

THE EFFECT OF METOPIRONE ON THE SYNTHESIS OF
LUNG SURFACTANT IN FETAL RABBIT

BY

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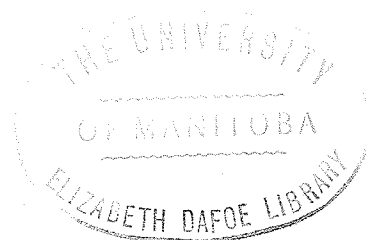
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INTRODUCTION

An intact fetal pituitary adrenal axis has been demonstrated to be important in the initiation of parturition. Prolonged gestation has been noted to be associated with human anencephaly (1), encephalitis and central nervous system malformation in cattle (2) and sheep (3). Experimentally gestation was prolonged by fetal hypophysectomy (4) and also by fetal adrenalectomy (5) indicating that steroids play an important role in the initiation of parturition in these animal models. Liggins (6) demonstrated that administration of adrenocorticotrophic hormone, dexamethasone, or cortisol to the fetal lamb resulted in premature delivery; he also noted that fetal lambs born as early as 115 days of gestation (term 147 days) were viable. DeLemos, et al (7) postulated that if the fetal pituitary adrenal axis is responsible for the initiation of parturition it also might play a role in the maturation of the lung, an organ system necessary for survival in extrauterine life. They found on administration of corticosteroids to one of the twin immature fetal lambs during a one to six day period that lung maturation was accelerated as measured by stable pressure volume curves and low surface tensions in treated fetal lungs compared to the twin control. Kotas et al (8) injected fetal rabbits with a single dose of long acting steroid, 9-fluoroprednisolone, at the 24th day of gestation (term 30-31 days). They found that at the 26th and 27th days of gestation the injected fetuses had lungs that were more mature than expected at that age. Saline treated controls did not show similar changes. Motoyama et al (9) studied the effect of corticosteroids on fetal rabbit lung maturation by injecting pregnant does. There was no acceleration of maturation of lung in the fetuses born of

of mothers who were given steroids, however in fetuses given steroids directly lung maturation was accelerated.

These studies indicate that exogenous steroids when given to the developing fetus accelerate lung maturation. If endogenous production of corticosteroids in the fetus do play an important role in the maturation of the lung it should be possible to delay maturation by the administration of a drug which interferes with fetal cortisol production. Metopirone (SU 4885) is an 11 Beta steroid hydroxylase inhibitor which when given to pregnant rats produces congenital adrenal hyperplasia of the newborn (10) simulating congenital adrenal hyperplasia of human infants characterized by reduced adrenal steroid production. We therefore designed a study to elucidate the role of endogenous steroids in lung maturation by administration of a metopirone to pregnant rabbits at various stages of gestation.

REVIEW OF LITERATURE

Surface tension is a measure of the tendency of an interface between dissimilar material, for example air and water, to contract to a minimal area. It arises from the cohesive forces of the molecules in and near its interface. Surface tension causes the pressure inside a soap bubble to be greater than atmospheric pressure, the higher the tension, the greater the internal pressure in accordance with LaPlace relationships for a sphere $P = 2T/r$, where P transmural pressure, T surface tension and $r =$ radius. At high internal pressures eg, with high surface tension, gas will diffuse out of a bubble, if the film is gas permeable, the bubble will contract. As the bubble contracts its radius decreases and its internal pressure increases for any given surface tension. However when surface tension is low the internal pressure is low and diffusion is minimal. As surface tension approaches zero the effect of radius and therefore of gas diffusion becomes negligible and the size of the bubble remains nearly constant. Since the lung may be considered as a number of branching tubes, the airways, ending up in bubbles (the alveolus) surface tension is an important determinant of the stability of alveoli.

The internal surface of the mammalian lung is lined with a lipoprotein complex capable of achieving a very low surface tension and therefore important in stabilizing alveoli during respiration. Macklin (11) was the first to suggest that the mucoid film of the alveolar wall is secreted by granular pneumonocytes or the so-called type II alveolar epithelial cells. Campiche et al (12) showed electron dense material being discharged from type II cells into the alveolar space. The studies of Kikkawa and associates (13) further provided

evidence that the inclusion bodies contained phospholipid and they were the precursors of the alveolar lining layer.

Pattle (14) suggested after observing the life span of foam bubbles from the saline filled lung that alveoli must be lined by some substance with a much lower surface tension than that of plasma. Clements (15) first reported low minimum surface tension and the low minimum surface compressibility for lung extracts. He used a modified Wilhelmy balance to measure surface tension of lung extracts minced with saline. Both saline washings and lung minced in saline were used with identical results of surface tension. He also noted a marked hysteresis when surface tension was plotted against surface area.

It is now presently hypothesized that surfactants exist in two phases at the lung air liquid interface: a) dissolved or dispersed in the liquid hypophase, b) a monomolecular film of phospholipids, associated with proteins, at the air-liquid interface with hydrophobic part of the phospholipid molecule pointing toward the air phase. The so-called lining complex of saline dispersible lipoprotein serves as reserve material.

Weibel and Gil (16) demonstrated by electron microscopy that the alveolar epithelial lining is coated by an extracellular lining layer composed of the two phases, a base layer containing proteins and mucopolysaccharides in aqueous solution as well as some lipids covered by a lamellar superficial layer consisting essentially of polar lipids and water. These observations support that of Pattle and Clements.

The recognition of the presence of the surface active material described so far has been an important achievement in understanding pulmonary mechanics. Thus surfactant is important in determining the

geometric configuration of the internal surfaces of the alveoli. The alveolar surface tension varies inversely with alveolar size.

The influence of surface active material on the stability of air spaces was discussed by Mead (17). He suggested that the surface forces and geometry of the terminal lung units are the primary determinants of the force required to initiate inflation of the lungs from the degassed state or from extremely low volumes. On inflation of the degassed lung once the opening pressure is exceeded tissue forces offer the major resistance to continued inflation of the lung. Surface and viscous forces are of primary importance to the stability of the lungs during deflation and at small lung volumes.

Von Neergard (18) in 1929 first demonstrated that surface forces contribute significantly to the retractive force of the lung. Measuring static pressure volume curves after filling lungs first with air and then with a gum arabic solution he concluded that $2/3 - 3/4$ of the retractive force of lung was due to surface forces and felt that surface tension of the alveoli was lower than that of active physiological fluids. In 1957 Mead et al (17) again noted that hysteresis was marked during air inflation but small during saline inflation and much less pressure was required to inflate the lung with saline than with air. A typical pressure volume curve of a degassed lung is shown in the Figure 1. On inflation the opening pressure is high, once the lung is opened the change in volume is proportional to change in pressure. On deflation the volume changes for each given pressure are smaller, and at zero pressure there is retention of air. Thus the pressure volume curve with air gives a deflation curve which is different from the inflation curve, that is, it shows hysteresis.

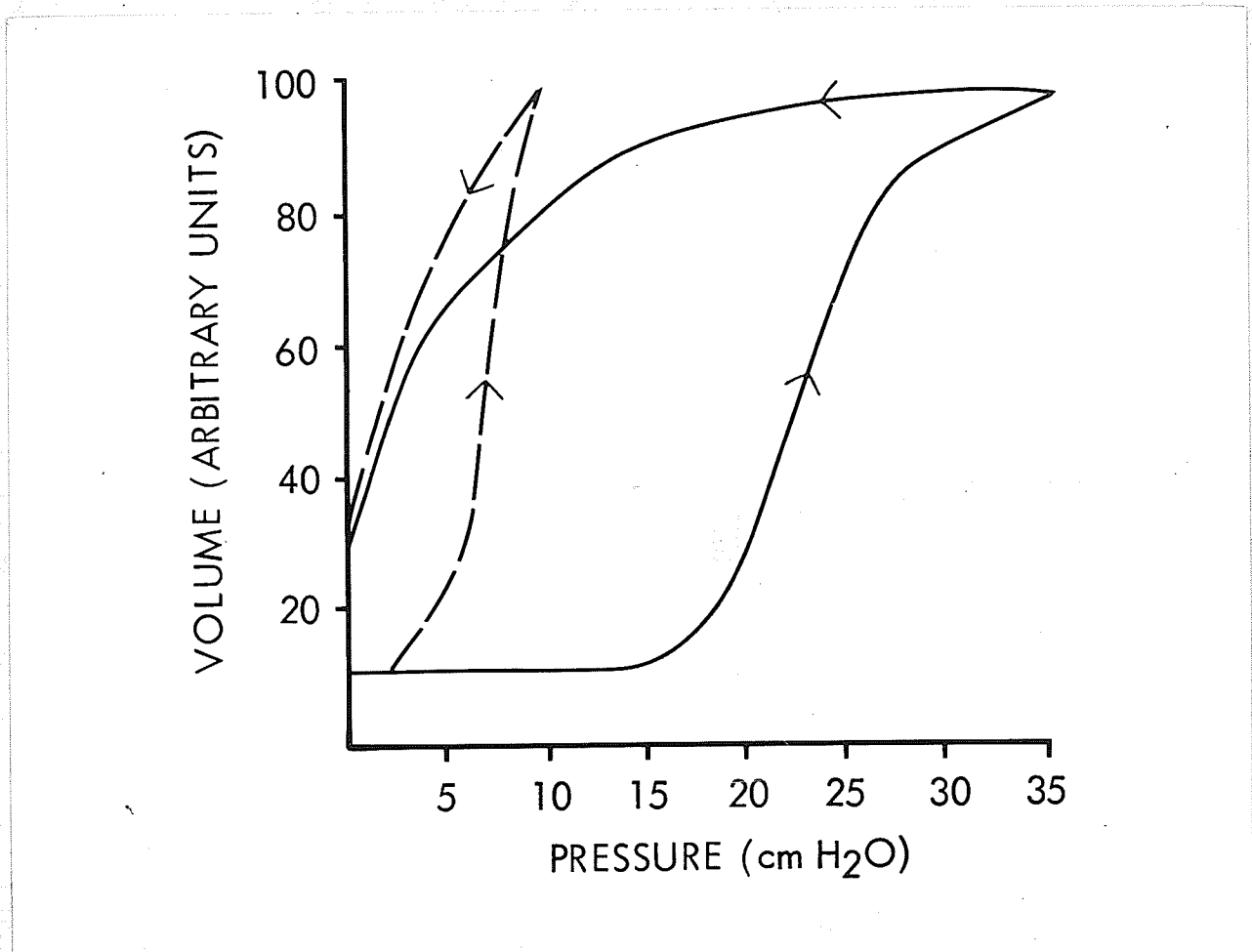


Figure 1. Saline and air volume pressure curves during inflation and deflation of mammalian lung. Interrupted line indicates the curve obtained from a saline filled lung. Continuous line is that of air filled lung. Inflation was begun after the lung was rendered airless in a vacuum jar. The initial volume represents tissue volume. Note the lower opening pressure, and small hysteresis of the saline filled lung. Comparison of the deflation air and saline curves shows that at high lung volumes about two-thirds of the retractive force of the lung is due to surface tension.

Liquid filled curves (Fig. 1) are characterized by low opening pressures and minimal hysteresis, that is, the difference between inflation and deflation is small. Mead and colleagues (17) noted that this hysteresis can be observed in humans in vivo and suggested that it was related to the influence of surface tension. They felt that the gross appearance of the lungs during the procedure was consistent with the observation that surface forces influences inflation of the lung with air. During saline inflation and deflation volume changes appeared to be uniformly distributed while during air inflation, expansion of the lungs is irregular or sequential with some regions remaining completely collapsed while others appeared to pop open one after another.

The relationship of surfactant and pressure volume curves of degassed lungs was further explained by the studies of Clements (19) on lung extracts. He measured a low surface tension when the surface of a lung extract was compressed to 15% of the original surface area. He constructed a surface tension diagram for the lung and found that it was similar to those obtained by direct pressure volume measurements. The surface tension of extracts decreased rapidly as the film was compressed and increased rapidly during the early phases of expansion giving rise to hysteresis. That the surface active material at the air-liquid interface was in a dynamic equilibrium with the hypophase was shown by Mendenhall (20). When lung extracts placed on surface tension balance were submitted to rapid compression and expansion of surface films at a frequency of 12/minute, they found that they could not produce a minimum surface tension unless surfactants were added to the hypophase continuously. Ionic concentration in the hypophase of lung extracts also plays a role in the production of hysteresis (21). When lung extracts

were prepared in distilled water the hysteresis loops were narrow which improved when NaCl, KCl or CaCl_2 was added to hypophase.

The functional significance of surfactant has been summarized recently by Clements (22). The unique property of surfactant is that surface tension changes with changes in alveolar diameter (smaller the diameter lower the tension) thus giving stability to the alveoli even at low volumes such as at the end of expiration. Because of stability of alveoli at low volumes less pressures are needed to expand the lung again. This fact is well borne out in the newborn. The pressures necessary to open the alveoli at first breath are very high in the order of 40 to 100 cm H_2O , however with each successive respiration the opening pressures diminished. Other functions that surfactant may play a role have been suggested. The work of Bruderman and colleagues (23) show that the suction effect produced by the alveolar surface reduces precapillary pressure in the lung. This allows the pulmonary vasculature to be perfused at a pulmonary artery pressure slightly lower than airway pressure in an air-filled lung but not in a liquid filled lung. They postulated an autoregulatory mechanism in which normal surface tension insures normal geometry, liquid flux, surfactant flux and local perfusion. Further well regulated perfusion is important in providing necessary metabolic precursors for the synthesis of the components of lung surfactant and maintenance of normal surface tension.

Surfactant has been implicated in functions such as that it might assist in clearance of particles by sliding up to the ciliated airways, and also in facilitation of phagocytosis but neither of these functions are supported with positive evidence. So one may conclude that the major role of surfactant system is in pulmonary mechanics.

BIOCHEMISTRY

Surface activity is shared by several phospholipids in lung but is related principally to the lecithin fraction of phospholipid. It has been found that the surface tension of disaturated lecithin and sphingomyelin is similar to that obtained from saline extracts of minced whole lung proproteins. Klaus et al (24) showed in the beef lung the lipid fraction of surface active material to be 75% phospholipid, 8% cholesterol, 10% triglycerides, 8% fatty acids. The lipid composition of the lining layer differs from that of whole lung tissue in that it contains less cholesterol, more lecithin, a higher concentration of saturated fatty acid residues, more surface active lecithin and phosphatidyl dimethylethanolamine (PDME). Klaus (25) suggested that the type II alveolar epithelial cell mitochondria may be the source of surfactant in the lung. He found presence of strong surfactant in the washed mitochondrial fraction of mammalian lung.

Turnover of phospholipids in the lung especially lecithin is extremely rapid; the half life in rat lung has been estimated at about 17 hours (26). C^{14} labelled palmitate appears in lung phospholipids of dogs within 2 minutes after its intravenous injection. The high turnover rate of lung lecithin may be due to the rapid formation and discharge of surfactant into the alveolar space. Studies indicate that lung tissue can incorporate acetate in vitro into lipids. When radioactive labelled acetate was perfused the lung was found to incorporate labelled acetate into phospholipid, the major activity being noted in the lecithin component (27). Felts (28) investigated the role of carbohydrate and lipid precursors in the synthesis of phospholipid in

rabbits. He found that lung slices readily oxidise plasma free fatty acids, and triglycerides and incorporate these into lecithin. Lung also metabolizes glucose in the absence of added insulin.

De novo synthesis of lung lecithin has been extensively studied, in various mammals. Two major pathways have been described. 1) Choline can be incorporated into lecithin via phosphoryl-choline and cystidine diphosphate (CDP-choline) (29) and 2) Conversion of phosphatidyl-ethanolamine to lecithin by stepwise methylation using S-adenosyl-1-methionine (Fig. 2) (30). In the adult rat lung the major pathway has been found to be the CDP-choline whereas in adult dog lung the major pathway is via methylation. In the fetal rabbit methylation is a major pathway which reaches a peak just prior to delivery, but early in gestation it is by way of choline pathway.

Lung tissue is also known to be highly active in the incorporation of free fatty acids into lecithin (28). In vivo and in vitro studies in several species indicate that labelled (^{14}C , or tritium) fatty acids appear in the phospholipid fraction. In terms of specific activity, tissue free fatty acids, diglyceride, triglyceride phospholipid respectively are found in decreasing order (31). Inhibition of oxidative energy production by hypoxia, cyanide, or low temperature markedly depresses esterification of palmitate ^{14}C .

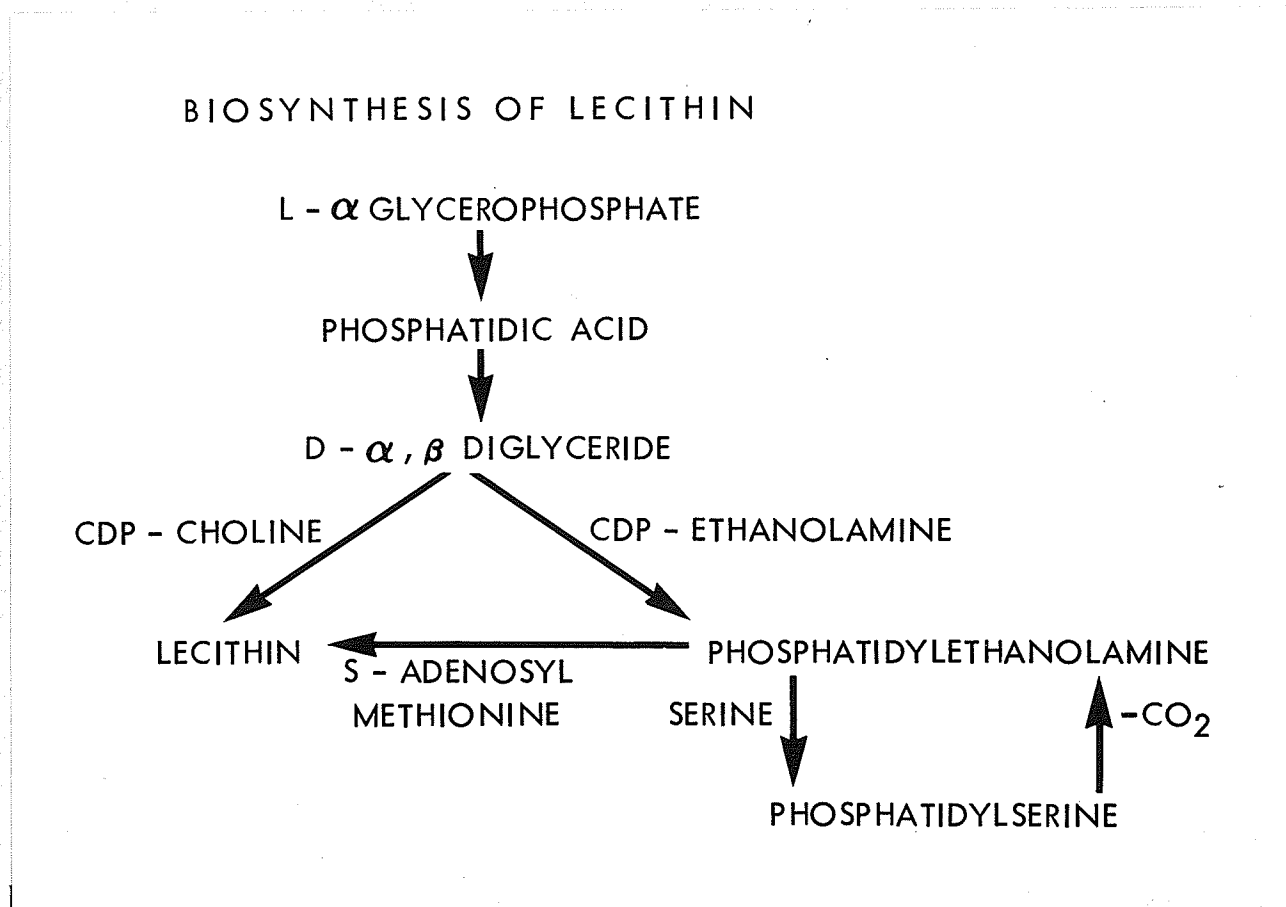


Figure 2. The major pathways in the biosynthesis of lecithin in the lung.

DEVELOPING LUNG AND SURFACE ACTIVITY

The mammalian fetal lung undergoes marked changes in the last part of gestation. The surface active material as measured by surface tension of lung extracts, pressure volume characteristics of the lung and biochemical assay of minced lung extracts reveal that it tends to increase with gestational age. Kikkawa et al (13) found that in fetal rabbits the content of lecithin in the lungs rose steadily from 2.2% of dry weight at two-thirds gestation to 8.4% at term. In lambs, a similar rise during gestation of disaturated lecithin has been measured by Brumley et al (32). Peculiar osmiophilic inclusions in the type II cells were noted by Karrer (33) in the mouse lung, they appear late in developing mouse at 18 days of gestation (term 21 days). The time of appearance in the human fetal lung is not well known, although the inclusions were noted in the lung of an 840 gm fetus (12). In lambs before 120 days of gestation (term 147 days) the lungs do not retain air on deflation to atmospheric pressure and no surfactant is demonstrable (32). Between 120-130 days the upper lobes are stable and surfactant is present. A few days later the lower lobes mature in this respect. In infants under 1.2 kg birth weight surfactant is rarely found by use of a surface film balance. However Gruenwald (34) found stable pressure volume characteristics in 40% of fetal lungs under 500 gm and in 50% in those weighing 750-1000 gm. Boughton et al (35) report that it is likely that in the human surfactant is first detectable between 20-24 weeks gestation and that thereafter it tends to increase in amount as gestation proceeds but with a large variability.

There are several factors, intrinsic and extrinsic which

alter surface active properties of lung (Table I). It is well known that hyaline membrane disease of the newborn, which is mainly a problem of prematurity is associated with diminished surfactant. Recently deprivation of food and water in adult rats was shown to alter lung phospholipids (36). Inhalation of hydrocarbons, anesthetics and high oxygen concentration also affect lung surfactants. Considerable evidence has been gathered, at least in fetus, showing that corticosteroids enhance surfactant production. There are indications that when lungs are ventilated at a constant transpulmonary pressure surfactant may be conserved (22).

DRUGS AND MICROSOMAL ENZYME INDUCTION

The accidental finding that the spraying of animal rooms with halogenated hydrocarbon insecticides alters the action of drugs led to the discovery that these insecticides stimulate drug metabolizing microsomal enzymes. Drugs like phenobarbital stimulate the various pathways of metabolism by liver microsomes including oxidation, reduction, glucuronamide formation and de-esterification. Studies in animals show that when an inducer is given there occurs an increase in the weight of liver and increase in the amount of protein (37). An increase in the number of cells has been shown to occur as evidenced by increased rate of mitosis in the liver (38). More important changes are seen in the smooth endoplasmic reticulum. Phenobarbital causes marked proliferation of the endoplasmic reticulum resulting in increase in the number of smooth membranes (39). Further phenobarbitone had effect on the phospholipid content of microsomes; causing an increase in the phospholipids particularly phosphatidylcholine (40). Enzyme induction has been shown to occur in many animals including man. Furthermore, microsomal induction occurs in tissues other than liver. Polycyclic hydrocarbon have been shown to induce microsomal enzymes in lungs, intestine, kidney, skin and placenta (41). Androgens, estrogens, progestational steroids, glucocorticoids, anabolic steroids, norepinephrine, insulin, thyroid hormone also alter drug metabolizing enzymes in liver microsomes. Testosterone given to female rats increased the activity of liver microsomal system that metabolize hexobarbital (42). Adrenalectomy of either male or female rats decreases the activity of liver microsomal enzymes that oxidize hexobarbital (43). Administration of prednisone to adrenalectomized male rats for several days restored

the activity of these enzymes to control values. Corticosteroids have also been implicated as enzyme inducers thereby accelerating maturation of organ systems. Moog (44) found that corticosteroids enhanced duodenal mucosal cell maturation and increased alkaline phosphatase activity in these cells. Margolis (45) et al demonstrated that phenyl-ethanolamine-N methyl transferase which methylates norepinephrine to epinephrine was decreased on decapitation but restored on administration of corticosteroids or ACTH. Buckingham (46) found that steroids increased the alkaline phosphatase activity of lung epithelial cell during maturation. More definitive evidence for the influence of steroids in lung maturation have been presented recently by others (7,8).

Microsomal enzyme induction results in an increase in the amount of enzyme produced. This is evident from studies showing increased amino acid incorporating after the administration of inducing agents (47). With phenobarbitone it is also known that it causes decreased breakdown of protein thus having a dual mechanism.

Microsomal Enzyme Induction in Fetus

Some of the inducing agents when injected to mother cross the placenta and influence the fetus. Since it is known that younger animals have a greater potential capacity to increase synthesis the subject of enzyme induction in the foetus has gained considerable importance in recent years. Catz and Yaffe (48) studied the effect of barbiturate administration on the newborn. Pregnant mice were injected with barbiturate for 6 days preceding labour. The livers of the offspring born of drug treated mice had twice the bilirubin conjugating capacity. Similar induction has been reported in human fetuses (49),

and newborn (50). Arias and co-workers (51) studied the effect of 3-4 benzopyrene and chloroquin on fetal enzymes. Chloroquin induced liver conjugating enzymes while 3-4 benzopyrene failed.

These studies indicate that induction can take place in the fetus if the mother is given the inducing agents during pregnancy. However the studies of Motoyama et al (9) showed that while steroid accelerated the appearance of surfactant when given directly to the fetus, no such acceleration was noted when given to the mother.

METOPIRONE (SU 4885)

Metopirone (Fig. 3) is a pinalcoline type ketone first synthesized by Bencze and Allen (52). It was synthesized in an attempt to find an inhibitor of adrenal cortical secretion with great specificity to

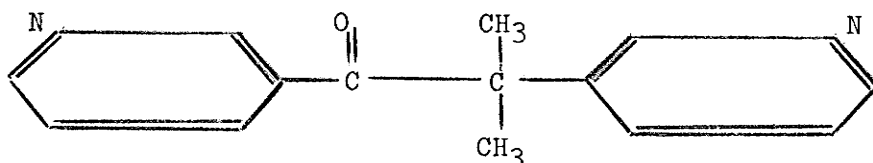


Fig. 3. Structure of Metopirone

inhibit at the 11 B hydroxylase position. In man this compound has been extensively used to test the function of the pituitary-adrenocortical axis.

Individuals with normally functioning pituitary and adrenals when given metopirone actually respond with a decrease in plasma cortisol and its urinary metabolites. Since pituitary ACTH secretion is dependent upon the circulating levels of cortisol, an intense stimulation of ACTH secretion occurs in an attempt to increase the plasma cortisol levels to normal. But in the presence of continued blockade by metopirone of B hydroxylation the precursors of cortisol are increased. It has been shown that the inhibition of metopirone of 11 B hydroxylase activity is competitive (53). Williamson and O'Donnell(54) found that the affinity of metopirone for 11 B hydroxylase was 56 times greater than than of desoxycorticosterone. Sanzari and Peron (55) suggest that inhibition may be substrate dependent involving endogenous

pyridine nucleotide co-factors, particularly NADPH. Others (56) linked inhibition to interaction of metopirone with cytochrome P-450, the oxygen activating and steroid linking component of the 11 B hydroxylase system.

The absorption of metopirone after oral administration is extremely variable. In man appreciable plasma levels may be reached within 30 minutes after drug injection. After 4 hours the plasma levels are very low. Intramuscular injections were used in one study to induce adrenal hyperplasia in the fetus. There are no reports showing the plasma metopirone levels when given subcutaneously. Placental transfer of metopirone to the fetus has not been studied directly previously, although Goldman (10) reported fetal adrenal hyperplasia after administration of metopirone to the mother.

Metopirone is converted to a reduced metabolite, 2-methyl-1, 2 bis (3 pyridyl)-1-propanol (SU 5236) by the adrenal, kidney and liver. It is apparently excreted both free and as the glucuronide in urine. There are indications that the reduced form (SU 5236) is equally potent inhibiting 11 B hydroxylase system (57). Only when the reduced form is conjugated in the liver does it lose its activity.

SUMMARY

In summary alveoli are lined with a material surfactant, which has unique properties of maintaining alveolar stability particularly during expiration. This material has been identified as a phospholipid, principally lecithin. Evidence has been presented showing that during fetal lung development, surfactant appears sometime before birth. Administration of corticosteroids to fetal lambs and rabbits has been shown to accelerate the appearance of surfactant but it is unclear whether endogenous steroid production is required for normal surfactant development. The present study, therefore was devised in an attempt to elucidate the influence of endogenous corticosteroid production on the appearance of surfactant in the fetal rabbit using metopirone, a B hydroxylase inhibitor, which blocks adrenal corticosteroid production.

MATERIALS AND METHODS

Pregnant New Zealand rabbits were used for this study. The rabbits were divided into four groups for treatment with drugs: 1) a control group received normal saline 1 ml/kg subcutaneously, 2) a metopirone group which received 100 mg/kg (100 mg/ml), 3) a third group received 100 mg/kg of cortisone, and 4) the fourth group received metopirone 100 mg/kg and cortisone 100 mg/kg injected at different sites.

Initially injections were started on the 23rd day of gestation since the studies of Gluck et al (58) indicated that surfactant in the fetal rabbit appeared at this stage of gestation and animals sacrificed on day 30 (term 31 days). Later during the course of our studies it was reported by Kotas et al (8) that pulmonary surfactant in the fetal rabbit appears at about the 29th-30th day of gestation. Hence in our later studies injection was started 3 days before sacrifice and animals were sacrificed on 26th to 27th day, 29th day or 30-31 day gestation so that we could study the effective blockage of endogenous steroid before, during and after the usual time of appearance of surfactant. Studies were also done on non-pregnant doe lungs.

The fetuses were delivered by Cesarean section shortly thereafter and fetal blood for metopirone estimation was collected by decapitation.

Deflation Pressure-Volume Curves

In the does the chest was opened by bisecting the sternum and the ribs were separated by a retractor to isolate the right lung.

After tying with an umbilical tape, the hilum was cut and the right lung removed for biochemical estimation and surface tension measurement. The left lung was left intact and care was taken to avoid puncturing the lung. The trachea was then cannulated in the neck region. The whole animal was then placed in a vacuum jar and the lung degassed before pressure-volume curves were obtained. Care was taken to avoid any spillage of blood into the trachea. Once degassed the whole animal was placed on a flat tray and the tracheal cannula connected to a water manometer and air displacement system as previously described (59). The lung was first inflated to a maximum pressure of 40 cm H₂O allowing 1-2 minutes for volume equilibration. This volume was considered as the maximum (V_{max}). The lung was then deflated in a stepwise manner and the volume recorded at each transpulmonary pressure was expressed as a percentage of the maximum volume. Thirty seconds were allowed at each pressure during deflation for volume equilibration.

In the fetuses a special technique was used for the measurement of quasi-static deflation pressure-volume relationships (Fig. 4). Upon delivery the fetal trachea was clamped to prevent air breathing. The fetus was then fixed in position and a midline incision made over the anterior part of neck and the trachea was isolated. A blunted 22 gauge needle 0.5 cm in length was placed into the trachea and tied with a suture. During the first part of the study the fetal chest was opened but this caused a high incidence of inadvertant rupture of the lung. Since the fetal chest wall is very compliant, the chest was not opened in the latter half of the study. There was no difference in lung compliance whether or not the chest wall was intact. The hub of the needle was connected to a specially drilled three-way

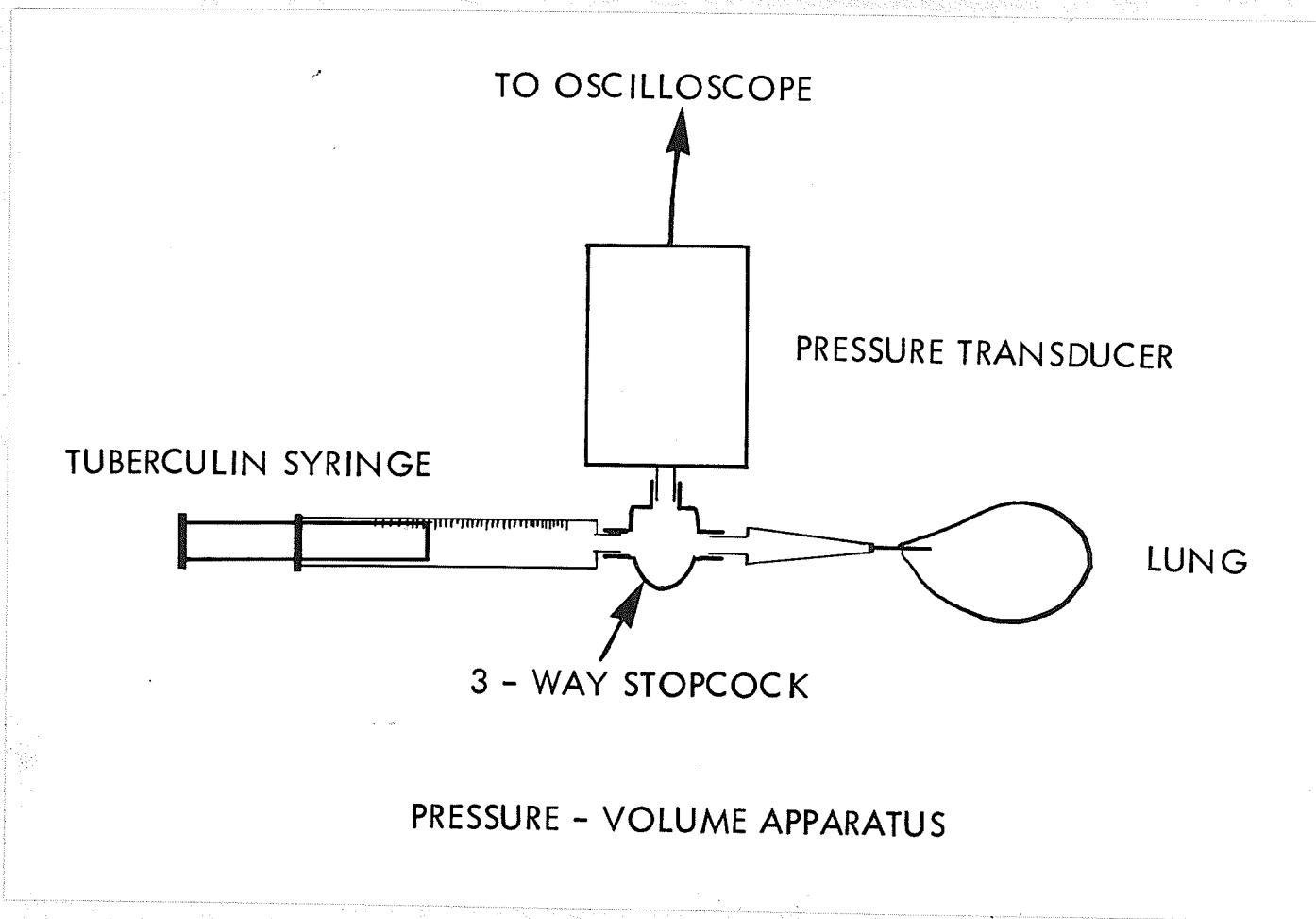


Figure 4. Technique used for the measurement of deflation pressure-volume curve in the fetuses. The three-way stopcock was open in all three directions.

stopcock which had all the three sides opened. To the stopcock were connected a pressure transducer (Statham P23DB) and a 2 ml syringe with the barrel coated with silicone to prevent air leak. The transducer was connected to a multichannel oscillograph to display pressure changes. The transducer and oscillograph was calibrated with a water monometer before the experiments were started. The maximum inflation pressure used in the fetal rabbits was 30 cm H₂O. The syringe barrel was drawn upto a known volume before connecting to the three-way stopcock. Air was injected slowly into the fetus, while the changes in the pressure were being noted visually. Once the pressure of 30 cm H₂O was reached one minute was given to equilibrate and if no fall in pressure occurred the amount of air injected at 30 cm H₂O pressure was considered as the maximum volume (V_{max}). The deflation curve was obtained by withdrawing air sufficient to cause a stepwise fall of 5 cm H₂O in pressure. The volume at each pressure was noted. The volumes at each pressure were calculated as percentage of volume reached at the maximum pressure.

Surface Tension

Shortly after the removal of the lung from the chest, 2 grams of aerated lung tissue was minced finely in 10 ml of saline first and then the volume made up to 40 ml. This mixture was then stirred using a teflon coated magnet for 10 minutes. The surface tension of this extract was measured at room temperature on a modified Wilhelmy surface tension balance (Kimray-Greenfield surfactometer) (60), which compresses the surface area to 15% of the maximum surface area. The extract was allowed to age for 10-15 minutes then the surface area alternately compressed and expanded at a rate of one cycle per three

minutes for 3 hours. The surface tension recorded at minimum surface area was designated as the minimum surface tension (γ min).

It was noted during this study that with fetal lungs of 27 days gestation fetus of 27 day or older it was essential to stir the minced lung extract for 10-15 minutes before putting on the surfactometer. Unstirred lung extracts of fetuses gave considerably high γ min. surface tensions. In contrast for extracts of adult lungs, stirring did not make any difference in the final minimum surface tension readings.

Lung Phospholipids

One half gram of lung tissue was homogenized in chloroform-methanol solution (2:1 V:V) and the lipid extract was washed according to the method of Folch et al (61). The extract was dried in a water-bath at 40-50°C under nitrogen and the dried extract was reconstituted to one ml with chloroform. An aliquot of lipid extract was used for lipid phosphorous determination by Brante's (62) modification of the method of Fiske and SubbaRow (63).

A second aliquot 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). The plate was then exposed to iodine vapor to identify the constituent lipid spots. After the iodine vapor had evaporated each spot was aspirated into a separate test tube and the lipid phosphorous determination on each was repeated. Phosphorous concentration was multiplied by 25 to convert to mg each lipid.

¹⁴C-Palmitate Uptake

One half gram of lung tissue was finely minced in 10 ml Krebs-Ringer bicarbonate buffer solution containing 5 mg of glucose and with a pH adjusted to 7.40. Samples were taken in duplicate. To each sample ¹⁴C labelled palmitate was added with a specific activity of .50 $\mu\text{c}/\text{m-mole}$. The tubes were gently agitated for two hours in a water bath at 37°C with 5% CO₂ in 95% oxygen continuously bubbling through the solution. Lipids were then extracted and separated into various components as described above. The activity of lecithin and other components was measured in a scintillation counter with an internal standard. Counts were expressed as disintegrations per minute (DPM) /mg lecithin.

Lung DNA Content

0.5 gm samples of lung were taken for deoxyribonucleic acid (DNA) determination by the method of Schneider (64).

Lung Dry Weight

A known amount of lung tissue was weighed soon after the lungs were removed. The tissue was placed in tin foil and dried in an oven at 110°C temperature for 48 hours or more until there was no change in weights of tissue. The dry weight was expressed as a percentage of the original weight.

Placental Transfer of Metopirone

Experiments were carried out to establish simultaneous plasma metopirone levels in the fetus and the mother at 0, 1, 2, 4, 6 and 24 hours after injecting the mother with metopirone (100 mg/kg subcutaneously).

Rabbits were sacrificed by giving pentobarbital 50 mg/kg intravenously. Maternal blood was drawn from the abdominal aorta immediately after anesthesia and placed in a heparinized tube. Plasma was separated and frozen at -20°C for later metopirone estimation.

Plasma metopirone was measured using a modification of the fluorometric method of Meikle et al (65). Plasma was alkalinized with 0.1 ml of 1N NaOH and extracted twice with three volumes of ether. At each extraction it was important to wait for 10 minutes. The ether extracts were evaporated to dryness and then dissolved in dichloromethane saturated with 0.1 N NaOH. The extract in dichloromethane was then passed through a fluorosil column, which was previously washed with methanol. The flow rate was adjusted one to two ml per minute. The extract was eluted with one ml of dichloromethane followed by 2% methanol in dichloromethane and finally 3.5% methanol in dichloromethane. The elution was evaporated to dryness. 0.5 molar cyanogen bromide in 5% KH_2PO_4 was added to the dried extract and incubated at $60 \pm 2^{\circ}\text{C}$ in a water bath for 10 minutes. After cooling at 20°C for 5 minutes one ml of 2% p-aminoacetophenone in 2 N HCl was added. The solution was then left in the dark for 30 minutes. Fluorescence was then measured with Aminco Bowman spectrofluorometer (activation wave length 395 m μ and emission at 462 m μ). A IP 28 photomultiplier tube and xenon lamp was used. Control plasma samples were used as a reagent blank. The method described above differs from Meikle et al (65) in that ether extraction was done over a 20 minute time period and secondly the elution of the extract was done slowly at a flow rate of one to two ml/minute. These modifications considerably improved the recovery of metopirone. Standard curve was obtained from control samples of plasma

to which metopirone was added to give concentrations of 0.5 µg/ml to 4.0 µg/ml. After adding metopirone in methanol the plasma was shaken and allowed to stand for 1-2 minutes. Plasma without added metopirone was used as a blank. The estimations had a good overall correlation between the levels and galvanometer reading, $t = .944$, $p < .001$ (Fig.5). Plasma metopirone levels from both does and fetuses were ready against the individual standard curve done on the day of estimation. Plasma metopirone recovery studies were also carried on two occasions. 92.3% of added metopirone was recovered after eluting the extract through the column.

Statistical Analysis

Groups were compared using an analysis of variance and Duncan test (66), where applicable. Other comparisons were made using a Student's T-test or, chi square analysis (67).

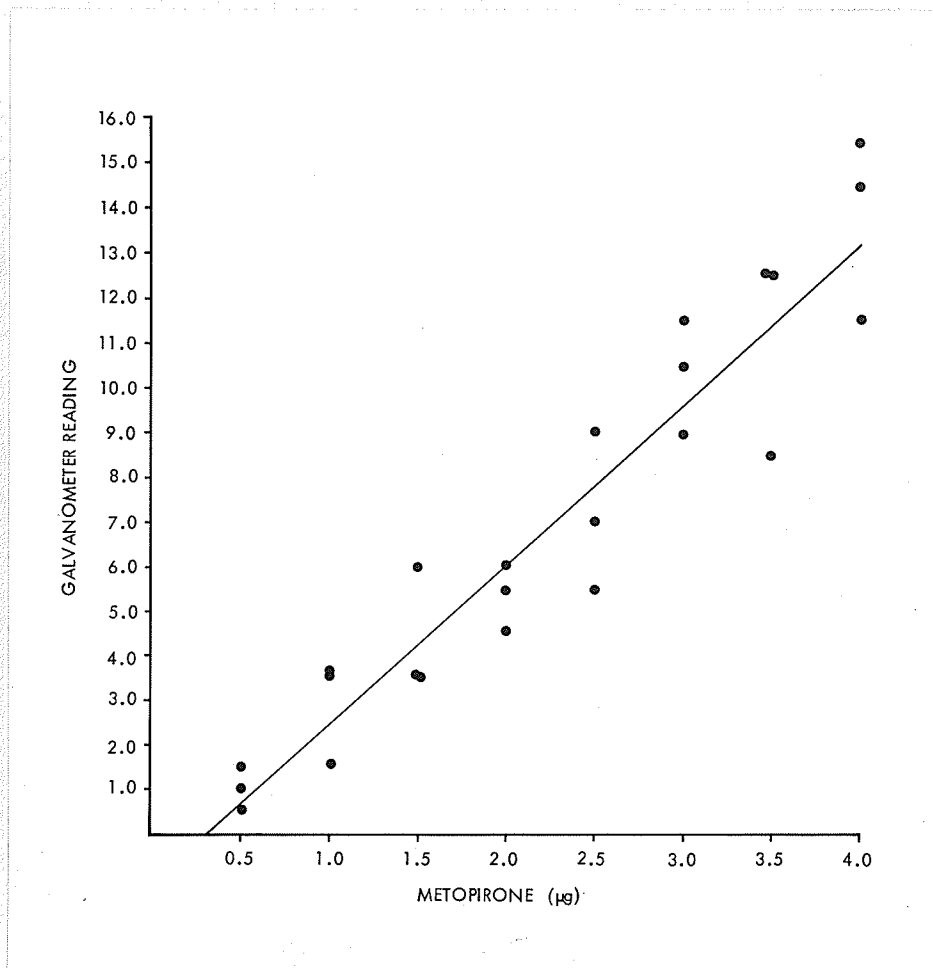


Figure 5. Galvanometer deflections for different known metopirone concentrations. Three sets of determinations from 0.5 to 4 Mq/ml were done on different days, at the time the unknown samples were estimated. The curve for each day was used to calculate the concentration in the known sample. The straight line in the figure represents the regression equation by the least squares technique for all the points ($y = 3.59x - 1.15$; $r = .944$, $p < .001$). The method appears to be accurate to within 0.5 µg/ml, particularly below 3 µg/ml. When the unknown sample was above this level it was appropriately diluted with plasma in order to increase the accuracy of the estimate.

RESULTSMATERNAL DATA

Table II shows data obtained in four groups of does. The mean body weights and total lung weights were similar in each group. Steroid treated does had a higher percent dry weight of lungs when compared with other groups, however the difference was not statistically significant ($p > .05$). Lung DNA content (mg/gm of lung) was not significantly different between control, steroid and metopirone plus steroid groups. However, DNA content (mg/gm) was significantly higher in the metopirone group when compared to controls ($p < .05$).

Surface Tension

Surface tension obtained from the lung extracts of the does are shown in Table III. The lowest minimal surface tensions (γ min) were obtained in the does who had received the combination of steroid and metopirone ($p < .05$). The maximum surface tension (γ max) also was lowest in this group ($p < .05$). Both min and max were similar in the other three groups.

Deflation Pressure-Volume Curves

The lung volume at 40 cm H₂O transpulmonary pressure (V_{max}) when expressed per gm of lung showed that there was no difference between any of the groups (Table IV). The values shown in Table IV are the volume retained at each transpulmonary pressure expressed as the percent of lung volume at a transpulmonary pressure of 40 cm H₂O. There were no differences in the percentages of volume retained at any given transpulmonary pressure between each of the four groups.

Lung Phospholipids

Table V and Figure 6 shows the mean values of lung total phospholipids and constituents expressed per mg DNA. There was a significant difference in total phospholipids between four groups with the highest values in the control group ($p < .005$). The other three groups had comparatively low values. Whereas the control group was significantly different from the metopirone treated does ($p < .01$), steroid treated ($p < .05$) and does treated with metopirone plus steroid ($p < .01$), the differences between the latter three groups were not significant. The control group had significantly higher content of lecithin compared to metopirone treated does ($p < .05$) steroid treated does ($p < .05$) and does treated with metopirone and steroid together ($p < .05$)!

Phosphatidyl-ethanolamine (PE) fraction showed similar results. The PE content in the control group was highest of the four groups ($p < .05$). Significant differences existed between control and metopirone treated groups ($p < .05$), steroid group ($p < .01$) and metopirone and steroid treated group ($p < .05$).

C¹⁴ Palmitate Uptake

Table VI shows the results of C¹⁴ labelled palmitate uptake by the lung tissue (dpm/mg phospholipid) in the four groups. There were no differences found between the groups.

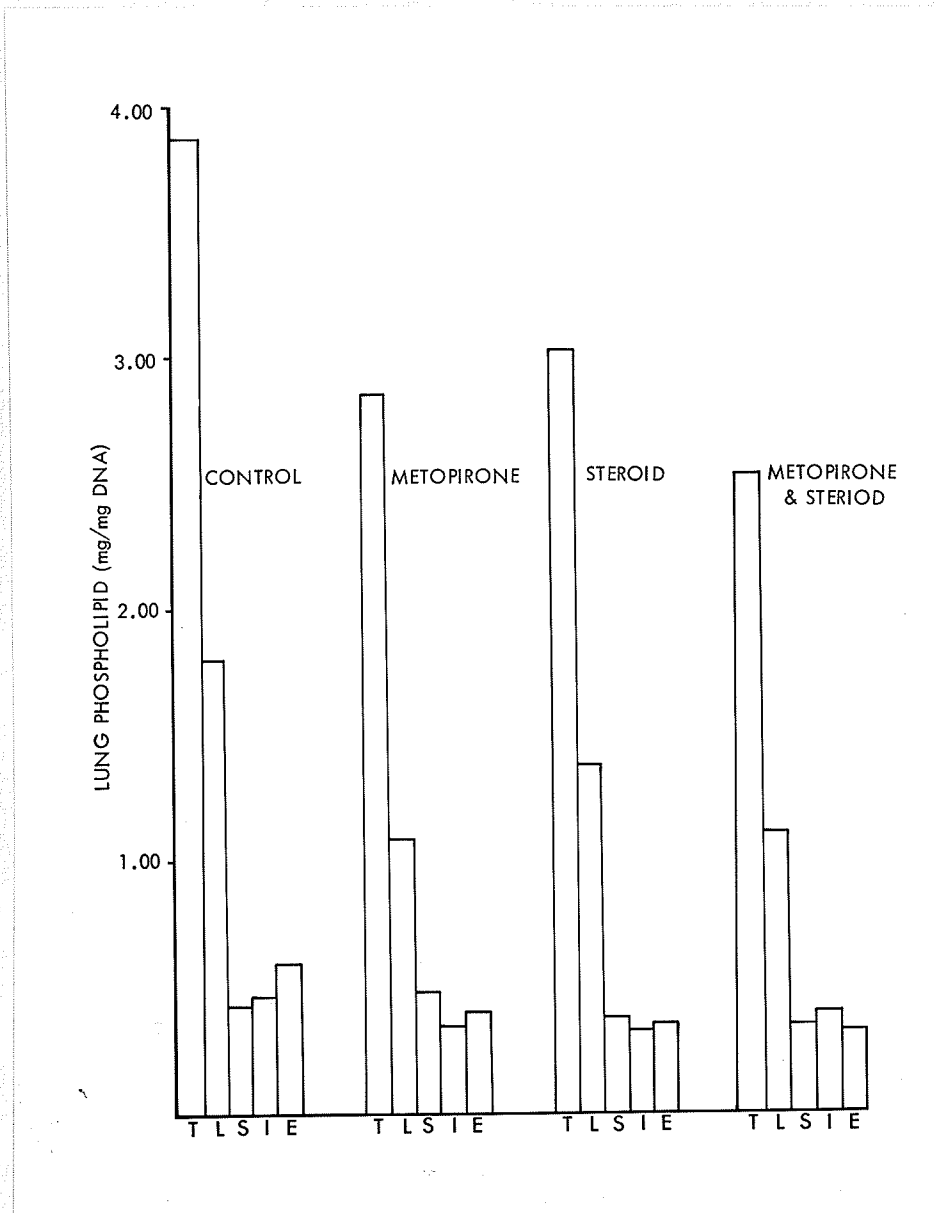


Figure 6. Phospholipid content of adult rabbit lung for the four treatment groups.

- T - total phospholipid
- L - lecithin
- S - sphingomyelin
- I - phosphatidyl-inositol-serine
- E - phosphatidyl-ethanolamine

FETAL DATA

The litter size, fetal weight, lung weight, percent dry weight and lung DNA content are shown for each gestational age from 26 to 31 days in Table VII.

Administration of metopirone did not influence litter size at any gestational age. Fetal weight increased with gestation as would be expected. Although the metopirone groups had smaller fetuses at both 29 days and 30-31 days gestation the differences were not statistically significant ($p > .1$). Lung weights were considerably higher in the control at 30-31 days gestation, but the numbers were too small for valid comparison. Dry weight expressed as percent of wet weight was not significantly influenced by metopirone.

Surface Tension Measurements

Table VIII summarizes the findings of surface tension studies done on fetal lung extracts. Earlier in gestation, ie. at 26 and 27 days gestational age, metopirone treatment of the mother did not alter the minimum surface tension of the fetal lung extracts. Similarly at 29 days gestation the mean minimum surface tensions in both groups were alike. The findings in the group at 30 and 31 days gestation were of interest. In the control group the minimum surface tension was very low. In the metopirone treated group at 30-31 days gestation 3 out of 8 fetal lungs had minimal surface tensions below the mean (4.6) of the control group. The other five had far higher values. A chi-square test (2x2) indicated that the occurrence of γ min above 6 dynes/cm (4.6 + 1 S.D.) was significant in the metopirone treated group ($\chi^2 = 4.90$; $p < .05$). Thus, a single daily metopirone injection of the mother

was associated with a significant increase in γ min. of mature fetuses although the effect was not universal.

Pressure-Volume Curves

The deflation curves are shown in Figures 7 and 8 and Table IX. These data indicate that with increasing gestational age the fetal lungs become more stable and the deflation curves moves to the left such that more volume is retained at each transpulmonary pressure on deflation. There were no significant differences between the control and metopirone treated group at any gestational age.

Lung Phospholipids

Total lung phospholipids increased with gestational age (Table X) in both the groups. Lecithin content paralleled the increase in total phospholipids. Metopirone did not influence the fetal lung phospholipid content or the content of lecithin, sphingomyelin, phosphatidyl inositol and phosphatidyl ethanolamine.

C¹⁴ Palmitate Uptake

Radioactive labelled palmitate uptake into lung phospholipids and its constituents was studied in 3 control and 3 metopirone treated fetuses at 30 days gestation (Table XI). The activity in the total phospholipid and the individual fractions were similar in each group but the number of observations were too small for statistical evaluation.

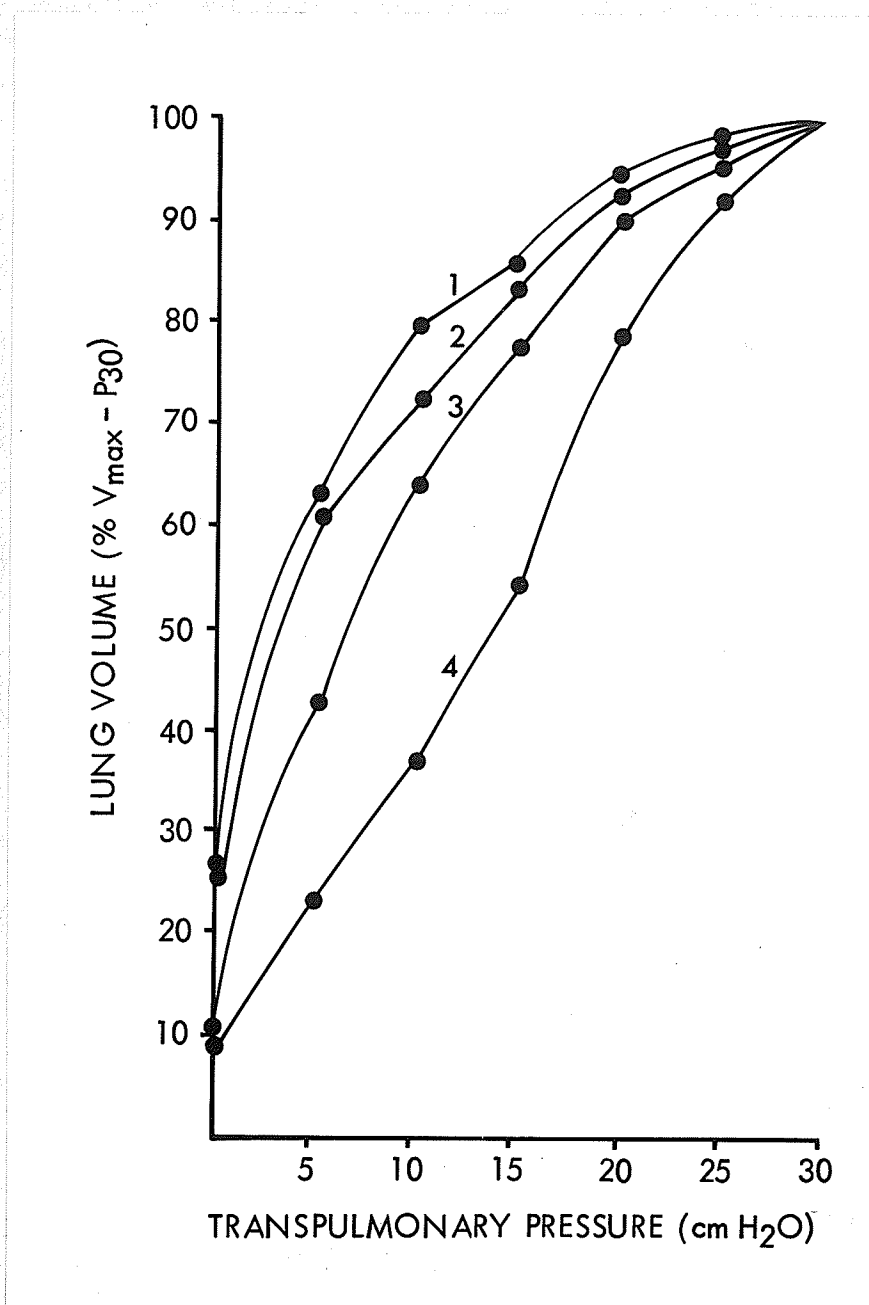


Figure 7. Deflation pressure-volume curves at different gestational ages of control fetal lung. Volume is expressed as percent of the maximum volume obtained at 30 cm H₂O transpulmonary pressure. 1. 31 days gestation, 2. 30 days, 3. 29 days, and 4. 26th and 27th days.

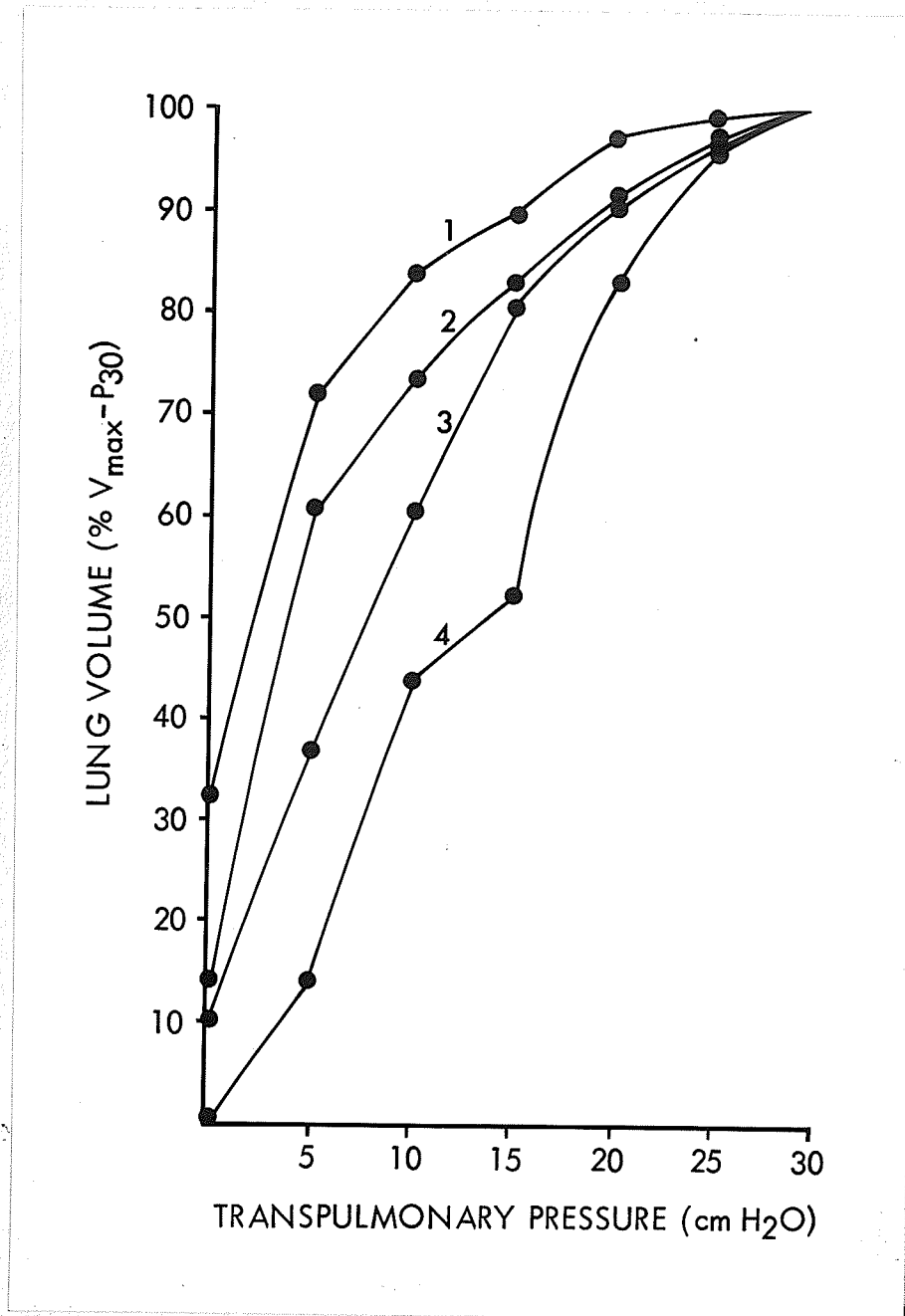


Figure 8. Deflation pressure-volume curve at different gestational ages of metopirone-treated fetal lungs. Volume is expressed as percent of the maximum volume obtained at 30 cm H₂O transpulmonary pressure. 1. 31 days gestation, 2. 30 days, 3. 29 days, and 4. 26th and 27th days.

PLASMA METOPIRONE LEVELS AND TIME COURSESTUDY

The time course study of plasma metopirone levels in the mother and the fetus are shown in Figure 9. Peak levels were reached both in the mother and the fetus at one hour following maternal injection indicating that metopirone readily crosses the placenta. The levels dropped sharply after 2 hours of age. By three to four hours the levels fell to low levels and at 6 hours only traces were found. At twenty-four hours there was no measurable metopirone in either maternal or fetal blood.

The method for plasma metopirone measurement was established late in the course of investigation. Because of the finding that metopirone completely disappears in 24 hours two rabbits were given 8 hourly injections of metopirone (100 mg/kg/dose) and surface properties of fetal lung studied. At 27 days gestation the minimum surface tension of the fetal lungs was similar to the controls. At 31 days gestation the fetal lung extract had a very high minimum surface tension (20 dynes/cm), indicating a delay in the maturation of the lung.

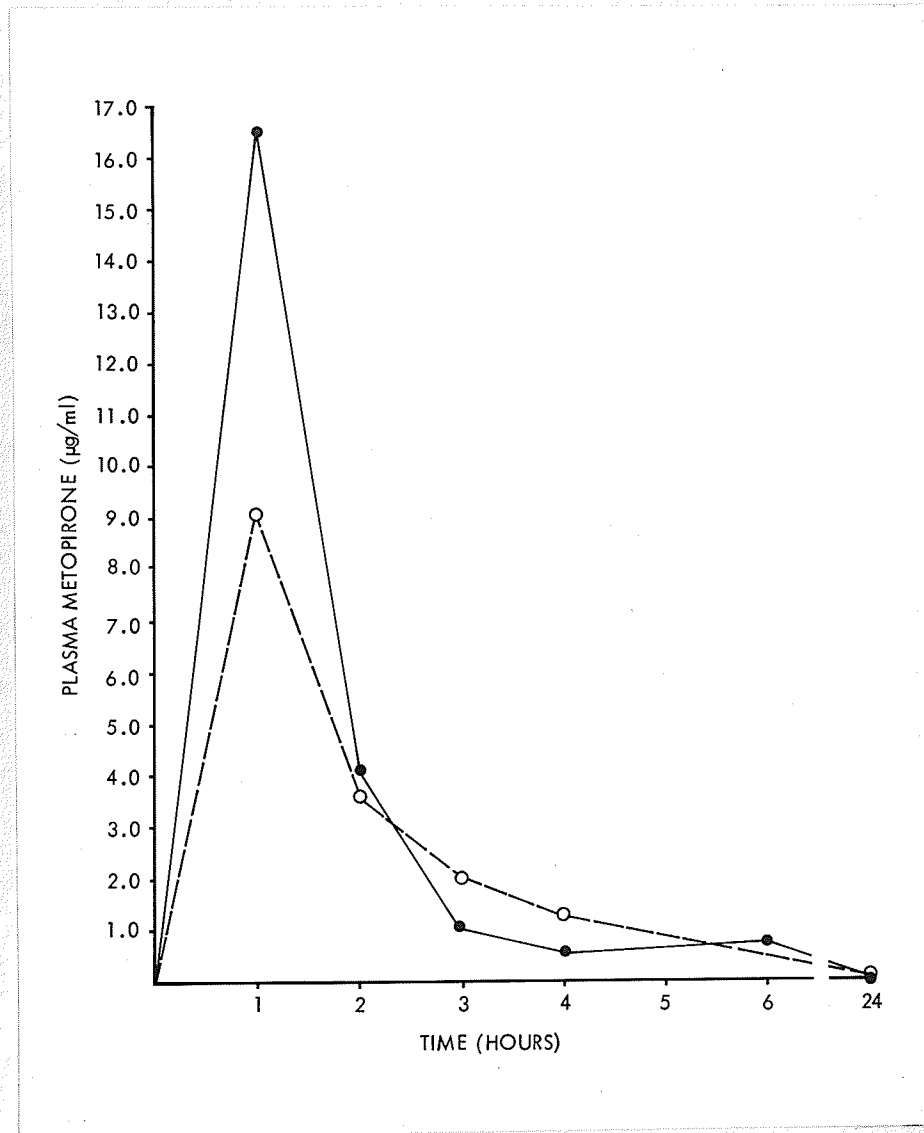


Figure 9. Metopirone concentration in maternal and fetal plasma. Time indicates the number of hours after subcutaneous injection of the mother of 100 mg/kg. Each point indicates mean of two values obtained from two different animals. The difference between individual values was a maximum of 2 µg/ml.

● ——— ● maternal plasma levels
○ - - - - ○ fetal plasma levels

DISCUSSION

Microsomal enzyme induction has become an important tool in manipulating the function of an organ system. Corticosteroids have been successfully employed in accelerating the appearance of phospholipids in fetal lamb and rabbit lung (7,8). The present study was designed to investigate the role of endogenous corticosteroids on lung maturation in both adult and fetal rabbits. Endogenous steroid production was suppressed by administering metopirone, a B-adrenal hydroxylase inhibitor. The administration of steroid, metopirone or a combination of steroid plus metopirone did not influence maternal lung pressure-volume curves, or palmitate incorporation. However, the lungs of the three treated groups had significantly lower total phospholipid, lecithin and phosphatidyl-ethanolamine content. This suggests that depression occurred at an early stage in the synthesis of phospholipids. It is not possible to speculate further on the reason for this depression since there is no direct data available to the present time on the influence of steroids or metopirone on lung phospholipid biosynthesis. Incorporation of C^{14} labelled palmitate into phospholipid indicated no difference between the four adult groups. Thus, cortisol and metopirone did not affect fatty acid incorporation into phospholipid.

Interesting changes were found in the surface tension measurements of the lung extracts of the adult rabbits. The lungs of metopirone plus steroid treated rabbits had both a lower γ min. and γ max. despite the finding of a lower total phospholipid and lecithin content. However, although the difference in surface tension was significantly different it was small and of dubious biological significance since there was no

change in the pressure-volume curves. Furthermore, the difference noted approached the error of estimation of about ± 1 dyne/cm. Changes in lung water content are known to influence surface tension (36). However, the dry weight of the metopirone and steroid treated lungs and the DNA content was similar to the control lungs.

There may be several reasons for the lack of any sizeable influence of metopirone or steroid on adult lungs. Corticosterone production in the adult rabbit may not be necessary for surfactant synthesis. In the mature lung additional steroid may not enhance surfactant production which is already optimal. On the other hand it has been noted that metopirone is not only an adrenal mitochondrial steroid hydroxylase inhibitor but it has inhibitory effects on mitochondrial respiratory chain in the liver. It reduces the oxygen uptake of liver tissue and has inhibitory effects on mitochondrial metabolism (68). Perhaps the reduction in lung phospholipid noted in this study can be ascribed to the inhibitory affect of metopirone on the lung mitochondria. In other words the effects may be local rather than through the pituitary adrenal axis. Further in vitro studies of the influence of steroid and metopirone on lecithin biosynthesis are required, to answer these questions. Of particular importance would be studies with labelled precursors for lecithin and P-ethanolamine.

The studies on fetuses were confined to the evaluation of the effect of metopirone administered subcutaneously to the maternal rabbits. Although indirect evidence has been provided by Goldman (10) suggesting placental transfer in rats, there have been no direct measurements of metopirone levels made in the fetus and the mother. A study of placental transfer of metopirone was therefore undertaken

during this investigation. Previous reports have shown plasma levels of metopirone after oral or intravenous administration only. In humans peak plasma levels are attained within one hour after oral ingestion (69). After four hours the plasma metopirone levels fall to very low levels. In the present study the plasma levels in the mother does and fetuses were estimated after varying intervals of time after injection. The levels peaked (Fig. 9) both in the mother and the fetus, at one hour after injection of metopirone, and fell by 2-3 hours. By six hours the levels in the fetus were not measurable. By 24 hours no detectable levels were found in the maternal or fetal plasma.

The fetal data show that metopirone treatment of the mother did not affect the litter size or fetal weight. The mean lung weights steadily increased with gestational age in the controls whereas in the metopirone treated group the lung weight at 30-31 days gestation was small. However, the number of observations was too few for statistical analysis. There were no differences found in percent dry weights or lung DNA content between the groups at any gestation.

The phospholipid content of lung expressed per mg DNA showed no differences between the control and metopirone treated fetuses at any gestation. The various components of phospholipids also remained unchanged. It is possible that the levels of metopirone in the fetus were not high enough and did not last long enough following a single daily injection to the mother to be effective. It is also possible that endogenous adrenal steroids play no role in the induction of lung phospholipid synthesis. Finally the direct inhibitory effect of metopirone must be considered. Radioactive labelled palmitate incorporation was studied at 30 days gestation in only a few lungs but no

apparent influence of metopirone was noted.

Lung stability studies did not reveal any differences between the two groups at any gestation. The most striking effect of metopirone was noted in the surface tension studies of lung extracts. No difference between the two groups was noted until after the 29th day of gestation. On day 30-31 a difference in minimum surface tensions become evident ($p < .05$) but this effect was not universal. The discrepancy between the findings of phospholipid content and minimum surface tension may be due to the fact that the number of observations were few. Furthermore phospholipid determinations were done on different litters than were the surface tension measurements because of a limitation in the amount of lung tissue obtainable. Thus, the average phospholipid content may reflect two populations of lungs, that is, those with normal surface tensions and those with abnormal surface tensions. Conclusions about the influence of metopirone on fetal lung lecithin biosynthesis are therefore difficult since the effect of the dose of drug used was sporadic.

The fetal data however does suggest that metopirone administration before the 30th day of gestation delays the appearance of normal surface tension characteristics of the mature fetal lung. The occurrence of a high surface tension in about 60% of the fetal lungs following a single daily injection of metopirone suggested that a more sustained level of metopirone may be required to demonstrate a more universal effect. Therefore in two instances does were given metopirone injections at 8 hourly intervals. One doe was sacrificed at day 27 and the other at day 31. On both occasions the minimum surface tension remained high suggesting that sustained levels of metopirone may show

definitive changes in the surface properties of the fetal lung. More studies are required to demonstrate this conclusively.

Obviously the mechanism of action of steroids and metopirone on surfactant biosynthesis requires further study. The present study however does suggest that endogenous corticosterone production influences the maturation of fetal lung.

SUMMARY

The possible role of endogenous corticosteroids in the induction of synthesis of lung surfactant in both maternal and fetal rabbits was investigated. Metopirone (SU 4885) an adrenostatic drug, which specifically inhibits B hydroxylation in the adrenal gland, thus preventing endogenous cortisol production was used in this study. Transfer of metopirone across the placenta was also quantitated.

Pregnant rabbits received one of four treatment regimens; saline, metopirone, cortisone or metopirone plus steroid for six days before delivery. Studies of the lungs of the adult rabbits revealed that lung weights and percent dry weight were not influenced by drug injection. However, lung DNA content (mg/gm) in the metopirone treated group was higher than the control group, indicating an increase in cell number. Compared to the saline treated group there was a significant decrease in lung phospholipid content in the other three groups ($p < .005$). A decrease in lung lecithin and phosphatidyl ethanolamine was also noted in these three groups indicating that the inhibition was in the early steps of biosynthesis of lung lecithin. The specific activity of ^{14}C labelled palmitate in lung lecithin was not altered by drug treatment indicating that saturated fatty acid incorporation into lung lecithin is not influenced by metopirone, steroid or a combination of both drugs.

Quasi-static deflation pressure-volume characteristics of the lung were also similar in all groups. However, lungs of animals receiving both metopirone and steroid had a small but statistically significant decrease in minimum surface tension ($p < .05$). The change in surface tension did not correlate with a change in lung retractive

forces or with lung lipid content and was therefore of dubious biological significance.

Metopirone administered subcutaneously to the maternal animals reached a peak plasma level at 1 hour of age and disappeared by 6 hours. The fetal plasma metopirone levels paralleled the maternal plasma levels but only reached about half the concentration of maternal plasma. Studies on control animals showed that with increasing gestation fetal lung total phospholipid and lecithin content increased. The deflation pressure-volume curves moved to the left indicating increased stability of alveoli. Minimum surface tension correspondingly falls to low levels by term (31 days).

Metopirone administration to maternal rabbits did not alter surface active properties in the fetus from the 26th to the 29th day of gestation when compared to control fetuses. At day 30-31 gestation metopirone treated rabbits had offsprings who had lung total phospholipid and lecithin contents, and deflation pressure-volume curves similar to control group, but minimum surface tension of minced lung extracts were high in about 60% of the fetal lungs studied ($p < .05$).

These findings indicate that in the pregnant rabbit metopirone crosses the placenta and plasma levels in the fetus parallel that of the mother. Since suppression of endogenous corticosterone production in the fetal rabbit increases the minimum surface tension of lung extracts of mature fetuses, it is suggested that endogenous steroid production plays a significant role in the maturation of the fetal lung.

TABLE ICONDITIONS ALTERING SURFACE ACTIVE PROPERTIES OF
LUNG

INTRINSIC FACTORS

- | | |
|------------------------------|--------------------------------|
| 1. Prematurity | 7. Extracorporeal circulation |
| 2. Pulmonary circulation | 8. Nutrition & hydration |
| 3. Pulmonary edema, embolism | 9. Hypoxia |
| 4. Emphysema | 10. Vagotomy |
| 5. Alveolar cell carcinoma | 11. Auto & homotransplantation |
| 6. Atelectasis | |

EXTRINSIC FACTORS

- | | |
|---------------------------|---|
| 1. Hydrocarbon | 7. Lipid solvent |
| 2. Volatile anesthetics | 8. Toxic inhalants |
| 3. Oxygen toxicity | 9. Phospholipids (decrease surface tension) |
| 4. Aerosol administration | 10. Steroids in fetus (decrease in surface tension) |
| 5. Detergents | 11. Constant transpulmonary positive pressure (decrease in surface tension) |
| 6. Lecithinase | |

TABLE II

MATERNAL DATA (Mean and S.E.)

	N	Body Wt. (kg)	Total lung Wt. (gm)	%Dry Wt.	DNA (mg/gm)
Control	12	3.43 (.124)	13.3 (.70)	19.7 (.36)	5.72 (.173)
Metopirone	9	3.31 (.553)	11.0 (.50)	20.8 (.50)	6.78** (.460)
Steroid	8	3.250 (.182)	11.3 (.65)	23.4* (2.03)	6.14 (.207)
Metopirone & Steroid	7	3.04 (.165)	12.6 (.58)	20.0 (.92)	5.86 (.265)

* t-test control vs steroid group ($t = -1.904$, $p > .05$)

** t-test metopirone vs control groups ($t = -2.516$, $p < .05$)

TABLE IIIMATERNAL DATASURFACE TENSION OF LUNG EXTRACTS (dynes/cm)

	Minimum (γ min)	Maximum (γ max)
Control (15)		
\bar{X}	12.6	51.5
S.E.	1.54	2.28
Metopirone (13)		
\bar{X}	11.8	51.5
S.E.	1.76	1.69
Steroid (11)		
\bar{X}	11.8	46.6
S.E.	1.58	3.46
Metopirone & Steroid (11)		
\bar{X}	8.4*	44.6**
S.E.	1.14	1.95

() Number of Observations

* t-test (metopirone & steroid) vs control group $t = p < .05$

** t-test (metopirone & steroid) vs control group $t = p < .05$
(metopirone & steroid) vs metopirone group $p < .02$

TABLE IV

DEFLATION PRESSURE-VOLUME CURVES OF MATERNAL LUNG.
Volume is expressed as a percent of the maximum in-
flation volume obtained at 40 cm H₂O transpulmonary
pressure.

	V_{\max} (ml/ gm lung weight)	30*	20*	15*	10*	5*	0*
Control (10)							
\bar{X}	3.74	94.93	93.50	90.55	83.46	63.24	11.80
S.E.	0.28	1.03	0.75	1.12	1.35	1.69	1.17
Metopirone (11)							
\bar{X}	4.4	94.82	91.96	88.81	81.69	62.27	12.66
S.E.	0.27	1.15	1.15	1.03	1.30	3.96	1.68
Steroid (8)							
\bar{X}	4.2	94.20	93.11	89.81	79.68	59.43	15.09
S.E.	0.41	1.29	1.155	1.55	3.66	4.06	.170
Metopirone & Steroid (5)							
\bar{X}	4.0	93.62	91.36	88.82	84.52	61.96	14.62
S.E.	0.11	2.28	2.46	2.41	3.13	3.59	3.03

() Number of Observations

* Transpulmonary pressure (cm H₂O)

TABLE V

MATERNAL LUNG PHOSPHOLIPIDS
(mg/mg DNA)

	Total	Sphingomyelin	Lecithin	Phosphatidyl- Inositol- Serine	Phosphatidyl- Ethanolamine
Control (10)					
\bar{X}	3.799	0.425	1.804	0.453	0.611
S.E.	0.183	0.030	0.142	0.030	0.069
Metopirone (5)					
\bar{X}	2.833	0.467	1.088	0.343	0.372
S.E.	0.185	0.123	0.193	0.053	0.082
Steroid (6)					
\bar{X}	3.032	0.356	1.367	0.327	0.346
S.E.	0.327	0.033	0.118	0.003	0.048
Metopirone & Steroid (6)					
\bar{X}	2.532	0.344	1.115	0.377	0.337
S.E.	0.09	0.052	0.066	0.003	0.055
Analysis of Variance					
F	7.080*	1.017	6.149**	2.571	4.620***
P	<.005	N.S.	<.005	N.S.	<.05

() Number of Observations
DUNCAN'S MULTIPLE RANGE TEST

* Groups	P	** Groups	P
(Met.&St.): Met.	N.S.	Met:(Met.&St.)	N.S.
(Met.&St.):St.	N.S.	Met:(St.)	N.S.
(Met.&St.): Control	<.01	Met:(Control)	<.05
Met. : St.	N.S.	(Met.&St.):(St.)	N.S.
Met. : Control	<.01	(Met.&St.):Control	<.05
St. : Control	<.05	(St.):(Control)	<.05
	*** Groups	P	
	(Met.&St.):St.	N.S.	
	(Met.&St.):Met.	N.S.	
	(Met.&St.):Control	<.05	
	St.: Control	<.01	
	Control:Met.	<.05	

TABLE VI

C^{14} PALMITATE INCORPORATION INTO
 MATERNAL LUNG TISSUE
 (Disintegration per minute/ mg phospholipid)

	Lecithin	Phosphatidyl- Inositol- Serine	Phosphatidyl- Ethanolamine	Spingomyelin
Control (6)				
\bar{X}	6636	2539	2272	2291
S.E.	1972	885	712	883
Metopirone (4)				
\bar{X}	9746	3038	3206	3039
S.E.	2818	979	882	1225
Steroid (4)				
\bar{X}	7393	2299	3586	2193
S.E.	1822	465	1118	535
Metopirone & Steroid (4)				
\bar{X}	10943	2902	4831	2603
S.E.	2624	338	986	431

() Number of Observations

TABLE VII
FETAL DATA

	Litter Size	Fetal Wt. (gm)	Lung Wt. (gm)	%Dry Wt.of Lung	DNA Content (mg/gm)
<u>Days 26 & 27</u>					
Control (3)					
\bar{X}	8.0	24.7	.822	9.26	5.12
S.E.	1.5	3.77	(2)*	(1)	(2)
Metopirone (10)					
\bar{X}	6.6	24.4	.845	11.52	4.41
S.E.	1.1	1.48	.043 (3)	1.22 (5)	.40 (6)
<u>Day 29</u>					
Control (5)					
\bar{X}	7.1	38.8	1.416	7.44	5.78
S.E.	0.7	2.54	(1)	.13 (5)	.12 (3)
Metopirone (3)					
\bar{X}	5.7	37.1	1.323	8.60	5.64
S.E.	0.3	1.37	(1)	.85 (6)	(2)
<u>Day 30 & 31</u>					
Control (12)					
\bar{X}	6.8	48.2	1.224	9.92	5.08
S.E.	0.7	2.62	.064 (8)	.40 (4)	.44 (9)
Metopirone (8)					
\bar{X}	7.6	40.1	.661	11.18	4.08
S.E.	0.8	4.4	(2)	.34 (4)	.26 (5)

() Number of Observations

TABLE VIII

MINIMUM SURFACE TENSION OF FETAL
LUNG EXTRACTS AT DIFFERENT GESTATIONAL
AGES (dynes/cm)

	Day 26 & 27	Day 29	Day 30 & 31
Control	18 21 16	8 8 9 10 8	5 6 4 6 2
\bar{X}	18.3	8.6	4.6*
S.E.	1.4	0.4	0.8
Metopirone	24 13 16 21 17 16	8 9 11	3 10 12 3 17 12 2 20
\bar{X}	18.2	9.3	9.9*
S.E.	0.1	0.9	2.4

* $\chi^2 = 4.90$ ($p < .05$)
(after Yates correction)

53
TABLE IX

DEFLATION PRESSURE-VOLUME CURVES OF
FETAL LUNG. Volume is expressed as per-
cent of the maximum volume obtained at
30 cm H₂O transpulmonary pressure

	25*	20	15	10	5	0
Day 26 & 27						
Control (9)						
\bar{X}	91.5	79.6	54.6	36.8	22.5	9.7
S.E.	1.90	3.10	3.30	3.20	1.90	1.40
Metopirone (3)						
\bar{X}	96.8	83.4	51.5	44.1	14.0	0
S.E.	3.0	2.20	1.90	4.80	0.64	0
Day 29						
Control (8)						
\bar{X}	95.3	90.1	77.9	64.5	43.4	9.6
S.E.	1.30	2.20	4.00	4.50	4.70	2.80
Metopirone (9)						
\bar{X}	96.5	91.3	81.5	61.0	37.4	10.5
S.E.	0.70	1.20	2.70	4.3	4.39	2.32
Day 30						
Control (7)						
\bar{X}	97.3	92.2	83.3	72.4	61.6	24.6
S.E.	0.47	1.60	2.20	3.60	3.30	2.50
Metopirone						
\bar{X}	95.8	90.2	81.5	72.6	59.8	14.1
S.E.	2.28	2.70	2.90	4.10	4.29	1.88
Day 31						
Control (12)						
\bar{X}	97.0	92.9	85.6	80.0	62.6	24.7
S.E.	0.47	0.77	1.20	1.20	2.30	2.50
Metopirone (5)						
\bar{X}	98.1	95.5	90.0	83.66	71.48	33.78
S.E.	0.14	0.51	0.41	0.96	0.86	3.66
() Number of Observations						

TABLE X

FETAL DATA: PHOSPHOLIPIDS (mg/mg DNA)

	N	Days 26&27	N	Day 29	N	Days 30&31
TOTAL						
Control	3		4		8	
\bar{X}		1.72		2.10		1.96
S.E.		0.81		.90		.10
Metopirone	5		4		4	
\bar{X}		1.46		1.82		2.59
S.E.		0.81		.10		.55
SPHINGOMYELIN						
Control	3		4		8	
\bar{X}		0.18		.21		.21
S.E.		.02		.05		.02
Metopirone	5		3		4	
\bar{X}		0.15		.27		.23
S.E.		0.034		.03		.03
LECITHIN						
Control	3		4		8	
\bar{X}		0.70		0.88		0.9
S.E.		.03		0.25		.06
Metopirone	5		3		4	
\bar{X}		0.61		0.79		1.00
S.E.		0.06		0.09		.08
PHOSPHATIDYL- INOSITOL						
Control	3		4		8	
\bar{X}		0.27		0.18		.25
S.E.		.09		0.05		.02
Metopirone	5		3		4	
\bar{X}		0.21		0.24		.22
S.E.		0.02		.01		.04
PHOSPHATIDYL- ETHANOLAMINE						
Control	3		4		8	
\bar{X}		0.38		0.32		0.366
S.E.		0.06				0.050
Metopirone	5		3		4	
\bar{X}		0.24		0.32		0.36
S.E.		.06				.12

TABLE XI

C^{14} PALMITATE INCORPORATION-FETAL
LUNGS
(counts per mg/phospholipid)

30 DAYS GESTATION

	Lecithin	Sphingomyelin	P-Inositol Serine	P-Ethanolamine
Control				
1	8396	955	1514	794
2	1646	387	438	719
3	2903	340	595	450
\bar{X}	4315	561	849	654
S.E.	2073	198	336	104
Metopirone				
1	2137	383	750	1000
2	3039	519	914	1436
3	3105	1091	1600	529
\bar{X}	2760	664	1088	988
S.E.	312	217	260	262

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