Assessment of Fetal Lung Maturity by Estimation of Amniotic Fluid Palmitic Acid

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Summary

The measurement of palmitic acid in amniotic fluid has been shown to be a rapid means of assessing the lecithin concentration. The level of palmitic acid increases quickly when the fetal lung matures at about 35 weeks' gestation, and the level in amniotic fluid obtained 24 hours or less before delivery clearly distinguishes which infants are likely to develop respiratory distress syndrome and which are mature.

Introduction

The usefulness of assessing phospholipid levels in amniotic fluid as a guide to the maturity of the fetal lung and to the probability of respiratory distress syndrome (R.D.S.) occurring in association with premature delivery seems to be established (Gluck *et al.*, 1971). Dipalmitoyl lecithin is the phospholipid component of surfactant, which is formed in increased amounts as the lung matures at about 35 weeks' gestation, and the changes result in rising concentrations of this substance in amniotic fluid.

The methods that have been described for monitoring changes in lecithin level have all included extraction of the amniotic fluid lipids followed by separation of the phospholipids by thin-layer chromatography. Usually the chromatogram is then stained for phosphate-containing compounds and the intensity of the lecithin spot (L) is compared with that of sphingomyelin (S), another phospholipid which is present in amniotic fluid in relatively constant amounts throughout pregnancy. The ratio L/S is used as the prognostic index (Gluck *et al.*, 1971; Macdonald and Isherwood, 1972; Whitfield and Sproule, 1972). Alternatively, the lecithin-containing spot can be removed from the thin-layer plate, the substance eluted, and the absolute concentration of lecithin phosphorous estimated (Nelson, 1972; Bhagwanani *et al.*, 1972). The former method has the disadvantage of being only semi-quantitative while the latter is rather time-consuming for routine use.

In an earlier work we suggested that the estimation of total palmitic acid levels in amniotic fluid by gas-liquid chromatography, which is both rapid and quantitative, could be used to predict fetal lung maturity (Warren *et al.*, 1973). It has been shown that, on average, 90% of the total palmitic acid in amniotic fluid originates from Lecithin (Warren *et al.*, 1974). Therefore, a close approximation of lecithin concentration is made by measuring the total palmitic acid. We have now extended our observations to confirm the clinical usefulness of the technique.

Patients and Methods

Most of the amniotic fluid samples were obtained by amniocentesis from rhesus-immunized patients, patients uncertain of gestational dates, or from others in whom an early delivery was being considered. Other samples were collected at caesarian section, at amniotomy to induce labour, or during labour itself. Only cases in which the amniotic fluid was obtained within 24 hours of delivery were included in the comparison of mature infants with those developing R.D.S. Samples which were badly contaminated with blood or meconium were considered unsuitable for the analysis.

The diagnosis of R.D.S. was made by using the following criteria: inspiratory retraction, expiratory grunting, poor alveolar air entry, oedema, and characteristic chest x-ray appearances. No attempt was made to compare the severity of the disease with the level of palmitic acid.

The method of extracting lipids from amniotic fluid, hydrolysing them, and then methylating the fatty acids has already been described (Warren *et al.*, 1973). The fatty-acid methyl esters were separated on a 5-ft. glass column filled with 10% diethyleneglycol succinate on acid-washed and silanized Chromosorb W (80-100 mesh), using a Pye 104 gas chromatograph with a dual-flame ionization detector. The optimum conditions for separating the fatty acids were a nitrogen and hydrogen flow rate of 33 ml/min each and an air flow rate of 400 ml/min. The attenuation was 2,000 and the chromatograph was operated at an oven temperature of 185°C. Full practical details are described by Warren *et al.* (1974).

Lecithin/sphingomyelin ratios were determined by using a modification of the method described by Gluck *et al.* (1971) in which the phospholipids were stained with bromothymol blue and the concentration was measured by the area of the spots (C. R. Whitfield, personal communication, 1973).

Analyses on the liquor were usually carried out immediately, but there was no significant change in the results when the samples were stored at 4°C for up to seven days. At -14°C the liquor appeared stable for much longer periods.

Results

Typical gas chromatography recordings for the fatty acids obtained from liquors early in pregnancy and at term are shown in figs. 1 and 2. The concentration of palmitic acid was obviously much higher when the fetus was mature. The variation of palmitic acid levels in 92 samples of liquor taken at different gestational ages is shown in table I and the mean

TABLE I—Variation of Palmitic Acid with Gestational Age

Gestational Age (Weeks)	No. of Samples	Mean Concentration of Palmitic Acid (mmol/l.)	Actual Range of Palmitic Acid Concentrations (mmol/l.)
28	8	0.030	0.017-0.066
29	3	0.030	0.021-0.039
32	3	0.032	0.017-0.047
33	3	0.028	0.019-0.034
34	10	0.065	0.016-0.106
36	3	0.120	0.031-0.226
37	9	0.115	0.074-0.354
38	7	0.273	0.090-0.752
39	12	0.267	0.123-0.377
40	22	0.206	0.099-0.589
41	12	0.203	0.102-0.429

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FIG. 1—Gas chromatographic tracing of fatty acids of amniotic fluid obtained at 33 weeks' gestation. Principal peaks are: 0 solvent, 1 palmitic acid (C16), 2 heptadecanoic acid (C17).

FIG. 2—Gas chromatographic tracing of fatty acids of amniotic fluid obtained at full term. Principal peaks are: 0 solvent, 1 myristic acid (C14), 2 palmitic acid (C16), 3 heptadecanoic acid (C17), 4 stearic acid (C18), 5 oleic acid (C18).

values at each period are plotted in fig. 3. The length of gestation was calculated from the last menstrual period and only those patients whose dates were known with certainty were included. The palmitic acid level began to rise at 34 weeks and was noticeably raised by 36 weeks.

The mean palmitic acid concentration for all samples obtained before 35 weeks' gestation is compared with those of greater maturity in table II. The difference in the means of the two groups was highly significant. There was also a highly significant difference between the palmitic acid concentrations obtained in cases where infants developed R.D.S. and the concentrations found in mature pregnancies (table III). There was no overlap between the values found in the two groups.

The palmitic acid concentration and L/S ratio values obtained on the same amniotic fluids from 49 pregnancies are compared in fig. 4. There is a reasonable linear correlation between the two values (correlation coefficient = 0.82) and the equation of the regression line was $y = 0.188x + 0.199$. There was no obvious discrepancy in the samples examined between the predictions of the different methods used.

FIG. 3—Palmitic acid concentrations in amniotic fluid at different gestational ages.

TABLE II—Comparison of Palmitic Acid Concentration in Amniotic Fluids obtained before and after 35 Weeks of Gestation

Gestation (Weeks)	No. of Samples	Mean $\pm$ S.D. for Palmitic Acid Concentration (mmol/L)*	Actual Range of Palmitic Acid Concentrations (mmol/L)
<35	30	0.041 $\pm$ 0.029	0.017–0.107
>35	65	0.21 $\pm$ 0.138	0.032–0.764

*Difference in means is significant ($P < 0.001$).

TABLE III—Comparison of Palmitic Acid Concentration in Amniotic Acid obtained at Delivery of Mature Babies and Babies who developed Respiratory Distress Syndrome (R.D.S.)

	No. of Samples	Mean $\pm$ S.D. for Palmitic Acid Concentrations (mmol/L)*	Actual Range of Palmitic Acid Concentrations (mmol/L)
Infants who developed R.D.S.	32	0.029 $\pm$ 0.018	0.006–0.068
Mature infants	60	0.215 $\pm$ 0.138	0.073–0.764

*Difference in means is significant ($P < 0.001$).

FIG. 4—Comparison of palmitic acid concentration and lecithin/sphingomyelin (L/S) ratio in 49 amniotic fluid samples.

Discussion

Previous work has suggested that estimating total palmitic acid is a useful method of assessing lecithin concentration in amniotic fluid. This is supported by the data contained in the graph of palmitic acid levels at different gestational ages (fig. 3) which is similar to the data of Gluck *et al.* (1971) on the variation of lecithin concentration with maturity. They both show rapid increases between 34 and 38 weeks' gestation and then a decline towards full term. Though the plot of lecithin concentration shows some increase beginning at 25 weeks' gestation, the level of palmitic acid does not rise at all until 35 weeks. This difference is most probably because the lecithin produced before 34 weeks is palmitoyl-myristoyl lecithin (Gluck *et al.*, 1971) and our measurement of palmitic acid would be less sensitive to changes in concentration of this substance. However, this is no disadvantage, since surfactant containing the palmitoyl-myristoyl lecithin is often ineffective in sustaining normal pulmonary function and it is the increase in dipalmitoyl lecithin at 35 weeks which is really important.

A comparison of the level of lecithin which Bhagwanani, *et al.* (1972) gave as an indication of lung maturity and the critical palmitic acid concentration suggested by the present study gives further confirmation of the validity of palmitic acid measurements in assessing liquor lecithin levels. By measuring lecithin phosphorus, Bhagwanani found that levels of lecithin above 3.5 mg/100 ml signified lung maturity. The lowest concentration of palmitic acid that we found in a specimen of liquor from a mature pregnancy was 0.073 mmol/l. If one assumes that this all comes from dipalmitoyl lecithin it corresponds to a lecithin concentration of 3.48

mg/100 ml. This supports our experimental findings that most palmitic acid in amniotic fluid originates from lecithin (Warren *et al.* 1974).

There is now much evidence that measuring the total palmitic acid concentration of liquor provides a good assessment of fetal lung maturity. There was a clear division between cases in which R.D.S. developed and our mature samples, and good agreement between the new method and the L/S ratio. The main advantage of measuring palmitic acid is the speed and simplicity of the method. Four specimens can be processed and the results obtained in one hour. The fact that it is quantitative is another possible advantage. This could be particularly valuable in borderline cases, where more than one liquor may be investigated to determine the trend in lecithin level, but this has yet to be shown in practice. The possibility that the degree of severity of R.D.S. could be predicted from the liquor palmitic acid level is also being examined.

We are greatly indebted to our obstetric and paediatric colleagues, who made this study possible.

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Further Studies of Gastric Hypersecretion in Chronic Renal Failure

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Summary

The effect of medical treatment has been studied in four patients with end-stage renal failure who suffered from severe spontaneous gastric hypersecretion, associated with very low levels of intraduodenal pH in two cases. Atropine decreased the overnight gastric acid output, but the residual acid secretion and duodenal content of acid remained dangerously high in one of the patients. Secretin inhibited the basal gastric secretion of acid in all cases and converted the pH of the duodenal contents to alkaline. While atropine cannot be used to control the gastric hypersecretion, secretin may prove to be valuable in the therapeutic management of the gastric hypersecretion of patients with chronic renal failure.

Introduction

A previous report (Shepherd *et al.*, 1973) confirmed the finding by Dekkers *et al.* (1972) that patients with chronic renal failure might suffer from a high incidence of duodenal ulceration, associated with pronounced nocturnal and basal gastric hypersecretion of acid. The present study was undertaken in order to investigate the pathophysiological basis of the duodenal ulceration and of the "spontaneous" gastric hypersecretion in more detail, and to study the effect of potential therapeutic measures on the gastric hypersecretory state.

Methods

The study involved four male patients who had been undergoing haemodialysis for end-stage renal failure (two chronic glomerulonephritis, one chronic pyelonephritis, and one unidentified chronic renal disease) for 12 to 46 months. Dialysis was carried out for an average of 17 hours a week in two sessions of equal length with Travenol "Ultra-flo 100" disposable coil dialysers.

The investigations of the alimentary tract were carried out between 24 and 48 hours after the preceding haemodialysis. On separate days four different tests were carried out on two of the patients (cases 3 and 4) and three of the tests on the other two patients. Informed consent was obtained for all the procedures.

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