LUNG DEVELOPMENT IN NEWBORN GUINEA PIGS AND THE EFFECTS OF ENDURANCE EXERCISE

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Katriona Anne Ross

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Name: Katriona Anne Ross

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Examining Committee:

Chairman: Dr. A. Chapman

Dr. E.W. Banister
Senior Supervisor

Dr. J. Wilkinson

Dr. W.M. Thurlbeck
University of British Columbia

Dr. J. Wright
University of British Columbia
External Examiner

Date Approved: Aug. 8, 1984.

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LUNG DEVELOPMENT IN NEWBORN GUINEA PIGS AND THE EFFECTS OF ENDURANCE EXERCISE.

Author: _____
(signature)

KATRIONA ANNE ROSS
(name)

AUGUST 8th, 1984
(date)

ABSTRACT

Interest in the timing of mammalian lung events has led to much research in the alteration of normal lung growth through a variety of manipulative techniques. These manipulations have established the importance of certain factors in controlling lung growth, one of which is oxygen consumption through increased metabolic demand. This study assessed the effects of daily endurance exercise on lung growth in newborn guinea pigs.

A morphological and biochemical comparison was made between lungs of exercised and sedentary animals after 1, 2 and 3 weeks of life. There were no morphological differences in lung structure between exercised and age-matched sedentary animals. Lung surface area was significantly different in exercised animals compared to the sedentary animals over the 3 week period. Also during this period, wet/dry lung weight ratio was significantly increased in exercised animals but not in sedentary controls. Significant differences were demonstrated in body weight, femur length, lung weights and lung volume after 2 weeks but not after 3 weeks in exercised animals, when compared to controls.

Significant differences due to growth were measured in most parameters in both groups. Wet lung weight, dry lung weight and lung volume increased at week 2. Relative lung weight (to body weight) decreased weekly, while relative lung volume and relative surface area decreased at week 1, then again at week 2. The mean chord length of alveoli increased over the 3 week period, as did surface area/lung volume

ratio. Absolute lung protein and DNA increased significantly at 3 weeks while protein/body weight and DNA/body weight ratios decreased at week 2. Body weight, heart weight and crown rump length increased at week 1 and week 2, while absolute femur length increased weekly.

It was concluded that daily treadmill running for 3 weeks 1) had no significant effect on the volume proportion of lung components 2) no significant effect on mean linear intercept, mean chord length of alveoli or mean chord length of ducts 3) no effect on relative surface area or surface/volume ratio 4) increased absolute surface area by a significant amount over controls (7%) and 5) had a significant effect on body weight, lung weight and lung volume after 2 weeks. Finally, the lungs of newborn guinea pigs appear to be in a phase of equilibrated growth, where the lungs are growing by enlargement of pre-existing alveoli rather than addition of new units.

D E D I C A T I O N

To my father and mother, brother and sisters, whose love, support and encouragement gave me the dedication to finish

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I. INTRODUCTION

1.1. General Objective

The purpose of this investigation was to study the influence of endurance exercise on the structural and biochemical aspects of postnatal lung growth and development in guinea pigs. Specifically, the objectives of this study were;

1) To describe normal lung growth in the guinea pig during the first 21 days of life
2) To test the hypothesis that daily endurance exercise has a significant effect on the dimensions of respiratory units in guinea pigs during the first 21 days of life

1.2. Effects of Increased Oxygen Consumption due to Exercise on Lung Growth

The postnatal growth of the lung involves both the rapid growth of preformed components and structural transformation due to the formation of new respiratory units or alveoli (Emery and Mithal, 1960; Boyden and Tompsett, 1961; Dunnill, 1962; Boyden and Tompsett, 1965; Emery and Wilcock, 1966; Boyden, 1967; Weibel, 1967; Burri, 1974; Amy et al, 1977). These transformations take place necessarily in a functioning organ in a new environment, therefore it may be expected that the environment and metabolic demands on the organism have some regulatory

influence on the process governing lung development. Among adult mammals of different species, respiratory surface area (Tenney and Remmers, 1963) and morphometric diffusing capacity (Weibel, 1970, 1971) have been shown to be well matched to metabolic requirements and the question arises whether imposed alterations in metabolic rate can influence lung development in young animals.

An increase in metabolic rate due to exercise has been hypothesized to have an effect on lung growth since the early 1900's, when effects were measured by changes in lung and chest volumes. A few German authors have attempted to demonstrate an effect of physical exercise on lung growth (Kuelbs, 1912; Tiemann, 1936; Gehrig, 1951; Clemens, 1956). Tiemann (1936) postulated that physical exercise lead to an enlargement of the chest cage and accompanying lung inflation, resulting in chronic distension of the alveoli which would be an adequate stimulus for proliferation of new interalveolar septa. Gehrig (1951) exercised mature rats and guinea pigs, 10 months of age, 210 minutes per day for 4 months using a swimming regimen. These experiments produced an acute over-distension of the alveoli, thickening of the alveolar walls and an increase in alveolar number in the exercised animals. Gehrig concluded that this distension triggered alveolar multiplication. However, Gehrig and coworkers did not inflate the lung tissue before quantitative analysis, therefore their results are difficult to interpret.

Over the past decade, with development of more reliable quantitative techniques, adaptive changes in lung structure have been more readily demonstrated (Weibel, 1967). Using these techniques, Bartlett (1970a) attempted to show changes due to daily exercise in rats.

Male Sprague Dawley rats, 4 weeks of age, were exercised on a rodent treadmill daily for 20 days, for 20 to 30 minutes at 0.5-2.0 mph, 7.5^{o} grade. Morphological measurements of the lungs demonstrated no change in relative or absolute lung volume or weight, alveolar surface area, total number of alveoli or mean alveolar diameter, due to exercise. However, closer examination of Bartlett's results reveal a definite trend towards an increase in number of alveoli and alveolar surface area in exercised animals. Any significant difference between the exercise and control groups may have been masked by the relatively small number of animals used in the study (8 exercised, 10 control). The above study is relevant only to the latter phase of lung growth in rats outlined by Burri (1974) as a period more of expansion and slower formation of new respiratory units. The author discussed whether an increase in metabolic demand made during the earlier growth phase of the lung, one of rapid formation of alveoli may have produced more significant results. It appears from recent work, however that rats continue to add respiratory units for up to 133 days after birth (Holmes and Thurlbeck, 1979). In a different study (Fu, 1976), 4 week old male rats were exercised by swimming and showed exercise-induced alveolar proliferation that was independent of training intensity, but was influenced by the age of the animal at the start of training. Alveolar proliferation was increased in rats exposed to swimming during the second month of postnatal growth but not in rats exposed to swimming during the third postnatal month.

Generally investigators have avoided using treadmill exercise to increase oxygen consumption, preferring the use of drugs or hormones which increase or decrease oxygen consumption (Bartlett, 1970a; Burri *et*

al., 1976; Bartlett and Areson, 1978; Hugonnaud et al., 1977). Bartlett (1970a) administered 1% thiouracil and L-thyroxine (10-20 gm) daily to two separate groups of 4 week old male rats for 20 days. Both thiouracil and L-thyroxine alter thyroid function, therefore change the oxygen consumption of the animal; thiouracil reduced oxygen consumption by 26% and L-thyroxine increased O_2 consumption by 35% (mL/min STPD/100 gm body weight). Neither group demonstrated a change in specific lung volume, alveolar surface area, alveolar number or mean alveolar diameter. There were significant differences between control and thiouracil treated rats in specific lung weight and differences between thiouracil and L-thyroxine treated groups in specific alveolar number. Bartlett suggested that a longer study period may have produced different results. Another possibility was that the lungs were insensitive to the thyroid hormones. Weiss (1957) demonstrated the oxygen consumption of lung tissue from hypothyroid rats to be no different from that of normal controls.

Burri et al. (1976) investigated the effects of altered O_2 consumption on lung growth by administering imino, ββ -dipropionitrile (IDPN) an inducer of increased oxygen consumption per minute ($\dot{V}O_2$). Three intraperitoneal injections of IDPN manifest a hyperkinetic syndrome consisting of choreoathetotic movements in normal white mice after 5 days. IDPN increased specific $\dot{V}O_2$ by 50% after a period of 4 months. Specific lung volumes were also increased by 23%. In a subsequent paper, IDPN treatment induced a reduction in air space volume density and an increase in tissue and capillary volume by 15% in mice (Hugonnaud et al., 1977). A 15% increase was also demonstrated in alveolar and capillary

surface areas which were in turn reflected in a 40% increase in the morphometric diffusion capacity. Hugonnaud et al. (1977) concluded that there was a possible alteration in the septal morphology, probably a lengthening and corrugation of the intralveolar septa.

Use of IDPN for increasing oxygen consumption has a number of disadvantages. The mechanism of the drug remains obscure. IDPN is known to be a powerful lathyrogen which effects the nervous system and has significant effects on overall growth. It also causes abnormalities in the skeletal system, retinal vasculature, platelet aggregation and connective tissue metabolism (Bartlett and Areson, 1978). The possibility of an effect of IDPN on the connective tissue metabolism of the developing lung would tend to cast some doubt on the use of this drug to study effects of increased $\dot{V}O_2$ on lung development.

The use of cold exposure to induce an increase in $\dot{V}O_2$ eliminates problems associated with drug side effects, but introduces other factors which must be taken into account when interpreting results. Thomson (1980) exposed 6 week old male hamsters to 4-5^{o}C for 28 days and recorded a 26% increase in O_2 consumption, a 24% increase in respiratory surface area, a 30% increase in lung volume, and a 21% increase in total alveolar number. Total DNA levels were also 30% higher than control values. In an earlier study, Gehr et al. (1978) raised 4 week rats for 3 weeks at 11^{o}C ambient temperature resulting in a $\dot{V}O_2$ increase of 64%, and an increase in specific lung volume of 24% due to an increase in air volume of 26%. Also reported was a 13% increase in capillary blood volume, 19% increase in tissue volume, and an 18% increase in specific surface area. It was shown in a recent study, that

cold exposure in 2 to 4 week old guinea pigs resulted in rapid increases in lung volume, alveolar surface area and capillary surface area. However, this initial acceleration in lung development did not persist, and similar pulmonary dimensions existed in adult cold exposed animals when compared to controls (Lechner and Banchero, 1980).

The relationship between lung size and increased whole body oxygen demand has also been studied in Japanese Waltzing Mice (JWM)(Geelhar and Weibel, 1970). These animals are kept in a continous waltzing motion due to a genetic defect of their vestibular apparatus, this demands an 80% increase in O_2 supply/100 gm body weight compared to control mice of a different species. Measurements on the dimensions of the pulmonary gas exchange apparatus and the pulmonary diffusion capacity were calculated morphometrically in such animals. Results indicated that the alveolar and capillary surface area and the capillary volume of JWM were 60% larger than those of normal mice. Geelhar and Weibel (1970) felt this reflected a reduction in the size of alveoli and an increase in their number. The air-blood barriers were also noticably thinner in JWM which contributed to their higher diffusion capacities over controls. In contrast to these interpretations, Hugonnaud et al. (1977) suggested that the thin air blood barriers of JWM could be the result of a genetically fixed adaptation due to selection, rather than a modification.

Bartlett and Areson (1978) attempted to eliminate the variable of evoluntionary adaptation in the JWM model by comparing lung dimensions of JWM with their phenotypically normal littermates, instead of normal lab mice. Body weights, lung volumes, surface area and alveolar number were measured in both groups as well as in normal laboratory mice. When

expressed relative to body weight, there was no significant difference between the lung volume and surface area and number of alveoli between the waltzing mice and normal littermates. Conflicting results to those of Geelhar and Weibel (1970) may have arisen due to the difference in ages of animals used in the two studies. Geelhar and Weibel (1970) used 8 week old rats whereas Bartlett and Areson (1978) used 12 week old animals. Discrepancies could also have arisen due to differences in lung fixation procedures.

From the literature it is evident that the timing of manipulative interference in lung growth is of great importance in the degree to which lung structure is altered. Burri and coworkers (Burri et al., 1974) reported three distinct postnatal periods in the rat lung which are presumed to be similar in all mammals with differences only in the timing of events (Burri and Weibel, 1977; Vaccaro and Brody, 1978; Brody and Vaccaro, 1979). The first 4 days constitute a period of lung expansion when lung volume increases, resulting in an 87% enlargement of existing airspaces. Morphometric data has suggested that little tissue is added during this phase (Burri et al., 1974). However, Amy et al. (1977) reported a rapid increase in lung weight after the first 24 hours while Das and Thurlbeck (1979) reported an increase in the rate of ^{3}H thymidine incorporation into DNA. These latter studies would tend to refute the argument that there is no tissue added during this phase.

Lung expansion is followed by the period of tissue proliferation from days 4 to 13 which is characterized by the subdivision of primary saccules into alveoli. Primary saccules are the smallest peripheral units in rat and mouse lungs. They are simple tubular components longer

than they are wide, lined with type I and type II alveolar epithelium, characteristically with a double capillary layer in their walls (Short, 1952; Weibel, 1967; Burri, 1974). These capillaries, one from each saccule, eventually fuse to form a single capillary. The subdivision of primary saccules during this phase increases alveolar and capillary surface areas. Alveolar surface area increases to the 1.6 power of lung volume (Burri et al., 1974). If the lung grew by expansion without proliferation the alveolar surface area would increase to the 2/3 power of the change in volume (Brody and Thurlbeck, 1984, in press).

The division of primary saccules is accomplished by secondary crests. These are characterized by elastic fibre, with attached collagen and basal lamina at the free margins. The crests contain a single capillary and numerous interstitial cells including type II cells which contain the surface active phospholipid 'surfactant' (Brody and Thurlbeck, 1984, in press). The elastic fibre seems to play an important role in the formation of alveoli. Their appearance in newborns has been compared to that of a "fishnet" (Emery, 1970; Emery and Fagan, 1970) "the apertures of which form the mouths of alveoli" (Emery and Fagan, 1970).

The final period of equilibrated growth is described from day 13 through to adulthood. Initially, there is a period of tissue mass re-distribution with septal lengthening and a further increase in alveolar and capillary surface area (Burri et al., 1974). There is an increase in the latter to the 0.71 power of lung volume, close to the value of 2/3 which would indicate growth by simple distension. There is an overall slowing of the increase in lung volume to the 0.7 power of body weight as compared to 0.99 power in the previous phase (Brody and

Thurlbeck, 1984, in press). The onset of the equilibrated growth phase was found to be between 4 and 14 weeks in the rat (Holmes and Thurlbeck, 1979). These investigators also indicated alveolar multiplication may continue to 133 days of age in the rat (Weibel, 1967) but the majority of lung growth between 10 and 14 weeks is by simple expansion (Buhain and Brody, 1973; Holmes and Thurlbeck, 1979).

Recently, Lechner and Banchero (1982) reported that guinea pig lungs appeared to be in the equilibrated growth phase at birth with no postnatal period of pronounced tissue proliferation, although some tissue is presumably added. This slower increase in tissue mass is accompanied by enlargement of air spaces. Up until this time there had been a tendency to presume that postnatal lung growth in mammals generally resembled that in the rat (Burri and Weibel, 1977; Bartlett, 1972; Vaccaro and Brody, 1978; Brody and Vaccaro, 1979). In this study, there is a suggestion that lung development in guinea pigs differs from the pattern in the neonatal rat. Structures resembling the pre-alveolar saccules of newborn rats were not observed, even 10 days pre-term in guinea pigs. The air blood barriers appeared mature by 7 days preterm and the double capillary layers were only rarely seen in septal walls. This tends to confirm the hypothesis of Engle (1953) that the degree of internal complexity of a mammal's lung at birth reflects the overall activity and maturity at birth. Guinea pigs are born with well developed locomotion, righting reflex, fur covering, eyes open and become independent of the mother after a few days.

Forrest and Weibel (1975) studied the mature guinea pig lung morphometrically. The only difference reported between guinea pig lungs

and other mammalian lungs were the quantitative differences in the mass of connective tissue in the alveolar-capillary barriers. They suggested that the level of physical activity or oxygen requirements may be an adaptive factor determining the size of the gas exchange apparatus. Recent studies have shown that the main morphometric parameters of the pulmonary gas exchange apparatus, namely alveolar surface area, capillary volume and diffusion capacity of oxygen, all scale linearly with body weight (Gehr et al., 1981; Taylor et al., 1980). These findings are in conflict with those of Tenney and Remmers (1963) who had found that alveolar surface area was linearly related to $\dot{V}O_2$. The differences seem to arise due to the selection of animals. Tenney and Remmers included both terrestrial and marine mammals, whereas the more recent studies have considered only terrestrial mammals. Clearly, the factors determining lung growth and development are complex, relating in part to the age, body weight, and the metabolic requirements of the mammal.

1.3. Rationale

In conclusion, the objective of this study is to determine whether or not daily endurance exercise has an effect on the dimension of the respiratory units in guinea pig lungs. A further objective is to add to the already existing knowledge of normal lung development in the guinea pig and confirm whether or not the maturity of the guinea pig at birth and shortly thereafter, parallels a similar maturity in lung development.

II. REVIEW OF THE LITERATURE

The lungs first appear as an outgrowth from the foregut which divides into two, to form the main airway to each lung. This airway then branches within its enclosing mesenchyme to form the lung. The events that transform this simple branched tube embedded in mesenchyme into the complex maze of small air chambers surrounded by capillaries, continue through from early embryonic life, well into the postnatal period (Burri and Weibel, 1977).

The most extensive account to date, of events in lung growth and development both prenatally and postnatally apply to the rat (Burri et al., 1974). However recent work on mice, rabbits, dogs, guinea pigs and humans has demonstrated similar stages of development to the rat, which differ only in the timing of the event. Birth, although a particularly critical event for the lung, does not appear to mark a specific point in the time sequence of lung development. However for purposes of this review it was convenient to separate prenatal events from post-natal events.

2.1 Prenatal Development

The lower respiratory system begins to develop, on day 26 after ovulation in humans (Boyden, 1977). It is first indicated by a median laryngotracheal groove in the endodermal tube which evaginates to form the lung bud which then branches into two buds at 26 to 28 days gestation. These endodermal buds, together with the surrounding

splanchnic mesenchyme, differentiate into the bronchi and proximal part of the respiratory tree. This first stage of lung development is termed the embryonic period and lasts from day 26 to day 52 in humans (Boyden, 1977).

Following the embryonic period in man and other species (rat, rabbit, sheep, guinea pig, opossum) three phases of lung development are recognized. These stages are a) pseudoglandular, during which the preacinar branching of airways is established b) canalicular, when mesenchyme rapidly increases and the respiratory portion of the lung develops and c) alveolar, when the functional unit of the lungs, the alveoli are formed from secondary subdivision of the saccular spaces by septae (Loosli and Potter, 1951; Boyden, 1972; Dubreil et al., 1936). These stages have been described by several investigators, however duration of each stage and terminology may differ from investigator to investigator and the transition from stage to stage is gradual. The alveolar stage is thought by some investigators to take place only after birth (Dubreil et al., 1936; Boyden, 1972).

The pseudoglandular (or glandular) period lasts from 6 weeks to approximately 4 months gestation in the human and from day 12 to day 18 after conception in the rat (Burri and Weibel, 1977). The lung buds continue to subdivide by dichotomous branching, specifically between the 10th and 16th week of gestation in humans when 65 to 75 percent of bronchial branching occurs (Brody and Thurlbeck, 1984, in press). At this stage, the lung has a distinctly glandular appearance with airways lined by columnar epithelial cells containing glycogen, separated from each other by primitive mesenchyme (Thurlbeck, 1975). By the end of 16

weeks, bronchial and bronchiolar development is complete as far as the terminal bronchioles (Buchner and Reid 1961). In the early pseudoglandular stage, the nuclei in the columnar epithelial cells are variable in size and shape, showing one or two prominent nucleoli, and frequent mitotic figures. Towards the latter part of this phase, the tubular epithelium becomes cuboidal and nuclei appear spherical. The basal surface of the epithelium is distinct from the mesenchyme by a narrow basement membrane during all three post embryonic stages, however, appears to be interrupted in the pseudoglandular phase (Burri and Weibel, 1977).

The canalicular stage occurs from the 17th week through to 26th week of gestation in humans, and from day 18 to 20 in the rat. This stage is characterized by the proliferation of mesenchyme and associated blood supply, together with a flattening of the epithelium that lines the airways. The epithelium takes on an irregular appearance in terms of thickness, and cellular continuity may only be apparent at cell bases (Thurlbeck, 1975). The gradual thinning of epithelial lining and protrusion of capillaries results in the close proximity of capillary lumen to airway surface in many areas. Capillary beds develope around each airway or saccule. As the saccules approach each other, and the interstitium thins, the saccule walls develop a double capillary layer - one from each saccule. The saccules are very simple structures, and continue to branch, developing a 'saw-tooth'-like appearance (Brody and Thurlbeck, 1984, in press).

The canalicular period is important biochemically for the synthesis of lecithin and the formation of a surface-active material. This

surface-active substance or surfactant, lines the airspaces and at birth decreases tension at the air-liquid interface and facilitates lung expansion (Boyden, 1977). Clinical evidence and autopsy studies (Brumley et al., 1967) have indicated that surfactant does not appear in sufficient quantity to sustain alveolar stability until 24 to 26 weeks gestation. The appearance of surfactant coincides with that of type I and type II alveolar epithelial cells, along with occassional osmiophilic bodies in the type II cells (Campiche, 1963). Respiration can be maintained during this period. At this point in lung development, birth occurs in rats and mice, whose lungs lack alveoli at birth (Burri, 1974; Burri et al., 1974; Amy et al., 1977). The interstitium of mice and rat lungs is also thinner and the septal divisions longer and thinner than in human lungs of 28 weeks gestation (Brody and Thurlbeck, 1984, in press).

The third stage of lung development has been classified as the alveolar stage by Dubreil et al., (1936) and Loosli and Potter (1951), as the terminal sac period by Boyden 1972, and a combination of a saccular phase followed by alveolar phase by Brody and Thurlbeck (1984, in press). There is controversy over the duration of this stage in the literature. Dubreil et al., (1936), classified alveolar development only after birth, Loosli and Potter (1951) between 6 months gestation and birth, Boyden (1972) after birth and Thurlbeck and Brody (1984, in press) from 28 weeks gestation (28 weeks to 36 weeks - saccular phase) up to 2 years postnatal (36 weeks to 2 yrs - alveolar phase).

The period from 28 to 36 weeks is characterized by the appearance of small low subdivisions in the saccules which elongate and produce small air spaces. These may have a double capillary layer ajacent to

them. By 32 weeks gestation, alveolar-like structures can be found in almost all lungs and alveoli are definitely present at 36 weeks (Brody and Thurlbeck, 1984, in press). Generally, investigators have observed a great variability in number of alveoli present at birth. Langston and Thurlbeck (1982) found a mean of 55 million and a range of 10-149 million. Dunnill (1962) found 20 x 10^6 in one infant, Hieronymi (1960; 1961) reported 70 x 10^6 and Thurlbeck and Angus (1975) found 71 x 10^6 alveoli at birth. Other investigators have reported that alveoli were absent at birth in humans and the units in question were saccules (Hislop and Reid, 1974). However this was based on a case study where the infant in question was below the 3rd percentile in body weight.

Reasons for these discrepancies could be a normal biological variation between infants, difficulty in recognizing and defining an alveolus or due to different methods of preparing lung tissue for quantitative assessment (Thurlbeck, 1975). Recent studies have also shown that pulmonary hypoplasia without associated congenital abnormalities such as renal agenesis and diaphragmatic hernia may be more frequent than generally thought (Swischuk et al., 1979; Wigglesworth et al., 1981; Page and Stocker, 1982). Wigglesworth and Desai (1982) have pointed out the critical relationship between normal lung growth and amount of amniotic fluid on intra-uterine respiration, which in turn are influenced by catecholamines, smoking, maternal hypoglycemia, alcohol and barbiturates. The wide variation in alveolar number at birth between individuals may represent this sensitivity to external stimuli (Brody and Thurlbeck, 1984, in press).

Vascularization of the lung occurs in a centripetal fashion with

the final stage being extension of vessels along the respiratory bronchioles (Perlman et al., 1981). The preacinar blood vessels follow the development of the airways, whereas intra-acinar arteries and veins develope in parallel with the alveoli. The main pulmonary veins develope later than arteries but the main pattern of vessels connecting heart and lungs as well as ductus arteriosus is established by the 7th week (Perlman et al., 1981). From the 7th to 16th week, the main feature of arterial growth is an increase in the number of branching, so by the end of the 16th post conceptual week, all pre-acinar vessels are present and will continue to grow in length and size only, while new vessels appear in intra-acinar regions. During fetal life, blood vessel diameters increase at a greater rate proximally than distally in contrast to postnatal growth. Arteries are also more muscular and veins less muscular in fetal lungs, compared to the mature lung (Hislop and Reid, 1973).

The development of the pulmonary nervous system follows the appearance of the structures that it innervates. These structures include perichondrial tissue, epithelial and specialized cells of the respiratory passages, mucous glands (appear between 12 weeks and 13 weeks and continue to grow until 28 weeks), smooth muscle cells of the trachea, bronchi and terminal units and smooth muscle cells of the blood vessels and lymphatics (Loosli and Hung, 1977).

2.2 Postnatal Development

Postnatal development of the human lung has been shown to comprise

the final phase of lung development - the alveolar phase (Dubreil et al., 1936; Emery and Mithal, 1960; Dunnill, 1962; Boyden and Tompsett, 1965; Emery and Wilcock, 1966; Reid 1967; Davies and Reid, 1970; Thurlbeck and Angus, 1975; Langston and Thurlbeck, 1982). Postnatal alveolar development has also been described in rats (Neuhauser and Dingler, 1962; Neuhauser, 1962; Weibel, 1967; Burri et al., 1974), mice (Amy et al., 1977), rabbits (Engle, 1953), cats (Engle, 1953; Dingler, 1958), and dogs (Boyden and Tompsett, 1961). Generally in rats and mice, during the first 24 hours there is little change in lung weight (Amy et al., 1977) or lung volume (Short, 1951) with negligible rates of ^{3}H-thymidine incorporation into DNA for the first few days (O'Hare and Towne, 1970; Kaufman et al., 1974; Crocker et al., 1970). The lungs then increase rapidly in weight (Amy et al., 1977; Nijjar, 1979) and rate of ^{3}H-thymidine incorporation into DNA (Das and Thurlbeck, 1979). A common characteristic growth pattern has emerged from these postnatal studies, comprised of three distinct stages, 1) a phase of lung expansion, where increase in lung volume is due to a gain in the volume of airspaces 2) a phase of tissue proliferation when alveolar surface area increases and 3) a phase of proportionate growth where the majority of alveoli have been formed and growth consists of enlargement of existing structures (Burri et al., 1974). In the rat lung, the first stage, as measured by change in lung volume shows lung volume to increase proportionally with body weight, during the first 10 days, then it increases to the power 0.7 of body weight thereafter. This biphasic pattern of volume shift suggests to some investigators that lung growth after birth is a function of chest growth and follows this growth

passively by an increase in residual volume (RV). This increase in RV could then trigger the proliferation of cells after 10 days (Burri and Weibel, 1977). The duration of alveolar multiplication appears to be variable from individual to individual within the same species. Dunnill (1962) concluded that alveolar multiplication was very rapid in the first few years of life slowing exponentially so that alveolar multiplication is slow at age 4 years and ceased by age 8 years. Dunnill counted 257 x 10^6 alveoli in one eight year old, which was a number close to the 300 million generally believed to be the adult complement of alveoli. Angus and Thurlbeck (1972) found however, that the adult range of alveoli was actually variable between 225 and 600 million alveoli. Radial counting, a technique for counting alveoli per acinus has suggested that alveolar formation continues until somatic growth ceases (Emery and Mithal, 1960). A recent study utilizing the radial count method suggests a slight but significant increase in alveolarization from 2 to 8 years (Cooney and Thurlbeck, 1982). However larger studies have indicated such wide variations in alveolar number in children that a definite statement on the cessation of alveolar development cannot be made (Thurlbeck and Angus, 1975; Thurlbeck, 1982). In a recent study, Thurlbeck (1982) observed a difference due to sex in postnatal lung growth. Alveolar dimensions and number of alveoli per unit area were similiar between the sexes before 5 years of age, however boys have bigger lungs for the same stature than girls. Lung growth events at puberty have not been adequately studied to date, to draw any conclusions as to the possible differences between sexes at a later age.

2.3 Control of Prenatal Lung Growth

Recent reports have established the importance of at least three factors in controlling lung growth and development in late gestation. These include, the distortion of lungs by external pressure, the amount of amniotic fluid and lung fluid, and intra-uterine respiration. Compression of the lung in utero has been shown clinically (Kitagawa et al., 1971; Reale and Easterly, 1973) and experimentally (DeLorimer et al., 1967) to disturb lung growth. These investigators observed hypoplasia in the developing lung associated with diaphragmatic hernia. The lung on the side of the hernia was more hypoplastic than the opposite side, although the exact nature of the hypoplasia is still in dispute. Kitagawa et al., (1971) considered that the number of alveoli per acinus was normal but the total number of alveoli per lung was insufficient because there were too few acinar units. These workers also observed significant alterations of the bronchial and arterial pathways. Kitigawa et al., hypothesized even after successful repair of the hernia, adequate alveolar multiplication would not occur. This was confirmed in 2 case studies by Thurlbeck et al., (1979). Results indicated that lung volume was restored to approximately that appropriate for body size, but lung structure was abnormal after successful repair of diaphragmatic hernia. In both cases there were fewer and larger alveoli on the herniated side and fewer bronchioles than normal. Interestingly, in one case, the sum of the number of alveoli from right and left lungs was normal, however the number in the herniated side was almost 50% that in the contralateral lung, indicating the possibility of compensatory alveolar multiplication

in the contralateral lung. Reale and Easterly (1973) using the radial count method for assessing lung maturity found decreased counts with congenital diaphragmatic hernia compared to normal values. This reduction in radial count however was insufficient to account for the small lung volumes, indicating a loss of major units. Cooney and Thurlbeck (1982) also noted a significant reduction in lung volume of contralateral lungs (41% of predicted) even though the acinar component of the hypoplasia was marginal, indicating loss of acinar units and hypoplasia of the peripheral bronchiolar tree.

Conclusions as to the cause of pulmonary hypoplasia in diaphragmatic hernia can be reflected in the technique used to measure lung maturity. Cooney and Thurlbeck have demonstrated that inflation of lungs prior to tissue fixation significantly affects radial count values and that there is an inherent interobserver error in the technique. Kitagawa et al., (1971) and Thurlbeck et al., (1979) inflated the lungs prior to fixation while the lungs were not inflated by Reale and Easterly (1973).

Compression of the lung is also the likely cause of hypoplasia in reported cases of thoracic dystrophy (Finegold et al., 1971), diaphragmatic atrophy (Briggs et al., 1973; Goldstein and Reid, 1980), and anencephaly (Reale and Easterly, 1973). In anencephaly, the thorax is characteristically small, shortened or abnormal resulting in possible compression of the lung. Compression in utero due to a deficiency of amniotic fluid, a conditon known as oligohydramnios may also be the mechanism behind pulmonary hypoplasia in this syndrome (Thomas and Smith, 1974; Perlman and Levin, 1974; Bain et al., 1964). The importance of

lung fluid and amniotic fluid in normal pulmonary development in utero has been demonstrated in tracheal ligation and tracheal drainage experiments. Tracheal ligation in sheep performed at 105-110 days gestation produced abnormally large lungs due to an increase in fluid and tissue mass. Also in these animals, alveolar walls were thinner, type II cells were rarely observed and lamellar bodies were diminished therefore differentiation did not parallel the increased tissue mass. In experiments where the lung fluid was chronically drained after ligation, reverse changes were observed (Alcorn et al., 1977).

The oligohydramnios syndrome may be considered somewhat analogous to tracheal drainage experiments. It is often associated with renal agenesis or other renal abnormalities, where lung hypoplasia is a characteristic complication. An experimental model of oligohydramnios in which amniotic drainage was performed in rats on days 16 and 17 in gestation showed a reduction in lung weight although histologically normal lungs (Symchych and Winchester, 1978). Hypoplasia was also indicated by diminished lung volume, lung weight and alveolar number as assessed by radial counting in cases with renal abnormalities and associated amniotic fluid reduction (Hislop et al., 1979; Reale and Esterly, 1973; Emery and Mithal, 1950).

The reverse of oligohydramnios, however, a greater than normal amount of amniotic fluid, or polyhydramnios is not necessarily associated with lung enlargement. Hypoplastic lungs from infants with polyhydramnios have been found to be structurally mature with normal phospholipid concentrations, indicating that amount of amniotic fluid during gestation may not be the most important factor regulating lung

growth (Wigglesworth et al., 1981; Wigglesworth and Desai, 1982).

Evidence exists that indicates a possible influence of the kidney on lung growth. Hislop et al., (1979) found a reduced number of pre-acinar airways and arteries in eight infants with renal anomalies. This was similar to findings of Reale and Esterly (1973) who found that lung weights were abnormal in 20 patients with renal anomalies. Since most of the distal airways develop in humans from the 12th to 16th week gestation, it would seem unlikely that amount of amniotic fluid would be important at this time in determining lung development. The kidney and lung begin development at the same time and it has been suggested that insult to the embryo at this time may lead to abnormalities in both kidney and lung. This could explain the characteristic of lung hypoplasia with kidney anomalies. An alternative explanation is the kidney may be directly involved in lung development. An experiment where nephrotoxins were injected into chick embryos resulted in decreased proline and collagen production and lung hypoplasia (Clemmons, 1977). Proline, an essential amino acid in lung development, is produced by renal arginase in the kidney during fetal development, while collagen maybe involved in the budding and branching of the lung (Alescio, 1973).

Another proposed mechanism behind control of lung development which may encompass those discussed is the force associated with intrauterine respiration. Compression of the lung in diaphragmatic hernia and thoracic abnormalities diminishes the distending force as does oligohydramnios or tracheal drainage reduce the distending pressure (Brody and Thurlbeck, 1984, in press). Wigglesworth et al., (1977) showed that high spinal cord section in fetal rabbits resulted in a 43%

loss of lung weight and 16% reduction in cell number. In another experiment, injection of tuborcurarine resulted in an 8% loss of lung weight in fetal rats (Moessinger et al., 1980). Bilateral phrenectomy in fetal sheep halted lung development structurally although epithelial differentiation appeared normal (Alcorn et al., 1980). These experiments certainly point to the importance of intrauterine respiration to normal prenatal lung growth.

2.4 Control of Postnatal Lung Growth

Interest in the timing of lung events has lead to research in the alteration of lung events by manipulative techniques postnatally as well as prenatally. Alterations in the chest wall dimensions, mechanical stretch of the lung, humoral influences and oxygen consumption have all been implicated in the control of postnatal lung development (Brody and Thurlbeck, 1984, in press). Unfortunately, there are few "natural" experiments pertaining to effects on the human lung and such data that are available are difficult to interpret due to the wide range of normal numbers and dimensions known to exist in the human lung (Thurlbeck, 1975). Consequently, the results of animal experimentation are often extrapolated to man which can never be done with certainty. Many experiments in lung growth manipulation have been performed on the rat, which may have a slightly different pattern of postnatal lung development than man due to continual enlargement throughout life which does not occur in man (Thurlbeck, 1975).

Pneumonectomy or resection of lung tissue in rabbits and rats

causes the remaining lung to increase in both volume and weight. The increase in lung weight is due to a rapid cellular proliferation and multiplication which begins a few days post-operatively, ceases in 1 to 2 weeks and is faster in younger animals than older animals. A closer review of the response to pneumonectomy illustrates an initial response of over expansion of the remaining lung. This is deduced because the increase in lung tissue is less than the increase in lung volume for the same period. In the dog, overexpansion is slight at first and increases during the 3rd to 4th week post operatively (Andrus, 1923; Phillips et al., 1941). The vacant space is partially filled by the expanding lung, together with a shift of the mediastinum, elevation of the diaphragm and inward movement of the chest wall (Andrus et al., 1923; Phillips et al., 1941; Edwards et al., 1939; Heuer et al., 1920).

Many investigators have shown that the remaining lung tissue increases in weight and volume after lung resection so that the remaining tissue approximately matches the weight and volume of both lungs of control animals (Buhain et al., 1973; Sery et al., 1969; Addis, 1928; Cohn, 1938; Cowan and Crystal, 1975; Nattie et al., 1974; Romanova, 1960; Tartter and Goss, 1973). Romanova and coworkers (1967) also showed increases in DNA and RNA in the contralateral lung, and Buhain and Brody (1973) also found that the amount of DNA was increased in the opposite lung after pneumonectomy, although the DNA/RNA ratio was unchanged, indicating hyperplasia rather than hypertrophy of cellular elements.

Cowan and Crystal (1975) noted a shift in protein synthesis towards collagen synthesis after pneumonectomy, followed by an accumulation of collagen. The total amount of collagen in the contralateral lung

doubled, but its concentration in the lung remained approximately the same. Cowan (1975) confirmed the work of Romanova et al., (1967) by showing synthesis of RNA and DNA reaching a maximum at days 4 and 5 after pneumonectomy and returning to nearly normal on day 7. The mitotic rate was doubled on days 3 and 4, increased to a maximum of approximately 5 times normal at day 5 and was twice normal at day 7 in one experiment (Romanova et al., 1967).

In a similar experiment Fisher et al. (1973) showed the peak of the mitotic response was reached on the 6th and 7th post-operative days and returned to nearly normal on the the 12th day. This experiment showed mitosis to occur particularly in the pleura and cells right below the pleura. Buhain and Brody (1973) demonstrated the response to pneumonectomy differed between young and old rats. The increase in DNA being greater in younger animals. In another experiment, the increase in weight occurred faster in young rats than in old rats and in the latter, the weight of the remaining lung did not increase to equal the weight of both lungs as was the case with younger animals (Nattie et al., 1974). This was not found in a similar experiment on guinea pigs where cell multiplication occurred only in young animals (Gnavi et al., 1970).

It also appears that all elements in the lung do not share equally in its expansion after pneumonectomy, and alveolar ducts expand more than alveoli. In one experiment the total lung capacity (TLC) of the remaining lung increased by approximately one third in adult rats but alveolar volume increased only 14% and alveolar duct volume 34% (Buhain and Brody, 1973). In rabbits, alveolar volume formed only 32% of the lung volume after pneumonectomy, compared to 40% in control animals.

Alveolar duct volumes were 46% to 47% and 30% to 32% in the post-pneumonectomy and control states respectively (Sery et al., 1969). In young rats a different response occurred; alveolar volume increased slightly more than alveolar duct volume after pneumonectomy (Buhain and Brody, 1973).

Manipulative techniques, other than pneumonectomy also seem to have a mechanical stimulus to lung growth. In rats, injection of wax into the pleural cavity decreased the ipsilateral lung weight in rats by 13 to 21%. Thoracoplasty decreased the ipsilateral lung weight by 4 to 6%, while phrenic nerve avulsion decreased lung weight by 11 to 15% (Cohn, 1938). Alveolar number was not obviously altered after phrenicectomy in rats (Cohn, 1940).

The hypothesis that mechanical stretch is of possible controlling influence in postnatal lung development has been suggested by Leung and coworkers (1977). Smooth muscle cells grown on an elastic membrane were measured for collagen and protein synthesis when cells were at rest or stretched rhythmically. It was found that stretching induced a 25% increase in collagen synthesis. Whether or not there was a similar control of elastin synthesis was unclear. While mechanical forces may play a role in controlling lung growth after pneumonectomy there is also evidence that a somatomedin-like substance appears in the blood that has the ability to make type II cells synthesize DNA in culture (Smith, et al., 1980), indicating a possible humoral factor.

Diminished lung volume is a natural occurrence in some diseased states in man. The lung is an organ which when fully inflated outside the chest, still shows impressions of the heart, aorta and superior vena

cava. Therefore it is not surprising that the lungs are permanently affected by the contents and shape of the thorax. The lungs of patients with kyphoscoliosis are distorted to fit into the deformed thorax and maintain this shape outside the body when inflated (Dunnill, 1970; Davies and Reid, 1971). Dunnill (1965) found that there were fewer alveoli in a patient with infantile onset of kyphoscoliosis than another patient with onset of the disease at 16 years. He postulated that the earlier the onset of disease, the more interference there was with alveolar multiplication. Davies and Reid (1971) noted an even greater diminution of lung volume and of number of alveoli in 4 patients with onset of kyphoscoliosis in childhood.

Despite the fact that a decreased space available for development affected the lungs in this way, Davies and Reid did not feel that this was the limiting factor in postnatal alveolar multiplication. Studies of congenital diaphragmatic hernia showed a severely hypoplastic lung with too few airways and arteries as well as too few alveoli (Kitagawa et al., 1971; Areechon et al., 1963). It appears then, that the timing of the herniation disturbed parallel development of the lung from that period on and the pathways that had already developed were relatively normal.

Investigators have long since studied the functional effects of high altitude, low barometric pressure and hypoxia, however relatively little experimentation has been done on these effects on lung structure. Much data on the effects of decreasing ambient PO_2 on lung dimensions is conflicting. Bartlett (1970b) exposed rats weighing 90 to 110 gms (approximately 4 weeks of age) at the beginning of the experiment to 10.4% O_2 at sea level (PO_2 of approximately 75 mm Hg) for 15 days.

The exposed animals were 19% lighter in weight than the control animals at the end of the experiment. Their lung weights and alveolar surface area, as well as the total number of alveoli were slightly less, although not significantly, than those of the controls and the lung volumes were the same. Specific lung weight and specific lung volumes were significantly increased, but specific alveolar surface area and specific number of alveoli were not. Bartlett concluded that "hypoxia had no demonstratable effect on the measured characteristics of lung morphology". Cunningham and associates (1973, 1974) demonstrated conflicting results. They exposed rats at birth and at 3 and 9 weeks of age to 12.5% O_2 (PO_2 of approximately 90 mm Hg) for 3 weeks. The results showed all groups of exposed animals were smaller than their control groups at the end of the period of exposure, but the effect on the organs depended on the age at which the animals were first exposed. Those exposed to hypoxia at birth had an absolute increase in heart and spleen weight, and the specific weight of every organ studied (lung, heart, liver, kidney and spleen) was increased. The results in rats exposed at 3 and 9 weeks of age were similar to each other; the absolute lung weight increased, as did the specific weight of heart and lungs. The total lung capacity (TLC) was increased in all animals exposed to hypoxia and there was no alteration in the elastic recoil properties of the lung. Morphometric analysis was performed on the left lungs of some of the animals and results demonstrated that rats exposed from birth showed more and larger alveoli and alveolar ducts than the control rats. Alveoli increased proportionately more in number and less in size than alveolar ducts. The number of alveoli and alveolar ducts were not

increased in rats exposed at 9 weeks of age but their dimensions were enlarged; alveolar ducts increased proportionately more in volume than did the alveoli. Exposure to hypoxia for 6 weeks rather than 3 weeks did not produce further changes. Cunningham and associates concluded that hypoxia affected lung structure. Because alveolar multiplication appeared to have occurred in the young animals, it seems likely that the increase in lung weight was due to hyperplasia of cells rather than hypertrophy.

Experimentation has been done on the effects of high altitude and hypobaric conditions on the lung in man and animals. In 1938, Cohn found a 41% increase in lung weight in rats exposed to a pressure of 500 mm Hg less than atmospheric (PO_2 of 50 mm Hg or 7% O_2). However, because lab animals at simulated altitude weigh less than the appropriate control animals the results are not easy to interpret. Specific weight of the lung, heart and spleen are also increased (Pepelko, 1970). A further complication is that pulmonary edema is a well recognized complication of exposure of humans to high altitude, so that edema may account, in part for the increase in lung weight. In fact, this has been suggested to be the only reason for an increase in lung weight and density of rats studied 1 week after exposure to a simulated altitude of 4,200 metres (Bartlett and Remmers, 1971). Tenney and Remmers (1966) concluded that there was no structural difference between the lungs of guinea pigs and sheep raised in New Hampshire compared to the lungs of the same species brought up in the Andes at an altitude of 4,500 meters. Thurlbeck (1975) noted that closer examination of their data showed trends for specific lung volume, alveolar surface area and diameter to be larger in the high

altitude animals. In 1971, Burri and Weibel demonstrated that rats being raised at 3,450 meters (ambient PO_2 of 100 mmHg) from 23 to 45 days of age had specific lung volumes, specific capillary volumes and specific alveolar and capillary surface areas that were significantly larger than those of control animals raised at 570 meters. The high altitude animals were 11% lighter and the results in absolute terms were not significantly increased although there was a trend in this direction. Bartlett and Remmers (1971) showed similar results in 1 month old rats placed in a hypobaric chamber at a simulated altitude of 4,200 meters (PO_2 of 95 mmHg) for 3 weeks. There were significant increases in absolute lung volume, surface area and lung weight and specific lung volumes. Specific lung weights were increased by approximately one-third. Alveolar diameter was largely unchanged despite the large increase in lung volume, suggesting that alveolar multiplication had occurred. Although alveolar surface area increased rather more closely to the two thirds power of the change in lung volume than to the absolute changes of lung volume, which would suggest that the increase in surface area was more by expansion than by alveolar multiplication.

Generally there seems to be fairly good agreement that specific lung dimensions increase under simulated or real high altitude conditions and in some cases, absolute dimensions also increase. The alterations are in agreement with those caused by hypoxia, which would be interpreted as brought about by hypoxia however the existance of a factor operating especially under hypobaric conditions cannot be excluded.

In man, there is evidence for real structural differences between high and low altitude dwellers. Hurtado (1932) found measurements of

chest cage girth were larger in residents living in the Peruvian Andes. The diaphragm muscle was found to be anatomically lower and the vital capacity bigger than those of lowlanders of comparable stature. Dempsey et al., (1971), and Guleria et al., (1971) found residents at high altitude to have an increased pulmonary diffusing capacity (DL_{CO}) attributable to a difference in the membrane component and increase in the capillary volume. This could be interpreted as a larger alveolar surface area due perhaps to an increase in alveolar number.

Evidence that hypoxia has an effect on lung structure could indicate that hyperoxic conditions may also have an effect as O_2 is a notable lung poison. Bartlett (1970b) exposed rats (90-110 gms) to 45.8% O_2 for 15 days. Although these rats weighed significantly more than the control rats at the end of the experiment, the absolute lung volumes and lung surface areas were less than the control values. Absolute lung weights were not diminished and total numbers of alveoli were not decreased, but obviously specific lung weight and alveolar number were significantly increased. In a similar experiment, Burri and Weibel (1971) exposed rats from 23 to 44 days of life to O_2 at a partial pressure of 290 mm Hg (45% O_2) and found similar results except that in this experiment there was no difference between the body weights of the experimental and control animals. Lung volumes were smaller with a normal distribution of tissue and air, thus all components of the lung were diminished.

Much of the work in manipulating lung growth was done by Brody in the early 1970's after it was observed that lung volumes were increased approximately 40% in male acromegalics. Physiological data on these

lungs suggested larger alveoli but no increase in number (Brody, 1970) Interestingly, the lungs in female acromegalics were functionally unaltered suggesting a hormonal influence. Jain et al., (1973) studied the lungs in patients with hypopituitarism and found the lung volumes decreased 20 to 40% of normal values.

The morphological events that correlate to these functional changes have been studied in animal experiments. Bartlett (1971) injected growth hormone into female rats weighing 250 gm After a period of 22 days they weighed 298.8 gm compared to 276.8 gm for the control animals. Absolute lung volumes, lung weights and surface area were all increased in the animals given hormone but were unchanged when expressed as specific dimensions. Bartlett could not demonstrate any increase in the total number of alveoli by direct counting but found alveolar surface area increased to the 0.85 power of the increase in lung volume. If the lung was merely expanding the surface area would increase to the 2/3 power of the increase in lung volume (Thurlbeck, 1975), thus suggesting alveolar multiplication.

Brody and Buhain (1972) implanted $MtTF_4$ tumors into 11 week old female rats. $MtTF_4$ tumors secrete growth hormone. The results demonstrated increased lung weights and volumes over the control values but the total amount of DNA (hence the number of nuclei) was the same in both groups. The RNA/DNA ratio increased, indicating the increase in lung weight could be due to cellular enlargement. When rats were given pure growth hormone similar but more subtle changes occurred. Interpretation of the results of this experiment are difficult due to the fact that $MtTF_4$ tumors secrete more adrenocorticotropic (ACTH) hormone

and prolactin than growth hormone. Adrenalectomy altered the response to tumor implantation because specific lung weight then increased, whereas specific organ weights of liver, kidney and heart were decreased. Specific lung weight increased 11 to 45% compared to 24 to 210% after tumor implantation without adrenalectomy. Animals that were adrenalectomized and tumor implanted, demonstrated twice as much lung DNA as control animals although lung volumes remained the same as tumor implanted animals. Thurlbeck (1975) interpreted the data as indicating adrenalectomized, tumor implanted animals had the appropriate number of nuclei and alveoli for their body size, as suggested by Bartlett on the basis of injection of growth hormone alone. Brody and Buhain believed the result could be taken to indicate a proliferation of connective tissue elements rather than parenchymal elements when refering to adrenalectomized tumor implanted groups.

Brody and Buhain (1973) also combined the effects of pneumonectomy with tumor implantation or hypophysectomy. When pneumonectomy was combined with the former, the animals were larger and had larger lungs than when pneumonectomy alone was performed but the amount of DNA in the lungs was smaller. Pneumonectomy plus hypophysectomy produced smaller rats with lungs that did not enlarge as much as after pneumonectomy alone but had increased specific lung weights and lung volumes. Clearly, different processes must be involved after pneumonectomy, growth hormone, $MtTF_4$ tumor implantation and hypophysectomy. Because the pattern of lung growth after pneumonectomy differs from that after growth hormone administration, the changes produced by growth hormone cannot be due solely to the enlargement of the chest cage.

The effects on postnatal growth of disease and drug treatment such as with steroid or cytotoxic drugs is not known in either animals or man. However certain observations have been described in various disease states. In the syndrome of congenital lobar emphysema (overinflation) in childhood, alterations in alveolar development have been observed. In this syndrome one lobe or part of a lobe expands very rapidly, shortly after birth, while the remaining lung may be compressed. One cause of the condition is hypoplasia of the bronchial cartilage. Hislop and Reid (1970,1971) observed a few cases with variable findings in the lung parenchyma. In one case, the lobe volume increased 5 times normal values, but alveolar number remained the same as that predicted, suggesting simple overdistention is not sufficient enough stimulus to induce alveolarization. In 3 other cases however, there were more alveoli than predicted; (in one case by a factor of 5) whereas lung volumes had increased 3 fold. Perhaps overinflation does contribute to alveolar multiplication but Hislop and Reid (1970) believe that the polyalveolar lobe produced the overinflation and not visa versa. They also observed too few arteries per unit volume therefore a diminished blood flow in the overinflated lobe. It was suggested blood flow may not be a determinant in alveolar multiplication.

Food deprivation has been shown to depress cellular multiplication in alveoli (Hackney et al., 1977) and actually produces emphysema-like changes in rats (Sahebjama and Wirman, 1981; Sahebjama and Vassalo, 1979). Food deprivation also aggravates emphysema caused by elastase administration (Sahebjama and Vassalo, 1980). These experiments were performed when alveolar multiplication had probably ceased. The mechanism

of this starvation induced 'emphysema' is unknown, although it is known that starvation diminishes oxygen consumption (Brody and Thurlbeck, 1984, in press). In a recent review on growth and aging in the lung, Brody and Thurlbeck (1984, in press) proposed a hypothetical mechanism for controlling postnatal lung growth which encorporates most factors discussed. This suggests that inhibitory processes are at work in the lung that affect interstitial fibroblasts, inhibiting lysyl oxidase and cellular multiplication and acting in a negative feedback loop to the anterior pituitary to inhibit growth hormone secretion. When the fibroblasts are in a compressed state, the inhibitory process are maintained but when they are stretched this feedback mechanism is switched off. Therefore, following pneumonectomy or with increased oxygen consumption the inhibitory processes are depressed and growth occurs. Increased growth hormone levels result in somatomedin secretion from the fibroblasts which results in proliferation of type II cells to form alveolar walls.

2.5 Morphometry - A Brief History**

Morphometry is a body of methods for obtaining quantitative information about macroscopic or microscopic anatomical structure. This information is usually in terms of quantities such as volume, surface area, number of components and size of components, and is of particular

** Most of this section was taken from Morphometry by W.A. Aherne and M.S. Dunnill, 1982.

value in correlating structure with function. Four general points can be made about morphological or stereological procedures. They derive information about three dimensional structure from measurements or analysis carried out on two-dimensional images or sections. Secondly, no quantity is measured directly, morphometry works in terms of estimates by repeated counting or measuring, and thirdly, each technique requires the use of test grids. Test grids are a set of lines for estimating surface area, a lattice of test points for estimating volume fraction and a discrete test area for counting actual components. Finally, it is usually necessary to measure the total volume of the organ in question, so as to calculate the volume fraction of a particular substructure. The methods of morphometry therefore, are indirect and essentially probabilistic. These methods are far more practical for determining quantitative information than the less flexible and infinitely more tedious direct methods such as serial section reconstruction.

Morphometry is founded upon ideas and procedures drawn from geometrical probability and its use in the fields of geology and metallurgy. The two subjects of geometry and probability were brought together for the first time, via integral calculus by the Comte de Buffon (1707-1788) who in 1777, challenged the members of the French Academy with his Needle Problem. 'Parallel lines, d units apart, are ruled on a plane surface. A needle of length ℓ units (where $\ell < d$) is thrown at random on the plane. What is the probability that it will meet one of the parallel lines?' Buffon correctly deduced the probability, p, that the needle would intercept one of the parallel lines as the fraction; $p = 2\ell/\pi d$ or, rearranging, that the length of the needle; $\ell = \pi/2\ pd$ where

p = n/N, the number of intersections (n) after N superimpositions. This model of a morphometric method illustrates three points. Firstly, any probabilistic method of determining a quantity involves repeated estimations, secondly, the probabilistic factor n/N is subject to chance fluctuation, although becomes consistent with increased repetition of measurement. Finally, the number of repetitions necessary to give the required precision can be calculated statistically.

The other major influence on the foundation of morphometry occurred in the field of geology. In the nineteenth century, geology had become a quantitative science but the volumetric analysis of rock into its mineral components was based on difficult separation techniques. The French geologist M.A. Delesse was the first to realize that volume fractions occupied by constituent minerals could be estimated from area profiles on rock surfaces. Delesse's method of estimating area was to match a particular component on a series of flat rock surfaces by suitably cut pieces of tin foil. These were then summed by weight to estimate the aggregate volume proportion occupied by that component. This concept that mean area is an unbiased estimator of volume went essentially unrealized until the development of area estimates by point counting almost a century later. Meanwhile another concept called linear analysis was being introduced by A. Rosiwal, 1898. He determined that volumetric analysis of mineral aggregates could be reduced to a study of one dimensional measurements, in fact by constructing a model of known chemical and quantitative composition, linear analysis could be made accurate to 1% (Thompson, 1930).

As is the case in many areas of science, new ideas often only

realize their full potential with the introduction of new instruments. Rosiwal's linear analysis procedure was a tedious measurement of linear intercepts over various rock components, one by one, with a micrometer. In 1916, however, S.J. Shand introduced the micrometer stage which was much faster and more efficient. In 1931, the ideas of Delesse were rejuvenated by the Russian petrographer A.A. Glagolev, who introduced a form of point counting and an integrating microscopical stage linked to a recording meter. Glagolev's method used a single fixed point (a crossed hairline) under which the image of the specimen was moved in a stepwise fashion. Each time the cross 'hit' a particular component, it was tallied on the meter. Glagolev discussed the probabilistic nature of this method and its sampling error in terms of the Bernoulli distribution. The conjunction of Delesse's principle and the technique of point counting put a powerful tool at the hands of morphometrists but there had been no mathematical proof that relative areas really do estimate relative volumes. However many theoreticians have succeeded in establishing this relationship since the times of Delesse and Glagolev. The following is from Hilliard (1968):

"Beginning with a cube of tissue of volume L^3 situated in the first octant of a co-ordinate system (Figure 1a) and containing a component body whose volume fraction we wish to determine. Imagine a plane traversing the cube of tissue, parallel to the x, y-plane and intersecting the z axis in the interval (z,z + dz) with uniform probability (Figure 1b), i.e., the plane is equally likely to cross the z-axis anywhere in the interval [0, L].

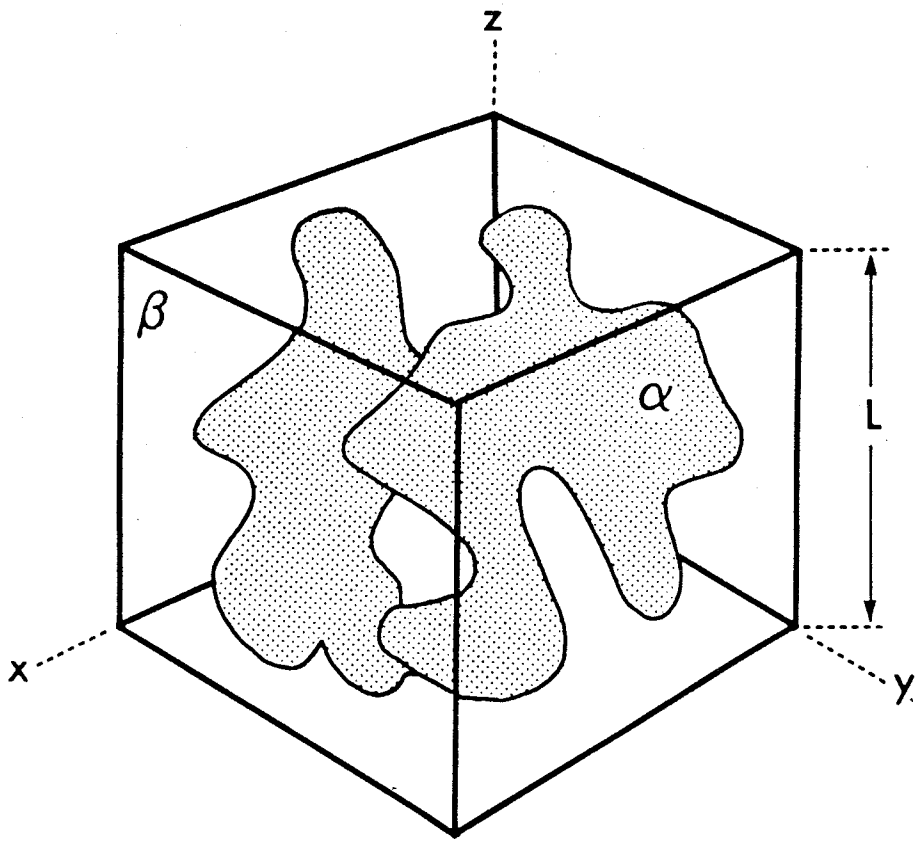


Figure 1a. A body of irregular shape, α , whose volume fraction we wish to determine is contained in a cube of tissue, β , of dimensions LxLxL.

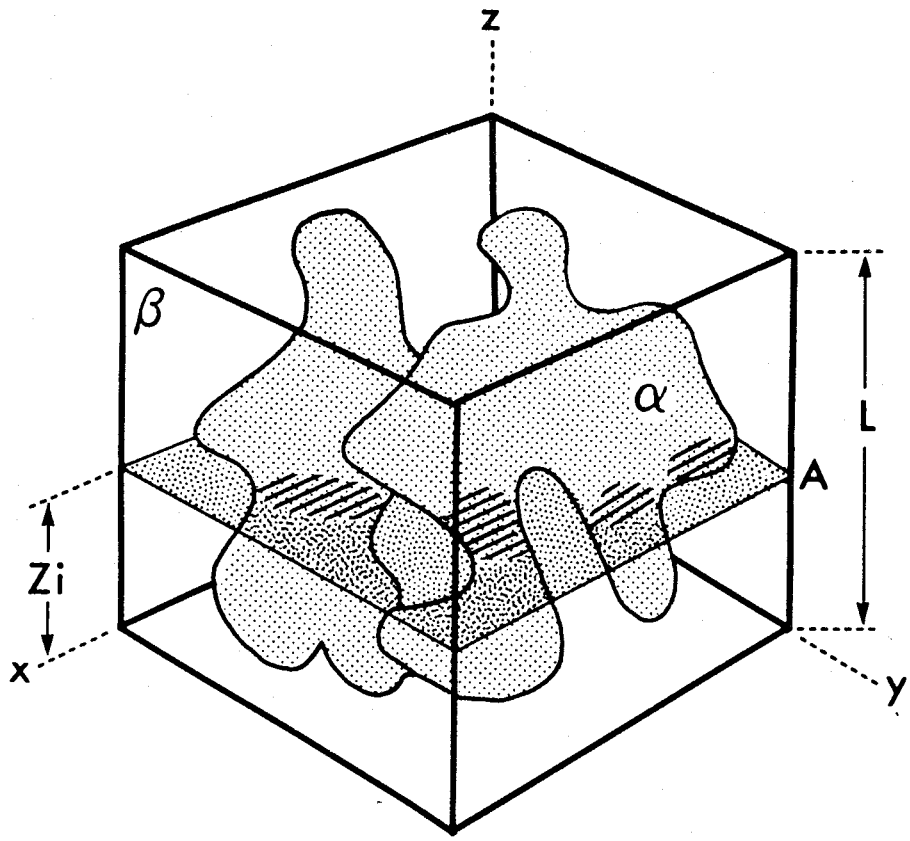


Figure 1b. A plane is passed through the cube of tissue, β , at A, in the x,y plane and contains two dimentional transections of the body α. By comparing the area fraction of these transections we determine the volume fraction of the body using the principle of Delesse (1848).

Profiles of the component body will appear on this plane. Let their aggregate area (A1 + A2 + ... + An) be A(z). The z in brackets signifies that the aggregate area of the profiles varies with (is a function of) the z coordinate.

By definition the area fraction is:

$$A_A = \frac{A(z)}{L^2} \tag{1.1}$$

Now, the average value of the area fraction over [0,L] is:

$$E(A_A) = \int_0^L A_A(z) \cdot f(z)dz = \bar{A}_A \tag{1.2}$$

Where the element f(z)dz is the probability that the plane intersects the z axis in (z, z + dz). But we have agreed that this probability is uniform, therefore;

$$f(z)dz = \frac{dz}{L} \tag{1.3}$$

Incorporating this in equation (1.2) we have:

$$E(A_A) = \int_0^L A_A(z). \frac{dz}{L} \tag{1.4}$$

Which, from equation (1.1)

$$= \int_0^L \frac{A(z)dz}{L^3} = \bar{A}_A \tag{1.5}$$

But A(z)dz is the fractional volume (dV) of component body contained in the volume element of the cube of thickness dz at position z.

Integration therefore gives:

$$\int_0^L \frac{A(z)dz}{L^3} = \frac{V}{L^3} = V_V \tag{1.6}$$

and so, from (1.5)

$$\overline{A_A} = V_V \qquad (1.7)$$ "

Between 1945 and 1953, four separate investigators demonstrated the relationship between the surface area of a metallic inclusion and the number of intersections which its two dimensional profile makes with a grid of randomly oriented test lines laid on the polished plane (Saltykov, 1945; Tomkeieff, 1945; Smith, 1952; Duffin et al., 1953). Tomkeieff considered a closed convex curve drawn on a plane, and constructed a one dimensional projection of it by dropping perpendiculars from its extremities to a line in the same plane, then measured the length L of this projection (Figure 2). Repeating this procedure in a number of different orientations gives a mean length of projection, $\bar{L}$.

Then it may be shown that

$$\bar{L} = C/\Pi$$

or $$C = \bar{L}\Pi$$

Where C is the perimeter of the closed convex curve. Moreover, the mean chord, $\bar{\ell}$, which can be found by drawing chords randomly inside the body, (Figure 3), is

$$\bar{\ell} = Ac$$

Where A is the area.

Tomkeieff applied these principles to a 3 dimensional body by drawing projections onto a 2 dimensional plane. Denoting mean projections of area by $\bar{A}_p$,

$$\bar{A}_p = S/4$$

where S is the surface area of the solid body. Moreover, if we denote

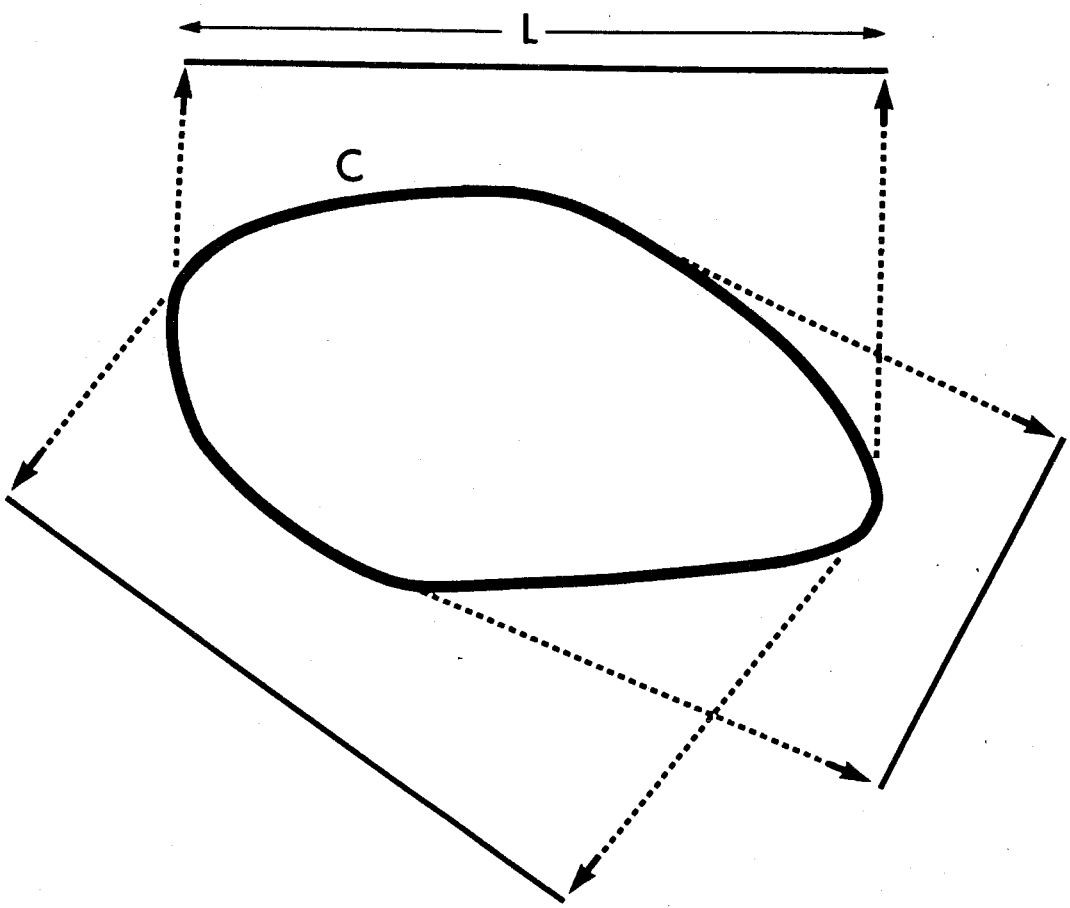


Figure 2. A closed convex profile c, and three one-dimensional vertical projections of it; each projection of length L; the mean projection length $\bar{L}$, is computed by making repeated vertical projections.

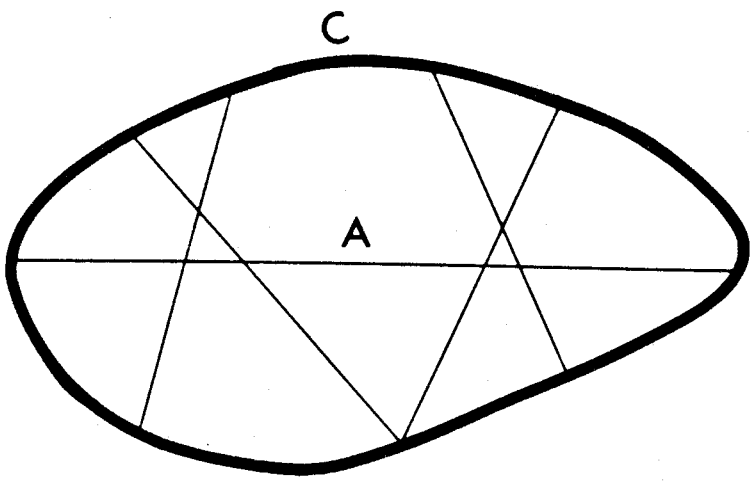


Figure 3. A closed convex profile c, of area A, as in Figure 2, in which a number of chords have been drawn. From a summation of chord lengths, the length of the mean chord is computed.

the mean chord of the projection by we have;

$$\bar{\ell} = 4V/S \quad \text{or} \quad S = 4V/\bar{\ell}$$

which is the equation for surface area. This equation has been derived at least eight times since 1945 by different investigators (Underwood, 1970).

All morphometric methods depend upon statistical analysis and central to this is the method of sampling. Non sampling errors include error due to varying section thickness, inaccurate measurement of grid lines and poor resolution of the microscope, however major sampling errors are more important to avoid. It is impossible to measure an entire organ morphometrically therefore methods must be followed to give truely representative samples. The lung is an organ with a structural gradient (DeHoff, 1967), meaning, there are variations in geometrical properties of lung components dependent on the position in the lung. Thus, correct sampling must be through a uniformly distributed set of planes obtaining representative samples of the whole organ. Grids are then placed at random on each slice, constituting a form of stratified random sampling. In the present investigation the sampling was more a stratified systematic sampling procedure due to the fact that each lung slice was very small and 90% (20 fields) of the slice was counted.

Once the representative tissue sections have been randomly picked, selection of fields is usually done systematically by placing the test area at equal intervals across the section. The next problem is calculating the optimal sample size and associated sampling error. The size of the standard error relative to the mean value of the count which estimates volume fraction (V_v) is;

$$RSE = \frac{\sqrt{1 - V_v}}{\sqrt{n}}$$

In this investigation, the maximum value for V_v alveolar air was 0.5841, therefore with a RSE of 5% or less,

$$.05 = \frac{\sqrt{1 - 0.5841}}{\sqrt{n}}$$ where n = the point count

so that n = 166. This is the number of points which must fall on alveolar air. Now, alveolar air constitutes 58.41% of lung, therefore, it follows that total number of points which must be superimposed on the lung slices is

166(100/58.41) = 285 points

In this investigation a range of 1600 to 3000 points were counted per lung for volume proportion of alveolar air which in theory would ensure a standard error less than 5%. The reason 20 fields were counted was to ensure a reasonable standard error for volume proportions of a lesser quantity, such as duct air (V_{vd} = .1724). Using the above calculations it was found that 1924 points should be counted per lung. In actuality a range of 600-900 points were counted per lung therefore the standard error should be closer to 8% for lesser volume proportions.

III. METHODOLOGY

3.1. Research Design

3.1.1. General

Lungs removed from each experimental and control animal were used for two types of experiments:

a) the left lung was processed to assess structural changes quantitatively by standard morphometric measurements (Weibel, 1979).

b) the right lung was processed to assess biochemical changes in protein and DNA content.

3.1.2. Animals

One hundred and ten male, newborn Hartley outbred albino guinea pigs (University of British Columbia Animal Care Facility) were randomly divided into seven groups; three exercise groups and four control groups (Table 1). The pups were born and housed with mothers at the Simon Fraser Animal Care Facility, Burnaby, British Columbia. The mothers (N=54) had been allowed to adjust to the environment from 2 to 4 weeks prior to delivery. All animals were allowed access to water and standard guinea pig chow, supplemented with alfalfa, ad libidum. Female pups were not studied.

Table 1. Outline of number of groups (4), treatments (exercised vs sedentary) and n's per group.

	Condition			
Treatment	Group 0 (0 week)	Group 1 (1 week)	Group 2 (2 week)	Group 3 (3 week)
Control	N=12	N=12	N=13	N=13
Exercise	N=0	N=20	N=20	N=20

3.1.3. Exercise protocol

Three exercise conditions were studied as outlined in Table 1. Group 1 was exercised daily for 1 week, group 2 daily for 2 weeks, and group 3 daily for 3 weeks. Group 0 was sacrificed within 12 hours of birth. All exercise groups and corresponding control groups were sacrificed 36 hours after the final exercise period. Experimental animals were exercised on a rodent treadmill (Quinton Instruments, Seattle, WA) at the Animal Care Facility, Simon Fraser University. Control animals were exposed to similar handling, were weighed daily and removed from the mother while experimental littermates were training on the treadmill. All experimental animals were initiated to treadmill running shortly after birth, followed by a 5 day learning period where the speed (meters per minute) and duration of exercise increased daily to a maxiumum speed of 25 meters per minute for 40 mintues at 0% grade. Group 2 reached a maximum exercise period of 60 minutes, while group 3 reached a maximum duration of 90 minutes (Appendix I).

3.2. Sacrifice and Lung Fixation

All animals were sacrificed by intraperitoneal injection of sodium pentobarbitone (.07 mg/gm body weight). The lungs, heart and trachea were exposed by a midsternotomy incision, an endotracheal plastic cannula was placed in the trachea and the lungs and heart were dissected from the thoracic cavity. The heart was dissected from the lungs, weighed and frozen in liquid nitrogen. All extraneous connective tissue was dissected from the lungs, then a ligature was tied around the right bronchus and the right lung was removed, weighed and frozen in liquid nitrogen. The left lung was attached to a 5 cc syringe filled with 10% phosphate buffered formalin, inflated by hand, then transfered to a formalin perfusion system for 72 hours where they were fixed at 25 cm H_2O transpulmonary pressure.

3.3. Estimation of Lung Volume

The left lung was removed from the perfusion system and the trachea clamped to prevent leakage of fixative. The lung was suspended from a laboratory stand and completely submerged in a beaker full of water, on a balance (Mettler 440) that had been previously tared to zero (Scherle, 1970). The increase in weight registered on the balance, by Archimedes Principle was equal to the volume of the lung thus:

> "a body partially or totally submerged in a body of fluid, experiences a buoyant force (F_B) equal to the weight of the fluid displaced by the volume of that body."

3.4. Lung Sampling and Processing

Six blocks of tissue approximately 1 cm^2, were taken from each left lung, three blocks from the upper lobe and three blocks from the lower lobe. The middle lobe was not sampled as it was too small. Each lobe was cut mid-saggitally and one block was taken from each mid-saggital surface. The third block was obtained by cutting the lateral half of the initial section saggitally, taking the sample from the lateral surface. All tissue blocks were trimmed and photographed. The tissue blocks were embedded in paraffin, sectioned in 5 micron-thick sections and stained with haematoxylin and eosin for light microscopy.

3.5. Estimation of Shrinkage

Tissue shrinkage due to fixation was not estimated as other investigators have shown this to be negligable. Fixed lung volume measurements were not significantly different from fresh lung volume measurements in rats (Kida and Thurlbeck 1980). Tissue shrinkage due to processing was estimated by comparing the total area of the lung section before embedding with total area after embedding. Fixed tissue blocks were photographed in embedding cassettes. An enlarged image of the negative was then projected onto a computer controlled digitizer and the area traced and recorded by planimetry. The area of each stained section was recorded similarly after embedding. Correction was made for magnification and the area and linear shrinkage factors were calculated;

$$\text{area shrinkage factor} = \frac{\text{area of tissue after embedding}}{\text{area of tissue before embedding}}$$

$$\text{linear shrinkage factor} = \sqrt{\frac{\text{area of tissue after embedding}}{\text{area of tissue before embedding}}}$$

3.6. Light Microscopy Morphometry

All slides were coded and measured morphometrically, without any knowledge on the part of the author, of the experimental group of animals from which the particular lung was derived (Weibel, 1969; Holmes and Thurlbeck, 1979; Kawakami, 1984). Each slide was placed on the automatic stage of a WILD M501 microscope and viewed through a square grid (108 mm x 108 mm), located on the screen of the microscope, using a 50x objective. Twenty random fields were then determined equidistantly on the section, using an imaginary 4 by 5 matrix (Appendix II). The square grid contained two diagonally placed cross test lines and 42 equidistant test points within its boundary.

Each test point was classified in one of five categories depending on which histological component it fell; i.e., alveolar air, duct air, alveolar wall, conducting airway and nonparenchyma. Alveoli were considered to be the smallest discrete air spaces encircled by alveolar wall, whereas the cylindrical core of air within alveolar ducts and sacs internal to the mouths of alveoli was referred to as alveolar duct air. Blood vessels, walls of conducting airways and connective tissue septa were grouped as nonparenchyma. The number of test points from 120 fields (20 fields x 6 slides) were summed for each of the tissue components and their volume proportion expressed as a proportion of the total number of points (42 x 120). No correction was made for tissue thickness, which

was presumed to be 5 microns.

The intercepts through alveolar wall structures (I_w) and intercepts between the mouths of alveoli and adjacent ducts (I_d) were also counted with the crossed test lines. An intersection through an alveolar wall was counted as 2 intercepts (2 gas exchanging surfaces) while an intersection that touched but did not pass through an alveolar wall was counted as 1 intercept (one gas exchanging surface). Similarly, 2 intercepts were counted for every intersection with alveolar duct walls (I_d) and 1 intercept if the test line touched but did not cross the wall. I_a represented the sum of $I_w + I_d$. Using the point counts and intercepts, calculations were performed as shown in Table 2 to obtain the morphometric values of mean linear intercept ($\bar{L}_m$), mean chord length of alveoli ($\bar{l}_a$), mean chord length of ducts ($\bar{l}_d$), alveolar surface area (S_w) and surface to volume ratio (S_{Vw}). Mean linear intercept was calculated by dividing the total length of the sum of both test lines by the total number of intercepts through alveolar walls. Total test line length was corrected for tissue shrinkage. The morphometric data were corrected for shrinkage by using the calculated shrinkage factors.

Since the absolute volume of alveolar air (V_a) is a product of total number of alveoli (N_a) and the mean volume of individual alveolus ($\bar{v}_a$), and $\bar{v}_a$ is related to the cube of the mean chord length of alveoli ($\bar{l}_a^3$) by a constant (j) which depends on the shape of the alveolus ($\bar{v}_a = j\bar{l}_a^3$), the ratio of $V_a:\bar{l}_a^3$ was estimated to represent N_{aT} assuming that the shape of the alveoli remained constant in the lungs (Kawakami, 1984; Kida et al., 1984, in press).

Table 2. Calculation of Morphometric Parameters of Lung Growth*

Parameters	Abbreviations	Calculations
Fixed lung volume	V_L	
Volume proportion of		
alveolar air	V_{Va}	
alveolar duct air	V_{Vd}	
alveolar wall	V_{Vw}	
conducting airways air	V_{Vb}	
nonparenchyma	V_{Vnp}	
Total volume of		
alveolar air	V_a	$V_L \times V_{Va}$
alveolar duct air	V_d	$V_L \times V_{Vd}$
alveolar wall	V_w	$V_L \times V_{Vw}$
conducting airways air	V_b	$V_L \times V_{Vb}$
nonparenchyma	V_{np}	$V_L \times V_{Vnp}$
Total projected length of test line	L_T**	
Mean linear intercept (µm)	$\bar{L_m}$	$2\ L_T/I_w$
Mean chord length of alveoli (µm)	$\bar{l_a}$	$2\ L_T \times V_{Va}/I_a$
Mean chord length of alveolar duct	$\bar{l_d}$	$2\ L_T \times V_{Vd}/I_d$
Alveolar surface area (cm^2)	S_w	$4\ V_L/L_m$
Sw per unit lung volume (cm^{-1})	S_{Vw}	$S_w/V_L \times V_{Va}$

* Further explanation of calculations in Appendix II 1.2
** Corrected for shrinkage

For I_a, I_w, and I_d see text. $I_a = I_w + I_d$

3.7. Extraction of Protein and DNA

The right lung was lyophilized in a Virtis Freezemobile 12 Freezer Dryer for 24 hours at -30^{0}C, followed by 24 hours at room temperature. This process was repeated until a constant dry weight was obtained (± .00020 gm). Paper thin sections of dried lung tissue were weighed to 5 decimal places on a Sartorius 2004 MP balance and transferred to pyrex homogenizers. Tissue was homogenized by hand for 5 minutes in 1 mL ice cold phosphate buffered saline (PBS). One mL aliquots of ice cold 30% trichloroacetic acid (TCA) was added to the homogenate, followed by centrifugation at 1000 x g for 4 minutes. The supernantant was discarded and the process repeated with 3 mL of 15% TCA. The resulting precipitate was incubated for 1 hour in 2 mL 37^{0}C 1 N NaOH. A .25 mL aliquot of the resulting solution was removed for non-connective tissue (NCT) protein estimation where NCT protein is the protein content of lung parenchyma excluding connective tissue elements such as collagen and elastin. The remainder was precipitated with 10% TCA to yield the acid insoluble DNA fraction for estimation of DNA content (Appendix III).

3.8. Estimation of Protein Content

NCT protein was measured by the method of Lowry et al., (1951) using bovine serum albumin (BSA)(Sigma A-4378) dissolved in 0.1 M NaOH as standard. The colour reagent (copper tartrate/sodium carbonate) was prepared accordingly, prior to assay. Two hundred and fifty µL aliquots of the protein samples in 1 N NaOH were diluted 1:1 with distilled water. The colour reagent was added to 100 µL of this solution and the reaction mixture allowed to sit at room temperature for 10 minutes. Five

hundred µL of 1 N Folin's reagent (Fisher Scientific Co. SO-P-24) was added to the mixture and the reaction was allowed to continue for a further 90 minutes. The colour which developed was read at 660 nm in a Pye Unicam SP6-550 Spectrophotometer. Protein content of the samples and protein/dry lung weight in mg/gm were then determined from a standard curve prepared from the absorbance readings of standard BSA processed similarly to the lung samples. The standard curve was linear in the range 10 to 250 µg protein, and the absorbance readings of the lung samples which reflected NCT protein content were all within the linear range (Appendix III). All samples and standards were duplicated.

3.9. Estimation of DNA Content

Deoxyribonucleic acid was measured according to the method of Burton (1956) using calf thymus (Sigma D-1501) as standard. The standard DNA was processed identically to the DNA extracts from lung samples. One mL aliquots of the 8 mL total DNA extract from the lung was added to 2 mL of diphenylamine reagent (J.T. Baker Chemical Co.) and boiled for 10 minutes. The resultant reaction mixture was allowed to cool on ice and absorbance was measured at 600 nm on a Pye Unicam SP 550 spectrophotometer. The DNA content of a lung sample and DNA/dry lung weight in mg/gm were estimated from a curve prepared from the absorbance of standard DNA. The standard curve was linear in the range of 20 µg to 200 µg DNA and the absorbance readings of each lung sample was within the linear range (Appendix III). All samples and standards were duplicated.

3.10. Estimation of Succinate dehydrogenase enzyme activity (SDH)

Succinate dehydrogenase activity was measured according to the methods of Cooperstein et al., (1951) in excised gastrocnemius muscle. The muscle was removed within one minute of sacrifice and frozen in liquid nitrogen, then stored at -70°C until assayed. Approximately one hundred mg of frozen muscle tissue was weighed and placed in a glass homogenizer with 4 mL of ice cold .033 M phosphate buffer. The muscle was homogenized mechanically (Canlab, stirrer type, teflon pestle) for 2 minutes at 3000 rpm on ice. Sixty µ L of tissue homogenate was added to the assay cocktail (0.1 mL sodium succinate and 0.2 mL sodium cyanide in 0.17M phosphate buffer and 2.8 mL cytochrome C salt solution (Sigma Horseheart type III C-2506). The solution was mixed thoroughly for 1 minute and placed in a Pye Unicam SP6-550 spectrophotometer. Extinction of succinate was recorded at 30 second intervals for 6 minutes at 550 nm. The activity of SDH was calculated from the average extinction/minute (Δ absorbance) for each sample (Appendix IV). All samples were duplicated.

3.11. Statistical Analysis

Groups means ± the standard errors of the mean (SEM) were calculated for all variables. A two way analyses of variance (SPSS MANOVA) was performed for each variable between conditions (groups 0, 1, 2 and 3) and treatments (exercise, control). When the F ratio indicated significance at the 5% significance level, one way analysis of variance tests (SPSS ANOVA) were conducted for the specific variable: 1) condition by treatment and 2) treatment by condition. This statistical procedure was followed by Post Hoc Student Neuman Keuls (SNK) multiple range test in order to locate the significant differences at the 5% significance level. Missing data due to animal loss as well as technical problems were corrected for in the statistical analysis. Three animals were severely underweight at birth (< 58 gm) and died within a few days. From the remaining 107 animals, 3 lungs were poorly inflated and not included in the lung volume measurements. Finally, more than 50% of the muscle samples for measurement of SDH activity were thawed in a power failure of the refrigerator where they were stored, therefore omitted from the study.

IV. RESULTS

4.1 General Characteristics

Body weight, heart weight, crown to rump length and femur length are shown in Table 3. All values are means ± SEM's for the variable at time of sacrifice. Values are shown for both exercised and control (sedentary or non-exercised) animals. Significant differences between group means are at $p < .05$ levels. The average litter size for sows that did not abort was 3.0, with a higher percentage of males (62%). Stillbirths were a common occurrence in the larger litters (> 3). Gestation time was observed to increase with increasing litter size and was variable between 59 and 75 days, with an average of 68 days. The range of birth weights in this study was from 56 gm to 143 gm, with an average of 96 gm.

4.1.1 Body Weight

The mean body weight of 0 week guinea pigs a few hours (4 to 12) after birth was 111.05 ± 4.80 grams. Control (non-exercised) weights increased 23% after 1 week, whereas exercised animals only increased by 7%. This difference was not significant. There was no difference due to age between 0 week and 1 week old animals in either control or exercise groups. From week 1 to week 2, control animal body weights increased from 137.17 ± 6.49 grams to 210.97 ± 12.69 grams, representing a significant 54% increase due to growth. The mean weight of exercised

animals at the same age, increased from 118.62 ± 5.44 grams to 172.82 ± 8.16 grams, a significant 46% increase due to growth. By 2 weeks after birth, the body weights of exercised animals were significantly lower than those of non-exercised animals. At 3 weeks of age, both exercised and non-exercised animals attained similar body weights of 251.39 ± 11.30 grams and 260.86 ± 13.85 grams respectively. This represented a 46% increase from 2 weeks to 3 weeks for the exercised group and a 24% increase for the control group. The group mean body weight of exercised and non-exercised animals by the 3rd week was significantly different from the corresponding groups at the end of week 2. Figure 4 represents the increase in body weight with increase in age in both groups.

4.1.2 Heart Weight

No significant differences were observed in mean heart weight or mean heart weight to body weight ratios between exercised and control groups within any age group. The mean heart weights for week 1 controls (.53 ± .03 grams) and week 2 controls (.73 ± .04 grams) were greater than those for week 1 exercised (.45 ± .02 grams) and week 2 exercised (.65 ± .04 grams). This was probably a reflection of the lower mean body weights in the exercised groups, as heart weight to body weight ratios were not significantly different between treatment groups. After 3 weeks, mean heart weights of experimental and control groups were almost identical, (1.07 ± .04 grams and 1.06 ± .06 grams respectively) as were the mean heart weight to body weight ratios, (.0043 ± .0001 and .0041 ± .0001 respectively). There was no difference due to growth between 0

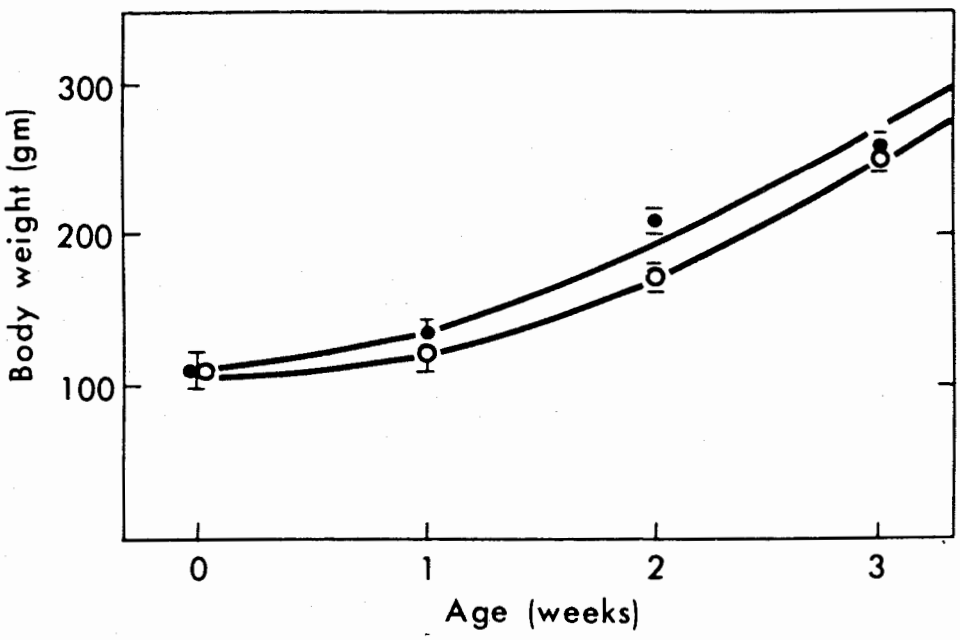


Figure 4. Mean body weight for non-exercised groups (●) and exercised groups (O) at week 0, week 1, week 2 and week 3.

week and 1 week animals in either parameter. There was a difference in growth of the heart between all 1 week and 2 week animals, and again between all 2 week and 3 week animals. The heart increased in weight by 45% in controls and 66% in exercised animals from week 2 to week 3. The heart weight to body weight ratio decreased from 0 weeks to 2 weeks in both treatment groups, then increased again at 3 weeks. This probably reflects the increased growth rate of the heart relative to the increase in body weight at this age.

4.1.3 Anthropometric Variables

Crown to rump (CR) lengths were measured for all animals except in 3 week groups. There was no significant effect on CR length due to exercise at any age tested. CR length increased predictably from 161±2 mm at birth to 190±5 mm at 2 weeks in controls and from 161±2 mm to 181±3 mm in experimentals. These represent an 18% and 12% increase respectively. Significant increases in CR length were observed due to growth between 1 week and 2 week animals, but not between 0 week and 1 week animals.

Femur length was measured as another index of whole body growth in all groups. Femur length increased in control animals from 2.25 ± .05 cm at birth to 3.20 ± .03 cm at 3 weeks, representing a 42% increase in 3 weeks. Exercise group femur lengths increased from 2.33 ± 0.04 cm to 3.15 ± .04 cm, a 35% increase in the same time period. Mean femur lengths were significantly different between each age group in both control and experimental animals. There was a significant difference

between control and exercised animals mean femur length at week 2, which were 2.74 $\pm$ .06 cm and 2.52 $\pm$ .05 cm respectively. These represent a 25% and a 17% increase respectively, over week 1 values, indicating perhaps a slower growth rate in exercised animals at this age.

Table 3. Group Means for Body Weight, Heart Weight, Crown to Rump Length and Femur Length in Control and Exercise Groups.

CONTROL GROUPS

AGE (wk)	N	W_b (gm)	W_h (gm) (wet)	W_h/W_b	CR (mm)	F (cm)
0	12	111.05^a ± 13.20	$.46^a$ $\pm .01$	$.0044^b$ $\pm .0003$	161^a ± 2	2.25^a $\pm .05$
1	12	137.17^a ± 6.49	$.53^a$ $\pm .03$	$.0039^b$ $\pm .0001$	173^a ± 3	2.45^b $\pm .04$
2	13	210.97^b ± 12.69	$.73^b$ $\pm .04$	$.0035^a$ $\pm .0001$	190^b ± 5	2.74^c $\pm .03$
3	13	260.86^c ± 13.85	1.06^c $\pm .06$	$.0041^c$ $\pm .0001$	-	3.20^d $\pm .03$

EXERCISE GROUPS

1	18	118.62^a ± 5.44	$.45^a$ $\pm .02$	$.0039^b$ $\pm .0001$	164^a ± 3	2.33^a $\pm .04$
2	19	172.82^{b*} ± 8.16	$.65^b$ $\pm .04$	$.0037^a$ $\pm .0001$	181^b ± 3	2.52^{b*} $\pm .05$
3	20	251.39^c ± 11.30	1.07^c $\pm .04$	$.0043^c$ $\pm .0001$	-	3.15^d $\pm .04$

Values are means $\pm$ SEM

* - means different due to treatment $p < .05$

a,b,c,d superscripts in a vertical column indicate means are different due to age at $p < .05$

abbreviations
W_b - body weight (gm)
W_h - heart weight (gm)
W_h/W_b - heart weight/body weight
CR - crown rump (mm)
F - femur (cm)

4.2 Lung Weights

Mean lung weights and lung weight to body weight ratios (W_L/W_b) $\pm$ SEM's are shown for all groups in Table 4 and illustrated in Figure 5. Predictably, both left lung weight (LW_L) and right lung weight (RW_L) increase with age. LW_L increased by 31% whereas RW_L increased by approximately 34% in both treatment groups. A RW_L/LW_L ratio of 1.21 $\pm$ .01 was maintained throughout the 3 weeks of exercise and growth. Significant growth occured between week 1 and week 2 controls and between week 2 and week 3 experimentals. A significant treatment effect was demonstrated in week 2 animals for both LW_L and RW_L. However, when expressed as a ratio over body weight, these differences did not persist. The LW_L/W_b ratio and the RW_L/W_b ratio both decrease significantly at each age level measured, reflecting the slower increase in lung weight relative to body weight during the first 3 weeks of life in guinea pigs. Lung weight was analyzed as linear, exponential and power functions variant with body weight. These equations are as follows:

	Non-exercised	r^2	r
Linear	$W_L=.0032W_b+.895$	.8909	.9439
Exponential	$W_L=.981e^{.0022W_b}$	.8945	.9458
Power	$W_L=.196W_b^{.39}$	.9238	.9611
	Exercised	r^2	r
Linear	$W_L=.0032W_b+.804$	.8966	.9469
Exponential	$W_L=.905e^{.0023W_b}$	.8924	.9447
Power	$W_L=.192W_b^{.38}$	.8370	.9149

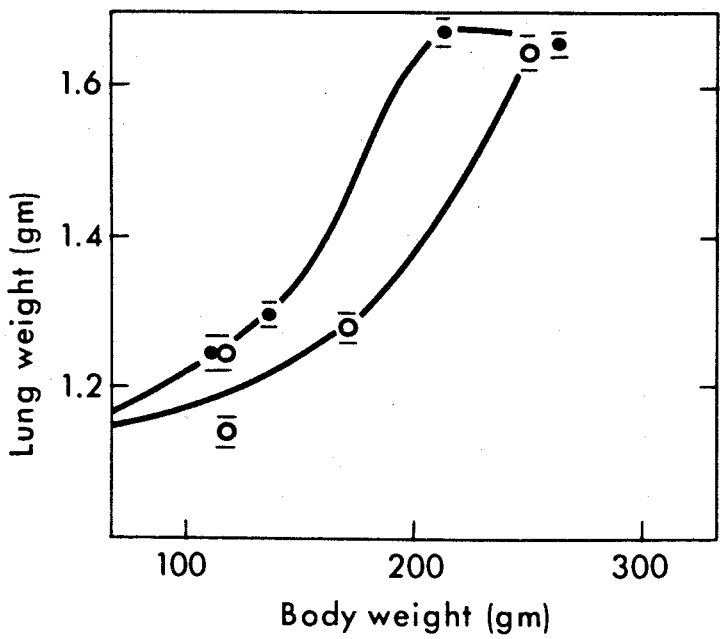


Figure 5. Mean lung weight as a function of mean body weight in non-exercised (●) and exercised (O) groups.

Table 4. Mean Lung Weights of Control and Exercise Groups Throughout the 1st 3 Weeks of Life.

AGE (wk)	N	LW_L (gm) (wet)	RW_L (gm) (wet)	W_L (gm) (wet)	LW_L/W_b	RW_L/W_b	W_L/W_b
CONTROL GROUPS							
0	12	$.57^a \pm .02$	$.68^a \pm .04$	$1.25^a \pm .06$	$.0054^a \pm .0002$	$.0063^a \pm .0002$	$.0112^a \pm .0010$
1	12	$.58^a \pm .03$	$.71^a \pm .03$	$1.29^a \pm .06$	$.0042^b \pm .0001$	$.0052^b \pm .0001$	$.0094^b \pm .0009$
2	13	$.75^b \pm .04$	$.93^b \pm .05$	$1.68^b \pm .09$	$.0036^c \pm .0001$	$.0044^c \pm .0001$	$.0079^c \pm .0009$
3	13	$.75^b \pm .02$	$.91^b \pm .03$	$1.66^b \pm .06$	$.0030^d \pm .0002$	$.0035^d \pm .0001$	$.0064^d \pm .0006$
EXERCISE GROUPS							
1	18	$.52^a \pm .02$	$.62^a \pm .03$	$1.14^a \pm .05$	$.0044^b \pm .0001$	$.0053^b \pm .0001$	$.0096^b \pm .0009$
2	19	$.58^{a*} \pm .02$	$.71^{a*} \pm .03$	$1.29^{b*} \pm .05$	$.0034^c \pm .0001$	$.0041^c \pm .0001$	$.0075^c \pm .0006$
3	17	$.75^b \pm .03$	$.90^b \pm .03$	$1.65^c \pm .06$	$.0031^d \pm .0001$	$.0037^d \pm .0001$	$.0066^d \pm .0005$

Values are means $\pm$ SEM

* - means are different due to treatment $p < .05$

a,b,c,d - superscripts in a vertical column indicate means are different due to age $p < .05$

abbreviations - LW_L - left lung weight (gm)
- RW_L - right lung weight (gm)
- LW_L/W_b - left lung weight/body weight ratio
- RW_L/W_b - right lung weight/body weight ratio

4.3. Lung Volumes

The volume of the left lung was measured directly by the method of water displacement (Scherle, 1970). The RW_L/LW_L ratio and the LV_L was extrapolated to give the volume of the whole lung as shown at the bottom of Table 5. There were no significant differences in lung volumes, due to the experimental treatment. The LV_L increased by 45% in control and 53% in experimental groups after 3 weeks. This was paralleled by a 47% increase in control group V_L and 55% increase in experimental group V_L, respectively. Significant growth occurred between week 1 and week 2 controls and week 2 and week 3 experimentals in absolute lung volume. This significance did not persist in experimental groups when lung volume was expressed as a fraction of body weight as illustrated in Figure 6. The LV_L/W_b fraction was reduced by 39% in controls and 33% in experimentals, while the V_L/W_b fraction was reduced by 43% in control groups and 32% in experimental groups. Significant differences occurred in LV_L/W_b fractions between week 0 and week 1 and again between week 1 and week 2 for both treatment groups. Lung volume (V_L) was analyzed as linear, exponential and power, functions variant with body weight. These equations are as follows;

	Non-Exercised	r	(r^2)	Exercised	r	(r^2)
Linear	$V_L=.019W_b+3.54$	.9945	.9890	$V_L=.023W_b+2.76$	.9512	.9048
Exponential	$V_L=4.21e^{.0027Wb}$	.9923	.9847	$V_L=3.75e^{.0033Wb}$	.9592	.9201
Power	$V_L=.58W_b^{.48}$	.9928	.9857	$V_L=.42W_b^{.54}$	.9240	.8538

Table 5. Mean Lung Volumes and Mean Relative Lung Volmes for Control and Exercise Groups.

AGE (wk)	N	LV_L (mL)	W_b (gm)	LV_L/W_b (mL/gm)	V_L** (mL)	V_L/W_b** (mL/gm)
CONTROL GROUPS						
0	12	2.62^a ±.19	111.05^a ± 4.80	$.0244^a$ ±.0013	5.73^a ±.96	$.0516^a$ ±.1090
1	12	2.70^a ±.20	137.17^a ± 6.49	$.0198^b$ ±.0013	6.02^a ±1.02	$.0439^b$ ±.0095
2	13	3.45^b ±.34	210.97^b ± 12.69	$.0161^c$ ±.0009	7.73 ±1.63	$.0367^c$ ±.0100
3	13	3.81^b ±.18	260.86^c ± 13.85	$.0149^c$ ±.0008	8.44^b ±.98	$.0296^d$ ±.0050
EXERCISE GROUPS						
1	18	2.49^a ±.17	118.62^a ± 5.44	$.0213^b$ ±.0013	5.45^a ±.87	$.0460^b$ ±.0095
2	19	2.74^a ±.14	172.82^{*b} ± 8.16	$.0163^c$ ±.0009	6.07^a ±.78	$.0351^c$ ±.0062
3	17	4.01^b ±.17	251.39^c ±11.30	$.0163^c$ ±.0008	8.85^b ±.98	$.0352^c$ ±.0055

Values are means ± SEM.

a,b,c,d superscripts in a vertical column indicate - means are different due to age $p < .05$.

* Means different due to treatment

** - V_L and V_L/W_b are extrapolated values and were not measured directly.

where $V_L = LV_L(1 + RW_L/LW_L)$

abbreviations: LV_L - left lung volume
LV_L/W_b - left lung volume/body weight
V_L - lung volume
V_L/W_b - lung volume/body weight

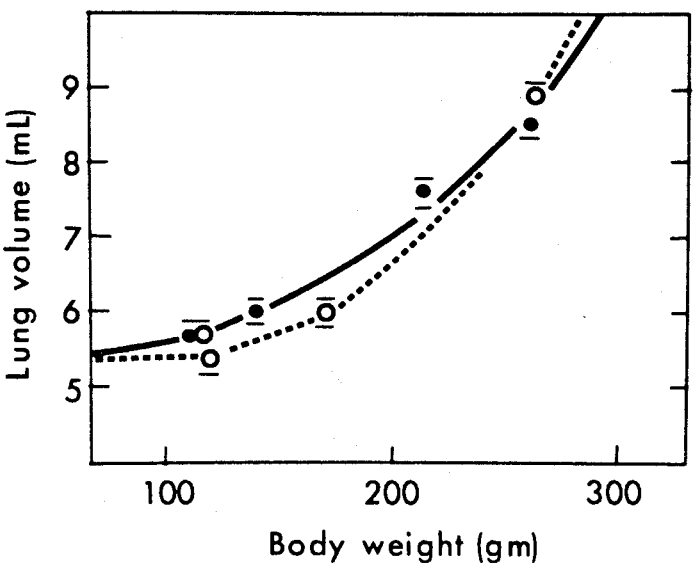


Figure 6. Mean lung volume as a function of mean body weight in non-exercised (●) and exercised (O) groups.

4.4 Quantitative Morphometry

The quantitative morphometric data obtained by point and intercept counting of tissue sections are shown in Tables 6-9. A representative subgroup of 6 cases was randomly selected from each group, with the exception of the 0 week control group (n=12), for the morphometric study.

4.4.1 Volume Proportions of Tissue Components

Table 6a shows the volume proportion of alveolar air (V_{Va}), duct air (V_{Va}) bronchial air (V_{Vb}) alveolar wall (V_{Vw}) and nonparenchyma (V_{Vnp}) in the lung sections analyzed. The volume proportions of tissue components shown in Table 6a, are used to calculate the total volume of tissue components in the lung when multiplied by the mean lung volume for that particular group as shown in Table 6b. Refer to Table 2 for the calculation. Nonparenchyma consists of non-respiratory tissue such as larger blood vessels, bronchiole walls and connective tissue fibres. There were no significant differences due to either treatment or growth in the volume proportions of tissue components. These proportions were maintained throughout the 3 week period. The volume proportion of bronchial air in the 1 week experimental animals was 57% greater than the mean value for the 3 age groups, although this was not significantly different at $p > .05$.

There were no significant differences due to exercise in the absolute values for all tissue components shown on Table 6b. There were significant differences due to growth in total volume of alveolar air

(V_a) duct air (V_d) and alveolar wall (V_w) in exercise groups and total volume of alveolar air (V_a) in control groups. V_a increased by 50% and 61% while V_d increased by 56% and 72% in control and exercise groups respectively during the 3 week period. V_w increased by 37% in control groups and 44% in exercise groups, the latter being a significant increase over the 3 week period. V_b increased, though not significantly by 7% in control groups and 37% in exercise groups. The high V_b value for group 1 exercise animals was due to an unusually large number of bronchial fields counted in one particular case, which has skewed the distribution. V_{np} increased by 26% and 20% in control and exercise groups respectively. This was not significant.

Table 6a. Mean Volume Fraction of Tissue Components Measured Morphometrically Per Group. In a Group of 6 Cases with 6 Slides/Case, This Represents the Mean Volume Fractions for 36 Sections.

CONTROL GROUPS

AGE (wk)	N	V_{Va}	V_{Vd}	V_{Vw}	V_{Vb}	V_{Vnp}
0	12	.563 ±.010	.152 ±.008	.170 ±.007	.015 ±.002	.100 ±.007
1	6	.562 ±.008	.161 ±.006	.165 ±.008	.017 ±.005	.089 ±.011
2	6	.573 ±.006	.169 ±.006	.161 ±.006	.013 ±.005	.068 ±.013
3	6	.583 ±.015	.166 ±.011	.157 ±.007	.011 ±.003	.084 ±.010
EXERCISE GROUPS						
1	6	.566 ±.012	.154 ±.001	.158 ±.008	.027 ±.006	.096 ±.011
2	6	.584 ±.015	.167 ±.015	.161 ±.010	.012 ±.004	.077 ±.005
3	6	.584 ±.013	.172 ±.015	.156 ±.005	.014 ±.002	.074 ±.011

Values are means ± SEM

abbreviations - refer to Table 2.

Table 6b. Mean total volume of lung components/lung of control and exercise groups in cm^3.

Age (wk)	N	V_a	V_d	V_w	V_b	V_{np}
CONTROL GROUPS						
0	12	$1.49^a \pm .12$	$.41 \pm .04$	$.44 \pm .03$	$.04 \pm .01$	$.25 \pm .02$
1	6	$1.50^a \pm .14$	$.43 \pm .04$	$.44 \pm .03$	$.05 \pm .01$	$.24 \pm .03$
2	6	$1.90 \pm .26$	$.58 \pm .10$	$.54 \pm .09$	$.05 \pm .02$	$.23 \pm .05$
3	6	$2.23^b \pm .16$	$.63 \pm .07$	$.60 \pm .04$	$.04 \pm .01$	$.32 \pm .04$
EXERCISE GROUPS						
1	6	$1.45^a \pm .12$	$.40^a \pm .04$	$.40^a \pm .03$	$.08 \pm .02$	$.26 \pm .05$
2	6	$1.63^a \pm .10$	$.49 \pm .08$	$.45 \pm .03$	$.04 \pm .01$	$.22 \pm .02$
3	6	$2.36^b \pm .09$	$.70^b \pm .07$	$.63^b \pm .01$	$.06 \pm .01$	$.30 \pm .05$

Values are means ± SEM
Abbreviations - refer to Table 2.
a, b, c, d superscripts in a vertical column indicate means are different due to age $p < .05$.

4.4.2 Mean Linear Intercept and Mean Chord Lengths

The quantitative morphometric parameters of mean linear intercept ($\bar{L}_m$), mean chord length of alveoli ($\bar{l}_a$) and mean chord length of ducts ($\bar{l}_d$) are shown in Table 7, together with the mean lung volume for the groups studied. The mean lung volumes for the smaller morphometry groups were almost identical to the values reported in Table 5 for the whole population. Interestingly, a significant difference due to treatment was shown in the 2 week group, although in the larger population there was no difference at the $p > .05$ level. The effect of growth on lung volume was significant between week 1 and week 3 in control groups of animals.

The mean linear intercept ($\bar{L}_m$), a morphometric estimate of the mean size of peripheral air spaces at maximum inflation was not significantly affected by exercise or growth. There was however a general increase in control values of 11% and in experimental values of 12%. The size of the alveoli, as assessed by the mean chord length ($\bar{l}_a$) was not significantly affected by exercise, but the week 3 control mean was significantly different from the week 1 control mean, representing a 19% increase due to age. No parallel increases due to age were observed in the experimental groups, where the increase was only 7.5% in 3 weeks. The size of alveolar ducts as assessed by mean chord length remained fairly constant in both control and experimental groups over the 3 week period, with no differences between age groups. With the exception of 1 week controls, experimental values were marginally lower than control values for this variable.

Table 7. Mean Interalveolar Wall Distance ($\bar{L}_m$), Mean Chord Length of Alveoli and Mean Chord Length of Ducts in Control (non-exercise) and Exercise Groups.

AGE (wk)	N	LV_L (mL)	$\bar{L}_m$ (μm)	$\bar{l}_a$ (μm)	$\bar{l}_d$ (μm)
CONTROL GROUPS					
0	12	2.62[a] ±.19	73.2 ±1.1	34.8 ± .6	60.5 ±2.6
1	6	2.66[a] ±.22	73.1 ±1.4	33.8[a] ± .8	55.6 ±3.3
2	6	3.33 ±.48	79.4 ±1.7	37.2 ±1.0	61.0 ±4.7
3	6	3.82[b] ±.24	81.5 ± .6	40.2[b] ± .4	59.8 ±4.5
EXERCISE GROUPS					
1	6	2.58[a] ±.24	76.4 ±3.9	35.6 ± .3	58.0 ±5.4
2	6	2.82[a]* ±.23	74.5 ±3.3	35.5 ± .6	56.8 ±4.9
3	6	4.04[b] ±.14	81.8 ±2.1	38.2 ± .7	58.0 ±6.1

Values are means ± SEM.

* - means different due to treatment

a,b superscripts in a vertical column indicate - means different due to age

abbreviations - see Table 2.

4.4.3 Surface Area Measurement

Lung surface area in meters^2 (S_W), surface area to body weight fraction (S_W/W_b), and surface area to lung volume fraction (S_W/V_L) are shown in Table 8. No differences due to exercise were observed in any of these parameters. Surface area increased by 30% in control groups and 37% in experimental groups in 3 weeks. There was a significant increase between week 1 and week 3 experimental groups. Surface area to body weight ratio decreased by 46% in control groups and 39% in experimental groups. A significant reduction in S_W/W_b was observed between week 0 and week 1 control groups, and again between week 1 and week 2 control groups, due to increasing body weight relative to surface area. Once the exercise group started to exercise, S_W/W_b fraction remained fairly constant, with only a 20% reduction in comparison to a 36% reduction in control groups over the same time period. Figure 7 illustrates the relationship between lung surface area and body weight. Surface area to left lung volume decreased by 13% in control groups with the most significant difference between week 0 and week 3, and 10% in experimentals with no significant differences between age groups. Surface area to whole lung volume was calculated from the extrapolated V_L defined at the bottom of Table 5. A reduction of approximately 11% was observed in both experimental and control groups during the 3 week period compared to week 2 values, within the range of that calculated for S_W/LV_L above. Figure 8 illustrates the relationship between lung surface area and lung volume. Surface area was analyzed as linear, exponential and power functions variant with lung volume (V_L) and body weight (W_b). These equations are as follows;

S_w vs. V_L

	Non-Exercised	r	r^2	Exercised	r	r^2
Linear	$S_w=.015V_L+.054$	.9812	.9628	$S_w=.018V_L+.040$	.9963	.9926
Exponential	$S_w=.083e^{.094V_L}$	.9870	.9742	$S_w=.077e^{.107V_L}$	.9919	.9839
Power	$S_w=.046V_L^{.65}$	.9809	.9622	$S_w=.037V_L^{.77}$	.9957	.9914
S_w vs W_b						
Linear	$S_w=.0003W_b+.108$	.9880	.9761	$S_w=.0004W_b+.089$	.9530	.9082
Exponential	$S_w=.116e^{.002W_b}$	.9917	.9835	$S_w=.103e^{.003W_b}$	.9545	.9111
Power	$S_w=.032W_b^{.312}$	.9742	.9491	$S_w=.019W_b^{.415}$	.9236	.8530

Table 8. Mean Surface Area of the Gas Exchanging Components in the Lung Expressed in Absolute Terms, Relative to Body Weight and Relative to Lung Volume for Both Control and Exercise Groups.

Age (wk)	N	S_w (m^2)	S_w/W_b (m^2/mL)	S_{Vw} m^2/mL)
CONTROL GROUPS				
0	12	.1438 ±.0112	.0013[a] ±.0001	.0976[a] ±.0020
1	6	.1460 ±.0121	.0011[b] ±.0001	.0976 ±.0025
2	6	.1674 ±.0232	.0008[c] ±.0001	.0882 ±.0021
3	6	.1875 ±.0104	.0007[c] ±.0000	.0847[b] ±.0026
EXERCISE GROUPS				
1	6	.1340[a] ±.0068	.0010 ±.000	.0937 ±.0034
2	6	.1503 ±.0071	.0008 ±.0000	.0927 ±.0025
3	6	.1974[b] ±.0044	.0008 ±0000	.0841 ±.0022

Values are means ± SEM

a,b,c,d superscripts in a vertical column indicate - means are different due to age $p < .05$

abbreviations - see Table 2

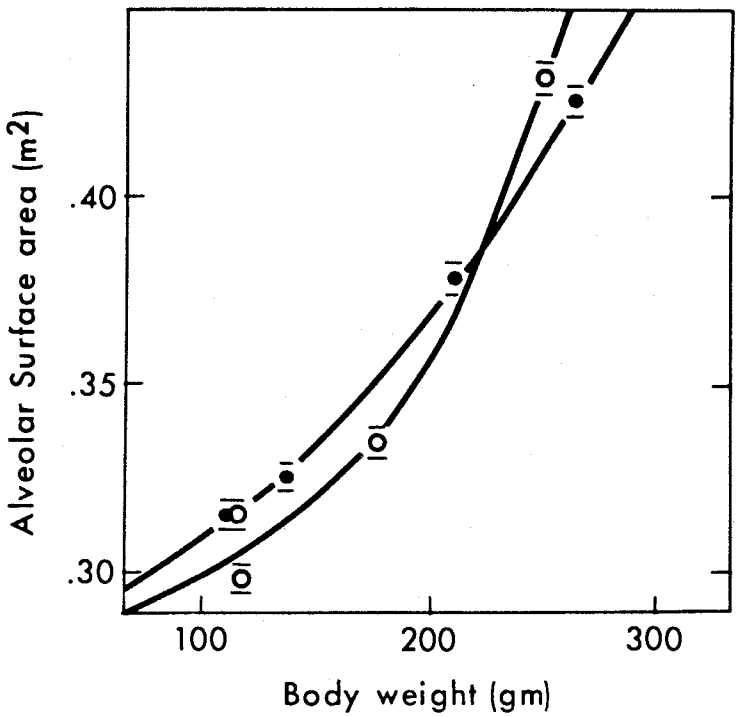


Figure 7. Mean alveolar surface area as a function of mean body weight in non-exercised (●) and exercised (O) groups.

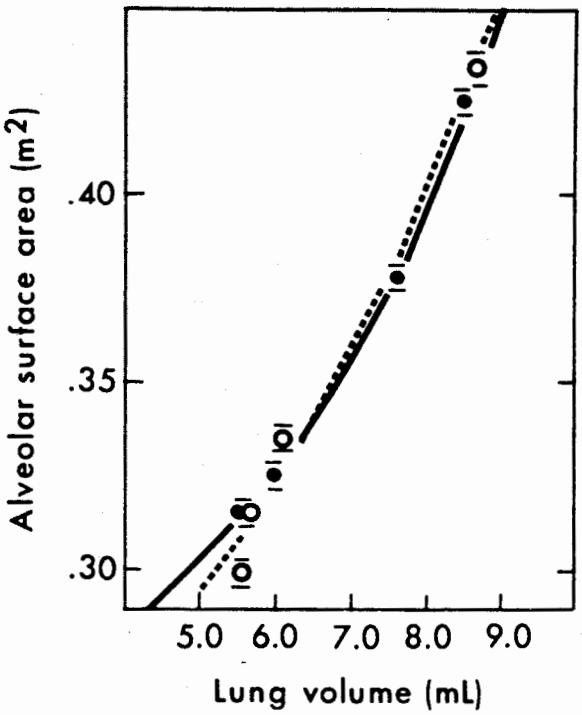


Figure 8. Mean alveolar surface area as a function of mean lung volume in non-exercised (●) and exercised (O) groups.

4.4.4 Total Alveolar Number

Table 9 shows the mean ratio of total volume to cubed mean chord length for alveoli ($V_a/\bar{l}_a^3$) in all groups. This ratio is an index of total alveolar number (refer to section 3.6). There were no significant differences due to exercise or growth in this parameter. The alveolar number index increased by 5.5% and 19% in control and exercise groups respectively, with an average of 81.6 million for all groups.

Table 9. Mean ratio of total volume to cubed mean chord length for alveoli and ducts in control and exercise groups.

Age (wk)	N	$V_a/\bar{l}_a^3 \times 10^6$
CONTROL GROUPS		
0	12	77.4 ±6.4
1	6	86.0 ±5.9
2	6	84.0 ±11.5
3	6	81.7 ±9.8
EXERCISE GROUPS		
1	6	72.3 ±5.3
2	6	81.3 ±3.5
3	6	92.4 ±3.3

Values are means ± SEM
Abbreviations - refer to Table 2, explanation - see text
a,b,c,d, superscripts in a vertical column indicate means are different due to age $p < .05$

4.5 Biochemistry

Freeze dried samples of right lung were homogenized and assayed for protein and DNA content. Table 10 shows the mean dry weight of right lung (DW_L) for each group, together with the dry weight/body weight ratio (DW_L/W_b) and wet lung weight/dry lung weight ratio (RW_L/DW_b). Table 11 and 12 show the mean protein content (mg) and DNA content (mg) per right lung for each group, along with the protein/DNA ratio, protein/body weight ratio and DNA/body weight ratio. Succinate dehydrogenase enzyme activity was also measured in gastrocnemius muscle samples, as an index of oxidative capacity. These results are reported in Table 13.

4.5.1 Dry Lung Weight

The mean dry lung weight was significantly affected by exercise in the 2 week group, being 22% lower than the mean control group value at the same age. Mean dry lung weight increased overall by 30% in control groups and 26% in experimental groups, reaching similar values at the end of 3 weeks, suggesting only a transient exercise effect. The increase roughly paralleled a 34% increase in RW_L noted in section 4.2. When expressed as a proportion of body weight, there were no significant changes due to exercise at any age. The DW_L/W_b proportion declined steadily in both treatment groups by 46%, paralleling the 43% decline in RW_L/W_b proportion reported in section 4.2. The RW_L/DW_L or wet/dry fraction was not affected by exercise and increased marginally through the 3 weeks (3.4% and 6.6% in control and experimental groups respectively). There was a small difference due to age between week 1 and week 3 exercise groups.

Table 10. Mean Dry Lung Weights Expressed in Absolute Terms, Relative to Body Weight and as a Wet Weight to Dry Weight Fraction for Control and Exercise Groups.

AGE (wk)	N	DW_L (gm)	DW_L/W_b	RW_L/DW_L
CONTROL GROUPS				
0	12	.1363[a] ±.0249	.0013 ±.0000	4.96 ±.04
1	12	.1422[a] ±.0066	.0010 ±.0000	5.01 ±.04
2	13	.1783[b] ±.0097	.0009 ±.0000	5.20 ±.03
3	13	.1777[b] ±.0068	.0007 ±.0000	5.14 ±.06
EXERCISE GROUPS				
1	18	.1263[a] ±.0055	.0011 ±.0000	4.91[a] ±.03
2	19	.1384*[a] ±.0054	.0008 ±.0000	5.12 ±.02
3	20	.1716[b] ±.0064	.0007 ±.0000	5.28[b] ±.05

Values are means ± SEM
5* - means are different due to treatment $p < .05$

a,b - superscripts in a vertical column indicate - means are different due to age at $p < .05$

abbreviations - see text.

4.5.2 Protein and DNA Content

No significant effect of exercise on either protein or DNA content in lung were measured (Table 11). Protein content increased by 43% in control and 32% in experimental groups respectively. This increase was significant between week 0 and week 3 control groups only, at $p > .05$. DNA content did increase with growth by 9% in control groups but only marginally in experimental groups (0.4%). These increases were not significant. The significant difference in week 0 and week 3 control protein content was reflected, predictably, in the protein/DNA fraction. This fraction increased by 32% in control and 28% in experimental groups respectively.

Protein and DNA content is expressed relative to body weight in Table 12. Again, there was no significant exercise effect. The protein/body weight ratio decreased significantly between week 1 and week 2 in both experimental and control groups. There was an overall reduction of 39% in controls and 42% in experimentals. DNA/body weight fractions also decreased significantly by 54% and 55% respectively between week 1 and week 2 for experimental and control groups.

Table 11. Mean NCT Protein content and mean DNA Content per dry weight and per gm dry weight and protein/DNA ratio in control and exercise groups.

AGE (wk)	N	PROTEIN (mg)	PROTEIN mg/gm	DNA (mg)	DNA mg/gm	PROTEIN/DNA
CONTROL GROUPS						
0	12	43.41[a] ±3.14	319.2[a] ±81.7	3.88 ±.34	28.5 ±7.7	11.52[a] ± .40
1	11	52.64 ±3.41	370.7[a] ±42.3	4.36 ±.28	30.7 ±3.5	12.10 ± .28
2	10	50.81 ±3.61	285.4[b] ±33.3	3.84 ±.33	21.6 ±3.1	13.45 ± .49
3	10	62.001[b] ±3.50	348.3[a] ±33.4	4.23 ±.24	23.8 ±2.3	14.99[b] ± .59
EXERCISE GROUPS						
1	10	46.55 ±2.71	369.4 ±39.1	3.59 ±.25	28.5 ±3.3	13.25 ± .70
2	10	49.35 ±2.98	357.6* ±30.5	3.64 ±.22	26.4 ±2.6	13.49 ± .57
3	14	57.16 ±4.37	332.2 ±37.0	3.90 ±.26	22.7 ±2.3	14.74 ± .63

Values are means ± SEM

a,b, superscripts in a vertical column indicate - means different due to age $p < .05$

Table 12. Mean NCT Protein Content and Mean DNA Content per Lung Dry Weight Expressed Relative to Body Weight for Control and Exercise Groups.

AGE (wk)	N	PROTEIN/W_b (mg/gm)	DNA/W_b (mg/gm)
CONTROL GROUPS			
0	12	$.390^a \pm .025$	$.035^a \pm .003$
1	11	$.377^a \pm .013$	$.032^a \pm .001$
2	10	$.251^b \pm .013$	$.019^b \pm .001$
3	10	$.238^b \pm .013$	$.016^b \pm .001$
EXERCISE GROUPS			
1	10	$.390^a \pm .021$	$.030^a \pm .002$
2	10	$.262^b \pm .011$	$.020^b \pm .001$
3	14	$.226^b \pm .009$	$.016^b \pm .001$

Values are means $\pm$ SEM

a,b superscripts in a vertical column indicate - means are different due to age $p < .05$.

4.5.3. Succinate dehydrogenase activity

Succinate dehydrogenase enzyme (SDH) activity is shown in Table 13, for all groups. A 26% increase in activity was demonstrated in the 2 week exercise group greater than control group values, however this was not significant at $p > .05$. Succinate dehydrogenase activity appears to increase with age by approximately 11% in controls and 40% in experimentals. No significant differences were reported due to exercise or age, however a definite trend towards higher activities in exercise groups is apparent.

Table 13. Mean Activity Values for Succinate Dehydrogenase Enzyme (μ g/gm/min) in Gastrocnemius Muscle (wet) of Control and Exercise Groups.

AGE (wk)	N	Activity of N (μg/gm/min)
CONTROL GROUPS		
0	10	1.60 ±.19
1	9	1.92 ±.15
2	6	1.78 ±.34
3	0	n/a
EXERCISE GROUPS		
1	10	1.76 ±.21
2	12	2.24 ±.18
3	0	n/a

Values are means ± SEM

V. DISCUSSION

The effects of endurance exercise on the newborn guinea pig's somatic growth and lung dimensions are best approached after first discussing normal growth events and related factors in the guinea pig during the period studied.

5.1. Effect of Growth on General Characteristics

Several studies have shown postnatal growth of the guinea pig to be intrinsically (genetically) determined, although birth weight, weanling weight and weight after 28 days are highly dependent on litter size and perinatal mortality (Eaton, 1932; Haines, 1931; McPhee and Eaton, 1931). The average litter size for Hartley albino guinea pigs is 3.0 (Dunkin et al., 1930). In the present study, average litter size for sows that did not abort was 3.0 with a higher percentage of males (62%). Stillbirths were a common occurrence in the larger litters (>3) and gestation time was observed to increase with increasing litter size. This direct relationship has been reported previously (Haines, 1931; Goy et al., 1957; McKeown and MacMahon, 1956).

Gestation time in this study was variable between 59 and 75 days, average 68 days, which was a slightly longer period than the ranges previously reported by Ediger (1976) as 59 to 72 days, average 63 days, by Harper (1976) as 65 to 70 days, average 68 days or by Rowlands (1949) and Labhsetwar and Diamond (1970) as 59 to 72 days, average 68 days. The range of birth weights of guinea pigs in this study was from 56 to 143 grams, average 96 grams. The average weight was similar to that reported

for previous studies as between 85 and 95 grams for a litter of 3 to 4 (Ediger, 1976; McKeown and MacMahon, 1956). Although it has been reported that young from large litters grow more rapidly in relation to birth weight than young from small litters, body weight after 28 days is still less in animals from large litters (McKeowen and MacMahon, 1956). This study confirmed these results in postnatal growth in guinea pigs studied for 21 days after birth. It is interesting to note that some studies have shown human infants with a smaller birth weight gain weight more rapidly in the postnatal period than larger infants and have a strong tendency to increase in crown rump length more rapidly (Thomson, 1955). However in a recent study, Pomerance and Krall (1984) found a near zero correlation between birth weight and infant weight rate gain and an essentially zero relationship between birth length and infant rate of length growth.

The wide range of body weights within a particular age group in this study, necessitated presentation of most of these data relative to body weight for easier comparison between groups. Interestingly with each group the range of body weights was normally distributed, confirmed by frequency distribution plots. Body weight was observed to decrease by 1 to 6 grams immediately post partum in most litters. This weight was usually regained by day 4 or 5. Similar observations have been noted both in guinea pigs delivered by Caesarian section (Wagner and Foster, 1976) and vaginally-delivered human infants (Nelms and Mullins, 1982), presumably due to water loss. After day 5, guinea pigs gained between 5 and 10 grams per day. Ediger (1976) reported an average weight gain of 2.5 to 3.5 grams daily for the first 2 months so it is possible that the

first month of growth is accelerated compared to the second month. Body weights of guinea pigs in this study at 2 and 3 weeks of age are similar to those previously reported (Lechner and Banchero, 1980).

5.2 Changes in Lung Weight

The guinea pig lung is comprised of three left lobes; cranial, middle and caudal and four right lobes; cranial, middle, caudal and accessory (Breazile and Brown, 1976). This study showed a ratio of approximately 1.21 for right lung weight/left lung weight ratio. Webster and Liljegren (1949) reported lung weight was proportional to body weight to the power .73 in guinea pigs. The study was performed on guinea pigs up to 1400 grams in weight, indicating that as long as the guinea pig increased in body weight, lung weight also increased. An adult weight for guinea pigs is usually 600 to 800 grams (Wagner and Manning, 1976) so presumably, these animals were force-fed. The present study demonstrated a significant increase in lung weight between week 1 and week 2 in both right and left lungs, however the increase in lung weight was proportional to body weight to the power .39 in control groups ($r = .9611$ control). The lower exponent compared to that of other investigators may be due to the short time period studied and the small gain in lung weight and body weight between birth and week 1. As discussed previously, the neonatal guinea pig loses weight after birth and does not regain this weight for approximately 5 days. During this period the lung does not gain weight appreciably. The different results found in this study may also be due to the changing relationship between W_L and W_b during the period studied. From the equations reported in section 4.2 for linear,

exponential and power functions, there appears to be no one function that fits the data better than the others. All three functions demonstrate good relationships between W_L and W_b. The highest correlation is with the power function in control groups.

As with other organs in the guinea pig, the relative weight of the lungs decreases as the body weight increases, however in a study comparing relative weight reduction in liver, heart, spleen, kidney and lungs, the lungs had the largest relative decrease with the trend towards lower ratios in lower weight groups. There is a large initial drop in relative lung weight during the 1st week of growth followed by a fairly steady decline up to adulthood (Webster and Liljegren, 1949). The present study confirmed this trend. Growth in lung weight may be accomplished by an increase in cell number and/or cell size or by an increase in the intercellular connective tissue matrix (Thurlbeck, 1975). The decreasing relative lung weights observed in this study indicate that body weight is increasing at a faster rate than lung weight. Dry lung weight was used as an index of tissue mass present in the lung. Tissue mass increases significantly between week 1 and week 2 as previously indicated by wet lung weight. The wet/dry lung weight ratio remains fairly constant in control group animals indicating increased cell growth.

5.3 Changes in Lung Volume

Lung growth is assessed by the measurements of both lung weight and lung volume. The lung has a unique ability to increase in size without a concomitant increase in weight. This is due to an increased filling of

the air spaces per gram of tissue. Growth in weight and volume of the lung may not necessarily be parallel due to alterations in its internal complexity (Thurlbeck 1975; Emery, 1970). In this study lung volume increased significantly with age between week 1 and 2 in control groups. Furthermore, lung volume relative to body weight increases significantly with age but occurs at different ages from absolute measurement increases. Significant differences occur between week 0 and 1, and between week 1 and 2 in relative lung volume measurement.

Figure 6 shows the increase in lung volume with increases in body weight in exercised and non-exercised groups. The mean lung volumes for non-exercised groups could be fit to linear, exponential and power functions equally well, so it is difficult to assess which function best describes the data. Difficulty describing the data in this study with one particular function can be attributed to the short time period studied. The immediate postnatal growth period in guinea pigs is likely best described by the initial linear portion of the exponental curve which describes growth to adulthood.

In this study, lung volume measurement refers to the fixed lung volume measurement after 72 hours of formalin perfusion. A question naturally arises about the correlation between any fixed lung volume measurement and total lung capacity it might reflect. Fixed lung volume in rats has been shown to approximate closely the volume of air which the lung may accommodate at maximum inflation (Berend et al., 1980; Kida and Thurlbeck 1980). In this study, pressure volume curve measurements were attempted on excised guinea pig lungs to determine their total lung capacity before fixation. These attempts were unsuccessful due to the

irreversible airway constriction which occurs in guinea pigs after death. This has been the experience of other investigators with guinea pigs (Pare and Kida, personal communication). It is unlikely however that the lungs were not fully inflated with formalin as each lung was inflated maximally by hand in order to overcome this high opening pressure, before being attached to the formalin perfusion system.

5.4 Changes in Quantitative Morphometry

Present interest in the alteration of lung growth by experimental and clinical factors started in the early 1960's with the application and development of modern morphometric technique to the lung by Weibel (1963) and Dunnill (1962). The term morphometry refers to a body of quantitative methods for assessing the anatomical structure of an organ (Section 2.5). In this study, the volume proportion of alveolar air, duct air, bronchial air and nonparenchyma, as well as surface area, mean linear intercept and mean chord length of alveoli and ducts were assessed.

A recent study on normal lung growth in the neonatal guinea pig demonstrated alveolar septa indistinguishable in appearance and dimensions from those of adult guinea pigs (Lechner and Banchero, 1982). This advanced stage of lung development at birth was observed in the present study and confirms the hypothesis of Engle (1953) that pulmonary development parallels the overall level of development at birth. Newborn guinea pigs are capable of thermoregulating and feeding in the absence of maternal care (Hill, 1959; Wagner and Manning; 1976; personal observations). Lechner and Banchero showed minimal evidence for any postnatal proliferative phase. Features that were considered typical of

neonatal lungs in rats, mice and humans such as double capillary layers and cresting septa arising from saccules were rarely seen in guinea pigs even at 10 days preterm (Burri, 1974; Hislop and Reid, 1974; Vaccaro and Brody, 1978). Prenatal lung development also appeared to be different in guinea pigs than other mammals. Terminal and respiratory bronchioles were present in prenatal guinea pigs whereas Burri (1974) reported no respiratory bronchioles in newborn rats until about 10 days of age. Elastin fibres appeared in bronchial walls, alveolar duct walls and 'alveoli' in the young guinea pigs, which usually indicates that alveolar walls have stabilized in size (Loosli and Potter, 1959; Emery, 1970). However this network was observed to be discontinuous in the younger animals, which may indicate some postnatal segmentation (Emery, 1970). Lechner and Banchero's data indicates some segmentation by demonstrating significant increases in lung surface area to volume ratio in newborn guinea pigs.

In reference to the three stages of postnatal lung growth described by Burri (1974) in the rat, and outlined in the Introduction section, it would seem the guinea pig is in the final phase, that of equilibrated growth. During this phase increases in lung volume, tissue and airspace volumes and surface areas are nearly evenly matched, so that septal dimensions and surface densities remain fairly constant.

In the rat, there is a biphasic relationship between body weight (W_b), V_L and S_w. For days 1-10, $V_L \alpha W_b^{.99}$, whereas for days 10-131, $V_L \alpha W^{0.70}$ and for days 1-21, $S_w \alpha V_L$ 1.6 then for days 21-31, $S_w \alpha V_L^{0.71}$. The first phase coincides with Burri's tissue proliferation period from day 3 or 4 to day 13, while the second

phase indicates the period of equilibrated growth. The present study demonstrates a monophasic relationship between the above parameters such that from days 1-21, $V_L \alpha W_b^{.48}$ and $S_w \alpha V_L^{.65}$ in normal controls. These exponents differ somewhat from those of Lechner and Banchero (1982) who demonstrated $V_L \alpha W_b^{.731}$ and $S_w \alpha V_L^{.957}$ in 17 post partum animals and $V_L \alpha W^{.694}$ and $S_w \alpha V_L^{.921}$ in guinea pigs from birth to adulthood (Lechner and Banchero, 1980). Forrest and Weibel (1975) reported $V_L \alpha W^{1.00}$ in adult guinea pigs.

The relationship between surface area and lung volume found in this study was similar to that demonstrated in the rat during the phase of equilibrated growth, however the relationship between lung volume and body weight was not as evident as in previous studies. As discussed in section 5.5, these differences are probably attributable to the time points at which lung volume is measured, relative to body weight. Postnatal growth in lung volume may be best described by a linear or exponential function for the 1st 21 days of life in guinea pigs. If surface areas within the lung increased with increasing V_L only through the enlargement of pre-existing alveoli, S_w would be proportional to $V_L^{.67}$, exponents greater than .67 would indicate the addition of new alveoli as well as alveolar enlargement during lung development (Bartlett, 1971; Burri et al., 1974; Thurlbeck, 1975). The relationship $S_w \alpha V_L^{.65}$ demonstrated in this study indicated enlargement of pre-existing alveoli or hypertrophy, rather than addition of new respiratory units, in contrast to findings of other investigators (Lechner and Banchero, 1982) who indicate addition of new alveoli

throughout growth.

While other investigators have described the surface area to lung volume relationship using a power function, the data in this study fitted an exponential and linear function equally well, in non-exercised and exercised groups (Section 4.4.3). It was therefore impossible to determine the best function to describe surface area to lung volume relationship in guinea pigs from data limited to the first 3 weeks of life. Figure 8 represents the relationship between alveolar surface area and lung volume found in this study. The relationship between these variables was best described by an exponential function in both non-exercised and exercised groups (section 4.4.3) although the r values for exponential functions were only marginally higher than those for linear functions.

If the lung grew by alveolar multiplication, the average distance between alveolar walls ($\bar{L}_m$) would remain the same, or increase slightly. If the lung grew by expansion of pre-existing alveoli, $\bar{L}_m$ would increase with age and lung volume (Thurlbeck, 1975). In this study, $\bar{L}_m$ increases by 11% over the 3 week period, but this increase was not significantly different at any age, therefore it is difficult to assess whether or not new respiratory units were forming at this time. Studies on hamsters have shown mean linear intercept ceases to increase by 6 weeks. This may be explained by the general growth pattern in these animals, namely, body weight and nosetail length cease to increase by 6 weeks of age and no growth is seen thereafter (Snider and Sherter, 1977).

The mean chord length of alveoli ($\bar{l}_a$) increased significantly

between week 1 and week 3, indicating an increase in size of alveoli, and therefore hypertrophy. Mean chord length of ducts remained constant with age. A progressive, though small increase in alveolar dimension during childhood has been noted by most investigators (Dunnill, 1962; Hieronymi, 1961; Nakamura et al., 1967; Wyatt et al., 1962) but others have found no increase in alveolar dimensions until 3 years of age (Davies and Reid, 1970) or 10 years of age (Emery and Wilcock, 1966). The consensus appears to be that lung volume always increases more than alveolar number so that alveoli progressively enlarge in humans.

In the present study surface area remained constant over the 3 week period however relative to body weight, surface area was significantly different due to age in control animals, presumably due to the increase in body weight. The constant surface area paralleled the constant volume proportions of alveolar air, alveolar wall and duct air. The volume proportion of non-parenchyma was also constant, therefore little deposition of connective tissue or blood vessel development is evident at this time. Predictably, the total volume of alveolar air (V_a) per mean lung volume (Table 6b) increased significantly between week 1 and week 3. There were no parallel increases in total duct air (V_d), alveolar wall (V_w) conducting airway (V_b) or non-parenchyma (V_{np}) which indicates that total lung volume increases may be due only to expanding alveoli. This was again indicated in the constant ratio $V_a/\bar{l}_a^3$, an index of total alveolar number per whole lung. There was a slight (5.5%) but insignificant increase in this estimate over the 3 week period in control animals. The average estimate of alveoli per guinea pig lung was 81.567 million.

5.5 Changes in Protein and DNA content

The details of organ growth and whole body growth have been investigated in rat. The lung appears to follow the same general growth pattern outlined by whole body growth (Enesco and Leblond, 1962; Winick and Noble, 1965; Thurlbeck, 1975). At birth, the weight of deoxyribonucleic acid (DNA), an index of nuclei number, does not quite match the increase in body weight during the first two weeks of life, thus nuclei per gm body weight (DNA/W_b) tends to decrease slowly. The amount of water in tissue and organs varies with age, therefore a better relationship is the change in amount of protein per nucleus (protein/DNA). Whole body protein increases faster than DNA, thus protein/DNA increases for most of the growing period. This appears primarily due to increased cell cytoplasm in fat and muscle and an increase in intercellular connective tissue (Thurlbeck, 1975).

In general, organs with expanding cell populations such as lung, brain, liver, kidney and heart follow this outline in three phases, in the rat. From birth to 14-17 days, organs grow by cell multiplication; the amount of DNA increases rapidly and the amount of protein per nucleus remains approximately constant. The second phase constitutes a period of slower cell multiplication relative to protein synthesis, thus whereas the amount of DNA and number of nuclei continue to increase, there is an overall increase in protein/DNA ratio. This phase continues up until 5-7 weeks of age when the final phase takes over. Cell proliferation is slow or stops altogether, cell enlargement is slight or may stop, thus the amount of protein/DNA remains fairly constant, as does amount of DNA. Relative to other organs with expanding cell populations the brain and

the lung demonstrate the largest increases in DNA in the early growth period, but whereas the brain adds few nuclei after day 14, the lung continues to add cells slowly (Thurlbeck, 1975).

The guinea pig lung after birth appears to be in the second phase described, that of slower cell multiplication relative to protein synthesis. Absolute DNA/lung increased by only 9% whereas NCT protein/lung increased significantly by 43% in controls. This relationship constitutes a significant overall increase in the protein/DNA ratio. There is a possibility however, that during the 1st postnatal week, the guinea pig is in the latter phase of the proliferative period. Between week 0 and week 1, DNA in mg/gm and mg/dry lung increases, though not significantly, before starting to decrease. The data suggests hyperplasia during week 1 and hypertrophy of cells from 1 to 3 weeks. Relative to whole body growth, lung protein decreased significantly between week 1 and week 2, as did lung DNA. For the first week, lung protein and DNA content approximately matched the increase in body weight, then between 1 week and 2 weeks, body weight increased at a faster rate than lung protein and DNA, then finally during the 2nd and 3rd week, the increase in body weight approximately matched lung DNA and protein content.

5.6 Effects of Exercise on General Characteristics

The mean body weight of exercised animals was consistently lower than the control counterparts for each age group. During the first week of exercise, body weight increased only 7%, compared to 23% in non exercised controls. This difference in weight gain became significant by

the end of 2 weeks exercise. The lower body weights of exercised animals is presumably due to a loss in body fat (Weltman et al., 1980; Tran et al., 1983), but may be attributable to an actual growth retardation due to exercise. Whatever the reason for weight loss, it is interesting to note that after 3 weeks, both exercised and control mean body weights are well matched indicating a rapid growth period during the 3rd week in the exercised group, possibly as a result of their acclimation to the exercise stimulus.

Significant weight loss shown in exercise groups after 2 weeks is also reflected in significantly smaller femur lengths in exercised animals. Femur length is an index of whole body growth so that linear growth also appears to be retarded by the exercise regimen during the first 2 weeks. However, during the 3rd week of exercise, animal femur lengths from both control and exercise groups are within the same range, although exercise lengths were marginally smaller. Hansen (1982) has recently reported epiphyseal changes in the proximal humerus of a young baseball pitcher, perhaps due to similar stress on growing bones. A marked widening and concomitant osteoporosis of the proximal humeral epiphyses was first reported by Dotter (1953) and termed "Little Leaguers' shoulder". Crown to rump length, another measure of whole body growth was not significantly affected in the exercise groups, but there was a trend towards a reduced length of this variable. Predictably crown rump length increased significantly with age.

5.7 Effects of Exercise on Heart Weight

Cardiac hypertrophy due to endurance exercise training is a normal

physiological response (Rost, 1982). Morganroth and Maron (1975) together with others, described the endurance althlete's heart as dilated without an increase in myocardial wall thickness. In contrast, weight training athletes exhibit hypertrophied cardiac muscle (especially left ventricular wall thickness). In haemodynamic terms this observation is consistent. Isometric physical exercise results in predominantly pressure work, whereas endurance work affects flow characteristics and increases cardiac output. Rost (1982) and Longhurst et al., (1981), clearly demonstrated that the endurance athletes' heart had both increased left ventricular diameter as well as some myocardial wall thickness increase compared to normal whereas increases in left ventricular size observed resulting from excessive muscular isometric work, occurred only in relation to increases in body mass. In this study, there appeared to be no changes in mean heart weight or heart weight/body weight proportion between exercised and control animals. In fact, absolute heart weights in exercised animals were less than their control group counterparts at week 1 and 2 respectively, although this was probably a reflection of reduced body weights in the exercised groups. The heart weight/body weight proportion was higher in exercised animals at both week 2 and 3 probably resulting from some cardiac hypertrophy although the differences observed were not significant. Heart weight increased over the 3 week period by 66% in exercised animals and 45% in control animals, a difference which indicates possible hypertrophy over the 3 week period in exercised animals.

Keul et al., (1982) demonstrated different adaptations in humans resulting from moderate endurance training (> 3 hours running per week)

compared to intensive endurance training (> 10 hours running per week) but found no increase in heart size with moderate training. An argument that the intensity of exercise training in this study was moderate for guinea pigs is unconvincing, because other investigators have demonstrated histochemical changes in skeletal muscle following treadmill exercise of similar intensity to that used in this study on adult guinea pigs (Faulkner et al., 1972; Maxwell et al., 1973; Barnard et al., 1970). These investigators trained animals for longer periods than used in the present study, so it is possible 3 weeks was not a sufficiently long training period to produce significant increases in heart weight .

5.8 Effects of Exercise on Lung Weight

The increase in lung weight was proportional to body weight to the power .38 (r=.9149), nearly identical to that found in control groups. However, as with control groups there appeared to be no one function that fit these data better than any other (section 4.2) although the linear function demonstrated the best correlation (r=.9469) this was different from that found in controls, where the power function demonstrated the best correlation (r=.9611). Figure 5 shows mean lung weight increases with increasing body weight at the same rate in both exercised and control groups evidenced by identical slopes in the respective linear equations. However, the control group curve reaches a plateau at a lung weight equal to 1.675 gm and body weight of 210.97 gm. The exercise group curve continues to rise exponentially, and shows no sign of reaching a plateau. It may be that lung weight does not exhibit a growth 'plateau' until it reaches a 'critical weight. In this study, growth in

the exercised animals appears to be retarded, so this 'critical' weight would be reached at a later time compared to control animals.

After 2 weeks of exercise a significant decrease in absolute lung weight was observed in exercised animals although there was no differences in relative lung weight. This is in agreement with Bartlett (1970a) who did not demonstrate significant changes in lung weight following treadmill exercise in rats, although his data show a trend towards lower lung weight in exercised animals. In rats trained for 4 and 8 weeks by swimming, lung weight was not significantly affected (Fu, 1976). Hugonnaud et al., (1977) failed to show any differences in absolute or specific lung weight in mice made hyperkinetic with imino dipropionitrile. Two way ANOVA demonstrated an interactive effect between exercise and growth in the parameters of lung weights. One way ANOVA showed the exercise treatment to have a greater influence than growth.

Dry lung weight was used as an index of tissue mass present in the lung. Tissue mass increases significantly between week 2 and week 3 in exercise groups. This significant increase in tissue mass appears to be delayed in exercise groups compared to control groups where the increase takes place between week 1 and week 2. The significantly decreased wet lung weight in week 2 exercised animals compared to control counterparts was paralleled by a significantly reduced dry lung weight but relative dry lung weights were no different between these groups. Interestingly, the protein content per gram dry weight of lung in week 2 exercise animals was significantly greater than in control animals, although total protein per lung was not different between these groups. This suggests a

greater percentage of dry weight is due to protein content in the exercise group when compared to the control group at week 2. The reduction in wet and dry lung weight in the week 2 group cannot be accounted for by a reduced NCT protein fraction which suggests a possible reduction in the CT protein fraction (collagen and elastin) which was not measured. The wet/dry lung weight ratio was significantly different between week 1 and week 3 exercised groups, in contrast to the fairly constant wet/dry weight ratio in control groups. The higher ratio in exercise groups could be due to decreased tissue H_2O in the dried tissue, or a slightly greater range of both wet and dry lung weights between week 1 and week 3 in exercise groups when compared to control groups.

5.9 Effects of Exercise on Lung Volume

No differences were demonstrated in lung volume due to exercise for any age group, although the total increase in lung volume over 3 weeks was 8% greater in exercised animals than in control animals. These results are in agreement with Bartlett (1970a). After 20 days of daily exhaustive treadmill running the lung volumes of exercised rats were not different from control animals. In the present study, relative lung volume decreases significantly with age. This decrease occurs between week 0 and week 1 and again between week 1 and week 2 in both exercise and control groups. Figure 6 shows the increase in lung volume with increases in body weight in exercised and non-exercised groups. The exponential function fit the exercise group data marginally better than the linear function with r values of .9592, and .9512 respectively.

Exercise has been reported to change the functional capacity of the lung. In a cross-sectional study of lung dimensions of 30 female swimmers, Engstrom et al., (1971) found that 12 to 16 year old girls could be trained to an exceptionally high functional capacity. In a similar but longitudinal study over 8 years, with 29 girls, they found significant increases in static lung volumes and vital capacity. Increased vital capacity and maximal flow rates have also been reported in trained adults with endurance exercise (Bannister et al., 1960; Freedman et al., 1955; Grimby and Saltin, 1966; Yost et al., 1981).

5.10 Effects of Exercise on Quantitative Morphometry

In this study, the volume proportion of alveolar air, duct air, bronchial air and non-parenchyma, as well as surface area, mean linear intercept, mean chord length of alveoli and ducts, and an index of alveolar number were assessed for exercise and control groups.

Exercise had no discernible effect on any of these parameters, when compared to control groups. Again, this is in agreement with the work of Bartlett (1970a) who found no morphological differences between rats trained for 20 days and control animals. There were differences between exercise and control groups in total volume of lung components per mean lung volume, due to growth. Whereas in the control animals, only V_a was significantly increased; in exercise animals, V_a, V_d and V_w were all significantly increased due to growth (Table 6b). In the exercised animals increased lung volumes may be due to expansion of alveoli and ducts in contrast to controls where increased lung volume is due only to expansion of alveoli. The index of alveolar number

($V_a/\bar{l}_a^3$) increases, though not signifncatly by 19% in exercise groups between week 0 and week 3, in contrast to only 5.5% in control groups. This trend towards a greater number of alveoli in exercised animals was also observed in Bartlett's study of exercised rats. Fu (1976) demonstrated significantly larger surface area to volume ratios and alveolar density in young rats exercised by swimming.

Interest in the role of exercise on lung development had its origins in the hypothetical relationship between metabolic needs for oxygen and morphometric diffusion capacity ($D_L(m)$) proposed by Weibel (1970/1971). Weibel examined the lung architecture of six mammalian species and confirmed an already existing hypothesis that mammalian lung volume increases proportionally with body weight (Tenney and Remmers, 1963; Weibel, 1967). Weibel also found that alveolar surface area and pulmonary diffusing capacity ($D_L(p)$) increased nearly linearly with body weight, whereas the morphometric pulmonary diffusing capacity ($D_L(m)$) related to the power 1.28 of oxygen consumption. Weibel distinguished $D_L(p)$ from $D_L(m)$; the former referred to the physiological parameter, while the latter referred to the maximum possible transfer rate of ventilated gas. Weibel demonstrated higher $D_L(m)$ values in the physically active 'wild" mammals compared to those in captivity. This was in agreement with previous work which showed physically active waltzing mice had a larger alveolar surface area and a larger diffusing capacity than normal laboratory mice. In mice with a 50% increase in oxygen consumption due to imino ββ dipropionitrile (IDPN) administration, a 23% increase in specific lung volume, an increase in the specific gas exchange area and a 40% increase in $D_L(m)$ was observed

over control values (Burri et al., 1976; Hugonnaud et al., 1977). In this study, daily treadmill exercise raised the animals' oxygen demands above resting levels for 1 to 1 1/2 hours, which was perhaps not equivalent to the continually increased demand in Japanese waltzing mice and IDPN-treated mice.

Figure 8 represents the relationship between alveolar surface area and lung volume found in this study. The function which best described the data in exercise groups, was the linear function, although the r-values were only marginally higher than those for the power and exponential functions at .9963 and .9957 respectively, compared to .9812. Interestingly absolute surface area measurements were significantly different between week 1 and week 3 exercise groups indicating possible overexpansion. Relative to body weight, however, this difference did not persist in exercised groups. There were no significant differences due to age in relative surface area in exercise groups, in contrast to control groups where differences were found between week 0 and week 1, then between week 1 and week 2.

5.11 Effects of Exercise on Protein and DNA Content

There were no significant differences between exercised and control animals at any age in; total protein/dry lung, DNA/dry lung, DNA mg/gm dry lung, protein/DNA ratio or relative parameters. However there was a greater protein/dry lung (mg/gm) value in week 2 exercise animals compared with control animals. As discussed in section 5.8 this suggests that the reduced wet and dry lung weight in the exercise group may be due to a decrease in CT protein. In the exercised animals there was an

overall trend towards lower absolute protein and DNA content/lung and reduced overall growth in the 3 week period which was reflected in a 32% increase in protein content and 0.4% increase in DNA content compared to 43% and 9% in control group animals. The constant ratio of protein/DNA over the 3 week period reflects neither cell proliferation nor cell enlargement in the exercised groups, characteristic of the third phase of organ growth described above. A different growth pattern may be evident in the lungs of exercised animals.

5.12 Exercise Effects on Succinate Dehydrogenase Activity

Membrane bound succinate dehydrogenase is present in all aerobic cells, is linked to the respiratory chain and is an enzyme in the Krebs cycle. It is a complex enzyme containing nonheme iron, acid labile sulfur and covalently bound flavin adenine dinucleotide (FAD). Muscle mitochondrial SDH is located on the matrix side of mitochondrial inner membranes and catalyzes the oxidation of succinate to fumerate and transfers the resultant reducing equivalents directly to the respiratory chain. SDH activity is modulated by a variety of activators and inhibitors (Hederstedt and Rutberg, 1981).

In this study the rate of reduction of oxidized cytochrome C by SDH was measured as a marker for oxidative capacity of guinea pig gastrocnemius muscle (Cooperstein et al., 1951). Endurance exercise has long been known to increase the oxidative capacity of skeletal muscle and is a good indicator of the degree to which an animal is trained (Holloszy, 1967; Gollnick and King, 1969; Kowalski et al., 1969; Edgerton et al., 1969; Barnard et al., 1971; Faulkner et al., 1971; Faulkner et

al., 1972). A 26% increase in SDH activity was demonstrated in week 2 exercised animals above mean control group values, although this difference was not statistically significant. An overall increase in SDH activity of 11% in control and 40% in experimental groups* respectively, indicated a definite trend towards increased oxidative capacity in the exercise groups, however it was not a significant difference. Unfortunately no values for 3 week age groups were obtained so it was impossible therefore to determine whether SDH activity was significantly different between exercise and control groups after a further week's training.

Barnard et al., (1970) demonstrated no mitochondrial adaptations in adult guinea pig gastrocnemius and plantaris muscle after 9 weeks of daily treadmill running. However after 18 weeks, mitochondrial protein concentration per gram muscle increased significantly with training. In another study, daily treadmill running from 6 to 14 weeks had no effects on guinea pig soleus or psoas muscle fibre composition, but a higher proportion of high oxidative fibres were reported in the plantaris muscle after similar activity (Maxwell et al., 1973). The differences between studies appears to depend upon the fibre composition of the muscle assayed. Plantaris and gastrocnemius muscle have a larger portion of the muscle cross-section composed of red fibres (Barnard et al., 1970; Edgerton et al., 1969; Faulkner et al., 1971) and have a greater capacity

* All 3 week samples and many 0,1 and 2 week samples were inadvertently destroyed after a power failure in the refrigerator where they were stored.

for oxidative metabolism. Red fibres are classified as highly oxidative fibres. It is possible that significant changes in the oxidative capacity of skeletal muscle cannot be demonstrated in guinea pigs until after a much longer training period.

5.13 Summary

Three weeks of daily endurance treadmill running in guinea pigs appeared to have little or no effect on lung morphology, in agreement with previous investigators (Bartlett, 1970a). A 37% increase in lung surface area in exercised animals compared to 30% in sedentary animals was significant which may mean slight distension of air spaces was occurring in the lung, although this was not indicated by an increased length of alveolar mean chord ($\overline{l}_a$)or mean linear intercept ($\overline{L}_m$). Exercise significantly reduced body weight, femur length, lung weight (wet and dry) and lung volume at week 2, but this difference did not persist at week 3. Treadmill running appeared to initially retard overall growth of the animals but the effect was temporary because after 3 weeks, no differences were found between exercised and sedentary groups.

Table 16 summarizes the parameters which were significantly affected purely by growth, rather than exercise. Previous investigators (Lechner and Banchero, 1982) have indicated the lungs of newborn guinea pigs are in the phase of equilibrated growth described by Burri (1974). This is the third and final stage of postnatal development where the lung is growing more by expansion of pre-existing alveoli rather than addition of new respiratory units. A phase of equilibrated growth is also indicated by the present study, although it appears that alveolar

formation has ceased, which is contrary to previous findings (Lechner and Banchero, 1982). Absolute surface area increases in proportion to body weight to the .65 power, very close to .70 power demonstrated by Burri (1974) in the rat, but different from .954 power demonstrated by Lechner and Banchero (1980; 1982) in guinea pigs. If the exponent is larger than .67, new alveolar formation is indicated (Bartlet 1971; Burri et al., 1974; Thurlbeck, 1975). There was a slight increase in $\bar{L}_m$ (11%) and a significant increase in $\bar{l}_a$ (19%) which probably represents expansion of air spaces. Lung volume was found to increase in proportion to body weight to the .48 power, a much lower exponent than previously demonstrated, perhaps due to the slow initial increase in body weight in the 1st week of life. Significant increases in whole body growth and organ growth were demonstrated over the 3 week period (Table 16).

TABLE 14. SUMMARY OF SIGNIFICANT GROWTH PARAMETERS IN ALL GROUPS

		Age (weeks)		
		1	2	3
General Growth:	W_b		↑	↑
	F	↑	↑	↑
	CR		↑	not measured
Organ Growth: (Absolute)	W_L wet		↑(C)	↑(E)
	RW_L dry		↑(C)	↑(E)
	LV_L		↑(C)	↑(E)
	DNA			
	Protein			↑(C)
	Protein/DNA			↑(C)
	W_h		↑	
Organ Growth: (Relative to W_b)	W_L wet	↓	↓	↓
	W_L dry			
	LV_L	↓	↓	
	DNA		↓	
	Protein		↓	
	S_w/W_b	↓	↓	
	W_h		↓	↑
Absolute to Lung	S_w			↑ (E)
	l_a			↑ (C)
	S_{Vw}			↓ (C)
	V_a			↑
	V_d			↑ (E)
	V_w			↑ (E)

All arrows represent significant growth effects.
*(E) - exercise group only, (C) - Control group only
Abbreviations - refer to Tables 3-5.

CONCLUSIONS

1. There was no evidence for addition of new respiratory units after birth in the guinea pig. It is proposed that this animal is already in the phase of equilibrated growth at birth.

2. There were no significant differences in the dimensions of respiratory components of guinea pigs exercised for 1, 2, or 3 weeks when compared to the dimensions of respiratory components of sedentary control animals at 1, 2, or 3 weeks.

3. Daily endurance treadmill running had a significant effect on body weight, femur length, absolute lung weight (wet and dry) and lung volume at week 2, but not at week 3.

4. There was a significant increase in alveolar surface area, duct volume, alveolar wall volume and wet/dry lung weight ratio in exercise groups, but not in control groups during the 3 week period.

Limitations of Study

1. Control guinea pigs were not totally sedentary as they were allowed to move freely in the cages at all times.

2. The protein content/lung measured by the methods of Lowry et al. (1951) only measured acid insoluble protein, denoted in this study as non-connective tissue (NCT) protein. The connective tissue protein (stromal protein fraction) was discarded.

APPENDIX I - EXERCISE PROTOCOL

All experimental animals were trained on the same rodent treadmill (Quinton Instruments, Seattle, WA) and were introduced to treadmill running within 12 hours of birth. The first 5 days were used as a learning period during which the treadmill speed was increased to a maximum of 25 m/min and the animals exercised for longer intervals each day, as illustrated in Table 15 below. Group 1 was sacrificed 40 hours after the exercise session on day 6.

Table 15 Exercise Protocol - Week 1

Day	Speed (m/min)	Time (min)	Total Time (min)
1	5.5	5 W*	10
	7.8	5 E**	
2	7.8	7 W	15
	10.5	8 E	
3	10.5	5 W	20
	13.4	10 E	
	17.2	5 E	
4	17.2	10 W	30
	21.0	20 E	
5	21.0	10 W	40
	25.0	30 E	
6	25.0	50 E	50

* W - warmup period

** E - exercise period

Groups 2 and 3 continued to exercise daily from day 8 to day 13 as illustrated in Table 16. Days 7 and 14 were rest days and the animals did not exercise. All exercise sessions were preceeded by 2-3 minute warmup periods at a treadmill speed of 20 m/min and no incline. The treadmill speed remained constant at 25 m/min for the remainder of the training period. Group 2 was sacrificied 40 hours after the exercise session on day 13 while group 3 continued to exercise from day 15 to day 21. Group 3 was sacrificed 40 hours after the exercise session on day 21. The treadmill speed was recalibrated every 7 days.

Table 16 Exercise Protocol - Week 2 and 3

Day	Time (mins)
8	55
9	60
10	70
11	75
12	60
13	50
14	Rest
15	30
16	40
17	50
18	60
19	70
20	80
21	90

APPENDIX II - MORPHOMETRIC PROTOCOL

1.1. Determination of Fields

The determination of 20 random fields on a tissue section is illustrated in Figure 9. Five fields are along the x axis and four fields are along the y axis. Using a 10x objective the coordinates xy, xy', x'y and x'y' were determined, and equidistant coordinates were calculated within this rectangle. A 2 mm border of tissue was excluded in the determination of coordinates to avoid inclusion of compressed areas or edge artifacts in the measurements. The reason for determining specific coordinates was to ensure non overlapping of counted fields.

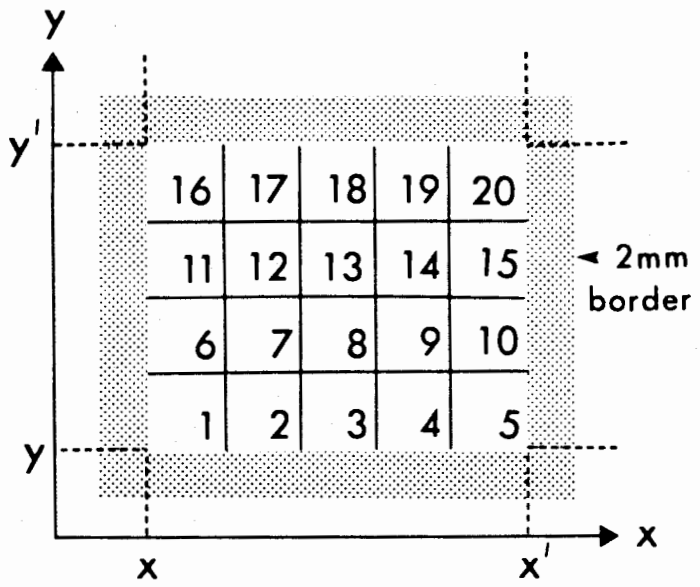


Figure 9. Determination of field coordinates on a tissue section, showing 20 fields in a 4 x 5 matrix.

1.2. Morphometric Calculations

The basis of the counting principle used in measuring lung parameters on random sections is the Delesse principle (Delesse, 1848), which states;

"area proportions are equivalent to volumetric proportions"

or "the planimetric fractions of a section occupied by sections of a given component correspond to the fraction of the tissue volume occupied by this component."

Thus, from two dimensional area measurements it is possible to calcuate three dimensional parameters including volume proportions:

i.e. Volume Proportions

$$Vvx = \frac{\text{number of test points on structure x}}{\text{total number of test points*}}$$

*5040 points per case (6 slides/case x 20 fields/slide x 42 points/field)

Refer to Section 2.5

APPENDIX III - PROTEIN AND DNA BIOCHEMISTRY

1.1. Protein and DNA Extraction

Reagents:

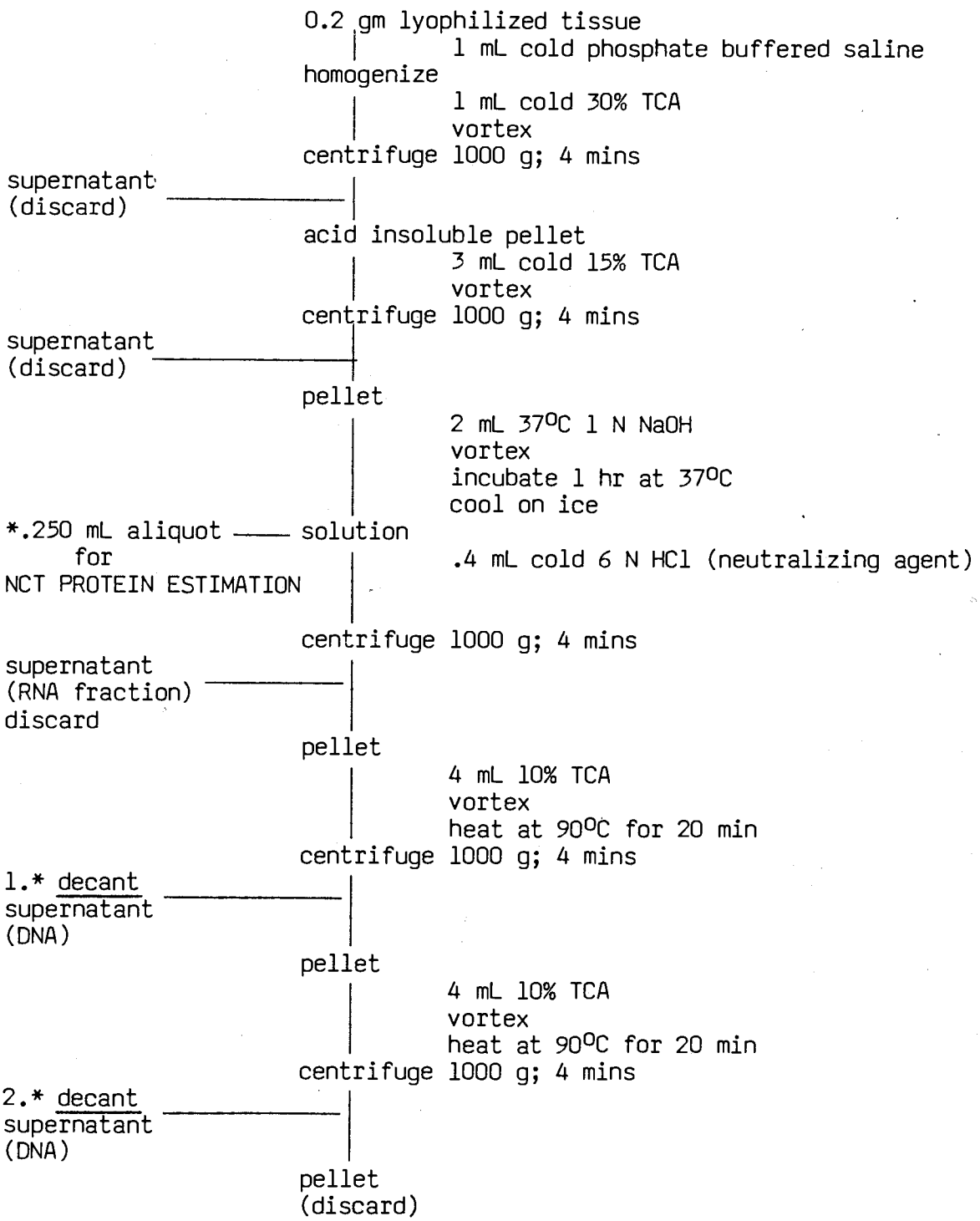
1. Phosphate buffered saline: .145 M NaCl

2. Trichloroacetic acid: 30%, 15%, 10% and 5% solutions

3. Sodium Hydroxide: 1.0 N

4. Hydrochloric Acid: 6.0 N

Procedure



* pool DNA fractions for a total of 8 mLs for DNA ESTIMATION.

1.2. Protein Estimation

Reagents:

1. Bovine Serum Albumin: (BSA)(Sigma A-4378) dissolve 50 mg in 100 mL 0.1 M NaOH.

2. Sodium Hydroxide: .1 M

3. Sodium Carbonate: dissolve 20 gm in 1000 mL distilled water to yield 2% solution.

4. Copper Sulphate: dissolve .5 gm in 50 mL distilled water to yield 1% solution.

5. Sodium Tartrate: dissolve 1 g in 50 mL distilled water to yield 2% solution.

6. Colour Reagent: (copper tartrate/sodium sulphate) Mix 2 mL of (4) and 2 mL of (5) then add to 200 mL of (3). Prepared freshly for each assay.

7. Phenol Reagent: 1 N Folin-Coicalteau (Fisher Scientific Co.) Prepared immediately before use by diluting 1:1 with distilled water.

Procedure (Lowry et al. 1951)

Samples

1. Add 0.1 mL of protein sample to each tube and duplicate

2. Add 0.4 mL of .1 N NaOH to each tube

3. Add 5.0 mL of colour reagent (Lowry Solution) and allow samples to sit at room temperature for 10 minutes

4. Add .5 mL 1 N Folin's Reagent and vortex immediately after its addition

5. Allow samples to sit in a dark place for 90 minutes at room temperature

6. Read optical density at 660 nm on Pye Unicam SP6-550 spectrophotometer using a blank as reference

Standards

7. Add: 10, 50, 75, 100, 125, 150, 175, 200, 225 and 250 μg of BSA solution to ten different tubes and duplicates

8. Makeup each solution to a total volume of 0.5 mL with 0.1 N NaOH

9. Repeat steps 3 through 6 for the standards

Calculation for Total Protein/Right Lung

$$\text{Total protein (mg)} = \frac{\text{Assay value } (\mu g)}{\text{Dilution Factor}} \times \frac{\text{sample volume (mL)}}{\text{assay volume (mL)}} \times \frac{1 \text{ mg}}{1000\ \mu g}$$

$$= \frac{\text{Assay value } (\mu g)}{\text{Dilution Factor}} \times \frac{2.0 \text{ mL}}{0.1 \text{ mL}} \times \frac{1 \text{ mg}}{1000\ \mu g}$$

$$= \frac{\text{Assay value } (\mu g)}{2} \times .02$$

$$= \text{Assay value } (\mu g) \times .04$$

* Assay value is determined from the standard curve.

1.3. DNA Estimation

Reagents:

1. Deoxyribonucleic acid - calf thymus: (Sigma D-1501) dissolve 40 mg in 100 mL .005 M NaOH

2. Sodium Hydroxide : .005 M

3. Trichloroacetic Acid: dissolve 20 mg in 200 mL distilled water to yield 10% solution

4. Diphenylamine Reagent: dissolve 1 gm diphenylamine (J.T. Baker Chemical Co.) in 100 mL glacial acetic acid. Then add 2.75 mL of concentrated H_2SO_4. Reagent was prepared freshly before each assay.

5. Diphenylamine: used for (4) above (J.T. Baker and Co.)

6. Acetic Acid - Glacial: Fisher Scientific Co. used for (4) above

7. Sulfuric Acid: Fisher Scientific Co. concentrated, used for (4) above

Procedure

Samples:

1. Add 1.0 mL of DNA sample to tubes and duplicates

2. Add 2.0 mL of diphenylamine reagent

3. Vortex all tubes and cap loosely with glass marbles

4. Heat for 10 minutes in a boiling water bath

5. Cool on ice

6. Read optical density at 600 nm on Pye Unicam SP6-550 spectrophotometer using a blank as reference

Standards

7. Add 20, 40, 60, 80, 100, 120, 160, 200 µg DNA standard solution to eight different tubes and duplicates

8. Make up each solution to a total volume of 1.0 mL with 10% TCA

9. Repeat steps 2 through 6 for the standards

Calculation for Total DNA/Right Lung

$$\text{Total assay DNA (mg)} = \text{assay value } (\mu g) \times \frac{\text{sample volume (mL)}}{\text{assay volume (mL)}} \times \frac{1 \text{ mg}}{1000\ \mu g} \times C$$

$$C = \frac{\text{sample volume before removal of aliquot for protein assay}}{\text{sample volume after removal of aliquot for protein assay}}$$

$$C = \frac{2.0 \text{ mL}}{1.75 \text{ mL}} = 1.14$$

$$\text{Total assay DNA (mg)} = \text{assay value } (\mu g) \times \frac{8.0 \text{ mL}}{1.0 \text{ mL}} \times \frac{1 \text{ mg}}{1000\ \mu g} \times 1.14$$

DNA mg/gm = DNA mg/assay / assay weight of lung tissue

Total DNA/Right lung (mg) = DNA mg/gm x dry lung weight (gm)

APPENDIX IV - SUCCINATE DEHYDROGENASE ACTIVITY

Reagents:

1. Phosphate Buffer: 0.17 M, pH adjusted to 7.4 buffer made up from;
   $Na_2HPO_2 \cdot 7H_2O$ - 36.19 gm/796 mL deionized distilled H_2O
   $Na_2PO_4 \cdot H_2O$ - 4.63 gm/200 mL deionized distilled H_2O

2. Sodium phosphate dibasic - hepatahydrate; Fisher Scientific Co. used for phosphate buffer

3. Sodium phosphate monobasic: Fisher Scientific Co. used for phosphate buffer

4. Sodium Succinate - hexahydrate; 0.5 M, dissolve 6.75 g in 50 mL phosphate buffer (Fisher Scientific Co.)

5. Sodium Cyanide: 0.06 M, dissolve 73.5 mg in 25 mL phosphate buffer (J.T. Baker Chemical Co.)

6. Cytochrome C - horseheart type III; .2 mM, dissolve 49.6 mg in 20 mL phosphate buffer (Sigma C-2506)

7. Aluminum Chloride - 4 mM; dissolve 53.3 mg in 100 mL deionized, distilled water

8. Calcium Chloride 4 mM; dissolve 44.4 mg in 100 mL deionized distilled water

9. Cytochrome C salt solution: made by mixing 2 mL deionized distilled water, 0.5 mL cytochrome C in 0.17 M phosphate buffer (6), and 0.3 mL of a water solution containing equal volumes of (7) and (8) above. Mix freshly before assay.

Procedure

Set up directly in cuvettes;

1. .06 mL tissue homogenate (cold)

2. .10 mL sodium succinate solution

3. mix and leave for 2 minutes

4. add .20 mL sodium cyanide solution

5. mix and leave for 2 minutes

6. add 2.8 mL cytochrome C salt solution

7. mix and leave for 1 minute

8. place cuvette in spectrophotometer and record absorbances every 30 seconds for 6 minutes at 550 nm, against a reference cuvette

Principle

1. Succinate $\xrightarrow{SDH}$ fumerate

FAD $\rightleftharpoons$ $FADH_2$

3. Cytochrome C Fe^{++} $\xleftarrow{CN}$ Cytochrome C Fe^{+++}

Calculation of SDH activity

$$\text{Activity } (\mu g/gm/min) = \frac{\text{total homogenate volume } (\mu L)}{\text{fraction of homogenate used for assay } (\mu L)} \times \frac{\text{total assay volume (mL)}}{\text{wet weight of tissue used for assay (mL)}} \times \frac{1000 \text{ g/gm}}{\text{extinction coefficient for cytochrome C}} \times \Delta E^{*}$$

* ΔE = average change in absorbance/minute

$$\text{Activity } (\mu g/gm/min) = \frac{4000 \mu L}{60 \mu L} \times \frac{3.16 \text{ mL}}{100 \text{ mL}} \times \frac{1000 \mu g/gm}{18.8} \times \Delta E$$

$$= 112.06 \ \Delta E$$

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