ADAPTIVE LUNG GROWTH FOLLOWING EXPOSURE TO SIMULATED HIGH ALTITUDE.

by

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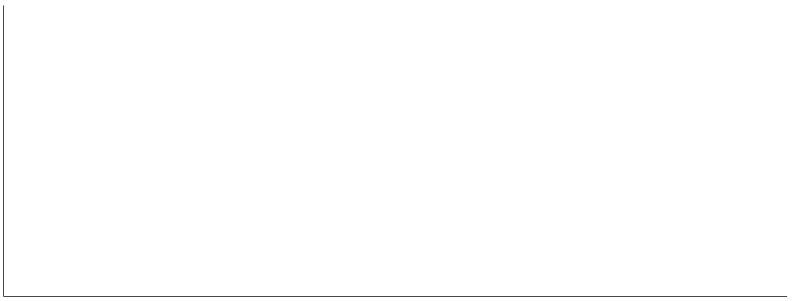

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ABSTRACT

Altered oxygen balance in the body at high altitude and in some physiological and pathological conditions may induce adaptive changes in the body which may be organ specific. Because gas exchange is the primary function of the lungs, they may undergo structural changes to adapt to the imbalance of oxygen. High altitude residents have large lungs and short body stature. Whether these adaptive changes are caused by hypobaric pressure or hypoxia (low oxygen) or hypobaric hypoxia is not known. In hypoxic conditions, somatic growth retardation occurs due to undernutrition, but the effect of undernutrition on growth of lung and other organs is not known. In this thesis, the influence of hypobaric normoxia (410 mm Hg, oxygen enriched to correspond the fraction of oxygen (Fo_2) to 0.21 at sea level), normobaric hypoxia (Fo_2 0.11), hypobaric hypoxia (410 mm Hg, Fo_2 equivalent to 0.11 at sea level) and diminished somatic growth (equivalent to that occurs in hypobaric hypoxia, food restriction) on lung growth (including biochemical, morphometric, cytokinetic and functional aspects) in rats from 4 to 7 weeks of age was studied.

After 3 weeks of exposure, somatic growth was diminished in hypobaric hypoxic and normobaric hypoxic animals, but lung growth was accelerated. All absolute biochemical and morphometric measurements in hypobaric hypoxic and normobaric hypoxic rats were higher than undernourished animals indicating that augmented lung growth occurred by hyperplastic and hypertrophic changes with increased accumulation of collagen and elastin. Lung weight, lung volume, DNA, RNA, protein and desmosine were also increased compared to general controls. Maximal tritiated thymidine uptake occurred on day 3 and declined thereafter suggesting that lung growth stimulation occurred during early exposure. With the exception of endothelial cells (alveolar wall, arterial), the maximum response in other cells (type II pneumonocytes, interstitial cells, unidentifiable cells) in the central alveoli wall cells lagged behind and was lower than in the peripheral alveoli. After 3 day recovery, lung DNA synthesis reached the control levels. Pulmonary function tests showed that hypobaric hypoxia caused a decrease in expiratory flow rates (FEF corrected for FVC) and an increase in specific upstream airway resistance while in normobaric hypoxia, $FEV_{0.1}$/FVC%, expiratory flow rates (absolute and FEF

corrected for FVC, and PEFR) decreased but both absolute and specific upstream airway resistance increased. However, static compliance remained unchanged. In addition to above parameters, hypobaric hypoxia also caused an increase in collagen and alveolar surface area. These changes did not occur in normobaric hypoxia instead enlargement of airspaces occurred indicating overinflation of the lungs. The collagen concentration and elastic lung recoil at high lung volumes were also decreased in normobaric hypoxic animals.

Hypobaric normoxia caused slight reduction in somatic growth which was associated with decreased lung volume, but biochemical and morphometric parameters did not change. Morphometric unit structures were smaller. Peak lung growth stimulation occurred on day 5 in all the main cell types, but not in endothelial cells. Undernutrition impaired both somatic and lung growth as lung weight and volume, cell number and size, accumulation of connective tissue proteins, alveolar number, and alveolar surface area were decreased compared to controls. DNA synthetic activity in all the main cell types diminished. However, body weight normalized lung weight, DNA, alveolar surface area and total alveolar number were higher in undernourished animals than general controls.

These data suggest that lung growth stimulation occurs in normobaric hypoxia and hypobaric hypoxia despite an inhibitory effect of undernutrition. Accelerated lung growth at high altitude is primarily induced by low oxygen tension. However, the differences in hypobaric hypoxia and normobaric hypoxia and changes in normobaric hypoxia indicate that hypobaric pressure *per se* may play a role in lung growth adaptation at high altitude. Geometrical location of the central alveoli may limit their adaptive response compared to the peripheral alveoli. Synchronous endothelial cell stimulation in each component of the lung suggests that endothelial cells may respond to hemodynamic changes while the other cells respond to functional demand of the lungs. Although lung growth was increased in normobaric hypoxic and hypobaric hypoxic animals, pulmonary function tests observations suggested that it may be dysanaptic. The response to hypoxic stress is organ specific as growth of lung, heart and spleen increased, but liver and kidney followed the growth patterns of undernourished animals. Increased specific parameters of lung growth in undernourished animals

suggest that in conditions which compromise somatic growth, corrections made for body weight may lead to misinterpretation of true changes.

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DEDICATED

to

MY WIFE SATNAM

and to

OUR CHILDREN MYPINDER AND INDEEP

CHAPTER 1

INTRODUCTION

Prenatally, the lungs have no role in gas exchange. During birth they undergo alterations in functional status: a fluid filled organ begins to function as a gas exchanging organ. The fundamental architecture of the lung is established during fetal development, but the main changes in the terminal unit occur postnatally (214). In humans and other mammalian species, various environmental, nutritional and hormonal factors have been implicated as potential modulators of structural and biochemical changes associated with lung growth and development during postnatal life. These up and/or down, external and/or internal modulators control lung growth by stimulating various biochemical events. These events primarily include cellular proliferation and connective tissue accumulation with concomitant remodelling of the existing framework of the respiratory structures to form new functional respiratory units (6, 47, 94, 151, 173, 261).

Since efficient diffusion of gases between blood and alveolar air is a primary function of the lungs, a substantial role of oxygen as a regulator of postnatal lung growth has been postulated (45). A sizeable portion of world's population which resides at high elevations (>3000 m) has larger lungs and chest size, but smaller body weight and stature than sea level residents (273). An increase in altitude is closely associated with a proportional decrease in both ambient pressure and oxygen tension which may disturb a balance of oxygen supply and demand in the body. Although postnatal lung development may be genetically programmed, an imbalance of oxygen supply and demand in the body at high altitude may produce structural and functional alterations in the lung in order to facilitate adequate gas exchange between alveolar and vascular compartments. The high altitude model has also been commonly used to investigate the effect of hypoxia which occurs in a number of clinical conditions (e.g. pulmonary and cardiovascular diseases). The insight so gained by studying high altitude adaptation not only has direct implications, but it can also be applied to the more

complicated clinical problems.

A brief overview of normal human lung growth will be followed by a review of postnatal lung growth with an emphasis on rat studies. This will be followed by adaptive lung growth at high altitude. Because the effect of hypobaric hypoxia and normobaric hypoxia may or may not be similar, their effect on lung growth adaptation will be reviewed separately. Thereafter, the influence of other high altitude variables on lung growth and the effect of hypoxic stress on somatic growth and other organs will be discussed.

1.1. NORMAL LUNG GROWTH

At birth, in various animal species, the lungs are immature. They lack alveoli and other definitive components of adult lung structures. Investigators studying postnatal lung growth and development have observed that at birth, animals such as rats (47, 194, 275), mice (6), rabbits (97), cats (87, 97) and dogs (33) do not possess alveoli, which are the terminal air exchanging units in adult lungs. Instead, the lungs consist almost exclusively of primitive thick walled primary saccules (47, 275) or terminal sacs (33). These smooth walled primary saccules are large and lack surface complexity. The walls of these primary saccules have a double capillary network which runs on either side of the connective tissue framework (47). Postnatally, considerable maturation of the lung is required to attain full functional ability. After birth, the primary saccules undergo rapid subdivision by the formation of secondary crests which results in structural rearrangement to form true alveoli.

In humans, some researchers (33, 34, 94, 135, 214) have suggested that alveoli are absent in the fetus at the time of birth and the structures then present are saccules (135). However Loosli and Potter (173) observed that from 7½ months gestation until term, development of respiratory channels into alveolar ducts produced small but definitive alveoli. Alveoli-like structures can be found in the fetal lungs by 32 weeks gestation and definitive alveoli are present at 36 weeks of gestational age (41). While Dunnill (91) found 20 million alveoli in one infant, Thurlbeck and Angus (262) reported

71 million. Langston and Thurlbeck (159) documented a mean of 55 million and a range of 10-149 million alveoli at the time of birth. The conflicting results could be due to biological variations, difference in tissue preparation or difficulty in defining and recognizing alveoli (261). Whether the complement of acini at birth increases postnatally by simple enlargement or due to formation of new structures, has been controversial.

In adults, there is a range of 200-600 million alveoli (7). Alveolar surface area increases 12 fold and lung volume 20 fold from birth to adulthood. The constant number of alveoli per unit volume and alveolar size up to 2-4 years of age in children is an indication of alveolar multiplication; subsequently it has been noted that little or no increase in total alveolar number occurs (263). some investigators have suggested that later in childhood, alveolar multiplication continues, but at a slower rate and ceases entirely with somatic growth (261) while other have demonstrated that alveolar multiplication ceases between age 4 and 11 years (82, 91).

Postnatal lung growth and development has been more extensively studied in animals (6, 46, 47, 87, 97, 151, 242, 243, 275) especially in rats (46, 47, 151, 182, 242). According to Burri (46, 47), postnatal lung growth and development in the rat has three phases: lung expansion, tissue proliferation, and equilibrated growth. A fourth phase of lung growth has also been suggested to occur in some species (261).

(i) LUNG EXPANSION PHASE: This phase spans days 1 to 4 of postnatal life. During this period, lung growth occurs primarily by distension of the pre-existing structural units. Increase in lung tissue is minimal. Thus the volume proportion of air increases while the volume proportion of the lung tissue decreases (46). An increase in lung volume is similar to body weight (47, 182). Tritiated thymidine incorporation into DNA decreases on the first two days after birth in rats, with a subsequent increase on the third day (199).

In contrast, studies conducted on mice show an increase in lung tissue mass during this phase. The elongation of secondary crests, increased collagen and elastin, and enhanced mitotic index of interstitial cells by the third and fourth day of postnatal life also suggest an

increase in lung tissue (6). In another study (81), increased labelling index in the lungs of mice during the early period of postnatal life indicated that some cellular multiplication must have occurred. In rats, a prominent increase in the endothelial cell labelling index has been reported which remained at elevated levels until day 10 of life (151). These studies suggest that an increase in the lung tissue mass occurs during this growth phase in both mice and rats, but lung growth occurs predominantly by an increase in lung volume.

(ii) TISSUE PROLIFERATION PHASE: (days 4-13). In this phase, the subdivision of primary saccules occurs to form definitive alveoli (46). The rate of lung tissue growth is faster than that in the lung expansion phase. During this growth phase, specific lung volume is increased because lung volume increases relatively more than body weight. Septation increases alveolar surface area to the 1.6 power of increase in the lung volume (46). If the lung grew by simple expansion only, the surface area would increase to the two-thirds power of the change in lung volume. Therefore, the greater increase in alveolar surface area during this phase indicates the marked increase in complexity of lung structure and rapid multiplication of alveoli. Alveolar surface area and capillary surface area increase steadily from day 4 to 21 (46). During this phase, the first part of increase in lung tissue is due to tissue proliferation and the second part is due to rearrangement of the tissue mass (46). The subdivision of the primary saccules occurs by the formation of secondary crests. The secondary crests have elastin fibers in their free margins, a single capillary layer and interstitial cells. ^{3}H-Thymidine incorporation in the crest cells is 1.4 times that occurring in other parts of the saccule (151). Elastic tissue is thought to play an important role in the formation of alveoli. It has been shown that by day 3 alveoli are already being formed and by day 8 the rate has quickened so that there is a three-fold increase in alveolar density (182).

(iii) EQUILIBRATED GROWTH PHASE: This is the third phase of alveolar growth and begins at approximately 2 weeks of postnatal age (47). This phase is characterized by a slower increase in lung volume, resulting in a specific lung volume decrease. The rate of cell

proliferation falls but there is a rapid increase in alveolar and capillary surface areas as the tissue mass undergoes redistribution. However, there is a decrease in alveolar surface area per unit body weight. New alveoli continue to be added and the alveolar surface area increases directly with lung volume increase rather than to the two-thirds power as would be expected on the basis of simple expansion. The secondary crests lengthen, and only a single capillary layer can be found in the walls of the airspaces. Between days 21 and 131, lung volume increases six-fold; half of this increase occurs between days 21 and 44 of postnatal life in rats (46).

(iv) FOURTH PHASE: This phase only exists in some species (261). This phase of simple expansion of airspaces begins when alveolar proliferation ceases. However, the time at which the alveolar multiplication ceases in various species is under dispute. Continued somatic growth and lung growth throughout the life span are characteristic of rats (243). However, it has been claimed that alveolar multiplication is complete by 10 weeks of age in Sprague-Dawley rats (141). Whether this is true in all strains of rats is unknown.

The pattern of biochemical changes that occurs during growth and development in most body organs is the same. An increase in tissue mass of an organ may either occur by proliferation of cells, by hypertrophic changes in the cells or by both cellular hyperplasia and hypertrophic alterations (283). According to Winick and Noble (283), biochemical aspects of postnatal growth of an organ may also be divided into three phases in rats. During the first phase (birth to 17th day of life), the amount of DNA and protein increase rapidly, while the amount of protein per nucleus remains constant. In the second phase (3 to 5-7 weeks), DNA synthesis is lower than protein synthesis, resulting in an increase in protein to DNA ratio. In the third phase, both DNA synthesis and protein synthesis diminish significantly, thus, the protein to DNA ratio remains unchanged.

Besides hypertrophic and hyperplastic changes in the lung, lung weight may also increase due to accelerated connective tissue protein accumulation. Connective tissue comprises approximately 25% of the adult human lung. Collagen and elastin are the major elements of connective tissue.

Collagen represents 60-65% and elastin represents 20-25% of the total connective tissue; the rest being comprised of proteoglycans and glycoproteins. The amount of collagen varies widely; for example, in mice it comprises 6% of dry lung weight (162) and in humans it comprises 20% (37). Collagen is distributed throughout the extracellular space of the lung. In the lung parenchyma, collagen is present in the alveolar interstitium as well as epithelial and endothelial basement membranes. In the adult, most lung collagen is insoluble because of the extracellular covalent bonding of the collagen molecules, as well as between collagen and other components of the extracellular matrix (76, 126). Although collagen content (amount/dry weight) in the tracheobronchial tree and pulmonary vasculature is greater than that in the lung parenchyma, the bulk of collagen is present in the alveolar structures because the total mass of the parenchyma is much larger (109). The bulk of elastin is its amorphous component comprising approximately 90% of total mature elastin; the rest being the microfibrillar component. Similar to collagen, elastin content also has interspecies variations ranging from as low as 2% in rodents to about 25% in man (212).

The relationship between synthesis of connective tissue constituents and their accumulation in lung and alveolar formation is unclear. Emery has suggested that elastic tissue plays an important role in alveolar development (94, 95). Elastin fibers along with collagen are consistently found at the free margins of the secondary crests which subdivide primary saccules, and around the mouths of alveoli. The elastin and collagen network has often been referred to as the "fishnet" by investigators (95, 173). Emery and Fagan have postulated that alveolar multiplication occurs by three methods: segmentation, alveolarisation and compoundment.

(i) During segmentation, the primary saccules divide to form smaller units, which finally develop into alveoli. Emery and Fagan (95) found that after birth there is a progressive development of a "fish-net" of elastic-collagen structure, the apertures of which form mouths of alveoli. They suggested elastic-collagen tissue formation as the beginning of the development of alveoli. Therefore, Emery and Fagan (95) conceived of alveoli as appearing in the developing lung as though a balloon with a very pliant wall was inflated within and through a container

formed by a semirigid mesh.

(ii) Loosli and Potter (173), and subsequently Emery and Fagan (95) stressed the importance of connective tissue in the process of alveolarisation. The process of alveolarisation is also thought to develop in the non-alveolated walls of the airways. The respiratory bronchioles are converted to alveolar ducts and terminal bronchioles to respiratory bronchioles. However, the duration of alveolarisation and its relative importance is uncertain.

(iii) During compoundment, the elastic tissue fibers form and alveoli protrude between the elastic-collagen net, and the alveoli may grow into either of the saccules between which the wall lies.

Primarily, lungs function as gas exchangers. Unlike other organs, the lungs have a unique characteristic to gain air content per unit lung weight with or without an increase in weight (261). An increase in lung volume and finer subdivision of the structural units occur to provide larger gas exchanging area. Efficient gas diffusion across the alveolar wall is mainly determined by thickness of tissue barrier and gas exchanging surface area. During postnatal lung growth and development, the formation and maintenance of a gas diffusion barrier requires highly coordinated regulatory mechanisms. In order to determine potential regulators for the effective control of postnatal lung growth, a variety of experimental models (e.g. lung resection, blood flow alteration, hormonal treatments, exposure to various ambient gases, thoracic cage restrictions, ambient pressure changes, avulsion of nerve supply etc.) have been used. Although a number of studies have maintained that stretch is a major determinant of postnatal lung growth, it has also been suggested that lung growth may be largely controlled by oxygen consumption (45). To examine the effect of oxygen on lung growth, a variety of physiological (high altitude conditions, hyperactivity), pharmacological (drugs affecting body metabolism) and pathological (e.g. interstitial lung diseases, chronic bronchitis, asthma, pulmonary hypertension, congestive heart diseases) conditions have been studied. A number of experimental models have been used to understand the adaptive response of the body to hypoxic conditions. Unfortunately, the metabolic, structural and functional alterations which occur in the lungs

in pathological conditions cannot be reproduced using existing models. High altitude is a common and naturally occurring model to study the effect of chronic hypoxia. About 400 million people reside in mountainous regions; of those, a sizeable fraction dwell at high altitude (>3000 m). At an elevation of >3000 m, the majority of the individuals who live for short or prolonged duration exhibit clinical, physiological, anatomical and biochemical changes (273). Hence, to examine the sequence of adaptive structural and functional changes in the lungs and other body organs induced by high altitude exposure is of a significant importance.

1.2. ADAPTIVE LUNG GROWTH AT HIGH ALTITUDE

Adaptation is "a change which reduces the physiological strain produced by a stressful component of the total environment" (26). Adaptive changes which occur due to translocation from low to high altitude vary with time. The initial responses to acute change in the environment such as quick ascent or exposure in a hypobaric chamber results in *accommodation* which lasts minutes to hours. The physiologic adaptation response that occurs over a period of days to weeks or months exposure to a single factor of high altitude conditions is referred to as *acclimation*, while adaptation to a natural high altitude environment is called *acclimatization* (31). High altitude environment is a complex set of conditions including low ambient oxygen, low ambient pressure, cold, low relative humidity and rugged terrain. Although hypoxia is considered as a major stressor at high altitude, each condition (independently or in combination with others) may potentially affect the adaptive response of the body.

With regard to lung growth adaptation to one or a combination of high altitude factors, various approaches have been employed, including: exposure to normobaric hypoxia(18, 77, 164, 186, 198, 210, 247), hypobaric hypoxia (20), transporting species in question to high altitude (45), or studying the species inhabiting at high altitude over generations and comparing them to their counterparts at low altitude or sea level (163, 204, 205, 258). The observations, however, are controversial. Aside from differences in conditions, the disparity in results may also occur due to differences in methods,

such as: type of exposure (continuous or intermittent), severity of conditions (mild, moderate, severe), and duration of exposure (acute, prolonged, chronic). The age of animals at the time of exposure (newborn, young or adult), different species of animals, the methods of nursing the animals, and sampling and manipulation of the tissue, can all introduce inconsistency in the results.

Although conclusions made from controlled environmental studies regarding high altitude may not provide direct answers to biological questions, high altitude simulated studies help to analyze the effect of various conditions at high altitude in a known strain of a particular animal species. The effect of controlled environment, therefore, may be regarded as a study of metabolic adaptation. When animals indigenous to high altitude are compared to their low altitude counterparts, some differences may be detected due to evolutionary selection. The strain of animals at high altitude may be different from that at lower levels. This may be the result of genetic adaptation. Therefore, the inherited differences in the data may compound the problem when results from one study are extrapolated to another.

High altitude environment comprises of a number of variables which may influence the adaptive lung growth response. In the following text, the effect of each condition on lung growth adaptation will be reviewed separately.

1.2.1. EFFECT OF LOWER OXYGEN TENSION

The processes by which organisms alter their body structure and function to fit new environments have intrigued biologists since the initial finding by Babak (10). He found that when tadpoles or salamander larvae were raised in water with low oxygen tension, the gills became enlarged. Drastich (89) studied the functional and structural changes in gills of *Salamander Maculosa* after prolonged maintenance in 11% O_2 for one month. The enlargement of gills was also associated with flattening of the epidermis and distension of blood vessels. Increased average cell area and mitosis in gills of animals exposed to low oxygen have also been reported (29, 89). Oxygen tension falls with an increase in altitude. It is possible that adaptive growth of gas exchanging surface at

higher elevations is solely regulated by changes in ambient oxygen.

Lung adaptation to high altitude is characterized by a variety of structural and functional changes which collectively facilitate oxygen transport from the ambient air to the body tissue. The transfer of oxygen from the surrounding environment to blood is partially controlled by diffusion resistance in the lung. Thus, the larger the gas exchanging surface area the smaller the resistance to gas diffusion. An increase in internal surface area is dependent upon an increase in lung volume and internal partitioning of the respiratory units. Through internal partitioning, the lung attains a gas exchanging surface area approximately 40 times as great as body surface area (274). According to Tenney and Remmers (257), the postnatal compartmentalization of the lung appears to be so controlled that the metabolic requirements (which varies with body surface area) and the alveolar surface area for O_2 uptake are matched quantitatively in all mammalian species.

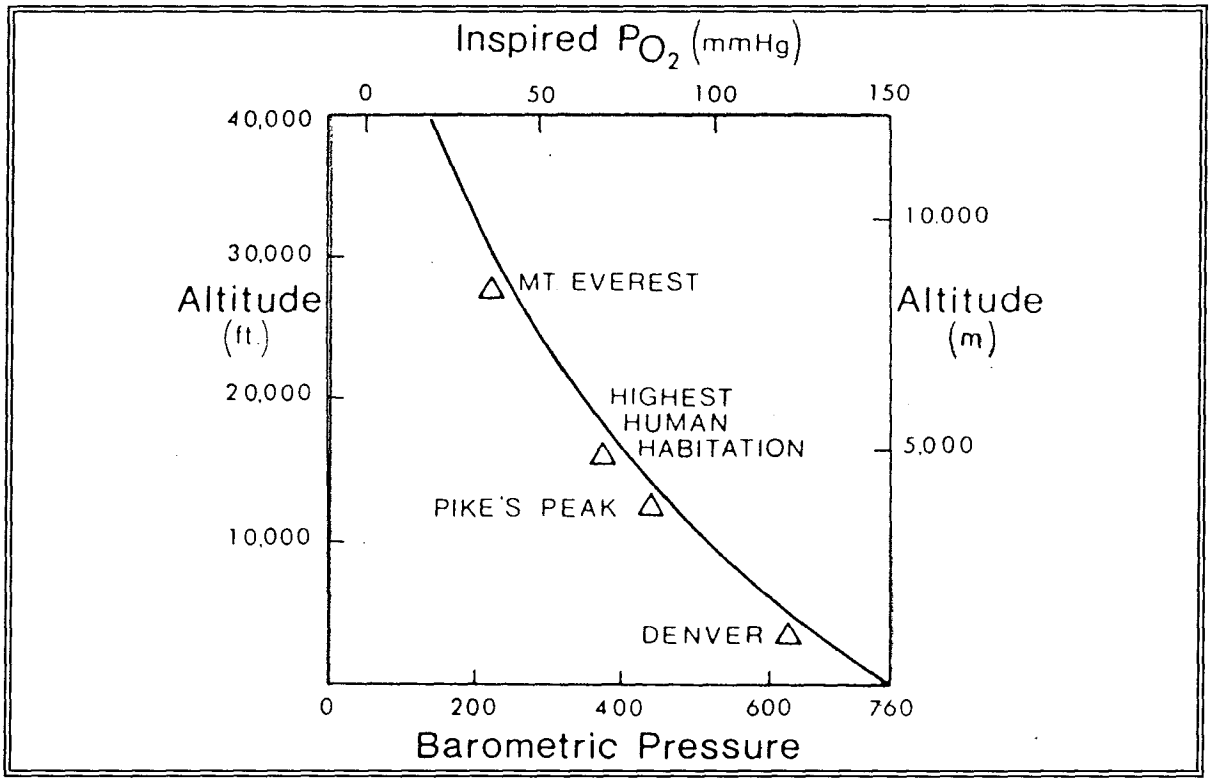


Figure 1. Relationship between altitude, barometric pressure and oxygen tension [West (281)].

Since ambient oxygen tension decreases automatically with a decrease in barometric pressure (Figure 1), both hypoxia and hypobaric pressure are considered together to study the effect of low

oxygen, but their effect may be different. Therefore, with regard to structural and functional adaptations in the lung, the effect of hypobaric hypoxia will be considered separately from normobaric hypoxia.

1.2.1.1. HYPOBARIC HYPOXIA

1.2.1.1.1. STRUCTURAL ADAPTATIONS

In earliest investigations, studies relating to high altitude acclimatization documented that Cholos Indians of Peru (native to high altitude), had short stature, low body weight, wide barrel-shaped chests and larger lungs compared to their counterparts at sea level (12, 134). Later, using Wistar male rats, it was shown that in animals exposed to hypobaric hypoxia (390 mm Hg), the length of the chest was smaller, but the width was not (chest radiographs), thus giving a *barrel-shaped* appearance to animals with stunted somatic growth (145). The authors suggested that this may have occurred because the lungs of hypobaric hypoxic rats were being held at a higher volume. Subsequent studies performed on humans have confirmed that high altitude residents have higher lung volumes (32, 84, 107, 122, 130, 146, 215). Using animals, investigators have demonstrated that translocation to higher elevations (45) and exposure to experimental hypobaric hypoxia (20) produce increases in absolute or specific (relative to body weight) lung weight and lung volume.

An increase in lung volume may be accompanied by the addition of alveoli or enlargement of existing airspaces. Increase in alveolar number may occur at a higher rate compared to lung volume, thus increasing the complexity of the lung, or at a slower rate which may be proportional to or lower than an increase in lung volume. In the latter case, surface to volume ratio may remain unchanged or decrease. Among the earlier studies, Hurtado (146) examined the lungs of rabbit, dog and human at high altitude, and found that alveolar dimensions were bigger than in the lungs of sea-level counterparts, but no units or methods of inflation and fixation of tissue were described. Later Cohn (66) observed increases in lung weight and alveolar surface area in rats following 2 weeks exposure

to high altitude simulated conditions, and remarked that these structural changes occur as a part of accommodation to high altitude.

Using comprehensive stereological techniques, Bartlett and Remmers (20), and Burri and Weibel (45) performed quantitative assessment of lung growth in rats exposed to high altitude and experimental hypobaric hypoxia and drew similar conclusions that exposure to hypobaric hypoxia stimulates lung growth but inhibits somatic growth. Bartlett and Remmers (20) observed that one month old rats exposed to simulated altitude of 4200 m (450 mm Hg) showed a significant increase in lung weight after 7 to 21 days of exposure. After 21 days of exposure, significant increases in lung volume and alveolar surface area were also observed, but mean alveolar diameter was not influenced, suggesting that lung growth occurred by formation of new alveoli rather than by enlargement. In the authors' opinion, the response to hypobaric hypoxia at day 7 was an increase in lung density (lung weight per unit lung volume) without alveolar proliferation. If the exposure continued, the density of the lung returned to the normal level and alveolar proliferation occurred at a faster rate than controls. Although they found no increase in lung blood volume following exposure to hypobaric hypoxia, they did not rule out the possibility of residual blood contributing to the lung weight increase. Burri and Weibel (45) transported 23 day old rats to high altitude (3450 m) for 21 days and compared the adaptive lung growth to those at low altitude (570 m). Absolute lung volume increased and body weight gain decreased in high altitude rats. The authors suggested that the unchanged relative volume proportions of the lung compartments favoured the argument that increased lung volume occurred due to the addition of new respiratory units. In high altitude rats, the specific alveolar, capillary and tissue volume increased by approximately 20% and so did alveolar surface area. Although conditions leading to hypoxia in both studies (20, 45) were different (hypobaric hypoxia vs high altitude), the adaptive lung growth response was similar. This may suggest that at high altitude, hypobaric hypoxia may be the main regulator of lung growth.

Tenney and Remmers (258) suggested that the diffusing capacity of the lung is proportional to the internal alveolar surface area. Since capillaries occupy a major fraction of the alveolar wall area,

a measure of the area of blood-filled pulmonary capillaries will be a more direct assessment of increased pulmonary diffusing capacity (258). Weibel (276) developed a model to calculate pulmonary diffusing capacity using structural parameters: alveolar and capillary surface areas, capillary volume, and the harmonic mean thickness of the tissue barrier and the blood plasma layer (which separates red blood cells and endothelial cells). Burri and Weibel (45) estimated morphometrically determined pulmonary diffusing capacity for oxygen in rats exposed to hyperoxia and high altitude. They found that after 3 weeks, the pulmonary diffusing capacity for oxygen in high altitude rats (PO_2 100 mm Hg) increased from 0.6 ml.min $^{-1}$.mmHg^{-1}.100 g^{-1} to 0.72; whereas it decreased to 0.51 in hyperoxic rats (PO_2 290 mm Hg). This indicates that alterations in ambient O_2 produces structural changes in the lung during the growth period.

Although the information obtained from animals following translocation to high altitude or exposure to experimental hypobaric hypoxia helps to understand the process that regulates the physiological adaptive response, information regarding genetic adaptation in populations residing at higher elevations is lacking. Lechner (163) conducted an interesting study to investigate the differences in genetic and metabolic adaptation. Lechner (163) studied adaptive lung growth in adult sea-level native fossorial pocket gophers (*Thomomys bottae* at 265 m, weight-matched, age unknown) following exposure to normobaric hypoxia (equivalent to the oxygen concentration at 3400 m) for three months. Both sea-level and normobaric hypoxic pocket gophers were compared with high altitude native pocket gophers (*Thomomys umbrinus melanotis* at 3200 m). Compared to sea-level gophers, the alveolar fraction and specific alveolar surface area were greater in high altitude native and normobaric hypoxic pocket gophers. Although these findings suggest that lung growth in high altitude natives is influenced mainly by decreased oxygen, the author concluded that the mechanism of adaptation at the tissue and blood level differed in high altitude natives and normobaric hypoxia-acclimated animals. Hematocrit increased only in normobaric hypoxic gophers, whereas blood oxygen carrying capacity and skeletal muscle myoglobin concentration were higher in high altitude native gophers. The variable response may either be attributed to difference in environment [hypobaric

hypoxia (high altitude) and normobaric hypoxia] or to difference in genetic and metabolic adaptations. In another study, lung volume of high altitude (4660 m) native mice (*Phyllotis Darwini*) was greater than the same species at sea level (204). Since the number of Type II pneumonocytes, interstitial cells and alveolar macrophages, and capillary leucocytes nuclei per unit alveolo-capillary volume was equivalent in both groups, the authors speculated that higher lung volume in high altitude native mice may have resulted from a greater number of structural units in the lung.

Most of the information available relating to high altitude adaptation in humans has been obtained from pulmonary function tests. Only one group of investigators has performed morphometric analysis on lungs of highlanders to determine lung structural alteration at high altitude. Saldana and Garcia-Oyola (230) studied pulmonary structural differences in males who lived at high altitude (altitude 12771 feet) and compared them to males of same age at sea level. They observed that males at high altitude had a greater number of alveoli and alveolar surface area. The mean alveolar diameter in high altitude subjects was also larger than in sea level subjects. The authors concluded that a larger residual volume of the lung and hyperventilation at high altitude may be associated with a greater number of alveoli, larger alveolar surface area and larger alveoli.

1.2.1.1.2. INTERSPECIES DIFFERENCES

The capability to acclimatize to high altitude conditions varies from species to species and even within species (interstrain) due to genetic anatomical and physiological differences. For example, guinea pigs are unique animals to study for lung growth adaptation to high altitude. They have inhabited the Andean mountains for thousands of years at elevations up to 4500 m (244) and have also been raised at sea level for many generations. Compared to guinea pigs, the lungs of rats are immature at birth. Furthermore, guinea pigs have the advantage of higher oxygen affinity for arterial blood than rats. At an altitude of 5000 meters, the arterial blood oxygen saturation in guinea pigs is more than 75%, but in rats, it is about 55% (16). Arterial blood of llamas at an altitude of 5000 m is more than 90% saturated with oxygen (16). Such structural and functional variations between the

species may determine the ability of an animal to adapt to variable ambient conditions.

Postnatal lung growth adaptation in newborn rats and guinea pigs to simulated conditions equivalent to 3000 m and 5000 m elevation was studied until the age of 50 days (16). In rats, absolute lung weights increased at 3000 m but were decreased at 5000 m at the age of 21, 35 and 49 days. However, compared to the controls, the specific lung weight in both 3000 and 5000 m groups was lower on day 21 and higher on days 35 and 49. On the other hand, in guinea pigs exposed to 3000 meters, lung weight increased on days 11 and 23 but body weight remained unaffected; whereas lung weight of guinea pigs exposed to the equivalent of 5000 m remained unchanged, but body weight decreased. In another study, when interspecies comparisons were made, the llama (inhabits at high altitude) had smaller mean alveolar diameters compared to other species of similar body size such as the domestic cow, western pronghorn antelope, and domestic goat (258). Therefore, it appears that animals such as guinea pigs and llamas, perhaps due to their anatomical and physiological differences, are able to withstand stress of severe simulated high altitude conditions better than rats.

1.2.1.1.3. CELL KINETICS AND MORPHOLOGICAL CHANGES

The lung tissue mass may increase due to hyperplastic and/or hypertrophic changes in the cells, enhanced accumulation of connective tissue matrix proteins and lung water content. No information is available about biochemical alterations which occur in lung structure during the high altitude adaptive response. Cellular kinetics and morphological changes in the lung have been studied but information is limited. The heterogeneous cell population of the lung of approximately 40 different cell types is mainly distributed in three compartments: conducting airways, pulmonary vasculature and the gas exchanging apparatus. Besides migratory cells, the cellular composition of the gas exchanging surface mainly consists of alveolar epithelium (type I pneumonocytes, type II pneumonocytes), alveolar wall capillary endothelium and interstitial cells (fibroblasts, smooth muscle cells, monocytes, plasma cells, macrophages, pericytes and a number of indeterminant cell types).

The alveolar epithelial type I cells are anatomically specialized to provide a minimum barrier to gas diffusion as their very thin cytoplasmic extensions line approximately 97% of the alveolar surface area. They are considered as terminal cells and thus do not have the ability to proliferate and replicate. The type II epithelial alveolar cells which represent ~15% of total lung cells in the parenchyma have two major functions that are critical in maintaining the integrity of alveoli. They have the ability to readily proliferate and differentiate into type I cells, and they produce surfactant which prevents alveolar collapse. These cells provide approximately 3% of the alveolar surface area and have a relatively long turnover time which ranges from 20 to 84 days (100, 245). Pearson and Pearson (205) demonstrated that aside from having larger nuclei and greater volume of mitochondria, type II pneumonocytes also have more and larger lamellar bodies in mice (*Phyllotis Darwini*) native to high altitude (4660 m) compared to those at sea level, resulting in four times more surfactant. Species having a high respiratory frequency rate have more surfactant. Increased surfactant in mice native to high altitude may occur due to hyperventilation.

Endothelial cells line the extensive intra-alveolar capillary network and make up ~36% (distribution of cells in the alveolar wall differs among various species) of total lung cells, and have a higher turnover rate compared to epithelial cells. These cells are permeable to water and electrolytes, but to some extent form a barrier to high molecular weight solutes. Mesenchymal cells are found within the interstitial compartment of the alveolar wall and represent ~38% of the total lung cells. These cells are important to the synthesis of the major connective tissue proteins found in the alveolar wall.

The kinetics of cells is generally studied either by pulse labelling DNA with a radio-labelled thymidine such as tritiated thymidine (^{3}H-TdR), or using stathmokinetic agents such as colchicine and vinca alkaloids (Vinblastine, Vincristine) to arrest dividing cells in metaphase. Thymidine is incorporated into cellular DNA while the cell is in S-phase of the cell cycle preceding mitosis. The uptake and incorporation of ^{3}H-TdR by the cell nuclei, however, does not necessarily reflect cell division, but is mainly considered an indicator of DNA synthesis. Increased DNA synthesis per se

does not indicate cellular proliferation or an increase in cell number. Binucleate cells appear after birth in certain organs and increase in frequency with age (287). Multinucleate cells and polyploidy may occur as a result of increased DNA synthesis without an increase in cell replication. Tritiated thymidine incorporation can be evaluated by radiochemical or autoradiographic techniques. The generation cycle and duration of individual phases of the cell cycle can be analyzed by ^{3}H-TdR pulse labelling and treatment with stathmokinetic agents simultaneously. Volkel et al. (271) studied the onset and kinetics of cellular proliferation following exposure to hypobaric hypoxia (440 torr) in adult female Wistar rats after 3, 6, 9, 12, 20 and 35 days of exposure, and then compared this with the results of 3, 12 and 35 day controls of similar starting age. They found that maximum in vitro DNA synthesis occurred on day 9 of exposure and measured 220-345% of controls extrapolated from days 3 and 12. Lung DNA synthesis reached control levels between day 15 and 20 of exposure.

The various cell populations in the lung may respond to the hypobaric hypoxic stimulus differently. Niedenzu and coworkers (193) observed an 85% increase in ^{3}H-TdR incorporation in rats exposed to 440 mm Hg for 9 days. The labelling index of bronchial epithelial cells and vascular smooth muscle cells increased in hypobaric hypoxic animals compared to the normobaric animals. The labelling index of alveolar interstitial cells remained unchanged. In the authors' opinion, the cells with normally very low labelling index (bronchial epithelial cells) were stimulated, whereas the cells with already high labelling index (interstitial cells) remained unaffected. Since DNA synthesis was only increased in the lung and not in the liver and kidney, the effect of hypoxia, in the authors' opinion was organ specific. Another group of investigators (181) observed that in adult rats, hypobaric hypoxia induced maximal stimulation in cells of arteries and alveolar walls on days 3 and 5 of exposure. On the other hand, Hunter and coworkers (145) found no significant change in lung cells labelled with ^{3}H-TdR in young mice or rats exposed to hypobaric hypoxia (390 mm Hg) for 1 and 4 weeks. By contrast, the labelling index of lung cells decreased in old rats (56 days old) compared to young rats (35 days old). Therefore, the sequence of cytokinetic events in the lung following exposure to hypobaric hypoxic stress remains unclear.

1.2.1.1.4. FUNCTIONAL ADAPTATION

As stated earlier, highlanders have large thoracic cages relative to sea-level residents (12, 134). Lung growth adaptation to high altitude has been assessed by using a variety of physiological techniques in humans and animals born and raised at high altitude and then comparing the results to their counterparts at sea level. Others have observed the effect of sojourn at high altitude on lowlanders. However, the results described in the literature are controversial.

A number of investigators have reported that high altitude natives have higher vital capacities (32, 39, 84, 107, 122, 130, 146, 215) whereas others (56, 75, 153, 256) observed reduction in vital capacity. Cerretelli (56) found that vital capacity (VC) was reduced by 10% during a Himalayan expedition, after 30 days sojourn between 3800 and 5000 meters. The reduction in VC was not influenced by oxygen breathing. In the author's opinion, this reduction in VC could be attributed to an increased inspiratory tone as once described by Peyser et al. (209), which could also have increased residual lung volume. Changes in FRC that take place in humans following exposure to high altitude have been controversial; while some investigators have reported an increase in FRC (75, 146, 232, 256), others did not find any change (11, 70, 153).

Brody et al. (39) studied lung adaptation in 17 to 20 year old residents of the Peruvian mountains (3850 m), and found that the lung volume of highlanders was 30-35% greater than lowlanders (600 m) of similar race, age and body size. The vital capacity of Peruvian lowlanders was only 84% of Caucasians but vital capacity of mountain dwellers was 116% of Caucasians. Lung elastic recoil at functional residual capacity and size-corrected pressure-volume curves were similar in Peruvian highlanders, lowlanders and Caucasian subjects of the same age, suggesting that neither increased muscle strength nor alterations in connective tissue properties of the lung were primarily responsible for large vital capacities in highlanders. Despite higher lung volumes in highlanders, absolute maximum expiratory flow rates were not increased and, when expressed as a function of lung volume, flow rates were actually decreased. In the authors' opinion, the crossectional area or volume of the airways did not participate in lung growth enlargement in highlanders. The authors suggested

that the difference in flow rates may have been an expression of the manner in which hypoxia stimulates the growth of the lung. Brody et al. (39) concluded that greater lung volumes observed in Peruvian mountain dwellers resulted from postnatal acclimatization to the hypoxic environment, rather than from genetically determined lung growth. Other investigators have found no difference in lung compliance between high and low altitude residents, or when either lowlanders translocated to high altitude or highlanders moved to sea level (39, 75). Lahiri et al. (157) studied respiratory control and vital capacity in highland infants, children, and adults and in the authors' opinion, the environment is the major determinant of pulmonary acclimatization at high altitude.

Table 1. Summary of previous studies: somatic and lung growth adaptation following chronic exposure.

	Previous studies					
	1	2	3	4	5	6
Experimental conditions						
Ambient Po_2 (Torr)	78	95	105	91-98	80	80
Ambient P_B (Torr)	750	450	497	760	630	630
Species	Rats	Rats	Rats	Rats	Guinea pigs	
Age before exposure (weeks)	4	4	3	9	3	3
Age after exposure (weeks)	6	7	6	12	5	17
Observations						
Body weight	⇓	⇓	⇓	⇓	⇒	⇒
Lung weight	⇑	⇑	n/a	⇑	n/a	n/a
Lung volume	⇒	⇑	Sp⇑	Sp⇑	⇑	⇒
Alveolar surface area	⇒	⇑	Sp⇑	⇑	⇑	⇒
Capillary surface area	⇒	n/a	Sp⇑	n/a	⇑	⇒
Alveolar multiplication	No	Yes	Yes	No	n/a	n/a

References: 1. Bartlett (18); 2. Bartlett and Remmers (20); 3. Burri and Weibel (45); 4. Cunningham and coworkers (77); 5-6. Lechner and Banchero (164, 167). Sp (per unit body weight), n/a (not available).

1.2.1.2. NORMOBARIC HYPOXIA

Although yet to be precisely determined, it is widely believed that the adaptive lung growth

response to decreased ambient oxygen is the same, whether low oxygen is delivered by decompression or by gas mixture. Aside from studying lung growth adaptation at high altitude, normobaric hypoxia has also been used as a model to study lung growth alterations in various conditions which may result in hypoxia and/or hypoxemia. While studying the adaptive response to normobaric hypoxia, some investigators have concluded that it accelerates lung growth (77, 101, 102, 164, 167) whereas others have either found no change in lung growth (18, 247) or conversely, that it was inhibited (187). The controversies may exist due to the reasons mentioned earlier.

Bartlett (18) made a quantitative assessment of lung growth in month old male Sprague-Dawley rats after 15 days of exposure to normobaric hypoxia (10.4% O_2) and normobaric hyperoxia (45.8% O_2). The author found that except for depressed somatic growth and increased specific lung weight, lung growth in hypoxic conditions remained unaffected. The investigator (18) attributed the increase in specific lung weight to an increased residual volume of blood remaining in the pulmonary vessels of the low oxygen rats. On the other hand, somatic growth of hyperoxic animals was not affected, but lung growth was diminished. The author commented that prolonged normobaric hypoxic exposure could have caused structural changes in the lung. However, this hypothesis was not supported by Cunningham and associates (77), who showed that the adaptive lung response to normobaric hypoxia (12-13% oxygen) is dependent upon the age of the animal at the time of exposure, and that exposure beyond 3 weeks did not cause further increase in lung growth parameters. The authors (77) found that whereas three weeks of exposure caused significant increase in size and number of alveoli in newborn rats, it produced an increase in alveolar size but not number in adult rats. An increase in alveolar surface area was also less in adults than in young animals. It has been demonstrated that even a short exposure of six days produced an increase in mean chord length of alveoli (198). It appears that normobaric hypoxia causes an increase in size of alveoli in both adult and young animals but it produces an increase in number of morphometric unit structures only in growing animals. This suggests that the type of adaptive response to normobaric hypoxia varies with age, but it remains to be shown whether there is a lower limit of environmental oxygen below which lung growth regulation

no longer functions.

The observation made by Cunningham and associates (77) that the adaptive lung growth response is limited despite continuous exposure to low oxygen, was further confirmed by Lechner and Banchero (164). It is possible that hypoxic stress accelerates lung growth to reach adult lung dimensions, and that physical restrictions may limit further lung growth. Lechner and Banchero (164) tested this hypothesis and showed that male guinea pigs acclimated to normobaric hypoxia (Po_2 80 Torr) for 3 weeks had significantly increased lung volume (32%) and alveolar surface area (27%) compared to controls of similar body mass. The differences between the normobaric hypoxic and normoxic guinea pigs progressively reduced with an increasing exposure period. The hypoxia-induced effect on lung growth adaptation ceased despite continued hypoxic exposure when the body weight was equivalent to or more than 900 g. The authors concluded that maximal size of the chest which is achieved at an age of 16-20 weeks ultimately constrained accelerated lung growth because no thoracic space was available for further lung development.

Morphometrically calculated pulmonary diffusion capacity remains unchanged following acute (7 days) exposure to normobaric hypoxia in rats (247) or chronic (14 weeks, Fo_2 0.126) exposure (164) in guinea pigs. The interstitial tissue thickness increased after acute exposure (164, 247) but it was no longer evident if exposure to hypoxia was prolonged. This change in the interstitium may have occurred due to the appearance of small electron-lucent areas indicating fluid accumulation in subendothelial locations (247). It is likely that initial thickening of interstitium is followed by thinning of tissue barrier as the lung undergoes reconstruction and structural remodelling.

Low oxygen has been shown to inhibit DNA, RNA and protein synthesis (57, 191, 252) in organs other than lung and heart. Sjostrom and Crapo (247) reported that rats maintained in 10-11% O_2 and mice in 10% O_2 for 7 days showed increases in lung weights of 31% and 28% respectively. Even though morphometrically no differences were found, in rats the total amount of protein and DNA was increased by 17% and 21% respectively. Increase in protein in part may be due to pulmonary edema, but the investigators (247) found increased endoplasmic reticulum and free polyribosomes in

the capillary endothelium suggesting increased protein metabolism. These findings may indicate that normobaric hypoxia increased protein synthesis and cellular proliferation. Another group of investigators (101) also found that an exposure to normobaric hypoxia for 14 days produced a significant increase in lung DNA in pregnant rats indicating hypoxia-induced lung growth stimulation in both young and adults. However, the other changes in the biochemical parameters such as connective tissue proteins are yet to be investigated. On the contrary, lung DNA decreased in newborn rats exposed to normobaric hypoxia, indicating that newborns may not have developed the required mechanisms to tolerate the hypoxic stress. This requires further investigation (187).

An increase in FRC in rats (13), rabbits (209), cats (209), dogs (30) has been reported following exposure to acute or chronic normobaric hypoxia. After exposing 4 week old male Wistar rats to hypoxia of 8%, 10%, and 12% O_2 in isobaric chambers for 3 weeks, Barer et al (13) observed an increase in FRC of 34-62%. In the authors'(13) opinion, chronic hypoxia did not increase FRC by making the lungs more compliant because static compliance did not change. They speculated that structural factors in the chest wall could have been responsible for the increase in FRC. In another study (77), lung compliance also remained unchanged regardless of age of the animals at the time of exposure to normobaric hypoxia. These findings suggest that normobaric hypoxia may not cause any structural changes in lung tissue.

Why decreased ambient oxygen stimulates growth only in some organs and not in others remains still an enigma. With regard to initiation and control of lung growth in a reduced oxygen environment, various biological and physical factors have been proposed but the mechanisms by which these factors regulate lung growth are yet to be understood. Hypoxic exposure also results in hyperventilation as the rate of breathing and tidal volume are increased (197, 200). In normal conditions as well as in conditions which stimulate lung growth, stretch has been implicated as a major regulator of lung growth. In hypoxic conditions hyperventilation may induce lung growth due to mechanical distortion of the lung tissue. Although other investigators (20, 207) studying lung growth in hypercapnic animals have ruled out the role of hyperventilation, Faridy and Yang (102)

demonstrated that the effect of hypoxia produces lung growth both by direct and indirect stimulation. The authors showed that while normobaric hypoxia enhanced DNA synthesis prior to an increase in lung volume, hyperventilation produced by higher ambient CO_2 increased only lung volume. In their opinion, lung tissue growth stimulation in hypoxia occurs by a direct effect of low PO_2, but an increase in lung volume occurs as a consequence of hyperventilation due to mechanical stimulation. This sequence of events is in sharp contrast to those proposed by Berger and Burri (23). The authors claim that after partial pneumonectomy, air space distension is followed by cell proliferation, tissue remodelling, and finally restoration of normal tissue architecture. This suggests that mechanical distortion of the tissue induced the cellular stimulation. Therefore, the role of hyperventilation and stretch in lung growth in hypoxic stress remains unclear.

It has been well documented that an exposure to low oxygen produces hemodynamic alterations (e.g. increased heart rate, cardiac output, central blood volume, hemopoiesis, hematocrit, hypertension). Since blood flow is increased in hypoxic conditions, alterations in organ blood flow characteristics have been suggested as regulating organ growth in hypoxic stress. Lechner and Banchero (164) suggest that a transient increase in pulmonary blood flow may occur during hypoxic exposure until hematological changes are achieved to compensate for reductions in ambient or arterial oxygen. Oxygen carrying capacity of blood increased by only 40% (166); this would not by itself account for increased lung growth. They suggested that increased pulmonary blood flow during early exposure may have enhanced lung growth. Another study supports the view that increased blood flow influences lung growth because lung weight increased (48%) four weeks subsequent to ligation to the right main pulmonary artery (254). Tucker and Horvath (266) subjected Sprague-Dawley albino rats to 440 mm Hg from 31 to 68 days of age. Increases in hematocrit and hemoglobin concentration, and increases in absolute weights of ventricles of the heart, lungs, spleen and adrenals occurred, while kidneys remained unaffected. Blood flow per g tissue and total organ blood flow increased in the lungs, heart and adrenals, but remained unchanged in kidneys. The authors inferred that an increase in organ weight is associated with circulatory adaptations in order to prevent the hypertrophied organs

from hypoperfusion during hypoxic exposure. Alternatively, augmented growth in organs which receive increased blood flow in hypoxia may support the hypothesis that increased blood flow removes or lowers the concentration of local tissue-specific growth inhibitors or chalones (103). Consequently, growth of a respective organ is stimulated. It has been suggested that increased pulmonary blood flow and pressure act directly or indirectly to stimulate endothelial cells to release endothelial-derived growth factor(s). These act as paracrine factors to increase mitogenic activity and connective tissue synthesis in the pulmonary arteries (221).

1.2.2. EFFECT OF REDUCED AMBIENT PRESSURE

It has been well known from balloon ascents that barometric pressure decreases as the height increases. This decrease in pressure of air on body was once described by De Saussure in 1786 as a mechanism for acute mountain sickness by causing "relaxation of blood vessels". More than a century ago, Bert ruled out the possibility that low ambient pressure plays any significant role in causing acute mountain sickness (characterized by headache, fatigue, dizziness, palpitation, nausea, loss of appetite, and insomnia (282)) as it could be relieved by oxygen breathing. However, it has recently been shown that a rapid descent, or use of Gamow bag (patient is exposed to increased pressure by means of a pump) is more effective in relieving mountain sickness than simple administration of supplemental oxygen (124, 282). This implies that low ambient pressure plays a significant role in the etiology of mountain sickness along with hypoxia and hypocapnic alkalosis (121). The effect of hypobaria per se is not known, but it appears that it causes hypoxemia. Levine and associates (171) found low arterial Po_2 in hypobaric normoxic and hypobaric hypoxic sheep compared to normobaric normoxic and normobaric hypoxic animals respectively. The same group of investigators later observed that hypobaric hypoxic sheep required 65% oxygen to make them hypobaric normoxic instead of 49.7% oxygen (137). How hypobaria affects the oxygenation of blood is not known.

No direct evidence is available that hypobaria per se at high altitude induces change in lung

or body growth adaptation. A group of investigators using the same rat species have observed that while normobaric hypoxia (18) produced no significant change in any of the morphometric parameters of the lung, hypobaric hypoxia (20) accelerated lung growth because lung weight, lung volume and alveolar surface area increased. Although ambient Po_2 used and duration of exposure in both studies were not the same, one may speculate from the variable lung growth response in normobaric hypoxia and hypobaric hypoxia that hypobaric pressure may have induced changes in the lungs by itself or in association with hypoxia. However, the effect of low ambient pressure on lung growth remains to be investigated (Table 1).

1.2.3. EFFECT OF EXERCISE

The rugged terrain at higher elevations may increase work load and whole body oxygen consumption. The internal alveolar surface area of the lung correlates in a linear fashion with the rate of resting O_2 consumption of mature animals (257). In mammals, lung volume is proportional to body weight, but the extent of internal partitioning is directly proportional to the metabolic rate (257). Thus, animals with greater oxygen demand have the smallest alveoli and the internal surface area per unit of lung volume is large.

The influence of exercise on human and animal lung structure and function has been investigated (17, 65, 108, 112, 265). Tiemann (265) postulated that physical exercise led to enlargement of the chest cage and accompanying lung inflation, resulting in chronic distension of the structural units, which in turn stimulated proliferation of interalveolar septa. It has been shown that after subjecting 10 month-old rats and guinea pigs to strenuous swimming (210 minutes/day) for 122 days, extensive pulmonary changes occurred, including increases in total lung weight and number of alveoli attributed to finer subdivisions, and thickening of the alveolar walls (112). By contrast, rats subjected to exhaustive exercise on a treadmill for 20 days showed no change in lung growth or compartmentalization as all parameters of lung growth (lung weight, lung volume, alveolar surface area or alveolar number) remained unchanged (17). In another study (108), investigators showed that rats

subjected to swimming during the second month of postnatal life had greater alveolar densities and alveolar surface area. They concluded that swimming induced alveolar proliferation. Alveolar proliferation was not related to the intensity of training but was influenced by the age of the animal at the time of training.

The effect of increased oxygen demand on lung growth has also been studied by using hyperactive animal models. Geelhaar and Weibel (111) demonstrated that increased alveolarization in Japanese Waltzing Mice was influenced by hyperactivity (JWM, exhibit a continuous waltzing motion due to a genetic defect in their vestibular apparatus) compared to control mice which were of different species. Contradicting that hypothesis, Bartlett and Arenson (21) suggested that increased lung growth in JWM is genetic rather than induced by sustained hyperactivity because they found no differences in lung dimensions between JWM and their phenotypically normal litter mates. Using IDPN (imino-BB'-dipropionitrile) which is known to cause hyperkinesia, Burri et al. (48) and later Hugonnaud et al. (142) showed increased lung growth in hyperactive mice, thus supporting the hypothesis put forth by Geelhaar and Weibel (111). They found increased specific lung volume and morphometrically calculated pulmonary diffusion capacity. However, one might argue that these results might just as well reflect a direct influence of this drug on lung development, rather than a true adaptation to increased oxygen consumption. Hence, experimental evidence is yet insufficient to establish convincingly that altered oxygen consumption can induce significant changes in lung growth pattern.

1.2.4. EFFECT OF COLD

Although various experimental approaches such as drugs, hormones, or genetically determined hyperactivity have been undertaken to study the effect of modified oxygen consumption and demand, cold is considered as more of a "physiological" experimental model to assess the adaptive response to increased oxygen consumption (3, 131, 203). Cold is an important parameter at high altitude which is often overlooked. The temperature falls by about 0.6^{o} C for every 100 meter increase in altitude, regardless of latitude (132, 273). Somatic growth observations made following exposure to cold in

small mammals are controversial. Some investigators have observed decreased somatic growth in lower temperatures (14, 58, 133, 250), whereas others have not (8, 113, 129, 167, 196). It has been suggested that skeletal growth retardation possibly occurs due to reduction of blood flow to the skeletal tissue during exposure to cold (168).

Increased oxygen utilization during exposure to cold acts as a stimulus to cause accelerated lung growth in order to meet the body's oxygen consumption requirements by increasing the pulmonary diffusing capacity. Gehr et al. (113) tested this hypothesis and showed an increase in oxygen consumption without any change in body growth in seven week old rats exposed to 11° C for three weeks. The specific lung volume of cold-exposed rats increased by 24% and was also associated with larger alveolar and capillary surface area, but the capillary volume density and the barrier thickness remained unchanged. In the authors' opinion, this may occur by an increase in the number of structural units and their complexity in proportion to lung volume. They suggested that increased stress, to which the growing lung tissue is subjected, may directly or indirectly cause lung growth alterations.

Guinea pigs which have been inhabitants of higher altitude for generations also showed accelerated lung growth following chronic exposure to cold temperature (5° C) from 2-18 weeks of age when body weight was <600 g (165). In the authors' opinion, lung growth occurred because of a continuously elevated oxygen consumption and the absence of anatomic limitations on thoracic cage size while the animals were in the rapid growth phase. Although lower oxygen and cold temperature go hand in hand at high altitude, the lung growth response mechanism to these variables appears to be common (167). The body growth rate of guinea pigs exposed to cold (6° C) and normobaric hypoxia (Po_2 85 Torr) remained unaffected. Following 3 weeks of exposure, specific lung volume of cold plus hypoxia animals increased, but by 16 weeks that increase disappeared. Similar relationships were observed in specific alveolar epithelial and capillary endothelial surface areas. The authors claimed that lung growth adaptation to cold plus hypoxia is similar to that of hypoxia or cold alone indicating that both cold and hypoxia operate through a common, rather nonspecific mechanism.

1.2.5. SOMATIC AND ORGAN (other than lungs) GROWTH ADAPTATION

A stressor which potentially disturbs the functional capacity of body organs mediated by its direct or indirect adverse effects, eventually disrupts the normal rate of body growth. In an attempt to adjust to the altered state of homeostasis, the body undergoes various adaptive changes and the net result is reflected in body weight. Body growth retardation has been observed in humans and animals following exposure to high altitude, acute or chronic hypobaric hypoxia or normobaric hypoxia (18, 20, 45, 85, 125, 143, 144, 146, 185, 191, 267).

It has been claimed that at high altitude, hypophagia and hypodipsia are mainly produced by lower oxygen (156). Reduced food consumption results from decreased appetite because an exposure to hypobaric hypoxia lowers caloric intake related to metabolic body weight (Appetite quotient = Cal/day/body weight$^{0.73}$) (5). The efficiency of protein utilization (daily weight gain/ daily protein intake) remains unchanged (5), but glucose utilization is impaired in hypoxic conditions (31). Besides decreased food and water intake, somatic growth retardation in response to high altitude has also been attributed to increased water loss (211), changes in food digestibility and utilization (59, 238), alteration in body composition primarily due to decreased deposition, or loss of body fat (59, 125, 237) and disturbances in protein metabolism (154). The underlying mechanisms which result in somatic growth changes are unknown, but a possible role of reduced body metabolic rate in an attempt to decrease oxygen utilization in the body in hypoxic stress has been suggested (144). In hypoxic conditions, initial body weight loss due to decreased food and water intake occurs within the first 24 hours (62). Subsequently, low body weight gain or weight loss may occur due to depletion of fat depots and decreased lean body mass. These differences further complicate the interpretations of the adaptive body response.

It has been shown that while the rate of somatic growth slows down in younger animals, older animals lose body weight after translocation to high altitude (104), or following exposure to hypoxic conditions (144, 145, 274). Besides body weight reduction, skeletal growth reduction has also been reported in animals subjected to hypoxic stress, but its effect on growth of various bones is not uniform

(143). Long bones grow relatively more than the axial skeleton. This may occur due to blood flow differences, skeletal maturity at the time of exposure or individual bone growth regulation (143).

A number of investigators (16, 55, 64, 93, 125, 148, 185, 191, 207, 223, 253, 267, 266) have studied the effect of exposure to high altitude, hypobaric hypoxia or normobaric hypoxia on quantitative or qualitative growth response in various organs such as lungs, heart, spleen, adrenals, liver, kidneys, thymus, thyroid, pituitary and reproductive organs, but the observations reported are inconsistent. In spite of inconsistent observations reported in the literature, it appears that the adaptive response to hypoxic stress in all body organs is variable. It has been demonstrated that despite decreased body weight gain in hypobaric hypoxic or normobaric hypoxic conditions, absolute and/or specific (per unit body weight) weights of organs such as lungs, heart, spleen and adrenals increased (16, 55, 64, 125, 148, 185, 191, 267, 266), while absolute or specific weights of liver and kidney decreased or remained unchanged (55, 64, 93, 125, 148, 185, 191, 207). Some investigators have even reported that hypoxia increased absolute and/or specific weights of liver and kidney (101, 267, 266). It appears that while hypoxic stress induces positive allometric growth of organs like the heart, lungs, spleen and adrenals, it either reduces or has no effect on growth of other organs.

1.3. HYPOTHESIS

It is evident that following exposure to hypoxic conditions, lung growth is either increased or remains unchanged while somatic growth is diminished. However, at high altitude, a decrease in oxygen tension is closely associated with a drop in barometric pressure. The effect of hypobaric hypoxia and normobaric hypoxia on lung growth may not be the same, and the effect of low ambient pressure on the adaptive response of the body is not known. Somatic growth retardation in hypoxic conditions complicates interpretations of results regarding organ growth and specific (per unit body weight) observations may not provide reflections of true changes in organ growth. As a consequence the following hypotheses were tested:

- In hypobaric hypoxia, low oxygen may be the main operating factor in producing adaptive

structural changes in body organs, but decreased ambient pressure may alter the adaptive lung growth response.

- The effect of hypobaric hypoxia may be immediate. If exposure is prolonged, lung growth stimulation may continue. All types of cells in the lung may participate equally in the adaptive response to hypobaric hypoxia.
- Decreased food intake and associated diminished somatic growth in hypobaric hypoxia may produce structural alteration in the lung and growth of other organs.
- Lung growth in hypobaric hypoxia may not be proportional in all compartments of the lung (parenchyma growth vs conducting airway growth) and lung function may be affected.

1.4. RATIONALE

In humans and animal species, acclimatization at high altitude is associated with structural and physiological changes in the body in order to adapt to new environment. High altitude environment is a complex set of variables. Since body metabolism is oxygen dependent, low ambient oxygen at high altitude has been considered as a main stressor. Exposure to high altitude or hypoxic conditions results in somatic growth retardation which occurs due to undernutrition. Despite decreased somatic growth, positive allometric growth occurs in some organs such as lungs heart and spleen, and as a result, specific weights (per unit body weight) of these organs show increases. In conditions which suppress somatic growth, interpretation of findings of specific results become rather complicated. Increase in specific parameters may occur either due to direct or indirect growth stimulation of some organs, while body growth is normal, or due to diminished somatic growth, while organ growth is less impaired. Therefore, the effect of high altitude or hypoxic conditions may be under or overestimated. In addition, undernutrition equivalent to that which occurs in hypoxic conditions may produce changes in the structure of the lung and other organs and its effect may be variable in different organs. Hence, organ growth should ideally be assessed by comparing the experimental group with age and weight-matched cohorts.

The adaptive lung growth response at high altitude has been evaluated by exposing animal species in question to either normobaric hypoxia, hypobaric hypoxia or high altitude, but results were controversial. In some studies, the results of highlanders have been compared with lowlanders. Beside these differences in conditions, differences in the age of animals, duration of exposure, severity of conditions and different species of animals may be attributed to the controversies. In utero cellular proliferation is high in the lung, but following birth, it declines rapidly (199). In utero, normally occurring low oxygen tension may act as a potent stimulant for cellular multiplication. An abrupt increase in arterial oxygen tension occurs at the time of birth; spontaneous respiration may largely be responsible for this change. Decreased oxygen at high altitude may be the leading stimulus of lung growth, but the effects of normobaric hypoxia (18) and hypobaric hypoxia (20) on lung growth appear to be different. This may suggest that low ambient pressure may play some role in the adaptive lung growth response by itself, or when delivered with hypoxia, which has not yet been examined.

Previous studies regarding adaptive lung growth in normobaric hypoxia or hypobaric hypoxia have been primarily limited to morphometric observations. Conclusions regarding alveolar multiplication at high altitude, hypobaric hypoxia and normobaric hypoxia have been drawn based on indirect observations, rather than direct alveolar count. Little is known about biochemical aspects of the adaptive lung growth response to high altitude conditions and no information is available regarding quantitative changes connective tissue compartment of the lung. The sequence of events with regard to cytokinetics and cellular biochemical changes following exposure to hypobaric hypoxia, normobaric hypoxia and hypobaric normoxia as well as in undernourished conditions have not been studied. If hyperplastic changes do occur in high altitude conditions, it is appropriate to assess cellular dynamics of various lung cell populations that participate in the adaptive response. Due to geometrical location, lung structures in the central part of the lung may respond differently to the stimulus or the inhibitor in comparison to the peripheral part of the lung.

Lung growth alteration in high altitude inhabitants has been assessed by lung function techniques, but the relevance of functional changes in the lung to biochemical or morphometric

changes has not been studied. Therefore, once the biochemical and morphological changes have been determined, it will be appropriate to establish the relevance between structural and functional changes in normobaric hypoxia, hypobaric normoxia, hypobaric hypoxia and undernutrition by performing lung function tests.

The effect of hypoxic stress appears to be organ specific. However, the time course of organ growth in hypoxia and the effect of undernutrition on various organs during hypoxia is not known. A number of mechanisms may play a part in ameliorating the effect of hypoxia. Lack of somatic growth may reduce oxygen consumption and changes in pulmonary, cardiac and hematological parameters may increase oxygen supply. Once the hypoxic stimulus is removed, structural changes may take time to reverse. By contrast, changes at the cellular level may show an immediate effect. This also remains to be studied.

Specific aims of present research are:

- To determine the alterations in somatic and lung growth (extent and nature) adaptation to normobaric hypoxia, hypobaric normoxia and hypobaric hypoxia in young rats using biochemical and morphometric techniques.
- To study the effects somatic growth retardation (equivalent to that occurring in normobaric hypoxia and hypobaric hypoxia) on lung growth in normobaric hypoxia and hypobaric hypoxia.
- To determine the onset and persistence of lung growth stimulation and biochemical alterations in normobaric hypoxia, hypobaric normoxia, hypobaric hypoxia and undernourishment.
- To evaluate the dynamics of various cell populations involved in the lung growth adaptive response in the central and the peripheral part of the lung in normobaric hypoxia, hypobaric normoxia, hypobaric hypoxia and undernutrition.
- To determine if lung growth in normobaric hypoxia, hypobaric normoxia, hypobaric hypoxia and undernutrition is normal or dysanaptic (disproportionate growth of lung parenchyma compared to conducting airways) by performing lung function tests.

- To examine the effect of hypobaric hypoxia, normobaric hypoxia, hypobaric normoxia and undernutrition on the growth of various organs.
- To study the effect of recovery on lung growth after returning hypobaric hypoxic, normobaric hypoxic and hypobaric normoxic rats to room air, and providing food ad libitum to undernourished rats.

CHAPTER 2

MATERIALS AND METHODS

2.1. PROJECT DESIGN

The study consisted of three sections:

I. Lung growth adaptation

II. Organ response and lung cytokinetics

III. Lung adaptation: physiological aspects

2.1.1. MATERIALS

2.1.2. CHEMICALS

All the reagents used in this study were of analytical grade and were obtained from Fisher Scientific Limited (Fairlawn, New Jersey, USA), Sigma Chemical Company (St. Louis, Miss., USA) or J.T. Baker Chemical Company (New Jersey, USA), unless otherwise mentioned. Elastin and desmosine standards were purchased from Elastin Products Limited (Pacific, Miss., USA). Iodinated Bolton Hunter Reagent, which was used to label the desmosine for desmosine radioimmunoassay, was bought from New England Nuclear Laboratories (Boston, Massachussetts, USA). The desmosine antibody raised in rabbits for the use in radioimmunoassay was received as a gift from Dr. Shiv Y. Yu, then at Veterans Administration Hospital, St. Louis, Miss., USA. Tritiated thymidine was purchased from Amersham Canada Limited (Oakville, Ontario, Canada) and autoradiography emulsion (NBT 2)

was received from Kodak (Toronto, Ontario).

2.1.3. ANIMALS

One hundred, three week old male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Guelph, Ontario, Canada) separated by litters. They were housed in the Academic Pathology Animal Care Unit, UBC, and were given free access to standard Purina^{R} rat chow and water. They were allowed to adapt to the laboratory environment for one week until used at four weeks of age.

2.2. LUNG GROWTH ADAPTATION

2.2.1. EXPERIMENTAL DESIGN

Four week old male, litter and body weight-matched Sprague-Dawley rats were randomly divided into six groups, each consisting of 12 animals.

* Group 1. Baseline Controls (BC) (4 weeks old, sacrificed at the beginning of the experiment)
* Group 2. General Controls (GC)

  Exposure: Sea level, room air, food and water ad libitum
* Group 3. Hypobaric Normoxic (HBNO)

  Exposure: Ambient pressure 410 mm Hg with enriched oxygen to correspond the fraction of oxygen (Fo_2) to 0.21 at sea level, food and water ad libitum
* Group 4. Normobaric Hypoxic (NBHY)

  Exposure: At sea level, air mixed with nitrogen to decrease Fo_2 to 0.11, food and water ad libitum

* Group 5. Hypobaric Hypoxic (HBHY)

Exposure: Ambient pressure 410 mm Hg with Fo_2 equivalent to 0.11 at sea level, food and water ad libitum

* Group 6. Weight-matched Controls (WMC)

Exposure: Sea level, room air, restricted food to approximate the body weight to those of the hypobaric hypoxic group, water ad libitum

2.2.2. EXPOSURE TO ALTERED AMBIENT CONDITIONS

2.2.2.1. Exposure chambers

Four hypobaric chambers, each with a capacity of 3 cages, were designed (Academic Pathology Workshop, UBC) from polyvinyl chloride cylinders. Each of the hypobaric chambers was equipped with an air intake for individual cages, a pressure gauge, a temperature and hygroscopic sensor hygrometer unit (Airguide, Chicago, Illinois) and an on-line port for carbon dioxide and oxygen tension measurements. Similarly, the normobaric chambers were also partitioned into compartments for every cage with a separate air inlet and outlet. The air inflow was adjusted to achieve 12-15 air changes per hour. An on-line vacuum was used for hypobaric conditions (hypobaric hypoxia and hypobaric normoxia).

2.2.2.2. Exposure procedure

The baseline group of rats was sacrificed at the beginning of the experiment at 4 weeks of age to obtain baseline somatic and lung parameters. Groups 2-6 were exposed to respective ambient conditions and all rats were kept in individual cages. All animals were fed standard Purina^R rat chow. The general control rats (GC) were housed in continuously air flushed chambers and were given food and water ad libitum. The chambers of the hypobaric normoxic (HBNO) group were connected to the on-line vacuum source to reduce the ambient pressure to 410 mm Hg. Simultaneously the air inflow into the HBNO chambers was supplemented with 100% oxygen to correspond Fo_2 to 0.21 at sea level

which was identical to that of the general control group. The oxygen tension was monitored with a polarographic oxygen analyzer (Hudson Vantronics, Temecula, CA, USA), pre-calibrated to the room environment as 0.21 fraction of oxygen. The HBNO rats were given free access to food and water. For normobaric hypoxic (NBHY) animals, the chambers were flushed with a mixture of air and nitrogen to obtain 0.11 Fo_2. In the case of hypobaric hypoxic (HBHY) rats, the ambient pressure was decreased to 410 mm Hg which decreased Fo_2 equivalent to 0.11 at sea level. Except for the weight-matched control group, all animals were given food and water ad libitum. The weight-matched group of rats were pair-matched for body weight to the hypobaric hypoxic group. They were allowed restricted food access but free access to water. On the first day, they were given 50% of the food consumed by them one day before the experiment was commenced. Later the weight-matched animals were given approximately the amount of food consumed by the hypobaric hypoxic rats the previous day, but depending upon their body weight gain some adjustments in the amount of food given were required.

All groups of rats were exposed to a 12 hour light/dark cycle. Temperature and relative humidity were monitored continuously. The gas flow through the chambers was adequate to maintain the ambient temperature at 22 $\pm$1° C and relative humidity at 60 $\pm$5 %. No condensation of water inside the chambers was noticed. Because water was placed in the chambers in open containers, calcium chloride was also placed in the chambers to control the humidity as a precautionary measure. O_2 and CO_2 concentrations were measured 3 times a day. The CO_2 concentration was controlled by placing soda lime inside the chambers. Using a Fryrite carbon dioxide analyzer (Bacharach Instrument Co., Pittsburgh, PA., USA) CO_2 did not increase to a detectable level of 0.5%. The chambers were opened daily for 15-20 minutes to change the shavings and to replenish food and water. The food intake and body weight of each rat was measured daily.

2.2.2.3. Animal termination

Following 3 weeks of continuous exposure to respective ambient conditions, the rats were

anaesthetized by intraperitoneal injections of sodium pentobarbital (5 mg/100 g body weight). Oxygen absorption atelectasis has been shown to be a more effective procedure to remove trapped lung gas than vacuum degassing the lung (248). The rats were given 100% oxygen to breathe for 3-5 minutes to remove gases from the lungs for pressure-volume measurements [as O_2 is readily absorbed giving the atelectic lungs a liver-like appearance (79)]. The abdomen was opened and the diaphragm was incised to cause pneumothorax and lung collapse. The thoracic cavity was opened and a sample of blood was taken in a capillary tube from the right ventricle of the heart for hematocrit measurement. Immediately, the trachea was clamped and cannulated. The rats were sacrificed by exsanguination, severing the abdominal aorta to reduce the contribution of pulmonary blood volume to lung weight and biochemical measurements. Lungs, heart and extrapulmonary tissue were dissected from the thoracic cavity.

2.2.3. PRESSURE-VOLUME CURVES

The lungs and heart en bloc preparation was placed in a plethysmograph with a heat sink designed for lungs. The cannulated trachea was attached to a connector in the lung chamber, connected to a pre-calibrated pressure-volume curve plotting equipment (Validyne, Model MC; Hewlett Packard, Model 7041 A X-Y recorder). The tracheal clamp was then removed. When the pressure changes within the lung chamber stabilized, the lungs were inflated in a step-wise fashion with a motor-driven air syringe to a transpulmonary pressure of 25 cm water and then deflated to a transpulmonary pressure of 0 cm water. During this maneuver, if the pressure did not stay constant at 25 cm of water after inflation for 15-20 seconds, air leakage was assumed and the lungs were not included for analysis. Following the initial hysteresis, two pressure-volume curves were recorded for each animal and the deflation limb of the second curve was used for lung elastic recoil analysis. Maximal lung volume was defined as the amount of air in the lung at a transpulmonary pressure of 25 cm of water. Recoil pressure was calculated from the pressure-volume plots at 10 percentiles of maximal lung volume. According to the method of Colebatch and associates (68), all the data points

over 30% of maximal lung volume were analyzed by fitting a single exponential to the pressure-volume curve with the help of a digital computer. The single exponential expression was:

$V = V_{max} - Be^{KP}$

where V is the volume at pressure P, V_{max} is the theoretical volume of air at infinite transpulmonary pressure, B the difference between V_{max} and the intercept on the volume axis and K is a constant that describes the shape of the curve. In this equation V_{max} is unknown, therefore, it was necessary to repeat the computations using variable values of V_{max}. Since it is well known that the slope of the pressure-volume curve is near zero in the region of V_{max} (206), the starting value for V_{max} was assumed to be close to the observed maximum lung volume. The value V_{max} was then increased positively or negatively until r^2 no longer increased with the tenth of the unit change of V_{max}. Then the change of V_{max} was further reduced to the 1/100th and iterative computations were performed until the best fit of the curve was achieved. The best fit was determined by no further increase in the value of r^2. The quality of the fit of the function is assessed from the r^2 value, which represents the proportion of total variance of points attributable to the mathematical regression (206). The r^2 value in the present study was >0.98 in all cases.

2.2.4. BIOCHEMISTRY

Following pressure-volume curve maneuvers, the extrapulmonary tissue was removed. The right lung was ligated and separated from the left lung. Our preliminary studies indicated that the time lapse during pressure-volume procedure did not affect DNA, RNA and protein estimations of the lungs. Lungs were washed with phosphate buffered saline to remove blood. After blotting, the wet lung weights of the right lung and the cannulated left lung (after taring the balance with a cannula and a portion of trachea) were recorded. The right lungs were frozen in liquid nitrogen and stored at -70° C until used. When required, the frozen lung samples were lyophilised (VirTis Preservator model 10-pr, Freezermobile 12 Freezedryer) until constant weights were attained and then the dry lung weights were measured. The lung samples were thinly sliced, rehydrated in 2 ml phosphate-buffered saline

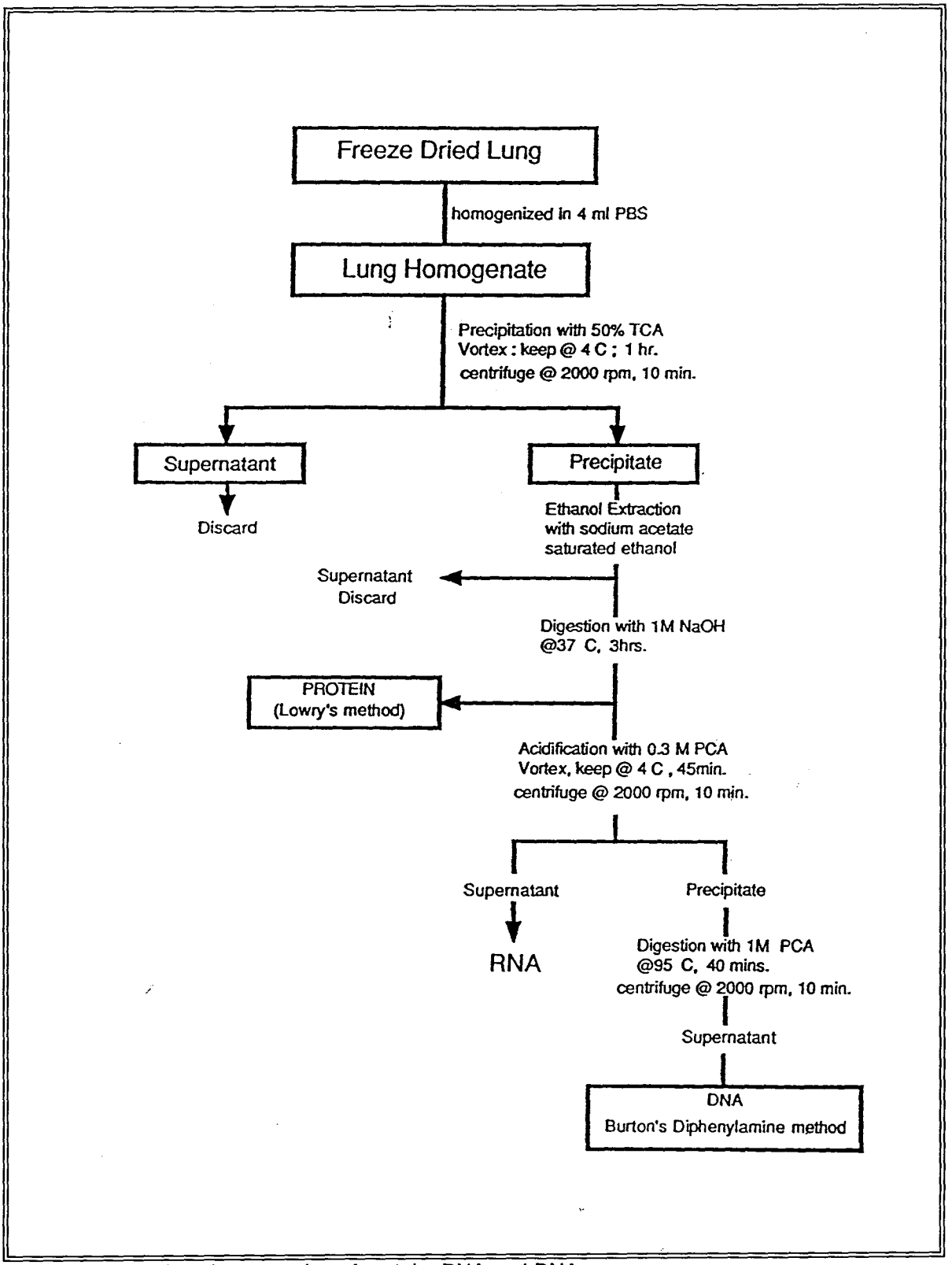


Figure 2. Flow chart for extraction of protein, RNA and DNA.

(PBS), and stored in a refrigerator overnight. The samples were homogenized with a homogenizer (Caframo, Wiarton, Ontario) and the final volumes were made up to 4 ml with phosphate buffered saline (PBS). One ml of the final lung homogenates were used for extraction of DNA, RNA and alkali soluble protein, and 3 ml were used for extraction and estimation of hydroxyproline and desmosine.

2.2.4.1. DNA, RNA AND PROTEIN EXTRACTION

The procedure of Schmidt-Thanhauser (236) as modified by Wannemacher (272) was used for extraction of DNA, RNA and alkali soluble protein (Figure 2). In order to precipitate the macromolecules, one ml of the sample homogenate was mixed with 50% trichloroacetic acid (TCA) to achieve the final concentration of 15% of TCA. The TCA precipitated lung samples were vortexed and placed in a refrigerator at 4° C for one hour. The lung samples were then centrifuged (Beckman, model J-68) at 2000 rpm at 4° C for 10 minutes. The supernatants were discarded. The TCA precipitates were then washed 3 times with sodium acetate saturated ethanol to remove lipids. Two ml of 1M sodium hydroxide (NaOH) was added to each of the residues and were placed in a 37° C water bath for one hour. After centrifugation at 2000 rpm for 10 minutes, the supernatants were then decanted into clean tubes and digestion of the residues was repeated with 2 ml of NaOH. The supernatants of the first and second digestion of samples were pooled. One ml aliquots of pooled supernatants were taken for protein estimation. Cold (4° C) 6 ml perchloric acid (PCA) was added to the remainder of the NaOH digest, to give a final concentration of 0.3M PCA. The samples were vortexed and kept at 4° C for 45 minutes. The PCA treated samples were then vortexed and centrifuged at 2000 rpm for 10 minutes and the supernatants were then decanted into clean tubes and were used for RNA estimation. 1 ml of PCA was added to the residues and digested at 90° C for 20 minutes. The digests were then cooled and centrifuged. The supernatants were removed and the residues were digested again. After pooling the supernatants from the first and second digests, an aliquot of each sample was used to determine the total amount of lung DNA and assessment of DNA synthesis.

2.2.4.1.1. Alkali soluble protein estimation

Alkali soluble protein content was measured by the method of Lowry and coworkers (174). Bovine serum albumin (BSA, Sigma A-4378, fraction V) dissolved in 0.1M NaOH was used as a standard stock solution (500 µg BSA/ml). The stock solution was divided into aliquots and stored at -70° C until required for an assay. For the assay, one aliquot was thawed and pipetted in duplicates in assay tubes to obtain a standard curve ranging from 10-200 µg of BSA. From the diluted samples 150 µl aliquots were taken and total volumes of standards and samples were made to 500 µl. Five ml of freshly prepared Lowry's solution (2% sodium carbonate in 0.1M NaOH solution, 1% copper sulphate solution, 2% sodium tartrate solution and all mixed in 100:1:1 ratio) was added to the tubes, vortexed and allowed to stand at room temperature for 15-20 minutes. A half milliliter of freshly prepared 1M Folin's phenol reagent (2N Folin-Coicalteau phenol reagent solution diluted with distilled water and 1M NaOH solution to obtain final pH of 1.8) was added to each assay tube, vortexed and left at room temperature for half an hour. The absorbance was read at 660 nm in a spectrophotometer (Philips Pye Unicam SP6-550 UV/Vis). The absorbance reading of all lung samples were within the linear range of the standard curve. By approximating the absorbance on the standard curve, protein content per sample was calculated.

$$\text{Protein/lung (mg)} = \frac{\text{Protein µg from the standard curve} \times \text{DF (dilution factor)}}{1000}$$

$$\text{Dilution factor (DF)} = \frac{\text{Total volume of the aliquot after dilution}}{\text{Fraction of sample taken}} \times \frac{\text{Total volume of NaOH soluble supernatant}}{\text{Volume of aliquot taken}}$$

2.2.4.1.2. Estimation of DNA

DNA content estimation was done according to the method described by Burton (43) using calf thymus DNA (Sigma D-1501) as standard. A standard stock solution (0.4mg/ml) was prepared, divided into 1 ml aliquots and stored in -70° C. An aliquot was thawed, diluted 1:1 with 1.7M PCA and incubated at 90° C for 10 minutes. After cooling, aliquots were pipetted in duplicate to obtain

standards containing 16, 32, 48, 64, 80, 100 and 120 µg of DNA. All standards and sample volumes were made up to 1 ml with 1.7M PCA. Two milliliters of freshly prepared diphenylamine reagent (1 g diphenylamine, 100 ml of glacial acetic acid, 2.73 ml concentrated sulphuric acid) were added to the samples and standards and then vortexed. The samples and standards were then incubated in a water bath at 90° C for 10 minutes. After cooling, the absorbance of the mixture was read at 600 nm using a spectrophotometer (Philips Pye Unicam SP6-550 UV/VIS). The standard curve used for DNA estimation was linear and all the samples fell within the limits of the standard curve. The DNA content per lung was calculated as follows:

$$\text{DNA /lung (mg)} = \frac{\text{µg DNA calculated from standard curve X DF}}{1000}$$

$$\text{Dilution factor (DF)} = \frac{\text{Total volume of supernatant after PCA incubation}}{\text{Volume of fraction taken for DNA assay}} \times \frac{\text{Total volume of NaOH supernatant X 4}}{\text{Volume taken for DNA precipitation X 3}}$$

2.2.4.1.3.. Estimation of RNA

The amount of RNA was measured by using a method of Wannemacher (272). Calf liver (type IV) ribonucleic acid (RNA, Sigma R-7250) was dissolved in distilled water to prepare a stock solution (400 µg RNA/ml) and stored at -70° C until required for estimation. One ml of stock solution was thawed and diluted to 10 ml with distilled water to obtain a final concentration of 40 µg/ml for assay. Standards were pipetted in duplicate concentrations with a range of 5, 10, 15, 20, 25 and 30 µg of RNA. Distilled water was added to 500 µl of the diluted samples and standard duplicates to make final volume up to 5 ml. The light absorbance of standards and samples was read in a spectrophotometer (Philips Pye Unicam SP6-550 UV/Vis) at 260 nm. The absorbance of standards and samples was also read at 280 nm to correct for protein interference. The absorbance reading of standards was linear and all sample readings fell within the range of the standards. By matching the absorbance on the standard curve, the RNA content per sample was calculated as follows:

$$\text{RNA /lung (mg)} = \text{µg RNA calculated from standard curve X DF}$$

$$\text{Dilution factor (DF)} = \frac{\text{Total volume of diluted supernatant after PCA precipitation (µl)}}{1000} \times \frac{500}{\text{Total volume of NaOH supernatant} \times 4} \over \text{Volume taken for DNA precipitation} \times 3$$

2.2.4.2. PROCEDURE FOR HYDROXYPROLINE AND DESMOSINE EXTRACTION

Hydroxyproline and desmosine were extracted by using the method of Laurent and associates (161), in which cyanogen bromide essentially solubilized all the lung collagen and left behind an insoluble residue with amino-acid content similar to elastin (Figure 3). Three milliliters of lung homogenate were centrifuged at 5000 rpm for 10 minutes and the supernatants retained. The residues were washed twice with 1.5 ml of phosphate buffered saline and centrifuged. The washing was repeated twice with 1.5 ml of 2% sodium dodecyl sulphate (SDS) and centrifuged. Finally, the residues were washed again with PBS in order to remove excess SDS. All the supernatants were then pooled and kept for the soluble fraction of hydroxyproline estimation. The residues were then washed with 2 ml acetone and left overnight in the fume hood to dry. The residues were again homogenized in 2 ml of 70% (w/v) formic acid. 0.5 ml of a 0.1 g/ml solution of cyanogen bromide in 70% formic acid was added to the residues to achieve a final cyanogen bromide concentration of 20 mg/ml. The solutions were vortexed and nitrogen gas was bubbled through the samples for 15 seconds. The tubes were then sealed and incubated in a shaker water bath at 37° C and the reaction was allowed to take place for 24 hours. The resulting digests were centrifuged at 5000 rpm for 20 minutes. The supernatants were decanted into 50 ml wide mouthed tubes and the residues were washed 4 times with 4 ml of distilled water. All supernatants were pooled in the 50 ml tubes and the final volumes were made up to 30 ml with distilled water. Both the residues and supernatants were frozen at -70° C and lyophilized till constant weights were achieved.

2.2.4.2.1. Estimation of hydroxyproline

(i). Sample preparation for hydroxyproline quantification

The samples recovered from the SDS/PBS extraction represented soluble collagen and cyanogen bromide soluble samples represented insoluble cross-linked collagen. In order to release hydroxyproline from peptide linkages, both soluble and insoluble collagen samples were hydrolysed in 6N hydrochloric acid. Hydrolysis was performed at 110° C for 24 hours. After hydrolysis, two drops of 0.02% methyl red indicator were added to the hydrolysates and 2.5M NaOH was added slowly and vortexed until a faint straw color was obtained. The hydrolysates were then filtered with Whatman #1 filter paper and the total volume was made up to 15 ml with distilled water.

(ii). Hydroxyproline assay procedure

Hydroxyproline content was measured in the hydrolysates by using the method of Woessner (285). Series of standards containing 0, 2, 4, 6, 8 and 10 µg of hydroxyproline and a known volume of sample hydrolysates were added to the assay tubes. The total volume of standards and sample hydrolysates were made to 2 ml with distilled water. One ml of freshly prepared chloramine-T solution was added to each tube , vortexed and allowed to stand at room temperature for 20 minutes. In order to eliminate excess of chloramine-T and to stop the oxidizing reaction, one ml of 3.15M PCA was added to the tube in the same order. The assay tubes were then vortexed and let stand sit for another 5 minutes. Finally, one ml of Ehrlich's reagent (20 g of p-dimethylamine benzaldehyde in 100 ml of n-propanol) was added, vortexed and incubated for 20 minutes in a water bath at 60° C. The assay tubes were cooled in tap water and the absorbance of the samples were read in a spectrophotometer at 561 nm. The standard curve prepared was linear to 6 µg hydroxyproline, and the absorbance of the lung samples were all within the linear range of the curve. The hydroxyproline contents of the lung samples were estimated directly from the standard curve.

Hydroxyproline content of soluble and insoluble collagen fractions was estimated separately and the sum of both was referred to as the total hydroxyproline content present in the lung. The percent of soluble or insoluble content was also calculated. Laurent and coworkers (161) have indicated that lung collagen contains 12.2% (w/w) hydroxyproline based on amino acid analysis of collagen

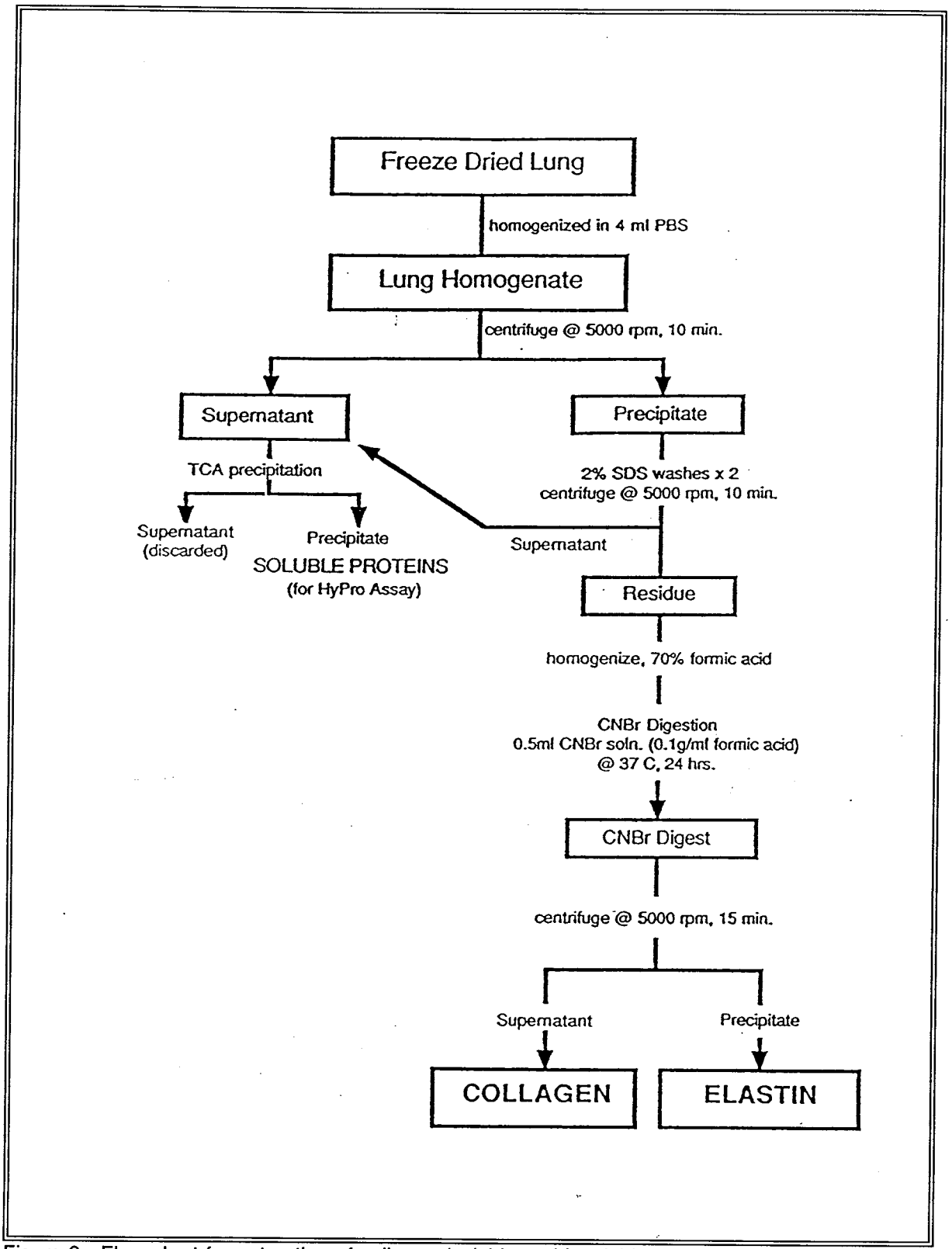


Figure 3. Flow chart for extraction of collagen (soluble and insoluble fractions) and elastin.

standards and lung tissue collagen extracted in cyanogen bromide. We did not convert our hydroxyproline content into collagen content.

2.2.4.2.2. Estimation of desmosine

Desmosine is unique to elastin. Measurement of the elastin content in the lung was done by measuring the desmosine by radioimmunoassay procedure. Peptide bound desmosine does not react with the antibody, therefore, samples must first be hydrolysed in 6N hydrochloric acid in order to release the bound desmosine.

(i). Desmosine preparation from lung samples

Two ml of 6N hydrochloric acid was added to the cyanogen bromide treated residues and were hydrolysed at 110^{o} C for 48 hours to liberate desmosine. Following hydrolysis of the samples, aliquots of 200 µl were taken, diluted 10 times and were lyophilized to dryness. The dried samples were then reconstituted in 1 ml of pH 7.6 potassium-phosphate buffer. The final Ph of the solution ranged between Ph 7.2-7.4. These solutions were further diluted to required dilutions so that they fell on the linear part of the desmosine radioimmunoassay curve. One hundred µl aliquots of these diluted samples were used for measurements of desmosine by radioimmunoassay.

(ii). Desmosine radioimmunoassay

A. Preparation of ^{125}I-labelled desmosine (Conjugation of desmosine with iodinated Bolton Hunter Reagent)

The labelled desmosine molecule which is used for the quantitation of desmosine samples, initially requires conjugation with radioactive iodinated reagent. The Bolton Hunter reagent (^{125}I-BHR) is unstable if left unused for more then 48 hours, therefore, the conjugation procedure needs to be conducted immediately. Once the conjugation is done, the labelled product is stable for 4-6 weeks

at 4° C.

The reaction procedure was performed in a well ventilated fume hood in accordance with the radiation safety measures. The di-iodo (^{125}I) Bolton Hunter Reagent {N-succinidyl-3 (4 hydroxy, 3, 5 [^{125}I] diiodo-phenyl) proprionate}, total activity of 0.5 mCi (Specific activity 4400 Ci/mmol) was purchased in two 2-250 uCi combi-V-vials in 250 µl anhydrous benzene, from New England Nuclear (NEN) Research Products, Boston, MA., USA. Both combi-V-vials were placed on ice and two hypodermic needles were inserted in through the rubber stopper. One needle was connected to the charcoal trap for any radioactive material which might escape during the evaporation procedure and the other needle was connected to a dry nitrogen gas supply. The benzene was carefully evaporated to dryness with a very gentle stream of nitrogen. Then 30 µl of 1 mg/ml desmosine (Elastin Products, Pacific, Miss., USA) solution was added to each Bolton Hunter reagent combi-V-vial. The reaction mixture was then transferred to the cold room and was left overnight on a gentle shaking platform with proper shielding. Next day, the conjugation reaction was stopped by adding 0.5 ml of 0.2M glycine solution. The reaction mixture was then transferred to the top of a 100 cm long Bio-Gel P2 (200-400 mesh) (Bio-Rad laboratories, Richmond, CA) packed column (which was packed the previous day) and was eluted with 0.1M acetic acid. One hundred 3 ml fractions were collected in siliconized vials. Two µl of solution from each vial was removed and placed in marked polypropylene (12 mm) tubes, and radioactivity was measured using a LKB gamma counter. Once the peak with the desmosine conjugates was determined, all the fractions from the peak were pooled and divided into 0.5 ml fractions and stored at 4° C. For each assay one aliquot was taken and diluted 12 times to get a total radioactivity in 50 µl that ranged between 20,000 and 30,000 cpm.

B. Desmosine standards preparation

The standard desmosine solution of 1µg/ml concentration was prepared in distilled water and one ml aliquots were then stored at -70° C. For each assay, one tube was thawed and serial dilutions were made of 500, 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.9 ng/ml with 7.2 pH potassium-phosphate

buffer. Internal standard preparations from rat lung elastin hydrolysates, desmosine recovery samples and control lung samples were used following every 10 samples to assess variations and precision within and between the assays.

C. Desmosine assay procedure

Desmosine in the lung tissue hydrolysates was assayed by the method of Harel and associates (128) with some modifications. One hundred µl of the lung hydrolysates, serially diluted standards (ranging from 3.9-500 ng/ml), internal elastin standards, lung controls and desmosine recovery samples were added to the assay tubes. They were diluted with 100 µl 7.2 pH phosphate buffer to get a total volume of 200 µl. The solution was mixed with 100 µl of anti-desmosine rabbit antibody (diluted 1:100 with 7.2 phosphate buffer). The mixture was gently vortexed and then incubated at 30° C for 30 minutes (this allows for the antigen-antibody complexes to form between desmosine from the lung samples and the anti-desmosine antibody). Fifty µl of the radioactive desmosine (labelled with ^{125}I-BHR (containing 20,000-30,000 cpm in 50 µl of phosphate buffer solution) was then added to the assay tubes. The assay tubes were vortexed and then further incubated at 30° C for one hour. This allows the competitive binding of the radioactive desmosine to the desmosine antibody in the reaction mixture. Therefore the reaction mixture at the end of second incubation contains Ab-Des complexes, Ab-Des(^{125}I) complexes, free desmosine ^{125}I and free desmosine. Fifty µl of a 10% (w/v) solution of pansorbin (Staphylococcus aureus cells, binding: 2.2 mg of human IgG/ml of cell suspension) was added to the assay tubes, vortexed and incubated at 30° C for one hour [Pansorbin cells adsorb on the surface of the antibody which is already bound to the antigen {Des or Des(^{125}I)} and precipitates the Ag-Ab complexes while the free desmosine and radioactive desmosine remains in the supernatant]. The tubes were then centrifuged at 4° C for 30 minutes at 5000 rpm. The supernatants were carefully aspirated. The residues were washed with 0.5 ml of 7.2 pH phosphate buffer and radioactivity of the precipitates was counted in an LKB gamma counter. All the assays were carried out in duplicate. From the results obtained, a standard curve was plotted with the assistance of a

computer and the average of the duplicate cpm (counts per minute) was automatically read from the standard curve. The sample dilutions were made such that all the readings fell within the linear part of the standard curve. Three assays were done for each sample and the average of all three observations was used for analysis.

The sensitivity of the desmosine radioimmuno assay was optimal and linear between 15.62 to 250 ng. Isodesmosine was only weakly cross-reactive (<0.1%). In order to check the non-specific interference from the other proteins, varying amounts of hydrolysates of collagen and bovine serum albumin were added to a measured amount of desmosine. There was no significant interference in the assay by any of the hydrolysates up to 200 μg in concentration. The dilution of the lung hydrolysates used for the assay had desmosine concentrations of less than 100 μg.

2.2.5. MORPHOMETRY

2.2.5.1. Lung fixation and lung volume determination

After plotting the pressure-volume curves, the left lung with the cannulated trachea was submerged and distended gently with a 5 cc syringe using 10% neutral buffered formaldehyde. The cannula was then connected to the instillation system for 72 hours at a constant transpulmonary pressure of 25 cm of water. After fixation, the trachea was clamped (to prevent fixative leakage) and detached from the instillation system. The hilum of the lung was ligated and the trachea was removed. A beaker full of water was placed on a balance and the lung was suspended and submerged completely in the water without touching the sides or the bottom of the beaker. The lung volume (cm^3) due to water displacement was estimated as equal to the weight in grams as described by Scherle (235). Archimedes principle states:

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

Weight of fluid displaced = Buoyant force (FB)

$$\text{Volume of lung (Vl)} = \frac{\text{FB}}{\text{Specific gravity of fluid}}$$

2.2.5.2. Lung sampling and tissue processing

Two mid-sagittal blocks, one from cephalic and the other from the caudal end of each left lung were cut. The edges of the blocks were trimmed at right angles for accurate digitization which was used for tissue shrinkage assessment that occurred during tissue processing and embedding. The blocks were then photographed and contact prints were made from the negatives. The blocks were embedded in paraffin. Two 5 micron sections were cut from each block and stained with hematoxylin and eosin for light microscopic morphometric measurements.

2.2.5.3. Assessment of tissue shrinkage

The areas of the blocks were measured by a computer assisted digitizer (Apple IIe, Bioquant II, R. & M. Biometrics) from the contact prints of the blocks before tissue processing and embedding (pre-processed). After tissue processing and embedding (post-processed), the area of the stained section was also digitized in a similar fashion. The shrinkage factor was determined by dividing the area of the tissue sections (post-processed) by the area of the pre-processed lung tissue. Area shrinkage and linear shrinkage factors were calculated as follows:

$$\text{Area shrinkage factor (asf)} = \frac{\text{Area of post-processed tissue}}{\text{Area of pre-processed tissue}}$$

$$\text{Linear shrinkage factor (lsf)} = \sqrt{\frac{\text{Area of post-processed tissue}}{\text{Area of pre-processed tissue}}}$$

No corrections were made for the thickness of the sections.

2.2.5.4. Morphometric measurements

All the slides were coded to eliminate bias in morphometric measurements. A microscope (Wild, M501, Heerbrugg, Switzerland) equipped with an automated stage and an overhead screen

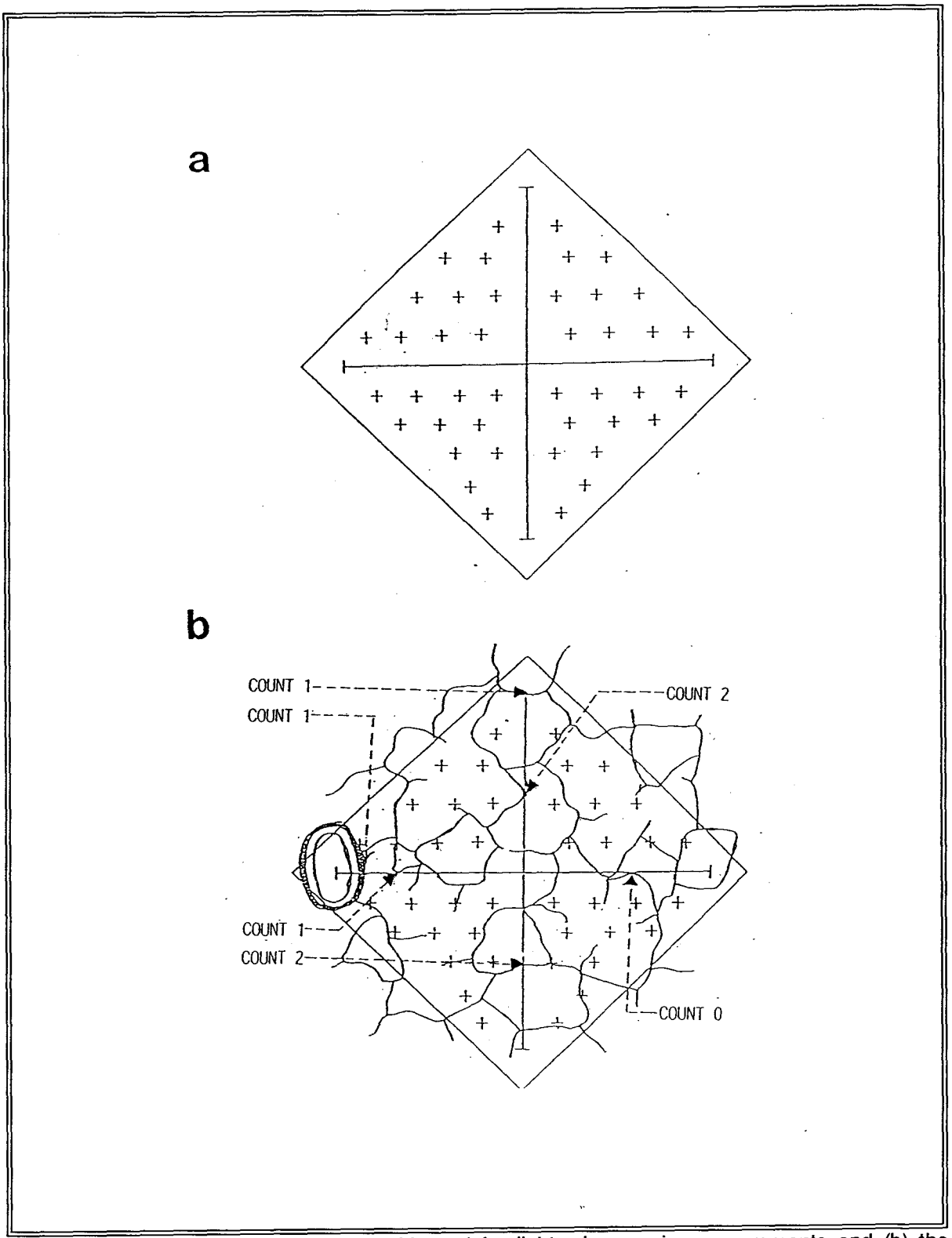


Figure 4. Illustrations of (a) the test grid used for light microscopic measurements and (b) the intercept counting technique.

fitted with a square grid (a 42 equidistant test points and two diagonally placed cross hair test lines) was used for morphometry (Figure 4a).

Using a X40 objective, the area and the total length of the two diagonally placed cross hair test lines was determined to be 0.0625 mm^2 and 0.64 mm respectively. Leaving a 1 mm distance along the margins, the rest of the area was used to choose 20 predetermined equidistant fields on a 4 X 5 matrix. Each of the 42 test points were counted according to their placement on the histological structures, i.e. alveolar air, alveolar duct air, alveolar wall, bronchial air and non-parenchymatous tissue. The smallest discrete structures surrounded by alveolar walls were considered as alveoli. Alveolar duct air was considered to be the cylindrical core of air within the alveolar duct and sacs internal to the mouth of alveoli. Conducting airway air was referred as bronchial air.

The counting principle used in measuring random sections was based on Delesse principle (86) which states:

> "Area proportions are equivalent to volumetric proportions" and " 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, number of test points for each of the tissue components were expressed as a fraction of the number of test points. This represents volume fraction of the tissue structure.

$$V_{vx} = \frac{\text{Number of test points falling on a structure x}}{\text{42 or total number of test points}}$$

Intercepts were counted either as wall intercepts (Iw) or duct intercepts (Id) and the sum of the two (Iw+Id) represented total number of intercepts (Ia). If the wall of the alveoli fell across the test line, two intercepts were counted (2 air exchanging surfaces), but if the alveolar wall touched the upper or the right side of the test lines or the end of the test line lodged in the wall of the alveolus or in any other tissue structure, it was counted as one intercept (Figure 4b). For the duct intercept (Id), an interception of the test line by an imaginary line across the mouth of the alveolus was counted as two intercepts. The total number of intercepts per case was used to calculate the mean linear intercept

Table 2. Morphometric calculations.

Parameters	Calculations
Alveolar wall intercept	= Iw
Duct mouth intercept	= Id
Total intercepts (Ia)	= Iw+Id
Volume fraction of :	
Alveolar air	= Vv_{alv}
Alveolar duct air	= Vv_{duct}
Alveolar wall	= Vv_{wall}
Bronchial air	= Vv_{br}
Non-parenchyma	= Vv_{np}
Number of alveoli in a field	= N
Total length of the test line (corrected for linear shrinkage factor {lsf})	= $L_{\wedge T}$
Area of the grid (corrected for shrinkage {asf})	= A
Mean linear intercept (MLI)	= $\frac{2L_T}{Iw}$
Mean chord length of alveoli (I_{alv})	= $\frac{2L_T \text{ X } Vv_{alv}}{Ia}$
Alveolar surface area (S_W)	= $\frac{4 \text{ X } V_L}{MLI}$
Alveoli per unit area (N_A)	= $\frac{N}{A}$
Number of alveoli per unit volume (N_V)	= $\frac{J \text{ X } \sqrt{NA^3}}{ß \text{ X } \sqrt{Vv_{alv}}}$
Distribution constant (J)	= 1
Shape constant (ß)	= 1.55
Total number of alveoli (N_{at})	= $N_V \text{ X } V_T$
Average alveolar volume ($Valv_{ave}$)	= $\frac{V_T \text{ X } Vv_{alv}}{N_{at}}$

as follows:

$$\text{Mean linear intercept (MLI)} = \frac{N_{fi} * L_T * 2}{I_i(\text{w or a})}$$

where N_{fi} is number of fields on slide i, L_T is total length of test line after correcting for linear shrinkage during tissue processing, 2 is used because every wall or duct intercept was counted as 2 intercepts, I_i is total of intercepts on slide i (w is alveolar intercept and a is sum of alveolar wall and alveolar duct

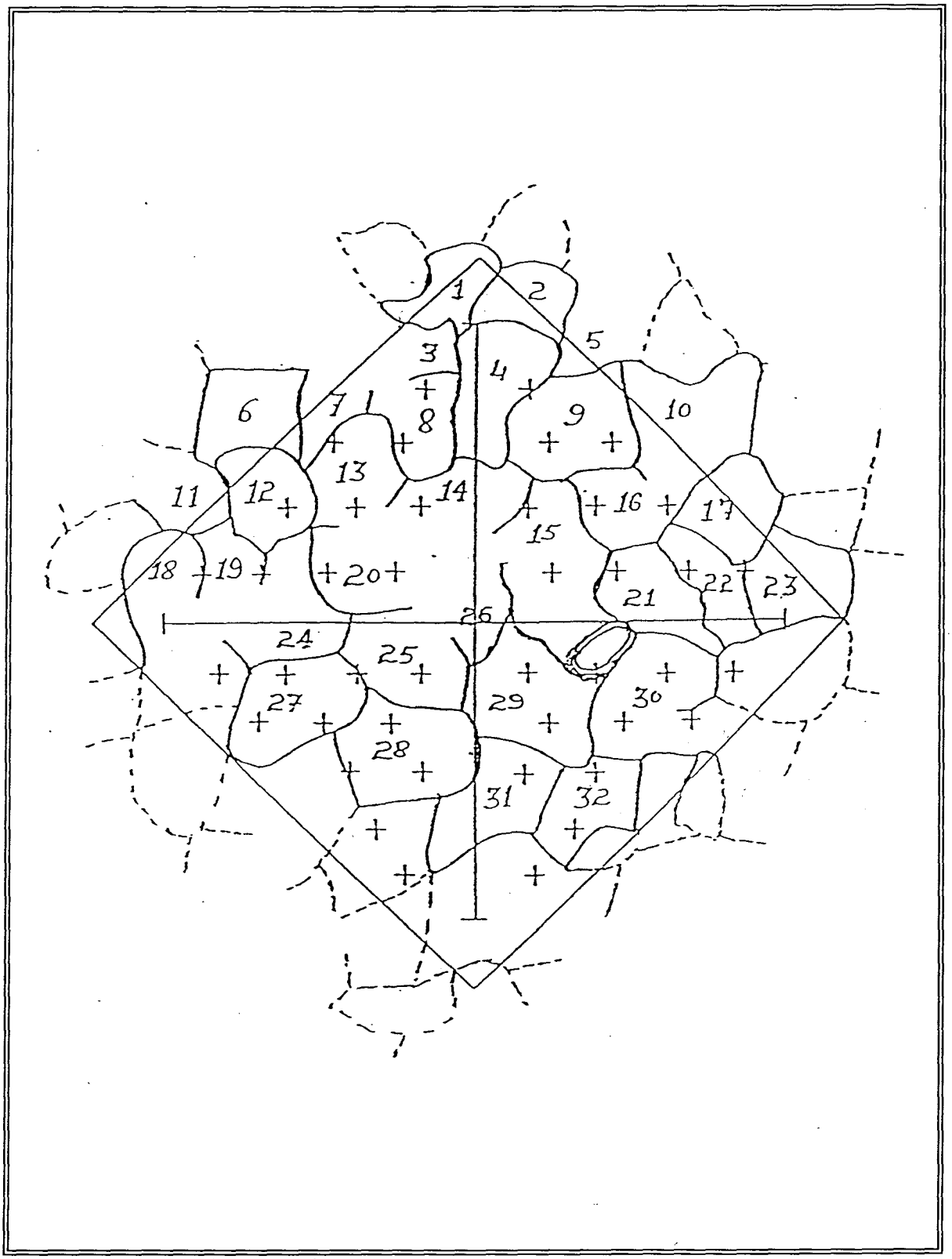


Figure 5. Illustration of a direct alveolar count.

intercept.

From these morphometric values, the mean linear intercept of airspaces or average interalveolar wall distance (MLI), mean chord length of alveoli (l_{alv}), and alveolar surface area (S_W) were calculated. The total length of the test line on the grid was corrected for tissue shrinkage in order to project onto the fixed tissue. The mean linear intercepts were calculated by dividing the total projected length of test lines by the number of wall intercepts (Table 2).

The alveoli that lay within the grid and on the upper left and right sides of the grid were counted (Figure 5). The known area (A) of the grid was corrected for tissue shrinkage during processing. The counted number of alveoli were used to calculate the number of alveoli per unit area (N_A), number of alveoli per unit volume (N_V), total number of alveoli (N_{at}) and average volume of an alveolus ($Valv_{ave}$). The distribution constant of the characteristic linear dimension of alveoli (J) was considered as one and shape constant (B) of the alveolus was taken as 1.55 (278).

2.3. ORGAN RESPONSE AND LUNG CYTOKINETICS

2.3.1. EXPERIMENTAL DESIGN

A total of 240 male Sprague-Dawley rats were used in this study. At 4 weeks of age, body weight and litter-matched rats were randomly divided into 5 groups:

* Group 1. General Control Group (GC)
* Group 2. Hypobaric Normoxic Group (HBNO)
* Group 3. Normobaric Hypoxic Group (NBHY)
* Group 4. Hypobaric Hypoxic Group (HBHY)
* Group 5. Weight-matched Control Group (WMC)

The rats were exposed to ambient conditions as detailed earlier. Six rats from each group 1-5 were sacrificed on days 1, 3, 5, 7, 10, 14 and 21 of exposure. Six rats in each of the groups 2-4 were then returned to room air for three days, and group 5 was given free access to food and water after 21 days for the subsequent 3 days. This was done to study the effect of post-exposure and refeeding. On days 1, 3, 5, 7, 10, 14 and 21 of exposure, and day 3 of post-exposure, the rats were anaesthetized with halothane. A 5 mm long skin incision was made at the mid groin and then femoral vein was carefully exposed by excising the femoral sheath. Using a 30G needle, 2 μCi/g body weight of tritiated thymidine (^{3}H-TdR) (specific activity 23 mCi/mmol) was injected very slowly (a fast injection appeared to be lethal to the rats). Pressure was applied at the injection site for approximately two minutes, which was found to be adequate to stop any further bleeding and no attempt was made to suture the wound.

2.3.1.1. Termination of rats

One hour after intravenous injections of ^{3}H-TdR, injections of sodium pentobarbital (50 mg/Kg body weight) were given intraperitoneally to anaesthetize the animals. Body weight and nose-tail length were recorded. The rats were terminated by exsanguination from the abdominal aorta. The spleen, kidneys and liver were removed, washed, blot dried and weighed. The spleen, the right kidney and a part of the ileum (positive controls for autoradiography) were fixed with Karnofsky's solution and stored for future use. The thoracic cage was opened and the trachea was cannulated. The right lung was clamped and ligated at the hilum and removed. After weighing of the right lung, liver and left kidney, they were immediately frozen in liquid nitrogen and stored at -70° C until further use.

2.3.1.2. Heart and lung preparation

The cannulated trachea was then connected to the lung instillation system in situ. The left lung was distended with Karnofsky's solution (150) containing 4% glutaraldehyde at 12 cmH_2O transpulmonary pressure. Simultaneously, the left lung vascular bed was also perfused through the

heart via the right ventricle with Karnofsky's solution containing 1% glutraldehyde at 20 cm water pressure (this was done to flush excess ^{3}H-TdR in the pulmonary blood volume and leukocytes, and to fix the distended pulmonary capillaries). Following 2 hours fixation of the left lung, the heart of each animal was dissected out, blot dried and weighed. Along with the left lung, the heart was also kept submerged in Karnofsky's solution and stored until required.

2.3.2. LUNG BIOCHEMISTRY

The right lungs were freeze-dried to constant weights. DNA, RNA and total alkali soluble protein extraction and amount estimation was done by similar methods as have been explained earlier.

2.3.2.1. Measurements of DNA synthesis

Specific radioactivity of DNA was estimated by adding 0.5 ml of DNA extracts to 9.5 ml of scintillation medium contained in standard scintillation vials and counting the [^{3}H] activity using a β-counter (Beckman, LS-6800). The incorporation of ^{3}H-TdR into DNA (dpm/μg DNA) was considered to be equivalent to the net rate of DNA synthesis during injected ^{3}H-TdR.

2.3.3. AUTORADIOGRAPHIC TECHNIQUES AND OBSERVATIONS

2.3.3.1. Techniques

2.3.3.1.1. Tissue sampling

An approximately 1 mm thick mid-sagittal slice of the whole left lung was cut. From that slice, two central (one cephalic and one caudal) and two peripheral (one cephalic and one caudal) blocks were cut as shown in Figure 6. The peripheral blocks of 3-4 mm in width were cut from the lateral edge of the lung slice such that they had a visceral pleural cell layer on one side and contained no major vessels or bronchi. The central blocks were about 3-4 mm in width and 5-6 mm in length and consisted of main bronchi and vessels. All blocks were stored in Karnofsky's solution until processed.

2.3.3.1.2. Tissue processing and emulsion coating

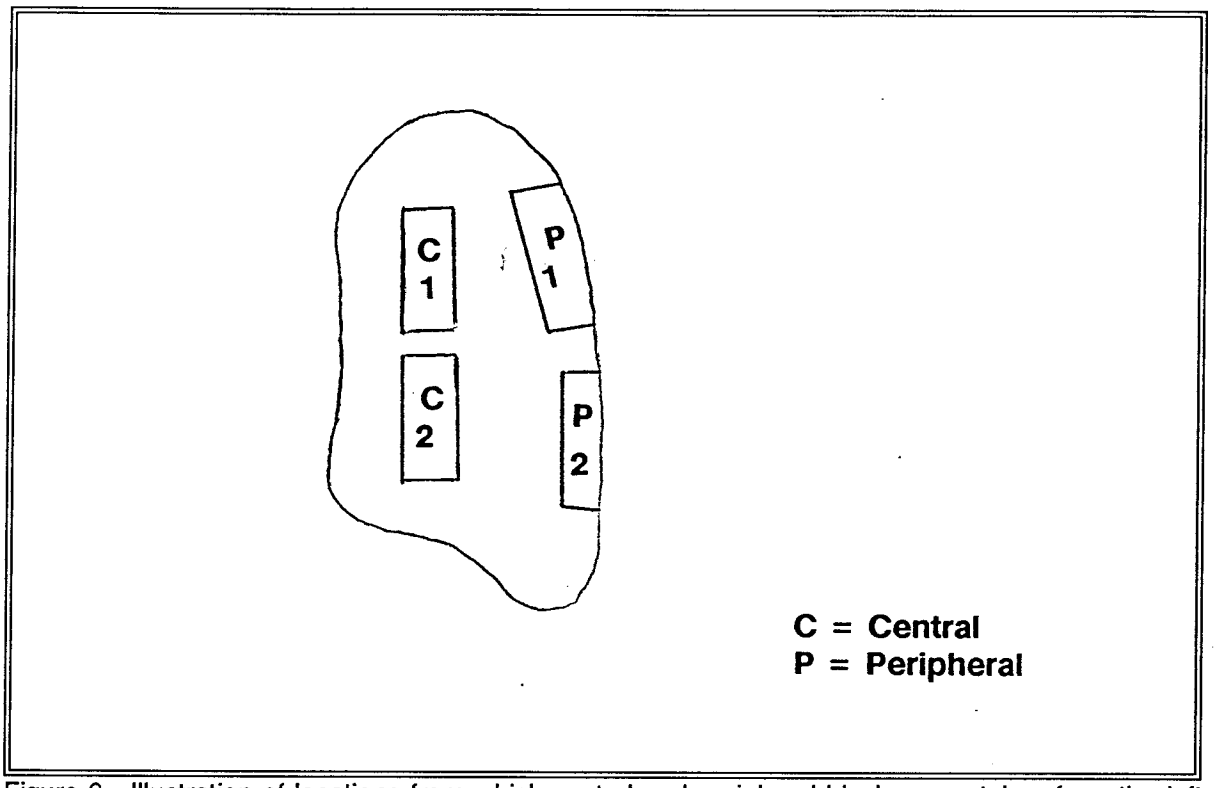


Figure 6. Illustration of locations from which central and peripheral blocks were taken from the left lung for autoradiographic measurements.

All blocks from each lung were processed and then embedded in methacrylate (JB4 embedding kit, Polysciences inc., Warrington, PA., USA). Two, one micron thick sections from each block were cut and mounted on a clean slide. All slides were coated by dipping in autoradiographic emulsion (Kodak NTB 2, cat# 165 4433) which was maintained at 47° C in a water bath in a darkroom (any light source can increase the background noise on a developed slide). The slides were then placed in racks to drain excess emulsion. The slides were allowed to dry for an hour and then wrapped carefully in aluminum foil and black paper to avoid any light exposure. The sealed slides were incubated in a cold room at 4° C for 3 weeks (3 weeks of incubation was found to be optimal, as it produced high resolution of labelled cells and low background).

2.3.3.1.3. Development of autoradiographic slides

The incubated slides were removed from the cold room and allowed to warm up for 4 hours to room temperature. The slides were then immersed in D19 developer for 4 minutes at 16° C and were gently agitated. The developing process was stopped by transferring the slides in distilled water. The distilled water was changed twice, each time for 20-30 seconds duration. Finally, the developed sections were fixed by submersion in Kodak fixative at 16° C for 5 minutes and with gentle agitation. The fixative was washed with cold distilled water for 5 minutes and then with cold tap water for 30 minutes. The slides were washed with tap water and allowed to dry. The dry slides were counterstained with 1% toluidine blue stain prepared in 1% sodium tetraborate. The radiolabelled cells appeared to be heavily laden with black granules with sporadic single granules in the background (Figure 41).

2.3.3.2.ANALYSIS OF AUTORADIOGRAPHS

2.3.3.2.1. Cell counting

Autoradiographs were examined using a Nikon microscope (100X objective) fitted with an eye piece graticule dividing the test area into four quarters. This made keeping accurate record of counted cells possible. Two sections mounted on the same slide were used for tritiated thymidine incorporation analysis. After discarding 2 test fields along the margins of the sections, every fifth adjacent field on the sections was counted until a total of 40 test fields on a slide was achieved. A field with any artifacts was discarded and the next adjacent field was included for analysis. From both the central and peripheral sections, labelled and unlabelled cells in the alveolar wall and free alveolar macrophages were identified and counted. The alveolar wall cells included type I pneumonocytes, type II pneumonocytes, alveolar wall capillary endothelial cells, interstitial cells, mast cells and unidentifiable cells. Apart from the central and peripheral alveolar wall cells, approximately 1000 cells in the walls of subpleural alveoli were also counted from the peripheral sections. An arbitrary distance

of about 100 micron along the pleural surface was defined as the subpleural zone of the lung. From the peripheral sections labelled and unlabelled mesothelial cells (<200> cells) were also recorded.

On a separate occasion, the non-parenchymal labelled and unlabelled cells were also counted. In the central sections, the major arteries and bronchi with an internal diameter of more than 400 μ were included in analysis. In the arteries, more than 150 labelled and unlabelled endothelial cells and 500 arterial wall cells were counted. In case of bronchi, more than 300 bronchial epithelial cells and more than 500 bronchial wall labelled and unlabelled cells were analyzed for cytokinetics. With regards to peripheral sections, 200-300 bronchiolar epithelial cells were counted along approximately 300-400 μ of terminal bronchiolar surface (internal diameter <200 μ) starting at the junction of alveolar duct and terminal bronchioles. A total of 6,000-8,000 labelled and unlabelled cells were counted in each animal for cytodynamic study.

2.3.3.2.2. Criteria for cell identification

The alveolar wall capillary endothelial cells were identified as cells lining and often protruding into the capillary lumen with attenuated cytoplasm adjacent to the nucleus. Very few nucleated cells were present within the lumen as the capillaries were flushed during the fixation process of the vascular bed. Type II pneumonocytes were identified as polygonal cells with vacuoles and furred surface and were often located at the corners of the alveoli. Type I pneumonocytes were located at the alveolar surface, distinguished by elongated nuclei with long cytoplasmic extensions and had a "fried egg" appearance. All cells in the interstitium (fibroblasts, septal cells, pericytes, monocytes, lymphocytes) were counted as interstitial cells because it was difficult to identify various cell populations. Leukocytes in capillary lumen were not taken into account. This was done to avoid the influence of the labelling index of leukocytes on the analysis. The mast cells were distinct with metachromatic staining. The bulk of mast cells were located in the subpleural area or in the walls of vessels and bronchi. The cells which were either in cross section (tiny nucleus with lots of cytoplasm or a large nucleus with no cytoplasm) or difficult to categorize were classified as unidentifiable cells.

Free alveolar macrophages were easy to recognize in airspaces.

2.3.3.2.3. Labelling indices

The cells which were, or entered, the s-phase of the cell generation cycle at the time of ^{3}H-TdR injection, incorporated labelled thymidine into the cell DNA, showed more than 10-20 granules on the nuclear area on the histological slides. No other criterion was considered necessary for the number of the granules present over the labelled cells because the background was low and the labelled cells were heavily laden with granules. The percent labelling index for each cell type population was calculated as follows:

$$\% \text{ Labelling index} = \frac{\text{Total number of labelled cells} * 100}{\text{Total number of cells counted (labelled + unlabelled)}}$$

A total of 4000-6000 alveolar wall cells were counted in the central and peripheral sections. The combined alveolar wall cell number was computed as the sum of type I pneumonocytes, type II pneumonocytes, alveolar wall capillary endothelial cells, interstitial cells, unidentifiable cells and mesothelial cells from central, peripheral and subpleural area. The labelling index for each category of cells was calculated by dividing the labelled cells of a particular category by the total number of that category of alveolar wall cells separately for the central, peripheral and subpleural alveoli. Since the labelling indices of the alveolar wall cells in the peripheral and subpleural areas of the lung were the same, the cells from both areas were pooled for respective cell categories and the results were referred as alveolar wall cells in the peripheral part of the lung.

2.4. LUNG ADAPTATION: PHYSIOLOGICAL ASPECTS

2.4.1. EXPERIMENTAL DESIGN

Forty, four week old male Sprague-Dawley littermate rats were randomly sorted into five groups as described earlier:

* Group 1. General Control Group (GC)
* Group 2. Hypobaric Normoxic Group (HBNO)
* Group 3. Normobaric Hypoxic Group (NBHY)
* Group 4. Hypobaric Hypoxic Group (HBHY)
* Group 5. Weight-matched Control Group (WMC)

Exposure protocol was the same as has been detailed in the first section of methods.

2.4.2. PULMONARY FUNCTION TESTS

Pulmonary function tests were performed as described by Wright and coworkers (286).

2.4.2.1. Animal preparation

After three weeks of chronic exposure to the respective conditions, the rats were anaesthetized by injecting urethane (100 mg/100 g body weight, i.p.). After recording the somatic parameters, an approximately 5 mm long skin incision was made along the median plane distal to the larynx. The trachea was exposed by dissecting the muscles and a tracheostomy was done. The trachea was cannulated with a polyethylene cannula (1.5 mm internal diameter). A gag was placed in the mouth to protect the esophageal catheter. To measure pleural pressure, an eight cm long (internal diameter 1 mm, polyethylene) water-filled, multiple side-holed catheter was carefully inserted into the esophagus just proximal to the heart (premarked) of the anaesthetized rat. The animal was then placed supine inside a pressure sensitive whole body plethysmograph consisting of a 7.5 liter chamber connected to a bottle containing copper mash used as a body heat sink. All the holes for connections to the plethysmograph were plugged to prevent air leaks. The esophageal catheter was flushed with a small amount of water and was then connected to the pressure transducer.

2.4.2.2. Lung volume measurements

The rats were allowed to breathe on their own. If the respiration frequency was irregular or too high, they were attached to a respirator and were hyperventilated for 1-2 minutes. This helped to reduce the respiratory rate to within the desired range (40-60 breaths/minute). At the end of expiration the tracheal opening was occluded with a finger and the rat was allowed to exert effort against the occlusion (end-expiration could be monitored by carefully watching the thorax movement or the recording pen). The difference between esophageal and airway opening pressure was recorded with the help of a differential transducer (DP 42-32, Validyne Corp., Northridge, CA) and the volume signal was obtained by using a pressure sensitive transducer (Validyne DP 45-16). For FRC measurements airway pressure was used. FRC was measured using Boyle's Law:

"At a constant temperature, the volume of any gas varies inversely as the pressure to which the gas is subjected."

$P_1V_1 = P_2V_2$

where P_1 is the initial pressure, V_1 is the initial volume, P_2 is changed pressure and V_2 is changed volume, while temperature remains constant. With the help of a digital computer, a pressure/volume loop was obtained. The slope of this loop was obtained with the assistance of a computer. The slope was then matched to read the functional residual capacity (FRC) of the rat from a calibrated chart. FRC was taken as the mean calculated from 3-4 consecutive maneuvers.

Following FRC measurements, an intraperitoneal injection of succinylcholine was given to paralyse the rat (0.1 ml/100 g body weight). The animal was connected to the respirator and a tidal volume of 2 ml was set at 60 breaths/minute frequency. When the rat was completely paralysed (the rat did not resist the ventilator), all the ports of the plethysmograph were sealed and the pressure-volume curves were recorded. First the lungs were deflated to -30 cm water transpulmonary pressure and the lung volume at this pressure was defined as residual volume (RV). The decrease in volume recorded after deflation was assumed as expiratory reserve volume (ERV). The residual volume was then calculated by subtracting ERV from FRC. The lungs were then inflated to +30 cm water

transpulmonary pressure and this volume from residual volume was defined as vital capacity (VC). Total lung capacity (TLC) was calculated as a sum of VC (obtained from the pressure volume curve recording) and RV (calculated as shown above). Following an inflation at +30 cmH_2O transpulmonary pressure, the lungs were deflated to -30 cm water pressure. This whole maneuver was performed once without recording and then two pressure-volume curves were plotted. The deflation limb of the pressure-volume recording was used for pressure-volume analysis. From the deflation limb recordings, static lung compliance (Cst) was calculated as change in lung volume per unit change in transpulmonary pressure at functional residual capacity at +5 cmH_2O transpulmonary pressure.

2.4.2.3. Flow-volume relationships

After recording the pressure-volume curves, the lungs were inflated to +30 cmH_2O pressure and were then rapidly deflated at an airway pressure of -50 cmH_2O pressure until expiratory air flow ceased. Using this procedure forced expiratory volume and simultaneous transpulmonary pressure changes were recorded. From these recordings peak expiratory flow (PFER), forced expiratory flow (FEF) at percentiles of forced vital capacity (FVC), FEF corrected for vital capacity at percentiles of forced vital capacity, maximum midexpiratory flow ($FEF_{25-75\%}$) at 25%-75% of forced vital capacity, absolute forced expiratory volume in 0.1 second ($FEV_{0.1}$) and $FEV_{0.1}$ percent of forced vital capacity ($FEV_{0.1}$/FVC%) were computed. Upstream resistance (Rus) was calculated (transpulmonary pressure/ flow) at 50% FVC.

In a normal lung, the lung compliance and airway resistance are influenced by the size of the lung (71). Therefore, in order to eliminate the effect of lung size, compliance and airway resistance were corrected for lung volume at which these measurements were made. Specific lung compliance (sCst) was calculated by dividing the static lung compliance by lung volume and specific upstream airway resistance (sRus) was calculated by multiplying upstream resistance with lung volume at 50% forced vital capacity (71).

From the expiratory flow rates and forced vital capacity recordings, flow-volume curves were

constructed. Using the pressure-volume curve tracings, pressure at percentiles of lung volume (FRC to TLC) was calculated and then pressure-volume characteristics for elastic lung recoil were analyzed. As described in section I, the pressure-volume curves were also used to fit a monoexponential function in the range of lung volume from FRC to TLC (68).

2.5. STATISTICAL ANALYSIS

Group means ±standard errors of the means (SEM's) were calculated. For section I (Lung growth adaptation) and III (Lung adaptation: physiological aspects) of the project, the statistical evaluation of the groups was performed by using a single factor analysis of variance to test the hypothesis that the means of all groups were equal. If the hypothesis was rejected at $p<0.01$, then the Student Newman Keuls (SNK) multiple range test was used in order to locate a significant difference at $p<0.05$ level between the groups. Statistical calculations were done using SPSS:X and Number Cruncher Statistical System.

For section II (Organ response and lung cytokinetics) of the project, a two-way analysis of variance was applied to detect the effect of treatments (undernutrition, hypobaric normoxia, normobaric hypoxia and hypobaric hypoxia) and duration of exposure (days) and their interaction on somatic variables, organ weights, lung biochemical measurements and ^{3}H-TdR incorporation into various types of cells in the lung. If an interaction between the two factors was detected for a variable, then further analysis was done separately for treatments and days using linear contrast test to locate a significant difference. In the case of groups, eight possible contrasts (GC vs WMC, GC vs HBHY, GC vs HBNO, GC vs NBHY, WMC vs HBHY, WMC vs NBHY, HBNO vs HBHY and HBHY vs NBHY) were made to detect the effect of various conditions. The rate of organ growth was evaluated by comparing the values of 3, 5, 7, 10, 14 and 21 days in each group (GC, WMC, HBNO, NBHY and HBHY) to the value of day 1 of general controls. This was done to determine the duration of time required in each experimental condition to produce a significant change in organ weights. Depending upon the number of contrasts between the groups or the days, the Bonferroni correction was applied to keep the total

chance of erroneously rejecting the hypothesis that no change occurred between two groups or two time intervals below 5 percent (288). This required significant levels of $p<0.006$ between the groups and $p<0.008$ between the days before applying Bonferroni correction to achieve a significance of $p<0.05$. The p-values shown in the results are p-values obtained by linear contrast times number of contrasts made in that category. To analyze the effect of the recovery period, a t-test was performed to compare the values of body weight, nose-tail length, organ weights, lung biochemical variables and radiochemically measured ^{3}H-TdR uptake on day 21 and 24 (3 days after returning the animals to room air and allowing free access to food) of experimental conditions. The effect of post-exposure and refeeding among the groups was analyzed as described above.

CHAPTER 3

RESULTS

3.1. LUNG GROWTH STUDY

3.1.1. GENERAL

Since changes in somatic growth occurred in the experimental groups compared to the general controls, and the experiments described here are complicated, the data will be presented in the following way. The general control group will be compared to the weight-matched control group to assess the effect of undernutrition. To evaluate the effect of low ambient pressure, the hypobaric normoxic rats will be compared to general control rats, and the hypobaric hypoxic rats will be compared with normobaric hypoxic rats. For the effect of low oxygen, comparisons will be made between the normobaric hypoxic group and the weight-matched control group, the normobaric hypoxic group and the general control group, and the hypobaric hypoxic group and the hypobaric normoxic group. Adaptive response to hypobaric hypoxia will be determined by comparing the hypobaric hypoxic group to the general control group, but more emphasis will be placed on the comparison with weight-matched controls.

The normobaric hypoxic and hypobaric hypoxic rats appeared to be less active on days 0 and 1 of the experiment relative to general control, weight-matched control and hypobaric normoxic rats but after day 1 regardless of the condition they were subjected to, no obvious difference in activity among rats was noticed.

3.1.1.1. NUTRITIONAL ASSESSMENT

As shown in Figure 7, during the first week of exposure, the hypobaric normoxic and general control animals consumed similar amounts of food, but thereafter the food intake of the hypobaric

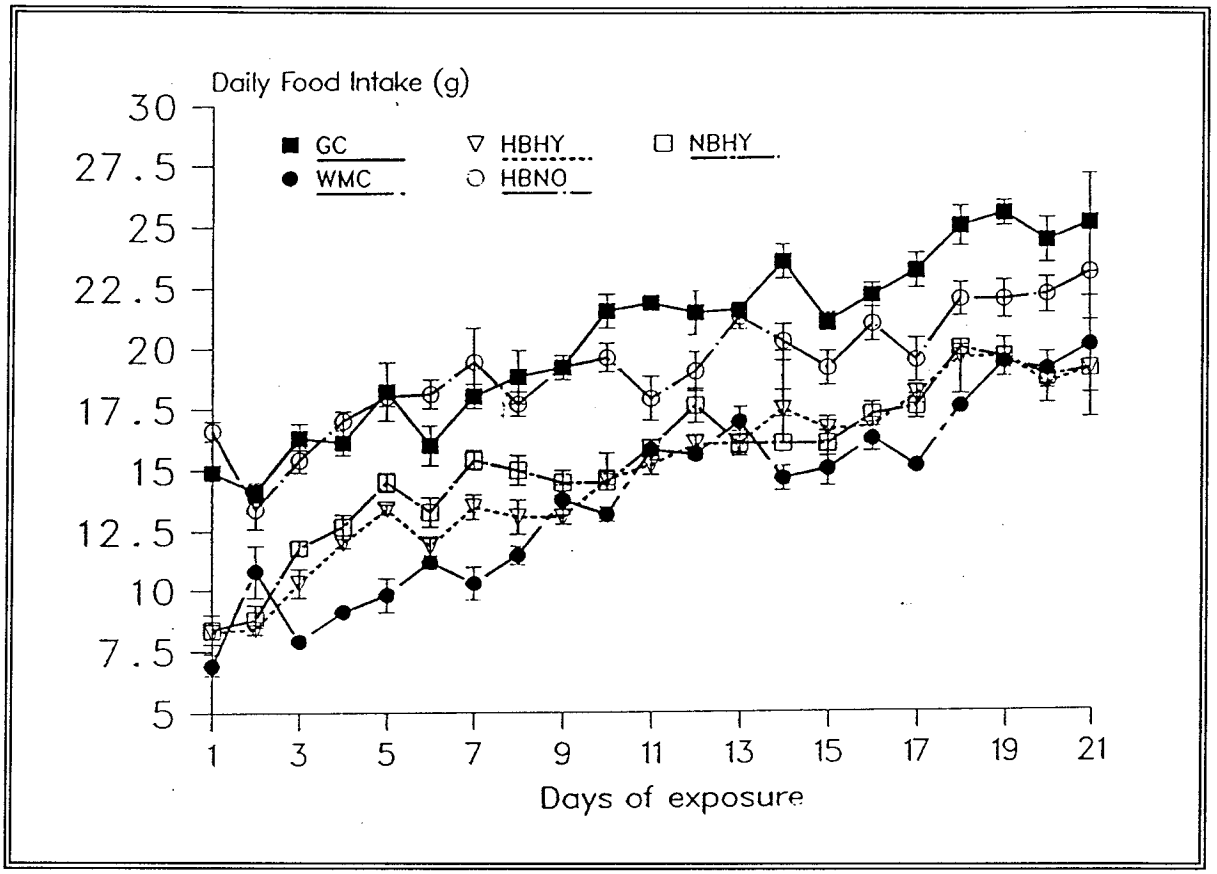


Figure 7. Daily food consumption of the general control (GC), weight-matched control (WMC), hypobaric hypoxic (HBHY), hypobaric normoxic (HBNO) and normobaric hypoxic (NBHY) groups.

normoxic animals gradually decreased and was significantly lower on day 10 and afterwards (except on days 13, 16, 20 and 21). The total food intake of hypobaric normoxic rats during 21 days of exposure was also significantly less compared to general controls (Table 3).

The exposure to hypobaric hypoxia or to normobaric hypoxia caused a significant drop in food consumption on day 1, which appeared to be the point of divergence. Thereafter the daily food consumption of hypobaric hypoxic and normobaric hypoxic rats increased gradually but it never reached the amount of daily food intake of general control rats during the experimental period (Figure 7). During three weeks of exposure, the total amount of food consumed by the hypobaric hypoxic and normobaric hypoxic groups was significantly less (25% and 27% respectively) than that of the general control group (Table 3). No difference in food consumption was found between the normobaric hypoxic and hypobaric hypoxic groups.

The weight-matched control and hypobaric hypoxic groups were paired for body weight gain. The weight-matched control rats were allowed limited access to food to keep their daily body weight gain similar to the body weight gain of paired hypobaric hypoxic rats (Figure 7). During three weeks, the total food consumed by the weight-matched control group was significantly lower (12%) than that of the hypobaric hypoxic group (Table 3).

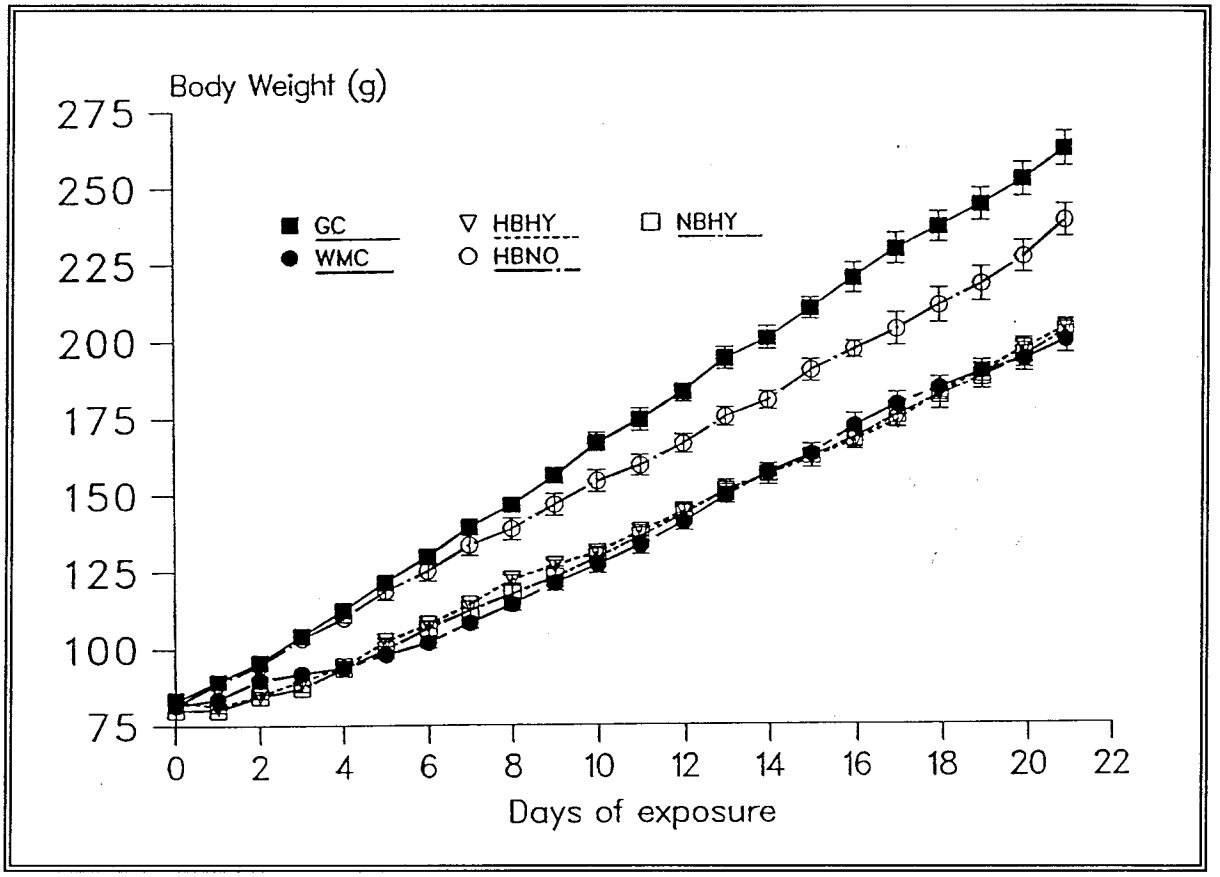


Figure 8. The effect of undernutrition, hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia on body weight gain.

3.1.1.2. SOMATIC GROWTH

Body weight was recorded as a measure of body size and nose-tail length as a determinant of skeletal growth of rats during the experimental period. The general control rats gained 214% body weight and 48% nose-tail length during 4 to 7 weeks of age (Table 3).

During the first week of exposure, no difference in body weight gain was detected between the hypobaric normoxic and general control groups, but thereafter, the body weight of the hypobaric normoxic group lagged behind the general control group. After day 12, the hypobaric normoxic animals were significantly smaller than the general control rats (Figure 8). At termination, the hypobaric normoxic rats were significantly lighter in body weight (9%) and shorter in nose-tail length compared to those measured in the general control group (Table 3).

As illustrated in Figure 8, in normobaric hypoxic and hypobaric hypoxic rats, the body weight decreased slightly on day 1 of exposure. However, from day 3 until the end of exposure, a consistent but slow body weight increase was observed in normobaric hypoxic and hypobaric hypoxic animals but they remained significantly smaller compared to the general control animals (Figure 8). During 3 weeks of exposure, both the normobaric hypoxic and the hypobaric hypoxic groups gained 142% in body weight. At the time of termination, the normobaric hypoxic and hypobaric hypoxic rats were significantly smaller in body weight (23%) and nose-tail length (7% and 9% respectively) compared to the general control group (Table 3). When the results for body weight and nose-tail length of weight-matched control, hypobaric hypoxic and normobaric hypoxic groups were compared, no differences were found among three groups (Table 3).

3.1.2. HEMATOCRIT

Before terminating the animals, blood samples were taken from the right ventricle and hematocrit was measured. After 21 days of exposure, the hematocrit values increased significantly in the normobaric hypoxic and hypobaric hypoxic groups compared to both the general control and weight-matched control groups (Table 3). Hematocrit in weight-matched animals remained unchanged compared to general controls. No difference was found between the hematocrit of hypobaric hypoxic and normobaric hypoxic rats. A slight but significant increase of 9% was also observed in rats after exposure to hypobaric normoxia compared to general controls (Table 3).

Table 3. Food intake, somatic growth and hematocrit measurements.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
TOTAL FOOD INTAKE (g)	----	402.2 ± 3.7^{a}	274.7 ± 4.2^{b}	376.9 ± 8.1^{c}	292.1 ± 3.3^{d}	307.0 ± 5.1^{d}
Wb g	83.2 ± 0.6^{a}	261.0 ± 2.6^{b}	199.0 ± 2.3^{c}	238.0 ± 3.2^{d}	203.0 ± 2.2^{c}	202.0 ± 1.5^{c}
NTL cm	26.0 ± 0.2^{a}	38.5 ± 0.2^{b}	35.1 ± 0.2^{c}	37.2 ± 0.3^{d}	35.7 ± 0.2^{c}	34.6 ± 0.3^{c}
Hct (%)	39.0 ± 0.9^{a}	41.4 ± 0.4^{a}	41.4 ± 0.6^{a}	45.1 ± 0.6^{b}	59.0 ± 0.7^{c}	60.1 ± 1.0^{c}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

Wb (body weight), NTL (nose-tail length), Hct (hematocrit), BC (baseline controls, 4 week old), GC (general controls), WMC (weight-matched controls), HBNO (hypobaric normoxic), NBHY (normobaric hypoxic) and HBHY (hypobaric hypoxic).

3.1.3. LUNG GROWTH

The morphometric results related to size of the airspaces or alveoli {mean linear intercept (MLI), mean chord length of alveoli (l_{alv}), average alveolar volume ($Valv_{ave}$)} and structures per unit volume or area {number of alveoli per unit area (Na), number of alveoli per unit volume (N_V), alveolar surface area to lung volume ratio (S_w/V_L)} will be referred to as unit structure morphometric parameters. The total lung weight was used to calculate the total lung volume from left lung volume.

3.1.3.1. NORMAL LUNG GROWTH (4-7 weeks of age)

3.1.3.1.1. EXTENT OF LUNG GROWTH

The lung weight and lung volume increased by 87% and 146% respectively during 4 to 7 weeks of age general controls (Table 4), but specific (relative to per unit body weight) lung weight and lung volume decreased significantly. Since lung volume increased more than lung weight, a significant increase in lung volume per unit lung weight was observed at 7 weeks of age compared to 4 week old animals (Table 4).

3.1.3.1.2. BIOCHEMICAL CHANGES

A significant increase in the dry lung weight (WI_{dry}) (89%), DNA (57%), RNA (69%) and alkali soluble protein (73%) content was found between 4 weeks to 7 weeks of age (Table 5). The amount of total hydroxyproline which comprised SDS-soluble and SDS-insoluble (cyanogen bromide soluble) fractions also increased by 120% (Table 5). An increase in the insoluble fraction of hydroxyproline was greater (130%) than that of the soluble fraction (53%), thus causing a significant decrease in the ratio of soluble to insoluble fraction of hydroxyproline at 7 weeks of age compared to 4 weeks of age. A greater increase in the amount of insoluble fraction of hydroxyproline at 7 weeks of age indicated increased formation of mature collagen. A marked increase (86%) occurred in desmosine content during 4-7 weeks of age (Table 5). Specific amounts of DNA, RNA, protein, hydroxyproline and

Table 4. Results of lung growth adaptation.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
W_L g	0.604 ± 0.011^a	1.130 ± 0.024^b	0.948 ± 0.014^c	1.063 ± 0.029^b	1.307 ± 0.02^d	1.326 ± 0.028^d
V_L ml	4.64 ± 0.12^a	11.40 ± 0.52^b	9.30 ± 0.24^c	10.57 ± 0.17^d	12.94 ± 0.21^e	12.26 ± 0.26^e
W_L/100 g Wb g	0.725 ± 0.011^a	0.433 ± 0.010^b	0.476 ± 0.006^c	0.445 ± 0.009^b	0.646 ± 0.008^d	0.656 ± 0.014^d
V_L/100 g Wb ml	5.58 ± 0.19^a	4.35 ± 0.17^b	4.70 ± 0.12^b	4.43 ± 0.09^b	6.38 ± 0.08^c	6.07 ± 0.12^c
V_L/W_L (ml/g)	7.72 ± 0.24^a	10.10 ± 0.44^b	9.87 ± 0.31^b	9.85 ± 0.24^b	9.87 ± 0.13^b	9.29 ± 0.29^b

Means ±sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

W_L (total lung weight), V_L (total lung volume), BC (baseline controls), GC (general controls), WMC (weight-matched controls), HBNO (hypobaric normoxic), NBHY (normobaric hypoxic) and HBHY (hypobaric hypoxic).

Table 5. Biochemical assessment of lung growth after three weeks of exposure to various conditions.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
Dry/Wet W_L %	21.35 ± 0.18^a	21.23 ± 0.23^a	21.43 ± 0.12^a	21.82 ± 0.33^a	21.02 ± 0.33^a	21.48 ± 0.16^a
WI_{dry} mg	74.0 ± 1.5^a	139 ± 3.0^b	120 ± 2.0^c	138 ± 3.0^b	168 ± 4.0^d	173 ± 3.0^d
DNA mg	2.47 ± 0.07^a	3.88 ± 0.07^b	3.37 ± 0.07^c	3.73 ± 0.09^b	4.41 ± 0.11^d	4.39 ± 0.11^d
RNA mg	2.34 ± 0.07^a	3.96 ± 0.11^b	2.82 ± 0.11^c	3.76 ± 0.09^b	4.52 ± 0.12^d	4.45 ± 0.12^d
PROTEIN mg	37.57 ± 0.66^a	65.08 ± 1.37^b	52.94 ± 0.91^c	64.99 ± 1.48^b	80.55 ± 2.51^d	84.97 ± 1.97^d
OHP(SOL) mg	0.079 ± 0.002^a	0.121 ± 0.004^b	0.105 ± 0.004^c	0.124 ± 0.004^b	0.141 ± 0.006^d	0.155 ± 0.003^d
OHP(INS) mg	0.525 ± 0.029^a	1.209 ± 0.029^b	0.985 ± 0.030^c	1.210 ± 0.030^b	1.258 ± 0.05^b	1.376 ± 0.03^d
OHP mg	0.605 ± 0.030^a	1.334 ± 0.033^b	1.090 ± 0.033^c	1.334 ± 0.035^b	1.400 ± 0.051^b	1.531 ± 0.033^d
DES ug	12.05 ± 0.47^a	23.21 ± 1.57^b	20.42 ± 1.15^b	26.41 ± 2.28^b	33.14 ± 2.18^c	40.15 ± 2.52^d

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

Dry/Wet ratio (dry lung weight to wet lung weight ratio), WI_{dry} (dry weight of the right lung), OHP (hydroxyproline [SOL] soluble fraction, [INS] insoluble fraction), DES (desmosine)

Table 6. Lung biochemical measurements normalized to 100 grams of body weight.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
DNA mg	2.96 ± 0.08^{a}	1.47 ± 0.03^{b}	1.70 ± 0.03^{c}	1.56 ± 0.03^{b}	2.17 ± 0.05^{d}	2.17 ± 0.06^{d}
RNA mg	2.99 ± 0.08^{a}	$1.52\pm0.03^{b,c}$	1.41 ± 0.04^{b}	1.58 ± 0.03^{c}	2.23 ± 0.07^{d}	2.19 ± 0.06^{d}
PROTEIN mg	45.12 ± 0.67^{a}	24.94 ± 0.45^{b}	$26.60\pm0.41^{b,c}$	27.21 ± 0.66^{c}	39.60 ± 1.26^{d}	42.07 ± 1.07^{d}
OHP mg	0.725 ± 0.038^{a}	0.510 ± 0.014^{b}	0.547 ± 0.016^{b}	0.558 ± 0.011^{b}	0.689 ± 0.024^{a}	0.757 ± 0.017^{a}
DESMOSINE ug	14.5 ± 0.5^{a}	8.9 ± 0.6^{b}	10.2 ± 0.5^{b}	11.1 ± 0.9^{b}	16.4 ± 1.1^{a}	19.9 ± 1.3^{c}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

desmosine were significantly higher in 4 week old compared to those in 7 week old rats (Table 6).

The concentrations (biochemical measurements per unit dry weight of the lung tissue) of DNA and RNA were significantly less at 7 week of age compared to 4 week old animals (Table 7). The concentration of hydroxyproline increased significantly as age increased, but the desmosine concentration remained constant indicating that a dynamic equilibrium of elastin was achieved before 4 weeks of age (Table 7). Except for a significant increase in 7 week old animals for the hydroxyproline to DNA (OHP/DNA) ratio, no differences in other ratios [RNA/DNA, desmosine to DNA (DES/DNA), desmosine to hydroxyproline (DES/OHP)] were observed between the two age groups (Table 8).

3.1.3.1.3. MORPHOMETRIC CHANGES

Results of unit structure morphometric measurements showed that between 4 and 7 weeks of age, significant increases in mean linear intercept of airspaces (MLI), mean chord length of alveoli (l_{alv}) and average volume of alveoli ($Valv_{ave}$), and associated decreases in the number of alveoli per unit area (Na), number of alveoli per unit volume (N_V) and surface to volume ratio (S_w/V_L) were observed (Table 9).

Seven week old rats had a significantly increased volume fraction of alveolar air (Vv_{alv}) compared to the 4 week old rats (Table 10). Although the volume fraction of duct air (Vv_{duct}), volume fraction of alveolar wall (Vv_{wall}), volume fraction of non-parenchymatous tissue (Vv_{np}) and volume fraction of bronchial air (Vv_{br}) did not change (Table 10), absolute volume of alveolar air (V_{alv}), alveolar duct (V_{duct}), alveolar wall (V_{wall}), non-parenchymal tissue (V_{np}) and bronchial air (V_{br}) increased significantly in 7 week old animals (Table 11).

The total number of alveoli (N_{at}) obtained by direct alveolar count and internal surface area (S_w) increased significantly (70% and 133% respectively) between 4 and 7 weeks of age (Table 12). Despite significant increases in absolute values of the S_w and N_{at} in 7 week old rats, body weight normalized values were significantly smaller in 7 week old animals compared to 4 week old animals

(Table 12).

The results showed that during 4 to 7 weeks of postnatal age, lung air volume increased more than the lung weight. Absolute biochemical and morphometric measurements increased with age but did not keep up with body growth. A decrease in DNA concentration and an increase in hydroxyproline concentration indicated that with age, the intercellular matrix increased more than the cellular component of the lung. Interestingly, elastin appeared to plateau before 4 weeks of age. The unit structure morphometric results showed that even though enlargement of the airspaces occurred during 4 to 7 week of age, alveolar number continued to increase.

3.1.3.2. EFFECT OF UNDERNUTRITION

3.1.3.2.1. EXTENT OF LUNG GROWTH

Three weeks of undernutrition caused reductions in lung weight (16%) and lung volume (18%) compared to the general control group (Table 4). On the other hand, specific lung weight of the undernourished group was significantly higher than that of the general control group. Lung volume per unit lung weight remained unchanged in undernourished rats (Table 4).

3.1.3.2.2.BIOCHEMICAL CHANGES

The rats given restricted food had significantly lower WI_{dry}, DNA, RNA, protein and hydroxyproline content (14%, 13%, 29%, 19% and 18% respectively) compared with those found in the general control group (Table 5). When biochemical parameters were corrected for body weight, except for a significant increase in DNA content, other parameters in the weight-matched control group remained similar to those in the general control group (Table 6).

The concentrations of DNA, hydroxyproline and desmosine remained unaffected in undernourished animals, but the concentrations of RNA and protein decreased significantly (Table 7). None of the biochemical ratios changed except for RNA/DNA ratio which was significantly decreased (Table 8).

Table 7. Biochemical variables per gram of dry lung weight.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
DNA mg	32.2 ± 0.5^{a}	28.0 ± 0.5^{b}	28.4 ± 0.6^{b}	$27.0\pm0.6^{b,c}$	$26.4\pm0.9^{b,c}$	25.3 ± 0.4^{c}
RNA mg	31.7 ± 0.60^{a}	28.5 ± 0.35^{b}	23.5 ± 0.53^{c}	27.3 ± 0.54^{b}	27.0 ± 0.5^{b}	25.7 ± 0.53^{b}
PROTEIN mg	501.4 ± 9.7^{a}	470.0 ± 6.3^{a}	443.6 ± 5.5^{b}	471.1 ± 8.5^{a}	480.1 ± 7.6^{a}	491.0 ± 7.7^{a}
OHP mg	8.12 ± 0.33^{a}	9.60 ± 0.16^{b}	$9.12\pm0.20^{b,c}$	9.65 ± 0.12^{b}	8.36 ± 0.26^{a}	$8.84\pm0.12^{a,c}$
DESMOSINE ug	163.0 ± 6.0^{a}	166.2 ± 11.4^{a}	170.8 ± 8.5^{a}	192.5 ± 15.7^{a}	196.4 ± 9.2^{a}	232.9 ± 13.1^{b}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

Table 8. Relationships between biochemical changes in different experimental groups.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
RNA/DNA (mg/mg)	1.00 ± 0.03^{a}	1.02 ± 0.02^{a}	0.83 ± 0.03^{b}	1.01 ± 0.02^{a}	1.03 ± 0.03^{a}	1.02 ± 0.02^{a}
PROTEIN/DNA (mg/mg)	15.27 ± 0.47^{a}	16.81 ± 0.34^{b}	15.65 ± 0.31^{a}	$17.43\pm0.63^{b,c}$	18.41 ± 0.65^{c}	19.39 ± 0.57^{c}
OHP/DNA (ug/mg)	243.2 ± 11.9^{a}	$342.2\pm6.8^{b,c}$	320.0 ± 10.3^{b}	358.1 ± 6.8^{c}	318.0 ± 9.6^{b}	349.3 ± 5.1^{c}
DES/DNA (ug/mg)	4.91 ± 0.20^{a}	$5.98\pm0.4^{a,b}$	$6.22\pm0.34^{a,b}$	7.18 ± 0.49^{b}	7.44 ± 0.5^{c}	$9.25\pm.51^{c}$
DES/OHP (ug/mg)	$21.0\pm1.5^{a,b}$	17.5 ± 1.5^{a}	18.8 ± 1.0^{a}	$20.1\pm1.7^{a,b}$	$23.7\pm1.5^{b,c}$	$26.5\pm1.8^{c,d}$
OHP (Sol) %	15.57 ± 0.92^{a}	10.03 ± 0.34^{b}	10.71 ± 0.47^{b}	10.29 ± 0.29^{b}	11.46 ± 0.55^{b}	11.27 ± 0.47^{b}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

OHP (hydroxyproline), DES (desmosine), OHP(sol) % (percent of soluble fraction of total hydroxyproline content).

3.1.3.2.3. MORPHOMETRIC CHANGES

Undernutrition did not cause changes in the unit structure morphometric parameters such as MLI, I_{alv}, N_A, N_V and $Valv_{ave}$, when compared to those in the general control group (Table 9), but the S_w/V_L ratio was higher in undernourished animals. The volume fractions of the lung structures remained unchanged (Table 10) but absolute volumes of alveolar air, alveolar duct air and alveolar wall decreased significantly (Table 11). Even though, the morphometric measures of the lung unit structures remained unchanged, S_w and N_{at} which are functions of lung volume were significantly lower (16% and 12% respectively) and the specific values for these parameters were significantly higher in the weight-matched group compared to the general control group (Table 12).

The above results showed that undernutrition diminished somatic growth as well as lung growth assessed by lung weight and volume, and also by biochemical and morphometric analyses. Somatic growth was affected more than lung growth. On the other hand, lung structure based on biochemical measurements (except for RNA) and morphometric results remained unchanged by food restriction, therefore, indicating diminished lung growth without altering the structure of the lung.

3.1.3.3. ADAPTATION TO LOW AMBIENT PRESSURE (HYPOBARIC NORMOXIA)

3.1.3.3.1. EXTENT OF LUNG GROWTH

As recorded in Table 4, in low ambient pressure conditions, the lung volume decreased significantly compared to the general control group, but lung weight did not. No differences between the general control and hypobaric normoxic groups were found when the values for lung weight and lung volume were normalized for body weight (Table 4). No change in lung volume per unit lung weight was found in hypobaric normoxic rats.

When the hypobaric hypoxic and normobaric hypoxic groups were compared to assess the effect of hypobaric pressure neither absolute nor specific lung weight and lung volume showed any

changes (Table 4).

3.1.3.3.2. BIOCHEMICAL CHANGES

No significant differences between the general control and hypobaric normoxic groups were found in any of the lung biochemical parameters (DNA, RNA, alkali soluble protein, hydroxyproline and desmosine) measured (Table 5). To observe the effect of low ambient pressure, the normobaric hypoxic and hypobaric hypoxic groups were compared and it was found that the biochemical parameters in normobaric hypoxic and hypobaric hypoxic groups were also similar, except for a marked increase (21%) in desmosine content in hypobaric hypoxic animals, which was significant (Table 5).

The specific amount of lung DNA, RNA, protein, hydroxyproline or desmosine were also the same in the hypobaric normoxic and general control groups. No differences were found for specific biochemical estimations between the normobaric hypoxic and hypobaric hypoxic groups, except that the amount of desmosine was higher in the hypobaric hypoxic group (Table 6).

The concentrations of all biochemical measurements remained unchanged following exposure to hypobaric normoxia (Table 7). When the hypobaric hypoxic and normobaric hypoxic groups were compared, only the concentration of desmosine was different and significantly higher in the hypobaric hypoxic group. DES/DNA was also significantly increased in the hypobaric hypoxic group compared to the normobaric hypoxic group (Table 8).

3.1.3.3.3. MORPHOMETRIC CHANGES

The hypobaric normoxic rats had significantly smaller MLI and $Valv_{ave}$, and greater N_A, N_V and S_w/V_L ratio compared to those in general controls (Table 9). Absolute volume of alveolar air, alveolar duct air and alveolar wall decreased significantly (Table 11). When the hypobaric hypoxic and normobaric hypoxic groups were compared, the values of MLI and $Valv_{ave}$ were significantly lower and N_A and N_V were higher in hypobaric hypoxic animals. No differences were found in absolute values

Table 9. Changes in morphometric parameters following exposure to different conditions.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
MLI (μm)	78.6 ± 1.2^{a}	85.4 ± 1.5^{b}	$81.9\pm1.3^{b,d}$	76.5 ± 1.1^{a}	91.1 ± 1.6^{c}	$84.3\pm1.8^{b,d}$
l_{alv} (μm)	34.9 ± 0.6^{a}	40.9 ± 0.8^{b}	38.6 ± 0.6^{b}	38.4 ± 0.6^{b}	45.1 ± 0.7^{c}	43.5 ± 1.3^{c}
N_A (10^{+3})	22.6 ± 0.41^{a}	18.1 ± 0.38^{b}	$19.1\pm0.32^{b,c}$	19.7 ± 0.37^{c}	16.7 ± 0.33^{d}	18.4 ± 0.42^{b}
N_V (10^{+5})	30.0 ± 0.85^{a}	20.9 ± 0.73^{b}	$23.0\pm0.55^{b,c}$	24.2 ± 0.67^{c}	18.3 ± 0.51^{d}	20.9 ± 0.71^{b}
$Valv_{ave}$ (10^{-8})	18.1 ± 0.6^{a}	$27.3\pm1.3^{b,e}$	$24.4\pm0.7^{b,c}$	23.9 ± 0.7^{c}	32.0 ± 0.8^{d}	28.7 ± 1.1^{e}
S_w/V_L ratio	0.095 ± 0.002^{a}	0.082 ± 0.002^{b}	0.089 ± 0.002^{a}	0.091 ± 0.002^{a}	$0.075\pm0.001^{c,d}$	$0.080\pm0.002^{b,d}$

Means ±sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

MLI (mean linear intercept), l_{alv} (mean chord length of alveoli), N_A (number of alveoli per unit area), N_V (number of alveoli per unit volume), $Valv_{ave}$ (average volume of alveoli), S_W/Vl ratio (surface to volume ratio of alveoli $\{S_w/V_L*Vv_{air}\}$).

Table 10. Alterations in volume proportions of lung morphological structures following 3 weeks of exposure to different conditions.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
Vv_{alv}	0.539 ± 0.008^{a}	$0.568\pm0.008^{b,c}$	$0.553\pm0.007^{a,b}$	$0.576\pm0.008^{b,c}$	$0.578\pm0.006^{b,c}$	0.595 ± 0.012^{c}
Vv_{duct}	0.217 ± 0.007^{a}	$0.204\pm0.008^{a,b}$	$0.202\pm0.009^{a,b}$	0.182 ± 0.008^{b}	0.178 ± 0.007^{b}	0.176 ± 0.008^{b}
Vv_{wall}	0.161 ± 0.005^{a}	0.151 ± 0.004^{a}	0.163 ± 0.005^{a}	0.158 ± 0.005^{a}	0.160 ± 0.003^{a}	0.161 ± 0.005^{a}
Vv_{np}	$0.050\pm0.004^{a,b}$	0.036 ± 0.006^{a}	$0.049\pm0.006^{a,b}$	$0.048\pm0.005^{a,b}$	0.061 ± 0.003^{b}	$0.055\pm0.005^{a,b}$
Vv_{br}	0.029 ± 0.005^{a}	0.037 ± 0.006^{a}	0.031 ± 0.006^{a}	0.030 ± 0.004^{a}	0.020 ± 0.003^{a}	0.023 ± 0.004^{a}
Vv_{duct}/Vv_{air}	0.403 ± 0.017^{a}	$0.361\pm0.017^{a,b}$	$0.367\pm0.020^{a,b}$	0.316 ± 0.016^{b}	0.308 ± 0.016^{b}	0.297 ± 0.016^{b}
Vv_{wall}/Vv_{air}	0.301 ± 0.011^{a}	0.266 ± 0.010^{a}	0.292 ± 0.010^{a}	0.277 ± 0.012^{a}	0.276 ± 0.005^{a}	0.271 ± 0.010^{a}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

Vv_{alv} (volume proportion of alveolar air), Vv_{duct} (volume proportion of alveolar duct air), Vv_{wall} (volume proportion of alveolar wall), Vv_{np} (volume proportion of non-parenchymatous tissue), Vv_{br} (volume proportion of conducting airway air).

of S_w and N_{at}, but the specific values were significantly higher in the hypobaric normoxic group compared to those in the general control group (Table 12).

In short, a decrease lung volume in hypobaric normoxia indicated that lung growth was diminished. On the other hand, in absolute terms biochemical analysis indicated that lung growth remained unaffected by hypobaric pressure. Despite decreased lung volume the internal surface area and total number of alveoli were equivalent to that of general controls and this appeared to occur because of smaller unit structures and increased complexity of the lung in hypobaric normoxic conditions.

When hypobaric hypoxic and normobaric hypoxic animals were compared for the effect of lower ambient pressure in conjunction of low oxygen, hypobaric pressure did not appear to affect lung growth pattern, except that desmosine accumulation was increased in the presence of decreased pressure in hypobaric hypoxic animals. Compared to the normobaric hypoxic animals, the changes in unit structure morphometric parameters in the hypobaric hypoxic animals were similar to those observed in hypobaric normoxic rats compared to general controls. These findings suggested that elastin accumulation increased and the size of airspaces decreased in hypobaric pressure when delivered with low oxygen.

3.1.3.4. ADAPTATION TO NORMOBARIC HYPOXIA

3.1.3.4.1. EXTENT OF LUNG GROWTH

Three weeks of exposure to normobaric hypoxia caused significant increases in lung weight (38%) and fixed lung volume (39%) compared to weight-matched controls. Lung weight and lung volume were also significantly higher (16% and 14% respectively) in normobaric hypoxic animals when compared with those in general controls (Table 4). No change in fixed lung volume per unit lung weight was observed in the normobaric hypoxic group compared to weight-matched controls or general controls. Since normobaric hypoxic animals had a lower body weight than the general control animals, specific lung weight and volume increased markedly in the normobaric hypoxic group (Table 4).

3.1.3.4.2. BIOCHEMICAL CHANGES

(i). NBHY vs WMC: The results of biochemical analysis showed that normobaric hypoxia produced marked increases in WI_{dry} (40%) and lung DNA (31%), RNA (60%) and protein (52%) compared to weight-matched control rats (Table 5). DNA concentration remained unchanged but RNA and total protein concentrations were significantly higher in normobaric hypoxic animals compared to those observed in weight-matched controls (Table 7). This resulted in significant increases in RNA/DNA and protein/DNA ratios in animals exposed to normobaric hypoxia compared to the animals subjected to food restriction (Table 8).

The total amount of hydroxyproline which is a sum of soluble and insoluble fractions of hydroxyproline increased significantly (28%) following three weeks of exposure to normobaric hypoxia compared with weight-matched controls (Table 5). Although an increase in the soluble fraction of hydroxyproline which is a measure of immature or newly synthesized collagen (34%) was greater than the insoluble fraction which represents mature or cross-linked collagen (28%), it did not raise the soluble/insoluble hydroxyproline ratio. The amount of desmosine increased markedly (62%) in normobaric hypoxic rats than that of weight-matched controls. Despite increased accumulation in connective tissue proteins in normobaric hypoxic animals, the amount of hydroxyproline relative to dry lung weight fell significantly but desmosine concentration remained undisturbed (Table 7). This unequal effect of normobaric hypoxia on connective proteins caused a significant elevation in the desmosine/hydroxyproline ratio over that found in weight-matched controls whereas OHP/DNA and DES/DNA ratio remained unchanged (Table 8).

(ii). NBHY vs GC: The amounts of WI_{dry}, DNA, RNA, protein and desmosine were significantly higher in the normobaric hypoxic group (21%, 13%, 14%, 24% and 43% respectively) than those observed in the general control group (Table 5). Since the amount hydroxyproline did not increase equivalent to the increase in dry lung weight, its concentration fell significantly compared to general controls but concentration of other biochemical measurements (DNA, RNA, protein, and desmosine) remained the

same (Table 7). The Protein/DNA, DES/DNA and DES/OHP ratios were elevated in normobaric hypoxic animals compared to those observed in general controls (Table 8).

The biochemical measurements expressed relative to body weight were significantly higher in normobaric hypoxic group compared to those in general controls (Table 6).

(iii). HBHY vs HBNO: When a comparison was made between hypobaric normoxic and hypobaric hypoxic groups to assess the effect of low oxygen tension, increases in all biochemical parameters were similar to those found between the general control and normobaric hypoxic groups (Table 5). In addition, the amounts of hydroxyproline and desmosine also increased significantly in hypobaric hypoxic animals compared to hypobaric normoxic animals.

Compared to the hypobaric normoxic animals, the concentrations of lung DNA, RNA and protein in the hypobaric hypoxic animals did not change but that of hydroxyproline concentration decreased whereas desmosine increased significantly (Table 7). These changes led to significant increases in the DES/DNA and DES/OHP ratios (Table 8).

3.1.3.4.3. MORPHOMETRIC CHANGES

(i). NBHY vs WMC: As shown in Table 9, following three weeks of exposure to normobaric hypoxia, MLI, l_{alv} and $Valv_{ave}$ increased while N_A, N_V and S_w/V_L ratio decreased significantly compared to the weight-matched control group. Since fixed lung volume was significantly larger in normobaric hypoxic animals and the volume fractions of lung structures did not change, absolute volumes of alveolar air, alveolar wall and non-parenchymal tissue increased significantly compared with weight-matched controls (Table 11). Despite larger unit structures and fewer alveoli per unit volume in normobaric hypoxic animals, the S_w and N_{at} increased significantly (17% and 12% respectively) compared to those in weight-matched control rats (Table 12).

(ii). NBHY vs GC: When normobaric hypoxic animals were compared for unit structure morphometric

parameters to those of general controls, MLI, l_{alv} and $Valv_{ave}$ increased and N_A, N_V and S_w/V_L decreased significantly in normobaric hypoxic animals. Except for a significant increase in the Vv_{np} in the normobaric hypoxic group compared to the general control group, other volume fractions of lung structures did not differ (Table 10). Absolute volumes of alveolar air, alveolar wall and non-parenchyma also increased significantly in normobaric hypoxic rats (Table 11). No differences were found between the normobaric hypoxic and general control groups for absolute S_w and N_{at} (Table 12). Specific S_w and N_{at} measurements were greater in normobaric hypoxic rats compared to general control rats.

(iii). HBHY vs HBNO: In order to examine the effect of hypoxia, the morphometric results of hypobaric hypoxic and hypobaric normoxic rats were also compared. The changes dimensions of unit structures, and volume fractions and absolute volumes of lung structures were similar to those observed by comparing the normobaric hypoxic and general control groups (Table 9-Table 11). No differences were found between hypobaric hypoxic and hypobaric normoxic rats for S_w and N_{at} (Table 12). Only specific S_w was higher in the hypobaric hypoxic group compared to the hypobaric normoxic group (Table 12).

In summary, these observations demonstrated that all absolute biochemical as well as morphometric variables of lung growth increased during adaptation to normobaric hypoxia compared to the animals of similar body weight suggesting that lung growth was enhanced by low oxygen. The amount of hydroxyproline fell relative to an increase in lung weight but concentrations of RNA and protein were elevated in normobaric hypoxic animals. Increased DNA content indicated a hyperplastic response, but increases in RNA/DNA and protein/DNA ratio showed that cellular hypertrophy also occurred in normobaric hypoxia. Unit structures enlarged along with addition of alveolar structures.

Compared to general controls, with the exception of hydroxyproline, all biochemical parameters increased along with lung weight and volume, indicating accelerated lung growth. Morphometric results indicated enlargement of unit structures with no changes in alveolar surface area

and alveolar number. Except for a significant decrease in the concentration of hydroxyproline, other biochemical concentrations remained unchanged. On the other hand, increases in DES/DNA and DES/OHP ratios indicated changes in connective tissue equilibrium with increased elastin accumulation. Comparison between hypobaric hypoxia and hypobaric normoxia also revealed similar changes, suggesting that effect of hypoxia was similar whether delivered with or without hypobaric pressure.

3.1.3.5. ADAPTATION TO HYPOBARIC HYPOXIA

3.1.3.5.1. EXTENT OF LUNG GROWTH

During three weeks of hypobaric hypoxic exposure, the adaptive changes in lung growth parameters were quite similar to those observed in normobaric hypoxia. Significant increases in lung weight and fixed lung volume were recorded in hypobaric hypoxic rats (40% and 32% respectively) compared to those in the weight-matched control group and they were also higher (17% and 8% respectively) than those in the general control group (Table 4). Lung weight per unit volume did not change compared to both weight-matched and general controls. When the results were expressed per unit body weight, significant increases in lung weight and lung volume were found in hypobaric hypoxic rats compared to those in general controls (Table 4).

3.1.3.5.2. BIOCHEMICAL CHANGES

(i). HBHY vs WMC: The rats exposed to hypobaric hypoxic conditions displayed a large increase in WI_{dry}, DNA, RNA and protein content (44%, 30%, 58% and 60% respectively) compared to their weight-matched controls (Table 5). The amount of DNA per unit of dry lung weight was significantly lower in hypobaric hypoxic rats, whereas RNA and protein concentrations were higher than those observed in weight-matched controls (Table 7) and as result of this the RNA/DNA and protein/DNA ratios elevated significantly (Table 8).

The connective tissue proteins were also increased in hypobaric hypoxic rats compared to

weight-matched controls. Significant accumulation of hydroxyproline (soluble and insoluble fractions) and desmosine occurred which increased 41% and 97% respectively (Table 5). The soluble fraction of hydroxyproline increased slightly more (48%) than the insoluble fraction (40%) but the soluble/insoluble fraction ratio remained unchanged. Since an increase in desmosine content was greater than an increase in lung weight, its concentration was elevated, whereas the concentration of hydroxyproline did not change (Table 7). As a result of these alterations in connective tissue accumulation, the DES/OHP ratio increased in hypobaric hypoxic animals compared to weight-matched controls (Table 7). Hypobaric hypoxia increased the DES/DNA and OHP/DNA ratios (Table 8).

(ii). HBHY vs GC: Significant increases of 13%, 12% and 30% were found in DNA, RNA and protein content respectively compared to those in general control rats (Table 5). The amounts of hydroxyproline and desmosine also increased significantly (15% and 73% respectively) in the hypobaric hypoxic group. Hypobaric hypoxia caused a drop in the concentrations of DNA and hydroxyproline but the concentration of desmosine was elevated while RNA and protein concentrations remained unchanged compared to general controls (Table 7). Since DNA and hydroxyproline concentration decreased following three weeks of exposure to hypobaric hypoxia, the protein/DNA, DES/DNA and DES/OHP ratios increased significantly (Table 8).

When all biochemical measurements (DNA, RNA, protein, hydroxyproline and desmosine) were expressed per unit body weight, specific values in the hypobaric hypoxic group were significantly greater than those calculated for the general control group (Table 6).

3.1.3.5.3. MORPHOMETRIC CHANGES

(i). HBHY vs WMC: Three weeks of hypobaric hypoxic exposure resulted in significant increases l_{alv} and $Valv_{ave}$ and a decrease in S_w/V_L compared to the weight-matched control group but MLI, N_A and N_V remained unchanged (Table 9). The volume fraction of alveolar air increased significantly, but in addition to the absolute volume of alveolar air, the volumes of alveolar wall and non-parenchymal

tissue also increased significantly in hypobaric hypoxic animals compared to weight-matched controls (Table 10-Table 11). Since the values of MLI and N_V did not alter, but lung volume increased, the alveolar surface area and total number of alveoli were increased (28% and 21% respectively) in rats exposed to hypobaric hypoxia (Table 12).

(ii). HBHY vs GC: With regards to morphometric changes in hypobaric hypoxia compared to general controls, with the exception of mean chord length of alveoli which increased significantly, the values of unit structure morphometric parameters remained the same as those in general controls (Table 9). Absolute volumes of alveolar air, alveolar wall and non-parenchymal tissue were greater in hypobaric hypoxic rats (Table 11). Exposure to hypobaric hypoxia also caused a significant increase (10%) in alveolar surface area but not in total alveolar number (Table 12).

The S_w and N_{at} expressed per unit body weight were found to be higher in the hypobaric hypoxic group than those in the general control group (Table 12).

In brief, marked increases in absolute lung weight, lung volume, and biochemical and morphometric measurements in hypobaric hypoxic rats compared with weight-matched controls showed that hypobaric hypoxia produced enhanced lung growth. Unlike normobaric hypoxia, except for slight enlargement of alveolar size, the unit structure dimensions remained unaltered. Connective tissue proteins accumulated markedly and accumulation of elastin was greater than accumulation of collagen. The results showed that hypobaric hypoxia produced hyperplastic as well as hypertrophic alterations in the lung compared to weight-matched controls.

Although the percentage increases of absolute lung growth parameters in hypobaric hypoxic animals were small compared to the general control group, this indicated that lung growth was accelerated by hypobaric hypoxia. Increase in lung growth occurred by increase in cell number and cell size without disturbing the dimensions of morphomatric unit structures. However, disturbance in connective tissue protein balance was found as accumulation of elastin was relatively greater than collagen as estimated by measuring hydroxyproline and desmosine contents.

Table 11. Absolute volumes of parenchymal and non-parenchymal lung structures.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
V_{alv} ml	2.50 ± 0.07^{a}	6.48 ± 0.37^{b}	5.13 ± 0.13^{c}	6.10 ± 0.16^{b}	7.52 ± 0.12^{d}	7.29 ± 0.19^{d}
V_{duct} ml	1.00 ± 0.04^{a}	2.37 ± 0.15^{b}	1.93 ± 0.12^{c}	1.92 ± 0.10^{c}	$2.27\pm0.11^{b,c}$	$2.14\pm0.09^{b,c}$
V_{wall} ml	0.75 ± 0.03^{a}	1.70 ± 0.07^{b}	1.50 ± 0.05^{c}	1.67 ± 0.05^{b}	2.08 ± 0.05^{d}	1.98 ± 0.08^{d}
V_{np} ml	0.23 ± 0.02^{a}	0.44 ± 0.06^{b}	0.47 ± 0.06^{b}	0.50 ± 0.05^{b}	0.79 ± 0.06^{c}	0.67 ± 0.07^{c}
V_{br} ml	0.13 ± 0.02^{a}	0.37 ± 0.06^{b}	$0.26\pm0.05^{a,b}$	0.32 ± 0.04^{b}	$0.25\pm0.04^{a,b}$	$0.27\pm0.04^{a,b}$

V_{alv} (volume of alveolar air), V_{duct} (volume of duct air), V_{wall} (volume of alveolar wall), V_{np} (volume of non-parenchymatous tissue), V_{br} (volume of bronchial air)

Table 12. Absolute and specific morphometric parameters after 3 weeks of exposure.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
S_w (m^2)	0.236 ± 0.011^{a}	0.527 ± 0.018^{b}	0.454 ± 0.007^{c}	$0.553\pm0.011^{b,d}$	$0.566\pm0.01^{b,d}$	0.582 ± 0.012^{d}
N_{at} (10^{+6})	13.9 ± 1.2^{a}	23.7 ± 0.8^{b}	21.1 ± 0.6^{c}	25.5 ± 0.8^{b}	23.6 ± 0.9^{b}	25.6 ± 0.9^{b}
S_w/100 g Wb (m^2)	0.284 ± 0.01^{a}	0.202 ± 0.006^{b}	0.229 ± 0.004^{c}	0.232 ± 0.005^{c}	0.279 ± 0.006^{a}	0.288 ± 0.007^{a}
N_{at}/100 g Wb (10^{+5})	16.7 ± 1.1^{a}	9.1 ± 0.3^{b}	10.7 ± 0.3^{c}	$10.7\pm0.5^{c,d}$	$11.6\pm0.5^{c,d}$	12.6 ± 0.4^{d}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.
S_w (internal alveolar surface area), N_{at} (total number of alveoli).

3.2. LUNG CYTOKINETICS STUDY

Two normobaric hypoxic rats died during injection of tritiated thymidine. The histological sections of the lungs and gross appearance of other organs showed no evidence of infection in control or experimental animals used in this study.

3.2.1. SOMATIC GROWTH

During three weeks of exposure, body weight changes in all groups were similar to those observed in the first experiment (Figure 8). In this experiment, the recovery from hypobaric normoxia, hypobaric hypoxia, normobaric hypoxia and undernutrition was also studied. Hypobaric normoxic, normobaric hypoxic and hypobaric hypoxic animals were returned to room air and weight-matched controls were fed ad libitum for 3 days. During three days of recovery period (day 24 of the experiment), body weight increased significantly in normobaric hypoxic (214.0 ±4.0 g), hypobaric hypoxic (217.2 ±3.7 g) and weight-matched control (218.8 ±6.4 g) rats compared to their body weights on day 21 (196.2 ±5.7, 193.0 ±3.0 and 189.8 ±2.7 g respectively) of the experiment. In general controls, body weight increased form 252.1 ±3.9 g to 273.6 ±7.2 g during three days of the recovery period. Despite significant increases in body weights, the normobaric hypoxic , hypobaric hypoxic and weight-matched control groups had smaller body weight compared to the general control group. The hypobaric normoxic rats which also weighed less after 21 days of exposure (231.5 ±5.0 g) failed to catch up (251.3 ±3.5 g) to the general control group after 3 day recovery.

Compared to the general control group, the nose-tail lengths of the hypobaric hypoxic, normobaric hypoxic and weight-matched control groups were also smaller but not until day 10 of exposure was a significant difference ($p<0.05$) found (Figure 9) and they remained smaller even after 3 days of recovery period. Although, the hypobaric normoxic group also had smaller nose-tail length measurements, the difference was not significant compared to the general control rats at any time interval studied (Figure 9).

3.2.2. ORGAN RESPONSE

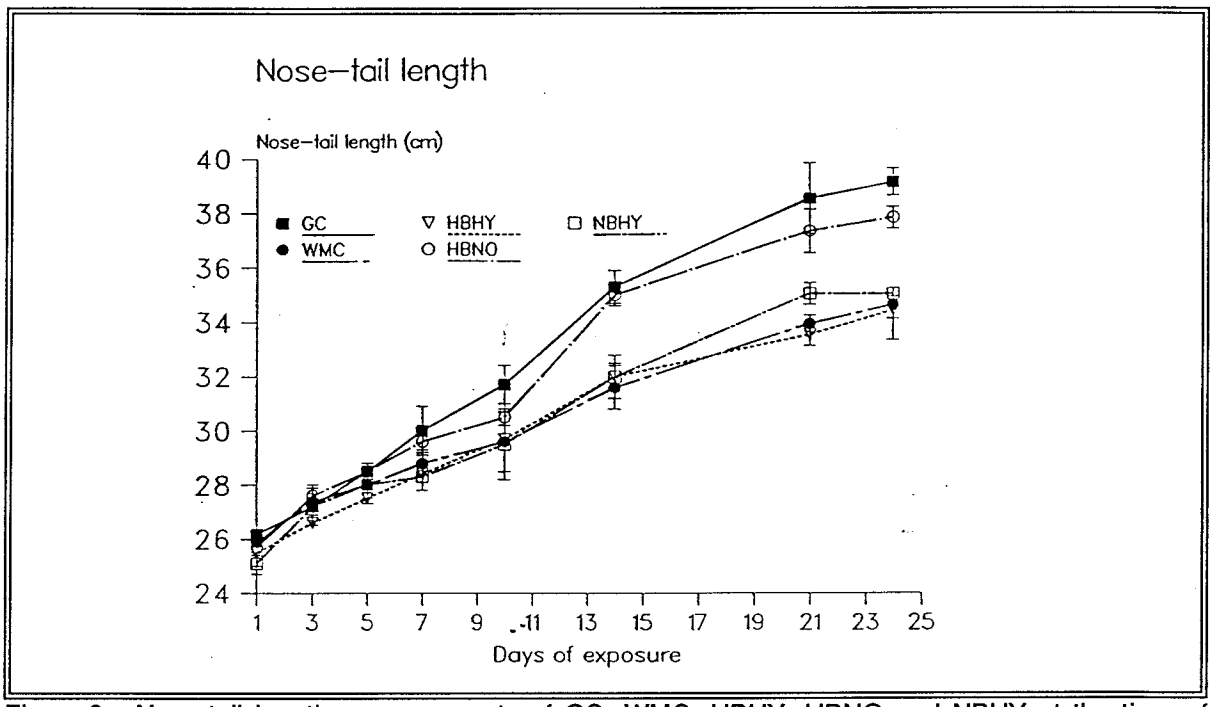


Figure 9. Nose-tail length measurements of GC, WMC, HBHY, HBNO and NBHY at the time of termination on days 1, 3, 5, 7, 10, 14, 21 and 3 days after returning to room air.

3.2.2.1. NORMAL ORGAN GROWTH

In general controls, a significant increase in lung weight was found by day 7 (Figure 10), heart weight by day 10 (Figure 11) and spleen weight by day 7 compared to the values on day 1 of the experiment (Figure 12). The weights of both kidney and liver appeared to increase more rapidly as they were significantly higher on day 5 of experimental period (Figure 13 and Figure 14).

3.2.2.2. EFFECT OF UNDERNUTRITION

The results of right lung, heart, spleen, kidney and liver growth are shown in Figure 10-Figure 14. Significant increases in weights of the lung, kidney and liver did not occur until day 14 in weight-matched animals while heart and spleen weights increased significantly by day 21 compared to day 1 of general controls. After 21 days of undernutrition, free access to food for 3 days resulted

in significant increases in lung, heart, kidney and liver weights but not in spleen weight (Figure 10-Figure 14).

Compared to general controls, lung weights of weight-matched controls decreased on days 3 and 5 but a significant difference was first noted on day 7 ($p=0.008$) and thereafter (Figure 10). The heart and kidney weights were affected as early as day 5 and day 3 respectively of the experiment and were significantly smaller ($p=0.008$ and $p=0.01$ respectively) and continued to be smaller afterwards (Figure 11, Figure 13). Spleen weight also decreased during the early period of undernutrition but a significant difference was not observed until day 14 (Figure 12), however, liver weight reduced significantly on day 1 ($p=0.001$) and remained so thereafter (Figure 14). After day 21 of the experiment, food ad libitum for 3 days produced significant increases in liver and kidney weights but failed to reach the values of general control rats. The increases in the weights of lung, heart and spleen did not reach significant levels (Figure 10-Figure 14).

3.2.2.3. EFFECT OF HYPOBARIC NORMOXIA (low ambient pressure)

Gradual increases in weights were observed in all organs during exposure to hypobaric normoxic conditions. Lung and liver weights increased significantly by day 7, heart and spleen by day 10 while kidney weight increased by day 5 (Figure 10-Figure 14).

The pattern of organ growth in hypobaric normoxic animals was similar to that in general controls. No significant differences were found between hypobaric normoxic rats and general control rats at any time interval during the exposure (Figure 10-Figure 14).

Comparisons made between the hypobaric hypoxic group and normobaric hypoxic group to assess the effect of low ambient pressure also did not reveal any changes in growth of organs. When the animals were returned to room air following chronic hypobaric normoxic exposure, no significant increases in organ weights were observed

3.2.2.4. EFFECT OF NORMOBARIC HYPOXIA

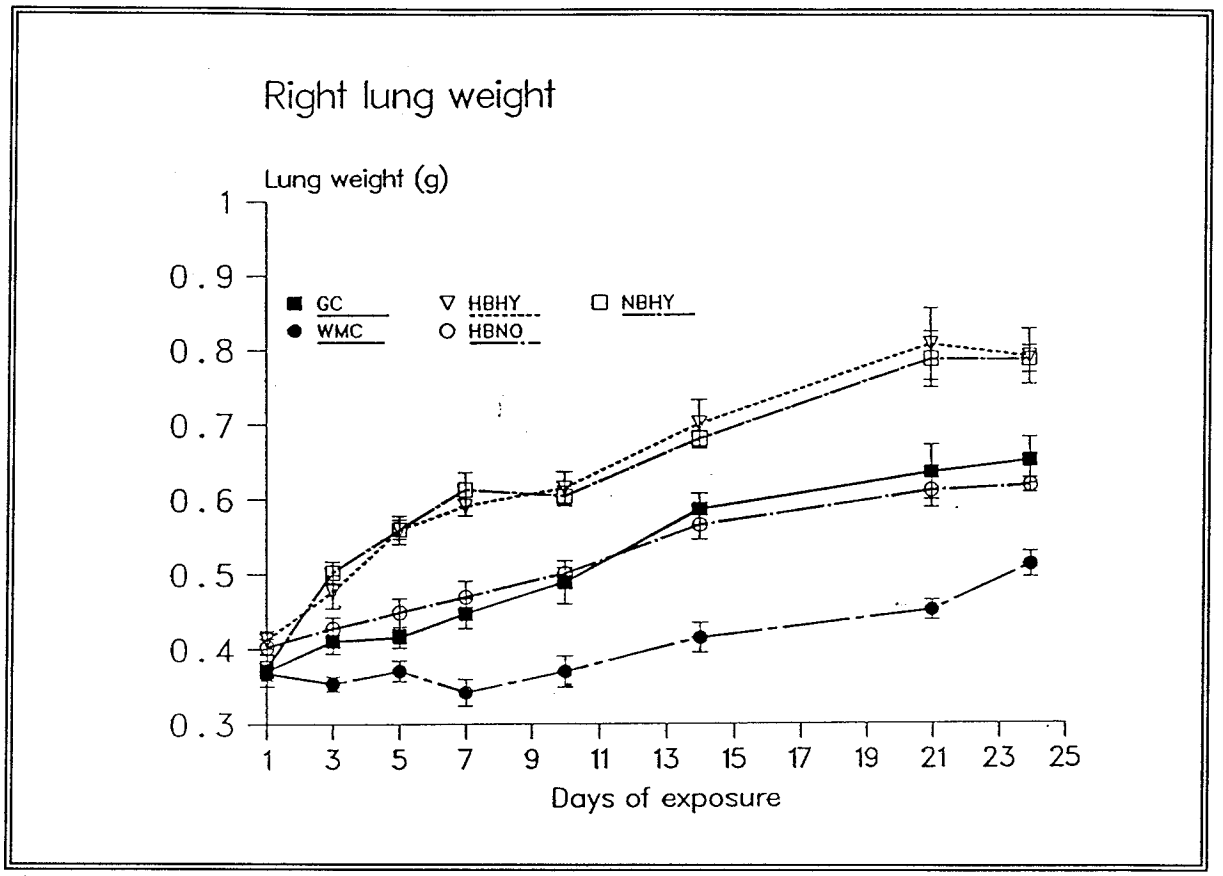


Figure 10. Right lung weights of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as animals 3 days after returning to room air.

Compared to the values of day 1 general controls, the weights of lung and spleen increased significantly on day 3 of exposure to normobaric hypoxia while a significant increase in heart weight was found on day 7 (Figure 10-Figure 12). Growth of kidneys and liver appeared to be slow as significant increases in weights were found on day 14 of normobaric hypoxic exposure (Figure 13 and Figure 14).

When comparison was made between the normobaric hypoxic and weight-matched control groups, an increase in lung growth was evident by day 3 of exposure as lung weight was significantly higher in normobaric hypoxic rats (Figure 10). This increase continued during the rest of the exposure. The weights of spleen and heart were significantly greater on days 5 and 7 respectively in the normobaric hypoxic group compared to those in weight-matched controls and continued to be higher thereafter until the end of the experiment (Figure 11, Figure 12). However, neither kidney nor liver

weight differed from those recorded in weight-matched control rats (Figure 13, Figure 14).

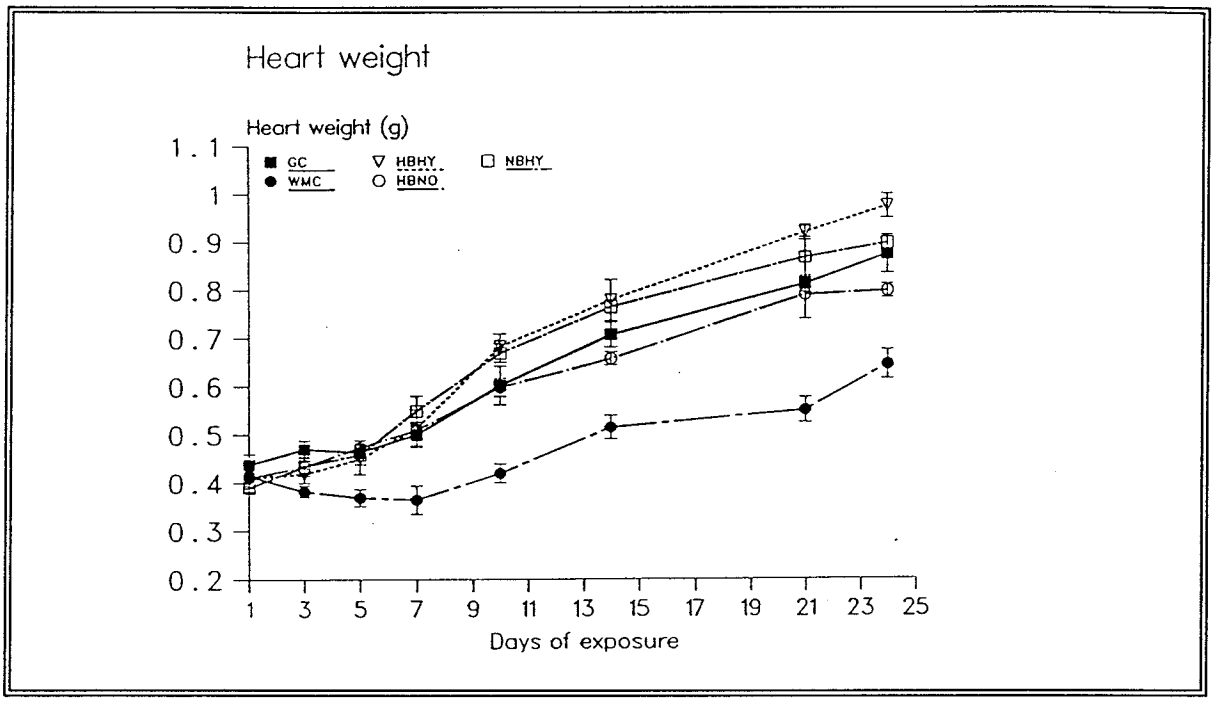


Figure 11. Heart weights of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as 3 days after returning to room air.

Compared to general controls, lung weight showed a significant increase from day 3 of exposure and thereafter (Figure 10). Heart and spleen weights also increased compared to the general control group but never reached a significant level (Figure 11, Figure 12). As displayed in Figure 13 and Figure 14, the kidneys and liver gained less weight and were significantly smaller on day 3 of exposure and remained significantly smaller during the rest of the period compared to general controls.

Following removal of normobaric hypoxic stress for three days, an increase in growth of lung, heart and spleen was no longer apparent (Figure 10-Figure 12). Although slight increases in kidney and liver weights occurred during the recovery period, they did not reach a significant level and remained significantly smaller than those of general controls.

3.2.2.5. EFFECT OF HYPOBARIC HYPOXIA

The changes in organ growth rate were similar to those that occurred in normobaric hypoxic

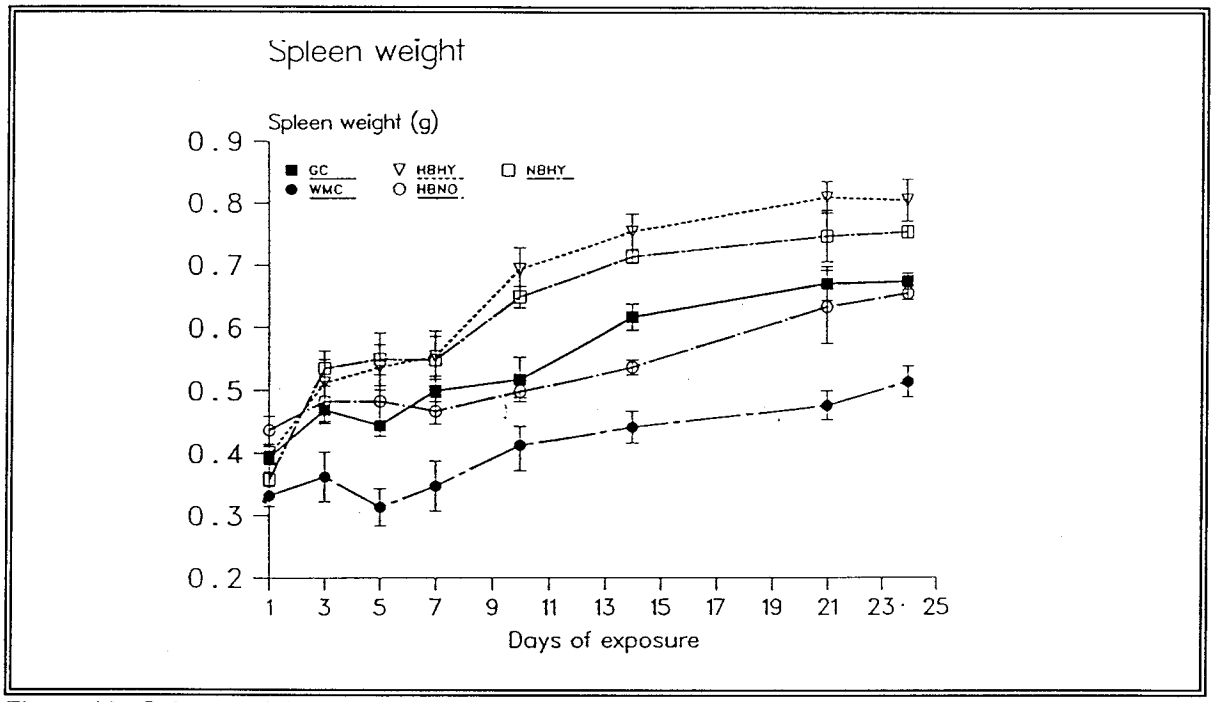


Figure 12. Spleen weights of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as 3 days of recovery.

conditions. A significant increase in lung weight occurred by day 3 of exposure to hypobaric hypoxia compared to general control day 1 lung weight (Figure 10). Hypobaric hypoxia produced increases in heart and spleen weights on days 10 and 5 respectively (Figure 11-Figure 12). Significant increases in the weights of liver and kidney were found on day 14 of hypobaric hypoxic exposure compared to the day 1 values of general controls (Figure 13, Figure 14).

The results of lung growth are displayed in Figure 10. Compared to weight-matched controls, lung weight increased significantly in hypobaric hypoxic rats on day 3 of exposure and continued to be greater thereafter (Figure 10). The heart and spleen weights in hypobaric hypoxic rats were significantly higher in hypobaric hypoxic rats from days 7 and 5 respectively onwards compared to weight-matched animals (Figure 11, Figure 12). On the other hand, growth of both kidney and liver declined and the weights were equivalent to those noted in weight-matched control rats throughout the exposure (Figure 13, Figure 14).

Compared with general controls, the lung weight increased on day 5 of hypobaric hypoxic exposure and continued to be higher until the end of the experiment (Figure 10). Relative to lung

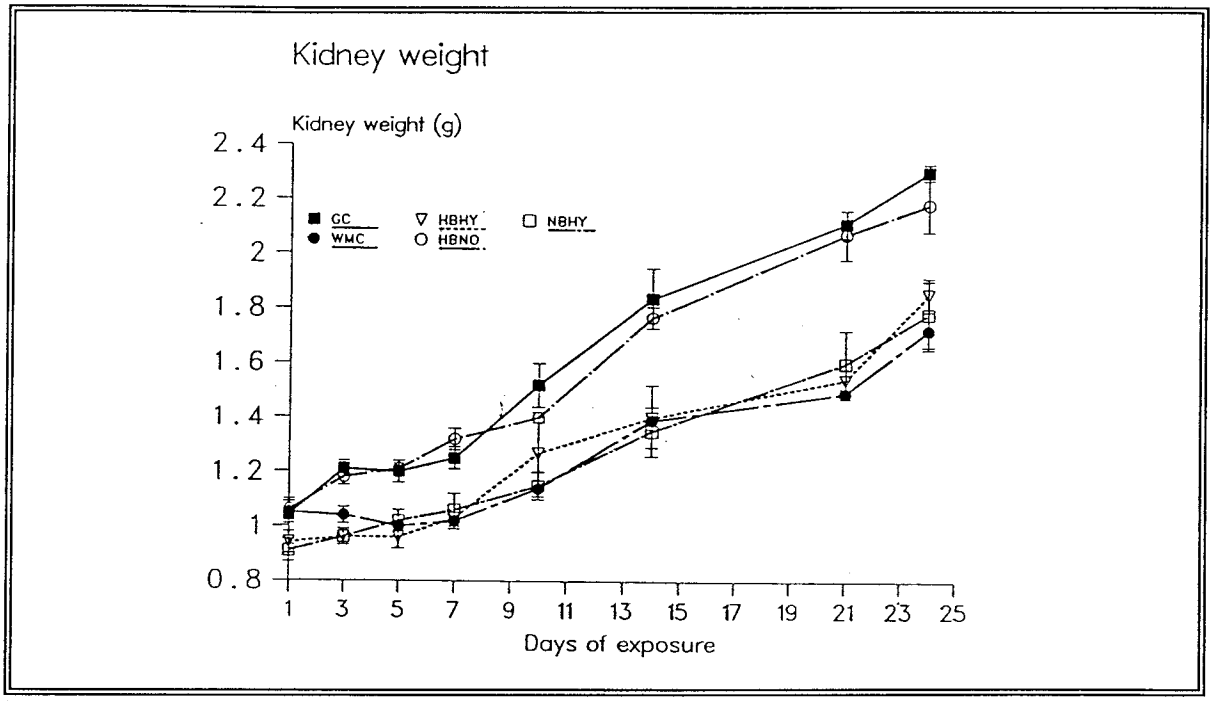


Figure 13. Kidney weights of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as 3 days following recovery.

growth, heart and spleen growth adaptation occurred at a slower rate in hypobaric hypoxic rats as their weights increased gradually and were significantly higher ($p=0.008$ and $p=0.04$ respectively) only on day 21 (Figure 11, Figure 12). On the other hand, the kidney and liver weights fell on day 1 of hypobaric hypoxic exposure and were significantly lower on day 3 onwards (Figure 13, Figure 14).

During three days of recovery following chronic hypobaric hypoxic exposure, lung and spleen growth stopped while the rate of heart growth remained unaffected (Figure 10-Figure 12). On the other hand, the weights of kidney and liver increased markedly during 3 days of recovery period compared to those on day 21 of exposure, but did not reach the values of general controls (Figure 13, Figure 14).

In short, it was evident that liver and kidney growth was diminished in normobaric hypoxic and hypobaric hypoxic conditions during the early period of exposure. These changes were similar to those occurred in weight-matched controls and never showed an adaptive response during the exposure. On the contrary, despite a decrease in somatic growth, the heart, spleen and lung weights increased markedly compared to weight-matched control animals. The lungs appeared to adapt earlier

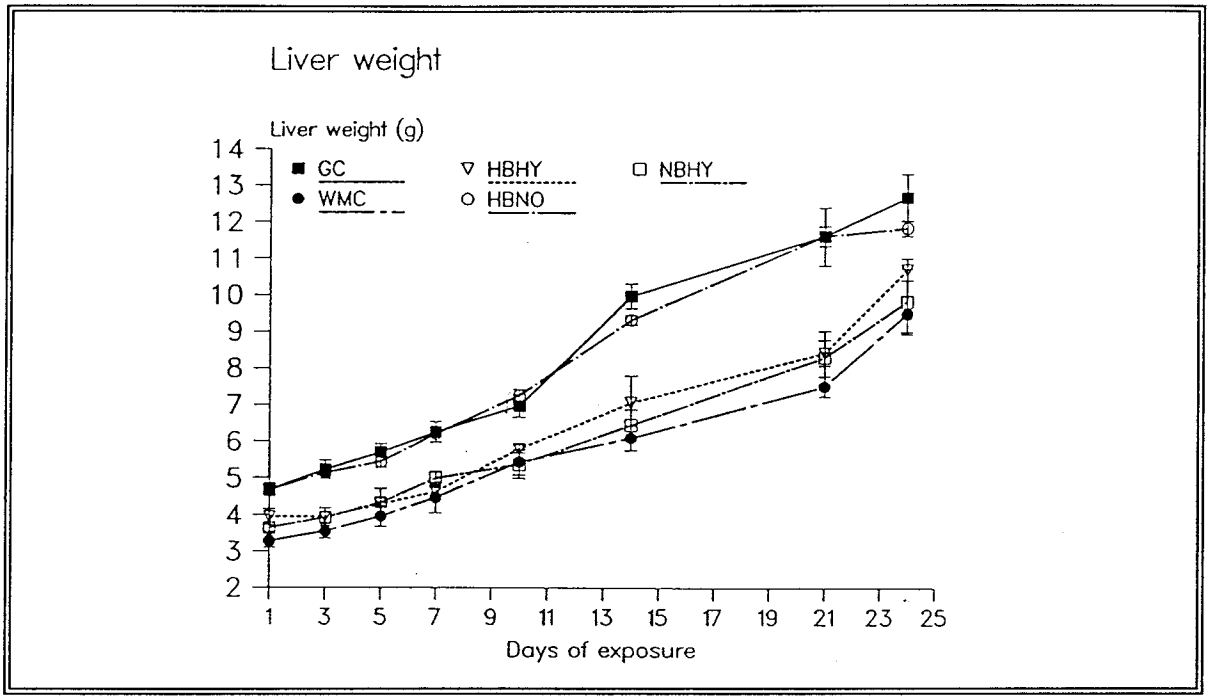


Figure 14. Liver weights of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as 3 days following recovery.

than heart and spleen to both hypobaric hypoxia and normobaric hypoxia. Low ambient pressure did not show a specific growth effect on any of the organs studied. In hypobaric hypoxic rats, 3 days of recovery period enhanced kidney and liver growth, but appeared to diminish spleen and lung growth rates and heart growth remained unaffected. In normobaric hypoxic rats, during the recovery period, the increase in growth of lung, heart and spleen stopped while liver and kidney showed growth stimulation. When food was provided ad lib to the weight-matched control rats, growth of all organs except the spleen was augmented.

3.2.3. BIOCHEMICAL ALTERATIONS IN THE LUNG

3.2.3.1. EFFECT OF UNDERNUTRITION

Dry to wet weight ratio of the lung remained unchanged (Figure 15). Food restriction caused a significant decrease in RNA and protein on day 3 ($p=0.05$ and $p=0.016$) and these measurements stayed lower than those of general controls until the end of the experiment (Figure 16, Figure 17). The

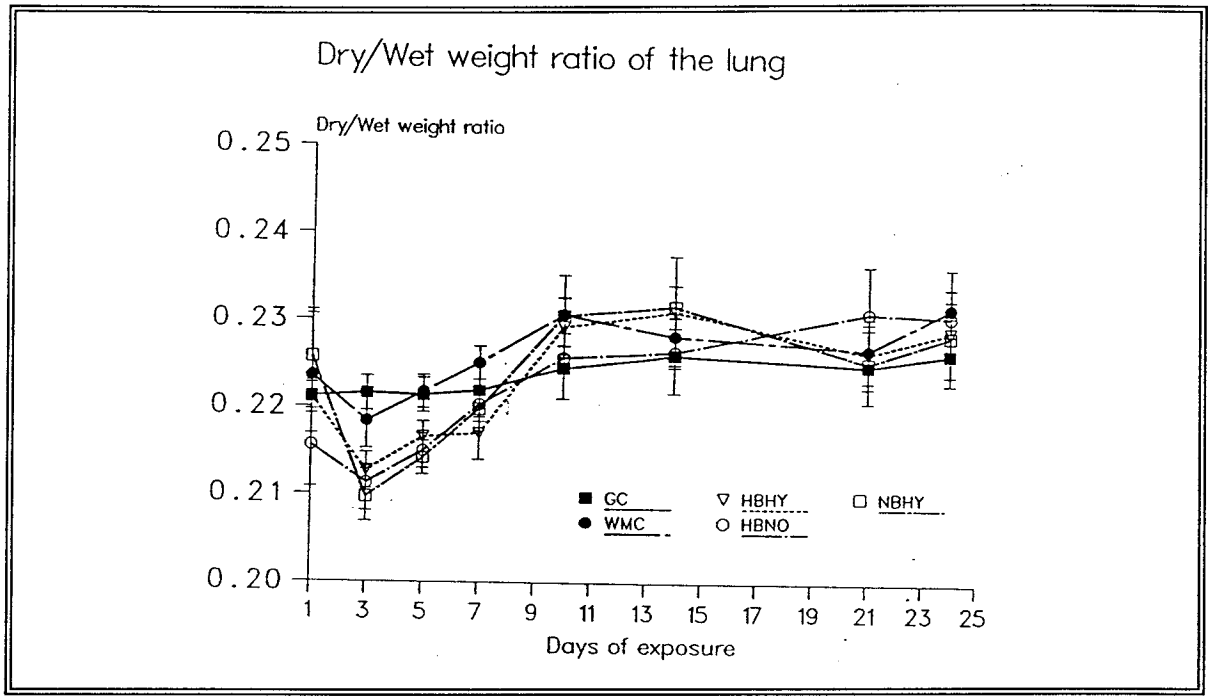


Figure 15. Changes which occurred in dry to wet weight ratio of the lung during exposure to different conditions.

amount of DNA also decreased on day 7, but a significant difference only appeared on day 14 ($p=0.004$) and remained lower until day 21 (Figure 18). The RNA/DNA ratio fell significantly on day 5 and remained consistently lower for the rest of the undernourished treatment (differences were not significant on days 10 and 21) (Figure 19). The protein/DNA ratio decreased significantly on days 3 ($p=0.003$) also remained low afterwards (differences were not significant on day 10)(Figure 20).

When weight-matched control rats were given free access to food for three days, the amount of DNA, RNA and protein content increased markedly (Figure 16-Figure 18). Increases in the RNA/DNA and protein/DNA ratios were apparent but the RNA/DNA ratio stayed significantly lower.

3.2.3.2. EFFECT OF HYPOBARIC NORMOXIA (low ambient pressure)

Dry to wet weight of the lung fell in hypobaric normoxic animals on day 1 but a significant difference was observed on day 3 (Figure 15). No significant differences existed between hypobaric normoxic rats and general controls for absolute (RNA, DNA and protein) or relative (RNA/DNA, protein/DNA) parameters (Figure 16-Figure 20). When hypobaric hypoxic animals and normobaric

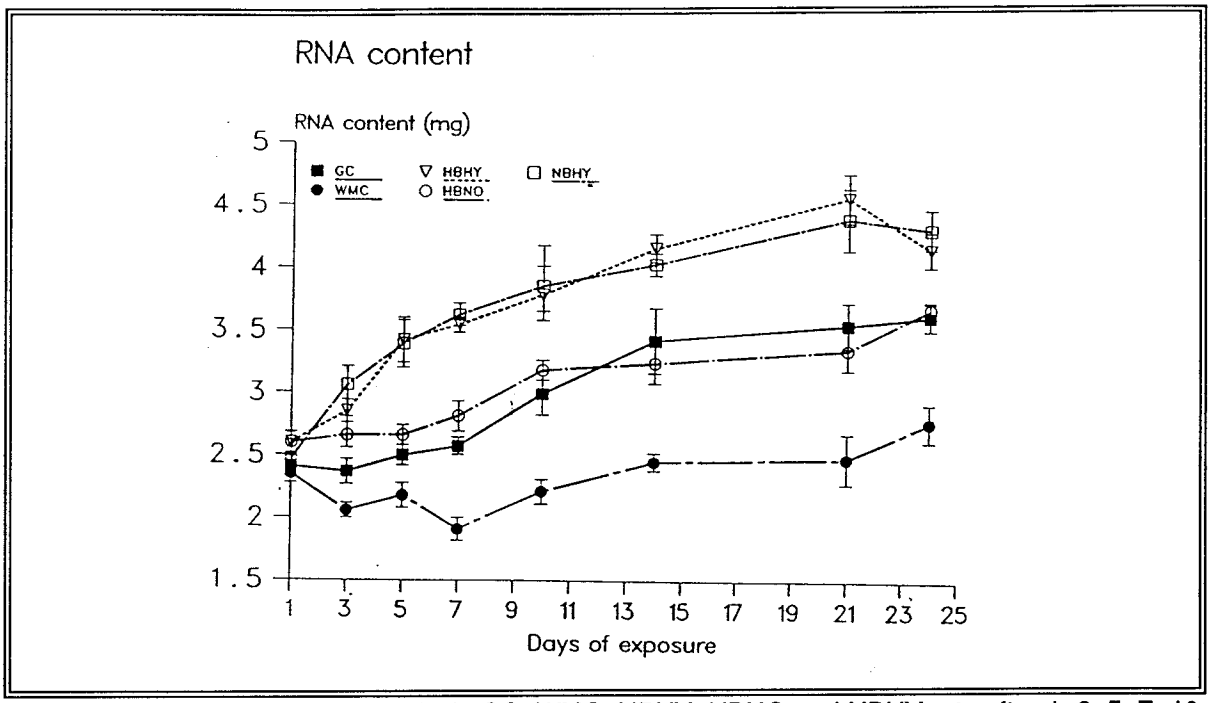


Figure 16. The amount of lung RNA in GC, WMC, HBHY, HBNO and NBHY rats after 1, 3, 5, 7, 10, 14 and 21 days of exposure to different conditions as well as 3 days following recovery.

hypoxic animals were compared for biochemical estimations of lung growth adaptation, the changes were not different.

3.2.3.3. EFFECT OF NORMOBARIC HYPOXIA

Compared with the weight-matched group, the RNA and protein content increased significantly ($p < 0.0008$) on day 3 of exposure and continued to be higher thereafter (Figure 16, Figure 17). Normobaric hypoxia also produced an increase in the amount of DNA of the lung but it occurred at later than RNA or protein. The amount of DNA was found to be significantly elevated on day 5 ($p=0.0008$) and remained higher until the end of the exposure (Figure 18). The RNA/DNA and protein/DNA ratios were significantly greater in hypobaric hypoxic animals from day 3 to 14 (Figure 19, Figure 20). On day 21, only the protein/DNA ratio was significantly higher in normobaric hypoxic animals.

Compared to the general control rats, dry to wet weight ratio of the lung fell on day 3 and 5 but did not reach a significant level (Figure 15). The amount of RNA and protein in normobaric

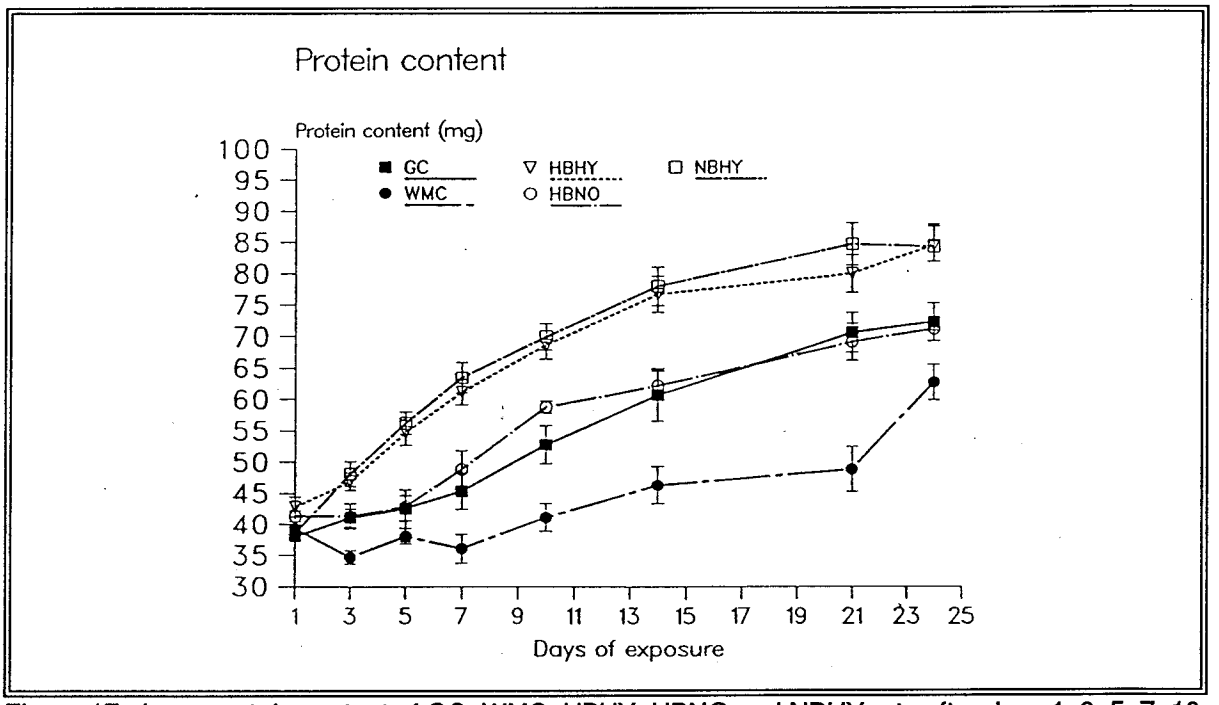


Figure 17. Lung protein content of GC, WMC, HBHY, HBNO and NBHY rats after days 1, 3, 5, 7, 10, 14 and 21 days of exposure to different conditions as well as 3 days following recovery.

hypoxic rats increased significantly ($p=0.002$ and $p=0.04$ respectively) on day 3 of exposure and afterwards,but an increase in the amount of DNA was only noted on days 7-14 (Figure 18). As result of the delayed increase in DNA content, a significant increase in the RNA/DNA ratio occurred on days 3, 5 and 7 (Figure 19), and in the protein/DNA ratio on days 3, 5, 7 and 14 (Figure 20).

Following removal of the rats from normobaric hypoxic conditions, no further increase in the amount of RNA, DNA and protein occurred (Figure 16-Figure 18). The RNA/DNA and protein/DNA ratios fell and reached general control levels (Figure 19 and Figure 20).

3.2.3.4. EFFECT OF HYPOBARIC HYPOXIA

Exposure to hypobaric hypoxia resulted in marked increases in biochemical parameters compared to weight-matched animals. Significant increases were observed in RNA and protein content in hypobaric hypoxic animals on day 3 ($p<0.0008$) and in DNA content on day 5 ($p=0.04$), and these increases continued thereafter until the end of exposure (Figure 16-Figure 18). The RNA/DNA

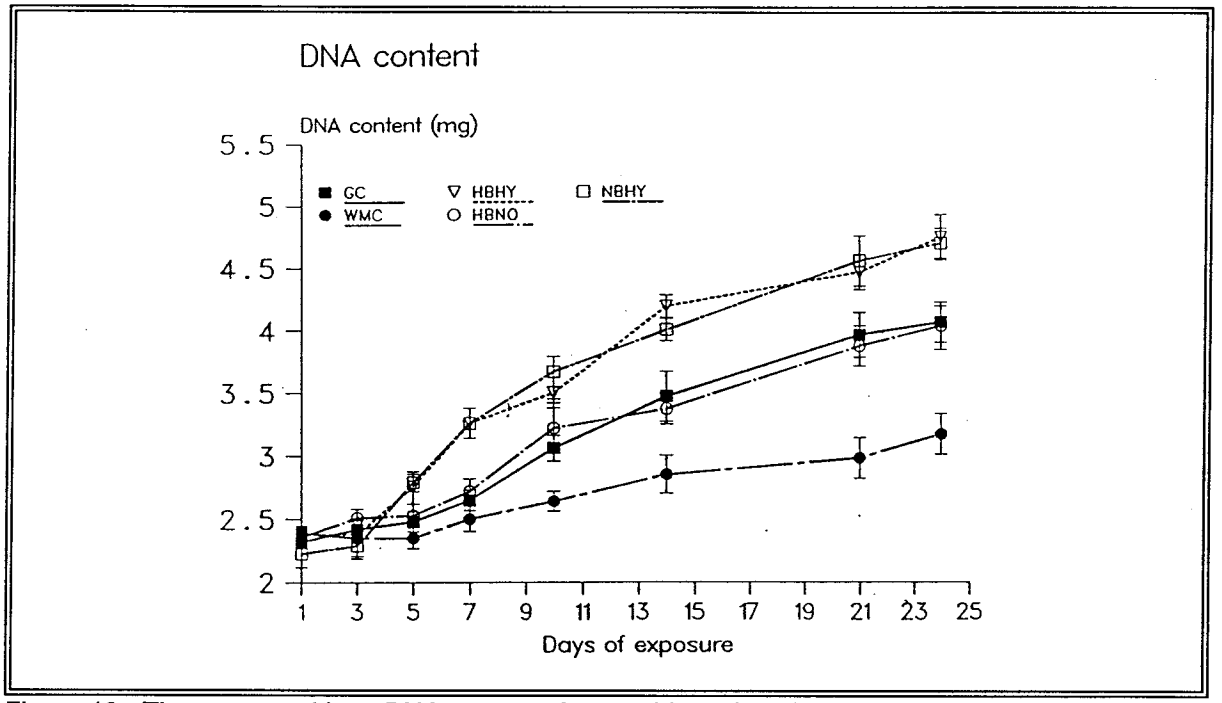


Figure 18. The amount of lung DNA content of rats subjected to different conditions for 1, 3, 5, 7, 10, 14 and 21 days as well as 3 days following recovery.

and protein/DNA ratios were also elevated on day 3 and continued to be so afterwards.

When results were compared to those noted in general controls, dry/wet weight ratio of the lung decreased on day 3 and 5 but not significantly (Figure 15). RNA and protein content increased significantly on day 3 ($p=0.1$ and $p=0.45$ respectively) and thereafter (Figure 16, Figure 17). The absolute amount of DNA also increased and reached a significant level on day 7 ($p=0.008$) and continued to be greater (Figure 18). As illustrated in Figure 19 and Figure 20, the RNA/DNA and protein/DNA ratios were significantly increased on days 3 and 5.

After three days of recovery, the rate of increase in the amount of total protein and DNA in hypobaric hypoxia appeared undisturbed but marked decrease in the amount of RNA was found (Figure 16-Figure 18). Hence a fall in RNA content produced a sharp drop in the RNA/DNA ratio, which reached the level found in weight-matched control rats (Figure 16, Figure 19). The protein/DNA ratio also fell and reached the general control level (Figure 20).

In brief, it was evident that lung growth adaptation in normobaric hypoxia and hypobaric hypoxia occurred earlier during the exposure in terms of RNA and protein contents and this was

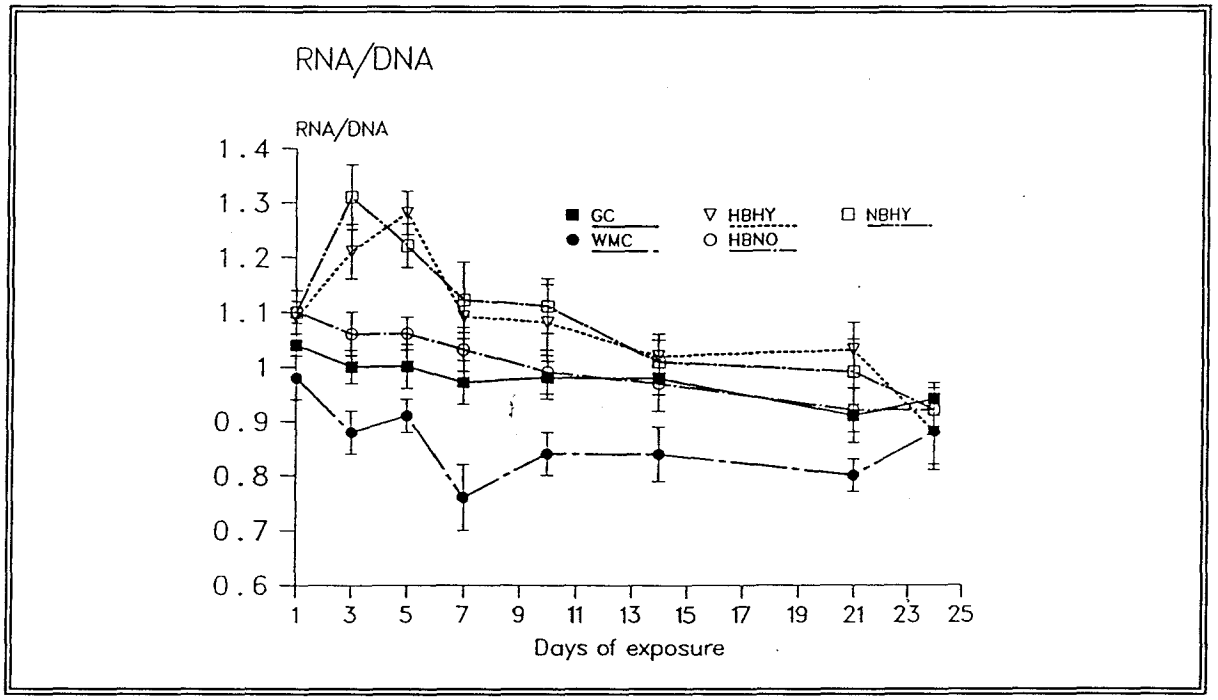


Figure 19. The lung RNA/ DNA ratio of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals as well as 3 days following recovery.

followed by hyperplastic changes as an increase in which DNA content lagged behind the changes that occurred in RNA and protein. On the other hand, a reduction in all biochemical parameters was found in undernourished rats compared to general controls rats. Therefore, increases in biochemical measurements were much greater in hypobaric hypoxic and normobaric hypoxic groups compared to the weight-matched group than compared to the general control group. The RNA/DNA and protein/DNA ratios, which are considered as indicators of cellular dimensions, also increased during the first week of exposure and remained slightly higher for the remaining period. The amount of RNA, protein and DNA did not alter following exposure to low ambient pressure. The three day recovery period appeared to stimulate lung growth in the undernourished rats, whereas it appeared to reduce increased lung growth in hypobaric hypoxic and normobaric hypoxic rats.

3.2.4. DNA SYNTHESIS

Tritiated thymidine (^{3}H-TdR) incorporation in DNA was estimated biochemically and expressed

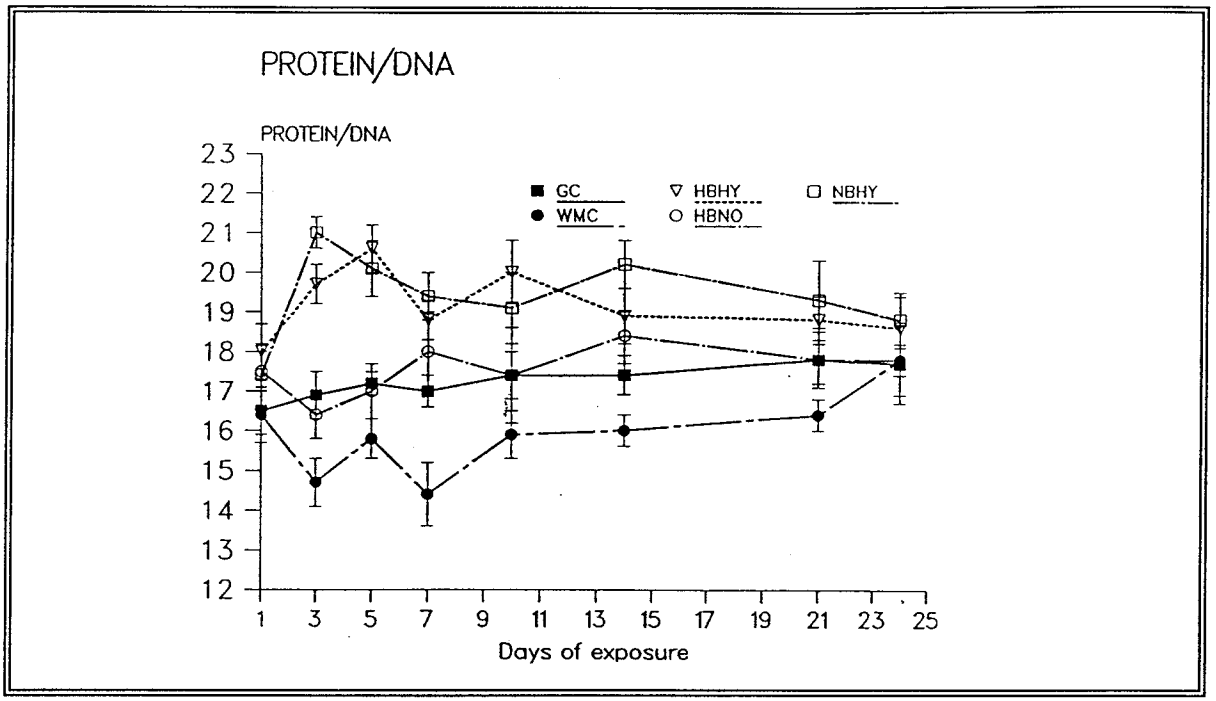


Figure 20. The effect of undernutrition, hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia on the lung protein/DNA ratio.

as counts per minute/ μg of DNA (Figure 21).

3.2.4.1. EFFECT OF UNDERNUTRITION

^{3}H-TdR incorporation was significantly decreased in the weight-matched control rats on day one of the experiment. DNA synthesis fell further between day 3 and 5 and it remained depressed from then onwards. When the weight-matched control rats were given free access to food after 21 days of undernutrition, a three fold increase in the rate of DNA synthesis was noted on day 24 of the experiment.

3.2.4.2.EFFECT OF HYPOBARIC NORMOXIA

The hypobaric normoxic rats, showed an increase in the DNA synthetic rate on days 1 to 7, but a significant change was only found on day 5 of exposure compared to that of general control animals. The rate of ^{3}H-TdR incorporation reached general control levels on day 10 of exposure. When hypobaric hypoxic and normobaric hypoxic animals were compared for the effect of reduced

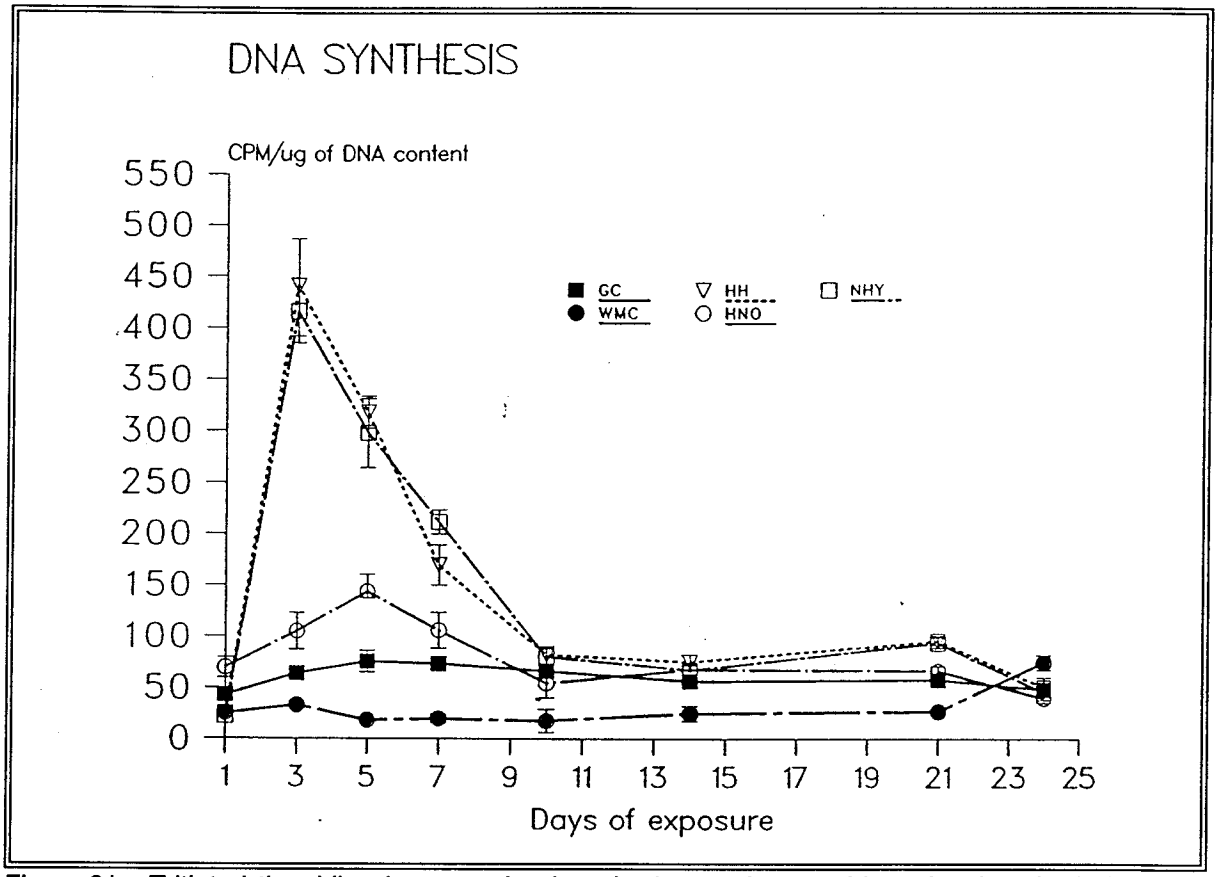


Figure 21. Tritiated thymidine incorporation into the lungs of rats subjected to hypobaric hypoxia, hypobaric normoxia, normobaric hypoxia and undernutrition for 1, 3, 5, 7, 10, 14 and 21 days as well as 3 days following recovery.

ambient pressure, no differences were found between the groups for DNA synthetic activity.

3.2.4.3.EFFECT OF NORMOBARIC HYPOXIA

When the results of normobaric hypoxic group were compared to those found in the weight-matched controls, ^{3}H-TdR incorporation was similar on day 1, increased more than twelve times on day 3, seventeen times on day 5 (because DNA synthesis dropped further on day 5 than on day 3 in the weight-matched control group), nine times on day 7 and remain significantly higher for rest of the exposure (Figure 21).

Compared to the general control animals, the rate of DNA synthesis in normobaric hypoxic animals was significantly diminished on day one. It increased sharply (six and a half fold) on day 3

of exposure. The increase fell to four fold on day 5, two fold on day 7 and then declined further. Although the rate of ^{3}H-TdR incorporation reached general control levels, a significant increase was observed on day 21 in normobaric hypoxic animals.

When the normobaric hypoxic animals were returned to room ambient conditions for three days, ^{3}H-TdR incorporation declined and reached normal levels.

3.2.4.4. EFFECT OF HYPOBARIC HYPOXIA

The results of ^{3}H-TdR incorporation in the hypobaric hypoxic rats were similar to those noticed in the normobaric hypoxic rats. Following a drop in the rate of DNA synthesis on day 1, it increased twelve fold on day 3, seventeen fold on day 5, eight fold on day 7, four fold on day 10, three fold on day 14 and four fold on day 21 compared to that in weight-matched controls.

To summarize, exposure to hypobaric hypoxic or normobaric hypoxic conditions stimulated DNA synthesis in the lungs. After an initial fall on day 1, it peaked on day 3 followed by a gradual decline until day 10 when it reached close to normal levels but stayed consistently higher until the end of exposure compared to weight-matched animals. On day 21, DNA synthesis in hypobaric hypoxic and normobaric hypoxic animals showed a significant increase. After returning the hypobaric hypoxic and normobaric hypoxic animals to room air, ^{3}H-TdR incorporation reached normal levels (general controls). An increase in DNA synthesis in hypobaric hypoxic and normobaric hypoxic rats was much greater when comparison was made with those of weight-matched control rats, where it was depressed throughout the exposure. ^{3}H-TdR incorporation increased in weight-matched control rats when they were given food ad libitum. DNA synthesis also increased in the rats exposed to low ambient pressure alone and peaked on day 5, but the percentage increase was lower than that observed in the normobaric hypoxic or hypobaric hypoxic rats.

3.2.5. AUTORADIOGRAPHY

Autoradiographs of general controls and rats subjected to hypobaric normoxia, normobaric hypoxia, hypobaric hypoxia and undernutrition for 1, 3, 5, 7 and 21 days were analyzed for pulmonary cell kinetics. ^{3}H-TdR labelled and unlabelled alveolar wall cells were counted in three different regions (central, peripheral and subpleural) of the lung (see materials and methods). Alveolar wall cells including, type I pneumonocytes, type II pneumonocytes, alveolar wall capillary endothelial cells, interstitial cells and unidentifiable cells were counted and labelling indices were computed. Since the results of subpleural and peripheral alveolar wall cells were similar, the observations of both sites were pooled and referred as peripheral alveolar wall cells.

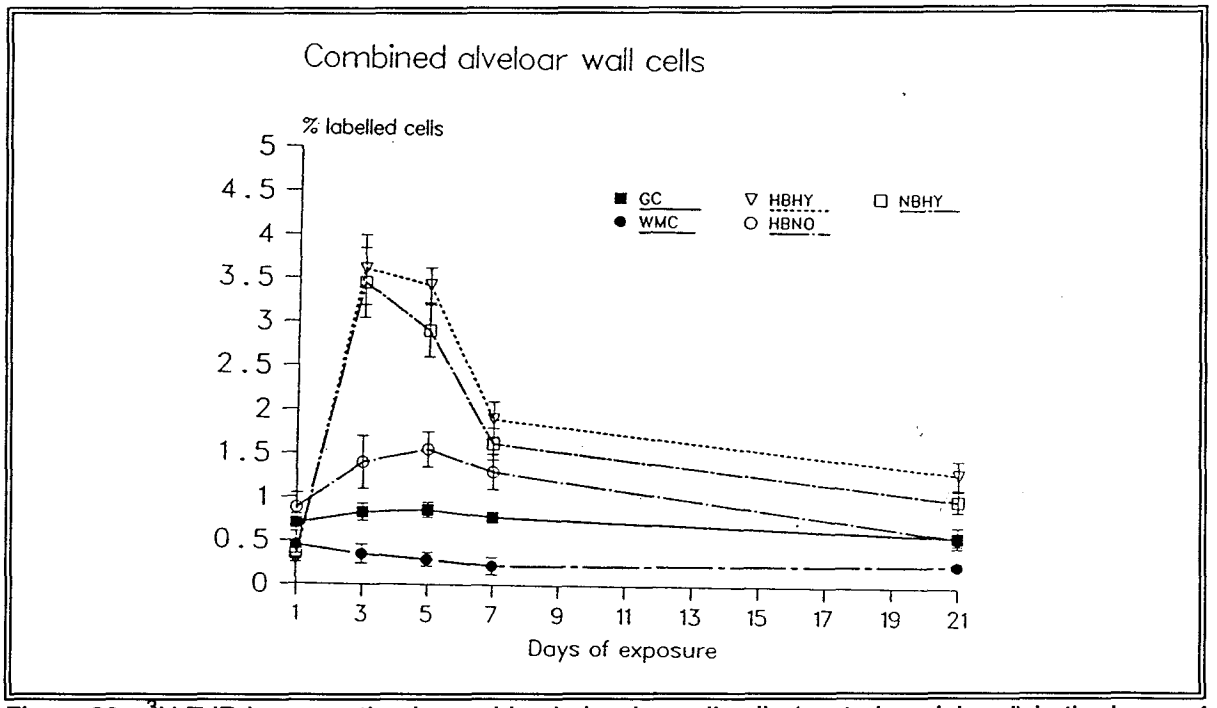


Figure 22. ^{3}H-TdR incorporation in combined alveolar wall cells (central+peripheral) in the lungs of GC, WMC, HBHY, HBNO and NBHY animals subjected to different conditions for 1, 3, 5, 7 and 21 days.

3.2.5.1. EFFECT OF UNDERNUTRITION

The combined alveolar wall cell labelling index (LI) declined on day 1, but a significant decrease were found on days 3, 5, 7 and 21 of exposure ($p=0.0016$, $p=0.0008$, $p=0.016$ and $p=0.016$ respectively) in undernourished rats compared to general control rats (Figure 22). The LI of free

alveolar macrophage and mast cells remained unaffected (Figure 23, Figure 24).

3.2.5.1.1. CENTRAL ALVEOLI

No detectable changes were found in the LI of type I pneumonocytes (Figure 25). Although the LI of type II pneumonocytes was diminished during the experimental period, no significant difference was detected between the weight-matched control group compared to the general control group (Figure 26). The LI of endothelial cells fell and reached a significant level only on day 5 ($p=0.0008$) (Figure 27). Compared to general controls, except for day 1, ^{3}H-TdR incorporation in interstitial cells was significantly decreased throughout the experiment (Figure 28). The LI of unidentifiable cells remained unchanged (Figure 29).

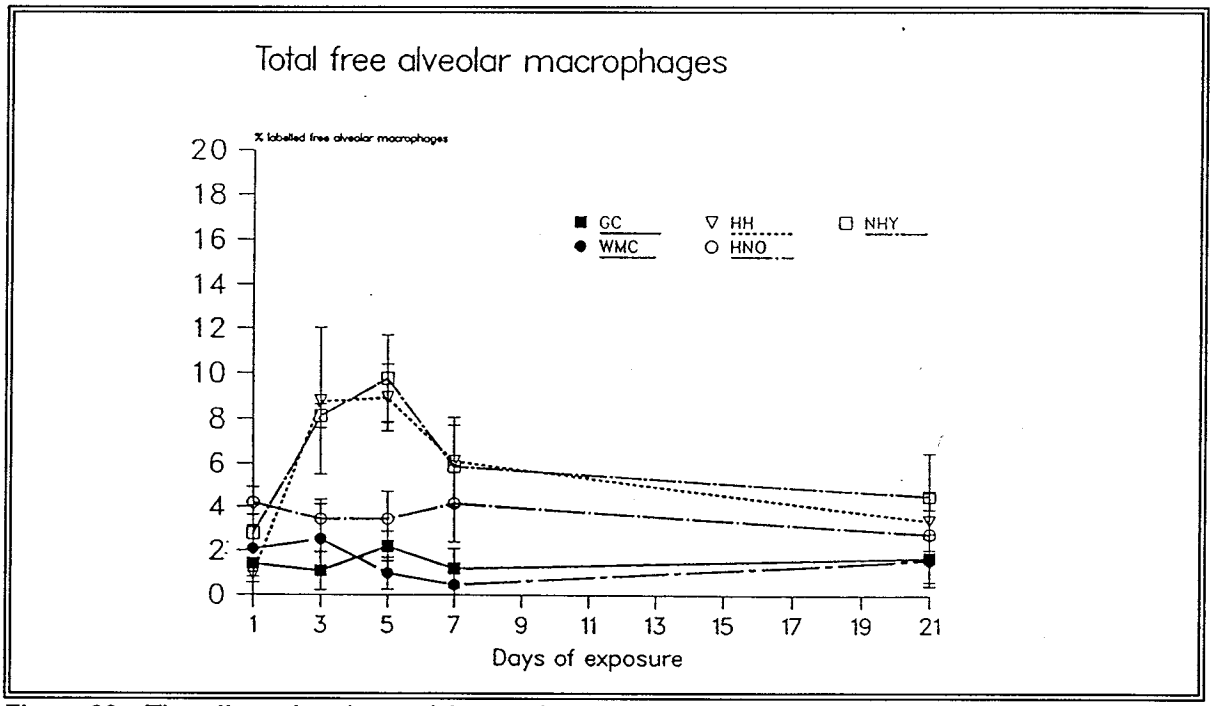


Figure 23. The effect of undernourishment, hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia on the labelling index of free alveolar macrophages.

3.2.5.1.2. PERIPHERAL ALVEOLI

No changes were found in the labelling indices of mesothelial cells or type I pneumonocytes (Figure 30, Figure 31) compared to general controls. The labelling indices of type II pneumonocytes, endothelial cells and interstitial cells fell in weight-matched animals and were significantly lower on

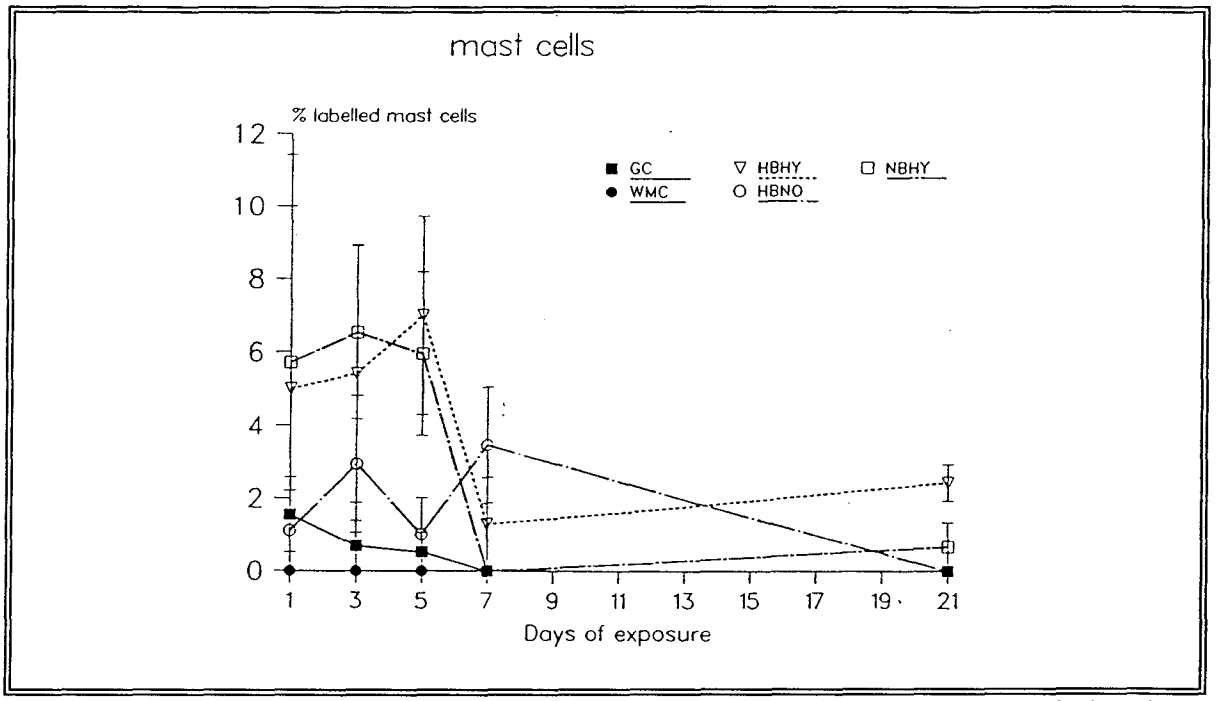


Figure 24. The mast cell labelling index in the lungs of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals.

days 5 and 7 (Figure 32, Figure 33, Figure 34) but the decline in the LI of unidentifiable cells did not reach a significant level (Figure 35).

3.2.5.1.3. NON-PARENCHYMA

A significant decrease in ^{3}H-TdR incorporation in bronchiolar epithelium was observed on day 7 and in bronchial epithelium on day 3 and 7 in animals subjected to limited food access compared to the free fed animals (Figure 36-Figure 37). The labelling indices of bronchial wall cells, arterial endothelium and arterial wall cells declined on day 3 of the experiment and remained low thereafter (Figure 38, Figure 39, Figure 40).

In short, the cytokinetic results showed that ^{3}H-TdR incorporation in combined alveolar wall cells fell due to undernutrition in the weight-matched animals which was mainly a reflection of depressed DNA synthesis in type II pneumonocytes, endothelial cells and interstitial cells. The effect of undernutrition was apparent on day 1 but became more apparent on day 5 and 7 of the experimental period.

3.2.5.2. EFFECT OF HYPOBARIC NORMOXIA

Compared to the general control group, the rats exposed to the low ambient pressure, showed a significant increase in the combined alveolar wall cell labelling index on days 5 and 7 of the experiment (Figure 22). However, when the hypobaric hypoxic and normobaric hypoxic groups were compared to observed the effect of low pressure, no significant difference was found. The LI of free alveolar macrophage and mast cells did not change in hypobaric normoxic rats (Figure 23, Figure 24).

3.2.5.2.1. CENTRAL ALVEOLI

Compared to the general control group, except a significant increase in the interstitial cells on day 7 ($p=0.0008$) (Figure 28), the labelling indices remained unchanged in all cell types. The LI of the type II pneumonocytes peaked on day 7 but never reached a significant level (Figure 26).

3.2.5.2.2. PERIPHERAL ALVEOLI

Compared with the general control group, the number of labelled mesothelial cells in the hypobaric normoxic group was significantly higher ($p=0.04$) on day 3 of hypobaric normoxic exposure (Figure 30). The LI of type II pneumonocytes was also increased and reached a significant level on day 5 (Figure 32) whereas the interstitial cell labelling index was significantly higher on days 5 ($p=0.03$) and 7 ($p=0.0008$) of exposure to hypobaric normoxia (Figure 34).

3.2.5.2.3. NON-PARENCHYMA

The LI of bronchial wall cells increased significantly on day 5 (Figure 38) whereas the LI of arterial wall cells (Figure 40) increased on days 3 and 5 in hypobaric normoxic rats compared to general controls. The labelling indices of bronchiolar and bronchial epithelium (Figure 36, Figure 37) and arterial endothelial cell did not change (Figure 39). On the other hand, low ambient pressure did not cause any significant change in cellular dynamics of non-parenchymal cells when the hypobaric hypoxic and normobaric hypoxic groups were compared (Figure 36-Figure 40).

In summary, the combined alveolar wall cell labelling index appeared to peak on day 5 of low ambient pressure exposure. Mesothelial cell ^{3}H-TdR incorporation was maximum on day 3. Peripheral type II pneumonocytes, interstitial cells and unidentifiable cells showed maximum ^{3}H-TdR incorporation on day 5, whereas central type II pneumonocytes and interstitial cells reached their maximum ^{3}H-TdR incorporation on day 7. ^{3}H-TdR incorporation in endothelial cells remained unaffected both in the central and peripheral regions of the lung. DNA synthesis in arterial and bronchial wall cells was stimulated by lower ambient pressure while in arterial endothelial and conducting airways epithelial cells it remained unaltered.

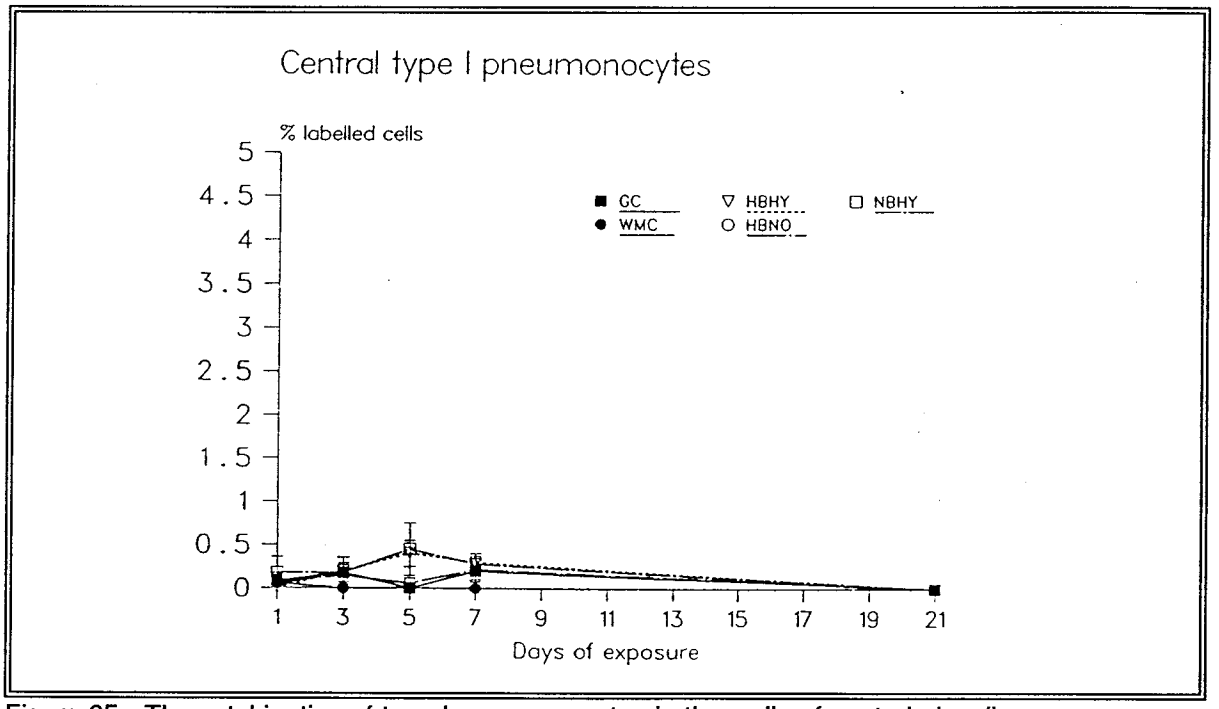


Figure 25. The cytokinetics of type I pneumonocytes in the walls of central alveoli.

3.2.5.3. EFFECT OF NORMOBARIC HYPOXIA

As displayed in Figure 22, ^{3}H-TdR incorporation in combined alveolar wall cells was significantly decreased on day 1 of exposure to normobaric hypoxia compared with the general control rats and reached the value of weight-matched control rats. On day 3 of exposure to normobaric hypoxia, DNA synthesis in alveolar wall cells increased markedly and was nine times higher compared

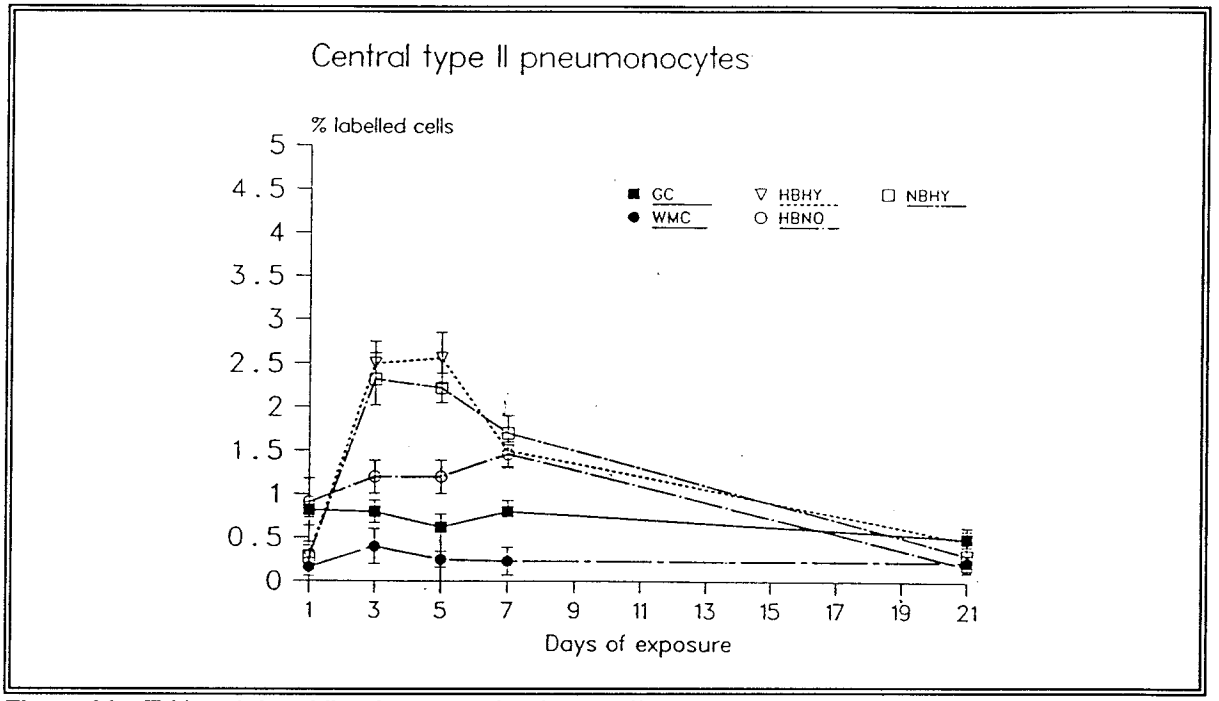


Figure 26. Tritiated thymidine incorporation in type II pneumonocytes in the walls of central alveoli.

to weight-matched controls and four times higher than general controls. After day 3, the labelling index of combined alveolar wall cells in normobaric hypoxic rats began to decline gradually but remained consistently higher than weight-matched control animals. On day 21 of exposure, their LI remained higher compared to both weight-matched controls ($p=0.0008$) and general controls ($p=0.024$). The LI of free alveolar macrophage cells increased significantly on day 5 ($p=0.04$) compared to weight-matched animals (Figure 23). The mast cell labelling index in normobaric hypoxic rats was significantly elevated on day 1 and remained higher on days 3 and 5 compared to that of weight-matched controls but not compared to general controls (Figure 24).

3.2.5.3.1. CENTRAL ALVEOLI

No change in the proportion of labelled type I pneumonocytes was observed in normobaric hypoxic animals (Figure 25). As shown in Figure 26, the rate of ^{3}H-TdR incorporation in type II pneumonocytes fell on day 1 ($p=0.05$) compared to general controls and was indistinguishable from that of weight-matched controls. It was followed by a sharp rise on day 3 ($p=0.016$) which sustained

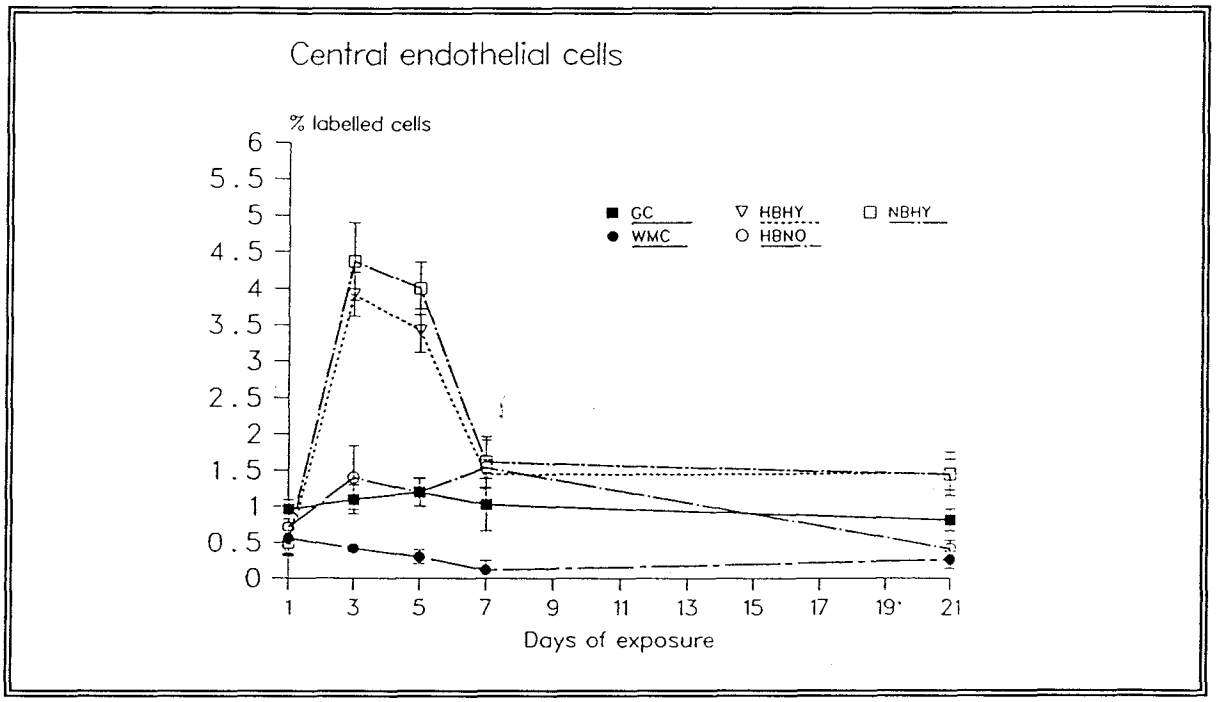


Figure 27. The capillary endothelial cell labelling index in the walls of central alveoli.

until day 5 (p=0.0008) of exposure (the % increase was greater on day 5 than day 3) compared to that of the weight-matched control group. Thereafter the labelling index of type II pneumonocytes fell on day 7 but was significantly higher compared to the weight-matched control (p=0.003) and reached normal levels on day 21. Endothelial cell ^{3}H-TdR incorporation also dropped after one day in normobaric hypoxic conditions but a significant increase was observed on day 3 (11.5 times weight-matched controls and 4.5 times general controls) and on day 5 it began to decline (Figure 27). A precipitous fall in the labelling index of endothelial cells did not occur until day 7 when it reached general control levels but remained higher than weight-matched controls.

Following a small drop on day one of exposure to normobaric hypoxia, ^{3}H-TdR incorporation in interstitial cells rose significantly on day 3. After maximal stimulation on day 5 it fell on day 7 of exposure but was significantly higher compared to both weight-matched and general controls (Figure 28). On day 21, the rate of ^{3}H-TdR incorporation was similar as that of general and weight-matched controls. The LI of the unidentifiable cells was significantly higher on day 3 (p=0.048) and 5 (p=0.002) compared to the weight-matched control group, and day 5 compared to the general control

(p=0.008) group but thereafter it reached normal levels (Figure 29).

3.2.5.3.2. PERIPHERAL ALVEOLI

Upon exposure to normobaric hypoxia, the labelling indices of all cells fell on day 1 of exposure and such changes were identical to those found in weight-matched animals. Although the maximal increase in the proportion of labelled mesothelial cells was found on day 3, this change, however, was not significant (because of the large variance). A significant change was only observed on day 5 (p=0.03) compared to weight-matched animals (because of a continued decrease in labelling index of mesothelial cells in the weight-matched group) but not compared to general controls (Figure 30). After day 3, the mesothelial cell labelling index fell gradually and was similar to that of weight-matched or general controls on days 7 and 21 of exposure.

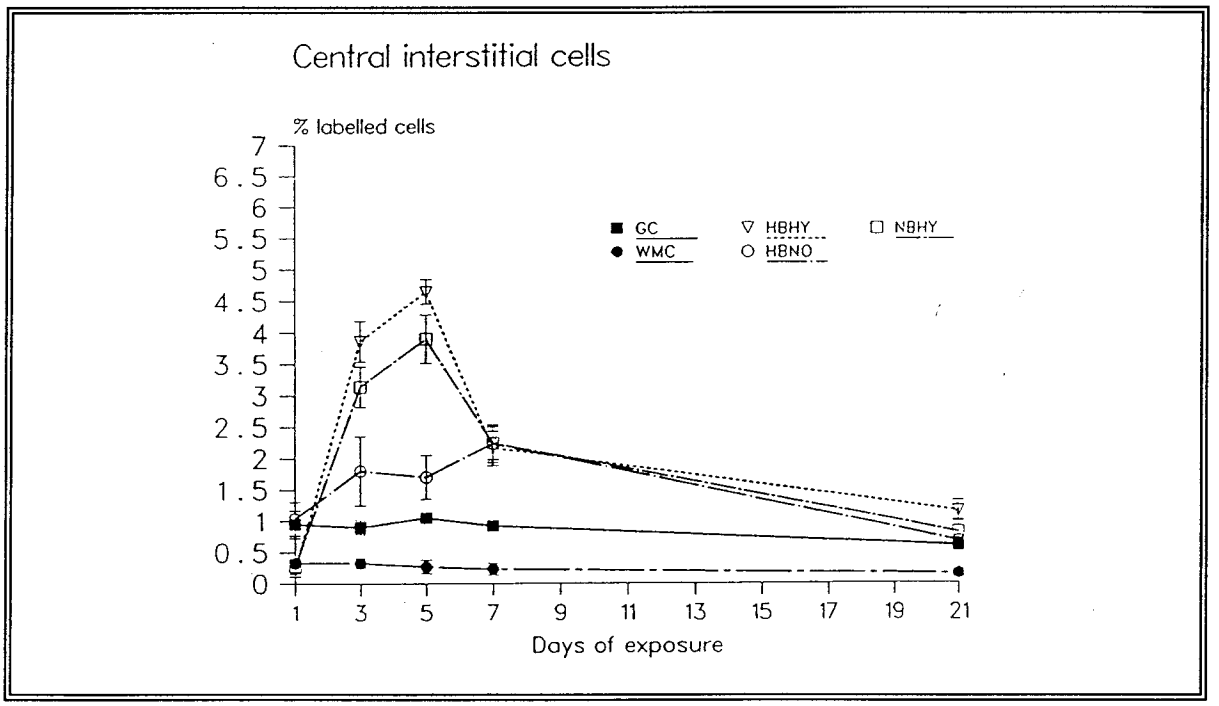


Figure 28. Cytodynamics of interstitial cells in the walls of central alveoli of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic rats.

Autoradiographs showed that maximal incorporation of ^{3}H-TdR into the peripheral type II pneumonocytes occurred on day 3 compared to both weight-matched (p=0.007) and general control (p=0.01) groups (Figure 32). On day 5, the type II pneumonocyte LI fell sharply and this was followed

by a further decrease on day 7 and finally reached the general control level at day 21 of exposure. Similar to the central alveoli, on day 3 of exposure, the LI of the capillary endothelial cells showed a ten fold increase compared to weight-matched controls and a six fold increase compared general controls. It fell sharply on day 5 but was significantly higher on day 21 compared to both weight-matched and general control rats (Figure 33).

The pattern of the rate of ^{3}H-TdR incorporation in the peripheral interstitial cells was also similar to those of peripheral type II pneumonocytes and endothelial cells. Following a decrease on day 1, the LI of interstitial cells peaked on day 3 of exposure and then declined gradually to reach the level of general controls on day 21 of exposure but was significantly higher compared to that in weight-matched animals (Figure 34). The proportion of unidentifiable labelled cells also peaked on day 3 and then decreased sharply on day 5 and continued to fall and reached the level of general controls on day 7 but was significantly higher compared to weight-matched controls ($p=0.03$). No difference was found between both control groups and normobaric hypoxic rats for the LI of unidentifiable cells on day 21 of exposure (Figure 35).

3.2.5.3.3. NON-PARENCHYMA

The proportion of bronchiolar and bronchial epithelial cells, bronchial wall cells, arterial endothelial cells and arterial wall cells which incorporated ^{3}H-TdR in normobaric hypoxic animals increased significantly on days 3, 5, 7 and 21 of exposure compared to weight-matched controls (Figure 36-Figure 40). The maximal response was observed on day 3 and thereafter it declined gradually. Compared to general controls, significant increases in the labelling indices of bronchiolar epithelial cells on day 3 (280%), bronchial epithelial cells on days 3 (88%) and 5, bronchial wall cells on days 3 (262%)and 5, arterial endothelial cells on days 3 (247%), 5 and 7, and arterial wall cells on days 3 (483%), 5 and 7 were also observed (Figure 36-Figure 40).

In summary, it was evident from the results that DNA synthesis activity in all cells was depressed on the first day of exposure to normobaric hypoxia which was similar to that of weight-

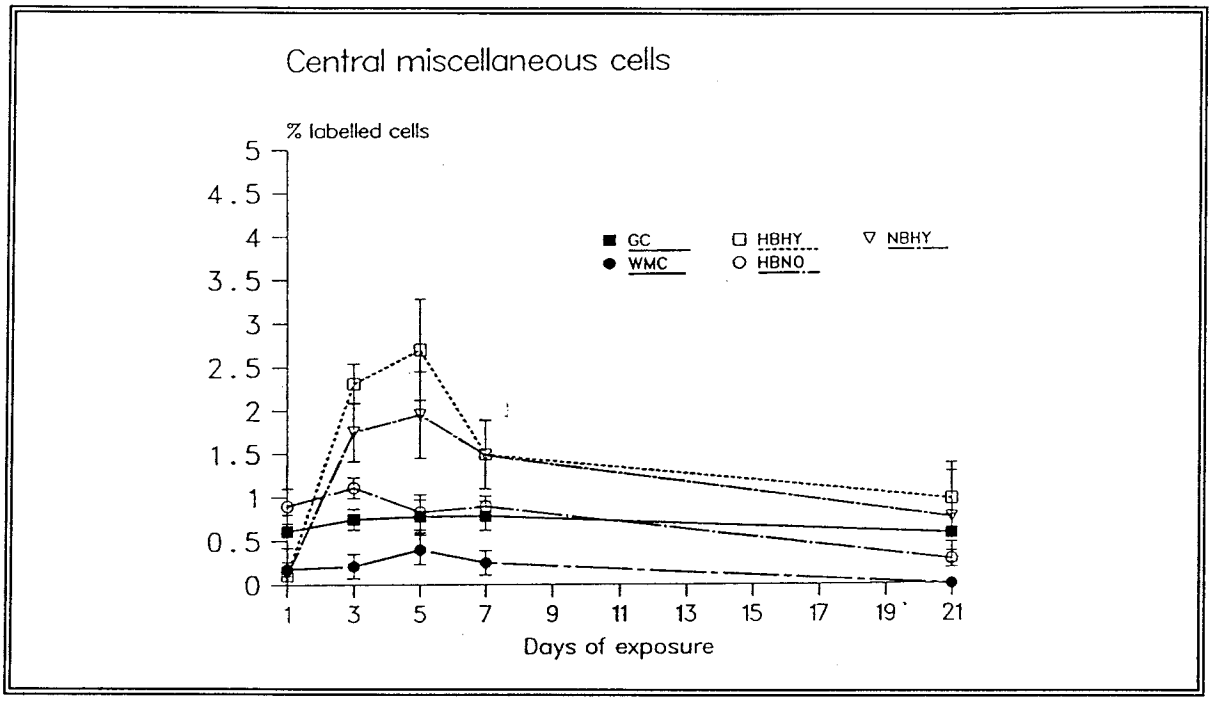


Figure 29. The labelling index of unidentifiable cells in the walls of central alveoli.

matched control animals but on day 3 a dramatic increase in the proportion of ^{3}H-TdR labelled cells occurred. Thereafter ^{3}H-TdR incorporation in the combined alveolar wall cells gradually decreased but stayed slightly higher during the rest of the exposure period. ^{3}H-TdR incorporation in peripheral type II pneumonocytes, interstitial cells and unidentifiable cells peaked on day 3 of exposure. The LI of the central type II pneumonocyte, interstitial cells and unidentifiable cells reached a peak on day 5, but the LI of both central and peripheral alveolar wall endothelial cells peaked on day 3. At the time of maximal stimulation, the percentage increase in the LI in the wall of peripheral alveoli was slightly higher compared to the central alveoli. The results of autoradiographs showed that peak stimulation mesothelial cells occurred on day 3 while free alveolar macrophage were stimulated on day 5 of normobaric hypoxic exposure. The mast cells appeared to be affected by normobaric hypoxia from the very first day of exposure and remained consistently higher before sharply falling after day 5.

3.2.5.4. EFFECT OF HYPOBARIC HYPOXIA

The results of combined alveolar wall cell ^{3}H-TdR incorporation is shown in Figure 22. As

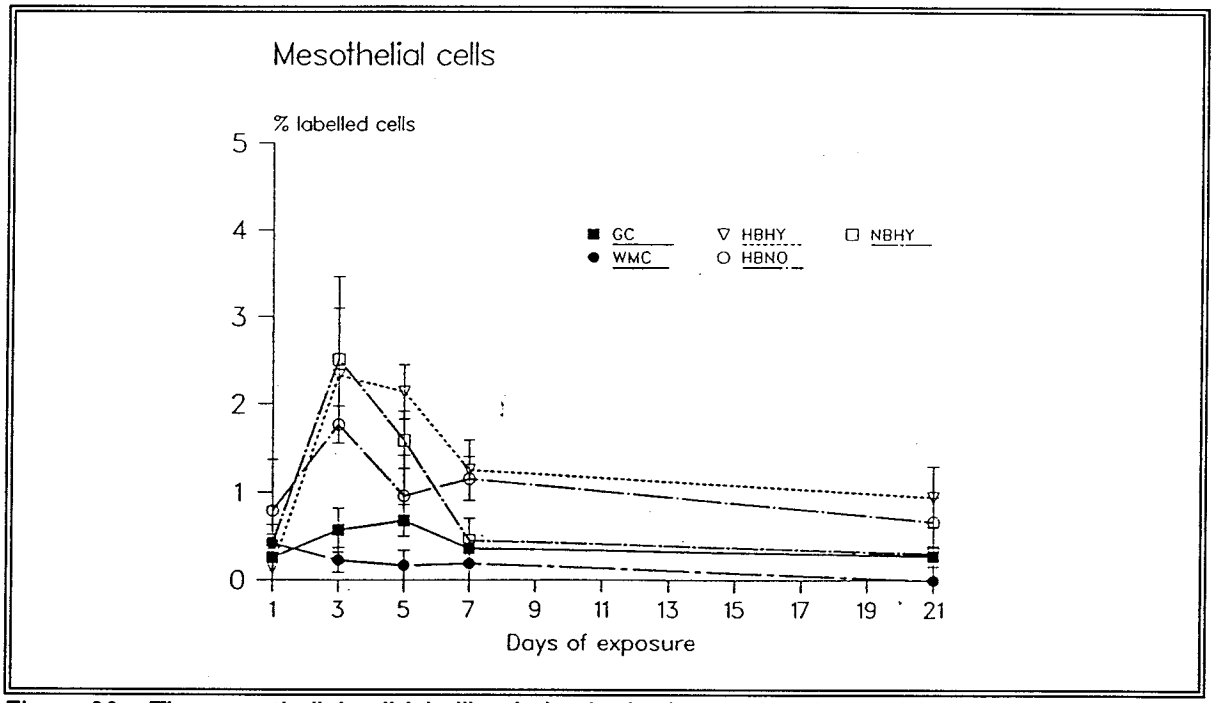


Figure 30. The mesothelial cell labelling index in the lungs of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals.

noted in normobaric hypoxic animals, the number of labelled combined alveolar wall cells dropped significantly ($p=0.02$) on day 1 of exposure to hypobaric hypoxia compared to that of general controls and was equivalent to that of weight-matched controls. On day 3, a sharp and significant increase (331%) in the labelling index was observed which dropped to 295% on day 5, 142% on day 7 and 84% on day 21 of exposure compared to the general control group. Since the labelling index of combined alveolar wall cells was depressed throughout the experimental period in weight-matched animals, except for day 1 an increase in the labelling index of alveolar wall cells in the hypobaric hypoxic animals was highly significant compared to the weight-matched group. The labelling index of free alveolar macrophages was significantly increased in hypobaric hypoxic animals on day 5 compared to both weight-matched controls ($p=0.008$) and general controls ($p=0.02$) (Figure 23). The proportion of mast cells which incorporated ^{3}H-TdR increased significantly on days 1, 3 and 5 compared to weight-matched controls (Figure 24).

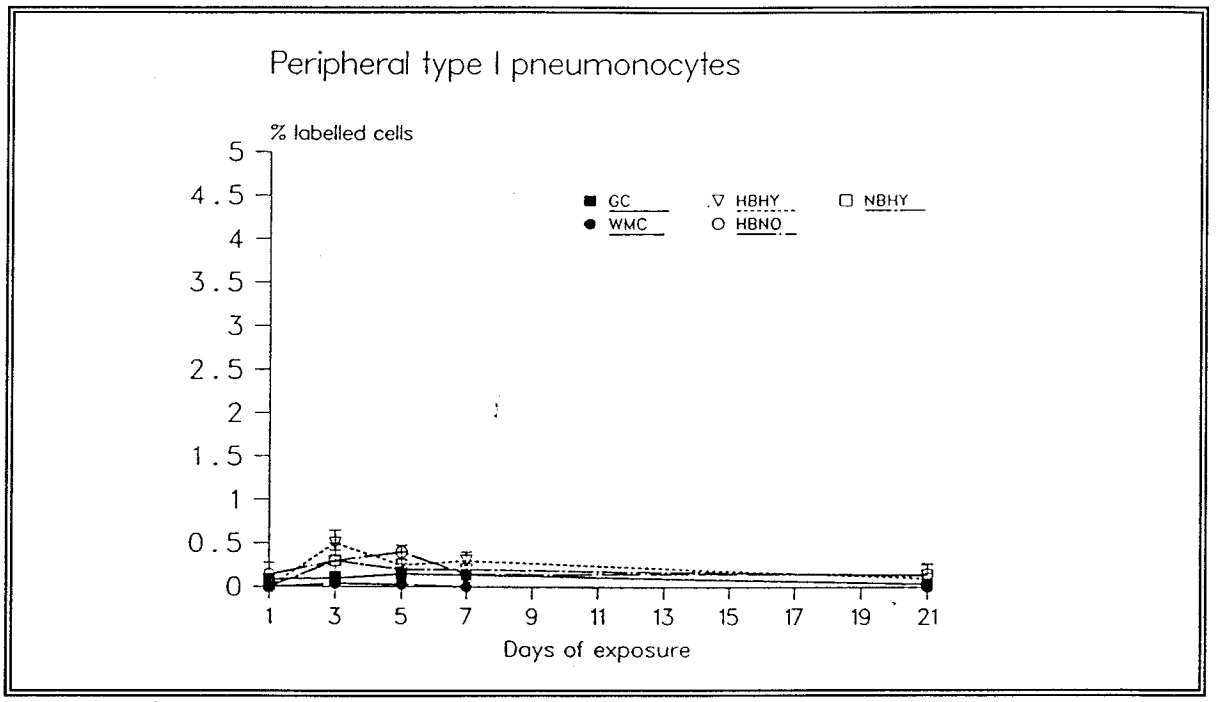


Figure 31. Cytokinetics of type I pneumonocytes in the walls of peripheral alveoli.

3.2.5.4.1. CENTRAL ALVEOLI

No change in the labelling index of type I pneumonocytes was noticed (Figure 25). Compared to weight-matched controls, except for day 1, the labelling index of type II pneumonocytes was significantly higher ($p<0.007$) throughout the exposure in hypobaric hypoxic animals (Figure 26). When comparison was made with the general control group, a significant increase in the labelling index of type II pneumonocytes was observed on days 3 ($p=0.008$) and 5 ($p=0.002$) and following a sharp drop on day 7, it eventually reached at the general control group levels on day 21. Except on days 1 and 21, the endothelial cell labelling index was significantly higher ($p <0.007$) compared to the weight-matched rats (Figure 27). Compared to general controls, endothelial cell ^{3}H-TdR incorporation was significantly lower ($p=0.048$) on the first day but increased markedly on day 3 (256%), 184% on day 5 and dropped thereafter to reach the control levels on days 7 and 21 (Figure 27).

Compared to general controls, apart from slightly less ^{3}H-TdR incorporation in the interstitial cells on day 1, a significant increase on day 3 ($p=0.0008$) and further increment on day 5 were observed. On day 7, the rate of increased ^{3}H-TdR incorporation declined but remained significantly

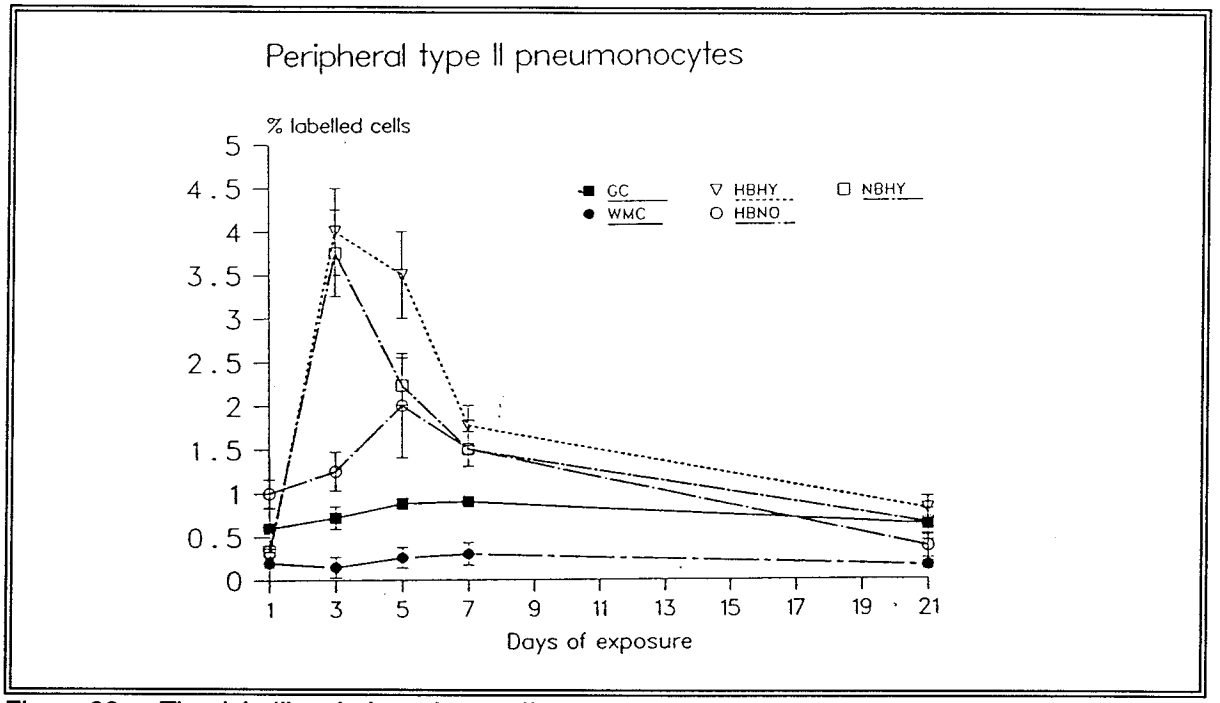


Figure 32. The labelling index of type II pneumonocytes in the walls of peripheral alveoli of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic rats.

higher (p=0.04) and reached the general control group values on day 21 (Figure 28). On the other hand, compared to the weight-matched group, the labelling index of the interstitial cells in hypobaric hypoxic group remained significantly higher from day 3 through day 21 of exposure (Figure 28). The LI of the unidentifiable cells were also significantly greater on day 5 than general controls, and on days 3 and 5 compared to the weight-matched controls (Figure 29).

3.2.5.4.2. PERIPHERAL ALVEOLI

Similar to normobaric hypoxic animals, ^{3}H-TdR incorporation in all cells in the walls of peripheral alveoli was depressed on day 1 of exposure and labelling indices were identical to those recorded in weight-matched controls. The LI of mesothelial cell increased on days 3, 5 and 7, and maximal ^{3}H-TdR incorporation occurred on day 3 but a significant increase (p=0.003) was achieved only on day 5 (Figure 30) compared to weight-matched controls. After day 1 of exposure to hypobaric hypoxia, the labelling indices of type II pneumonocytes, alveolar wall endothelial cells, interstitial cells and unidentifiable cells increased markedly on day 3 of exposure and remained significantly high

Table 13. Cell composition of the walls of the peripheral alveoli.

VARIABLE	GC	WMC	HBHY	HBNO	NBHY
Type I pneumonocytes (%)					
Day 1	9.98+0.37	10.78+0.59	9.96+0.4	10.33+0.59	10.16+0.37
day 3	10.01+0.17	9.54+0.38	10.01+0.58	10.31+0.34	10.02+0.16
Day 5	10.11+0.39	9.85+0.57	9.53+0.46	10.01+0.51	9.77+0.24
Day 7	10.08+0.36	9.59+0.35	9.43+0.47	9.62+0.22	9.06+0.45
Day 21	10.08+0.25	9.88+0.22	10.58+0.32	10.09+0.4	10.07+0.17
Type II pneumonocytes (%)					
Day 1	14.47+0.55	15.49+0.47	14.22+0.43	15.09+0.39	14.85+0.57
Day 3	15.51+0.20	14.51+0.65	16.04+0.48	15.66+0.6	14.57+0.87
Day 5	15.53+0.4	15.37+0.2	14.32+0.46	14.73+0.6	14.03+0.27
Day 7	14.56+0.77	13.75+0.93	16.65+0.76	14.80+0.55	15.28+0.73
Day 21	14.69+0.66	15.46+0.56	15.83+0.49	15.64+0.28	15.05+0.51
Capillary endothelial cells (%)					
Day 1	29.93+0.28	28.62+1.37	27.14+1.89	29.17+1.94	30.58+0.74
Day 3	30.59+0.45	31.27+0.87	29.23+1.31	30.28+0.92	30.66+0.69
Day 5	30.43+0.3	30.92+0.25	30.14+0.75	30.76+1.89	33.56+2.11
Day 7	29.96+0.53	29.91+0.62	28.99+0.75	28.69+0.26	30.49+1.07
Day 21	31.25+1.52	29.87+1.84	29.72+1.35	28.52+1.07	31.85+1.25
Interstitial cells (%)					
Day 1	34.00+0.46	33.27+1.06	33.79+0.85	33.76+1.56	31.85+0.55
Day 3	32.60+0.31	34.14+2.11	32.84+0.36	30.94+0.47	32.11+0.82
Day 5	32.24+0.38	32.44+0.71	34.71+1.21	32.66+1.3	32.27+1.76
Day 7	31.22+1.64	32.85+0.46	32.68+0.83	35.64+0.51	31.58+1.12
Day 21	31.19+1.04	32.63+1.22	33.25+0.7	33.31+1.12	31.02+0.75
Unidentifiable cells (%)					
Day 1	11.25+0.22	11.81+1.52	14.89+1.58	11.64+1.19	12.54+0.78
Day 3	11.29+0.36	10.53+0.54	11.86+0.25	11.80+0.6	12.62+0.73
Day 5	11.67+0.43	11.40+0.32	11.29+0.49	12.83+1.06	10.35+0.55
Day 7	15.18+0.56	13.89+0.99	12.24+1.29	12.25+0.36	12.59+0.36
Day 21	12.79+0.71	12.17+0.84	10.61+0.29	12.42+0.44	12.01+0.35
Type I/Type II pneumonocyte ratio					
Day 1	0.69+0.05	0.70+0.04	0.71+0.04	0.70+0.05	0.69+0.04
Day 3	0.65+0.02	0.66+0.03	0.62+0.03	0.66+0.02	0.70+0.05
Day 5	0.65+0.02	0.64+0.03	0.67+0.04	0.67+0.04	0.70+0.03
Day 7	0.69+0.04	0.71+0.06	0.57+0.06	0.65+0.03	0.59+0.06
Day 21	0.69+0.02	0.64+0.03	0.67+0.03	0.65+0.02	0.67+0.02

No differences were found between the groups.

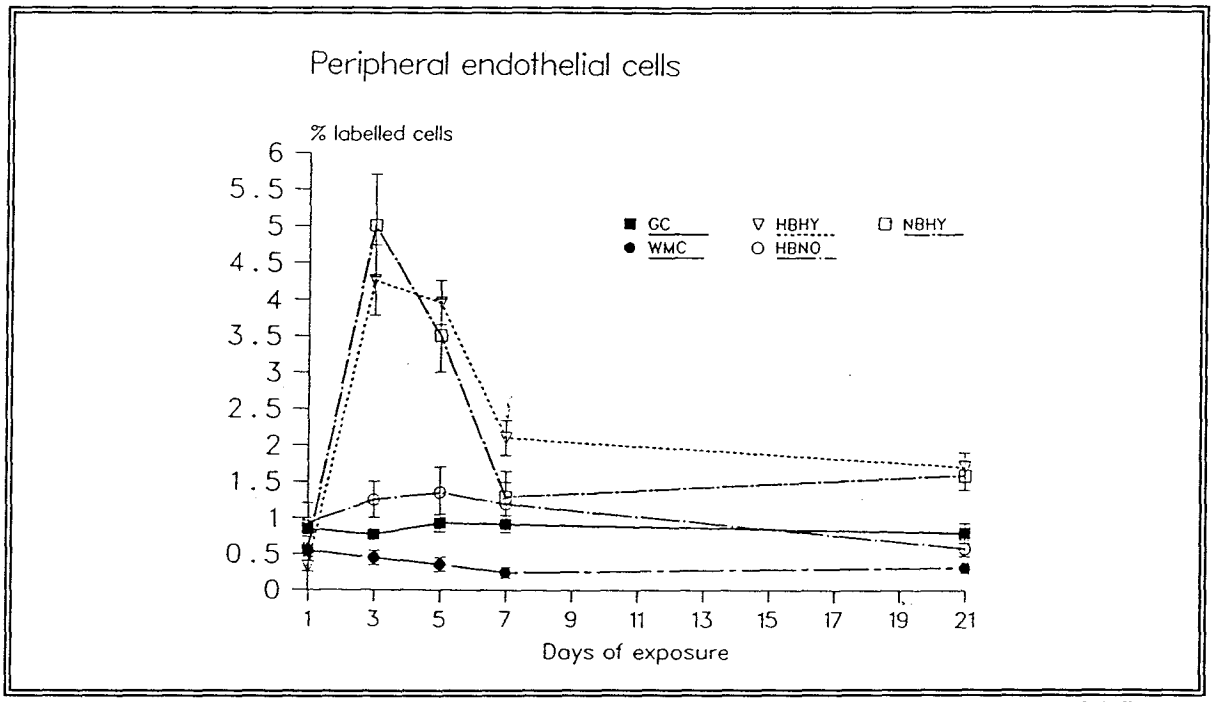


Figure 33. The capillary endothelial cell labelling index in the walls of peripheral alveoli following exposure to different experimental conditions.

($p<0.01$) until the end of exposure (except unidentifiable cells were not significantly different on days 7 and 21) compared to weight-matched animals (Figure 32-Figure 35).

When hypobaric hypoxic animals were compared to general controls, ^{3}H-TdR incorporation in type II pneumonocytes was enhanced in hypobaric hypoxic animals on day 3 by 449%, and on day 5 by 303%. It decreased to 95% on day 7 and reached at a normal level on day 21 (Figure 32). The endothelial and interstitial cell labelling indices also declined significantly ($p=0.02$ and $p=0.05$ respectively) on day 1, but they rose sharply on day 3 and remained significantly higher on days 5,7 and 21 ($p<0.03$) in hypobaric hypoxic rats (Figure 33). ^{3}H-TdR incorporation in unidentifiable cells also increased but a significant change was only found on days 3 ($p=0.016$) and 5 ($p=0.0008$) (Figure 35).

3.2.5.4.3. NON-PARENCHYMA

The number of bronchiolar and bronchial epithelial cells, bronchial wall cells, arterial endothelial cells and arterial wall cells which incorporated ^{3}H-TdR in hypobaric hypoxic animals peaked

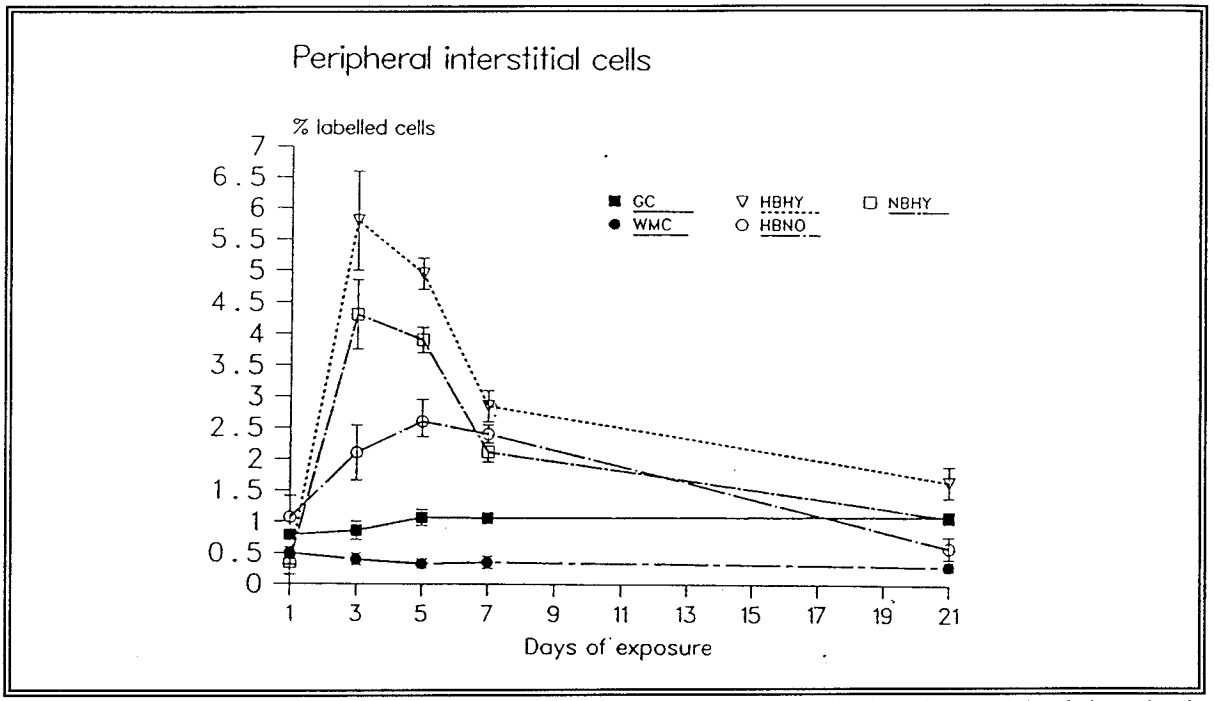


Figure 34. The interstitial cell labelling index in the peripheral alveoli of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals.

on day 3 of exposure and afterwards declined but remained significantly higher than those of weight-matched animals (Figure 36-Figure 40).

Compared to the general control group, the labelling indices of bronchiolar and bronchial epithelial cells increased by 340% and 133% respectively on day 3. The arterial endothelial cell and arterial wall cell labelling indices increased significantly on days 3 (222% and 550% respectively), 5 and 7 while the bronchial wall cell labelling index increased on days 3 (287%) and 5 in hypobaric hypoxic rats compared to general controls (Figure 38-Figure 40). At the end of exposure, the labelling indices of all cells reached at the normal level.

In brief, these findings have demonstrated that alveolar wall cells or cells in the non-parenchymal compartment of the lung were stimulated during the first week of exposure to hypobaric hypoxic conditions. On day one, the labelling indices of all cells decreased and the change was similar to that observed in the undernourished animals. Thereafter, the labelling indices of cells in weight-matched animals continued to fall but in hypobaric hypoxic animals increased dramatically resulting in marked increases. Although the combined alveolar wall cell labelling index peaked on day

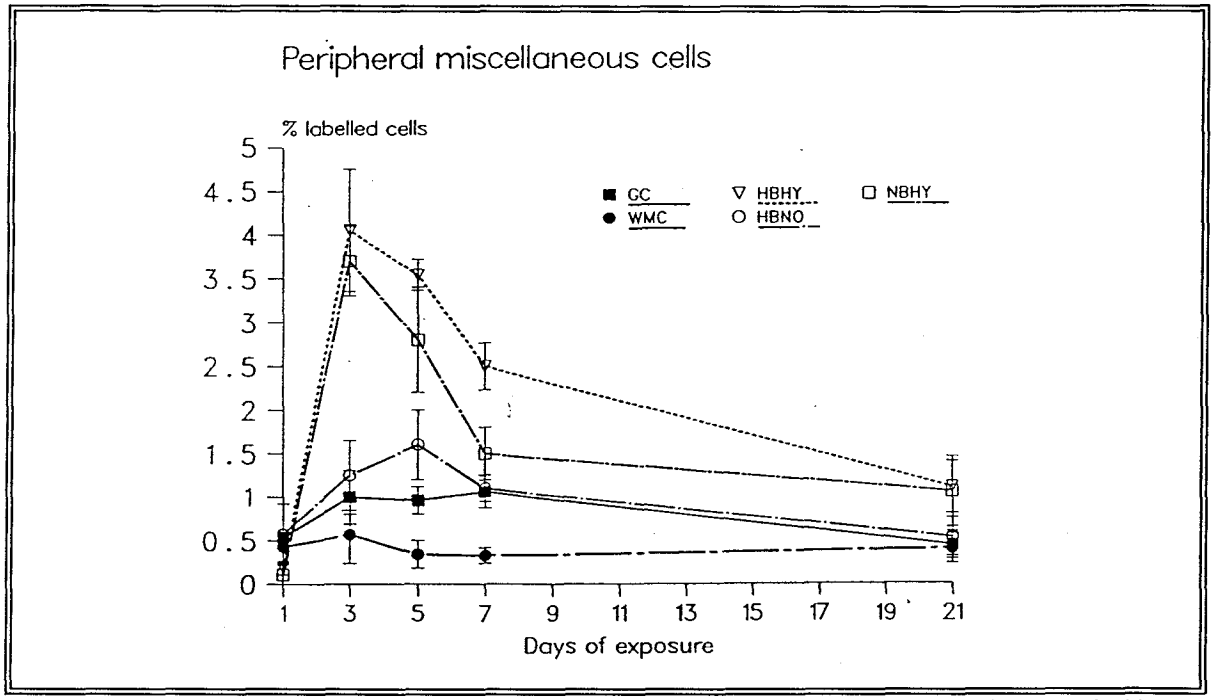


Figure 35. The labelling index of unidentifiable cells in the peripheral alveoli of GC, WMC, HBHY, HBNO and NBHY rats.

3, the percentage increase in the labelling index on day 5 was greater than day 3 when compared to weight-matched controls. This occurred because the labelling index in weight-matched controls continued to fall until day 7 of the experiment. Following a peak stimulation of combined alveolar wall cell on day 3, DNA synthetic activity begun to declined gradually thereafter to reach the general control levels on day 7 and stayed at a slightly higher level until the end of the exposure period. Similar to normobaric hypoxia, the mesothelial cells, type II pneumonocytes, alveolar capillary endothelial, interstitial and unidentifiable cells in the walls of peripheral alveoli also had maximum incorporation of ^{3}H-TdR on day 3 of exposure to hypobaric hypoxia. In the walls of central alveoli, the labelling index of endothelial cells peaked at day 3 but the labelling index of type II pneumonocytes, interstitial and unidentifiable cells peaked on day 5. The overall assessment revealed that ^{3}H-TdR incorporation in type II pneumonocytes, interstitial and unidentifiable cells in peripheral alveoli, and type II pneumonocytes and interstitial cells in central alveoli in hypobaric hypoxic animals was higher compared to those in normobaric hypoxic animals. The labelling indices of endothelial cells and unidentifiable cells in the walls of peripheral and central alveoli were lower in the hypobaric hypoxic

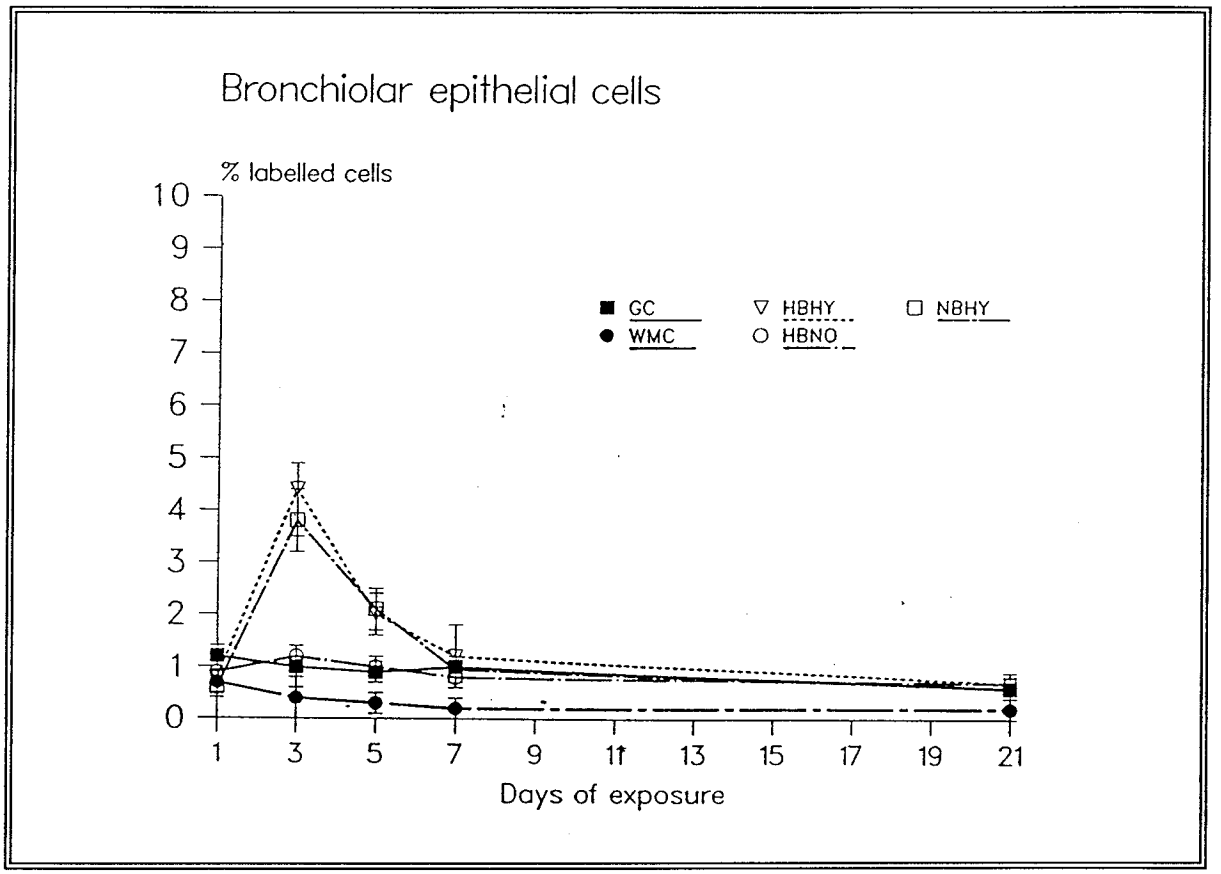


Figure 36. Tritiated thymidine incorporation in bronchiolar epithelial cells following exposure to different experimental conditions.

group than in the normobaric hypoxic group. The results also showed that DNA synthesis in free alveolar macrophage cells and mast cells increased during early exposure to hypobaric hypoxia.

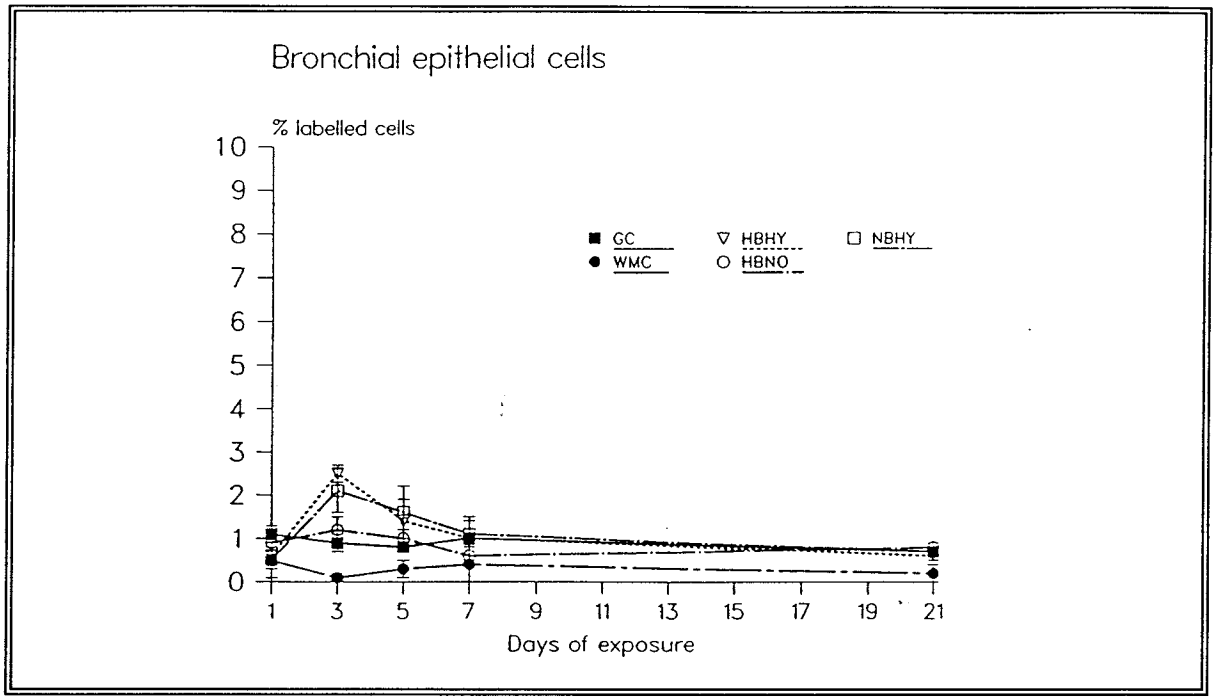


Figure 37. The bronchial epithelial cell labelling index after subjecting animals to different experimental conditions.

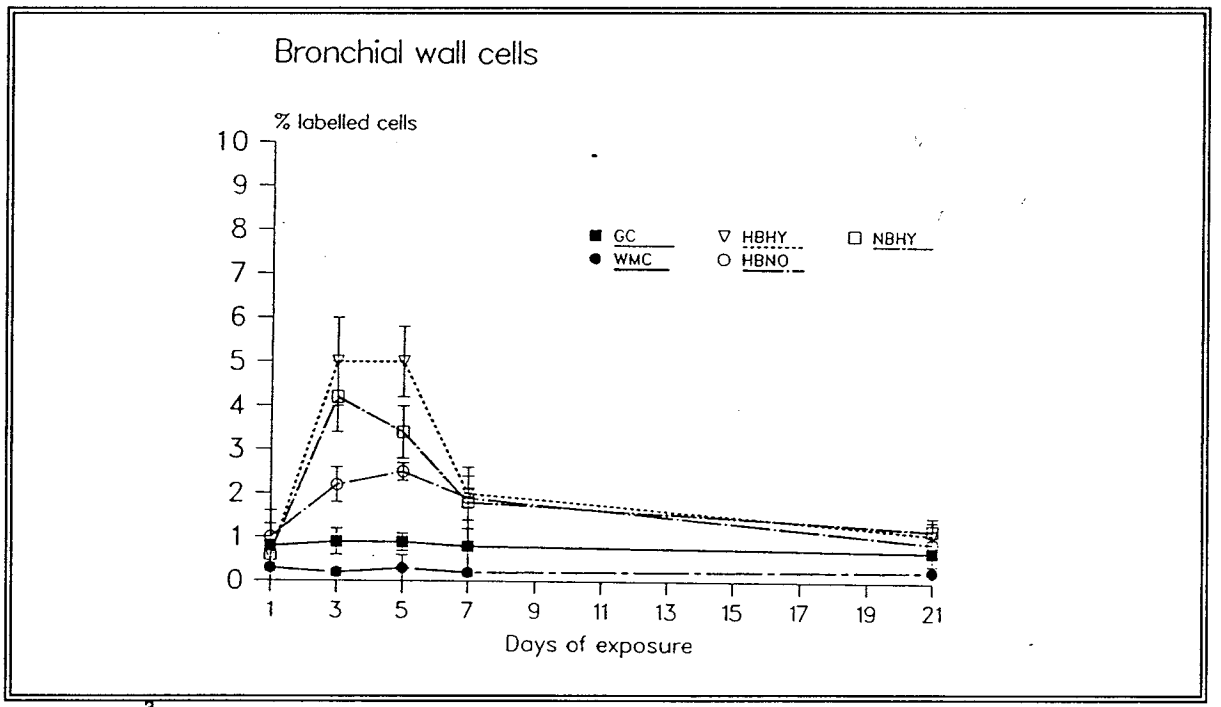


Figure 38. ^{3}H-TdR incorporation in the bronchial wall cells following exposure to different conditions.

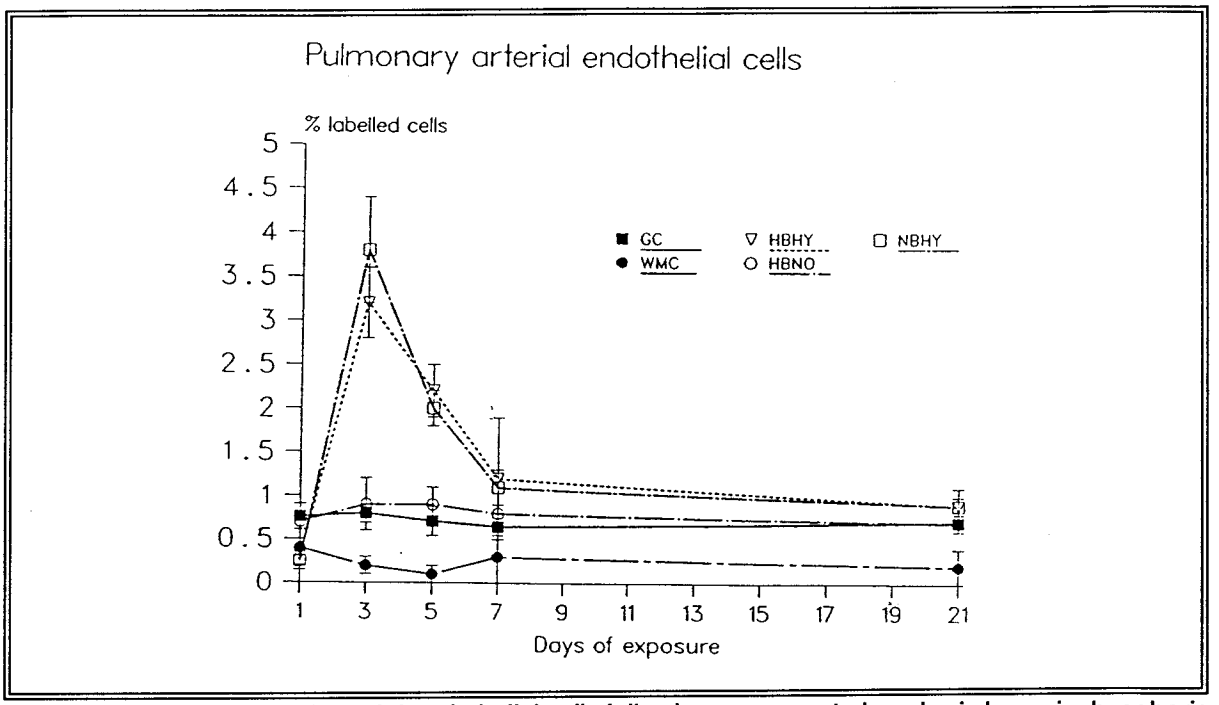


Figure 39. Cytokinetics of arterial endothelial cells following exposure to hypobaric hypoxia, hypobaric normoxia, normobaric hypoxia and undernutrition.

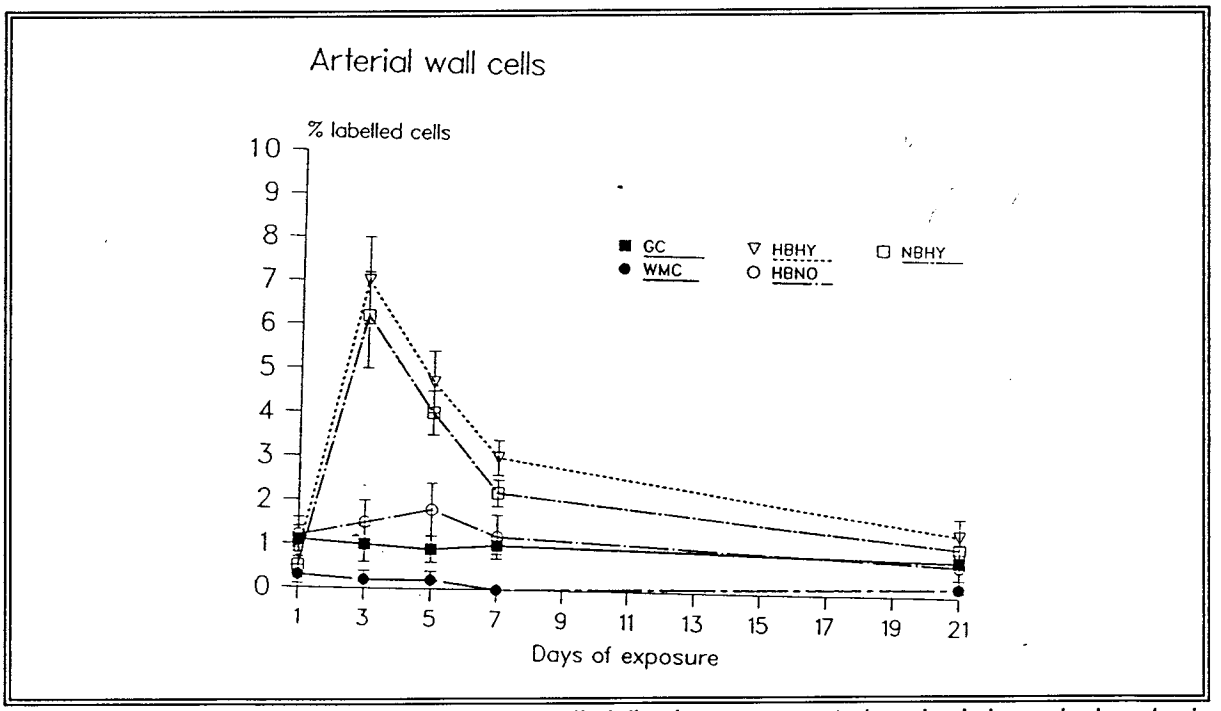
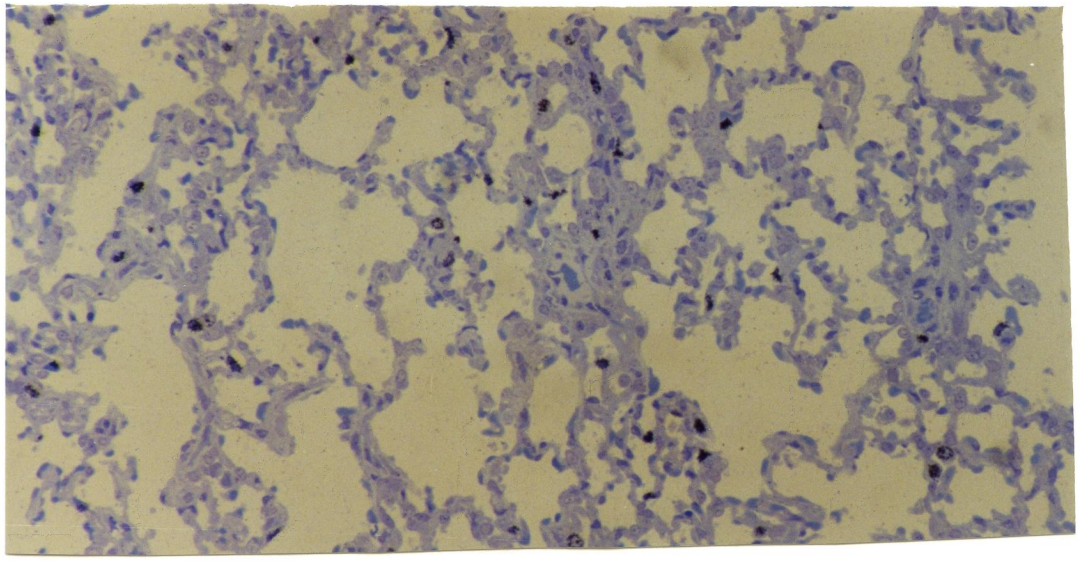
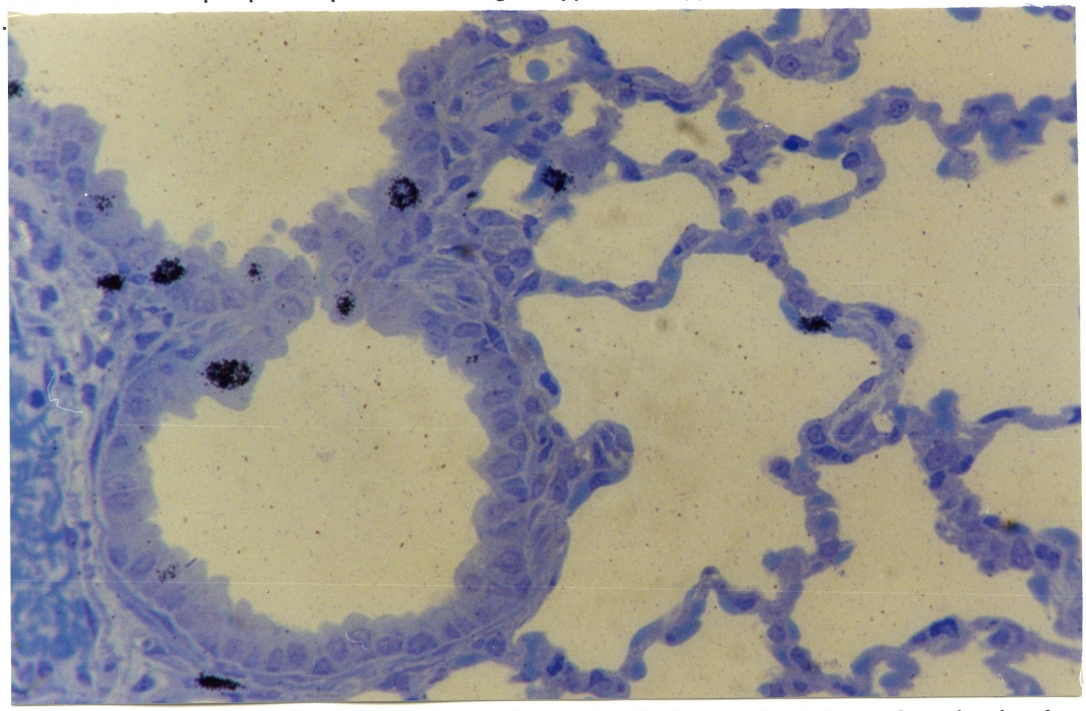


Figure 40. The labelling index of arterial wall cells following exposure to hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia, and restricted feeding.



A. Labelled cells in the peripheral part of the lung of hypobaric hypoxic rats after 3 days of exposure (X200).



B. Labelled bronchiolar epithelial cells and alveolar wall cells in hypobaric hypoxic animals after 3 days of exposure (X800)

Figure 41. Autoradiographs showing cells heavily laden with black granules following tritiated thymidine incorporation.

3.3. LUNG PHYSIOLOGY

3.3.1. LUNG VOLUMES

3.3.1.1. EFFECT OF UNDERNUTRITION

The weight-matched control rats were 22% smaller in body weight compared to the general

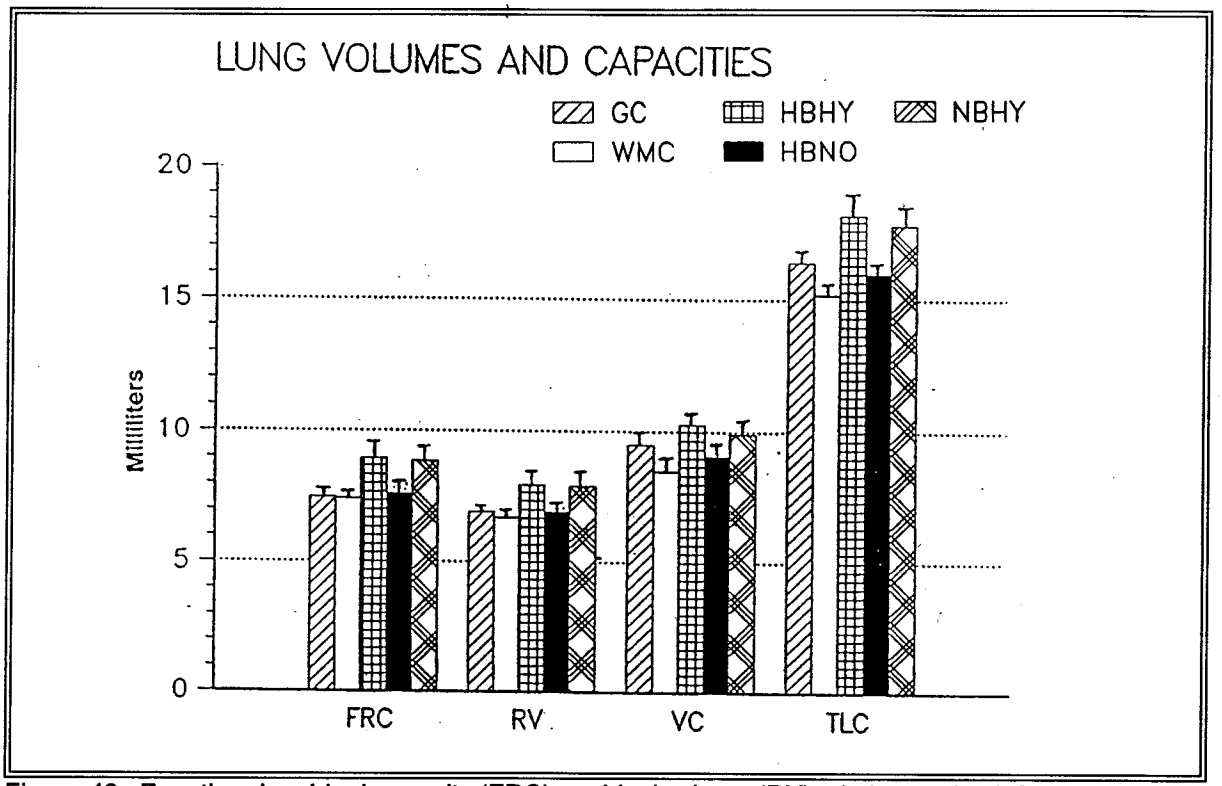


Figure 42. Functional residual capacity (FRC), residual volume (RV), vital capacity (VC) and total lung capacity (TLC) of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic animals exposed to different conditions for 21 days.

control group (201 $\pm$6 and 257 $\pm$8 g respectively) . The functional residual capacity (FRC), residual volume (RV), vital capacity (VC), FRC/TLC and RV/TLC % remained unchanged compared to general controls, but total lung capacity (TLC) was significantly lower in undernourished animals (Figure 42, Figure 43).

3.3.1.2. EFFECT OF HYPOBARIC NORMOXIA

The rats exposed to low ambient pressure were smaller in size (234 ±8 g) compared with the general control group (257 ±8 g) but not significantly. The values of FRC, RV, VC, TLC, FRC/TLC and RV/TLC of hypobaric normoxic animals did not differ from those of general control rats (Figure 42, Figure 43). When hypobaric hypoxic and normobaric hypoxic animals were compared to detect the effect of low ambient pressure, no differences were found between the groups.

3.3.1.3. EFFECT OF NORMOBARIC HYPOXIA

The hypoxic rats were significantly smaller (198 ±5 g) in body weight than general controls (257 ±8 g). FRC, RV, VC and TLC were significantly increased (19%, 18%, 16%, 17% respectively) during 3 weeks of normobaric hypoxic exposure compared to those in the weight-matched control group (Figure 42). Significant increases in FRC (17%) and TLC (8%) were also observed compared to general control values. FRC/TLC% or RV/TLC% did not alter compared to those in both weight-matched and general controls (Figure 43).

When comparison was made between hypobaric hypoxic and hypobaric normoxic rats, FRC and TLC were significantly greater in hypobaric hypoxic animals.

3.3.1.4. EFFECT OF HYPOBARIC HYPOXIA

The adaptive response to hypobaric hypoxia resulted in a significant increase in FRC, RV, VC and TLC (21%, 18%, 21% and 20% respectively) compared to weight-matched controls (Figure 42). Increases in FRC (19%) and TLC (11%) in hypobaric hypoxic rats also reached a significant level ($p<0.009$) when compared to the general control group. FRC/TLC% and RV/TLC% remained the same as in weight-matched control and general control rats (Figure 43).

3.3.2. FLOW-VOLUME RELATIONSHIPS AND LUNG MECHANICS

Flow resistance and static compliance are lung size dependent (71). Therefore, specific

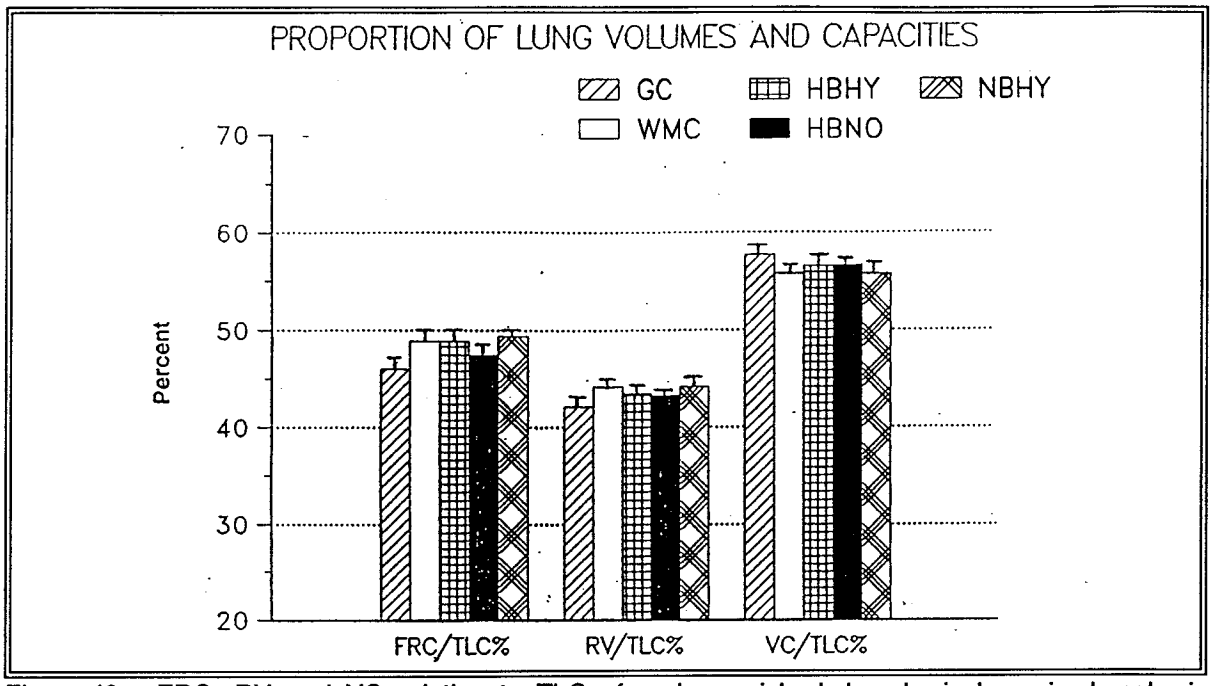


Figure 43. FRC, RV and VC relative to TLC of undernourished, hypobaric hypoxic, hypobaric normoxic and normobaric hypoxic rats.

upstream resistance (sRus) and specific static compliance (sCst) were calculated as described in materials and methods.

3.3.2.1. EFFECT OF UNDERNUTRITION

Neither Rus nor sRus in undernourished animals differed from those in general controls (Figure 44). Forced expiratory volume in 0.1 second ($FEV_{0.1}$) and forced expiratory volume/forced vital capacity % ($FEV_{0.1}$/FVC %) also remained unchanged in weight-matched animals compared to general controls (Figure 45). The peak expiratory flow rate (PEFR), forced expiratory flow rates (FEF) and FEF corrected for FVC were also similar to those in the general control group (Figure 46, Figure 47, Figure 48). Cst as well as sCst remained unchanged (Figure 49). As shown in Figure 50, the pressure-volume characteristics and exponential shape constant K in the weight-matched control and general control groups were also the same.

3.3.2.2. EFFECT OF HYPOBARIC NORMOXIA

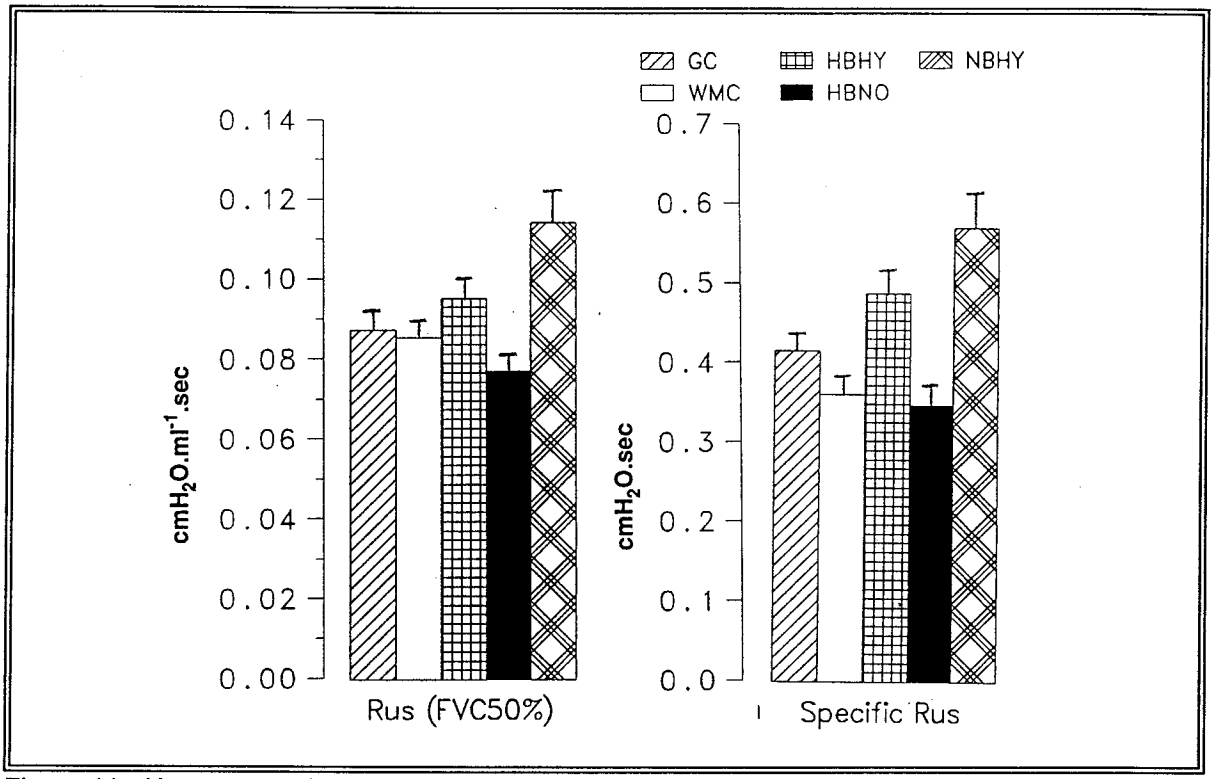


Figure 44. Upstream resistance (Rus) and specific upstream resistance (sRus) at FVC 50% in GC, WMC, HBHY, HBNO and NBHY rats following 3 weeks of exposure to different conditions.

The Rus, sRus, flow rates and the flow-volume relationship in hypobaric normoxic animals were similar to those in general controls (Figure 44, Figure 47, Figure 48). The Cst, sCst and the pressure-volume curve characteristics in hypobaric animals also remained unchanged (Figure 49, Figure 50). When comparison between hypobaric hypoxic and normobaric hypoxic rats was made to evaluate the effect of hypobaric pressure, $FEV_{0.1}$/FVC%, $FEF_{25-75\%}$, and PEFR were significantly higher in hypobaric hypoxic animals. No differences in pressure-volume characteristics, Cst and sCst were detected between the two groups.

3.3.2.3. EFFECT OF NORMOBARIC HYPOXIA

NBHY vs WMC: Compared to weight-matched controls, the Rus and sRus were significantly increased (Figure 44). The $FEV_{0.1}$ of normobaric hypoxic animals was equivalent to that in weight-matched control animals (Figure 45), but FEV_1/FVC% decreased significantly in normobaric hypoxic animals

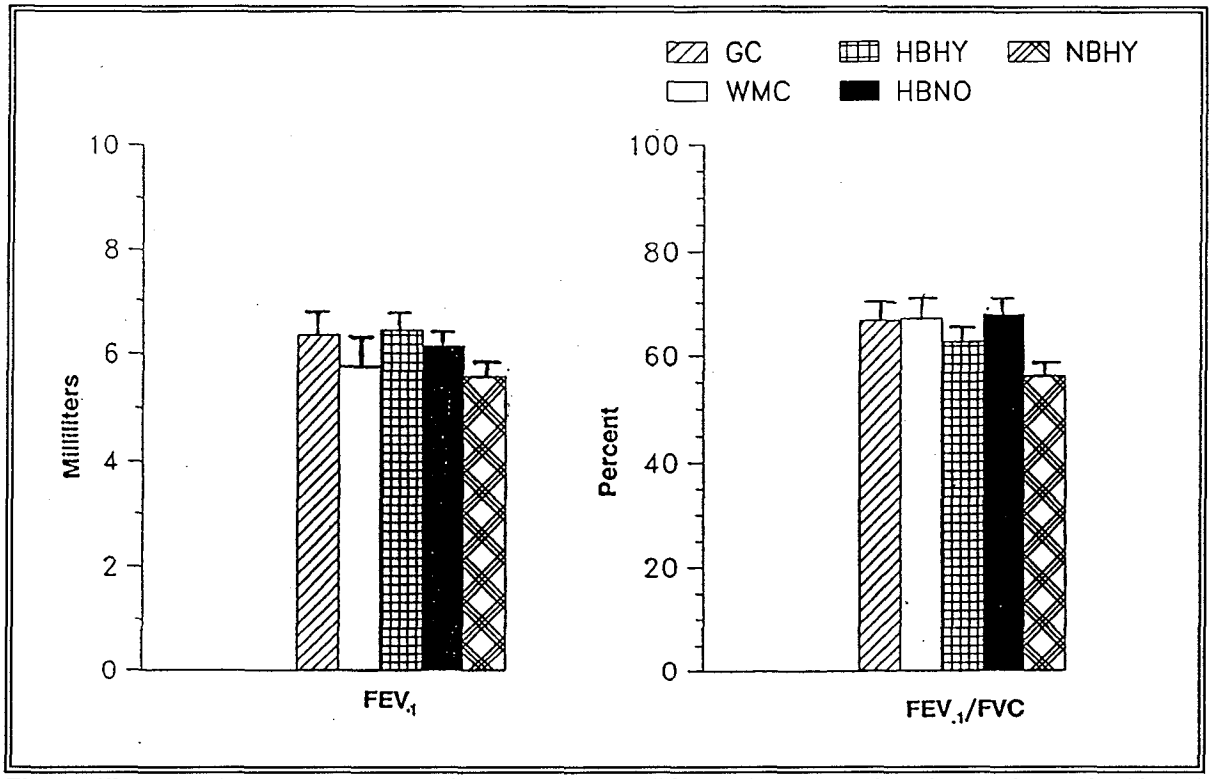


Figure 45. Forced expiratory volume in 0.1 second ($FEV_{.1}$) and percent of vital capacity expired in 0.1 second ($FEV_{0.1}$/FVC%) of rats following exposure to different experimental conditions.

(Figure 45). The peak expiratory flow rate and $FEF_{(25-75\%)}$ decreased significantly in normobaric hypoxic animals compared to those in weight-matched animals (Figure 46). Significant reductions in FEF at 50% and 70% of FVC and predicted FEF at 50-90% of FVC were observed in normobaric hypoxic rats compared to weight-matched control rats (Figure 47, Figure 48). No significant differences were found in the values of Cst and sCst between normobaric hypoxic animals (0.303 $\pm$0.013 ml.cmH_2O^{-1} and 0.0306 $\pm$0.0011 cmH_2O^{-1} respectively) and weight-matched controls (0.25 $\pm$0.02 ml.cmH_2O^{-1} and 0.0291 $\pm$0.0011 cmH_2O^{-1} respectively). Pressure-volume curves and the shape constant K of normobaric hypoxic rats also did not differ from those of weight-matched controls (Figure 49, Figure 50).

NBHY vs GC: Compared to general controls, Rus and sRus were significantly higher in normobaric hypoxic animals (Figure 44). $FEV_{0.1}$/FVC%, PEFR and $FEF_{25-75\%}$ were significantly lower in normobaric hypoxic animals than general controls (Figure 45, Figure 46). Absolute and corrected (for

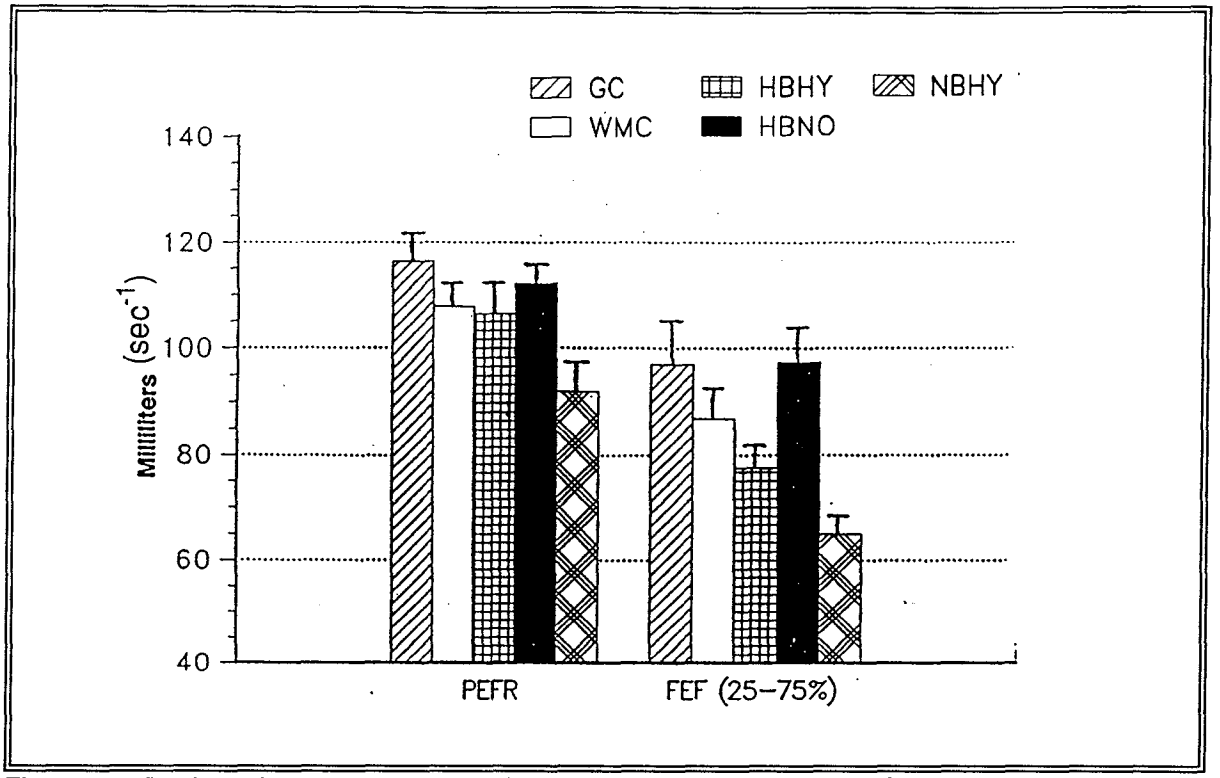


Figure 46. Peak expiratory flow rates and midexpiratory flow rates at $FVC_{75-25\%}$ in rats subjected to undernutrition, hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia.

FVC) forced expiratory flow rates also decreased significantly at 50-90% of FVC following exposure to normobaric hypoxia (Figure 47, Figure 48). Increases in Cst, sCst and the constant K in normobaric hypoxic animals (0.303 $\pm$0.013 ml.cmH_2O^{-1}, 0.0306 $\pm$0.0011 cmH_2O^{-1} and 0.209 $\pm$0.011 respectively) did not reach levels of significance compared to general controls (0.208 $\pm$0.014 ml.cmH_2O^{-1}, 0.0272 $\pm$0.0014 cmH_2O^{-1} and 0.18 $\pm$0.008 respectively) (Figure 49). However, transpulmonary pressure at 50-90% of lung volume (FRC to TLC) was significantly decreased compared to that in general control rats (Figure 50).

HBHY vs HBNO: To assess the effect of low oxygen, the hypobaric hypoxic and hypobaric normoxic animals were also compared. Rus was significantly higher (Figure 44) and $FEF_{25-75\%}$ was lower in hypobaric hypoxic animals than hypobaric normoxic animals (Figure 46). Hypobaric hypoxia also produced a significant reduction in expiratory flow rates corrected for FVC at 60-80% of FVC (Figure 48). No differences in the pressure-volume relationships between the two groups were noticed

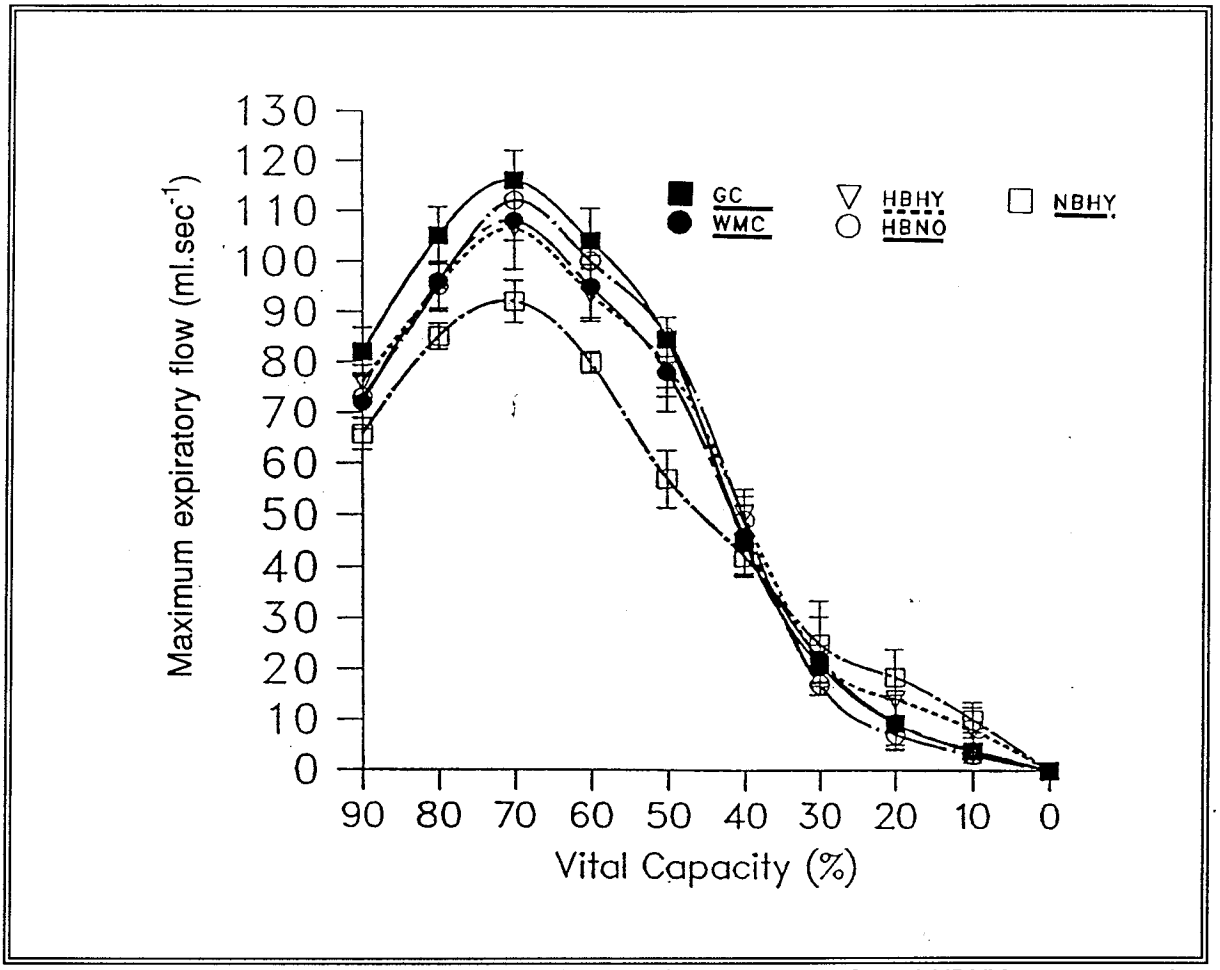


Figure 47. The flow-volume relationships in GC, WMC, HBHY, HBNO and NBHY rats exposed to different experimental conditions for 21 days.

(Figure 49, Figure 50).

3.3.2.4. EFFECT OF HYPOBARIC HYPOXIA

HBHY vs WMC: As shown in Figure 44, Rus (0.0953 $\pm$0.0062 $cmH_2O.ml^{-1}.S^{-1}$) in hypobaric hypoxic animals was not different from that in weight-matched controls (0.0857 $\pm$0.0041 $cmH_2O.ml^{-1}.S^{-1}$). However, sRus (0.489 $\pm$0.034 $cmH_2O.S^{-1}$) was significantly increased compared to weight-matched controls (0.362 $\pm$0.018 $cmH_2O.S^{-1}$). The changes occurred in FEV_1, FEV_1/FVC%, PEFR and FEF in hypobaric hypoxic animals during the three weeks of exposure were not significant (Figure 45, Figure 46, Figure 47). When the FEF were normalized for VC, it was significantly lower at 60-80%

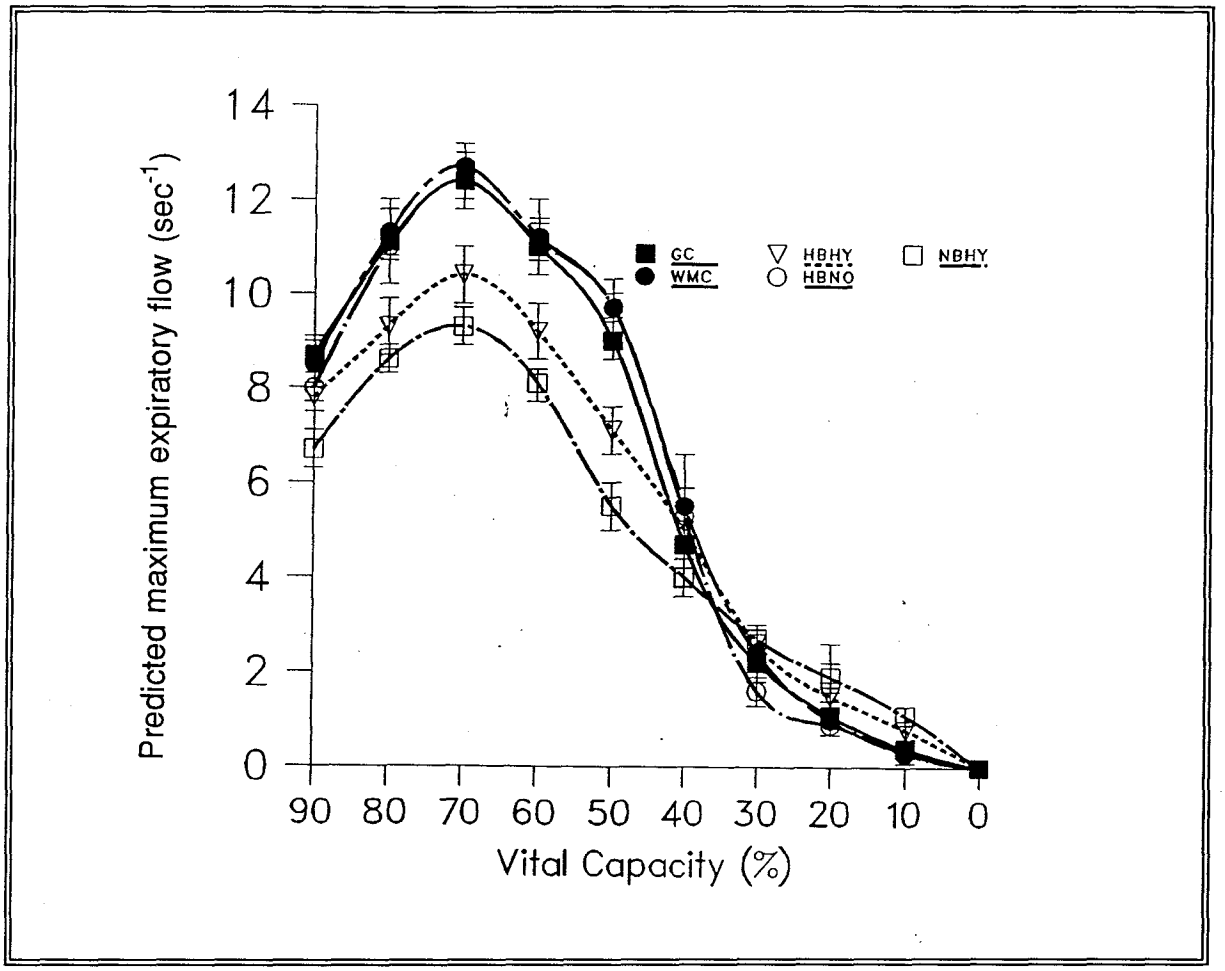


Figure 48. The flow-volume curves using normalized forced expiratory flow rates for vital capacity of animals exposed to hypobaric hypoxia, hypobaric normoxia, normobaric hypoxia and undernutrition for three weeks.

of FVC in hypobaric hypoxic rats compared to those found in weight-matched control rats (Figure 48).

HBHY vs GC: Compared with general control animals, sRus increased following exposure to hypobaric hypoxia (Figure 44). A significant reduction in $FEF_{25-75\%}$ was found in hypobaric hypoxic animals (Figure 46). Expiratory flow rates were also decreased but significant decreases were only detected when expiratory flow rates were corrected for FVC at 60-80% FVC (Figure 48).

As shown in Figure 49, the Cst and sCst were not effected by exposure to hypobaric hypoxic conditions. There was no change in the constant K value (Figure 49) or pressure-volume characteristics of rats exposed to hypobaric hypoxia compared to both weight-matched and general controls (Figure 50).

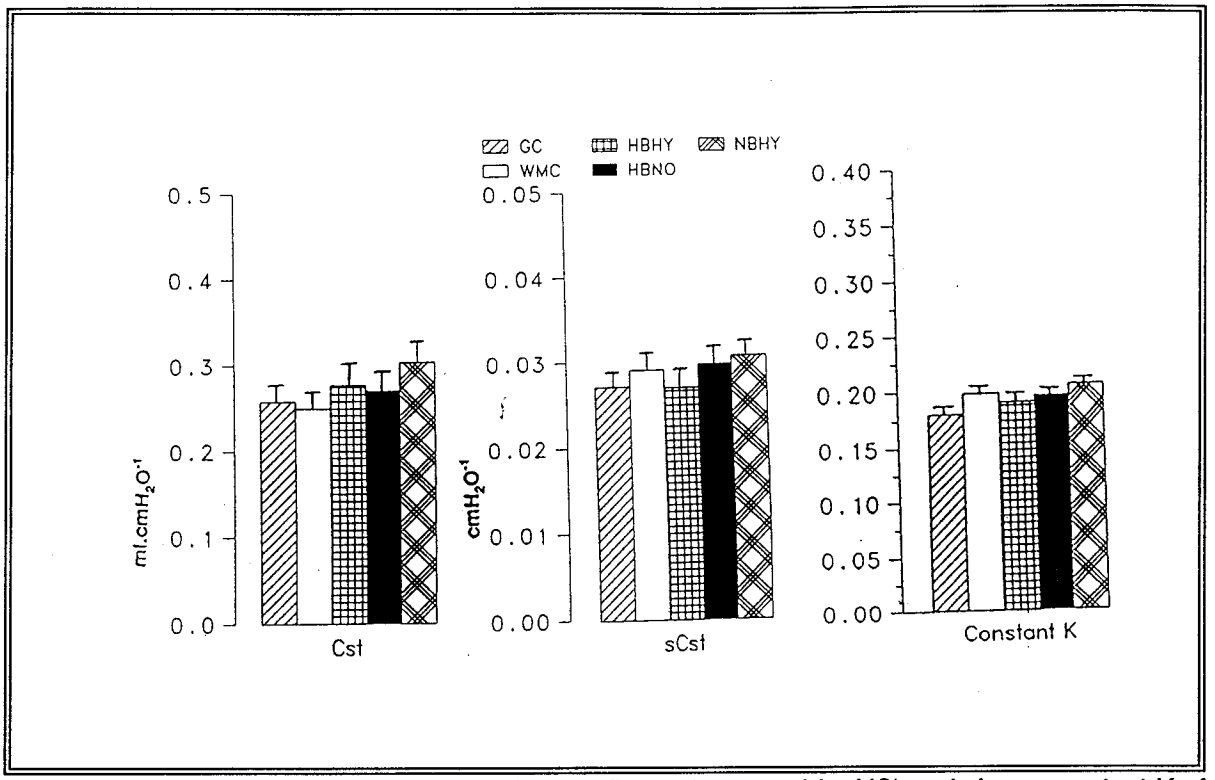


Figure 49. Static compliance, specific static compliance (corrected for VC) and shape constant K of P-V curves of GC, WMC, HBHY, HBNO and NBHY rats following 3 weeks of exposure to different conditions.

3.3.3. PRESSURE-VOLUME CURVE CHARACTERISTICS IN EXCISED LUNGS

Total lung capacity and static pressure-volume curves were recorded in the excised lungs. As shown in Table 14, the total lung capacity (TLC) increased by 114% between 4 weeks to 7 weeks of age. Compared to the baseline control group (BC), TLC in the normobaric hypoxic and hypobaric hypoxic group increased by 134% during three weeks of exposure and was not different from the general control group. On the other hand, the TLC gain in the weight-matched animals during three weeks was only 66%, which was significantly lower than that in the normobaric hypoxic and hypobaric hypoxic groups. The TLC in the hypobaric normoxic group was significantly smaller compared to the general control group. The values of specific TLC in general controls, weight-matched controls and hypobaric normoxic rats were similar and were significantly lower compared to the normobaric hypoxic and hypobaric hypoxic groups (Table 14). When TLC calculated per unit lung weight was compared,

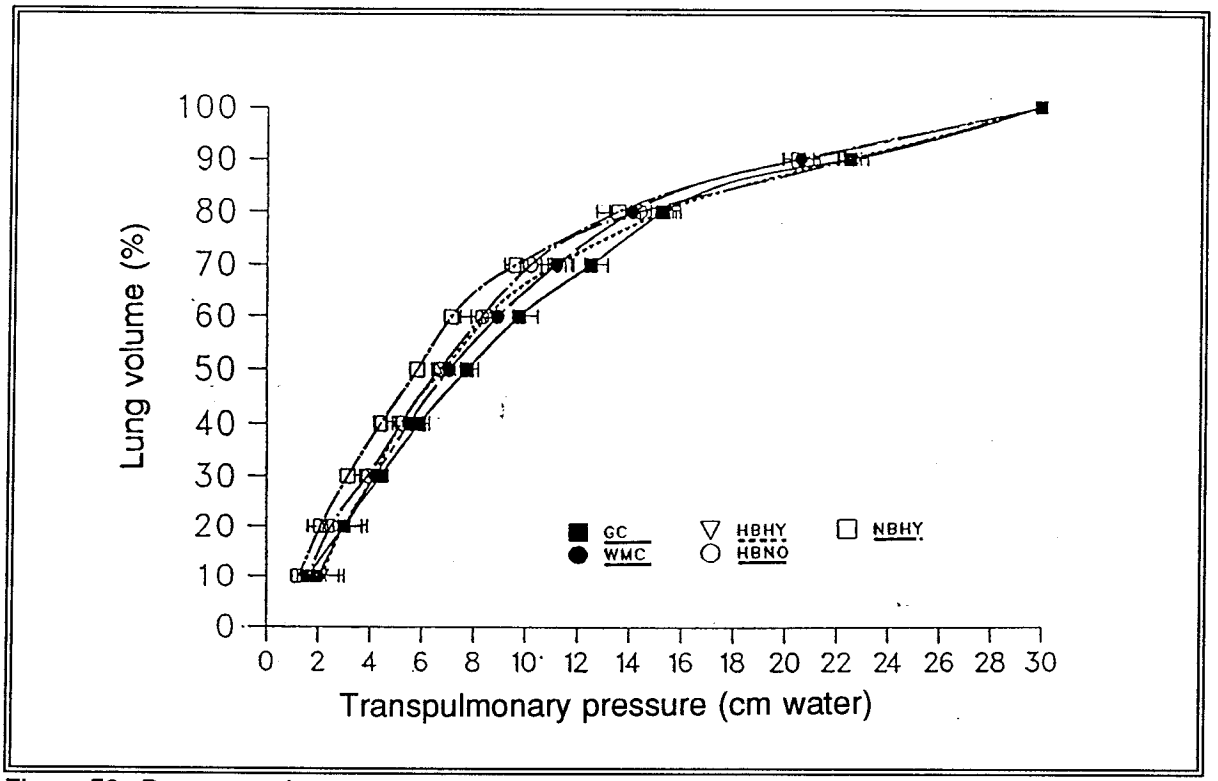


Figure 50. Pressure-volume curves recorded in intact animals which were subjected to undernutrition, hypobaric hypoxia, hypobaric normoxia and normobaric hypoxia for 21 days.

no differences were detected among the groups. The exponential shape constant K of pressure-volume curves also remained unchanged following exposure to the various experimental conditions (Table 14). As illustrated in Figure 51, the transpulmonary pressure was calculated at 10 percentiles of total lung capacity and pressure-volume curves were constructed and were similar in all groups suggesting that elastic lung recoil remained unaltered after subjecting animals to hypobaric normoxia, normobaric hypoxia, hypobaric hypoxia and undernutrition for three weeks.

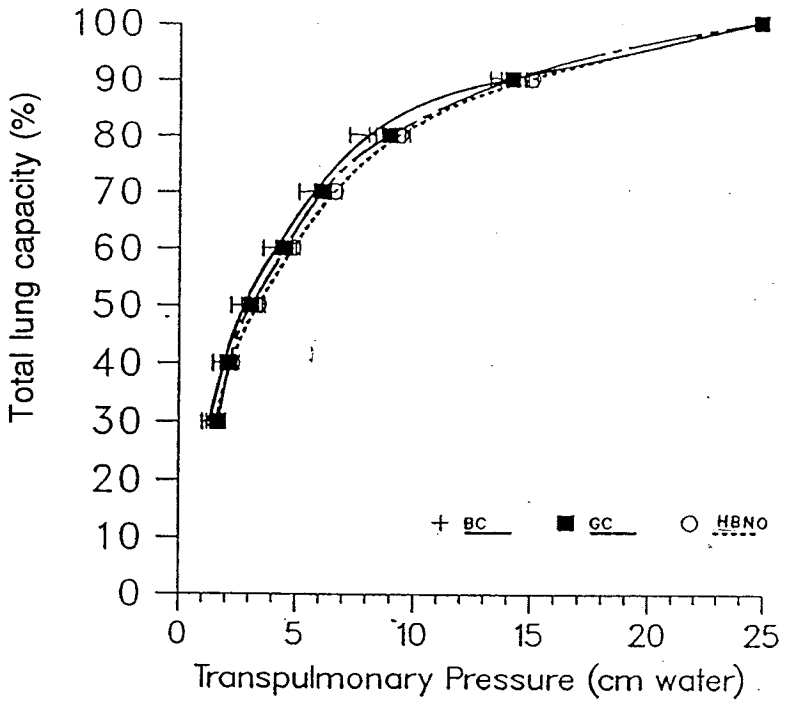
In summary, following exposure to normobaric hypoxia, $FEV_{0.1}$/FVC%, PEFR, $FEF_{(25-75\%)}$, and absolute and FVC corrected FEF at 50-90% of FVC were significantly reduced compared to weight-matched controls. In addition to above parameters, $FEV_{0.1}$ was also decreased in normobaric hypoxic animals compared to general controls. Upstream resistance and specific upstream resistance increased. Elastic lung recoil decreased at high lung volumes in normobaric hypoxic animals compared to general controls. The hypobaric hypoxic group showed similar changes in their flow-

Table 14. Alterations in total lung capacity and shape constant k after 3 weeks of exposure.

VARIABLE	BC	GC	WMC	HBNO	NBHY	HBHY
TLC (ml)	4.21 ± 0.23^{a}	9.01 ± 0.45^{b}	6.98 ± 0.45^{c}	7.27 ± 0.34^{c}	9.86 ± 0.43^{b}	9.85 ± 0.73^{b}
TLC/100 g Wb	5.00 ± 0.31^{a}	3.46 ± 0.20^{b}	3.47 ± 0.28^{b}	3.17 ± 0.13^{b}	4.85 ± 0.18^{a}	4.85 ± 0.35^{a}
TLC/g $W_L T$	6.99 ± 0.54^{a}	8.19 ± 0.50^{a}	7.37 ± 0.59^{a}	7.02 ± 0.30^{a}	7.47 ± 0.30^{a}	7.80 ± 0.67^{a}
Constant k	0.124 ± 0.005^{a}	0.124 ± 0.005^{a}	0.123 ± 0.006^{a}	0.123 ± 0.003^{a}	0.127 ± 0.007^{a}	0.119 ± 0.007^{a}

Means $\pm$sem's are shown and those without a common superscript in a horizontal row are significantly different at $p<0.05$.

Total lung capacity (TLC), Body weight (Wb), Lung weight ($W_L T$), Constant K (shape constant of pressure-volume curve)



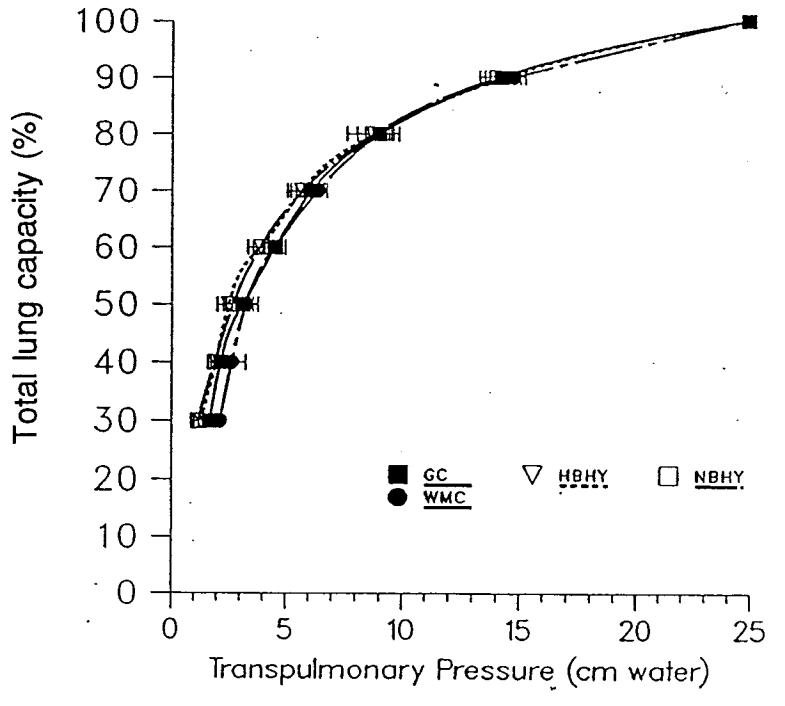


Figure 51. Pressure-volume curves of excised lungs of rats exposed to different experimental conditions for 21 days.

volume relationship as those found in the normobaric hypoxic rats but these changes did not reach levels of significance except for the $FEF_{25-75\%}$ and FEF at 60-80% of FVC corrected for FVC. In hypobaric normoxic or weight-matched control rats neither flow-volume relationships nor static pressure-volume characteristics showed any alteration suggesting that lung structure was not affect by low ambient pressure or undernutrition.

CHAPTER 4

DISCUSSION

4.1. GENERAL POINTS

The main emphasis of this research was to examine the process of acclimation to several of the changes associated with high altitude in growing animals, with particular interest in lung growth and development. Thus, we studied structural and functional aspects of adaptive lung growth to hypobaria, hypoxia and hypobaric hypoxia. The structural aspects were studied using biochemical and morphometric techniques. Functional adaptation of the lungs was assessed by pulmonary function tests. The adaptive lung growth was further studied using radiochemical and autoradiographic techniques after administration of a radiolabelled DNA precursor. Through such analysis, we attempted to quantify the cellular response in various compartments of the lung. The effects of hypobaria and hypoxia on food consumption and somatic growth were also studied. In addition, we evaluated the effect of hypobaria, hypoxia and hypobaric hypoxia on heart, spleen, liver and kidney growth, to see whether the effect of high altitude on growth was organ specific.

The results of organ growth are often normalized for body size in experiments which produce changes in somatic growth. With regards to body dimensions, however, no definitive parameter is available as an indicator of body size. An increase in body weight with time is a widely accepted measure of somatic growth, but body weight may also change because of altered fat and water metabolism without any associated change in skeletal dimensions or lean body mass. Some studies have used nose-tail, or tail and femur length as assessments of body size. Although a good correlation between stature and lung size exists (264), skeletal growth measurements may not provide accurate information of body growth and size in some circumstances. A study conducted by Hunter and Clegg (143) revealed that skeletal growth was non-uniform in rats exposed to 390 mm Hg as long bones grew relatively more than the axial skeleton. Similar findings have also been reported by

Acheson and MacIntyre (1). Thus, caution should be used while interpreting allometric results. To avoid complications of corrections for body size, we used weight-matched animals as appropriate reference controls for estimating the specific effects of hypoxia and hypobaria. This made it unnecessary to normalize our data for body weight. The deleterious effect of acute or chronic hypobaric hypoxia and normobaric hypoxia on somatic growth (31) has never been debated, but it raises an important question; does diminished somatic growth, equivalent to that expected to occur in hypobaric hypoxia, also cause identical reductions in growth of all body organs? In order to determine if lung growth in undernourished animals was different from that of control animals, we compared specific (per unit body weight) variables (i.e. lung weight, volume, alveolar surface area, total alveolar number) between the two control groups.

Even though it would have been interesting to examine adaptive changes in suckling rats, we chose not to use rats yet to be weaned for the following reasons: (1) It is difficult to effectively control body weight of individual suckling rats to use as weight-matched controls. (2) Diminished milk production in hypobaric hypoxic conditions has also been reported (185). (3) In addition, the mothers may secrete some growth-related factors in milk. We preferred to study lung growth in rats from 4 to 7 weeks of age as during this time a marked increase occurs in lung weight and lung volume (46), alveolar multiplication continues (141), and lung growth has been extensively studied in this age group (261).

4.2. FOOD CONSUMPTION AND SOMATIC GROWTH

A fall in daily food intake of hypobaric hypoxic and normobaric hypoxic rats was observed on the first day, but food intake began to increase after day 3. This may indicate that within 2 days of exposure the rats were able to adjust to the new environment. After day 3, food intake increased gradually but the amount of food consumed by both hypobaric and normobaric hypoxic rats remained lower than general controls. Similar observations have been made by other investigators (5, 62, 59, 92, 125, 237, 274).

At the end of three weeks exposure, the body weight gain relative to the total food intake {referred to as the efficiency of food utilization (total weight gain/total food consumed)} fell in rats exposed to normobaric hypoxia (0.411 ±0.008) and hypobaric hypoxia (0.392 ±0.005) relative to general controls (0.447 ±0.006). The efficiency of food utilization was also lower in hypobaric hypoxic and normobaric hypoxic animals compared to the weight-matched animals (0.430 ±0.012). Gloster and coworkers (114) also found that when the amount of food consumed by experimental animals was given to pair-fed adult male Sprague-Dawley rats, they were bigger than the rats exposed to 400 mm Hg for two weeks. These observations indicate that decreased oxygen availability not only decreases appetite and food intake, but also the efficiency of food utilization.

Carbohydrate and lipid metabolism may be impaired more than protein metabolism in hypobaria. Evidence for this was shown by Alippi and associates (5) as the efficiency of protein utilization in Wistar rats exposed to 342 mm Hg did not change for 23 days. Hochachka et al. (138) hypothesized that some amino acids may function as an anaerobic source of energy but on the other hand, oxygen is required for utilization of fats and carbohydrates (31). Barrie and Harris (15) found decreased glucose utilization in the hearts of guinea pigs kept at a simulated altitude of 5000 m for 28 days. Similar observations have been made in the liver (61). Furthermore, reduced gastrointestinal absorption of carbohydrates and fats has been found in humans following exposure to high altitude (36). Despite a lower food intake, the weight-matched controls gained the same weight as hypobaric hypoxic animals. Enforced food restriction results in meal eating (eating all food in one meal) which is known to cause lipogenesis (170, 259) rather than nibbling (eating small amounts of food throughout the day) which occurs in hypobaric hypoxic animals. This may be one of the reasons for lower efficiency of food utilization in hypoxic animals.

Previous studies have documented that chronic hypobaric hypoxic and normobaric hypoxic conditions lead to a decrease in body weight and this occurs in animals and humans of all age groups (5, 18, 45, 57, 77, 92, 267). It has often been postulated that at high altitude or in high altitude simulated conditions, low oxygen concentration is responsible for poor body weight gain. However,

no direct evidence is available. In the present experiment we observed that hypobaric hypoxic and normobaric hypoxic animals lost weight slightly on day 1 but they recovered by day 3 of exposure. Similar changes have been reported by Clegg and Harrison (62), who conclude that animals adapt to hypobaric hypoxic conditions after 24 hours. The initial body weight loss at high altitude or simulated conditions has been attributed to anorexia (106, 238, 274), food absorption (106), digestibility (59), disturbances in protein metabolism (154) and dehydration due to hypohydration, hyperventilation and other water loss (211).

Following 3 days of exposure, both normobaric hypoxic and hypobaric hypoxic rats showed a sign of acclimatization as they started to gain weight. Although the rate of weight gain increased progressively with time, it lagged behind general control rats, and thus the animals weighed less than general controls at the end of the exposure. These findings implied that even though the rats began to acclimatize to hypobaric and normobaric conditions earlier during the exposure, they did not adapt fully to the altered conditions. Although our findings are in agreement with other investigators (5, 18, 45, 57, 77, 92, 267), we cannot rule out the argument that further exposure might have resulted in complete somatic adaptation. The few studies which explored adaptation to extended exposure periods have reported conflicting results. It appeared that while rats kept at high altitude for 10 months failed to maintain a normal rate of growth (267), the rate of body growth of normobaric hypoxic guinea pigs remained unchanged for 14 weeks (164). The discrepancy may be related to biological variations between the species.

Nose-tail length is often used as a measure of skeletal growth. Concomitant with body weight decrease, a decrease in nose-tail length was noticed after 10 days of exposure to hypobaric or normobaric hypoxia. Thereafter the skeletal size of both hypobaric hypoxic and normobaric hypoxia groups remained stunted and was similar to that of the weight-matched group. This observation is in contrast to Cunningham and coworkers (77), who exposed rats to normobaric hypoxic conditions, but it is in agreement with Hunter and Clegg (143). They found that reduction in axial growth occurred within one week of exposure to hypobaric hypoxia.

Our results showed that skeletal growth was identical in both hypobaric hypoxia and normobaric hypoxia. Therefore, our observations demonstrated that low body weight gain and reduced skeletal growth leading to stunting of somatic growth occurred under both hypobaric hypoxia and normobaric hypoxia. The adverse effect of these conditions on body weight appeared immediately following exposure while skeletal retardation occurred slowly. These findings also indicated that reduced somatic growth in hypobaric hypoxia is mainly caused by decreased oxygen.

Animals exposed to hypobaric normoxia ate less food (7%) than general controls but this decrease was less compared to hypobaric hypoxia (24%). Low ambient pressure also appeared to affect the efficiency of food utilization because it was lower in hypobaric normoxic animals (0.418±0.005) compared to general controls (0.447±0.006). Similarly the hypobaric hypoxic animals had a lower efficiency of food utilization (0.392±0.005) than normobaric hypoxic animals (0.411±0.008).

Hypobaric normoxia slowed the rate of body weight gain and at the end of exposure hypobaric normoxic rats weighed less than general controls. Following the first week of exposure to hypobaric normoxia, skeletal growth was also stunted, but significant reduction in nose-tail length was apparent only after three weeks of exposure compared to the general control group. On the other hand, no differences were found in body weight and nose-tail length between hypobaric hypoxic and normobaric hypoxic animals. Studying the response of hypobaric pressure per se, Epstein and Saruta (98) found decreased body weight of 8 men kept for 9 days in hypobaric normoxia. Therefore, the findings of the present study implied that low ambient pressure itself interfered with somatic growth but its role in the presence of hypoxia (in hypobaric hypoxia) was not clear. It is possible that the effect of low ambient pressure was masked by a greater effect of low ambient oxygen in hypobaric hypoxia. Alternatively, decreased oxygen concentration and hypobaric pressure may act through the common mechanism.

4.3. ORGAN GROWTH ADAPTATION: OTHER THAN LUNG

We found that both hypobaric hypoxia and normobaric hypoxia stunted somatic growth.

Table 15. Changes in organ weights in weight-matched control (WMC), hypobaric hypoxic (HBHY), normobaric hypoxic (NBHY) and hypobaric normoxic (HBNO) animals after 21 days of exposure.

Organs	WMC	HBHY	NBHY	HBNO
A. Compared to general controls.				
Heart	⇓	⇑	⇑	⇒
Spleen	⇓	⇑	⇑	⇒
Hematocrit	⇒	⇑	⇑	⇑
Liver	⇓	⇓	⇓	⇒
Kidney	⇓	⇓	⇓	⇒
B. Compared to weight-matched controls.				
Heart		⇑	⇑	⇑
Spleen		⇑	⇑	⇑
Hematocrit		⇑	⇑	⇑
Liver		⇒	⇒	⇑
Kidney		⇒	⇒	⇑

Undernourishment equivalent to that observed in hypobaric hypoxia and normobaric hypoxia, produced liver, heart, spleen and kidney growth retardation in weight-matched rats. It was of interest that in undernourished animals, significant decrease in weight occurred in liver on day 1, kidney on day 3, heart on day 5 and spleen on day 14, and subsequently remained lower thereafter compared to general controls. Following diminished growth of all organs during the first week of food restriction, organ weights increased steadily thereafter but remained lower compared to animals fed ad libitum. It showed that a decrease in somatic growth, equivalent to that in hypobaric hypoxic or normobaric hypoxic animals, was associated with suppression of the growth rate of all the organs studied. However, the time course of the effect of food restriction on growth of organs was not identical. If we

consider day 1 values of general controls as a baseline, after 21 days heart, spleen, kidney and liver weights in undernourished animals increased 14%, 21%, 33% and 60% respectively while body weight increased 93%. This suggested that, not only was the effect of undernutrition on growth of body organs variable, organs such as the liver which showed an immediate effect also has a capacity to adapt and grow better than other organs. The variable effect of undernutrition on organ growth has been also reported by other investigators (191, 284). Winick and noble (284) also concluded that the effect of malnutrition is age dependent.

The weight of heart and spleen increased significantly by day 7 of hypobaric hypoxic exposure, and remained higher compared to weight-matched controls until the end of exposure. The absolute weights of heart and spleen also increased compared to general controls but significant differences were only found in hypobaric hypoxic animals on day 21. Although we exsanguinated the animals to reduce the amount of residual blood in the organs, a contribution of blood to the increase in the weight of spleen due to higher hematocrit in hypobaric hypoxia and normobaric hypoxia cannot be ruled out. Our heart and spleen growth observations are in agreement with those reported in previous studies (16, 55, 64, 93, 125, 148, 185, 191, 207, 223, 253, 267, 266). We have shown that the level of dietary deficiency equivalent to that in hypobaric hypoxia and normobaric hypoxia suppressed growth of heart and spleen. This provided evidence that heart and spleen growth in hypobaric hypoxia and normobaric hypoxia was greater when compared to the general control animals. Hunter and coworkers (145) have shown that tritiated thymidine (^{3}H-TdR) incorporation into DNA increased in the heart after one week when four week old rats were subjected to 390 mm Hg. In our study, no differences in heart and spleen growth response were found between hypobaric hypoxia and normobaric hypoxia. This suggested that heart and spleen growth adaptation in hypobaric hypoxia was primarily induced by reduced oxygen.

Contrary to heart and spleen growth, the weights of kidney and liver in rats kept in hypobaric hypoxia and normobaric hypoxia were reduced on day 3 of exposure compared to general controls and were equivalent to those of weight-matched animals. Thereafter, kidney and liver growth pattern

in hypobaric hypoxia and normobaric hypoxia were similar to that in undernourished rats. Results of our experiment clearly indicated that the effect of hypobaric hypoxia or normobaric hypoxia is organ specific. Regardless of hemodynamic changes which occur in hypobaric hypoxia or normobaric hypoxia, liver and kidney growth appeared to be influenced by undernourishment and followed the pattern of somatic growth. Our findings are in agreement with some investigators (57, 77, 64, 93, 125) but are in contrast with others (101, 185, 207) who found no difference or an increase in weights of liver and kidney. Reduced or no change in the rate of ^{3}H-TdR incorporation in kidney and liver after 9 days of exposure to hypobaric hypoxia has been reported (193). However, it has been pointed out that undernutrition and normobaric hypoxia appear to affect liver growth differently (191) in that normobaric hypoxia reduced cell number, whereas undernutrition produced a greater decrease in cytoplasmic mass than cell number. Therefore, an effect on liver growth in hypoxic conditions may not be purely alimentation dependent but could also be induced by a direct effect of hypoxia.

Hypobaric hypoxia and normobaric hypoxia are known to produce changes in hemodynamics such as increased hematopoietic activity (125), blood volume (31), hematocrit (31), heart rate and concomitant hypertension. It is conceivable that such alterations increase cardiac work load and in turn produce cardiac hypertrophy. Since the spleen is mainly a storage organ, the increased hematocrit in hypobaric hypoxia or normobaric hypoxia can increase spleen weight in these conditions. Tucker and Horvath (266) postulated that altitude-induced changes in organ weights were related to concomitant alterations in organ blood flow. They showed that blood flow increased in lungs, heart and spleen but was reduced in the kidney in hypobaric hypoxia. Besides the effects of hemodynamic alterations, decreased intracellular oxygen may also act directly to stimulate or suppress growth of organs. Hypoxia has been shown to induce cellular proliferation in culture but its affect does not appear to be cell specific (140). Therefore, the mechanism by which hypoxia stimulates organ growth remains elusive. The liver being the main organ involved in enzyme synthesis for carbohydrate, fat and protein metabolism has higher RNA/DNA than other tissues and is very sensitive to nutrient intake (234). Cheek and associates (57) suggested that hypoxia not only prevented DNA accumulation in

the liver but also reduced protein synthesis and interfered with RNA production. In another study, hypoxia reduced liver ATP by 40% (231). It is conceivable that liver growth reduction in the hypoxic environment may occur because of decreased food consumption.

Hypobaric normoxia did not show any striking changes in growth of either heart, spleen, liver or kidney. When results of hypobaric hypoxia and normobaric hypoxia were examined for the effect of hypobaric pressure, organ growth remained unchanged. These findings suggested that hypobaric pressure by itself or in the presence of hypoxia did not affect growth of the organs which were examined in the present study.

4.4. LUNG GROWTH ADAPTATION

Adaptive lung growth response will be discussed as follows. First, the effect of food restriction on lung growth will be discussed. The effect of hypobaric hypoxia will be assessed mainly by comparing it to weight-matched controls. Where appropriate, the results will also be compared to the general controls. Lung growth response may vary in decreased oxygen tension delivered by lowering the ambient pressure and by mixing the oxygen with nitrogen. Therefore, the effect of normobaric hypoxia will be evaluated both by comparing the normobaric hypoxic group to weight-matched and general controls, and by comparing the hypobaric hypoxic animals to hypobaric normoxic animals. Later, the role of low ambient pressure at high altitude in regulating the adaptive lung growth response will be discussed by comparing the hypobaric normoxic rats to general controls and hypobaric hypoxic rats to normobaric hypoxic rats. Finally, the residual effect of all conditions after a 3 day recovery period will be analyzed.

4.4.1. EFFECT OF UNDERNUTRITION

This study provided an opportunity to examine the effect on lung growth of the degree of undernutrition which was similar to that in hypobaric hypoxia. The effect of food deprivation on lung growth was assessed by comparing the weight-matched control group with the general control group

which was given free access to food.

During 4-7 weeks of age, lung weight and lung volume increased less in undernourished animals (57% and 100% respectively) compared to general controls (87% and 145% respectively). The absolute amounts of lung DNA, RNA, total protein, hydroxyproline and desmosine increased less during 4-7 weeks of age in undernourished rats (36%, 20%, 41%, 80% and 69% respectively) compared to general controls (57%, 69%, 73%, 120% and 92% respectively). Increases in absolute alveolar surface area and total alveolar number during the experimental period were also lower in weight-matched controls (92% and 52% respectively) compared to general controls (123% and 70% respectively). Undernutrition suppressed the rate of DNA synthesis in the lung. This was further confirmed by autoradiographic results as ^{3}H-TdR incorporation fell following diet restriction and all the main cell types in the lung tissue were equally affected. Although total lung capacity measured in vivo in undernourished rats decreased, lung function remained unaffected.

DNA is confined almost entirely to cell nuclei, and its amount within the diploid cell of any species is constant (96). A reduction in DNA content in undernourished rats indicated that the number of cells in the lung decreased at day 21. This occurred as a result of decreased DNA synthesis. ^{3}H-TdR incorporation into DNA dropped 50% on day one and continued to fall until day 7 before showing a small increase. Autoradiography results also showed that except for cells which normally undergo minimal proliferation (mesothelial cells, free alveolar macrophages and mast cells), 3H-TdR into other cell types (type II pneumonocytes, endothelial cells, interstitial cells and bronchial epithelial cells) fell on day one and continued to fall until 5-7 days of food restriction. This may imply that even though body weight began to increase by day 3 of food restriction, the effect of undernutrition on lung growth was prolonged. A decrease in DNA occurs due to interference with cell division which is time dependent. DNA did not appear to be affected during the early period of food deprivation and did not decrease significantly until day 14. Since lung weight was reduced, cell density (DNA relative to per unit dry lung weight) increased during the earlier period but it approached the control level at the end of the experiment. This may have occurred due to greater decrease in other parameters of the lung

(see below). We did not starve our rats, but similar observations have been reported in food deprived rats (118, 228, 229).

The rate of protein synthesis in the lungs of undernourished rats appeared to slow down as total RNA and soluble protein content fell on day 3 and reached their lowest level on day 7 and then slowly increased but never reached the control levels. As mentioned earlier, DNA content did not decrease significantly until day 14, the RNA/DNA and protein/DNA ratios dropped gradually until day 7 and remained consistently lower than general controls. No changes were found in dry/wet weight ratio of the lung in undernourished animals. It appeared that reduced lung growth in undernourished rats did not occur due to variable hydration but due to reduction in the normal increases in cellular cytoplasmic constituents. During the first week, lack of lung growth resulted primarily from diminished cell size and later from both hypoplasia and decreased cell size. Our findings with regards to decreased cell size in response to undernourishment are consistent with those reported by other investigators (118, 195, 228, 284).

Previous studies have shown significant reduction in collagen and elastin in undernourished and starved animals (195, 228). In the present study, there was no difference in soluble and insoluble fractions of hydroxyproline. Since the collagen equilibrium remained undisturbed, a reduction in the amount of total hydroxyproline may have occurred because of diminished collagen synthesis. The amount of desmosine did not change in undernourished animals compared to general controls. Using weanling rats, Myers and associates (189) studied the influence of restricted food. Although the extent of food deprivation was more severe than ours, the results for collagen and elastin were similar. It has been documented that the bulk of elastin is synthesized during the first few weeks of postnatal life in rats and later reaches a plateau, whereas collagen synthesis extends well into adulthood (90, 190). Moreover, lung elastin turnover is slow (90). Neither hydroxyproline nor desmosine concentration changed due to undernutrition indicating that the integrity of lung tissue structure was not disrupted. This was further supported by hydroxyproline/DNA, desmosine/DNA and desmosine/hydroxyproline ratios as they also remain unchanged.

Morphometric results showed that a trend towards smaller size and increased number of structural units per unit lung volume was apparent in undernourished animals compared to general controls. Although undernourished animals had decreased alveolar surface area and fewer alveoli, both parameters increased (92% and 52% respectively) between 4-7 weeks of age. These findings suggested that alveolar multiplication continued at a reduced rate in undernourished animals, but the unit structures in the lung were smaller than the general controls. This may have occurred due to decreased cell number (reduced cellular proliferation) and cell size. With regards to morphometric assessment, our results were partly in agreement with those observed by other investigators (195, 177, 226, 227) but we did not find emphysematous changes in the lung due to starvation (226, 227).

Volume per unit lung weight did not change. This indicated that food deprivation did not alter the distensibility of the lung. This was further substantiated by the results obtained from the excised and in vivo static pressure-volume curve characteristics and the flow-volume relationship of the lung, as those remain unchanged. In contrast, loss of elastic lung recoil has been reported while studying the effect of starvation (229) or a low protein diet (177). The undernourished rats in the present study did not lose weight but the rate of weight gain was slower than controls. Therefore, physiological assessment further supported the evidence obtained from biochemical measurements that, in the present investigation, undernutrition did not alter the structure of the lung. It was interesting to observe that the degree of inanition in hypobaric hypoxia in the present study did not alter normal lung structure, but rather offset the rate of normal lung growth.

In previous studies, the effect of decreased food consumption leading to somatic and lung growth retardation following exposure to normobaric hypoxia, hypobaric hypoxia or high altitude has either been ignored or an attempt has been made to correct lung growth relative to somatic growth. Using weight-matched control animals to evaluate the influence of ambient environment on lung growth, we avoided introduction of an assumption of linear correlation of lung growth to body size. Body weight is often considered as a reference variable for body size to compare lung growth within or between species. Although it was not necessary in the present study to normalize lung growth

parameters to that of body weight, we did so to explore whether a relationship between lung and somatic growth is maintained in conditions where food consumption is compromised. We found that all specific (relative to per unit body weight) lung growth variables were higher in undernourished rats compared to the general control rats, but only the increases in specific lung weight, DNA, alveolar surface area and total alveolar number reached significant levels. This is not unexpected since it has been shown that with increasing age and increasing body weight, specific lung parameters decrease (240). There is no definitive explanation for this disturbance in somatic and lung growth due to undernourishment. One may argue that body growth was more affected by inanition or that lung growth was less compromised. It was evident from the results that while body weight decreased on day one, significant decreases in lung weight and absolute lung DNA were not found until days 7 and 14 respectively. This showed that a divergence in the normal relationship between lung growth and body size occurred during the early period of food restriction. This also may have occurred due to relatively changed water content or decreased fat deposits in the body. The effect of undernutrition may be time dependent. Short term food deprivation may result from decreased body water content and depletion of fat depots while prolonged undernutrition may also decrease lean body mass. Whatever the reason, it was evident from our findings that body weight may not be an appropriate representation of body size and normalization of lung growth variables to body weight may lead to misinterpretation of results. On the other hand, the observations made in undernourished animals imply that conditions which diminish somatic growth also have associated reduction in growth of body organs and such reductions should be accounted for when conclusions are drawn for organ specific response.

4.4.2. EFFECT OF HYPOBARIC HYPOXIA

Decreased food consumption and diminished somatic growth are common findings following translocation to high altitude or exposure to hypobaric hypoxia. However, lung growth adaptation has never been compared to animals which have been subjected to a similar degree of undernutrition.

The effect of decreased food intake on somatic and lung growth in hypobaric hypoxia can be debated and may not be exactly similar to animals subjected to undernutrition at room air, but weight-matched controls are probably better reference than general controls. We compared our results of hypobaric hypoxic animals to both weight-matched animals and general control animals.

With regards to adaptive lung growth to chronic hypobaric hypoxia for three weeks, compared to weight-matched animals, lung weight (40%), lung volume (32%), DNA (30%), RNA (58%), total soluble protein (60%), hydroxyproline (40%), desmosine (97%), alveolar surface area (28%) and total alveolar number (21%) increased significantly. The mean linear intercept of airspaces and number of alveoli/unit volume remained unchanged, but mean chord length of alveoli and the volume fraction of alveolar air increased in hypobaric hypoxic animals compared to weight matched controls. The cytokinetic study revealed that biochemically analyzed ^{3}H-TdR incorporation showed a sharp increase on day 3 of exposure and gradually declined thereafter but remained higher than weight-matched controls during the rest of the exposure. Analysis of autoradiographs confirmed the above findings as alveolar wall cell ^{3}H-TdR incorporation peaked on day 3 of exposure. The results of pulmonary function tests showed that FRC, RV, VC and TLC increased significantly (21%, 18%, 21% and 20% respectively) in animals exposed to hypobaric hypoxia for three weeks compared to weight-matched controls. Except for a significant increase in specific airway resistance and a decrease in forced expiratory flow rates corrected for forced vital capacity (FRC), no other changes in pulmonary function tests were found in hypobaric hypoxic animals.

Absolute variables of lung growth in hypobaric hypoxic animals were also significantly increased compared to general controls but the percentage changes were lower than those found by comparing the hypobaric hypoxic and weight-matched control groups. Compared to general controls, exposure to hypobaric hypoxia produced significant increases in lung weight (17%), lung volume (13%), DNA (13%), RNA (12%), total soluble protein (30%), hydroxyproline (15%), desmosine (73%) and alveolar surface area (10%). Except for a significant increase in mean chord length of alveoli, no other morphometric parameter changed in hypobaric hypoxia. Alveolar surface area (11%) increased

Table 16. Morphometric changes in weight-matched control (WMC), hypobaric hypoxic (HBHY), normobaric hypoxic (NBHY) and hypobaric normoxic (HBNO) animals after 21 days of exposure to different conditions.

Variables	WMC	HBHY	NBHY	HBNO
A. Compared to general controls.				
V_L	⇓	⇑	⇑	⇓
MLI	⇒	⇒	⇑	⇓
I_{alv}	⇒	⇑	⇑	⇒
$Valv_{ave}$	⇒	⇒	⇑	⇓
N_V	⇒	⇒	⇓	⇑
N_{at}	⇓	⇒	⇒	⇒
S_W	⇓	⇑	⇒	⇒
B. Compared to weight-matched controls				
V_L		⇑	⇑	⇑
MLI		⇒	⇑	⇓
I_{alv}		⇑	⇑	⇒
$Valv_{ave}$		⇑	⇑	⇒
N_V		⇒	⇓	⇑
N_{at}		⇑	⇑	⇑
S_W		⇑	⇑	⇑

V_L (lung volume), MLI (mean linear intercept), I_{alv} (mean chord length of alveoli), $Valv_{ave}$ (average alveolar volume), N_V (number of alveoli/unit volume), N_{at} (total number of alveoli), S_W (alveolar surface area.

significantly in hypobaric hypoxic animals but the increase in total alveolar number (8%) did not reach a significant level. FRC and TLC in intact hypobaric hypoxic animals increased. Expiratory flow rates corrected for FVC (60-80% FVC) decreased and specific airway resistance increased in animals following exposure to hypobaric hypoxia for three weeks. Some investigators have observed

increases in absolute lung weight and volume in hypobaric hypoxia (20, 218) whereas others have found increases only in specific lung weight and volume in animals raised at (258) or translocated to (45) high altitude, or exposed to simulated high altitude (136, 219).

An increase in lung growth in terms of lung weight may be brought about by hyperplastic or hypertrophic changes in lung tissue, accumulation of intercellular connective tissue matrix in parenchyma or non-parenchyma, extracellular or intracellular water content, and blood volume in the pulmonary vasculature. Bartlett and Remmers (20) observed that after 7 days of exposure to hypobaric hypoxia, the non-blood lung water increased to 35%, compared to 20% after 21 days of exposure. In the present study, a trend of decreased dry/wet weight ratio of the lung was apparent only during the first week of exposure but did not reach a significant level. This ruled out the possibility that water accumulation was responsible for an increase in lung weight after three weeks of exposure. In our study, we observed an increase in hematocrit which is one of the commonly observed adaptive changes that occur at high altitude (31). Increases in central blood volume in high altitude natives (184), and in central and pulmonary blood volume in lowlanders who have resided at high altitude for prolonged periods (224) have been reported. It is conceivable that an increase in blood volume contributed to the observed increase in lung weight of hypobaric hypoxic animals.

The animals in this experiment were exsanguinated prior to the removal of lungs to minimize the contribution of residual pulmonary blood volume in lung weight measurements. In this study, the lungs were not flushed free of blood for two reasons. Firstly, a recent study (88) has revealed that exsanguination reduces the residual blood volume in the lungs to 15% and further flushing with saline decreased the contribution of blood volume ranging from 5-9%. This suggested that flushing cannot completely remove the blood as it may remain sequestered in certain areas of lung. In addition, flushing also results in a variable amount of fluid retention in the pulmonary vasculature which may also alter wet lung weight measurements. Secondly, the excised lungs were also used for pressure-volume curves and total lung capacity measurements before using them for biochemical and morphometric estimations. Flushing may also alter mechanics of the lung as fluid may leak into the

airspaces.

If the contribution of blood volume to lung weight is considered to be substantial, then an increase in lung weight could be misinterpreted. If that is so, then the contribution of increased hematocrit can be corrected for. Hematocrit in the present study increased by 50% following exposure to hypobaric hypoxia. In that case, the contribution of residual blood volume following exsanguination to lung weight will be 22% instead of 15%. However, lung weight increased by 40% and 17% compared to those of the weight-matched controls and general controls. If a 7% of an increase in lung weight is attributed to the hematocrit increase, lung weights were still higher than weight-matched and general controls. Furthermore, an increase in hematocrit at high altitude is in part, due to decreased plasma volume (4, 252). In addition, Bartlett and Remmers (20) did not find any change in residual lung blood volume in rats sacrificed by exsanguination from the abdominal aorta following three weeks of exposure to hypobaric hypoxia. It seems unlikely that increased hematocrit, in the range observed in the present studies would contribute significantly to the observed lung weight increase. Thus, an increase in lung weight may be presumed as a true change in tissue weight that occurred in hypobaric hypoxic conditions.

4.4.2.1. STRUCTURAL ADAPTATIONS

Alterations that occur in freeze-dried lung weight most likely represent changes in the number and/or size of cells and/or contributions of intercellular connective tissue proteins. From biochemical measurements in the lung, cell size is often determined by calculating the RNA/DNA and protein/DNA ratios (225, 261, 283). The use of protein/DNA ratio as a measure of cell size may not be an accurate one as the amount of protein includes both cellular and extracellular proteins such as blood and newly synthesized intra- and extra-cellular connective tissue proteins. Thus, the extracellular protein contribution may complicate the interpretations of protein/DNA ratio as an indicator of cell size. The RNA/DNA ratio, on the other hand, may provide a more precise index of cell size as it is not affected by extracellular factors.

DNA synthetic activity fell on day one of hypobaric hypoxic exposure and reached the level of weight-matched controls and this coincided with a decrease in body weight. A sudden change in ambient environment may have produced this adverse effect while animals were struggling to adapt to new conditions. On day three, ^{3}H-TdR incorporation in hypobaric hypoxic rats increased sharply and was twelve fold higher than weight-matched controls. Following a DNA synthesis peak on day 3, the difference on day 5 increased to seventeen fold (because the rate of DNA synthesis in weight-matched group fell further) and then fell to eight fold on day 7. Thereafter, it dropped gradually to reach the general control level but remained higher than that in weight-matched controls. These observations indicated that a brief depression in DNA synthesis in hypobaric hypoxic rats was followed by maximum lung growth stimulation and the bulk of the cellular adaptive response was completed within the first week. It was noteworthy that the lungs showed a remarkable potential to adapt to hypobaric hypoxic stress in the presence of the secondary stress of undernutrition which diminished DNA synthesis. Although DNA synthesis fell after an initial surge, continued stimulation of lung growth was evident as the rate of ^{3}H-TdR incorporation remained high during the rest of the exposure. However, an increase in synthetic activity of DNA does not reflect accelerated cellular multiplication to produce greater number of cells. Cells may go through the S-phase of the cell generation cycle and not enter into the G_2 and M-phase (mitotic period), and result in multinucleated cells or increased ploidy. Although decreased oxygen tension induces mitochondrial proliferation, mitochondrial DNA accounts for less than 1% of the total DNA (119). Despite exsanguination of animals prior to sacrifice, accumulation and margination of leukocytes and their contribution to ^{3}H-TdR incorporation in the lung of hypobaric hypoxic animals cannot be ruled out. In spite of the early elevation of DNA synthesis, the amount of lung DNA did not increase significantly until day 7. This lag between maximum DNA synthesis and an increase in the amount of DNA could be expected. An increase in DNA is dependent upon the duration of the cell cycle which may vary in various cell types in the lung. In the present study, increased ^{3}H-TdR incorporation and concomitantly higher amount of DNA compared to general controls suggested that chronic hypobaric hypoxia not only protected lung growth at the

normal level but induced a hyperplastic response.

In our study, maximum ^{3}H-TdR incorporation occurred on day 3 of exposure to hypobaric hypoxia which is in conflict with the results of Voelkel and associates (271) who found that cellular proliferation peaked (345% increase) on day 9 of exposure to hypobaric hypoxia. They (271) studied in vitro ^{3}H-TdR incorporation in the middle lobe of right lung slices in adult female Wistar rats previously exposed for 3, 6, 9, 12, 20 and 35 days to hypobaric hypoxia compared with the results extrapolated from 3, 12 and 35 day controls. The authors suggested that DNA synthesis may be the terminal event in a chain of other metabolic processes that result in a lag between the hypoxic stimulus and DNA synthesis. Later, in the same laboratory, using similar conditions, age, sex and strain of rats, Niedenzu and coworkers (193) found only an 85% increase in DNA synthesis on day 9 after injecting ^{3}H-TdR intraperitoneally.

The discrepancy between the above studies and our's regarding timing of maximal DNA synthesis may be due to various reasons. The route of injection of radioactive tracer is of importance (208). Oral administration of labelled thymidine leads to incorporation into DNA but is only about 20% of that found following intravenous injection. In our laboratory, we observed that DNA incorporation of ^{3}H-TdR following intraperitoneal injection was less than half of that obtained by intravenous route and the variation was also greater. On the other hand, one can argue against in vitro radioactive tracer incorporation because physiological conditions are disturbed. Baserga (22) reported that 80% cells in vitro can be stimulated to synthesize DNA by changing the culture medium. According to Niedenzu and coworkers (193), higher specific DNA activities are obtained in lungs if the in vitro method is used. However, Voelkel and coworkers also used approximately 20 times as much ^{3}H-TdR as used by Niedenzu and associates. We used young growing male animals and it has been demonstrated by Cunningham and associates that the adaptive response to hypoxia is not similar in young and adult animals (77). It has been reported that males and females of the same species show a different hemodynamic adaptive response to hypoxic conditions (219), thus lung growth adaptation to hypobaric hypoxic stress may also be variable in the two sexes. Lung growth is increased following

pneumonectomy and recently we have found that compensatory lung growth is also different in male and female rats (240).

From our results, it is evident that hypobaric hypoxia increased production of RNA preceding and during the phase of maximal DNA synthetic activity. The amount of RNA increased slightly on day one of exposure when DNA synthesis was depressed. The amount of RNA increased significantly on day 3 and then continued to be higher than both weight-matched and general controls. Therefore, maximal increase in RNA and the relatively smaller increase in the amount of total DNA produced a marked increase in RNA/DNA ratio during the first week of exposure. Low oxygen concentrations produce an increase in ^{3}H-uridine incorporation into RNA 6-8 hours prior to ^{3}H-TdR incorporation into nuclear DNA and incorporation of radiolabelled amino acids into proteins in cultured chick embryo heart cells (139). The authors suggested that this occurred as a result of a nuclear transcriptional process rather than a cytoplasmic event. Similar phenomena may have taken place in our hypobaric hypoxic animals The total amount of RNA increased on day 3 but the amount of DNA did not increase until day 7 compared to general controls. This provided evidence that the initial adaptive lung growth response occurred by cellular hypertrophy which was followed by hyperplasia. An increase in the RNA/DNA ratio during the early exposure period may have accelerated protein synthesis. Increased protein production was apparent as the amount of total protein also increased on day 3 and continued until the end of exposure. This may also explain why enhanced lung growth continued long after ^{3}H-TdR incorporation declined and may in part have occurred by cellular hypertrophy. A pattern of cellular response to hypobaric hypoxia was suggested from these findings. Initially the hypobaric hypoxic stimulus triggered RNA production which was followed by DNA synthesis. Once adequate cellular adaptation was achieved, DNA synthesis fell but cellular hyperactivity continued to maintain elevated lung growth which largely occurs by protein accumulation. Ultrastructural studies have also shown an increase in cell size in the lungs of mice at high altitude (204). According to Winick and Noble (283), tissues that are actively engaged in protein synthesis are rich in RNA and a high RNA/DNA ratio is reached long before the high protein/DNA ratios are reached in the same tissue.

High RNA/DNA ratios have also been shown in tissue subjected to stress such as cardiac hypertrophy which indicate the capacity to produce more RNA per cell (115). It is interesting to note that even in the presence of undernutrition when lung growth is retarded and cell size in the lung is decreased, hypobaric hypoxia causes an increase in lung growth by enhancing cell replication and increasing cell size. One may conclude that lung growth in hypobaric hypoxia appears to be different from that which occurs in undernourished animals. It not only makes up for the lung growth that may occur in undernourishment but also results in an increase in absolute terms.

Connective tissue proteins, particularly collagen, are heterogeneous in type and anatomical localization. After three weeks of hypobaric hypoxic exposure, increased amounts of hydroxyproline and desmosine indicated accumulation of connective tissue proteins which would probably occur as a result of shift in the protein synthesizing machinery towards more collagen and elastin production. We did not measure hydroxyproline during the exposure. The soluble/insoluble hydroxyproline ratio did not differ from general or weight-matched controls on day 21 of exposure. One may conclude that a dynamic equilibrium in collagen metabolism was achieved by three weeks of exposure or it remained undisturbed in hypobaric hypoxia. In pneumonectomized rats DNA synthesis peaks after 3-5 days (38, 53) but collagen synthesis does not increase until the second week (72). This may suggest that connective tissue protein production occurs after cell replication. Similar connective tissue protein changes may have occurred in hypobaric hypoxic animals.

Interestingly, the concentration of hydroxyproline decreased following exposure to hypobaric hypoxia compared to the general control group. This may suggest that collagen production lagged behind lung growth. A similar decrease in hydroxyproline concentration in the right ventricle of the heart has been reported in rats exposed to simulated high altitude (175). The hydroxylation of proline to hydroxyproline is an aerobic reaction in vitro (60). It has further been shown that the oxygen incorporated into the hydroxyl group originates from atmospheric oxygen rather than from water. Thus, the inspired oxygen may be sufficiently reduced in our animals to interfere with this reaction. The effect of undernutrition in hypobaric hypoxic conditions cannot be ruled out. It has been reported

that food deprivation reduces hydroxyproline and lysyl oxidase activity (a cross-linking enzyme for collagen and elastin) (181). Therefore, the effect of undernutrition, decreased hydroxylation of proline due to low oxygen and increased non-connective tissue proteins in hypobaric hypoxia may have caused a decrease in hydroxyproline concentration.

Unlike collagen, elastin per unit dry weight of the lung increased. It was apparent from our results that elastin increased relatively more than collagen in hypobaric hypoxia. The amount of hydroxyproline and elastin relative to DNA content increased compared to weight-matched controls suggesting that structural changes in the lung tissue may have occurred following exposure to hypobaric hypoxia. However, compared to general controls only the desmosine/DNA ratio increased. The possible reasons for such an imbalance in elastin and collagen will be discussed later. It is tedious to estimate collagen and elastin in various components of the lung, but it has been shown histologically that elastic tissue increases in the walls of small blood vessels in hypobaric hypoxia (69, 145, 182). It is possible that the bulk of elastin accumulation may have occurred in vessel walls. Although we do not know the location of enhanced collagen and elastin accumulation in the lung tissue, such alterations in connective tissue dynamics could be expected to cause functional and structural changes in the lung.

Collagen and particularly elastin which are major components of the extracellular matrix in the lung are thought to have a critical role in defining lung structure, especially in the process of alveolarization. The morphometric analysis showed that hypobaric hypoxia increased the volume fraction and absolute volume of alveolar air compared to those of weight-matched controls. The absolute volume of alveolar air also increased compared to general controls. Increases in the volume fraction and the volume of alveolar air may occur if more alveolar units are added, or the alveoli have enlarged with or without alveolar multiplication. On the other hand, the volume fraction of parenchymal air (alveolar air + alveolar duct air) remained unchanged suggesting that rearrangement within the parenchymal structures took place as the volume fraction of alveolar duct air decreased and alveolar air increased. This remodelling of respiratory units may result from the conversion of former alveolar

ducts to bronchioles by a distal extension of bronchiolar epithelium (see below: the frequency of labelled epithelial cells increased in terminal bronchioles), or alveolarization of terminal bronchioles. From our data it is uncertain which mechanism could have operated during the exposure to hypobaric hypoxia. The absolute volume of alveolar wall increased compared to both weight-matched and general controls. This may occur as a result of alveolar multiplication or thickening of the alveolar walls. However, the volume fraction of alveolar wall remained unchanged, indicating that thickening of alveolar wall was unlikely. We cannot rule out the possibility that the restructuring of alveolar walls might have taken place during the exposure and was completed by the end of three weeks of exposure.

With regards to dimensions of unit structures, mean linear intercept (MLI) which is an indirect measure of airspace size, and the number of alveoli/unit volume (N_V) remained unchanged but mean chord length (l_{alv}) which is a measurement of alveolar size, increased in hypobaric hypoxic rats compared to weight-matched controls. Similar changes were also found when compared to general controls. The average volume of alveoli ($Valv_{ave}$) increased and the surface to volume ratio of alveoli decreased compared to those of weight-matched controls. These observations coupled with a larger fraction of alveolar air led us to believe that enlargement of alveoli with no or little increase in alveolar duct dimensions occurred in hypobaric hypoxia, more so compared to weight-matched than general controls. Bartlett and Remmers in simulated high altitude (20) and Tenney and Remmers at high altitude (258) found a slight increase in mean alveolar diameter of animals (this did not reach significant level). Burri and Weibel (45) also observed enlargement of alveoli by indirect measurement as the surface to volume ratio of alveoli was decreased in rats at high altitude. A significant change in our study could be due to more severe hypobaric hypoxic conditions and a larger sample size. Interestingly, the only morphometric study conducted on humans by Saldana and Garcia-Oyola (230) also found a greater mean chord length in highlander males than age-matched sea-level males.

Alveolar surface area increased (28%) in hypobaric hypoxic animals compared to weight-matched animals. An increase in gas exchange capacity of the lung can be brought about by three

mechanisms (113): 1) enlargement of lung volume while size of the alveoli remains constant, 2) increase in the density of gas exchanging surface by finer subdivisions of the air spaces, and 3) thinning of the air-blood barrier. An increase in alveolar surface area in this study was likely a reflection of increased fixed lung volume (32%). The surface/volume ratio of alveoli did not change, therefore, it was indicative that equilibrated lung growth occurred. However, the unchanged MLI may have been the result of altered volume fractions of alveolar air and alveolar duct air because the mean chord length of alveoli increased. Burri and coworkers (49) proposed that increased gas exchanging surface can occur by more initial anisotropic enlargement of alveolar ducts compared to alveoli and which was then followed by lengthening of alveolar walls, and thus restoring the original relationship in volume fractions. In the present study, one can speculate that prolonged hypobaric hypoxic exposure might have restored the original relationship in volume fractions of alveolar duct air and alveolar air. Compared to weight-matched controls, the unchanged N_V and increased total alveolar number (21%) assessed by a direct alveolar count supported the argument that alveolar multiplication indeed took place during hypobaric hypoxic adaptation. An increase in mean chord length of alveoli was observed indicating enlargement of alveoli. This could be due to overexpansion of alveoli or due to lengthening of alveolar walls. Therefore, an increase in gas exchanging area may be a combination of alveolar multiplication, overinflation of alveoli and/or lengthening of alveolar walls.

Alveolar surface area was also increased compared to general controls, indicating that lung growth was indeed accelerated in hypobaric hypoxia. High altitude male residents also have greater alveolar surface area and total alveolar number compared to age-matched sea level males (230). Rabinovitch and associates (218, 219, 220) found that alveolar concentration (alveoli/mm^2) remained unchanged with or without an increase in lung volume following exposure to hypobaric hypoxia in young or old Sprague-Dawley rats compared to room air controls. They suggested that hypobaric hypoxia either accelerated or maintained the normal rate of alveolar multiplication. In the present study, although alveolar surface area and total alveolar number increased, this change was lower than the observed increases in the amount of collagen and elastin. In a recent study, it has been shown

that 64% of the lung tissue is alveolar and 36% is nonalveolar (249). It is possible that the bulk of connective tissue protein accumulation most likely occurred in non-gas exchanging components (i.e. vascular and bronchial wall) of the lung.

4.4.2.2. CELL KINETICS

Autoradiography showed in hypobaric hypoxic rats, maximal ^{3}H-TdR incorporation in alveolar wall cells occurred on day 3 and then declined slowly to reach the general control values by day 7 but remained higher than weight-matched controls. These findings matched the lung synthetic activity determined by biochemical analysis. Increased uptake of ^{3}H-TdR in hypobaric hypoxic animals may not necessarily imply that cellular multiplication occurred, but the unchanged percentage distribution of all cell types and increased total lung DNA in hypobaric hypoxic animals compared to general controls support the argument that cellular multiplication occurred. One would perhaps expect a change in the percentage distribution of cells in the alveolar wall, since the rate of ^{3}H-TdR incorporation was not identical in these cells. One explanation may be that increased ^{3}H-TdR incorporation does not necessarily reflect cell replication. The labelling index of type II pneumonocytes increased but the labelling index of type I pneumonocytes did not. If we assume that the increased labelling index of cells is an indicator of cell replication then the type I/type II pneumonocyte ratio should have decreased. Such a change was not observed. One explanation may be that since type I pneumonocytes are in immediate contact with alveolar air, alterations in ambient environment may induce functional changes and ultimately affect the life span of the cell. One can also argue that hypobaric hypoxic stress may have accelerated the differentiation of type II pneumonocytes to type I pneumonocytes. Alternatively, changes in type I/type II pneumonocyte ratio may have occurred between days 7 and 21 of exposure which we did not study.

Cellular kinetics of various cell populations in the central and peripheral parts of the lung showed a variable response to hypobaric hypoxic stress. The labelling indices of type II pneumonocytes, capillary endothelial cells, interstitial cells and unidentified cells in the walls of central

alveoli increased on day 3 in hypobaric hypoxic animals compared to both weight-matched and general controls. On day 5, the labelling index either increased slightly (interstitial cells and unidentified cells), stayed at the same level (type II pneumonocytes), or slightly decreased (alveolar wall endothelial cells). Unlike the central alveoli, all types of cells in the walls of peripheral alveoli and mesothelial cells showed maximal ^{3}H-TdR incorporation on day 3 of exposure to hypobaric hypoxia.

From the results it was apparent that in hypobaric hypoxia maximal DNA synthetic activity in the peripheral part of the lung occurred earlier than in the central part. ^{3}H-TdR incorporation homogeneity in various cell types in the walls of peripheral alveoli and the heterogeneity in central alveoli of the lung were interesting observations. The reason for this discordant effect in the peripheral and central region of the lung is uncertain but it suggested that the response to hypobaric hypoxia may not be the same in all parts of the lung. Close examination of the data also revealed that regardless of the time, the percentage increase at maximal ^{3}H-TdR incorporation in the wall cells of the peripheral alveoli (type II pneumonocytes 449%, alveolar wall endothelial cells 447%, interstitial cells 574%, unidentified cells 307%) also appeared to be greater than the central alveoli (type II pneumonocytes 312%, alveolar wall endothelial cells 256%, interstitial cells 342%, unidentified cells 246%). This indicated that hypobaric hypoxic stress produced a greater effect on the peripheral part of the lung than the central part. The peripheral part of the lung is mainly comprised of pure respiratory unit structures without rigid axial non-parenchymatous structures while the unit structures in the central part are arranged in a more complex three dimensional geometrical pattern which may restrict growth in the central portion of the lung. Therefore, it is conceivable that the geometrical location may facilitate more growth in the peripheral part of the lung than the central part.

Mesothelial cell ^{3}H-TdR incorporation was synchronous with incorporation into peripheral cells. Recently a study conducted by Cagle and coworkers reported that following pneumonectomy, the pleural mesothelial cell ^{3}H-TdR incorporation peaked two days prior to other cells in the lung parenchyma (53). They suggested that early stimulation of mesothelial cells may have occurred due to closer proximity of these cells to the factors which stimulate lung growth or to their greater sensitivity

to such factors. Nattie and associates (192) documented that compensatory lung response following partial lung resection was not similar in central and peripheral parts of the lung in young rats. Surface alveoli showed evidence of multiplication whereas internal alveoli dilated. A similar type of response may have occurred in hypobaric hypoxic animals.

Autoradiographic observations of alveolar wall endothelial cells showed some unique features in the peripheral and central part of the lung. First, the maximal alveolar wall endothelial cell stimulation was found on day 3 of exposure regardless of central or peripheral alveoli. It was also noteworthy that the sequence of ^{3}H-TdR incorporation in arterial endothelial cells was also identical to that of alveolar wall endothelial cells. In the walls of central alveoli, the endothelial cells peaked earlier than the other cells. These observations suggest that the endothelial cell stimulation was related to changes in hemodynamics (discussed in normobaric hypoxia). Second, the hypobaric hypoxic stress appeared to induce a bimodal adaptive response in the alveolar wall endothelial cells. ^{3}H-TdR incorporation increased sharply and peaked on day 3 of exposure. Thereafter it fell gradually and reached control levels on day 7 but another significant increase in the endothelial wall labelling index was noted on day 21. Rabinovitch and coworkers (218) showed that the mean pulmonary artery pressure increased significantly after three days of exposure to hypobaric hypoxia and remained high until day 14. They believed that an initial increase in pulmonary artery pressure may have occurred due to changes in hemodynamics whereas a later sustained increase was a result of structural alterations in the pulmonary vasculature. It is possible that in our hypobaric hypoxic rats, the initial surge in endothelial cell DNA synthesis may have been mediated by immediate changes in hemodynamics (increased cardiac output, hematocrit) and the second increase occurred due to sustained hypertension secondary to vascular structural changes.

Peak stimulation of arterial wall cells did not synchronize with the arterial endothelial cells. In hypobaric hypoxic rats, maximum stimulation of arterial wall cells occurred on day 5 of exposure and it coincided with the response in bronchial wall cells. The reason for this variable response between arterial endothelial cells and arterial wall cells is unclear but geometrical location and delayed

availability of humoral mediators may be responsible.

4.4.2.3. FUNCTIONAL ADAPTATIONS

Whether functional changes in the lung occurred prior to or after structural adaptation to hypobaric hypoxia is uncertain. Observations made by Bartlett and Remmers (20) suggest that lung mass increases before lung volume. They found that dry lung weight/lung volume was higher on day 7 of exposure but not on day 20-21. We studied lung function alterations three weeks after exposure to hypobaric hypoxia. In hypobaric hypoxia, despite reduced somatic growth, biochemical, morphometric and cytokinetic evaluations provided evidence of enhanced lung growth. A wide and barrel-shaped chest has been regarded as characteristic of residents of high altitude. In our study, the TLC in intact and excised lungs was significantly increased compared to the undernourished rats. The TLC in intact hypobaric hypoxic animals was also higher than general controls. Our results are compatible with those found in humans by other investigators (146, 273).

FRC, RV and VC increased compared to both weight-matched and general controls. Such increases in lung volumes and capacities also suggested that lung growth was augmented in hypobaric hypoxia. An increase in FRC has also been reported in humans (39, 75, 146, 232, 256). Human data from Andean residents have shown that most of the increased total lung capacity is due to larger residual volume (270). It has been suggested that FRC may increase due to a sustained inspiratory position of ribs as a result of hyperventilation (13). Our findings did not support this hypothesis. The FRC/TLC, RV/TLC and VC/TLC ratios in hypobaric hypoxic animals were similar to weight-matched and general controls suggesting that increased lung growth in hypobaric hypoxia occurred by a proportional increase in all fractions of volume.

As in other organs, elastin and collagen are found in association with proteoglycans in the lung. The elastic fibers can stretch to 140% of their resting length before breaking while the tensile strength of the lung tissue is attributed to collagen which can stretch only about 2%. According to Weibel (280) the *axial connective tissue* of the bronchi, bronchioles and pulmonary vessels, the *peripheral*

connective tissue of the pleura and the interlobar and interlobular septa, and the *parenchymal connective tissue* of the alveolar septa are three functional components of the connective tissue of the lung. The axial connective tissue represents the immobile center while the peripheral connective tissue represents the oscillating part. Parenchymal connective tissue forms the elastic link between the oscillating and immobile part and thus any force exerted on the parenchyma is distributed throughout the lung.

Static lung compliance which is a measure of lung distensibility is a change in lung volume per unit change in the pulmonary transpulmonary pressure. Besides connective tissue forces and surface tension, static compliance may also be affected by pulmonary blood volume and viscosity, smooth muscle tone or thickening of the visceral pleura (71). In the present study, static compliance and pressure-volume characteristics in hypobaric hypoxic rats were indistinguishable from both weight-matched and general controls. Similar observations have been made in highlanders compared to lowlanders (39, 75). Static compliance may be influenced by lung size but when corrected for lung volume, called specific compliance, this also stayed unchanged. This suggested that in hypobaric hypoxia, elastic lung recoil at lower or higher lung volumes did not alter, and these largely reflect changes in connective tissue properties of the lung. Our biochemical analysis showed an increase in elastin concentration and an increase in elastic recoil was expected. However, elastin deposition might have occurred more in the vascular component of the lung and thus its predicted effect on the lung recoil was not observed.

The pressure-volume curves were also analyzed by using the exponential curve fitting technique (68) and the K value obtained in hypobaric hypoxic rats was similar to that of weight-matched and general controls. In rats as well as in other species, the shape constant K of the pressure-volume curve has been claimed to be directly related to the mean size of the airspaces as assessed by the mean linear intercept (123, 201). The mean linear intercept in the hypobaric hypoxic group did not change compared to the weight-matched and general controls. This further supported the evidence that in hypobaric hypoxia, the structural integrity of the lungs was maintained at a normal level.

Furthermore, high altitude native mice have type II pneumonocytes with larger lamellar bodies, resulting in four times more surfactant (205) but surfactant is decreased in undernutrition (83). Since undernourishment is a part of the adaptive response to high altitude, the equilibrium of surfactant may stay unaltered and may not exert any change in lung recoil properties.

The resistance to the respiratory apparatus in vivo is attributable to the chest wall, lung tissue and gas in the airways. The airways account for more than 90% of the total resistance of the lung and the remainder may be represented by the tissue component which is difficult to measure (71). We measured the upstream resistance (Rus) under conditions of maximal flow when the driving force is the static elastic recoil of the lung. The resistance is then independent of the expiratory effort. The animals were paralysed by an injection of succinylcholine. Gas flow is also dependent upon the size of the lungs. Therefore, expiratory rates and upstream resistance were corrected for lung volume (as described in materials and methods).

Three weeks after exposure to hypobaric hypoxia, the flow-volume curves revealed that absolute forced expiratory flow (FEF) rates at all percentiles of FVC (forced vital capacity) were similar compared to weight-matched and general controls, but when FEF was corrected for lung volume, a significant reduction was noticed at 60-80% of FVC in hypobaric hypoxic rats compared to both control groups. The $FEF_{25-75\%}$ (forced expiratory flow between 25-75% of forced vital capacity) was also decreased. These findings suggested air flow obstruction. Rus increased and forced expiratory volume relative to FVC ($FEV_{0.1}$/FVC%) decreased, but the changes were not significant. However, the specific Rus increased significantly compared to both weight-matched and general controls. We performed pulmonary function tests in room air and animals were exposed to room air for more than two hours before tests. This helped us to avoid inheritent assumptions for air density if tests are performed in hypobaric conditions. Brody and coworkers performed tests at high altitude and low altitude and made correction for decreased air density in high altitude subjects. Changes in flow rates in their highlanders compared to lowlanders were similar to our results (39). In another study, airway resistance increased when residents of high altitude were moved to sea level (75). It is interesting

to note that three weeks of exposure to hypobaric hypoxia in growing rats produced similar alterations in lung function to that of humans living at high altitude. This may imply that adaptations which occur at high altitude are phenotypic rather than genotypic.

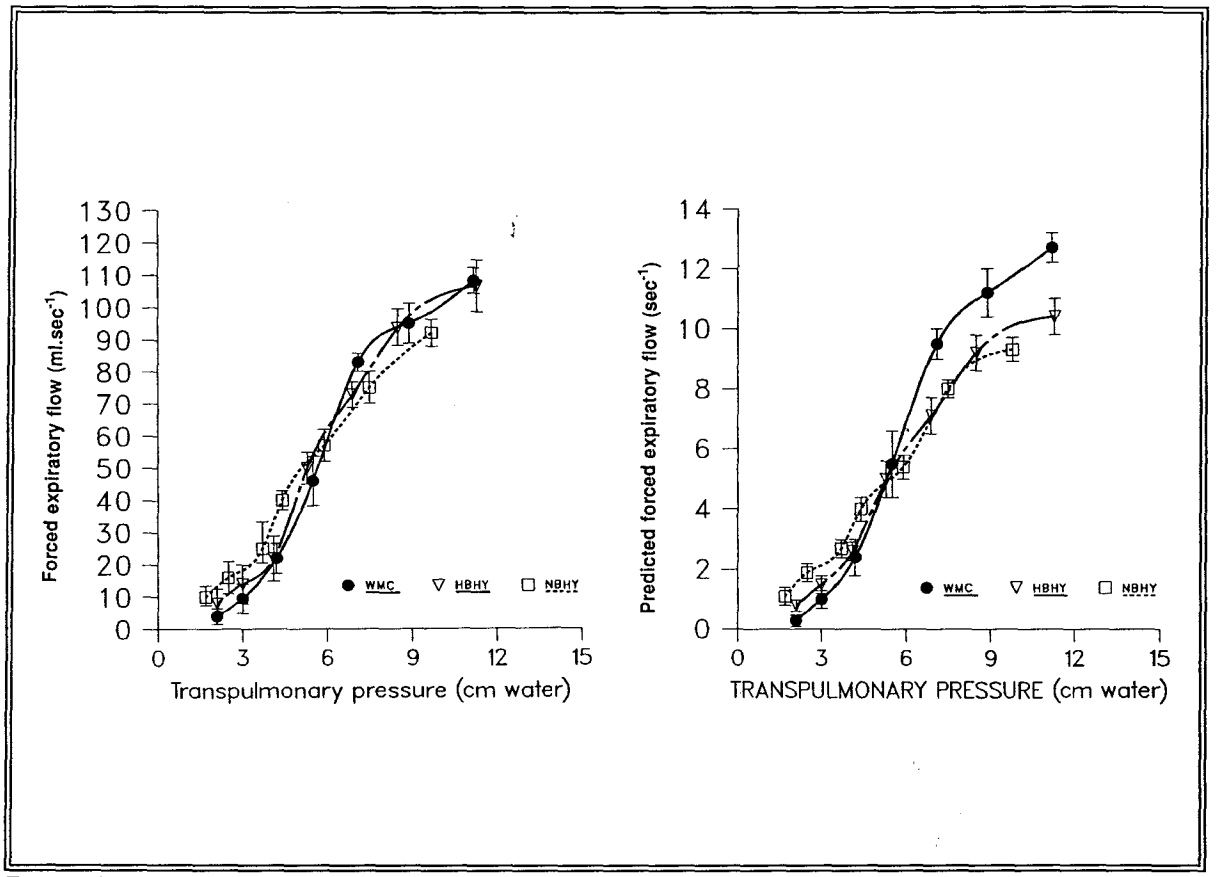


Figure 52. Absolute and corrected for FVC maximum flow-static recoil relationship in rats following exposure to different conditions.

In mammals, airway formation and alveolar partitioning takes place at different times (261). In humans, bronchial tree development occurs before 16 weeks of fetal gestational age, while alveolar formation continues during childhood (261). Therefore, it is possible that factors influencing one stage of development could cause dysanaptic growth (disproportionate growth pattern between constituent parts on an organ) of the lung, resulting in lung function changes. Green and coworkers (117) suggested that variability in maximum expiratory flow-volume measurements in healthy individuals may occur due to dysanaptic lung growth because variability in measurements could not be attributed to differences in bronchomotor tone or size of lungs. In the present study, static compliance and

pressure-volume characteristics did not alter in hypobaric hypoxic rats. When absolute and lung volume normalized expiratory flow rates at percentiles of vital capacity were plotted against transpulmonary pressure, predicted (corrected for FVC) flow-static recoil curve of hypobaric hypoxic animals shifted right (Figure 52). We presume that this shift in flow-static recoil curve and a decrease in predicted expiratory rates in hypobaric hypoxic animals would have occurred if airways failed to grow in size proportional to total lung growth.

Using morphometric techniques, the volume fraction of airway lumen was also assessed. In the present study, the volume fraction of conducting airways lumen has been referred to as the volume fraction of bronchial air (bronchial and bronchiolar air) and it was relatively small (ranged from 0.005 to 0.075). Stone and associates have recently reported that only 4% of lung air is occupied by the nonalveolar region of the lung (249). Only one group of investigators (50) has been able to find a significant decrease in volume fraction of conducting airways following partial pneumonectomy while the volume fraction of parenchyma did not change. The investigators suggested that such an imbalance in growth of lung structures may have functional implications. We found a trend to a slightly lower volume fraction of bronchial air in hypobaric hypoxic animals (0.023 $\pm$0.004) compared to both weight-matched (0.031 $\pm$0.006) and general controls (0.037 $\pm$0.006) whereas the volume fraction of alveolar air increased in hypobaric hypoxic animals. At the time of maximal response, the central airway epithelium DNA synthesis increased by 130% compared to 330% in alveolar wall cells. An inference can be drawn from these observations that lung growth in hypobaric hypoxia was dysanaptic as airways grew relatively less than the gas exchanging surface.

It was of interest that the labelling index of bronchiolar epithelium increased 210% more than bronchial epithelium. During childhood, distal airways increase in diameter more than proximal airways and the length of distal airways increases more than the diameter (261). As mentioned earlier we found a slight decrease in the fraction of alveolar duct air in hypobaric hypoxic animals. One explanation may be that part of the alveolar ducts were converted into bronchioles by extension of bronchiolar epithelium. Rabinovitch and coworkers (220) observed that hypobaric hypoxia produced

extension of muscularization of peripheral arteries which are not normally muscularized at the alveolar duct or wall level. The other explanation may be that peripheral airway growth stimulation occurred more than that of central airways. The peripheral airway growth may have been triggered by the similar mechanism which mediated growth of the gas exchanging surface, while geometric restrictions may also have limited the growth of central airways. McBride (178) found an increase in cross-sectional areas of both central and peripheral airways in pneumonectomized ferrets, whereas Boatman (28) noticed an increase in axial length of airways in rabbits. If peripheral airways increased in cross-section, then the resistance in the airways would have decreased but resistance increased in hypobaric hypoxic rats. Therefore, it is probable that hypobaric hypoxia induced lengthening of peripheral airways.

The present study affirmed the findings of previous investigations (20, 45, 204) that hypobaric hypoxia enhances specific lung growth. We also found evidence that absolute lung growth is increased even compared to the general controls and it is much greater than weight-matched counterparts. Since specific alveolar surface area and total alveolar number was higher in weight-matched controls than in general controls, corrections of morphometric results made for body weight may overestimate response of high altitude or hypobaric hypoxia in growing animals. Thus, caution must be used while interpreting specific data. Our data for morphometric analysis did not show any change in dimensions of lung structure in undernourished rats, and hence, they served as valid reference controls.

In brief, despite diminished somatic growth, lung growth was accelerated in hypobaric hypoxia. Lung growth occurred both by hyperplastic and hypertrophic changes. Although hypobaric hypoxia produced accumulation of collagen and elastin, collagen increase lagged but elastin increased more than the increase in lung weight. This may have occurred due to lack of oxygen to facilitate the hydroxylation of proline. Biochemical, morphometric and physiological observations showed that lung structure remained unaltered in hypobaric hypoxia. Along with other adaptive changes in the body (increased hematocrit, decreased somatic growth) hypobaric hypoxia also induced an increase in gas

exchanging surface area, perhaps sufficient to meet body oxygen requirements. Lung growth occurred both by alveolar enlargement and multiplication. Expiratory flow rates decreased and airways resistance increased. These changes may have occurred due to dysanaptic lung growth in hypobaric hypoxia.

4.4.3. EFFECT OF REDUCED AMBIENT OXYGEN (NORMOBARIC HYPOXIA)

This thesis provided us an opportunity to compare adaptive lung growth response to two types of hypoxia, either by dilution of oxygen or by lowering the ambient pressure. Dilution of oxygen with nitrogen reduces the concentration of oxygen content while the density of total gases remain unchanged, but a reduction in atmospheric pressure lowers the density of gases without changing the relative oxygen concentration. In this part of the discussion, the effect of low oxygen concentration and low oxygen density on lung growth adaptation will be discussed separately. We did not have weight-matched controls for the normobaric hypoxic group but their somatic growth was equivalent to hypobaric hypoxic animals. Therefore, the weight-matched group served as a suitable control group to study the effect of normobaric hypoxia without dealing with specific lung growth parameters.

4.4.3.1. NORMOBARIC HYPOXIA vs WEIGHT-MATCHED CONTROLS AND GENERAL CONTROLS

Compared to the weight-matched group, three weeks of exposure to normobaric hypoxia produced significant increases in absolute lung weight (38%), lung volume (39%), excised total lung capacity (41%), DNA (31%), RNA (60%), total soluble protein (52%), hydroxyproline (28%), desmosine (62%), alveolar surface area (24%) and total alveolar number (12%). Mean linear intercept (MLI) and mean chord length of alveoli (l_{alv}) increased and number of alveoli/unit volume (N_V) decreased in normobaric hypoxic animals. Biochemical analysis and autoradiographic measurements showed that normobaric hypoxia induced peak DNA synthetic activity in the lungs on day 3 of exposure. Thereafter, it fell gradually but remained consistently higher than weight-matched animals. Pulmonary

function tests performed after three weeks of normobaric hypoxic exposure also showed significant changes as functional residual capacity, residual volume, vital capacity and total lung capacity increased significantly. Although no differences were found in the elasticity of the lung, the peak expiratory flow rate decreased and upstream resistance increased in animals kept in normobaric hypoxic conditions. Our findings are not consistent with the only other study that used pair-weighed animals to assess the adaptive lung response. Naeye exposed suckling mice to normobaric hypoxia (12% oxygen) for 3 and 4½ weeks (191). Although undernourished and normobaric hypoxic (12% oxygen) mice were smaller (44% and 42% respectively) in body weight than controls, lung weights of undernourished and normobaric hypoxic mice were similar. This may suggest that the effect of undernutrition and normobaric hypoxia on lung growth in suckling mice may be different from that in growing rats.

Investigators in the past have usually studied the adaptive lung growth response to hypoxia by comparing the normobaric hypoxic animals and controls kept in room air and fed ad libitum. We also compared normobaric hypoxic rats to general controls and found significant increases in absolute lung weight (16%), lung volume (13%), DNA (13%), RNA (14%), total protein (24%) and desmosine (43%) On the other hand, the excised total lung capacity, total amount of hydroxyproline, alveolar surface area and total alveolar number remained unchanged. Significant increases in functional residual capacity and total lung capacity were also noticed in normobaric hypoxic animals. In addition, decreases in expiratory flow rates, increased flow resistance and decreased elastic lung recoil between 50-90% of vital capacity were observed. With regards to augmented lung growth in normobaric hypoxia, our results for lung weight and/or lung volume were in agreement with some investigators (13, 77, 101, 164, 247) but were in conflict with others (18, 191). Part of the controversy in the literature regarding lung growth response to decreased oxygen tension may be related to age and species of the animals, duration and severity of exposure, and techniques used to estimate lung growth.

In the present study, the unchanged dry/wet lung weight ratio and a significant increase in dry

lung weight in normobaric hypoxic animals compared to both weight-matched and general controls indicated that increased water retention was not responsible for the marked increase in lung mass. Increased hematocrit may have contributed to an observed increase in dry lung weight of normobaric hypoxic animals. The possibility of such a contribution was minimal in this study and the reasons have been discussed before. Therefore, an increase in lung weight in normobaric hypoxia was real.

Fixed lung volume and lung weight in normobaric hypoxic rats not only increased compared to weight-matched controls but also to general controls. Lechner and Banchero (164) found an increase in absolute lung volume in guinea pigs after three weeks of exposure to normobaric hypoxia and once guinea pigs reached 900 g body weight, an increase in lung volume no longer existed. They suggested that the maximum size of the thoracic cage may be the critical factor restraining further lung growth. In their view, lung growth accelerated to reach adult dimensions faster in hypoxic conditions. The effect of prolonged normobaric hypoxia may be different in rats, since in guinea pigs, normobaric hypoxia did not appear to affect somatic growth. However, besides mechanical restrictions, other factors such as oxygen requirements, growth inhibitors and directly (autocrine or paracrine) or humorally mediated factors may also help regulate growth of lungs in hypoxic conditions.

4.4.3.1.1. STRUCTURAL ADAPTATIONS

Lung DNA synthetic activity in normobaric hypoxic rats increased markedly on day 3 of exposure and dropped thereafter to reach the general control levels on day 7 but remained elevated compared to weight-matched controls. The total amount of DNA increased significantly on day 5 compared to weight-matched controls and on day 7 compared to general controls. The RNA/DNA ratio increased by day 3 in normobaric hypoxia and then declined gradually but remained slightly elevated until the end of exposure compared to weight-matched animals. These observations suggested that lung growth adaptation in normobaric hypoxia was brought about by both hyperplastic and hypertrophic changes and the bulk of adaptive response occurred during the first week of exposure. Compared to general controls, it was apparent that the initial adaptive response occurred

by an increase in cell size. As the number of cells increased, cell size began to decrease. One may speculate that the initial adaptive lung response to a hypoxic stimulus is an increase in synthesis of cytoplasmic constituents and if the stimulus is removed at this stage, the hypertrophic cells may return to normal size (see post-exposure recovery). On the other hand, if the stimulus persists, cellular proliferation occurs which may fulfill the functional demand. Thereafter the cytoplasmic enlargement is no longer required and cell size reaches the normal levels. Faridy and coworkers (101), and Sjostrom and Crapo (247) have also reported elevated DNA content in adult animals subjected to normobaric hypoxia. On the contrary, normobaric hypoxia produced a hypoplastic lung response in newborn rats (187). The differing lung growth adaptive response in newborn and adult animals may imply that lower oxygen availability may in fact compromise lung growth during the rapid growth phase (because tissue proliferation is maximal) but stimulate it when the lung growth rate is slow. A similar conclusion may be drawn from observations made by Cunningham and coworkers (77) as three week old rats showed no increased in lung weight following three weeks of exposure to normobaric hypoxia but in 6 and 12 week old rats lung weights increased by 16% and 22% respectively.

Connective tissue proteins showed a variable response to normobaric hypoxia. Despite increased lung tissue mass in normobaric hypoxia, the total amount of hydroxyproline did not increase compared to general controls. This led to a significant drop in hydroxyproline concentration compared to both general and weight-matched controls suggesting that a decrease in hydroxyproline/unit dry lung weight in normobaric hypoxia did not result from undernutrition. As has been mentioned earlier, the hydroxylation reaction of proline to hydroxyproline is aerobic and oxygen incorporation into the hydroxyl group comes from atmospheric oxygen rather than from water (213). This may imply that in this study, the decreased ambient oxygen tension may have been the main factor interfering with collagen formation and accumulation.

In contrast to collagen, the amount of elastin increased following exposure to normobaric hypoxia compared to both weight-matched and general controls indicating a greater effect of normobaric hypoxia on elastin than on collagen. Compare to general controls, the desmosine

concentration did not change, but the desmosine/DNA ratio increased significantly. This indicated that normobaric hypoxia induced excessive accumulation of elastin relative to cellular proliferation. It has been reported that lysyl oxidase, which is a key enzyme for cross-linking newly formed elastin and collagen, increased four to six fold in hamsters after 24 hours of exposure to 12-13% O_2 and remained high until the end of the exposure of 5 days (40). This may support the view that elastin formation and maturation was stimulated by acute normobaric hypoxic exposure. Since the total amount of elastin increased and collagen did not, it is possible that the mechanisms which regulate the dynamic equilibrium of these two connective proteins in decreased ambient oxygen tension may be different.

In the gas exchanging area, the connective tissue proteins are responsible for the framework of the lung structures. Compared to weight-matched controls, significant increases in absolute alveolar surface area and total alveolar number signifies that normobaric hypoxia might have increased gas exchanging area by subdivision of airspaces. Other investigators (164, 167) have also found significant increases in alveolar surface area in normobaric hypoxic guinea pigs compared to guinea pigs of similar weight. However, alveolar surface area and total alveolar number are functions of lung volume and did not increase in normobaric hypoxic animals compared to general controls. These findings did not support the view that normobaric hypoxia increased the surface complexity of the lung. Our observations are in agreement with Bartlett (18) who found no alterations in lung morphometric parameters of normobaric hypoxic rats compared to general controls but are in conflict with Cunningham and associates (77). They found an increase in total alveolar number when newborn rats were exposed to 12-13% O_2 for 3 weeks but not in those exposed from 9-12 weeks of age. It is conceivable that the nature of the adaptive response to normobaric hypoxia may vary with age and severity of hypoxia.

In the present study, it is unlikely that normobaric hypoxia induced an increase in lung surface complexity. The dimensions of unit structures indeed favoured the thesis that normobaric hypoxia caused enlargement of gas exchanging structures rather than multiplication. MLI, l_{alv} and $Valv_{ave}$ increased and N_V decreased significantly compared to the weight-matched control animals. Changes

in MLI, l_{alv}, average alveolar volume and N_V were also significant compared to general controls. If lung growth occurs only by enlargement of unit structures, then the linear dimensions of airspaces will increase with the cube root of the increase in volume and the number of alveoli per unit volume will decrease directly to the increase in volume (261). Increases in MLI and l_{alv} and a decrease in N_V in our normobaric hypoxic animals reached the dimensions expected by simple expansion of airspaces [observed (91.1 μm, 45.1 μm and 16.7 (10^5) respectively) vs those predicted from general controls (89.1 μm, 42.6μm and 16.0 (10^5) respectively), and vs those predicted from weight-matched controls (91.4 μm, 43.1 μm and 13.7 (10^5) respectively)]. No differences were found in the fractions of alveolar air, alveolar duct air and alveolar wall suggesting that enlargement of both alveolar ducts and alveoli was equivalent. The increase in alveolar surface area was also the same as predicted by simple expansion of the airspaces (observed 0.566 m^2 vs 0.573 m^2 predicted from general controls and vs 0.566 m^2 predicted from weight-matched controls) supporting the view that morphometric unit structures underwent simple enlargement in normobaric hypoxia. Regardless of age, increases in alveolar size (186, 198) and in airspaces (alveolar and alveolar duct size) (77) in rats following exposure to normobaric hypoxia have also been reported by other investigators.

Ventilatory adaptation to hypoxia is commonly regarded as a progressive hyperventilation of increasing magnitude which persists even after removal of hypoxic stimulus (31). Piazza and coworkers observed that normobaric hypoxia produced sustained hyperventilation accompanied by higher tidal volume (210). Hyperventilation may cause expansion of the lung unit structures. However, Bartlett (unpublished study in 20) after keeping young rats in 5% CO_2 for 20 days ruled out the possibility of hyperventilation being a stimulatory factor of lung growth in hypoxic conditions. Faridy and Yang (102) showed that lung hyperplasia in normobaric hypoxia resulted from the direct effect of low oxygen tension, whereas lung distension occurred due to mechanical stimulation of lung tissue by hyperventilation.

One can consider the possibility that the prolonged hyperventilation (210) might have acted as a mechanical stimulus to produce consistently greater response in the peripheral than central

airspaces. This notion was supported by the evidence that ^{3}H-TdR incorporation increased markedly in mesothelial cells and cells in the walls of peripheral alveoli on day 3 of exposure whereas the bulk of the cells in the walls of central alveoli showed maximum ^{3}H-TdR incorporation on day 5. This suggested that stimulation of cells in the walls of peripheral alveoli occurred before stimulation of cells in the wall of central alveoli. In addition, at the time of peak stimulation, the percentage increase in ^{3}H-TdR incorporation in the walls of peripheral alveoli was also relatively higher than in the central alveoli. Reasons for such changes which also occurred in hypobaric hypoxia have been discussed. The percentage distribution of cells in normobaric hypoxic rats also remained similar to those of general controls. This observations in conjunction with increased lung DNA supported the idea that hypoxic stress indeed increased cell division. From biochemical analysis it was evident that cellular hypertrophy occurred. However, the question remains whether hypertrophic changes occurred in all cells or whether the hypertrophic response in normobaric hypoxia was cell specific.

The pattern of kinetics in the walls of central and peripheral alveoli was also similar to hypobaric hypoxic rats. The maximal response in the walls of central alveoli (type II pneumonocytes, interstitial cells and unidentified cells) was delayed except in endothelial cells. Irrespective of location (in the walls of central or peripheral alveoli, or arteries), all endothelial cells showed maximal stimulation on day 3. Synchronous stimulation of all endothelial cells suggest that it was triggered by an endothelial cell specific mediator.

Tissue-specific mitotic Inhibitors, called chalones, have been proposed as being involved in tissue growth in the lung (42). It has been postulated that increased blood flow dilutes the local concentration of these chalones and thus diminishes their inhibitory effect (103). In part, alterations in hemodynamics in hypoxic conditions may also be responsible. A significant increase in hematocrit occurs by 36 hours of exposure to normobaric hypoxia (210). Since lungs handle the whole cardiac output, alterations in hemodynamics in hypoxic conditions may facilitate the removal of endothelial cell division inhibitors. One may argue that increased blood flow and volume may also supply a humoral promoter of cell division or produce a mechanical stress which may alter physical properties of the

cells to induce cell proliferation. Recently, an in vitro study has demonstrated that a 2% concentration of oxygen stimulated endothelial cell proliferation by 102% compared to a 20% O_2 concentration and it was mediated by increased adenosine release (180). Studies using models such as wound healing (155) and malignant tumors (105) which lack adequate oxygen supply have shown that reduced oxygen availability induces neovascularisation by releasing angiogenic factors. It has been suggested that increased blood volume in hypoxia produces pulmonary capillary recruitment by opening new capillaries in the lung tissue (54). These studies suggest that along with hemodynamic alterations, oxygen may also exert a direct chemical stimulus to regulate the proliferation of endothelial cells in hypoxic conditions. However, it remains speculative that low oxygen tension may stimulate and regulate growth of different cells through different mechanisms.

The number of free alveolar macrophages which incorporated ^{3}H-TdR increased in normobaric hypoxic rats on day 5 compared to the weight-matched controls. Similar changes were found in hypobaric hypoxic rats. This shows that macrophages which were until recently considered terminal cells (2), also respond to hypoxic stress by increased ^{3}H-TdR incorporation. What induces an increase in DNA synthesis in free alveolar macrophages is not understood. One may speculate that hypoxia stimulates the release of chemotactic substances such as macrophage activating factor which may stimulate the alveolar macrophages. It has been reported that hypoxia increased metabolic activity in alveolar wall endothelial cells (247), therefore, hypoxia may also directly activate the free alveolar macrophages. Alveolar macrophages are known to release two mediators, fibronectin and macrophage derived growth factor. Fibronectin stimulates resting fibroblasts to proliferate (25). Therefore, interstitial cell stimulation may also occur due to the paracrine effect of macrophages.

Previous studies have reported increased number of peribronchial and perivascular mast cells in animals exposed to normobaric hypoxia (152, 202). Thus, hypoxia induced hyperplasia of the amine containing cells in the lung may provide a pool of available mediators which could conceivably affect the pulmonary vasculature and airways. However, no direct relationship between mast cell hyperplasia and vasoconstriction in hypoxic conditions has been established. To our knowledge, ^{3}H-

TdR incorporation into mast cells in the lung in normobaric hypoxic or hypobaric hypoxic conditions has not been studied. Our autoradiographic results showed an increased mast cell labelling index in response to normobaric hypoxia in the lung. We found that the bulk of the increase in the number of ^{3}H-TdR incorporated mast cells occurred in the subpleural part of the lung. The significance of subpleural proliferation of mast cells is not clear. It has been suggested that pulmonary vascular resistance is more closely related to alveolar than to arterial oxygen tension and it is alveolar hypoxia rather than hypoxemia that causes structural changes in pulmonary mast cells (152). Therefore, an increase in subpleural mast cell DNA synthesis may have occurred due to the direct effect of alveolar hypoxia. Our findings showed that mast cell stimulation occurred immediately following exposure to hypobaric hypoxia or normobaric hypoxia suggesting that mast cells were responsive to low oxygen tension before any other cells in the lungs. Azizkhan and associates (9) have demonstrated that mast cells stimulate angiogenesis. It is possible that mast cell proliferation may be one of the regulatory mechanisms of endothelial proliferation to the increased blood volume load in hypoxic conditions. In addition to histamine release, mast cells are also associated with interstitial edema and connective tissue formation (188). Therefore, it may be that increased lung connective tissue protein accumulation and moisture content (during the early period of exposure) in hypobaric hypoxia and normobaric hypoxia were also partly mediated by mast cells.

4.4.3.1.2. FUNCTIONAL ADAPTATIONS

Rats exposed to three weeks of normobaric hypoxia showed significant increases in functional residual capacity, residual volume, vital capacity and total lung capacity compared to weight-matched animals. These findings suggest that normobaric hypoxia produced an increase in functional capacity of the lungs. An increase in functional capacity of the lungs in normobaric hypoxia may occur due to a raised inspiratory position of the thoracic cage, airway obstruction or due to loss of elasticity of the lung. Barer and associates (13) concluded that the raised inspiratory position of the thoracic cage was the possible reason for an increase in FRC of normobaric hypoxic rats as pressure-volume

characteristics remained unchanged. We did not find differences among any experimental and control groups for FRC, RV and VC relative to TLC suggesting that all components of the lung volume increased proportionally. An increase in FRC in our normobaric hypoxic rats was not due to persistently hyperinflated lungs.

Pressure-volume characteristics of excised normobaric hypoxic lungs were similar to those of weight-matched and general controls. Our findings are compatible with other investigators who studied elastic properties of excised lungs following chronic normobaric hypoxic exposure (13, 77, 101). Absolute and specific lung compliance in intact normobaric hypoxic animals also remained unchanged. This may suggest that decreased oxygen tension did not disturb structural integrity of the lungs. However, we found loss of elastic lung recoil in intact normobaric hypoxic animals between 50-90% of VC and a significant increase in airspace size compared to general controls. These alterations supported the view that normobaric hypoxia might have caused changes in lung structure.

Setnikar (241) and Mead (179) postulated that the function of collagen and elastin in lung were largely independent of each other. The coiled collagen fibers determine lung distension characteristics at higher lung volumes or near total lung capacity. The readily extendable elastin fibers are determinant of lung distensibility at low lung volumes, giving the steep part of the P-V curve. Collagen concentration in our normobaric hypoxic animals decreased compared to general controls and this decrease may have been responsible for the loss of lung elastic recoil at higher lung volume. Lung compliance was measured at low volumes where elastin fibers determine the lung recoil. Despite an increase in the amount of elastin in normobaric hypoxia, the elastin concentration did not change. Therefore, variability in elastin and collagen equilibrium in normobaric hypoxia may in part account for differences in elastic lung recoil at lower and higher lung volumes.

Besides tissue forces, elastic lung recoil in air-filled lungs is also influenced by surface forces of the lung. In order to assess the effect of tissue forces, fluid-filled P-V curve measurements could provide more definitive information. Unfortunately, we did not perform fluid-filled P-V curves. Food consumption diminished in normobaric hypoxic rats. Restricted food intake has been reported to

decrease disaturated phosphotydylcholine in the lung (83). It is possible that the changes in P-V curves of normobaric hypoxic animals may in part be the result of altered surface forces. Alterations in P-V curves were only found in the intact animals and not in excised lungs, therefore, a loss of lung elastic recoil may in fact reflect changes in the mechanical properties of the chest wall.

The $FEV_{0.1}$ expressed as percentage of FVC (FEV%) may decrease due to obstruction such as narrowing of airways, to chest wall abnormalities and loss of elastic recoil. Our results showed that FEV%, PEFR and $FEF_{25-75\%}$ decreased significantly in normobaric hypoxic animals compared to both weight-matched and general controls suggesting that ventilatory capacity fell in normobaric hypoxic animals. Absolute and volume normalized forced expiratory flow rates decreased at 50-90% VC in normobaric hypoxic rats compared to general controls. The absolute expiratory flow rates were also diminished at 50-70% of vital capacity compared to weight-matched controls. In addition, absolute and specific upstream airway resistance increased markedly following exposure to normobaric hypoxia. Airway obstruction may have contributed to such changes in flow-volume characteristics in normobaric hypoxic rats. As described by Green (117), reductions in air flow rates in the lung may occur due to dysanaptic lung growth where airway growth would be less than the gas exchange surface. It is likely that decreased flow rates in normobaric hypoxic rats resulted mainly from structural alterations in airways. Since elastic lung recoil decreased at high lung volume, one can argue that reductions in maximum expiratory flow rates may be attributed to decreased static recoil of the lung. However, maximum (corrected for FVC) flow-static recoil curve of normobaric hypoxic animals shifted far right compared to weight-matched controls (Figure 52). Although one can not rule out the possibility that decreased elastic recoil may have affected expiratory flow rates, flow-static recoil relationship supports the argument that expiratory flow rates may also have reduced due to dysanaptic lung growth in normobaric hypoxia. Autoradiographic results further confirmed these observations as the rate of DNA synthesis in the central airways was lower than that in the alveolar wall cells. Implications of such observations related to dysanaptic lung growth have been discussed in the preceding section.

4.4.3.2. HYPOBARIC HYPOXIA vs HYPOBARIC NORMOXIA

Comparison between hypobaric hypoxic and hypobaric normoxic rats allowed us to evaluate the effect of lower oxygen density. In both low oxygen density and low oxygen concentration, the number of oxygen molecules remains the same but it has been postulated that decreased gas density facilitates ventilation (75). Therefore, it is possible that lower oxygen tension achieved by lowering ambient pressure (similar to high altitude) may affect lung growth differently than low oxygen concentration.

Compared to hypobaric normoxia, lung weight, lung volume, DNA, RNA, protein, hydroxyproline and desmosine increased significantly in hypobaric hypoxic rats. With the exceptions of lung weight and connective tissue proteins which were greater in hypobaric hypoxia compared to hypobaric normoxia, increases in other lung growth parameters were almost equal to those found by comparing normobaric hypoxic animals to general controls (see page 177). This suggested that except for lung weight and connective tissue proteins, the adaptive lung growth response to reduced oxygen content was similar whether it is delivered by dilution or by lowering the ambient pressure.

Hydroxyproline content increased significantly in hypobaric hypoxic rats compared to hypobaric normoxic rats but such an increase was not found in normobaric hypoxic animals compared to general controls. This suggests that low oxygen, when delivered by reducing pressure can cause collagen accumulation. Although, hydroxyproline concentration (relative to the dry weight of the lung) was also lower in hypobaric hypoxic animals than general controls, the change was less dramatic than that found in normobaric hypoxic animals. Since hydroxylation of proline is oxygen dependent, better ventilation due to decreased gas density in lower ambient pressure may facilitate collagen formation (this will be discussed later). An increase in elastin was also greater in hypobaric hypoxic animals. Therefore, it is likely that low oxygen delivered by lowering pressure had a variable effect on connective tissue protein equilibrium.

Despite an increase in lung volume, alveolar surface area and total alveolar number did not change. This occurred because of significant increases in MLI, l_{alv} and average alveolar volume, and

decreases in N_V and surface/volume ratio in hypobaric hypoxic rats. This indicated that lung growth in decreased ambient oxygen density also occurred primarily by enlargement of airspaces. The data from pulmonary function tests showed that FRC and in vivo TLC were also higher in hypobaric hypoxic animals compared to hypobaric normoxic animals. $FEF_{25-75\%}$ and expiratory flow rates normalized to lung volume decreased at 60-80% of FVC, and upstream resistance increased in hypobaric hypoxic animals compared to hypobaric normoxic animals. Similar observations were made in normobaric hypoxic rats compared to general controls suggesting that structural alterations in the lung in hypobaric hypoxia were produced by decreased oxygen.

In summary, hypoxia produced by dilution or by lowering the ambient pressure, accelerated lung growth by increasing lung weight and lung volume. Compared to weight-matched controls, lung growth increased markedly in normobaric hypoxia. However, the pattern of lung growth was not normal. Although lung growth in normobaric hypoxia occurred by hyperplastic and hypertrophic changes, accumulation of collagen was hampered which may be the cause for loss of elastic lung recoil at higher lung volumes. Morphometric analysis showed that normobaric hypoxia induce simple expansion of airspaces without an increase in surface complexity of the lung. DNA synthesis in the walls of central alveoli increased less and was delayed relative to the peripheral alveoli. A fall in expiratory flow rates suggested that normobaric hypoxia might have induced dysanaptic lung growth. This was further supported by autoradiographic results. One can infer that low oxygen tension may have produced a decrease in collagen concentration, overinflation of alveoli and dysanaptic lung growth in hypobaric hypoxia.

4.4.4. EFFECT OF REDUCED AMBIENT PRESSURE (HYPOBARIC NORMOXIA)

Although our results have demonstrated that increased lung growth (i.e. lung weight, volume, DNA, RNA and protein) in hypobaric hypoxia was mainly induced by decreased oxygen, the effect of hypobaric pressure cannot be ignored. Our results showed that there were some differences in lung growth adaptation between hypobaric hypoxic and normobaric hypoxic animals which may be

attributed to lower ambient pressure. We assessed the effect of low ambient pressure by comparing the hypobaric normoxic group with the general control group and the hypobaric hypoxic group with the normobaric hypoxic group. The former provided information related to the hypobaric pressure acclimation without altering the ambient oxygen and the later comparison helped us to assess the adaptive response in low ambient pressure with decreased oxygen.

Three weeks of exposure to hypobaric normoxia produced a small but significant increase (9%) in hematocrit compared to general controls. In the present study, our objective was to study lung growth response to hypobaric normoxia rather than hypobaric normoxemia, but the increased hematocrit raised the important suggestion that hypobaric normoxia may have caused arterial hypoxemia. In preliminary studies, we did blood gas analysis of rats exposed to acute (30 minutes) hypobaric normoxia. Arterial Po_2 (88.5 ±4.5 mm Hg) and O_2 saturation (94.0 ±1.4 %) were not significantly different than normobaric normoxic animals (92.0 ±4.3 mm Hg and 96.2 ±1.9 % respectively).

A question naturally arises with regards to the accuracy and sensitivity of the measurements. It is unlikely that measurements of blood gases were inaccurate and insensitive since these were performed in a quality controlled clinical laboratory. Our oxygen measurements were not precise to a decimal point and had an accuracy of ±1%. The chambers were opened daily to replenish food and for general maintenance, and oxygen was measured three times a day. The possibility of consistently lower oxygen settings inside hypobaric normoxic chambers were minimal. It has been reported that an exposure to chronic mild normobaric hypoxia (19%) did not affect hematocrit values (187). Therefore, it is unlikely that a small variation in oxygen of ±1% may have produced consistent hypoxemia but an increased hematocrit in our hypobaric normoxic rats was surprising. However, other investigators have found hypoxemia following exposure to hypobaric normoxia. Levine and coworkers (171) found that following two hours of exposure to hypobaric normoxia, hypobaric hypoxia and normobaric hypoxia (simulated to 6600 m or 326 torr), arterial PO_2 decreased in awake sheep which were subjected to hypobaric normoxia. Hirai and associates (137) reported that it required 65%

ambient oxygen concentration to make hypobaric sheep normoxemic instead of 49.7% ambient oxygen concentration measured in similar hypobaric normoxic conditions. This issue is further complicated by the fact that an increase in hematocrit may also occur due to a decrease in plasma volume (251) which we did not measure. Thus, we have no convincing explanation for an increase in hematocrit in hypobaric normoxic rats.

4.4.4.1. HYPOBARIC NORMOXIA vs GENERAL CONTROLS

Lung volume and the TLC of excised lungs decreased significantly in rats exposed to hypobaric normoxia for three weeks. Dry weight of the lung, DNA, RNA, total soluble protein, hydroxyproline and desmosine remained the same in hypobaric normoxic rats compared to general controls. Despite a decrease in lung volume, alveolar surface area and total alveolar number did not differ from those of general controls. However, dimensions of morphometric unit structures were smaller in hypobaric normoxic animals. Biochemically estimated ^{3}H-TdR incorporation into DNA increased on day 5 of exposure and thereafter reached the general control level. Autoradiographs also revealed that maximal DNA synthetic activity occurred on day 5 in alveolar wall cells. Lung volumes and capacities measured in vivo hypobaric normoxic animals were the same as those in general controls. Hypobaric normoxia did not produce changes in expiratory flow rates and in pressure-volume or flow-volume characteristics.

Somatic growth of hypobaric normoxic animals was diminished. Hypobaric normoxic animals ate less and were significantly smaller on day 10 of exposure, and on day 21 their body weight was almost midway between general controls and hypobaric hypoxic animals. We did not have weight-matched controls for hypobaric normoxic animals and thus results in lung growth are hard to interpret. Although it is not appropriate, we also normalized our results for body weight. Despite diminished lung volume, specific lung weight and volume of hypobaric normoxic animals were equivalent to that of general controls. These findings suggested that lung growth was relatively less affected in hypobaric normoxia than somatic growth.

One may argue that in the present study, hypoxemia may have stimulated lung growth in hypobaric normoxic animals. This cannot be ruled out. However, Mortola and associates (187) found that mild (19% FIO_2) normobaric hypoxia for one week caused no effect on somatic or lung growth in rats. In addition, if we delivered a higher concentration of oxygen to make hypobaric normoxic animals normoxemic then, hyperoxia would have affected lung growth. Since we designed our experiments to study the effect of high altitude conditions, hypobaric normoxia was an appropriate experimental condition for the effect of low ambient pressure.

4.4.4.1.1. STRUCTURAL ADAPTATIONS

Even though hypobaric normoxic rats gained less body weight compared to general controls, biochemically estimated lung parameters were the same. RNA/DNA, protein/DNA, hydroxyproline/DNA and desmosine/DNA ratios also remained unchanged. These findings suggested that in spite of diminished somatic growth, hypobaric normoxia did not produce alterations in lung structure.

^{3}H-TdR incorporation measured by biochemical analysis doubled on day 5 of exposure to normobaric hypoxia indicating that decreased ambient pressure did stimulate lung growth. These findings were confirmed by analysis of autoradiographs as the labelling index of cells in the alveolar walls also peaked on day 5 of exposure. Cytodynamic heterogeneity was observed in various cell populations. Pleural mesothelial cell stimulation peaked on day 3 while type II pneumocytes, interstitial cells and unidentified cells in the peripheral part of the lung showed maximal stimulation on day 5. In the walls of central alveoli, ^{3}H-TdR incorporation in type II pneumonocytes and interstitial cells peaked on day 7. These results indicated that the peak effect of hypobaric normoxia was also delayed in the central part compared to the peripheral part of the lung. The reasons for relatively early stimulation of mesothelial cells are not clear.

Interestingly, a maximal increase was noticed in interstitial cells while alveolar wall and arterial endothelial cells remained unaffected by hypobaric normoxic stress. Although a small increase was found in hematocrit of hypobaric normoxic rats, it perhaps was not sufficient to cause hemodynamic

changes which could have stimulated endothelial cell DNA synthesis by removal of local cell-specific growth inhibitors or by supplying the cell growth promoters. On the other hand, acute exposure to hypobaria increases transvascular fluid flux due to a hydraulic pressure difference between capillary pressure and interstitial pressure (171). We also found an increase in dry/wet lung weight ratio on days 1 and 3 of exposure to hypobaric normoxia (0.2156 ±0.0038 and 0.2113 ±0.0019 respectively) compared to general controls (0.2212 ±0.0015 and 0.2218 ±0.0021 respectively)but it reached a significant level only on day 3. Transvascular fluid flux may be the reason for an increase in lung water during the early period of exposure and this may stretch the interstitial space. With altered hydrostatic pressure, changes in membrane shape and function, and cellular protein synthesis (158) have been documented. In vitro periodic stretch of the membrane on which fibroblasts were cultured stimulated cell proliferation (78). Therefore, changes in interstitial cells might have been induced by physical forces in the interstitium of the lung under hypobaric normoxia. Alternatively, with the movement of transvascular fluid into the interstitial space, it is possible that some growth promoting factors may also increase within the interstitium and hence stimulate growth related alterations. Therefore, results of autoradiographs of hypobaric normoxic animals support the notion that endothelial cells and other alveolar wall cells are stimulated through different mechanisms.

Bartlett (18) did not find convincing changes in morphometric lung growth variables in rats exposed to normobaric hypoxia for 15 days, whereas an exposure to hypobaric hypoxia for 21 days produced significant increases in lung volume and alveolar surface area (20). These studies (18, 20) suggest that hypobaric pressure may play an important role in lung growth adaptation. Although in the present study, biochemical changes suggested that hypobaric normoxia did not induce structural alteration in the lung, the morphometric results showed that it produced an increase in the surface complexity (increased surface/volume ratio and N_V, and decreased MLI and $Valv_{ave}$) of the lung. This supported the concept that hypobaric pressure may have a definitive role in high altitude lung growth adaptation.

Interestingly, despite decreased lung volume, alveolar surface area and total alveolar number

remained unchanged in hypobaric normoxic rats. This may suggest that even though lung growth diminished in hypobaric normoxia, alveolar proliferation was maintained at normal levels. This supports the hypothesis that alveolar number is genetically programmed. How low ambient pressure may bring about a decrease in the size of the respiratory units is not known. One explanation may be that alveolar proliferation occurred at the normal rate but concomitant enlargement of airspaces did not follow as lung volume did not increase. This may have occurred due to lack of mechanical stimulation such as hyperventilation that occurs in hypoxia (31, 210). Alternatively, reduced ambient pressure may have induced a direct effect on size of morphometric unit structures. It has been suggested that alveolar pressure is directly transmitted to the interstitium (27). Theoretically, the alveolar pressure equilibrates with ambient pressure as it falls in hypobaric conditions and such a change in ambient pressure should not produce structural alterations in the lung. However, the pleural cavity is a potential space between the lungs and the chest wall and the chest wall is a rigid structure. It is thus conceivable that pleural pressure may become more negative and that transpulmonary pressure may also become more negative. It can be speculated that an increased pressure gradient between the pleural cavity and respiratory units may in part be responsible for smaller dimensions of airspaces in hypobaric conditions.

4.4.4.1.2. FUNCTIONAL ADAPTATIONS

Although the values of absolute FRC, RV, VC, TLC were lower than those of general controls, the differences were not significant. Studies conducted during Skylab 4 mission showed that vital capacity of crew members exposed to hypobaric normoxia for 84 days was decreased without affecting other lung functions and upon recovery vital capacity returned to the normal level (233). The authors concluded that these changes in vital capacity may have occurred due to a direct effect of reduced ambient pressure. Similar observation have been made by other investigators (222, 268). In the present study, FRC/TLC, RV/TLC and VC/TLC ratios in hypobaric normoxic rats did not differ from those in general controls. These findings also suggested that lung growth in hypobaric normoxic

animals was not affected.

As mentioned earlier, connective tissue proteins such as collagen and elastin play a major role in elastic lung recoil and thus can influence lung flow rates. The amount of collagen and elastin and their concentration in the lung tissue remained unaltered in hypobaric normoxic conditions compared to general controls. $FEV_{0.1}$/FVC %, PEFR, $FEF_{25-75\%}$, absolute or specific upstream resistance, and pressure-volume and flow-volume characteristics in hypobaric normoxic animals did not differ from those of general controls. Theoretically, the decreased mean linear intercept of airspaces in hypobaric normoxic rats would have reduced the value of the shape constant K of pressure-volume curves but the constant K and static lung compliance remained unchanged. These observations supported the biochemical results suggesting that chronic hypobaric normoxia did not alter the integrity of the lung structure.

4.4.4.2. HYPOBARIC HYPOXIA vs NORMOBARIC HYPOXIA

Some differences were observed in the hypobaric hypoxic and normobaric hypoxic rats but otherwise the lung growth response was similar. The amount of desmosine and desmosine/unit dry lung weight increased significantly in hypobaric hypoxic animals compared to normobaric hypoxic animals. The changes that occurred in hydroxyproline were not as dramatic as desmosine. Although an increase in the amount of hydroxyproline in hypobaric hypoxia did not reach a significant level, hydroxyproline/DNA ratio increased significantly compared to normobaric hypoxic animals. These findings suggested that elastin and collagen accumulation was increased when low oxygen was delivered by lowering the ambient pressure. Hydroxyproline/unit dry weight of the lung fell in normobaric hypoxic animals compared to weight-matched controls but remained unchanged in hypobaric hypoxic animals suggesting that hypobaric pressure may have counterbalanced the inhibitory effect of low oxygen on hydroxyproline equilibrium. One may suggest that increased transvascular fluid flux in hypobaric hypoxia (171) may have stimulated connective tissue protein synthesis. Leung and associates (169) found that rhythmic stretch increased collagen and protein

synthesis in smooth muscle cells grown on an elastic membrane. In the present study, peripheral interstitial cell labelling index was significantly higher on day 5 ($p=0.04$) in hypobaric hypoxic animals than normobaric hypoxic animals. It suggests that low ambient pressure may have enhanced collagen synthesis by stimulating interstitial cells in hypobaric hypoxic animals. The other explanation is that low ambient pressure may facilitate hydroxylation of proline.

The density of air is directly proportional to the barometric pressure, thus, in hypobaric conditions the mass of air decreases with a fall in ambient pressure. It has been postulated that decreased mass of air in hypobaric conditions helps to reduce the resistance to turbulent flow of air in the airways (75). However, hypoxia is known to cause bronchoconstriction (31). Cruz (75) suggested that increased airway resistance due to bronchoconstriction may counterbalance the effect of the decrease in air flow resistance due to decreased air density at high altitude. It is possible that decreased airway resistance in hypobaric conditions may also help a greater mass of air to move into the lungs and thus establish better ventilation in hypobaric hypoxic animals compared to normobaric hypoxic animals. Therefore, relatively increased alveolar oxygenation may have helped the hydroxylation of proline and in essence, increased the collagen accumulation in the lung in the hypobaric hypoxic animals. In hypobaric normoxia, an adequate supply of oxygen was available and that may be the reason that no obvious change in the amount of hydroxyproline was observed. In hypobaric hypoxic conditions where oxygen was deficient, better ventilation might have increased the quantity of oxygen available for various processes. Therefore, with regards to collagen equilibrium, the role of hypobaric pressure remains elusive.

Morphometric analysis showed that changes occurring in hypobaric hypoxic animals compared to normobaric hypoxic animals were similar to those found in hypobaric normoxic animals compared to general controls. MLI and $Valv_{ave}$ increased and N_V decreased significantly in hypobaric hypoxic animals compared to normobaric hypoxic animals. Normobaric hypoxia produced simple enlargement of airspaces and hypobaric hypoxia did not. Airspaces were enlarged in normobaric hypoxia, reduced in hypobaric normoxia and remained unchanged in hypobaric hypoxia. Therefore, low ambient

pressure in hypobaric hypoxia may have counterbalanced the airspace enlargement of hypoxia, thus keeping the dimensions of airspaces at normal levels.

$FEV_{0.1}$/FVC%, PFER and $FEF_{25-75\%}$ were higher in hypobaric hypoxic animals than normobaric hypoxic animals. It was interesting to note that the values for $FEV_{0.1}$/FVC%, PFER, $FEF_{25-75\%}$, and absolute and corrected FEF of hypobaric hypoxic animals fell in between normobaric hypoxic rats and general controls. Elastic recoil of the lung was decreased at higher lung volumes in normobaric hypoxic animals compared to general controls but not in hypobaric hypoxic animals. Definitive reasons for these differences in hypobaric hypoxia and normobaric hypoxia are not known but alterations in lung structure (biochemical and morphometric) may be responsible. These observations lead us to believe that hypobaric pressure in hypobaric hypoxic conditions may have improved the lung function alterations produced by hypoxia alone. Compared to weight-matched controls, the amount of collagen and elastin increased more in hypobaric hypoxic rats than normobaric hypoxic rats. Postnatally, the bulk of collagen and elastin is present in lung parenchyma and is related to the formation of respiratory units (41). We do not know where in the lung the accumulation of collagen and elastin occurred in hypobaric hypoxia. Airways are the site of elastin and collagen fibers and act as guy lines (70), therefore increased collagen and elastin accumulation may assist expiratory flow rates in hypobaric hypoxic animals by making airways less compliant. Formation of new elastic lamina occurs in peripheral vessels in hypobaric hypoxia (145, 182) and these vessels run in close proximity of airways. They may also provide support to airways and reduce the airway resistance in hypobaric hypoxic animals.

In brief, hypobaric normoxia stunted somatic growth and diminished lung growth. Specific lung weight and volume in hypobaric normoxia did not differ from general controls suggesting lung growth was affected less than somatic growth. Biochemical assessment revealed that hypobaric normoxia did not induce any changes in lung structure but morphometric analysis showed that it caused reduction in size of unit structures of the lung. Hypobaric normoxia produced no functional alterations in the lung. Hypobaric normoxia stimulated lung growth which peaked on day 5. Lung growth

stimulation was relatively less and was also delayed compared to hypobaric hypoxia or normobaric hypoxia. Endothelial cells were least stimulated by hypobaric normoxia which perhaps correlated with a small increase in hematocrit. Low ambient pressure in the presence of low oxygen tension increased accumulation of elastin more than collagen. Low pressure appeared to reduce airspace enlargement and improve lung function changes caused by hypoxia alone.

The **similarities** in hypobaric hypoxia and normobaric hypoxia may be due to two reasons. One possibility is that greater effect of hypoxic stress may have masked the effect of low ambient pressure. Alternatively, hypoxia and low ambient pressure may stimulate lung growth through a common mechanism. Our results did not show a clear synergistic effect of hypobaric pressure and hypoxia on lung growth in hypobaric hypoxic animals. However, with regards to elastin accumulation some additive effect of hypobaric pressure and hypoxia was apparent in hypobaric hypoxia. The desmosine concentration increased 16% in hypobaric normoxic rats, 18% in normobaric hypoxic rats and 40% in hypobaric hypoxic rats compared to general controls. Similar changes were found in the amount of total desmosine and in the desmosine/DNA ratio. In other lung parameters such as collagen accumulation and airspace size, hypobaric pressure in hypobaric hypoxia appeared to help keep values approximately the same as in general controls. Flow-volume characteristics of hypobaric normoxic animals did not alter but expiratory flow rates fell and upstream resistance increased in normobaric hypoxic animals. In hypobaric hypoxic animals, the flow-volume characteristics showed a shift towards the general controls. These observations support the notion that low ambient pressure plays a significant role in lung growth adaptation at high altitude. One can speculate that in some respects such as connective tissue protein accumulation and size of morphometric unit structures, the mechanism of the effect of low ambient pressure may not be similar to that of low oxygen tension.

4.5. EFFECT OF 3-DAYS POST-EXPOSURE AND REFEEDING

Adaptive response to a stress includes quantitative or qualitative alterations in organs whose functions are directly or indirectly affected. If that stress is removed, structural and functional changes

which occur as a result of adaptation may or may not return to normal levels. The recovery of adaptive response in various organs may also be variable. We aimed to study somatic and organ growth changes following chronic exposure to hypobaric hypoxia, normobaric hypoxia and hypobaric normoxia, and restricted feeding. After subjecting the animals to respective experimental conditions for three weeks, they were returned to room air and were allowed free access to food for three days. We chose a three day recovery for following reasons: (1) hemodynamics and heart weight return to normal levels within one week after returning to room air (197), (2) changes at cellular level may occur early while the time course for structural remodelling following recovery may be prolonged, and (3) in the present study, maximal lung growth stimulation and cellular biochemical changes occurred after 3 days of exposure.

Since undernourishment caused impairment of body weight gain and axial skeletal growth, food ad libitum for three days produced substantial increase in body weight (16%) compared to general controls (8%) but skeletal growth appeared to be unaffected. Except for the spleen, growth of all organs (heart, lung, liver and kidney) showed a considerable increase. This suggested that organs whose metabolic needs and functions were affected by undernutrition showed accelerated recovery. Winick and Noble (284) showed that recovery in body and organ weights is dependent upon the age of the animals following undernourishment. They observed that while the body and organ weights of adult rats subjected to restricted food intake recover fully within seven weeks, young rats subjected to the same length of period to undernutrition did not.

When food restricted animals were fed ad libitum the rate of ^{3}H-TdR incorporation into lung tissue DNA more than doubled. In addition, the amount of lung DNA also increased slightly. The amounts of lung RNA and protein increased suggesting enhanced protein synthesis after refeeding the undernourished animals ad libitum. Cell size also increased as DNA/RNA and protein/DNA ratios were increased. Similar observations have been made by other investigators following refeeding the starved animals (228).

Hunter and Crapo reported that somatic growth recovery may vary depending upon the severity

and duration of hypobaric hypoxia conditions (144). Our results revealed that the rate of body weight change increased but nose-tail length was not affected by returning hypobaric hypoxic and normobaric hypoxic animals to room air for three days. Hunter and Clegg (144) showed that 28 day old mice exposed for 4 or more days to hypobaric hypoxia (390 mm Hg) caused permanent stunting of body growth.

Interestingly, the weights of liver and kidney increased during the three day recovery in animals previously exposed to hypobaric hypoxia and normobaric hypoxia for 3 weeks. During exposure, liver and kidney growth were similar to those of undernourished animals suggesting that their growth was not directly affected by hypoxia but was related to diminished food intake. Once the inhibitory effect of a down modulator (hypoxia) was removed, increased liver and kidney growth appeared to be associated with increased food consumption. On the other hand, following recovery, the weights of lung, heart and spleen either decreased slightly or remained unaffected in hypobaric hypoxic and normobaric hypoxic animals. This suggests that after removal of the growth stimulus (hypobaric hypoxic or normobaric hypoxic stress), augmented level of lung, heart and spleen growth was no longer maintained. This may have occurred due to an immediate fall in increased cellular proliferation and/or connective tissue protein accumulation during recovery.

We observed that after 3 day recovery, heart weight reached the general control levels while lung weight remained high. Our findings matched with those made by Okubo and Mortola (197) who found that in newborn rats, an exposure to 10% FIO_2 for six days produced changes in ventilation, oxygen consumption, hematocrit, body weight, heart, and specific lung weight. After returning to room air for one week, hematocrit and heart weight were back to control values but lung weight and ventilation were not. The authors suggested that differences in heart and lung growth recovery may have occurred due to long-term effects on the regulation of breathing. In addition, heart growth adaptation in hypoxic conditions primarily occurs by hypertrophic changes in myocardial fibers (191) whereas we found that lung growth occurred both by hyperplastic and hypertrophic changes. This may be the reason that heart weight returned to normal levels within three days of recovery period and

lung weight did not.

It has been shown that normobaric hypoxic exposure for 6 days produced permanent changes in neonatal rat lungs as lung weight, lung volume and mean chord length of alveoli remain increased after 44 days of recovery (198). However, the authors found that somatic growth recovery was complete. In the present study, ^{3}H-TdR incorporation into the lung tissue decreased during the three day recovery period in hypobaric hypoxic and normobaric hypoxic animals indicating that lung growth was no longer stimulated after removal of stimulus. During recovery, in hypobaric hypoxic and normobaric hypoxic rats, the RNA content fell, and RNA/DNA ratio decreased and reached the control level. These findings suggested that enhanced lung growth that occurred by continued stimulation, returned immediately to normal levels in some respects (i.e. cell size, DNA synthesis) when the hypoxic stress was discontinued. However, increased lung growth dimensions (i.e. lung weight, DNA, protein) did not appear to decrease, thus the residual effect of hypobaric hypoxia or normobaric hypoxia may be permanent. We did not study long-term recovery and it is possible that unlike the heart, structural changes in the lungs which occur as a part of the adaptive response to hypoxic stress may be permanent or require a prolonged period to recover.

4.6. RECAPITULATION AND CONCLUSIONS

It has been postulated that besides other factors, oxygen plays a substantial role in determining growth and development of the lungs as gas exchange is the primary function of the lungs. Lower oxygen tension has been shown to stimulate lung growth while somatic growth is diminished. However, results are controversial. Because the reduction in somatic growth occurs due to undernutrition, it is important to know the effect of impaired nutrition on the adaptive growth response in lungs or other organs in hypoxic conditions (hypobaric hypoxia and normobaric hypoxia) and for the first time it has been assessed systematically. Oxygen tension at high altitude is determined mainly by a decrease in ambient pressure, but the role of low ambient pressure in the absence (hypobaric normoxia) or presence of hypoxia (hypobaric hypoxia) on lung (or any other organ) growth adaptation has not been investigated. Lung connective tissue proteins which may alter in contents following exposure to hypobaric hypoxia and normobaric hypoxia have not been quantified before. The present study also provided us the opportunity to compare the adaptive lung response to normobaric hypoxia and hypobaric hypoxia which has not been done so far. Furthermore, the influence of normobaric hypoxia and hypobaric hypoxia on lung growth variables including biochemical (both cellular and connective tissue proteins), morphometric, cytokinetic and physiological parameters have not been studied in a single experiment.

We found that stunting of somatic growth (body weight and axial skeleton) in both hypobaric hypoxia and normobaric hypoxia occurred due to diminished food consumption which resulted mainly due to decreased oxygen. Low ambient pressure alone also reduced body size but this reduction was less than that occurring in hypobaric hypoxia or normobaric hypoxia. Hypobaric hypoxia and normobaric hypoxia produced substantial increases in hematocrit. Despite retarded somatic growth in hypobaric or normobaric hypoxia, the growth of lungs, heart and spleen increased whereas in the weight-matched animals their growth decreased. This indicated that growth of these organs increased profoundly in hypobaric and normobaric hypoxia when compared to weight-matched animals. The effect of hypobaric hypoxia and normobaric hypoxia on organ growth was the same, suggesting that

augmented growth of lungs, heart and spleen at high altitude is induced by low oxygen.

Undernutrition not only diminished somatic growth but also adversely affected lung growth because lung weight and volume, and all quantitative biochemical and morphometric parameters were decreased. DNA synthetic activity was inhibited by undernourishment in all the compartments of the lung. Despite reduced lung growth, pressure-volume and flow-volume characteristics remained unchanged in undernourished animals, indicating that the structural integrity of the lungs was not disturbed by undernutrition.

Since there were no visible differences in the structure of the lungs of undernourished and general controls, we can virtually rule out the effect of malnutrition at least in our hypobaric and normobaric hypoxic animals. Therefore, the changes occurred in lung structure and function in hypobaric hypoxia or normobaric hypoxia reflected the specific effect of respective environment. Adaptive lung growth response was similar in most aspects in hypobaric hypoxia and normobaric hypoxia compared to weight-matched controls.

- Striking increases in lung weight and volume were indicative of considerable lung growth in hypobaric hypoxia and normobaric hypoxia
- Increases in DNA indicated hyperplastic changes while the increase in RNA suggested enhanced protein synthesis. Cell size increased during the first week of exposure but reached normal levels by the end of exposure.
- Radiochemical analysis showed that maximal lung growth stimulation occurred on day 3 of exposure as ^{3}H-TdR incorporation into DNA increased 12-fold. Thereafter, the rate of ^{3}H-TdR incorporation fell gradually till day 7 but remained high until the end of exposure suggesting low but persistent cellular proliferation.
- Connective tissue protein accumulation was substantial, but an increase in collagen was less than cellular proliferation in normobaric hypoxia. On the other hand, the accumulation of elastin was higher relative to DNA in hypobaric hypoxia suggesting a shift in connective tissue protein dynamics.

- Airspace dimensions increased and surface complexity decreased more in normobaric hypoxia than hypobaric hypoxia. Alveolar enlargement was thus a more prominent feature in lung growth adaptation in normobaric hypoxia compared to hypobaric hypoxia. Furthermore, alveolar surface area and total alveolar number increased more in hypobaric hypoxia than normobaric hypoxia, thus indicating that lung growth occurred by addition of new structural units along with alveolar enlargement.
- Autoradiographic results showed that a high proportions of all endothelial cells (alveolar walls, or arterial) incorporated ^{3}H-TdR on day 3 of exposure. The rate of DNA synthesis of peripheral type II pneumonocytes, interstitial cells and unidentified cells, and mesothelial cells peaked on day 3 but the central cells (type II pneumonocytes, interstitial cells and unidentified cells) showed maximal stimulation on day 5. This suggested that endothelial cells stimulation may be secondary to hemodynamic changes but other cells react to the direct effect of the stress. In addition, the peripheral part of the lung responds earlier than the central part and this variation may be due to geometrical considerations. These are also new findings.
- The connective tissue framework of the lung was not affected by hypobaric hypoxia as pressure-volume characteristics and static lung compliance remained unchanged. Upstream airway resistance increased and expiratory flow rates at higher volumes decreased more in normobaric hypoxia than hypobaric hypoxia. This evidence combined with higher cell proliferation in the alveolar wall than conducting airways suggested dysanaptic lung growth during acclimatization to hypobaric hypoxia and normobaric hypoxia.

With regards to adaptation to hypobaric hypoxia and normobaric hypoxia, there was a clear indication of enhanced lung growth compared to general control animals. Absolute lung weight and volume, DNA, RNA, total protein and elastin increased in normobaric hypoxia. In addition, hydroxyproline and alveolar surface area also increased in hypobaric hypoxia. In addition to the above physiological changes (compared to weight-matched controls), elastic lung recoil at higher volumes in normobaric hypoxic rats decreased which may be attributed to alterations in tissue forces. The

similarities in lung growth response in hypobaric hypoxia and normobaric hypoxia provided evidence that low ambient oxygen is the main driving stimulus to enhance lung growth at high altitude.

In some aspects, lung growth differed in hypobaric hypoxia compared to normobaric hypoxia. Hydroxyproline and desmosine increased more in hypobaric hypoxia than normobaric hypoxia. With regards to morphometric analysis, airspace size and average volume of alveoli were smaller and the number of alveoli per unit volume was greater in hypobaric hypoxia demonstrating that surface complexity of the lung may have increased in hypobaric hypoxic animals compared to normobaric hypoxic animals. Physiological measurements (flow rates, upstream airway resistance) decreased less in hypobaric hypoxia than normobaric hypoxia. One can infer from these differences that lung growth diverged less in hypobaric hypoxia than in normobaric hypoxia. These differences may also be attributed to low ambient pressure in hypobaric hypoxic conditions.

In hypobaric normoxic animals, hematocrit increased. Apart from decreased lung volume, lung growth was similar to general control animals. DNA synthetic activity doubled on day 5. Autoradiographic results showed that the endothelial cells showed a minimal increase in ^{3}H-TdR incorporation and since there was a smaller increase in hematocrit suggesting that it is possible that endothelial cell stimulation depended upon alterations in hemodynamics. The size of airspaces was small and a greater number of alveoli were present per unit volume indicating increased surface complexity of the lung. Specific total protein, alveolar surface area and total alveolar number increased. Lung structure was not disturbed as lung function tests did not show any alterations following chronic hypobaric normoxic exposure. In addition to the differences noted from comparison of hypobaric hypoxia and normobaric hypoxia, the alterations caused by hypobaric normoxia suggest that, although low ambient oxygen is the major determinant of lung growth adaptation at high altitude, low ambient pressure also plays a role. Connective tissue protein accumulation increased and dimensions of morphometric unit structures remained smaller in hypobaric hypoxia than normobaric hypoxia.

Finally, specific lung growth, DNA, alveolar surface area and total alveolar number increased

in weight-matched animals compared to the general controls. Since exposure to hypobaric or normobaric hypoxia results in somatic growth reduction, from our results it was evident that comparison made for specific variables to assess lung growth adaptation in hypobaric hypoxic and normobaric hypoxic conditions or at high altitude may provide an overestimation of the lung growth response in such conditions.

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