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DETERMINATION OF FETAL LUNG MATURITY IN RATS BY FLUORESCENCE POLARIZATION

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Abstract—The fluorescence polarization technique was utilized to determine fetal lung maturity in rats. The study confirmed that changes in fluorescence polarization values with gestational age follow the pattern already seen in human beings. A sharp drop was observed on the afternoon of the 20th day of gestation which corresponds in the rat to the beginning of surfactant synthesis. This was confirmed by determination of phosphatidylglycerol. In order to verify the effectiveness of this animal model, a group of pregnant rats was treated with 100 μg/kg betametasone twice 48 and 24 hr before the 20th or 21st days of gestation. As expected, this significantly reduced fluorescence polarization on day 20 (4–6 p.m.) and day 21 of gestation, indicating increased surfactant synthesis in betametasone-treated rats. Another group of female rats was pretreated before mating with streptozotocin (40 mg/kg), inducing diabetes. Fluorescence polarization values in the amniotic fluid of the diabetic rats at the above intervals were significantly higher than controls, indicating reduced lung maturation.

Hyaline membrane disease (HMD), more commonly called respiratory distress syndrome (RDS), is the most common cause of neonatal death with an incidence of 0.5–1.1% of all live births [1]. Investigations by Avery and Mead in 1959 established that the lungs of infants who died of HMD had less surfactant than normal [2]. More recently, Gluck *et al.* [3] showed that lung maturity could be correlated with the levels of lecithin and sphyngomyelin present in the amniotic fluid [4].

Since the 1970s several chemical, biochemical and biophysical methods for determining lung maturation have been proposed [5, 6]. However, chemical and biochemical methods take too long to give results, and the biophysical ones were semiquantitative [7]. Shinitzky *et al.* [8, 9] proposed for the first time the use of fluorescence polarization, already used for complex membrane biochemistry studies, for the determination of lung maturity by measuring the microviscosity of amniotic fluid. The advantages of this technique are:

1. The instrumental value obtained, "P", can be easily transformed to microviscosity expressed in poise, a highly linear and quantitative parameter;
2. The results are obtained very rapidly (about 45 min from sample collection) and analysis requires small volumes (0.3–0.5 ml) of amniotic fluid;
3. Since this is a chemico-physical determination on the lipid micelles in the amniotic fluid, it is independent of the absolute value of lipids present. This is why the coefficient of variation of the method is below 2% whereas all other quantitative determinations range from 7 to 21% [10].

This method has aroused increasing interest in obstetric practice, especially for cases needing urgent pharmacological therapy [11].

MATERIALS AND METHODS

Virgin female albino rats (CD COBS, Charles River Italy, Calco, Como) 9 weeks old, after a 2-week acclimatization period in standard conditions (temperature 21 ± 1°, humidity 55 ± 5%, light from 6 a.m. to 6 p.m.), were placed with males for mating from 7 p.m. to 9 a.m. Vaginal smears were examined the next morning for the presence of spermatozoa. The mid-point of the dark cycle during which copulation occurred (12:00 midnight), was arbitrarily taken as time zero of pregnancy; fractions of hours are expressed as decimals. The mated females were individually caged in Makrolon shoeboxes with water and diet (Altromin MT, Rieper, Bolzano, Italy) *ad libitum*. Females were killed by cervical dislocation on day 20 or 21 of gestation and the uteri were immediately removed for amniotic fluid collection. Amniotic fluid samples from fetuses from the same litter were immediately frozen at −80° until analysis.

The induction of fetal lung surfactant synthesis was stimulated by treating the animals with 100 μg/kg betametasone i.m. (Schering A.G., Berlin, F.R.G.) 48 and 24 hr before killing. Control received i.m. an equal volume of saline solution at the same times. Diabetes was induced in female rats with a single i.m. dose of 40 mg/kg streptozotocin (The Upjohn Company, Kalamazoo, Michigan, U.S.A.) one week before mating. Blood glucose levels were assayed weekly and in the terminal animal group only those with blood glucose higher than 180 mg per 100 ml were taken into consideration.

Microviscosity was determined by fluorescence polarization according to Blumenfeld *et al.* [12]. Two milliliters of a dispersion of 1,6-diphenyl-1,3,5-hexa-

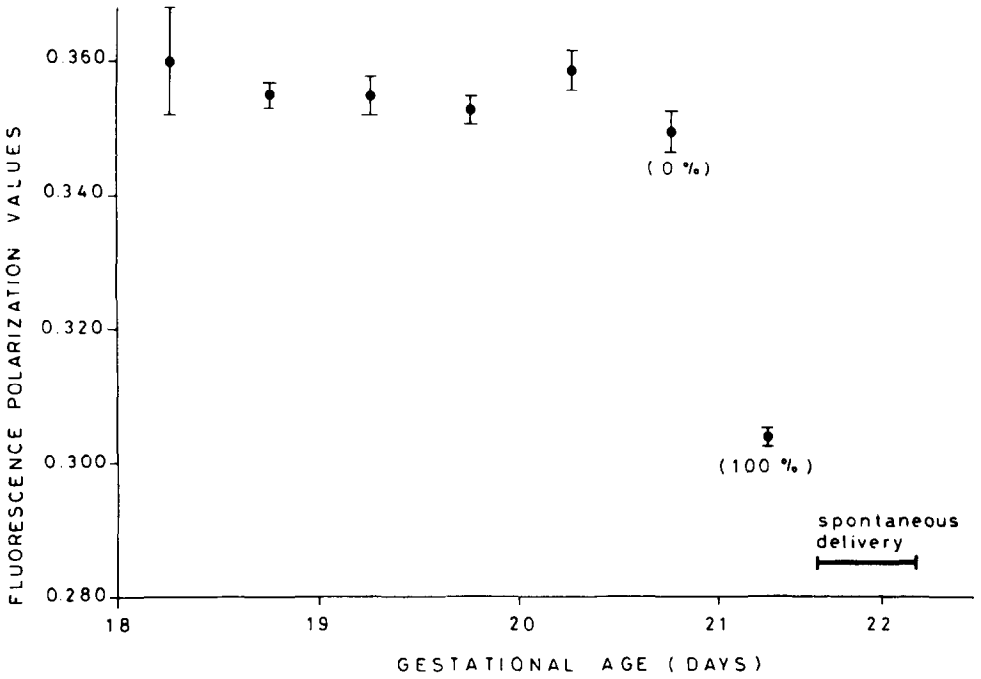

Fig. 1. Correlation between fluorescence polarization value and gestational age in untreated rats. Each point is the mean ± S.E. of 10–17 pregnant animals. The values in brackets indicate the percentage of survival of fetuses recovered by hysterectomy.

triene (DPH) in phosphate-saline buffer (PBS) were added to 0.3–0.5 ml amniotic fluid.

After 30 min of incubation at 37° the fluorescence polarization value was determined at 24° by a microviscosimeter MV-1 (Elscint, Haifa, Israel). Phosphatidylglycerol was determined enzymatically according to Muneshige *et al.* [13].

RESULTS AND DISCUSSION

Figure 1 shows the relationship between the fluorescence polarization value and gestational age in rats. The absolute fluorescence polarization values were lower than in human amniotic fluid, but not strikingly different. Between fluorescence polarization values of 0.330 and 0.300 the rat fetal lung displays an immature condition whereas only at values around 0.300 is maturity complete. Fluorescence polarization value of the bronchoalveolar lavage in the adult rat is P = 0.290 ± 0.002 (unpublished results). This value is in good agreement with the "mature" value of fluorescence polarization in rat pre-partum amniotic fluid. In human beings, during physiological pregnancies, the maturity threshold is attained at fluorescence polarization values of 0.340 [14]. The figure also shows that the synthesis of surfactant in rats reaches its crucial moment between the afternoon (4–6 p.m.) of the 20th day of gestation and the morning (8–10 a.m.) of the 21st day. In fact only in these 16–18 hr of gestation was there a marked drop in fluorescence polarization values. Moreover, all fetuses recovered through hysterectomy on day 21 of gestation and kept in an incubator survived, whereas all those recovered on the afternoon of the 20th day died (Fig. 1). This illustrates the importance of the last 16–18 hr of gestation in triggering lung surfactant synthesis in the rat.

Figure 2 shows that the trend of another biochemical index of lung maturity, phosphatidylglycerol, parallels that of fluorescence polarization. Levels rose sharply starting from the 20th day of gestation, reaching 18 nmol/ml on day 21. This is in good agreement with the trend known for phosphatidylglycerol in women: this phospholipid is considered an index of lung maturity in the sense that it starts to rise when lung maturation is triggered [15].

Table 1 shows the effect of betametasone (100 μg/kg) in pregnant rats treated 48 and 24 hr before the

Table 1. Effect of different pharmacological treatments on fetal lung maturity in the rat

	Day 20 (8–10 a.m.)	Day 20 (4–6 p.m.)	Day 21 (8–10 a.m.)
Control	0.359 ± 0.003	0.350 ± 0.003	0.304 ± 0.001
Betametasone (100 μg/kg)	0.350 ± 0.007	0.331 ± 0.004†	0.287 ± 0.001†
Streptozotocin (40 mg/kg)	0.360 ± 0.002	0.348 ± 0.007	0.318 ± 0.004*

Values are the mean ± S.E. of at least 10–17 determinations.
* $P \leq 0.05$ and † $P \leq 0.01$ compared to controls.

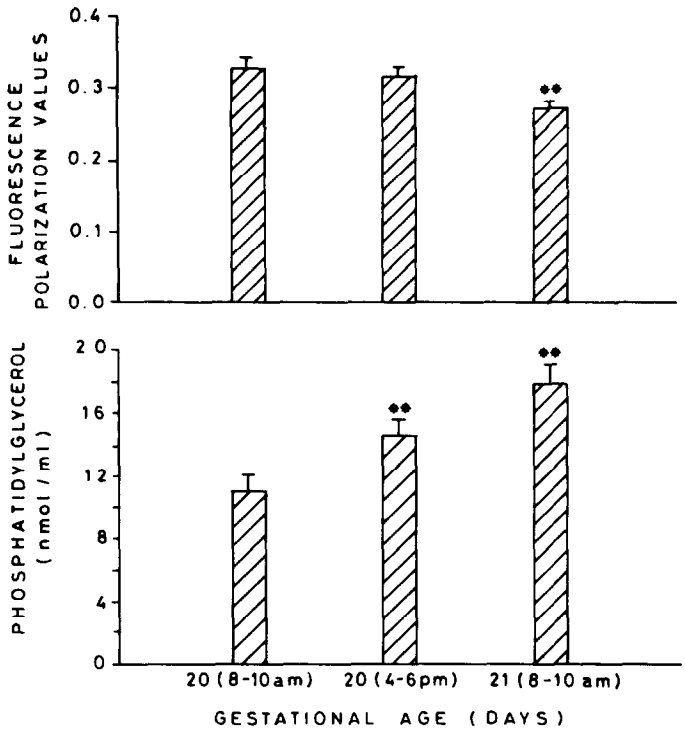

Fig. 2. Comparative determination of fluorescence polarization and phosphatidylglycerol in rat amniotic fluid on the 20th and 21st day of gestation. Each value is the mean ± S.E. of at least 10 pregnant animals. ** $P \leq 0.01$ compared to the 20th day (8–10 a.m.).

intervals considered. On day 20 of gestation, at 8–10 a.m., the drug had no effect on the lungs, but 8 hr later fluorescence polarization was significantly reduced. On the 21st day of gestation the drug was fully effective, reducing the fluorescence polarization value to 0.287. The lack of efficacy of betametasone on the rat fetus before the 20th day could be ascribed to lung immaturity and thus the absence of receptors for the drug. Table 1 also reports the results with pregnant diabetic rats. Fluorescence polarization values were significantly higher than control values on day 20, indicating slower lung maturity. This is in good agreement with the well-known effect of diabetes in man in slowing lung maturation in insulin-uncompensated patients [16].

In conclusion, the fluorescence polarization technique provides a good index of lung maturity in the rat and appears to be a useful approach in the development of an animal model of lung immaturity and in assessing the efficacy of new drugs or therapeutic regimens enhancing fetal surfactant production.

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