EVALUATION OF FETAL PULMONARY MATURITY BY GAS-CHROMATOGRAPHIC ANALYSIS OF AMNIOTIC FLUID PALMITIC ACID/STEARIC ACID (P/S) RATIO

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SUMMARY

Gas-chromatographic analysis of the fatty acids (P/S ratio) in 212 samples of amniotic fluid, 35 samples of vernix caseosa and 35 samples of the pellets obtained after centrifugation of the amniotic fluid were carried out to evaluate the effects of contaminants that might be present in amniotic fluid on the P/S ratio. The P/S ratio is used as an index of the degree of maturity of the fetal or neonatal lungs.

Analysis of variance applied to the means of the P/S values for each of above materials showed them to be significantly different and attests to the importance of centrifugation for obtaining valid results.

We propose a standard procedure of centrifugation for 60 minutes at 3500×g followed by extraction and gas-chromatography as a rapid, valid way to measure the P/S ratio.

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It has been known for some time that the concentration of lecithin in the amniotic fluid is closely correlated with the respiratory adequacy of the neonate's pulmonary alveoli ([2]), although L/S ratio and DSPC ([11]) are perhaps better indicators.

Among the various methods for evaluating the concentrations of lecithin in amniotic fluid gas-chromatographic analysis of the amniotic fluid fatty acids has been widely used ([13, 1, 10]).

Warren *et al.* ([12, 13]) recommend quantitative measurement of palmitic acid. Gautray and Vielh ([8]) suggest for reasons of simplicity to determine the ratio of palmitic acid to stearic acid (P/S). We have used the P/S ratio in this study, adopting Gautray and Vielh's value of 5 as the lower limit for maturity.

Studies of fatty acids in amniotic fluid require that there be no contaminants present that would invalidate the diagnostic results. Among such contaminants would be cells, cell fragments, meconium, hair and vernix caseosa.

For this reason in order to eliminate these contaminants and to obtain comparable results between specimens great emphasis has been placed on centrifugation of the amniotic fluid samples by standardized procedure before analysis ([7, 9]).

MATERIAL AND METHODS

a) *Subjects*

Analyses were made of 212 samples of amniotic fluid, 35 samples of vernix caseosa and 35 samples of amniotic fluid sediments (pellets).

Amniotic fluids were obtained by transcervical puncture of the amniotic sac with a Drew-Smythe catheter at the beginning of labour (68 cases), or by transparietal puncture of the uterus in cases of elective caesarian section (89 cases) or by late amniocentesis (55 cases) in women with gestational ages between 30 and 42 weeks.

The samples of vernix were obtained within five minutes after birth, directly from the neonate into small containers of flexible plastic.

b) *Preparation of the samples*

The standard conditions used were as follows: centrifugation at 3500×g for 60 minutes, sepa-

ration of supernatant fluid and pellet and storage of the samples after centrifugation at −20 °C until extraction.

Lipids were extracted from the amniotic fluid and the pellets with a mixture of chloroform and methanol to ensure complete extraction of the fats. The extracts were then saponified with 0,5 N NaOH in methanol and methylated with CH_4OBF_3 (Merck), as described in detail in our earlier publications ([3]).

We used two methods for preparing the methyl esters of fatty acids in vernix caseosa samples. One was methylation with BF_3 and the other was methylation with Zn-$ZnCl_2$ ([3, 4]).

c) *Gas-chromatographic analysis*

The methyl esters were analyzed by the GLC technique, with quali-quantitative evaluation from the acidogram. The apparatus used was the Carlo Erba Fractovap 2200 gas-chromatograph, with a column packed with 20% DEGS on silanized Chromosorb W 80/100 mesh.

Quantitative evaluation was made by measuring the areas under the peaks (base×height method, manual).

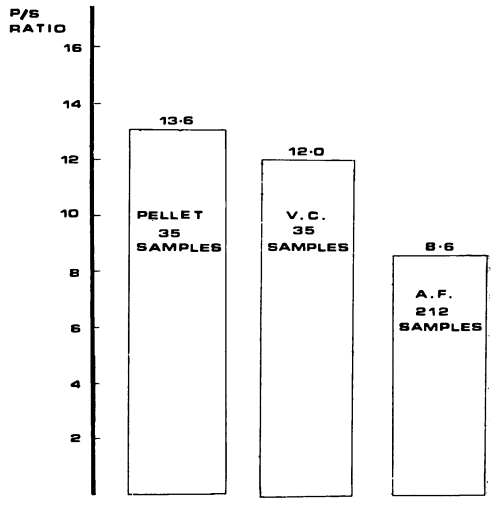

Fig. 1. — Plot of the mean values of P/S ratio in centrifuged amniotic fluid (A.F.), in sediment obtained after centrifugation (pellet) and vernix caseosa (V.C.). The differences between the means are highly significant ($P<0.01$).

RESULTS AND DISCUSSION

Figure 1 summarizes the mean values of the P/S ratios obtained by the standard procedure in amniotic fluid, in pellets and in vernix caseosa.

The highest P/S values were those in sediment (13.6). Next were those of the vernix caseosa (12.0) and the lowest were those of amniotic fluid (8.6). Analysis of variances showed that the differences between these values are significant ($P< 0.01$).

These data plus those of our earlier study ([7]) on the effects of different times of centrifugation on the P/S values, enphasize very strongly that if one wishes to use amniotic fluid fatty acids as an index of fetal pulmonary maturity one must pay strict attention to this part of the procedure. Certainly, without careful standardization of this step, the amniotic fluid will contain variable amounts of contaminants that will invalidate the clinical significance of the results.

From the 212 cases, in which we analised the amniotic fluid after standard centrifugation, we have been able to obtain clinical data to support the opinion that P/S values above 5 indicate that the lungs will be mature at birth, while those below 5 indicate that the lungs are immature.

In one of our earlier studies ([6]) we investigated the usefulness of a rapid method of determination of surface tension of the amniotic fluid, the Clements's test, without centrifugation of the amniotic fluid, as described in original method ([5]), and with centrifugation. We found that when the amniotic fluid had not been centrifuged, the results were not reliable.

Also with P/S determinations insufficient centrifugation and the presence of contaminants in the sample can give results that do not correspond to either the gestational age or the degree of maturity of the fetal lungs. We found, for example, that suspending 0.3 mg of vernix caseosa ($P/S=8.5$) in 1 ml of amniotic fluid ($P/S=4$) would give for the mixture a value of $P/S=5$, the critical value for maturity, and completely invalidate the clinical significance of the determination.

Even though the number of samples subjected to centrifugation for different time periods was small, it showed the effects of centrifugation on the values obtained and indicated that a good compromise between theoretical considerations and practicability is a centrifugation of 60 minutes at 3500 × g. When the results obtained in 212 samples by this procedure were checked against the clinical evaluations of gestational age and lung maturity, they were found to be valid.

There remains the limitation that our studies have for the most part been done with amniotic fluid samples taken from pregnancies at term, in which the possibility of contamination is greater than in amniotic fluids taken earlier in pregnancy.

It is possible that the P/S ratio could be measured in amniotic fluids centrifuged for shorter periods of time if they are obtained from patients earlier in pregnancy.

Nevertheless, it seems to us that since we cannot predict what the degree of contamination with solid material will be, it would be better to stick to the most rigorous standard procedure in all cases. This comparatively easy and valid procedure should make it possible to use amniotic fluid studies for evaluating the degree of the maturity of the lungs of the fetus, especially in cases in which it is necessary to induce delivery early.

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