

# OBSERVATIONS ON PROTEIN CONTENT OF LIQUOR AMNII AND THAT OF MATERNAL AND FOETAL SERUM IN SELECTED HEALTHY MOTHERS

by

H. B. SINHA,\* M.S. (Pat.), F.R.C.O.G.

D. K. SEN,\*\* M.B.B.S. (Pat.), D.T.M. (Cal.), D. Path. (Lond.), M.C. Path.

and

A. K. MUKHERJI,\*\*\* M.B.B.S. (Cal.), M.S. (Pat.).

Liquor amnii constitutes the vital intra-uterine medium in which the foetus grows. It protects the foetus from external injuries, maintains even temperature and humidity, allows enough space for free foetal movement, and supplies some amount of nutrient substances in the form of protein, carbohydrate and amino-acids. Zangemeister and Meissl (1903), and Makepeace *et al* (1931), considered liquor amnii as a serum dialysate mixed with foetal urine. Palliez (1956) by paper electrophoresis study concluded that liquor amnii was not a serum dialysate but the product of secretion of amniotic epithelium. Barbanti (1956), McKay, Richardson, and Hertig (1958), and Abbas and Tovey (1960) from paper electrophoresis came to the conclu-

sion that the proteins of amniotic fluid arose by filtration of maternal serum through semipermeable membrane. Derrington and Soothill (1961), by immuno-chemical study of the proteins, found that the pattern of protein constituents of liquor amnii resembled maternal serum more closely than the foetal serum. But Mentasti (1959), by using method of free electrophoresis, concluded that protein pattern of amniotic fluid bore more resemblance to foetal serum than to maternal serum. Brzezinski *et al* (1961) were also of the opinion that foetal circulation supplies proteins to liquor amnii. In the present work the protein constituents of liquor amnii were studied against similar study of maternal and foetal blood to see their reflections on the amniotic fluid.

\*Prof. & Head, Depart. of Obsterics & Gynaecology, P. W. Medical College, Patna.

\*\*In-Charge, Research Unit, Post-graduate Department of Paediatrics, Patna University.

\*\*\*Resident Surgeon, Hospital for Women, Patna.

Received for publication on 11-1-67.

## Material and Method

Ten healthy pregnant women admitted to Unit II of Hospital for Women, Patna, who had been under regular antenatal care, were selected for study.

Liquor amnii was collected in five cases by direct puncture of lower

uterine segment during laparotomy done for caesarean section. In five other cases collection was done during the first stage of labour with a polythene tube of 1 mm bore passed through a Drew-Smythe catheter in place of the stylet. The catheter was introduced above and behind the presenting part and the polythene tube was steadily pushed in, whence the tip of the tube shaped like a goose-quill punctured the hind bag of waters. The mid-stream clear sample of liquor amnii draining through the polythene tube was collected. Sample of maternal blood was obtained by cubital vein puncture. Foetal blood was collected from cord stump attached to the foetus immediately after it had been separated from the mother.

Investigations included haemoglobin estimation, total red cell count and haematocrit readings of maternal and foetal blood. Total protein and albumin—globulin ratio of the maternal and foetal serum and amniotic fluid, were determined by biuret method. Electrophoresis of maternal serum, foetal serum, and amniotic fluid were done on Whatman No. 1, filter paper strips in horizontal bath, with barbitone buffer at pH 6.8, ionic strength 0.1. Current used was 0.4 milli./amp per centimetre width of the paper. Strips were dried and stained with 1 per cent acidified Light-green and differentiated with 5 per cent acetic acid solution. Urea and creatinine determinations were done by Urease-Nesslerisation technique and by Jaffe reaction with alkaline picrate solution respectively. All colorimetric estimations were calibrated against 'Lab-trol' Lyophilised

Assay Serum standard. The quantitative estimation of serum proteins was done by biuret method as it was thought to give a more accurate result than the electrophoretic technique as such (Varley 1963). Filter paper electrophoresis was taken recourse to determine the qualitative pattern of different protein fractions as compared against known normal samples. Specific gravity determination was done in four cases with urinometer.

#### Observations

The cases selected for study were healthy pregnant women between 20 and 30 years of age. There were one primigravida, two second gravidae, three third gravidae, two fourth gravidae, one sixth gravida, and one eighth gravida in the series. Five cases were forty weeks pregnant, two cases forty-one weeks, one each forty-three and forty-four weeks. One case was terminated at thirty-four weeks pregnancy by caesarean section due to central placenta praevia and the baby weighed four pounds ten ounces. All the babies were live-born and their birth weight varied from five pounds ten ounces to seven pounds twelve ounces. Cases delivered vaginally were within normal limits of duration of labour except one primigravida whose labour lasted for 27 hours 5 minutes.

The average haemoglobin content, total red cell count and haematocrit values of maternal and foetal blood are shown in Table I.

The mothers were of good nutritional status, taking a non-vegetarian diet and were under regular antenatal supervision. The average serum pro-

TABLE I  
Showing Average Maternal and Foetal Blood Picture

	Haemoglobin Grams per cent	Total R.B.C. mille per c mm	Haematocrit per cent
Maternal blood	11.38	4.05	33.5
Foetal blood	16.48	5.72	50.37

tein content of these cases was 7.22 g per cent. When compared with findings of Menon (1965) and Kishore *et al* (1965), it is evident that the average serum protein content is higher in the present series. Moller-Christensen *et al* (1946), in a similar study, found that the serum protein content of healthy pregnant women varied from 5.57 to 7.04 gm. per cent with an average haematocrit value of 34 per cent. In the present series the protein content varied from 6.84 to 7.72 gm. per cent with an average haematocrit of 33.5 per cent (Table — II). Although pregnancy alters the total serum protein level with marked reduction of albumin fraction there is wide range of variation within normal limits much of which depends upon nutritional

status, dietary habits, nature of pregnancy, method of estimation, and perhaps many other factors yet to be investigated.

The total serum protein content in foetus is also subject to variation as stated by Silver *et al* (1963) and observed by Prasad *et al* (1965). It is seen in Table III that the average total serum protein in our series is about 1 gm per cent higher than the average figure obtained by Prasad *et al* (1965). While the cases selected for the present study were babies delivered by healthy pregnant women, there was no such selection in the series of Prasad *et al* (1965).

Proteins play an important role in the regulation of body fluids apart from their other vital functions. Total protein estimation and specific gravity

TABLE II  
Showing the Protein content of Serum and Average Haematocrit Reading of Mothers

Author	Miller-Christensen et-al (1946)	Menon (1965)	Kishore et al (1965)	Present series
Method of estimation	Chemical Thygesen et al (1946)	Electro- phoresis	Electro- phoresis	Chemical Biuret
Total protein gm. per cent	5.57 - 7.05	6.46 (average)	5.20 - 7.12	6.84 - 7.72
Albumin-globulin ratio	1.27 - 2.12	—	0.436 - 1.12	1.45 - 2.12
Haematocrit per cent	34	—	—	33.5

TABLE III  
Showing Serum Protein Content of Foetal Blood

Author	Silver et al (1963)	Prasad et al (1965)	Present series
Range of total protein Gm. p.c. .. ..	4.6 - 7.4	4.8 - 8.2	6.4 - 8.2
Average protein Gm. p.c. .. ..	—	6.5	7.4
Albumin-globulin ratio .. ..	—	2.1 - 1.82	2.5 - 1.9

determination are the two important criteria for analysis of any body fluid in health or disease. The total protein content of liquor amnii varied from 0.184 to 0.24 gm. per cent. Bonsnes (1966) reported the protein content of liquor amnii from world literature which is compared with the present series in Table IV.

(Table V). If foetal blood contributes to the formation of liquor amnii wholly or in part, the vascular plexus at the placental site can be compared with the choroid plexus of brain secreting the cerebrospinal fluid.

Vasicka (1966) studied the oxygen concentration in amniotic fluid and cerebrospinal fluid by polarographic

TABLE IV  
Showing Range of Protein Content of Liquor Amnii

Author	Bonsnes (1966)	Present series
Range of protein content Gm./litre	1.60 — 3.50	1.84 — 2.4

The specific gravity in our series varied from 1012 to 1022. According to Wright's Physiology (1965) the specific gravity of liquor amnii varies from 1007 to 1025. When compared with cerebrospinal fluid it is seen that the amniotic fluid is richer in protein and has a higher specific gravity

method, and found that just as an increased oxygen tension in the cerebrospinal fluid causes spasm of the cerebral vessels, so also an increased oxygen tension in amniotic fluid caused spasm of the placental vessels

In the present series 75 to 80 per cent of the amniotic fluid protein

TABLE V  
Showing Comparison of Protein Content and Specific Gravity of Liquor Amnii and Cerebrospinal Fluid

	Total Protein Gm. p.c.	Specific Gravity
Cerebrospinal fluid (Wright's Applied Physiology-1965)	0.02 — 0.035	1006
Amniotic fluid (present series)	0.184 — 0.24	1012 — 1022

was found to consist of albumin; and similar is the case with cerebrospinal fluid. Figure 1, illustrates the preponderance of albumin in liquor amnii with a trace of globulin in contrast to maternal and foetal serum in

concentration in maternal or foetal blood, the concentration of urea and creatinine are within their normal limits in the blood of the healthy pregnant women or the newborn foetuses as shown in Table VI.

TABLE VI

Showing Urea and Creatinine Contents of Blood of Pregnant Women, Foetuses, and Liquor Amnii

	Urea mg. p.c.	Creatinine) mg. p.c.
Blood of pregnant women	18.62 (calculated from blood urea nitrogen—figure by Sims & Krantz (1958))	0.46 (Sims & Krantz 1958)
Blood of foetus	10.7—32.1 (Silver et al 1963)	0.3—2.0 (Silver et al 1963)
Aminotic fluid	16.8—27 (present series)	0.56—1.2 (present series)

electrophoretogram of one of the cases.

The factors guiding the protein content of amniotic fluid are not known. No correlation could be found between total amniotic fluid protein and maternal or foetal serum protein levels. But whenever there was a rise of total protein in amniotic fluid, the urea and creatinine contents also increased as illustrated graphically in Fig. 2.

The probable source of urea and creatinine is the foetal urine which is diluted in a much larger volume of liquor amnii. As stated by Silver *et al* (1963) the new-born excretes 15 to 500 mg. of urea and 7 to 10 mg. creatinine per kilo body weight per day. Although the protein content of liquor amnii is but a fraction of its

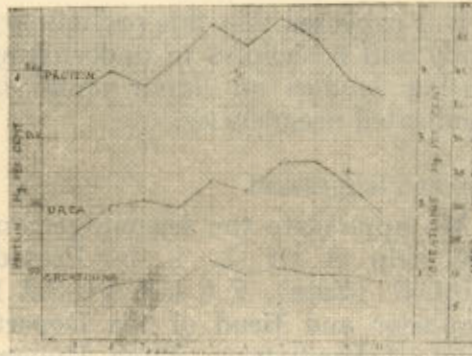


Fig. 2

Showing correlation between total protein, urea and creatinine contents of liquor amnii.

From such finding it is tempting to surmise that these excretory products are in equilibrium with the maternal or foetal blood.

*Summary*

An analytical study of protein content of maternal and foetal serum, and liquor amnii of identical cases was made in ten healthy pregnant women. The average protein content of maternal and foetal serum and of liquor amnii were 7.22, 7.43, and 0.207 gm. per cent respectively. Electrophoretograms of maternal serum, foetal serum, and liquor amnii showed an abundance of albumin in amniotic fluid with a trace of globulin. No direct relationship could be found between the protein content of liquor amnii and that of maternal or foetal serum.

Simultaneous estimations of urea and creatinine in liquor amnii were also done and on an average found to be 22.1 mg. and 0.94 mg. per cent respectively. They have been observed to be directly related to the protein content of liquor amnii.

It is expected that this preliminary study will be helpful in undertaking further studies on liquor amnii in complicated pregnancies.

*Acknowledgement*

We appreciate the encouragement and help of Dr. L. S. N. Prasad, F.R.C.P. (Edin), F.A.A.P. (U.S.A.), Professor and Head of the Department of Paediatrics, Patna University, in providing access to UNICEF AIDED RESEARCH LABORATORY of his department without which it would not have been possible to do this small piece of work.

*References*

1. Abbas, T. M. and Tovey, J. E.: *Brit. Med. J.* 1: 476, 1960.
2. Barbanti, A.: *Minerva Gynec. Torino.* 8: 708, 1956.
3. Bonsnes, R. W.: *Clin. Obst. & Gynec.* 9: 440, 1966.
4. Brzezinski, A., Sadovsky, E. and Shafir, E.: *Am. J. Obst. & Gynec.* 82: 800, 1961.
5. Derrington, M. M. and Soothill, J. F.: *J. Obst. & Gynec. Brit. Comm.* 68: 755, 1961.
6. Kishore, N. and Gupta, K.: *J. Obst. & Gynec. India.* 15: 1, 1965.
7. Krishna Menon, M. K.: *J. Obst. & Gynec. India.* 15: 127, 1965.
8. Makepeace, A. W., Fremont-Smith, F., Dailey, M. E. and Carroll, M. P.: *Surg. Gynec. & Obst.* 53: 635, 1931.
9. Mc Kay, D. G., Richardson, M. V. and Hertif, A. T.: *Am. J. Obst. & Gynec.* 75: 699, 1958.
10. Mentasti, P.: *Minerva Gynec. Torino.* 11: 547, 1959.
11. Moller, Christensen, E. & Thygesen, J. E.: *J. Obst. & Gynec. Brit. Emp.* 53: 328, 1946.
12. Palliez, R., Biserte, G., Savařy, J. and Montreuil, J.: *Bull. Soc. Belg. Gynec. & Obst.* 26: 446, 1956.
13. Prasad, L. S. N., Sinha, K. P. and Sen, D. K.: "A Study On Maternal & Foetal Health And Its Relation To Neonatal Morbidity And Mortality" Paper presented at the XI International Congress of Paediatrics, Tokyo, 1965.
14. Silver, H. K., Kemp, C. H. and Bruyn, H. B.: *Hand Book Of Paediatrics*, ed. 5, California, 1963, Large Medical Publications, Los Altos.
15. Sims, E. A. H. and Krantz, K. F.: *J. Clin. Invest.* 37: 1764, 1958.
16. Vasicka, A.: *Clin. Obst. Gynec.* 461, 1966.

- 17. Varley, H., Practical Clinical Biochemistry, ed. 3, New York, 1963, William Heinemann Medical Books Ltd. & Interscience Books Inc.
- 18. Wright's Applied Physiology, Ed. by Cyril A. Keele & Eric, Neil,
- 19. Zangemeister, W. and Meissl, J.: Munch. Med. Wschr. 1: 673, 1903.(x)

Note: 'x' indicates cross references.

Fig. on Art Paper I

J. Am. Med. Assoc. 1965, 193: 1000

severity of anemia—blood haemoglobin estimation, peripheral blood smear, leucocyte tube average, bone marrow smear, serum iron level, serum protein level, estimation of mean corpuscular haemoglobin, estimation of mean corpuscular haemoglobin concentration, reticulocyte count, and routine examination of stool and urine.

On the basis of the above investigations the patients of severe chronic anemia were treated with the total dose of iron as determined by computer and those of moderate anemia were treated with the dose supplemented by oral iron preparation according to the severity of the condition. 100% response was however, given as a routine to all patients.

The total dosage of iron dextran complex was calculated according to the following formula:

$$0.1 \times W \times X (100 - H) = \text{mg. of iron}$$

(W stands for weight of the patient in lbs.)  
 Precautions were taken before the infusion in order to avoid reaction and leakage. All allergic drugs were

Anemia is one of the commonest complications encountered in women in our part of the world. It is still a major problem and takes a heavy toll of material and social life. The treatment of mild and moderate anemia is not difficult but the real problem is to treat severe anemia of pregnancy. Such anemic patients die during or a few days after labour. A rapid and successful treatment has to be instituted to avoid the accident during this period. Attempts have been made in recent years to treat such patients by intravenous iron or exchange transfusion of packed blood cells. An assessment of the treatment of severe anemia with intravenous iron and dextran complex (Infusiron) has been made in this paper. Patients of cardiovascular heart failure and of kidney and liver damage were excluded.

The following investigations were carried out in the anemic patients in order to ascertain the type and

From the Laboratory Department of Obstetrics & Gynaecology, Datta Medical College, Bombay  
 Dr. P. W. Medical College, India  
 Received for publication on 22-1-65