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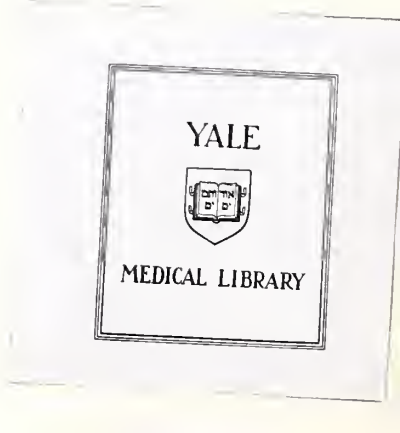

LUNG MATURITY: RESPIRATORY DISTRESS SYNDROME, PRENATAL METHODS OF ANALYSIS AND A NEW METHOD OF ASSESSMENT

BY

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... lovingly to my parents, Mom and Dad,
who never fail to listen or to care,
to my husband Jose, who constantly
inspires me to do and to be, to my
"Barrio", whose people are constantly
in my thoughts, and to my sister
Milagros, who at thirteen is just
beginning, ... as I am.

My gratitude is expressed to Dr. John C. Hobbins, for his enduring support and advice, Dr. Edward Cohart, for his unfailing inspiration, Ms. Inge Venus, for being a truly genuine person, and to Dr. Mary Keohane, whose internal support is ever present.

CONTENTS:

Introduction:

Air, like food and water, is a necessity to the continuance of life. Unlike the latter two, lack of air is not tolerated long by man and indeed death is evident when it is lacking for greater than five minutes.[1]

The major challenge facing an infant during birth is changing from one oxygen source to another.[2] This changeover can only be accomplished if and when the lungs are structurally and functionally mature, so as to prevent collapse of expanded airways in inspiration and allow gaseous exchange. Asphyxia of the newborn and immaturity account for 3.0 (1974) infant deaths per thousand live births,[3] while the death rate from all causes accounts for 16.7 per thousand live births. Demonstrations have indicated that Respiratory Distress Syndrome (RDS), a symptom complex that arises secondary to deficiency of surfactant or a surface tension-lowering substance in the lung, is strongly related to prematurity, insofar as this is related to shortened gestational age or immaturity.[4-6] Respiratory Distress Syndrome or Hyaline Membrane Disease (HMD), the pathologic diagnosis, account for an estimated twelve thousand neonatal deaths per year.[7] RDS/HMD is therefore, the leading cause of neonatal deaths,[2-7] with congenital anomalies of the newborn and other diseases of early infancy being ranked second.

This thesis highlights epidemiological risks and factors of the Respiratory Distress Syndrome, focuses on prenatal methods utilized in evaluation of fetal lung maturity, and attempts to disclose a new

simple method of assessing lung maturity of the fetus.

The Normal Lung:

In the normal lung development is in a glandular fashion at approximately twenty-eight days post conceptual age. The "canalicular stage,"[8] or development of premature airways, lasts from the sixteenth week to approximately the twenty-fourth week. Thereafter, the "alveolar stage,"[8] with development of alveolar ducts and bronchioles begins and continues until birth.

The lung is fluid filled from the beginning of the "canalicular phase,"[8] until birth. This fluid must be either expelled during delivery, with thoracic compression as it passes through the birth canal, or absorbed by capillaries and lymphatics, by movement of the fluid into the interstitial space, following initial inflation of the lung after delivery.[7]

The alveolus is the portion most likely to be underdeveloped secondary to prematurity. During its development, there is a change in the appearance of the epithelial cells of its lining. These cells at approximately eighteen to twenty weeks are vacuolated and contain cytoplasmic inclusion bodies. The cells which line the alveolar ducts at this time are termed "alveolar epithelial Type II cells."[2,6] Their presence has been noted to occur simultaneously with the occurrence of pulmonary surfactant in the extracts of lung tissues and indeed, production of cellular surfactant can be enhanced by certain agents, in particular glucocorticoids.[6,9-18]

Surfactant:

The greatest resistance to aeration or inflation of fetal lungs is produced by the surface tension of the lung liquid. Pulmonary surfactant in the lung liquid of the mature infant lowers the surface tension and thereby lowers the pressure needed to inflate the smallest units of the lung or alveoli.[20] This tension lowering capacity is in keeping with the Law of Laplace,[21] where pressure (P) is equal to tension (T) divided by the radius (r) multiplied by two, or $P = 2T/r$. The pressure required to inflate the lung depends on the size of the radius, and on the presence or absence of surfactant, since this substance lowers the surface tension (T).

Since demonstrations[6] suggested that fetal lung movements resulted in extrusion of endotracheal fluid containing pulmonary surfactant into amniotic fluid, many investigators began to try to unravel the composition of amniotic fluid and surfactant. The basic surfactant composition is a complex of many lipids, approximately eighty-five percent of which are phospholipids. Of these phospholipids, dipalmitoyl phosphatidylcholine (DPPC), or lecithin, make up approximately seventy-five percent.[6,9,19,22]

Not only was DPPC the principal component of surfactant, it also appeared to be the principal functioning component as well. By 1968, a detailed composition of human amniotic fluid had been reported. Nevertheless, it was not until 1971, that Gluck and coworkers suggested that the concentration of amniotic fluid

phospholipids might reflect formation of pulmonary surfactant and fetal lung maturity. There are five pathways of DPPC synthesis in the mammalian body.[19] The cytidine diphosphate (CDP) pathway has been shown to be the major pathway of all lecithin synthesis. This synthesis has been shown to be active at approximately thirty-five weeks gestational age. There appears to be a smaller amount of lecithin synthesized by an alternative methylation pathway, at approximately twenty-two to twenty-four weeks.[19] This production is of little significance.[19] On the assumption that increasing amounts of amniotic fluid lecithin reflected increasing production of surfactant and hence maturation of the fetal lung, Gluck et al. suggested that at various ratios of lecithin to sphingomyelin in amniotic fluid, one could estimate lung maturation. Physiological control of pulmonary surfactant was investigated, and demonstrated that glucocorticoids tend to accelerate lung maturation. The hypothesis that RDS may therefore result from a deficiency in glucocorticoids has been examined. Indeed, infants who develop RDS tend to have lower cortisol levels than infants of comparable gestational ages. Investigators have also noted good correlation of cortisol levels with the L/S ratio. Some studies estimate that RDS will not develop at cortisol levels of greater than sixty nanograms per milliliter.[24] These lines have led to treatment of mothers with premature rupture of membranes or other high risk problems with glucocorticoids in an effort to increase pulmonic maturation in the infants.[25] Studies thus far have been encouraging, although further confirmation is necessary.

In summary, surfactant has been said to be the pulmonary surface-active material located in the alveolar lining layer. This anti-atelectasis factor provides a low and variable surface tension and serves the two fold function of decreasing the pressure needed to distend the lung, and maintains alveolar stability in a wide range of local volumes.[6]

Lung Pathology of Hyaline Membrane Disease:

For infants with immature lungs each inhalation is as difficult and demanding as the first because of the tendency, at exhalation, for the alveoli of the lungs to deflate completely and collapse producing atelectasis. This pathology known as Hyaline Membrane Disease, is manifested clinically as RDS.

Criteria for diagnosis of Hyaline Membrane Disease differ among pathologists. Some pathologists require as criteria atelectasis, membrane formation, or both.[26,27] Not surprisingly, investigators of the same case material may disagree in their calculations of incidence.[28]

At autopsy, the lungs appear deep purplish red and are liverlike in consistency. Microscopically, the process is uniform throughout the lung with extensive atelectasis and engorgement of the interalveolar capillaries and lymphatics. Individual alveoli are rarely distinguished. Membranes are rarely present in those infants who live less than a few hours. Of those infants who live longer, a number of alveolar ducts and respiratory bronchioles are

lined with acidophilic homogeneous or granular membranes. Leukocytes are usually present in these infants, but are rarely found in those who live a short period of time. Epithelial necrosis of the respiratory bronchioles is an additional reaction that may suggest injury.[29] Amniotic fluid, intra-alveolar hemorrhage, pneumonia and interstitial emphysema are additional but inconsistent findings in the lungs of those infants who succumb after seven to ten days secondary to other complications.[30]

Electro-microscopically, the membrane is composed of a cellular matrix of fibrin and cellular debris. The basement membrane of alveolar cells is disrupted and epithelial cells tend to have a variable appearance.[31,32] The type II granular pneumocytes, where surfactant production is thought to take place, contain a large number of osmiophilic inclusion bodies. Since these inclusion bodies are thought to relate to pulmonary surfactant, many questions arise as to their possible pathology.[33,34]

Respiratory Distress Syndrome

Epidemiology:

Respiratory Distress Syndrome (RDS) is manifested in approximately one percent of infants during the first days of the neonatal period. It continues to be the greatest killer of infants during that time interval.

Up until 1968, mortality statistics were based on estimated figures. Farrell and Wood[7] were the first to compile nationwide statistics to determine the extent of the disease. These results have shown an estimated national mortality rate of 28.8 per thousand premature births for 1968, that has risen to 35.9 for 1973. Mortality rates associated with HMD/RDS increased during the six year period from 2.36 per thousand live births, to 2.73 per thousand live births. On the basis of mortality rates, a trend toward an increased incidence of fatal HMD/RDS was established from 1968 to 1973. This is attributed to greater diagnostic ability. Analysis of the mortality data during the 1968-1973 time period reveals a rather uniform seasonal distribution of RDS that clusters around the summer months, May through August, and shows, correspondingly, low points during the winter months, January through February. This occurrence is noteworthy, in terms of alerting clinicians to a greater mortality threat during particular time periods. Age at time of death from RDS decreased in an exponential fashion between the first and fourth days. Ninety-two percent of all deaths occurred by four days while ninety-six percent occurred by seven days of age. There appears to be an associated male predominance in fatality from RDS, at a ratio of 1.7/1. A greater number of white fatality, eighty-one percent, compared to eighteen percent for black fatality was noted. Black fatality was noted to be disproportionately high when one takes into account that they contribute only fifteen percent to the total birth rate. This higher rate is attributed to their greater incidence of prematurity.

Diagnosis:

Respiratory Distress Syndrome as the clinical expression of an immature lung, leads to extremely labored breathing and perfusion of non-ventilated areas in the neonate. This pathology, evident within two hours of birth,[35] is clinically manifested in the newborn as cyanosis,[36] chest wall and sternal retractions, tachypnea, systemic hypotension,[37] and expiratory grunting associated with whining and nasal flaring, that complete the picture of an infant valiantly increasing its efforts to increase oxygenation.[39] A characteristic chest film of a diffuse reticular granular pattern with air bronchograms is usually evident.[38]

Physical examination can be helpful when signs of hypotension, fine bibasilar inspiratory rales, edema, ileus, and oliguria are present.

Complicating the syndrome is a persistent Patent Ductus Arteriosus (PDA), leading to the possibility of development of severe congestive heart failure and pulmonary edema. This complication is more than likely secondary to prematurity. Although PDA's usually close spontaneously after birth, it is estimated that the ductus remains patent in approximately ten to fifteen percent of all premature infants.[3] In these infants, the ductus usually closes spontaneously at approximately twelve weeks. In infants with RDS, this complication may greatly incapacitate the infant, requiring ligation of the ductus or closure by indomethacin. Since infants who suffered Premature Rupture of the Membranes (PROM) for greater

than seventy-two hours were noted to have a decreased persistence of PDA's,[40] it has been postulated that perhaps PROM should be tolerated for this period of time in premature infants to increase closure of the ductus.

Intracranial hemorrhage has been associated with HMD.[41-43] This association may be due to tissue hypoxia prior to death, a decrease in clotting factors associated with hypoxia, or to elevated cerebral venous pressure secondary to grunting and increased respiratory effort.

The exact diagnosis of HMD as a pathological diagnosis can only be verified at autopsy. There is nevertheless, a high correlation between the clinical findings, described as RDS, and the pathological findings of HMD.[6,9,22]

Etiology:

One of the most frequently cited associations with RDS is prematurity/immaturity.[6,9,22] Dunn,[44] noted that of those neonatal deaths linked to RDS, eighty-one and one-half percent were among infants less than thirty-seven weeks gestation. Seventy-three and one-half percent were less than twenty-five hundred grams. An attempt was made to define prematurity and immaturity. The former encompassed low birth weight infants, of less than twenty-five hundred grams, whereas the latter comprised those infants of shortened gestational age who nevertheless might be heavier than average. On the basis of this separation, Dunn concluded that RDS was related to low birth

weight, only as this was related to immaturity, a conclusion that is still undisputed.[6] Usher and coworkers[45] similarly correlated relationships of prematurity to the development of RDS. Unfortunately, much confusion has been perpetuated in the exact definition of prematurity, immaturity, and low birth weight. Authors continually use them interchangeably. Drillien and coworkers[46] in fact define low birth weight and not prematurity, as less than twenty-five hundred grams and/or a gestational age of less than thirty-eight weeks. RDS appears in immature and/or low birth weight infants whose weights are low secondary to prematurity and is a symptom of developmental immaturity.[49]

Immaturity/prematurity occur in approximately eight percent of all live births, to as high as fifteen percent in certain hospitals.[50] It seems inevitable with its high association to RDS, that investigators should turn to the many causes of prematurity. These are primarily environmental and maternal.

Environmental factors have led to evidence that prematurity is greatest among poor, non-white, unmarried and older mothers with inadequate prenatal care.[7,51,52] It seems not at all surprising that low socio-economic factors, poor health, poor nutrition and obstetrical complications such as pre-eclampsia, infection and ante-partum hemorrhage also lead to prematurity. Naeye[52] and Blanc noted RDS to have an increased severity in white male infants, infants of Puerto Rican mothers who worked actively during their pregnancies, and among the offspring of the poor.

Of interest, is a possible genetic link to the development of RDS. Lankernau and Opitz,[53] noted an interesting relationship among premature first cousins of affected infants. Only the offspring of maternal aunts showed an increased incidence of RDS. This finding suggests the occurrence of a maternal dependent genetic factor predisposing the infants of certain mothers to developing RDS. RDS was increased in siblings by approximately twelve to nineteen percent. That incidence was increased to thirty to fifty percent, if these siblings were also of low birth weight or premature. There was a relative absence of RDS among identical first born twins, while there appeared to be a greater occurrence among second born twins. This finding, correlates well with Verduzco and coworkers[54] finding that RDS occurs in twins and is predisposed by prematurity, with greater risk to twin B secondary to asphyxia.

Maternal diseases add further to the risk of development of RDS. One important disease that has been implicated is maternal diabetes.

Prior to the utilization of insulin, in 1921, most diabetic females were too ill to conceive, let alone bring to term the product of a conception. The precise etiology of this infertility is not clear. Of those mothers fortunate enough to conceive, during those times, a maternal mortality of twenty-five percent as well as a fifty percent infant mortality faced them. Diabetes was and still appears to be deleterious to a pregnancy in several ways. For instance, the incidence of pre-eclampsia and eclampsia is increased approximately fourfold.[55] A greater susceptibility

to infection and difficult delivery secondary to macrosomia of the infant are also evident. The fetus has a tendency to succumb before the onset of spontaneous labor and is, therefore, at greater risk for cesarean section and its potential complications. Postpartum hemorrhage is a very common occurrence in vaginal deliveries. Dystocia, birth trauma, and/or RDS and metabolic derangements that include hypoglycemia and hypocalcemia are common. The fetus of the diabetic mother, therefore, suffers a greater perinatal mortality and morbidity risk. Prognosis is, therefore, guarded, with mortality and morbidity related generally to maternal classification of diabetes. Cruz et al.[56] noted that the only complication which added to the risk of development of RDS with any significance was insulin dependency. Although this study and others with similar conclusions attempt to show diabetes as a risk factor to the development of RDS, others, such as a large study at the Joslin Clinic,[57] indicate that only prematurity and not diabetes, nor any other severity of disease, influences the development of RDS. Class A or chemical diabetes is estimated to involve an infant mortality of 3.6 percent, while all other classes range from sixteen to twenty-three percent. Roberts and coworkers[60] favored the finding that RDS is associated with certain classes of diabetes.[58,59] They hypothesized that insulin present in greater amounts in the fetus, secondary to maternal diabetes, may in fact antagonize cortisol by a blocking mechanism on its enzyme induction system capacity in the lung, leading to poor synthesis of surfactant and thereby predisposing these infants to the development of RDS. In keeping with this

philosophy is a study by Smith, Giroud, and Avery,[61] where it is noted that, although cortisol enhanced lecithin synthesis and reduced cellular growth, addition of insulin abolished this stimulation effect. Therefore, hyperinsulinemia of the infant of the diabetic mother may interfere with the physiological process of glucocorticoid induced pulmonary maturity and may be a causal factor in the development of RDS in this group of infants. Although it would seem that a causal relationship might be established, there continues to be much debate on past and present evidence. Regardless of the disputes, it seems unfailingly clear that infants of diabetic mothers are at greater risk in developing RDS, be it secondary to insulin antagonism of cortisol or prematurity.

Much controversy has centered around the incidence of development of RDS in the infant of the pre-eclamptic or eclamptic mother. Pre-eclampsia,[62] the development of hypertension with proteinuria and/or edema, usually occurs after the twentieth week of gestation and is induced by pregnancy. Eclampsia, commonly is the occurrence of convulsions, not secondary to any neurological disorder, but due to the underlying hypertension. Studies by Hendricks et al.[63] and Lee and associates[64] have noted the occurrence of the disorder to be associated with a higher incidence of prematurity, but a lower incidence of developing RDS. This lower incidence is most likely related to the glucocorticoid theory of maturation. Lee noted that those infants, of eclamptic mothers, less than thirty-three to thirty-six weeks or less than fifteen hundred grams had an incidence of RDS similar to uncomplicated

pregnancies of similar gestational age, inferring that lung maturation cannot be induced at very premature gestational ages.

Premature rupture of membranes (PROM),[62] classically defined as rupture of membranes prior to the onset of labor, has caused controversy regarding management of the expected fetus. Some authors advocate aggressive termination of the pregnancy to avoid the ever present danger of severe maternal and/or fetal infection. The possibility that the infant will then be capable of subsequent normal development is then questioned. Numerous studies[62-63] have shown a tendency of increased pulmonic maturation associated with delayed birth following PROM. Unfortunately, much dispute has continued with regard to the actual amount of time necessary to allow this maturation to occur. Chiswick and associates[64] noted RDS to be decreased in infants born after forty-eight hours of PROM, when compared to those born after twenty-four to forty-eight hours. Others[65,66] noted this decrease in RDS to occur at greater than twenty-four hours after PROM, while Bauer et al.[67] noted this time period to be greater than sixteen hours. Lee et al.[49] considered the relationship to gestational age and noted a decrease in RDS only if infants weighed fifteen hundred to twenty-five hundred grams or were of a gestational age of thirty-three to thirty-six weeks. Berkowitz et al.[68] noted no decreased incidence of RDS in gestational ages between thirty-two to thirty-six weeks regardless of the length of PROM, but noted a significant decrease in RDS in infants less than thirty-two weeks who had suffered PROM for greater than sixteen hours. It may be concluded from these

and various other studies, that there is indeed an accelerating effect of PROM on the maturation of the fetal lung. Prolongation of PROM to sixteen to twenty-four hours may be of value to some infants in avoiding RDS, but other underlying factors must be involved in the maturation of the lung in other less fortunate infants. Clarification of management is therefore needed.

Heroin and alcoholism have been investigated as to their effect on RDS. It has been noted[69-70] that infants of heroin addicted mothers did not suffer from an increased incidence of RDS, irrespective of gestational age. Infants of alcoholic mothers conversely not only suffered from a higher incidence of RDS but also of perinatal and postnatal growth deficiency, developmental delays and also congenital anomalies.

Iatrogenic cesarean section is a potential complication that increases the incidence of RDS. Studies by Hack et al.[71] and Goldenberg and associates[72] have brought the implications to the foreground. These studies noted that fifteen to eighteen percent of infants develop RDS secondary to obstetrical misjudgement of gestational age. This common dilemma, secondary to inadequate means of verifying clinically the gestational age of pregnancies at high risk, must be avoided. Fundal examination is obviously an inadequate measure of gestational age. New simple methods are, therefore, needed so as to safeguard the delivery of a term infant.

Complications of Amniocentesis:

Prenatal analysis of amniotic fluid has become widespread following the discovery that amniotic fluid composition reflected fetal lung maturation and therefore, could be utilized to predict maturity.

The majority of these techniques for analysis are based on the invasion of the uterus with withdrawal of amniotic fluid for analysis. Although this is a widely used obstetrical procedure for both diagnosis and therapy, most complications from the technique are associated with therapeutic indications. There are, nevertheless, real risks associated with amniocentesis for diagnostic purposes, such as evaluation of fetal maturity during late pregnancy. Of the complications reported, most have been associated with injury to the fetus, placenta, or umbilical cord. Occasionally, there have been those reported cases where the mother has been involved. Mid-trimester amniocentesis has been known to be relatively safe as compared to those performed at or close to term. It is these latter procedures that are generally utilized to assess fetal maturity, that warrant discussion. Numerous studies have noted risks that include, pneumothorax,[73] pneumopericardium,[74] splenic rupture or laceration,[75] ocular trauma,[76] fetal exsanguination,[77] intra and extraperitoneal major bleeding,[78,79] amniotic fluid embolism,[80] chorioamnionitis,[81] premature labor,[82] abruptio placenta,[83] maternal rigors and pyrexia,[84] fetal cardiac tamponade,[85] and, the most lethal, fetal and maternal death.[86] These occurrences though rare

are real. In one study,[87] spontaneous labor followed 9.8 percent of amniocentesis, with cesarean section for fetal distress necessary for seven percent. The most serious complication, fetal death, occurred in .41 percent of patients. Regardless of these complications, amniocentesis for evaluation of fetal lung maturation has decreased the incidence of RDS in most institutions, and has eliminated the element of surprise postnatally for the obstetrician as well as for the pediatrician. Because of the potential complications, it seems imperative that obstetricians carefully evaluate clinically gestational age in all patients and limit the utilization of this valuable technique for high risk patients.

Methods of Prenatal Diagnosis:

Prediction of the risk of development of RDS is important for often delivery can then be deferred or glucocorticoids administered in hopes of accelerating lung maturation. Following Avery and Mead's[88] observation that lungs from infants dying of HMD as well as from immature neonates were deficient in surfactant, more rational approaches to research were developed, especially by way of laboratory studies.

Lecithin/Sphingomyelin:

During the 1960's, the composition of surfactant was demonstrated. Composed almost totally of phospholipids, studies revealed

that these lipids increased with gestational age and that lecithin was diminished in amniotic fluid of infants with RDS.[89,90] Using this information, Gluck and coworkers[23] reported the early prenatal prediction of fetal lung maturation, by evaluation of the lecithin/sphingomyelin ratio (L/S). It has been confirmed that at a ratio of two, the likelihood that an infant will develop RDS, if delivery is attempted, is minimal,[91] and if such an infant does develop RDS, morbidity is greatly reduced.[92] Although confirmation has led to acceptance of its correlation with fetal lung maturity, it has been noted by Gluck and coworkers[55] and other researchers,[93] that the L/S ratio in many high risk pregnancies does not yield as good a correlation as expected. These findings, suggest that perhaps biochemical maturation of the fetal lung may be either accelerated or delayed depending upon the maternal disease. Gluck and Kulovich noted two different means in the measurement of L/S ratios during gestation in various maternal diseases. Results indicated that pregnancies complicated by hypertension, eclampsia, renal disease, cardiovascular disease, diabetes mellitus of the D, E, F class, Sickle cell disease, and diseases of the placenta tended to have an accelerated maturation curve. The L/S ratio in these diseases would be at two at approximately thirty-two to thirty-three weeks as compared to normal maturation with an L/S of two at approximately thirty-five weeks. Other disease entities such as diabetes mellitus class A, B, C, chronic nonhypertensive glomerulonephritis and fetal diseases such as hydrops fetalis of Rh^- disease, and the smaller twin of non-parasitic monoclonal twins, tended to have delayed

maturation. The average fetal maturation of an L/S ratio of two was then approximately thirty-seven weeks gestation to as much as forty weeks. This last category is of great interest because these infants are at higher risk of development of RDS if delivered during a gestational age that would otherwise represent maturity.

The L/S ratio is still the single best assessment of fetal lung maturity,[94,95] since many problems in its determination have been elucidated.

Centrifugation speed markedly affects the L/S ratio, and therefore may vary studies depending on preparation methods. It has been noted that the L/S ratio of mature fluid falls with increasing gravitational force according to Cherayil et al.[97] According to Gluck's method, sample preparation is done at 5,400 rpm's for five to ten minutes and an L/S of two derived from this method is considered mature. If one lowers the gravitational force, interference with separation of lecithin and sphingomyelin spots may result on thin-layer chromatography and yield falsely high L/S ratios. It is not surprising that other studies evaluating L/S and RDS, have not provided similar results since changing Gluck's original methodology may lead to error of prediction.[96,98]

Other factors that have been implicated in accelerating or delaying L/S ratios are contamination of the fluid by blood or meconium. Buhi and Spellacy,[99] as well as Gluck and Kulkarni,[100] noted that serum contained an L/S of approximately 1.31 to 1.46. Therefore, addition of serum to amniotic fluid decreases a high L/S ratio while it may increase a very low L/S ratio. Addition

of meconium decreased or increased the L/S ratio in a similar fashion. Because of these problems workers have investigated other means of determining fetal lung maturation or surfactant development. Gluck and coworkers,[101] noted the correlation of levels of phosphatidylinositol and phosphatidylglycerol. The former was noted to increase during early gestation, the latter during late gestation paralleling lung maturity. These two parameters can add further to assessment of fetal lung maturation, and may lead to greater support of the mature L/S ratio report. A mature report at present does not guarantee that RDS will not occur since one to three percent of infants will develop RDS despite the report of maturity. The immature report, indicates that the risk of development of RDS is high and probable but not one hundred percent.

Other Amniotic Fluid Methods:

Total phospholipid concentration has been evaluated in order to devise a simple method of measuring fetal pulmonic maturity. Nelson and Lawson,[102] Schreyer and associates,[103] as well as Singh et al.,[104] have noted a strong correlation of this method with the L/S ratio. These determinations unlike the L/S ratio are not dependent on a ratio or on the level of sphingomyelin. They are instead dependent on volume and have been shown to lack precision in determining intermediate zones of maturity when compared to the L/S ratio.

Clements and coworkers,[105] introduced a fast, simple technique, the foam test, dependent on pulmonary surfactant's ability to generate stable foam in the presence of ninety-five percent ethanol. The foam test appears to have high predictive value[105] when applied to non-contaminated amniotic fluid, for meconium and blood tend to mature the foam leading to false positive results. This rapid test has been applied postnatally as the gastric aspirate shake test for assessment of an infant's possibility of developing RDS,[106] and has become a further aid in the assessment of lung maturation.

Recently, new literature on various methods of evaluation of amniotic fluids has arisen.

Measurement of surface tension of amniotic fluid by Goldkrand and coworkers[107,108] yields good correlation with the L/S ratio. A surface tension of less than fifty-six dynes per cm. at 120 microliters of extract and less than 46 dynes/cm., at 220 microliters of extract denotes pulmonic maturity or an L/S ratio of two. Blood and meconium contamination also appear to mature results. Their study revealed no false positive results, in comparison to the L/S ratio while 13.6 percent false negative results were attained. This method of measuring the surface tension lowering properties of amniotic fluid lipid extract appears to be a good direct method of assessment of fetal lung maturity. Since this method correlates well with the L/S ratio yet is quicker, and more direct, it seems logical that future advances in this method may add to its precision and to our clinical knowledge of fetal lung maturation.

A similar analysis derived from the above method is the lipid globule technique.[109] This method based on surface tension, involves the utilization of surface tension lowering properties of amniotic fluid lipid extract and its ability to form a globule when the surface tension reaches 36.9 dynes per centimeter. Since the amount of extract required to create a globule varies dependent on the degree of fetal maturity, the volume required can be used to differentiate pulmonic maturity. The RDS predictive value was one hundred percent but resulted in nine out of seventy or a twelve percent false negative value. Thus far the predictive value of surface tension analysis seems encouraging, but tends to have false negative results that may create difficult decision making problems for the obstetrician. Perhaps, with further analysis, advances will be made to avoid this pitfall.

Optical density analysis at 650mm of amniotic fluid introduced by Sbarra et al.,[110] provides a very simple measure that is thought to correlate with the L/S ratio. An optical density of 0.15 or greater, correlates one hundred percent with an L/S ratio of two or greater, below 0.15 correlates ninety-four percent with an L/S ratio of less than two but resulted in a value of six percent false negatives. In this small and first study, more data is needed to evaluate further the findings suggested.

The L/S ratio continues to command the obstetrical scene at the moment; however many problems that have been discussed have arisen that make further investigations for a simple and more precise method warranted.

Analysis of other components of amniotic fluid such as fetal cells,[111] creatinine,[112] as well as hormonal levels of estrogens[113] and thyroid[114-117] have not yielded information that can precisely aid the clinician in a decision making position. However, many have added to the understanding of lung development such as the relationship between cortisol and fetal maturation. However, further investigation is needed in all areas to establish precise evaluation and understanding both clinically and physiologically.

A New Method of Analysis of Fetal Lung Maturity:

The foregoing discussion of RDS, as well as review of prenatal methods of analysis, show advances in medicine that reflect much thought and time. These thoughts are a fitting introduction to the analysis of a new method for assessment of fetal lung maturity, which takes into account the whole surfactant composition and is independent of the lipid level of amniotic fluid.

The following study is based on a specially designed instrument, the "Elscint Fetal Lung Maturity Analyzer."[118] This instrument evaluates surfactant composition based on fluorescent polarization (FP).[119] The analysis of the recorded signal, FP, is a function of the intrinsic microviscosity of lipid aggregations in amniotic fluid.[119]

I Methodology:

A. Technique:

The fluorescent polarization technique utilizes a hydrocarbon chemical probe, 1,6-diphenyl 1,3,5,-hexatiene (DPH), and is a good measure of the microviscosity of cell membranes and liposomes.[119] The values obtained are determined by measuring the amount of rotation of the DPH dye probe in a sample of amniotic fluid. This rotation is dependent on the microviscosity of the hydrocarbon region of the lipids. It has been shown that polarization of DPH, decreases as the L/S ratio increases. The FP value is a measure of the intrinsic microviscosity of the lipids. Under experimental conditions, the value of a DPH labelled specimen can extend between 0.000 to 0.460. The FP is specifically defined as:

$$FP = \frac{I_{II} - I_I}{I_{II} + I_I}$$

Where:

I_{II} = fluorescent intensities directed through an analyzer with the direction of polarization parallel to the direction of polarization of the excited light.

I_I = fluorescent intensities directed through an analyzer with the direction of polarization perpendicular to the direction of polarization of the excited light.

B. Instrumentation:

A specially designed instrument is utilized whereby light is polarized by a Glan-Thompson[119] polarizer and excites the DPH probe dissolved in the dispersion of amniotic fluid and phosphate buffered saline (PBS). Microviscosity is dependent on temperature. Therefore, the cuvette containing the amniotic fluid to be sampled, is placed in a thermoelectrically temperature stabilized compartment with a temperature of twenty-five degrees centigrade. The emitted light from the probe is polarized as a function of the microviscosity. This polarization is detected by measuring two perpendicular channels that tend to measure the intensity of light. This measurement is amplified, balanced, and fed to a calculating unit. The calculated depolarization value is digitalized and displayed on the front panel of the instrument. This value, FP, is related to lung maturation, by correlation with the L/S ratio and gestational age.

C. Amniotic Fluid Samples:

Samples of amniotic fluid were made available for this study from patients who underwent amniocentesis for prenatal evaluation at the Perinatal Unit of Yale New Haven Hospital, during 11/76 through 2/78. Each sample was labelled with type of disease, and gestational age. Almost all samples were sent for L/S ratios except many of those from Rh^- patients. Samples were examined immediately or frozen to be done later. Long term freezing and thawing at any

time up to three months did not seem to affect the FP value in any uniform way.

D. Materials:

1. DPH Fluorescent Probe:

Sealed ampules of fluorescent molecular probe EF-101 were obtained from Elscint LTD. These were stored at room temperature in aluminum foil.

2. Dulbecco's Phosphate Buffered Saline (PBS):

Each of the following:

Nacl	8.5 gm./liter
NaH_2PO_4	0.1 gm./liter
Na_2HPO_4	0.4 gm./liter

were added to 100 mls. of distilled water. A pH of 7.4 was obtained by titration with either 1N HCL or 1N NaOH.

Of the above solution, 20 mls. were removed at each run of samples, to this 180 mls. of distilled water was added to bring the total volume to 200 mls. After termination of the experiments that day, the remaining amount of this solution was discarded. The remaining 80 mls. of PBS was stored at room temperature for further use.

3. DPH Probe Preparation:

One sealed ampule of DPH (D 1.) was dispersed by pipette into

200 mls. of vigorously stirring PBS solution (D 2.). The obtained solution is clear and practically devoid of fluorescence. A fresh dispersion is made each day prior to the series of experiments.

E. General Procedure:

1. Preparation of Elscint Microviscosimeter:

Twenty minutes prior to preparing the samples, the instrument's ON switch, located on the instrument panel, is depressed. The ON switch on the Auxiliary Power Supply, or START switch is also depressed for the Mercury Arc Lamp.

2. Amniotic Fluid Preparation:

Each sample of amniotic fluid is centrifuged at 1500 g's for 10 minutes. Then, 0.5 mls. of clear supernatant is pipetted off. To this is added 2.0 mls. of the DPH dispersion (D 3.). Inherent in the assessment of the FP value is a twenty percent dilution of amniotic fluid. The mixture thus prepared is shaken vigorously and incubated in a water bath at 37° C for twenty minutes, after which the samples are left at room temperature for fifteen minutes.

3. Analysis:

The sample to be analyzed is placed into a clean fluorescent quartz cuvette and placed in the sample holder of the instrument. The temperature is checked to be 25°C with the appropriate controls on the instrument. The cover for the sample is properly positioned

and the sample is analyzed according to Elscint Directions.

This basic methodology was used on all the experimental investigations performed.

II Experiments:

A. Experiments were carried out to study the effect of the following manipulations on the FP value.

1. Dilution of amniotic fluid utilizing:
    a. H_2O
    b. PBS--phospate buffered saline
2. Addition of maternal serum
3. Addition of meconium utilizing:
    a. mature meconium from a term infant
    b. immature meconium from a preterm infant
4. Freezing and thawing

Inherent to the test is a 20% dilution of amniotic fluid (2 mls. of PBS dye solution). This is not considered a dilutional effect for it is necessary to calculate the FP.

1. Effect of dilution on FP value utilizing PBS without dye and H_2O without dye:

Although a 20% dilution of amniotic fluid exists in order to calculate the FP value, further dilution of amniotic fluid by phosphate

buffered saline and distilled water was assessed to evaluate any change that this might incur on the original FP value. This dilutional change might be associated with technical error in preparation of the samples for analysis.

Five amniotic fluid samples of varying FP values were examined. Two of the five samples were made from pooled amniotic fluid samples (Table 1). Each of the specimens was treated as per protocol in the methodology section. The results are tabulated in Tables 1, 2, and 3, while they are graphically displayed in Figure 1. In the initial experiment (Table 1) no significant change in FP value was demonstrated until a five fold dilutional level. Thereafter in the remaining experiments the earlier dilutions (less than five fold) were eliminated.

Diluting the amniotic fluid matured or decreased the FP value. This maturation tendency was especially evident at a tenfold dilution (10%), except for sample #4 (Table 1) where the maturation occurs at a fivefold dilution (20%). Above these values, the FP remained stable at approximately the initial FP value. This decrease in FP value may cause false positive interpretation of maturity at critical decision levels if they were to occur. This possibility is, however, unlikely if one adheres to the selected proportion of amniotic fluid and dispersion solution required to carry out the analysis.

2. Addition of Maternal Serum:

Fetal and/or maternal blood may be an inadvertent contamination

of amniotic fluid during amniocentesis. Because of this potential problem, evaluation of the effect of varying amounts of serum contamination were made on the FP value. Serum was used because all samples are subjected to centrifugation for the determination of the FP value. The effect of different blood preparations was also assessed because of the possibility that samples may not have been immediately frozen and clotting may have occurred.

Blood was collected from pregnant females, allowed to clot, or immediately frozen, or placed in heparin to avoid clotting. From these three sources, serum was removed after centrifugation. Two amniotic fluid samples were used to assess the results. Each specimen of amniotic fluid was analyzed according to the methodology section.

The results are tabulated in Tables 4 and 5 with graphical display in Figure 2.

Diluting amniotic fluid with even very small amounts of maternal serum tended to decrease (mature) the FP value in a similar fashion as did the PBS and H_2O dilutions. This maturation tendency was especially evident at a tenfold dilution or at ten percent amniotic fluid. Above this level, the FP value remained stable at approximately the initial FP value. Thus, substitution of two percent serum to amniotic fluid-DPH dispersion mixture caused the FP value to fall significantly.

Amniotic fluid increases rapidly to an average volume of 50 mls. at 12 weeks gestation and 400 mls. at midpregnancy; it reaches a maximum of about a liter at 36 to 38 weeks gestation.

The volume then decreases as term approaches, with variations of 200-800 mls. At ten percent amniotic fluid, 0.40 mls. of serum is equivalent to contamination of 200 mls. of blood per liter of amniotic fluid.

This important effect created by serum contamination, varies from the others in that contamination with blood is unavoidable in some cases, and unlike the previous experiment, cannot be attributed to technical error. This result, therefore, should be noted to avoid depending on the FP when gross amounts of contamination of amniotic fluid have occurred.

Evaluation of non-maternal serum was assessed because other sources had utilized this source in determining the effect of contamination on amniotic fluid. This result is tabulated in Table 6 and graphically displayed in Figure 2.

Dilution or contamination of amniotic fluid with non-maternal serum tended to increase (immature) the FP value, in keeping with its effect in other studies on L/S ratio.

This effect, though interesting is highly unlikely to occur.

3. Addition of meconium utilizing term and preterm meconium:[1*]

Meconium contamination of amniotic fluid is similar to serum contamination in that it is unavoidable when it occurs. To evaluate any change that this contamination might have on the original FP value, the following study was performed.

Preterm and term meconium were added to term and preterm (mature and immature) amniotic fluid to assess the change or effect

on the FP value.

Pooled samples of amniotic fluid were prepared to yield two types of amniotic fluid pools, immature (greater than 0.310) and mature (less than 0.310). Meconium was collected from two infants, one preterm at 32 weeks, and one term at 39 weeks. To facilitate handling, a dilutional scheme was developed whereby meconium was diluted with amniotic fluid at 1 gm./10 mls. of amniotic fluid. This led to two stock mixtures:

1. immature meconium diluted with immature amniotic fluid
2. mature meconium diluted with mature amniotic fluid

The two types of meconium, immature and mature, were therefore added to the similar types of amniotic fluid that would represent contamination, if it had occurred. The stock solutions of meconium were diluted with the same gestational type of amniotic fluid pool to be tested to avoid any changes in the FP value from the diluting medium.

These results are tabulated in Table 7 and graphically displayed in Figure 3.

From these data, which were repeated and yielded similar results, it seems apparent that: meconium, term or preterm, raises the FP value indicating immaturity; with addition of meconium, mature amniotic fluid tended to have a greater tendency to reach this immature state; and preterm meconium created less change than term meconium.

Meconium contamination, be it of mature or immature amniotic fluid, tends to raise the FP to more immature values. This alteration

appears much greater in mature amniotic fluids. This may be due to greater sensitivity to contamination with meconium or perhaps this effect is due to a different lipid composition of the meconium. Because of this problem, perhaps gestational age should be taken into account if one is analyzing fluid that is contaminated with meconium, for false positives, indicating immaturity, are possible.

4. Freezing and Thawing:

Samples from various patients and from pooled samples were frozen and thawed and refrozen or left at room temperature for varying lengths of time to assess any change in the FP value.

These results are tabulated in Table 8.

It is clear from the data, that caution should be taken with freezing and thawing of samples; changes of up to +.020 and -.026 were observed without any predictable trend. However, it should be noted that not freezing a particular sample, or forgetting to freeze a sample for up to three days does not alter the FP value significantly.

II Experiments:

B. Analysis of Data Collected on Patients:

For this study, attempts were made to ascertain FP in different disease states, to compare FP with the L/S ratio, gestational age, and ultimate infant outcome.

1. Materials:

Samples included amniotic fluid from patients with various disease states as well as with normal pregnancies as follows: samples from 10 patients with normal pregnancies between 34-41 weeks gestation; 9 samples from Rh^- patients between 21-39.5 weeks gestation; a 37.5 week sample from one patient with cardiac disease; samples from 17 diabetic patients between 35-40 weeks; 9 samples from eclamptic patients 31-41 weeks; two samples from PROM 37-39 weeks; four samples of postmaturity all greater than 40 weeks; six samples of hydramnios at 33-40 weeks; and 5 IUGR samples.

Gestational age was assessed from obstetrical data, including LMP, estimated uterine size, and ultrasound with BPD. Amniotic fluid was obtained by trans-abdominal amniocentesis. All samples were labelled with date of delivery and ultimate outcome of infant, retrieved from records retrospectively. L/S ratios and FP values were made on each sample of amniotic fluid.

2. Method:

All samples were centrifuged and the FP value measured as per protocol in the methodology section. Permission was obtained for utilization of two "Elscint Fetal Lung Maturity Analyzers." Samples were analyzed with both instruments and for L/S ratios if enough fluid was obtained. The results for the two instruments are labelled as FP(1) for the analyzer at YNHH and FP(2) for the analyzer located in N.Y.C. Samples analyzed in N.Y.C. were frozen immediately at YNHH at $-2^{\circ}C$ after centrifugation and taken to the

area on dry ice. These samples were then analyzed identically, in accordance with protocol.

An FP(1) value of .310 is the upper limit threshold above which RDS may occur while an FP(2) value of .336 is the upper limit threshold of the N.Y.C. instrument. A value less than or equal to .310 and .336 for FP(1) and FP(2) respectively have therefore, been claimed to indicate lung maturity.

3. Results:

A. Histogram:

A histogram (Figure 4) was created to show the distribution of patients for each particular L/S ratio and to emphasize the number of patients (frequency) actually used in evaluating FP values for particular L/S ratios.

B.$_1$ FP values and increasing gestational age:

Table A shows tabulated data of FP values and L/S ratio for increasing gestational age. This gives easy evidence to all results and to corresponding L/S ratios. The L/S ratio shows the usual trend of increasing maturity with advancing gestational age, with some scatter noted for particular disease states. This similar trend of increasing maturity is noted for the FP value. IUGR and Eclampsia, tend to have higher L/S ratios at an earlier gestational age than other diseases at comparable gestational ages. This is evident at approximately 34 weeks. This same trend was noted in

Class A and B diabetics when compared to Class C and D diabetics. L/S ratios for the former at approximately 38 weeks were much higher. Class A diabetics, in fact at 39 weeks had higher L/S ratios than other diseases at comparable gestational age. The L/S ratio surprisingly seemed to be especially low for samples with hydramnios at 40 weeks if compared to other disease states.

B.$_2$ Correlation of FP and L/S to ultimate fetal outcome:

Three patients whose infants developed RDS, #6, 13 and 48, are shown in Table A. Their gestational ages are 31, 34, and 40 weeks respectively. At forty weeks gestation, development of RDS is extremely rare, but #48 was noted to have mild RDS as diagnosed in Poughkeepsie, New York. FP(1) and the L/S ratio predicted #6, but insufficient fluid was obtained to calculate FP(2). The L/S ratio and FP(1) failed to predict the outcome in #13 while FP(2) did predict immaturity. Bot FP values failed to predict immaturity in #34 while the L/S ratio estimated this result.

B.$_3$ Evaluation of false positives and false negatives were assessed to compare FP results to the L/S ratio.

The false positive rate was defined as all those samples with an L/S greater than or equal to two with an immature FP value (greater than .310 (FP(1) and .336 (FP(2)). The total false positive rate for the FP was 10.9% for FP(1) and 24.2% for FP(2). To evaluate ultimate outcome of all false positive infants, all those infants with false positive values who were delivered within three days of

the test were evaluated. The three day period was picked to avoid maturation that would have occurred if longer periods of time were used. A total of five false positive infants were found within that time period. One did develop RDS #13, not predicted by the L/S ratio while four, three from FP(2) and one from FP(1) did not, even though the FP value predicted that they should.

The total false negative rates were evaluated. This rate is defined as all those infants with immature L/S ratios and FP values of less than or equal to .310 (FP(1)) and .336 (FP(2)). The false negative rate was assessed to be 21.8% and 17.74% for FP(1) and FP(2) respectively. Again to evaluate infant outcome, all false negative infants delivered within three days of the test were evaluated. In this case, again one #48 developed RDS, while four, #'s 11, 20, 29, and 49 did not. These four were therefore, not false negatives since the L/S erred and these infants did not develop RDS. This consideration was taken when calculating the false negative rate. The results were estimated as a false negative rate of 14.5% and 11.3% for FP(1) and FP(2) respectively, a change of 7.3% and 6.4% respectively.

The true positive rates for FP(1) and FP(2) were calculated as 40% and 32.25% respectively while the true negative rate, taking again into account the four new patients (#'s 11, 20, 29 and 49), was 34.5% and 32.2% respectively.

All values that did not correlate with the L/S ratio were totaled. These were 25.4% for FP(1) and 35.5% for FP(2).

Table B was analyzed for false positives and false negatives to evaluate any correlation to disease states. The false positive rate was especially greater in diseases such as IUGR and Class A diabetics, while the false negative rate tended to cluster in diseases such as Class C diabetics and hydramnios.

B.$_4$ Predictive values

Disregarding RDS or infant outcome, the probabilities for L/S ratios at a given FP were assessed. This was calculated to establish the trend of the instrument with regards to L/S ratio. The probability of a mature L/S ratio given a mature FP value (less than or equal to .310 or .336) was 64.7% and 64.5% for FP(1) and FP(2) respectively. The probability of an immature L/S ratio given a mature FP value was 35.3%, FP(1) and 35.5%, FP(2). The probability of a mature L/S ratio given an immature FP value (greater than .310 and .336) was 28.5%, FP(1) and 48.3%, FP(2). The probability of an immature L/S ratio given an immature FP value was 71.4%, FP(1), and 51.6%, FP(2).

C. FP values correlated with L/S and gestational age

The average FP values with standard deviations were tabulated for an L/S ratio greater than or equal to two and for an L/S ratio less than two, to demonstrate the trend of the instrument. When the mean FP values are compared to the L/S ratio, Figures 4A and 4B, they show good association. FP(1) and FP(2) differ in their slope of increasing maturity. FP(1), Figure 4A, shows a rapid and early

increase in maturity at an L/S ratio of 1.1-1.9 while FP(2) shows a more gradual increase in maturity that is evident at an L/S ratio of 2.9-3.7 (Figure 4B). Both values show a wide standard deviation (S.D.) at an L/S of 2.9-3.7 and especially at an L/S greater than 5.6 for FP(2).

Because maturity was reached at different L/S ratios for FP(1) and FP(2), a correlation with gestational age was done. FP values compared to gestational age, Figure 4C, show that average maturity was not reached for FP(1) until 34-35 weeks, and 38-39 weeks for FP(2). In comparison, when the average L/S was tabulated, maturity was noted at a mean gestational age of 39.2 weeks. Both FP values, therefore, matured earlier than the L/S ratio. They, nevertheless, show a gradual decline with increasing L/S ratio and advancing gestational age, indicating maturity.

D.$_1$ Maternal Diseases:

To evaluate any underlying trend that might be altering the FP value, average values for maternal diseases were plotted at immature and at mature L/S ratios. The diseases plotted against mean FP values are in Figure 5A, at an L/S greater than or equal to two, and Figure 5B, at an L/S less than two.

The mean FP values at a mature L/S were also mature for all evaluated disease states in FP(1). Nevertheless, cesarean section, pre-eclampsia and post-maturity tended to have lower mean FP values, indicating increased maturity, when compared to those samples of IUGR, class A diabetics and hydramnios. To evaluate this discrepancy,

mean gestational ages for each of these disease states at an L/S of greater than two was analyzed from the data in Table B. These ages were, 37.5, 38.6, and 38.5 for IUGR, class A and hydramnios compared to the mean gestational ages of 39.16, 38.16 and 40.8 for cesarean section, eclampsia and post-maturity respectively. FP(2) at an L/S greater than or equal to two, tended to have mean values that indicated maturity for cesarean section, eclampsia, and post-maturity. Again, a similar trend was noted as in FP(1) where disease states such as IUGR and class A diabetics tended to have higher mean FP values. In fact, the mean values of these two states were in the immature FP range (greater than .336).

Evaluation of mean FP(1) values at an L/S less than two (Figure 5B) shows immature values for Rh disease and eclampsia yet, class C and D diabetics and hydramnios show mean values in the mature range. Mean FP(2) values show a similar trend, with class D diabetics and hydramnios samples at a mean maturity range yet at an L/S of less than two.

$D._2$ Evaluation of Maturation Trend:

To evaluate the trend of maturity, eclampsia was analyzed. The mean value for eclampsia at an L/S of less than two, Figure 5B, was .312 for FP(1) and .340 for FP(2). This mean value changed at an L/S greater than two to .270 for FP(1) and .305 for FP(2), Figure 5A. The maturation change confirms correlation with the L/S ratio. Other diseases were analyzed in a similar fashion. Surprisingly, in comparison the mean values for hydramnios at an

L/S less than two were .280 for FP(1) and .300 for FP(2), yet changed very little to .290 and .310 for FP(1) and FP(2) respectively, at an L/S ratio of greater than two. This lack of maturation usually associated with a maturing L/S ratio is also seen in diabetic samples, plotted in Figure 6A. There is also little change in the mean FP values for all diabetics at an L/S ratio of greater than or less than two. These diabetic and hydramnios samples were therefore, compared to gestational age, Figure 6B, and Table B. It is worth noting, that for hydramnios, the patients' mean gestational ages were the same for both mature and immature L/S ratios (38 weeks). For the diabetic sample, in comparison, the mean gestational age did show a change from 35-38 weeks with maturation of the L/S ratio.

Discussion:

Since surfactant lowers surface tension, which is a function of microviscosity, it seems likely that an instrument that could measure this function could be of use in evaluating fetal lung maturation and therefore, RDS.

Our experimental data showed fairly good correlation of the FP value with the L/S ratio. The FP tends to mature earlier than the L/S ratio, showing better association to known fetal maturity at approximately thirty-five weeks.

Experimental contamination of amniotic fluid samples with dilutional factors tended to alter the FP value and caused false maturation to occur. This dilutional effect is not in keeping with current published data[118] where no change was noted up to a ten fold dilution.

Experimental contamination with term or preterm meconium caused falsely elevated (immature) FP values. This effect was especially pronounced in fluids with mature FP values, perhaps indicating a particular sensitivity of this fluid to alteration. Term meconium rather than preterm meconium tended to increase this effect, perhaps secondary to varying amounts of lipids or changes in its composition.

Experimental contamination with blood is of interest because alteration of the FP was dependent on the type of serum used, but maternal blood matured FP.

In summary, our study shows that false positive results secondary to meconium contamination and false negative results secondary to blood and dilutional changes may develop in the analysis of the FP value. This tendency must be carefully evaluated in the event of unavoidable contamination. Dilutional error are, however, unlikely to occur and therefore does not require such caution unless extremely poor technique is applied. Almost all amniotic fluid measurements including the L/S ratio are affected by blood and meconium. Nevertheless, reliance on the FP value in samples that are contaminated with greater than 20% serum or as little as 0.5 mg/ml of meconium should be cautiously appraised at present.

The disease states of diabetes and hydramnios tended to alter the FP value. The stable maturation means reflected in these two diseases can only be explained in hydramnios. This effect may have been due to the dilutional change of the amniotic fluid that may have falsely lowered the L/S ratio without a similar effect on the FP, or perhaps there was greater correlation of FP values with gestational age. Mean gestational age, however, did change for diabetic samples while the mean FP values remained constant. There is no explanation for this result. One may speculate that perhaps the microviscosity of the amniotic fluid was altered in the diabetic state.

Gluck and coworkers noted[58] two mean maturation slopes for different disease states. L/S ratios in classes D, E, and F matured earlier at 32-33 weeks while, classes A, B, and C diabetics

tended to mature late at 37-40 weeks. Our L/S ratios did not demonstrate this trend. Interestingly, the mean FP value does show this trend, not correlating well with the L/S ratio in this disease, and demonstrates low (mature) values for classes D and C at an L/S of less than two, and high (immature) values for classes A and B at an L/S greater than two. Thus, the FP correlates with Gluck's data.

Although fairly good correlation with the L/S was achieved, the false positive rate of 10-24% for FP(1) and FP(2) and the false negative rate of 14.5% and 11.3% for FP(2) and FP(1) are disturbing. Utilization of this instrument without or instead of the L/S ratio would therefore allow the delivery of 11-14% of infants who would not be demonstrated as mature by L/S standards. Whether these infants would in fact develop RDS is an unanswerable question at present. Similarly, 10-24% of infants considered mature by the L/S ratio would be considered immature by FP standards and would not be delivered. Their maturity, or the effect of delayed delivery also cannot be assessed.

Time and much evaluation will be our only tools to answer these questions. At present, our analysis is not conclusive. Correlation with gestational age and L/S is good save for two disease states. Nevertheless, further evaluation in a setting where the results can be analyzed in terms of infant outcome (RDS) is needed to evaluate not only efficiency but worth.

The procedure for measuring FP with the Elscint instrument is simple and highly reproducible but, further evaluation of the

instrument is needed as well as more stringent and safer quality controls. Adequate standardization levels were not available during this study which created uncertainties with regards to daily mechanical functions.

Given that the instrument measures the microviscosity of phospholipid liposomes, sonication of the amniotic fluid samples to enhance the uniformity of liposomes could be of value.

Conclusion:

Epidemiological data have shown RDS to be the leading cause of neonatal deaths. The L/S ratio is at present the leading laboratory method utilized for prediction of lung maturation. The Elscint Fetal Lung Maturity Analyzer appears to have potential in its prediction of lung maturity. Therefore, its potential usefulness for prediction of RDS or lung maturity has been assessed. Alteration of the FP value by contamination and changes imposed by certain high-risk patients, may lead to problems in its overall use by clinicians. At present, further evaluation is needed to establish its efficiency in the prediction of lung maturation, and its use in the prediction of RDS.

TABLES

AND

FIGURES

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Table 1 Effect of Dilution on FP Value

1 Dilution of Amniotic Fluid, Sample 3, Utilizing Distilled H_2O:*

Sample #'s	H_2O in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.0	0.50	.317	
2	0.05	0.45	.315	-.002
3	0.10	0.40	.318	+.001
4	0.20	0.30	.320	+.003
5	0.30	0.20	.318	+.001
6	0.40	0.10	.315	-.002
7	0.45	0.05	.302	-.015
8	0.50	0.00	.275	-.042

*
DPH dispersion is added (2 mls.) to all samples to be analyzed

A.F.= amniotic fluid ; H_2O = distilled water ; FP = fluorescent polarization

Dilution of Amniotic Fluid, Sample 4, Utilizing Phosphate Buffered Saline (PBS):*

Sample #'s	H_2O in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.0	0.50	.332	
2	0.05	0.45	.331	-.001
3	0.10	0.40	.331	-.001
4	0.15	0.35	.326	-.006
5	0.20	0.30	.323	-.009
6	0.25	0.25	.323	-.009
7	0.30	0.20	.315	-.017
8	0.40	0.10	.306	-.026
9	0.45	0.05	.293	-.039
10	0.50	0.00	.277	-.055

*
DPH dispersion (2 mls.) is added to all samples

A.F.=amniotic fluid ; FP=fluorescent polarization

Table 2 Effect of Dilution on FP Value

1 Dilution of Amniotic Fluid, Sample 2, Utilizing Distilled H_2O:*

Sample #'s	H_2O in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.352	
2	0.40	0.10	.346	-.006
3	0.45	0.05	.332	-.020
4	0.475	0.025	.302	-.050
5	0.50	0.00	.267	-.085

* DPH dispersion (2 mls.) is added to all samples

A.F.= amniotic fluid ; H_2O= distilled water; FP = fluorescent polarization

1 Dilution of Amniotic Fluid, Sample 1, Utilizing Phosphate Buffered Saline (PBS):*

Sample #'s	H_2O in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.308	
2	0.40	0.10	.303	-.005
3	0.45	0.05	.288	-.020
4	0.475	0.025	.266	-.042
5	0.50	0.00	.255	-.053

* DPH dispersion (2 mls.) is added to all samples

A.F. = amniotic fluid

FP = fluorescent polarization

PBS = phosphate buffered saline

Table 3 Effect of Dilution on FP Value

1 Dilution of Amniotic Fluid, Sample A, Utilizing 1) distilled H_2O

2) PBS-phosphate buffered saline

Distilled H_2O:*

Sample #'s	H_2O in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.274	
2	0.10	0.40	.274	.000
3	0.20	0.30	.280	+.006
4	0.40	0.10	.268	-.006
5	0.45	0.05	.255	-.019
6	0.475	0.025	.242	-.022
7	0.50	0.00	.240	-.024

* DPH dispersion(2 mls.) is added to all samples

A.F.= amniotic fluid; H_2O = distilled water; FP= fluorescent polarization

Phosphate Buffered Saline:*

Sample #'s	PBS in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.274	
2	0.10	0.40	.276	+.002
3	0.20	0.30	.280	+.006
4	0.40	0.10	.268	-.006
5	0.45	0.05	.257	-.017
6	0.475	0.025	.243	-.021
7	0.50	0.00	.240	-.024

* DPH dispersion(2 mls.) is added to all samples

A.F.= Amniotic Fluid; FP= fluorescent polarization; PBS= phosphate buffered saline

Figure 1

Effect of Dilution on FP Value, Utilizing Distilled Water and Phosphate Buffered Saline

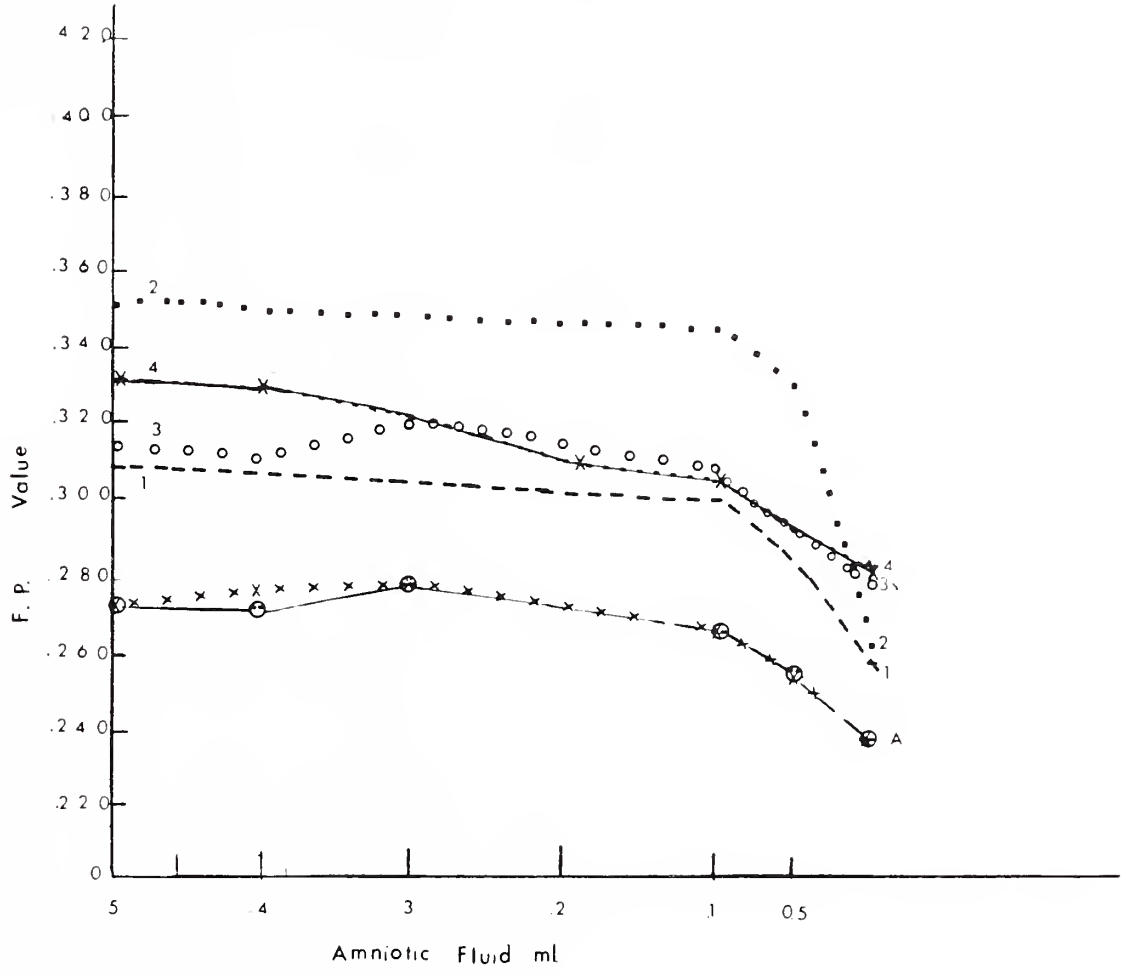


Table 4 Effect of Maternal Serum on FP Value *

A. Clotted Blood (Utilizing Serum):

Sample #'s	Serum in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.310	
2	0.01	0.49	.293	-.017
3	0.05	0.45	.288	-.032
4	0.10	0.40	.283	-.027
5	0.50	0.00	.261	-.049

B. Heparinized Blood (Utilizing Serum):

Sample #'s	Serum in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.310	
2	0.01	0.49	.291	-.019
3	0.05	0.45	.286	-.024
4	0.10	0.40	.285	-.025
5	0.50	0.00	.258	-.054

C. Immediately Frozen (Utilizing Serum):

Sample #'s	Serum in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.310	
2	0.01	0.49	.287	-.023
3	0.05	0.45	.280	-.030
4	0.10	0.40	.281	-.029
5	0.50	0.00	.254	-.056

* DPH dispersion (2mls.) is added to all samples

A.F.= amniotic fluid

FP = fluorescent polarization

Table 5 Effect of Maternal Serum on FP Value*

D. Immediately Frozen (Utilizing Serum):

Sample #'s	Serum in mls.	A.F. in mls.	FP Value	Change from Initial FP
1	0.00	0.50	.311	
2	0.01	0.49	.285	-.026
3	0.025	0.475	.285	-.026
4	0.05	0.45	.285	-.026
5	0.10	0.40	.285	-.026
6	0.15	0.35	.289	-.022
7	0.20	0.30	.295	-.016
8	0.25	0.25	.280	-.031
9	0.40	0.10	.270	-.041
10	0.50	0.00	.250	-.061

*DPH dispersion (2 mls.) is added to all samples

A.F. = amniotic fluid

FP = fluorescent polarization

Table 6 Effect of Non-Maternal Serum on the FP Value *

E. Immediately Frozen Non-Maternal Serum:

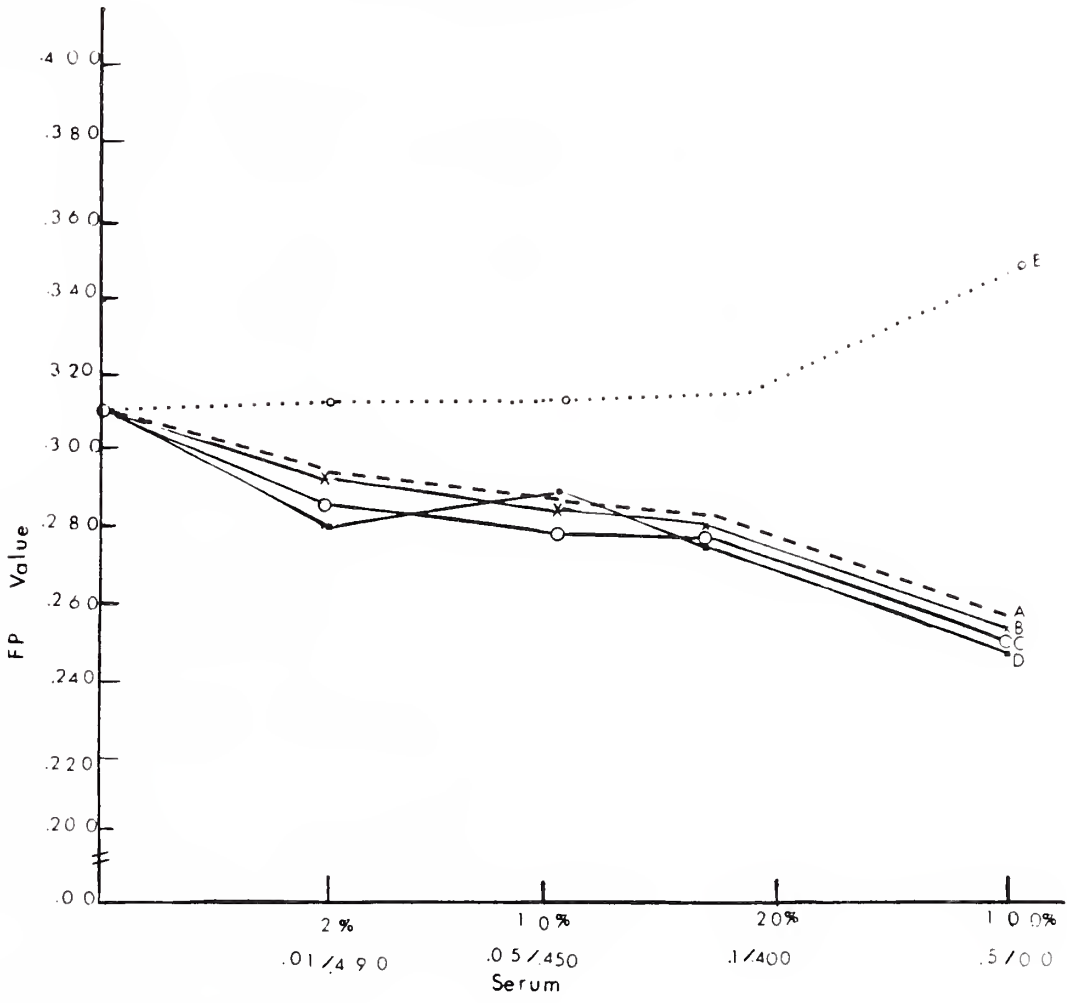
Sample #'s	Serum in mls.	A.F. in mls.	FP Value	Change from initial FP
1	0.00	0.50	.310	
2	0.01	0.49	.312	+.002
3	0.05	0.45	.318	+.008
4	0.10	0.40	.320	+.010
5	0.40	0.10	.332	+.022
6	0.50	0.00	.347	+.037

* DPH dispersion (2 mls.) is added to all samples

A.F.= amniotic fluid

FP = fluorescent polarization

Figure 2



A Clotted
B Heparinized
C Immediately frozen
D
E Non maternal

Effect on FP Value With Addition of Maternal and Non-Maternal Serum.

Table 7 Effect of addition of term and preterm meconium on the FP value *

1 Preterm Meconium :

Sample #'s	Meconium stock in mls.	Amniotic fluid mls.	Meconium mgm/ml	FP Value	Change from initial FP
1	0.00	0.50	0.00	.343	
2	0.01	0.49	2.00	.352	+.009
3	0.025	0.475	5.00	.351	+.008
4	0.05	0.45	10.00	.350	+.007
5	0.10	0.40	20.00	.348	+.005
6	0.25	0.25	50.00	---	---

---= no data was obtained from instrument

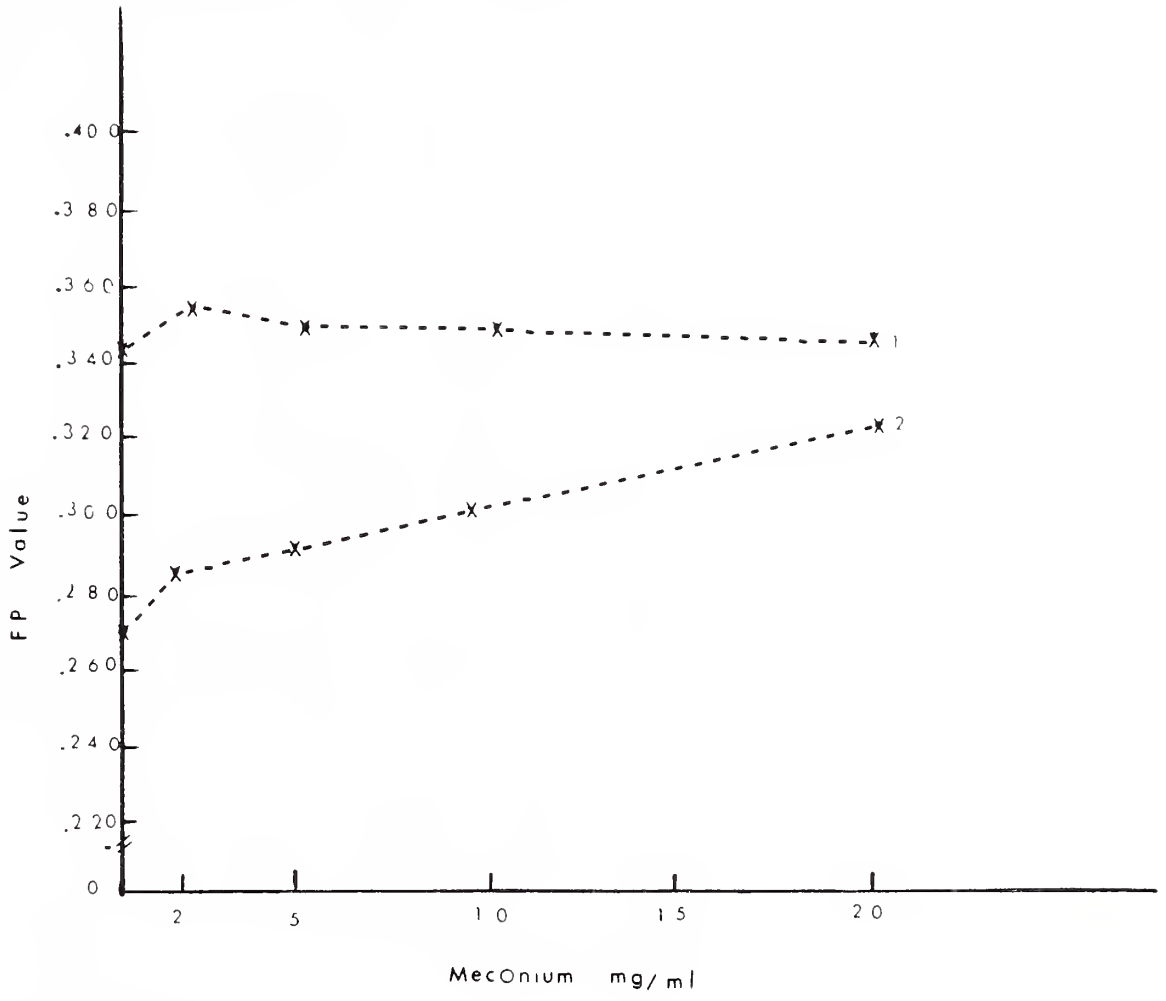
2 Term Meconium:

Sample #'s	Meconium stock in mls.	Amniotic fluid mls.	Meconium mgm/ml	FP Value	Change from initial FP
1	0.00	0.50	00.00	.272	
2	0.01	0.49	02.00	.285	+.013
3	0.025	0.475	05.00	.290	+.018
4	0.05	0.45	10.00	.300	+.028
5	0.10	0.40	20.00	.322	+.050
6	0.25	0.25	50.00	---	----

--- = no data obtained from instrument

* DPH dispersion (2 mls.) is added to all samples

Figure 3 Effect on FP Value With Addition of Preterm and Term Meconium



1 Preterm

2 Term

Table 8 Effect of Temperature and Time on the FP Value

IV Freezing and Thawing:* (A)

Sample #	Initial FP	Time Frozen in Days	New FP	Change	Disease of Patient
1	.345	99	.350	+.005	Rh^- Disease
2	.342	97	.362	+.020	Rh^- Disease
3	.304	73	.304	.000	PROM
4	.334	57	.320	-.014	Diabetic
5	.314	51	.306	-.008	Pre-eclampsia
6	.330	36	.342	+.012	Rh^- Disease
7	.234	8	.234	.000	Hydramnios
8	.308	8	.304	-.004	Diabetic
9	.308	21	.282	-.026	Normal
10	.315	14	.315	.000	Hydrammios
11	.292	10	.303	+.011	Diabetic

(B) Effect of Time

Sample #	Initial FP	24 Hours	Change	48 Hours	Change	72 Hours	Change
1	.317	.317	.000	.315	-.002	.316	-.001
2	.234	.234	.000	.234	.000	.235	+.001
3	.304	.310	+.006	.295	-.009	.310	+.006
4	.272	.270	-.002	.274	+.002	.272	.000
5	.314	.315	+.001	.314	.000	.315	+.001
6	.303	.304	+.001	.309	+.006	.304	+.001

Average days frozen = 43
Average change = .009 $\pm$ .008 S.D.
* DPH dispersion was added in the constant amount of 2 mls. to all samples to be analyzed.

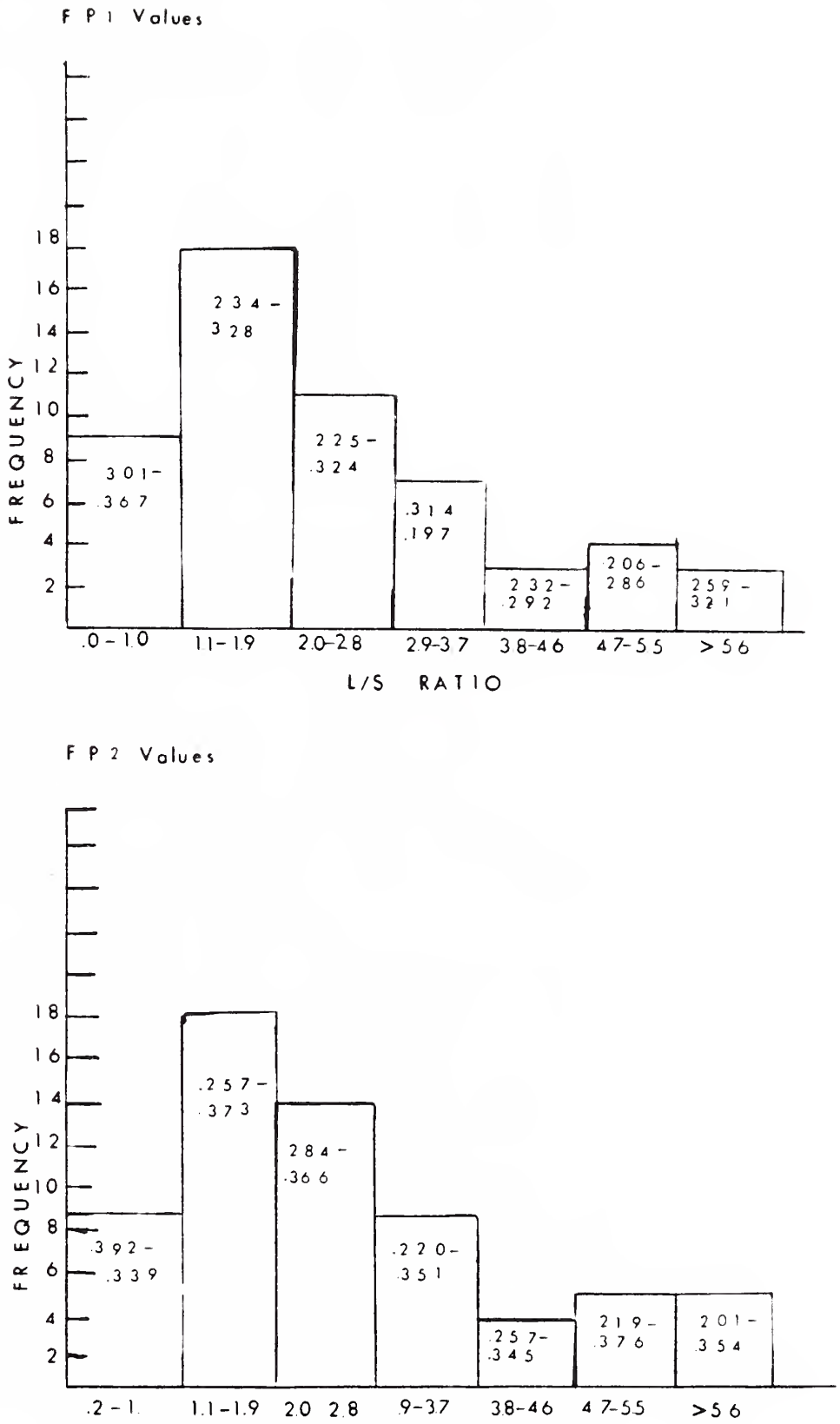


Figure 4 FP Values at various L/S ratios

Table A Tabulation of all data

Sample #'s	Gest. Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Disease	Apgar #	# days post test
1	21	0.5	.367	.390	+.023	Rh^- Dis.		
2	25	0.5	.351	.392	+.041	Rh^- Dis.		
3	26	1.0	.342	.383	+.041	Rh^- Dis.	8/9	62
4	28	1.2	.298	.334	+.036	Rh^- Dis.		
5	30	0.5	.301	.339	+.038	Rh^- Dis.		
6	31	0.7	.316			Pre-eclam.	1/3	0 *
7	31	1.3	.340	.373	+.033	Pre-eclam.	8/6	20
8	32	0.2		.369		Rh^- Dis.		
9	33	0.5	.315	.343	+.028	Hydram.	5/8	8
10	34	0.5	.322	.365	+.043	Pre-eclam.	4/6	4
11	34	1.5	.280	.320	+.040	Rh^- Dis.	6/7	2
12	34	2.0	.324	.366	+.042	IUGR	9/9	1
13	34	2.1	.225	.341	+.116	Norm. C/S	7/9	0 *
14	34.5	1.6	.320	.355	+.035	Rh^- Dis.	8/9	14
15	34.5	2.9	.304	.334	+.030	Pre-eclam.		
16	35	1.6	.321	.354	+.033	Class A	7/8	6
17	35	1.6	.326	.355	+.029	Class B	7/9	7
18	35	1.6	.285	.316	+.031	Class C	8/8	5
19	36	0.5	.312	.375	+.063	Class C		
20	36	1.4	.283	.312	+.029	Class D	1/7	2
21	36	2.4	.312	.347	+.035	IUGR	9/9	11
22	37	0.3	.342	.378	+.036	PROM	8/8	35
23	37	1.1	.294	.345	+.051	Class C		
24	37	1.9	.276	.300	+.024	Pre-eclam.	6/8	6
25	37	3.4	.314	.351	+.037	Class A	8/9	9
26	37.5	1.6	.328	.347	+.019	Pre-eclam.		
27	37.5	3.4		.339		Cardiac	9/9	3

Table A continued

Tabulation of all data

Sample #'s	Gest. Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Disease	Apgar #	# days post term
28	38	1.1	.304	.329	+.025	Class D	8/9	10
29	38	1.2	.311	.330	+.019	Pre-eclam.	5/8	0
30	38	1.8	.318	.358	+.040	Class C	9/9	7
31	38	1.9	.293	.322	+.029	Class C	8/9	10
32	38	2.0	.268	.284	+.016	Hydram.	8/9	1
33	38	2.3		.364		Norm. C/S		
34	38	2.9	.309	.351	+.042	Class A	8/9	6
35	38	4.5		.345		Class B	9/9	0
36	38	8.3		.319		Class A		
37	39	1.4	.283	.320	+.037	Hydram.	7/8	6
38	39	2.1	.304	.337	+.033	PROM	9/9	1
39	39	2.7	.319	.325	+.006	IUGR	9/9	10
40	39	3.2	.308	.350	+.042	Class A	8/8	6
41	39	2.7	.319	.336	+.017	Hydram.	7/8	6
42	39	3.3	.290	.338	+.048	IUGR	8/8	4
43	39	5.0	.286	.332	+.046	IUGR	9/9	10
44	39	5.5		.263		Norm. C/S		
45	39	7.7	.282	.339	+.035	Pre-eclam.	7/9	11
46	39.5	2.4	.274	.332	+.058	Class C	9/9	3
47	39.5	5.9		.201		Rh^- Dis.	9/9	0
48	40	1.8	.284	.302	+.018	Hydram.	7/8	6 *
49	40	1.8	.234	.257	+.023	Hydram.	9/9	0
50	40	2.0	.291	.315	+.024	Norm. C/S	4/9	4
51	40	2.0	.279	.319	+.040	Norm. C/S	9/10	0
52	40	2.4	.255	.331	+.076	Norm. C/S	9/9	0
53	40	2.9	.251	.321	+.070	Norm. C/S	9/9	3

Table A continued

Tabulations of all data

Sample #'s	Gest. Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Disease	Apgar #	# days post term
54	40	3.1		.298		Norm. C/S	8/9	3
55	40	3.6	.197	.220	+.023	Postmatur.	9/9	1
56	40	3.9	.268	.333	+.065	Postmatur.	9/9	0
57	40	4.5	.292	.338	+.046	Class A	9/9	6
58	40	4.8	.217	.252	+.035	Norm. C/S		
59	40	5.7	.321	.354	+.033	Class A	7/8	6
60	41	4.2	.232	.257	+.025	Pre-eclam.	9/9	1
61	41	4.8	.206	.219	+.013	Postmatur.	9/9	2
62	41	6.8	.259	.294	+.035	Norm. C/S	9/9	0
63	42/5	4.7	.270	.376	+.106	Postmatur.	9/9	8

Gest. Age= gestational age in weeks

FP = fluorescent polarization

* = infant developed RDS

Table B Tabulation of data according to disease states of patients, in order of increasing L/S ratio

Rh Disease:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
32	0.2		.369			
21	0.5	.367	.390	+.023		
25	0.5	.351	.392	+.041		
25	0.5		.373			
30	0.5	.301	.339	+.038		
26	1.0	.342	.383	+.041	8/9	62
28	1.2	.298	.334	+.036		
34	1.5	.280	.320	+.040	6/7	2
34.5	1.6	.320	.335	+.035	8/9	14
39.5	5.9		.201		9/9	0

Mean: at L/S > 2; at L/S < 2

Gest. Age: 39.5; 28.4

FP(1): ; .322, false (-) 3/7

FP(2): .201 ; .361, false (-) 2/9 No false (+)

Normal Cesarean Section:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
40	2.0	.279	.319	+.040	9/10	2
40	2.0	.291	.315	+.024	4/9	4
34	2.1	.225	.341	+.116	7/9	0*
38	2.3		.364			
40	2.9	.251	.321	+.070	9/9	3
40	3.1		.298		8/9	3

Table B Tabulation of data according to disease states of patients, in order of increasing L/S ratio

Normal Cesarean Section: continued

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
39	5.5		.263			
41	6.8	.259	.294	+.035	9/9	0
40	2.4	.255	.331	+.076	9/9	0
40	4.8	.217	.252	+.035		

Mean: at L/S > 2 ; at L/S < 2

Gest age: 39.2 ; 00, L/S false (+) 1/10 with infant developing RDS

FP(1): .253 ; 000, false (+) 1/7 with infant developing RDS

FP(2): .309 ; 000,false (-) 1/10, No false (+)

Pre-eclampsia:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
34	0.5	.322	.365	+.043	4/6	6
31	0.7	.316			1/3	0 *
38	1.2	.311	.330	+.024	6/8	6
31	1.3	.340	.373	+.033	6/8	20
37.5	1.6	.328	.347	+.019		
37	1.9	.276	.300	+.024	6/8	6
34.5	2.9	.304	.334	+.030		
41	4.2	.232	.257	+.025	9/9	1
39	7.7	.282	.336	+.054	7/9	11

Mean: at L/S > 2 ; at L/S < 2

Gest. Age: 38.2 ; 34.75

FP(1): .272 ; .315 , false (-) 1/6, No false (+)

FP(2): .309 ; .343 , false (-) 2/5, No false (+)

Table B Tabulations of data according to disease states of patients, in order of increasing L/S ratio

Cardiac Disease:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
37.5	3.4		.339		9/9	3

FP(2) false (-) 1/1

PROM:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
37	0.3	.342	.378	+.036	8/8	35
39	2.1	.304	.336	+.032	9/9	1

Mean: at L/S > 2 ; at L/S < 2

Gest. Age: 39 ; 37

FP(1): .304 ; .342, No false (+) or (-)

FP(2): .336 ; .378, No false (+) or (-)

IUGR:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
34	2.0	.324	.366	+.042	9/9	1
36	2.4	.312	.347	+.035	9/9	11
39	2.7	.319	.325	+.006	9/9	10
39	3.3	.290	.338	+.048	8/8	4
39	5.0	.286	.332	+.046	8/9	1

Mean: at L/S > 2

Gest. Age: 37.4

FP(1) .306 , false (+) 3/5

FP(2) .341 , false (+) 3/5

Table B Tabulation of data according to disease states of patients, in order of increasing L/S ratio

Diabetes: Class "A"

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
35	1.6	.321	.354	+.033	7/8	6
38	2.9	.309	.351	+.042	8/9	6
39	3.2	.308	.350	+.042	8/8	1
37	3.4	.314	.351	+.037	8/9	9
40	4.5	.292	.338	+.046	9/9	6
40	5.7	.321	.354	+.033	6/8	2
38	8.3		.319			

Mean: at L/S > 2 ; at L/S < 2

Gest. Age: 38.6 ; 35

FP(1): .308 ; .321, false (+) 2/5, No false (-)

FP(2): .343 ; .354, false (+) 5/6, No false (-)

Diabetes: Class "B"

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
35	1.6	.326	.355	+.029	7/9	6
38	4.5		.345		9/9	0

FP(2) false (

Diabetes: Class "C"

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
36	0.5	.312	.375	+.063		
37	1.1	.294	.345	+.051		
35	1.6	.285	.316	+.031	8/8	5
38	1.8	.318	.358	+.040	9/9	7

Table B Tabulation of data according to disease states of patients, in order of increasing L/S ratio

Diabetes: continued Class "C"

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
38	1.9	.293	.322	+.004	8/9	10
39.5	2.4	.274	.332	+.058	9/9	3

Mean: at L/S > 2 ; at L/S < 2

Gest. Age: 39.5 ; 36.8

FP(1): .274 ; .300, false (-) 3/5, No false (+)

FP(2): .332 ; .343, false (-) 2/5, No false (+)

Diabetes: Class "D"

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
38	1.1	.304	.329	+.025	8/9	10
36	1.4	.283	.312	+.029	1/7	2

Mean: at L/S < 2

Gestational age: 37

FP(1): .293, false (-) 2/2, No development of RDS

FP(2): .320, false (-) 2/2, No development of RDS

Post Maturity:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
40	3.6	.197	.220	+.023	9/9	1
40	3.9	.268	.333	+.065	9/9	0
42.5	4.7	.270	.376	+.106	9/9	8
41	4.8	.206	.219	+.013	9/9	2

Mean: at L/S > 2

Gest. age: 40.8

FP (1): .235 while FP(2) .287 with false (+) 1/4

Table B Tabulation of data according to disease states of patients, in order of increasing L/S ratio

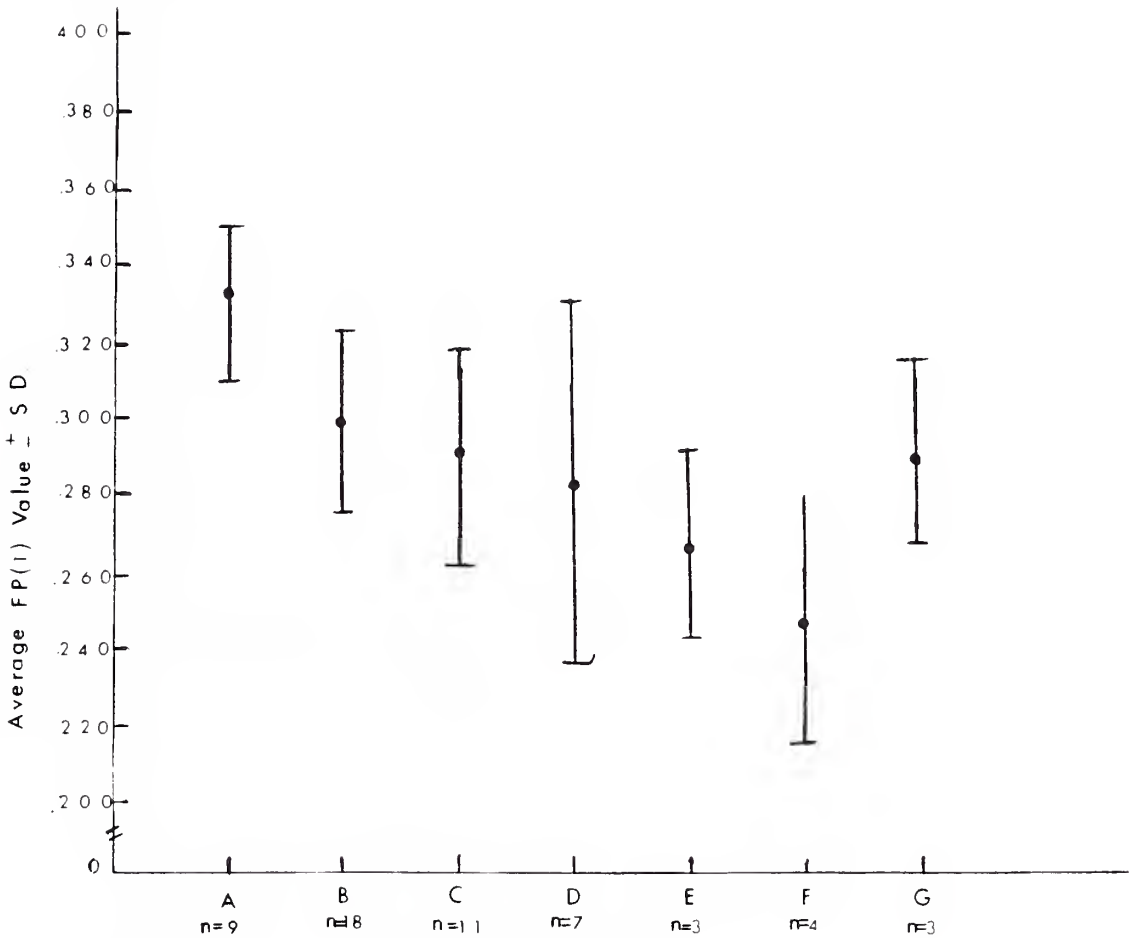
Hydramnios:

Gestational Age in weeks	L/S ratio	FP(1) value	FP(2) value	Change	Apgar	days post test
33	0.5	.315	.343	+.028	5/8	8
39	1.4	.283	.320	+.037	7/8	6
40	1.8	.284	.302	+.018	7/8	6 *
40	1.8	.234	.257	+.023	9/9	0
38	2.0	.268	.284	+.016	8/9	1
39	2.7	.319	.336	+.017	9/9	9

Mean:	at L/S >2	;	at L/S < 2
Gest. age:	38.5	;	38
FP(1):	.293	;	.279, false (+) 3/4, one (*) developed RDS
FP(2):	.310	;	.305, false (+) 3/4, one (*) developed RDS

Note* = infant developed RDS

Figure 4A FP Values at increasing L/S ratios



A - 0.2 - 1.0

B- 1.1 - 1.9

C - 2.0 - 2.8

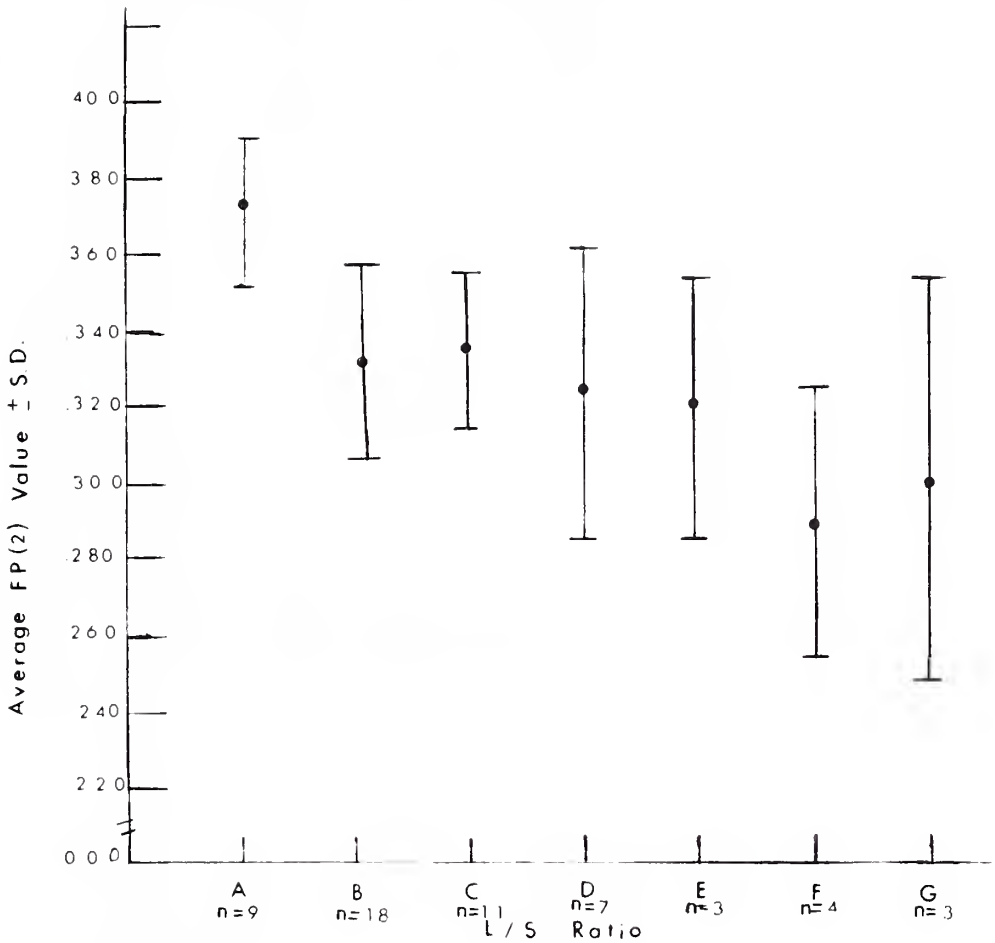
D - 2.9 - 3.7

E - 3.8 - 4.6

F - 4.7 - 5.5

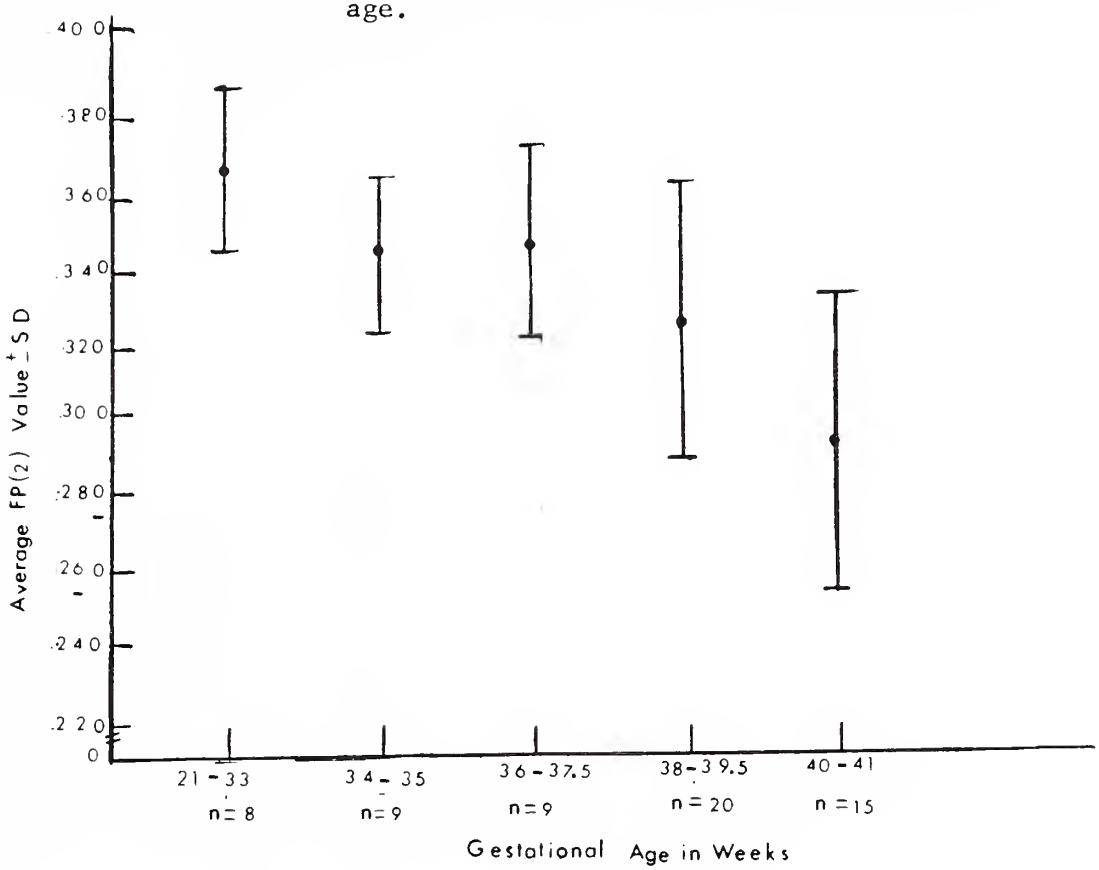
G - > 5.6

Figure 4B FP Values at increasing L/S ratios



A - 0.2 - 1.0
B - 1.1 - 1.9
C - 2.0 - 2.8
D - 2.9 - 3.7
E - 3.8 - 4.6
F - 4.7 - 5.5
G - > 5.6

Figure 4C Average FP Values at increasing gestational age.



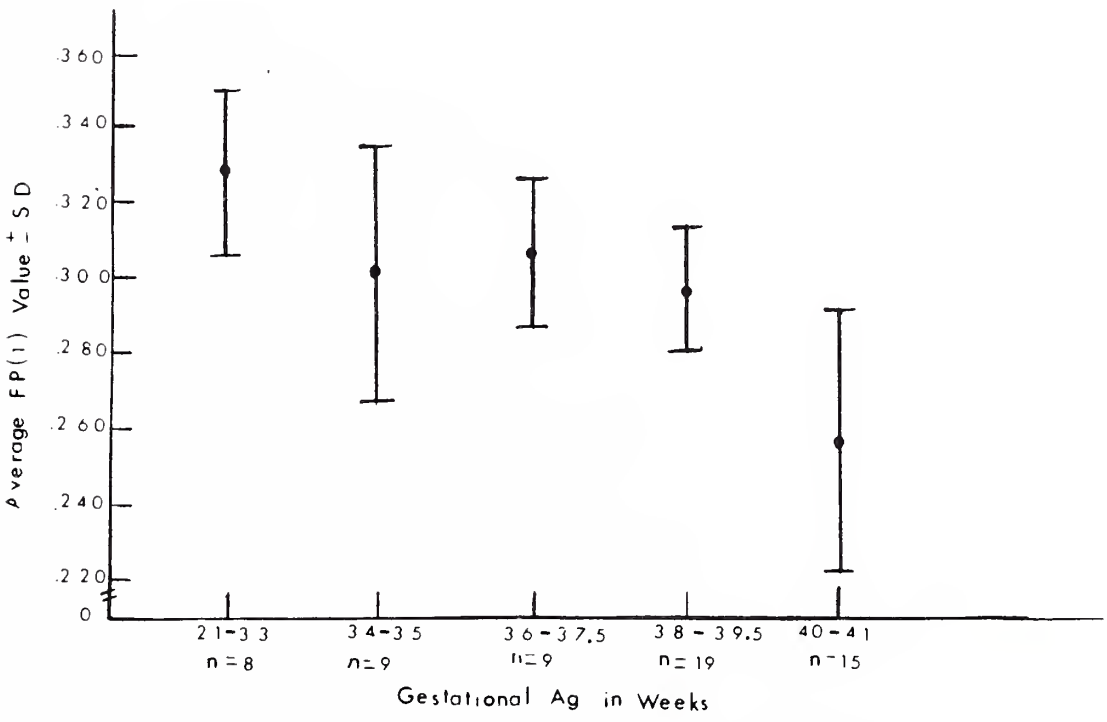
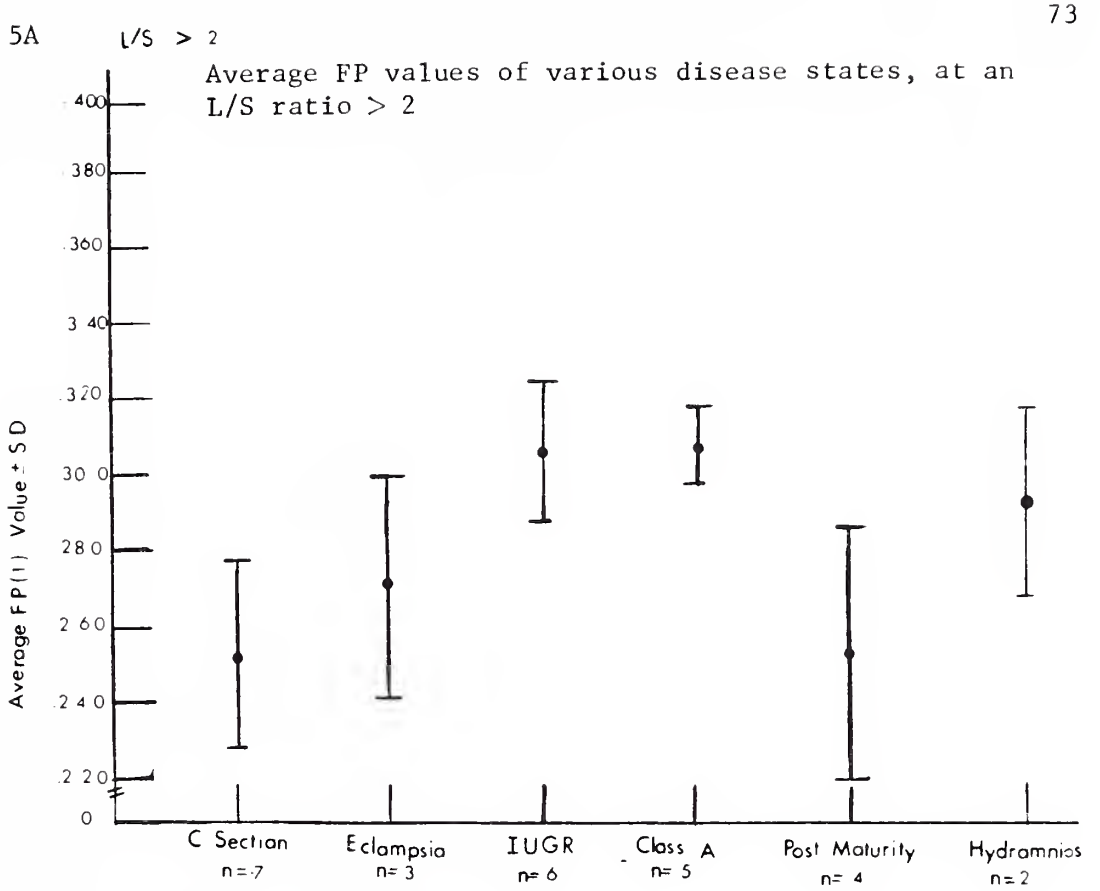


Figure 5A


L/S > 2
Average FP values of various disease states, at an L/S ratio > 2
Average F P(1) Value ± S D
400
380
360
3 40
320
30 0
280
2 60
240
2 20
0
C Section n = 7
Eclampsia n= 3
IUGR n= 6
Class A n= 5
Post Maturity n= 4
Hydramnios n= 2

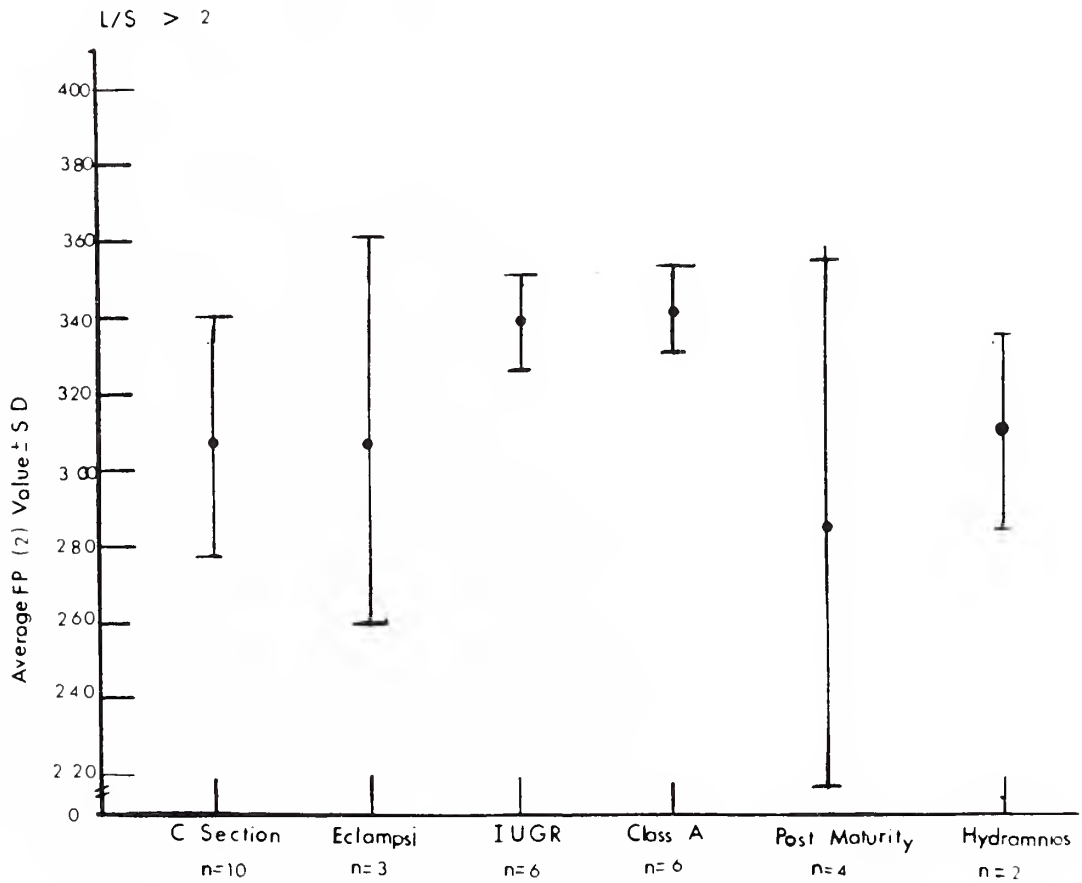
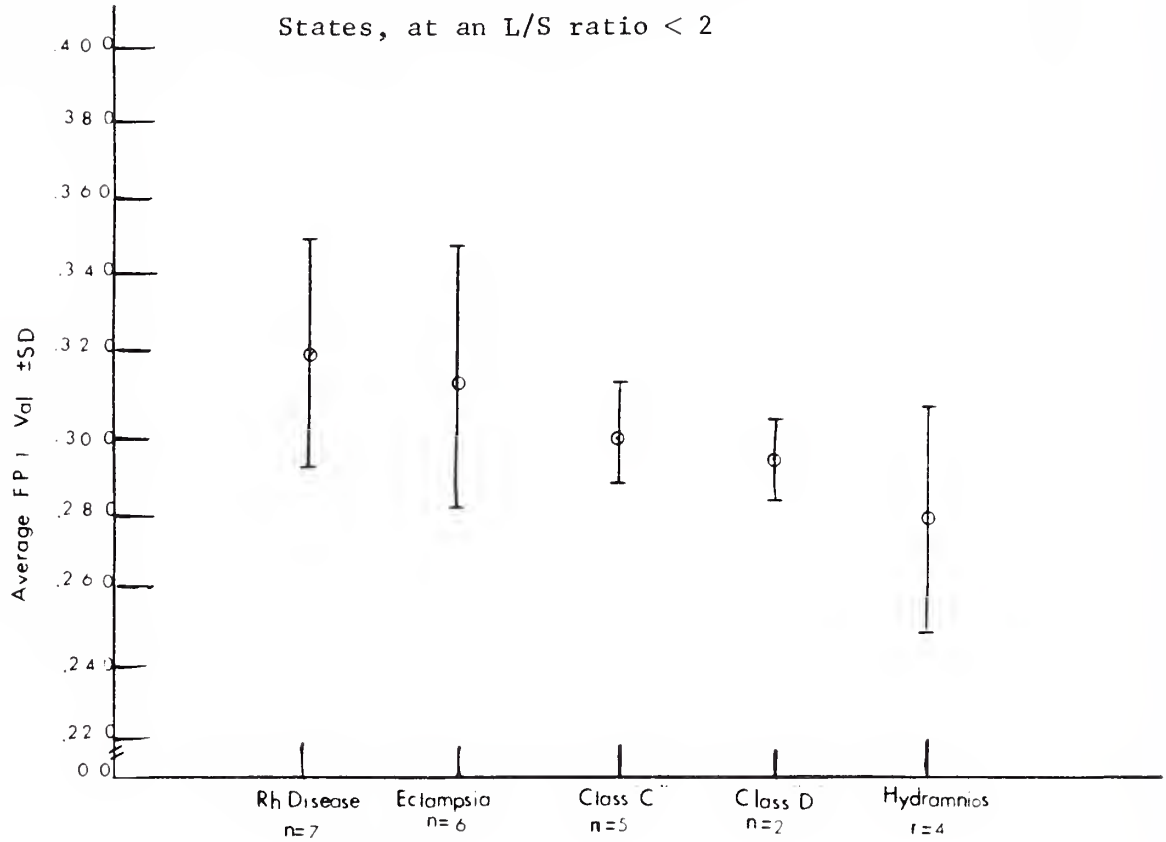

L/S > 2
Average FP (2) Value ± S D
400
380
360
340
320
3 00
280
260
240
2 20
0
C Section n=10
Eclampsi n= 3
IUGR n= 6
Class A n= 6
Post Maturity n= 4
Hydramnios n = 2

Figure 5B L/S < 2 Average FP Value of Various Disease States, at an L/S ratio < 2



L/S < 2

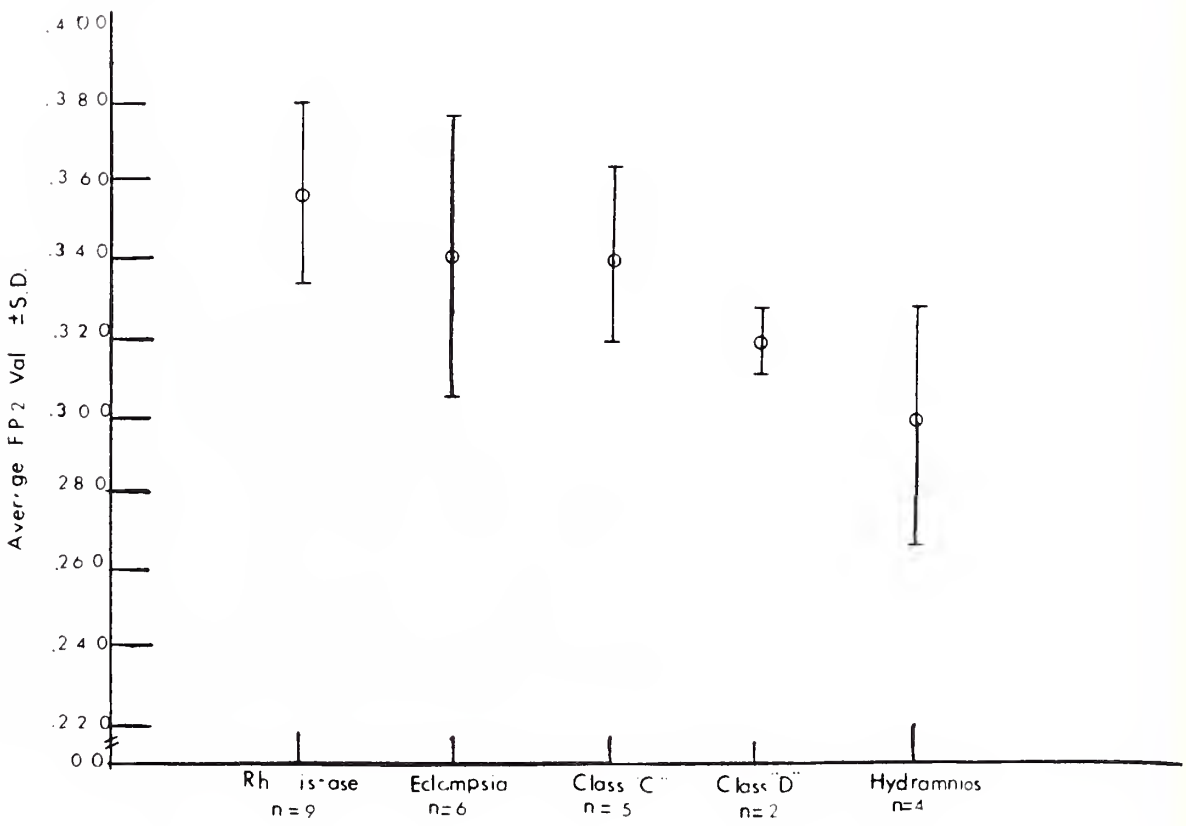
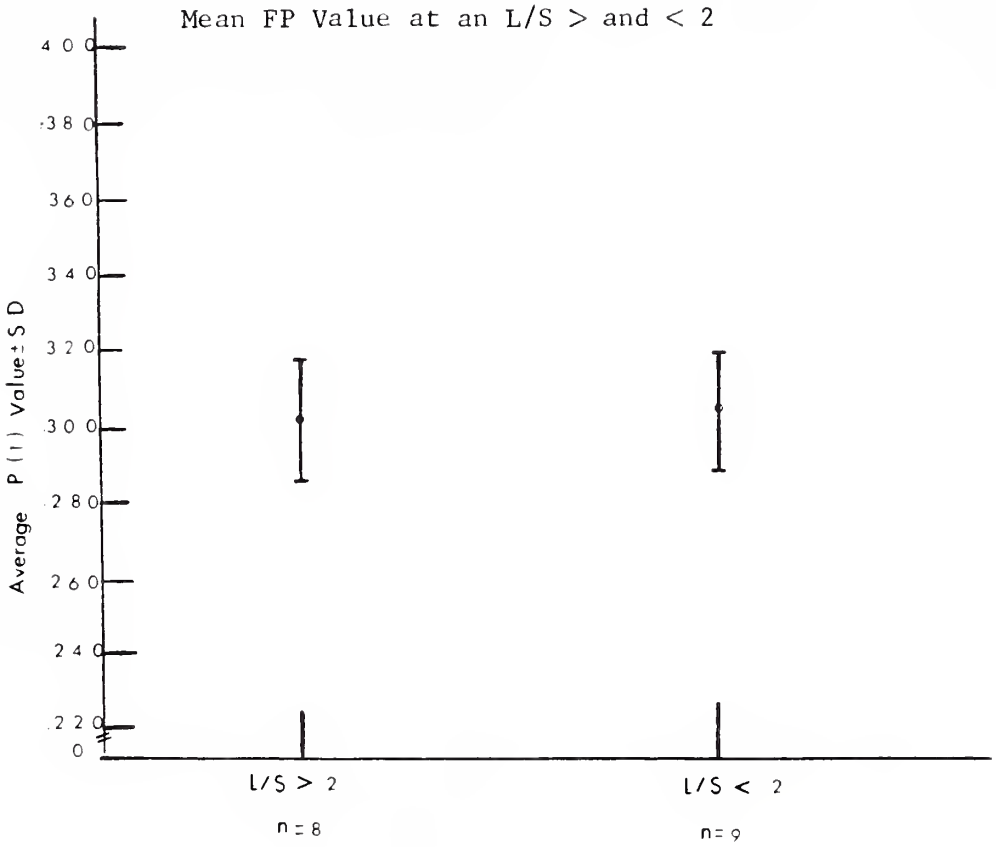


Figure 6 A

DIABETES

Mean FP Value at an L/S > and < 2



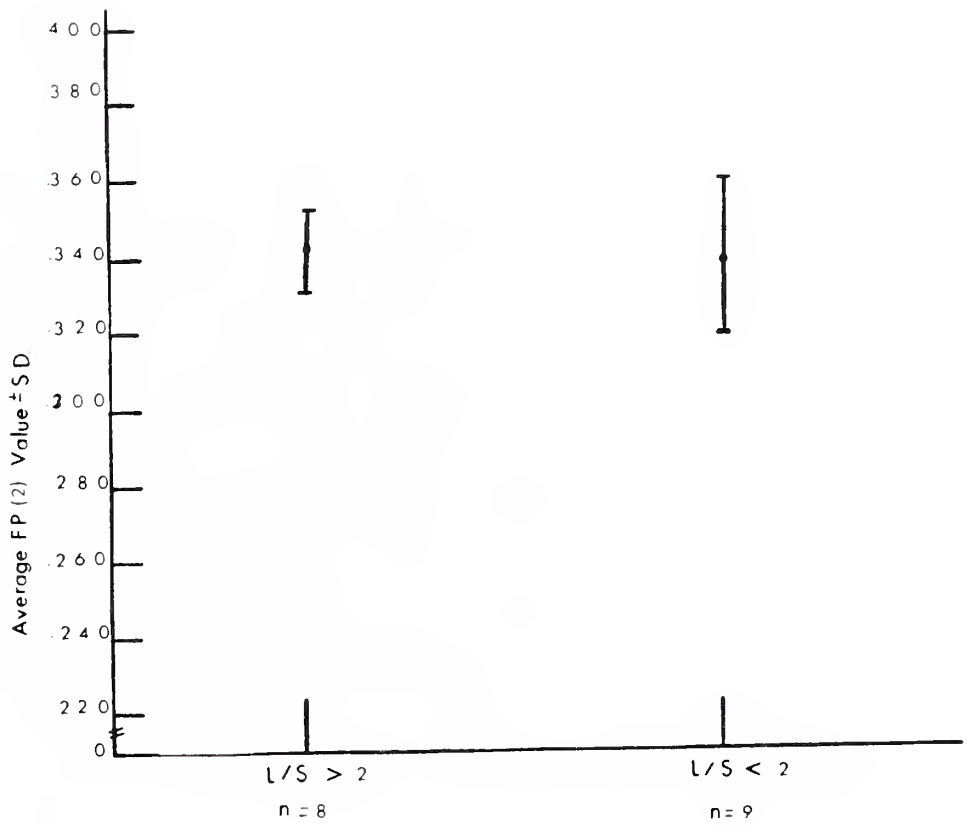
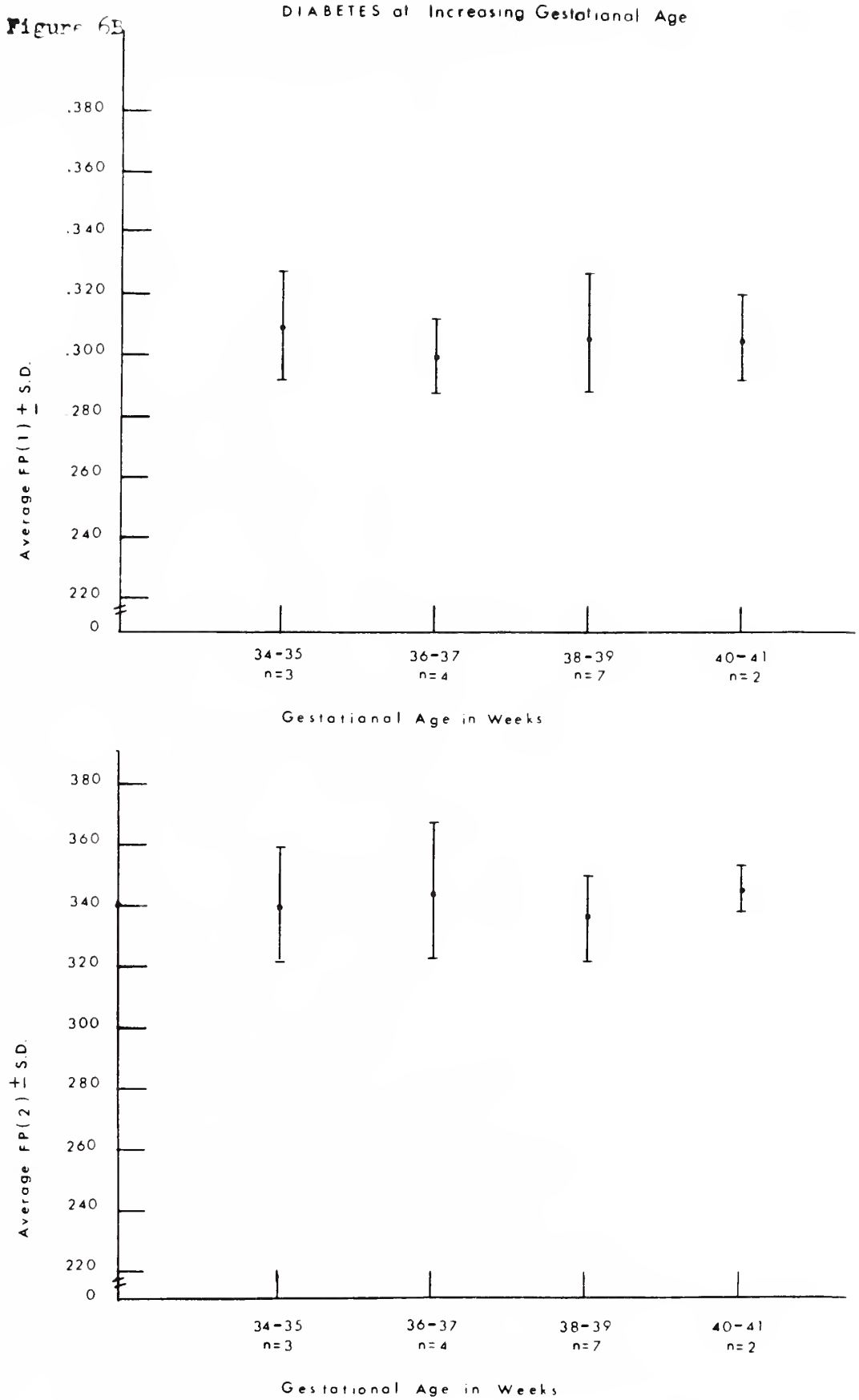


Figure 65


DIABETES at Increasing Gestational Age
Average FP(1) ± S.D.
.380
.360
.340
.320
.300
280
260
240
220
0
34-35
n=3
36-37
n=4
38-39
n=7
40-41
n=2
Gestational Age in Weeks
Average FP(2) ± S.D.
380
360
340
320
300
280
260
240
220
0
34-35
n=3
36-37
n=4
38-39
n=7
40-41
n=2
Gestational Age in Weeks

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