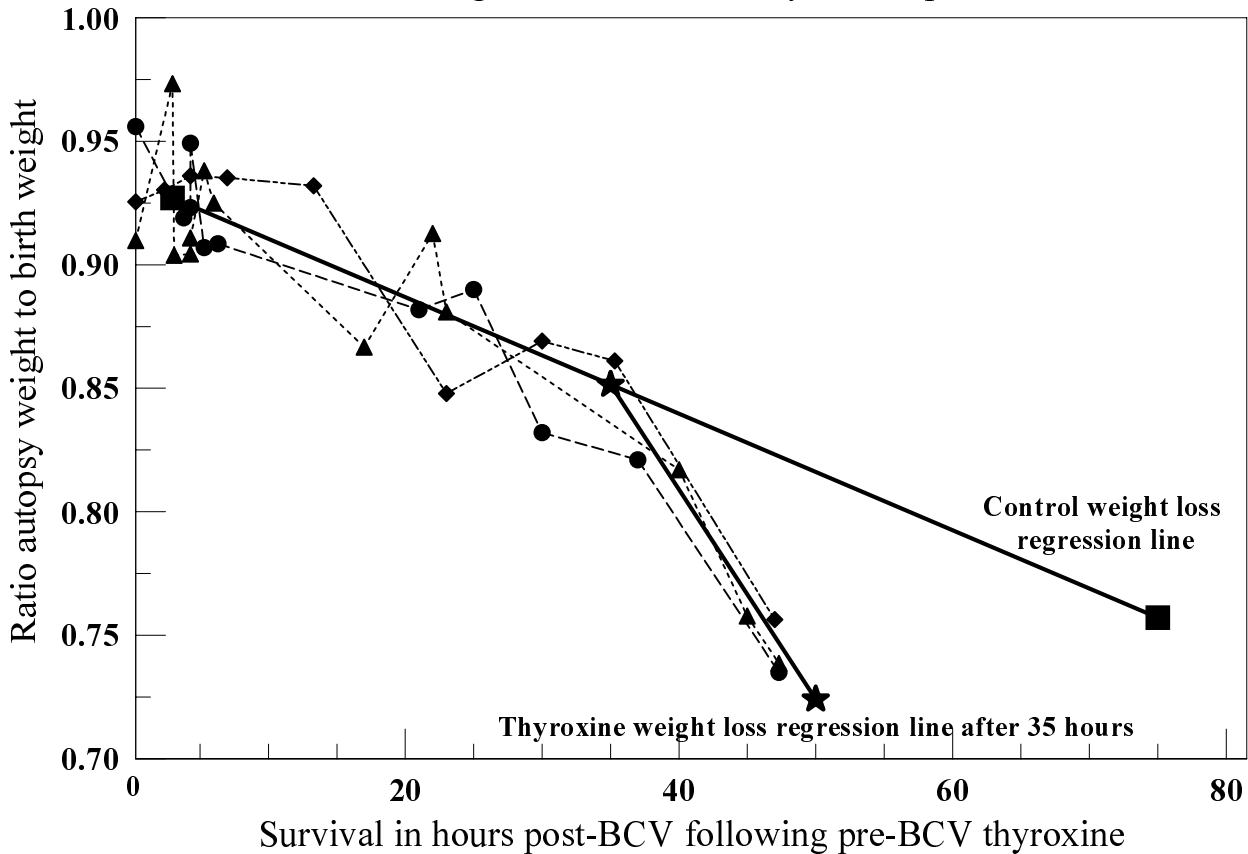
Post-Thyroidectomy Survival Experience



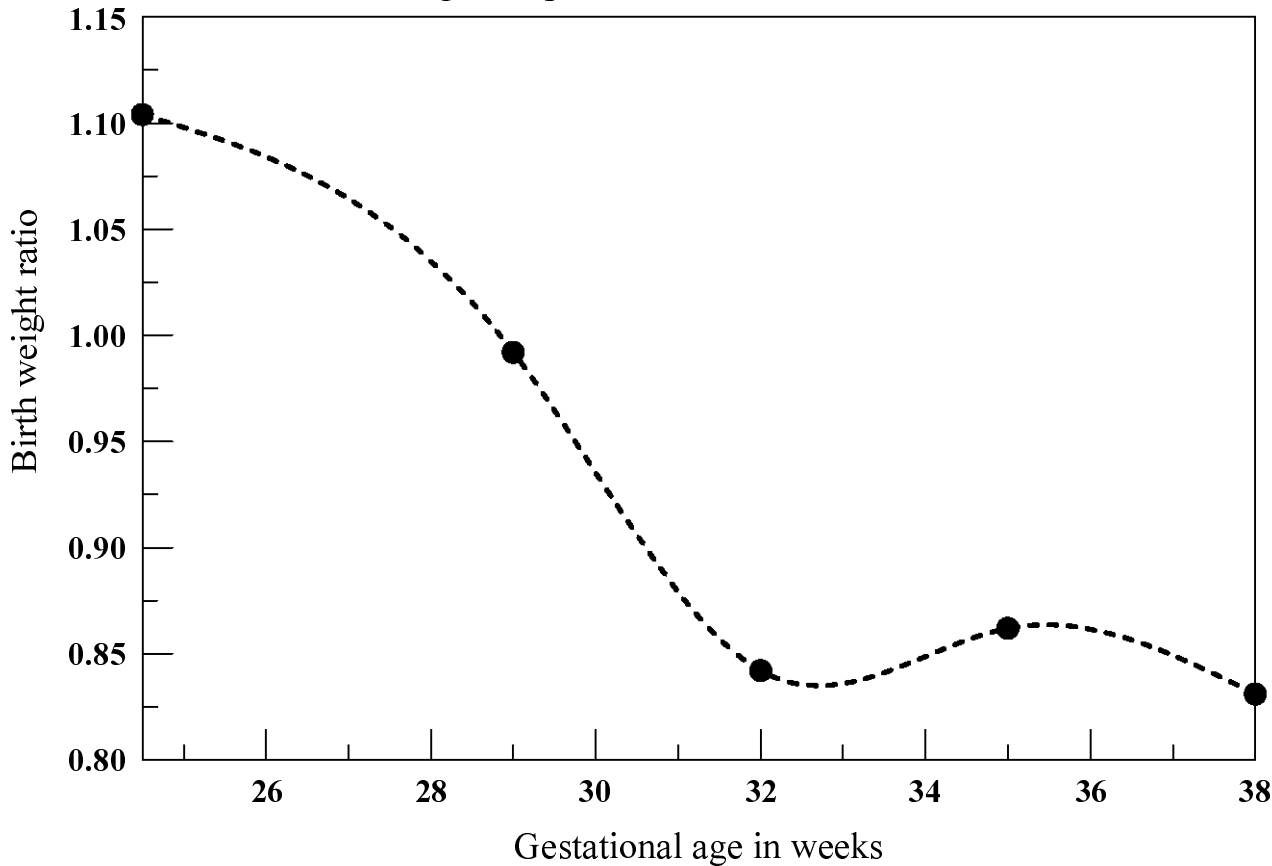
Ratio of Thyroid Weight (mg) to Body Weight (g)



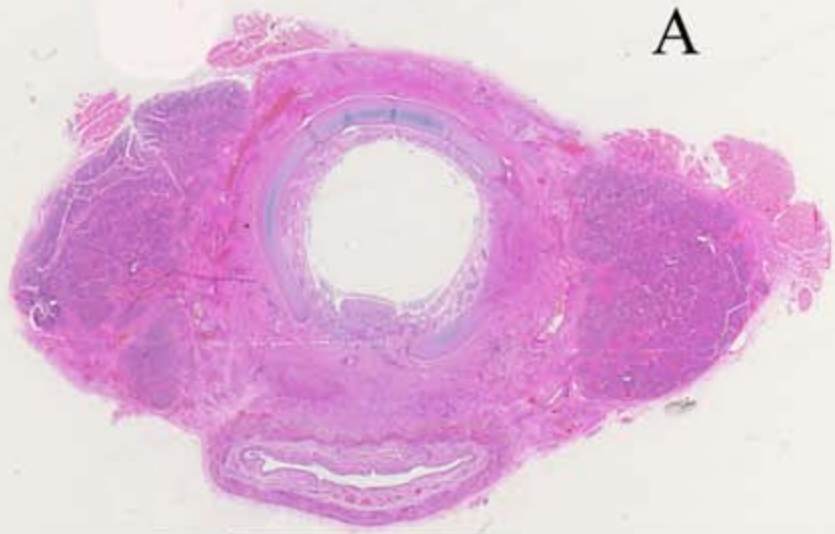
Latent weight loss effect of thyroxine post BCV



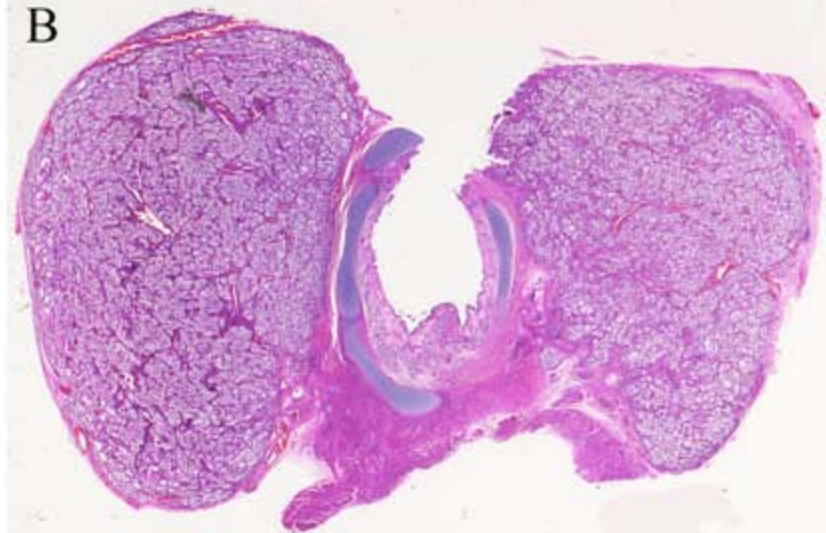
Ratios birth weight of premature infants with HMD vs. controls



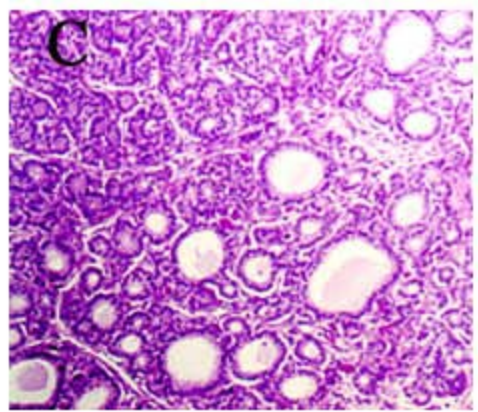
A



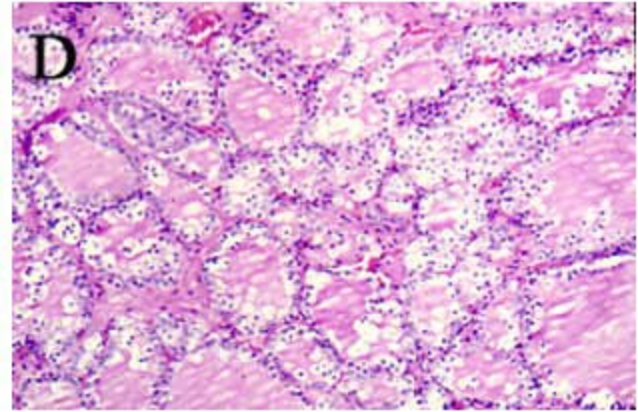
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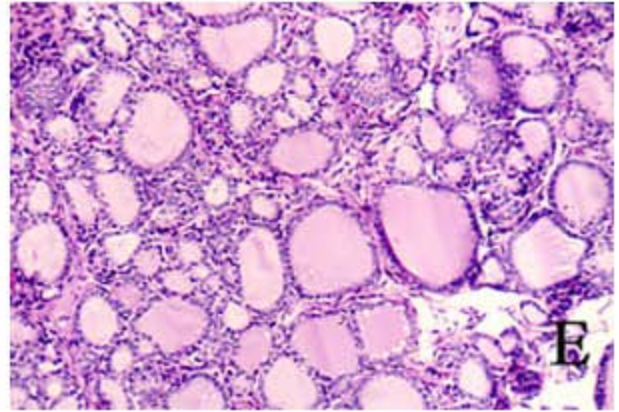
C



D



E



Thyroxine effect on survival and lung injury in phase B post BCV

