THE UNIVERSITY OF MANITOBA

INFLUENCE OF MATERNAL LUNG GROWTH DURING PREGNANCY ON GROWTH AND DEVELOPMENT OF FETAL LUNG

BY

MOHAMMAD REZA SANII

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ABSTRACT

Knowledge of factors controlling the development of the prenatal and postnatal lung is essential to formulate ways and means of correcting pulmonary problems arising from prematurity and lung growth retardation in newborns.

This study focuses on understanding the relationship between the maternal and fetal lung, and the influence the maternal lung growth during pregnancy might have on the fetal lung. Pregnant rats were used for this study. To stimulate maternal lung growth during pregnancy, rats were exposed to the following experimental conditions at different days of gestation: hypoxia (10-14% O_2) starting at gestation day (GD) 7 or GD 14, cold (10°C) starting at GD 3 or GD 14, daily ten minute swimming exercise, and left pneumonectomy (PN) at GD 3, 7, 9, 12, 14, 16 and 18. In all conditions, the fetuses were harvested by caesarean section on GD 21 (term 22 days). Maternal lung growth was assessed by measuring the lung air volume (at 30 cm H_2O transpulmonary pressure), weight and DNA content. Liver, kidney and heart weights were also recorded. Fetal lung growth was assessed by measures of DNA content and total protein. Fetal lung maturity was determined by phospholipids (DSPC) and electron microscopic study. The results indicate that: (1) hypoxia, cold environment and partial pneumonectomy stimulate lung growth in pregnant rats, but swimming exercise does not affect the maternal lung (2) pregnant rats with large litter size have larger lungs than rats with small litter size (3) the maternal lung either was the only organ enlarged,

such as in pneumonectomized rats and in pregnant rats with a larger litter size, or was enlarged in conjunction with other organs (hypoxia) or as part of overall body growth (cold). (4) There is a direct relationship between cellularity (DNA content) of the fetal lung and maternal lung when the latter undergoes a growth change during pregnancy (PN and large litter size). (5) In no circumstances was a relationship in cellularity found between the maternal lung and placenta and between the fetal lung and placenta.

Fetal lung enlargement following pneumonectomy suggests that a humoral factor which stimulates the maternal lung cell proliferation has been released in maternal blood circulation and was able to cross the placenta and stimulate the fetal lung development simultaneously. It also suggests that this factor may be specific for the lung growth, since organs other than the lung (kidney and liver) were not affected. With fetal lung enlargement there was no enhancement of lung maturity which suggests that lung growth (cell proliferation) and maturity are two different phenomena, controlled by separate factors and that lung growth factor has no influence on lung maturity. The findings of the present study point out an important phenomenon: that the maternal organs (in this case, the lung) can influence the fetal organ and perhaps that the well-being of maternal organs during pregnancy is important. Furthermore, it has opened a new area in research concerning the growth of the fetal lung. The processes involved in regulating the organ growth through the mother is an exciting field of endeavour.

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CHAPTER I

LITERATURE REVIEW

(A) INTRODUCTION

The most critical moment in the life of a mammal is that of its birth. During gestation, organs develop to a functional and anatomical level where they are prepared for the abrupt transition from intrauterine life, during which time the fetus is sustained by the mother, to extrauterine life when survival depends on the neonate's own vital functions. The most urgent demand to be met at birth is the effective exchange of gases, therefore, the lung plays a major role in the infant's adaptation to extrauterine life. Anything which adversely affects the ability of the lung to function is a threat to survival.

Most of the lifelong neurological damage suffered by humans occurs during the perinatal period and the major cause is asphyxia. Asphyxia is caused by several diseases, one of which is the respiratory distress syndrome (RDS). RDS results in 10-15% of perinatal and 20-35% of neonatal deaths. Children who survive severe RDS show an incidence of neurological impairments much greater than the normal population.[1]

Prematurity is the major cause of RDS and neonatal death. Without adequate time to develop, a premature individual possesses lungs which have inadequate surface area, insufficient vascularization and poor metabolic capability to sustain ventilation and oxygenation during the neonatal period.

Knowledge of the anatomical, physiological and biochemical development of pre- and postnatal lung becomes essential in order to assist the infant who is born prematurely or one who is born

with structural or functional lung impairment. The factors affecting these aspects of lung development are poorly understood but a number of experiments indicate that there may be a close correlation between fetal growth and maternal hormones, nutritional state and environmental situation.[2,3,4]

This study was undertaken to better understand the relation between maternal and fetal lung. In an attempt to understand the physiology of lung development in both fetuses and adults, a pregnant animal model was used to study this relationship. Alteration or stimulation of maternal lung growth during pregnancy at different stages by changing the metabolic rate, environmental O_2 concentration or surgical removal of part of the lung and its effects on the fetal respiratory apparatus is a reliable model for this study. Although a large body of information is available regarding lung growth at different body states and environmental conditions in adults, little information is available to show the fetal-maternal lung relationships. The present study not only can provide information to fill this gap in lung biology but also is a good physiological model that can be used to study the factors which can control the lung growth and development.

(B) PRENATAL LUNG GROWTH

(1) Lung Development in Utero

Growth of the lung begins when the pulmonary rudiment forms as an out-pouching of the foregut in the 24-26th days of the human embryo and 9-12th days of the rat fetus. Growth of the primitive lung bud forms a tree-like tubular structure embedded in mesenchymal tissue. These tubular structures are lined with a single layer of epithelial cells having a tall columnar shape. The epithelial cells at the distal ends of the tubes undergo structural changes and differentiate into two types of cells: Type II cells containing lamellar inclusion bodies and Type I cells which are flatter, have cytoplasmic extensions and form the major part of the alveolus. The differentiation between Type I and Type II cells begins at late gestational age, for example gestation day 27 in rabbit (term 30 days), 110 in sheep (term 150 days) and 190 in human (term 270 days).

Although the original source(s) of pulmonary tissue is a point of contradiction between investigators, it seems that most agree that the epithelial cells of both airways and alveoli originate from the epithelial buds derived from the foregut. Emery[5] claims that the epithelial cells are responsible for the bronchial lining, alveolar membrane and mucous glands and that mesenchyme is responsible for the cartilage, muscle, elastic tissue and lymphatic tissue of the lung. Alescio[6,7] believes that the growth rate of the epithelial cells in culture media in the presence of mesenchyme is enhanced, as measured by the mitotic index. He con-

cluded that mesenchyme in the early stage of lung development has more than just a structural role.

The pattern of formation of the respiratory system has been divided into four periods in humans:[8,9]

(a) Embryonic or glandular period

This period encompasses the first 7 weeks after ovulation, and it includes the earliest phases of lung development mentioned above. The growth and development of the pulmonary blood vessels is closely linked to the bronchial tree and by the 3rd week of gestation the primitive heart, along with its systemic arterial and venous communications, is present.

Pseudoglandular period

This period extends from the 52nd day to the end of the 16th week. Growth of bronchopulmonary segments continues by dichotomous branching of lung buds forming a structure lined by cuboidal epithelium similar in appearance to a gland. By the end of this period all axial generations of the bronchial tree (i.e. the non-respiratory portions) have been formed. By the 7th week the adult pattern of the pulmonary circulation linking the heart and lungs is established as well as the ductus arteriosus. From the 7th to the 16th week the main feature of arterial growth is an increase in number of branches. By the 16th week virtually all pre-acinar vessels are present. These vessels grow further by increasing their length and diameters but new pre-acinar vessels do not appear after this time. New vessels

appear only in the intra-acinar region.

Canalicular period

This period (17-26 weeks) is characterized by the delineation of the pulmonary acini and the initial invasion of their peripheral branches by capillaries. Differentiation of the airways begins with widening of lumina and gradual thinning of the epithelium, giving the lung the appearance of canals. A rich vascular supply occurs and a relative decrease in mesenchyme brings the capillaries closer to the airway epithelium. Primitive respiratory bronchioles begin to form, thus delineating the gas-exchanging portion of the lung including the respiratory bronchioles, alveolar ducts, sacs and alveoli. Another important feature of the period is the synthesis of lecithin resulting in the formation of surfactant which lines the air spaces and which, at birth, decreases tension at the air-liquid interface facilitating expansion of the lung.

Terminal sac stage (26th week to term)

This period is characterized by further differentiation of the respiratory region of the lung with saccules having thin septae becoming prominent and leading to a marked increase in the internal surface area of the lung.

The glandular, canalicular, and terminal sac stages of human fetal lung development also occur in the rat. The glandular stage is the most extensive period of intrauterine lung growth and continues until the end of gestation day 19. The canalicular stage

occurs between day 20 and 21 of gestation and the appearance of surfactant in the fetal lung fluid between day 20 and 21 indicates that alveolar cell differentiation has occurred and Type II cells have begun secreting surfactant. Between days 21 and 22 the third stage of lung development occurs.

Analysis of fetal rat lung DNA at 24[10] and 48 hour intervals[11] from the 16th day of gestation through birth indicates that there is a period of rapid pneumocyte proliferation which begins on day 17, continues through day 20 and then slows down on day 21. In the 24 hours preceding birth, there is little change in lung cell numbers. This agrees with structural information which has shown that the number of recognizable Type II cells increases rapidly from days 20 to 21 but that there is little or no change in their concentration during the last 24 hours before birth. The change in rate of increase of DNA content on day 21 shows that cell proliferation is decreasing while cytodifferentiation[11] increases producing biochemical and functional differentiation as well.

The rapid accumulation of glycogen during the proliferation period, its peak on day 20 and subsequent disappearance from the lung are important features of differentiation of the fetal rat lung.[10,11] Since the loss of the lung glycogen occurs at the time when: (a) lecithin is being rapidly synthesized, (b) lamellar bodies accumulate in Type II cells, and (c) DPL is increasing in concentration; it is believed these events are related.[11] Because of this, it is thought that a significant portion of fetal lung glycogen is utilized for the initial synthesis of surfactant by

Type II cells.[10]

(2) Fetal Lung Maturation

The lung is considered to be mature upon formation of lamellar inclusions in Type II cells and the production, storage and release of surface active material in the alveolar space. Surface active material, by reducing the surface tension in alveoli, helps them to open more easily, uniformly, and remain stable. Babies born prematurely develop respiratory distress syndrome (RDS) and hyaline membrane disease. Avery[12] noticed that the surface behaviour of lung extracts obtained from infants with RDS and infants smaller than 1200 g is different from that of infants dying from causes other than respiratory distress syndrome. Following a foam stability test, Pattle[13] suggested that bubbles from the lung extract are covered by a material which causes an extremely low surface tension and absence of this material in the lungs of premature infants has a role in atelectasis and hyaline membrane disease. Clements and Brown[14,15] found that the tension of the surface film from the lung was high when the surface was stretched, but when the surface area was decreased the tension fell.

The functional behaviour of surfactant is due to its unique composition. The composition of surfactant isolated by lavage from the lungs of several species varies from 53-93% lipid with the remainder being protein. The highest percentage of lipid is found in rabbit lung. Rats have slightly lower amounts than rab-

bits and human lung washings contain approximately 62% lipids.[16] The major portion of the lipids are phospholipids. Pulmonary surfactant also contains a lesser amount of cholesterol, neutral lipid, and other phospholipids such as phosphatidylglycerol, phosphotidylinositol, phosphatidylethanolamine and phosphatidylserine. Dipalmitoyl lecithin (DPL, dipalmitoyl phosphatidyl choline) is the predominant component, making up 88% of the total phospholipid fraction of surfactant.

Lecithin is composed of a 3-carbon glycerol backbone with the C-1 and C-2 carbon atoms esterified to fatty acids; this portion of the molecule alone constitutes 1,2-diacylglycerol or a diglyceride, the third alcoholic carbon is linked as a phosphate ester and the latter is in turn esterified to a quatenary amine choline. Two pathways proposed for denovo lecithin biosynthesis are: (a) a choline incorporation pathway in which choline is phosphorylated by choline kinase and subsequently activated by choline phosphate cytidyl-transferase. Finally, lecithin is formed by choline phosphotransferase,[17] (b) a methylation pathway, in which ethanolamine undergoes similar steps of phosphorylation, activation, and linkage to diglyceride to form phosphatidylethanolamine. It then undergoes three successive methylations, with S-adenosyl methionine serving as the methyl donor, to form lecithin.[18] The choline incorporation pathway is the major pathway responsible for nearly all de novo surfactant lecithin synthesis in the lung. In the fetal rat lung, lecithin is synthesized almost exclusively via this pathway.[19] Evidence indicates that lung lecithin is also formed by acylation of 2-lysolecithin.[1,20]

(3) Factors Affecting Fetal Lung Maturation

Maturity of the lung can be controlled by pharmacologic agents and hormones which can affect the synthesis and release of surfactant.

(a) Glucocorticoids

Glucocorticoids have an effect on a variety of organs. Morphological maturation of the small intestine, the exocrine secretion of pancreas, as well as enzymes such as intestinal alkaline phosphatase content are stimulated by glucocorticoids.[1,21] Glucocorticoids also affect lung maturation.

Glucocorticoids stimulate alkaline phosphatase in the gut of the suckling mouse. Lung alkaline phosphatase, located exclusively in the Type II cells, is seen to increase with gestational age. This led Buckingham[22] to speculate that glucocorticoids might have a similar effect on developing lung since the lung, intestine and pancreas originate from the same embryological tissue.

Direct administration of dexamethasone, ACTH or corticosterone to the fetal rabbit accelerates pulmonary maturation. This has been assessed by electron microscopic studies which revealed maturation of Type II cells by abundant lamellar inclusion bodies one or two days early compared with control.

Decapitation of fetal rabbits showed delayed appearance of lamellar bodies in Type II cells.[23] This delay could be reversed by administraon of steroids at the time of decapitation.

Besides increasing the number of Type II cells and the lamellar inclusions,[24-27] glucocorticoids also accelerate maturation of the surfactant system by increasing phospholipid synthesis. Ballard[28] and Giannopoulos[29] showed that lungs of fetal rat and rabbit contain a specific receptor system for glucocorticoids which is present in the cytoplasm of the Type II cells. The glucocorticoid-receptor complex enters the nucleus of the cells and initiates the synthesis of RNA which ultimately directs the synthesis of enzymes involved in the production of phospholipids such as choline phosphotransferase.[30,31] However, Smith[32] believes that glucocorticoids can accelerate the maturation of Type II cells through their effect on lung fibroblasts. The fibroblasts secrete a factor which, when injected, can stimulate Type II cell maturation in fetal rats.

Glycogen deposition will be potentiated under the influence of glucocorticoids in early gestation but at the end of gestation glycogen depletion will be enhanced.[33,34]

(b) Catecholamines

The catecholamines are also agents which can be considered as stimulators of lung development.

Evidence that catecholamines enhance the anatomical development and secretory process of alveolar Type II cells includes: (a) an increase in catecholamines during birth in blood circulation, (b) increase of pulmonary fluid and surfactant release by administration of epinephrine,[10,35] (c) light and electron microscopic studies of lung tissue after pilocarpine administration and

(d) advanced lung maturation estimated by L/S ratio of amniotic fluid after injection of catecholamines in fetal body.[36]

(c) Thyroid hormone

Since thyroid hormones have an important role in lipid metabolism, many investigators examined the effects of these hormones on regulation of surfactant metabolism and lung maturation.

In humans, thyroid hormone levels in cord serum from term and premature newborns as well as in fetuses of gestational age 33 to 37 weeks showed a progressive elevation of serum thyroxine occurs between 32 weeks gestation and term. Newborns with RDS had significantly lower cord concentrations of triiodothyronine and thyroxine.[37,38] Redding et al.[39] reported that L-thyroxine injected into adult rats increased the number and size of lamellar bodies as well as the amount of surfactant recovered by alveolar washing. Wu et al.[40] found that thyroxine injected into rabbit fetuses at 24 days of gestation resulted in accelerated maturation of the surfactant system 2-3 days later, as judged by the increased number of lamellar bodies and an increased bubble stability ratio on alveolar washing. These results led Redding and Wu to believe that thyroxine might be an important hormone for lung maturation. However, Adamson and Bowden[41] failed to show such an effect. Using isolated Type II cells in cell culture, Sommers[42] found stimulation of surfactant phospholipid synthesis with thyroxine. Smith and Torday[43] found that thyroid hormones stimulated choline incorporation into phosphatidyl choline in fetal lung cell cul-

tures. However, Mason et al.[44] found no change in the disaturated phosphatidyl choline content of whole lung lipids with thyroxine treatment. Thyroxine analog (Dimit) injection in pregnant rabbits stimulated lecithin synthesis in fetal lung.[45]

Although these experimental results are contradictory, the presence of triiodothyronine receptors in rat lung nuclei[46] can be considered as strong support for a regulatory function of thyroid hormones on lung growth and maturation.

(d) Estrogen

Premature infants suffering from RDS have lower levels of estrogen in their first voided urine.[47] This suggests that estrogen may play a role in the prevention of RDS.

Administration of estradiol in pregnant rabbits increased the fetal acetylcholine content of the lung[48] and 17-beta-estradiol increased the total phospholipid and phosphatidylcholine by four times in lung lavage of neonates.

The influence of estrogen, especially 17-beta-estradiol on the synthesis of phospholipids by fetal rat has been examined in organ culture.[49] A significant increase in the rate of incorporation of choline into disaturated PC, as well as a doubling of the rate of incorporation of acetate into phosphatidylglycerol was found.

Specific receptors for 17-beta-estradiol in fetal lung[50] and enhanced activity of cholin phosphate cytyltransferase, lyso-PC acyltransferase, and glycerol phosphate phosphatidyltransferase, suggest that estrogen plays a role in lung maturation and in the

production of surfactant.

(e) Prolactin

The available literature cannot support a major role for prolactin on maturation of the fetal lung.

A sharp increase of serum prolactin prior to the rise in pulmonary surfactant has been documented in humans.[51] Hamosh and Hamosh[52] injected ovine prolactin into 24 day rabbit fetuses and saw a 67% increase in lung tissue PC two days after injection. These results led them to speculate that prolactin may trigger the synthesis of surfactant in fetal rabbits. Cox and Torday[53] have presented data indicating that exposure of fetal rabbit mixed lung cell cultures to prolactin increases incoroporation of choline into disaturated PC but not into total PC. Hauth[54] found decreased prolactin concentration in cord blood of infants with RDS. However, Ballard and associates[55] did not find any stimulation of synthesis of PC or surfactant release in the lung by prolactin in fetal rabbit and sheep.

(f) Insulin

It is well known that glucose can serve as a substrate for pulmonary lipid synthesis. It can be used for both the glyceride backbone and fatty acid moiety for surfactant phospholipids[56] or used as a source of energy necessary for phospholipid synthesis. It is also known that insulin regulates glucose metabolism in tissues. Therefore, its effect on lung maturation, specifically surfactant synthesis, has been hypothesized.

Specific receptors for insulin in membrane preparations of normal rat lung[57] suggest that insulin regulates the transport and utilization of glucose in the rat lung. Salisbury-Morphy et al.[58] studied the metabolism of glucose, palmitate and acetate by rabbit lung slices. They showed that lung slices oxidized glucose to CO_2 and converted glucose into glycerol and fatty acid moieties of glycerides. This oxidation was not affected by insulin. Results of Perez-Diaz et al.[59] demonstrated high glycolytic rates in suspended lung cells; but this activity was not affected by the presence of insulin. Weber and Visscher,[60] however, found an elevated glucose uptake by isolated perfused lung following administration of insulin. Stubbs et al.[4] perfused the rat lung with glucose with and without insulin. They found that physiologic concentration of insulin (50 u/ml) increased lactate production by 1.5-fold. On the other hand, when Neufeld et al.[61] exposed the lung slices to 100 u/ml of insulin, no change in glucose conversion to glycogen or lactate was observed.

Investigations by Moxley and Longmore[62,63] revealed that isolated perfused rat lung of experimentally-induced diabetic animals had a 60-80% decrease of (u-^{14}C)-glucose incorporation into neutral lipids and phospholipids of surfactant. The experiments of Smith et al.[64] with insulin and glucocorticoids led them to speculate that insulin antagonizes the glucocorticoid stimulation of lung PC synthesis in the fetuses.

Morphological changes have been studied in experimentally induced diabetic rats by injection of streptozotocin.[65] The most affected cells are Type II cells which show dilation of granular

endoplasmic reticulum but no change in the number of lamellar bodies.

Gross and Smith[66] explanted fetal lung at 19 days gestational age with and without insulin. The controls continued to mature showing larger alveolar spaces, less interstitial mesenchyme, decreased glycogen content and increased numbers of lamellar bodies. Insulin treated tissues had very tall alveolar lining cells filled with glycogen, more interstitial tissue and fewer lamellar bodies. This indicates that insulin delays the maturation of fetal lung. Further evidence of this is seen in fetuses of diabetic mothers. These fetuses are exposed to high levels of endogenous insulin because maternal glucose can cross the placenta but not maternal insulin. This high glucose level stimulates insulin production in the fetal pancreas. Because infants born to diabetic mothers have an incidence of RDS 5- to 6-fold greater than age and weight matched controls, the effect of the high insulin level is evident.

In summary, pulmonary carbohydrate and lipid metabolism are regulated at least partially by the action of insulin.

(g) Malnutrition

In malnutrition there is a reduction in available nutrients necessary for normal energy production and a decrease in availability of material for DNA and protein synthesis. It has been shown that there is a relationship between malnutrition and subsequent retarded lung development.

Animals subjected to postnatal malnutrition show a reduction

of up to 50% in both body and lung weight.[69] Malnutrition at early age (0-21 days) produced signs of irreversibly impaired cell division as assessed by decreased amounts of DNA. However, malnutrition at a later stage of growth (21-42 days) resulted only in reduction of cell size from which the animal could recover.[69] Lungs of rabbits fed with fats have a higher proportion of granular alveolar cells which contained greater numbers of inclusion bodies when compared with control.[70]

Gross et al.[71] studied the inhibition of enzymes involved in pulmonary fatty acid and phospholipid synthesis after acute nutritional deprivation in three week old rats. It was found that enzymes necessary for pulmonary surfactant production are reduced in activity from 36-55%.

Faridy[72] has studied the effects of maternal malnutrition on fetal lung growth and development of the lung surfactant system. He demonstrated that the fetuses of rats subjected to food deprivation, low protein or fat free diets were smaller and the lung weight/body weight ratios were less than control in all groups, but especially so in those treated at late gestational age (GD17-21). He also noticed a reduction in fetal lung DNA in all groups except those receiving the fat free diet which had reduction only in lung phospholipids. He concluded that the nutritional state of the pregnant rat influences the growth of the fetal lung and that maternal nutritional status should be maintained at a normal level when studying the effects of different factors on lung growth.

(h) Other Factors

A number of other factors can enhance maturation of the fetal lung.

Delivery by cesarean section will increase the risk of development of RDS.[20]

Rooney et al.[67] found that labor induced in rabbits by oxytocin administration increased phospholipid and choline phosphotransferase activity in lung lavage fluid. But, it is generally believed that the process of labor, rather than oxytocin, is the key factor in stimulating the synthesis and secretion of phospholipid in the lung.

Toxemia of pregnancy, and prolonged rupture of the membranes also enhance fetal lung maturation. Prostaglandins (E2 and F2) can also stimulate the incorporation of choline palmitate in lecithin.[68]

(C) POSTNATAL LUNG GROWTH

Some investigators believe that expansion of pre-existing cells is responsible for postnatal lung growth, while others suggest that multiplication and enlargement occur in the lung in the same way as other organs.

Regardless of enlargement or cell proliferation, postnatal lung growth is characterized by formation of alveoli in humans. There are 20 million air spaces (saccules) at birth, reaching the adult value of 300 million by the age of 8 years. The alveolar

surface area increases from 2.8 m^2 to 32 m^2 in the same period and 75 m^2 by 25 years. The volume increases 28-fold from 200 ml at birth to 2.2 liters at 8 years and 5.5 liters at 25 years.

The pattern of body and lung growth has been studied in detail in rats by Thurlbeck.[20] He described three phases of general organ growth after birth:

1. From birth to 14-17 days of age organs grow primarily by cell multiplication.
2. From 5 to 7 weeks of age cell multiplication is slower than protein synthesis.
3. After 7 weeks of age when cell proliferation stops, or it is very slow, cell enlargement is slight or may even stop. The amount of protein per nucleus stays approximately constant as does the amount of DNA. Brain and lung show the largest increases in DNA in the early phase of growth compared with the other organs.

Postnatal development of the rat lung also follows this pattern.[8] The first phase, from day 1-4 comprises the phase of lung expansion. During this time the lung grows mainly by expansion, and morphometric studies have shown that little lung tissue is added. Lung volume increases less than body weight. Therefore, the lung weight to body weight ratio decreases at this time.

The second phase has been designated as the tissue proliferation phase and lasts from the 4th to the 13th postnatal day. Subdivision of primary saccules by secondary crests takes place and definitive alveoli are formed. Lung growth is relatively faster than the previous phase with lung volume increasing faster

than body weight so that the specific lung volume is increased. The secondary crests are characterized by an elastic fiber in their free margins, a single capillary layer and numerous interstitial cells. The elastic tissue plays a critical role in development of alveoli with each fiber demarcating the mouth of future alveoli. The precise process by which alveoli are formed is disputed.[73,74]

The third phase of growth, commencing at about the second week of age, has been termed the phase of equilibrated growth. The increase in lung volume slows down with a consequent reduction in specific volume and surface area per unit weight. New alveoli continue to be added and the interstitium matures. Secondary crests lengthen and a single capillary layer is found in the walls of air spaces. The rat lung has approximately 70 X 10^6 cells at birth, 200 X 10^6 cells at 14 days of age and 350 X 10^6 cells at 31 days.

The question of whether the development of the lung will stop at birth or continue during the postnatal period in human remains to be answered, because the results of the morphometric studies are contradictory. Many authors suggest that growth after birth is only enlargement of the pre-existing structures[75-77] while others believe that new respiratory units are formed during this period.[78]

Pulmonary cell turnover assessed by thymidine incorporation into DNA or autoradiography revealed a low degree of mitotic activity and slow turnover of the respiratory cells in mouse after birth.[78] In rats the number of labelled cells decreases in the

last day of gestation and the first 2 days of life with a subsequent increase by day 3.[79] Autoradiographic studies by Kauffman et al.[80] indicate that in rats the labelling index is the highest between days 4 and 7. Thereafter, it declines and reaches the adult level at 2 weeks of age.

When rats are sacrificed within hours of tritiated thymidine injection, labelling of only Type II cells is seen.[80] If the rats are allowed to live for several days after injection, labelling of both Type II and Type I cells is seen. This indicates that only the Type II cells synthesize DNA and incorporate tritiated thymidine. The Type II cells divide, producing both Type I and Type II cells. The Type I cells produced have nuclei which contain the tritiated thymidine incorporated by the progenitor cells.

(D) COMPENSATORY LUNG GROWTH

(1) Hypoxia

Since gas exchange is the main function of the lung, many investigators speculated that the environmental O_2 concentration can influence or even dictate postnatal lung development.

(a) Effect on Lung Growth

The effect of hypoxia on lung volume and weight is not quite clear because of contradictory results in the literature. This may stem from the fact that different techniques and

animals of various ages were used by investigators to assess the pulmonary effect of hypoxia.

Bartlett[81] exposed 3 groups of one month old male rats (body weight of 90-100 g) to 12 (hypoxia), 21 (control) or 50% (hyperoxia) oxygen for 15 days. He observed that the hypoxic group had heavier lungs with no change in lung volume, alveolar surface area or alveolar number when expressed per body weight in comparison with controls. In contrast, in hyperoxic group, lung weight, volume, alveolar surface area and alveolar number decreased by 28 to 35%. Because the lungs were heavier without morphometric changes, he suggested that the elevation of lung weight in the hypoxic group was due to the increased lung blood volume resulting from hypoxia.

Animals living at high altitudes, such as Peruvian domestic sheep and guinea pigs have morpholigically the same lung as sea level animals for the same body size.[82] Cunningham[83] exposed newborn, 3 and 9 week old rats to 12.5% oxygen for 21 days, and showed that body, liver, kidney and spleen weights decreased but the heart and lung weights increased in all hypoxic animals when compared with body-size matched controls. Morphometric studies revealed that the newborns had an increase in both the size (15%) and the number of alveoli, while the adults responded to hypoxia only by an increase in the size (20%) of alveoli. Further exposure beyond 21 days did not cause additional changes in the lungs. One of the interesting points in Cunningham's report is the effect of hypoxia on the body length. Although the body weight was less than control in all groups, the body length was the same for all,

meaning skeletal size is not influenced by hypoxia.

Similar results were obtained by morphometric analysis of light and electron micrographs.[84] It was shown that varying O_2 tension had a significant effect on lung growth in young rats. Hypoxia increased the specific lung volume and specific alveolar surface area by 20% and these parameters were depressed by 16% with hyperoxia.

Pepelko[85] examined the effects of O_2 and CO_2 separately and combined on rat's body, lung, spleen, heart, kidney, liver and adrenal weights. He found both hypercapnia and hypoxia can impair body growth and this impairment can be intensified by a combination of both. He also noticed greater lung, heart and spleen weights as a result of hypoxia but this increase was unaffected by concomitant hypercapnia.

(b) Vasoconstriction as a Result of hypoxia

Arterial vasoconstriction during acute alveolar hypoxia is a well known phenomenon which may have an effect on lung perfusion. The mechanisms involved in this activity have been the focus of intense study.

Lloyd[86] examined the effect of PO_2 and pH on muscle strips from the fourth and fifth generation of pulmonary arteries *in vitro*. He was not able to find any evidence that hypoxia is responsible for increased excitability or contractility of the muscle. This led to speculation that a mechanism within the lung itself controls vasoconstriction during hypoxia.[87] This hypothesis

of local hormone involvement was tested by antihistamine (histaminase - inhibiting compound) to abolish and potentiate the hypoxia response in absence and presence of histamine. A role for histamine was successfully demonstrated.[87]

Since the mast cells are a known source of histamine, Haas and Bergofsky[88] examined the extent of granulation of mast cells located around the vessels following hypoxia. They found a markedly increased degranulation of mast cells and a positive correlation between the amount of histamine released during hypoxia and vasoconstriction.

It has been suggested that there are chemoreceptors located in the parenchyma of the lung.[89] These receptors could be the pulmonary mast cell which might respond to alveolar hypoxia by releasing histamine and causing vasoconstriction.

In spite of these results, several investigators have been unable to demonstrate such a relation between histamine release and hypoxia. For example, blood samples collected from the pulmonary artery and left ventricle of anesthetized dogs after 10 minutes of hypoxia showed no difference in histamine concentration.[90] Dawson et al.[91] believed that histamine is not necessary for hypoxic vasoconstriction. Finally, Tucker et al.[92] looked at the mast cell distribution in several species following hypobaric hypoxia. They found that the only species which responds to high altitude by mast cell hyperplasia was calves. The responses of pig, rat and sheep were not significant.

It has been accepted as a fact that pulmonary vasoconstriction takes place in the presence of alveolar hypoxia. However,

the precise role of histamine and other substances such as Ca^{++}[93], adenosine,[94] ATP and ADP and hypothermia[95] in this response is still unclear.

(c) Histological Changes in Heart and Vessels

The effects of hypoxia in causing right ventricular hypertrophy and an increase in thickness of pulmonary blood vessels has been widely demonstrated. Abraham et al.[96] observed a progressive rise in right ventricular pressure that was in close proportion to the progressive increase in ventricular hypertrophy and development of muscular media between internal and external elastic lamina in pulmonary arterioles and arteries. Other experiments[97] with adult rats indicated right ventricular hypertrophy and thickening of the pulmonary trunks, but no change at arterial or arteriolar levels. Hypoxia does not cause ventricular hypertrohy in all species - Tucker et al.[92] found a negative result for dogs.

Smith et al.[98] pointed out rats of different sex and age show different responses to hypoxia. Although both adult and young are inactive and tachypnoeic during the first day of exposure, the adult can adjust very quickly, but young rats continue to have tachypnoea for 4 days and only recovered after day 7 of exposure. More pronounced right ventricular hypertrophy was found in the adult males, but more muscularisation was noticeable in the pulmonary arterial tree in adult females. Pulmonary trunk medial thickness has no relation with age or sex.

People living at high altitude who suffer from so-called chronic mountain sickness (Monge's disease) and patients with hypoxia due to chronic bronchitis and emphysema have clinical, physiological and anatomical disorders very much like experimental animals exposed to hypoxia.[99,100] The ventricular hypertrophy and pulmonary vascularization have been shown to be reversible in both patients after treatment with oxygen[101,102] and high altitude natives after living at sea level for some time.[103,104]

Williams et al.[105] tried to explain the mechanism of pulmonary hypertension by hypothesizing a relation between pulmonary hypertension and mast cell hyperplasia. They observed a high density of these cells around the pulmonary blood vessels and alveolar septae of acutely hypoxic rats. Mungall[106] demonstrated differently by showing that in hypoxic rats proliferation of mast cells does not occur until 3 weeks of exposure but right ventricular hypertrophy is noticeable after only 14 days.

One might expect that vasoconstriction during hypoxia will decrease the efficiency of the lung for gas exchange because of elimination of some surface area and decreased diffusion capacity. Wagner et al.[95] observed more capillaries were perfused during hypoxia and Dugard[107] showed redistribution of blood flow from the bottom to the top of the lung by hypoxia. This indicates that derecruitment is taking place at the bottom of the lung and recruitment occurs at the top of the lung.

Carbon monoxide[108] diffusion capacity also increases during hypoxia. This is also due to recruitment. The recruitment is a result of an increase of pulmonary vascular resistance. This is

illustrated by administration of a vasodilator substance which causes derecruitment and redistribution of the blood toward the bottom of the lung. Measuring diffusion capacity by morphometric technique[84] indicates there is an increase of 0.12 ml/min in hypoxia compared with controls and a decrease of 0.01 ml/min in hyperoxia. Therefore, there is no loss of surface area for gas exchange during hypoxia.

(d) Development of pulmonary edema in hypoxia

People ascending rapidly to a high altitude or animals kept in hypobaric hypoxic chambers develop a syndrome known as mountain sickness. In humans, the symptoms of this are dry cough, dyspnoea, palpitations, headache and vomiting. Both humans and animals show signs of cyanosis of lips and nails and finally development of respiratory distress. One of the more obvious clinical features of mountain sickness is pulmonary edema. After description of mountain sickness by Hepburn[109] in 1895, many authors investigated the mechanism of edema incidence in such conditions. There are controversial results in the literature.

Ultrastructural changes which occur in capillary endothelial cells as a result of acute exposure to hypobaric hypoxia have been described as a possible mechanism of edema generation. Pulmonary capillaries of rats kept in a hypobaric hypoxic (265 Torr) chamber for 12 hours contain multiple extrusions filled with fluid extending into the lumen, sometimes large enough to occlude capillaries.[110] In contrast it has been reported that pulmonary arterial

hypertension as a result of hypoxic vasoconstriction is the cause of edema.[111] Since hypoxic vasoconstriction does not increase the resistance to the blood flow as a result of recruitment of collapsed capillaries, one can assume that development of vesicles in the capillary lumen can be considered as the resistance site to the flow. Natives of high altitude are adapted to their environment and have no sign of pulmonary edema. But edema will develop in acclimatized subjects who live at high altitude and then return to the mountains after a short period living at lower levels.[112] Scott, Barer and Leach[113] exposed 3 groups of rats to hypoxia. Two groups were previously acclimated to low O_2 tension for 80 or 38 days. All 3 groups showed ultrastructural changes in the lung. The acclimatized rats developed interstitial but not intra-alveolar edema. The capillary endothelial cells and alveolar Type I cells showed evidence of micropinocytosis. The rats exposed without adaptation developed vesicles arising from endothelial cells in the capillary lumina of the alveolar walls but no interstitial or intra-alveolar edema.

From these reports, regardless of the mechanism or the site of edema, it is clear that accumulation of fluid in lung tissue following exposure to normobaric hypoxia or hypobaric hypoxia is taking place.

(e) Metabolic Changes in Hypoxia

(i) Protein

Generally, it has been accepted that oxygen consumption and basal metabolism in adult humans does not fall even in severe hypoxia.[114-117] In contrast, animals at high altitude have reductions of up to 20% in O_2 consumption.[118,119] Also, hypoxia retards the rates of growth and food consumption in animals. Exposing them to 9-11% oxygen for 3-6 weeks reduced the food intake up to 77% of the control value.[120]

Cheek et al.[120] exposed newborn (1-7 days of age) rats to 12% O_2 and examined the brain DNA and protein synthesis after 7, 21 or 35 days following exposure. Brain DNA and protein content remained constant, but there was a reduction in body weight, cerebellar weight, liver weight, muscle mass, muscle cell number and skeletal collagen by the end of 35 days. The RNA content was low in these organs which indicates that hypoxia prevents cell multiplication and impairs protein synthesis in young rats.

Since animals under malnutrition show the same degree of impaired protein synthesis, it has not been determined whether decreased food intake or hypoxia per se is responsible for the impaired protein synthesis.

Sanders et al.[121] described tissue ATP level reduction as the main factor responsible for retardation of protein synthesis in hypoxia. They demonstrated that ventilating adult rats with 5% O_2 for 105 minutes will reduce the brain and liver ATP by 20% and 40% of normal values respectively, but has no effect on kidney ATP.

Leucin - ^{14}C incorporation into protein was moderately depressed in brain and kidney but almost completely abolished in liver. It has been shown that hypoxia is associated with a marked reduction in hepatic artery blood flow and an increase in coronary as well as cerebral blood flow. Therefore, severe reduction of liver leucine incorporation and ATP explained by Sanders could be due to this alteration of blood circulation in hypoxic conditions.

(ii) Carbohydrate

Changes of carbohydrate metabolism in hypoxia appear as reduction of liver glycogen and blood glucose. A short term (few days) exposure of rats to hypoxia at 3800 meters (PO_2 approximately 107 torr O_2) above sea level produced a progressive decrease in liver glycogen content for 6 months, while blood glucose had returned to normal values by this time.[122] Blume and Pace[123] studied the time course of changes in liver glycogen and blood sugar as well as glycolytic activity in mice translocated from sea level to an altitude of 3800 meters. They found a 50% reduction in liver glycogen content and a 25% reduction in blood sugar values after 10 days (short term). Thirty days following exposure the conversion rate of labelled glucose to CO_2 was reduced but there was no change in glycerol, pyruvate, acetate and succinate conversion to CO_2. However, the total energy metabolism was found to be unchanged during the altitude exposure. Decreased rate of conversion of glucose to CO_2 led them to conclude that there is a hexose monophosphate shunt activity in hypoxia.

(iii) Lipids

Decrease in body weight under hypoxic conditions is mainly due to loss of body fat. Studies of the effect of hypoxia on the metabolism, absorption and catabolism of fat have suggested intestinal malabsorption of lipid[124,125] and anoxic inhibition of propulsive motility of the small intestine.[126] Klain et al.[127,128] measured serum total lipid, free fatty acids and phospholipids in a group of soldiers at 14, 100 feet (PO_2 approximately 89 torr O_2) altitude. They showed a rapid decrease of total lipids and cholesterol between 4 and 7 days, but phospholipids and free fatty acid levels were increased during this period, which indicates an increase of fat mobilization from depot fat. Aerobic and anaerobic in vitro studies of adipose tissue collected from rats which have been kept in a low pressure chamber (14,500 feet altitude) for 6 weeks revealed that there is a free fatty acid mobilization from adipose tissue.

An increase of both mobilization and utilization of fat has been confirmed by Blume and Pace.[123] They injected ^{14}C-labelled palmitic acid, DL-aspartic-^{14}C acid and DL-alcinine-1-^{14}C acid in mice, previously kept at an altitude of 3800 m for one month. One half hour, 1 hour and 2 hours after injection they found that the rate of oxidation of palmitate was elevated and increases occurred in the amount of labelled material in the liver, muscle and heart. Louhija[129] failed to see any significant increase in serum free fatty acid, phospholipids or cholesterol concentration, only the level of triglyceride was higher in hypoxia.

Since the lung uses plasma free fatty acids or fatty acids from plasma lipoprotein, hypoxia can impair lung lipid metabolism and surfactant synthesis. As Chander[130] pointed out, rats in hypobaric-hypoxic conditions have a reduction in lung triglyceride, total phospholipid and phosphatidyl choline. Oxidation of labelled palmitic acid in lung slices, plus esterification of this acid to form triglycerides, phosphatidylcholine and phosphatidylethanolamine is lower in hypoxic conditions[131] which indicates a reduction in utilization of fatty acids in such an environment.

(2) Body Metabolism and Lung Growth

According to Tenney and Remmers,[132] alveolar surface area is in direct proportion to O_2 consumption in adult animals. Therefore, if O_2 requirement is altered by any means, one should expect a proportional change in the respiratory system. In other words, alteration of metabolic rate can influence lung development.

It is well documented that the metabolic rate can be changed by exposing the animals to low ambient temperature or exercise.

(a) Cold Environment

When warm-blooded animals are exposed to cold, their response is to produce heat, which can be measured by their oxygen consumption.

Oxygen consumption for rats under normal or resting

conditions in 24°C is 13.5 ml/kg. min measured by the open-circuit[133] and 13 ml/kg. min measured by a closed-circuit method.[134] The oxygen consumption increased 2-fold at 5°C up to 27.5 ml/kg. min. Bartlett calculated 2.91 ml/min/100 g body weight for rats at room temperature.[135] Pasquis et al.[136] measured O_2 consumption at rest and maximum activity in different T° for rats, mice, hamsters and guinea pigs. They showed that rats acclimatized to 30°C had an O_2 consumption of 26.7 ml/min kg. Acute exposure of mice to 0°C for one hour increased the oxygen consumption by 82% in young (4-6 weeks old) and 57% in old (1 year) animals.[137]

(i) Lung Growth

Investigators have been working for years to find a relation between the increase of metabolism (or O_2 consumption) and growth of mammalian lungs.

Morphometric analysis of young rat lungs after three weeks exposure to 11°C was carried out by Gehr et al.[138] They demonstrated significant differences of air volume, capillary volume, alveolar surface area and pulmonary diffusion capacity as compared to control, even though the body weight was different from the control. The lung volume expressed per body weight was 26% greater in cold exposed rats. Since no changes have been found in alveolar and capillary surface densities, capillary volume density and the barrier thickness, they concluded that the size of structural units of the lung parenchyma (alveoli and capillaries) has

not changed but that their number has increased in proportion to lung volume.

Lechner and Banchero[139] studied lung morphometry in weanling guinea pigs exposed to various durations of cold (5°C). They showed an accelerated increase in lung volume (27%) alveolar surface area (29%) and capillary endothelial surface area in animals up to 600 g body weight. Once the body weight exceeds 700 g, continuation of cold exposure has no effect on lung parameters beyond predicted size for control. This means the role of cold environment is only to accelerate lung development in young animals toward normal adult dimensions.[139]

(ii) Thyroid Hormone and Cold Exposure

Increases of thyroid hormone levels in blood circulation during the metabolic adaptation to cold have been demonstrated directly or indirectly by several laboratories. Increase of these hormones not only has been tested in experimental animals but in humans as well.

Healthy young men between 23 and 28 years of age have been exposed to 6.6°C and assessed for T3 and T4 levels. Triiodothyronine (T3) concentration was increased 14% on day two and thyroxine was elevated by 24% on day 4 of exposure. Both T3 and T4 return to normal levels within 48 hours following removal to a warm environment.[140] Raud and Odell[141] measured TSH in 12 subjects during 7 days exposure to cold. TSH rose and reached the maximum level in day 3 of exposure which shows that there is a

close correlation between TSH and T3, T4 secretion during the cold adaptation period. Since TSH secretion by the pituitary is under the control of the hypothlamus through thyrotropin releasing factor (TRF),[142-144] the hypothalamic-pituitary-thyroid axis should be involved in hormone release in cold conditions and this has been shown in rats exposed to 4°C.[145]

Cold adapted rats which have been kept at 4°C for 3 months have a thyroid iodine uptake and hormonal secretion rate 3-fold more than control.[145] However, there are contradictory reports on the role of the thyroid in cold. Thyroid hormones have been postulated to be increased to compensate the increased loss of these hormones through the GI tract of the cold adapted rat[146,147] as demonstrated by direct measurement of the amount of thyroid hormones in tissue, serum and excreted urine and feces.[148]

Balsman and Sexton[149] have shown that both urinary and fecal clearance of labelled hormones were elevated in rats after 2 weeks at 4°C. They also noticed intracellular iodothyronine and radioiodine concentrations were reduced in liver and kidney 24 hours after injection of tracer hormones. These results led them to conclude that disappearance of hormones from plasma, tissue and total body pools are due to increased hormone deiodination and fecal disposition.

Despite the well established fact that deiodination of T4 and T3 by hepatic and kidney cell homogenates are increased in cold adapted rats,[150,151] the mechanism of this conversion is uncertain. The role of sympathetic activity in increasing T3 was negative[152] and increased beta-adrenergic activity has no effect in

this proces.[151] However, Storm[153] and Benedict[154] showed that not only T3 and T4 are increasing in rats at 4°C, but plasma noradrenaline concentration has a synchronized elevation as well, whereas no change was distinguished for adrenaline. NA infusion in animals via the jugular vein for 7 days significantly increased both T3 and T4. The interrelation of thyroid hormones and catecholamines provide evidence to believe that T4 metabolism is under the control of the sympathetic nervous system.

Cold influence on thyroid activity may be considered as a possible mechanism for lung structural changes and DNA synthesis. Exposure of rats to 4°C causes an increase of TSH by 2-fold over control value,[155] which stimulates the release of thyroid hormones. Daily injection of 3.5-triiodothyronine (T3) for 28 days results in 30% increase of DNA, 125% increase of dry weight, surface area, lung volume and alveolar number.[156] On the other hand, administration of an antithyroid drug (1-methylimidazole-2-thiol) had no effect on lung structure or DNA synthesis in hamster lung. Administration of thyroid hormones (T3) can increase the metabolic rate and O_2 consumption by 26% above control which is similar to that of cold exposure (4-5°C). Furthermore, there is also a close similarity in other parameters such as alveolar surface area (24%), lung volume (30%), alveolar number (21%) and DNA (30%) between the cold and T3 injected animals. Therefore, it is conceivable that the action of T3 might account for comparable effects of cold exposure and T3 injection. The lack of effect of the antithyroid drug suggests that the lungs grow to a certain size in spite of lower oxygen demands by the system.

(iii) Acclimation to Cold

It has been shown that chronic exposure to cold increases the ability of the subject to survive in an environment with a temperature that would be ultimately lethal.[157,158]

Hart[158] found no difference in O_2 consumption in 3 groups of mice that had been acclimated to 10, 20 and 30°C respectively. He then exposed these mice to -11° and -20°C. The mice acclimated to 30°C, showed a fall in O_2 consumption and died. Those acclimated to 20°C survived for a longer period but finally died as well. Those acclimated to 10°C had a high metabolism and survived for the duration of the experiment. This clearly indicated that the difference in survival at lethal temperatures is entirely due to differences in metabolic capabilities. The heat production by cold acclimated animals at any lower temperature is greater compared with non-acclimated animals. It usually takes 2 to 6 weeks for acclimation to be developed in most of the species studied. During this period there is a gradual increase in cold resistance, food intake and basal metabolic rate.

Sellers et al. in 1951 published a series of papers in which they studied the possible mechanism of acclimatization to cold.[157,159] Adrenalectomy or thyroidectomy was performed in rats which had been acclimatized to cold and room temperature. Exposure of these animals to cold showed a longer survival period for both operated groups which had been subjected to cold before operation. This result led Sellers to conclude that adrenocortical and thyroid hormones are essential for survival in a cold

environment and the amount of these hormones left in the body after removal of the gland is sufficient to supply the physiological requirement for survival for several days in cold. They were even able to increase the survival period in the cold indefinitely by injection of adrenocortical hormones or thyroxin in adrenalectomized or thyroidectomized animals.

Sellers et al. also examined the survival period in artificially acclimatized animals by injection of one of the following substances: cortisone, thyroxine, insulin, glucose, combined glucose and insulin, desoxy corticosterone acetate (DCA) or combinations of two of them. They found that acclimatization did not occur in any groups except those which received a combination of cortisone and thyroxin. This study indicated that in addition to other factors such as muscular activity (shivering), the thyroid and adrenal hormones play an important role in development of acclimatization in animals. However, it has been hypothesized that after acclimatization much less thyroid and cortical hormones are required for survival. Some investigators speculate that no cortical hormone is required for survival after animals have been adapted to cold.

Acclimatization is a reversible phenomenon. It takes 4 to 6 weeks to reach the maximum, but only 4 days following the return of the animals to room temperature there will be a considerable reduction in their level of acclimatization.

(iv) Brown Adipose Tissue and Cold

Fat is stored mainly in the form of triglycerides in adipose tissue which can be drawn whenever needed to be utilized as a source of energy. Two different kinds of fat are present in the body: yellowish or white fat which generally refers to subcutaneous fat depots, and brown fat which is characterized by various sizes of droplets in the granular cytoplasm.

Brown fat occurs in the neck, back (especially interscapularly), the inguinal region, around the kidneys and large vessels in the chest and on both sides of the spine and sternum.

Microscopically, brown fat is present as small regular droplets within the cells with the nucleus at or near the center of the cell. In white adipose tissue the fat is found as one large vacuole within the cell, pushing the nucleus to one side against the membrane. Brown fat is rich in mitochondria and its brown color is believed to be due to its high cytochrome content. Analysis of lipids from rat brown and white fat by thin-layer or gas-liquid chromatography showed that triglycerides of brown fat have more saturated fatty acids and phospholipids which contain more linoleic acid.

Differences in fatty acid composition of the phospholipid and triglyceride fraction between the two tissues led Chalvardjian[160] to postulate two different metabolic pathways in lipogenesis or lipid mobilization or both in these two tissues.

Brown and white fat are not only different in microscopic appearance but also chemically. Glycogen breakdown and fatty acid

release from both types of adipose tissue is under the influence of epinephrine, ACTH, glucagon and TSH.[161] Fain et al.[162] demonstrated a different response of brown and white fat to growth hormone and dexamethasone. These substances can increase lipolysis in white fat cells but have no effect on release of fatty acid or glycerol from brown fat tissue. In fact, these hormones increased the amount of free fatty acid taken up from the medium by brown fat cells. ACTH has the same effect as epinephrine on white fat cell lipolysis but no effect on brown fat cells. Some authors indicated that large doses of ACTH have a lypolytic effect on brown adipose tissue.

Brown adipose tissue was shown to have an increased O_2 consumption and played a thermogenic role in cold-adapted rats. This tissue is highly innervated by adrenergic nerves, judged on the basis of the presence of significant amounts of norepinephrine. It has been shown that brown adipose tissue (BAT) has a higher content of norepinephrine (NE) in cold acclimated rats.[163] Release of norepinephrine controls the thermogenic function of this tissue by means of lipolysis. The amount of BAT in rats was unchanged for 3 hours but then started to decline and reached about 2/3 of its initial value after 40 hours at 4°C. By the end of the third day it began a rapid increase which continued up to the 7th day of exposure.

Despite the general agreement concerning the role of NE in thermogenesis in cold adapted animals, the exact mechanism of its effect on brown fat tissue is uncertain. Kennedy et al.[164] are in favor of a possible interaction between the thyroid and norepi-

nephrine metabolism of brown fat.

Although some investigators have demonstrated a role for brown tissue in heat production which may be responsible for replacing shivering thermogenesis with non-shivering thermogenesis, this issue is still in doubt.

Non-shivering thermogenic mechanisms are effective in rats after 4 to 6 weeks of exposure to cold. Fuller et al.[165] believed that the hypothalamus is controlling the shivering and non-shivering response in cold because warming the preoptic anterior area of the hypothalamus decreases O_2 consumption and shivering. This can be reversed on cessation of warming.

(b) Exercise

The effects of exercise on the development of the lung have been extensively studied.

Tenney and Remmers[166] have shown that in mature animals, alveolar surface area is directly proportional to body O_2 consumption. They raised the question of whether high or low levels of O_2 consumption can cause proportional alteration of structural parameters of the respiratory system. Bartlett[135] examined the effect of daily exhausting treadmill exercise (2-30 minutes for 20 days) on one-month old rats. He failed to see any change in lung growth judged by measuring body weight, lung weight, lung volume, alveolar surface area, alveolar number and mean alveolar diameter.

Chronic alteration of the metabolic rate by manipulation of thyroid function also had no influence on lung development.

Bartlett and Areson[167] saw similar results in three-month old Japanese waltzing mice (JWM) which are in continuous motion because of a genetic defect in the vestibular apparatus and in the brain. Even though the oxygen consumption of waltzing mice was significantly higher than their heterozygous non-waltzing litter mates, the lung volume, surface area and alveolar number were less in waltzing mice. The body weight of waltzing mice was 18% less but specific lung volume, surface area and alveolar number were the same as control. Therefore, this study as well failed to provide any data to support the hypothesis that higher O_2 consumption due to physical activity enhances lung growth.

Geelhaar and Weibel[168] compared two-month old Japanese waltzing mice with normal white laboratory mice. Waltzing mice had 80% more O_2 consumption per gram body weight than control and morphometric analysis revealed that the alveolar and capillary surface area and capillary volume of JWM were 70% larger than those of normal mice. Since lung volume increased only 40% but alveolar surface area was enlarged by 70%, it indicates that the number of alveoli in JWM is greater. Despite the differences observed in those mice, it is difficult to judge whether higher activity and O_2 consumption is the cause of lung enlargement in JWM or if it is due to genotype mismatch in these animals. Burri et al.[169] defended their hypothesis of the relation between O_2 consumption and lung growth by injecting imino-beta, beta-dipropionitrile (IDPN) into a group of normal white mice and increased their activity and O_2 consumption by 50% of control. Since these animals showed no differences of specific heart, liver, kidney and

viscera weight and an increase of specific lung volume by 23%, they were quite convinced that increased $\dot{V}O_2$ by any means was always followed by a proportional change of the respiratory apparatus.

The effect of different types of exercise on different animals has yet to be studied. However, as indicated by many reports, swimming might have a different effect on pulmonary diffusion capability in both humans and rats.

Mostyn et al.[170] found that champion swimmers have a significantly higher steady-state pulmonary diffusion capacity than normal. Fu[171] subjected one month old rats to exhaustive and non-exhaustive swimming exercise for 4 and 8 week periods. He found that swimming exercise did not change the lung blood volume, lung weight, or body weight. However, alveolar density and the ratio of alveolar surface area to lung volume was increased compared to control. Two interesting points in this study were the similar results for 4 and 8 week period of exercise and that non-exhaustive swimming was as effective as exhaustive. Therefore, exercise does affect lung growth and Fu's result showed that alveolar proliferation occurred during the second month of postnatal growth in trained rats.

(i) Catecholamines and Glucagon

During exercise and emotion the catecholamine activity will increase, which in turn influences the plasma glucagon, free fatty acids, glucose and insulin. Physical activ-

ity increases norepinephrine whereas epinephrine levels will be elevated under general emotional arousal.[172] Dimsdale and Moss[173] have shown that secretion of epinephrine by the adrenal medulla increases 2-fold during public speaking while secretion of norepinephrine by the sympathetic nerve endings increased 3-fold during physical exercise. Exhaustive swimming exercise in both trained and untrained rats also stimulates the release of catecholamines, particularly norepinephrine.[174] Plasma NE concentration rose from 2.6 ug/L to 4.4 after 40 minutes of exercise and 4.2 at exhaustion in 20 and 24 year old men. Epinephrine levels were significantly elevated above resting values at exhaustion.[175]

Increased energy requirements of the muscle during exercise is accompanied by elevation of uptake and metabolism of different substrates, among them, circulating glucose is essential. It has been shown that the rate of gluconeogenesis in the liver and/or the muscle and glucose utilization by the muscle are in balance with each other. As a result, the plasma glucose level remains unchanged during exercise.[176,177] Bergstrom et al.[178] clearly showed the importance of carbohydrate diet in the onset of exhaustion, which provided evidence that factors controlling the glucose level of plasma have a dramatic role in exercise performance. Catecholamines can be considered as the key issue in this activity.

Insulin and glucagon are responsible for keeping the blood glucose level within the physiological range. Exercise can increase glucagon secretion.[179] Studies of the factors that stimulate elevated secretion of glucagon following initiation of exer-

cise indicate that pancreatic alpha cell stimulation by catecholamines is responsible for glucagon increases, at least in animals.[180-182]

Luycky[183] demonstrated the role of sympathetic stimulation of the beta-adrenergic receptors by administration of the beta-blocker propranolol to rats. Propanolol inhibited glucagon release during exercise.

Studies on humans after administration of propanolol[184] showed that the rate of increase of both glucagon and epinephrine concentrations during exercise was closely related to the rate of decline in plasma glucose concentration. This is an indication that stimulation of adrenergic receptors is not the major determinant for the exercise-induced glucagon secretion in man. Rather, it is the reduction in glucose concentration which enhances the secretion of epinephrine and glucagon during exercise.

Since catecholamines and glucagon are not the only hormones that are affected by physical activities, perhaps it would be necessary to consider the possible role of other hormones, such as growth hormone, which increases during moderate exercise and declines just prior to exhaustion in humans,[185] and insulin which is either unchanged or decreased in the circulation during physical activity.

One of the most interesting characteristic features of hormonal changes during exercise is the response of trained and untrained subjects to stimuli. Bloom et al.[185] measured different hormones in venous blood samples of trained and untrained cyclists at rest and after 5 minutes of exercise. They found an increase

of catecholamines in both groups but the rise was significantly less in trained cyclists. Insulin was decreased to a greater extent in trained subjects. Glucagon rose to a greater extent during strenuous exercise and remained elevated after the end of exercise in the untrained group. Growth hormone rose more during exercise and remained elevated after the end of exercise in the untrained group.

Galbo et al[186] demonstrated that trained and untrained rats have the same response as humans. Their blood glucose increased by 60% in trained animals while it decreased by 20% in controls. Glucagon and insulin did not change in trained animals, whereas glucagon increased and insulin declined markedly in samples obtained from control rats. Epinephrine and NE were lower in trained than control rats. Despite the obvious capability of both humans and animals for adaptation to physical activity, especially with regard to hormones, the mechanism of this adaptation is uncertain.

(ii) Glucocorticoids

The principal function of glucocorticoids such as cortisol, hydrocortisone and corticosterone can be summarized as follows:

1. stimulate gluconeogenosis: increase liver conversion of amino acids to glucose, thereby increasing liver glycogen and blood glucose,
2. mobilize amino acids from tissues and increase liver amino

acids,

3. mobilize fatty acids from adipose tissue and increase blood free fatty acids.

Reports on changes of plasma cortisol levels induced by muscle activity in different animals are contradictory. Although increase, decrease and steady state of glucocorticoids in moderate and light exercise have been reported, there is general agreement that exhaustive exercise in both rats and humans will raise the glucocorticoid concentrations.[175],[187] That means the increase in plasma glucocorticoids is related to the intensity of activity.[188] Many studies provide evidence that glucocorticoids do contribute to the ability of muscles to perform work.

Ingle et al.[189] reported that the muscle activity of normal rats could be increased by intravenous infusion of cortisone or ACTH. On the other hand, muscles of adrenalectomized rats were found to have greatly decreased work times which were improved to near normal when glucocorticoids were injected.[190]

Prolonged exercise produces some adaptations which result in changes in glucocorticoid secretory responses in the same manner as for other stresses.[191] These glucocorticoid secretion changes resemble those which can be seen in adaptation syndrome response to any stress.

Several authors have reported in both trained animals and humans that the plasma glucocorticoid levels are lower after exercise. White et al.[192] and Frenkl[193] have shown that after muscular activity the plasma steroid level of humans trained by regular swimming was less than that of untrained controls. The same

results for rats trained by swimming exercise were seen.[194] The rise in adrenocortical hormone production that was usually observed in the second and third weeks of training is either normal or below normal values after 6 weeks of training. The mechanisms involved in this adaptation of glucocorticoid secretion with training are not fully understood.

In vitro responsiveness of adrenals obtained from trained animals to adrenocorticotropic hormone was decreased[195] and adrenal hypertrophy similar to that produced by any type of stress[196] suggests that decreases in plasma glucocorticoid level in response to exercise training were due to changes within the adrenal glands.

Frenkl's[197] experiments provide sufficient reason to assume that the smaller increase in corticosterone level in the trained rats was the result of decreased mobilization of ACTH from the pituitary gland. Therefore, he concluded that it is the pituitary-adrenal axis that is involved in this adaptation, not the adrenocortical hormone production. In any case, it is well documented that adaptation is taking place in all types of stress including exercise.

(c) Pneumonectomy

The studies of compensatory lung growth following PN started more than half a century ago. Addis in 1927[198] pointed out that removal of the left lung of albino rats (which constitutes 35.5% of the total lung at age of 200 days) is fol-

lowed by an average increase of more than 40% in the remaining lung tissue 60 days after the operation.

Since the remaining lung after PN undergoes a heavy workload, to compensate for the removed portion, it has been postulated that this excess work stimulates the growth. However, Cohn[199] showed that negative pulling pressure exerted by the thoracic cage on the remaining lung as the air absorbed from the operated side stretched the tissue and stimulated lung growth. He kept the rats in a tank with a pressure of -500 mm Hg to increase the size of the thoracic cage for a period of 14 days. He found a heavier lung in these animals. On the other hand, the prevention of chest pulling by inserting a bolus of wax prevented the lung tissue from growing. He also showed that in a period of 14 days the remaining lung will grow and completely replace the removed part in rats of various sizes (50-300 g body weight). The lung growth following pneumonectomy was accompanied by a new growth of alveoli.[200]

Buhain and Brody[201] performed PN in both young (3 weeks) and adult (10 weeks) rats. The morphometric results indicated that in both groups compensatory growth of the lung occurred as a result of growth of pre-existing gas exchange units. The only noticeable difference between young and adult rats was equal enlargement of both alveolar ducts and alveoli in young rats, whereas in adults enlargement of alveolar ducts was the dominant response. The enlargement of either alveoli or alveolar ducts in young and adults is due to cell proliferation rather than cell hypertrophy because DNA increased by 33% in young and 27% in adult lungs after PN.

Nattie et al.[202] had reason to believe that different parts of the lung have different responses to PN. Pneumonectomized and sham-operated 30 and 80 day old rats showed a lung weight increase, but the alveoli on the pleural surface were equal in size. This indicates that there must be alveolar proliferation. On the other hand, internal alveoli were larger in the PN group. It is known that surface alveoli are smaller than internal alveoli in 4 week old rats but that this difference disappears as the rats aged.[203] Finally, at the age of 6 to 10 weeks, no difference between internal and surface alveoli is noticeable.

According to Brody,[204] lung DNA synthesis in mice was increased and reached a peak by day 6 post-pneumonectomy. Despite the increase of DNA synthesis and [^{3}H]-thymidine incorporation into the DNA 3 days after operation, and 38% and 40% increases of lung weight and tissue volume respectively one week after pneumonectomy, total lung volume does not change until the second post-operative week.[205] Unlike the lung, DNA synthesis in liver and kidney is not changed by pneumonectomy[204] which indicates that the growth stimulus is specific for the lung only.

Autoradiographs of operated lung show that alveolar Type II cells are the cells which account for the alveolar wall proliferation with evidence that some of these cells transform into alveolar Type I cells. In vitro study of alveolar Type II cells indicated that serum from pneumonectomized rabbits collected 9 and 21 days after operation significantly enhances incorporation of [^{3}H]-thymidine into DNA.[206] Increased thymidine incorporation into DNA and participation of different right lung lobes equally in

compensatory growth and cell hyperplasia has also been confirmed by Rannels et al.[207] Brody[205] has shown that both PN and hypoxia separately stimulate lung growth. He then combined these to accelerate the growth in PN rats by exposing them to 17% or 14% hypoxia. While hypoxia increased both total lung volume and weight of PN lung, hyperoxia did not affect the rate of growth.

Lately, Davies et al.[208] showed the number of alveoli is the same in PN and controls 5 years after operation. They believed proliferation of alveoli shortly after PN is just a normal developmental pattern which can be seen in early lung development. Although alveolar multiplication in the remaining right lung has been shown to increase by 60% in 10-week old rabbits 4 weeks after operation,[209] a few reports indicated no change of alveolar number will take place. Sery et al.[210] found that pneumonectomy in both young and adult rabbits produced enlargement of existing respiratory units without any increase in number. Morphometric study of adult rabbits (3 months of age) by Boatman[211] also demonstrated no change in number of alveoli despite the increase in the volume of the right lung which became equal to total lung volume 4 weeks after removal of the left lung.

The studies of Thurlbeck et al. on rats, rabbits and puppies showed different responses to PN. Rats[203] underwent PN at 4, 8 and 12 weeks of age. Lung volume, lung weight, surface area and lung protein increased significantly 2 weeks after PN. The number of alveoli increased significantly only in the animals pneumonectomized at 4 weeks of age. They suggested if PN is performed after alveolar multiplication has ceased, the adaptive response is

primarily an enlargement of alveoli. Ten-week old rabbits[212] showed elevation of DNA synthesis 5 days after operation which became significant by day 9. Unlike rats, rabbit compensatory lung growth occurs in the second week following PN. Nine-week old pneumonectomized puppies[213] also showed increases in weight, volume, surface area and number of alveoli 11 weeks following operation.

Different lobes have different responses to PN. In dogs, the right lower lobe has a greater response than the middle or cardiac lobes. Morphometric study 45 days following resection of the upper and middle lobes of the right lung in 23-day old rats showed that both the left lung and the remaining right lung participated proportionally in the restoration of the original lung volume.[214]

Pneumonectomy is not the only growth stimulus. Any other situation which increases the working load or makes the functioning portion insufficient will also stimulate growth. For example, lung collapse performed by injection of dental plastic in the left bronchi of young (3-week old) and adult (10-week old) rats causes the same effect on cell hyperplasia in the right lung, but to a greater extent in the young than in the adults.[215] Left lung collapse in adult mice also stimulated mitotic activity in the right lung four days after collapse. This activity can be prevented by packing the left thoracic cavity with cotton wool.[216] These findings support the idea that hyperinflation of the right lung causes change in the mitotic activity.

Perhaps Romanova et al.[217] best demonstrated that functional load following PN is not responsible for stimulating or initiating

the cell proliferation and DNA elevation. Increasing the functional duty of the right lung by ligation of left pulmonary artery did not change the cell proliferation in the hyperactive side.

One of the major components of lung tissue is collagen which is fundamental for functional and structural behaviour of the lung. Analysis of lung P-V curves revealed that lung recoil is increased especially at low volume one week following PN. This suggests that synthesis of both lung elastin and collagen were increased. In vitro study of collagen synthesis by lung tissue slices obtained from pneumonectomized rabbits measured by [^{14}C] proline incorporation into [^{14}C] hydroxyline demonstrated a 100% increase compared with controls.[218]

Lysyl oxidase is an enzyme responsible for deamination of lysine and hydroxylysine leading to formation of compounds which produce the crosslinks of elastin and collagen. Lysyl oxidase activity in the right lung of an adult hamster that had undergone left PN started to increase only 2 hours after surgery and reached a peak of 200% of control after 24 hours. This shows that collagen synthesis begins shortly after PN.

CHAPTER II

EXPERIMENTAL STUDY

(A) MATERIALS AND METHODS

Four hundred-forty-six rats and 2,152 fetuses were used for this study. Virgin female Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (St. Constant, Que.) weighing 160-180 g. They were placed in separate cages and housed under controlled lighting conditions with 12 hours of light alternating with 12 hours of darkness. Rats were allowed food (Purina rat chow) and water ad libitum. They were mated when their body weight was between 195-230 grams. Vaginal smears were taken daily, and while rats were in the proesterus cycle[228] they were placed with male rats for four hours (during the period of darkness). After the four hour period, females were smeared again and the presence of spermatozoa indicated that mating had occurred. Gestation day one was designated as commencing 24 hours after successful mating had occurred. The body weights were measured at day zero, at least twice a week and at the time of sacrifice. Food intake was measured during pregnancy.

Pregnant rats were divided into 6 groups, each submitted to one of the following experimental conditions.

(1) Control

Pregnant rats were either kept singly in wire cages throughout pregnancy or were transferred into an environmental chamber in a group of not more than 12 on gestation day seven until the day of sacrifice. The rats kept in the chamber were to

be used as control for the hypoxia experiment. The environmental chamber was made of plexiglass and had a volume of 80 litres. A wire cage within the chamber had sufficient space to accommodate 12 pregnant rats. By closing or removing the cover of the chamber, the rats could be exposed to the gas within the chamber or to the outside environment (i.e. room air). When in the covered environmental chamber, the rats were breathing a gas mixture containing 20.9% O_2 and the balance of N_2. A continuous flow of gas (air) from a cylinder washed out the CO_2 and excess humidity from the chamber. The gas flow ranged between 5-7 litres depending on the number of rats in the chamber. The chamber air was continuously cycled by a noiseless fan, and passed over a temperature controlled copper coil. The in and out flow of gas was continuously monitored by O_2 and CO_2 analyzers (Beckman). A temperature of 23°C $\pm$ 1 and O_2 concentration of 20.9% and a CO_2 of less than 0.3% were maintained over the duration of the experiment, with a short daily interruption of 15-20 minutes when food was changed and the cage cleaned. The group of rats transferred into the chamber on GD 14 remained in the chamber 24 hours a day and the GD 7 group were exposed to a covered environmental chamber for 10 hours a day and remained in the uncovered cage in a group for the rest of the day. Since the data obtained from the three above groups were similar, they were combined to be used as control for all experimental conditions.

(2) Hypoxia

This group of pregnant rats was divided into two sugroups:

a). Gestation day 7: These rats were placed in the environmental chamber as mentioned above, but only for 10 hr/day during lighting period, until the day of sacrifice. The concentration of O_2 within the chamber was maintained at 10% by inflow of gas from a cylinder containing 10% O_2 and 90% N_2. The temperature, humidity and CO_2 concentration were similar to that of the control condition as described above.

b). Gestation day 14: These rats were transferred from the wire cages to the environmental chamber on GD 14. They were kept in the chamber 24 hr/day with a short cleaning period of 15-20 minutes a day until the day of sacrifice.

The O_2 concentration of gas within the chamber was kept at 14% on GD 14 and 15, at 13% on GD 16, 12% on GD 17 and thereafter at 11% until GD 21. In all other aspects, the conditions were similar to that of control.

(3) Swimming Exercise

Pregnant rats were subjected to daily swimming exercise for a 10 minute duration, starting either from GD 3 or GD 11 until the day of sacrifice. A load equal to 5% of the body weight was attached to the base of the tail. The dimensions of the swimming pool were 60 cm across and 40 cm deep. The water temperature

was maintained at 30°C throughout the exercise. At the end of exercise, the rat was dried and returned to its wire cage.

(4) Cold

The pregnant rats in their separate cages were transferred to a special temperature and humidity controlled room either at GD 3 or GD 14 until the day of sacrifice. The room temperature was maintained at 10°C and the humidity at 70%. The light and darkness cycle was the same as for control rats. The rats had free access to food and water.

(5) Pneumonectomy

A left PN was performed in pregnant rats on gestation days 3, 9, 12, 14, 16 and 18. The animals were anaesthetized with ether and placed in a right lateral position. After shaving and cleansing of the skin, an incision along the left fifth intercostal space was made. Prior to surgical intervention, a rubber glove attached to a small animal respirator was placed tightly around the neck. Upon opening the chest, the respirator was turned on to assist the ventilation of the right lung. The ribs were retracted. The left lung was carefully freed from ligamentous attachments, and a silk ligature was passed around the hilum of the lung and tied. The left lung was then resected distal to the ligature. Following lung resection, the ribs were approximated with silk sutures, followed by closing the wound in layers.

The surgical procedures, skin to skin, were completed within 5 minutes. After one hour of post-operative observation, the rat was returned to its wire cage.

(6) Sham - Operated

This group was subjected to a surgical procedure similar to that mentioned above with the exception of manipulation and excision of left lung. The days of operation were also similar to that of PN rats.

Dissection

At the end of each experimental condition, the rats were anesthetized with a subcutaneous injection of sodium pentobarbitol (5 mg/100 g of body weight) on the day of sacrifice. Laparotomy was performed and fetuses were then harvested by ceasarean section. The umbilical cord was ligated and cut. Fetuses were dried with gauze before their body weight was recorded. Only fetuses from mothers free of respiratory infection were included in this study. The fetuses were then decapitated and using a dissecting microscope, the lungs were removed, separated from the extrapulmonary airways and weighed. Determination of DNA, dry weight, and EM studies were done on 5 individual lungs from litter-mates. The rest of the lungs were pooled and used for the following determinations: DNA, total protein content, total lung lipids, phospholipids, lecithin and DSPC.

Lungs that appeared to contain blood or air due to fetal

gasping were discarded. In addition, liver, spleen and kidney were removed, weighed and their DNA measured. Placentae collected at the time of ceasarean section were dissected free of the umbilical cord, placed on a paper towel to absorb any excess blood and weighed. Five placentae from each mother, belonging to the fetuses used for individual lung DNA assessment, were used for DNA determination. Placenta and liver dry weights were also collected. The uterus and its contents were removed from some rats and subjected to microwave irradiation to inactivate the enzymes responsible for glycogenolysis. Lungs and liver were dissected from these irradiated fetuses and used for glycogen determination. The mothers were exsanguinated by cutting the abdominal aorta. A pneumothorax was produced by opening the diaphragm adjacent to the xyphoid. The opening in the diaphragm was enlarged and the lungs were inspected under a magnifying glass. If the lungs were not uniformly pink in color and showed signs of possible infection, the rat, including the fetuses, were excluded from the study. The lungs were used for measurements of air pressure-volume curve and DNA.

At the end of pressure-volume measurements, liver, kidneys, heart and uterus were removed, cleaned of extra tissues, blotted on a paper towel and weighed. Dry weights were obtained on liver, kidneys and heart.

Air Deflation Pressure-Volume Curve

The lungs were left intact in the chest. This precaution was taken to prevent accidental puncture of the lung which may occur

in the process of excision. The trachea was cannulated and the lungs were degassed by placing the animal in a vacuum jar. The degassing procedure was repeated if the lungs were not completely degassed during the first attempt. The chest was then opened by bisecting the sternum. The ribs were separated by forceps. The lungs were again inspected under a magnifying glass. If signs of infection, such as grey spots, were noted the lungs were excluded from the study. The large vessels were ligated at the base of the heart. The cannulated, degassed lungs were then attached to a pressure-volume apparatus similar to that described by Gribetz et al.[218]

The lungs were inflated with air to 30 cm of pressure. This inflation pressure was maintained until the lungs were fully inflated and the air volume remained constant for 15 sec. The air volume observed at this transpulmonary pressure, considered as maximal lung air volume, was designated as 100% and each volume subsequently observed after deflation to a predetermined transpulmonary pressure (20, 15, 10, 5 and 0 cm H_2O) was expressed as a percentage of maximal lung air volume. These pressures were maintained for 20 sec. before the volumes were read at each pressure. If, during the procedure, the lung air volume did not remain constant at high pressures, air leaks were assumed to be present, and such lungs were excluded from the study. All pressure-volume measurements were performed at room temperature. At the end of air PV measurements, the lungs were separated from the extrapulmonary airways, weighed and used to measure DNA and dry weight.

Dry Weight

Dry weight measurements were made by placing the tissues on preweighed pieces of tinfoil which were then kept in an oven at 60°C for one week.

DNA and Total Protein

Samples of lung tissue were homogenized in 2.5 ml of normal saline in a Kontest all glass homogenizer. One ml aliquots (in duplicate) were used for the extraction and determination of lung deoxyribonucleic acid by the method of Schneider.[220] One hundred microliters of above homogenized sample was diluted 1/20 in normal saline and 25 or 50 microliters of the dilution were added to 1 N NaOH and digested for 18-20 hours. The total protein content was then determined by the Lowry method.[219]

Phospholipids

Samples of lung tissue were homogenized in chloroform:methanol (2:1) in a Kontes all glass homogenizer. The lipid extract was washed according to the method of Folch et al.[221] The samples were dried in a waterbath at 47°C under nitrogen and the dried extract reconstituted to 1 ml with chloroform:methanol (2:1). An aliquot (200 microliters) of the lipid extract was dried on a hot plate to determine total lipids. A second aliquot (25 microliters) of the lipid extract was used to determine lipid phosphorus according to Brante's modification[222] of the method of Fiske and Subbarow.[223] A third aliquot (50 microliters) of the original lipid extract was plated on an activated silica gel-H plate and the lipid fractions separated using a solvent system

containing chloroform-methanol-acetic acid-water (25:15:4:2).[224] The plate was then exposed to iodine vapor. The phosphatidylcholine (lecithin) spot was identified and then aspirated into test tubes for measurements of lipid phosphorus as described above. A fourth aliquot (50 microliters) of the original lipid extract was plated on an activated silica gel-H plate and only the lecithin spot was identified and aspirated into a test tube to isolate the disaturated lecithin (DSPC) by mercuric acetate adduction[225] and the lipid phosphorus determined.

Glycogen

The uterus with the fetuses in was removed, placed in a microwave oven and irradiated for 10-15 sec. Lungs and liver were dissected from the fetuses and lyophilized overnight. They were then weighed and stored at -20°C until used. Tissues were then homogenized in 0.05 M acetate buffer (pH 4.7) to extract the glycogen. Glycogen was measured using amylo-alpha-1, 4-alpha-1, 6-glucosidase according to the method of Passonneau and Lauderdale[226] and tissue glycogen calculated using the equation: A/6.22 X volume of cuvette (ml) X [total extraction volume (ml)]/[volume of extract assayed (ml)] X 1/[tissue dry weight (mg)], where A = change of absorbance at 340 nm and 6.22 = extinction coefficient of NADPH at 340 nm.

Electron Microscopy

Fetal left lungs were divided into upper, middle and lower portions. Each portion was cut into 6-8 blocks, and fixed for 2 hours at 4°C in 3% gluteraldehyde in 0.1 M phosphate buffer (pH

7.4). Tissues were rinsed for 24 hours at 4°C in 0.1 M phosphate buffer (pH 7.4) containing 0.2 M sucrose. Tissues were then post-fixed for 2 hours at 4°C in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). After rapid dehydration in ascending concentrations of ethanol, the tissues were embedded in Epon 812.[227] Thin sections were made from 4 blocks of each portion (3 grids per block), stained with uranyl acetate and lead citrate. From a total of 36 grids prepared from each lung, 6 grids were randomly selected, viewed and photographed in a Phillips EM 201 electron microscope. In order to eliminate observer bias, tissues were examined using coded grids without foreknowledge of their source.

Thirty-six random exposures were taken from each grid. Four fetal lungs were studied from each experimental condition. Electron micrographs of lungs (magnification X 14,300) were used to estimate the ratio of Type II cells to total number of lung cells. Only those cells containing a nucleus were counted. Cells were considered Type II pneumocytes if they contained lamellar inclusion bodies. Cells found in capillaries were not included in the cell count.

Micrographs from each lung were counted 3 times and the average used as the cell count. Lamellar bodies were also counted in Type II cells and the average number of observed lamellar bodies per Type II cell was calculated.

Statistics: Statistical analysis of the data was carried out using a T-test of paired or unpaired variates and a multiple range test for analysis of variance by Duncan's method, when applicable.

(B) RESULTS

(1) Control

The average age and body weight of 70 pregnant rats on gestation day zero (date of mating) were 56.7 days ± 0.8 SE and 207.2 g ± 1.48 SE, respectively. There was no relationship between the age of the time of mating and the litter size, but there was a significant correlation between the body weight at gestation day zero (BWo) and the litter size in these primiparous rats (Fig. 1, $n = 70$, $r = 0.340$, $P < 0.01$). The larger the body weight (BWo) the greater the litter size. Increase in body weight during pregnancy is partly due to growth of the rat with age and partly due to litter size. This is shown in Fig. 2 where the body weight of the pregnant rats having a litter size of 9-14 is compared with that of non-pregnant rats of the same age. The body weight at gestation day 21 (GD 21) minus the fetus and placenta weight (BW-fp) is much greater ($P < 0.001$) than that of non-pregnant rats of the same age (35.4% weight gain in 21 days versus 13.4% in non-pregnant rats of the same age) (Fig. 2). However, the percentage gain in body weight from the initial body weight (BWo) is independent of the litter size (Fig. 3).

The daily food intake increased slightly as pregnancy progressed (11.5 g/100 g BWo), then decreased to about 1/2 during the last two days of pregnancy. Oxygen consumption also increased progressively with pregnancy. The value was 28% greater at late gestation when compared to non-pregnant rats of the same age. This was due to an increase in body weight. When O_2 consumption

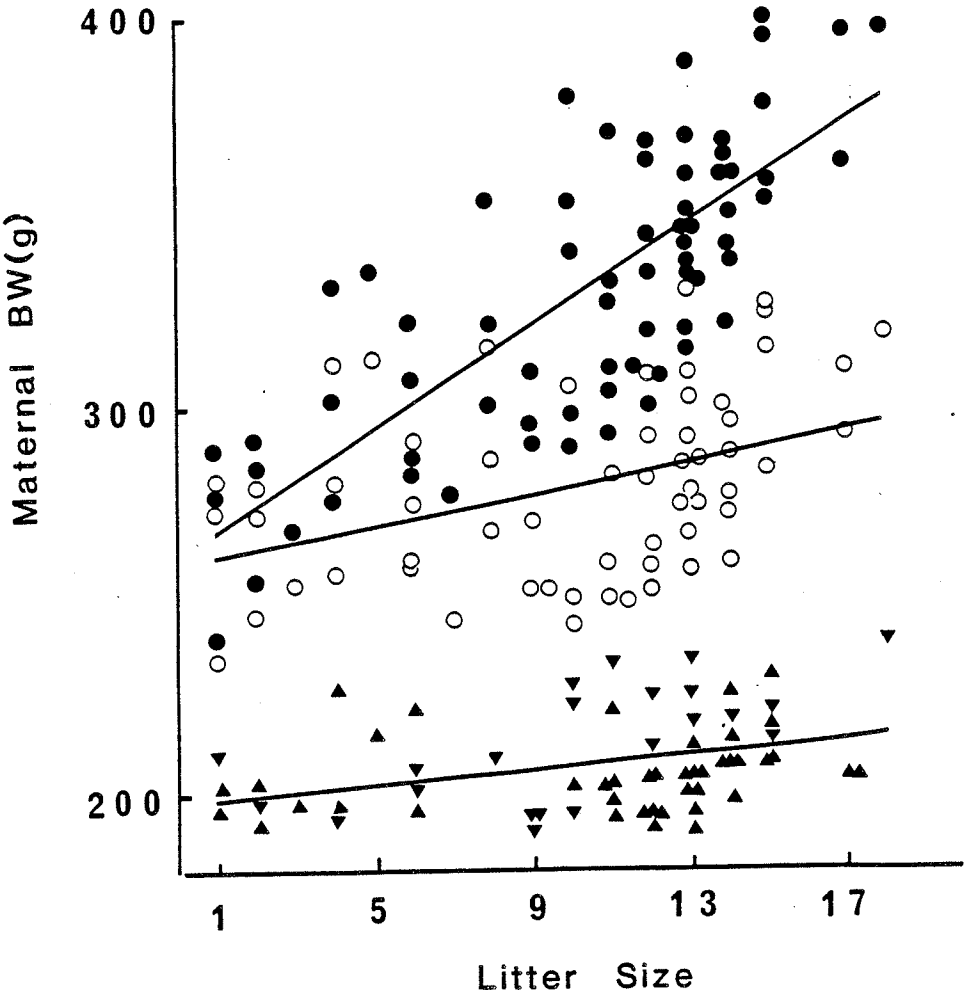


Figure 1: Relationship between maternal body weight (BW,g) and litter size. The closed circles and the upper line represent body weight of pregnant rats at GD 21 (BW_{21}; $r = 0.77$, $P < 0.001$). The open circles and the middle line are body weight at GD 21 minus fetal and placenta weight (BW_{-fp}; $r = 0.406$, $P < 0.01$). The triangles and the lower line are for body weight at GD 0 (BW_0; $r = 0.334$, $P < 0.01$). Each point is one pregnant rat. Regression lines calculated by least square technique.

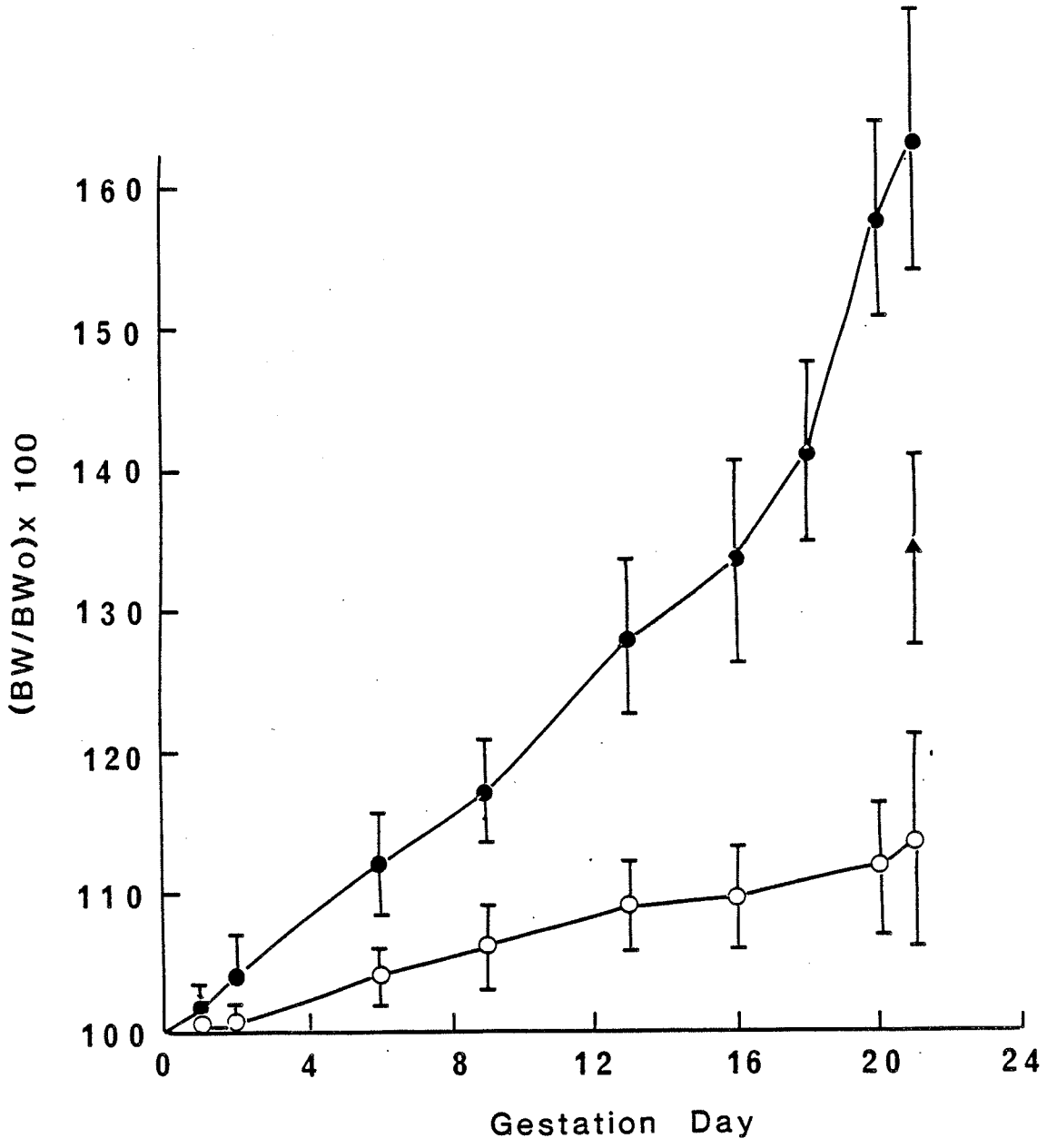


Figure 2: Body weight of pregnant rats with litter size of 9-14 fetuses expressed as a percentage of BW_0 (closed circles, upper line). Open circles and lower line are for non-pregnant rats in that same period. The triangle represents BW_{-fp}. Each point is the mean of 4 to 41 measurements. The vertical lines are ± 1 SD.

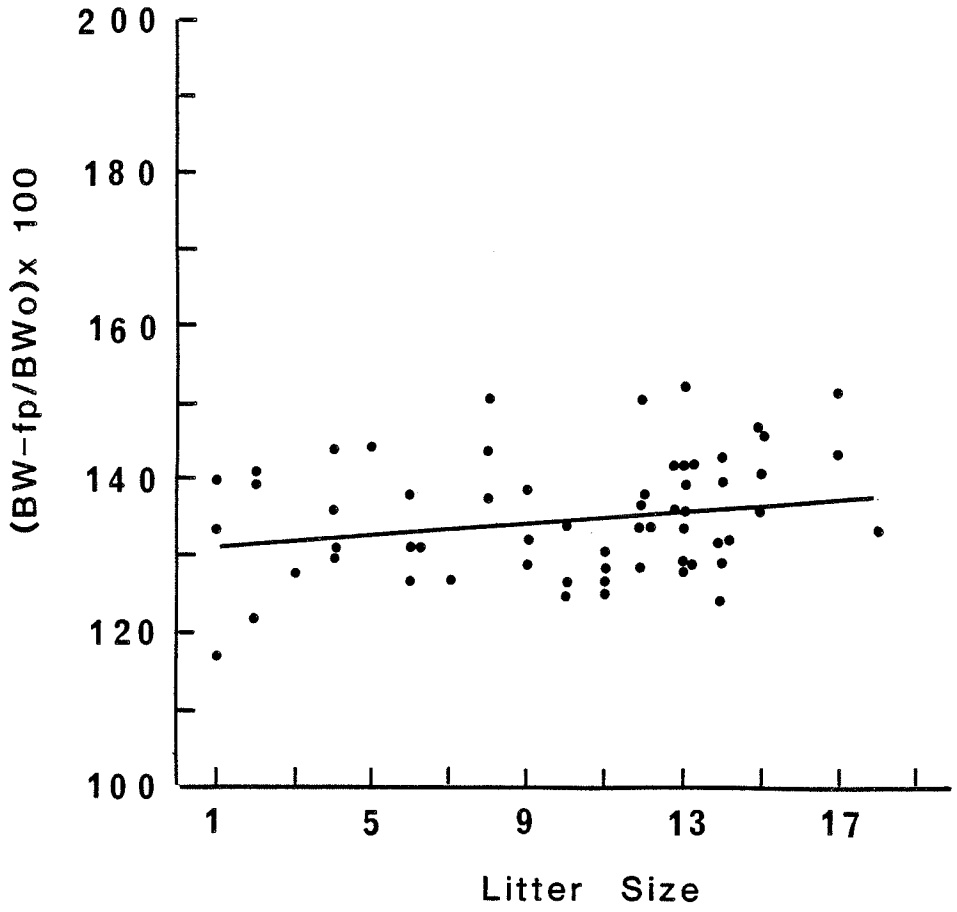


Figure 3: Relationship between BW-fp, expressed as a percentage initial body weight (BW_0) and litter size. Each point represents one rat. Regression line calculated by least square had a slope of 0.399 ± 0.215, which was not significant.

was expressed per body weight, no differences were noticed between pregnant and non-pregnant rats.

Fetal death or absorption was at a rate of 0.96% in control rats. Litter size ranged between one and nineteen. The distribution of litter size among 446 pregnant rats used for this study (as control and for the experimental conditions; pneumonectomy, hypoxia, cold and exercise) is shown in Fig. 4. Seventy-one percent of the rats had litters of 10-14 fetuses, the peak being 13; 17% had less than 10 fetuses and 11.8% had more than 14.

To assess the influence of rapid body growth during pregnancy on lung weight, lung DNA content and lung air volume (ml air volume at 30 cm H_2O inflation pressure, Vmax), comparisons were made between pregnant and non-pregnant rats of comparable age and body weight. The data shown in Table 1, Fig. 5 and 6, indicate that the lung increases in weight, DNA content and air volume (Vmax) with increased body weight, but the rate of body growth exceeds that of the lung resulting in a lower lung weight and lung volume per body weight. This phenomenon is more exaggerated in pregnant rats. However, the rate of increase of lung DNA appears to match with the overall rate of body growth in pregnancy.

The relationship between litter size and maternal lung weight, lung DNA content and lung air volume are shown in Figs. 7, 8 and 9. It appears that the maternal lung size is directly related to the litter size; the greater the litter size the larger the lung weight, DNA content and Vmax. In Table 2, the lungs of mothers with small litter size (1-3) are compared with those of larger litter size (15-18). Since there was also a direct

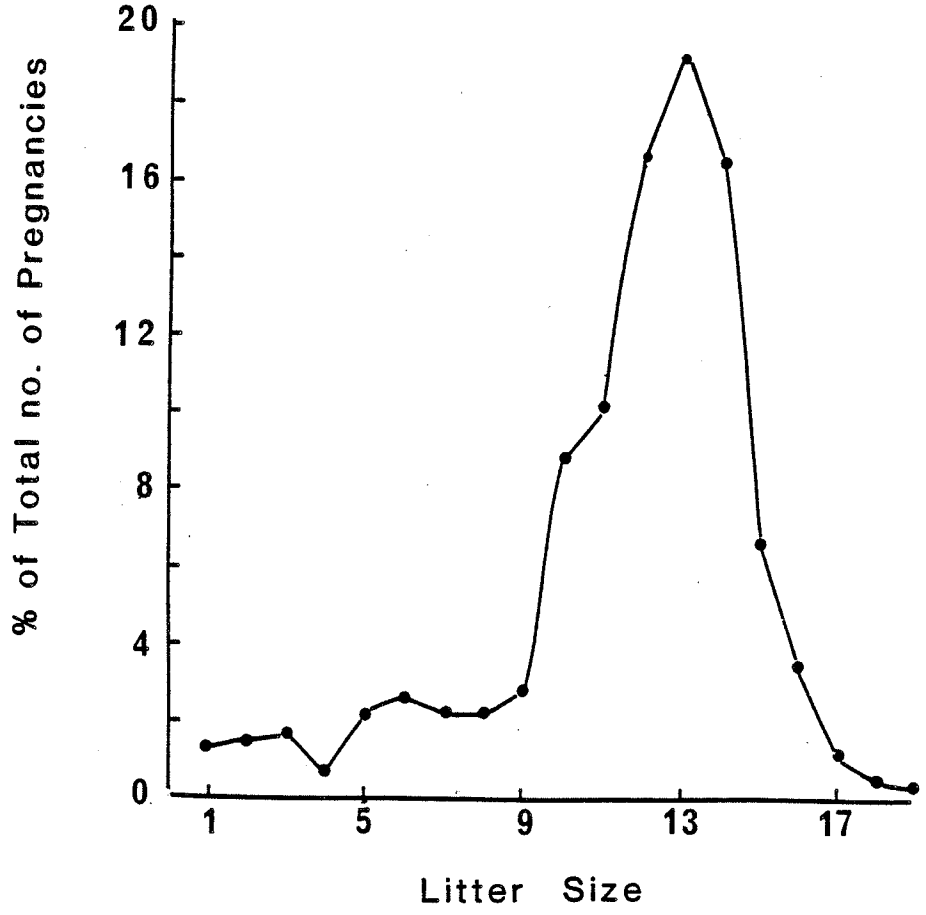


Figure 4: Distribution of litter size in 446 pregnant rats.

Table 1: Lung Measurements in Pregnant and Non-Pregnant Rats

	Pregnant (control)	Non-Pregnant Day 0	Non-Pregnant Day 21	Non-Pregnant Weight Matched
Body weight (g)	279.5 ± 3.06 (60)	210.09 ± 3.39 (11)	235.4 ± 6.79* (8)	273.7±7.84 (12)
Lung weight (g)	0.953 ± 0.011 (56)	0.877 ± 0.019 (11)	0.909 ± 0.026 (8)	1.034±0.026† (12)
Lung weight (g)/100 g BW	0.343 ± 0.003 (56)	0.418 ± 0.008 (11)	0.386 ± 0.005* (8)	0.381±0.01† (11)
Lung DNA (mg)	7.672 ± 0.177 (34)	7.013 ± 0.121 (10)	6.73 ± 0.32 (7)	7.52±0.187 (12)
Lung DNA (mg) /g lung	8.024 ± 0.125 (34)	7.996 ± 0.151 (10)	7.42 ± 0.137* (7)	7.28±0.091† (12)
Lung DNA (mg) /100 g BW	2.74 ± 0.047 (34)	3.35 ± 0.07 (10)	2.85 ± 0.05* (7)	2.76±0.069 (12)
Lung Volume (ml)	13.4 ± 0.217 (51)	11.64 ± 0.344 (10)	12.97 ± 0.331* (7)	14.13±0.386 (12)
Lung Volume (ml) /g lung	14.15 ± 0.223 (50)	13.24 ± 0.362 (10)	14.3 ± 0.49 (7)	13.7±0.316 (12)
Lung Volume (ml) /100 g BW	4.814 ± 0.079 (50)	5.55 ± 0.119 (10)	5.5 ± 0.184 (7)	5.2±0.167† (12)
Lung Volume (ml) /mg lung DNA	1.77 ± 0.047 (32)	1.66 ± 0.053 (10)	1.94 ± 0.087* (7)	1.89±0.052 (12)
Cell no., in millions per lung	1237.42 ± 28.55 (34)	1131.13± 19.52 (10)	1085.48 ± 51.61 (7)	1212.9±30.16 (12)

Data expressed as mean ± 1 SE; number in parentheses indicate number of animals studied. Body weight for pregnant rats is after exclusion of fetal and placental weights (BW_{-fp}). Comparisons were made between non-pregnant day 21 and zero, and between non-pregnant body weight match and pregnant rats. The non-pregnant body weight match rats were about 6 to 7 weeks older than non-pregnant day zero rats.

* different from non-pregnant day zero ($P<0.001$ to < 0.02).

† different from pregnant rats ($P<0.001<$to<0.05).

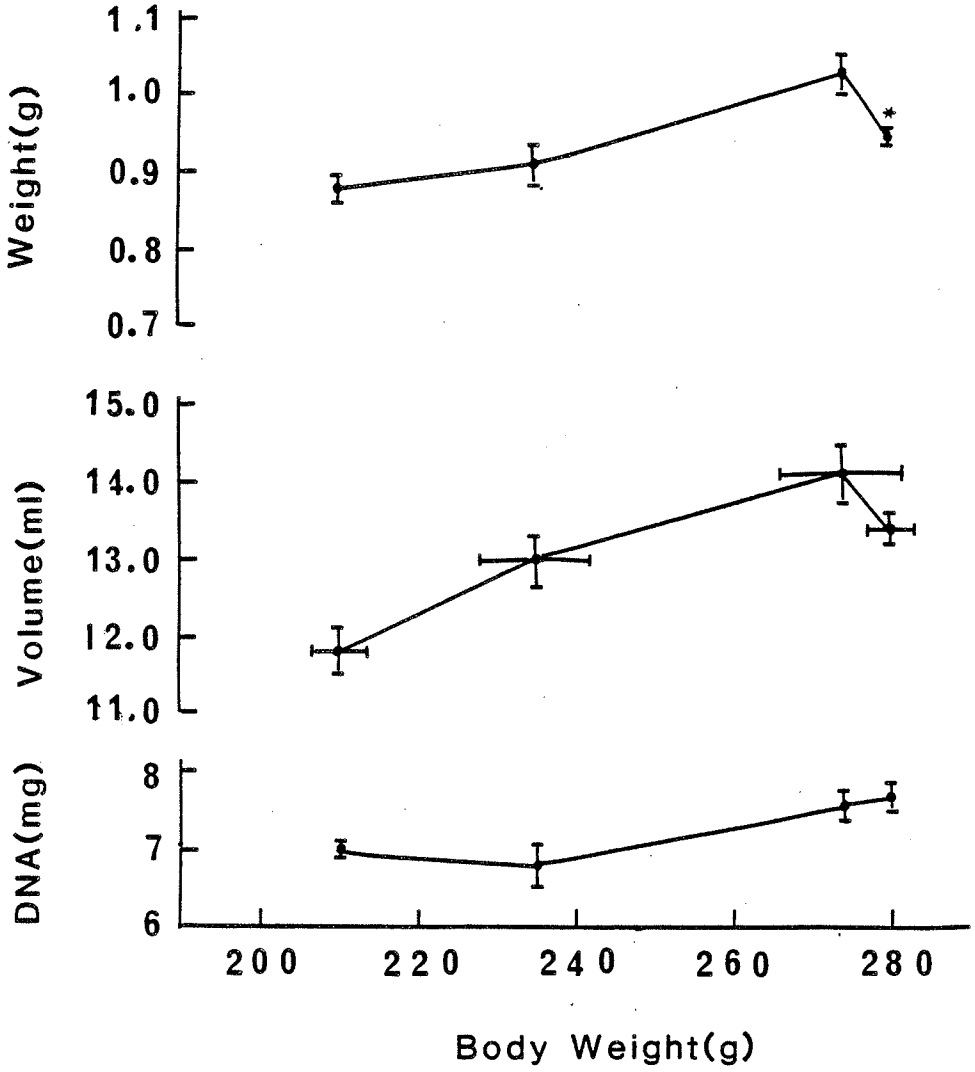


Figure 5: Relationship between lung weight, air volume and DNA, and body weight. Each point is the mean ± 1 SE for a group of rats (n = 6 to 25), representing, from left to right, non-pregnant day zero, non-pregnant day 21, non-pregnant body weight-matched and pregnant rats GD 21 (BW_{-fp}). The line connecting these points indicates the direction of change of lung parameter with increase in body weight. Only the comparison between pregnant and non-pregnant body weight-matched is shown here. * = different from non-pregnant body weight-matched ($P < 0.01$).

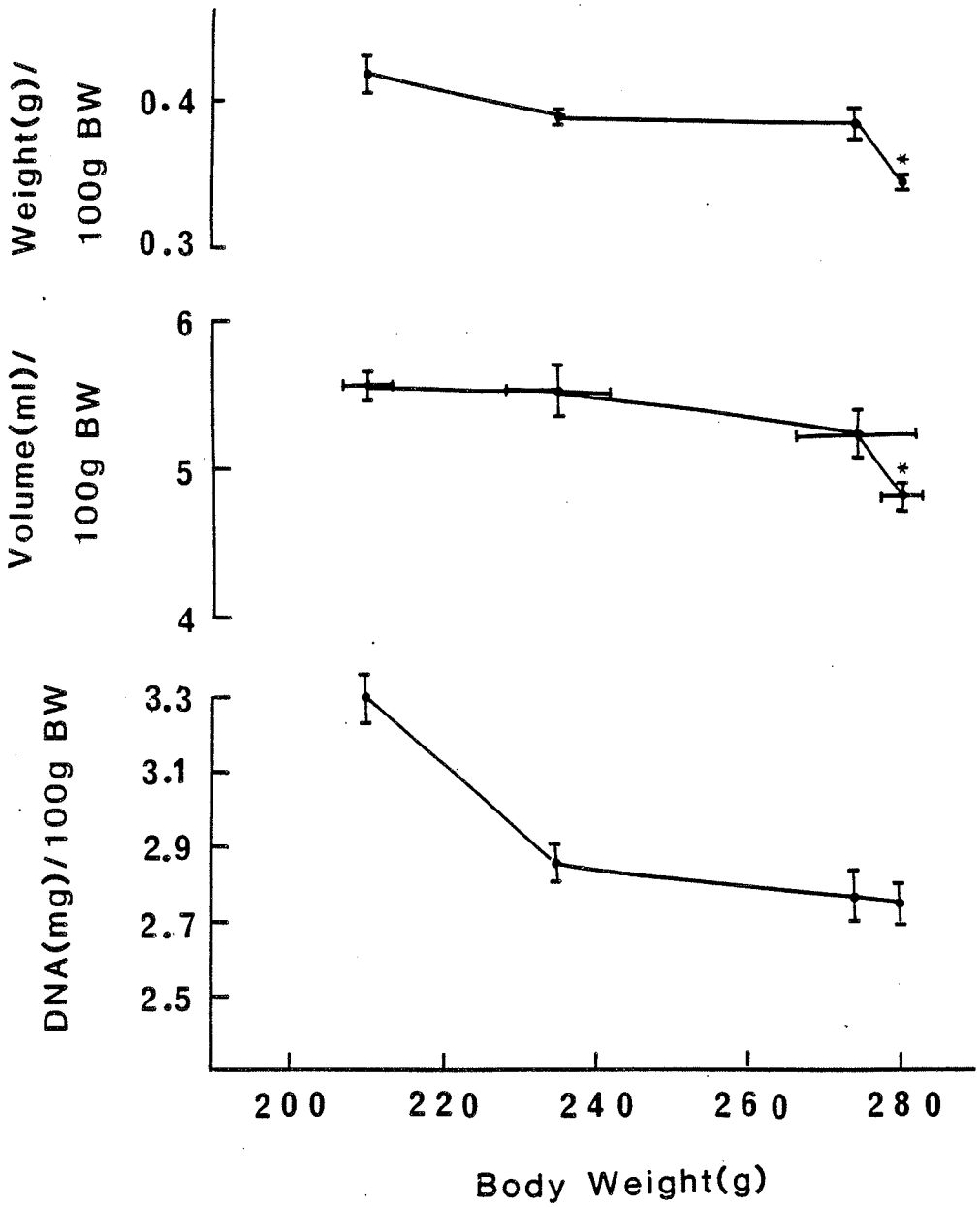


Figure 6: Relationship between lung weight, volume and DNA expressed per body weight, and body weight for the four groups of rats described in Figure 5. * = different from non-pregnant body weight-matched ($P < 0.001$ to $P < 0.05$).

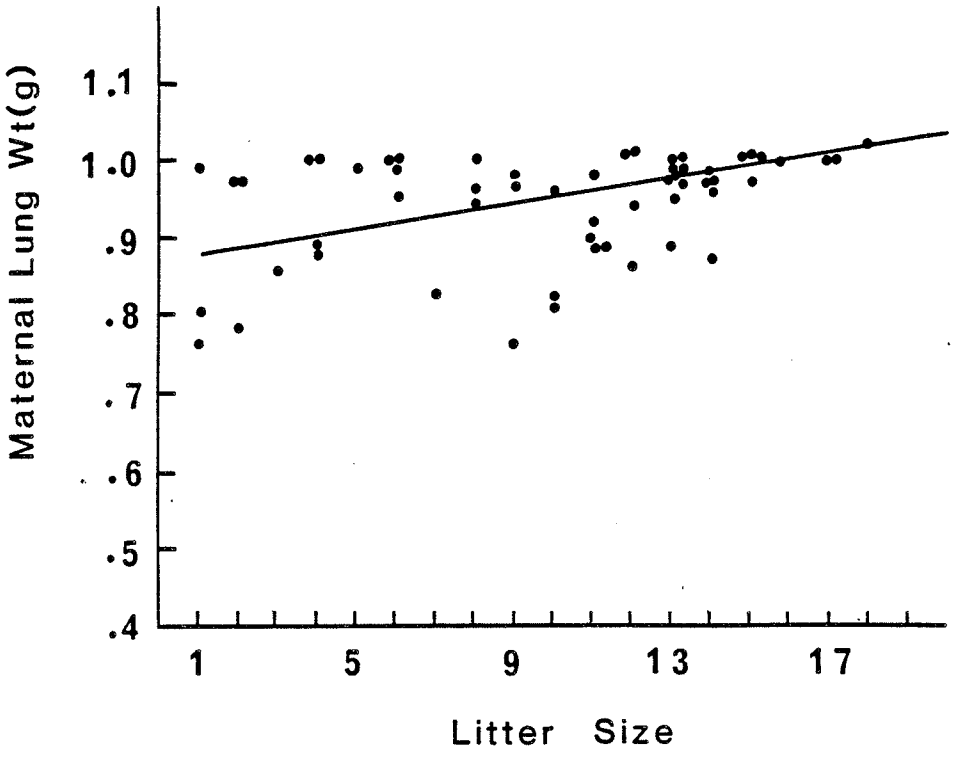


Figure 7: Relationship between lung weight and litter size in 56 pregnant rats. Each point represents one animal. Regression line calculated by least square method, $r = 0.428$ and $P < 0.001$.

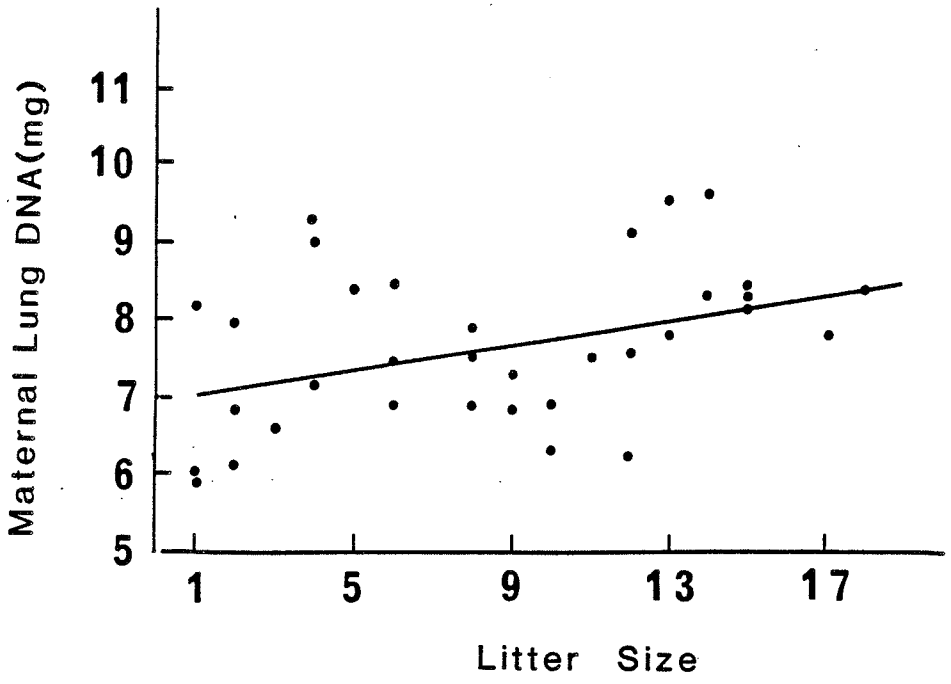


Figure 8: Maternal lung DNA in relation to litter size for 34 pregnant rats. Each point represents one rat. Regression line calculated by least square method, $r = 0.397$, $P < 0.02$.

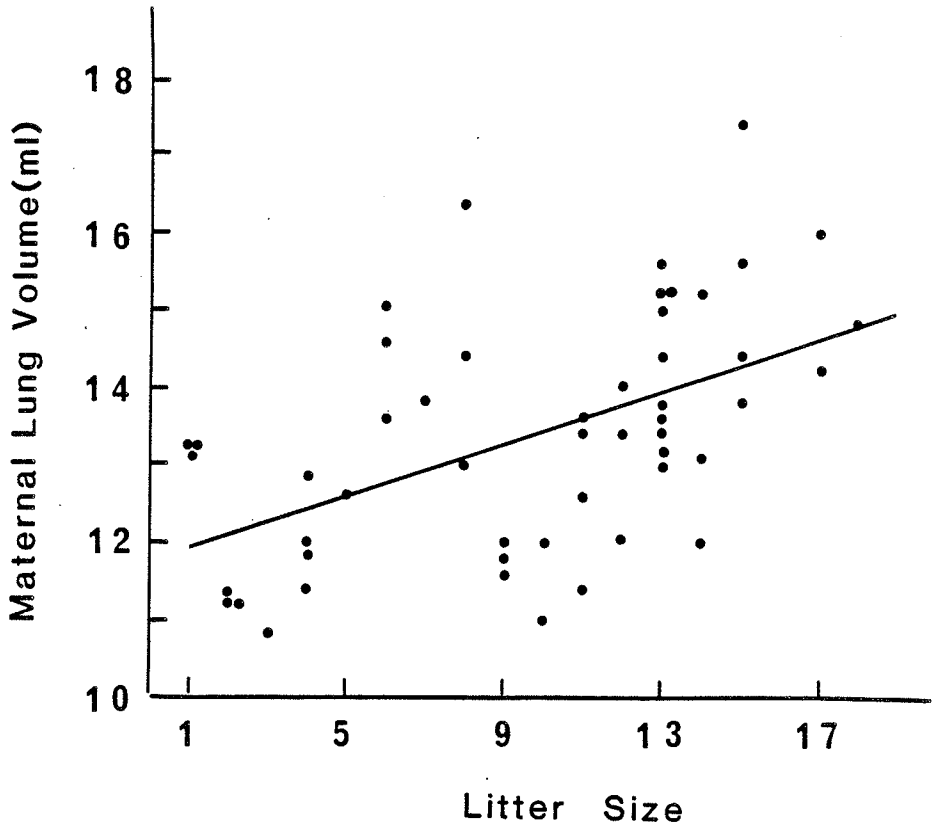


Figure 9: Relationship between maternal lung volume and litter size for 51 pregnant rats. Each point represents one rat. Regression line calculated by least squares, $r = 0.523$, $P < 0.001$.

Table 2: Lung Measurements of Pregnant Rats with Small and Large Litter Size

	Litter Size	
	1-3	15-18
BW-fP (g)	261.8 ± 7.2 (7)	310.4 ± 6.1* (7)
Lung wt (g)	0.875 ± 0.038 (7)	1.058 ± 0.023† (7)
Lung DNA (mg)	6.792 ± 0.351 (7)	8.232 ± 0.115† (5)
Lung volume (ml)	12.08 ± 0.46 (7)	15.18 ± 0.47 * (7)
Lung wt (g)/ 100 g BW-fP	0.333 ± 0.01 (7)	0.341 ± 0.005 (7)
Lung DNA (mg)/ 100 g BW-fP	2.58 ± 0.096 (7)	2.65 ± 0.073 (5)
Lung V (ml)/ 100 g BW-fP	4.58 ± 0.203 (7)	4.91 ± 0.23 (7)

BW-fP = body weight at GD 21 excluding fetal and placenta weights. Data expressed as mean ± 1 SE numbers in parentheses indicate number of animals studied. Different from rats with small litter size: * = $P<0.001$, † = $P<0.01$

relationship between maternal body weight at the time of mating and litter size, the increase in lung size with litter size may be interpreted as a function of maternal body weight. The influence of litter size on lung size may be detected if lung weight, DNA content and volume were expressed per BW-fp. This would be plausible if the ratio of lung size to body weight was constant.

Figs. 10, 11 and 12 show that as the body weight increases the lung weight, DNA content and air volume expressed per body weight declines. Figs. 13 and 14 show the ratios of lung air volume and lung DNA per body weight for pregnant rats with small and large litters and for non-pregnant rats. For both pregnant and non-pregnant rats the ratios decline with increasing body weight. However, when the fetal and placental weights are excluded from the body weight of the pregnant rats, these ratios for pregnant rats with large litter size are higher than expected, even higher than that of pregnant rats with small litter size whose body weights are smaller.

The influence of litter size on the lung could be measured by comparing the lungs of mothers with comparable BW-fp but with either small or large litters. Figs. 15 and 16 illustrate such comparisons between eight pairs of iso-body weight pregnant rats. The relationship for lung weight was poor ($0.1 < P < 0.05$) but for lung volume it was highly significant ($P < 0.001$). Initial body weights (BWo) of these eight pairs of rats were not different from each other. A similar comparison could not be made for maternal lung DNA because the number of samples measured were far less than those of lung weight and lung volume. However, since the ratios

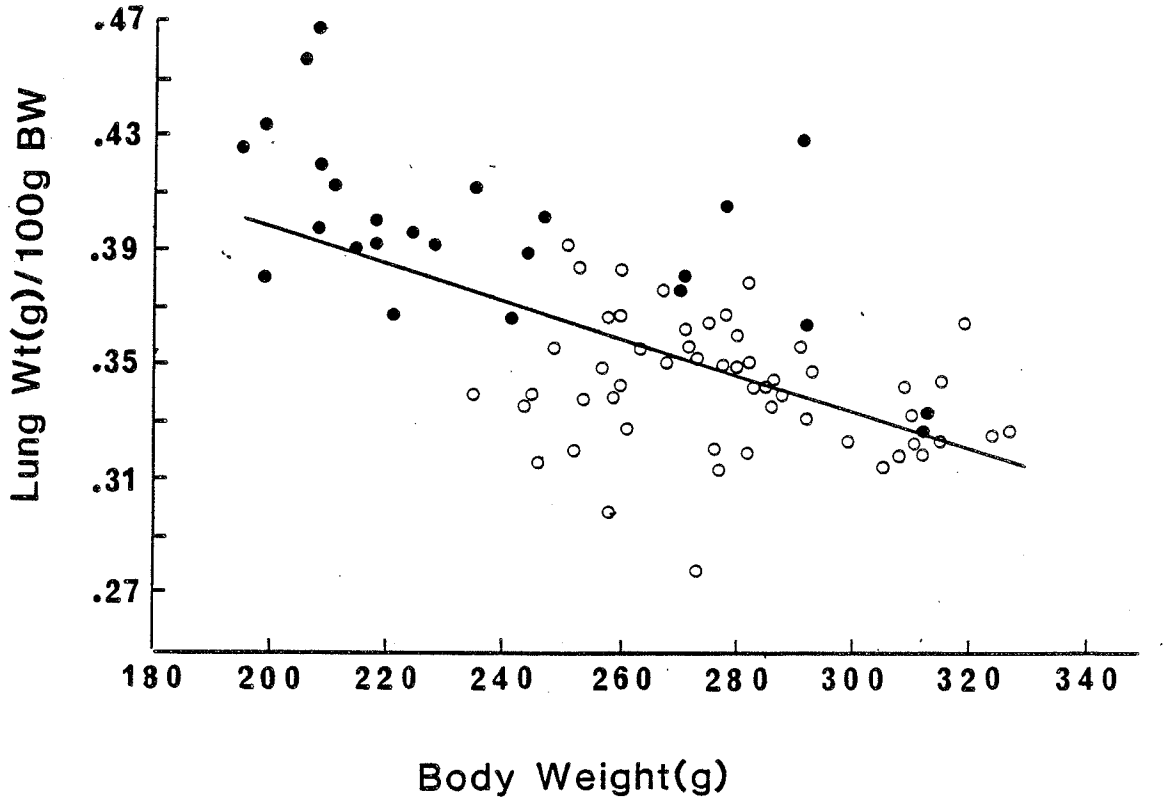


Figure 10: Relationship between lung weight expressed per body weight and body weight in non-pregnant rats (closed circles) and pregnant rats (open circles). Each point represents one rat. Body weight for pregnant rats is after exclusion of fetal and placenta weights (BW_{-fp}). Regression by least squares, $r = -0.649$, $P < 0.001$.

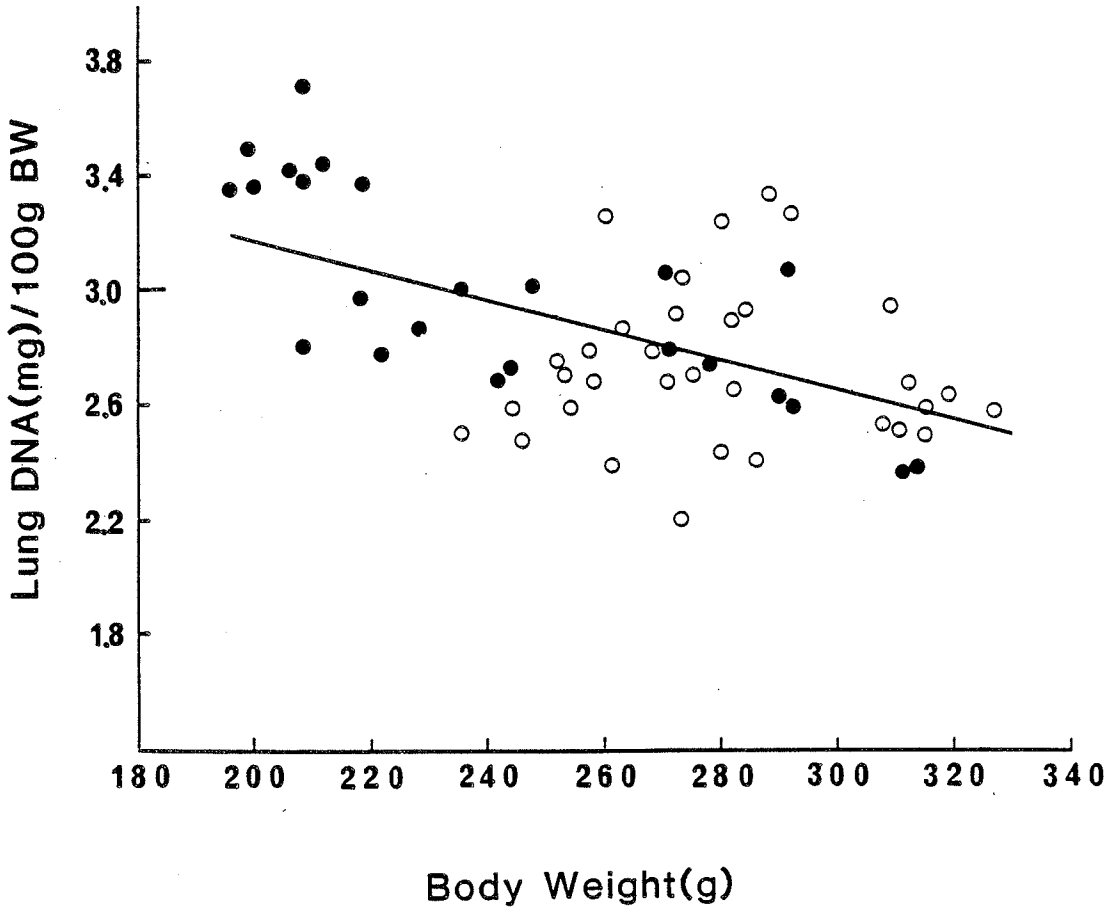


Figure 11: Lung DNA expressed per body weight in relation to body weight for non-pregnant (closed circles) and pregnant (open circles) rats. Each point represents one animal. Body weight for pregnant rats is BW_{-fp}. Regression line by least square, $r = -0.548$, $P < 0.001$.

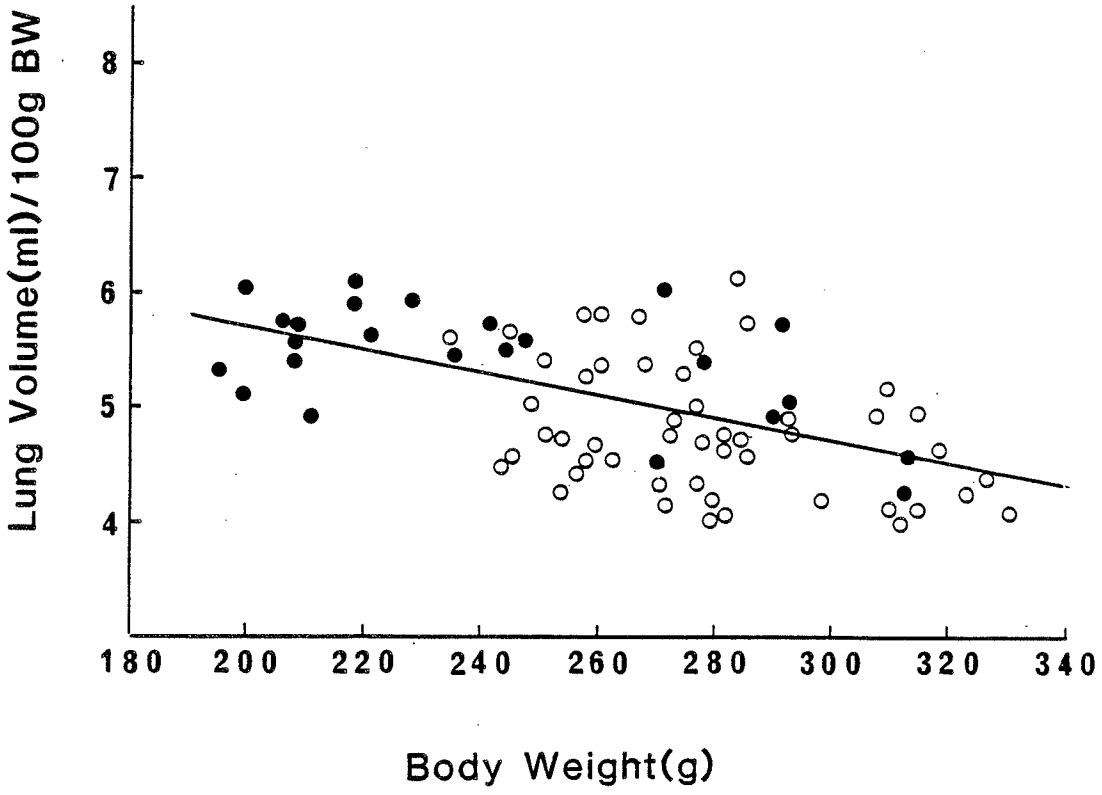


Figure 12: Lung air volume expressed per body weight in relation to body weight for non-pregnant (closed circles) and pregnant (open circles) rats. Each point is one animal. $r = -0.558$, $P < 0.001$.

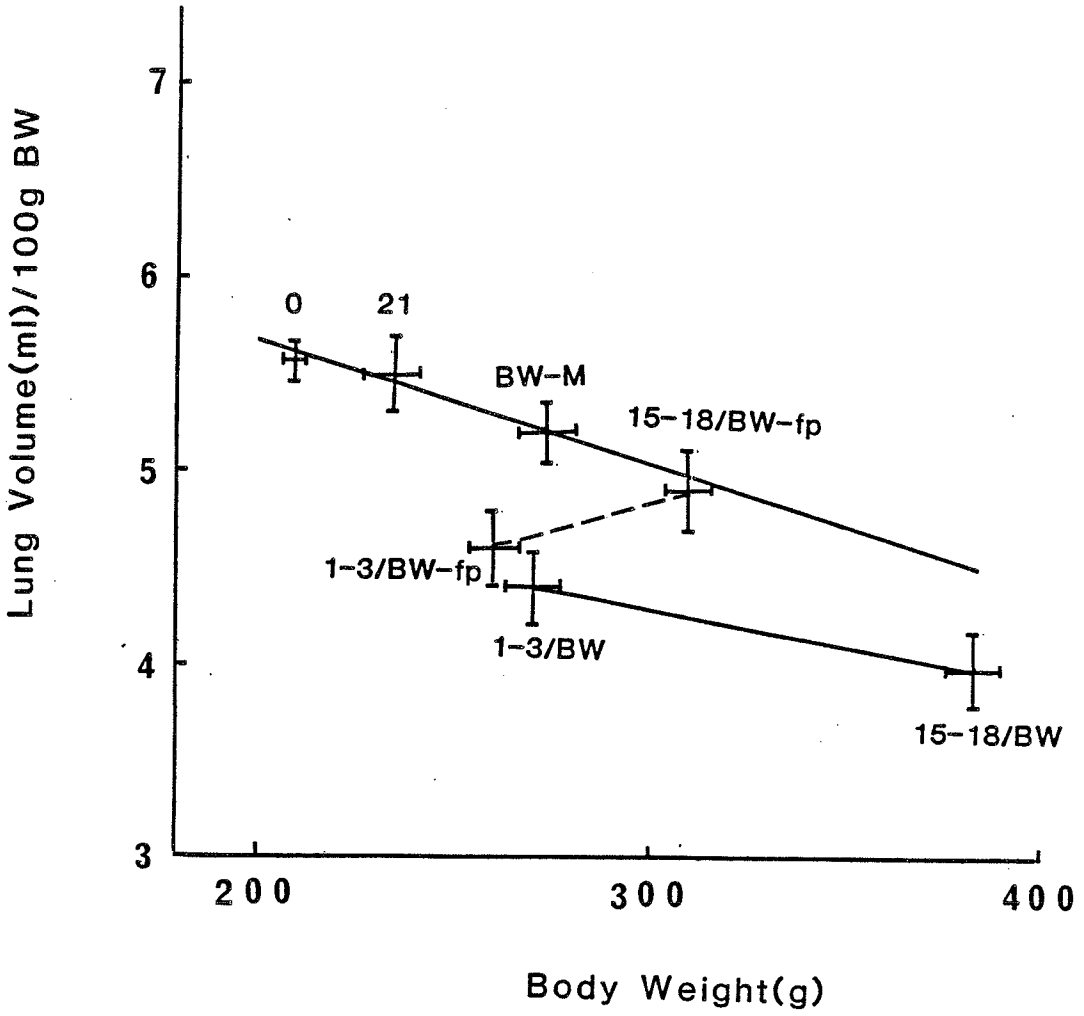


Figure 13: Relationship between lung volume expressed per body weight and body weight for 5 groups of non-pregnant and pregnant rats (n = 6 to 11). Each point with bars represents the mean ± 1 SE for lung volume and body weight. The upper solid line shows the decline in ratio of lung volume per body weight for non-pregnant rats (from left to right: day 0, day 21 and body weight matched). The lower solid line shows the decline in this ratio for pregnant rats with small (1-3) or large (15-18) litters when total maternal body weight was considered in the ratio. The dotted line connects the ratios for pregnant rats of small or large litters when only maternal body weight excluding fetal and placental weight (BW_{-fp}) is taken into account.

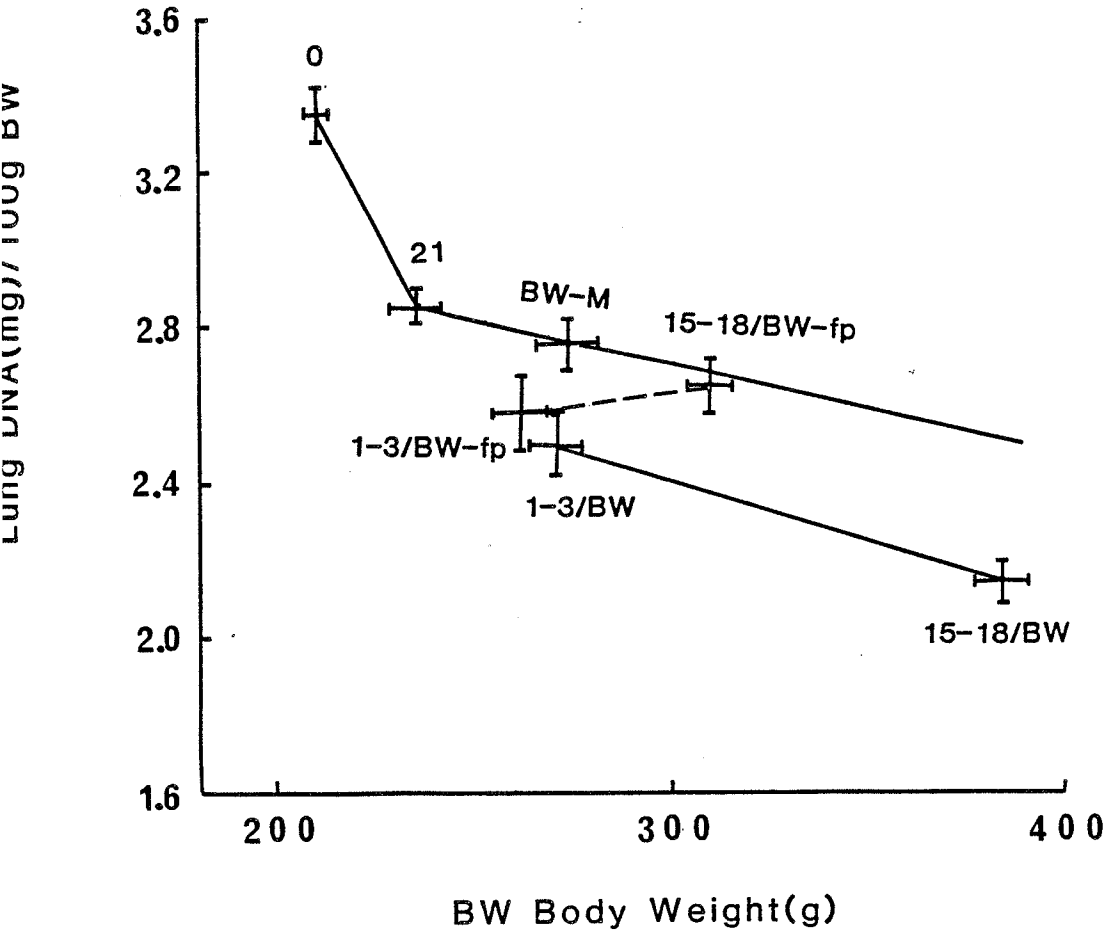


Figure 14: Relationship between lung DNA expressed per body weight and body weight for 5 groups of rats described in Figure 13. For description, see legend of Figure 13.

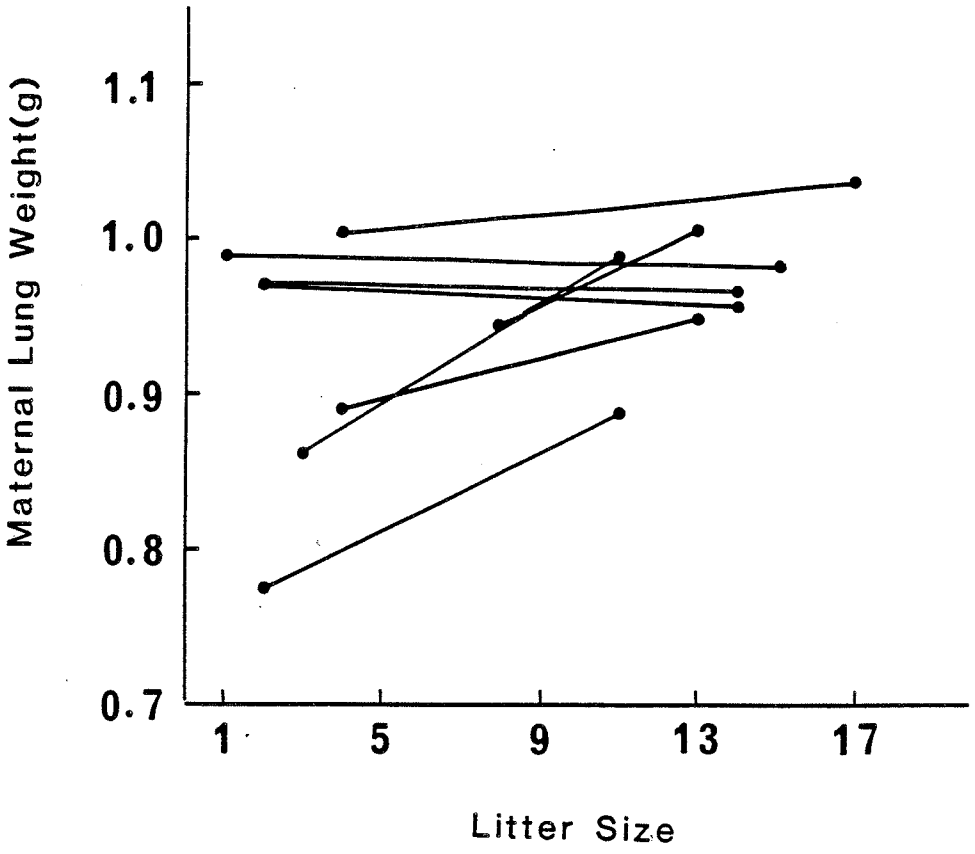


Figure 15: Lung weight for eight pairs of isobody weight (BW_{-fp}) pregnant rats with different litter sizes. Each line connects two pregnant rats of equal body weight. A T-test of paired variates demonstrated a small difference ($0.1 < P < 0.05$) between the lung weight of small and large litters, an unpaired T-test revealed no difference between the two groups.

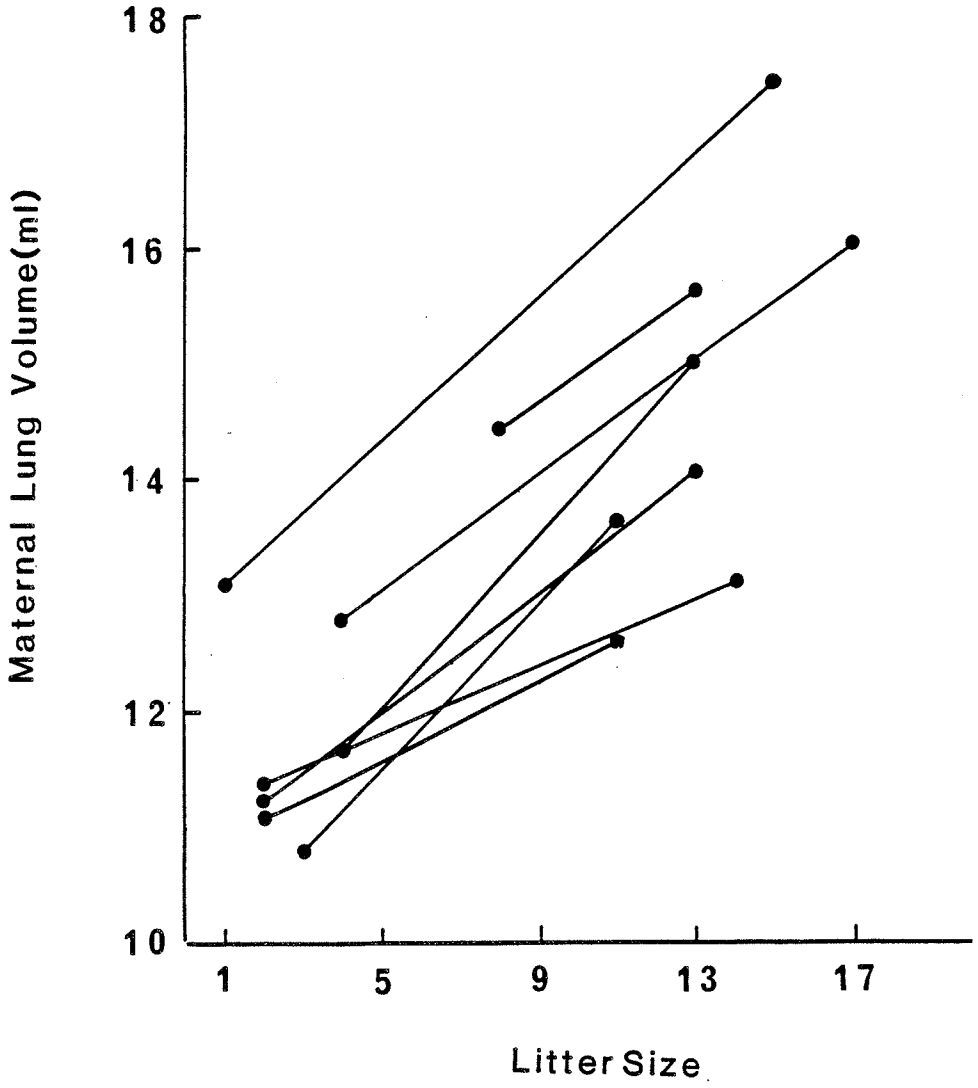


Figure 16: Lung volume for eight pairs of isobody weight (BW_{-fp}) pregnant rats with different litter sizes. Each line connects two pregnant rats of equal body weight. A significant difference in lung volume was found between rats with small and large litters by employing a T-test of paired variates ($P < 0.001$), and unpaired variates ($P < 0.01$).

of lung volume per lung weight and lung volume per DNA are not influenced by body weight (Figs. 17, 18), it is justified to assume that the maternal lung DNA also increases with increasing litter size.

Comparison of lung weight, DNA content and lung air volume of pregnant rats (with small and large litter size) with that of non-pregnant rats (day zero, day 21 and body weight-matched) indicate that the lung in pregnant rats only grows when the litter size is large, since no difference is noted between lungs of non-pregnant rats and pregnant rats with small litter size (Tables 1 and 2).

Placenta

As shown in Figs. 19 and 20, placenta weight declines as litter size increases from one to about 9 or 10, thereafter it remains constant. The placenta weight corresponds to fetal body weight in that the larger the fetus the larger the placenta weight (Fig. 21). The ratio of placenta weight per body weight is almost constant for different fetal body weights. As fetal body weight increases the ratio decreases, but not significantly. Although placenta weight per fetal body weight is indirectly related to maternal body weight (BW-fp:Fig. 22, and BWo:not presented), because a similar relationship exists between litter size and maternal body weight, it is difficult to distinguish whether it is the maternal body weight or the litter size which influences the placenta weight. When these factors are tested on fetuses of similar body weights (e.g. 4.4-4.5 g), variations in placenta weight appear to be inversely related to litter size (Fig. 23) and not

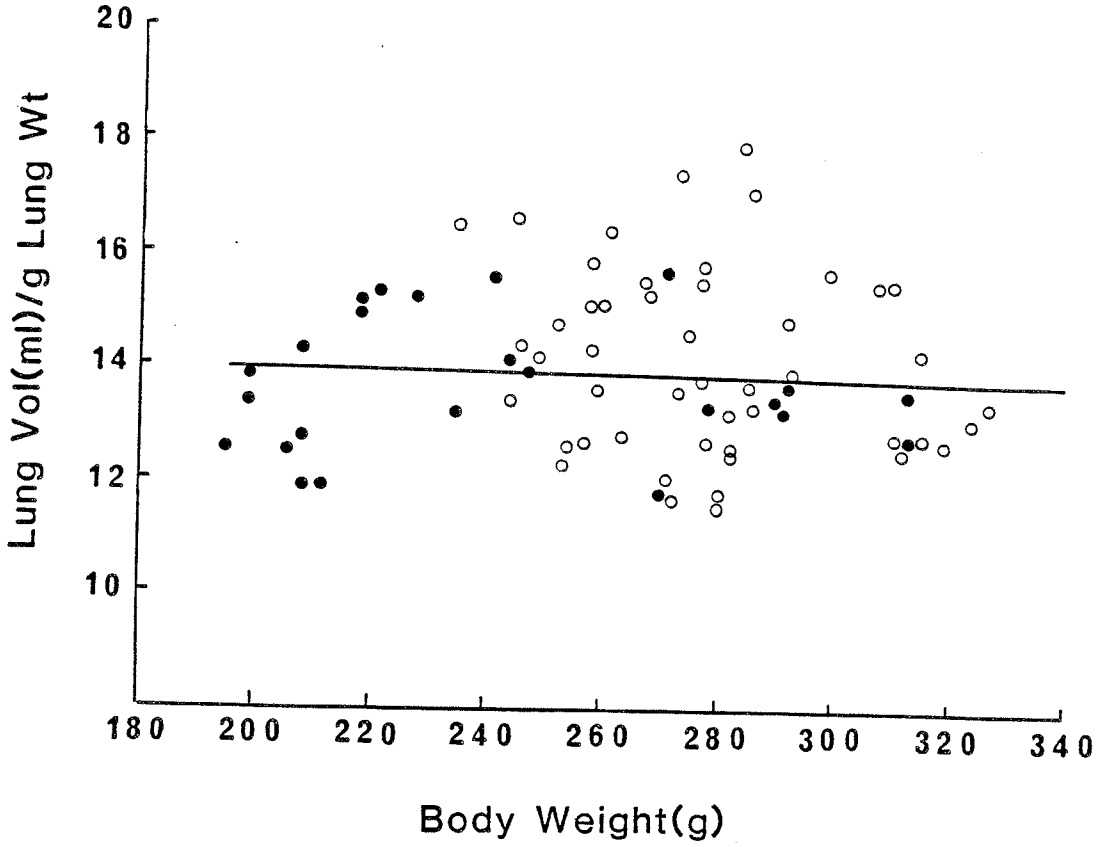


Figure 17: Relationship between lung volume expressed per gram lung weight and body weight for non-pregnant (closed circles) and pregnant (open circles) rats. Body weight for pregnant rats is after exclusion of fetal and placenta weights (BW_{-fp}).

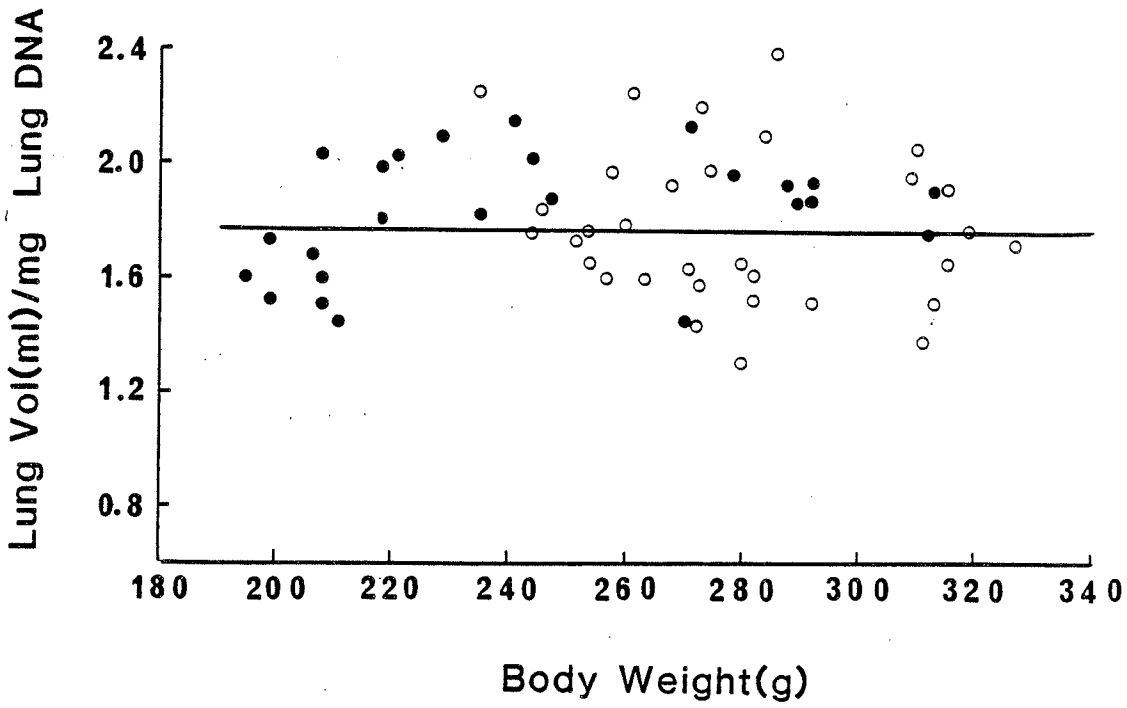


Figure 18: Relationship between lung volume expressed per lung DNA and body weight for non-pregnant rats (closed circles) and pregnant rats (BW_{-fp}, open circles).

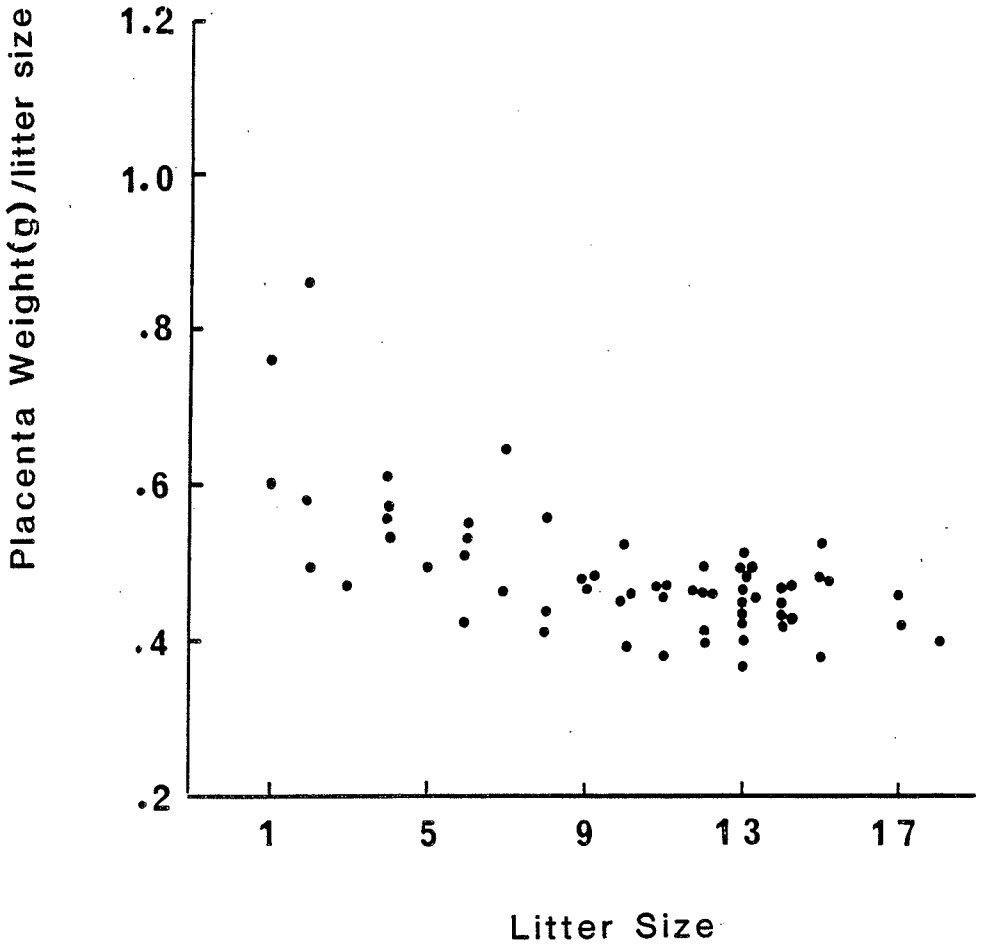


Figure 19: Placenta weights for different litter sizes. Each point represents the average placenta weight for a pregnant rat (total placenta weight/litter size).

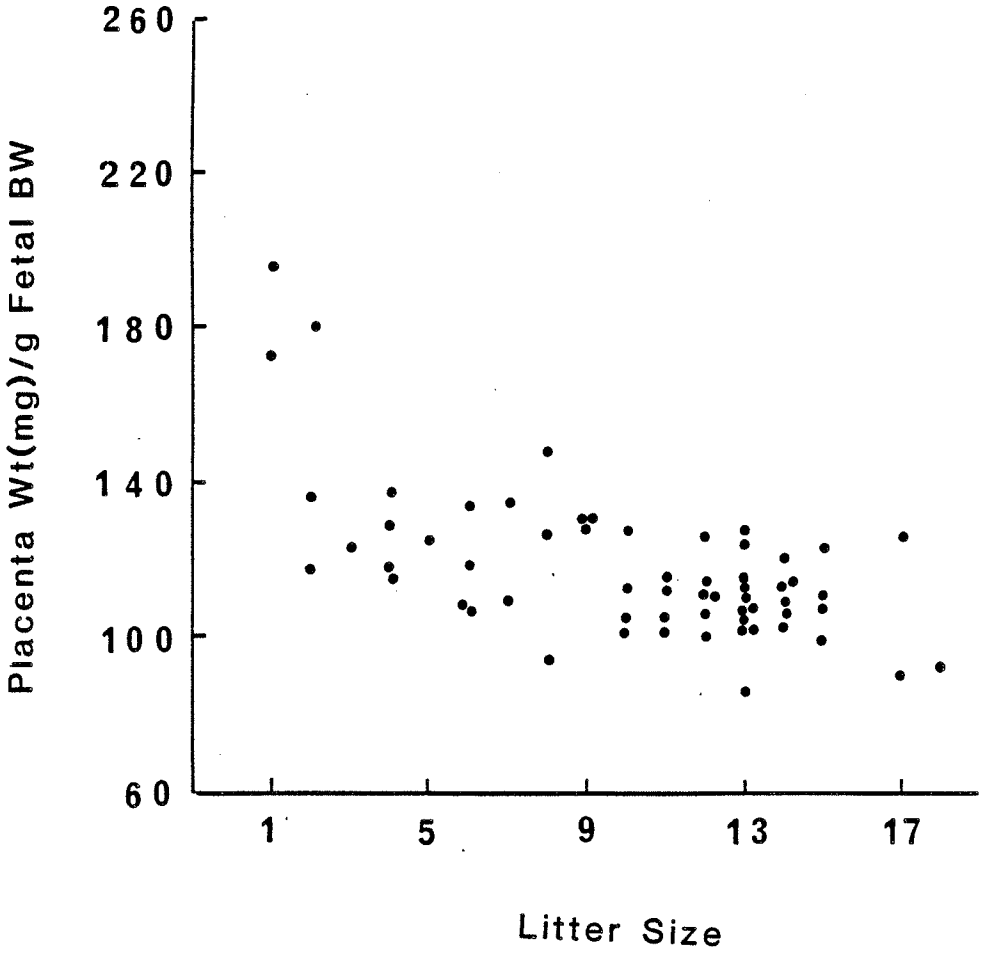


Figure 20: Relationship between placenta weight expressed per fetal body weight and litter size. Each point represents the average placenta weight per fetal body weight for a pregnant rat.

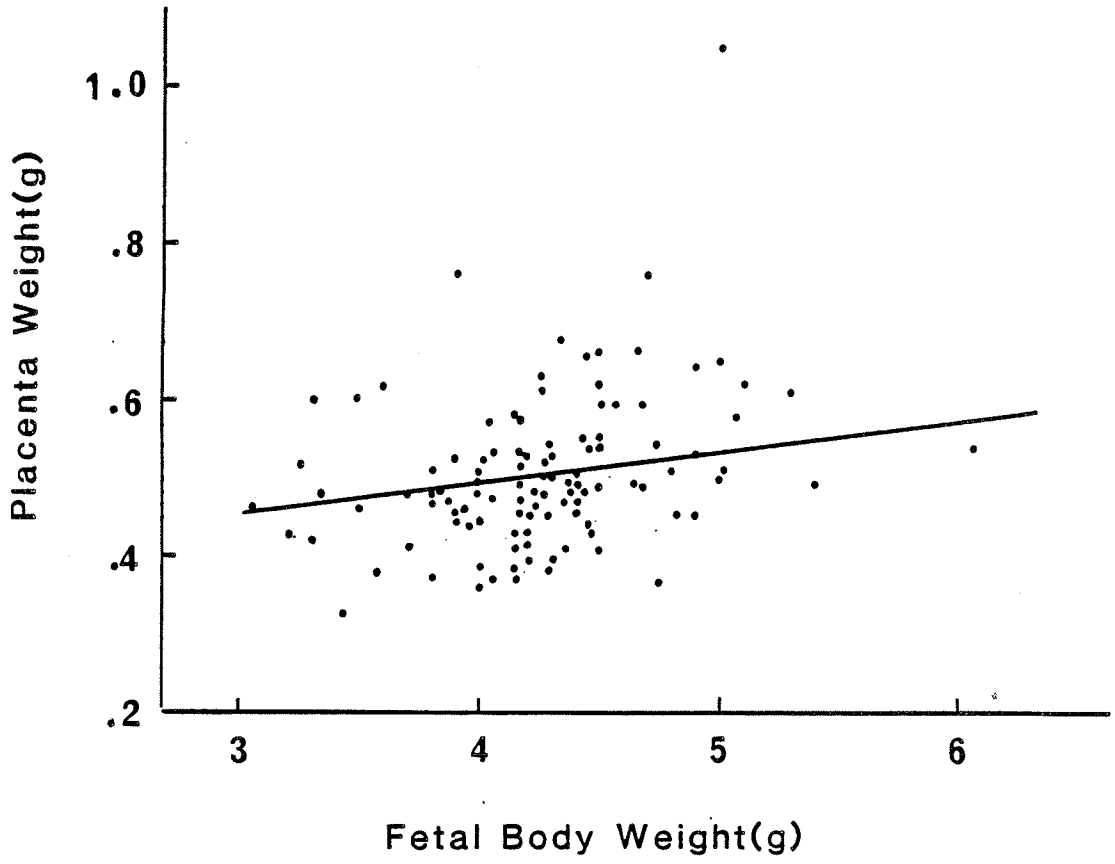


Figure 21: Relationship between placenta weight and fetal body weight. Each point represents one fetus. Regression line by least squares, $r = 0.248$, $P < 0.02$.

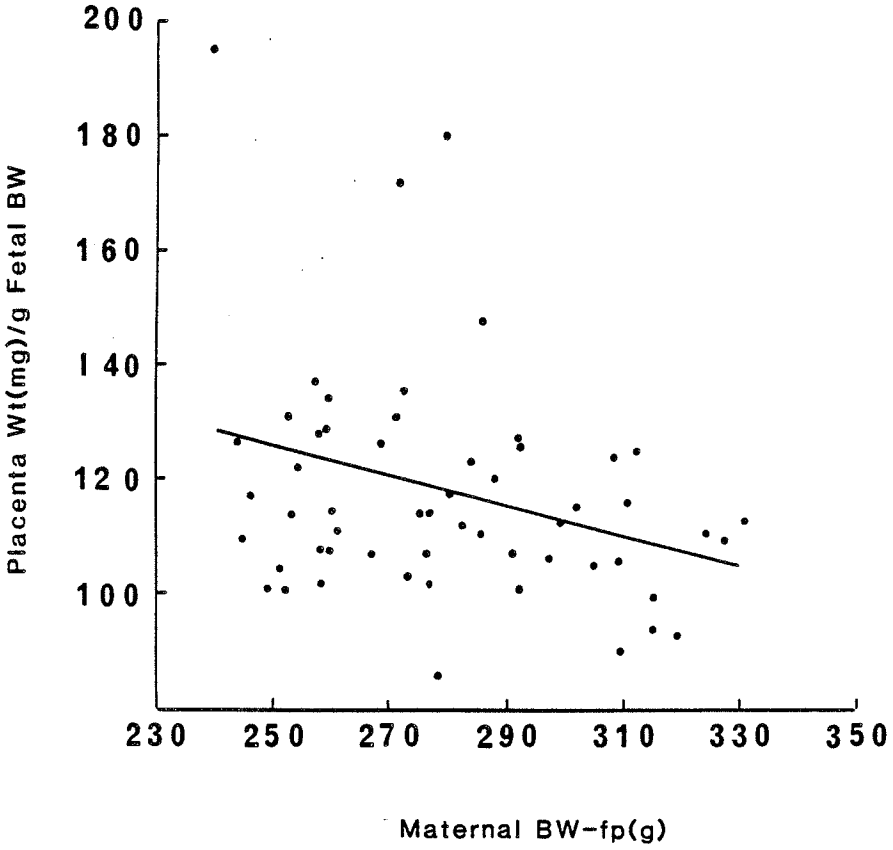


Figure 22: Relationship between placenta weight per fetal body weight and maternal body weight (BW_{-fp}). Each point is the average placenta weight per fetal body weight for a pregnant rat. $r = -0.298$, $P < 0.05$.

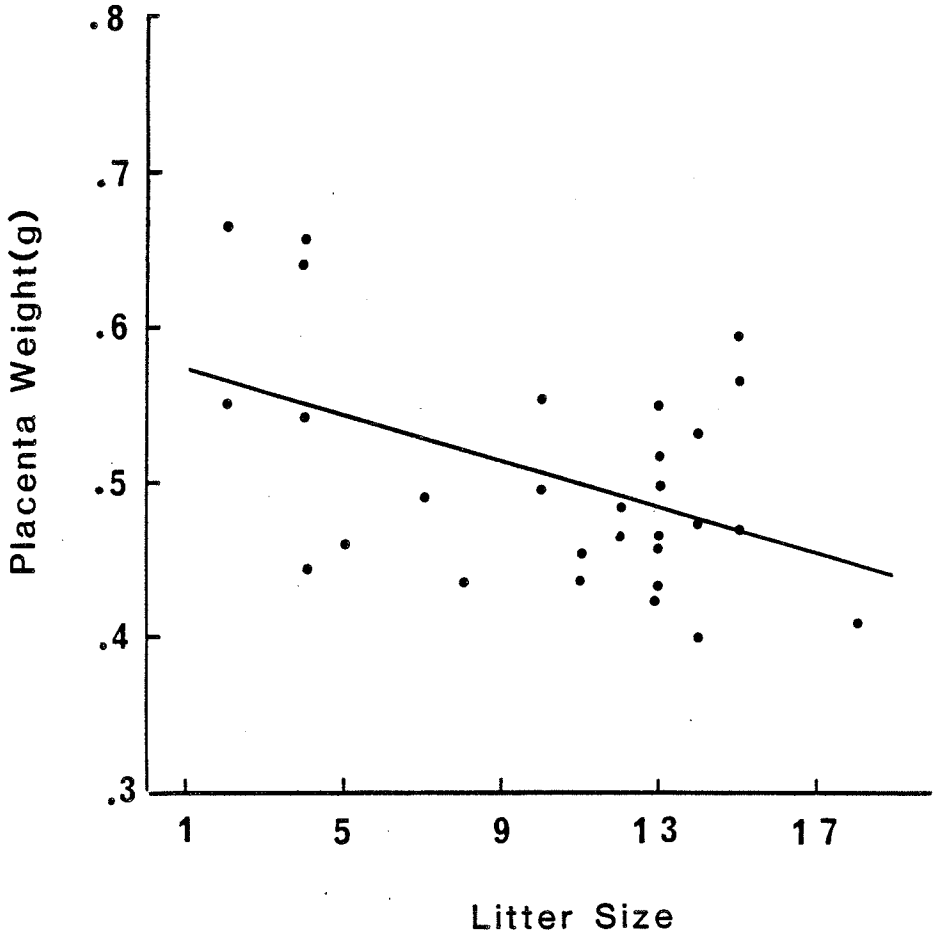


Figure 23: Relationship between placenta weight and litter size for fetuses with body weights ranging between 4.4 and 4.5 g. Each point represents one fetus, $r = -0.467$, $P < 0.02$.

to maternal body weight (BWo or BW-fp). Furthermore, variations in placenta weight for a given litter size (analyzed for litter size of 13) were independent of maternal body weight (BWo or BW-fp).

Since there is a highly significant correlation between the placenta DNA and placenta weight (Fig. 24), all the above relationships for placenta weight could be applicable for placenta DNA. Indeed Figure 25 shows that placenta DNA also decreases with increasing litter size.

The growth of the maternal lung and the placenta in relation to litter size appear to be in opposite directions. While the maternal lung enlarges in volume and in DNA content with increasing litter size, the placenta decreases in weight and in DNA (Fig. 26). This phenomenon is independent of maternal or fetal body weight (Fig. 27).

Placenta DNA content is compared between body weight-matched fetuses from small (1-4) and large (13-18) litters in Fig. 28. It shows that placenta DNA content decreased in the larger litters. The same was true for placenta weight ($n = 20$, $P < 0.01$). There was no relationship between maternal lung DNA (expressed per lung or per body weight) and average placenta DNA (expressed per placenta or per average fetal body weight).

Fetus

Fetal body weight ranged between 2.69 and 5.28 g. Average fetal body weight per litter was constant at different litter sizes (Fig. 29). Although fetal lung DNA increased with body

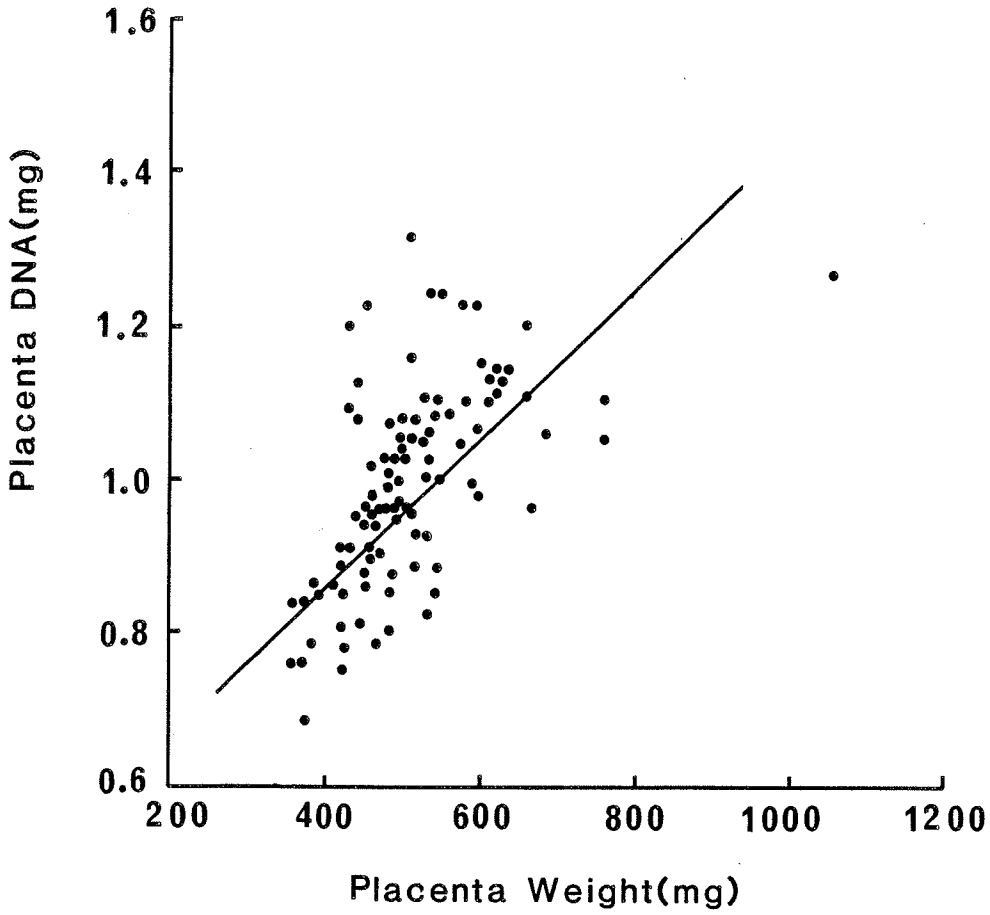


Figure 24: Relationship between placenta DNA and placenta weight. Each point represents one placenta, $r = 0.602$, $P < 0.001$.

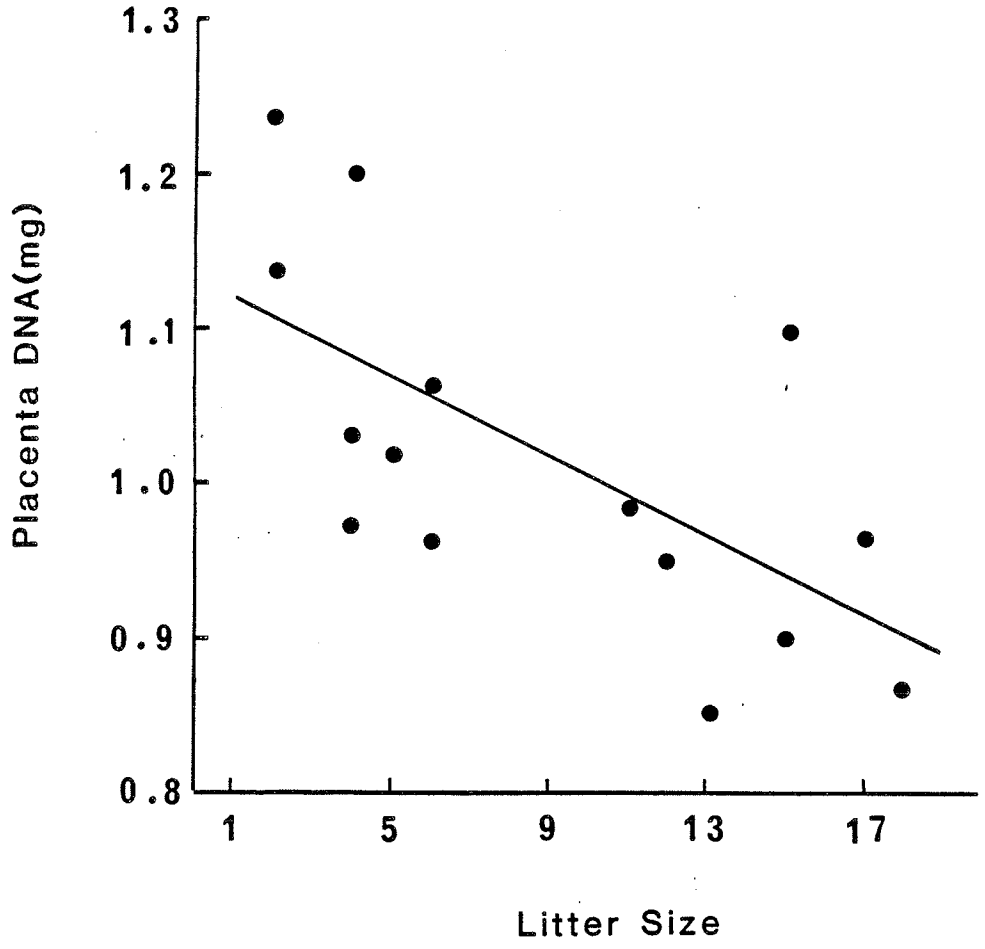


Figure 25: Placenta DNA in relation to litter size for fetuses with body weight ranging between 4.25 and 4.5 g. Each point is one placenta, $r = -0.664$, $P < 0.01$. A similar relationship was also found for fetal body weight between 4.0 and 4.25 g.

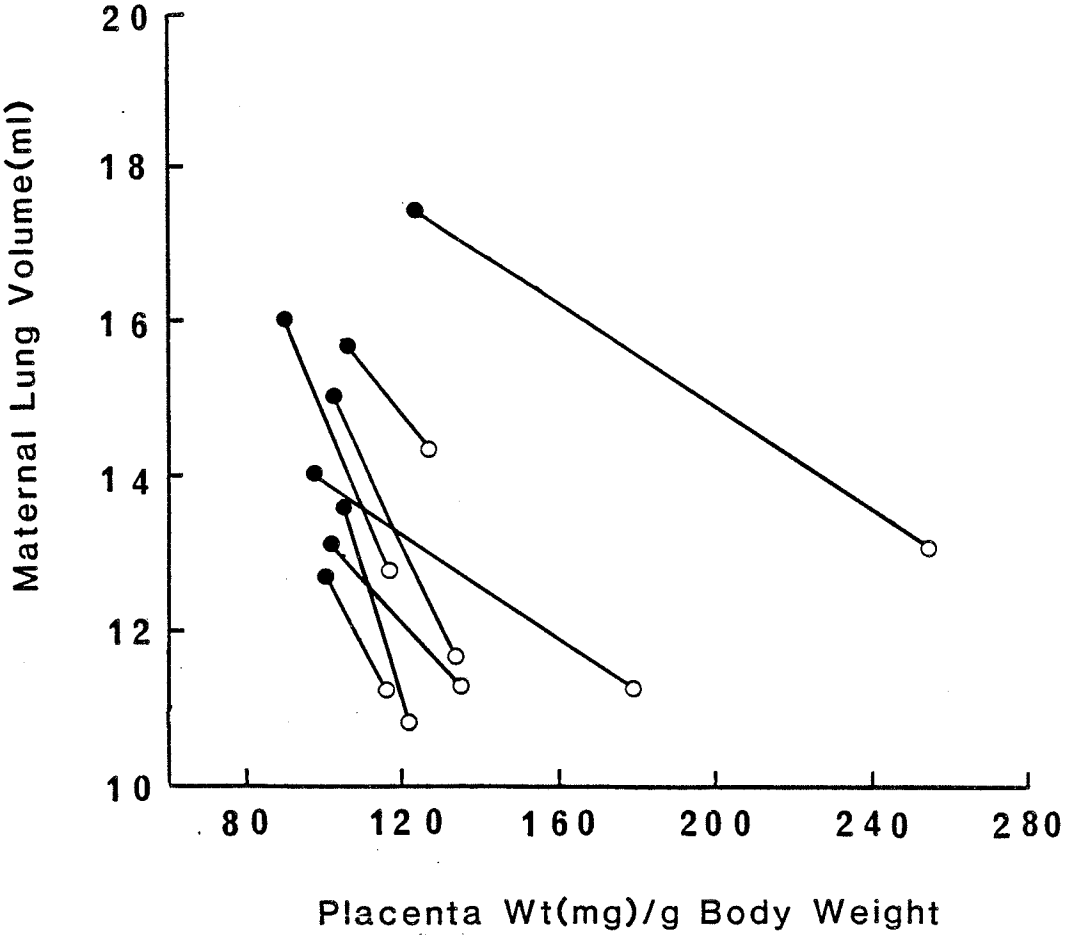


Figure 26: Relationship between maternal lung volume and placenta weight per fetal body weight for the same 8 pair of weight matched pregnant rats from Figure 16. Each line connects two pregnant rats of equal body weight (BW_{-fp}) with large (closed circles) and small (open circles) litter sizes. Placenta weight per body weight is the average of all placentae in a pregnant rat. The difference in placenta weight/BW between the two groups of rats was significant by both paired ($P < 0.02$) and unpaired ($P < 0.05$) T-test.

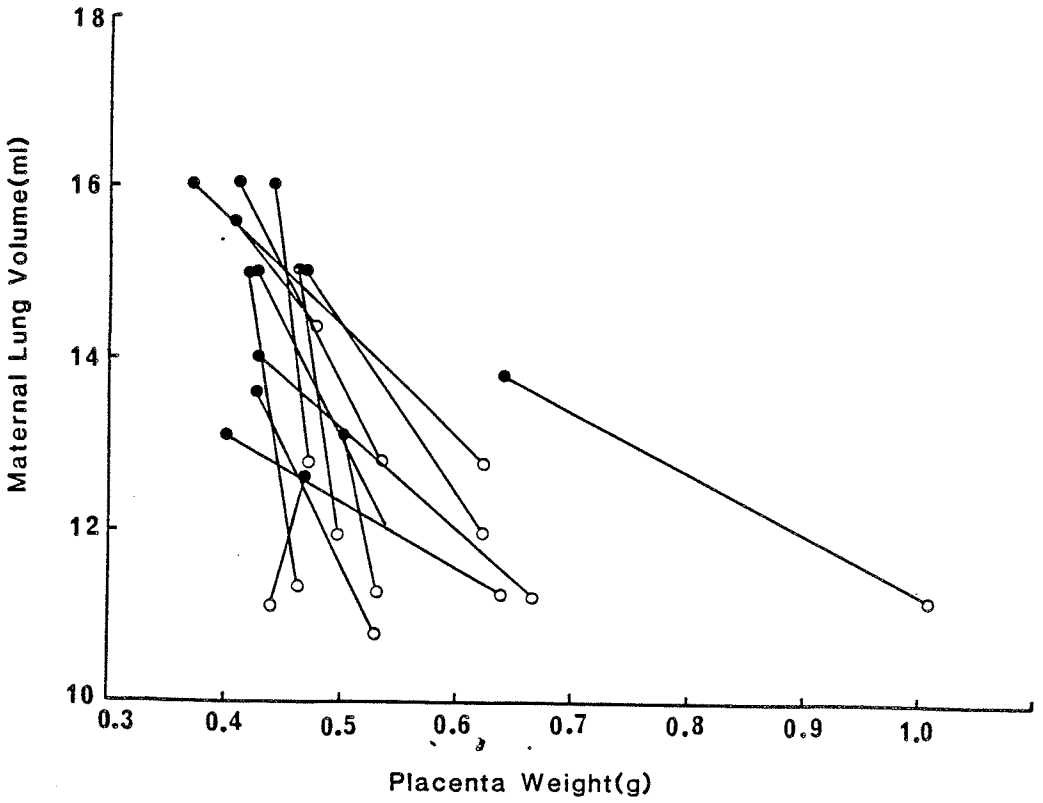


Figure 27: Relationship between maternal lung volume in pregnant rats of equal body weight (BW_{-fp}) but with small or large litters, and placenta weight in fetuses of equal body weight. Each line connects the placenta weight of two fetuses of equal body weight from two mothers of equal body weight with large (closed circles) and small (open circles) litter sizes. The difference in placenta weight between the two groups of rats is significant by both paired ($P< 0.01$) and unpaired ($P< 0.01$) T-test.

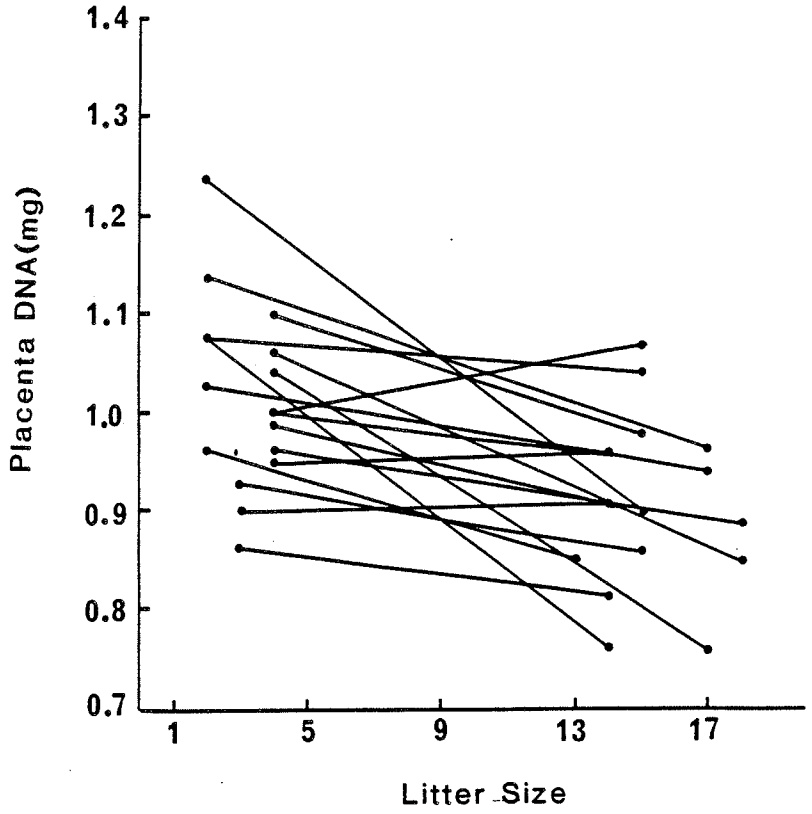


Figure 28: Relationship between placenta DNA and litter size in 17 pairs of fetuses of equal body weights but from small (1-4) and large (13-18) litters. Each line connects two fetuses of equal body weight. The difference in placenta DNA content between the two groups of fetuses was significant by both paired ($P < 0.01$) and unpaired ($P < 0.001$) T-tests.

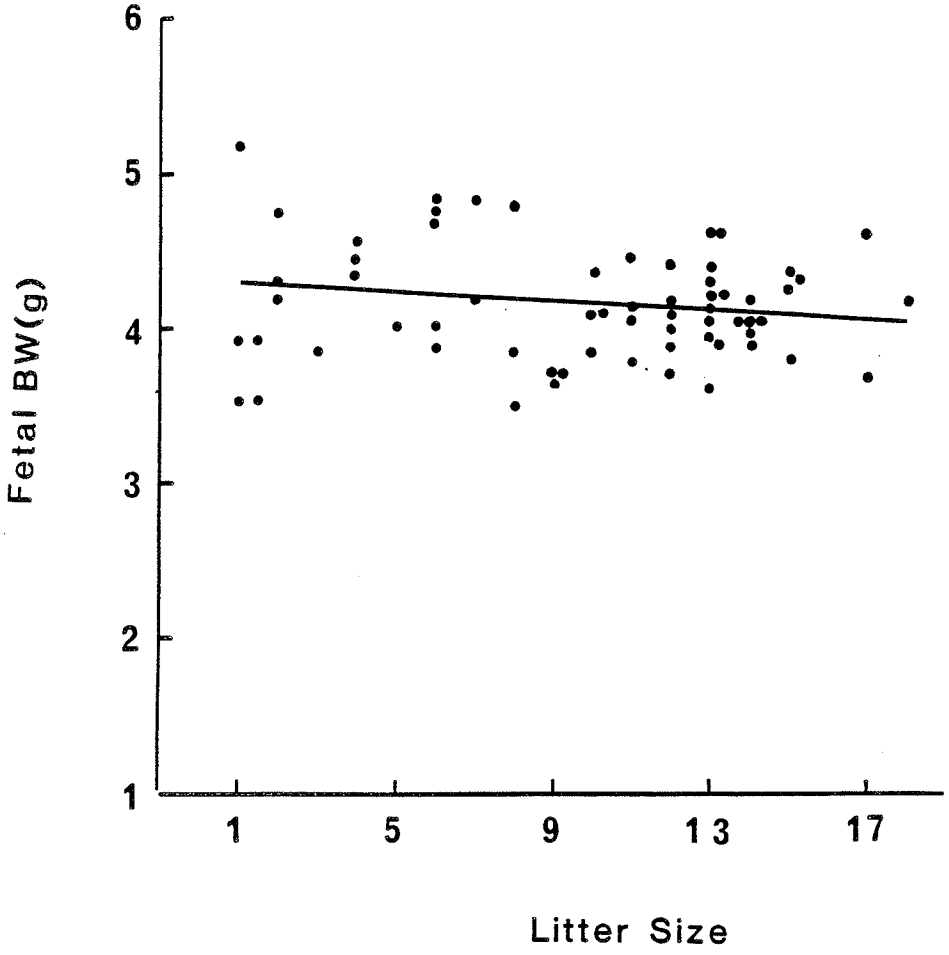


Figure 29: Fetal body weight in relation to litter size. Each point represents the average body weight of all fetuses in a pregnant rat. Regression by least squares did not show any significant change in average body weight with litter size.

weight, the ratio of lung DNA to body weight significantly decreased with increasing fetal body weight (Fig. 30). In addition, the DNA content of the fetal lung decreased when the litter size increased. This relationship for a given fetal body weight (e.g. between 4.25 and 4.5 g) is demonstrated in Fig. 31.

The data were analyzed to determine whether the size (DNA content) of the three gas exchange organs (maternal lung, placenta and fetal lung, the latter to be a gas exchange organ after birth) were related to each other. The results of these analyses for a fetal body weight between 4.25 and 4.5 g indicated no relationships between fetal lung DNA and placenta DNA and between maternal lung DNA and placenta DNA content. However, a significant direct relationship was found between maternal and fetal lung DNA content (Fig. 32). These same relationships were also found for fetal body weights of 4.0 to 4.25 g. These two body weights were chosen because of the large number of fetal rats in these two body weight ranges. When maternal lung DNA content expressed per body weight was plotted against fetal lung DNA per body weight, a similar direct relationship as above was found in rats with large litters (10-18) (Fig. 33), but not in rats with small litters (1-4) (Fig. 34).

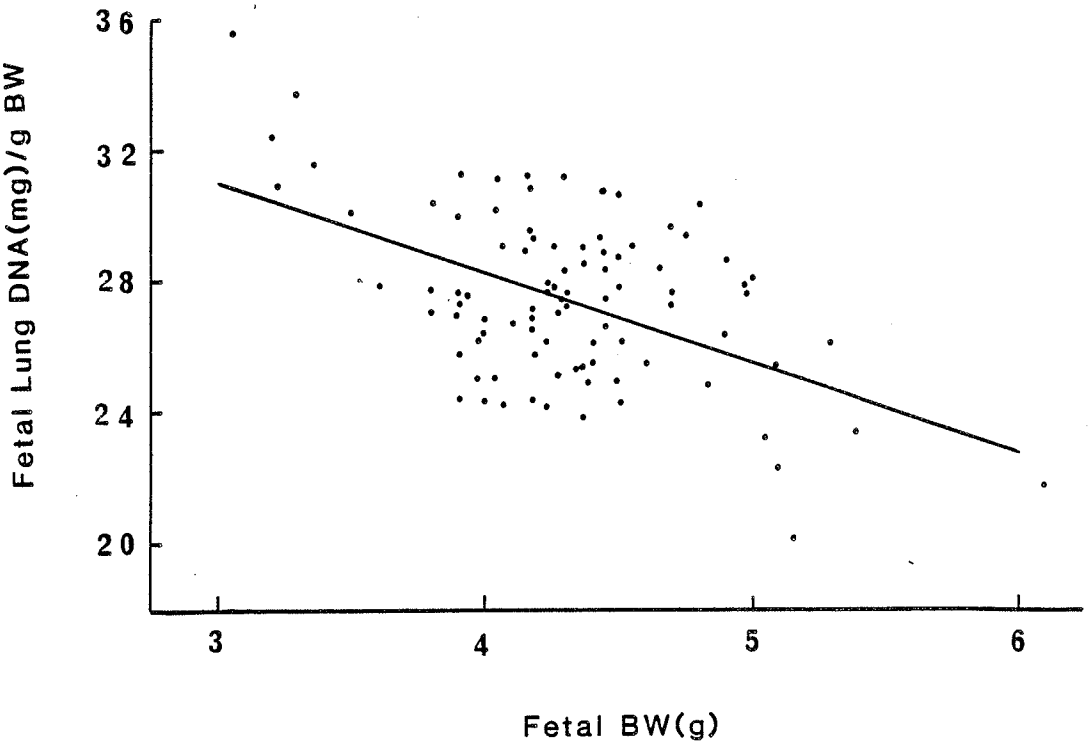


Figure 30: The ratio of fetal lung DNA per body weight in relation to fetal body weight. Each point is a fetus. Regression by least squares, $r = -0.517$, $P < 0.001$.

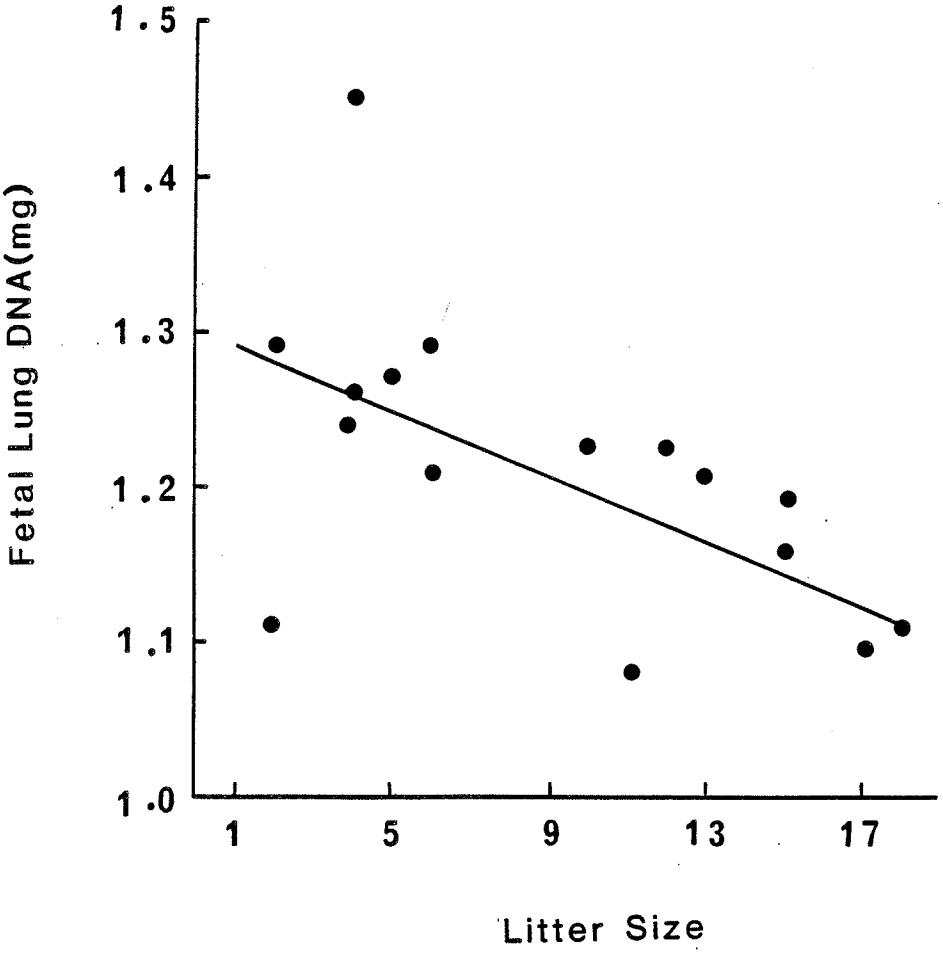


Figure 31: Relationship between fetal lung DNA and litter size for fetuses with body weights ranging between 4.25 and 4.5 g, $r = -0.577$, $P < 0.02$.

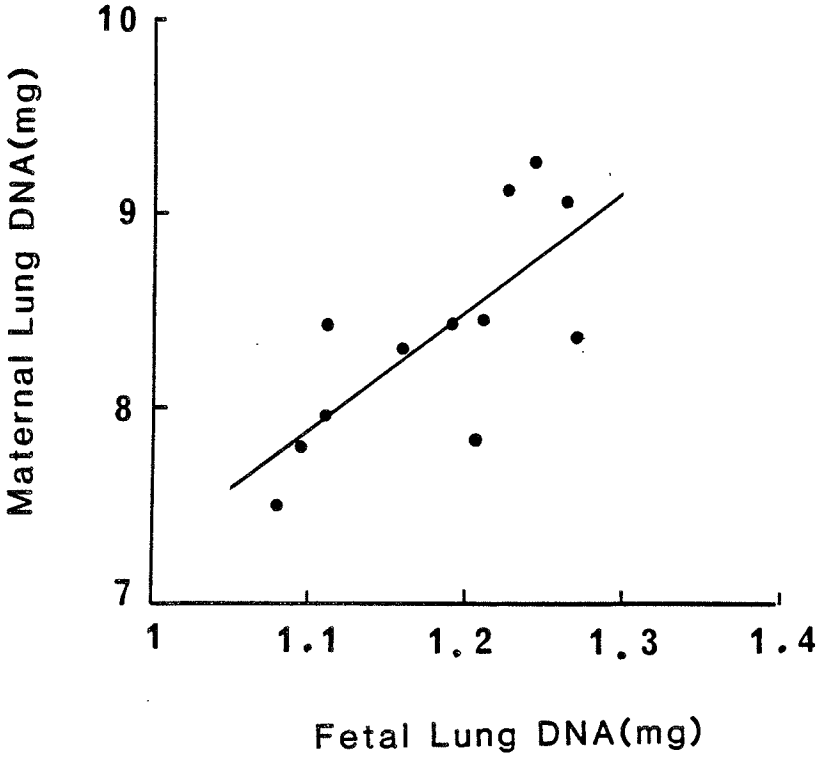


Figure 32: Relationship between maternal lung DNA and fetal lung DNA for fetuses with body weights between 4.25 and 4.5 g. Each point is one fetus, $r = 0.719$, $P < 0.01$.

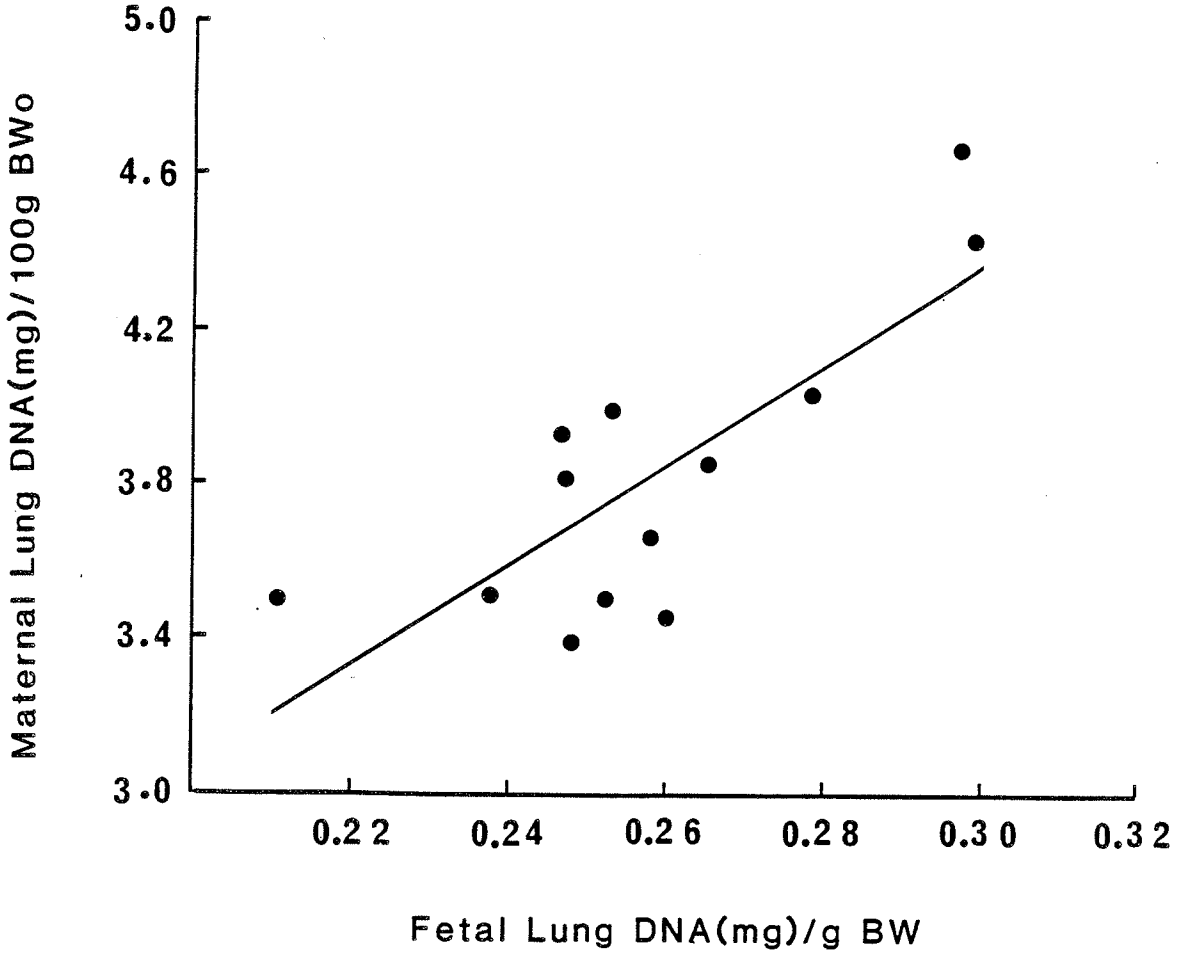


Figure 33: Relationship between maternal lung DNA expressed per body weight at GD 0 and fetal lung DNA expressed per body weight in pregnant rats with litter size of 10-18 fetuses. Each point is one fetus, $r = 0.784$, $P < 0.01$.

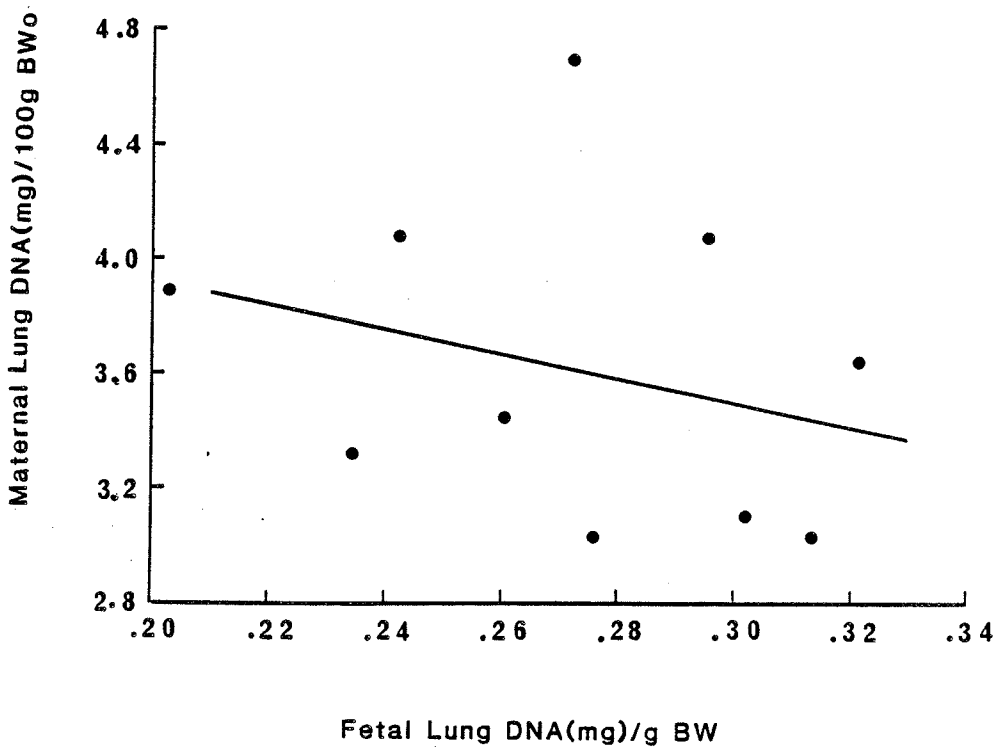


Figure 34: The same relationship as in Figure 33 but for rats with a litter size of one to four fetuses, r = -0.282, not significant.

(2) Hypoxia

Mother:

The body weight and food intake of rats exposed to continuous hypoxia for one week beginning on gestation day 14 (hypoxia 14) were similar to that of controls (Table 3). Fetal absorption was slightly higher than control (1.3%).

Figure 35 and Table 3 show the body weight of pregnant rats exposed to intermittent hypoxia for 2 weeks beginning on GD 7 (hypoxia 7). Although food intake was similar to that of control, the body weight was significantly decreased. The rate of fetal absorption was up to 9.1%.

Table 4 shows lung weight, DNA content and lung air volume (at 30 cm H20 inflation pressure) of rats exposed to intermittent or continuous hypoxia. It appears that intermittent hypoxia caused only an increase in maternal lung weight while continuous hypoxia for one week increased lung weight, DNA content and lung air volume. In addition, the lung weight of hypoxia 14 rats was greater than that of those experiencing intermittent hypoxia.

Table 3 shows the influence of hypoxia on the liver, kidney and heart of hypoxia 14 rats. All three organs were significantly larger than control.

Placenta:

As shown in Table 5, hypoxia 7 rats had smaller placentae (weight and DNA content) than controls. No reduction in

Table 3: Measurements in Pregnant Rats

Condition	Control	Hypoxia (7)	Cold (3)	Exercise (3)	Hypoxia (14)	Cold (14)
Body wt at gestation day 0 (BW_0)	207.43±1.9 (42)	209.78±2.89 (18)	208.75±2.16 (24)	205.6±1.99 (18)	203.2±4.1 (5)	206.2±3.71 (5)
Body wt (gestation day 21)/BW_0 x 100	161.5±1.31 (41)	148.85±1.77 * (18)	159.45±1.54 (24)	157.3±1.80 (18)	165.9±3.72 (5)	156.2±2.97 (5)
BW_{-fP}/BW_0 x 100	134.24±1.24 (32)	128.45±1.85 ‡ (11)	132.45±1.22 (18)	128.77±1.31 † (15)	140.37±4.18 (5)	128.52±1.98 (5)
Liver wt (g)	10.868±0.26 (20)		12.65±.34 * (13)	10.89±0.19 (14)	12.27±0.53 Φ (5)	12.09±0.342 Φ (5)
Liver wt (g)/ 100 g BW_0	5.369±0.117 (20)		6.11±0.14 * (13)	5.276±0.124 (14)	6.046±.271 ‡ (5)	5.878±0.231 (5)
Liver wt (g)/ 100 g BW_{-fP}	4.010±0.061 (19)		4.59±0.09 * (12)	4.061±0.078 (12)	4.306±.129 Φ (5)	4.568±0.127 * (5)
Kidney wt (g)	1.568±0.034 (20)		1.816±0.043 * (13)	1.639±0.031 (14)	1.846±0.06 † (5)	1.697±0.037 (5)
Kidney wt (g)/ 100 g BW_0	0.773±0.015 ((20)		.879±0.023 * (13)	.797±0.017 (14)	.9098±.032 * (5)	.824±0.024 (5)
Kidney wt (g)/ 100 g BW-fP	0.578±0.009 (19)		.658±0.014 * (12)	0.618±0.010 † (12)	0.648±0.014 † (5)	0.641±0.011 † (5)
Heart weight (g)	0.710±0.015 (20)		0.827±0.012 * (13)	0.783±0.014 † (14)	0.854±0.031 * (5)	0.791±0.017 (5)
Heart wt (g)/ 100 g BW_0	0.349±0.005 (20)		.400±0.006 * (13)	.381±0.007 † (14)	0.420±0.012 * (5)	.384±.005 † (5)
Heart wt (g)/ 100 g BW-fP	0.262±0.003 (19)		.299±0.004 * (12)	.296±0.004 * (12)	0.299±0.007 * (5)	.299±.004 * (5)

BW_0 = body weight at GD.0. BW_{-fP} = body weight at GD 21 excluding fetal and placenta weights. Data expressed as mean ±1 SE; numbers in parentheses indicate number of animals studied. Comparisons were made between each experimental condition and control.
* = $P < 0.001$, † = $P < 0.01$, ‡ = $P < 0.02$, Φ = $P < 0.05$

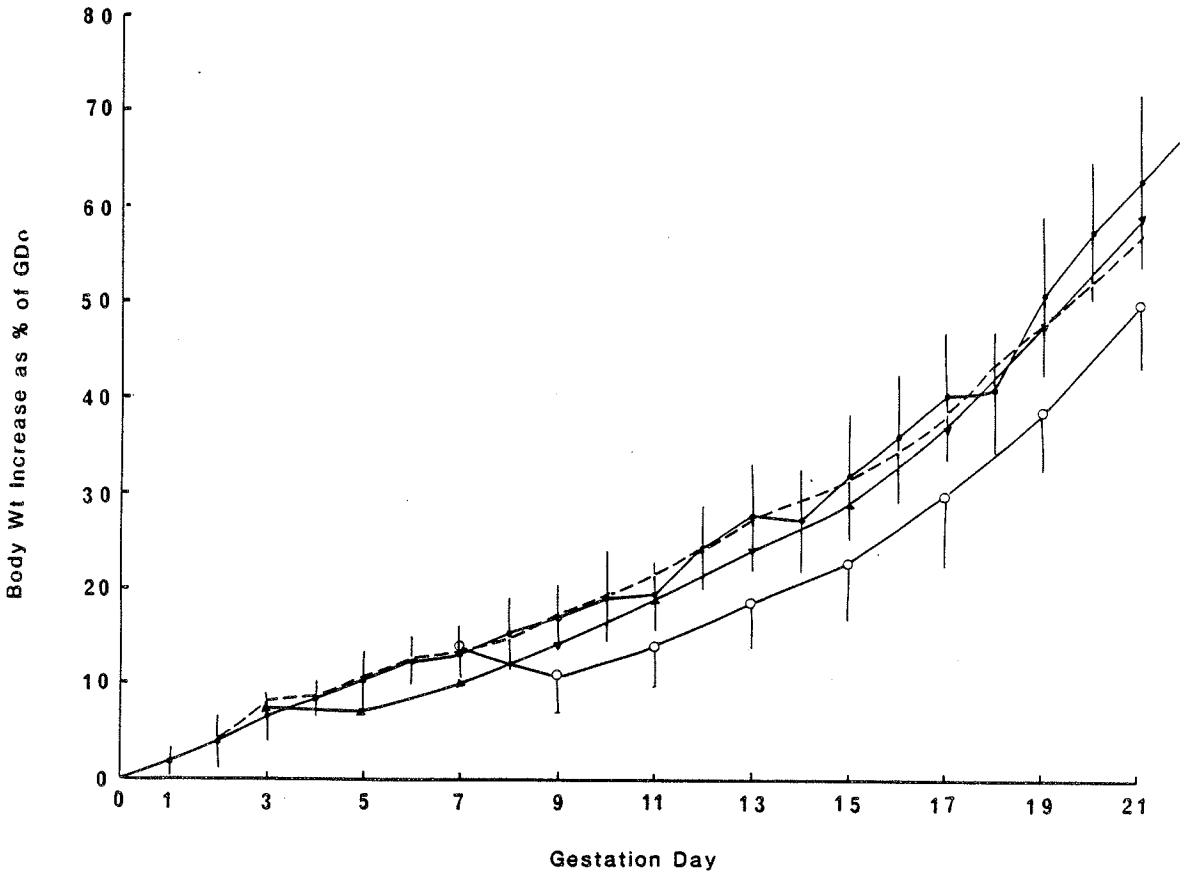


Figure 35: Maternal body weight expressed as a percentage of BW_0 for rats exposed to intermittent hypoxia beginning on GD 7 (open circles), cold beginning on GD 3 (triangles) or subjected to exercise (dashed line). Control rats (closed circles) are shown for comparison. Data are given as mean ± 1SD (n = 11 to 50).

Table 4: Lung Measurements of Pregnant Rats Exposed to Hypoxia

	Control	Hypoxia 7	Hypoxia 14
Lung wt (g)	0.9421±0.0132 (29)	1.084±0.028 * (14)	1.302±0.057 * (5) a
Lung wt (g)/ 100 g BW_0	.4564±.0066 (29)	0.514±0.011 * (14)	.642±0.032 * (5) a
Lung wt (g)/ 100 g BW_{-fP}	.344±0.005 (27)	0.399±0.009 * (11)	0.456±0.011 * (5) b
Lung DNA (mg)	7.762±0.34 (12)	8.071±0.288 (9)	8.99±0.392 Φ (5)
Lung DNA (mg)/ g lung	8.273±0.261 (12)	7.467±0.234 Φ (9)	6.918±0.196 † (5)
Lung DNA (mg)/ 100 g BW_0	3.741±.133 (12)	3.781±0.143 (9)	4.442±0.257 ‡ (5)
Lung DNA (mg)/ 100 g BW_{-fP}	2.817±0.084 (12)	2.968±0.102 (9)	3.157±0.122 Φ (5)
Lung Volume Vmax (ml)	13.27±0.268 (25)	14.46±0.322 † (14)	15.52±0.8 † (5)
Lung vol. (ml)/ g lung	14.174±0.264 (24)	13.39±0.334 (14)	11.94±0.48 † (5) d
Lung vol. (ml)/ 100 g BW_0	6.468±0.126 (25)	6.867±0.196 (14)	7.657±0.44 † (5)
Lung vol. (ml)/ 100 g BW_{-fP}	4.813±0.101 (24)	5.264±0.162 ‡ (11)	5.442±0.208 ‡ (5)
Cell no. ($X10^6$) /lung	1251.94±54.84 (12)	1301.77±46.45 (9)	1450.0±63.23 Φ (5)

Hypoxia 7 = pregnant rats exposed to intermittent hypoxia (10 hr/day) starting gestation day 7. Hypoxia 14 = pregnant rats exposed to continous hypoxia as of gestation day 14. BW_0 = body weight at gestation day zero, BW_{-fP} = body weight at gestation day 21 excluding fetal and placenta weights. Vmax = lung air volume at 30 cm H_2o inflation pressure. Data are expressed as mean ± 1 SE, number in parentheses indicate number of animals studied. Different from controls: * = $P < 0.001$, † = $P < 0.01$, ‡ = $P < 0.02$, Φ = $P < 0.05$. Different from hypoxia 7: a = $P < 0.001$, b = $P < 0.01$, d = $P < 0.05$

Table 5: Placenta Measurements

	Control	Hypoxia 7	Hypoxia 14	Cold 3	Cold 14	Exercise 3	Exercise 11
Fetal Body Weight	4.09±0.017 (406)	3.24±0.047 * (146) a	3.86±0.046 * (60)	4.41±0.028 * (227) b	4.19±0.048 (61)	4.18±0.043 Φ (184) d	3.56±0.045 * (41)
Placenta Wet Weight (mg)	452.4±3.29 (385)	408.3±6.24 * (148)	460.4±7.57 (60)	480.9±4.7 * (230)	483.7±16.7 Φ (23)	428.8±5.24* (179)	416.5±10.34 * (41)
Placenta Wet Weight/Body Weight (mg/g)	111.05±0.83 (373)	127.9±2.2 * (164)	119.96±2.12 * (60)	109.5±1.1 (227)	113.9±5.32 (23)	103.1±1.30* (179)	116.6±2.2 Φ (41)
Placenta Dry Weight (mg)	73.87±2.57 (21)	66.5±2.21 Φ (26)	----	77.14±2.99 (21)	----	77.3±2.63 (26)	----
Placenta Dry Weight/wet wt x 100	16.84±0.12 (21)	17.18±0.16 (26)	----	16.86±0.114 (21)	----	17.23±0.112 Φ (26)	----
Placenta DNA (mg)	0.947±0.023 (35)	0.866±0.017 † (45)	0.983±0.02 (17)	0.929±0.014 (50)	1.05±0.027† (23)	0.886±0.022 (30)	.876±0.036 (12)
Placenta DNA(mg)/ g tissue	1.97±0.053 (35)	2.04±0.044 (45)	2.15±0.05 Φ (17)	1.92±0.0265 (50)	2.23±0.05 † (23)	2.07±0.04 (30)	2.21±0.07 Φ (12)
Placenta DNA (mg)/ g Body wt	0.239±0.007 (35)	0.262±0.007 Φ (45)	0.253±0.006 (17)	0.211±0.004 * (50)	0.243±0.006 (23)	0.224±0.006 (30)	0.246±0.007 (12)

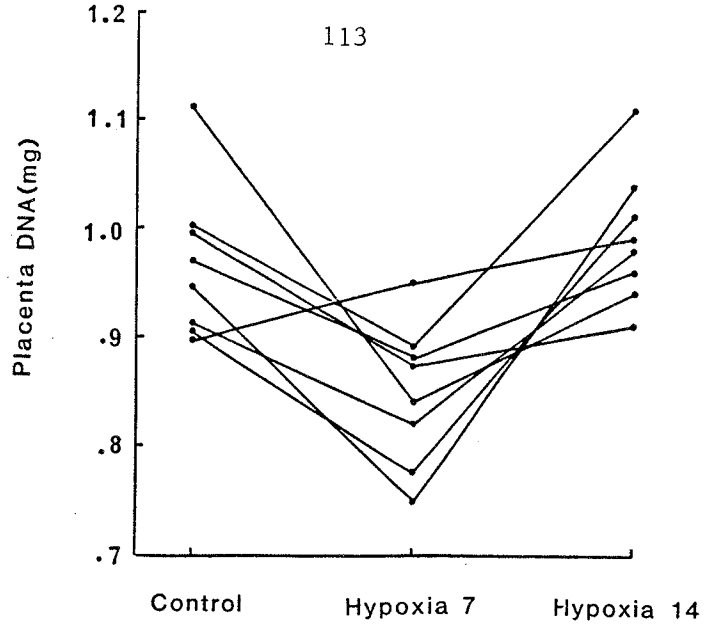
Hypoxia 7 = placentae obtained from pregnant rats exposed to intermittent hypoxia beginning on gestation day 7. Hypoxia 14 = placentae obtained from pregnant rats exposed to continuous hypoxia as of gestation day 14. Cold 3 = placentae obtained from pregnant rats exposed to cold beginning on gestation day 3. Cold 14 = placentae obtained from pregnant rats exposed to cold as of gestation day 14. Exercise 3 = placentae obtained from pregnant rats subjected to swimming exercise starting gestation day 3 and daily thereafter until gestation day 20. Exercise 11 = placentae obtained from pregnant rats subjected to 10 minute daily exercise starting gestation day 11 until gestation day 20. Data are expressed as mean ± 1 SE; number in parentheses indicate number of placentae studied. Different from control: * = $P < 0.001$, † = $P < 0.01$, Φ = P 0.05, a = different from hypoxia 14, b = different from cold 14, d = different from exercise 11.

the placenta size was noted in hypoxia 14 rats. Because of the low fetal body weight in both hypoxia 7 and hypoxia 14, the placenta weight and DNA content expressed per body weight were significantly larger than control.

In Figure 36, placenta DNA content of hypoxia 7, hypoxia 14 and control rats are compared for fetuses of similar body weights. Hypoxia 7 placenta DNA content was significantly lower than both hypoxia 14 and controls. In hypoxic rats, similar to control rats, placenta weight and DNA content increased with an increase in fetal body weight (but this was only significant for hypoxia 14: $n = 17$, $r = 0.41$, $P < 0.05$). The ratio of placenta weight or DNA content to fetal body weight decreased with an increase in fetal body weight, as in controls. This decrease in ratio is significant in hypoxia 7 rats for placenta weight ($n = 40$, $r = -0.475$, $P < 0.01$) and in both hypoxic conditions for placenta DNA (hypoxia 7: $n = 40$, $r = -0.555$, $P < 0.001$; hypoxia 14: $n = 17$, $r = -0.565$, $P < 0.02$). In both hypoxic groups there is a significant relationship between placenta weight and DNA content (hypoxia 7: $n = 40$, $r = 0.645$, $P < 0.001$, for hypoxia 14: $n = 17$, $r = 0.504$, $P < 0.05$). Similar to control rats, no significant relationship was found between maternal lung DNA (per lung, per BWo) and placenta DNA (per body weight or for a group of fetuses of similar body weight, 3-3.25 g for hypoxia 7, 3.8-4 g for hypoxia 14).

The relationship between fetal lung DNA and placenta DNA was analyzed in three ways: a) average fetal lung DNA versus average placenta DNA of a litter, b) individual fetal lung DNA versus individual placenta DNA, and c) lung DNA versus placenta DNA among

Figure 36: Upper panel: comparison of placenta DNA content in fetuses of equal body weight from control, hypoxia 7 and hypoxia 14 rats. Lines connect the 3 fetuses of equal body weight. Hypoxia 7 placenta DNA is significantly lower than both control and hypoxia 14 (paired: $P < 0.02$ and $P < 0.01$, respectively; unpaired: $P < 0.01$ and $P < 0.001$, respectively). Middle panel: same comparison as upper panel but for rats exposed to cold at GD 3 and GD 14. Cold 3 placenta DNA content is significantly lower than both control and cold 14 (paired: $P < 0.001$; unpaired: $P < 0.02$ and $P < 0.01$, respectively). Lower panel: rats subjected to exercise at GD 3 or GD 11. Exercise 3 placenta DNA is significantly lower ($P < 0.05$) than control (unpaired T-test).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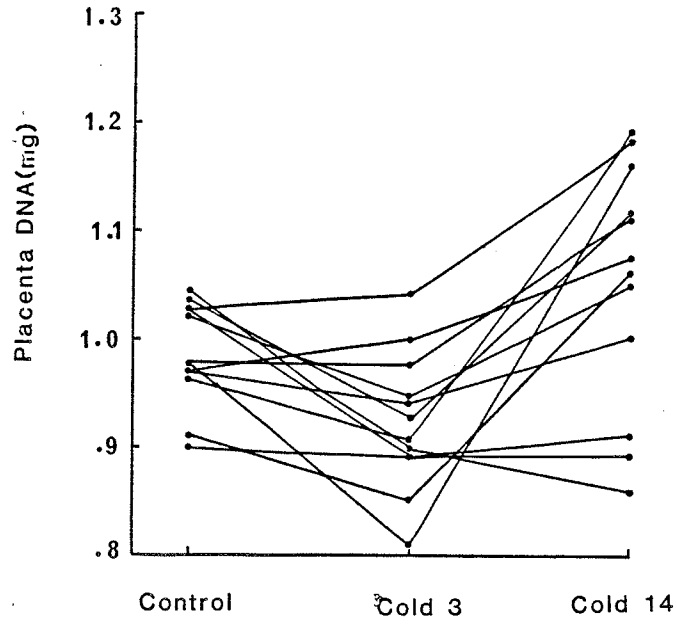
.8
.7
Placenta DNA(mg)
Control
Hypoxia 7
Hypoxia 14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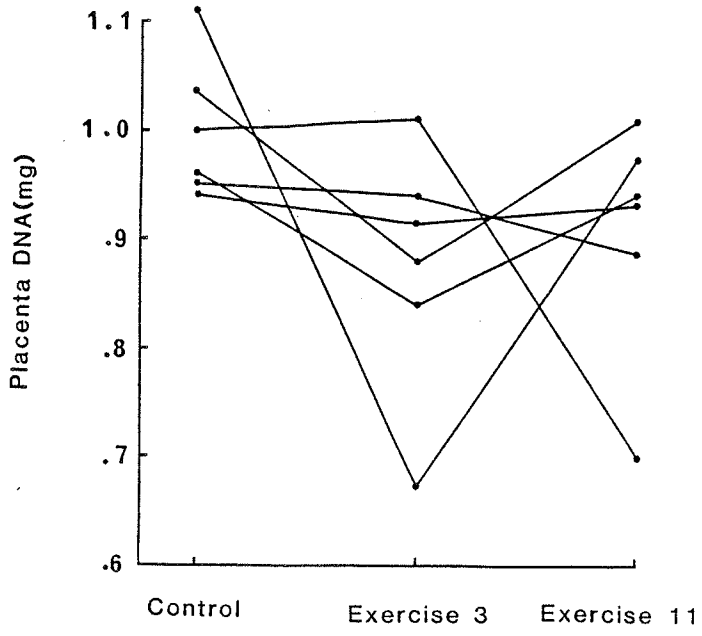
Placenta DNA(mg)
Control
Cold 3
Cold 14


1.1
1.0
.9
.8
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.6
Placenta DNA(mg)
Control
Exercise 3
Exercise 11

fetuses of similar body weight (hypoxia 7: 3-3.25 g, hypoxia 14: 3.8-4 g body weight). The three methods of analysis did not indicate any significant relationship between fetal lung DNA and placenta DNA in either hypoxia 7 or hypoxia 14 rats.

Fetus:

Fetal body weight of hypoxia 7 and hypoxia 14 rats ranged between 1.65 and 4.25 g and 3.14 and 4.52 g, respectively. As shown in Table 5, they were both significantly ($P < 0.001$) smaller than control fetuses, hypoxia 7 fetuses being the smallest. Only the fetal lung of hypoxia 7, but not hypoxia 14 rats, was smaller than the control (Table 6). In Figure 37, fetal lung DNA of hypoxia 7 and hypoxia 14 are compared with controls for fetuses of equal body weights. The DNA content of hypoxia 7 fetal lungs is less than both hypoxia 14 and control lungs.

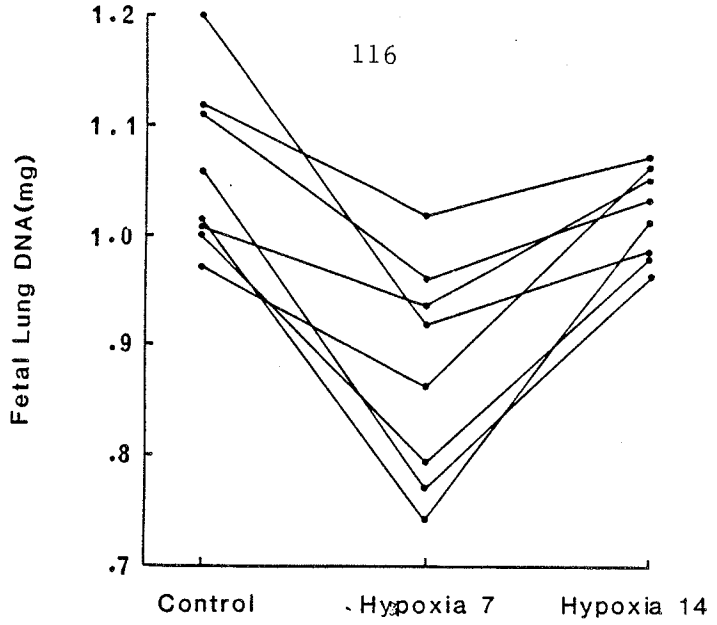
Analysis of maternal and fetal lung DNA content revealed a lack of relationship between the two in hypoxia 7 rats, but did show a significant direct relationship in hypoxia 14 rats. The greater the hypoxia 14 maternal lung DNA content, the greater the fetal lung DNA. The regression coefficient for hypoxia 14 maternal lung DNA per BWo versus fetal lung DNA per body weight is 0.907 ($n = 5$, $P < 0.05$), and for hypoxia 14 maternal lung DNA per BWo versus fetal lung DNA for fetuses of similar body weight (3.8-4.0 g) is 0.872 ($n = 4$ and $P < 0.05$).

The weight and DNA content of liver and kidney of fetuses of hypoxia 14 were compared with controls in fetuses of equal body

	Control	Hypoxia 7	Hypoxia 14	Cold 3	Cold 14	Exercise 3	Exercise 11
Lung wt (mg)	123±1.33 (190)	87.02±1.34* (105)	111.6±1.32* (56)	130.3±1.103* (202)	124.14±1.9 (36)	119.2±1.23 (184)	104.73±3.7* (21)
Lung wt (mg)/ g body wt	29.7±0.2 (190)	27.6±0.33* (104)	29.05±0.4 (56)	29.55±0.2 (202)	29.2±0.27 (36)	28.6±0.2* (185)	29.2±0.64 (21)
Lung dry wt (mg)	18.15±0.52 (21)	10.59±0.32 (24)	----	17.39±0.46 (15)	17.14±0.42 (13)	15.9±0.4 (5)	15.78±0.96 (9)
Lung dry wt (mg)/ g body wt	4.23±0.096 (21)	3.44±0.118 (24)	----	3.98±0.098 (15)	4.05±0.074 (13)	4.30±0.139 (5)	4.37±0.103 (9)
Lung dry wt/ wet wt x 100	14±0.124 (21)	13.29±0.19 (24)	----	14.03±0.203 (15)	14.12±0.182 (13)	14.71±0.558 (5)	14.5±0.092 (9)
Lung DNA (mg)	1.098±0.018 (57)	0.822±0.014* (47)	1.033±0.018 (22)	1.148±0.016 Φ (64)	1.214±0.033† (23)	0.993±0.024* (42)	1.04±0.053 (12)
Lung DNA (mg)/ g lung	9.184±0.14 (57)	9.44±0.114 (47)	9.25±0.072 (22)	8.59±0.076* (64)	9.52±0.153 (23)	8.55±0.12† (42)	10.18±0.107† (12)
Lung DNA (mg)/ g body wt	0.269±.004 (57)	.26±0.004 (47)	0.267±0.006 (22)	0.259±.004 (64)	0.281±0.005 (23)	0.25±0.005† (42)	0.291±0.01† (12)

Hypoxia 7 = fetuses obtained from pregnant rats exposed to intermittent hypoxia starting gestation day 7. Hypoxia 14 = fetuses obtained from pregnant rats exposed to continuous hypoxia starting gestation day 14. Cold 3 = fetuses obtained from pregnant rats exposed to cold starting gestation day 3. Cold 14 = fetuses obtained from pregnant rats exposed to cold starting gestation day 14. Exercise 3 = fetuses obtained from pregnant rats subjected to swimming exercise starting gestation day 3 and daily thereafter until gestation day 20. Exercise 11 = fetuses obtained from pregnant rats subjected to daily exercise starting gestation day 11 until gestation day 20. Data are expressed as mean ±1 SE; number in parentheses indicate number of fetuses studied. Different from control: * = $P < 0.001$, † = $P < 0.01$, ǂ = $P < 0.02$, Φ = $P < 0.05$

Figure 37: Fetal lung DNA compared in the same manner as placenta DNA in Figure 36. Upper panel: hypoxia 7 fetal lung DNA is significantly less than both control ($P < 0.001$) and hypoxia 14 ($P < 0.01$) for both paired and unpaired T-test. Middle panel: cold 3 fetal lung DNA significantly lower than cold 14 ($P < 0.01$ paired; $P < 0.02$ unpaired), cold 14 significantly higher than control ($P < 0.05$, paired). Lower panel: exercise 11 fetal lung DNA is significantly greater than exercise 3 ($P < 0.02$, paired), exercise 3 is significantly lower than control ($P < 0.05$, unpaired T-test).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1.2
1.1
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.9
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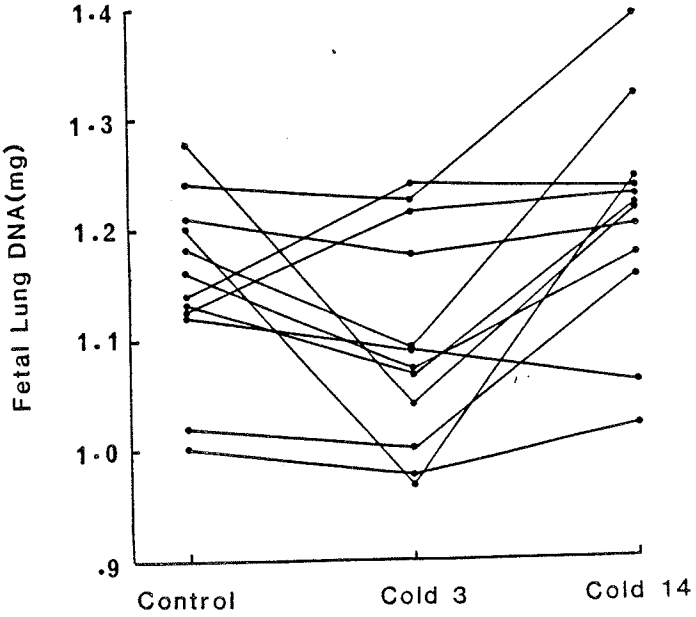
Control
Hypoxia 7
Hypoxia 14


Fetal Lung DNA(mg)
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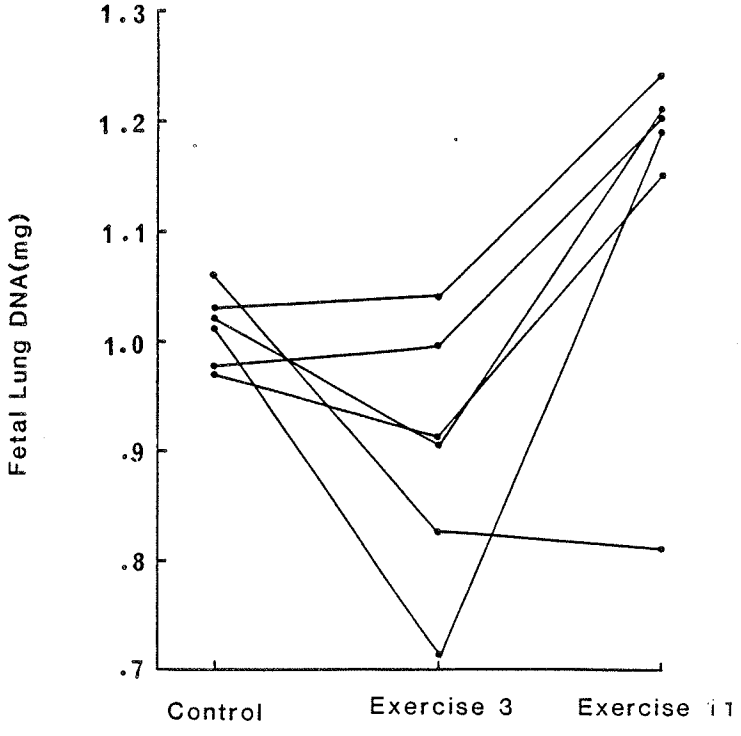
1.3
1.2
1.1
1.0
.9
Control
Cold 3
Cold 14


Fetal Lung DNA(mg)
1.3
1.2
1.1
1.0
.9
.8
.7
Control
Exercise 3
Exercise 11

weights. A paired T-test analysis revealed only a significant ($P < 0.05$) reduction (24.5%) in liver DNA content of hypoxia 14 in comparison with control. No data on liver and kidney is available for hypoxia 7 fetuses.

(3) Cold

Rats exposed to 10°C either at GD 3 or GD 14 tolerated the environment but appeared to be less active compared to control rats kept at room temperature. Their food intake was significantly increased (27.7%), but their body weights (BWo and BW-fp, Table 3) and the rate of fetal absorption (0.6%) were similar to that of control. Only the maternal rats exposed to 10°C at GD 3 had larger lungs (lung weight, DNA content and air volume, expressed either per BWo or BW-fp, Table 7) but the liver, kidney and heart of both groups of cold exposed rats were larger than the controls (Table 3).

Fetal body weight of cold 3 and cold 14 rats ranged between 3.25 and 5.42 g, and 3.67 and 4.62 g, respectively. As shown in Table 5, cold 3 fetuses were significantly ($P < 0.001$) larger than both control and cold 14, while cold 14 was not different from control.

In both groups of cold exposed rats, the placenta weight was larger than control, but only in cold 14 rats did the placenta DNA content exceed the control value ($P < 0.01$). In Fig. 36, the placenta DNA content of cold 3 and cold 14 rats are compared with control rats for fetuses of equal body weight. Cold 3 placenta

Table 7: Lung Measurements of Pregnant Rats Exposed to Cold

	Control	Cold 3	Cold 14	
Lung wt (g)	0.9421±0.0132 (29)	1.041±0.016* (20)	0.961±0.025 (5)	d
Lung wt (g)/ 100 g BW_0	.4564±.0066 (29)	.5002±.009* (20)	.467±.017 (5)	
Lung wt (g)/ 100 g BW_{-fP}	.344±0.005 (27)	.378±0.007* (18)	.363±0.011 (5)	
Lung DNA (mg)	7.762±0.34 (12)	8.672±0.219Φ (9)	7.514±0.267 (5)	b
Lung DNA (mg)/ g lung	8.273±0.261 (12)	8.294±0.184 (9)	7.82±0.203 (5)	
Lung DNA (mg)/ 100 g BW_0	3.741±.133 (12)	4.159±0.121Φ (9)	3.657±0.186 (5)	d
Lung DNA (mg)/ 100 g BW_{-fP}	2.817±0.084 (12)	3.152±0.074† (9)	2.841±0.112 (5)	d
Lung Volume Vmax (ml)	13.27±0.268 (25)	15.22±0.261* (16)	13.44±0.63 (5)	b
Lung vol. (ml)/ g lung	14.174±0.264 (24)	14.69±0.303 (15)	14.11±0.91 (5)	
Lung vol. (ml)/ 100 g BW_0	6.468±0.126 (25)	7.269±0.171* (15)	6.533±0.37 (5)	
Lung vol. (ml)/ 100 g BW_{-fP}	4.813±0.101 (24)	5.493±0.121* (13)	5.078±0.247 (5)	
Cell no. ($X10^6$)/ lung	1251.94±54.84 (12)	1398.7±35.3Φ (9)	1211.94±43.06 (5)	

Pregnant rats exposed to temperature of 10°C starting gestation day 3 (Cold 3) or gestation day 14 (Cold 14). BW_0 = body weight at gestation day zero, BW_{-fP} = body weight at gestation day 21 excluding fetal and placenta weights. Vmax = lung air volume at 30 cm H_2O inflation pressure. Data are expressed as mean ±1 SE, number in parentheses indicate number of animals studied. Different from control: * = $P < 0.001$, † = $P < 0.01$, ‡ = $P < 0.02$, Φ = $P < 0.05$. Different from Cold 3: a = $P < 0.001$, b = $P < 0.01$, d = $P < 0.05$

DNA content was significantly ($P < 0.01$) lower than the other two groups. In both cold-exposed groups, there was a direct relationship between placenta weight and DNA content (cold 3: $n = 50$, $r = 0.667$, $P < 0.001$, for cold 14: $n = 23$, $r = 0.594$, $P < 0.01$); only in cold 3 rats the placenta weight and DNA content increased with an increase in fetal body weight (placenta weight: $n = 50$, $r = 0.425$, $P < 0.01$; placenta DNA: $n = 50$, $r = 0.496$, $P < 0.001$). The ratio of placenta weight or DNA content to fetal body weight decreased with an increase in fetal body weight similar to controls. This decrease in ratio is only significant in cold 3 rats (for DNA: $n = 50$, $r = -0.553$, $P < 0.001$, for weight: $n = 50$, $r = -0.238$, $P < 0.05$). No significant relationship was found between maternal lung DNA (per lung, or per body weight) and placenta DNA (per body weight or for a group of fetuses of similar body weight, 4.1-4.35 g for cold 3, 4.2-4.45 g for cold 14).

The relationship between fetal lung DNA and placenta DNA was analyzed in 3 ways: 1) average fetal lung DNA versus average placenta DNA for a litter, 2) individual fetal lung DNA versus individual placenta DNA, 3) lung DNA versus placenta DNA among fetuses of similar body weight (cold 3 = 4.1-4.35 g, cold 14 = 4.2-4.45 g). In the first two modes of analysis, a direct relationship was found between fetal lung DNA and placenta DNA content (1- for cold 3: $n = 10$, $r = 0.554$, $P < 0.05$, for cold 14: $n = 5$, $r = 0.964$, $P < 0.001$; 2- for cold 3: $n = 50$, $r = 0.496$, $P < 0.001$ and for cold 14: $n = 23$, $r = 0.453$, $P < 0.05$). However, the 3rd method of analysis revealed no relationship between placenta and lung DNA content. The lung DNA content of both cold groups was larger than

control (Table 6), but only cold 3 fetuses had lung weights that exceeded the control values ($P < 0.001$).

In Figure 37, the fetal lung DNA of cold 3 and cold 14 rats are compared with controls for fetuses of equal body weight. Cold 14 fetal lung DNA content was significantly higher than control and cold 3 ($P < 0.05$, $P < 0.01$, respectively). Analysis of maternal and fetal lung DNA content failed to show any relationship between the two.

The weight and DNA content of liver and kidney of fetuses of cold 3 and 14 were compared with controls in fetuses of equal body weights. A paired T-test analysis revealed a significant ($P < 0.02$) increase in both weight (17.2%) and DNA (20.4%) of cold 14 fetal kidneys and a decrease (13%, $P < 0.01$) in cold 3 fetal liver DNA, in comparison to control.

(4) Exercise

Normally, it took 2 to 3 days for rats to learn to swim. They were able to carry out the daily 10 minute swimming exercise throughout pregnancy. Only two significant changes were observed in rats subjected to exercise on day 3, a reduction in BW-fp and an increase in heart weight (Table 3). Swimming exercise had no significant influence on the lungs of pregnant rats (Table 8).

Fetal body weight of exercise 3 and exercise 11 rats varied between 2.66 and 5.66 g, and 3.00 and 4.04 g, respectively. The fetuses of exercise 11 were signficantly smaller than both

Table 8: Lung Measurements of Pregnant Rats Subjected to Exercise

	Control	Exercise 3	Exercise 11
Lung wt (g)	0.9421±0.0132 (29)	0.921±0.016 (15)	0.9773±0.0198 (3)
Lung wt (g)/ 100 g BW_0	.4564±.0066 (29)	.448±0.009 (15)	0.471±0.0016 (3)
Lung wt (g)/ 100 g BW_{-fP}	.344±0.005 (27)	.348±0.007 (15)	0.344±0.0014 (3)
Lung DNA (mg)	7.762±0.34 (12)	7.808±0.258 (6)	6.8501 (2)
Lung DNA (mg)/ g lung	8.273±0.261 (12)	8.253±0.304 (6)	7.0219 (2)
Lung DNA (mg)/ 100 g BW_0	3.741±.133 (12)	3.801±0.173 (6)	3.301 (2)
Lung DNA (mg)/ 100 g BW_{-fP}	2.817±0.084 (12)	2.979±0.135 (6)	2.409 (2)
Lung Volume Vmax (ml)	13.27±0.268 (25)	13.43±0.3 (14)	14.3 0.058 (3)
Lung vol. (ml)/ g lung	14.174±0.264 (24)	14.64±0.393 (14)	14.65±0.36 (3)
Lung vol. (ml)/ 100 g BW_0	6.468±0.126 (25)	6.503±0.15 (14)	6.903±0.153 (3)
Lung vol. (ml)/ 100 g BW_{-fP}	4.813±0.101 (24)	5.049±0.13 (14)	5.042±0.109 (3)
Cell no ($X10^6$)/ lung	1251.94±54.84 (12)	1259.35±41.62 (6)	1104.85 (2)

Pregnant rats subjected to swimming exercise starting gestation day 3 (Exercise 3) or gestation day 11 (Exercise 11). BW_0 = body weight at gestation day zero, BW_{-fP} = body weight at gestation day 21 excluding fetal and placenta weight. Vmax = lung air volume at 30 cm H_2O inflation pressure. Data are expressed as mean ± SE; number in parentheses indicate number of animals studied. No significant differences between exercise and control rats.

exercise 3 and control (Table 5). Placenta weights of both exercise 3 and 11 were significantly smaller ($P < 0.001$) but the DNA content was similar to controls (Table 5). Placenta DNA content of exercise 3, exercise 11 and control rats of fetuses with equal body weight are compared in Figure 36. No differences were found between control and either of the exercise groups.

In both groups of rats subjected to exercise, a highly significant relationship exists beteen placenta weight and DNA content (exercise 3: $n = 30$, $r = 0.775$, $P < 0.001$), exercise 11: $n = 12$, $r = 0.855$, $P < 0.001$). There is no correlation between placenta weight or placenta DNA, and fetal body weight in exercise 3 rats. However, similar to control rats, placenta weight and DNA content of exercise 11 increased with an increase in fetal body weight (for weight: $n = 12$, $r = 0.858$, $P < 0.001$, for DNA: $n = 12$, $r = 0.868$, $P < 0.001$). In exercise 3 rats, the ratios of placenta weight per fetal body weight and DNA content per fetal body weight significantly decreased as the fetal body weight increased (for weight: $n = 30$, $r = -0.476$, $P < 0.01$, for DNA: $n = 30$, $r = -0.387$, $P < 0.05$). Conversely in exercise 11 rats, these ratios significantly increased with an increase in fetal body weight (for weight: $n = 12$, $r = 0.732$, $P < 0.01$, for DNA: $n = 12$, $r = 0.623$, $P < 0.05$). As in controls, no significant relationship was found between maternal lung DNA and placenta DNA content in exercise 3 rats, even when this was tested for fetuses of similar body weight (3.85-4.0 g). However, placenta DNA/fetal body weight was directly related to maternal lung DNA per BWo ($n = 8$, $r = 0.755$, $P < 0.05$). Such a relationship could not be analyzed for exercise

11 rats because of the small number of rats in that group.

The relationship between fetal lung DNA and placenta DNA was analyzed in three ways:

1. average fetal lung DNA versus average placenta DNA for a litter,
2. individual fetal lung DNA versus individual placenta DNA.
3. lung DNA versus placenta DNA among fetuses of similar body weight (3.85-4.10 g).

No relationship was found between the placenta and fetal lung DNA content in the first and third methods of analysis. But the second mode of analysis showed a significant relationship between individual fetal and placenta DNA content (exercise 3: $n = 30$, $r = 0.921$, $P < 0.001$ and exercise 11: $n = 12$, $r = 0.921$, $P < 0.001$).

The fetal lung weight and DNA of exercise 3 rats were smaller than the controls (Table 6), while the DNA of exercise 11 rats was greater.

In Figure 37, fetal lung DNA of exercise 3 and exercise 11 were compared with controls for fetuses of equal body weights. Fetal lung DNA of exercise 11 exceeds that of exercise 3. Analysis of maternal and fetal lung DNA content did not reveal any relationship between the two.

No significant differences were noted in fetal liver and kidney weight and DNA content in fetuses of equal body weights between exercise 3 and control rats.

(5) Pneumonectomy

Mother:

Pregnant rats tolerated the surgical procedure well. Five to ten minutes after surgery, they were awake and moving about. Some rats did die during surgery but not post-operatively. There was no fetal abortion following surgery. The frequency of fetal absorption was similar to controls in both pneumonectomized (PN, 0.92%) and sham-operated (0.95%) rats. Respiratory infection, intrapulmonary hemorrhage and adhesion of the lung to the chest wall were observed frequently. These were all excluded from the study. The body weight at GDo was similar in both PN and sham-operated rats. The rats lost between 1 to 5 g weight during the first day after surgery. On the second day post-operatively, they began to gain weight and by gestation day 21, their body weights (BW21 and BW-fp) were similar to control rats. The food intake was only decreased on the day of operation and thereafter returned to normal.

Figures 38 and 39 show lung weight, lung DNA content and lung air volume expressed per initial body weight and BW-fp for rats after pneumonectomy and sham-operation. Weight, DNA content and volume of the right lung in pneumonectomized pregnant rats progressively increased post-operatively. Eighteen days post-operation (operated on GD 3), the values for these parameters approached those of both lungs of sham-operated rats. Of interest is the gradual and significant ($n = 8$, $r = 0.732$, $P < 0.05$) reduction in lung DNA in sham-operated rats with days after operation

Figure 38: Lung weight (upper panel), lung DNA content (middle panel), and lung air volume (lower panel) expressed per body weight at gestation day zero for pneumonectomized (closed circles) and sham operated (open circles) pregnant rats in relation to the day of surgery. Controls are shown by open triangles on gestation day 21. Each point and bar represents the mean ± 1 SE (n = 2 to 12). The points without bars are the average of 2 rats. The upper group of open circles in each panel is the combined left and right lungs of sham operated rats and the lower group of open circles represent only the right lung. The lines (solid for PN, dashed for sham rats) drawn through the corresponding points are hand-drawn approximations.

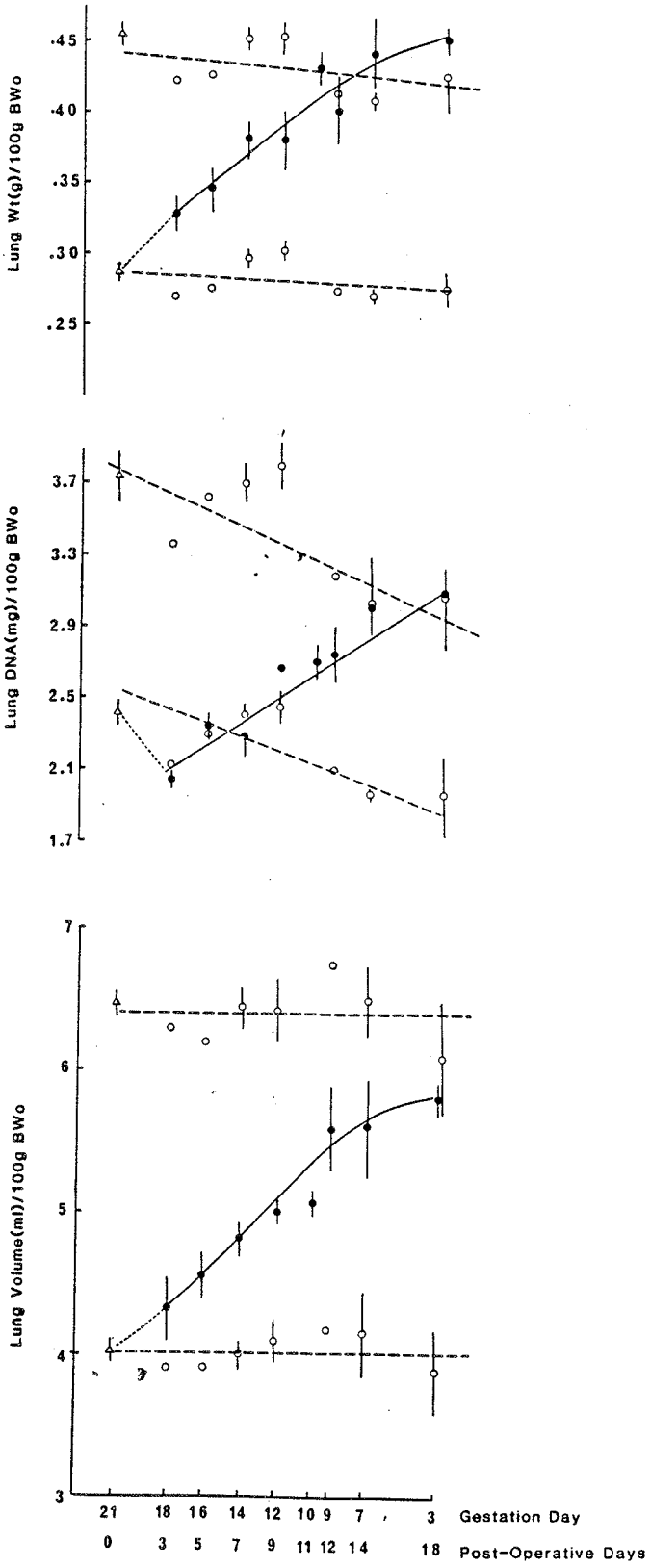
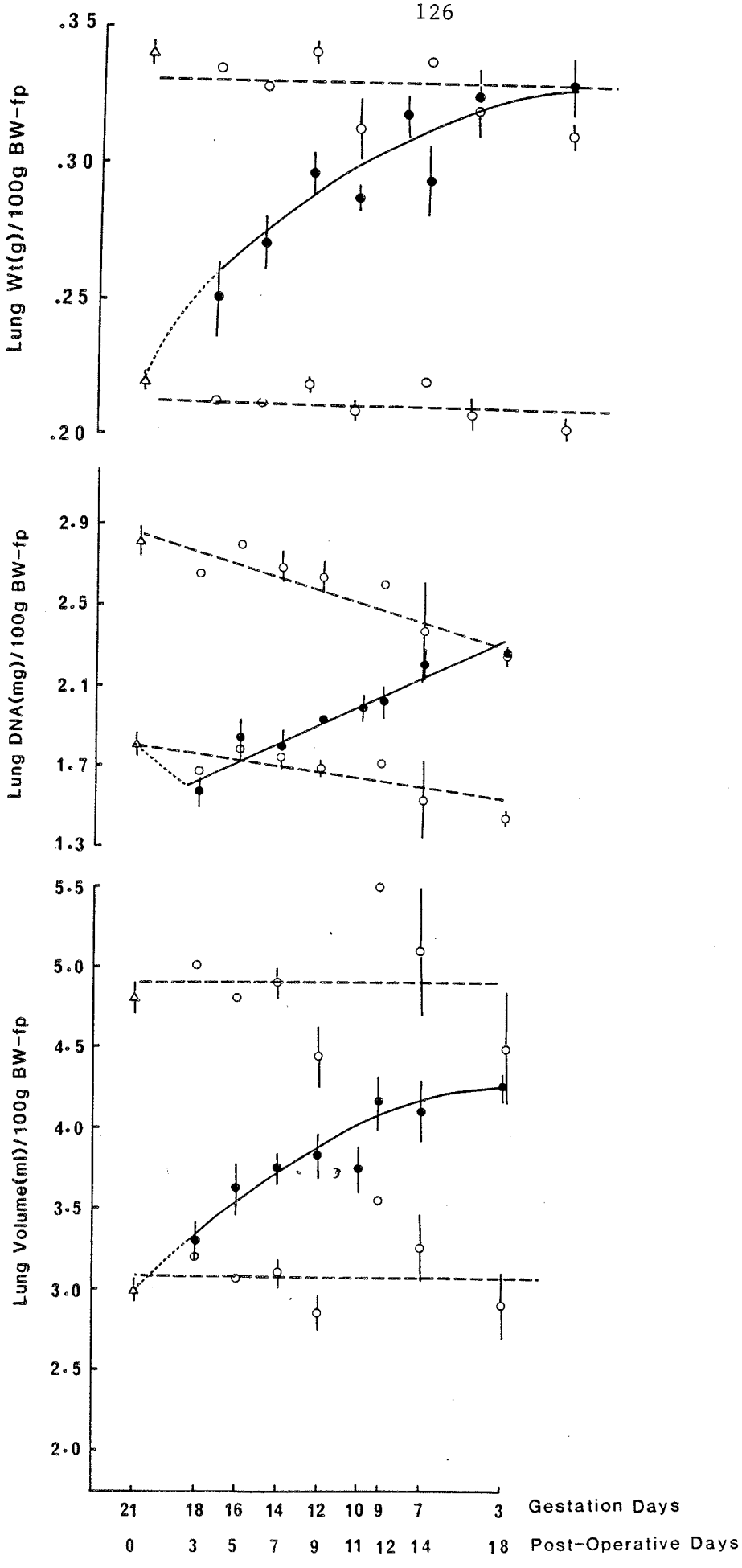

Lung Wt(g)/100g BWo
.45
.40
.35
.30
.25
Lung DNA(mg)/100g BWo
3.7
3.3
2.9
2.5
2.1
1.7
Lung Volume(ml)/100g BWo
7
6
5
4
3
21 18 16 14 12 10 9 7 3 Gestation Day
0 3 5 7 9 11 12 14 18 Post-Operative Days

Figure 39: For description see legend of Figure 38. Lung parameters in this case are expressed per body weight at GD 21 minus fetal and placenta weights.


.35
.30
.25
.20
Lung Wt(g)/100g BW-fp
2.9
2.5
2.1
1.7
1.3
Lung DNA(mg)/100g BW-fp
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Lung Volume(ml)/100g BW-fp
21 18 16 14 12 10 9 7 3
Gestation Days
0 3 5 7 9 11 12 14 18
Post-Operative Days

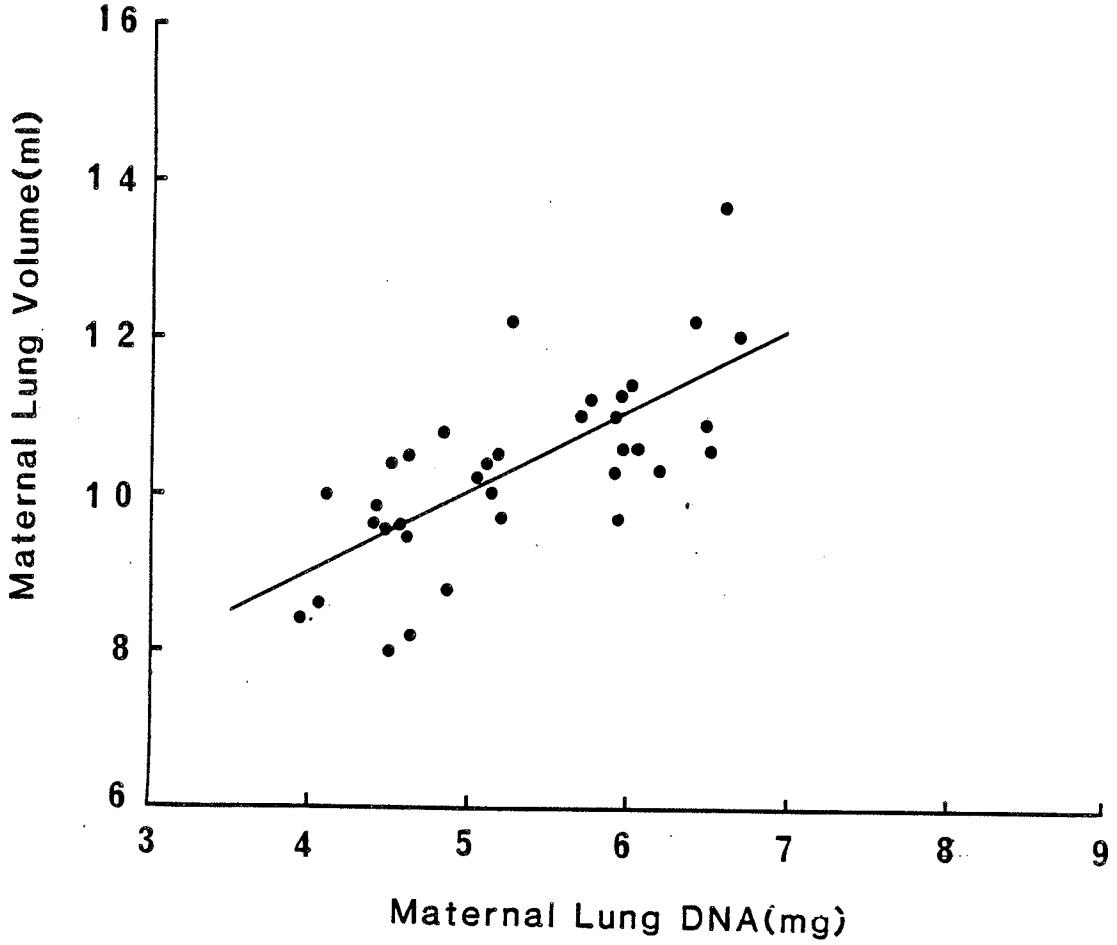


Figure 40: Relationship between maternal lung volume and lung DNA in pneumonectomized rats. Each point represents one animal ($r = 0.714$, $P < 0.001$).

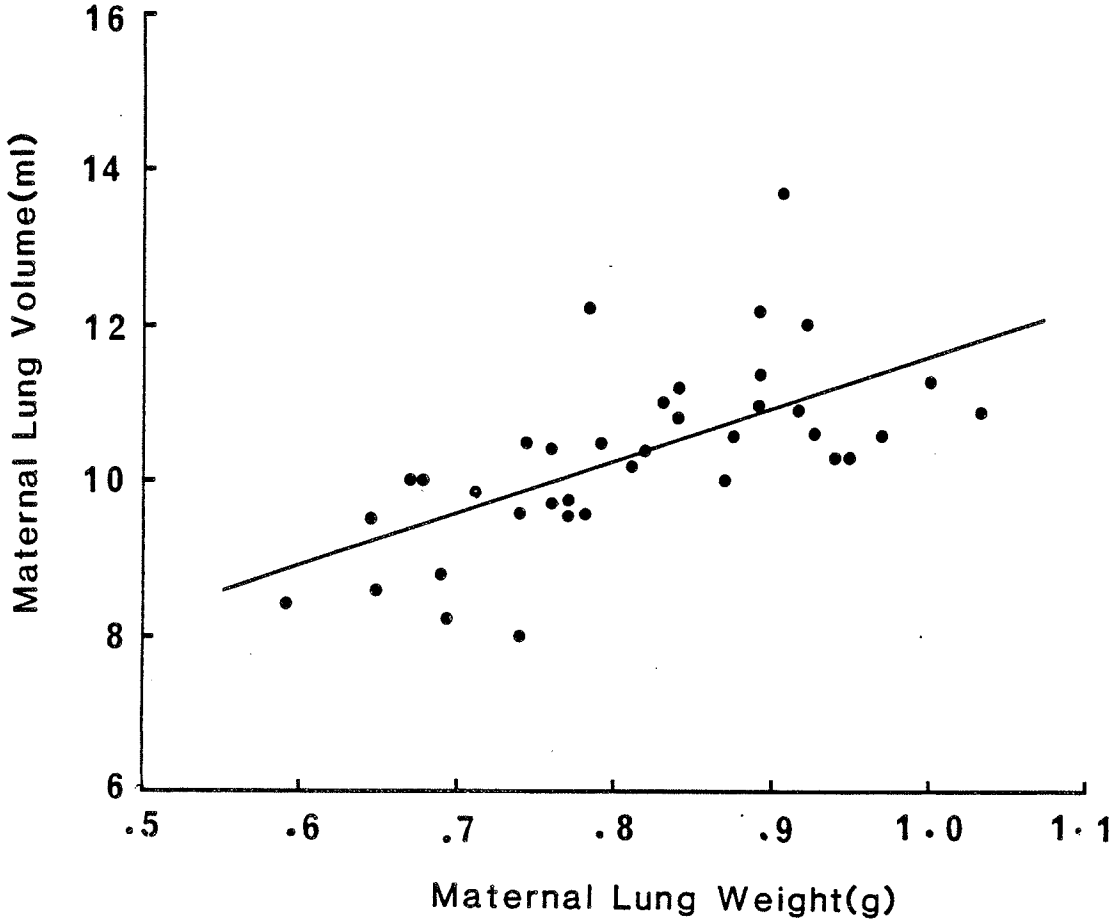


Figure 41: Relationship between maternal lung volume and lung weight in pneumonectomized rats. Each point is one animal. ($r = 0.646$, $P < 0.001$).

even though neither the lung weight nor the lung volume underwent any significant change. The reduction in lung DNA was in both lungs since the ratio of right to left lung remained constant.

A significant relationship was noted between lung volume and lung DNA and between lung volume and lung weight in pneumonectomized rats (Figs. 40, 41). Such relationships were not found in sham-operated lungs. No significant differences were noted in liver, kidney and heart weights between PN and sham-operated rats.

Placenta:

Placenta weight and DNA for PN and sham-operated rats are shown in Table 9. Surgical intervention in pregnant rats does not appear to have a significant effect on the size of the placenta (weight and DNA). Figure 42 compares the ratio of placenta DNA per body weight in PN and sham-operated rats. It appears that this ratio is larger in PN rats when the operation is performed in the first 2/3 of pregnancy, but this may be due to the influence of fetal body weight. Indeed, this ratio decreases with an increase in fetal body weight in sham-operated rats ($n = 81$, $r = -0.42$, $P < 0.001$), but remains constant for PN rats. When the ratio of placenta DNA per fetal body weight was compared between PN and sham fetuses of equal body weight, no significant differences were observed (Fig. 43).

There was a direct relationship between placenta DNA and placenta weight in both sham-operated ($n = 81$, $r = 0.537$, $P < 0.001$) and PN rats ($n = 137$, $r = 0.737$, $P < 0.001$).

Table 9: Fetal and Placenta Measurements

Condition	Body Weight (g)		Fetal Lung Weight (mg)		Fetal Lung DNA (mg)		Placenta Weight (mg)		Placenta DNA (mg)	
	PN	Sham	PN	Sham	PN	Sham	PN	Sham	PN	Sham
3	4.235±0.062 Φ (36)	4.068±0.048 (23)	125.68±1.926 ǂ (36)	119.06±1.9 (23)	1.082±0.023* (18)	0.959±0.017 (12)	492.84±15.57 Φ (36)	447.7±9.87 (23)	0.936±0.028 (15)	0.901±0.015 (10)
7	4.027±0.054 (67)	4.119±0.037 (35)	118.64±2.057 (67)	0.119±0.007 (35)	1.104±0.035 * (21)	0.969±0.014 (18)	465.61±8.06† (67)	431.6±9.06 (35)	0.974±0.02 Φ (16)	0.925±0.019 (15)
9	3.76±0.036 * (54)	4.332±0.04 (24)	119.33±1.36 (53)	123.32±2.12 (24)	1.083±0.015 * (24)	0.9922±0.0143 (12)	450.98±8.4 † (53)	415.9±9.97 (24)	0.901±0.0153 (20)	0.936±0.029 (10)
10	3.842±0.038 * (82)	----	109.64±1.59 * (82)	----	1.047±0.02 Φ (23)	----	479.88±8.11 * (82)	----	1.028±0.022 † (16)	----
12	3.674±0.054 * (48)	4.143±0.037 (43)	106.49±1.49 * (48)	115.63±1.44 (43)	0.977±0.025 (22)	1.01±0.017 (18)	430.36±7.58 * (48)	495.02±10.25 (43)	0.966±0.0401 (10)	0.936±0.03 (15)
14	3.763±0.033 † (139)	3.908±0.037 (116)	113.79±1.346 (112)	116.43±1.15 (103)	0.973±0.024 Φ (38)	1.038±0.017 (34)	439.42±9.165 † (102)	466.56±5.97 (116)	0.871±0.024 (30)	0.9097±0.016 (26)
16	3.518±0.048 (38)	3.532±0.048 (24)	95.67±1.89 Φ (38)	101.64±2.22 (24)	0.891±0.015 † (18)	0.97±0.013 (12)	444.8±7.82 * (38)	395.57±9.12 (24)	0.8842±0.049 (15)	0.969±0.025 (10)
18	3.864±0.056 † (36)	3.594±0.063 (23)	111.49±1.665 † (36)	102.24±3.078 (23)	0.909±0.028 (18)	0.928±0.02 (12)	440.74±9.46 (36)	433.7±12.84 (23)	0.881±0.031 Φ (15)	0.984±0.036 (10)
Control	4.094±0.017		123.01±1.133		1.098±0.0184		452.37±3.29		0.947±0.023	

Fetuses and placentae obtained from pregnant rats operated on different gestational days (3, 7, 9,...18). Data are expressed as mean ± 1 SE; number in parenthesse indicate number of fetuses and placentae studied. PN 10 was compared with Sham 9. Different from sham: * = $P < 0.001$, † = $P < 0.01$, ǂ = $P < 0.02$, Φ = $P < 0.05$

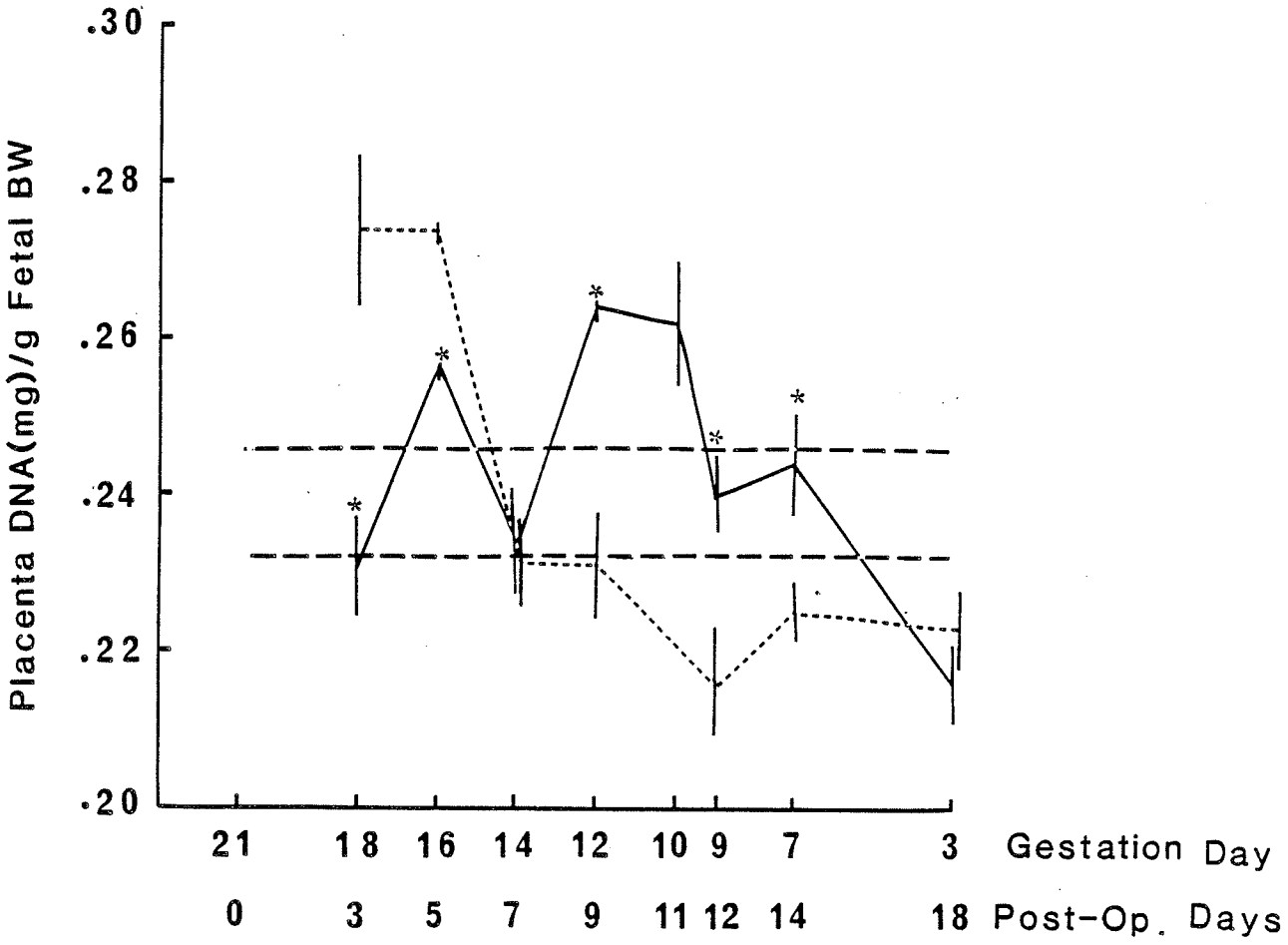


Figure 42: Placenta DNA expressed per fetal body weight in relation to day of surgery during pregnancy. The horizontal dashed lines represent the mean ± 1 SE for control rats. Solid line = pneumonectomized rats; dotted line = sham operated. Bars represent mean ± 1 SE (n = 10 to 30). * = significantly different from sham.

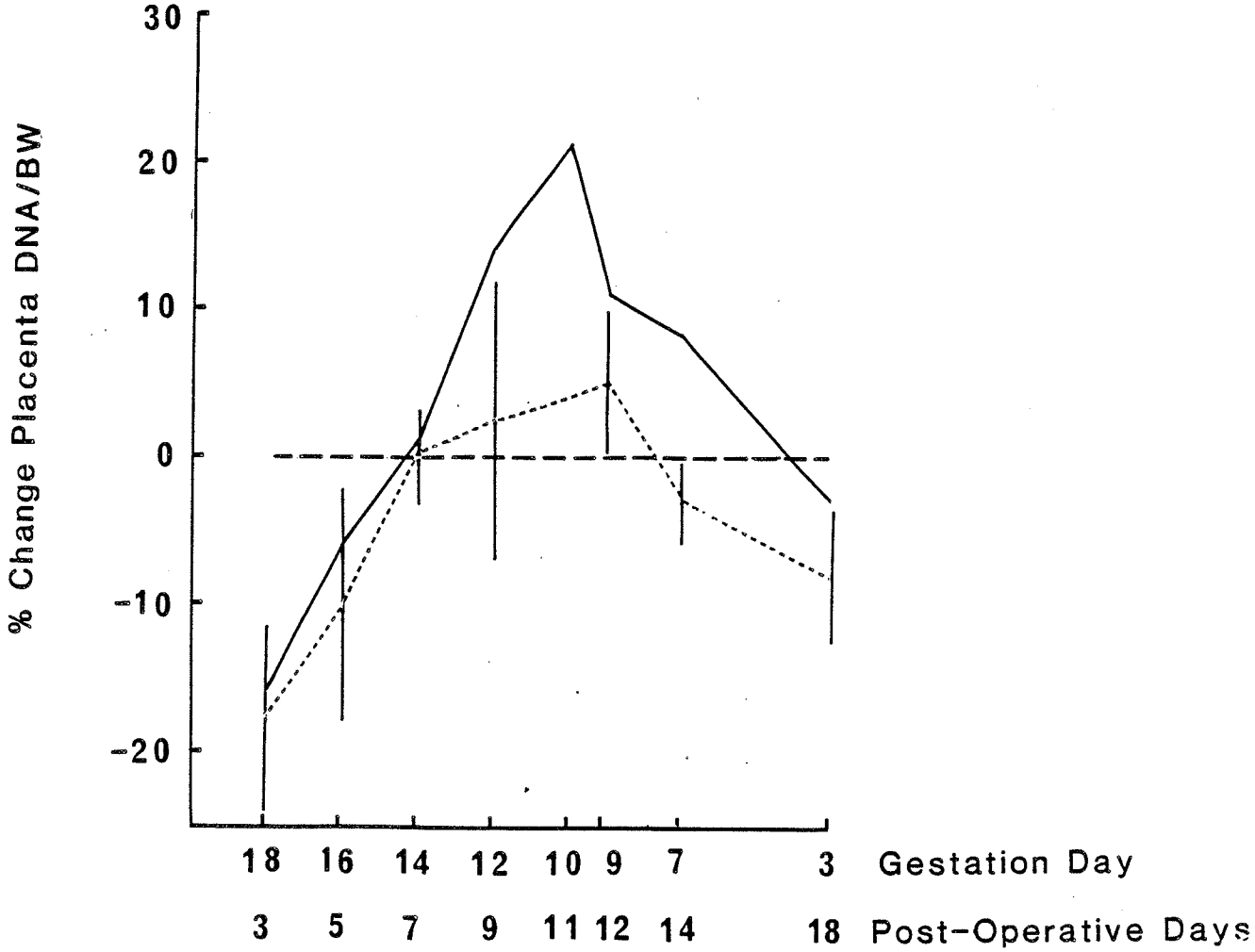


Figure 43: Difference in placenta DNA per fetal body weight between pneumonectomized and sham operated rats expressed as a percentage of sham in relation to day of surgery. The solid line shows this difference calculated from Figure 42. The dotted line is percentage difference in PN versus sham fetuses of equal body weight. Vertical bars represent mean ± 1 SE (n = 3 to 8). The differences between the two groups of fetuses with equal body weights were not significant.

The relationship between maternal lung DNA and placenta DNA was analyzed as follows:

1. Maternal lung DNA versus average placenta DNA of the corresponding mother.
2. Maternal lung DNA versus placenta DNA of her fetuses of a given body weight (3.85-4.05 g).
3. Average lung DNA of a group of rats which were operated on a given gestation day versus the average placenta DNA of the same group of rats.

No significant relationship was found between placenta DNA and maternal lung DNA in either PN or sham-operated rats.

When the relationship between placenta DNA and fetal lung DNA was tested for all the fetuses of PN rats, a significant direct relationship was found ($n = 138$, $r = 0.263$, $P < 0.02$). Such a relationship did not exist among fetuses of sham-operated rats. To rule out the influence of lower fetal body weight in PN rats on this relationship, placenta DNA and fetal lung were analyzed in fetuses of similar body weight (3.85-4.05 g). No relationship was found in either PN or sham-operated rats.

Fetus:

In Table 9 and Figure 44, fetal body weight from PN and sham-operated rats are compared with that of control. The fetal body weight in general was lower than controls when the surgical intervention was after midpregnancy. This reduction in fetal body weight was more exaggerated with pneumonectomy than

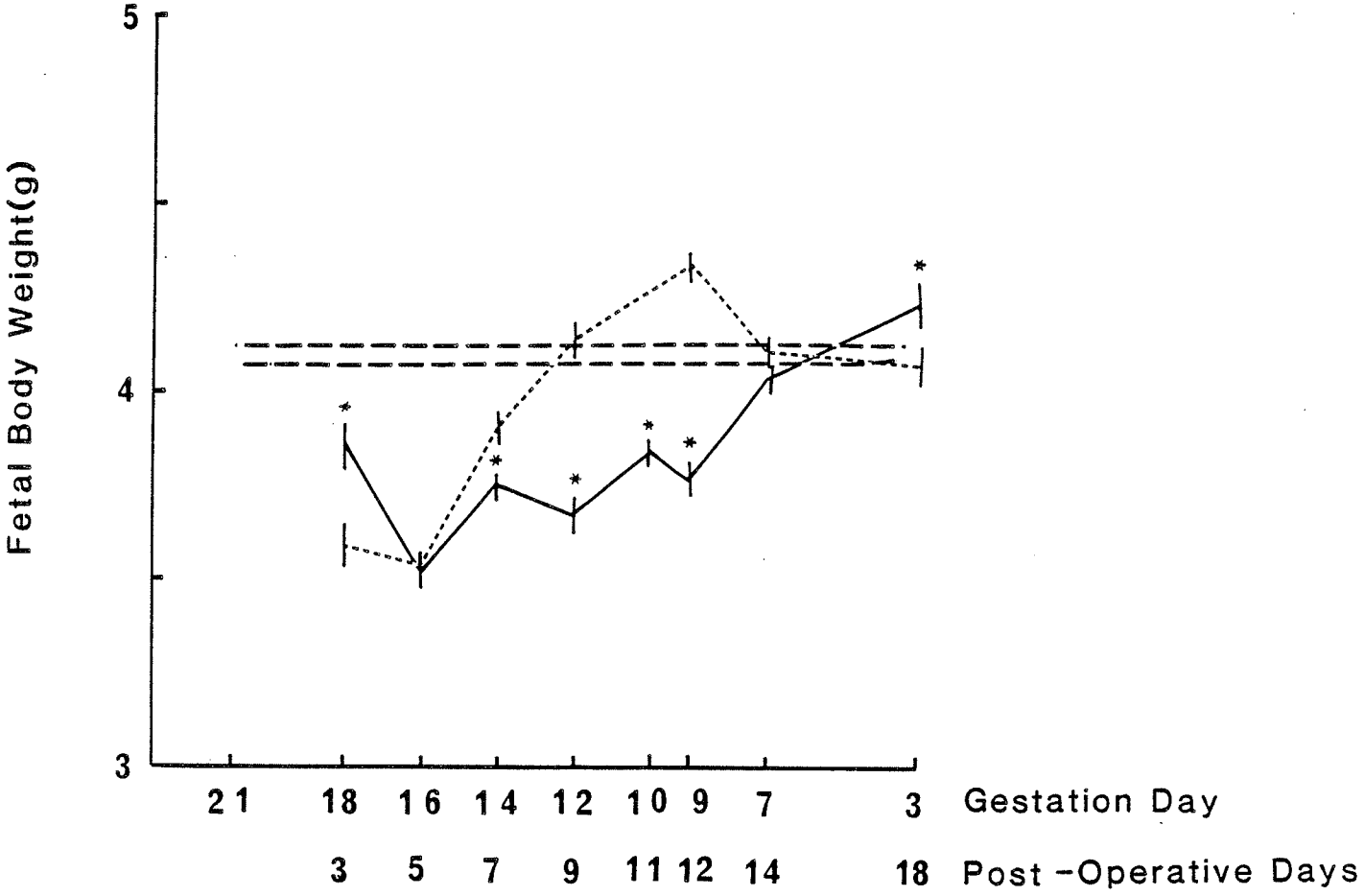


Figure 44: Fetal body weight for pneumonectomized (solid line) and sham operated (dotted line) rats in relation to the day of surgery. Dashed horizontal lines are mean ± 1 SE for controls. Each point with bar represents mean ± 1 SE (n = 23 to 139) of a group of rats subjected to surgery on a given gestation day shown on abscissa.

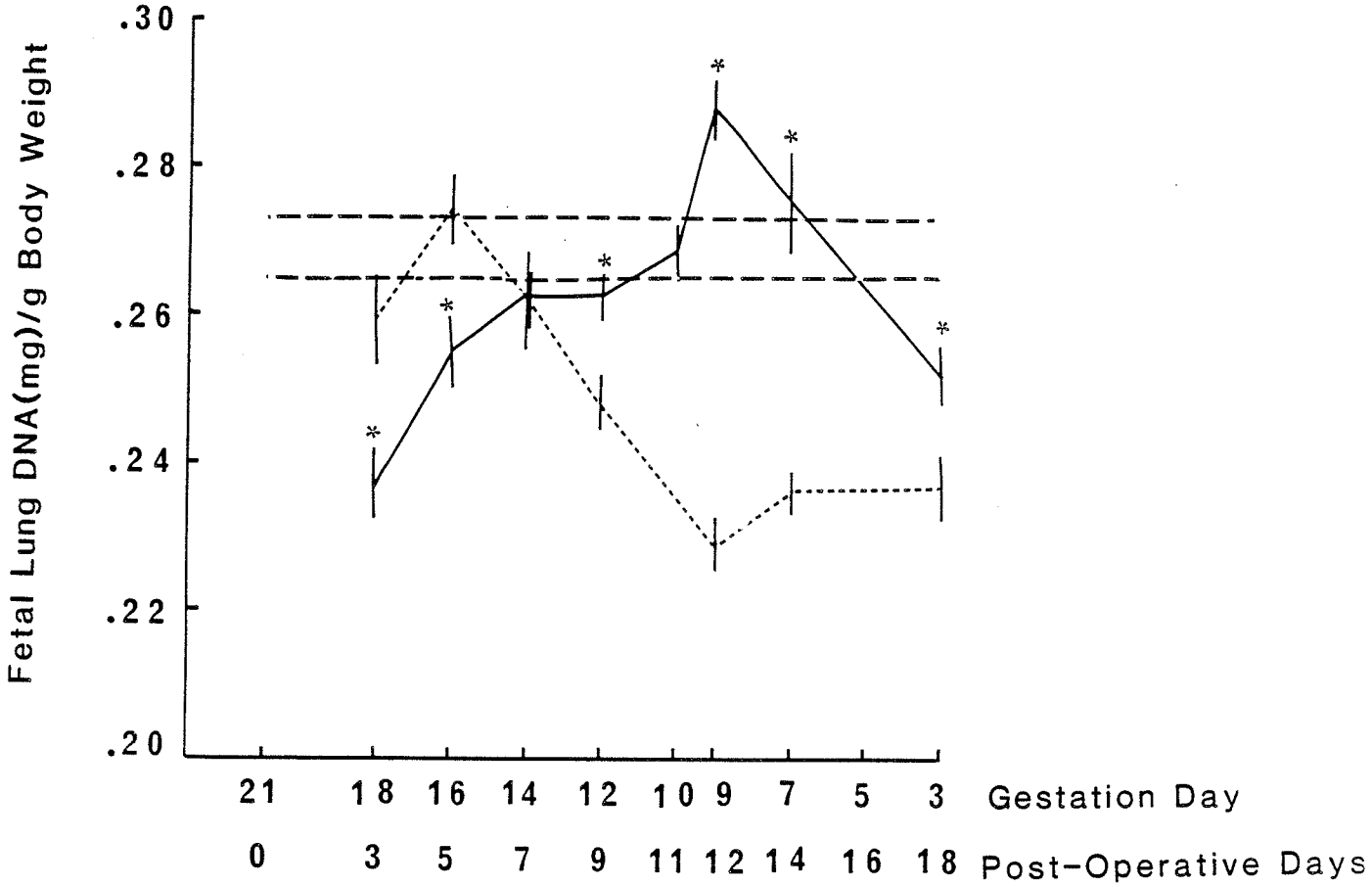


Figure 45: Fetal lung DNA content expressed per body weight for pneumonectomized (solid line) and sham operated (dotted line) rats in relation to day of surgery. The horizontal lines are the mean ± 1 SE for controls. Each point with bar is the mean ±1 SE (n = 12 to 38) for a group of rats subjected to surgery on a given gestation day shown on abscissa.

with sham operation.

The ratio of fetal lung DNA per body weight (Fig. 45) varied depending on the day of surgery during gestation. When surgery was performed in the first 2/3 of gestation, the ratio of fetal lung DNA to body weight was greater in fetuses of PN rats than in sham-operated rats. This was reversed in the last third of pregnancy in that the fetuses of PN rats had lower ratios than the sham groups.

The percentage difference in fetal lung DNA per body weight between the fetuses of PN and sham rats is compared in Figure 46. The largest value is seen when pneumonectomy was performed at gestation days 7, 9 and 10, the highest point occurring on day 9. Since this ratio is influenced by body weight (as shown in control fetuses, Fig. 30), a true comparison between PN and sham could only be made in fetuses of equal body weights. This comparison is shown in Figure 47 among isobody weight fetuses from mothers subjected to surgery at different gestational days. Fetuses of PN rats of gestation day 10 are compared with that of sham of gestation day 9 since sham operation was not performed on gestation day 10. The percentage difference in fetal lung DNA per BW between PN and sham fetuses of equal body weights is shown in Figure 46. The largest ratio in this analysis was shifted from gestation day 9 to gestation day 7. Values for PN rats operated on gestation days 3, 7, 9 and 12 were significantly higher ($P < 0.01$ to $P < 0.05$) than those of sham of corresponding gestation days.

The relationship between the maternal and the fetal lung DNA in pneumonectomy and sham is analyzed in three ways:

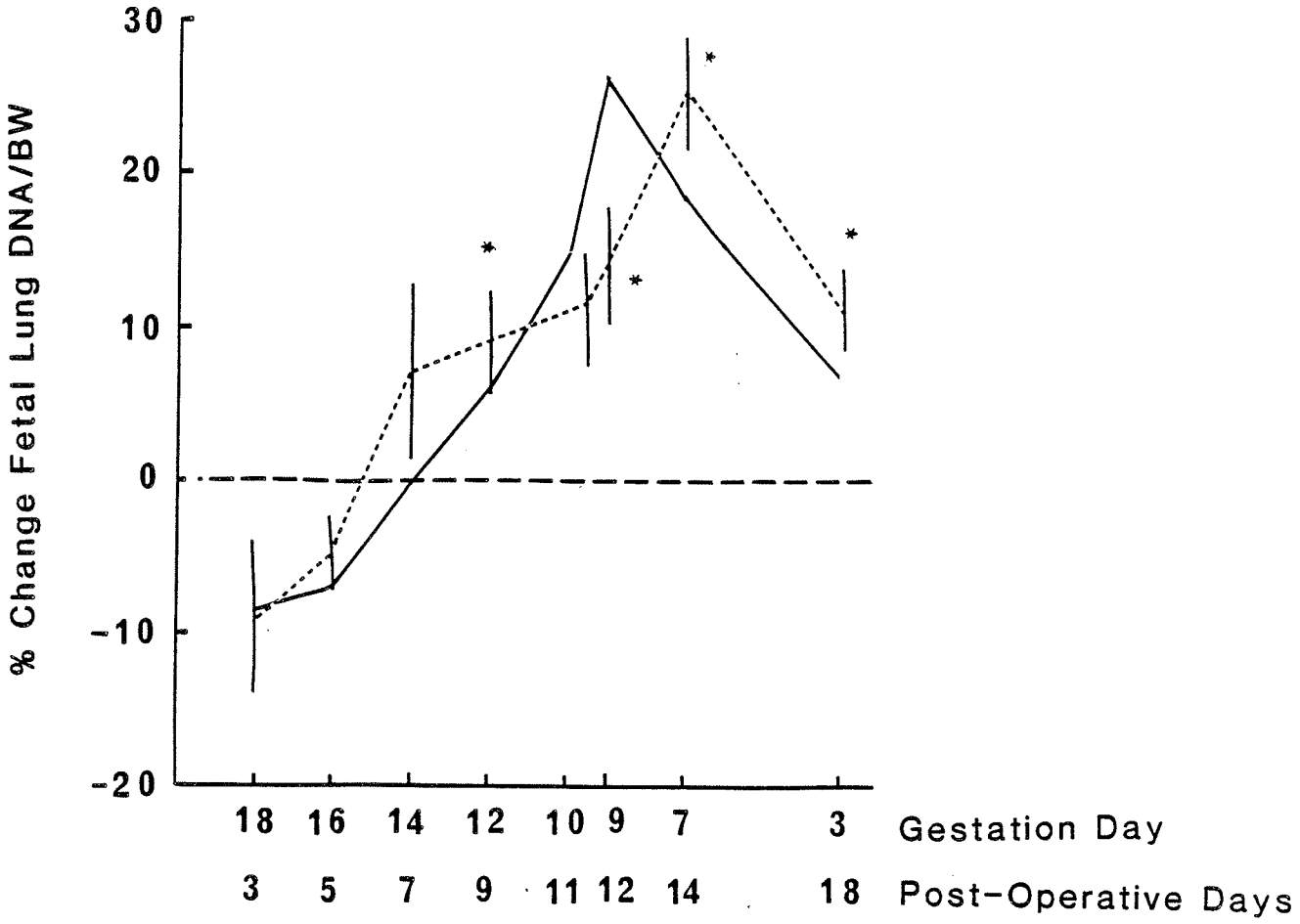
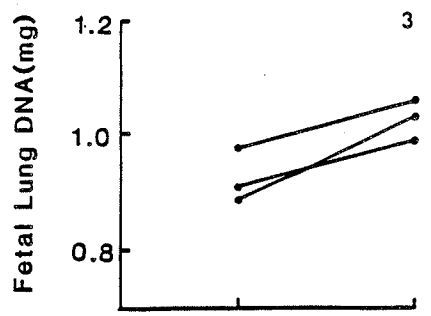
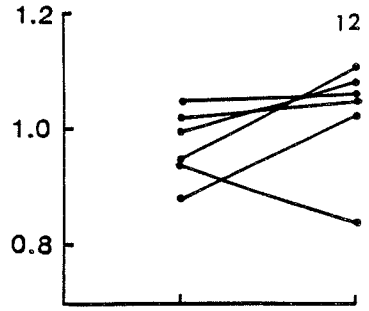
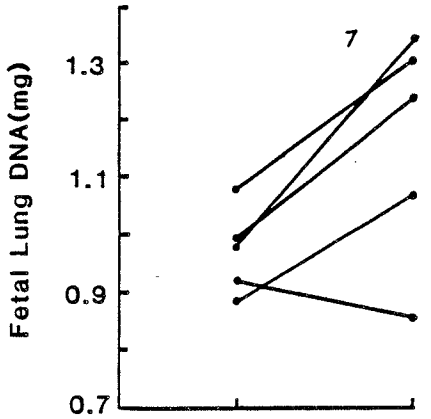


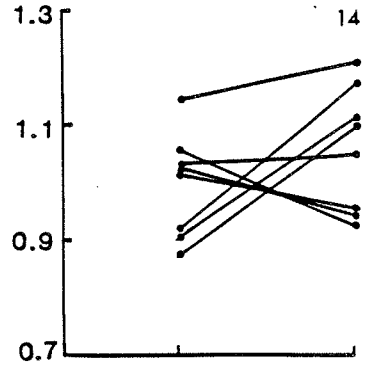
Figure 46: Difference in fetal lung DNA per body weight between PN and sham operated rats expressed as a percentage of sham in relation to day of surgery. The solid line shows this difference calculated from Figure 45. The dotted line is percentage difference in PN versus sham fetuses of equal body weight. Bars represent mean ± 1 SE (n = 3 to 8). * = indicates a significant difference ($P < 0.01$ to $P < 0.05$) in lung DNA per body weight between the two groups of fetuses with equal body weights.

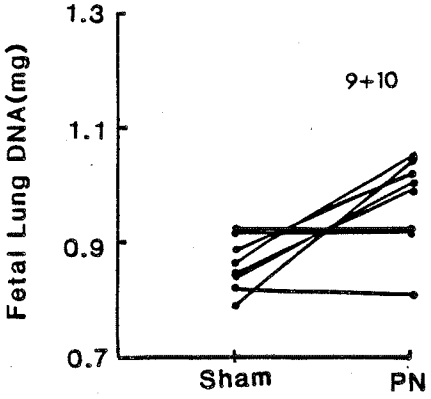
Figure 47: A comparison of fetal lung DNA between fetuses of PN and sham operated rats with equal body weights. Each panel represents this comparison for groups of rats subjected to surgery on a given gestation day (3, 7, 9...18) indicated on corresponding panels. Each line connects two fetuses of equal body weight. T-test of paired variates indicates a significant difference between two groups of rats at GD 3 ($P < 0.05$), at GD 7 ($P < 0.01$), at day GD 9 and 10 ($P < 0.02$), and at GD 12 ($P < 0.05$).

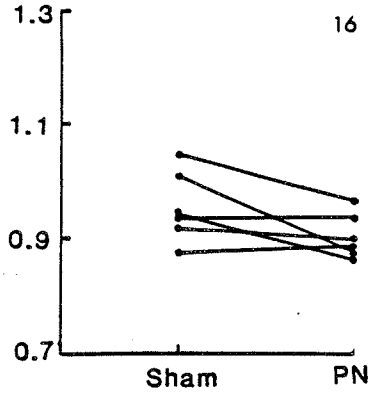

Fetal Lung DNA(mg)
1.2
1.0
0.8
3

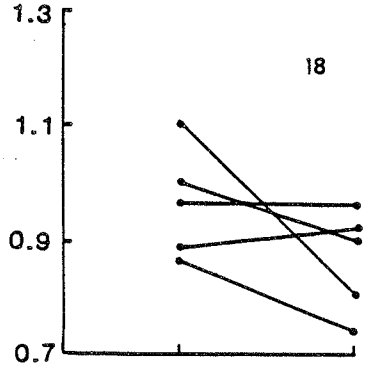

1.2
1.0
0.8
12


Fetal Lung DNA(mg)
1.3
1.1
0.9
0.7
7


1.3
1.1
0.9
0.7
14


Fetal Lung DNA(mg)
1.3
1.1
0.9
0.7
9+10
Sham
PN


1.3
1.1
0.9
0.7
16
Sham
PN


1.3
1.1
0.9
0.7
18

1. Maternal lung DNA versus average fetal lung DNA of corresponding mother.
2. Maternal lung DNA versus lung DNA of her fetuses of a given body weight (3.85-4.05 g).
3. Average lung DNA of a group of rats operated on a given gestation day versus the average fetal lung DNA of the same group of rats.

A significant relationship between maternal lung DNA and fetal lung DNA in sham-operated rats was found only when the average maternal lung DNA of rats operated on a given gestational day was compared with the average lung DNA of their fetuses ($n = 8$, $r = 0.757$, $P < 0.05$) (Fig. 48). In PN rats, the relationship between maternal lung DNA and fetal lung DNA was highly significant in all three methods of analysis (Figs. 49, 50, 51). This relationship in Figure 51 is not linear. The largest fetal lung growth occurs when pneumonectomy is performed between gestation day 7 and 9. When pneumonectomy is performed within the second half of pregnancy, the relationship between maternal and fetal lung enlargement is linear. As the maternal lung increases in size (DNA), the fetal lung also increases. Thereafter, in spite of a further increase in maternal lung size (DNA) the fetal lung decreases in size (DNA content). No significant differences were noted in fetal liver and kidney weights between PN and sham-operated fetuses. The comparisons were made in fetuses of equal body weights from mothers subjected to PN or sham operation at a given gestational day.

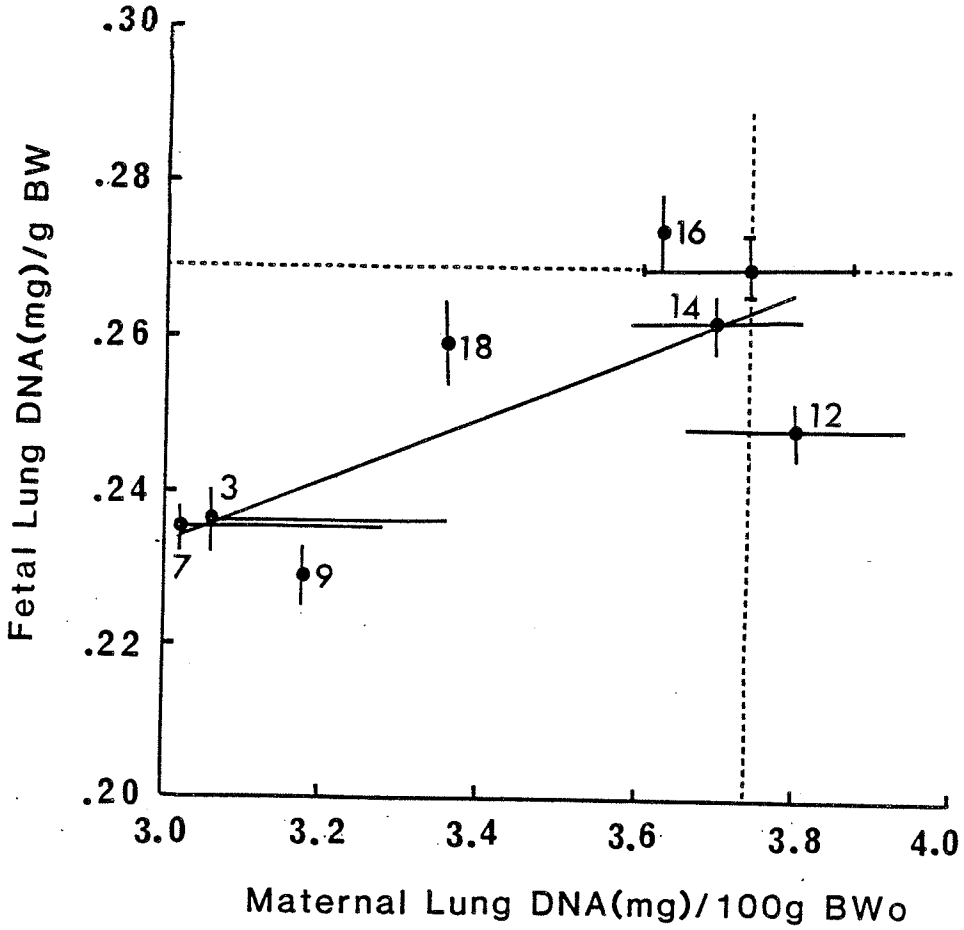


Figure 48: Relationship between fetal lung DNA and maternal lung DNA expressed per body weight for sham operated rats. Each point is the mean for a group of rats subjected to surgery at a given gestation day. Numbers adjacent to the points indicate the day of surgery (gestation day). The values for control rats are shown by dotted lines. Regression by least squares, $r = 0.757$, $P < 0.05$.

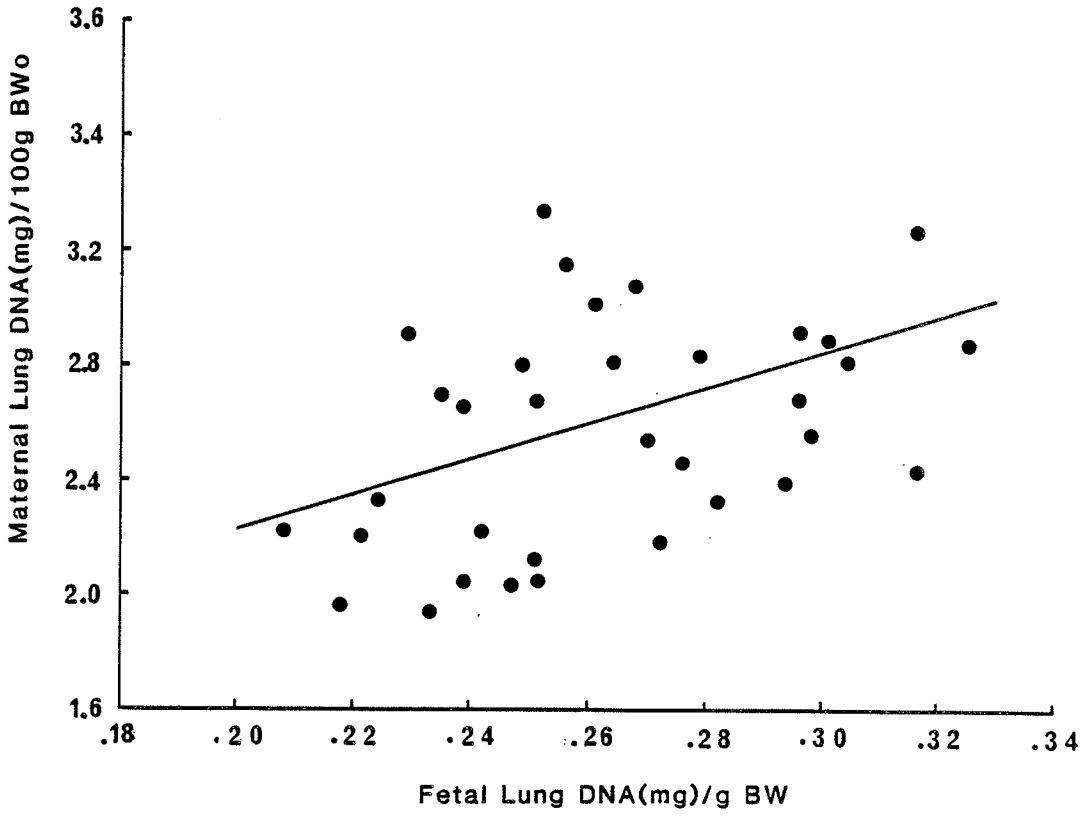


Figure 49: Relationship between maternal lung DNA and fetal lung DNA content expressed per body weight at GD 0 in PN. Each point represents the lung DNA of one pregnant rat and the average fetal lung DNA for that mother. Regression by least squares, $r = 0.488$, $P < 0.01$.

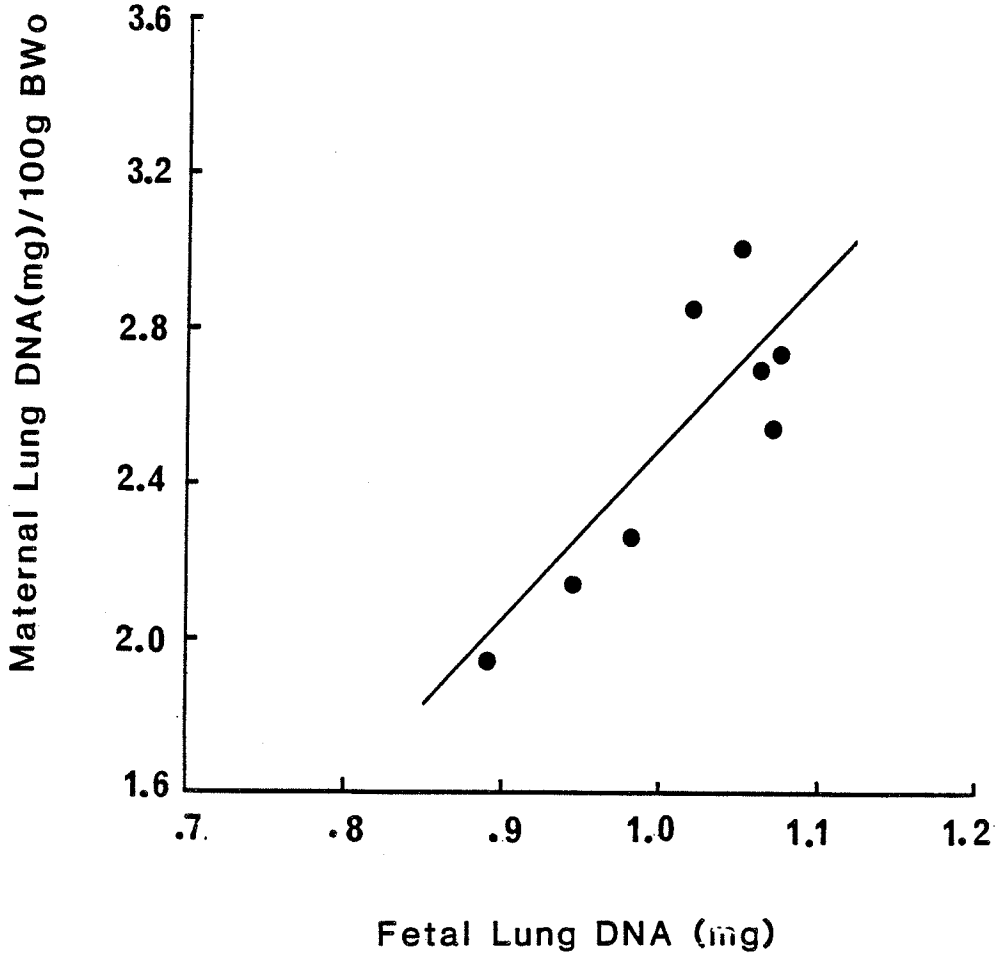


Figure 50: Relationship between maternal lung DNA expressed per body weight at GD 0 and lung DNA of fetuses of a given body weight (3.85 - 4.05 g) in PN. Each point is one pregnant rat and one fetus ($r = 0.809$, $P < 0.02$).

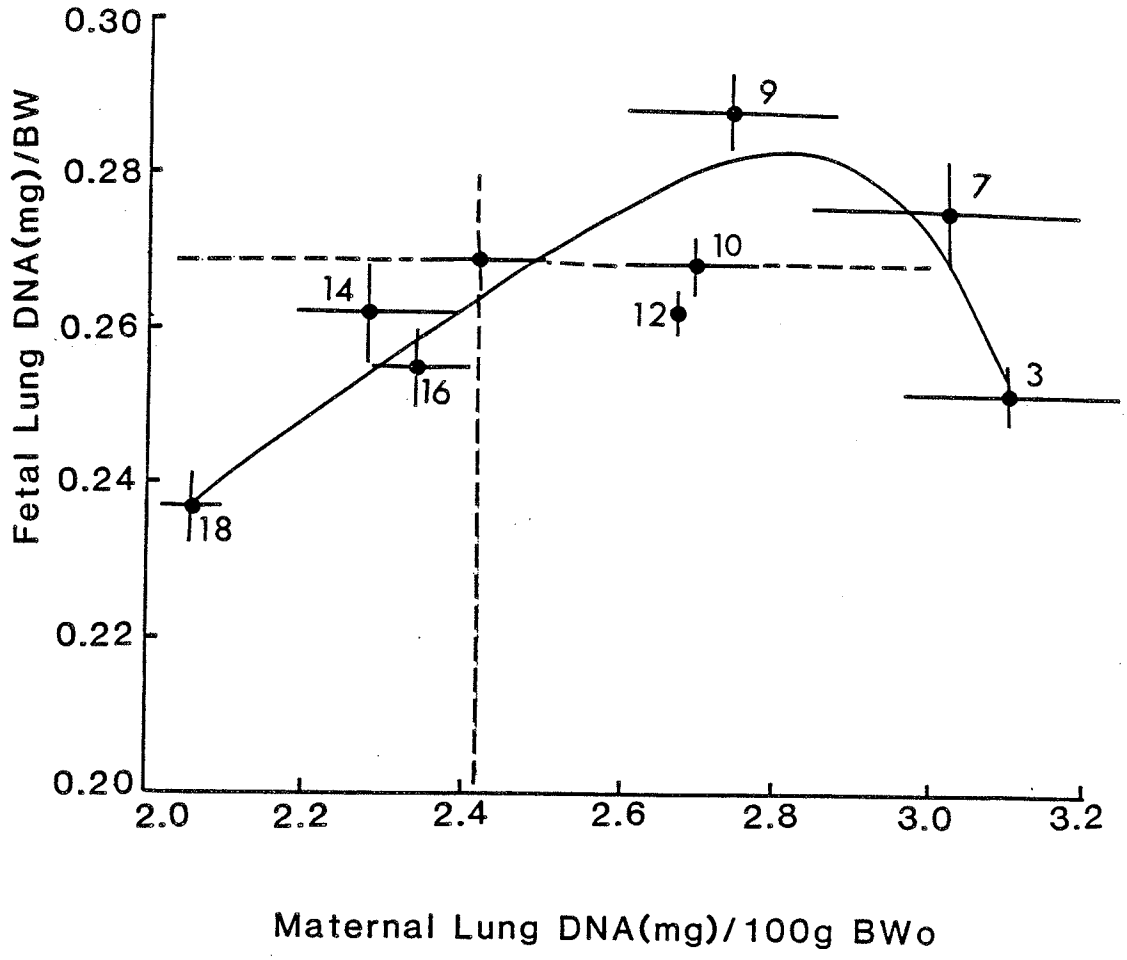


Figure 51: Relationship between fetal lung DNA and maternal right lung DNA expressed per body weight of PN rats at GD 0. Each point is the mean for a group of rats subjected to surgery at a given gestation day. Numbers adjacent to the points indicate the day of surgery (GD). The values for control rats (right lung only) are shown by broken lines. The line is hand-drawn approximation through corresponding points.

TABLE 10: MEASUREMENTS OF FETAL LUNG AT GESTATION DAY 21.

CONDITIONS	LUNG TOTAL PROTEIN (mg)/ mg LUNG DNA	LUNG TOTAL LIPID mg/mg LUNG DNA	LUNG PHOSPHOLIPID (mg)/mg LUNG DNA		
			TOTAL	LECITHINE	DISATURATED DSPC
CONTROL	7.79 ± 0.29 (15)	2.40 ± 0.09 (5)	1.64 ± 0.07 (5)	0.82 ± 0.04 (5)	0.41 ± 0.02 (5)
HYPOXIA 14	9.2 ± 0.11 * (21)	1.9 ± 0.05 * (5)	1.52 ± 0.04 (5)	0.73 ± 0.02 (5)	0.37 ± 0.02 (5)
COLD 3	7.63 ± 0.18 (11)	2.43 ± 0.04 (15)	1.67 ± 0.03 (5)	0.81 ± 0.19 (5)	0.40 ± 0.01 (5)
EXERCISE 3	8.6 ± 0.3 ϕ (21)	2.31 ± 0.07 (6)	1.59 ± 0.06 (6)	0.80 ± 0.02 (6)	0.39 ± 0.01 (6)
SHAM 14	7.9 ± 0.25 (21)	2.1 ± 0.07 (6)	1.46 ± 0.02 (6)	0.70 ± 0.02 (6)	0.33 ± 0.01 (6)
PN 14	9.73 ± 0.25 [a] (20)	2.26 ± 0.04 (5)	1.58 ± 0.07 (5)	0.77 ± 0.037 (5)	0.37 ± 0.02 (5)
PN 7	-	-	-	-	0.27 ± 0.01 [a] (5)
PN 9	-	-	-	-	0.31 ± 0.03 (4)
PN 18	-	-	-	-	0.28 ± 0.02 [d] (5)

DATA ARE EXPRESSED AS MEAN ± 1SE; NUMBERS IN PARENTHESES INDICATE NUMBER OF FETAL RATS STUDIED. FETUSES OF COLD, EXCERCISE AND HYPOXIA ARE COMPARED WITH CONTROL (* = $P<0.001$, ϕ = $P<0.05$) AND FETUSES OF PN RATS ARE COMPARED WITH SHAM OPERATED 14 ([a] = $P<0.001$, [d] = $P<0.05$).

(6) Lung Phospholipids

Measurements of fetal lung total protein and phospholipids on GD 21 expressed per lung DNA for control, cold 3, exercise 3, hypoxia 14, PN (7, 9, 14, 18) and sham 14 are shown in Table 10. It appears that none of the experimental conditions had any significant influence on fetal lung phospholipid content (a measure of fetal lung maturity). However, the ratio of lung total protein per DNA (an index of cell size) was significantly increased in fetuses of rats subjected to exercise, hypoxia 14 and PN 14.

(7) Electron Microscopy

Electron microscopic (EM) studies were performed in control, cold 3, exercise 3, hypoxia 7, PN 14, and sham 14. There were no visible structural changes in the lungs of the fetuses of mothers exposed to various experimental conditions when compared to that of controls. Measurements derived from EM figures indicated that the changes in the percentage and the total number of Type II cells and the number of lamellar inclusion bodies per Type II cell were minimal. The lack of enhancement of lung maturity (indicated by the number of Type II cells and the number of lamellar bodies per Type II cell) was further substantiated by analysis of these indices in fetuses of comparable body weights.

TABLE 11: GLYCOYEN MEASUREMENTS ON FETAL ORGANS ON GESTATION DAY 21.

CONDITIONS	LUNG	LIVER
CONTROL	86.5 ± 4.67 (10)	267.1 ± 8.3 (10)
HYPOXIA (7)	92.8 ± 8.97 (8)	162.3 ± 9.9 * (8)
COLD (3)	80.3 ± 4.32 (10)	266.7 ± 10.88 (10)
EXERCISE (3)	65.9 ± 3.78 † (8)	191.1 ± 18.02 * (8)
SHAM 14	86.9 ± 4.53 (12)	223.9 ± 11.62 (12)
PN 14	99.5 ± 4.52 (13)	245.8 ± 7.49 (13)

DATA (MG GLYCOGEN PER GRAM TISSUE DRY WEIGHT) ARE EXPRESSED AS MEAN ± ISE; NUMBER IN PARENTHESES INDICATE NUMBER OF FETAL RATS STUDIED. HYPOXIA, COLD AND EXCERCISE FETUSES ARE COMPARED WITH CONTROL (* = $P < 0.001$, † = $P < 0.001$) AND FETUSES OF PN 14 ARE COMPARED WITH SHAM 14.

(8) Glycogen

In Table 11 is shown fetal lung and liver glycogen content expressed in mg per gram tissue dry weight. The glycogen content was significantly reduced in the liver of both hypoxia 7 and exercise 3 fetuses but it was only decreased in the lungs of exercise 3 rats. Surgical procedure (left pneumonectomy and sham operation) on mother had no influence on fetal lung and liver glycogen content.

(C) DISCUSSION

It is accepted that environmental O_2 concentration, metabolic O_2 requirements and partial resection of the lung can operate as adaptive and compensatory stimuli for lung growth in rat. In this study, hypoxia (10-14% O_2), cold (10°C), swimming exercise, and left pneumonectomy were chosen as stimuli for lung growth. The pregnant rats were exposed to these conditions to further stimulate lung growth during pregnancy and to determine the effect of maternal lung growth on fetal lungs.

Since the appearance of the lung bud in the embryonic period in rats begins at about gestation days 10-11, the consequences of maternal lung growth on the fetal lung may depend upon the timing of the stimulation of maternal lung growth during pregnancy. Therefore, two different times were selected to begin the stimulation of maternal lung growth, early gestation (GD 3, 7) and late gestation (GD 14).

In order to reduce the variations in our results, only rats with a litter size of 9-14 (observed most frequently) were taken for comparison. All rats with the slightest gross appearance of possible respiratory infection (a common disease in rats) such as a single red or grey spot, excessive amounts of secretion in the trachea, pulmonary congestion, etc. were excluded from this study.

Food deprivation during pregnancy[72] results in a reduction in the rate of growth of the fetus. In the present study reduction of food intake was of short duration (such as in PN rats) and not

to an extent to influence fetal growth.

It has been shown[81,82] that continuous exposure of rats to hypoxia leads to a decrease in body weight and food intake. To minimize the effect of hypoxia on food consumption, the rats were exposed to hypoxia at early gestation only during the day time when they are less active and to room air at night when they consume food. Another reason for implementing intermittent hypoxia was to reduce the incidence of fetal absorption.

Although rats going through an uneventful normal pregnancy were used as controls for different experimental conditions, one could easily foresee the difficulty in accepting these rats as true controls for each experimental condition, since the control pregnant rats were not subjected to stress at a level comparable to that of each experimental condition. Although there are numerous methods of exposing the rat to stress (such as immobilization, noise),[229] these methods were not used for two reasons: first, it is difficult to create stress in the control rats at levels comparable to that of experimental rats, and secondly because of the findings of Rooney et al.[230] which indicated that daily injections of glucocorticoids in maternal rats had no influence on growth and maturity of the fetal lung. Among the hormones that can influence lung maturation are glucocorticoids which increase in concentration in the maternal blood with stress. The experimental rats in this study were all under some degree of stress. A rapid adaptation to continuous stress occurs in rats resulting in normal function of adrenal glands and their glucocorticoid secretory responses.[192-194] This adaptation is delayed in daily repeated stress,

such as daily swimming exercise and intermittent hypoxia. The results of this study indicate that the fetal lung growth and maturation were not affected by the daily stress experienced by the mother. Indeed, the results of phospholipid measurements and electron microscopic studies of fetal lungs indicate that the fetuses were under little stress, if any at all, and certainly not to an extent which would result in enhancement of lung maturity.

Sham-operated rats serve as a good control for PN rats since both nutritional status and the degree of stress were similar. In many studies the sham operation consisted only of anesthesia and thoracotomy without opening the pleural cavity. These investigators had considered that lung collapse may stimulate cell multiplication in the contralateral lung.[203-205] Without opening the chest cavity the animal would not experience a general condition and stress similar to that of PN rats. In this study, in contrast to the results of the above investigators, lung collapse in sham-operated pregnant rats did not stimulate cell proliferation but significantly decreased the lung DNA content. It may be that reduction in lung size occurred as a result of restriction of chest wall expansion.

A number of different ways have been used to assess lung growth: lung weight, lung tissue volume, lung air volume, cell number and morphometric and biochemical measurements (DNA). In this study lung weight, lung air volume and DNA content were employed. Lung weight can be influenced by the volume of blood remaining in pulmonary vessels after lung excision, by secretions in pulmonary airways or by the volume of fluid in air sacs (fetal

lung). Therefore, lung weight was not considered a good measure for lung growth and emphasis was placed on lung air volume and on lung cell number (DNA). No attempt was made to differentiate various cell types or to evaluate specific influence on each cell type, with the exception of Type II cells which are used to assess lung maturity.

There are a number of different ways that one can express the results of lung weight, lung volume and lung DNA content, such as: per animal, per body weight, per age, per body length, or per gram lung wet or dry weights. Body length was not measured. Since fetuses were all of the same age but of different weights, the results on lungs were expressed per body weight. To maintain the uniformity the same was done for maternal lungs. However, both in adults and fetuses, the ratio of lung measurements per body weight declines as the body weight increases. This necessitated making the comparisons of lung growth between different conditions in fetuses of equal body weight. This method was employed since a correction factor to nullify the influence of body weights on the ratio could not always be accurately calculated due to the limited number of experiments in certain conditions.

The results indicate that: 1) hypoxia, cold environment and partial pneumonectomy stimulate lung growth in pregnant rats but swimming exercise does not affect the pregnant lung, 2) pregnant rats with large litter size have larger lungs than rats with small litter size, 3) there is a direct relationship between cellularity (DNA content) of the fetal lung and maternal lung when the latter undergoes a growth change during pregnancy, 4) in no circumstances

was a relationship in cellularity found between the maternal lung and placenta and between the fetal lung and placenta.

In this study emphasis was placed on lung growth. The relationship found between the pregnant rat and the fetus was more clear cut for the lung than for other organs such as kidney, liver and heart. In spite of the fact that both the pregnant rat and fetus experienced changes in these organs under different experimental conditions, further studies are required to substantiate whether the growth of organs other than the lung in the pregnant rat does influence the growth of the same organ in the fetus.

Factors that increase the maternal lung DNA and volume and surface area for gas exchange have no significant influence in increasing placenta cell number. Since the placenta serves as a gas exchange organ for the fetus, theoretically the O_2 needs of the fetus and not the mother should dictate its size. No evidence is available to substantiate this hypothesis. Whether or not the metabolic rate of the fetus under these experimental conditions had been changed concomitant with that of the mother is also not known.

Comparisons made among fetuses of equal body weight from pregnant rats subjected to hypoxia, cold, swimming exercise and sham operation at early and at late gestation suggest that any disturbance in the physical condition of the pregnant rat at early gestation has a deleterious effect on fetal lung and placenta in that both organs become hypocellular. This interpretation may be correct because these same conditions, when applied at late gestation, have no effect on either organs. This phenomenon occurs

regardless of whether the maternal lung size increases (cold 3), decreases (sham 3, 7, 9), or remains unchanged (hypoxia 7).

The mechanism(s) by which the fetal lung growth is suppressed is not clear. One may suspect that the appearance of the lung bud in the embryonic period is delayed when the mother is under stress at early gestation. This means that at gestation day 21 the fetal lung is at a younger stage, hence smaller. For two reasons this may be unlikely: first, the index of lung maturity (DSPC/DNA, EM studies) indicates that the level of lung maturity is comparable to that for gestation day 21, and secondly, a lack of correlation between the maternal lung and fetal lung is not supportive of this view because the fetal lung DNA content increases almost linearly from gestation day 20 to 22.

The result of cold exposed rats showed that the placentae were larger than control, if we were to take the average placenta weight (cold 3 and cold 14) and DNA (cold 14) without consideration of fetal body weight. However, for fetuses of a given body weight, the placenta weight and DNA content is in fact decreased with cold at early gestation. The same occurs with hypoxia 7 and exercise (at early or late gestation). If stress is responsible for reducing placenta cell number, it is not clear why reduction has not occurred following surgical intervention at early gestation (pneumonectomy, sham). The influence of experimental conditions at early gestation on other organs (such as liver and kidney) is not conclusive from the present study.

For four reasons, hypoxia, cold and exercise may not be suitable to enlarge the lungs of pregnant rats for the purpose of

determining a relationship between the maternal and fetal lung growth. First, these stimuli are not specific for the lung since they also cause enlargement in the other organs (such as kidney, liver and heart). Secondly, they may directly affect the fetus (hypoxia). Thirdly, a proper control is difficult, if not impossible, to devise for these conditions as mentioned previously. Fourthly, in order to significantly enlarge the maternal lung, these stimuli have to be applied continuously for a period of at least two weeks.

The only way by which the maternal lung could influence the fetal lung is by a humoral factor which is released in the maternal blood circulation and crosses the placenta. Therefore, to assess the influence of maternal lung growth on the fetal lung, it is imperative that the stimuli applied to the pregnant rat cause enlargement of growth only in the lung. This would mean that the humoral factor would be specific for the lung.

In this study it appears that in pregnant rats the lung enlarges by different processes:

1. as a result of an increase in overall metabolic rate of the rat (cold environment),
2. by hypoxia (the mechanism of organ enlargement is not clear). In these two conditions, organs other than lung also grow.
3. following partial removal of lung and availability of space for compensatory growth of the remaining lung.
4. possibly, as a result of an increase in total $\dot{V}O_2$ where excess O_2 is consumed not by the maternal, but by the fetal tissues. The enlargement of the lung, therefore, depends on

litter size.

In the latter two conditions, the lung is the only organ which grows. The mechanism(s) responsible for enhancement of lung growth may be different when lung enlargement is part of an overall growth or when it is specific to the lung.

The work of Smith et al.[206] shows the presence of a growth factor in serum following pneumonectomy which stimulates growth of Type II cells in tissue culture. Whether a similar factor is also present in serum with lung enlargement as a result of hypoxia and cold environment is not known. Our pneumonectomy experiments which resulted in fetal lung enlargement is suggestive that a growth factor has been released in the circulation and has crossed the placenta. It also suggests that this factor may be specific for lung growth, since organs other than lung (kidney and liver) have not been affected. Whether or not this factor could also have an influence on fetal organs derived from the same embryonic origin as the lung (such as bladder, intestine, etc.) has to be tested. The variations observed in fetal lung enlargement in response to maternal pneumonectomy performed at different gestational ages may be difficult to explain, because of the paucity of information in this field. For example, it is not known how long after the first stimulation for lung growth (in this case, PN) the lung growth factor continues to be released into the blood; what its concentration is; when the concentration decline reaches the non-effective level; whether with continuation of stimulation there is a continuous release of this factor in the blood and whether a certain concentration in the blood is required for this

substance to cross the placenta. It may be that following PN, a few days is required before sufficient quantities of lung growth factor is released in the maternal blood. It appears from Figures 38 and 39 that following PN, there is an initial reduction in right lung DNA content. Perhaps during this short period (3 to 5 days) when the right lung DNA content is similar to control value, lung growth factor, if released is in minute and non-effective quantities to stimulate fetal lung growth. This being the case, one can appreciate why pneumonectomy at gestation day 7 greatly enhances growth in the fetal lung, but pneumonectomy at late gestation does not. Since PN performed earlier than gestation day 7 (GD 3) has caused less fetal lung growth it may be an indication that the concentration of the lung growth factor in the serum declines about two weeks after surgery. With fetal lung enlargement (hypercellularity) there was no enhancement in fetal lung maturity (DSPC per DNA, lamellar inclusion body per Type II cells). This suggests that lung growth and maturity are two separate phenomena governed by separate factors and that the lung growth factor in question has no influence on lung maturity.

The lung hypocellularity in fetuses of pregnant rats subjected to PN at late gestation in comparison to sham is not clear. It may be that PN rats experienced a longer period of starvation and hypoxia than the sham-operated rats.

Most experiments are subjected to criticism mainly because of improper controls. This study is no exception. Fortunately, normal, control pregant rats with large or small litters provided results which were not fully anticipated. Since the C_2 consump-

tion per Kg body weight is constant in pregnant rats, the total body $\dot{V}O_2$ would change with litter size; the larger the litter size, the greater the $\dot{V}O_2$. If the lung size is dictated by $\dot{V}O_2$, one would expect large litters to increase the maternal lung size. This indeed turned out to be the case. Therefore, two populations of pregnant rats, one with small litters and small lungs and the other, with large litters and large lungs became available for evaluating the effect of maternal lung growth on the fetal lung. The lung size of the fetuses of these groups of rats followed that of the mother. The larger the maternal lung, the larger the fetal lung. This direct relationship between the fetal and maternal lung size is only observed when the maternal lung changes in size during pregnancy. The basis for this assumption is the lack of correlation between the fetal and maternal lung in rats with small litters. In these rats, lung size (weight, volume and DNA) must have remained unchanged since it was not different from non-pregnant rats of the same age and body weight. The fact that only the maternal and fetal lung and not the other organs (liver, kidney and placenta) were enlarged with large litter size suggests the possibility of the presence of a specific lung growth factor in the serum of rats with large litters, perhaps similar to that observed after pneumonectomy. This model in nature is important because it can be used to demonstrate the role of lung growth factor under normal physiological conditions.

The findings of the present study point out an important phenomenon, that the maternal organs (in this case the lung) can influence the fetal organ; and that perhaps, the well-being of

maternal organ during pregnancy is important. Furthermore, it has opened a new area in research. Not only the growth of the fetal lung, but the processes involved in regulating the organ growth through mother is an exciting field to endeavour. The first step taken to achieve these goals should be aimed at isolation and purification of this lung growth factor in PN rats; to find if this substance is also present in rats exposed to hypoxia, cold, environment, exercise and in pregnant rats, with large litter size; to inject the substance in pregnant rats at different gestational days to assess its effect on fetal lung; and finally, to determine the role of this factor on fetal lung growth in culture media.

(D) SUMMARY

The effect of maternal lung growth on the fetal lung was studied in pregnant rats. In conditions where the maternal lung was the only organ enlarged (DNA content), such as following PN and in pregnancy with large litter size, the fetal lung was also enlarged. In contrast, the fetal lung was not affected when the maternal lung enlargement followed the overall body growth (such as in cold) or was in conjunction with enlargement with other organs (such as in hypoxia). This synchronization between maternal and fetal lung growth suggests that a humoral factor, which stimulates the maternal lung cell proliferation, is released in the maternal blood circulation and crosses the placenta and stimulates fetal lung development simultaneously.

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