DETERMINATION OF GESTATIONAL AGE
AND LUNG MATURITY
Phospholipids, Creatinine and
Phosphatase in Amniotic Fluid

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by

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Umeå Universitet
Umeå 1976
"Now that we can conquer outer space it is high time that we begin to conquer the intrauterine space."

Eric Saling
The Perinatal Congress in London, 1970

To
Carin and Cecilia
The present thesis is based on the following publications which will be referred to by the Roman figures I-IV.


INTRODUCTION

The perinatal medical field embraces pregnancy, labor, and neonatal life. During this period fetal and neonatal health may be jeopardized in several ways. Mention may be made of utero-placental insufficiency during pregnancy accompanied by nutritive disturbances with fetal malnutrition and growth retardation, acute or chronic and progressive oxygen deprivation during labor and respiratory distress after birth. These conditions are often linked together, presenting insurmountable difficulties to the obstetrician and the pediatrician. There can be no doubt, therefore, that a precise determination of gestational age and fetal maturity is an urgent task for the obstetrician and of the utmost importance to the future health of the child.

In addition to the clinical signs, a body of methods is available to this end - endocrinological, roentgenological, cytological, chemical, and physical - all of which, however, have the disadvantage that quite considerable errors of measurement can occur (28, 49). The menstrual history is, however, fundamental, but often difficult to evaluate in practice, as irregular periods and anovulatory bleeding are frequently encountered in the era of the pill. Routine monitoring by diagnostic sonor of the biparietal diameter and other parameters may become the favoured method. But on the one hand it still belongs to the future and has to be further evaluated, and on the other, there is no doubt that complementary methods will be needed.
In high risk pregnancy the determination of gestational age and fetal maturity, and especially of pulmonary maturity, is indeed of great importance. Knowledge of gestational age is also of considerable value to the pediatrician for correct management of the individual infant.

A questionable gestational age or an unknown degree of feto-maternal disease might indeed make it very difficult to decide the optimal timing of delivery. If labor is induced too early the risk of hyaline membrane disease (HMD) is present, and the state is often aggravated by nutritional difficulties. Should, however, the labor be delayed too long the fetus may die due to extensive placental or fetal lesions.

Amniotic fluid has been the subject of interest since the fifties as an aid to estimating fetal condition. Fairweather and Eskes (25) have recently reviewed amniotic fluid studies. In brief, the findings which have been relevant to the present investigation, the aim of which will be explained below, are as follows.

From the classical isotope studies of Vosburgh et al (87) it is known that the pool of liquid is a reservoir with interchange of water and chemical constituents between the amniotic sac and the maternal and fetal circulation. The volume and composition of amniotic fluid undergo considerable variations during the course of pregnancy (56).

Brosens and Gordon (11) were the first to use amniotic fluid
cytology as a diagnostic tool in determining gestational age. Pitkin and Zwirek (65) concluded that the rise of creatinine concentration in amniotic fluid as pregnancy progresses has important clinical applications. Foulds and Pennock (30) found, however, creatinine concentration to be an unreliable index of gestational age.

HMD is responsible for more deaths in the neonatal period than any other disease (23). Avery and Mead (2) found in 1959 that HMD is associated with the absence or delayed appearance of a substance capable of attaining a low surface tension at expiration thus preventing alveolar collapse. This surface active material was shown to contain as a major component phospholipids, mainly dipalmitoyl lecithin, synthetized and secreted to the respiratory tract by the type II alveolar cells (41, 59, 77).

In 1971 Gluck et al (38) established that a positive correlation existed between fetal lung maturity and amniotic fluid phospholipid pattern expressed in lecithin/sphingomyelin ratio. He showed that a sudden increase in this L/S-ratio took place at or after 35 weeks’ gestation and interpreted this as an expression of fetal lung maturation.

As far as complicated pregnancy is concerned Pomerence et al (67) found no specific alteration of the amniotic fluid lipids in toxemia of pregnancy. Whitfield et al (89) found that the expected terminal rise in amniotic fluid lecithin did not always take place in cases of maternal diabetes. These findings are not quite in accordance with those of Gluck and Kulovich (37).
It has been established that the activity of certain enzymes in the amniotic fluid varies as a function of gestational age. Alkaline phosphatase activity was shown by Bonsnes (10) to increase steadily until the last trimester and then remain consistent until the time of delivery. Pathak et al (63) found a raised level of heat-stable alkaline phosphatase in serum, which was interpreted as a reflection of inadequate placental function. Fennefrohn (27) suggested that increased levels of heat-stable alkaline phosphatase in amniotic fluid indicated retarded fetal growth or placental insufficiency.
THE AIMS OF THE PRESENT INVESTIGATION

To study if amniotic fluid phospholipid concentration, lecithin/sphingomyelin concentration ratio (L/S-ratio), creatinine concentration and acid- and alkaline phosphatase concentration, as well as combinations of these parameters, could be used as an index of gestational age in normal and complicated pregnancy.

To evaluate the prognostic value of lecithin/sphingomyelin concentration ratio in the antenatal prediction of Hyaline Membrane Disease.
PATIENT MATERIAL

The present series consisted of 185 samples of amniotic fluid from 174 healthy women between the 15th and 43rd week of gestation (I, IV). 176 samples from 69 patients with complicated pregnancy in the 27th-43rd week were also assayed (II, IV). Gestational age was defined and estimated according to criteria given in paper I.

253 samples were obtained from normal and complicated pregnancies within three days before delivery. 246 live children were born and in 7 cases the infants were dead at birth. Gestational age was not known in many cases (III). All women were examined and delivered at the University Hospital of Umeå.

ASSESSMENT OF PREGNANCY COMPLICATION (II, III, IV)

Diagnosis and classification of toxemia was made according to recommendations of the U.S. Committee on Maternal Welfare (42). The White classification was used for diabetic mothers (88). All mothers with Rhesus-isoimmunization had antibodies demonstrated by the indirect Coombs' test. The liveborn infants had a positive direct Coombs' test. The degree of isoimmunization was expressed according to Jonasson (51).

ASSESSMENT OF THE NEONATES

All afflicted children were examined and treated at the Neonatal Ward of the Department of Pediatrics. Hyaline membrane disease (HMD), often called idiopathic respiratory distress
syndrome (IRDS), was defined according to Hutchison’s criteria (50). Respiratory distress (RD) was considered if the criteria for the diagnosis of HMD were not fulfilled in cases with respiratory symptoms.

METHODS

SAMPLING. Amniotic fluid was obtained by abdominal amniocentesis (90) or by vaginal puncture of the amniotic sac under visual control through an amnioscope. Samples containing blood or meconium were not included.

HANDLING THE SAMPLE. The specimens were centrifuged for ten minutes and then filtered through filter paper. One part of each sample was frozen and kept at -18°C until analysed for phospholipids and enzymes. The other part was kept in a refrigerator until analysed for creatinine, mostly within 24 hours.

ANALYTICAL METHODS

Creatinine concentration was determined by an autoanalyser according to Jaffe’s method (15).

Phospholipid concentration and L/S-ratio were determined as is described in detail in paper I. The determination of L/S-ratio was a modification of Gluck’s technique (18). To 5 ml of the filtrate 12.5 ml methanol and, after shaking, 12.5 ml chloroform were added. The homogenous phase was allowed to stand for about 30 min at room temperature and then 6.25 ml 0.9% NaCl was added and the mixture shaken. After phase separation the lower chloroform phase containing the lipids
was recovered and dried under a stream of nitrogen. The lipids were dissolved in 100 microliters of chloroform and 10-20 microliters applied to 0.25 mm TLC plates coated with silica gel H. The plates were developed with chloroform/methanol/acetic acid/water 25/15/4/1 (by vol), the spots visualized by charring with conc. \( \text{H}_2\text{SO}_4: \text{H}_2\text{O} 1:1 \) (v/v) and the height times width of the lecithin and sphingomyelin spots were measured.

**Acid- and alkaline phosphatase** were determined according to Beckman et al (6). Double-diffusion experiments in agarose and starch gel electrophoresis were used to identify different isoenzymes.

**STATISTICAL METHODS**

Only conventional statistical assumptions and calculations have been used with the exception of publication II. In that paper a special method for testing possible differences between normal and complicated pregnancy was applied. Under the assumption that the values in the normal series were normally distributed, prediction intervals for each time period of gestation were calculated as

\[ M \pm \text{S.D.} \sqrt{\frac{n+1}{n}} \times t_{0.975}(n-1) \]

where \( t_{0.975}(n-1) \) is the 97.5:th percentile of a t-distribution with \( n-1 \) degrees of freedom. The probability that the value of a presumptive "normal" individual falls outside this interval is approximately 5%.

For each of the pathological groups and for each time period,
the number of cases with values outside the corresponding normal prediction interval was recorded. This number follows a binominal distribution with parameters $n$ and 0.05, where $n$ is the number of individuals in the pathological group during the specific time interval. The probability of obtaining the observed result or a more extreme one was calculated. A small probability value indicates that the pathological group has a distribution of values which should not be regarded as "normal".

RESULTS AND DISCUSSION

METHODOLOGICAL STUDIES (I)

The centrifugation and subsequent filtration were found to remove a considerable fraction of the phospholipids. The absolute value of phospholipid concentration was strongly dependent on the rate of centrifugation and on whether filtration was performed or not. On the other hand the relative phospholipid composition was not affected. The L/S-ratio is therefore probably constant, independent of the rate of centrifugation and filtration, as was earlier presumed by Quinlivan et al (68). In other methods quantifying the amount of surfactant (7, 16, 60) the rate of centrifugation as well as the volume of amniotic fluid plays an important role, which is why misleading conclusions may sometimes be drawn.

The accuracy of height x width determinations of L/S-ratio in our method was tested against standard mixtures and amniotic fluid samples with "known" L/S-ratios (determined by
phosphorus determination). The linear correlation coefficients were 0.83 and 0.86 respectively, which agrees with figures obtained by Roux et al (74). The precision of this planimetric procedure for L/S determination is thus reasonably good if the real ratios are not larger than 4-5. This limitation is, however, of minor importance from a clinical point of view.

Meconium and blood are sometimes contaminants of amniotic fluid samples. It is important to know the degree of influence on the L/S-ratio in order to interpret the results. Thus meconium analysis showed only small amounts of phospholipids and a low L/S-ratio of about 1.5. These data indicate that contamination with meconium might lower the L/S-ratios, particularly after the 35th week. The high L/S-ratios reported by Kulkarni et al (53) cannot thus be confirmed. The L/S-ratio in plasma is about 4 (46, 64) and thus contamination with blood mainly affects the ratio before the 35th week.

PHOSPHOLIPID PATTERNS IN AMNIOTIC FLUID (I)

A "surfactant rich" fraction from adult human lung samples was analysed and its phospholipid content compared with that of amniotic fluid samples obtained in the second trimester and at term. The phospholipids and fatty acid composition of lecithin were very similar in the lung fraction and in the amniotic fluid at term. In early gestation a different pattern with considerably less dipalmitoyl-lecithin and a higher amount of sphingomyelin was found. These data are in accord with the view that the phospholipids that accumulate in amniotic fluid late in pregnancy, derive from fetal lung
according to Biggs et al (8, 9) and Reynolds et al (69). Condorelli et al (17), however, revealed the possibility that urine and fetal serum may be major contributors of amniotic fluid phospholipids.

The concentration of phospholipid phosphorus reflecting the total amount of phospholipids in amniotic fluid, showed a slight increase with advancing gestation. The large dispersion makes this determination a poor parameter in assessing gestational age, which does not agree with earlier reports (61, 78). The different results may be due to methodological reasons.

The distribution of amniotic fluid phospholipids was determined in 47 samples. Both the percentage of sphingomyelin and lecithin were significantly correlated to gestational age, which agrees well with data of others (38, 61, 74).

ASSESSMENT OF GESTATIONAL AGE IN NORMAL AND COMPLICATED PREGNANCY BY MEANS OF L/S-RATIO AND CREATININE CONCENTRATION (I, II)

A. L/S-ratio

In normal pregnancy the L/S-ratio increased slowly up to around the 35th week after which there was a very rapid increase. The ratio was closely correlated to the gestational age ($r = 0.712, p < 0.001$). The change in L/S-ratio with increasing gestational age agreed well with previous investigations (18, 19, 34, 38, 46). The ratios found were similar to those found by Spellacy and Buhi (81), who used a similar
technique, but differed from those reported by Quinlivan et al (68).

A L/S-ratio of $\geq 2.25$ indicated all healthy women with uncomplicated pregnancy to be in $\geq$ the 34th week of pregnancy. 64 per cent of the pregnancies had attained the 38th week.

The L/S-ratio was found to be significantly higher in the toxemia group during the 34-35th week than in normal pregnancy ($p < 0.05$). No statistical difference was found in the other time periods. This is in accordance with the findings of Gluck et al (37, 40) who also reported an early increase in L/S-ratio in pregnancy complicated by maternal disease other than toxemia. Other investigators, however, have found no increase in L/S-ratio (18, 19, 22) or in amniotic fluid lecithin concentration (5, 7) in complicated compared with normal pregnancy. This lack of a significant deviation from normal pregnancy may be due to differences in classification of toxemia.

In a pregnancy complicated by isoimmunization or maternal diabetes the L/S-ratio did not deviate significantly from that in normal pregnancy in accordance with findings of Doran et al (22). This is not in agreement with other reports, where the increase of L/S-ratio was found to be delayed in Class A, B and C diabetes (37, 66, 89), but accelerated in Class D, E and F diabetes (37). The number of patients in most of the investigations was, however, relatively small and sometimes the compared nondiabetic group consisted of cases with toxemia.
B. Creatinine concentration

The creatinine concentration increased slowly during gestation with a highly significant correlation to gestational age ($r = 0.886, p < 0.001$) in normal pregnancy. The increase from week to week was more pronounced towards term. A creatinine concentration of $\geq 1.8 \text{ mg} \%$ was found to represent a gestational age of $\geq 38$th week in 75 per cent of the cases. The remaining ones were all $\geq 35$th week of gestation. These figures are in agreement with most of the earlier reports (20, 33, 65, 85). According to Foulds and Pennock (30), however, creatinine concentration is an unreliable index of gestational age. Possibly the poor reliability could be explained by the heterogeneous material consisting of both normal and complicated pregnancies.

From the 32nd week until term the mean level of creatinine was significantly higher ($p < 0.05$) in toxemia than in normal pregnancy of corresponding age. This supports the findings of Roopnarinesingh (71) and O’Leary et al (62) but are in conflict with many others (21, 22, 47). The reason for the higher concentration of creatinine has been suggested by Roopnarinesingh and Morris (72) to be a decreased rate of transport of creatinine from the amniotic fluid. The possibility that maternal substances pass through the placenta affecting fetal renal function or that placental insufficiency causes increased fetal catabolism may also be considered.

A creatinine concentration $\geq 1.8 \text{ mg} \%$ in amniotic fluid was found in 64 per cent of the preterm cases of pregnancy with toxemia ($<36$th week) but only in 10 per cent of normal pregnancy $<36$th week.
but >30th week. If this difference between normal and toxemia pregnancy is overlooked, there is thus a considerable risk that the gestational age may be overestimated possibly with severe consequences for the fetus.

In diabetes the creatinine concentration was within the normal range except in the 36-37th week, where a significant rise was found. Whether this difference is an expression of a superimposed toxemia is difficult to say. A slight increase in creatinine concentration was reported and interpreted by Roopnarinesingh (70) as a sign of accelerated maturation. In our series the corresponding lung maturity as indicated by L/S-ratio was, however, not accelerated (II, III).

From the 32nd week the mean creatinine concentration in pregnancy with isoimmunization was in the lower range of the confidence interval for normal pregnancy. This, however, could not be verified statistically. The finding is in line with results by Hinkley et al (48). If, therefore, the estimation of gestational age is based mainly on the creatinine concentration some cases might incorrectly be placed in a low zone according to Liley. The consequences for the fetus might be severe.

C. Combined L/S-ratio and creatinine concentration

There was a wide scattering of both L/S-ratio values and of creatinine concentrations at a certain gestational week. On the other hand, a combination of borderline values made it possible to discriminate better between a preterm pregnancy and that at term. In normal pregnancy, all cases had reached
the 36th week and in 71 per cent the pregnancy had reached the 38th week, if the criteria of a creatinine concentration >1.8 mg% as well as a L/S-ratio >2.25 were fulfilled. Thus no normal pregnancy <36th week fulfilled the above criteria. In high risk pregnancy an increased precision was also obtained when discriminating between different gestational ages. The pregnancy was always ≥35th week when the above criteria were used.

AMNIOTIC FLUID LECITHIN/SPHINGOMYELIN RATIO AND PREDICTION OF HYALINE MEMBRANE DISEASE (III)

The L/S-ratio was correlated with the clinical course of the neonate. The L/S-ratio had a high degree of reliability in predicting fetal lung maturity, a ratio ≥2.0 indicating that functional pulmonary maturity had advanced to the stage that permitted extrauterine life to proceed normally with no likelihood of HMD. Creatinine concentration seemed to be of minor clinical value in predicting HMD.

When the L/S-ratio was <1.75 50% of the 16 children developed HMD. Ten newborn babies developed HMD and the highest L/S-ratio in this group was 1.82. Most investigators (12, 19, 34, 37, 46, 52, 78, 81) find L/S-ratios 1.5-2 to be values above which HMD is uncommon with the exception of Schulman et al (79). Minor differences may be due to methodological and diagnostic variations.

HMD developed in 50% of the preterm infants with birth-weights <2000 g in contrast to the nine low birth-weight infants in the toxemia group (<2000 g), where no case of HMD occurred.
This difference may be due to the fact, that some infants in the latter group were small-for-date and that lung maturity is accelerated in toxemia (37, II) or in utero-placental insufficiency due to other reasons (39).

The risk of HMD is considered to be increased in infants delivered by Caesarean section and among infants of diabetic mothers (1, 86). This opinion is refuted by others (19, 32). In this report there seems to be a higher rate of HMD among children delivered by Caesarean section. The gestational age was, however, found to be incorrectly estimated to be near term in two of the three cases of HMD. Corrected data suggest that the incidence of HMD is not increased by Caesarean section or by diabetic pregnancy if, according to L/S-ratio, fetal lung is mature prior to delivery.

The incidence of respiratory distress (RD) and HMD showed no appreciable difference between children after induced and after spontaneous labor. Conclusions concerning a possibly increased risk of HMD can not be drawn in cases of intrapartum hypoxia as the number of infants afflicted was too small.

Fetal maturity is usually related to a gestational age of \(>36\) weeks. This investigation, however, shows that lung maturation is subject to large biological variations, even among twins, and can be affected by complicating feto-maternal diseases.
In normal pregnancy heat-labile alkaline phosphatase showed two "peaks" of activity, one in the 15th-22nd week and the other one at term. Both heat-stable alkaline- and acid-phosphatase significantly increased after the 36th week. These alterations in phosphatase levels are in agreement with findings by Sutcliffe et al (83) and Hahnemann and Sørensen (45).

Gestational age was also divided into four periods. No significant difference in the levels of acid phosphatase was found between normal and complicated pregnancy. In isoimmunization heat-stable alkaline phosphatase was decreased between the 36th and 40th week (p < 0.01). Geyer and Schneider (35) also found a lowered level of alkaline phosphatase in immunization, but the observed decrease in heat-stable alkaline phosphatase level has not been reported before. In toxemia the activity of heat-stable alkaline phosphatase was within the normal range. The observation by Roopnarinesingh et al (73) and Fennerfrohn (27) of an increased level of heat-stable alkaline phosphatase in pre-eclampsia or placental insufficiency can thus not be confirmed in our study. The finding (27, 73) might be explained by the fact that meconium is sometimes let out in cases of fetal distress. Even a slight contamination by meconium may give a pronounced increase in the phosphatase level. In this investigation 11 cases of toxemia with placental insufficiency showed neither increase nor decrease in the activity of alkaline phosphatase compared
with the normal range.

Estimation of gestational age or fetal maturity by means of phosphatase in amniotic fluid was not possible due to an appreciable overlapping between the levels in early and late pregnancy. Studies of acid- and alkaline phosphatase also seemed to be of little value in the clinical management of complicated pregnancy.

23 meconium-contaminated samples showed a complex pattern of the phosphatase enzymes. Mostly one or two of the enzymes had very high levels.

Samples with high levels of heat-stable alkaline phosphatase reacted in double-diffusion experiments with an antiserum prepared against purified placental alkaline phosphatase. This is also a property of intestinal alkaline phosphatase.

The electrophoretic pattern of acid- and alkaline phosphatase showed an activity similar to that found in meconium. Alkaline phosphatase in amniotic fluid did not coincide in its electrophoretic mobility with any of the common phenotypes of placental alkaline phosphatase.

The origin of phosphatase in amniotic fluid is of great interest. Sutcliffe and Brock (82) suggested heat-stable alkaline phosphatase in amniotic fluid to be of placental origin. Changed levels could therefore reflect disturbances in placental function (27, 73). In this study the alkaline phosphatase in amniotic fluid was found to be different from common
placental alkaline phosphatase phenotypes. Furthermore, cases with placental insufficiency had levels of alkaline phosphatase within the normal range. Heat-stable alkaline phosphatase is thus most likely not of placental but probably of fetal intestinal origin.

ASPECTS OF MANAGEMENT OF COMPLICATED PREGNANCY

Much evidence has accumulated suggesting that the maturation of the fetal lung may be accelerated by corticosteroids. These hormones are possibly important physiological inducers of the synthesis of surface active material in the lung (24, 26, 54, 84). Furthermore, Giannopoulos (36) found that human fetal lung contains specific glucocorticoid receptors, which implies that these hormones probably play a regulatory role in lung maturation.

Controlled human trials indicate that antepartum administration of steroids to pregnant women might be associated with a substantial reduction in HMD, particularly infants of less than 32 weeks gestation (14, 55). Postnatal administration of corticosteroids is, on the other hand, ineffective as a therapeutic agent in HMD (3).

Hazards connected with prenatal administration of corticosteroids were reported by Liggins and Howie (55). They found an increased perinatal mortality in cases of toxemia, fetal growth retardation or low urinary estrogen excretion compared with an untreated group. Furthermore corticosteroid treatment is associated with a reduction in the secretion of estrogen precursors by the fetal adrenal glands (80). It might thus
be very difficult to interpret whether a decrease in urinary estrogen in high risk pregnancy, such as placental insufficiency and small-for-date, is due to a depressed placental function or to the profylactic corticosteroid drug. Corticosteroid therapy should possibly be avoided in these cases as lung maturity seems in any event to be accelerated in toxemia (37, II, III), fetal growth retardation or ablatio placentae (39, III) and prolonged preterm rupture of the fetal membranes (4, 58). Steroid therapy might therefore be suitable in women with imminent premature labor and possibly in preterm rupture of the membranes as well as in severe isoimmunization, with a low L/S-ratio calling attention to pulmonary immaturity of the fetus.

In high risk pregnancy such as toxemia, small-for-date or progressive placental insufficiency the obstetrician will obtain valuable information by amniocentesis to help him to time the delivery. Sometimes the finding of meconium contamination or oligoamnios is a serious sign of fetal distress (31, 57). Fujikura and Klionsky (31) recently found that the incidence of HMD in infants weighing 1500 g or less at birth was significantly lower in a group with meconium staining of the amniotic fluid compared with a group without staining. The rate of neonatal death was, however, increased. There is also evidence that damage to the central nervous system can be demonstrated later in life in many children with prolonged exposure to poor intrauterine environment (29, 43, 44, 91). Sabel et al (76) and Rubin et al (75) found evidence of increased manifestation of neurological and psychological impairment over a period of time for small-for-date children.
when compared with children for whom low birth-weight was appropriate to gestational age. Delivery, usually by Caesarean section, should therefore be considered in certain high risk pregnancies as soon as the L/S-ratio predicts lung maturity (III) to avoid an increased risk of intrauterine death or cerebral sequele. Routinely the L/S-ratio should be determined before an elective Caesarean section, so that delivery may be postponed until lung maturity is obtained (III).

The concentration of cortisol in amniotic fluid should be investigated. Raised cortisol levels may be an expression of physiological stress secondary to the natural process of fetal maturation as well as to fetal distress during gestation or labor.
GENERAL SUMMARY

1. A method of analysing samples of amniotic fluid (centrifugation, filtration and lipid extraction) and of determining the L/S-ratio is described and its limitations defined and discussed.

2. The relative amounts of lecithin and sphingomyelin as well as the L/S-ratio and creatinine concentration in amniotic fluid were highly correlated to gestational age and made discrimination possible between gestational ages <36th week and ≥38th week.

3. Combination of L/S-ratio and creatinine concentration increased the precision in estimating gestational age.

4. The total amount of phospholipids showed only a slight increase as well as large variation during gestation and was therefore unreliable in assessing gestational age.

5. The mean level of creatinine in toxemia was significantly elevated (p <0.05) from the 32nd week until term and in diabetes significantly elevated in the 36-37th week compared with normal pregnancy.

6. In toxemia the L/S-ratio was significantly higher (p <0.05) in the 34-35th week compared with normal pregnancy. This is possibly an expression of accelerated lung maturity due to intrauterine fetal distress.
7. The L/S-ratio correlated well to functional pulmonary maturity of the neonate and is a very useful predictive tool in assessing the risk of HMD prior to a planned induction of labor or an elective Caesarean section.

8. When the L/S-ratio was $\geq 2.0$ HMD did not occur irrespective of birth-weight and such a ratio is regarded as a sign of maturity. When the L/S-ratio was $< 1.75$ 50% of the 16 children developed HMD.

9. In toxemia the creatinine concentration may be very high, while the L/S-ratio still is low with risk for development of HMD if delivery occurs.

10. Caesarean section was not found to be associated with HMD if the L/S-ratio prior to the operation indicated lung maturity.

11. In contrast to low birth-weight infants born to women with toxemia or chronic hypertensive disease, where no case of HMD appeared, children with birth-weights $< 2000$ g developed HMD in about 50 per cent of the cases. The difference in incidence might partly be due to the fact that infants in the former group were small-for-date, partly depending on an accelerated lung maturity.

12. Induction of delivery, usually by Caesarean section, should be performed in certain very high risk pregnancies as soon as the L/S-ratio indicates lung maturity.
13. The activity of heat-labile alkaline phosphatase (HLAP), heat-stable alkaline phosphatase (HSAP) and acid phosphatase (AcP) significantly increased during gestation. Due to the large dispersion phosphatase determinations were unreliable in assessing gestational age.

14. No difference in phosphatase activity was found between normal and complicated pregnancy apart from a lowered level of HSAP in the 36-40th week of isoimmunization (p <0.01).

15. HSAP was most likely found not to be of placental but of intestinal origin.
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