

The Effect of Corticosteroid on the Fetal Pulmonary Maturation of Rats with Streptozotocin-Induced Diabetes

Kwang-Gil Lee, Myung Sook Yoo and In Joon Choi

The effect of dexamethasone on the maturation of the fetal lungs of rats with streptozotocin-induced diabetes was studied morphologically and biochemically. By light and electron microscopy there was little difference in fetal pulmonary maturation between the untreated control group and the untreated diabetic group, but when both groups were treated with dexamethasone the fetuses showed accelerated pulmonary maturation, approximately one day earlier with an increase of air spaces per unit area and an earlier appearance of type II pneumocytes. The number of osmiophilic inclusion bodies per alveolus and per type II pneumocyte, and the lecithin/sphingomyelin ratio in amniotic fluid increased markedly and they were statistically significant in both groups injected with dexamethasone, but were decreased in the untreated diabetic group, though only the L/S ratio of the animals of the 19th day gestation was statistically significant. Phosphatidylglycerol was present in the amniotic fluid of the groups injected with dexamethasone one day earlier than the untreated control and the untreated diabetic groups. However, the intensity of phosphatidylglycerol tended to be lower in the untreated diabetic group. It is concluded that the prenatal administration of dexamethasone to the diabetic pregnant rats will accelerate fetal pulmonary maturation morphologically and promote the synthesis of surfactant biochemically.

Key Words: Corticosteroid, fetal pulmonary maturation, streptozotocin-induced diabetes, type II pneumocytes

It has been well known that the lecithin/sphingomyelin (L/S) ratio in amniotic fluid is generally lower in diabetic mothers and the increased incidence of respiratory distress syndrome (RDS) in infants of diabetic mothers (IDM) is related to a deficiency of pulmonary surfactant due to delayed fetal pulmonary maturity (Harlap and Polishuk 1973; Polishuk *et al.* 1973; Singh *et al.* 1974). Robert and colleagues (1976), in a recent retrospective study of infants of diabetic mothers, reported that even when gestational age, mode of delivery, weight, type of labor and Apgar score were controlled, the incidence of RDS was increased more than sixfold in IDM. Their study suggests that the diabetes itself is related to RDS in infants.

Since phosphatidylcholine is the major component of pulmonary surfactant, the L/S ratio in amniotic fluid has been widely accepted as a test of pulmonary maturity of the fetus. Recently it has been suggested that phosphatidylglycerol (PG) may be an important

supplemental index of fetal lung maturation based on the fact that PG is a second surface active component of surfactant representing 10-14% of the total amount of phospholipid lining the alveoli (Rooney *et al.* 1974; King 1982) and appears in amniotic fluid as term approaches (Hallman *et al.* 1975).

The possibility that fetal steroid may influence pulmonary epithelial cell maturation was suggested by Buckingham *et al.* (1968) and supported by the observation of Liggins (1969) and others (DeLemos *et al.* 1970; Kotas and Avery 1971; Tausch *et al.* 1972). In a well controlled study on humans, Liggins and Howie (1972) reported the use of betamethasone in mothers as being effective for the prevention of RDS in premature infants, and results of subsequent studies by others also supported the effectiveness of prenatal corticosteroid therapy (Ballard and Ballard 1976; Caspi *et al.* 1976; Farrell and Kotas 1976; Tausch *et al.* 1979). It is, therefore, of interest to study the effect of glucocorticoid on the morphology of pulmonary maturation in IDM, but experimental studies in this field have been limited mainly to the biochemical aspect of the changes in the pulmonary surfactant and to the functional delay of fetal lung (Sosenko *et al.* 1980). Moreover, the study of the effect of cor-

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Department of Pathology, Yonsei University College of Medicine,
CPO Box 8044, Seoul, 120, Korea

Reprint request to Dr. In Joon Choi (Chairman).

ticosteroid on fetal pulmonary maturation in diabetic animals, particularly on the morphological aspect of it, has not been reported as yet. The present work has been designed to study the effect of preterm glucocorticoid on the fetal pulmonary maturation morphologically and on surfactant in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

A total of 535 mature Wistar albino rats, weighing 150-200 gms, were impregnated. Among them, 57 rats and 171 fetuses obtained from these were usable in the experiment. The first group was a control group consisting of 16 pregnant rats and 48 fetuses. The second was a dexamethasone (DXM)-treated group which consisted of 12 pregnant rats and 36 fetuses. The third group was a diabetic group consisting of 16 rats and 48 fetuses. The fourth was a DXM-treated diabetic group which consisted of 13 rats and 39 fetuses.

The confirmation of pregnancy was made by identification of sperms in vaginal smears. The day when sperms were first identified was considered to be the first day of gestation. On the fourth day of gestation, the diabetes was induced by the injection of streptozotocin, 55 mg/kg, via the tail vein. Controls were injected with an equal volume of acidified saline (pH 4.5). Dexamethasone was injected intramuscularly with 6 µg/gm per day for 2 days before delivery. Urine sugar was examined with reagent strip (Ames Co.) to confirm the induction of diabetes 2 and 10 days after the streptozotocin injection and right before their sacrifice. The does were killed with ether anesthesia at 18, 19, 20 and 21 days gestation, following which the fetuses were delivered by hysterotomy and killed by severing cervical cord to prevent air breathing. Whole blood, 1-2 ml, was collected for glucose concentration. The number of fetuses including the dead one was recorded and the total body weight of 5 fetuses were measured. About 1-2 ml of amniotic fluid was collected from a litter for a measurement of surfactant phospholipid.

Three fetuses were randomly selected from a brood for examination. The thorax was opened and the lungs, together with trachea, were dissected out of the thoracic cage. The lung tissue was fixed in 10% neutral formalin and processed for routine light microscopic study. The apex of the right upper lobe was chosen for study by electron microscopy. The fresh tissue was minced with a sharp razor. Blocks were fixed in 3% glutaraldehyde solution buffered with 0.1 M phosphate at pH 7.4, postfixed in 1% osmium tetroxide solution buffered with 0.1 M phosphate at

pH 7.4, dehydrated with graded ethanol, and embedded in Epon 812. Sections were cut with glass knives on an MT 2-B ultratome. Ultrathin sections for electron microscopy were examined after conventional staining.

One micron sections of Epon-embedded tissues of fetal lung at 20 and 21 days gestation were examined by light microscopy after staining with PAS-Azur II and the number of type II cells and OIB were counted in 20 alveoli at magnification of $\times 1,000$. Five photomicrographs of each H-E stained sections at magnification of $\times 200$ were used for a morphometric study of the air spaces. The areas of air spaces in the photomicrograph were measured by IBM-PC Digitalizer DT-3100 and calculated as percentage of area of air spaces per total.

The amniotic fluid obtained was promptly centrifuged for 10 min. at $1,500 \times g$ and the supernatant was stored at -70°C . Then, according to Painter (1980), phospholipid was extracted with methanol/chloroform solution and developed on a TLC plate pretreated with cupric chloride dihydrate (1 g/L) solution for 4 seconds. The plates were sprayed with a cupric acetate (350 g/L)/phosphoric acid (80 ml/L) reagent and charred on a hot plate to make the phospholipids visible. The L/S ratio and the presence of PG were determined by Quick scan densitometry.

RESULTS

Blood Sugar, Number of Fetuses from a Doe, and Body Weight of 5 Fetuses

The level of blood sugar was 80-129 mg% in the control and 67-237 mg% in the DXM-treated group. The sugar level was markedly higher ranging from 280 mg% to 574 mg% in the diabetic and from 332 mg% to 629 mg% in the DXM-treated diabetic. In the DXM-treated group, 5 out of 12 does were hyperglycemic (Table 1). There was little difference among the 4 groups in the number of fetuses from a doe. The body weight of the fetuses was less in the DXM-treated, the diabetic and the DXM-treated diabetic groups, but much less in the latter 2 groups.

Light Microscopic Observations

Histological findings

The control group: The fetal lungs showed the glandular stage at 18 days gestation. The glands were embedded in an abundant mesenchymal stroma, round in shape and lined by tall columnar cells. Capillaries were scattered in the stroma. At the 19th day of gestation, the lungs developed into the early

Table 1. Blood sugar, total number of fetuses from a doe, and body weight of 5 fetuses

Group	Gestation Day	Blood Sugar*	Total Number of Fetuses*	Body Weight of 5 Fetuses**
I	18	109	10	5.8±0.5
	19	98.5	9.8	8.4±0.2
	20	79.7	10	12.1±1.5
	21	114.3	10.5	20.8±0.8
II	18	169.8	10.5	4.8±0.3▲
	19	118	9.7	7.2±0.3▲
	20	102.3	12.3	10.5±1.5
	21	109.3	10.5	18.0±1.0
III	18	403.3	13	3.8±0.3▲
	19	440.4	12.6	6.6±0.8▲
	20	507	9.5	10.9±1.9
	21	378.1	11.5	15.3±3.4▲
IV	18	417.5	14	3.5±0.5▲
	19	535.8	9.7	5.5±1.0▲
	20	342.7	10	8.5±1.8▲
	21	327.3	12.5	11.9±1.9▲

* Values shown are mean.

** Values shown are mean ± S.D.

▲ p<0.05 when compared with group I.

canalicular stage; respiratory bronchioles and alveolar ducts were partially formed, the epithelial cells were cuboidal, and relatively the amount of the stroma decreased with an increase of capillaries. At day 20, most of air passages developed into alveolar ducts with formation of alveolar sacs in part. At day 21, the lung was composed mainly of alveolar sacs. The stroma decreased in amount much more. Capillaries became numerous and came into close contact with the alveolar surface.

The DXM-treated group: The fetal lung showed the early canalicular stage at 18 days gestation. The airways were mainly round tubules, but a small number of respiratory bronchioles and alveolar ducts were observed. All of the air passages were much wider and this distension persisted until the 21st gestational day. At day 19, most of the tubules differentiated into respiratory bronchioles and alveolar ducts, forming alveolar sacs in part. At day 20, the air passage was composed of alveolar sacs, for the most part, and at day 21, almost all of them were alveolar sacs with marked decrease in the amount of the stroma. As compared with the controls, the fetal pulmonary maturation of the DXM-treated group appeared ac-

celerated about one day with distension of air passages.

The diabetic group: There were no detectable differences in maturation when compared to fetuses of the same gestation of the control group (Fig. 1 and 3).



Fig. 1. Lung of 19-day-gestation fetus in the diabetic group. The lung is composed mainly of round respiratory tubules. Respiratory bronchioles are found in part. (H & E × 200)

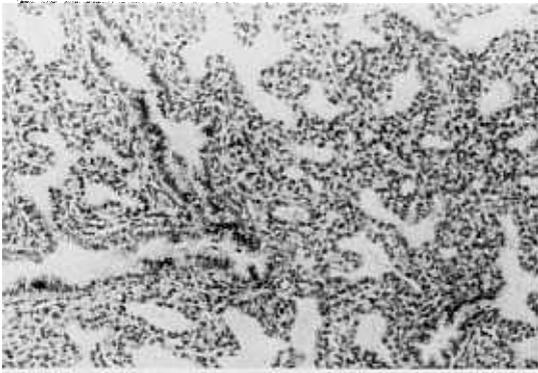


Fig. 2. Lung of 19-day-gestation fetus in the DXM-treated diabetic group. The lung consists mainly of respiratory bronchioles and alveolar ducts with distension of the air spaces. (H & E, $\times 200$)

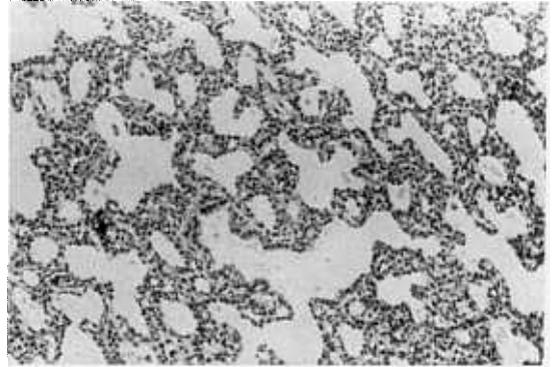


Fig. 4. Lung of 21-day-gestation fetus in the DXM-treated diabetic group. The lung consists mostly of alveolar sacs that are distended. (H & E $\times 200$)

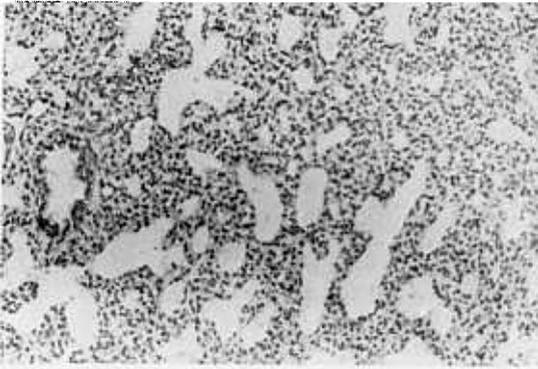


Fig. 3. Lung of 21-day-gestation fetus in the diabetic group. The lung is composed mainly of alveolar sacs. (H & E, $\times 200$)

showed accelerated maturation of approximately one day with persistent distension of airways (Fig. 2 and 4).

The morphometry of air space area of the fetal lung

The area of air space was calculated as percentage of the total area of the lung (Table 2). In the control group, the area of air space increased as gestation days went on, particularly higher from day 20. The diabetic group was generally similar to the control, although somewhat decreased at day 20 as compared to the control. In the DXM-treated group, the area of air space increased markedly, more than 3 times at day 18 and day 19 as compared to that of the control. This prominent increase persisted until the 21st gestational day with a statistical significance throughout the gestation. The DXM-treated diabetic group, as compared with the diabetic, showed a marked increase in the area of air space throughout the period. The increase was similar to that of the DXM-treated group.

The DXM-treated diabetic group: The lung showed similar maturation to the DXM-treated group. They

Table 2. Area of air spaces/total of fetal lung*

Gestation Day	Group I	Group II	Group III	Group IV
18	5.2 \pm 0.8	16.0 \pm 2.2▲	5.2 \pm 0.8	11.7 \pm 4.0▲▲
19	7.9 \pm 1.9	28.4 \pm 11.1▲	6.5 \pm 1.3	25.2 \pm 10.7▲▲
20	16.6 \pm 4.7	42.9 \pm 8.8▲	11.1 \pm 1.8▲	42.6 \pm 6.1▲▲
21	31.4 \pm 5.9	46.0 \pm 5.0▲	31.4 \pm 6.3	47.9 \pm 5.2▲▲

* Values shown are mean \pm S.D.: all measurements are per cent.

▲ $p < 0.05$ when compared with group I.

▲▲ $p < 0.05$ when compared with group I and III.

Table 3. Distribution of type II cells and OIB in alveoli*

Group	Gestation Day	Type II Cell/Alveolus	OIB/Alveolus	OIB/Type II Cell
I	20	17.61±2.86	11.71±4.92	0.66±0.21
	21	13.16±2.13	9.90±2.67	0.81±0.37
II	20	18.32±5.69	19.35±5.10▲	1.09±0.25▲
	21	15.12±3.25	15.92±2.32▲	1.07±0.17
III	20	17.64±1.81	10.41±3.15	0.59±0.17
	21	13.45±2.60	9.58±3.57	0.71±0.20
IV	20	18.59±1.23	18.96±4.23▲	1.02±0.28▲▲
	21	14.67±2.49	16.14±3.78▲▲	1.10±1.04▲▲

* Values shown are mean ± S.D.: all measurements are per cent.

▲▲ p<0.05 when compared with group I.

▲▲ p<0.05 when compared with group III.

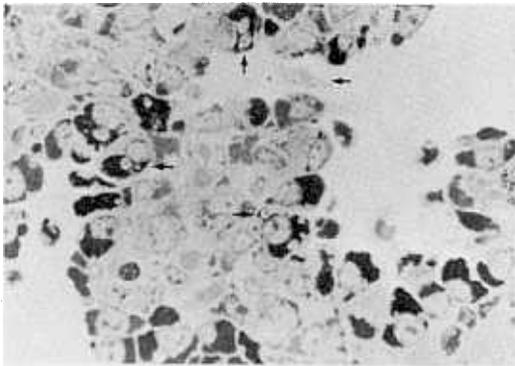


Fig. 5. Lung of 21-day-gestation fetus in the diabetic group. Many OIB (arrow) can be seen in the alveolar space and type II cells. (PAS-Azur II, × 1,000)

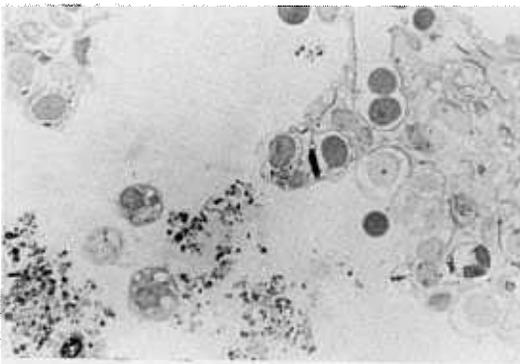


Fig. 6. Lung of 21-day-gestation fetus in the DXM-treated diabetic group. A large amount of OIB are present in the alveolar space. (PAS-Azur II, × 1,000)

The number of type II cells and osmiophilic inclusion bodies (OIB) in the alveoli

In the PAS-Azur II stain, the OIB were seen as coarse, deep blue granules. The cells containing the OIB in their cytoplasm or the cuboidal cells with cytoplasmic microvilli at their alveolar luminal border were regarded as type II cells. We counted the OIB and type II cells present in 20 alveoli at day 20 and day 21 (Table 3).

In the control, the numbers of type II cells per alveolus and OIB per alveolus were smaller at day 21 than at day 20, but the number of OIB per type II cell increased at day 21. The diabetic group showed similar findings to the control, although OIB per alveolus and OIB per type II cell were slightly decreased in number with no statistical significance. In the DXM-treated group, the number of type II cells per alveolus increased as compared with those of the control but it had no statistical meaning. The numbers of OIB per alveolus and OIB per type II cell increased markedly with a statistical significance in all except the number of OIB per type II cell at day 21. The DXM-treated diabetic group showed marked an increase in the number of type II cells per alveolus at day 20 and day 21 with a statistical significance (Fig. 5 and 6).

Electron Microscopic Observation

The control group: The epithelium on day 18 was mostly columnar. The glycogen within the epithelium was diffusely scattered. There were a few rough endoplasmic reticula and a scarce number of mitochondria. At day 19, cuboidal epithelium was observed

frequently. The glycogen zone within the cytoplasm was larger and, glycogen granules, in part, were more tightly packed as compared to those in the lung of the 18 day-old fetus. Occasional, round, clear spaces

were seen in the glycogen zone but lamellar bodies were not detected. At day 20, the type II cell could be readily recognized by the presence of well-formed lamellar electron-dense inclusions. There was still a

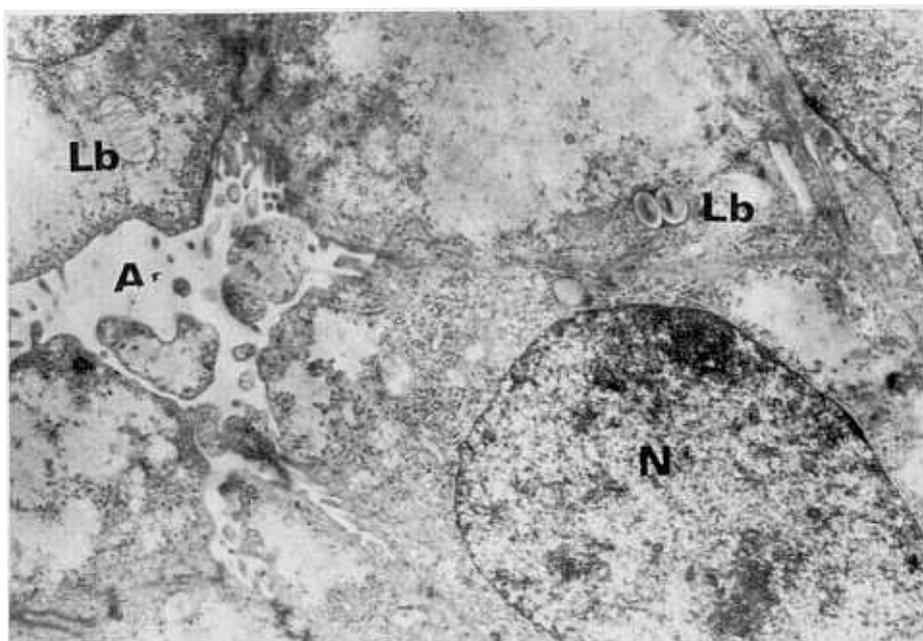


Fig. 7. Lung of 19-day-gestation fetus in the DXM-treated group. A type II cell containing lamellar bodies (Lb) in the cytoplasm is seen. As, alveolar space; N, nucleus. (Uranyl acetate & lead citrate, $\times 10,000$)

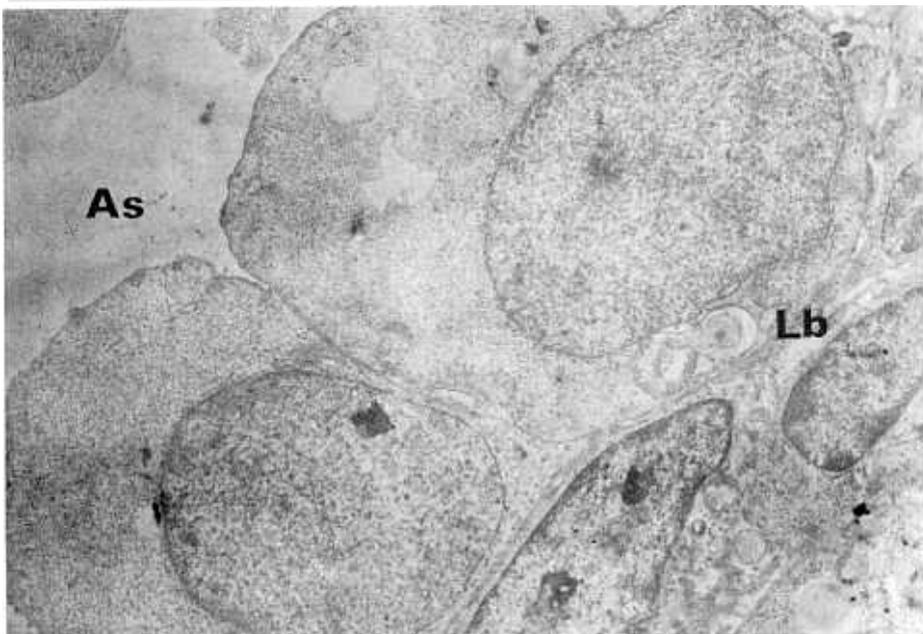


Fig. 8. Lung of 19-day-gestation fetus in the DXM-treated diabetic group. A type II cell with lamellar bodies (Lb) is observed. As, alveolar space. (Uranyl acetate & lead citrate, $\times 10,000$)

large amount of glycogen within the type II cells, although less than that at day 19. A small number of lamellar bodies were sometimes present within the alveolar space. In the lung at day 21, type I and type II cells were differentiated. Many lamellar bodies were often seen in the type II cells and alveolar spaces. The amount of glycogen within the type II cells was much more decreased.

The DXM-treated group: Type II cells containing lamellar bodies in their cytoplasm could be seen already on day 19 (Fig. 7). Glycogen granules decreased in amount as compared to those in the lung of the 19-day-old control fetus. At day 20, type I cells could be recognized by their thin, flat cytoplasm and the absence of lamellar bodies. A large number of lamellar bodies were present not only in the cytoplasm of type II cells but in alveolar lumina. There were no detectable abnormalities in the shape and structure of lamellar bodies as compared to those of the control.

The diabetic group: Type II cells appeared at day 20 and type I cells at day 21. There were no detectable differences in the ultrastructure of the lamellar bodies when compared to those of the control group.

The DXM-treated diabetic group: Like the DXM-treated group, type II cells were visible at day 19 (Fig. 8) and type I cells at day 20.

Biochemistry of the Phospholipid in the Amniotic Fluid

L/S ratio: The L/S ratio in the control group increas-

ed as gestation days went on; 0.7 at day 18, 1.5 at day 20, and 2.2 at day 21. In the diabetic group, as compared to the control group, the L/S ratio decreased throughout the gestation period, becoming statistically significant only at day 19. In the DXM-treated group, it increased markedly with a statistical significance at day 19 and day 20. As compared to the control, the L/S ratio decreased in the diabetic group throughout the gestation period, becoming statistically significant only at day 19 and in the DXM-treated group, increased markedly with a significance at day 19 and day 20. In the DXM-treated diabetic group, it increased throughout gestation and as compared to that of the diabetic, was significant statistically at day 19 and day 20 (Table 4).

The presence of PG: PG was present at day 20 both in the control group and in the diabetic group. Its appearance was somewhat low in intensity in the diabetic, but became distinct at day 20. It was present already at day 19 in the DXM-treated group and the DXM-treated diabetic group (Table 5).

DISCUSSION

In 1969, Liggins, in his study with a lamb, postulated that corticosteroid treatment induces lung maturation and causes the appearance of surface active material in alveolar spaces. Subsequent studies in the fetal rat, lamb, rabbit and monkey supported

Table 4. L/S ratio of amniotic fluid*

Gestation Day	Group I	Group II	Group III	Group IV
18	0.7±0.2	Failed	Failed	0.8±0.1
19	1.1±0.1	1.7±0.1▲	0.9±0.2▲	1.6±0.2▲▲
20	1.5±0.3	2.5±0.7▲	1.4±0.3	2.5±0.9▲▲
21	2.2±0.2	2.8±1.0	1.7±0.3	2.5±0.9

* Values shown are mean ± S.D.; all measurements are its number

▲ p<0.05 when compared with group I.

▲▲ p<0.05 when compared with group III.

Table 5. Phosphatidylglycerol in amniotic fluid

Gestation Day	Group I	Group II	Group III	Group IV
18	----	Failed	Failed	---
19	---	---	----±	±±±
20	+++	±±±	±±±±	±±±
21	+++	±±±	+++++	±±±±

—: negative ±: trace +: positive

this proposal (DeLemos *et al.* 1970; Kotas and Avery 1971; Motoyama *et al.* 1971; Kling and Kotas 1975; Kim *et al.* 1981). A study by Kikkawa *et al.* (1971) in rabbits demonstrated morphologically accelerated maturation, approximately of one and half day, in lungs of fetuses injected with corticosteroids two days before delivery. In our study there were no detectable morphological differences in maturation of fetal lungs and area of air spaces between the control and the diabetic groups when compared each other according to the gestation day. The DXM-treated and the DXM-treated diabetic groups, however, showed accelerated lung maturation of approximately one day along with distension of air spaces throughout the gestation. These results indicated that corticosteroid accelerated lung maturation associated with an increase in area of air spaces not only in the normal fetuses but also in the fetuses of diabetic rats. Ultrastructurally as the lung matures, type II cells appear first with formation of lamellar bodies in the cytoplasm. And then these cells differentiate into type I cells with flattened cytoplasm devoid of the lamellar bodies (Campiche *et al.* 1963; Williams 1977; Robbins *et al.* 1984). In this study, the appearance time of type II cells and type I cells in the diabetic group was the same as that in the control, but in the DXM-treated and the DXM-treated diabetic groups, they appeared one day earlier than in the control and the diabetic groups. These results suggest, as the study by Wang *et al.* (1971) did, that corticosteroid accelerates the appearance of lamellar bodies and pulmonary maturation. It seems that these ultrastructural findings are in agreement with the accelerated histological maturation of approximately one day. There were no detectable ultrastructural abnormalities in the shape and content of the lamellar bodies in the fetal lungs of diabetic rats.

Exogenous steroids have been noted to influence pulmonary epithelial differentiation and to increase the synthesis, storage, and release of surfactant but not to cause epithelial proliferation (Kikkawa *et al.* 1968; Kikkawa *et al.* 1971; Wang *et al.* 1971). In our experiment, the groups injected with DXM showed accelerated differentiation of air sacs and appearance of type II cells one day earlier. There was, however, no difference in the number of type II cells per alveolus between the groups that received the injection of DXM and those that did not receive the injection of DXM, indicating that corticosteroid does not invoke the proliferation of epithelial cells. The increased number of OIB per alveolus and per type II cell, and higher L/S ratio in the amniotic fluid of groups injected with DXM seems to be in agreement with the view that the steroid promotes the synthesis and

release of surface active material.

As shown in our study, the fetal lung of the diabetic group shows no evidence of delay in maturation morphologically, but the number of OIB per type II cell and the amniotic fluid L/S ratio were lower as compared to the controls. Although these were not statistically significant, it still seems to be possible that the synthesis of surfactant is inhibited in the fetuses of diabetic rats. These findings are in agreement with those of some others (Boutwell and Goldman 1979; Kim *et al.* 1984). Unlike this, Sosenko *et al.* (1980) were unable to demonstrate the decrease of L/S ratio in the fetuses of diabetic rabbits but able to show only a functional delay in lung maturation, which suggests the possibility that a defect in surfactant other than lecithin may be present.

Liggins and Howie (1972) first reported the use of betamethasone in mothers as effective for the prevention of RDS in premature infants, and results of subsequent studies by others have supported the effectiveness of prenatal corticosteroid therapy (Spellacy *et al.* 1973; Ballard and Ballard 1976; Caspi *et al.* 1976; Dluholucky *et al.* 1976; Block *et al.* 1977; Taeusch *et al.* 1979). Caspi *et al.* (1976) noted a rising of the L/S ratio to mature values as early as 48 hours after treatment with DXM was begun. However, Liggins and Howie (1974) observed no changes within several days in the amniotic fluid L/S ratio of most treated women in their study. In this study the amniotic fluid L/S ratio increased markedly in the groups injected with DXM whether the groups were diabetic or not. This finding indicates that the administration of corticosteroids restores the decreased amniotic fluid ratio in fetuses of diabetic rats to mature values. However, Sosenko *et al.* (1980), in their study on fetal lung maturation of alloxan diabetic rabbits, found no accelerating effect of glucocorticoids on lung lavage lecithin or L/S, but found only an improvement in pressure volume characteristics and functional surfactant determined by surface balance. Further experiments are necessary to resolve the difference between their findings and ours.

The major surface active lipid in lung wash is phosphatidylcholine. PG is the second most abundant surface active lipid in the human lung accounting for up to 14% of the total phospholipid (Rooney *et al.* 1974; King 1982). Although it is unlikely that PG plays a primary role in lowering surface tension at the alveolar surface, it may be important in maintaining the structural integrity of surfactant lipid-protein complex after secretion into the alveoli or in its lamellar storage phase (Rooney *et al.* 1974; Godinez *et al.* 1975; Cunningham *et al.* 1978). Therefore it is possi-

ble that the absence of PG in amniotic fluid indicates that the surfactant may not be quite mature. PG appears at 35 weeks of gestation when the L/S ratio exceeds 2 and thereafter increases progressively as days of gestation pass by (Hallman *et al.* 1976; Kulovich *et al.* 1979). Hence analysis of PG in amniotic fluid as a marker of surfactant seems to be of value as an additional index of prenatal evaluation of lung maturity. Cunningham *et al.* (1978) warned that the absence of PG following 35 weeks of gestation should merit caution in the interpretation of an otherwise mature L/S ratio. Hallman *et al.* (1975) found an absence of PG in the tracheal and gastric aspirate of infants with RDS and the appearance of PG during recovery. They concluded that in RDS, despite adequate elaboration of lecithin, the absence of PG appears to be the most prominent defect in the phospholipid composition of the surfactant complex and this may be the principal deficiency in mild to moderate RDS. In our experiment, PG was found to be present in the amniotic fluid at the same gestation day in the control and the diabetic but its intensity tended to be lower in the diabetic group. Although PG was not analyzed quantitatively in this study, the results suggest that the synthesis of PG may be decreased in fetuses of diabetic rats and that PG may have an important role in the increased incidence of RDS in IDM. There was also a tendency for PG to be present one day earlier in groups injected with DXM than in those not injected with DXM. This finding indicates that the administration of corticosteroid may promote the synthesis of PG as well as of lecithin. This is in agreement with the study by Rooney *et al.* (1975), which showed that administration of cortisol to fetal rabbits resulted in a significant increase in the activity of pulmonary glycerophosphate phosphatidyltransferase, an enzyme involved in the synthesis of PG.

The mechanism for the increased incidence of RDS in IDM is not clear. Boutwell and Goldman (1979) suggested that maternally-mediated hyperglycemia or reactive fetal hyperinsulinemia depresses the nuclear uptake of the pulmonary steroid-receptor complex, with an ensuing depression of the enzymes necessary for choline incorporation into lecithin. Sosenko *et al.* (1980) suggest a similar mechanism but they have proposed that maternal diabetes may affect some component of the surfactant other than lecithin. Smith *et al.* (1975) found in their experiment that insulin alone stimulates choline incorporation into lecithin, while in the presence of cortisol its effect is to suppress this activity. A report of Draisey *et al.* (1977) indicates that there is an apparent inverse relation between lecithin and insulin levels in amniotic fluid in late pregnancy.

These results tend to support the hypothesis that insulin inhibits lecithin synthesis. Hence, estimation of amniotic fluid insulin appears to be of value in all pregnancies where there is risk of fetal immaturity. These experimental results could, therefore, suggest that the hyperinsulinemia, characteristic of the IDM, may interfere with the physiologic process of glucocorticoid-induced pulmonary maturation and thereby increase the risk of the RDS (Farrell and Zachman 1973; Ballard and Ballard 1974; Murphy 1974). Based on these results, it seems that the decrease in the number of OIB in the alveoli and in the amniotic fluid L/S ratio in fetuses of the diabetic group in our experiment is related to maternal diabetes. The reversal of these in fetuses of diabetic rats following administration of DXM also may be explainable by the following: the inhibition of lecithin synthesis due to fetal hyperinsulinemia is restored by the injection of DXM causing acceleration of lung maturation. Further experiments using quantitative examination of insulin and cortisol in the blood and amniotic fluid are necessary to substantiate this explanation.

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