0031-3998/85/1903-0253$02.00/0
PEDIATRIC RESEARCH

Vol. 19, No. 3, 1985
Printed in U.S.A.

Fetal Lung Development in the Diabetic Pregnancy

JACQUES R. BOURBON AND PHILIP M. FARRELL

Laboratoire de Physiologie du Développement, Collège de France, Paris, France and Department of Pediatrics, University of Wisconsin, Madison, Wisconsin

INTRODUCTION

Statement of the problem. RDS[1] due to pulmonary surfactant deficiency (10) remains a major cause of perinatal mortality and morbidity despite progress in prevention and treatment (209). An increased incidence of RDS in IDM was initially reported in 1959 by Gellis and Hsia (94). Since that time, the influence of maternal diabetes on fetal lung development has become a field of intensive clinical and experimental animal research. These investigations have sometimes led to conflicting results and opinions. Whether or not maternal diabetes mellitus by itself has a direct effect on fetal lung maturity was the first controversial question to arise (80, 272). Even if it is accepted that the diabetic state per se increases the incidence of RDS (at least in some defined classes of hyperglycemic mothers), the exact nature of the alterations in lung development and the mechanism(s) by which the metabolic disturbances impair the process of lung maturation remain poorly understood. In particular, the respective role of increased blood glucose and of fetal hyperinsulinism have not been clarified.

Clinical studies have usually been descriptive at best and have not provided cellular or molecular clues as to pathogenetic mechanisms. This is largely attributable to the limitations in clinical research since only observations on infants with lung disease and indirect studies using many different amniotic fluid tests have been possible. In basic research, a major limitation has resided in the problem of creating appropriate animal models that reproduce the characteristic features of human diabetic pregnancies. Thus, although clinical and basic information has led to improvements in both diagnosis and prevention, the abundance of sometimes contradictory data and the discrepancy between interpretations render the literature extremely confusing and call for an overall synthesis of experimental information dealing with both clinical and biological research. The purpose of this paper, therefore, is to review information from the literature and describe the different experimental approaches being used at present. Recommendations are made for improvements in and standardization of experimental design. Whenever possible, we present conclusions about what has been clearly established and what remains questionable. Additionally, we have proposed explicative hypotheses concerning implicated mechanisms.

Normal process of lung development. The human lung differentiates early in gestation from a ventral bud of primitive foregut epithelium and surrounding mesenchyme. A series of asymmetric branchings gives rise to the bronchial tree which is completed on the 16th postconceptional week. Epithelial cells of bronchi and alveoli derive from the primitive epithelium. During the saccular phase (from 25–26 wk until the postnatal period), the future alveoli are formed and the terminal epithelium differentiates into two principal cell types: 1) type I pneumocytes which form the thin wall of the alveolar sacs and facilitate gas exchange; 2) type II pneumocytes which during the last 10% of gestation show increased numbers of characteristic osmiophilic lamellar bodies, the intracellular storage form of lung surfactant. Simultaneous with appearance of lamellar bodies, the glycogen stores of these cells disappear.

When the lung is mature, pulmonary surfactant appears as a lipoprotein complex which facilitates efficient gas exchange by lowering surface tension at the alveolar-air interface (159), and possibly by inducing water repellency in alveoli (133). Biochemically, the major components of surfactant are phospholipids. The principal constituent is PC, particularly DSPC, which is otherwise called disaturated lecithin (81). Other phospholipids such as PG also are thought to play a functionally important role (81, 120).

Increasing amounts of phospholipids are observed in lung tissue in association with accumulated lipid in type II pneumocytes. Similar changes in phospholipid concentrations are seen in tracheal and amniotic fluids as pregnancy progresses to term (81). On the basis of several studies in animals (reviewed in Ref. 81), the elevated concentration of phospholipids has been attributed to increased *de novo* synthesis via the CDPcholine pathway. Biochemical changes underlying lung maturation are exceedingly complex, and although metabolic pathways for production of lipids such as PC have been identified, many important maturational processes and regulatory factors remain to be elucidated (81, 101, 104, 107, 122, 155, 204, 253). Moreover the timing of key biological events in lung maturation varies among species.

To the extent that the results of Jobe *et al.* (148) in the rabbit fetus can be extended to the human species, it appears that newly synthesized PC reaches the alveolus after a long lag period, and alveolar stability would be entirely dependent during the early postnatal period upon phospholipids stored in anticipation of birth. Any impairment in phospholipid production in late gestation could, therefore, have prolonged consequences upon alveolar stability, even when an underlying maternal or intrauterine abnormality has ceased to be influential because of birth.

CLINICAL DATA

Heterogeneity of the diabetic pregnancy. Diabetes mellitus in pregnancy is not a unique pathological condition but rather a family of conditions whose common factor is glucose intolerance. Included among many variables are the time of appearance of glucose intolerance, variation of insulin requirements, and the severity of metabolic disturbances and of organ damage. Various classifications of diabetes mellitus exist. For the diabetic pregnancy, the system used widely is that of White (281), which takes into account age of onset, duration, and severity of diabetes and has proved useful in predicting the outcome of diabetic pregnancies and in individualizing obstetrical care (86). One condition,

Received November 30, 1983; accepted September 26, 1984.
Address all correspondence and reprint requests to Jacques R. Bourbon, Ph.D., Laboratoire de Physiologie du Développement, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05, France.
This work was partly supported by grants from Institut National de la Sante et de la Recherche Medicale (PRC 135002) and the National Institutes of Health (HD 11429 and P50-HL-27358.

[1] Abbreviations: RDS, respiratory distress-syndrome; IDM, infants of diabetic mothers; PC, phosphatidylcholine; DSPC, disaturated phosphatidylcholine; PG, phosphatidylglycerol; L/S, lecithin/sphingomyelin; STZ, streptozotocin; PI, phosphatidylinositol; FFA, free fatty acids.

namely gestational diabetes (class A), merits further description because it has been most frequently associated in the literature with delay of fetal lung development. It is characterized by abnormal glucose tolerance during pregnancy and reversal to normal pospartum. Its transient nature reflects the inability of the β cell to keep pace with the increased demands for insulin generated by the hormonal milieu of pregnancy associated with relative insulin resistance (41, 157); islet cell function is subsequently sufficient for glucoregulation in the nongravid state.

Lung developmental abnormalities associated with diabetic pregnancies. RDS may be defined as an acute restrictive pulmonary disease characterized by generalized atelectasis which develops shortly after birth in susceptible premature infants; this is principally due to pulmonary surfactant deficiency and often leads to progressive ventilatory failure (78). The pathophysiology and epidemiology of the disease has been described in detail elsewhere (78). In addition to prematurity, several other risk factors for RDS influence perinatal lung function including: 1) fetal sex [males more frequently develop fatal RDS (84, 188)]; 2) mode of delivery [higher incidence of RDS after cesarean section delivery without antecedent labor (85, 272)]; 3) perinatal asphyxia and maternal hemorrhage [increased risk (80]; 4) maternal diabetes with hyperglycemia [see below]; 5) maternal hypertension, intrauterine growth retardation, and prolonged rupture of membranes [(lower risk (19)]. Although humoral substances are potential mediators of these effects (13, 242), the precise mechanism(s) remain unclear. Nevertheless, these factors provide clues as to how pulmonary surfactant synthesis may be regulated, particularly *in utero*.

In various clinical series the incidence of RDS in IDM has been as high as 37% (94, 139, 140, 279). Usher *et al.* (272) pointed out that elective premature birth by cesarean section was the usual mode of delivery for IDM and that maternal diabetes was only one risk factor for these babies. Robert *et al.* (227) subsequently delineated the risk of RDS for diabetic offspring delivered in Boston from 1958 to 1968. According to these authors, who compared 805 IDM to 10,152 infants of nondiabetic mothers, the uncorrected risk for RDS among IDM was 23.7 times those of infants born to mothers without diabetes mellitus. Even when corrected for gestational age, maternal age, type of labor, route of delivery, birth weight, sex, Apgar score, hydramnios, prepartum hemorrhage, and maternal anemia, the risk remained 5.6 times as great in IDM as in the control population. More recent statistical studies are confirmatory (4). It should be recalled that increased susceptibility to respiratory distress is only one of numerous clinical problems encountered with IDM, other disorders include macrosomia and its consequences on parturition, neonatal hypoglycemia, and congenital malformations (42, 137, 180).

Although the risk of lung immaturity leading to RDS at birth appears to vary in the different classes of diabetes, discrepancies exist among reported studies, probably because of the fact that not only RDS but a variety of biochemical and biophysical indices of lung maturity was used as endpoints. There is general agreement that inadequately controlled class A (gestational) diabetes can increase the risk of RDS or at least cause delayed fetal lung maturation as judged by phospholipid determinations or by biophysical measurements on amniotic fluid samples (88, 103, 110, 152, 161, 185, 237).

The situation is more complicated with classes B and C diabetes which have been considered to delay, to not affect, or to even accelerate lung maturation. Gluck and Kulovich (103) using the lecithin/sphingomyelin (L/S) ratio in amniotic fluid, Singh *et al.* (27) using the degree of saturation of amniotic fluid lecithin, Cruz *et al.* (43) considering L/S ratio and incidence of RDS, Goldkrand and Slattery (110) using globule formation from the amniotic fluid lipid extract, and Higuchi *et al.* (132) measuring surfactant lipoprotein concentration in amniotic fluid all concluded that delayed fetal lung maturation occurred in pregnancies with classes B and C diabetes. Delayed evolution of rising L/S ratios and occurrence of RDS with classes B and C diabetic pregnancies have also been reported by Mueller-Heubach *et al.* (190). On the other hand, Curet *et al.* (48) found no evidence of delayed maturation in carefully regulated diabetic pregnancies as estimated by amniotic fluid L/S data, and Kulovich and Gluck (161) reported even higher L/S ratios in fetuses of classes B and C diabetes than in age matched controls. Also, Farrell *et al.* (83) found no evidence for delayed lung maturation based on determination of saturated lecithin in amniotic fluid; also this group found no increased incidence of RDS in IDM. Signs of accelerated lung maturation have been reported in some chronically stressed pregnancies of classes B and C with accompanying hypertension, preeclampsia, or premature rupture of membranes (284). Nevertheless, one can conclude that classes B and C diabetes in pregnancy can delay fetal lung maturation in some instances, even if some biochemical data are discrepant. The discrepancies probably are due to clinical variables such as the success of hyperglycemia management (*i.e.* insulin therapy) and the degree of chronic intrauterine stress, as well as to the indirect and imprecise nature of endpoints studied.

For diabetes classes D, E, F, and R, conflicting results have also been reported. In these instances, accelerated rather than delayed fetal lung maturation was reported by most of the authors who studied L/S ratio or phosphatidylglycerol in amniotic fluid (36, 100, 103, 161, 186, 240). This acceleration was contested by Curet *et al.* (48) on the basis of L/S ratio determinations. Additionally, Lowensohn and Gabbe (168) found a similar incidence of RDS in classes B through R and in nondiabetic pregnancies when the L/S ratio was considered as mature, which would argue for an absence of differences between these classes and normal pregnancies with respect to fetal lung maturation.

Available data suggest that the worse the control of maternal blood glucose, the higher the risk of RDS and other morbidities (152). This also argues for the direct role of the maternal metabolic disturbances upon fetal lung development. Consequently, the incidence of RDS in diabetic pregnancies appears to be decreasing with improvements in maternal care during pregnancy (48, 83, 92). Nevertheless, RDS continues to occur (with significantly greater frequency) in pregnancies with gestational diabetes (88). Because this class of diabetes appears to be most associated with delayed fetal lung maturation, it is possible that these patients may not be as carefully managed as those known to be diabetic prior to pregnancy. Thus, their diabetic state could possibly be less well controlled or disclosed too late in pregnancy for optimal treatment. This risk of RDS also might be slightly higher in infants of prediabetic mothers whose diabetes develops later in life (4). These observations indicate the importance of screening for glucose intolerance during pregnancy.

In conclusion, a potentially increased risk of RDS due to delayed fetal pulmonary maturation appears associated with recent diabetic conditions without severe complications, *i.e.* class A and sometimes classes B and C, whereas longstanding, more complicated diabetic states, especially with accompanying vascular disease (*i.e.* classes D through R or classes B and C with stress), appear more often associated with accelerated maturation. It may be inferred, therefore, that maternal hyperglycemia per se, that is to say with elevated blood glucose as the sole pathological feature, can cause a delay in fetal pulmonary functional maturation. On the other hand, when diabetes is longstanding and/or when severe complications occur either before (vascular disease of classes E, F, and R) or during (classes B, C, and D) pregnancy, chronic intrauterine stress occurs whose effects can potentially counteract those of maternal hyperglycemia and lead to accelerated fetal lung maturation. This is in keeping with data from studies of animals indicating that stress hormones stimulate lung maturation (242).

Antenatal prediction of RDS in diabetic pregnancies by amniotic fluid analysis. Because of the lack of direct access to human fetal lung, amniotic fluid has been used extensively to assess lung maturation and to schedule elective deliveries to minimize the

risk of neonatal respiratory disease. Although this approach allows only an indirect and crude reflection of what is actually happening within the developing fetal lung, its reliability is generally well established (270). Various methods for measuring amniotic fluid surfactant have been reviewed recently (89, 202, 270). The L/S ratio in amniotic fluid has been widely accepted for 15 yr as a valid test for pulmonary maturity (105, 106) and is now supplemented with other determinations such as measurement of PG (118–120, 211), PI (121, 122), and DSPC (83)—all of which show characteristic changes during late gestation.

The reliability of the L/S ratio in diabetic pregnancies has been a source of controversy. A lower amniotic fluid L/S ratio in late gestation as compared to normal pregnancies of the same duration was mentioned by several investigators, especially in early studies (103, 127, 190, 216, 217, 237). More recently, others observed no significant difference (48, 83, 91, 123, 161, 221). Among the possible causes of these conflicting results are the large variations of L/S ratio in normal as well as in abnormal pregnancies, and technical factors which vary from study to study such as acetone precipitation (106), centrifugation (203), the use of one or two dimensional chromatography (47), and the method of phospholipid quantitation (202). Also, the degree of diabetic control may be an important factor affecting L/S ratio, which would explain why early observations reported differences which are no longer evident in more recent series with better control (48, 83). Therefore, whether maternal diabetes actually impairs the rise of amniotic fluid lecithin, remains an unsolved question. Moreover, a higher incidence of false-positive predictions of fetal lung maturity using L/S ratio has been reported for diabetic pregnancies (43, 49, 64, 79, 186, 190, 237), although a few investigators considered the test as reliable as in normal pregnancies (61, 91, 168, 263).

Diabetes mellitus in pregnancy has been shown to cause a marked reduction, or even an absence, of PG in amniotic fluid when compared with age-matched nondiabetic pregnancies (45, 46, 116, 117, 123, 124, 241, 268). According to Kulovich and Gluck (161) this was true only in class A diabetes, but Hallman and Teramo (123) also reported PG/PI ratios significantly lower than normal in diabetic pregnancies of classes B, C, D, and F. In the series of patients reported by Cunningham *et al.* (45) and of Hallman and Teramo (123), when RDS occurred with a L/S ratio over 2, PG was absent.

TOWARD THE UNDERSTANDING OF MECHANISMS, THE EXPERIMENTAL APPROACHES

Need for animal models. Understanding the mechanism(s) of impaired fetal lung biochemical development associated with maternal diabetes mellitus probably cannot be achieved solely with the aid of clinical data for several related reasons: 1) direct access to the lung is possible only postmortem, which allows study of only fatal cases in which secondary alterations are certain to be present; 2) generally only collection and analysis of amniotic fluid, or at the most of bronchopulmonary fluid at birth, is possible in humans; 3) it is impossible to control completely the important clinical variables in human pregnancies (*e.g.* changing care practices make the diabetic pregnancy of this era much different than 10–20 yr ago).

Use of animal models is necessary for control of the involved metabolic factors and a systematic analysis of their mode of action. Thus there is a crucial need to design animal models reproducing features of the fetal environment in the diabetic pregnancy. This would make it possible to determine conclusively if fetal lung surfactant is developmentally abnormal and, if so, what metabolic factors account for the disturbances associated with maternal diabetes.

Maternal hyperglycemia appears to be the precipitating cause of most the characteristic features of the fetus of diabetic mother (206). The most prominent feature of the "milieu interieur" of fetuses of diabetic mothers is the unique association between hyperglycemia and hyperinsulinemia. It seems reasonable to presume that these factors are primarily involved in the process leading to the potential delay of fetal lung maturation.

Hyperglycemia due to increased placental transfer of glucose has long been recognized in the fetus of the diabetic pregnant woman, and was proposed in the early years of relevant research to induce a hyperinsulinemic fetal state (63, 205). Direct demonstration of hyperinsulinemia in infants born to insulin-treated mothers has been difficult because of the interference in the radioimmunoassay for insulin caused by placentally transferred endogenous insulin antibodies (146). Because of this analytical problem, only indirect evidence of the fetal hyperinsulinemic state was available at first, including: 1) β cell hyperplasia and increased pancreatic insulin in fetuses of diabetic pregnancies (254), and 2) an increased rate of circulating glucose disappearance in newborn infants of diabetic mothers (145). More recently, direct demonstration of hypersecretion of insulin by the fetal pancreas in diabetic pregnancies has been obtained by measurement of cord blood C-peptide levels (250). Placental transfer of maternal insulin (endogenous or exogenous) is not involved in the increase of fetal blood insulin levels since insulin does not cross the human placenta (150). A primate animal model of maternal-fetal glucose relationships was used by Chez *et al.* (37) to show a rapid increase of fetal blood glucose and insulin, with a linear relationship between maternal and fetal plasma glucose, within minutes following maternal glucose loading (injection plus infusion). The insulin response of the fetal pancreas after the 12th wk of human gestation is thought to be induced by the increased blood glucose stimulating the β cell; this is reinforced by the ingestion of glucose from the amniotic fluid and its insulinogenic action on the fetal digestive tract (134).

To investigate the effects of high fetal serum glucose and/or insulin concentrations on fetal lung maturation, investigators have used both *in vitro* and *in vivo* approaches. *In vitro* experiments have been based on the property of lung cells, either in organ culture or in cell culture, to pursue their differentiation and maturation in relatively simple systems. These studies were initiated before *in vivo* experimentation of this problem with the observation by Smith *et al.* (243) that insulin inhibits the stimulatory effect of cortisol on DSPC synthesis in rabbit fetal lung cell cultures. *In vitro* studies subsequently focused attention on hyperinsulinemia as the major factor delaying fetal lung development. It was proposed (9, 259) that the increased insulin levels in the fetus of the diabetic pregnant woman retard the normal glucocorticoid–regulated stimulation of lung maturation. *In vivo* studies have included animal models of induced diabetes in pregnancy. They have led, on the contrary, to the conclusion that increased blood glucose is the main factor for the delay of lung maturation. The results of *in vitro* and *in vivo* studies need to be reviewed in detail for one to understand the basis of these conclusions.

Before examining fetal lung studies, it is useful to review the consequences of diabetes mellitus upon adult lung in order to help one better understand what makes fetal lung development in the diabetic pregnancy a special problem.

Experimental insulin deprivation in adult animals treated with STZ or alloxan results in lung abnormalities whose final consequence is a reduction of lung surfactant phospholipid production (188, 189, 283). Although the degree of lung lipid alterations varies, Engle *et al.* (70) recently found that decreases in DSPC concentration correlated with the severity of hyperglycemia. The reduction in pulmonary phospholipid levels is probably related to a decreased capacity of *de novo* fatty acid synthesis (32, 33, 52) and incorporation into PC. Surfactant phospholipids are also decreased in lung lavage fluid (260). Insulin was found by Sharp *et al.* (234) to directly stimulate PC synthesis in granular epithelial lung cells. The effects of insulin could be mediated, at least in part, through thyroid hormone action since chemically induced diabetes reduces nuclear triiodothyroine binding capacity of rat lung nuclei, according to Das and Ganguly (51). In the

adult lung, therefore, insulin appears to be a regulatory factor necessary for normal surfactant synthesis. On the contrary, an excess of insulin, as in the fetus of the diabetic pregnancy, may have an adverse consequence on surfactant production, as reviewed subsequently.

Insulin and fetal lung development—in vitro studies. Monolayer cultures prepared from fetal rabbit lungs of 28 days gestation (mixed population of lung cells) were first reported as insulin responsive in 1975 (243). Earlier, corticosteroids, whose stimulating effects on fetal lung maturation are well established (160, 164, 165), were shown to increase ^{3}H-choline incorporation into PC (244, 245). Although, insulin alone had a slight positive effect on choline incorporation, insulin added to cultures with cortisol significantly reduced the corticosteroid-stimulated PC synthesis (243).

In another isolated cell system used by Engle *et al.* (69), *i.e.* organotypic cultures of fetal rat type II pneumocytes, the presence of low concentrations of insulin (10–25 μU/ml) caused an increase in the incorporation of glucose into surfactant PC, but higher levels (100, 250, or 400 μU/ml) significantly decreased incorporation of both glucose and choline. Elevating the media glucose concentration from 5.6 to 20 mM caused a 2- to 2.5-fold increase in glucose utilization for phospholipid synthesis, but did not produce any changes in choline incorporation and thus apparently did not alter *de novo* synthesis of PC as assessed with an isolated surfactant fraction. On the other hand, addition of 400 μU/ml of insulin to media containing 20 mM glucose did result in significantly lowered choline incorporation into surfactant PC. These data suggest that insulin is an important hormone regulating fetal lung phospholipid metabolism, that its effects are dose (concentration) dependent, and that high levels of insulin predominate over glucose in causing an inhibition of surfactant formation.

Exposure of fetal rat lung explants to insulin for 24 h by Gross *et al.* (112) resulted in a significant increase in the glycogen content and the rate of glucose oxidation to CO_2 (112). No effect of insulin was observed on the rate of labeled choline incorporation into PC or DSPC, which conflicts with the results of Smith *et al.* (243). Insulin did reduce significantly the incorporation of acetate into DSPC, but increased the incorporation of acetate into general membrane phospholipids, namely phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin. This argues for a specific inhibiting action on the synthesis of DSPC which reflects surfactant phospholipid. One of the most interesting observations of that study was the delay of morphological maturation of the lung cells induced by insulin: the number of type II cells and lamellar bodies was significantly decreased in insulin-treated explants as compared with control explants cultivated without insulin. As a whole, this study suggests that insulin stimulates cellular growth and inhibits cellular differentiation and maturation of the fetal lung *in vitro*. In another study, insulin was shown to prevent in part the dexamethasone-induced stimulation of choline phosphate cytidylyltransferase in cultured fetal rat lung explants (230). This suggests that the apparent antagonism of insulin on corticosteroid-induced stimulation of fetal lung PC synthesis may be at least partly expressed at the level of this key enzyme of the CDPcholine pathway.

Bourbon *et al.* (29) recently reported a direct precursor-product relationship between fetal lung glycogen and phospholipids, a relationship that had long been postulated on the basis of morphological and biochemical observations (21, 31, 99, 159, 171). In this *in vitro* study, glycogen, which accumulates before the surge of surfactant production, appeared from their data to be used preferentially as a precursor for DSPC and PG, the phospholipids most characteristic of surfactant, while free glucose served as a precursor of many lipids including membrane phopholipids. Insulin both reduced DSPC and PG synthesis and decreased the transfer of radioactivity from previously *in vivo* labeled glycogen to DSPC and PG. Although a high glucose concentration in the medium had the same effects in the absence of insulin the effects of insulin and glucose were additive.

Other data on *in vitro* insulin effects have come from experiments with lung slices incubated directly (without culture) in the presence of labeled precursors. Neufeld *et al.* (199) studied the effects of insulin on the incorporation of labeled glucose and fatty acid residues into total PC and DSPC in lung slices of rabbit fetuses. When labeled glucose was used, the incorporation into PC and DSPC was reduced by insulin despite an increase in overall glucose utilization by lung tissue. Insulin also decreased labeled palmitate incorporation into DSPC.

Considered together, these *in vitro* studies suggest that insulin is capable of impairing fetal lung maturation both structurally and biochemically. This appears contrary to the situation in the adult lung in which insulin seems to favor the biosynthesis of surfactant phospholipids; however, the evidence for a dose-response relationship identified in fetal lung cells (69) must be kept in mind as the probable explanation for this apparent discrepancy. Nevertheless, the following criticisms suggest caution in the extrapolation of *in vitro* results to the clinical situation and indicate that more experimental data are needed.

1) The insulin concentrations used in the culture media generally range from 10 to 1000 μU/ml and are probably not representative of those in the lung environment *in vivo*.

2) In studies reporting measurement of the rate of incorporation of radiolabeled precursors added to the medium, there is uncertainty as to the exact significance of the observed rate of net surfactant PC or DSPC synthesis. In most studies, except for that of Engle *et al.* (69), total pulmonary PC or DSPC rather than that in surfactant PC or DSPC was measured.

3) Studies to date report measurement of radiochemical rather than biochemical rates because the pool sizes of the various precursors inside the cells and in the various subcellular compartments are not known. Tokmakjian and coworkers (265, 266) showed that because of the marked decrease in the pool size of cholinephosphate during development (demonstrated at least for the rat and rabbit fetus), a change in the incorporation of radioactive choline into PC may not be indicative of a change in PC production by the *de novo* biosynthetic pathway.

4) Incorporation of a precursor into a substance *in vitro* can be observed without net accumulation of this substance because of turnover processes with equal synthesis and degradation. This has been observed for instance in liver cells that incorporate labeled glucose into glycogen proportional to time with no change in glycogen concentration in the tissue (J. Bourbon, unpublished data). Thus an insulin-induced decrease of precursor incorporation into lung phospholipids does not definitely demonstrate that insulin affected the net synthesis of these phospholipids to the same magnitude. The ultrastructural observation of Gross *et al.* (112) that insulin decreased the number of lamellar bodies in fetal lung explants and the observation of Bourbon *et al.* (29) that insulin reduced the accretion of tissue DSPC during the culture period provide more convincing evidence.

5) Regarding the significance of the insulin-cortisol antagonism in isolated fetal lung cells by Smith *et al.* (243), it should be reiterated that Gross *et al.* (112) failed to observe this antagonism. Also, considering that antgonism as an explanation of the mode of action of insulin *in vivo* presupposes that endogenous corticosteroids effectively control fetal lung maturation. This point is controversial since studies in fetal lambs (14, 178) and monkeys (208) clearly showed that surfactant-related phospholipids appear in increased amounts before the rise in fetal corticosteroid levels. The same could also be true for the human fetus (35). Additionally, there seems to be little correlation between the level of amniotic fluid cortisol and the degree of fetal lung maturation in human neonates (221), although the late increase of amniotic fluid palmitrate/stearate ratio seems to correlate with the level of conjugated corticosteroids (194). One can wonder if the postulated insulin-cortisol antagonism is not of a pharmacological nature.

Animal models of diabetes for in vivo study of fetal lung development. Animal models for human diabetes mellitus, their appropriateness, and their availability have recently been reviewed extensively by an NIH Task Force (230). In brief, these models can be classified into two main categories: 1) animal strains with spontaneously occurring, genetically induced diabetes; 2) animals (rodents, dogs, or monkeys) with chemically induced diabetes due to infusion of pancreatic β-cell toxic agents such as alloxan or streptozotocin (STZ). In addition to these widely used models, studies on fetal development also have used nondiabetic animals chronically infused with glucose when pregnant, fetuses with reactional hyperinsulinism, and fetuses chronically infused with insulin.

The intrauterine injection of long-acting insulin into rat fetuses has been shown to reproduce some of the features of IDM, *i.e.* increased length and body weight, increased organ weight, higher fat, and nitrogen content (6, 213). In the fetal rhesus monkey, chronic fetal hyperinsulinemia achieved with aid of subcutaneously implanted, osmotically driven minipumps confirmed that several characteristics of IDM such as fetal overgrowth may be attributed to fetal hyperinsulinism (175, 261). The same group (3) and Rooney *et al.* (229) failed to observe any effect of chronic hyperinsulinemia upon morphological development and phospholipid content of fetal lung, but the time of gestation (141 ± 2 days and 134–148 days, respectively) chosen to study lung tissue was too early. Warburton *et al.* (277) using the same experimental approach in fetal lambs from 112 to 135 days of gestation observed a markedly decreased flux (about 26 times less) of surface active material into the tracheal fluid of insulin-infused fetuses as compared to controls. This finding is supported by the recent report of diminished L/S ratio values in amniotic fluid of rabbit fetuses rendered hyperinsulinemic by litter reduction *in utero* (163). Thus, chronic hyperinsulinism without accompanying hyperglycemia delays the secretion of surface active material by fetal lung. When secondary hyperinsulinemia was induced by chronic glucose infusion to fetal lambs, it inhibited the maturational response of fetal lungs to cortisol (276a).

There is little information regarding fetal development in genetically diabetic animals. Large for gestational age newborns have been observed in some pregnancies in the BB/W rat (166) but nothing is known about lung function at birth in these strains. On the contrary, animals with drug-induced glucose intolerance have been widely used to investigate the influence of the diabetic pregnancy state upon fetal lung development. It is unclear, however, which of the animal models of chemically induced diabetes reviewed below will prove most useful in elucidating the mechanisms of delayed fetal lung maturation. Ideally, an experimental model of diabetes in pregnancy would have to reproduce the characteristic features of the human fetus of the diabetic mother: hyperglycemia, hyperinsulinemia, and macrosomia. Hyperglycemia should not be extreme since this is unlikely to be encountered routinely in human pregnancies. Pitkin and Van Orden (25) observed that in STZ-treated pregnant rats, fetal hyperinsulinemia was present only when glycosuria was minimal. Such a model appears closer to human diabetic pregnancies than models with very marked glycosuria and no fetal hyperinsulinism. If fetal hyperinsulinemia is not present, the model is not a proper model of human diabetes but rather a model of chronically increased fetal blood glucose, with probable severe toxicity for the β cell which does not appear able to respond to glucose over-load. As for macrosomia, it should be noted that IDM may in fact be either oversized, small for dates, or normal, *i.e.* appropriate in size for gestational age. Oversized infants are usually observed in those forms of maternal diabetes associated with delayed lung maturation, *i.e.* in patients with hyperglycemia appearing during pregnancy (gestational diabetes) but without severe complications, whereas small for date infants are observed in those diabetic conditions with vascular disease (54) in which acceleration of lung maturation often occurs. Fetal hypertrophy has been directly correlated with fetal hyperinsulinism in humans (250), as well as experimentally in rhesus monkeys (3, 261). A model of diabetes most suitable for the study of lung development retardation would, therefore, include macrosomia of the fetus.

In fact, the rodent models used commonly for studies of lung development have generally failed to reproduce the features described above. Maternal hyperglycemia has frequently been quite pronounced (up to 35 mmol/liter or 640 mg/dl), while fetal hyperinsulinemia and macrosomia have generally been absent. The relevance of the animals to clinical situations is therefore questionable. However, models devoid of fetal hyperinsulinism present the potential advantage of dissociating the putative effects of high glucose and high insulin.

Another point of controversy is the time for administration of the β-cell toxic agent. Since STZ crosses the rhesus monkey placenta (234) and can alter the fetal rat pancreas (17), a direct effect of the drug on fetal organs is possible, independently of maternal metabolic disorders, when the drug is given to already pregnant animals. It was recommended by the NIH Task Force to infuse the toxic agent before mating (82). Nevertheless, it must be mentioned that STZ has not been associated with fetal lung toxicity thus far. In those studies in which animals were rendered diabetic prior to mating or immediately after mating, the consequences upon fetal lung development were similar to those in studies with drug injection during the course of pregnancy. Both types of experiments can therefore be compared.

The first studies of fetal lungs were performed using rhesus monkeys (term = 165 days) injected with STZ when 40–75 days pregnant. Gluck *et al.* (102) found elevated amniotic fluid L/S ratios in diabetic macaque pregnancies compared to matched controls. Epstein and Farrell (73) and Epstein *et al.* (74) reported no change in lung PC concentration but an increase in ^{14}C-choline incorporation into PC in lung slices prepared from fetuses of STZ-treated mothers as compared to control fetuses at 140–146 days gestation despite the presence of fetal macrosomia and β-cell hyperplasia. More recently, Kemnitz *et al.* (153) studied lung development in a small number of 145-day-old rhesus fetuses whose mothers were infused with STZ before mating. Observations on lung biochemistry and physiology have been inconclusive thus far. However, fetal lung glycogen concentrations were 28% higher in diabetic animals compared to normoglycemic controls. These results in monkeys are difficult to interpret. The stage for lung study was not ideal since the major changes occur between 145 and 165 days (208). Further investigation will be necessary to delineate the possible abnormalities in the process of fetal lung maturation in this model.

In rats (term = 22 days), inducing maternal diabetes at midgestation with STZ led to increased glycogen, DNA, and lipid contents of the lungs of neonates, no change in PC content, but a decrease in the percentage of DSPC (225). The neonatal body weights were reduced in the litters of diabetic rats. Serum glucose was increased 3-fold in the STZ-treated mothers at delivery, but no information was given regarding fetal blood glucose and insulin in this study.

In fetuses of rats made diabetic with STZ on day five of gestation, Boutwell and Goldman (30) observed significantly decreased labeled choline incorporation into PC in lung slices, as compared with control fetuses on day 20 of gestation. Moreover, the *in vivo* uptake of ^{3}H-dexamethasone by lung nuclei was significantly diminished in fetuses of STZ-treated mothers. This suggests the possibility of a decreased fetal pulmonary receptivity for corticosteroids in experimental diabetes. Tyden *et al.* (271) also used the STZ-diabetic rat, with mating being performed 2 to 4 wk after induction of hyperglycemia. Morphologically, the fetal lungs at day 20 of gestation were less developed in the STZ group compared to controls with more abundant mesenchyme, less completely developed alveolar ducts, and less well-differentiated pulmonary epithelium. Labeled choline incorporation by fetal lung slices was decreased in the diabetic group compared to controls; insulin treatment of the mothers abolished this dimi-

nution of choline incorporation. In this model, the fetuses were profoundly hyperglycemic but not hyperinsulinemic and their body weights were reduced.

Garcia-Miranda *et al.* (93) observed an absence of differentiation in alveolar lining stem cells to type I and II pneumocytes in the lungs of 15- to 21-day-old rat fetuses of alloxan diabetic mothers; however, no indication was given about the severity of the diabetic state. Gewolb *et al.* (95) reported a delay in degradation of previously stored pulmonary glycogen in fetuses of STZ diabetic rats studied from 16 through 22 days of gestation. This occurred in association with decreased amounts of PC and DSPC in fetal lung tissue on day 21 of diabetic gestations but not before or after that time. They suggested that substrate availability may be related to the delay in lung maturation in fetuses of diabetic mothers, in particular for phospholipid synthesis, which is consistent with the conclusions of Bourbon *et al.* (29). More recently, Gewolb *et al.* (96) reported that this decrease of PC and DSPC content occurred without any alteration in the activity of the enzymes involved in phospholipid synthesis, which may support the concept of an impairment in utilization of a precursor pool. However, this is in contradiction with decreased activity reported by others for choline phosphate cytidylyltransferase, choline phosphotransferase, and the acyltransferases in the lungs of fetuses or newborns from rats with STZ-induced diabetes (191, 239).

The decreased availability of glycogen and substrates derived from glycogenolysis for DSPC biosynthesis is still suggested by the findings of Bourbon *et al.* (28), Erickson *et al.* (75), Mulay and McNaughton (191), and Singh and Feigelson (238). It should be emphasized that increased fetal blood insulin was present in most of the rat models used in these studies (28, 191, 238).

Only recent studies of rats have included assessment of PG. Erickson *et al.* (76) and Tsai *et al.* (267) reported that fetal lung slices of STZ-treated rat pregnancies incorporated less radioactive glucose or glycerol into PG than controls at 20 and 21 days of gestation. Insulin treatment of the pregnant rats restored PG labeling (76) and dexamethasone treatment enhanced the labeling of PG but not to the same extent as noted in controls (267). In both of these studies, PG concentrations in fetal lung have not been established, but Pignol *et al.* (214) reported a 55% decrease of PG content in the lung of fetuses in manifest STZ-diabetic rat pregnancies at term; additionally, they found a concomitant 60% rise in PI content. In the model used for these three studies, maternal blood glucose levels were at least three times the normal value and fetuses were consistently small for gestational age; however, fetal blood insulin was below the normal level (214). The impairment of PG biosynthesis therefore appears as a direct consequence of raised blood glucose. The possible molecular mechanisms are considered subsequently.

Grant *et al.* (110) reported on a profound remodeling of lung basement membrane during type II cell development, with basal cytoplasmic foot processes extending through discontinuities of the basement membrane. The number of these foot processes was diminished in fetuses of diabetic rats (111) but the significance of these changes is thus far unclear.

Models of diabetes in the rabbit (term = 31 days) have led to similar observations as in rats. Bose *et al.* (26) induced diabetes in pregnant rabbits with alloxan, and studied lungs of fetuses that were hyperglycemic but had neither hyperinsulinemia nor macrosomia. On day 28 of gestation, fetal pulmonary maturity was assessed by measurement of pressure-volume relationships to determine deflation stability. Interpretation of the data is based on the fact that mature fetal lungs with adequate surfactant retain more air on deflation than immature ones, reflecting greater alveolar stability. Fetuses of diabetic rabbits demonstrated less retention of air on deflation than control fetuses of the same gestational age, but it has been suggested recently that the results of pressure-volume measurements in immature lungs are questionable because of the trapping of air by liquid which alters the air spaces on deflation (231). Additionally, levels of DSPC were diminished in the fluid obtained from lavage of the lungs in fetuses of diabetic mothers.

The rabbit with alloxan-induced diabetes was also the model used by Sosenko *et al.* (247, 251, 252) with the drug injected 24 h after mating. Pressure-volume curves at 27.5 days demonstrated less deflation stability in the fetuses of diabetic pregnancies compared to controls, but the difference was no longer observed on day 29.5 (251, 252). The surface activity of lung lavage liquid measured on a surface balance was less in fetuses of diabetic mothers at both 27.5 and 29.5 days of gestation, despite the observation that DSPC and L/S ratio in lung wash and DSPC in lung tissue were not significantly different in fetuses of diabetic mothers. The reason for this discordance is unclear. From a morphological point of view (247), the lungs of the fetuses of hyperglycemic mothers appeared less mature than control lungs with a decrease of air space density and a higher glycogen content of type II cells. However, the proportion of type II cells and the number of lamellar bodies per type II cell were similar in control and in alloxan fetuses.

Ultrastructural examination of capillaries demonstrated (247) that their migration and the fusion of their basement membrane with that of alveolar epithelium did not occur as frequently in fetuses of alloxan-treated does as in controls. This observation is of considerable potential significance since it implies the possibility of lesser substrate supply to the type II cells in the alloxan fetuses at the time of intense synthesis of surfactant material. In this model, no differences were observed in the incorporation of labeled choline into PC and DSPC in lung slices of fetuses from diabetic mothers in comparison to control fetuses (56, 251). The authors concluded that the previously observed functional abnormalities were not due to a defect of DSPC synthesis, which is consistent with the results of lung wash analyses. However, besides being in contradiction with other studies in rats and rabbits (26, 30, 225, 271), this result does not imply that *in vivo* incorporation of choline occurred at the same rate as *in vitro*. Placing the tissue *in vitro* in a metabolic and hormonal environment (Krebs-Ringer solution free of glucose and insulin) markedly different from that *in vivo* could have modified its metabolic behavior. It should also be noted that in these studies with alloxan diabetic rabbits, the animals were severely hyperglycemic throughout gestation, the fetuses were small for gestational age, and there were no measurements of blood insulin levels. MacFadyen (176) also studied phospholipids in the lungs of fetuses from alloxan diabetic rabbit pregnancies accompanied by reduced fetal body weight; the L/S ratio was lower than in controls, but phospholipid content was similar.

Sosenko *et al.* (248, 249) reversed the functional delay of lung maturation in fetuses of diabetic rabbits with cortisol. This does not imply that alloxan diabetes acts through an impairment of endogenous cortisol effects, but it could have clinical consequences as to the potential usefulness of corticosteroid treatment to prevent lung immaturity in diabetic pregnancies.

In two studies with rabbit models, the fetuses exhibited not only hyperglycemia but also hyperinsulinemia. One was reported by Merrit *et al.* (177) with rabbits rendered diabetic prior to gestation with STZ. Fetal weight on day 29 was normal, and the fetuses were only slightly hyperglycemic. Lung phosphatidylinositol metabolism was altered, but the significance for lung functional maturation is unclear since decreased phosphatidylinositol content has been proposed to signify an accelerated lung maturation. On the other hand, the observed increase of plasma myoinositol appears unfavorable to fetal lung maturation since in normal development the decrease of the percentage of phosphatidylinositol in surfactant has been correlated with a gestational decline in plasma myoinositol concentration (25). It is regrettable not to have information about DSPC and PG in this interesting model of diabetes.

Neufeld *et al.* (196, 198) also obtained hyperinsulinemic fe-

tuses with pregnant rabbits made diabetic with alloxan on day 14 of pregnancy. The lung concentrations of sphingomyelin, phosphatidylcholine (total and disaturated), and phosphatidylserine were significantly lower in fetuses of diabetic rabbits than in controls. For PG, the difference was not significant which contrasts with most of the clinical data concerning human diabetic pregnancies (based on amniotic fluid analyses). Treatment of the diabetic rabbits with 3,5-dimethyl 3′-isopropyl-L-thyronine, a thyroid hormone analogue, restored the phospholipids of fetal lung to normal (198).

Changes in fetal lung receptivity to insulin are also possibly involved, although contradictory data have been reported. An increased number of apparent receptors has been observed by some (196) in the lungs of fetuses from diabetic rabbits, as previously observed for monocytes and erythrocytes in humans (197, 207), whereas others found this number diminished in fetal lungs from rat diabetic pregnancies (193).

In conclusion, the fetal lungs in animals with induced diabetes showed some characteristics consistent with a delay of maturation including less deflation stability, reduced incorporation of precursors into PC and PG, immature cellular or ultrastructural aspects of lung parenchyma, delay in glycogenolysis, and sometimes decreased DSPC and/or PG content of lung tissue or lung fluid. Despite the criticisms which can be made about the significance of some of these measurements, all the studies suggest that some impairment of lung maturation occurred in these models. Since increased blood insulin in the fetus was often not observed in these induced diabetic pregnancies, one must conclude that fetal hyperglycemia alone can delay lung maturation.

Hyperglycemia versus hyperinsulinemia. From the above reported experiments, either excessive blood glucose or excessive insulin alone appear sufficient to cause a delay of fetal lung maturation. The question, therefore, arises as to which of them is effectively responsible for the impairment in lung development encountered in some human diabetic pregnancies.

It is clear that the adverse effects of excessive insulin upon lung development have been deduced mainly by *in vitro* experiments, often with greater than physiological concentrations of insulin. However, Engle *et al.* (69) demonstrated inhibition of surfactant phospholipid synthesis by organotypic cultures of fetal lung cells in the presence of 100–400 μU/ml, a level of insulinemia found in fetuses of diabetic pregnancies (153). On the other hand, the experimental animal models of diabetes suggest that fetal hyperinsulinism need not accompany hyperglycemia to cause a delayed lung maturation *in vivo*. It is therefore tempting to ascribe the important role to hyperglycemia. In most of the models of induced diabetes, however, maternal blood glucose was quite high (up to 35 mmol/liter or 640 mg/dl). The deleterious effects of hyperglycemia on fetal lung maturation could be less in humans who are not likely to reach such levels. Additionally, in those models of the diabetic pregnancy with fetal hyperinsulinemia (177, 198, 239), abnormalities in fetal lung development were observed in the presence of rather moderate hyperglycemia.

The putative implication of fetal hyperinsulinism is still supported by two additional kinds of indirect evidence. First, in a clinical study, Draisey *et al.* (62) found a reverse correlation between insulin concentration and lecithin concentration and degree of saturation in human amniotic fluid beyond 35 wk of gestation. Also, Beck *et al.* (20) observed a dramatic increase in maternal and fetal plasma insulin values when the pregnant rhesus monkey was treated with betamethasone. Contrary to previous reports in other species (55, 160, 164, 187) the corticosteroid in this model failed to stimulate the fetal lung surfactant system. The authors suggested that the betamethasone-induced hyperinsulinemia could have impaired the acceleration of surfactant production by the steroid. Further research is needed on this interesting proposal.

For the time being it does not seem possible to reach a judgment as to the relative importance of hyperglycemia and hyperinsulinemia. It is likely that both are implicated in human diabetic pregnancies, and that they act either synergistically or on different but additive mechanisms.

THE POSSIBLE BIOCHEMICAL MECHANISMS

General considerations. Unquestionably, both clinical and biological approaches indicate that the etiology of fetal lung developmental retardation due to diabetes in pregnancy relates in some way to altered pulmonary surfactant metabolism. Whatever the cause, hyperglycemia or hyperinsulinemia, the diabetic pregnancy must lead to biochemical disturbances in the developing lung which translate into a delayed functional maturation. Both the major surface active constituents of surfactant, DSPC and PG, appear to be present in inadequate amounts at birth. Although the pathogenetic mechanisms at the molecular level leading to this situation are not yet elucidated, available experimental data allow us to propose several hypotheses.

Figure 1 summarizes the putative mechanisms of abnormal fetal lung development due to the metabolic changes in diabetic pregnancies. The impairment may be either direct, *i.e.* at the levels of DSPC and PG biosynthetic pathway and/or secretion, or indirect, *i.e.* being the consequence of an inadequate substrate availability or utilization for surfactant synthesis.

Direct impairment of surfactant biosynthesis and/or secretion by insulin or glucose. Nothing is known about the activity of phospholipid biosynthetic pathways in the lung of human fetuses of diabetic mothers. In animals with induced diabetes, decreased enzyme activities have been observed in fetal lung only when the mother was severely diabetic (200, 253), a condition in which the fetuses are generally not hyperinsulinemic. Indications of a possible adverse effect of excessive insulin upon lung phospholipid biosynthesis have been gained exclusively from *in vitro* experiments. Insulin was shown to prevent the corticosteroid-induced increase of choline phosphate cytidylyltransferase activ-

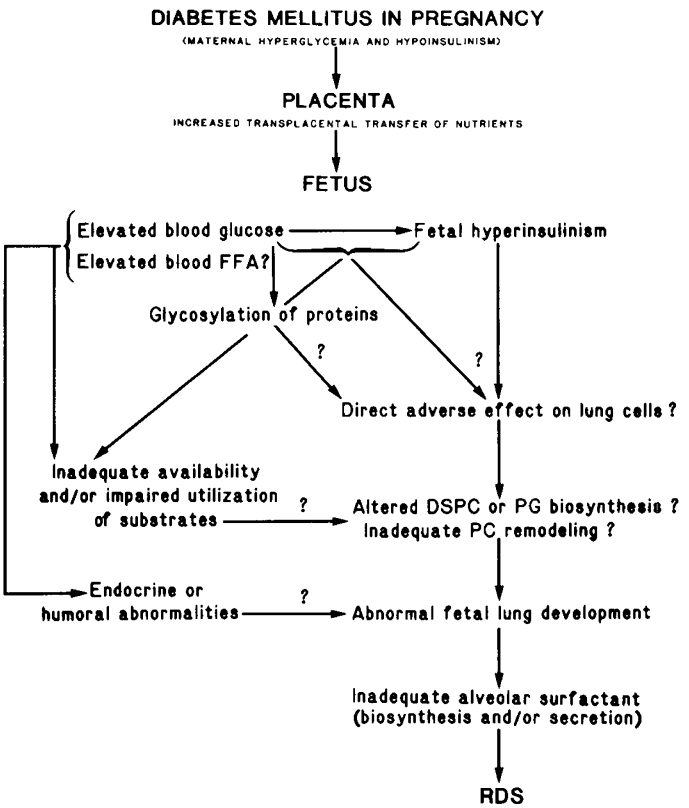

Fig. 1. Possible mechanisms of the impairment of fetal lung functional maturation in the diabetic pregnancy.

ity in fetal rat lung explants (230). However, decreased incorporation of choline into PC *in vitro* was not caused by insulin alone (112, 243). On the contrary, a decreased incorporation into DSPC (but not into membrane phospholipids) induced by insulin was observed for glucose, acetate, and palmitate (112, 199).

The effect of insulin on palmitate incorporation into PC suggests that one defect could take place at the level of the phosphatidylcholine-lysophosphatidylcholine remodeling mechanism described in Figure 2. The observations of Ishidate and Weinhold (144) strongly suggest that synthesis of disaturated PC from unsaturated PC and the disaturated species of diacylglycerol is a major route for the synthesis of surfactant dipalmitoylphosphatidylcholine via PC remodeling in fetal lung, and that diacylglycerols would represent obligatory donors of saturated fatty acids (mainly palmitate) in the transacylation remodeling pathway. Additionally the pool of 1,2-diacyl-sn-glycerol increases 5-fold during the fetal and neonatal periods in the rat (265, 266). However, it must be kept in mind that the acyltransferase activities were not decreased in the lung of hyperinsulinemic rat fetuses of the mildly diabetic rat (238). On the other hand, insulin is known to stimulate the uptake of FFA from fetal blood (213a) and their incorporation into adipose tissue triglycerides (6, 213). It therefore appears possible that high fetal blood insulin levels in the diabetic pregnancy could favor the incorporation of FFA into a fetal lung pool of triglycerides, but in opposing lipolysis, could decrease the availability of diglycerides for PC transacylation. As for hyperglycemia, it is unlikely to interfere with this process since glucose stimulated the incorporation of ^{14}C-palmitate into PC in isolated perfused adult lung (18).

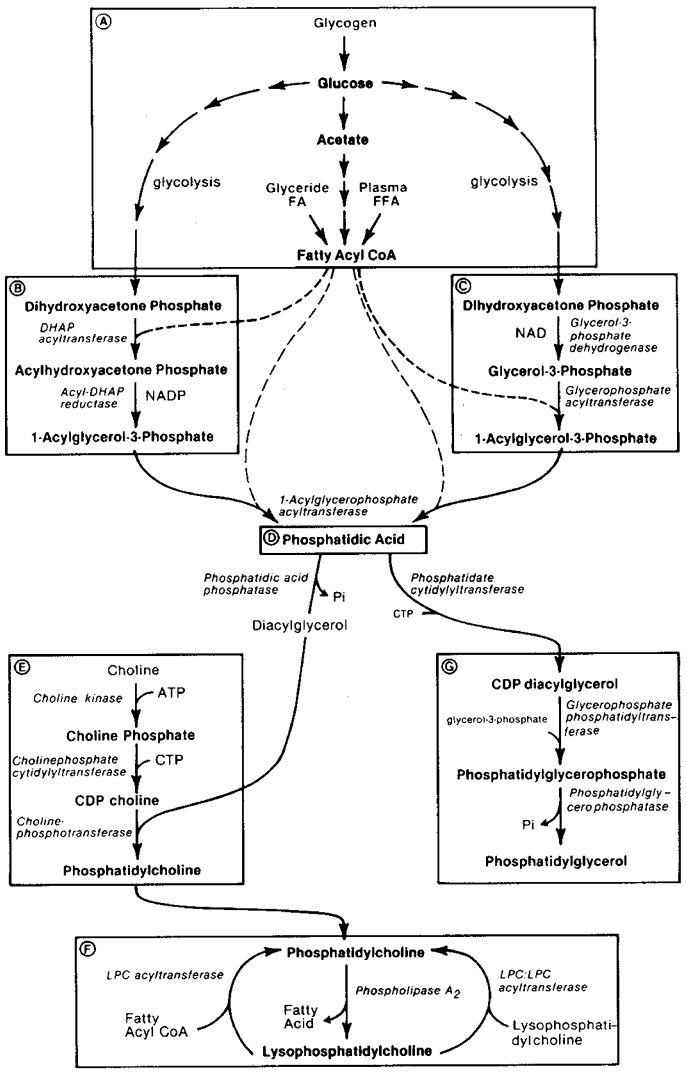

Fig. 2. Pathways for synthesis and remodeling of phosphatidylcholine and for synthesis of phosphatidylglycerol. Abbreviations: FA, fatty acids; DHAP, dihydroxyacetone phosphate; LPC, lysophosphatidylcholine.

In regard to the molecular mechanisms of insulin action, several propositions can be formulated at present. There is some evidence that the adrenergic system could participate in the control of surfactant biosynthesis (53, 179) and pulmonary maturation (2, 72, 151), and that this control is mediated by cyclic AMP (16, 17, 114, 233). β-Adrenergic receptors are present early in fetal lung (97, 282) and increase near term (282).

Not only biosynthesis but also secretion of surfactant could be defective in IDM as suggested by the observation of a decreased release of surface active material in the chronically hyperinsulinemic sheep fetus (295). Many observations suggest that surfactant release into alveoli is under β-adrenergic control. β-Sympathicomimetic agents stimulate surfactant release from fetal lung *in vivo* (2, 23, 67, 71, 72, 128), as well as from adult rat alveolar type II cells *in vitro* (60). Epinephrine infusion to the sheep fetus also increases surfactant efflux (162). Labor promotes surfactant secretion from fetal rabbit lung, probably mediated through β receptor stimulation since β blocking agents abolish this effect of labor (174). Finally, a recent study (58) established a significant positive correlation between human amniotic fluid levels of catecholamines and the L/S ratio, thus corroborating the results of animal experiments.

In other tissues such as liver or muscle, the antagonist effect of insulin *versus* the stimulation of adenylate cyclase by catecholamines or glucagon is well established. In the lung it appears possible that insulin could impair the maturational process as well as surfactant secretion by opposing cyclic AMP synthesis. An alternative mechanism would involve a disturbance in prostaglandin metabolism in the fetus of diabetic mother, as discussed subsequently.

Insulin could still inhibit the production of fibroblast-pneumocyte-factor (36a), a factor produced by lung fibroblasts, which stimulates pneumocyte maturation and whose production is corticosteroid responsive (241a).

Another possible explanation at the molecular level involves an effect of excessive amounts of myoinositol upon PG synthesis. It has been reported that fetal lung biochemical maturation is accompanied by a change from the production of a phosphatidylinositol (PI)-rich surfactant to one rich in PG (121, 122). The reciprocal changes in the relative proportions of PI and PG in broncho-alveolar surfactant suggest a regulation at the level of a common precursor of both these lipids, most likely CDPdiacylglycerol (see Fig. 2). This intermediary substrate is present in minute amounts in mammalian cells (280). There is evidence on one hand that limited availability of CDPdiacylglycerol may restrict the biosynthesis of PG and PI and, on the other hand, that a competition exists between the pathways of PG and PI biosynthesis for the amount of CDPdiacylglycerol available (66, 77, 90, 115). An increase in the extracellular concentration of myoinositol in experiments with various tissues studied *in vitro* (including lung) was followed by an enhancement of PI biosynthesis at the expense of PG biosynthesis (66, 77, 115). The availability of myoinositol therefore appears to be a potential regulatory factor for surfactant composition in the developing lung (115). This is consistent with the observation that the plasma concentration of myoinositol is much higher in the fetus than in the adult and falls dramatically toward the end of gestation in rats (34) and rabbits (25). The activity of pulmonary myoinositol-1 phosphate synthase (cyclase) also is elevated in the rabbit fetus as compared to the adult and decreases near the end of gestation, *i.e.* between days 25 and 28 (25).

An increase of fetal plasma myoinositol level has been reported in two experimental rabbit models of the diabetic pregnancy, both of which were accompanied by fetal hyperglycemia and hyperinsulinemia. One was STZ-induced diabetes (177); the other involved continuous glucose infusion to the pregnant rabbit

between days 27 and 29 (125). On the contrary, chronic hypoglycemia in the pregnant rabbit and its fetuses by continuous insulin infusion to the doe was accompanied by a decrease of fetal serum myoinositol concentration and a concomitant stimulation of surfactant phospholipid production (particularly PG) by fetal lung (125). Moreover, glucose was shown to stimulate myoinositol uptake by lung slices *in vitro*, an active transport system (25). Fetal hyperglycemia and/or hyperinsulinemia therefore appear able to increase myoinositol availability, uptake, and utilization by the developing lung. The absence, or delay in appearance, of PG in fetal lung surfactant reported in human diabetic pregnancies may therefore be the consequence of the presence of excessive amounts of myoinositol in fetal blood at the time when it normally declines.

A last hypothesis that can be formulated for molecular mechanisms concerns a possible deleterious effect of protein glycosylation by excessive glucose. One type of glycosylated proteins is Hb A_{1c} whose level is abnormally high in diabetic subjects (222). Whether or not the presence of glycosylated proteins influences fetal lung maturation is unknown but cannot be excluded. Proteins other than Hb can presumably be glycosylated in the presence of high blood glucose concentrations, including enzymes or hormone receptors and their biological activity could potentially be modified. Such events may intervene in the lung of the developing fetus of the diabetic mother and affect functional maturation.

Alterations in utilization of precursor pools. In comparing fetal to adult lung from a biochemical point of view, one major difference is the presence in the former of high amounts of reserve substances, such as glycogen (mainly in epithelial cells) and mono-, di-, and triglycerides in lipofibroblasts. Although the potential importance of this may not be immediately apparent, it must be kept in mind that the lung only receives about 10% of the cardiac output *in utero*, whereas the adult lung is perfused with virtually the entire cardiac output and thus is continuously presented with an abundant supply of substrates and nutrients. The fetal lung stores may therefore compensate for lower blood flow. In addition to the low proportion of the cardiac output perfusing fetal lung, the necessity of glycogen utilization for the synthesis of phospholipids of surfactant is perhaps linked to the environment of epithelial cells in developing lung. As shown in the rat fetus, the majority of type II epithelial cells have no contact until birth with capillary endothelial cells, from which they are separated by mesenchymal cells, namely the lipofibroblasts (173). This is probably the cause of the rather low exchange between blood and fetal lung epithelium and a possible reason for the apparent necessity to use previously stored precursors for the intense synthesis of phospholipids just prior to birth in short gestational species. If these substrate stores are indeed necessary for the normal process of surfactant phospholipid elaboration, the effect of maternal diabetes could be exerted through inhibition of the mobilization of stored molecules when they are needed to support augmented phospholipid biosynthesis. It is well known of course that both hyperglycemia and hyperinsulinemia inhibit glycogenolysis and lipolysis. In fact, glycogen stores rather than glycerides may be primarily concerned since glycogen in lung epithelial cells begins involution and utilization *in utero* (154, 171, 235), whereas the utilization of glycerides stored in lipofibroblasts appears to take place shortly after birth (39, 273).

It should be emphasized that an impairment of glycogen breakdown in fetal lung was a constant finding in models of induced diabetes. Also obvious is the fact that insulin or high glucose concentration impaired glycogenolysis (29, 112) and glycogen utilization for synthesis of the phospholipids of surfactant in fetal rat lung tissue studied *in vitro* (29). The increased blood glucose might therefore stimulate lung glycogen accumulation but prevent glycogenolysis as it does in fetal rat liver (98).

Bourbon and Jost (27) showed that the surge of corticosteroids of fetal origin appears to be the primary signal controlling fetal lung glycogen breakdown. This confirmed previous assumptions of Blackburn *et al.* (24) and Gilden *et al.* (99). In fact, the effect of corticosteroids could be indirect. It has been shown that aminophylline, an inhibitor of phosphodiesterase which enhances cellular cyclic AMP levels, and cyclic AMP itself, stimulate both glycogenolysis and the synthesis of phospholipids in fetal rat or rabbit lung *in vivo* and *in vitro* (17, 172, 200, 233). Corticosteroids have been shown to inhibit phosphodiesterase activity and to increase cyclic AMP concentration in rabbit fetal lung (17), and to increase the number of β-adrenergic receptors in rat fetal lung explants *in vitro* (170). In addition, it is well established that corticosteroids are partly responsible for the maturation of the fetal adrenal medulla (228). Epinephrine increases during late gestation in fetal blood of sheep (149) and rats (22) and in human amniotic fluid (59, 212). For fetal lung, the cascade of events could therefore be: 1) surge of fetal corticosteroids: 2) maturation of fetal adrenal medulla and secretion of epinephrine into fetal blood in increasing amounts; 3) enhancement of cyclic AMP production in fetal lung; and finally 4) glycogenolysis. Cyclic AMP would be responsible not only for phosphorylase activation, according to its usual mode of action, but also would lead to glycogen synthase inactivation (27, 172, 178) and enhancement of autophagic activity (27). The importance of autophagic hydrolysis of glycogen for surfactant synthesis is also suggested by the existence of numerous lysosomal structures in fetal lung epithelium (12) and by the fact that lamellar bodies in type II pneumocytes are related to lysosomal structures and possess acid hydrolases (57, 109, 136).

Sodoyez-Goffaux *et al.* (246) observed a high concentration of insulin receptors in rat lung at the pseudoglandular stage (day 17 of gestation) but a much lower concentration later (days 19 and 21). They speculated that insulin receptors may modulate lung glycogen metabolism, their presence favoring accumulation of glycogen during the pseudoglandular stage, whereas their partial disappearance would later allow glycogen breakdown and surfactant synthesis. The same is true in the rabbit fetus in which the maximal insulin receptor number of lung tissue was observed on day 29 of gestation with an abrupt fall the day after (56a), although glycogen breakdown in rabbit fetal lung is already detectable on day 28 of gestation (27). Taking into account the increase of blood insulin and the subsequent prevention of decay of lung insulin receptors in the fetus of diabetic mother, this would explain the delay of lung glycogenolysis.

Bourbon *et al.* (28) reported a much more impaired glycogen utilization in the lung of fetuses of mildly diabetic rats than in the lung of fetuses of severely diabetic rats despite the fact that DSPC biosynthesis was impaired to the same extent in both cases. Since enzyme activities of phospholipid biosynthesis are decreased in severe but not in mild diabetes (253), it appears that the mechanisms leading to delayed lung maturation could be different according to the severity of diabetes. Impaired glycogen utilization seems directly related to fetal hyperinsulinemia and is present in mild but not in severe diabetes. This suggests that this mechanism could be predominant in the human fetus of diabetic mothers with reactional hyperinsulinemia.

Alterations in the intermediary metabolism of glucose also have been suggested by Stubbs and Stubbs (259) who proposed that stimulation of pyruvate dehydrogenase by hyperinsulinism in the lung of the fetus of the diabetic mother may increase the conversion of glucose into lactate and acetyl-CoA, thus decreasing the availability of glycerol-3-phosphate for phospholipid synthesis. This assumption has not received demonstration but is supported by the following observations. The rate of lipid production in the lung could be partially regulated by the availability of two intermediates of glycolysis, glycerol-3-phosphate, and dihydroxyacetone phosphate (see Fig. 2). If insulin increases lactate production in the fetal lung as in the adult lung (258), this means that hyperinsulinemia in the fetus of diabetic mother may stimulate the glycolytic pathway increasing lactate production, and thus decreasing the accumulation of glycerol-3-phosphate for lipid synthesis.

The impairment of biosynthesis of surfactant phospholipids could still be an indirect consequence of altered lipid metabolism, particularly lipogenesis. It has been shown that both adult rat lung (224, 264) and fetal rabbit lung (126) take up FFA from blood and incorporate them into neutral lipids and phospholipids. The availability of FFA could potentially influence pulmonary maturation, since alterations in maternal dietary fat affect the concentration of saturated PC in fetal rat lung tissue (187). However, recent investigations in rats (147, 169) suggest that in late fetal life *de novo* fatty acid synthesis is the major source of saturated fatty acids for phospholipid biosynthesis in fetal lung, and that exogenous palmitate inhibits *de novo* FFA synthesis. Any increase of fetal blood FFA, which might at first glance appear favorable for fatty acid incorporation into newly synthesized phospholipids, could in fact inhibit the necessary *de novo* fatty acid synthesis in type II pneumocytes and finally lead to decreased DSPC and PG production.

In insulin-dependent diabetics, plasma FFA and triglycerides often are elevated. If this is reflected in fetal plasma in the diabetic pregnancy, it could conceivably affect fetal lung phospholipid metabolism. Although fatty acids can cross the placenta readily from mother to fetus in most species [including rat (141), rabbit (68, 274), guinea pig (129), sheep (275), monkey (218), and man (50, 262)], the quantitative importance of this placental transfer is not clear (201). In the pregnant rat, the work of Koren and Shafrir (158) indicated that the transfer of palmitate, stearate, and linoleate is very small and that the maternal circulation cannot be an important source for direct transfer of FFA. In other species, such as rabbit and guinea pig, a much greater proportion of FFA seems to be transfered from mother to fetus (65, 129). The case of the human placenta is unclear. Measurement of plasma fatty acids in human cord blood suggest that there is limited transfer of FFA throughout the last trimester of pregnancy (135).

The situation is complicated by the fact that not only maternal plasma FFA but also plasma triglycerides (as very low-density lipoproteins) seem to be a source of fetal fatty acids, at least in the rat (142). Recent examination of triglyceride levels in cord blood of human newborns (38) however, seems to indicate that it is independent of the maternal serum triglyceride concentration. In the pregnant diabetic rat, on the contrary, placental transfer of triglycerides and/or FFA is clearly increased (233a).

The consequence of the elevation of plasma lipids in the pregnant diabetic upon fetal lipidemia is therefore an unsolved question. Systematic examination of lipid levels in cord blood and cord arteriovenous differences at birth could perhaps help to answer this question.

Circulating glycerol is also a precursor for pulmonary PC in the developing mammalian lung (182). The concentration of blood glycerol is increased in diabetic states (167) and also seems elevated in umbilical venous blood of IDM (210). Although this situation would appear rather favorable for phospholipid synthesis, Scholz *et al.* (232) have shown that glucose decreased the apparent utilization of glycerol by the isolated-perfused adult rat lung, suggesting that glucose and glycerol share a common metabolic pool in rat lung. In the presence of hyperglycemia, the use of circulating glycerol for phospholipid synthesis could therefore be reduced despite its higher availability. Additionally, because the common metabolite in the metabolic fate of glucose and glycerol is glycerol-3-phosphate, the use of circulating glycerol could also be affected at this level by the previously described possibility of pyruvate dehydrogenase stimulation in the presence of high insulin.

Endocrine or humoral abnormalities. The probable implication of the β adrenergic system (mediated by cyclic AMP) in lung biochemical maturation and surfactant release also allows one to propose another possible cause of impaired fetal lung development in the diabetic pregnancy, namely altered fetal (and neonatal) sympathoadrenal status. Any impairment of epinephrine secretion or of norepinephrine activity in the fetus would have consequences upon surfactant biosynthesis and/or secretion. Conflicting reports have been published in the literature as to the level of sympathoadrenal activity in IDM. Blood catecholamines have been reported to be elevated at birth by some observers (8, 131, 285), while others observed reduced catecholamine secretion in the first days of life (130, 255). Metanephrine, a metabolite of epinephrine, is present in lower amounts in amniotic fluid in some diabetic pregnancies (7). Artal *et al.* (7, 8) proposed a synthesis of these findings, namely that there would be a decreased sympathoadrenal activity or a delayed maturation of the system in fetuses of diabetic mothers, while at birth these infants would react excessively to the stress of labor and delivery, thus secreting excessive amounts of catecholamines; this would temporarily deplete them of catecholamines in the subsequent period.

It is difficult to reach a judgment about the possible consequences of this situation upon lung surfactant synthesis and release. Lower sympathoadrenal activity during pregnancy could be unfavorable to lung maturation through several mechanisms (activation of enzyme activities for surfactant biosynthesis, availability of diacylglycerol, glycogen breakdown and utilization, etc.). High levels of catecholamines at birth would appear rather favorable for surfactant secretion. Catecholamine depletion, however, could thereafter cause an inadequate surfactant secretion in the next several hours after birth. On the other hand, reabsorption of lung liquid, which is controlled in part by increased fetal epinephrine secretion in the perinatal period (276), would be enhanced by high catecholamine levels at birth. This appears contradictory to reports of transient tachypnea of the newborn in IDM and with Strang's (256) proposal of impaired lung liquid reabsorption at birth in the etiology of respiratory distress. These aspects of lung physiology in IDM remain to be clarified by further clinical and physiological investigations.

Many other hormones including corticosteroids, estrogens, prolactin, and thyroxin are able to stimulate fetal lung maturation (242), although the exact role of endogenous hormones is still unclear. Low plasma concentrations of estrogens, prolactin, and thyroid hormones have been reported in infants with RDS (40,44 108, 138) as compared with age-matched controls, which suggests that fetal lung immaturity could be attributable to an insufficient action of stimulating hormones.

Unfortunately, the potential role of maternal diabetes in fetal hormone abnormalities is unknown, since in the pertinent clinical studies either there were no indications about maternal diabetes in the cases studied or maternal diabetes was a criterion for exclusion and the differences compared to controls were clearer after exclusion of IDM. Very little is known about the hormonal status of IDM for hormones other than insulin, glucagon, and catecholamines. Aarskog (1) found no difference in cortisol production rate between IDM and newborns of normal mothers. Cortisol levels in amniotic fluid increase normally in diabetic pregnancies (221). Measurement of adrenal and thyroid gland weights at autopsy revealed no difference between IDM and controls but pituitary weight was slightly reduced in IDM (143). However, in fetuses of experimentally diabetic rabbits, plasma cortisol was lower than in normal fetuses (113). Corticosterone was similarly diminished in fetuses of STZ diabetic rats (192). Further documentation on this point appears necessary.

In one case report (5), RDS occurred in an IDM in the presence of a mature amniotic fluid phospholipid profile, including the presence of phosphatidylglycerol. This baby also had congenital hypothyroidism and supplemental thyroxine led to normalization. The representativity of that case, however, is questionable.

Recently, an involvement of prostaglandin metabolism has been implicated in the delay of lung maturation associated with the diabetic pregnancy. Prostaglandins are known to regulate cyclic AMP levels in many tissues and the lung is an active organ of arachidonic acid metabolism for prostaglandin synthesis (183). It is therefore possible that cyclic AMP levels in fetal lung and their developmental consequences were modulated by prostaglandin production in fetal lung. This assumption is reinforced

by the existence of a high capacity for prostaglandin E_2 biosynthesis beginning around 23 days of gestation and peaking at 28 days in fetal rabbit lung (219), *i.e.* at the very time of fetal lung biochemical maturation. Moreover, prostaglandins seem to be involved in the increased flux of surfactant occurring in late gestation (156).

The observation of an altered vascular arachindonic acid metabolism in IDM (257) raises a question about maternal diabetes impairing lung maturation, at least partly, through altered fetal lung production of prostaglandins. Tsai *et al.* (269) assessed arachidonic acid metabolism in lung homogenates of fetuses from alloxan diabetic rabbits. They observed a decreased conversion of arachidonic acid to prostaglandin E_2 as compared with control fetuses, whereas all other metabolites were produced in similar quantities. The authors suggested that the decreased production of prostaglandin E_2 could be partially responsible for the functional delay of lung maturation in offspring of alloxan diabetic rabbit. However, it must be pointed out that the degree of difference from controls, although significant, was small and that the consequence of this reduction upon cyclic AMP biosynthesis are unknown. It does not seem likely that this reduced prostaglandin E_2 production may account for the totality of the features which characterize the delay of fetal lung maturation observed in diabetic rabbits.

SUMMARY AND FINAL PERSPECTIVE

It seems quite likely that the normal process of fetal lung biochemical maturation is delayed by maternal diabetes and that abnormalities in the pulmonary surfactant system are involved. The appearance of PG in amniotic fluid and possibly in fetal lung is impaired or at least delayed. The same is possibly true for DSPC, the main constituent of surfactant, but recent discrepant data call for further clarification of this specific point.

Careful determination of the fetal lung phospholipid profile by amniotic fluid analysis helps predict and prevent RDS in IDM, along with a careful control of the maternal diabetic condition. A study of alveolar surfactant at birth, if it could be performed in addition to amniotic fluid analysis, would help to better characterize surfactant deficiency in IDM.

On the basis of both *in vivo* and *in vitro* experimental approaches, it seems clear that hyperglycemia and fetal reactional hyperinsulinism are both involved in the processes delaying fetal lung maturation. Further advances in the understanding of cellular and molecular mechanisms leading to this delay will be conditional on the availability of animal models reproducing the features of the metabolic and hormonal environment of human fetuses in diabetic pregnancies. The appropriateness of *in vivo* models needs to be defined by two kinds of criteria: 1) presence of simultaneous hyperglycemia and hyperinsulinemia in the fetus; 2) the presence of delayed fetal lung maturation as judged by morphology and morphometry of epithelial lung cells, by physiological assessment of surfactant, and by the phospholipid composition of the lung (and including lung tissue per se, bronchoalveolar lavage fluid, lamellar bodies, and/or isolated surfactant fractions). Therefore, future studies must necessarily be comprehensive in scope and include information indicating that fetal growth, blood glucose, and circulating insulin are all increased.

Such models already exist in rats and rabbits. Rat models are possibly not the best because of the high basal level of fetal blood insulin in this species and the relatively rapid rate of lung maturation that is not analogous to the human. Monkey models are of interest, because of their close relationship with the human pregnancy, and need to be studied further. They are particularly attractive also because primary fetal hyperinsulinism can be studied (268), as well as the combination of hyperglycemia and hyperinsulinemia in pregnancies of STZ-treated monkeys (152).

An appropriate model of the diabetic pregnancy could provide answers to the following questions.

1) Are the biosynthetic pathways of surfactant phospholipids directly impaired?

2) If so, what step(s) is (are) impaired and what molecular mechanism(s) is (are) involved?

3) Alternatively, or concomitantly, is the availability of substrates for phospholipid biosynthesis insufficient?

4) If so, what precursor is involved: glycogen, glycerol, *de novo* synthesized fatty acids, etc?

5) Is surfactant secretion into fetal and newborn terminal respiratory spaces impaired?

Other models would have to be studied to determine more precisely what fetal alterations (hyperglycemia, hyperinsulinism, increased blood FFA, or other metabolic or hormonal abnormalities) cause the delay in lung maturation, and if several alterations are involved together, what are their roles and relative importance.

Many investigations have already been reported that indicate the direction for furture research, but the understanding of mechanisms is only at its very beginning. Undoubtedly, much progress will be achieved in the next several years in the understanding and management of this important problem of neonatal biology and medicine.

Acknowledgments. The authors are grateful to Professor A. Jost, Dr. M. Rieutort, and Dr. M. Engle for critical review of this paper and helpful suggestions for revisions. We also thank J. Beard for typing and editorial assistance.

REFERENCES

1. Aarskog D 1963 Cortisol production rate in newborn infants of diabetic mothers. J Pediatr 62:807–814
2. Abdellatif MM, Hollingsworth M 1980 Effect of oxotremorine and epinephrine on lung surfactant secretion in neonatal rabbits. Pediatr Res 14:916–920
3. Adamsons K, McCormick KL, Susa JB, Widness JA, Singer DB, Schwartz R 1981 Effects of insulin on the biochemical and morphologic maturation of the fetus. J Perinatal Med 9 (suppl 1):104–105
4. Allen RD, Palumbo PJ 1981 Respiratory distress and neonatal mortality in infants of diabetic and prediabetic mothers. Acta Diabet Lat 18:101–106
5. Anderson, CW, Conrad L, Cordero L 1982 Neonatal respiratory distress in the presence of amniotic fluid phosphatidylglycerol. Am J Obstet Gynecol 143:223–234
6. Angervall L, Karlsson K, Martinsson A 1981 Effects on rat fetuses of intrauterine injections of insulin. Diabetologia 20:558–562
7. Artal R, Platt LD, Kammula RK, Strassner HT, Gratacos J 1980 Concentrations of metanephrine in the amniotic fluid as an index of fetal stress. Dev Neurosci 8:449
8. Artal R, Platt LD, Kammula RK, Strassner HT, Gractacos J, Golde SH 1982 Sympathoadrenal activity in infants of diabetic mothers. Am J Obstet Gynecol 142:436–439
9. Avery ME, Frantz I 1975 Intrauterine developmental retardation. J Pediatr 87:956–957
10. Avery ME, Mead J 1959 Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 97:517–523
11. Balint JA, Kyriakides EC, Devas Guna Wardhane G, Risenberg H 1978 Surfactant lecithin fatty acid composition and its relationship to the infantile respiratory distress syndrome. Pediatr Res 12:715–719
12. Balis JU, Conen PE 1964 The role of alveolar inclusion bodies in the developing lung. Lab Invest 13:1215–1229
13. Ballard PL 1982 Hormonal aspects of fetal lung development. In: Farrell PM (ed) Lung Development: Biological and Clinical Perspectives, Vol II. Academic Press, New York, pp 205–253
14. Ballard PL, Gluckman PD, Brehier A, Kitterman JA, Kaplan SL, Rudolph AM, Grumbach MM 1978 Failure to detect an effect of prolactin on pulmonary surfactant and adrenal steroids in fetal sheep and rabbits. J Clin Invest 62:879–883
15. Barela TD, Wogenrich J, Hayek A 1983 Fetal B-cell destruction after administration of streptozotocin (STZ) to pregnant rats. Pediatr Res 17:128A (abstr)
16. Barrett CT, Sevanian A, Kaplan SA 1974 Adenylate cyclase activity in immature rabbit lung. Pediatr Res 8:244–247
17. Barrett CT, Sevanian A, Lavin N, Kaplan SA 1976 Role of adenosine 3′,5′-monophosphate in maturation of fetal lungs. Pediatr Res 10:621–625
18. Bassett DJP, Hamosh M, Hamosh P, Rabinowitz JL 1981 Pathways of palmitate metabolism in the isolated rat lung. Exp Lung Res 2:37–47
19. Bauer CR, Stern L, Colle E 1974 Prolonged rupture of membranes associated with a decreased incidence of respiratory distress syndrome. Pediatrics 53:7–12
20. Beck JC, Johnson JWC, Mitzner W, Lee PA, London WT, Sly DVM 1981 Glucocorticoids, hyperinsulinemia, and fetal lung maturation. Am J Obstet Gynecol 139:465–470

21. Benito M, Lorenzo M, Medina JM 1982 Relationship between lipogenesis and glycogen synthesis in maternal and fetal tissues during late gestation in the rat. Biochem J 204:865–868
22. Ben Jonathan N, Maxson RE 1978 Elevation of dopamine in fetal plasma and the amniotic fluid during gestation. Endocrinology 102:649–652
23. Bergman B, Hedner T, Samsioe G 1982 Terbutaline and pulmonary surfactant release in the rabbit fetus. Gynecol Obstet Invest 13:44–54
24. Blackburn WR, Kelly JS, Dickman PS, Travers H, Lopata MA, Rhoades RA 1973 The role of the pituitary adrenal-thyroid axes in lung differentiation. II Biochemical studies of developing lung in anencephalic fetal rats. Lab Invest 28:352–360
25. Bleasdale JE, Maberry MC, Quirk JG 1982 Myo-Inositol homeostasis in foetal rabbit lung. Biochem J 206:43–52
26. Bose CL, Manne DN, D'Ercole AJ, Lawson EE 1980 Delayed fetal pulmonary maturation in a rabbit model of the diabetic pregnancy. J Clin Invest 66:220–226
27. Bourbon J, Jost A 1982 Control of glycogen metabolism in the developing fetal lung. Pediatr Res 16:50–56
28. Bourbon JR, Pignol B, Rieutort M 1984 Rat models of diabetic pregnancy for study of fetal lung maturation. Diabetologia 27:259A (abstr)
29. Bourbon JR, Rieutort M, Engle MJ, Farrell PM 1982 Utilization of glycogen for phospholipid synthesis in fetal rat lung. Biochim Biophys Acta 712:382–389
30. Boutwell WC, Goldman AS 1979 Depressed biochemical lung maturation and steroid uptake in an animal model of infant of diabetic mother. Pediatr Res 13:355 (abstr)
31. Buckingham S, Heineman HO, Sommers SC, McNary WF 1966 Phospholipid synthesis in the large pulmonary alveolar cell. Am J Pathol 48:1027–1041
32. Buechler KF, Rhoades RA 1980 Fatty acid synthesis in the perfused rat lung. Biochim Biophys Acta 619:186–195
33. Buechler KF, Rhoades RA 1981 De novo fatty acid synthesis in the perfused rat lung. Incorporation of palmitate into phospholipids. Biochim Biophys Acta 665:393–398
34. Burton LE, Wells WW 1974 Studies on the developmental pattern of the enzymes converting glucose 6-phosphate to myo-inositol in the rat. Dev Biol 37:35–42
35. Bustos R, Giussi G 1981 L/S ratio and cortisol in amniotic fluid according to gestational age. Pediatr Res 15:996–998
36. Bustos R, Kulovich MV, Gluck L, Gabbe SG, Evertson L, Vargas C, Lowenberg E 1979 Significance of phosphatidylglycerol in amniotic fluid in complicated pregnancies. Am J Obstet Gynecol 133:899–903
36a. Carlson KS, Smith BT, Post M 1984 Insulin acts on the fibroblast to inhibit glucocorticoid stimulation of lung maturation. J Appl Physiol 57:1577–1579
37. Chez RA, Mintz DH, Reynolds WA, Hutchinson DL 1975 Maternal-fetal plasma glucose relationships in late monkey pregnancy. Am J Obstet Gynecol 121:938–940
38. Chow SN, Chen HY, Ouyang PC, Lee TY, Wei PY, Chen JS 1982 Serum triglyceride levels in newborns and mothers at parturition. Biol Res Pregnancy 3:30–42
39. Collet AJ, Desbiens G 1975 Evolution of mesenchymal cells in fetal rat lung. Anat Embryol 197:273–292
40. Conly PW, Le Maire WJ, Monkus EF, Cleveland WW 1973 Plasma estriol concentration in infants with respiratory distress syndrome. J Pediatr 83:851–853
41. Costrini NV, Kalkhoff RK 1971 Relative effects of pregnancy, oestradiol and progesterone on plasma insulin and pancreatic islet secretion. J Clin Invest 50:992–999
42. Cornblath M, Schwartz R 1976 Disorders of carbohydrate metabolism in infancy. W. B. Saunders Company, Philadelphia
43. Cruz AC, Buhi WC, Birk SA, Spellacy WN 1976 Respiratory distress syndrome with mature lecithin/sphingomyelin ratios:diabetes mellitus and low Apgar scores. Am J Obstet Gynecol 126:78–82
44. Cuestas RA, Lindall A, Engel RR 1976 Low thyroid hormones and respiratory distress syndrome of the newborn. Studies on cord blood. N Engl J Med 295:297–302
45. Cunningham MD, Desai NS, Thompson SA, Greene JM 1978 Amniotic fluid phosphatidylglycerol in diabetic pregnancies. Am J Obstet Gynecol 131:719–724
46. Cunningham MD, Greene JM, Thompson SA, Desai NS 1976 Antenatal reduction of surfactant phosphatidylglycerol (PG) in infants of diabetic mothers (IDM) with respiratory distress (RD). Pediatr Res 10:460 (abstr)
47. Cunningham MD, McKean HE, Gillispie DH, Greene JW Jr 1982 Improved prediction of fetal lung maturity in diabetic pregnancies: a comparison of chromatographic methods. Am J Obstet Gynecol 142:197–204
48. Curet LB, Olson RW, Schneider JM, Zachman RD 1979 Effect of diabetes mellitus on amniotic fluid lecithin/sphingomyelin ratio and respiratory distress syndrome. Am J Obstet Gynecol 135:10–13
49. Dahlenburg GW, Martin FIR, Jeffrey PR, Horacek I 1977 Amniotic fluid lecithin/sphingomyelin ratio in pregnancy complicated by diabetes. Br J Obstet Gynaecol 84:294–299
50. Dancis J, Jansen V, Kayden HJ, Schneider H, Levitz M 1973 Tranfer across perfused human placenta. II Free fatty acids. Pediatr Res 7:192–197
51. Das DK, Ganguly M 1981 Diabetes, hypophysectomy, or thyroidectomy reduces nuclear L-triiodothyronine-binding capacity of rat lung. Endocrinology 109:296–300
52. Das DK, Kumar S 1975 Nutritional and hormonal variations alter de novo fatty acid synthesis in mammalian lung. Fed Proc 34:673 (abstr)
53. DeCamara DL, Moss GS, Das Gupta TK 1979 Influence of sympathetic nervous system on surfactant production. Ann Surg 189:416–425
54. DeGasparo M, Hoet JJ 1970 Normal and abnormal fetal weight gain. Excerpta Med Intern Congr Ser No 231:667–677
55. DeLemos RA, Shermeta DW, Knelson JH, Kotas R, Avery ME 1970 Acceleration of appearance of pulmonary surfactant in the fetal lamb by administration of corticosteroids. Am Rev Respir Dis 102:459–461
56. Demottaz J, Epstein MF, Frantz ID 1980 Phospholipid synthesis in lung slices from fetuses of alloxan diabetic rabbits. Pediatr Res 14:47–49
56a. Devaskar SU, Ganguli S, Devaskar UP, Sperling MA 1982 Glucocorticoids and hypothyroidism modulate development of fetal lung receptors. Am J Physiol 242:E-384–E-391
57. DiAugustine RP 1974 Lung concentric laminar organelle. Hydrolase activity and compositional analysis. J Biol Chem 249:584–593
58. Divers WA, Babaknia A, Hopper BR, Wilkes MM, Yen SS 1982 Fetal lung maturation: amniotic fluid catecholamines, phospholipids and cortisol. Am J Obstet Gynecol 142:440–444
59. Divers WA Jr, Wilkes MM, Babaknia A, Yen SSC 1981 An increase in catecholamines and metabolites in the amniotic fluid compartment from middle to late gestation. Am J Obstet Gynecol 139:483–486
60. Dobbs LG, Mason RJ 1979 Pulmonary alveolar type II cells isolated from rats. Release of phosphatidylcholine in response to β-adrenergic stimulation. J Clin Invest 63:378–387
61. Donald IR, Freeman RK, Goebelsmann U, Chan WH, Nakamura RM 1973 Clinical experience with the amniotic fluid lecithin/sphingomyelin ratio. Am J Obstet Gynecol 115:547–552
62. Draisey TF, Gagneja GL, Thibert RJ 1977 Pulmonary surfactant and amniotic fluid insulin. Obstet Gynecol 50:197–199
63. Dubreuil G, Anderodias J 1920 Ilots de Langerhans géants chez un nouveau-né issu de mère glycosurique. Comptes Rendus Soc Biol 83:1490–1493
64. Duhring JL, Thompson SA 1975 Amniotic fluid phospholipid analysis in normal and complicated pregnancies. Am J Obstet Gynecol 121:218–220
65. Edson JL, Hudson DG, Hull D 1975 Evidence for increased fatty acid transfer across the placenta during a maternal fast in rabbits. Biol Neonate 27:50–55
66. Eichberg J, Gates J, Hauser G 1979 The mechanism of modification by propranolol of the metabolism of phosphatidyl-CMP (CDP-diacylglycerol) and other lipids in the pineal gland. Biochim Biophys Acta 573:90–106
67. Ekelund L, Burgoyne R, Brymer D, Enhörning G 1981 Pulmonary surfactant release in fetal rabbits as affected by terbutaline and aminophyllin. Scand J Clin Lab Invest 41:237–245
68. Elphick MC, Hudson DG, Hull D 1975 Transfer of fatty acids across the rabbit placenta. J Physiol (Lond) 252:29–42
69. Engle M, Langan SM, Sanders RL 1983 The effects of insulin and hyperglycemia on surfactant phospholipid synthesis in organotypic cultures of type II pneumocytes. Biochim Biophys Acta 753:6–13
70. Engle MJ, Perelman RH, McMahon KE, Langan SM, Farrell PM 1983 Relationship between the severity of experimental diabetes and altered lung phospholipid metabolism. Proc Soc Exp Biol Med 176:261–267
71. Enhörning G, Chamberlain D, Contreras C, Burgoyne R, Robertson B 1977 Isoxsuprine-induced release of pulmonary surfactant in the rabbit fetus. Am J Obstet Gynecol 129:197–202
72. Enhörning G, Chamberlain D, Contreras C, Burgoyne R, Robertson B 1979 Isoxsuprine-infusion to the pregnant rabbit and its effect on fetal lung surfactant. Biol Neonate 35:43–51
73. Epstein MF, Farrell PM 1975 Primate fetal lung in gestations complicated by maternal glucose intolerance. Pediatr Res 9:395 (abstr)
74. Epstein MF, Farrell PM, Chez RA 1976 Fetal lung lecithin metabolism in the glucose-intolerant rhesus monkey pregnancy. Pediatrics 57:722–728
75. Ericksson U, Tyden O, Berne C 1983 Glycogen content and lipid biosynthesis in the lungs of fetuses of diabetic rats. Biol Res Prog 4:103–106
76. Eriksson UJ, Tyden O, Berne C 1983 Development of phosphatidylglycerol biosynthesis in the lungs of diabetic rats. Diabetologia 24:202–206
77. Esko JD, Raetz CRH 1980 Mutants of chinese hamster ovary cells with altered membrane phospholipid composition. Replacement of phosphatidylinositol by phosphatidylglycerol in a myo-inositol auxotroph. J Biol Chem 255:4474–4480
78. Farrell PM, Overview of Hyaline Membrane Disease. In: Farrell PM (ed) Lung Development: Biological and Clinical Perspectives, Vol II. Academic Press, Inc., New York, pp 23–46
79. Farrell PM 1976 Indices of fetal maturation in diabetic pregnancy. Lancet 1:596
80. Farrell PM, Avery ME 1975 Hyaline membrane disease. Am Rev Respir Dis 111:657–688
81. Farrell PM, Hamosh M 1978 The biochemistry of fetal lung development. Clin Perinatol 5:197–229
82. Farrell PM, Engle MJ, Frantz JD, Goldman AS, Kalkhoff R, Kemnitz JW, Perelman R, Stern JS, Susa JB 1982 Complications of pregnancy and fetal development. Diabetes 31(suppl 1):89–94
83. Farrell PM, Engle MJ, Curet LB, Perelman RH, Morrison JC 1984 Saturated phospholipids in amniotic fluid of normal and diabetic pregnancies. Obstet Gynecol 64:77–85
84. Farrell PM, Wood RE 1976 Epidemiology of hyaline membrane disease in the United States. Pediatrics 58:167–176
85. Fedrick J, Butler NR 1972 Hyaline membrane disease. Lancet 2:768–769
86. Felig P 1981 The endocrine pancreas:diabetes mellitus. In: Felig P, Baxter JD, Broadus AE, Frohman LA (eds) Endocrinology and Metabolism. McGraw-Hill Book Co., New York, pp 761–868
87. Filler DA, Rhoades RA 1982 Lung phosphatidate phosphatase: activity during altered physiologic states. Exp Lung Res 3:37–46

88. Frantz ID, Epstein MF 1980 Fetal lung development in pregnancy complicated by diabetes mellitus. In: Markutz JR, Adams AAS (eds) Diabetic Pregnancy: A Perinatal Perspective of Glucocorticoids. Grune & Stratton, Inc., New York, pp 227–233
89. Freer DE, Statland BE 1981 Measurement of amniotic fluid surfactant. Clin Chem 27:1629–1641
90. Freinkel N, El Younsi C, Dawson RMC 1975 Inter-relations between the phospholipids of rat pancreatic islets during glucose stimulation and their response to medium inositol and tetracaine. Eur J Biochem 59:245–252
91. Gabbe SG, Lowensohn RI, Mestman JH, Freeman RK, Goebelsmann, U 1977 Lecithin/sphingomyelin ratio in pregnancies complicated by diabetes mellitus. Am J Obstet Gynecol 128:757–760
92. Gabbe SG, Mestman JH, Freeman RK, Goebelsmann UT, Lowensohn RI, Nochimson D, Cetrulo C, Quilligan EJ 1977 Management and outcome of pregnancy in diabetes mellitus, classes B to R. Am J Obstet Gynecol 129:723–729
93. Garcia-Miranda JL, Otero-Gomez A, Martin-Herrera AI, Gonzalez-Espinosa C, Bullon A Jr 1980 Morphogenesis of the epithelial cells in the pulmonary alveolus of fetuses from Wistar rats with alloxanic diabetes. Ultrastructural aspects and biochemical correlation. Morfol Normal Patolo [Sect A] 4:179–189
94. Gellis SS, Hsia DY-Y 1959 The infant of diabetic mother. Am J Dis Child 97:1–41
95. Gewolb IH, Barrett C, Wilson CM, Warshaw JB 1982 Delay in pulmonary glycogen degradation in fetuses of streptozotocin diabetic rats. Pediatr Res 16:869–873
96. Gewolb IH, Rooney SA, Barrett C, Wilson C, Light D, Ingleson L, Gross I, Warshaw JB 1983 Delayed fetal lung development in the fetus of the diabetic rat. Pediatr Res 17:376A (abstr)
97. Giannopoulos G 1980 Identification and ontogeny of β-adrenergic receptors in fetal rabbit lung. Biochem Biophys Res Commun 95:383–394
98. Gilbert M, Bourbon J 1980 Effects of acute variation of fetal glycemia on glycogen storage and on glycogen synthase and phosphorylase activities in the liver of the rat fetus. Diabetes 29:266–271
99. Gilden C, Sevanian A, Tierney DF, Kaplan SA, Barrett CT 1977 Regulation of fetal lung phosphatidylcholine synthesis by cortisol: role of glycogen and glucose. Pediatr Res 11:845–848
100. Gluck L 1971 Biochemical development of the lung: clinical aspects of surfactant development, RDS and the intrauterine assessment of lung maturity. Clin Obstet Gynecol 19:710–727
101. Gluck L 1979 Fetal lung development. In: The Surfactant System and the Neonatal Lung. Mead Johnson Symposium on Perinatal and Developmental Medicine, No. 14. Mead Johnson & Co., Evansville, IL, pp 40–49
102. Gluck L, Chez RA, Kulovich MV, Hutchinson DL, Niemann WH 1974 Comparison of phospholipid indicators of fetal lung maturity in the amniotic fluid of the monkey (*Macacca mulatta*) and baboon (*Papio papio*). Am J Obstet Gynecol 120:524–530
103. Gluck L, Kulovich MV 1973 Lecithin/sphingomyelin ratios in amniotic fluid in normal and abnormal pregnancy. Am J Obstet Gynecol 115:539–546
104. Gluck L, Kulovich MV 1975 Fetal lung development. In: Cheek DB (ed) Fetal and Postnatal Cellular Growth, Hormones and Nutrition. J. Wiley and Sons, New York, pp 273–282
105. Gluck L, Kulovich MV, Borer RC Jr, Brenner PH, Anderson GG, Spellacy WN 1971 Diagnosis of the respiratory distress syndrome by amniocentesis. Am J Obstet Gynecol 109:440–445
106. Gluck L, Kulovich MV, Borer RC Jr, Keidel WW 1974 The interpretation and significance of the lecithin/sphingomyelin ratio in amniotic fluid. Am J Obstet Gynecol 120:142–155
107. Gluck L, Kulovich MV, Eidelman AI, Cordero L, Khazin AF 1972 Biochemical development of surface activity in mammalian lung. IV. Pulmonary lecithin synthesis in the human fetus and newborn and etiology of the respiratory distress syndrome. Pediatr Res 6:81–99
108. Gluckman PD, Ballard PL, Kaplan SL, Liggins GC, Grumbach MM 1978 Prolactin in umbilical cord blood and the respiratory distress syndrome. J Pediatr 93:1011–1014
109. Goldfischer S, Kikkawa Y, Hoffman L 1968 The demonstration of acid hydrolase activities in the inclusion bodies of type II alveolar cells and other lysosomes in the rabbit lung. J Histochem Cytochem 16:102–109
110. Grant MM, Cutts NR, Brody JS 1983 Alterations in lung basement membrane during fetal growth and type II cell development. Dev Biol 97:173–183
111. Grant MM, Cutts NR, Brody JS 1983 Lung basement membrane alterations and type II cell development in fetuses of diabetic rats. Fed Proc 41:859
112. Gross I, Walker-Smith GJ, Wilson CM, Maniscalco WM, Ingleson LD, Brehier A, Rooney SA 1980 The influence of hormones on the biochemical development of fetal rat lung in organ culture. II. Insulin. Pediatr Res 14:834–838
113. Guleff PS, Beck RR 1981 Maternal and fetal adrenocortical function in the diabetic rabbit. Am J Physiol 240:E217–E225
114. Hallman M 1977 Induction of surfactant phosphatidylglycerol in the lung of fetal and newborn rabbits by dibutyryl adenosine-3′, 5′-monophosphate. Biochem Biophys Res Commun 77:1094–1102
115. Hallman M, Epstein BL 1980 Role of myo-inositol in the synthesis of phosphatidylglycerol and phosphatidylinositol in the lung. Biochem Biophys Res Commun 92:1151–1159
116. Hallman M, Feldman B, Gluck L 1975 RDS: the absence of phosphatidlyglycerol in surfactant. Pediatr Res 9:396 (abstr)
117. Hallman M, Feldman BH, Kirkpatrick E, Gluck L 1977 Absence of phosphatidylglycerol (PG) in respiratory distress syndrome in the newborn. Pediatr Res 11:714–720
118. Hallman M, Gluck L 1974 Phosphatidylglycerol in lung surfactant: I. Synthesis in rat lung microsomes. Biochem Biophys Res Commun 60:1–7
119. Hallman M, Gluck L 1975 Phosphatidylglycerol in lung surfactant: II. Subcellular distribution and mechanism of biosynthesis *in vitro*. Biochim Biophys Acta 409:172–191
120. Hallman M, Gluck L 1976 Phosphatidylglycerol in lung surfactant: III. Possible modifier of surfactant function. J Lipid Res 17:257–262
121. Hallman M, Gluck L 1980 Formation of acidic phospholipids in rabbit lung during perinatal development. Pediatr Res 14:1250–1259
122. Hallman M, Kulovich M, Kirkpatrick E, Sugarman RG, Gluck MD 1976 Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: indices of lung maturity. Am J Obstet Gynecol 125:613–617
123. Hallman M, Teramo K 1979 Amniotic fluid phospholipid profile as a predictor of fetal lung maturity in diabetic pregnancies. Obstet Gynecol 54:703–707
124. Hallman M, Teramo K 1981 Measurement of the lecithin/sphingomyelin ratio and phosphatidylglycerol in amniotic fluid: an accurate method for the assessment of fetal lung maturity. Br J Obstet Gynecol 88:806–813
125. Hallman M, Wermer D, Epstein BL, Gluck L 1982 Effects of maternal insulin or glucose infusion on the fetus: study on lung surfactant phospholipids, plasma myoinositol, and fetal growth in the rabbit. Am J Obstet Gynecol 142:817–882
126. Hamosh M, Schechter Y, Hamosh P 1978 Metabolic activity of developing rabbit lung. Pediatr Res 12:95–100
127. Harlap S, Polishuk WZ 1973 Respiratory distress syndrome and diabetes. Lancet 2:577
128. Hayden W, Olson EB, Zachman RD 1977 Effect of maternal isoxsuprine on fetal rabbit lung biochemical maturation. Am J Obstet Gynecol 129:691–694
129. Hershfield MS, Nemeth AM 1968 Placental transport of free palmitic and linoleic acids in the guinea pig. J Lipid Res 9:460–468
130. Hertel J, Andersen GE, Christensen NJ 1980 Plasma noradrenaline in infants of diabetic mothers. Diabetologia 19:484
131. Hertel J, Christensen NJ, Pedersen SA, Kühl C 1982 Plasma noradrenaline and adrenaline in infants of diabetic mothers at birth and at two hours of age. Acta Paediatr Scand 71:941–945
132. Higuchi M, Hirano H, Maki M 1980 Surfactant lipoprotein concentration in amniotic fluid of abnormal pregnancies and premature deliveries. Acta Obst Gynaecol Jap 11:1767–1774
133. Hills BA 1982 Water repellency induced by pulmonary surfactants. J Physiol (Lond) 325:175–186
134. Hoet JJ, Reusens B 1976 Etude de l'autonomie du pancreas fetal et de ses implications cliniques. Bull Mem Acad R Med Belg 131:193–205
135. Holman RT, Smythe L, Johnson S 1979 Effect of sex and age on fatty acid composition of human serum lipids. Am J Clin Nutr 32:2390
136. Hook GER, Gilmore LB 1982 Hydrolases of pulmonary lysosomes and lamellar bodies. J Biol Chem 257:9211–9220
137. Horger EO, Miller MC, Conner ED 1975 Relation of large birthweight to maternal diabetes mellitus. Obstet Gynecol 45:150–159
138. Ho Yuen B, Phillips WDP, Cannon W, Sy L, Redford D, Burch P 1982 Prolactin, estradiol and thyroid hormones in umbilical cord blood of neonates with and without hyaline membrane disease: a study of 405 neonates from midpregnancy to term. Am J Obstet Gynecol 142:698–703
139. Hubbell JP Jr, Drorbaugh JE 1965 Infants of diabetic mothers: neonatal problems and their management. Diabetes 14:157–161
140. Hubbell JP Jr, Muirhead DM Jr, Drorbaugh JE 1965 The newborn infant of the diabetic mother. Med Clin North Am 49:1035–1052
141. Hummel L, Schirrmeister W, Zimmerman T 1975 Transfer of maternal plasma free fatty acids into the rat fetus. Acta Biol Med Ger 34:603–605
142. Hummel L, Schwartze A, Schirrmeister W, Wagner H 1976 Maternal plasma triglycerides as a source of fetal fatty acids. Acta Biol Med Ger 35:1635–1641
143. Hultquist GT, Olding LB 1981 Endocrine pathology of infants of diabetic mothers. Acta Endocrinol 97(suppl 241):1202
144. Ishidate K, Weinhold PA 1981 The content of diacylglycerol, triacylglycerol and monoacylglycerol and a comparison of the structural and metabolic heterogeneity of diacylglycerols and phosphatidylcholine during rat lung development. Biochim Biophys Acta 664:133–147
145. Isles TE, Dickson M, Farquhar JW 1968 Glucose tolerance and plasma insulin in newborn infants of normal and diabetic mothers. Pediatr Res 2:198–208
146. Isles TE, Farquhar JW 1967 The effects of endogenous antibody on insulin-assay in the newborn infants of diabetic mothers. Pediatr Res 1:110–115
147. Jobe A, Ikegami M, Sarton-Miller I 1979 Palmitic acid synthesized by the developing lung is a preferred precursor of surfactant phosphatidylcholine. Pediatr Res 13:536
148. Jobe A, Kirkpatrick E, Gluck L 1978 Lecithin appearance and apparent biologic half-life in term newborn rabbit lung. Pediatr Res 12:669–675
149. Jones C 1980 Circulating catecholamines in the fetus, their origin, actions and significance. In: Parvez S, Parvez H (eds) Biogenic Issues in Development. Elsevier North-Holland, Biological Medical Press, Amsterdam, pp 63–86
150. Kalhan SC, Schwartz R, Adam PAJ 1975 Placental barrier to human insulin-I^{125} in insulin-dependent diabetic mothers. J Clin Endocrinol Metab 40:139–142
151. Kanjanapone V, Hartig-Beecken I, Epstein MF 1980 Effect of isoxsuprine on fetal lung surfactant in rabbits. Pediatr Res 14:278–281
152. Karlsson K, Kjellmer I 1972 The outcome of diabetic pregnancies in relation to the mother's blood sugar level. Am J Obstet Gynecol 112:213–220

153. Kemnitz JW, Perelman RH, Engle MJ, Farrell PM An experimental model for studies of fetal maldevelopment in the diabetic pregnancy. Pediatr Pulmonology (in press)
154. Kikkawa Y, Kaibara M, Motoyama EK, Orzalesi MM, Cook CD 1971 Morphologic development of fetal rabbit lung and its acceleration with cortisol. Am J Pathol 64:423–443
155. King RJ 1979 Pulmonary surface active material: basic aspects. In: The Surfactant System and the Neonatal Lung. Mead Johnson Symposium on Perinatal and Developmental Medicine, No. 14. Mead Johnson & Co. Evansville, IL. pp 3–11
156. Kitterman JA, Liggins GC, Clements JA, Campos G, Lee CH, Ballard PL 1981 Inhibitors of prostaglandin synthesis, tracheal fluid, and surfactant in fetal lambs. J Appl Physiol 51:1562–1567
157. Knopp RH, Ruder HJ, Herrera E, Freinkel N 1970 Carbohydrate metabolism in pregnancy. VII: Insulin tolerance during late pregnancy in the fed and fasted rat. Acta Endocrinol 65:352–360
158. Koren Z, Shafrir E 1964 Placental transfer of free fatty acids in the pregnant rat. Proc Soc Exp Biol Med 116:411–414
159. Kotas RV 1982 Physiologic assessment of lung surfactant. In: Lung Development: Biological and Clinical Perspectives, Vol I. Farrell PM (ed) Academic Press, New York, pp 57–86
160. Kotas RV, Avery ME 1971 Accelerated appearance of pulmonary surfactant in the fetal rabbit. J Appl Physiol 30:358–361
161. Kulovich MV, Gluck L 1979 The lung profile. II. Complicated pregnancy. Am J Obstet Gynecol 135:64–70
162. Lawson EE, Brown ER, Torday JS, Madansky D, Taeusch HW 1978 The effect of epinephrine on tracheal fluid flow and surfactant efflux in fetal sheep. Am Rev Respir Dis 118:1023–1026
163. Levine DH 1983 Insulin effects on surfactant production. Pediatr Res 17:136A (abstr)
164. Liggins GC 1969 Premature delivery of foetal lambs infused with glucocorticoids. J Endocrinol 45:515–523
165. Liggins, GC 1976 Adrenocortical-related maturational events in the fetus. Am J Obstet Gynecol 126:931–939
166. Like AA, Butler L, Williams RM, Appel MC, Weringer EJ, Rossini AA 1982 Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 31(suppl 1):7–13
167. Lin ECC 1977 Glycerol utilization and its regulation in mammals. Ann Rev Biochem 46:765–795
168. Lowensohn RI, Gabbe SG 1979 The value of lecithin/sphingomyelin ratios in diabetes: a critical review. Am J Obstet Gynecol 139:702–704
169. Maniscalco WM, Finkelstein JN, Parkhurst AB 1982 De novo fatty acid synthesis in developing rat lung. Biochim Biophys Acta 711:49–58
170. Maniscalco WM, Shapiro DL 1983 Effects of dexamethasone beta-adrenergic receptors in fetal lung explants. Pediatr Res 17:274–277
171. Maniscalco WM, Wilson CH, Gross I, Gobran L, Rooney SA, Warshaw JB 1978 Development of glycogen and phospholipid metabolism in fetal and newborn rat lung. Biochim Biophys Acta 530:333–346
172. Maniscalco WM, Wilson CM, Gross I 1979 Influence of aminophylline and cyclic AMP on glycogen metabolism in fetal rat lung in organ culture. Pediatr Res 13:1319–1322
173. Marin L, Dameron F, Relier JP 1982 Changes in the cellular environment of differentiating type II pneumocytes. Biol Neonate 41:172–182
174. Marino PA, Rooney SA 1981 The effect of labor on surfactant secretion in newborn rabbit lung slices. Biochim Biophys Acta 664:389–396
175. McCormick KL, Susa JB, Widness JA, Singer DB, Adamsons K, Schwartz R 1979 Chronic hyperinsulinemia in the fetal rhesus monkey. Effects on hepatic enzymes of lipogenesis and carbohydrate metabolism. Diabetes 28:1064–1068
176. McFadyen RJ 1981 Fetal surfactant phospholipids in chemically diabetic pregnancies. Proc. Meet. Med. Scientif. Section Brit. Diabetic Assoc. Diabetologia 20:671–672
177. Merritt TA, Curbelo V, Gluck L, Clements RS 1981 Alterations in fetal lung phosphatidylinositol metabolism associated with maternal glucose intolerance. Biol Neonate 39:217–224
178. Mescher EJ, Platzker ACG, Ballard PL, Kitterman JA, Clements JA, Tooley WH 1975 Ontogeny of tracheal fluid, pulmonary surfactant and plasma corticoids in the fetal lamb. J Appl Physiol 39:1017–1021
179. Mettler NR, Gray ME, Schuffman S, Lequire VS 1981 β-Adrenergic induced synthesis and secretion of phosphatidylcholine by isolated pulmonary alveolar type II cells. Lab Invest 45:575–586
180. Mickal A, Begneaud WP, Weese WH 1966 Glucose tolerance and excessively large infants. Am J Obstet Gynecol 94:62–64
181. Miller HC, Futrakul P 1968 Birth weight, gestational age and sex as determining factors in the incidence of respiratory distress syndrome of prematurely born infants. J Pediatr 72:628–635
182. Mims LC, Mazzuckelli LF, Kotas RV 1975 The significance of circulating glycerol as a precursor of pulmonary phosphatidylcholine in the developing mammalian lung. Pediatr Res 9:165–167
183. Moncada S, Vane JR 1979 Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. N Engl J Med 300:1142–1147
184. Morishige WK, Uetake CA, Greenwood FC, Akaka J 1977 Pulmonary insulin responsivity: *in vivo* effects of insulin on the diabetic rat lung and specific insulin binding to lung receptors in normal rats. Endocrinology 100:1710–1722
185. Morrison JC, Schneider JM, Whybrew WD, Bucovaz ET 1980 Effect of corticosteroids and fetomaternal disorders on the L:S ratio. Obstet Gynecol 56:583–590
186. Morrison JC, Whybrew WD, Bucovaz ET, Wiser WL, Fish SA 1977 Amniotic fluid tests for fetal maturity in normal and abnormal pregnancies. Obstet Gynecol 49:20–24
187. Motoyama EK, Orzalesi MM, Kikkawa Y, Kaibara M, Wu B, Zigas CJ, Cook CD 1971 Effect of cortisol on maturation of fetal rabbit lungs. Pediatrics 48:547–555
188. Moxley MA, Longmore WJ 1975 Studies on the effects of alloxan and streptozotocin-induced diabetes on lipid metabolism in the isolated rat lung. Life Sci 17:921–926
189. Moxley MA, Longmore WJ 1977 Effect of experimental diabetes and insulin on lipid metabolism in the isolated perfused rat lung. Biochim Biophys Acta 488:218–224
190. Mueller-Heubach E, Caritis SN, Edelstone DI, Turner JH 1978 Lecithin/sphingomyelin ratio in amniotic fluid and its value for the prediction of neonatal respiratory distress syndrome in pregnant diabetic women. Am J Obstet Gynecol 130:28–34
191. Mulay S, McNaughton L 1983 Fetal lung development in streptozotocin-induced experimental diabetes: cytidylyltransferase activity, disaturated phosphatidylcholine and glycogen levels. Life Sci 33:637–644
192. Mulay S, Solomon S 1983 Influence of streptozotocin-induced diabetes in pregnant rats on plasma corticosterone and progesterone levels and on cytoplasmic glucocorticoid receptors in fetal tissues. J Endocrinol 96:335–345
193. Mulay S, Solomon S 1983 Influence of maternal diabetes on fetal rat development: alteration of insulin receptors in fetal liver and lung. J Endocrinol 98:401–410
194. Murphy BEP 1978 Conjugated glucocorticoids in amniotic fluid and fetal lung maturation. J Clin Endocrinol Metab 47:212–219
195. Nelson GH, McPherson J, Perling L, Ciechan R 1980 The effect of maternal dietary fat on fetal pulmonary maturation in rats. Am J Obstet Gynecol 138:466–467
196. Neufeld ND, Corbo LM, Kaplan SA 1981 Plasma membrane insulin receptors in fetal rabbit lung. Pediatr Res 15:1058–1062
197. Neufeld ND, Kaplan SA, Lippe BM, Scott M 1978 Increased monocyte receptor binding of [^{125}I] insulin in infants of gestational diabetic mothers. J Clin Endocrinol Metab 47:590–595
198. Neufeld N, Melmed S 1981 3,5-Dimethyl-3′-isopropyl-L-thyronine therapy in diabetic pregnancy. Stimulation of rabbit fetal lung phospholipids. J Clin Invest 68:1605–1609
199. Neufeld ND, Sevanian A, Barrett CT, Kaplan SA 1979 Inhibition of surfactant production by insulin in fetal rabbit lung slices. Pediatr Res 13:752–754
200. Nijjar MS 1979 Role of cAMP and related enzymes in rat lung growth and development. Biochim Biophys Acta 586:464–472
201. Noble RC, Shand JH 1981 The placenta. Its role in the relationship between the lipids of mother and foetus. IRCS J Med Sci 9:174–177
202. Olson EB, Graven SN 1974 Comparison of visualization methods used to measure the lecithin/sphingomyelin ratio in amniotic fluid. Clin Chem 20:1408–1415
203. Oulton M 1979 The role of centrifugation in the measurement of surfactant in amniotic fluid. Am J Obstet Gynecol 135:337–343
204. Pattle RE 1969 The development of the foetal lung. In: Ciba Foundation Symposium. Wolstenholme GEW, O'Connor M (eds) Foetal Autonomy. J. & A. Churchill Ltd., London, pp 132–146
205. Pedersen J, Bojsen-Möller B, Poulsen H 1954 Blood sugar in newborn infants of diabetic mothers. Acta Endocrinol 15:33–52
206. Pedersen J, Osler M 1961 Hyperglycemia as the cause of characteristic features of the foetus and newborn of diabetic mothers. Dan Med Bull 8:78–83
207. Pedersen O, Beck-Nielsen H, Klebe JG 1981 Insulin receptors in the pregnant diabetic and her newborn. J Clin Endocrinol Metab 53:1160–1166
208. Perelman RH, Engle MJ, Kemnitz JW, Kotas RV, Farrell PM 1982 Biochemical and physiological development of fetal rhesus lung. J Appl Physiol 53:230–236
209. Perelman RH, Farrell PM 1982 Analysis of causes of neonatal death in the United States with specific emphasis on fatal hyaline membrane disease. Pediatrics 70:570–575
210. Persson B, Lunell NO 1975 Metabolic control in diabetic pregnancy. Variations in plasma concentrations of glucose, free fatty acids, glycerol, ketone bodies, insulin and human chronic somatomammotropin during the last trimester. Am J Obstet Gynecol 122:737–745
211. Pfleger RC, Thomas HO 1971 Beagle dog pulmonary surfactant lipids. Arch Intern Med 127:863–872
212. Phillipe M, Ryan KJ 1981 Catecholamines in human amniotic fluid. Am J Obstet Gynecol 139:204–208
213. Picon L 1967 Effects of insulin on growth and biochemical composition of the rat fetus. Endocrinology 81:1419–1421
213a. Picon L, Irondelle MM, Prudont G 1974 Action de l'insuline et du diabète sur le taux plasmatique des acides gras non estérifiés chez le foetus et le norveau né de rat. CR Acad Sci (Paris) [Ser D] 279:2067–2069
214. Pignol B, Bourbon J, Rieutort M 1983 Diminution du phosphatidylglycerol pulmonaire chez les foetus de rattes rendues diabétiques par la streptozotocine. CR Acad Sci (Paris) [Ser III] 297:339–342
215. Pitkin RM, Van Orden DE 1974 Fetal effects of maternal streptozotocin-diabetes. Endocrinology 94:1247–1253
216. Polishuk WZ, Anteby S, Bar-on H 1973 Lecithin/sphingomyelin ratio in amniotic fluid of diabetic mothers: a warning of respiratory distress in newborn? Lancet 1:36–37
217. Polishuk WZ, Anteby S, Stein Y, Bar-on H 1974 Lecithin/sphingomyelin ratio in amniotic fluid of diabetic and latent diabetic pregnancies. Intern. J Gynaecol Obstet 12:49–53
218. Portman OW, Behrman RE, Soltys P 1969 Transfer of free fatty acids across the primate placenta. Am J Physiol 216:143–147
219. Powell WS, Solomon S 1978 Biosynthesis of prostaglandins and thromboxane

B_2 by fetal lung homogenates. Prostaglandins 15:351–363
220. Proceedings of a task force on animals appropriate for studying diabetes mellitus and its complications 1982. Diabetes 31(suppl 1):1–102
221. Pschera H, Björkhem I, Carlström K, Lantto O, Lunell NO, Persson B, Somell C, Stangenberg M, Wager J 1979 Total cortisol and L/S ratio in amniotic fluid in late pregnancies complicated by diabetes mellitus. Horm Metab Res 11:612–615
222. Rahbar S 1968 An abnormal hemoglobin in red cell diabetics. Clin Chim Acta 22:296–298
223. Reynolds WA, Chez RA, Bhuyan BK, Neil GL 1974 Placental transfer of streptozotocin in the rhesus monkey. Diabetes 23:777–782
224. Rhoades RA 1974 Net uptake of glucose, glycerol and fatty acids by the isolated perfused rat lung. Am J Physiol 226:144–149
225. Rhoades RA, Filler DA, Vannata B 1979 Influence of maternal diabetes on lipid metabolism in neonatal rat lung. Biochim Biophys Acta 572:132–138
226. Rimmer S, Fawcitt J 1982 Delayed clearance of pulmonary fluid in the neonate. Arch Dis Child 57:63–67
227. Robert MF, Neff RK, Hubbell JP, Taeusch HW, Avery ME 1976 Association between maternal diabetes and the respiratory-distress syndrome in the newborn. N Engl J Med 294:357–360
228. Roffi J 1968 Influence des corticosteroides sur la synthèse d'adrénaline chez le foetus et le nouveau-né de rat et de lapin. J Physiol (Paris) 60:455–494
229. Rooney SA, Chu AJ, Gross I, Marino PA, Schwartz R, Seghal P, Singer DB, Susa JB, Warshaw JB, Wilson CM 1983 Lung surfactant in the hyperinsulinemic fetal monkey. Lung 161:313–317
230. Rooney SA, Ingleson LD, Wilson CM, Gross I 1980 Insulin antagonism of dexamethasone-induced stimulation of cholinephosphate cytidyltransferase in fetal rat lung in organ culture. Lung 158:151–155
231. Scarpelli EM, Kumar A, Doyle C, Clutario BC 1981 Functional anatomy and volume-pressure characteristics of immature lungs. Respir Physiol 45:25–41
232. Scholz RW, Woodward BM, Rhoades RA 1972 Utilization in vitro and in vivo of glucose and glycerol by rat lung. Am J Physiol 223:991–996
233. Sevanian A, Gilden C, Kaplan SA, Barrett CT 1979 Enhancement of fetal lung surfactant production by aminophylline. Pediatr Res 13:1336–1340
233a. Shafrir E, Khassis S 1982 Maternal-fetal fat transport versus new fat synthesis in the pregnant diabetic rat. Diabetologia 22:111–117
234. Sharp MJ, Borer RC, Vadnay L, Douglas WHJ 1980 Choline incorporation into lecithin in response to insulin or dexamethasone in homogenous cell cultures of rat lung epithelial cells and fibroblasts. Pediatr Res 14:899–900
235. Shelley HJ 1961 Glycogen reserves and their changes at birth and in anoxia. Br Med Bull 17:137–143
236. Shelley SA, Kovacevic M, Paciga JE, Balis JU 1979 Sequential changes of surfactant phosphatidylcholine in hyaline-membrane disease of the newborn. N Engl J Med 300:112–116
237. Singh EJ, Mejia A, Zuspan FP 1974 Studies of human amniotic fluid phospholipids in normal, diabetic, and drug abuse pregnancy. Am J Obstet Gynecol 119:623–629
238. Singh M, Feigelson M 1983 Effects of maternal diabetes on the levels, synthetic rates and activities of synthetic enzymes of surface-active phospholipids in perinatal rat lung. Biochim Biophys Acta 753:53–59
239. Singh M, Feigelson M 1983 Effects of maternal diabetes on the development of carbohydrate metabolizing enzymes, glycogen deposition and surface active phospholipid levels in the fetal rat lung. Biol Neonate 43:33–42
240. Skjaeraasen J, Lindback T 1976 Phospholipid concentrations in amniotic fluid from diabetic pregnant women. Acta Obstet Gynecol Scand 55:225–232
241. Skjaeraasen J, Stray-Pedersen S 1979 Amniotic fluid phosphatidylinositol and phosphatidylglycerol. II. Diabetic and eclamptic pregnancies. Acta Obstet Gynecol Scand 58:433–438
241a. Smith BT 1979 Lung maturation in the fetal rat: acceleration by injection of fibroblast–pneumonocyte factor. Science 204:1094–1095
242. Smith BT, Bogues WG 1980 Effects of drugs and hormones on lung maturation in experimental animal and man. Pharmacol Ther 9:51–74
243. Smith BT, Giroud CJP, Robert M, Avery ME 1975 Insulin antagonism of cortisol action on lecithin synthesis by cultured fetal lung cells. J Pediatr 87:953–955
244. Smith BT, Torday JS 1974 Factors affecting lecithin synthesis by fetal lung cells in culture. Pediatr Res 8:848–851
245. Smith BT, Torday JS, Giroud CJP 1974 Evidence for different gestation dependent effects of cortisol on cultured fetal lung cells. J Clin Invest 31:1518–1528
246. Sodoyez-Goffaux FR, Sodoyez J, Devos CJ 1981 Insulin receptors in the fetal rat lung. A transient characteristic of fetal cells? Pediatr Res 15:1303–1307
247. Sosenko IRS, Frantz ID, Roberts RJ, Meyrick B 1980 Morphologic disturbance of lung maturation in fetuses of alloxan diabetic rabbits. Am Rev Respir Dis 122:687–696
248. Sosenko IRS, Hartig-Beeken I, Frantz ID 1979 Glucocorticoid effects on delayed lung maturation in fetuses of alloxan diabetic rabbits. Pediatr Res 13:506 (abstr)
249. Sosenko IRS, Hartig-Beeken I, Frantz ID 1980 Cortisol reversal of functional delay of lung maturation in fetuses of diabetic rabbits. J Appl Physiol 49:971–974
250. Sosenko IR, Kitzmiller JL, Loo SW, Blix P, Rubenstein AH, Gabbay KH 1979 The infant of the diabetic mother. Correlation of increased cord C-peptide levels with macrosomia and hypoglycemia. N Engl J Med 301:859–862
251. Sosenko IRS, Lawson EE, Demottaz V, Frantz ID 1978 Delayed lung maturation in fetuses of alloxan diabetic rabbits. Pediatr Res 12:569 (abstr)
252. Sosenko IRS, Lawson EE, Demottaz V, Frantz ID 1980 Functional delay in lung maturation in fetuses of diabetic rabbits. J Appl Physiol 48:643–647
253. Stahlman MT, Gray ME 1978 Anatomical development and maturation of the lungs. Clin Perinatol 5:181–196
254. Steinke J, Driscoll SG 1965 The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes 14:573–578
255. Stern L, Ramos A, Leduc G 1968 Urinary catecholamine excretion in infants of diabetic mothers. Pediatrics 42:598–605
256. Strang LB 1979 Heterogeneity of pathogenetic mechanisms in hyaline membrane disease. Mead Johnson Symposium on Perinatal and Developmental Medicine, No. 14. Mead Johnson & Co., Evansville, IL, pp 53–58
257. Stuart MJ, Sunderji SG, Allen JB 1981 Decreased prostacyclin production in the infant of the diabetic mother. J Lab Clin Med 98:412–416
258. Stubbs WA, Morgan I, Lloyd B, Alberti KGMM 1977 The effect of insulin on lung metabolism in the rat. Clin Endocrinol 7:181–184
259. Stubbs WA, Stubbs SM 1978 Hyper-insulinemia, diabetes mellitus, and respiratory distress of the newborn: a common link? Lancet 1:308–309
260. Sugahara K, Ezaki K, Kaneko T, Morioka T, Maeda H 1981 Studies of the lungs in diabetes mellitus. II. Phospholipid analyses on the surfactant from broncho-alveolar lavage fluid of alloxan-induced diabetic rats. Biochem Biophys Res Commun 98:163–168
261. Susa JB, McCormick KL, Widness JA, Singer DB, Oh W, Adamsons K, Schwartz R 1979 Chronic hyperinsulinemia in the fetal rhesus monkey. Effects on fetal growth and composition. Diabetes 28:1058–1063
262. Szabo AJ, Grimaldi RD, Jung WF 1969 Palmitate transport across perfused human placenta. Metabolism 18:406–415
263. Tchobroutsky C, Amiel-Tison C, Cedard L, Eschwege E, Rouvillois JL, Tchobroutsky G 1978 The lecithin/sphingomyelin ratio in 132 insulin-dependent diabetic pregnancies. Am J Obstet Gynecol 130:754–760
264. Thomas T, Rhoades RA 1970 Incorporation of palmitate-1-^{14}C into lung tissue and "alveolar" lecithin. Am J Physiol 219:1535–1538
265. Tokmakjian S, Haines DSM, Possmayer F 1981 Pulmonary phosphatidylcholine biosynthesis. Alternations in the pool sizes of choline and choline derivatives in rabbit fetal lung during development. Biochim Biophys Acta 663:557–568
266. Tokmakjian S, Possmayer F 1981 Pool sizes of the precursors for phosphatidylcholine synthesis in developing rat lung. Biochim Biophys Acta 666:176–180
267. Tsai MY, Josephson MW, Donhowe J 1983 Delayed pulmonary phosphatidylglycerol synthesis and reversal by prenatal dexamethasone in fetal rats of streptozotocin-diabetic mothers. Exp Lung Res 4:315–323
268. Tsai MY, Marshall JG 1979 Phosphatidylglycerol in 261 samples of amniotic fluid from normal and diabetic pregnancies, as measured by one-dimensional thin-layer chromatography. Clin Chem 25:682–685
269. Tsai MY, Schallinger LE, Josephson MW, Brown DM 1982 Disturbance of pulmonary prostaglandin metabolism in fetuses on alloxan-diabetic rabbits. Biochim Biophys Acta 712:395–399
270. Tsao FHC, Zachman RD 1982 Prenatal assessment of fetal lung maturation: a critical review of amniotic fluid phospholipid tests. In: Farrell PM (ed) Lung Development: Biological and Clinical Perspectives, Vol II. Academic Press, New York, pp 167–203
271. Tyden O, Berne C, Eriksson U 1980 Lung maturation in fetuses of diabetic rats. Pediatr Res 14:1192–1195
272. Usher RH, Allen AC, McLean FH 1971 Risk of respiratory distress syndrome related to gestational age, route of delivery and maternal diabetes. Am J Obstet Gynecol 111:826–832
273. Vaccaro C, Brody JS 1978 Ultrastructure of developing alveoli. I. The role of the interstitial fibroblast. Anat Rec 192: 467–481
274. Van Duyne CM, Havel RJ, Felts JM 1962 Placental transfer of palmitic acid-1-C^{14} in rabbits. Am J Obstet Gynecol 84:1069–1074
275. Van Duyne CM, Parker HR, Havel RJ, Holm LW 1960 Free fatty acid metabolism in fetal and newborn sheep. Am J Physiol 199:987–990
276. Walters DV, Olver RE 1978 The role of catecholamines in lung liquid absorption at birth. Pediatr Res 12:239–242
276a. Warburton D 1983 Chronic hyperglycemia with secondary hyperinsulinemia inhibits the maturational response of fetal lamb lungs to cortisol. J Clin Invest 72:443–440
277. Warburton D, Lew CD, Platzker ACG 1981 Primary hyperinsulinemia reduces surface active material flux in tracheal fluid of fetal lambs. Pediatr Res 15:1422–1424
278. Warren C, Allen JT, Holton JB 1973 Assessment of fetal lung maturity by amniotic fluid fatty acid analysis. Clin Chim Acta 44:457–459
279. Warrner RA, Cornblath M 1969 Infants of gestational diabetic mothers. Am J Dis Child 117:678–683
280. White DA The phospholipid composition of mammalian tissues. In: Ansell GB, Hawthorne JN, Dawson RMC (eds) Form and Function of Phospholipids. Elsevier, Amsterdam, pp 441–482
281. White P 1974 Diabetes mellitus in pregnancy. Clin Perinatol 1:331–338
282. Whitsett JA, Manton MA, Darovec-Beckerman C, Adams KG, Moore JJ 1981 β-Adrenergic receptors in the developing rabbit lung. Am J Physiol 240:E351–E357
283. Wolfe RR, Snowden JM, Burke JF 1979 Influence of insulin and palmitic acid concentration on pulmonary surfactant synthesis. J Surg Res 27:262–267
284. Yambao TJ, Clark D, Smith C, Aubry RH 1981 Amniotic fluid phosphatidylglycerol in stressed pregnancies. Am J Obstet Gynecol 141:191–193
285. Young JB, Cohen WR, Rappaport EB, Landsberg L 1979 High plasma norepinephrine concentrations at birth in infants of diabetic mothers. Diabetes 28:697–699