

**OBSERVATIONS ON THE PROTEIN CONTENT OF THE LIQUOR  
AMNII IN NORMAL PREGNANCY, PRE-ECLAMPTIC TOXAEMIA  
AND ECLAMPSIA**

by

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The protein content of the liquor amnii has been investigated more extensively than any other single constituent of this fluid and the results obtained vary widely (Prochownick, 1877; Zangemeister and Meissl, 1903; Makepeace *et al*, 1931; Cantarow *et al*, 1933; Shrewsbury, 1933; Bevis, 1953; Abbas and Tovey, 1960; Derrington and Soothill, 1961; Sozanskii, 1961; Stander, *et al*, 1964; Heron 1966; Sinha *et al*, 1967; Crosignani and Polvani, 1969). This variation might be due to the condition under which the fluid was collected, the method applied for the estimation of protein or this may be the physiological character of this fluid. The liquor amnii also contains moderate amount of mucoprotein (Bevis, 1963) and it is likely that the mucoprotein might also be included when the estimation was done by biuret method (Winzler *et al*, 1948), leading to higher results. The present work is an observation on the changes in the total protein content and its fractions in the amniotic fluid in pre-eclamptic toxæmia and eclampsia compared with normal pregnancy. An attempt has also

been made to elucidate the factors responsible for the wide variation in the results.

*Method of study*

One hundred and twenty three women between 38 to 41 weeks of pregnancy were included in this study. The cases were randomly selected from the labour room and were of the following types:

(i) Normal pregnancy (35 cases). Blood pressure upto or below 120/80 mmHg. without oedema, proteinuria, Rh-incompatibility, diabetes mellitus or systemic diseases complicating pregnancy.

(ii) Mild and moderate pre-eclamptic toxæmia (29 cases): Blood pressure upto or below 160/110 mmHg. with oedema and/or albuminuria. In 7 of the cases the upper limit of blood pressure was 140/90 mmHg. with detectable oedema and proteinuria which were grouped as mild pre-eclamptic toxæmia.

(iii) Severe pre-eclamptic toxæmia (26 cases): Blood pressure above 160/110 mmHg. with marked oedema and/or proteinuria.

(iv) Eclampsia (33 cases). All were emergency admissions and had many fits before being hospitalised.

The liquor amnii was collected by the following methods taking care to avoid contamination with blood.

(i) From the hind waters, using a

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Drew-Smythe catheter as described in a previous communication (Sinha, Sen and Mukerjee (1967).

(ii) By aspiration of the fore waters under vision, using a dry syringe and thick-bore needle.

(iii) Transuterine amniocentesis during laparotomy for caesarean section.

(iv) Trans-abdominal amniocentesis.

Protein estimation of the fluid was done by turbidimetric method using 3 per cent Salicyl Sulphonic acid at 450 millimicrons in a Junior Coleman Spectrophotometer, using a suitable blank for

next application (Smith, 1965). The relative proportion of the protein fractions were estimated by elution of the bromophenol blue-stained paper strips in 0.01 N sodium hydroxide for 30 minutes and reading the amount of the dye so extracted in spectrophotometer at 590 millimicrons (Block, Durrum and Zweig, 1964; Varley, 1967).

*Results*

The mean difference between the results of protein estimation by biuret method and the mucoprotein content in 27 cases is shown in Table I. The same

TABLE I  
*Difference in Protein Content of A.F. Due to the Application of Different Methods*

I. Mean difference between Protein content of A.F. by biuret method and mucoprotein content (27 cases) (Mg/100 ml.)	II. Mean protein content of those 27 cases by turbidimetric method (Mg/100 ml.)	t-value of the difference between I and II (df = 2)
235 mg. (S.D.=105.41; S.E.=20.34)	219 mg. (S.E.=94.63; S.E.=18.25)	0.58 Statistically insignificant

correction of the increased optical density due to the normal turbidity of this fluid (Wootton, 1964). In 27 cases the protein estimation was also done by the biuret method (Varley, 1969). Estimation of the mucoprotein content of these samples were done by the methods of Winzler, Devor, Mehl and Smyth (1948) and Varley (1967). The albumin and globulin contents of the liquor amnii were separated by paper electrophoresis. Because there was no arrangement for concentrating the liquor amnii which had a low protein content, at least 6 applications of 10 microlitres each were made at the selected site on the paper strip, every time drying it at room temperature before

table also indicates the mean protein content of the same samples of amniotic fluid as observed by using the turbidimetric method with 3 per cent Salicyl Sulphonic acid.

Table II shows the total protein content in normal pregnancy, pre-eclamptic toxæmia and eclampsia and the statistical significance of the difference of the mean values between them. The percentage of albumin and globulin, and albumin-globulin ratio are shown in Table III.

Table IV indicates the statistical significance of the difference in albumin-globulin ratio in normal pregnancy, pre-eclamptic toxæmia and eclampsia.

TABLE II  
Comparison of the Mean Protein Content (mg. per 100 ml.) of the Amniotic Fluid in Normal Pregnancy as Against Different Degrees of Toxaemias as well as Between Increasing Degrees of Toxaemia

Groups of cases	No. of cases	Mean	S.D.	S.E.	t	df	P-value
Normal pregnancy	25	136	43.38	8.67	3.64	51	Less than 0.001
Mild and mod. P.E.T.	28	185	53.83	10.30			
Normal pregnancy	25	136	43.38	8.67	3.82	49	Less than 0.001
Severe P.E.T.	26	186	50.28	9.80			
Normal pregnancy	25	136	43.38	8.67	8.06	55	Less than 0.001
Eclampsia	32	212	21.26	3.7			
Mild and mod. P.E.T.	28	185	53.83	10.30	0.07	52	Not significant
Severe P.E.T.	26	186	50.28	9.80			
Severe P.E.T.	26	186	50.28	9.80	2.57	56	Less than 0.05
Eclampsia	32	212	21.26	3.7			

P. E. T. Pre-eclamptic toxaemia.

TABLE III  
Mean Percentage of Albumin and Globulin and Mean A/G Ratio of Liquor Amnii in Normal Pregnancy Pre eclamptic Toxaemia and Eclampsia

Cases	N	Albumin			Globulin			A/G Ratio		
		Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
Normal pregnancy	25	68	3.80	0.76	32	3.85	0.77	2.19	0.61	0.12
Mild and Mod. P.E.T.	20	67.64	2.62	0.59	32.36	2.24	0.50	2.09	0.32	0.07
Severe P.E.T.	19	66.94	3.16	0.41	33.06	3.00	0.42	2.04	0.31	0.07
Eclampsia	16	67.29	2.64	0.80	32.71	4.28	0.72	2.00	0.59	0.14

P. E. T. Pre-eclamptic toxaemia.

TABLE IV

*Comparison of the Mean Albumin-Globulin Ratio of the Amniotic Fluid Between Normal and Toxaemic Pregnancies and Between Different Degrees of Toxaemia*

Groups of cases	No. of cases	Mean	S.D.	S.E.	t	df	P-value
Normal Pregnancy	25	2.19	0.61	0.12	0.71	43	Not significant
Mild & Mod. P.E.T.	20	2.09	0.32	0.07			
Normal pregnancy	25	2.19	0.61	0.12	1.07	42	Not significant
Severe P.E.T.	19	2.04	0.31	0.07			
Normal pregnancy	25	2.19	0.61	0.12	1.05	39	Not significant
Eclampsia	16	2.00	0.59	0.14			
Mild and Mod. P.E.T.	20	2.09	0.32	0.07	0.55	37	Not significant
Severe P.E.T.	19	2.04	0.31	0.07			
Severe P. E. T.	19	2.04	0.31	0.07	0.26	33	Not significant
Eclampsia	16	2.00	0.59	0.14			

Table V shows the birth weight of the newborn in normal pregnancy, pre-eclamptic toxaemia and eclampsia and statistical significance of the difference of the birth weight in different degrees of toxaemia. The product moment correla-

tion co-efficient between the birth weight and protein content in individual cases was calculated and the inverse correlation between the two variables are shown in Table VI.

TABLE V

*Comparison of the Mean Birth Weight (kilograms) Between Normal Pregnancy and Different Degrees of Toxaemia*

Cases	No. of cases	Mean	S.D.	S.E.	t	df	P-value
Normal Pregnancy	35	3.00	0.50	0.08	—	—	—
Mild and Mod. P.E.T.	29	2.66	0.45	0.08	3.09	62	Less than 0.001
Severe P.E.T.	25	2.66	0.71	0.14	2.12	58	Less than 0.05
Eclampsia	20	2.64	0.63	0.15	2.25	53	Less than 0.05

TABLE VI

*Correlation Between the Birth Weight of the Newborn and the Protein of the Corresponding Amniotic Fluid, which is Statistically Significant*

Variables	No. of cases	r	t	df	P-value
Birth weight	97	-0.51	5.43	95	Less than 0.001
Protein content of amniotic fluid					

### Discussion

Protein in biological fluids is commonly estimated either by biuret method or by turbidimetric method. Table I shows that the total protein value of liquor amnii obtained by the biuret method, excluding the mucoprotein value, lies close to the value of total protein obtained by the turbidimetric method. The mean difference between them is statistically insignificant ( $t = 0.58$ ,  $df = 25$ ). Mucoprotein can be estimated by biuret reaction like other proteins (Winzler, *et al*, 1948). In the estimation of amniotic fluid protein by the biuret method, there is likelihood of mucoprotein also being estimated. Looney and Walsh (1939) and Mawson (1942), have expressed their views in favour of using salicyl sulphonic acid for the turbidimetric determination of protein. Further, Winzler *et al* (1948) demonstrated that in precipitating protein with salicyl sulphonic acid or perchloric acid most of the non-dialysable nitrogen is retained in the filtrate as mucoprotein.

The total protein of liquor amnii was raised in pre-eclamptic toxæmia and eclampsia compared with normal pregnancy (Table II). Similar results were also reported by Abbas and Tovey (1960) and Sozanskii (1961). Rise of mean protein content was not related with the severity of toxæmia, but a significant rise of mean protein content has been

observed in eclampsia compared with mild and moderate or severe degrees of pre-eclamptic toxæmia. The birth weight of the newborn in different degrees of toxæmia was significantly lower than in normal pregnancy (Table V). An inverse co-relation ( $r=0.51$ ,  $P<0.001$ ) between the protein content of the amniotic fluid and the birthweight was observed (Table IV, Fig. I). The placental ischaemia and dysfunction in toxæmia of pregnancy is responsible for the lowering of the birthweight and may be related with the higher protein content of liquor amnii. Similar observation was also reported by Queenan *et al* (1970).

Some difference in the relative proportion of albumin and globulin in amniotic fluid was observed in toxæmia cases compared with normal pregnancy (Table III). But the difference on statistical analysis was found to be insignificant (Table IV). However, Abbas and Tovey (1960) and Heron (1966) reported a diminution of the albumin content with relative rise of globulin in toxæmia of pregnancy, but the present study does not confirm the earlier observations.

### Summary

Although the total protein content of liquor amnii was higher in pre-eclamptic toxæmia and eclampsia compared with normal pregnancy, the albumin-globulin ratio did not show any significant change

in toxæmia. There was an inverse correlation between the birth weight and protein content of amniotic fluid in near-term cases. The inclusion of mucoprotein, while estimating total protein content, might be responsible for the wide variation of the results reported by various authors.

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