ANALYSIS OF AMNIOTIC FLUID PROTEINS BY ISOELECTROFOCUSING (IEF)

F. DIANI (*), I. CONTI (*),
A. COSTA (**), G. A. BOTTA (**)

(*) Third Department of Obstetrics and Gynaecology, University of Genoa, School of Medicine, Genoa (Italy)

(**) Institute of Microbiology, University of Genoa, School of Medicine, Genoa (Italy)

SUMMARY

Among the most recent methods for investigation of proteins in biological fluids SDS Polyacrilamide-gel-electrophoresis and Isoelectrofocusing (IEF) have recently been introduced into laboratory practice.

The present investigation has been performed on 20 samples of amniotic fluid obtained during normal pregnancies in the first and in the third trimester.

The obtained results suggest that IEF analysis seems to have a selective advantage in allowing the separation of bands which can not be easily recognized with SDS electrophoresis. These bands detected by IEF and present in amniotic fluid during late pregnancy seem to be related to some low molecular weight lipoprotein fractions and we suggest that they might be used as a possible marker for monitoring fetal lung maturation.

In conclusion we think that it would be of great interest to evaluate the usefulness of IEF analysis in examining A.F. obtained during pregnancies complicated by infections.

INTRODUCTION

Between 1958 and 1978 several studies on the protein composition of amniotic fluid (A.F.) have been published (for a review see Queenan) [9].

The following points have been investigated in more detail:

1) Total protein content of A.F. according to gestational age [11, 13, 17].

2) Relationship between protein concentration in A.F. and maternal serum; the ratio is close to 1/20 in early pregnancy, rises to 1/10 around 25-28 weeks gestational age and drops again to 1/20 at term [9].

3) The origin, characteristics and possible functions of A.F. proteins [4, 5, 15].

Among the most recent methods for investigation of proteins in biological fluids Sodium - Dodecyl - Sulfate Polyacrilamide - gel - electrophoresis (SDS - PAGE) and Isoelectrofocusing (IEF) have recently been introduced into laboratory practice [10].

In the present paper we report the protein concentration of normal A.F. at various gestational ages as determined by IEF and SDS-PAGE.

MATERIAL AND METHODS

The present investigation has been performed on 20 samples of A.F. obtained during normal pregnancies.

A.F. samples were obtained by transcervical puncture of the amniotic sac by means of a Drew-Smythe catheter at the beginning of labor (6 cases), or by transparietal puncture of the uterus in case of elective caesarean section (4 cases), or by transabdominal amniocentesis (10 cases) in women of gestational age between 16 and 42 weeks.

The standard procedure was as follows: centrifugation at 3500 × g for 60 minutes followed by separation of supernatant fluid and pellet. All the above described procedures were performed with the greatest care because many Authors and particularly Viergiver *et al.* [16] have stressed the importance of a properly performed centrifugation of A.F. samples before

Clin. Exp. Obst. Gyn. - ISSN: 0390-6663
IX, n. 4, 1982

analysis in order to eliminate all contaminating factors. The samples were divided into small fractions (1 ml each) and stored at −20 °C until extraction.

– *Preparation of the plates for IEF*

Polyacrylamide gels (6% final concentration) were prepared by mixing 10 ml acrylamide (29.1%) and 10 ml of bisacrylamide (0.9%) with 7 ml of 87% glycerol solution. After addition of N, N, N′, N′ Tetramethylendiamine (TMED) (50 µl) and Ampholines as specified below (BIO RAD, Milan, Italy), the volume of the solution was brought to 60 ml with twice distilled water.

After degassing the solution for 10 minutes under vacuum, ammonium persulfate was added (1.5 ml, 1% stock solution).

To obtain a wide pH range (3.5-9) the following Ampholines solutions were used: 0.3 ml Ampholine (pH 4-6), 0.3 ml Ampholine (pH 5-7) and 3 ml Ampholine (pH 3.5-10). In a second set of experiments a narrower pH range (4.5-7) was obtained by adding Ampholine pH 5-7 (2.25 ml) and pH 7-9 (0.75 ml) to the components listed above.

After casting, the gel was allowed to polymerize at room temperature for 20 minutes. Before running the experiment the gel was maintained at 4 °C for at least 1 hour.

– *IEF conditions for samples of amniotic fluid*

Samples of amniotic fluid were concentrated to obtain equal amounts of proteins and were dispensed with a microsiringe onto small pieces of filter paper (25 µl each containing 70 ug of proteins).

Samples were applied on the gel near the cathode. Electrode solutions were 1 M H_3PO_4 and 1 M NaOH, for the anode and cathode respectively.

IEF separation was achieved in 90 minutes at constant power (30 W) under the following conditions: initial voltage 300 V, 1000 mA; final conditions 1200 V, 150 mA. For the experiments in which a narrower pH range has been used, final voltage was equal to 1000 V.

– *SDS-PAGE electrophoresis*

Separation of proteins was obtained as previously described ([1]). Final concentration in the running gel of the acrylamide-bisacrylamide solution (30 : 0.6) was 10%. Equal amounts of protein were layered onto the stacking gel after boiling in SDS-sample buffer.

– *Staining of the gels*

IEF gels were stained in a solution containing 25% ethanol, 10% acetic acid, 400 mg/l of Blau R (SERVA, Heidelberg, W. Germany) and 1 g/l $CuSO_4$.

Destaining was carried out overnight in a solution containing 12% ethanol, 7% acetic acid and 1 g/l of $CuSO_4$. SDS-PAGE gels were stained in a solution containing 50% methanol, 7.5% acetic acid and 0.08% of Blau B. Destaining of the gels was obtained in 2.5% methanol and 7.5% acetic acid.

– *Chemicals*

Acrylamide, bis-acrylamide and Ampholines were obtained from BIO RAD (Milan, Italy). Samples of bovine serum albumin, ceruloplasmin and transferrin were from SIGMA (Saint Louis, Missouri, USA). Human lyophilized IgG were from Miles Laboratories (New York, USA). A.F. samples were concentrated five times in Amicon concentrator (Amicon, Elkhart, Indiana, USA).

RESULTS AND DISCUSSION

The protein composition of normal A.F. evaluated by means of SDS-PAGE and IEF is presented in fig. 1 and 2 (first trimester of pregnancy) and fig. 3 and 4 (third trimester). Electrophoretic patterns of A.F. in the second trimester were not determined because amniocentesis is usually not performed during this period in normal women.

The protein compositions of A.F. samples obtained in the first and third trimester of normal pregnancies and examined by SDS-PAGE appear similar. By this method a clear separation of the three major proteins (i.e. albumin, ceruloplasmin and transferrin) can be achieved. These proteins have been identified on the gel by co-migration with commercially obtained markers. (Bovine serum albumin, used as a marker, has a slower migration in comparison with human serum albumin; fig. 1 and 3).

No significant changes have been detected in the relative composition of these components in the A.F. during different periods of normal pregnancies. Thus, despite the good separation of albumin, ceruloplasmin and trasferrin by SDS-PAGE, the absence of apparent modifications in the relative composition of these components during pregnancy makes this tech-

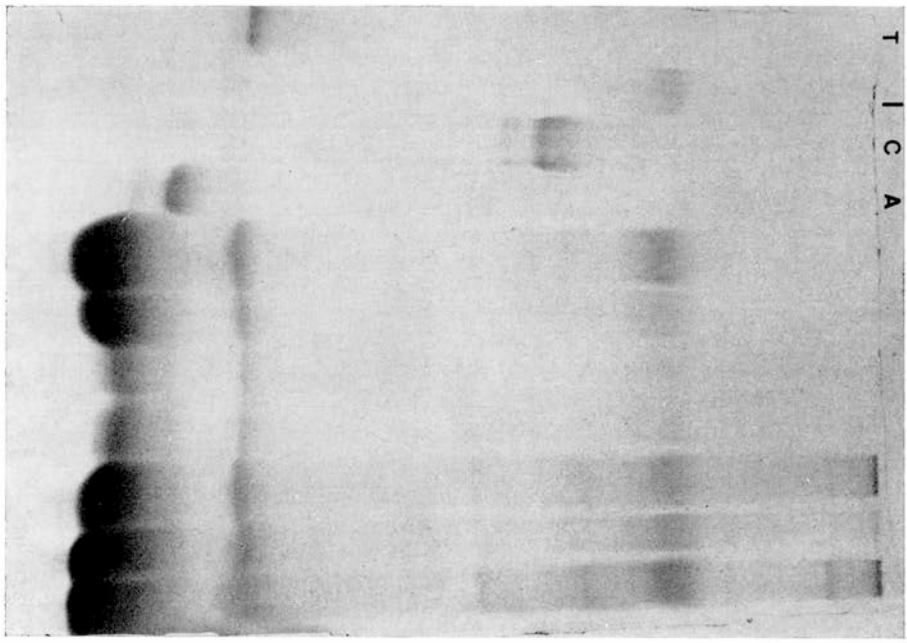

Fig. 1. — SDS-PAGE of amniotic fluids collected during the first trimester of normal pregnacies. (The markers used are shown on the left). T = Transferrin; I = Immunoglobulins; C = Ceruloplasmin; A = Bovine Serum Albumin.

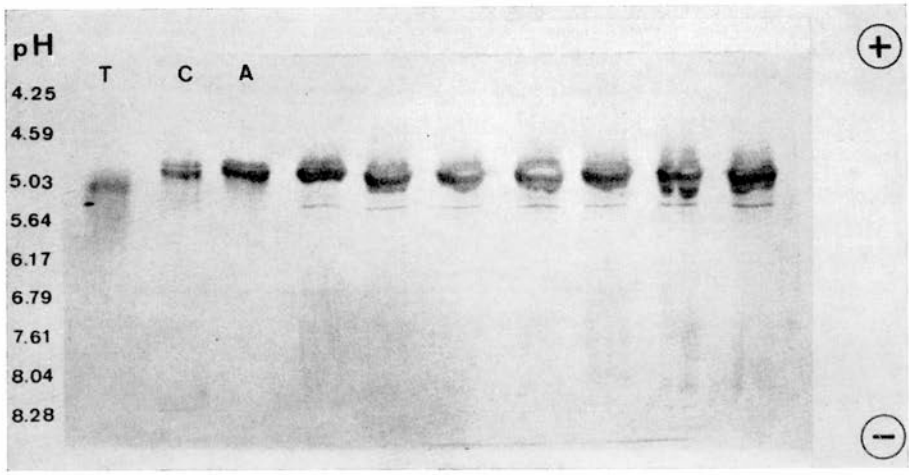

Fig. 2. — SDS-PAGE of amniotic fluids collected during the third trimester of normal pregnancies. (Abbreviations as in the legend of figure 1).

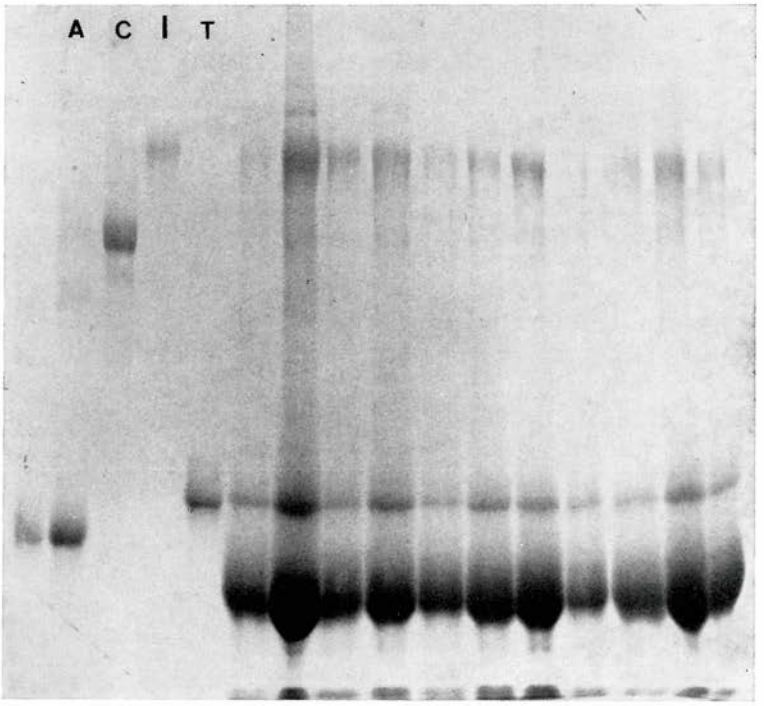

Fig. 3. — IEF of amniotic fluids collected during the first trimester of normal pregnancies. (Abbreviations as in the legend of figure 1).

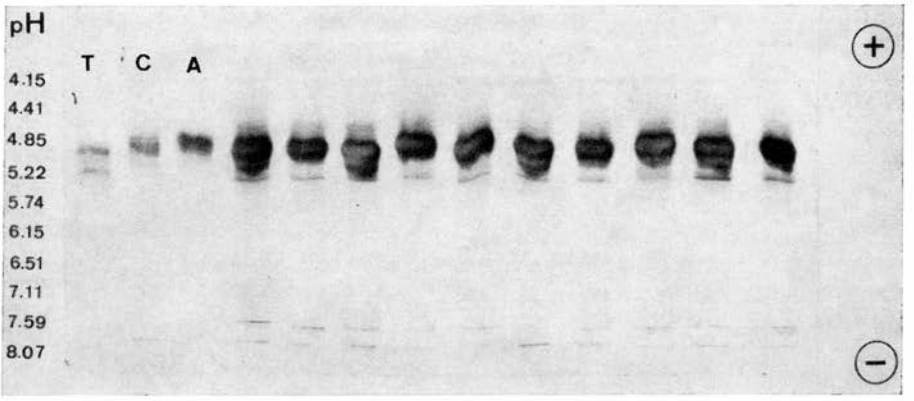

Fig. 4. — IEF of amniotic fluids collected during the third trimester of normal pregnancies. (Abbreviations as in the legend of figure 1).

nique unsuitable for a careful analysis of changes in protein composition of A.F. during pregnancy. Interestingly enough, a band with molecular weight lower than albumin is more evident in the third trimester samples than in A.F. becomes more abundant late pregnancy. However the significance of such a small change in the amount of this component is presently very difficult to assess.

few bands (two to four) were exclusively detected in A.F. samples obtained during the third trimester (fig. 4). These bands are completely lacking in the corresponding region of the gels of A.F. from early pregnancies (fig. 2).

A better separation of these bands was achieved by using gels with pH ranging from 4.5 and 7 (fig. 5).

These bands do not seem to correspond

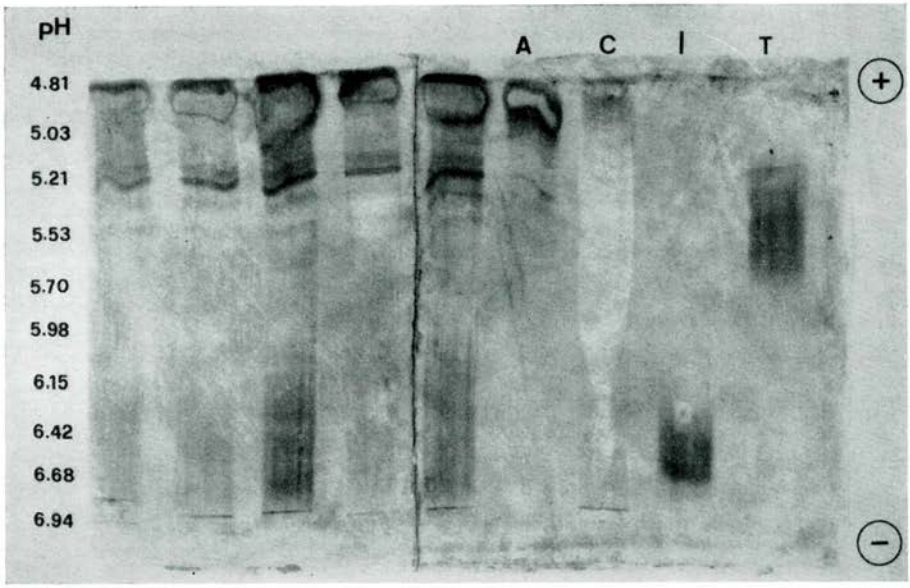

Fig. 5. — IEF of amniotic fluids collected during the third trimester of normal pregnancies; pH range of this gel was 4.5-7. (Abbreviations as in the legend of figure 1).

The results obtained by means of IEF are shown in fig. 2 and 4. It is evident that albumin, ceruloplasmin and transferrin do not separate well within the pH range used (3.5-9). Since these components have not been shown to undergo any modification during pregnancy as detected in SDS-gels, the lack of separation in IEF does not represent a major limitation of the method. Indeed the informations obtained by IEF appear to be of particular interest for other reasons. In the pH region between 5.20 and 5.98 a

to any of the proteins detected in our SDS-PAGE system and cannot be identified, on the basis of the isoelectric point of the markers used, with the major components usually described in A.F.

Previous studies (Pantarotto *et al.*, 1973; Casu *et al.*, 1975) have shown a possible relationship between fetal pulmonary maturation and the appearance in the A.F. of a low molecular weight lipoprotein fraction (studies done by means of disc-electrophoresis on poliacrylamide gel).

Gas-chromatographic analysis of fatty acids and evaluation of the palmitic/stearic acid (P/S) ratio ([3]) has been performed in all the A.F. samples investigated in the present study, and the bands uniquely observed in A.F. of third trimester cases present only in cases where the P/S ratio indicated fetal lung maturation.

Therefore it is possible that the previously described lipoprotein related to pulmonary maturation corresponds to one of the bands detected with IEF. Furthermore we cannot exclude the possibility that the single band observed with SDS-PAGE electrophoresis may be made up of several proteins corresponding to the bands detected with IEF and having the same molecular weight but differing in their isoelectric points, possibly due to the presence of different lipid polar heads ([10]).

The results suggest that IEF analysis seems to have a selective advantage in allowing the separation of bands which can not be easily recognized with SDS electrophoresis. These bands detected by IEF and present in A.F. during late pregnancy seem to be related to the previously described lipoproteins ([2, 8]) and we suggest that they might be used as a possible marker for monitoring fetal lung maturation.

Recent reports ([10]) suggest the importance of IEF in separating immunoglobulin fractions in spinal fluid ([7, 14]) during chronic inflammatory neurological disease. It would be of great interest to evaluate the usefulness of this procedure in examining A.F. obtained during pregnancies complicated by infections.

BIBLIOGRAPHY

1) Botta G. A., Park J. T.: *J. Bact., 145*, 333, 1981.
2) Casu A., Grimaldi M., Monacelli R., Pantarotto M. F., Vicino P., Pecorari D.: *Pathologica, 67*, 395, 1975.
3) Diani F., Pecorari D.: *Biol. Res. Preg., 1*, 90, 1980.
4) Fischbacher P. H., Quinlivan W. L.: *Am. J. Obst. Gyn., 108*, 1051, 1970.
5) Heron H. J.: *J. Obst. Gyn. Brit. Cwlth., 73*, 91, 1966.
6) Josimovich J. B.: *The role of placenta in the synthesis and metabolism of proteins.* In: "Diagnosis and Treatment of Fetal Disorders", Adamsons (ed.), Springer, New York, p. 102, 1967.
7) Kjellin K. G., Siden A.: *Eur. Neurol., 15*, 40, 1977.
8) Pantarotto M. F., Casu A., Monacelli R., Pala V., Pecorari D.: *Pathologica, 65*, 943, 1973.
9) Queenan J. T.: *Amniotic fluid proteins, amniotic fluid aminoacids and their clinical significance.* In: "Amniotic Fluid. Research and Clinical Application", Fairweather D. V. I., Eskes T. K. A. B. (eds.), Excerpta Medica, Amsterdam, p. 187, 1978.
10) Righetti P. G., Drysdale J. W.: *Isoelectric focusing.* In: "Laboratory Techniques in Biochemistry and Molecular Biology", Work T. S., Work E. (eds.), Publ. Co., North Holland, 1979.
11) Shapiro A. L., Vinuela E., Maizel J. V.: *Res. Commun., 28*, 815, 1967.
12) Schulman J. D., Queenan J. T., Doores L.: *Am. J. Obst. Gyn., 114*, 243, 1972.
13) Schulman J. D., Queenan J. T., Doores L., Holland B.: *Obst. Gyn.*, 1972 (in press.).
14) Siden A., Kjellin K. G.: *J. Neur. Sci., 42*, 153, 1979.
15) Usategui-Gomez M., Morgan F. D., Toolan H. W.: *Proc. Soc. Exp. Biol., 123*, 547, 1966.
16) Viergiver E., Stroup P. E., Sheff M. F., Westphal M.: *Obst. Gyn., 19*, 664, 1963.
17) Waddel W. J.: *J. Lab. Clin. Med., 48*, 311, 1956.