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FETAL LUNG DEVELOPMENT IN PROLONGED GESTATION IN THE RAT

by

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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to my parents

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ABSTRACT

Infants suffering from the postmaturity syndrome often develop the respiratory distress syndrome (RDS). Information regarding the pathophysiology of RDS is lacking. To understand the factors that predispose the postmature lung to RDS, postmaturity was induced in an animal model (rat). Pregnancies were prolonged by daily subcutaneous injection of progesterone to the maternal rats from gestation days (GD) 20 through 24 (term is 22 days). The fetuses were harvested by ceasarean section on GD 21 to GD 25. Post-term fetuses displayed characteristic features of the postmaturity syndrome. Prolongation of gestation was associated with an increased fetal mortality rate. Fetal lung DNA content (cell number) was highest on GD 23 but by GD 25 there was a 20% reduction in lung cellularity and a 90% reduction in lung glycogen content. Lung phospholipids (lecithin and disaturated lecithin) expressed per lung increased up to GD 23 and subsequently decreased by GD 25. However, when phospholipids were expressed per mg lung DNA the value was constant throughout GD 21 to GD 25. Preliminary electron microscopic studies revealed that there was a progressive accumulation of lamellar bodies in the terminal sacs as gestation was prolonged. The results of this investigation suggest that lung hypocellularity, depletion of lung glycogen and reduction in intracellular reserve of phospholipids may be predisposing factors for the development of respiratory distress in postmaturity.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PART I

LITERATURE REVIEW

A. Introduction

The postmaturity syndrome is often the consequence of prolonged pregnancy. The basis for the syndrome is the characteristic physical condition of the infant at birth. This physical condition has been well documented but the actual physiological state of these infants has not been thoroughly studied. In an attempt to understand the physiology of the postmature infant, pregnancy has been artificially prolonged in animal models to investigate the effect of prolonged gestation on fetuses and neonates. These studies have pertained to the development of organs other than the lung. Although a large body of information is available regarding lung development during normal gestation and its relation to the respiratory distress syndrome in premature infants, none is available regarding lung development during prolonged pregnancy. This is surprising since the incidence of the respiratory distress syndrome is high in postmature neonates of prolonged pregnancy.

B. Clinical Aspects of Prolonged Pregnancy and the Postmaturity Syndrome

1) Definition

Post-datism, dysmaturity, prolonged pregnancy, delayed labor, small-for-dates, post-term and runting; these terms have been used as pseudo-synonyms of the postmaturity syndrome in the literature. Anderson (1) states that post-datism should refer to a gestation which has reached 42 weeks while postmaturity should refer to the syndrome of dysmaturity occurring in a post-date gestation. If semantics are ignored, what is basically being dealt with are two separate and perhaps unrelated conditions. The first condition is simple prolongation of gestation, by an arbitrary period of time beyond the average duration of human pregnancy (280 days). The probability that birth will occur on the expected date of confinement (280 days) is 5%, but within ±3 days of the due date the probability is 29% and within ±2 weeks of the due date it rises steeply to 80% (2). Pregnancy is considered to be prolonged when it exceeds 294 days as calculated from the first day of the last menstrual period (3). But the definition of prolonged pregnancy is not as clear cut as the 294 day period may lead one to believe. Many "prolonged" pregnancies are nothing more than term pregnancies in which an inaccurate menstrual history has been provided, or 4 to 6 weeks of amenorrhea or anovulatory cycles (4,5). A pregnancy that is prolonged is not necessarily abnormal or dangerous to mother and fetus, although in some instances this may be the case.

The second condition is a clinical syndrome, referred to as the postmaturity syndrome. It occurs most frequently, but not exclusively in prolonged pregnancy and thus the two conditions are not necessarily synonymous. In the most simple terms, prolonged pregnancy can

be considered a description of the maternal condition while the postmaturity syndrome describes the fetal condition during prolonged pregnancy. A detailed description of fetal manifestations in the postmaturity syndrome will be dealt with later.

2) Incidence

Prolonged pregnancy affects a small but significant group of total pregnancies. Vorherr (3) has stated that 10 - 12% of gravidas suffer from prolonged pregnancy, with approximately 7% of all pregnancies extending beyond 42 weeks and 5% beyond 43 weeks of gestation. Others have reported an incidence of 8% beyond the forty-second week (4,6,7,8), 7.3% beyond 43 weeks (9) (somewhat higher than that reported by Vorherr), and 4.4% exceeding 43 weeks (10). In another study (11), 9% of gravidas delivered during the forty-second and forty-third weeks of pregnancy and 1.4% exceeded 44 weeks.

Prolonged pregnancy may not foster a postmature fetus however; 60 - 80% of fetuses carried beyond term are in good condition when delivered and do well thereafter. Various degrees of postmaturity are observed in the remaining 20 - 40% of fetuses (3). Strand (12) found the incidence of fetal postmaturity to be 3% at term and 20% post-term. A similar five fold increase of postmaturity in prolonged pregnancies was observed in another study (13).

3) Fetal Mortality in Prolonged Pregnancy

Although many cases of prolonged pregnancy do not involve any special difficulty or greater risk than pregnancies of normal duration, investigations have uniformly shown increases in both fetal and neonatal mortality when pregnancies extend substantially beyond the expected date of confinement (4,11,14,15). In general, 2 to 2½ times as many

perinatal deaths occur post-term compared to term (9,11), although perinatal mortality has been estimated to be as high as 5 - 7% in prolonged pregnancy compared to term values of 1 - 2% (3).

4) Placental Degeneration in Prolonged Pregnancy

It is generally agreed that the longer gestation extends beyond term, the greater is the likelihood for development of placental insufficiency due to placental degeneration. Placentas from prolonged pregnancies of up to 43 weeks of gestation display a moderate increase in incidence of infarction (3). There is usually no significant reduction of placental weight because the loss of functional placental tissues and water is compensated for by the deposition of calcium and fibrinoid material (16,17). Although no specific morphological basis exists for explaining the decline of placental function post-term (3, 10,18), a pattern of histologic abnormalities can be traced. Placentas from prolonged pregnancies display the following features (3):

1. thickened vasculosyncytial membranes or thin, partly lost syncytium, vacuolization of syncytial cells and increased formation of syncytial knots (syncytial knot formation is considered a sign of regressive villous changes caused by reduced fetal villous blood flow (19)),

2. degeneration of villi (villous fibrosis) containing edematous stroma (large placentas) or a dense-hardened stroma (small placentas),

3. decreased number of villous capillaries, thrombosis of villous stem vessels, and hyaline changes of vessel walls,

4. reduction in size of the intervillous space in 50% of the cases due to fibrin deposition.

All of these changes are increased several fold as compared to term placentas (20). In addition to these pathologic occurrances, a reduction of functional placental tissues has been observed in cases of fetal

postmaturity. Normal villous surface area (11 - 14 M^2) or even an increased one (14 - 15 M^2) was found in uncomplicated prolonged pregnancies, but in situations of fetal postmaturity it was reduced to 6 - 9 M^2 (21,22).

Clifford (23) has noted that in live births from pregnancies of 296 days or more, more than 40% of placentas showed gross abnormalities while in cases of fetal postmaturity and intrauterine fetal death 90% of the placentas showed gross abnormalities and 85% of the placentas were meconium stained.

5) Placental Insufficiency and Fetal Risk

The most important underlying cause of the postmaturity syndrome is placental insufficiency causing functional disturbances in the fetal and maternal sides of the placental circulation (18). The placental insufficiency has been attributed to degenerative morphological changes occurring in the placenta, resulting in inadequate nutrition and oxygenation of the fetus; this is believed to be the cause of the postmaturity syndrome in prolonged pregnancy (3,6,14,15,18,24,25). The functional deterioration of the placenta causes a significant and progressive decline in fetal oxygenation and a rise in fetal hemoglobin concentration occurs as pregnancy passes 41 weeks duration (26,27). Walker (8) observed this decrease in oxygen, studying umbilical cord blood PO_2. Fetal PO_2 levels fell slowly to 40 weeks and was 60 - 70% of normal at 43 weeks. Placental transfer studies using radioactive sodium showed that after the thirty-sixth week of gestation, placental exchange processes decrease, indicating a reduction in placental efficiency with aging (28).

More recent studies (29,30,31) have demonstrated that after the fortieth week of pregnancy, placental oxygen transport diminishes and

a critical reduction of fetal oxygen may occur after the forty-third week of gestation. Despite the decreasing oxygenation that the fetus is subjected to, the oxygen demand of the fetus increases with intrauterine maturity (8,26,32). The decreasing supply and increasing fetal oxygen requirements causes fetal hypoxia which, if severe enough, will produce fetal death. Other studies (33-36) however, have shown no difference in oxygen content in fetal blood of term and post-term fetuses. This illustrates that in many prolonged pregnancies (60 - 80%) no fetal hypoxia exists because intrauterine respiratory and nutritive placental functions are adequate and the fetus is born with no signs of postmaturity.

Autopsies done on postmature fetuses reveal signs of hypoxia. These include multiple petechiae of the pleura and pericardium and amniotic debris in the lungs (37), indicative of meconium aspiration due to asphyxia (3).

6) Appearance of the Postmature Infant

The most evident external features of the postmaturity syndrome is the absence of the vernix caseosa and development of dry, cracked, wrinkled and parchment-like skin that is green or yellow in color. This discoloration is due to meconium release into the amniotic fluid, and loss of the vernix allows the meconium to stain the skin. Saling (18) believes that the vernix is lost during periods of hypoxia. Cutaneous vasoconstriction occurs as an oxygen conserving circulatory adjustment to hypoxia and results in cessation of sebaceous secretion formation. The loss of protection afforded by the vernix causes maceration of the horney layer of the epidermis. In addition to the previous physical changes, the infants limbs appear long and thin, advanced hardness occurs

in the skull, and loss of lanugo hair is evident.

7) Stages of the Postmaturity Syndrome

Three different stages of the postmaturity syndrome have been described by Clifford (24):

a. Stage I of postmaturity is characterized by the loss of the vernix caseosa with resultant skin maceration. The skin is dry and cracking but meconium staining is absent. The general appearance is that of a malnourished infant which reflects the failure of the placenta to provide adequate nourishment for normal growth and development. Many babies appear much older than the usual term infant. Death does not occur in this stage of postmaturity.

b. Stage II displays all of the changes of Stage I but in addition there is evidence of degenerated placental function to the extent, that fetal distress or anoxia has occurred, with the liberation of large quantities of meconium into the amniotic fluid. Meconium covers the skin of the infant and the green meconium has stained the placental membranes and umbilical cord. Respiratory distress is seen in 50% of the infants. Autopsies done on some of the stage II neonates displayed large sections of atelectatic lungs and numerous emphysematous blebs. All showed marked aspiration of amniotic fluid with the alveolar ducts and bronchioles filled with cellular debris, squamous cells, and extravasated blood. Hyaline membranes were also seen. The mortality rate for these neonates was 35%.

c. Stage III postmature infants are assumed to have passed through the first two stages and to have had intrauterine anoxia with the passing of meconium days or weeks before birth. The majority of the fetuses (85%) die in utero, presumably as a result of anoxia. Only fetuses whose intrauterine anoxia is of a less severe degree survive in utero to be liveborn. Because the meconium has been passed some time before delivery, the green bile of the meconium is converted into a brilliant yellow that stains the nails and skin of the newborn. The mortality rate in this group of infants is only 15% and is explained on the assumption that because of the severe second stage in utero, fetuses would have been stillborn and only the milder cases would survive to livebirth. Severe respiratory distress was noted in 30% of the infants and at autopsy there was evidence of aspiration of meconium and bilateral patchy atelectasis.

8) Respiratory Distress in the Postmature Infant

Fetuses of prolonged pregnancies can be at risk in utero because

of placental insufficiency and development of the postmaturity syndrome that can lead to intrauterine death. But also of importance is the possibility that they may develop respiratory distress in the neonatal period as a consequence of the postmaturity syndrome. In fact, the respiratory distress syndrome, otherwise known as hyaline membrane disease, is the most frequent cause of death in postmature neonates (3).

Lieberman (38,39) has suggested that hyaline membrane disease may be a sequel of placental dysfunction. He demonstrated a potent inhibitor of plasminogen activator in normal placental tissue and that the lungs of infants with hyaline membrane disease lack plasminogen activator. Lieberman postulated that placental infarction or other abnormalities may release the inhibitor into the fetal circulation, preventing the dissolution of intra-alveolar fibrin, with formation of hyaline membranes. Other possible causes of respiratory distress in the postmature infant include aspiration of meconium filled amniotic fluid (23) and vasoconstriction of the pulmonary vessels as a circulatory adjustment to hypoxia (41), however no conclusive data exists which can explain the phenomenon of respiratory distress in postmature neonates.

9) Respiratory Distress Syndrome

Why postmature infants develop the respiratory distress syndrome (RDS) is a puzzling question, since RDS is predominantly a disease of immature infants such that the incidence increases with diminishing gestational age. As the baby approaches term, he is far less susceptible to this syndrome. But to speak of RDS and its relation to postmature infants is of little value without a basic understanding of normal lung mechanics at birth and pathophysiology of RDS. It is thus appropriate to discuss these subjects briefly.

a) Surfactant and Alveolar Stability

In the late 1950's, a number of investigators (41-44) rediscovered the observations of Van Neergaard (45), and confirmed that normal alveoli had to be coated by a surface-active material (surfactant) capable of reducing surface tension that would otherwise cause each alveolus to collapse during the end of expiration. Thus the surface forces operating at the extensive air-tissue interface of the lung and contributing to the retractive forces of the lung, are of a minimal magnitude at low lung volumes because of the presence of pulmonary surfactant. By reducing the surface tension of the alveoli at expiration, pulmonary surfactant stabilizes the air spaces, and allows the lung to retain a volume of air at end expiration. The retention of air at expiration decreases the pressure needed to distend the lung on the following inspiration and reduces the work of breathing to a minimum. This is predicted by the LaPlace equation which relates pressure (P) to surface tension (T) and radius of the curvature (r) of an alveolus as $P = 2T/r$. Thus the smaller the alveolus (alveolar collapse), the greater the pressure required to inflate it. The pressure required to inflate an already partially inflated alveolus is considerably less than that required to inflate a collapsed alveolus because the partially inflated alveolus has a much greater radius of curvature.

In summary, it can be seen that pulmonary surfactant is an anti-atelectasis factor which by providing a low surface tension to the air-tissue interface serves the 2-fold function of maintaining alveolar stability and decreasing the pressure needed to distend the lung (46). The functional behavior of surfactant is due to its unique composition. Dipalmitoyl lecithin (DPL, dipalmitoyl phosphotidylcholine)

is generally accepted as the major surface active component of pulmonary surfactant (47,48), although unsaturated phosphatidyl choline (lecithin), other lipids and phospholipid classes and small amounts of specific proteins also contribute to the complex mixture of pulmonary surfactant.

b) Pulmonary Fluid and the First Breath

Prior to birth the fetal lung airways are fluid filled. The composition of fetal lung liquid differs from that of both plasma and amniotic fluid (49) and it has been suggested that lung liquid is not an ultrafiltrate of plasma but a substance actively secreted by the fetal lung (50). The cellular source of the liquid is not known. The lung liquid has a low protein concentration and is rich in surface active material. At birth, extrauterine survival demands that the infant switch from dependence upon maternal placental oxygenation to complete reliance on its own pulmonary oxygenation. The first few breaths taken require a considerable force because of the high viscosity and inertia of the lung fluid as compared to air. Thus at birth the infant must expand the thorax by contractions of the respiratory muscles with sufficient force to (1) move air and a column of liquid ahead of it into the lungs, (2) overcome the forces of surface tension at the interface of the contiguous columns of air and liquid moving through small airways, and (3) distend the lung tissues (51).

During the first few breaths infants produce negative interstitial pressure and a hydrostatic pressure difference that favors movement of lung liquid from alveoli into contiguous interstitial spaces. From the interstitium, the liquid is returned to the bloodstream by either reabsorption into the alveolar capillaries or lymphatic channels

that are located in the interstitial spaces.

As the air-liquid interface approaches the smaller bronchioles, the forces of surface tension become operative and surfactant in the fluid reduces the surface tension at the interface. As the interface reaches the terminal lung units, the radius of curvature becomes minimal and the pressure required becomes maximal (opening pressure). As inflation continues and alveolar radius increases above minimal radius, the pulmonary air volume increases rapidly, as does fluid removal from the lung. On first expiration, the presence of surfactant in the alveoli, causes the surface tension to fall allowing the lungs to retain a significant amount of air at the end of expiration thus producing the functional residual capacity.

c) Pathophysiology of RDS

The salient features of RDS are the early postnatal onset of rapid, grunting respirations and the development of intercostal and sternal retractions, reflecting the tendency of the lung to collapse with expiration. Avery and Mead (43) were the first to suggest that lungs from infants with RDS have abnormal surface tension properties due to a deficiency of pulmonary surfactant, and other reports are in agreement (53-56). The lack of surfactant causes alveolar instability such that with expiration the alveolar diameter decreases, the alveolar surface tension rises steeply and the lung collapses. This lung lacks the ability to retain a sufficient volume of air upon expiration to keep the alveoli open and as a result, the infant must exert a high negative intrathoracic pressure to open the alveoli for each inspiration. Each breath resembles the "first breath". The mechanical disadvantage of decreased surface active material results in increased work of breathing.

Other functional abnormalities of RDS infants include a decreased tidal volume, a compliance (change in volume per unit change in pressure at points of no flow) that is reduced to 1/4 or 1/5 of normal and a reduced functional residual capacity.

Lungs of infants with RDS are rarely, completely devoid of surfactant (57,58). Quantitation of the amount of phospholipid in lungs of RDS infants has been reported by several investigators (56,59,60,61), who demonstrated that the amount of phospholipid, including surface active lecithin, is markedly lower than normal but not absent. The fact that surface-active phospholipids are present in the syndrome indicates that synthesis or secretion of surfactant in the lung is impaired but not abolished.

RDS is characterized by distention of certain alveoli and by collapse of others. Scarpelli (62) has described three probable populations of alveoli that the lungs of RDS infants contain at birth:

1. Alveoli that are aerated relatively well and retain a residual volume on expiration, presumably with sufficient surfactant to lower the surface tension. The number of these alveoli present in the lung is probably the most significant determinant of the severity of the disease.

2. Alveoli that are not aerated at all.

3. Alveoli that are aerated but collapse during expiration due to insufficient surfactant. These alveoli are highly unstable.

With the collapse of respiratory tissue there is ventilation-perfusion imbalance and concomitant hypoxia (63,64), but the insult to the infant does not end here and a series of events, likened to a vicious cycle occur (63,65,66). The hypoxia produces acidosis, acidosis reduces pulmonary blood flow (vasoconstriction), decreasing surfactant production and promoting further alveolar collapse. Hypoxia also damages capillary

endothelia, which along with the high negative intrathoracic pressure, help to promote transudation of fluid into the alveoli. Fibrin forms the matrix which entraps necrotic alveolar duct epithelium, red blood cells and serum proteins. These coalesce to form hyaline membranes that line dilated alveolar ducts and terminal bronchioles. The vicious cycle of atelectasis, hypoxia, acidosis, diminished pulmonary blood flow, transudate, inhibition of pulmonary surfactant, atelectasis, hypoxia etc., continues until recovery (from a few hours to 6 or 7 days), or death (at any time in the course of illness) occurs. The characteristic pathologic feature is one of diffuse atelectasis with dilation of the terminal bronchioles and alveolar ducts, congestion of the pulmonary capillaries and the presence of hyaline membranes.

C. Prolonged Pregnancy in Animal Models

It has been established that some fetuses of prolonged pregnancy develop the postmaturity syndrome, a term that applies basically to the physical condition of the infant at birth. Although placental degeneration and hypoxia have been attributed to the development of postmaturity, no information is available on the actual physiological or biochemical state of the fetus. A fetus that has suffered sufficient insult in utero to manifest an abnormal physical condition, will likely possess an altered physiological state also. In order to investigate any abnormal physiological and biochemical processes, it has been necessary to utilize animal models of prolonged pregnancy that might provide information applicable to the human situation. A review of the literature follows to illustrate that artificial prolongation of gestation in animals is a useful model to elucidate the condition of the postmature fetus in prolonged human pregnancy.

1) History

Studies in prolonged pregnancy were initially designed to determine what substance had the ability to prolong gestation. These experiments consisted of injecting corpus luteum extracts into pregnant rats, rabbits and mice and observing the length of the pregnancy thereby prolonged (67,68). Others (69,70) studied the role of intact corpora lutea in parturition by inducing ovulation with urine extract injections during the pregnancy of rabbits. As a result, fresh corpora lutea at the stage of maximal activity were present at term and prevented normal parturition. The onset of parturition was delayed 15 days after injection (gestation day 40), or until the end of the life span of the induced corpora lutea. Heckel and Allen (71) delayed parturition by

injecting progesterone into rabbits. The common findings of these experimentally prolonged pregnancies was that many fetuses were dead and macerated if pregnancy was prolonged too long, their size however was larger in comparison to fetuses at term. This indicated that in utero growth of the fetuses occurred despite the delayed parturition.

Boe (72) carried out the first systematic study of the effects of prolonged pregnancy in rats. Pregnancy was prolonged by injections of gonadotropic hormone from pregnant mare's serum, pregnant human's urine, estrone, progesterone, hypophysectomy or ligation of uterine horns. During prolonged pregnancy characteristic changes were seen in the fetal organs, especially the endocrine organs studied. Boe ascribed these changes to hyperemia, edema, and regressive cellular changes. These changes were more marked just prior to the intrauterine death of the fetuses. Hyperemia could be detected in all organs examined before any of the other changes occurred. Histologically, severe degeneration of the placenta was observed and the cause of intrauterine death of the fetuses during prolonged pregnancy was thought to be due to the cessation of placental function as term approached. This assumption was based on the regressive changes occurring in the placenta in the later stages of pregnancy, by the rapid degenerative changes appearing during prolonged pregnancy, and by the deteriorating condition of the fetuses in utero.

2) Placental Changes

The morphology of placentas obtained from prolonged pregnancies of rats has been described recently (3,73,74). Vorherr (3) using light microscopy found progressive degeneration of the placenta as pregnancy was prolonged from the normal period of 22 days to 26

days. As early as gestation day 23 pyknotic nuclei were seen in some syncytial cells and deposition of fibrinoid material beneath the chorionic plate was observed. Polymorphonuclear leukocytes were dispersed within the labyrinthine placenta and a layer of polymorphonuclear leukocytes could be seen beneath the chorionic plate. Placentas from gestation day 24 and 25 displayed increased degeneration (as indicated by fibrinoid deposition of large areas of the placenta including the area beneath the chorionic plate) and diffuse infiltration of polymorphonuclear leukocytes throughout the placenta. Interlabyrinthine placental spaces were empty and fetal capillaries contained few erythrocytes. By day 26 fibrinoid deposits were extensive, large areas of the placenta necrotic and few viable cells remained. Calcification was also in progress. Thliveris (73) noted similar ultrastructural changes as Vorherr (3) and interpreted the progressive loss of trophoblast integrity and deposition of fibrinous material as signs of reduced placental function. Although one report (74) describes no degenerative changes (minor degrees of trophoblast vacuolation and deposition of amorphous material) in placentas obtained on the fourth day of prolonged gestation, it may be attributed to the experimental procedure. Only one fetus was retained in utero beyond term and this was accomplished by a surgical ligature, placed prior to term. The other littermates were delivered spontaneously at term, nursed by the mother and consequently the retained fetus was maintained in utero presumably because uterine muscular activity was inhibited by endogenous progesterone secreted from the corpus luteum of lactation. Blood flow to the isolated gestational sac did not appear to be interrupted. In

Thliveris' study however, blood vessels to all gestational sacs were markedly congested. Thliveris believes the essential difference in the results may reside in the blood supply to the uterus (73).

3) The Postmaturity Syndrome in the Rat and Rabbit Fetus

Markedly postmature fetuses are obtained in both rats and rabbits if pregnancy is prolonged experimentally by 2 to 3 days (3,32, 73,75). There is extensive meconium staining of the wrinkled fetal skin, umbilicus, amniotic fluid, fetal membranes and placenta (3,73,76). In addition there is a reduction of the amniotic fluid volume (3) and the amnion often adheres to the fetus (76). These features indicate severe fetal distress (3,24) that is not compatible with life since in utero fetal death occurs after 2 to 3 days of prolonged pregnancy. The similarity of the clinical and experimental signs of postmaturity in humans and animals provide a means of studying the effects of prolonged gestation on fetal development.

4) Pathophysiology of Prolonged Pregnancy

Besides the obvious signs of fetal distress and postmaturity in fetal rats and rabbits post-term, the physiological state of the fetus is also abnormal. The oxygen saturation of blood taken from the cranial venous sinuses of rabbit fetuses, deteriorates progressively as gestation is prolonged (32). Thus the fetuses pass progressively through a period of moderate hypoxia to profound anoxia and finally death. Fetal survival during chronic hypoxia is likely due to high rates of glycolysis in fetal organs, which has been demonstrated in the fetal liver and heart of postmature rabbits, but the presence of new metabolic pathways in glycolysis and the oxidative cycle were not demonstrated (75).

Liver glycogen content of the postmature fetus is much lower

than that of term (75,77) and has been attributed to hypoxia and resultant increased rates of glycolysis (75). The glycogen depletion is not due to glucose-6-phosphatase (the enzyme that catalyzes the last step in the chain of reactions converting glycogen to glucose), since it is not increased in the postmature liver (78). Another contributing factor could be a decrease in placental transfer due to a loss of trophoblast integrity and deposition of fibrinoid material over the placental exchange area (79,80) causing a reduction of nutrients transferred from mother to fetus. Mobilization of the fetus's own glycogen reserves would be required, in an effort to meet its own energy requirements and maintain normal blood glucose levels. Thliveris (81), using electron microscopy has shown that gestation day 23 fetal rat hepatocytes have similar amounts of glycogen as term hepatocytes. Blood glucose levels are also similar on day 22 and 23 (73,77). On day 24 and 25 however, a marked glycogen depletion of the hepatocytes occurred along with a significant decrease in blood glucose (73, 81).

The role of insulin, glucagon and the catecholamines in regulation of fetal blood glucose levels and glycogen metabolism has been studied, but at present their actual roles are not known with certainty. Plasma insulin decreases sharply on gestation day 22 and 23 (77) but no significant reduction in fetal blood glucose levels occur at this time. An increase in pancratic insulin occurs between day 21 and 22 of gestation but no change in pancreatic insulin occurs between day 22 and 23, according to biochemical (77) and morphological (82) studies. However, pancreatic insulin did rise on gestation day 24 and 25 as measured by an increase in the number of secretory granules in pancreatic beta cells (82), indicating an accumulation rather than a

release of insulin stores by beta cells during prolonged gestation.

Pancreatic glucagon increases from day 22 to 23 (77), but the alpha cells on day 24 and 25 display a reduction in the number of secretory granules although some alpha cells contain numerous secretory granules concentrated at the cell surface in proximity to capillaries (82). The events occurring in the fetus of prolonged pregnancy are not clear, in regards to glycogen depletion, insulin and glucagon release or storage. It is also not certain whether or not glucagon is the mediator or co-mediator of glycogenolysis. Whether or not fetal adrenal catecholamines play a role in carbohydrate metabolism at this time is also speculative although they have been implicated (82), since the fetal rat adrenal in prolonged pregnancy releases catecholamines as indicated by the absence of catecholamine storage granules (83). Both epinephrine and norepinephrine promote hepatic glycogenolysis (84,85) while norepinephrine causes a decrease in plasma insulin as well as an increase in plasma glucagon when injected into rat fetuses at term (86).

The consequence of glycogen depletion in the fetal rat liver is not appreciated until the postmature fetus is forced to survive extrauterine. Portha et al (77), using term and postmature rat neonates (gestation day 23), monitored blood glucose levels and liver glycogen content during the first 6 hours of life with fasting. Term rat neonates displayed transient hypoglycemia in the first hour after birth but restoration of normal blood glucose occurred at 6 hours, while postmature neonate blood glucose levels progressively decreased in the 6 hour period. The liver glycogen stores decreased after birth in the postmature neonates and were almost completely depleted after 6 hours of fasting while the normal neonates had 16 times as much liver glycogen after the

same period of fasting. It is likely that the low blood glucose levels observed in postmature animals are related to the low liver glycogen content and is therefore an important feature of postmaturity. The restoration of normal blood glucose levels in the term neonate is well correlated with the development of glycogenolysis, glycogen depletion and gluconeogenesis in the early neonatal period (87-90). In the postmature neonate the mobilization of glycogen stores also occurs, but because of the very low glycogen content, glycogenolysis is unable to maintain glucose homeostasis (77). Although liver glycogen content of postmature human infants has not been measured, hypoglycemia is a common occurrance of postmature infants that display marked intrauterine fetal distress and meconium staining (91,92), demonstrating the similarity of clinical and experimentally induced postmaturity.

D. Fetal Lung Development

1) Human Lung Development in Utero

An outpouching of the foregut in the 26 day old human embryo is the precursor of the future lung. A series of asymmetric, dichotomous branchings of the growing lung bud initiate the development of the bronchial tree and by the sixteenth week bronchial development is complete (93). The dichotomous branches are lined by actively dividing, columnar ciliated epithelial cells but as the columnar epithelium extends peripherally to the level of the bronchioles they gradually change to a cuboidal shape. This period of lung development has been termed the glandular stage because of its distinctly glandular appearance. Although cartilage of the airways is present they are not fully developed and respiration is impossible.

After the sixteenth week, the onset of the canalicular phase is marked by proliferation of vascular tissues around the distal ends of the terminal bronchioles. Respiratory bronchioles which will be future alveolar ducts begin to differentiate by branching from the terminal bronchioles. The cuboidal epithelium lining these bronchioles flattens, becoming irregularly thinned and cellular continuity between the cells may be lost at the margins of the cells, maintained only at their bases. Capillaries protrude into the epithelium and occassional areas of thin blood-airway barrier, similar to those of the adult, appear.

The third stage of lung development has been termed either the alveolar stage or the terminal sac stage for reasons that are discussed below. This stage succeeds the canalicular stage at approximately 24 to 26 weeks of gestation, and it is at this time that res-

piratory tissue begins to appear as saccular structures, differentiating from the respiratory bronchioles. The respiratory portion of the fetal lung has thick interalveolar septa and relatively few capillaries in contact with the air spaces. Various types of alveolar lining epithelium (Type I and Type II cells) can be seen. Type I cells are characterized by cytoplasmic extensions lining the capillaries and forming the blood-gas barrier, while Type II cells are large and round in shape, containing lamellar inclusion bodies (osmiophilic bodies) that are characteristic of Type II cells. It is only after the differentiation of the alveolar epithelium into Type I and Type II cells that surfactant begins to appear in the fetal lung fluid of the pulmonary airways. Progressive thinning of the epithelium and protrusion of capillaries leads to many more areas where capillary lumens are in close proximity to airway surface. Respiration can now be maintained although alveoli are not present in the walls of the terminal sacs. It is for this reason that this stage has been called terminal sac stage rather than alveolar stage (94). Some species such as the rat, are born during this stage and true alveoli are not present at birth and thus the alveolar stage per se occurs postnatally. In humans however, a considerable number of alveoli are present at birth, in addition to the terminal sacs indicating that there may also be an intrauterine alveolar stage, but its time of onset is uncertain (94).

2) Rat Lung Development in Utero

The glandular, canalicular, terminal sac and alveolar 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 day 21 indicates that alveolar cell differentiation has occurred and Type II cells have begun secreting surfactant. Between day 21 and 22 the third stage of lung development occurs and whether or not it should be called the alveolar stage (that continues postnatally) or the terminal sac stage that is proceeded by the alveolar stage is a matter of semantics. Suffice it to say that fetal rat lung development continues after birth and is responsible for postnatal lung growth.

3) The Type II Alveolar Cell

Surfactant deficiency and concomitant development of RDS in infants born with immature lungs has been the impetus for extensive research in fetal lung development, with special attention being paid to the Type II cell. Macklin (95) in 1953 attributed the site of formation of the alveolar lining constituents (surfactant) to the large alveolar Type II cells. Since this original observation by Macklin, considerable evidence has accumulated to confirm that the Type II cells are responsible for the synthesis, storage and release of surfactant. The evidence supporting this comes from a variety of studies including morphologic, histochemical, autoradiographic, correlations between morphology and measurements of the surface tension of lung washings, and simultaneous appearance of lamellar bodies and surfactant (96,97). These studies have demonstrated that the cellular source of the surface-active lipids are the Type II cells and thus it has been only recently that one can speak of surfactant in relation to Type II cells with assurance instead of reservation.

4) Lamellar Body Formation

Autoradiographic studies using various labelled substrates have been used to trace the movement of phospholipid constituents from the Type II cell organelles to the lamellar bodies. Chevalier and Collet (98) and Dickie and Massaro (99) followed the incorporation of choline and leucine into various subcellular fractions of Type II cells. At very short times after the injection, the labelled choline was localized predominantly in the endoplasmic reticulum, suggesting that this cell organelle is the locus of phosphatidyl choline synthesis. Subsequently, the radioactivity was found to be rapidly transferred via the Golgi apparatus to the lamellar bodies for storage. A similar sequence of activity in subcellular organelles of Type II cells was observed after radioactive leucine injection, which suggests that lipid and protein may be stored as lipoprotein in the lamellar bodies and secreted as such. Other investigators (100) however, believe that the proteins are added to the lipids at a stage after lamellar body formation. There is no evidence for the transfer of phospholipids in lamellar form from the endoplasmic reticulum or Golgi apparatus. It is possible that newly synthesized phospholipid is transported intracellularly in a nonlamellar form, perhaps as a protein-phospholipid complex. Blackburn (96) has summarized the synthesis of surfactant in Type II cells as involving a number of cellular organelles in the following sequence: 1) endoplasmic reticulum, 2) transfer vesicle, 3) Golgi apparatus, 4) transfer ("coated") vesicle, 5) multivesicular body, 6) homogeneous granule, and 7) lamellar body.

5) Biochemical Aspects of Fetal Rat Lung Development

Besides the histological stages of fetal lung development, it

has also been beneficial to assess the development of the lung by biochemical and kinetic methods. Three periods of activity have been demonstrated. In the initial inductive period, little proliferative activity occurs and tissue composition is quite unspecialized. The proliferative period follows and is characterized by a lung cell number that rapidly increases, mostly by epithelial cell proliferation (101). The only notable biochemical change occurring at this time is an accumulation of glycogen. This is succeeded by the cytodifferentiation period where mitotic activity has receded and specific biochemical changes have occurred, particularly in phospholipid metabolism. The biochemical studies of fetal lung development have in general been concerned with either carbohydrate or lipid metabolism and related to the Type II cell population of the fetal lung.

Although morphological studies provide a visual means of documenting maturation of the fetal lung it is only through biochemical studies that much needed information on the metabolic events occurring in fetal lung development can be obtained. Biochemical studies have been concerned with changes in the tissue composition of glycogen, DNA, protein, and phospholipid (lecithin and DPL) at the end of gestation.

DNA analysis of fetal lung at 24 (96) and 48 hour intervals (102) from day 16 through birth have shown a period of rapid alveolar cell proliferation beginning after day 17, continuing through day 20, and then slowing down on day 21 of gestation. Little change in lung cell number occurs during the 24 hours preceding birth. These compositional studies agree with the structural information that has shown the number of cells recognizable as Type II cells to increase at a rapid rate during day 20 to 21, but during the 24 hours immediately preceding

birth little or no change in their concentration occurs. The change in rate of increase (on day 21) of DNA content, indicates that the rate of cell division decreased as cytodifferentiation proceeded during the last days of gestation (102). It is during this period of cytodifferentiation that biochemical and hence functional differentiation also occurs.

An important feature of differentiation of fetal rat lung is the rapid accumulation of glycogen during the proliferation period, its peak concentration on day 20 and subsequent virtual disappearance from the lung during the next 48 hours (102,103). This glycogen depletion correlates with other biochemical and morphological changes occurring during this period. Because the loss of lung glycogen occurs at the time when (1) lecithin is being rapidly synthesized, (2) lamellar bodies accumulate in Type II cells, and (3) DPL is increasing in concentration; it is reasonable to assume that these events are related (102). This has led some to believe that a significant portion of fetal lung glycogen is utilized for the initial synthesis of surfactant by Type II cells (96). At present however, neither the enzymatic basis for the large changes in glycogen content nor the precise mechanisms of regulation of carbohydrate metabolism in fetal lungs is known.

In the fetal rat, lipid begins to accumulate slowly after day 17 (96) and much more rapidly after day 19 (96,102). This same pattern is also true for lecithin (96,102) and DPL (102). These changes in lung lipids during the cytodifferentiation phase of fetal lung development, correlate directly with the appearance of surface activity in lung minces and with the appearance of Type II cells. In addition, the increase in quantity of extractable lecithin correlates with the rise in number of

Type II cells during in utero development.

In summary biochemical studies have shown that during fetal development, lung composition changes dramatically as seen by changes in: (1) DNA, (2) the increase and abrupt decrease in glycogen content and (3) an increase in phospholipid (especially DPL) content that occurred as glycogen concentration decreased.

6) Biosynthetic Pathways of Lecithin in the Fetal Lung

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 quaternary amine, choline. Without the choline moiety, the compound would represent phosphatidic acid and hence the term phosphatidylcholine for the final phospholipid product.

From the structure described, it is apparent that three key components are necessary for lecithin synthesis: glycerol, fatty acids and the nitrogenous base (choline). The former two however, enter the de novo pathways for lecithin synthesis as a diglyceride unit. There are two pathways responsible for de novo lecithin biosynthesis. In the choline incorporation pathway (pathway I), as elucidated by Kennedy and Weiss (104), choline is phosphorylated and then "activated" by conversion to the cytidine diphosphate choline derivative. The phosphorylcholine moiety of cytidine diphosphate is transferred to a diglyceride molecule to form phosphatidylcholine (lecithin). In pathway II, described by Bremer and Greenberg (105), ethanolamine undergoes similar steps of phosphorylation, activation, and linkage to diglyceride to form phosphatidylethanolamine. It then undergoes 3 successive methylations, with

S-adenosyl methionine serving as the methyl donar, to form lecithin.

In the fetal rat lung lecithin is synthesized almost exclusively via the choline incorporation pathway (106). The choline incorporation pathway in the lung produces mainly unsaturated phosphatidylcholine (107) yet the most surface active phosphatidylcholine is dipalmitoyl phosphatidylcholine, a disaturated lecithin. Thus there should be another mechanism, or mechanisms, that can remodel de novo synthesized unsaturated lecithin into disaturated lecithin. At present two mechanisms could possibly be involved in such transformations; the deacylation-reacylation process and the deacylation-transacylation process (107). There is increasing evidence that both processes may operate with sufficient specificity to account for the introduction of palmitate at the 2-position of dipalmitoyl lecithin (107). The relative importance of these two processes is still unknown.

E. Purpose of the Study

The purpose of this investigation is to examine fetal lung development during prolonged gestation. Although it has been documented that postmature infants of prolonged pregnancy develop RDS and a number of possible causes have been described (3,23), there is no definite evidence for any of these causes. Aspiration of meconium was thought to cause RDS (23), but recently Fujikura and Klionsky (108) found that meconium staining was associated with a decrease in the expected frequency of hyaline membranes and atelectasis, even in premature infants. Since bile pigments are one of the main components of meconium and has a strong surface-active action (109), aspiration of meconium could reduce surface tension in the alveoli and facilitate alveolar expansion, preventing the production of atelectasis and hyaline membranes (108).

It is generally agreed that RDS of the premature infant is due to an immature lung, characterized by surfactant deficiency (46). It is reasonable to assume that the postmature infant's lung has passed the stage of immaturity, yet both groups of RDS infants display the classic pathological features of RDS: lung atelectasis and hyaline membrane formation. No information is available that describes the basic biochemical characteristics of the postmature lung and it is not known whether alterations in the surfactant system or other biochemical alterations have occurred which might predispose the postmature infant to RDS. As a result, this project was undertaken to study fetal lung development during prolonged gestation with emphasis placed on the surfactant system of the lung. Parameters of fetal lung development that were studied include: 1) DNA, 2) RNA, 3) total protein, 4) total lipid and phospholipid, 5) lecithin and DSPC and 6) glycogen content of the

fetal lung. It is hoped that this study may help elucidate possible abnormalities of the lung that contribute to the development of respiratory distress in postmature infants.

PART II

EXPERIMENTAL STUDY

A. Methods

Virgin female Sprague Dawley rats (Biolab Co.) were received, having a weight of 160 - 180 grams. 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 and water ad libitum and mated when their body weight was between 195 - 225 grams. Vaginal smears were taken daily, and while rats were in proestrus 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 (GD) one was designated as commencing 24 hours after successful mating had occurred. Females, if allowed to deliver, usually did so on day 22 of gestation.

Gestation was prolonged 1, 2 or 3 days beyond term (GD 22) by daily subcutaneous injections of 5 mg progesterone (pregn-4-ene-3,20-dione, Prolutin, Schering Corp., New Jersey) in sesame oil from GD 20 through GD 24. Rats were divided into 4 groups: 1) non-injected (NI); 2) progesterone injected daily from GD 20 until the day before sacrifice (P_{20}); 3) progesterone injected daily from GD 14 to GD 21 - sacrificed on GD 22 (P_{14}) and; 4) sesame oil injected daily from GD 14 to GD 20 - sacrificed on GD 21 (S_{14}).

Pregnant rats were weighed on the day of sperm positivity (GD 0) and subsequently every second day up to GD 20 and every day thereafter. Rats were anesthetized with a subcutaneous injection of sodium pentobarbitol (5 mg/100g of body weight) on the day of sacrifice. Laparotomy was performed and fetuses were then harvested by ceasarean section.

Fetuses were dried with gauze before their body weight was recorded. Only live, normal littermates were used, and only litters with 9 to 14 fetuses per mother were included in this study. Litters outside this range were discarded. The fetuses were then decapitated and using a dissecting microscope, the lungs were removed, separated from the extrapulmonary airways and weighed. The lungs of littermates were pooled to provide sufficient sample size for one of the following determinations: 1) DNA, RNA and total lung protein content (0.5g sample); 2) total lung lipid and phospholipid, lecithin and DSPC (0.5g sample); 3) dry weight of individual lungs. Some lungs were immersed in fixative for histological studies. Lungs that appeared to contain blood or meconium due to aspiration, or air due to fetal gasping were discarded. In addition, liver, spleen, and kidneys were dissected from the fetuses and weighed. Placentas 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 then weighed. Placenta and liver dry weights were also collected. The uterus and its contents was removed from some rats and subjected to microwave irradiation to inactivate enzymes responsible for glycogenolysis (118). Lungs, liver, heart and muscle were dissected from these irradiated fetuses and used for glycogen determination.

Some females were allowed to deliver and nurse their pups for either 24, 48 or 72 hours, at which time the pups were weighed, anesthetized by sodium pentobarbitol and exsanguinated by cutting the abdominal aorta. The lungs were removed and weighed after the extrapulmonary airways had been dissected away. The lungs of littermates were pooled and samples taken to measure the following: 1) DNA, and total lung protein content (0.5g sample); 2) total lung lipid and phospholipid,

lecithin and DSPC (0.5g sample); 3) dry weight of individual lungs. Liver, spleen and kidneys were dissected from the fetuses and weighed. The dry weight of the liver was obtained. Some puppies were subjected to microwave irradiation. Lungs, liver, heart and muscle were dissected from these puppies and used to measure glycogen content. Dead puppies were excluded from the study and only litters of 9 to 14 puppies were used in this study.

Fetuses obtained from P_{20} pregnant rats at GD 23, 24 and 25 were compared to fetuses obtained from P_{20} pregnant rats at GD 22. This comparison was made to determine any differences in fetal development post-term to that of term. In addition post-term fetal development was compared to postnatal development, ie. GD 23 versus 24 hours, GD 24 versus 48 hours and GD 25 versus 72 hours.

Dry weight

Dry weight measurements were made by placing the lung, liver or placenta on pre-weighed pieces of tin-foil which were then kept in an oven at 60°C for one week.

Phospholipids

Pooled samples of lung tissue (0.5g) 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 (110). 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 (111) of the method of Fiske and Subba Row (112). 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) (113). The plate was then exposed to iodine vapor. The sphingomyelin-lysolecithin, phosphatidyl choline (lecithin) and phosphatidyl ethanolamine spots were 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 (114) and the lipid phosphorus determined.

DNA, RNA and total protein

Pooled samples of lung tissue (0.5g) were homogenized in 2.5 ml of normal saline in a Kontes all glass homogenizer. One hundred microliters of this suspension 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 hours. The total protein content was then determined by the Lowry method (115). From the above homogenized sample, 1 ml aliquots (in duplicate) were used for the extraction and determination of lung deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) by the method of Schneider (116).

Glycogen

Viability of fetuses, while in utero, was determined by pinching the tail with forceps. A fetus was considered dead if fetal movement did not occur and was marked by passing a needle through the body. The uterus was then removed and placed in a microwave oven and fetuses irradiated for 10 to 15 seconds. Occasionally fetuses would "burst" and

their organ types could not be identified. These fetuses were discarded. Postnatal rats were also irradiated for 10 to 15 seconds. Lung, liver, heart and muscle (from the thigh) samples were dissected from the fetuses and postnatal rats and lyophilized overnight. They were then weighed and stored at -20°C until used. Tissues were then homogenized in 0.05M acetate buffer (pH 4.7) to extract the glycogen. Glycogen was measured using amylo-α-1,4-α1,6-glucosidase according to the method of Passoneau and Lauderdale (117) and tissue glycogen calculated using the equation ΔA/6.22 X volume of cuvette (ml) X (total extraction volume (ml)/volume of extract assayed (ml)) X (1/tissue dry weight (mg)), where ΔA = change of absorbance at 340 nm and 6.22 = extinction coefficient of NADPH at 340 nm (118).

Light microscopy

The left lung of the fetus was cut into 3 pieces and fixed for one week at 4°C in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Tissues were then transferred to a 0.2 M sucrose solution containing 0.1 M phosphate buffer (pH 7.4) and stored at 4°C. The tissues were then postfixed for 2 hours in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at 4°C. After rapid dehydration in ascending concentrations of ethanol, the tissues were embedded in Epon 812 (119). One micron sections were cut on an ultramicrotome using a glass knife equipped with a water boat. The sections were then transferred from the water boat to drops of water on glass microscope slides and gently heated on a hot plate till dry. Sections were stained with toluidine blue for 2 minutes, then rinsed with water to remove the excess stain. A coverglass was mounted and allowed to dry.

Statistics

Statistical analysis of the data was carried out using a t-test of unpaired variates and a multiple range test for analysis of variance by Duncan's method, where applicable.

B. Results

1) Maternal Measurements

Tables 1 - 4 show the body weight and the number of fetuses or puppies for the 4 groups of rats. All rats were of a similar weight on GD 0 (day of mating) and of similar litter size. The body weight increased from GD 0 to GD 23 and then decreased on GD 24 and GD 25 (Figure 1 and Table 3). Fetal death was uncommon on GD 21 to GD 23 but by GD 24 fetal mortality increased to 21.7% and rose sharply to 71% on GD 25. If litters with 100% fetal death were excluded from GD 25 so that only litters containing at least one living fetus were considered, the mortality rate dropped to 57.5%. The number of live fetuses ranged from 1 to 10, in these litters. The mortality rate of the newborn rats is given in Table 4, but because deliveries were not observed, no information can be given in respect to the number of still-births, the percentage of pups that had died in the first few hours of the neonatal period, or the exact number of pups per litter since the mother will often ingest its dead puppies.

2) Fetal and Newborn Rat Measurements

While harvesting the fetuses and during their dissection a number of observations were made regarding their physical appearance. Fetuses obtained at GD 21 and GD 22 displayed no sign of passing meconium, while fetuses at GD 23 had passed meconium into the amniotic fluid but the fetal skin was not stained. By GD 24 and GD 25 the fetuses were meconium stained and after drying, the skin had a cracked, wrinkled appearance. Fetal amniotic fluid appeared to be greatly reduced on GD 24 and GD 25 as compared to term. These observations of the physical appearance of the fetus post-term are in general agreement with other

TABLE 1 MEASUREMENTS IN PREGNANT RATS AT GESTATION DAY 21

	Non-injected	Progesterone 20	Sesame 14
No. of animals	17	17	13
Body wt at gestation day 0, g	202.8 ± 2.00 (15)	205.1 ± 2.69 (11)	198.2 ± 1.93* (13)
(Body wt (gestation day 21)/Body wt (gestation day 0)) X 100	170.7 ± 2.28 (15)	164.5 ± 2.90 (10)	165.5 ± 3.43 (11)
No. of fetuses/rat	12.2 ± 0.25 (16)	11.5 ± 0.40 (14)	11.9 ± 0.46 (11)
Dead fetuses (% of total)	0 (195)	0 (161)	0 (131)

Progesterone 20 = rats injected with progesterone on gestation day 20. Sesame 14 = rats injected with sesame oil on gestation day 14 and daily thereafter until day 20. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of animals studied.

* Different from progesterone 20 ($P < 0.05$)

TABLE 2 MEASUREMENTS IN PREGNANT RATS AT GESTATION DAY 22

	Non-injected	Progesterone 20	Progesterone 14
No. of animals	13	21	13
Body wt at gestation day 0, g	201.7 ± 2.51 (11)	208.3 ± 2.61 (17)	204.8 ± 2.48 (13)
(Body wt (gestation day 22)/Body wt (gestation day 0)) X 100	175.3 ± 2.96 (10)	172.8 ± 3.44 (15)	169.2 ± 1.97 (13)
No. of fetuses/rat	11.9 ± 0.50 (12)	12.7 ± 0.30 (15)	11.3 ± 0.40* (10)
Dead fetuses (% of total)	0 (143)	0.5 (190)	0 (113)

Progesterone 20 = rats injected with progesterone on gestation day 20 and 21. Progesterone 14 = rats injected with progesterone on gestation day 14 and daily thereafter until day 21. Data are expressed as means ±1 SE; numbers in parentheses indicate number of animals studied.
* Different from progesterone 20 ($P < 0.05$)

TABLE 3 MEASUREMENTS IN PREGNANT RATS AT DIFFERENT DAYS OF GESTATION

	21	22	23	24	25
No. of animals	17	21	20	22	36
Body wt at gestation day 0, g	205.1 ± 2.69 (11)	208.3 ± 2.61 (17)	207.2 ± 2.16 (18)	207.7 ± 1.71 (20)	204.9 ± 1.82 (32)
Body wt/Body wt (gestation day 0) X 100	164.9 ± 0.98 (79)	170.8 ± 1.17* (72)	173.9 ± 1.34* (57)	172.0 ± 1.37* (43)	168.3 ± 2.14 (22)
No. of fetuses/rat	11.5 ± 0.40 (14)	12.7 ± 0.30 (15)	11.3 ± 0.43† (16)	12.2 ± 0.28 (19)	11.7 ± 0.23 (34)
Dead fetuses (% of total)	0 (161)	0.5 (190)	0.6 (180)	21.7 (231)	70.9 (399)

All rats injected with progesterone on gestation day 20, and daily thereafter until the day before sacrifice. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of animals studied. Body wt/Body wt (gestation day 0) X 100, represents the per cent increase in maternal body weight during gestation and includes the body weight of rats that were to be sacrificed at a later gestation day.

* Different from day 21 ($P < 0.001$)
† Different from day 22 ($P < 0.05$)

TABLE 4 MEASUREMENTS IN PREGNANT RATS ALLOWED TO DELIVER AND NURSE LITTERS FOR DIFFERENT PERIODS OF TIME

	24 hours	48 hours	72 hours
No. of animals	14	10	11
Body wt. at gestation day 0, g	202.4 ± 3.01 (10)	204.7 ± 2.60 (10)	205.9 ± 2.23 (11)
No. of pups/rat	11.9 ± 0.48 (10)	11.5 ± 0.50 (10)	11.3 ± 0.51 (11)
Dead pups* (% of total)	0.8 (119)	6.1 (115)	0 (124)

Data are expressed as means ±1 SE; numbers in parentheses indicate number of animals studied.

* Dead pups were observed 4 - 6 hours after birth

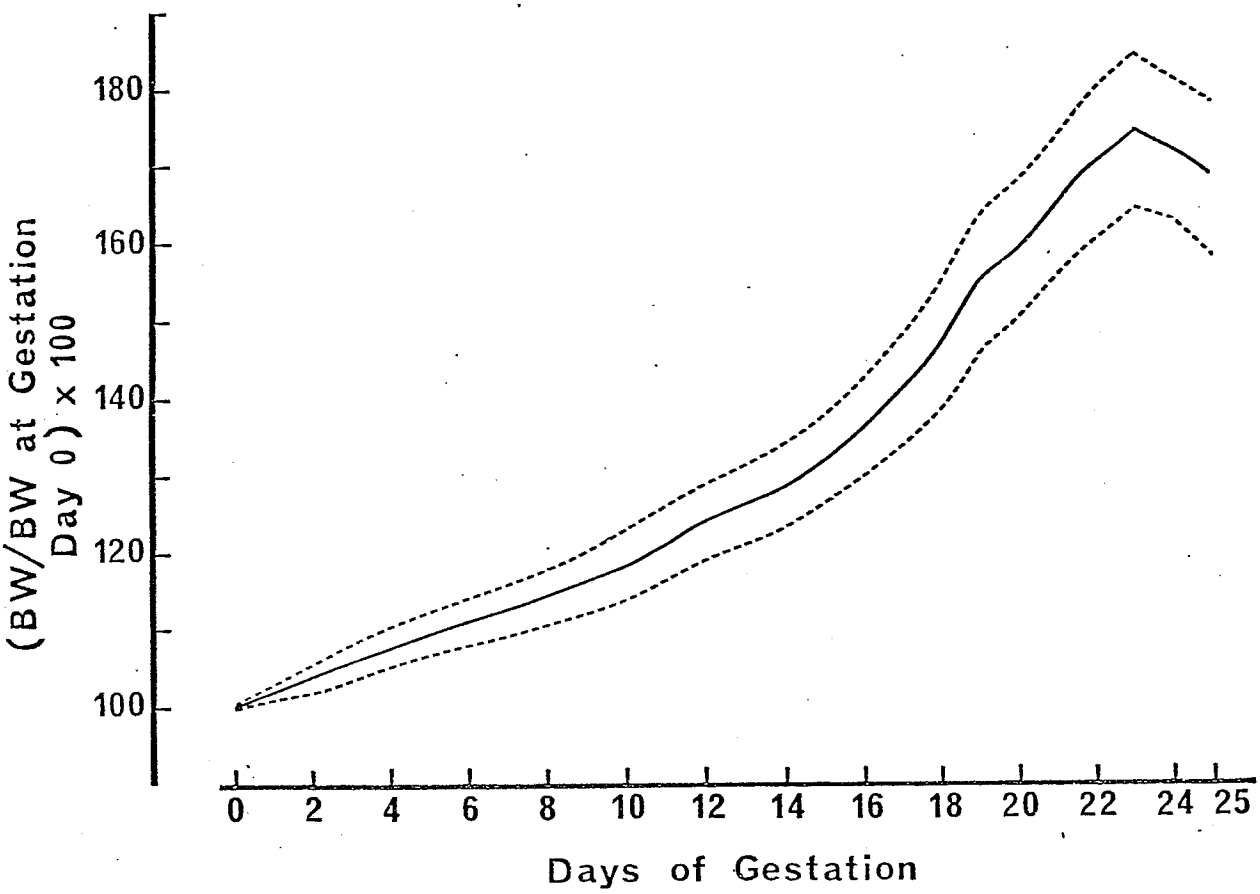

Figure 1. Maternal body weight as a percentage of weight at gestation day 0. Broken line represents one standard deviation to either side of the mean.

studies (3,73,76,79). In addition, because of the excessive size of the fetuses on GD 24 and GD 25, fetuses were often seen overlapping in their in utero position and when removed from the uterus they possessed a "humped" back. During the process of decapitation and dissection there appeared to be a marked increase of bone hardness in post-term fetuses.

Fetal body, lung and placental weights obtained from NI, P_{20}, P_{14} and S_{14} pregnant rats were similar on GD 21 and on GD 22 (Tables 5 and 6) with the exception of a lower P_{14} placental weight on GD 22 (Table 6). Fetal body weight increased progressively from GD 21 to GD 25 despite a decreasing placental weight during prolonged gestation (Table 7). Both lung wet and dry weights decreased post-term and resulted in a smaller lung in proportion to body weight. Fetal lungs at GD 25 were visibly smaller then those at term. As gestation was prolonged fewer distended terminal sacs were seen, as compared to GD 21 and GD 22 (Figure 2). Kidney and spleen weight increased significantly during prolonged gestation despite lower liver (Table 8) and lung weights (Table 7). The lower liver weight can be attributed to both loss of water and dry weight, but was largely due to loss of dry weight since the liver dry/wet weight ratio decreased as pregnancy was prolonged.

Table 9 shows the body and organ weights of neonatal rats at 24, 48 and 72 hour periods. Although the body weight of a GD 23 fetus was similar to a 24 hour neonate, at GD 24 and 25, fetal body weight was less then the weight of 48 and 72 hour neonates, respectively. Lung and liver weights were lower at GD 24 and 25 in comparison to 48 and 72 hour periods, while kidney and spleen weights of post-term fetuses either equaled or surpassed the kidney and spleen weights of neonates

TABLE 5 MEASUREMENTS IN FETAL RATS AT GESTATION DAY 21

	Non-injected	Progesterone 20	Sesame 14
Body wet wt, g	4.07 ± 0.03 (120)	4.01 ± 0.03 (101)	4.03 ± 0.04 (79)
Lung wet wt, mg	118.94 ± 1.39 (111)	119.20 ± 1.78 (97)	117.90 ± 1.41 (75)
Lung dry wt, mg	15.63 ± 0.43 (28)	16.58 ± 0.42 (22)	15.93 ± 0.22 (27)
(Lung dry/wet wt) X 100	13.60 ± 0.15 (28)	13.58 ± 0.18 (22)	13.06 ± 0.13*† (27)
Placenta wet wt, mg	466.98 ± 7.79 (58)	467.40 ± 9.08 (46)	461.44 ± 10.24 (45)
Placenta dry wt, mg	72.52 ± 1.27 (58)	72.37 ± 1.45 (46)	72.83 ± 1.76 (45)
(Placenta dry/wet wt)X 100	15.51 ± 0.09 (58)	15.50 ± 0.10 (46)	15.75 ± 0.08 (45)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20. Sesame 14 = fetuses obtained from pregnant rats injected with sesame oil on gestation day 14 and daily thereafter until day 20. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

* Different from non-injected ($P < 0.01$)

† Different from progesterone 20 ($P < 0.05$)

TABLE 6 – MEASUREMENTS IN FETAL RATS AT GESTATION DAY 22

	Non-injected	Progesterone 20	Progesterone 14
Body wet wt, g	5.49 ± 0.05 (85)	5.55 ± 0.04 (118)	5.58 ± 0.05 (61)
Lung wet wt, mg	135.50 ± 1.73 (76)	133.47 ± 1.64Φ (116)	138.68 ± 1.76 (61)
Lung dry wt, mg	16.85 ± 0.50 (26)	18.69 ± 0.48† (33)	17.44 ± 0.46 (20)
(Lung dry/wet wt)X 100	12.36 ± 0.29 (26)	13.70 ± 0.30* (33)	12.76 ± 0.14 (20)
Placenta wet wt, mg	504.90 ± 11.59 (52)	478.03 ± 8.62 (56)	453.77 ± 11.56* (40)
Placenta dry wt, mg	79.18 ± 1.79 (52)	76.74 ± 1.28Φ (47)	71.52 ± 1.84* (40)
(Placenta dry/wet wt)X 100	15.70 ± 0.06 (52)	15.64 ± 0.10 (47)	15.76 ± 0.07 (40)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and 21. Progesterone 14 = fetuses obtained from pregnant rats injected with progesterone on gestation day 14 and daily thereafter until day 21. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

* Different from non-injected ($P < 0.01$)
† Different from non-injected ($P < 0.05$)
Φ Different from progesterone 14 ($P < 0.05$)

TABLE 7 MEASUREMENTS IN FETAL RATS AT DIFFERENT DAYS OF GESTATION

	21	22	23	24	25
Body wet wt, g	4.01 ± 0.03* (101)	5.55 ± 0.04 (118)	6.52 ± 0.05* (125)	6.75 ± 0.10* (117)	7.01 ± 0.07* (82)
(Body dry/wet wt) X 100	13.66 ± 0.10 (19)	13.76 ± 0.05 (24)	14.53 ± 0.12* (25)	15.14 ± 0.14* (29)	14.62 ± 0.16* (21)
Lung wet wt, mg	119.20 ± 1.78* (97)	133.47 ± 1.64 (116)	126.02 ± 1.81Φ (110)	93.25 ± 1.63* (100)	84.27 ± 1.33* (83)
Lung dry wt, mg	16.58 ± 0.42* (22)	18.69 ± 0.48 (33)	18.23 ± 0.57 (30)	14.32 ± 0.33* (35)	12.94 ± 0.47* (18)
(Lung dry/wet wt) X 100	13.58 ± 0.18 (22)	13.70 ± 0.30 (33)	14.39 ± 0.23 (30)	15.51 ± 0.22* (35)	15.09 ± 0.61† (18)
Placenta wet wt, mg	467.40 ± 9.08 (46)	478.03 ± 8.62 (56)	464.70 ± 9.54 (43)	424.54 ± 10.05* (42)	425.56 ± 11.50* (55)
Placenta dry wt, mg	72.37 ± 1.45† (46)	76.74 ± 1.28 (47)	73.10 ± 1.45 (43)	60.56 ± 1.39* (40)	64.54 ± 1.66* (55)
(Placenta dry/wet wt) X 100	15.50 ± 0.10 (46)	15.64 ± 0.10 (47)	15.75 ± 0.07 (43)	14.40 ± 0.08* (40)	15.21 ± 0.10Φ (55)

Fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and daily thereafter until the day before sacrifice. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

* Different from day 22 ($P < 0.001$)
Φ Different from day 22 ($P < 0.01$)
† Different from day 22 ($P < 0.05$)

Figure 2: Micrographs of fetal lung at different days of gestation:

a) GD 21, NI
b) GD 21, P_{20}
c) GD 22, NI
d) GD 22, P_{14}
e) GD 22, P_{20}
f) GD 23, P_{20}
g) GD 24, P_{20}
h) GD 25, P_{20}.

X 210.

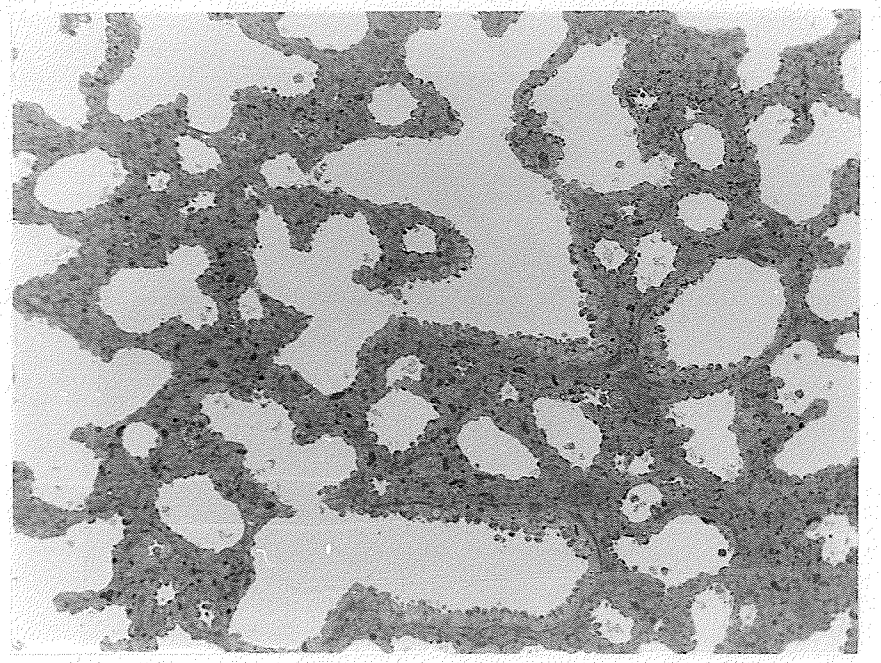

Figure 2a.

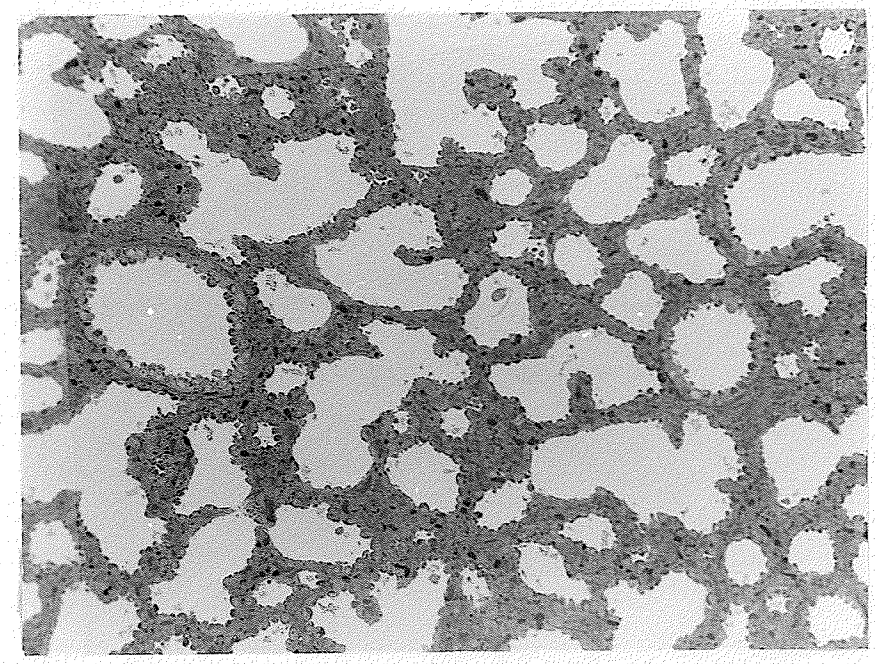

Figure 2b.

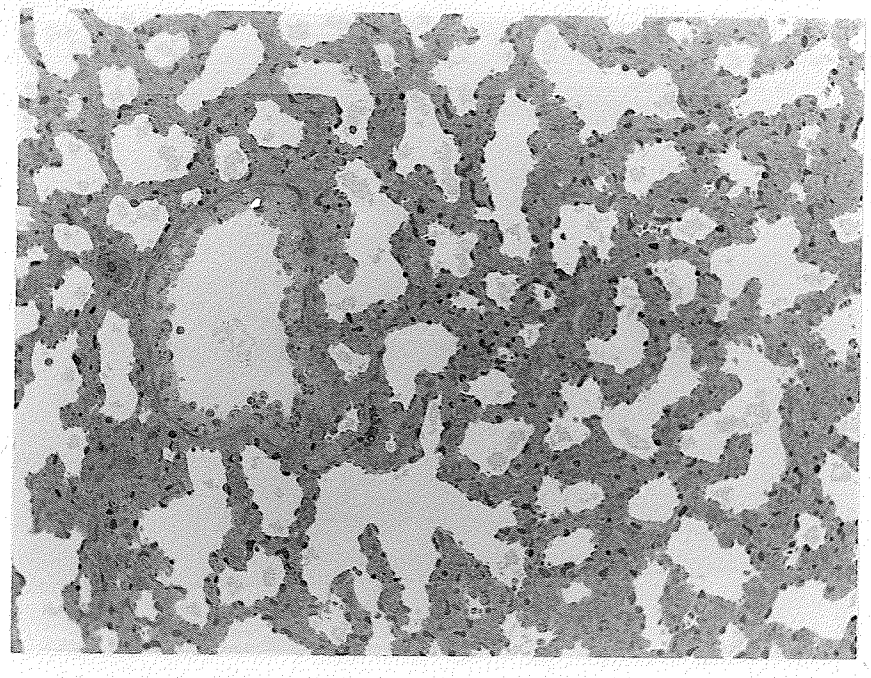

Figure 2c.

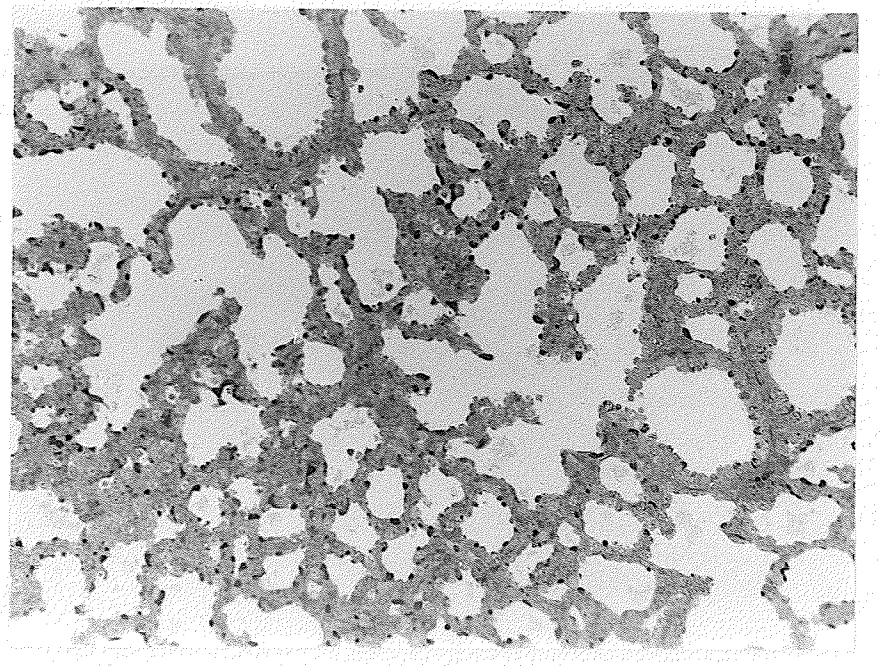

Figure 2d.

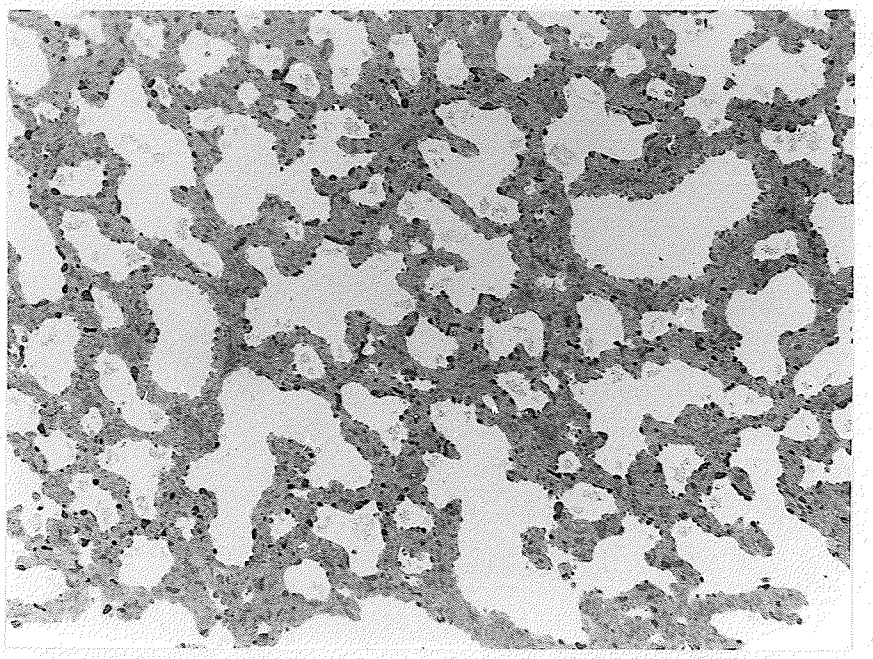

Figure 2e.

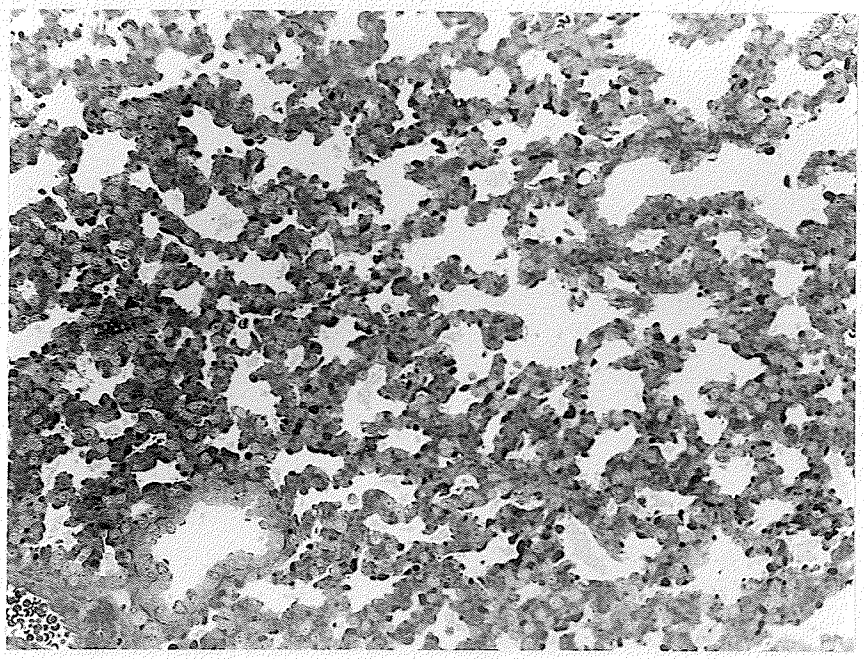

Figure 2f.

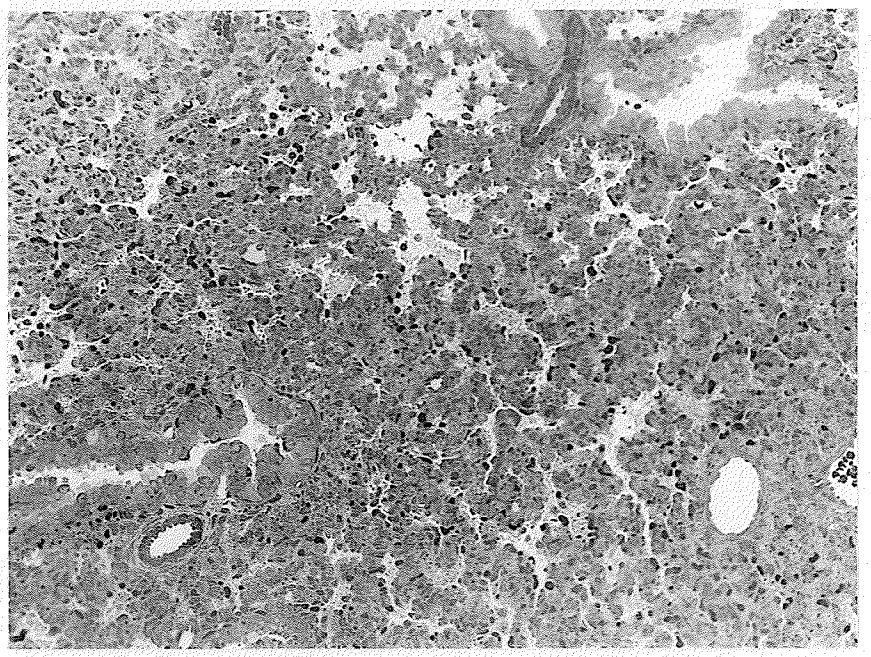

Figure 2g.

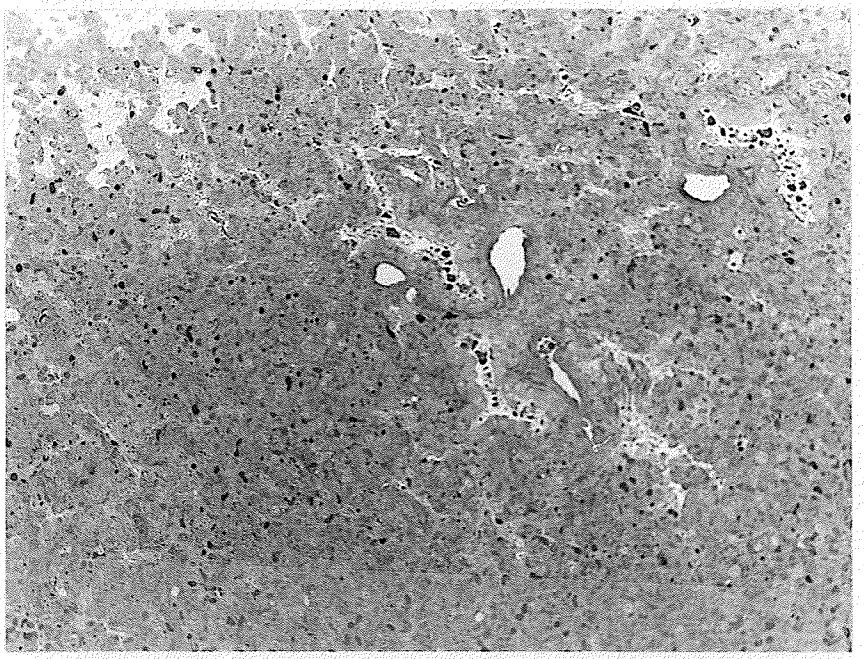

Figure 2h.

TABLE 8 ORGAN MEASUREMENTS IN FETAL RATS AT DIFFERENT DAYS OF GESTATION

	21	22	23	24	25
Liver wet wt, mg	310.82 ± 4.48* (27)	362.98 ± 4.16 (43)	338.73 ± 5.98† (25)	275.21 ± 7.95* (22)	271.38 ± 6.52* (24)
Liver dry wt, mg	71.34 ± 1.09* (27)	88.61 ± 1.03 (43)	73.98 ± 1.59* (25)	55.22 ± 1.65* (22)	57.15 ± 1.40* (24)
(Liver dry/wet wt) X 100	22.95 ± 0.12 (27)	24.42 ± 0.08 (43)	21.83 ± 0.24Φ (25)	20.07 ± 0.19* (22)	21.09 ± 0.25† (24)
Kidney wet wt, mg	26.62 ± 0.64* (26)	43.36 ± 0.72 (45)	59.52 ± 2.12* (24)	74.73 ± 2.22* (22)	85.07 ± 1.96* (24)
Spleen wet wt, mg	4.39 ± 0.31* (26)	10.39 ± 0.25 (45)	18.98 ± 1.18* (25)	31.93 ± 1.41* (22)	42.29 ± 1.65* (24)

Fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and daily thereafter until the day before sacrifice for days 23, 24 and 25. Days 21 and 22 had organ weights pooled from non-injected and progesterone-injected pregnant rats injected from gestation day 20 since fetal organ weights were not significantly different between injected and non-injected groups. Data are expressed as means ±1 SE; numbers in parentheses indicate number of fetal rats studied.

* Different from day 22 ($P < 0.001$)
† Different from day 22 ($P < 0.01$)
Φ Different from day 22 ($P < 0.05$)

TABLE 9 MEASUREMENTS OF NEWBORN RATS AT DIFFERENT POSTNATAL PERIODS

	24 hours	48 hours	72 hours
Body wet wt, g	6.64 ± 0.05 (105)	7.38 ± 0.06† (82)	8.03 ± 0.10§ (83)
Lung wet wt, mg	100.17 ± 1.14* (88)	113.52 ± 1.41† (68)	135.63 ± 2.16§ (73)
Lung dry wt, mg	18.67 ± 0.11 (24)	20.30 ± 0.34† (20)	25.57 ± 0.58§ (23)
(Lung dry/wet wt) X 100	17.70 ± 0.15* (24)	17.31 ± 0.15† (20)	17.75 ± 0.14§ (23)
Liver wet wt, mg	322.94 ± 10.60 (28)	296.88 ± 4.58o (27)	303.76 ± 8.40Φ (26)
Liver dry wt, mg	78.86 ± 2.82 (28)	73.64 ± 1.06† (27)	74.40 ± 2.35§ (26)
Liver dry/wet wt X 100	24.37 ± 0.21* (28)	24.84 ± 0.24† (27)	24.45 ± 0.15§ (26)
Kidney wet wt, mg	58.13 ± 1.45 (25)	70.68 ± 1.47 (26)	85.62 ± 2.46 (25)
Spleen wet wt, mg	14.05 ± 0.73* (28)	22.36 ± 0.89† (27)	25.13 ± 0.91§ (25)

Data are expressed as means ±1 SE; numbers in parentheses indicate number of newborn rats studied.

* Different from gestation day 23 ($P < 0.001$)
† Different from gestation day 24 ($P < 0.001$)
o Different from gestation day 24 ($P < 0.05$)
Φ Different from gestation day 25 ($P < 0.01$)
§ Different from gestation day 25 ($P < 0.001$)

at a comparable postnatal age.

Protein, DNA, RNA and phospholipid (lecithin and DSPC) analysis of fetal lungs obtained from NI, P_{20}, P_{14} and S_{14} pregnant rats at GD 21 and GD 22 are shown in Tables 10 and 11, respectively. The parameters analized are similar on GD 21 and on GD 22 among the groups studied. Table 12a shows the results obtained from P_{20} fetal lungs at GD 21 to GD 25. As gestation was prolonged from GD 22 to GD 25, total lung protein, DNA, lipid and phospholipid increased but RNA decreased when expressed per gram lung. When expressed per mg lung DNA, lung phospholipids and total protein of post-term fetuses were similar to GD 22, with only lung RNA and total lung lipid being lower than at term. In Table 12b the total lung protein, lung phospholipid (lecithin and DSPC), lung DNA, and the number of cells are expressed per lung. Since lung total protein, phospholipid and DNA contents were measured on 1 gram samples, protein, phospholipid and DNA per lung were based on the average lung weights in that particular sample. The number of cells per lung was calculated using the following formula: number of nuclei (in millions) = (total lung DNA (mg) X 10^3)/6.2 pg, where 6.2 is the amount of DNA in picograms in a single diploid rat nucleus (120). The constancy of the amount of DNA per nucleus has been established in the rat (147). At GD 23 lecithin, DSPC and DNA (cell number) appeared to reach a maximum level, but subsequently declined as pregnancy was prolonged to 25 days (Figure 3). The fetal lung at GD 25 may be considered hypocellular in comparison to GD 23 since 40 million lung cells were lost by GD 25.

Comparison of the postnatal and post-term lung showed that total protein, DNA and phospholipids were similar when they were expressed per gram lung or per mg lung DNA (Tables 12 and 13). Only when phospholipids and DNA were expressed per lung could differences be seen in

TABLE 10a BIOCHEMICAL MEASUREMENTS IN FETAL RATS AT GESTATION DAY 21

	Non-injected	Progesterone 20	Sesame 14
Lung total protein, mg/g lung	66.23 ± 0.18 (7)	65.63 ± 0.75 (6)	64.76 ± 1.26 (5)
Lung DNA, mg/g lung	7.62 ± 0.09 (10)	7.91 ± 0.18 (8)	7.25 ± 0.14* (5)
Lung RNA, mg/g lung	5.10 ± 0.07 (6)	5.11 ± 0.17 (5)	4.46 ± 0.22†Φ (5)
Lung total lipid, mg/g lung	22.20 ± 0.35 (10)	22.90 ± 0.43 (8)	22.50 ± 0.32 (5)
Lung phospholipid, mg/g lung			
Total	17.57 ± 0.40 (10)	17.30 ± 0.49 (8)	18.0 ± 0.24 (5)
Sphingomyelin-lyso-lecithin	1.95 ± 0.11 (7)	1.91 ± 0.14 (6)	1.72 ± 0.11 (5)
Phosphatidyl ethanolamine	3.93 ± 0.14 (7)	4.04 ± 0.19 (6)	3.54 ± 0.27 (5)
Phosphatidyl choline	7.65 ± 0.20 (10)	8.53 ± 0.35 (8)	7.62 ± 0.58 (5)
Disaturated phosphatidyl choline	3.72 ± 0.11 (5)	4.09 ± 0.39 (5)	3.33 ± 0.27 (5)
Lung phospholipid, mg/mg lung DNA			
Total	2.31 ± 0.05 (10)	2.19 ± 0.07 (8)	2.48 ± 0.04* (5)
Phosphatidyl choline	1.00 ± 0.02 (10)	1.08 ± 0.03 (8)	1.05 ± 0.07 (5)
Disaturated phosphatidyl choline	0.498 ± 0.01 (5)	0.524 ± 0.03 (5)	0.458 ± 0.03 (5)
(DSPC/phosphatidyl choline) X 100	50.62 ± 0.75 (5)	49.25 ± 1.94 (5)	44.01 ± 2.90 (5)
Lung total protein, mg/mg DNA	8.51 ± 0.11 (6)	8.14 ± 0.23 (6)	8.93 ± 0.27 (5)
Lung RNA, mg/mg DNA	0.65 ± 0.01 (6)	0.62 ± 0.03 (5)	0.62 ± 0.02 (5)
Lung total lipid, mg/mg DNA	2.92 ± 0.04 (10)	2.91 ± 0.07 (8)	3.10 ± 0.04 (5)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20. Sesame 14 = fetuses obtained from pregnant rats injected with sesame oil on gestation day 14 and daily thereafter until day 20. Data are expressed as means ±1 SE; numbers in parentheses indicate number of fetal rats studied.
* Different from progesterone 20 ($P < 0.01$). † Different from progesterone 20 ($P < 0.05$).
Φ Different from non-injected ($P < 0.05$).

TABLE 10b BIOCHEMICAL MEASUREMENTS OF FETAL RAT LUNGS AT GESTATION DAY 21

	Non-injected	Progesterone 20	Sesame 14
Total phospholipid per lung, mg	2.13 ± 0.05 (9)	2.07 ± 0.11 (8)	2.08 ± 0.03 (5)
Phosphatidyl choline per lung, mg	0.924 ± 0.04 (9)	1.02 ± 0.07 (8)	0.884 ± 0.07 (5)
DSPC per lung, mg	0.448 ± 0.03 (5)	0.492 ± 0.06 (5)	0.386 ± 0.03 (5)
Total DNA per lung mg	0.916 ± 0.03 (9)	0.948 ± 0.05 (8)	0.838 ± 0.02 (5)
Cell no., in millions per lung	147.84 ± 4.76 (9)	153.03 ± 8.52 (8)	135.16 ± 2.76 (5)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20. Sesame 14 = fetuses obtained from pregnant rats injected with sesame oil on gestation day 14 and daily thereafter until day 20. Data are expressed as means ±1 SE; numbers in parentheses indicate number of fetal rats studied.

TABLE 11a BIOCHEMICAL MEASUREMENTS IN FETAL RATS AT GESTATION DAY 22

	Non-injected	Progesterone 20	Progesterone 14
Lung total protein, mg/g lung	72.07 ± 1.05* (6)	68.11 ± 1.50 (7)	73.12 ± 0.46* (5)
Lung DNA, mg/g lung	8.22 ± 0.07 (6)	8.47 ± 0.11 (11)	7.99 ± 0.01† (5)
Lung RNA, mg/g lung	4.65 ± 0.10Φ (6)	4.71 ± 0.03Φ (7)	5.03 ± 0.05 (5)
Lung total lipid, mg/g lung	25.92 ± 0.15 (6)	26.14 ± 0.39 (11)	26.00 ± 0.16 (5)
Lung phospholipid, mg/g lung Total	19.12 ± 0.37 (6)	19.63 ± 0.36 (11)	19.34 ± 0.28 (5)
Sphingomyelin-lyso-lecithin	2.02 ± 0.06† (6)	2.25 ± 0.04 (7)	2.07 ± 0.07* (5)
Phosphatidyl ethanolamine	4.28 ± 0.15 (6)	4.42 ± 0.12 (7)	4.10 ± 0.08 (5)
Phosphatidyl choline	9.56 ± 0.12 (6)	9.65 ± 0.17 (11)	9.18 ± 0.22 (5)
Disaturated phosphatidyl choline	4.51 ± 0.07* (6)	4.89 ± 0.16 (5)	4.37 ± 0.06† (4)
Lung phospholipid, mg/mg lung DNA Total	2.33 ± 0.05 (6)	2.32 ± 0.04 (11)	2.42 ± 0.03 (5)
Phosphatidyl choline	1.16 ± 0.02 (6)	1.14 ± 0.02 (11)	1.15 ± 0.03 (5)
Disaturated phosphatidyl choline	0.548 ± 0.01 (6)	0.576 ± 0.01 (5)	0.550 ± 0.01 (4)
(DSPC/phosphatidyl choline) X 100	47.24 ± 0.93† (6)	52.85 ± 0.90 (5)	47.76 ± 1.37† (4)
Lung total protein, mg/mg DNA	8.78 ± 0.18† (6)	8.02 ± 0.13 (7)	9.15 ± 0.06† (5)
Lung RNA, mg/mg DNA	0.57 ± 0.02Φ (6)	0.56 ± 0.01Φ (7)	0.63 ± 0.01 (5)
Lung total lipid, mg/mg DNA	3.16 ± 0.04 (6)	3.09 ± 0.05 (11)	3.25 ± 0.02 (5)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and 21. Progesterone 14 = fetuses obtained from pregnant rats injected with progesterone on gestation day 14 and daily thereafter until day 21. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

† Different from progesterone 20 ($P < 0.01$). * Different from progesterone 20 ($P < 0.05$).
Φ Different from progesterone 14 ($P < 0.01$).

TABLE 11b BIOCHEMICAL MEASUREMENTS OF FETAL RAT LUNGS AT GESTATION DAY 22

	Non-injected	Progesterone 20	Progesterone 14
Total phospholipid per lung, mg	2.58 ± 0.11 (6)	2.55 ± 0.09 (11)	2.70 ± 0.08 (5)
Phosphatidyl choline per lung, mg	1.29 ± 0.04 (6)	1.25 ± 0.04 (11)	1.29 ± 0.05 (5)
DSPC per lung, mg	0.607 ± 0.01 (6)	0.674 ± 0.04 (5)	0.605 ± 0.02 (4)
Total DNA per lung mg	1.11 ± 0.03 (6)	1.12 ± 0.04 (11)	1.12 ± 0.02 (5)
Cell no., in millions per lung	178.77 ± 4.18 (6)	180.66 ± 7.10 (11)	180.64 ± 3.84 (5)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and 21. Progesterone 14 = fetuses obtained from pregnant rats injected with progesterone on gestation day 14 and daily thereafter until day 21. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

TABLE 12a BIOCHEMICAL MEASUREMENTS IN FETAL RATS AT GESTATION DAYS 21 TO 25

	21	22	23	24	25
Lung total protein, mg/g lung	65.63 ± 0.75 (6)	68.11 ± 1.50 (7)	82.98 ± 0.71* (8)	94.17 ± 0.94* (6)	96.26 ± 2.92* (6)
Lung DNA, mg/g lung	7.91 ± 0.18Φ (8)	8.47 ± 0.11 (11)	9.90 ± 0.14* (9)	11.52 ± 0.08* (6)	12.08 ± 0.24* (6)
Lung RNA, mg/g lung	5.11 ± 0.17Φ (5)	4.71 ± 0.03 (7)	4.55 ± 0.06Φ (8)	4.48 ± 0.02* (6)	4.11 ± 0.04* (2)
Lung total lipid, mg/g lung	22.90 ± 0.43Φ (8)	26.14 ± 0.39 (11)	30.11 ± 0.16* (9)	32.83 ± 0.32* (6)	34.86 ± 0.24* (5)
Lung phospholipid mg/g lung					
Total	17.30 ± 0.49† (8)	19.63 ± 0.36 (11)	22.18 ± 0.23* (9)	25.05 ± 0.29* (6)	27.42 ± 0.24* (5)
Sphingomyelin-lyso-lecithin	1.91 ± 0.14Φ (6)	2.25 ± 0.04 (7)	2.54 ± 0.06† (8)	3.46 ± 0.12* (6)	3.56 ± 0.09* (5)
Phosphatidyl ethanolamine	4.04 ± 0.19 (6)	4.42 ± 0.12 (7)	5.17 ± 0.07* (8)	6.51 ± 0.22* (6)	7.15 ± 0.07* (5)
Phosphatidyl choline	8.53 ± 0.35† (8)	9.65 ± 0.17 (11)	10.69 ± 0.19* (9)	12.23 ± 0.07* (6)	13.89 ± 0.13* (5)
Disaturated phosphatidyl choline	4.09 ± 0.39 (5)	4.89 ± 0.16 (5)	5.49 ± 0.05† (5)	6.24 ± 0.05* (6)	6.66 ± 0.38† (5)
Lung phospholipid, mg/mg lung DNA					
Total	2.19 ± 0.07 (8)	2.32 ± 0.04 (11)	2.24 ± 0.02 (9)	2.19 ± 0.03 (6)	2.25 ± 0.05 (5)
Phosphatidyl choline	1.08 ± 0.03 (8)	1.14 ± 0.02 (11)	1.08 ± 0.03 (9)	1.06 ± 0.01 (6)	1.17 ± 0.02 (5)
Disaturated phosphatidyl choline	0.524 ± 0.03 (5)	0.576 ± 0.01 (5)	0.562 ± 0.01 (5)	0.542 ± 0.01 (6)	0.558 ± 0.03 (5)
(DSPC/phosphatidyl choline) X 100	49.25 ± 1.94 (5)	52.85 ± 0.90 (5)	53.40 ± 1.15 (5)	51.0 ± 0.45 (6)	47.97 ± 2.74 (5)
Lung total protein, mg/mg DNA	8.14 ± 0.23 (6)	8.02 ± 0.13 (7)	8.28 ± 0.06 (8)	8.18 ± 0.12 (6)	7.97 ± 0.17 (6)
Lung RNA, mg/mg DNA	0.62 ± 0.03Φ (5)	0.56 ± 0.01 (7)	0.46 ± 0.32* (8)	0.39 ± 0.01* (6)	0.32 ± 0.01* (2)
Lung toral lipid, mg/mg DNA	2.91 ± 0.07 (8)	3.09 ± 0.05 (11)	3.05 ± 0.04 (9)	2.85 ± 0.04† (6)	2.93 ± 0.07 (5)

Fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and daily thereafter until the day before sacrifice. Data are expressed as means ±1 SE; numbers in parentheses indicate number of fetal rats studied.

† Different from day 22 ($P < 0.01$). * Different from day 22 ($P < 0.001$).
Φ Different from day 22 ($P < 0.05$).

TABLE 12b BIOCHEMICAL MEASUREMENTS OF FETAL RAT LUNGS AT GESTATION DAYS 21 TO 25

	21	22	23	24	25
Total protein per lung, mg	8.01 ± 0.43 (6)	8.69 ± 0.50 (7)	10.56 ± 0.46Φ (8)	8.85 ± 0.31 (6)	8.00 ± 0.14 (6)
Total phospho-lipid per lung, mg	2.07 ± 0.11† (8)	2.55 ± 0.09 (11)	2.80 ± 0.09 (9)	2.35 ± 0.10 (6)	2.34 ± 0.08 (5)
Phosphatidyl choline per lung, mg	1.02 ± 0.07† (8)	1.25 ± 0.04 (11)	1.35 ± 0.05 (9)	1.15 ± 0.05 (6)	1.18 ± 0.04 (5)
DSPC per lung, mg	0.492 ± 0.06Φ (5)	0.674 ± 0.04 (5)	0.724 ± 0.03 (5)	0.588 ± 0.03 (6)	0.566 ± 0.02 (5)
Total DNA per lung, mg	0.948 ± 0.05Φ (8)	1.12 ± 0.04 (11)	1.25 ± 0.05 (9)	1.09 ± 0.05 (6)	1.01 ± 0.03 (6)
Cell no., in millions per lung	153.03 ± 8.52Φ (8)	180.66 ± 7.10 (11)	202.14 ± 7.64 (9)	175.02 ± 7.96 (6)	162.63 ± 4.26 (6)

Fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and daily thereafter until the day before sacrifice. Data are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

† Different from day 22 ($P < 0.01$).
Φ Different from day 22 ($P < 0.05$).

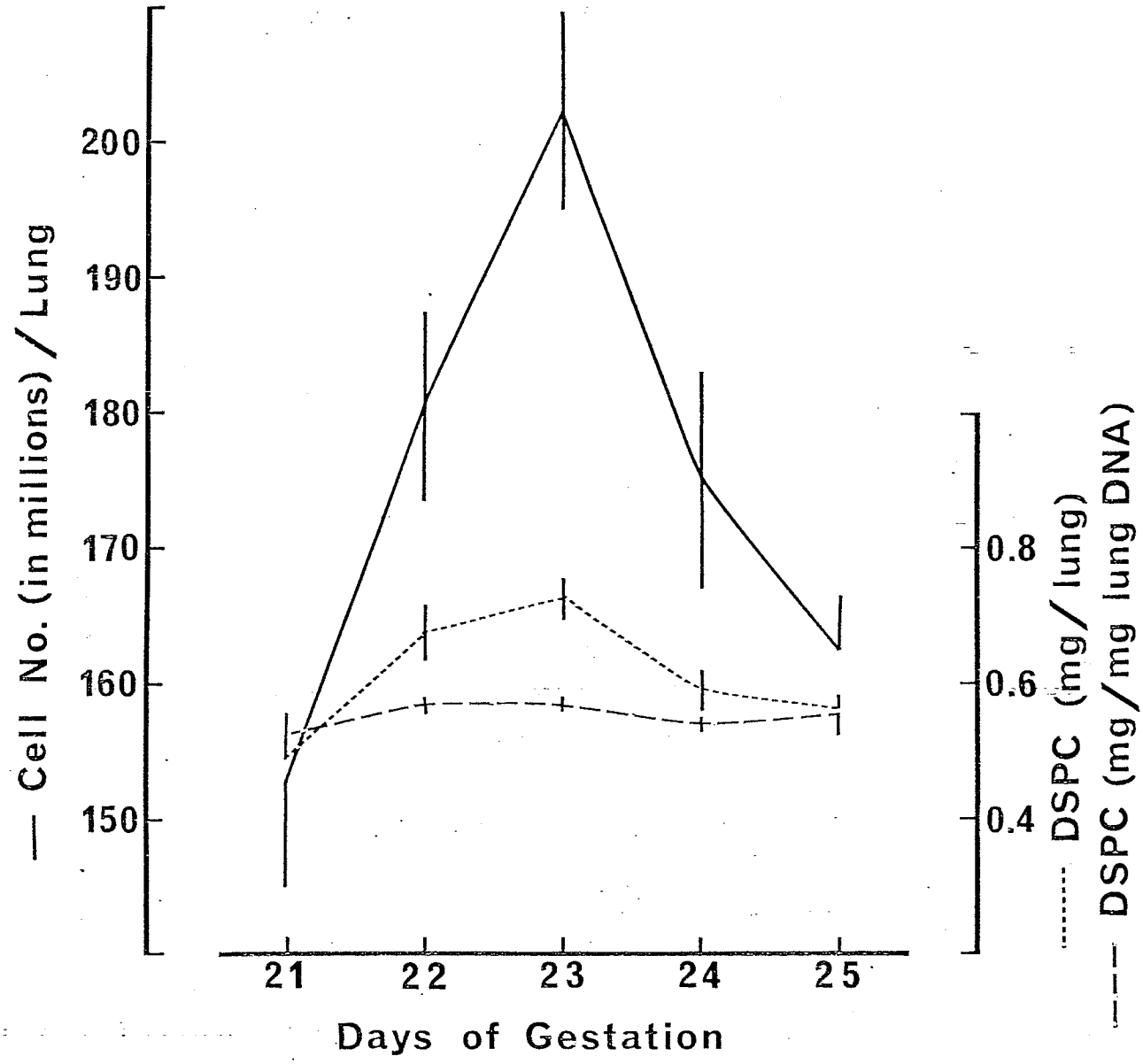

Figure 3. Comparison of cell number and DSPC content expressed per lung and DSPC content expressed per mg DNA at GD 21 through GD 25. Vertical lines represent ±1 SE. Cell number at GD 23 was significantly different from GD 21 and GD 25 ($P < 0.01$ to $P < 0.001$). DSPC per lung at GD 23 was significantly different from GD 21 and GD 25 ($P < 0.01$).

TABLE 13a BIOCHEMICAL MEASUREMENTS IN NEWBORN RATS AT DIFFERENT POSTNATAL PERIODS

	24 hours	48 hours	72 hours
Lung total protein, mg/g lung	82.80 ± 1.41 (5)	91.92 ± 1.84 (5)	97.04 ± 0.94 (5)
Lung DNA, mg/g lung	9.84 ± 0.34 (5)	10.81 ± 0.18§ (5)	11.99 ± 0.05 (5)
Lung total lipid, mg/g lung	33.86 ± 0.91Φ (5)	33.40 ± 0.19 (5)	37.10 ± 0.19* (5)
Lung phospholipid, mg/g lung			
Total	21.91 ± 0.20 (5)	24.36 ± 0.33 (5)	27.14 ± 0.15 (5)
Sphingomyelin-lyso-lecithin	2.69 ± 0.02 (5)	3.12 ± 0.06∇ (5)	3.63 ± 0.03 (5)
Phosphatidyl ethanolamine	5.32 ± 0.09 (5)	6.32 ± 0.11 (5)	7.12 ± 0.08 (5)
Phosphatidyl choline	10.78 ± 0.22 (5)	12.20 ± 0.12 (5)	13.53 ± 0.16 (5)
Disaturated phosphatidyl choline	5.16 ± 0.20 (5)	5.76 ± 0.29 (4)	6.49 ± 0.21 (5)
Lung phospholipid, mg/mg lung DNA			
Total	2.24 ± 0.07 (5)	2.26 ± 0.06 (5)	2.27 ± 0.01 (5)
Phosphatidyl choline	1.10 ± 0.03 (5)	1.13 ± 0.02∇ (5)	1.13 ± 0.01 (5)
Disaturated phosphatidyl choline	0.528 ± 0.02 (5)	0.538 ± 0.03 (4)	0.540 ± 0.02 (5)
(DSPC/phosphatidyl choline)X 100	47.89 ± 1.34° (5)	47.14 ± 2.80 (4)	47.94 ± 1.38 (5)
Lung total protein, mg/mg DNA	8.44 ± 0.21 (5)	8.51 ± 0.18 (5)	8.10 ± 0.10 (5)
Lung total lipid, mg/mg DNA	3.45 ± 0.13† (5)	3.09 ± 0.05§ (5)	3.09 ± 0.02Δ (5)

Data are expressed as means ± 1 SE; numbers in parentheses indicate number of newborn rats studied.

° Different from gestation day 23 ($P < 0.05$). † Different from gestation day 23 ($P < 0.01$).
Φ Different from gestation day 23 ($P < 0.001$). ∇ Different from gestation day 24 ($P < 0.05$).
§ Different from gestation day 24 ($P < 0.01$). Δ Different from gestation day 25 ($P < 0.05$).
* Different from gestation day 25 ($P < 0.001$).

TABLE 13b BIOCHEMICAL MEASUREMENTS OF NEWBORN RAT LUNGS AT DIFFERENT POSTNATAL PERIODS

	24 hours	48 hours	72 hours
Total protein per lung, mg	8.23 ± 0.24† (5)	10.40 ± 0.62 (5)	12.76 ± 0.69* (5)
Total phospholipid per lung, mg	2.18 ± 0.09Φ (5)	2.75 ± 0.15∇ (5)	3.56 ± 0.17* (5)
Phosphatidyl choline per lung, mg	1.07 ± 0.05† (5)	1.38 ± 0.07∇ (5)	1.77 ± 0.07* (5)
DSPC per lung, mg	0.514 ± 0.02Φ (5)	0.643 ± 0.04 (4)	0.852 ± 0.04* (5)
Total DNA per lung, mg	0.980 ± 0.05† (5)	1.22 ± 0.07 (5)	1.56 ± 0.08* (5)
Cell no., in millions per lung	158.08 ± 8.21† (5)	197.12 ± 11.17 (5)	252.26 ± 13.02* (5)

Data are expressed as means ± 1 SE; numbers in parentheses indicate number of newborn rats studied.

o Different from gestation day 23 ($P < 0.05$).
† Different from gestation day 23 ($P < 0.01$).
Φ Different from gestation day 23 ($P < 0.001$).
∇ Different from gestation day 24 ($P < 0.05$).
Φ Different from gestation day 24 ($P < 0.01$).
* Different from gestation day 25 ($P < 0.001$).

the post-term and postnatal lungs. Lecithin, DSPC and DNA (cell number) were higher at GD 23 than at 24 hours. This difference disappears at GD 24 and 48 hours. At 72 hours the postnatal lung contained much more lecithin, DSPC and DNA than at GD 25. The fetal lung at GD 25 was not only hypocellular in comparison to GD 23 but also to 72 hours.

The glycogen content of fetal lung, liver, heart and muscle samples obtained from NI, P_{20}, P_{14} and S_{14} pregnant rats at GD 21 and GD 22 is shown in Table 14. The glycogen content of each tissue was similar on GD 21 and on GD 22 despite the experimental condition. The only exception was the P_{14} fetal lung which had a higher glycogen content in comparison to the other groups studied on GD 22.

Table 15 shows that liver glycogen is the lowest of all tissues studied on GD 19 but by GD 20 it exceeded the glycogen content of the other tissues. This accumulation of glycogen in the liver continued until term, in contrast to the lung which had over 50% reduction of its glycogen by GD 22 (Figures 4 and 5). Loss of liver glycogen was greatest between GD 22 and GD 23 while heart and muscle glycogen remained relatively constant from GD 19 to GD 23. By GD 25, the glycogen content of the tissues reach very low levels. The glycogen content of the lung, liver, heart and muscle during the postnatal period is shown in Table 16. At 24 hours these tissues contained less glycogen than at GD 23, but by 48 hours liver and lung glycogen was similar to GD 24 (Figure 6) while heart and muscle glycogen was still higher in the GD 24 fetus. At 72 hours however, the lung and liver contained more glycogen in comparison to GD 25.

TABLE 14 GLYCOGEN CONTENT OF VARIOUS FETAL ORGANS ON GESTATION DAYS 21 AND 22

Experimental condition	Lung	Liver	Heart	Muscle
Gestation day 21 non-injected	117.49 ± 7.26 (11)	351.98 ± 12.91 (11)	85.79 ± 7.08 (8)	100.53 ± 3.55 (11)
progesterone 20	96.48 ± 9.50 (8)	353.50 ± 18.93 (7)	93.65 ± 7.93 (7)	117.84 ± 7.20Φ (10)
sesame 14	103.45 ± 6.44 (8)	321.58 ± 24.58 (8)	97.09 ± 6.34 (6)	115.98 ± 5.85 (9)
Gestation day 22 non-injected	53.48 ± 1.97 (15)	354.41 ± 11.37 (14)	85.10 ± 5.61 (8)	107.60 ± 3.86 (13)
progesterone 20	46.70 ± 2.34 (13)	327.88 ± 17.37 (12)	74.14 ± 10.51 (7)	98.90 ± 10.71 (10)
progesterone 14	67.47 ± 4.79*† (11)	349.42 ± 9.32 (12)	97.90 ± 8.91 (6)	112.11 ± 6.67 (8)

Progesterone 20 = fetuses obtained from pregnant rats injected with progesterone on gestation day 20 (GD 21 progesterone 20) and day 21 (GD 22 progesterone 20). Sesame 14 = fetuses obtained from pregnant rats injected with sesame oil on gestation day 14 and daily thereafter until the day before sacrifice. Progesterone 14 = fetuses obtained from pregnant rats injected with progesterone on gestation day 14 and daily thereafter until the day before sacrifice. Data (in mg/g dry wt) are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

Φ Different from day 21 non-injected ($P < 0.05$).
† Different from day 22 non-injected ($P < 0.01$).
* Different from day 22 progesterone 20 ($P < 0.01$).

TABLE 15 GLYCOGEN CONTENT OF VARIOUS FETAL ORGANS AT DIFFERENT DAYS OF GESTATION

Gestation day	Lung	Liver	Heart	Muscle
19	133.77 ± 26.97* (6)	38.15 ± 4.83 (7)	103.60 ± 4.28 (3)	97.57 ± 18.87 (3)
20	134.17 ± 14.94* (5)	179.54 ± 26.11 (9)	93.21 ± 5.21 (3)	100.02 ± 9.79 (6)
21	96.48 ± 9.50* (8)	353.50 ± 18.93 (7)	93.65 ± 7.93 (7)	117.84 ± 7.20 (10)
22	46.70 ± 2.34 (13)	327.88 ± 17.37 (12)	74.14 ± 10.51 (7)	98.90 ± 10.71 (10)
23	35.94 ± 2.10† (12)	111.67 ± 6.27* (10)	80.94 ± 3.70 (7)	79.59 ± 2.63 (12)
24	19.84 ± 1.36* (12)	64.11 ± 10.02* (11)	55.95 ± 2.63 (8)	53.46 ± 3.06* (12)
25	11.60 ± 1.14* (10)	56.98 ± 13.11* (10)	32.89 ± 4.45† (7)	29.75 ± 3.66* (10)

Fetuses obtained from pregnant rats injected with progesterone on gestation day 20 and daily thereafter until the day before sacrifice for days 21 to 25. Days 19 and 20 are from non-injected pregnant rats. Data (mg/g dry wt) are expressed as means ± 1 SE; numbers in parentheses indicate number of fetal rats studied.

† Different from day 22 progesterone 20 ($P < 0.01$).
* Different from day 22 progesterone 20 ($P < 0.001$).

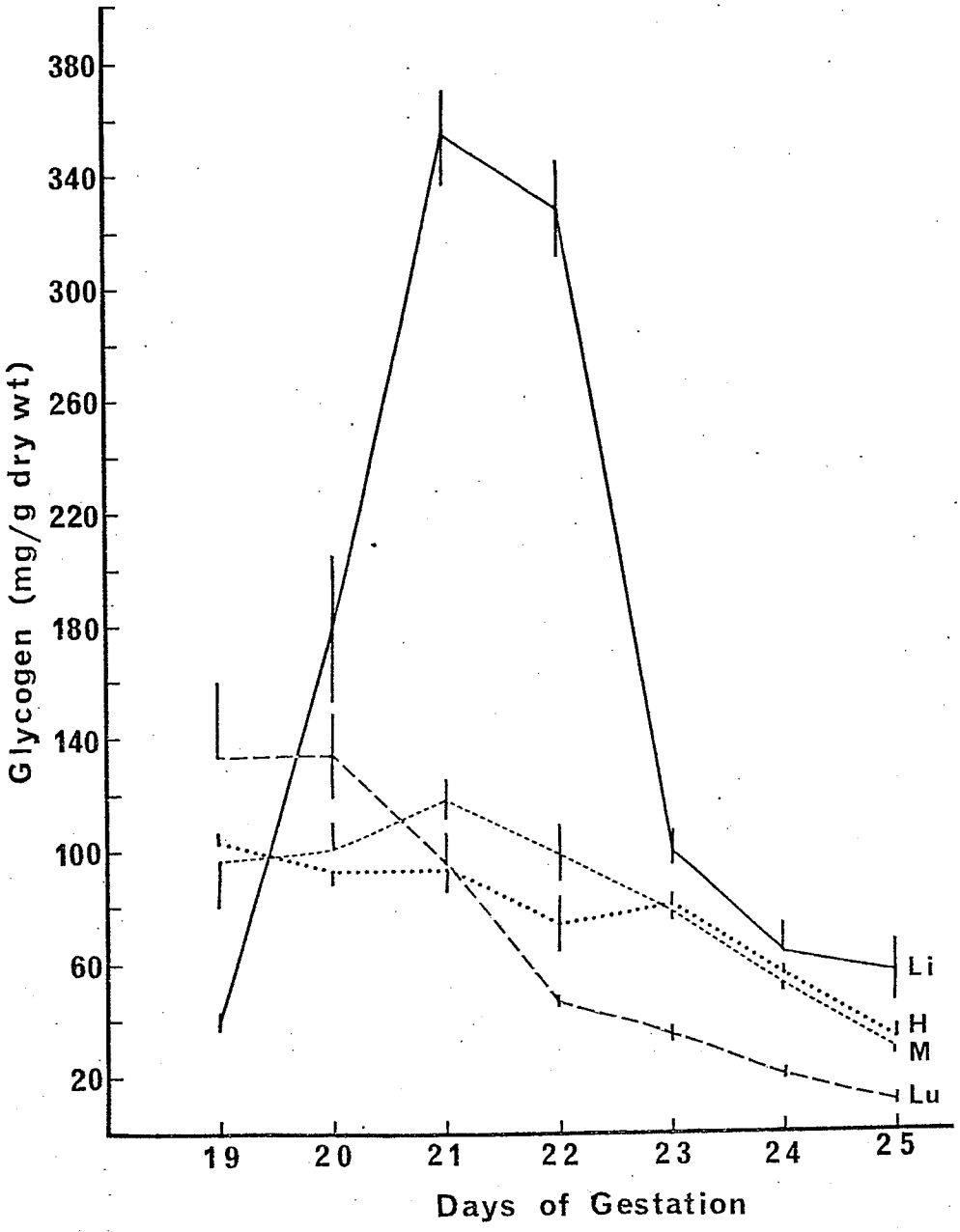

Figure 4. Glycogen content of lung (Lu), liver (Li), heart (H) and muscle (M) at GD 19 through GD 25. Vertical lines represent ±1 SE.

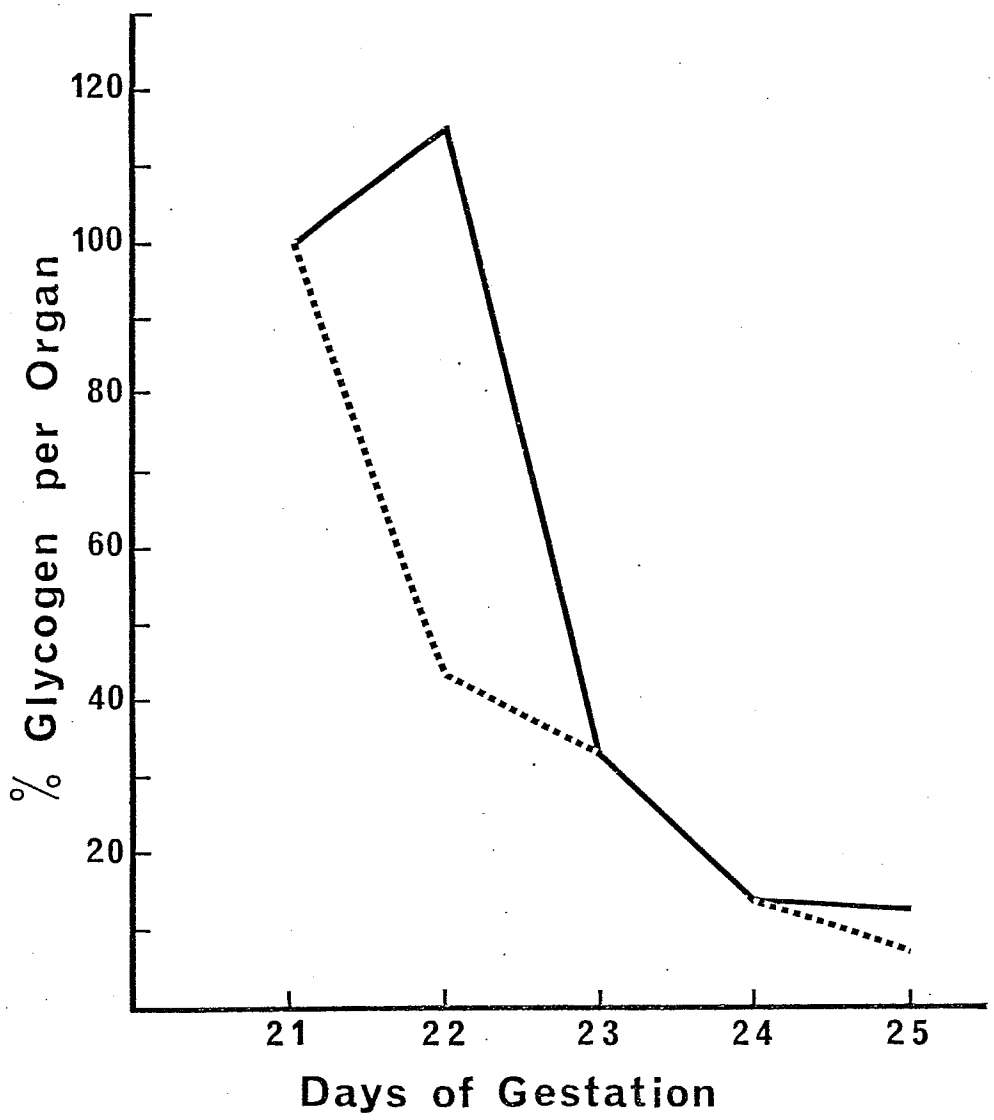

Figure 5. Glycogen content per lung (broken line) and per liver (solid line) as a percentage of glycogen content at GD 21.

TABLE 16 GLYCOGEN CONTENT OF VARIOUS ORGANS OF NEWBORN RATS AT DIFFERENT POSTNATAL PERIODS

Time after birth	Lung	Liver	Heart	Muscle
24 hours	21.33 ± 0.87* (9)	43.22 ± 10.27* (6)	41.87 ± 4.67* (4)	41.28 ± 3.83* (4)
48 hours	24.16 ± 1.52 (6)	73.90 ± 9.17 (6)	41.09 ± 3.63† (5)	32.65 ± 2.64Φ (5)
72 hours	28.05 ± 0.79o (10)	110.27 ± 7.33§ (8)	41.60 ± 3.92 (7)	29.72 ± 2.51 (6)

Data (mg/g dry wt) are expressed as means ± 1 SE; numbers in parentheses indicate number of newborn rats studied.

* Different from gestation day 23 (P 0.001).
† Different from gestation day 24 (P 0.01).
Φ Different from gestation day 24 (P 0.001).
§ Different from gestation day 25 (P 0.01).
o Different from gestation day 25 (P 0.001).

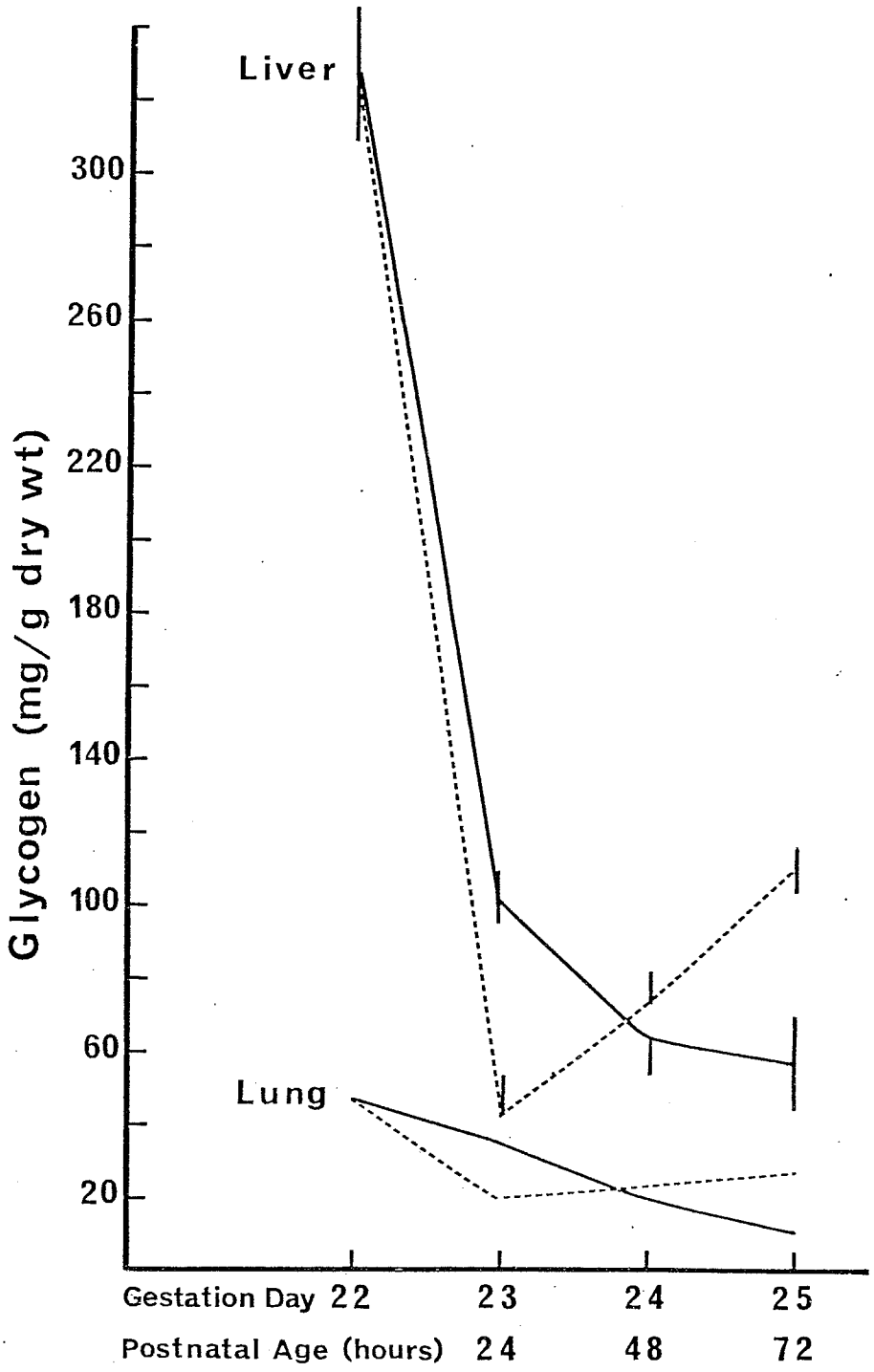

Figure 6. Comparison of the lung and liver glycogen content during prolonged gestation (solid line) and postnatal life (broken line). Vertical lines represent ±1 SE. In the lung SE. was less than 3 units.

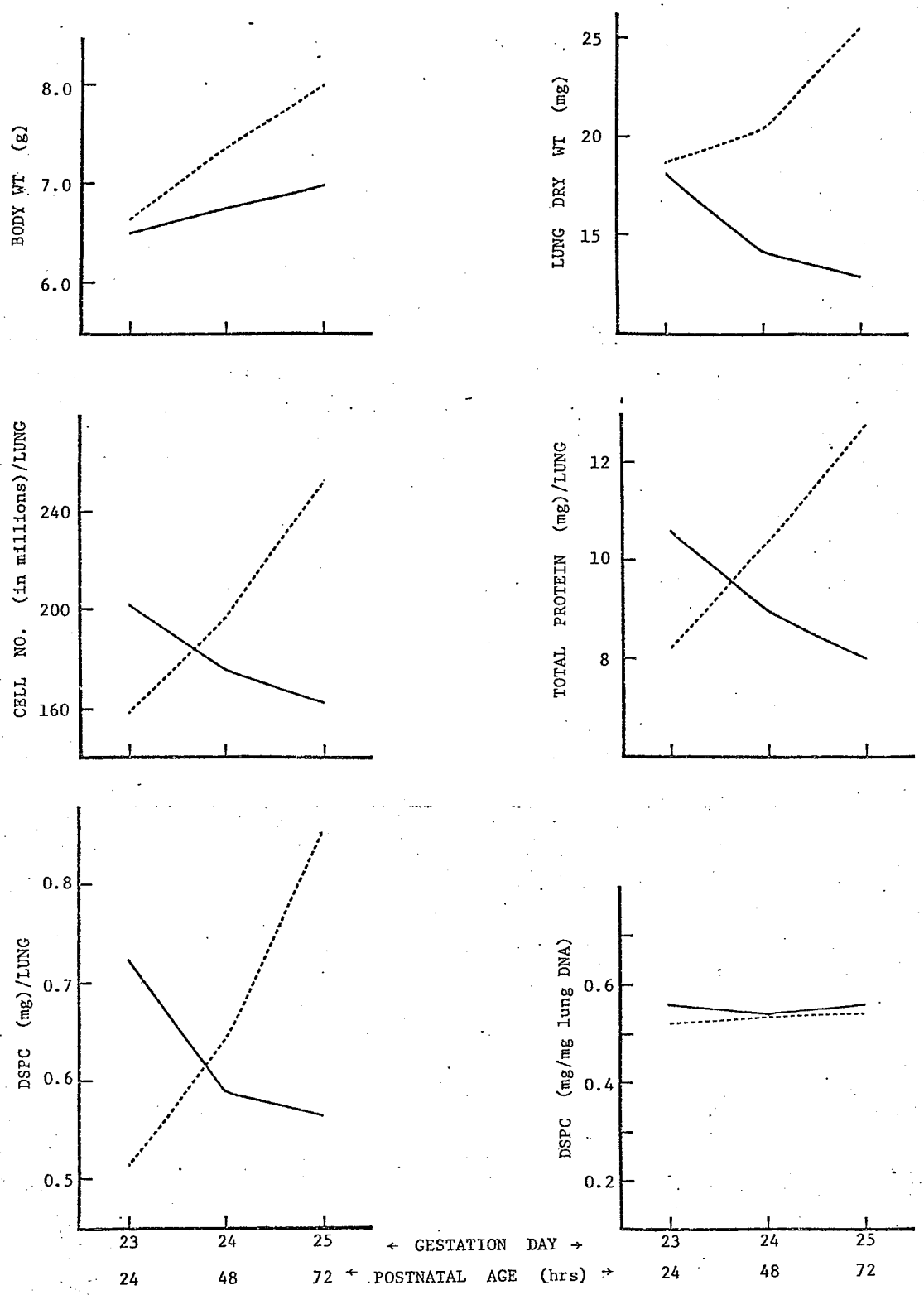

Figure 7. Comparison of post-term fetuses (gestation days 23, 24 and 25 - solid line) and newborn rats (postnatal age, 24, 48 and 72 hours - broken line). The solid and broken lines connect the mean values. Data obtained from Tables 7,9,12,13.

C. Discussion

Progesterone has been used to prolong pregnancy in a number of studies (73,77,81,82,83). The changes observed in fetal organs were attributed to prolonged gestation since no effect of exogenous progesterone was observed in term fetal organs. Because these previous studies did not include the fetal lung, it was necessary to distinguish the effects of prolonged pregnancy from the progesterone per se and the maternal stress created from the injection procedure. Our results suggest that fetal lung development was not influenced by progesterone or sesame oil injections. To determine the effect of prolonged pregnancy on fetal lung development, post-term fetuses were compared to fetuses at GD 22 (term). Because placental degeneration occurs post-term, resulting in deficient placental function (3,23, 24), it would have been ideal to have an experimental group with normal placental function in prolonged gestation to serve as a control for postmaturity. At present this is not possible. Therefore postnatal rats of comparable age were used as a guideline of age-dependent normal growth.

In this study measurements were made on lung samples pooled from the lungs of littermates. This was done in order to reduce the possible variation which may exist between littermates due to the uterine position of the littermates and differences in the rate of blood flow to the placentas (121,122). This method also allowed more reliable comparison between fetuses and neonates since it is not possible to predict the in utero position of the neonates.

These studies suggest that fetal lung growth was affected by prolonged pregnancy. Lung size and weight decreased post-term resulting in a smaller lung in proportion to the body weight. By GD 25 the lungs

were hypocellular with a reduction in lung phospholipid and glycogen content.

Fetal body weight increased with prolongation of gestation, in spite of the reduction in placental weight. Body weight at GD 23 was almost identical to the postnatal rat at 24 hours, but by GD 25 fetal body weight was one gram less than the neonate at 72 hours. This difference cannot be attributed exclusively to an increase in the body mass of the postnatal rat since some of the weight was due to ingested milk. Therefore it is difficult to state that an actual reduction in the rate of growth occurred in utero during prolonged pregnancy. It is well known that during prolonged pregnancy, the placenta degenerates and results in placental insufficiency. The reduction in placental weight and fetal glycogen stores observed in this study, and the fetal hypoglycemia observed by Thliveris (73), suggest that the post-term fetus was subjected to in utero nutritional deficiency. Malnutrition during pregnancy (123) or lactation (124) results in a reduction in the rate of growth of the fetus and neonate, respectively. The degree of malnutrition that the post-term fetus was exposed to is difficult to assess. It is tempting to speculate that if the fetus had been receiving sufficient maternal nutrients, the body weight of the post-term fetus could have been larger and perhaps exceeded that of the postnatal rat.

The fetal lung became progressively smaller as gestation was prolonged due to a loss of lung water and lung dry weight. Because the light microscopic studies provide visual evidence of fewer distended terminal sacs as gestation was prolonged, it is likely that a major portion of the water loss from the extracellular compartment was due

to the reduction in pulmonary fluid. The reduction of pulmonary fluid may result from a low secretion rate or a high removal rate of pulmonary fluid. In the lamb, the rate of fluid production was constant from 120 days gestation to near term (125), but at term there appeared to be a slight decrease. This suggests that a slowing of fluid production could be occurring and if pregnancy was prolonged, might become more apparent.

Normal lung growth in the rat proceeds entirely by cell division (hyperplastic growth) with a progressive increase in DNA content from mid-gestation until approximately 9 days postnatally (126). In this study, hyperplastic lung growth continued until GD 23 and subsequently regressed. By GD 25 the fetal lung was hypocellular in comparison to the GD 23 fetus and 72 hour neonate. In addition to this cell loss, there was a continual reduction of dry weight. This progressive decline is apparent when dry weight is expressed per cell number, such that at GD 21 the value is 0.1083 mg/1 million cells and at GD 25 is 0.0796. Since up to 26% of the mass of the adult lung is due to extracellular connective tissue (127), the above index (dry weight per cell number) can not be indicative of cell size (124,126). If we consider total lung protein per mg lung DNA as a valid index of cell size (124,126), the index is practically constant from GD 21 through GD 25 with only a 4% reduction at GD 25 as compared to GD 23. However, blood proteins contribute approximately 25% of the total lung protein in the term fetus (128) and not only influenced the above index but may have prevented the detection of intracellular protein loss. This masking effect by blood proteins could occur if their concentration per unit volume of blood increased or the actual volume of pulmonary blood increased. Irrespective of the source of protein for this index, the discrepancies between the index

of dry weight and total protein, per cell number simply indicates that the loss of lung dry weight is due to substances other than protein. Our data showed that a small portion of the dry weight loss could be due to the reduction of glycogen and lipids. A major portion could possibly be loss of connective tissues such as collagen and elastin, which could not be measured using the Lowry method.

Two possible factors may have been responsible for the reduction in lung cell number. The first is the effect of intrauterine nutritional deficiency occurring post-term (as discussed above), because of the similarities between the present work and that of Faridy's (123) which shows the effect of maternal malnutrition on fetal lungs. In both studies there were reductions in lung size per body weight and cellularity. In addition to these changes, there was a progressive decrease in the RNA/DNA ratio, which are all signs of malnutrition (124, 129). Therefore malnutrition must be considered a possible cause, but since body weight and kidney weight increased post-term, a phenomenon not observed in other malnutrition studies (123,124), it is unlikely that malnutrition is entirely responsible for the selective reduction in lung size. The second possibility is the direct effect of corticosteroids on lungs which has been shown to decrease cell mitosis of fetal lungs if administered to the fetus at late gestation (130). Because fetal distress occurs post-term, as suggested by the release of meconium (24) and the increased activity of the fetal adrenal gland (83), corticosteroid concentration may have been elevated. Cortisol measurements in human fetal umbilical cord blood showed that the levels of cortisol were elevated at term (131) and remained high in the postmature infant (132). By slowing mitosis, corticosteroids might disturb the cell renewal rate

of the lung in favor of a cell loss.

During prolonged gestation there was a reduction in the glycogen content of the tissues studied. The greatest reduction of glycogen occurred in the liver between GD 22 and GD 23 and was similar to the reduction of liver glycogen occurring during the first 24 hours after birth. Since mobilization of liver glycogen in the newborn is in response to hypoglycemia (133) it is possible that hypoglycemia was present in the post-term fetus, most likely as a result of a reduction in the rate of glucose transport from the mother to the fetus (placental insufficiency). Blood glucose was normal (54.6 mg %) at GD 23 which suggests that glycogen mobilization is capable of maintaining a normal blood glucose level during this time, but after GD 23 hypoglycemia (38.0 mg %) ensues (73) since the fetal glycogen stores (liver and other organs) were nearly exhausted. The function of glycogen in the fetal lung during late gestation has not been definitely proven. Although it was originally thought to be an energy source for mitosis of rapidly proliferating cells (134), it was later thought to be important in the general carbohydrate metabolism of the fetus (135). Recently it has been suggested that fetal lung glycogen was an energy source for the synthesis of lecithin, since the rate of glycogen utilization correlates with alveolar cell differentiation and an increase in lung phospholipid content (97). Glucose appears to be a major energy substrate for the adult lung (136) and might be important in the synthesis of the glycerol moiety of DSPC (137). The reduction of lung glycogen and blood glucose levels post-term may affect the general lung metabolism and phospholipid synthesis in the fetal lung and subsequently in the post-term neonate lung.

Our preliminary electron microscopic studies reveal two phenomena in the post-term fetal lung, namely an increase in the percentage of Type II cells and the number lamellar bodies in the terminal sacs. It is not conclusive at present whether the total number of Type II cells per lung has increased or decreased despite the 20% reduction of lung cell number post-term (GD 25). The fact that the DSPC/DNA ratio remained constant from GD 21 to GD 25 while the number of lamellar bodies in the terminal sacs progressively increased, suggests that the intracellular reserve of DSPC was decreased. Recently it has been shown in the rabbit, that ventilation accelerated the rate of release of DSPC into the alveoli (138). Since respiratory movements are known to occur in the fetus in utero (139), and fetal distress may increase the frequency of these respiratory movements, it is tempting to speculate that mechanical distension of the lung (140) might accelerate the secretion of lamellar bodies into the terminal sacs, resulting in a reduction of intracellular lecithin. However, distension of the lung in utero during breathing movements is not sufficient (141) to account for the excessive amount of phospholipid released. Another possible cause for the release of lamellar bodies was the presence of high levels of corticosteroids in the fetus. Exogenous steroid given to fetal lambs early in gestation, in a dose that would simulate the cortisol level of the lamb fetus at term, appeared to promote secretion of DSPC into the pulmonary fluid (142). It is known that fetal rats (143), rabbits (144) and possibly humans (56) store large quantities of lecithin intracellularly in the last 10 - 15% of gestation, establishing a lecithin "reservoir". The function of the reservoir has been studied by Gluck et al (145) who have shown that in

the newborn rabbit, large amounts of lecithin are rapidly released onto the alveolar surface after the onset of breathing. Clinical observations show that some newborn infants with normal respiration for several hours subsequently develop RDS (46). It has been suggested that these infants initially possess a sufficient amount of surfactant in the alveoli to allow normal respiration but with ventilation surfactant is depleted (146) and is not replenished at a sufficient rate to maintain the surfactant homeostasis at the alveolar surface (46). This could result from a low rate of surfactant secretion or a reduction in the intracellular reservoir of surface active material.

Clinical studies show that there is an increased incidence of respiratory distress in postmature infants. The cause of the postmature respiratory distress has not been clearly defined. However, it has been suggested that aspiration of meconium may play a role in predisposing the fetal lung to RDS (3,23). This hypothesis has been challenged by Fujikura and Klionsky (108) who observed a lower incidence of RDS in premature infants stained with meconium and suggested that the high surface activity of meconium (109) does not directly affect the activity of the pulmonary surfactant. Although it is difficult to definitely rule out the contribution of meconium aspiration to the development of respiratory distress in the postmature infant, because of the significant differences found between the post-term and term lungs, it is conceivable that these alterations may well be predisposing factors for RDS. Our results indicate that the lung of the post-term fetus is small for its body weight, is hypocellular, lacks glycogen and has diminished intracellular reserves of phospholipid. With these deficiences, the lung may not be able

to maintain the surfactant homeostasis and hence alveolar stability is lost. Assuming that the oxygen consumption of a post-term and term neonate (for a given body weight) is constant, then the post-term neonate is at a disadvantage in that the tidal volume, or frequency of breathing, is greater. This may further enhance the movement of surfactant into the airways (146). The diminished intracellular phospholipid reserve, perhaps in conjunction with reduced lung glycogen (and total body glycogen stores) may influence the rate of secretion and de novo synthesis of surfactant. Through this action the rate of replacement lags behind the rate of removal of surfactant, resulting in a net loss of surface active material in the alveoli.

D. Summary

The effect of prolonged pregnancy on fetal lung development was studied in the rat. Post-term fetuses displayed some characteristic signs of the postmaturity syndrome, specifically meconium staining and cracked, wrinkled skin. The post-term fetus possessed a lung that was smaller in proportion to body weight, hypocellular, lacking glycogen and possibly reduced in its intracellular reserve of surface active material. It is suggested that these deficiencies may prevent the maintenance of surfactant homeostasis in the post-term neonatal lung and be an important factor in the development of RDS in postmature infants.

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