the vasoconstriction which it produced was greater in patients with essential hypertension than in normal controls. Their controls, however, were healthy young subjects whose relative lack of response may thus quite possibly have been due merely to greater dilution and washing out of the adrenaline by tears.

Conclusions

The vascular response of the conjunctiva to locally instilled priscol has been recorded in 297 diabetics.

Analysis shows a significant correlation between this response and the concentration of the drug, and also with sex and age.

There was no relationship between the response and the adjusted blood-pressure score, the duration of diabetes, or the presence of retinal micro-aneurysms.

This variation of the response with age and sex may be related to the rate of tear production, which, in normals, has been shown to be greater in females and to decrease with age.

The diagnostic, therapeutic, and experimental implications of these findings are considered.

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"It is this matter of 'communication' which lies at the root of the National Council of Social Service's work; and assumes a greater importance as the machinery of the Welfare State is elaborated and its philosophy is developed. For there seems to be opposition between these two things. Each year sees additions to the already complicated structure which the State is erecting for welfare, education, health, and so on; and with every new job, defined in terms of duties and responsibilities over against a pre-existing job, the problem of communication is increased by the erection of new invisible boundaries. Yet the philosophy which seems at the same time to guide all present-day social thinking is based on the need for coming together, for community responsibility, for bringing our social misfits out of their specialized institutions into the everyday world. The whole structure of the National Council was designed to make easier this communication between the national voluntary organizations among themselves and with the statutory bodies, both at the centre and, by means of local groups, throughout the country. Some of the ways in which the Council's departments and associated groups have, in their different but interdependent fields, worked towards that purpose during the past year are shown in this year's report." (40th Annual Report, National Council of Social Service.)

PROTEINS OF THE LIQUOR AMNII

BY

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Despite all the available information concerning the composition, origin, and fate of the liquor amnii, our knowledge with regard to this fluid remains incomplete. This communication presents an evaluation of the results obtained with paper electrophoresis of the protein pattern in the amniotic fluid, maternal serum, umbilical cord blood, and *in vitro* dialysates. The study was primarily undertaken for the purpose of obtaining further data on the possible source, nature, and function of the protein in the liquor amnii. It was also hoped that it might elucidate certain important and related clinical problems, such as the nutritional value of the swallowed liquor to the foetus and the possible diagnostic value of any detectable atypical electrophoretic pattern in abnormal pregnancies.

A review of the meagre literature on electrophoresis of the liquor (McCarthy and Kekwick, 1949; Bramwell *et al.*, 1953; Candiani, 1956; Wirtschafter and Williams, 1957a, 1957b; McKay *et al.*, 1958) shows that much of this work has been done on experimental animals. These studies have yielded some interesting information. For instance, in rats Wirtschafter and Williams (1957a, 1957b) have shown that in contrast to the maternal serum the albumin/globulin ratio in the liquor of these animals is subject to characteristic fluctuations. High albumin readings were noted at parturition in normal cases and a correspondingly high globulin value in abnormal ones. Brambell *et al.* (1953), on the other hand, reported that β - and γ -globulin in immune rabbit serum enter foetal circulation when such a serum is injected into the pregnant uterine lumen. The clinical significance of such findings, if observed in the human, could be of inestimable value, but the only two available studies on the liquor in man (Candiani, 1956; McKay *et al.*, 1958) have been controversial and not entirely in agreement with one another. In view of this, and because of the primary role of protein in foetal metabolism, it was felt that a further study in this field might prove instructive.

Material and Method

Samples of liquor amnii and venous blood were collected from 11 essentially normal patients who were surgically induced at 40 weeks plus five days. To avoid contamination all samples of amniotic fluid were withdrawn from the hind-water with a Drew Smythe catheter.

In seven additional cases enough maternal blood was removed to provide an adequate quantity of serum for *in vitro* dialysis against normal saline across their respective "foetal" membranes. The membranes utilized in the dialysis were in each case selected from an area about $\frac{1}{4}$ in. (1.3 cm.) beyond the placental edge. Care was also taken to ensure that each specimen included both amniotic and chorionic layers. The electrophoretic findings in the resultant saline dialysate

were in each case compared with that of the original liquor aspirated from the same patient.

Electrophoretic studies were also conducted on liquor collected from five cases of severe pre-eclampsia, three cases of anencephaly with hydramnios, two cases of hydramnios with no demonstrable underlying abnormality, one case of uniovular twins with hydramnios, and another case of Rh incompatibility with hydrops foetalis. In addition the protein-distribution curves of samples of cord blood removed at delivery from most of the cases in this study, including that from a baby with oesophageal atresia, were similarly analysed.

The electrophoretic technique adopted for this study entailed the use of the inverted V apparatus, as described by Flynn and de Mayo (1951), a barbitone buffer (0.515 g. of sodium diethylbarbitone, 0.092 g. of diethylbarbituric acid, and 0.415 g. of sodium caprylate made up to 100 ml. with distilled water), and Whatman No. 1 filter-paper. Each electrophoretic strip was run in the apparatus for 18 hours at 150 volts, then dried at room temperature and stained with azocarmine B (Gurr). These stained strips were interpreted by the reflecting photodensitometer (Latner *et al.*, 1954), and all recordings finally corrected for non-linearity between the amount of dye present and the amount of protein.

Prior to each electrophoretic run the total protein content of the liquor amnii was estimated by the method of Denis and Ayer (1920), and in order to obtain readable patterns each sample was concentrated by dialysis through a Viskap membrane against 25% solution of polyvinylpyrrolidone made up in electrophoretic buffer.

To ensure accuracy, all electrophoretic tests in this study were duplicated.

Results

The mean total protein content of the amniotic fluid in the 11 five-days-postmature but otherwise normal cases varied from 160 to 285 mg., averaging 210 mg./100 ml. These results are comparable with the findings

TABLE I.—Mean Values of Protein Fractions in Maternal Sera, Liquor Amnii, and Cord Blood Collected from 11 Normal Patients

	Alb.	α_1	α_2	β	γ	Total Protein (g./100 ml.)
Maternal serum	54.4 (± 4.5)	4.9 (± 0.7)	10.0 (± 1.4)	15.8 (± 1.7)	14.9 (± 3.6)	6.6
Liquor	68.6 (± 7.6)	6.2 (± 1.3)	4.6 (± 2.2)	11.7 (± 3.7)	8.9 (± 2.8)	0.21
Cord blood	67.7 (± 3.2)	3.0 (± 1.0)	5.7 (± 2.2)	7.3 (± 2.4)	16.3 (± 3.1)	5.4

Units=percentage of dye present. S.D. (n-1) given in parentheses.

TABLE II.—Comparison of the Mean Electrophoretic Results of Liquor Amnii and Dialysates in 7 Cases

	Alb.	α_1	α_2	β	γ
Liquor amnii	67.0 (± 5.1)	6.1 (± 1.3)	5.6 (± 1.9)	13.9 (± 1.8)	7.4 (± 2.0)
Dialysate	67.5 (± 5.2)	6.0 (± 1.3)	5.1 (± 1.9)	14.6 (± 2.8)	6.8 (± 1.8)
P	0.8	0.9	0.6	0.6	0.6

Units=percentage of dye present. S.D. (n-1) given in parentheses. P=probability (Fisher's "t" test).

TABLE III.—Electrophoretic Patterns Seen in the Liquor Amnii in Certain Types of Abnormal Pregnancies

	No. of Cases	Alb.	α_1	α_2	β	γ	Total Protein (g./100 ml.)
Normal pregnancy	11	68.6 (± 7.6)	6.2 (± 1.3)	4.6 (± 2.2)	11.7 (± 3.7)	8.9 (± 2.8)	0.21
P.E.T.	5	63.2	9.0	5.7	12.7	9.4	0.23
Anencephaly with hydramnios	3	71.4	6.4	3.4	11.1	7.7	0.4
Hydramnios (with no other abnormality)	2	66.6	6.8	4.8	13.7	8.1	0.31
Twins with hydramnios	1 (a)*	71.2	7.1	6.2	8.6	6.9	0.22
Rh-incompatibility. Hydrops foetalis	1 (b)	70.6	9.4	5.8	8.4	5.8	0.22
	1	69.3	3.4	6.0	11.5	9.8	0.3

Units=percentage of dye present. S.D. (n-1) given in parentheses. * From amniotic sac with hydramnios.

FIG. 1.—Electrophoretic protein pattern and distribution curves as seen in (A) maternal blood, (B) foetal cord blood, (C) liquor amnii, and (D) maternal serum dialysate.

of Zangemeister (1908) and Makepeace *et al.* (1931), but they fall short of the values obtained by Taussig (1927), Cantarow *et al.* (1933), and McKay *et al.* (1958).

Fig. 1, A shows that maternal serum protein can be electrophoretically separated into five major fractions, digressing on the test strip according to their mobility in the following order—albumin followed by α_1 -, α_2 -, β -, and γ -globulin. Representative examples of electrophoretic protein patterns, as seen in foetal blood, liquor amnii, and maternal serum dialysate are also shown in Fig. 1.

Table I demonstrates the mean values for protein fractions in maternal sera, liquor amnii, and cord blood collected from 11 normal parturients.

Table II represents the mean electrophoretic results of seven samples of liquor amnii and of their corresponding number of dialysates obtained from maternal sera across their respective foetal membranes. It is interesting to observe the close similarity of these two groups of figures.

Table III shows the mean protein values in the liquor amnii in the five cases of severe pre-eclampsia, three cases of anencephaly with hydramnios, two cases of hydramnios with no demonstrable underlying abnormality, one case of uniovular twins with hydramnios, and one case of Rh incompatibility with hydrops foetalis.

Discussion

The technique of paper electrophoresis has provided a new approach to the study of protein in different body

fluids. The present investigation was designed to utilize this procedure to supplement our knowledge on the protein of the human liquor. The initial findings disclosed certain interesting features which induced us to extend the study, and this in turn led to several attractive observations.

The first of these observations concerns the findings in the liquor of a protein pattern suggestive of a serum dialysate. This feature was repeatedly seen on comparing electrophoretic results of the liquor amnii with that of the corresponding maternal serum. Table IV illustrates

TABLE IV.—*Electrophoretic Results of Liquor Amnii and the Corresponding Maternal Serum*

	Alb.	α_1	α_2	β	γ
Maternal serum	54.4	5.8	12.4	16.3	11.1
Liquor amnii ..	65.5	6.8	6.1	14.2	7.4

Units=percentage of dye present.

a typical example. The "small" albumin molecule with a weight of 69,000 appeared in the liquor in a relative concentration of 65.5% against 54.4% in maternal serum, while α_2 globulin with the most consistently high molecular weight of 300,000 showed a relative concentration of 6.1% in the liquor as against 12.4% in maternal serum. Similarly, the relative values of α_1 -, β -, and γ -globulin in the two fluids represented a comparable relationship which was apparently determined by the "size," and therefore the ease with which the different protein molecules can traverse a barrier such as the trophoblastic membrane. To provide further evidence in support of this hypothesis we dialysed maternal sera across the corresponding foetal membranes, and in each case the resultant dialysate pattern was found to be similar to that seen in the original liquor. To obviate any possible secretory mechanism the membrane in each of the seven *in vitro* tests carried out for this purpose was placed in such a manner that the foetal surface was positioned next to maternal serum. Other subsequent experiments showed that it did not matter which way the membrane was placed between the two fluids.

The result of one of these experiments is given in full detail in Table V, demonstrating the close similarities between the protein pattern of the liquor amnii and that

TABLE V.—*Electrophoretic Protein Pattern in Maternal Serum, Liquor Amnii and Saline Dialysate of Maternal Serum Across Foetal Membranes*

	Alb.	α_1	α_2	β	γ
Serum	55.7	4.0	11.4	15.0	13.9
Liquor amnii ..	72.0	3.5	4.4	11.9	8.2
<i>In vitro</i> dialysate	73.5	3.7	3.8	11.2	7.8

Units=percentage of dye present.

seen in the corresponding *in vitro* saline dialysate. The albumin content of the liquor was 72% against 73.5% in the dialysate, and the values for α_1 -, α_2 -, β -, and γ -globulin were 3.5, 4.4, 11.9, and 8.2 as against 3.7, 3.8, 11.2, and 7.8 in the liquor and the dialysate, respectively. Minor differences in these figures are well within the errors of the electrophoretic method.

According to these results one may reasonably conclude that the protein of the liquor is derived from maternal blood across the whole of the "foetal" membranes, which lie virtually in direct contact with the whole of the vascular uterine mucosa.

The second interesting observation concerned the probable nutritional value to the foetus of the swallowed

protein of the liquor amnii, especially during the later weeks of intrauterine life, when the functional capacity of the foetal alimentary tract is adequate and the placental efficiency is on the wane.

The difficulties and limitations of any inquiry about intrauterine nutrition are self-evident, but two lines of investigation in this respect proved helpful: (1) the finding of a subnormal standard of birth weight in babies born with congenital oesophageal atresia as shown in a group of 47 cases illustrated in the scattergram (Fig. 2); and (2) the demonstration of a low γ -globulin value in the cord blood of two infants born with this deformity (Table VI). No doubt the interpretation of these findings is open to several possibilities, but the teleological significance appears to suggest that the ability of the foetus to swallow and assimilate liquor amnii can influence its birth weight and possibly its serum γ -globulin level. This latter speculation is of special interest when one considers that the transmission of antibodies in man can occur from maternal circulation to the foetus either through the placenta and the vascular

FIG. 2.—Scattergram of birth weights and maturity of 47 cases of oesophageal atresia plotted against a normal birth-weight curve (Gibson and McKeown, 1952).

route or via the chorion laeve into the amniotic fluid and foetal gut. Brambell *et al.* (1947) have shown that, in the rabbit, "transfer of antibodies occurs by way of uterine lumen into the amniotic fluid and foetal stomach and not via the placenta." They also reported (Bramwell *et al.*, 1951, 1954) that antibodies are absorbed in quantity from "the gut contents of newborn ruminants, horses, pigs, dogs, mice, and rats." It would be in keeping with these findings to record that the same route of transmission occurs in man.

The third observation is concerned with the evaluation of atypical electrophoretic liquor patterns and its

TABLE VI.—*Electrophoretic Record of Cord Blood in Two Cases of Oesophageal Atresia*

	alb.	α_1	α_2	β	γ	Total Protein (g.)
Oesophageal atresia (1)	73.8%	4.1	6.6	7.9	7.6	5.6
(2)	76.1	2.2	4.9	9.3	7.5	6.0
Normal record	67.7 (± 3.2)	3.0 (± 1.0)	5.7 (± 2.2)	7.3 (± 2.4)	16.3 (± 3.1)	5.4

Units=percentage of dye present. S.D. (n-1) given in parentheses.

possible diagnostic import. Despite the limited number of abnormal cases studied (Table III), the results show only minor variations from the normal which could be explained on the grounds of changes in maternal serum proteins; for instance, the low value of serum albumin in toxæmia may well be responsible for the correspondingly low albumin reading in the liquor, and this may also apply to the high α_1 -globulin levels in the liquor of these patients.

The significance of the slightly elevated total protein in the liquor in anencephaly, hydramnios, and hydrops foetalis, and its possible relationship to foetal swallowing, can only be a matter of conjecture. On the other hand, the findings of identical protein patterns in the two amniotic sacs of uniovular twins where hydramnios was limited to the upper of the two sacs, as confirmed on abdominal paracentesis, shows that there is more to it than foetal swallowing, and protein dialysis in the complex processes involved in the formation and elimination of all the constituents of the amniotic fluid.

Summary

Proteins present in maternal serum, umbilical-cord blood, liquor amnii, and dialysates from maternal serum across "foetal membranes" have been studied with the aid of paper electrophoresis.

The protein pattern seen in the liquor amnii has the characteristics of a simple protein dialysate, and can be reproduced *in vitro*.

Evidence is presented to suggest that the swallowed liquor amnii contributes to foetal nutrition and may well provide a source of γ -globulin and antibodies to the foetus.

Despite minor variations, no diagnostically significant changes were detected in the electrophoretic patterns of the liquor in toxæmia, anencephaly, twins, hydramnios, and hydrops foetalis.

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DEMONSTRABLE DEFICIENCY OF CHRISTMAS FACTOR IN TWO SISTERS

BY

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Evidence indicates that Christmas disease is inherited as a sex-linked recessive defect. This was suggested by one of the family trees in the first seven cases of Christmas disease reported by Biggs *et al.* (1952). Numerous family trees published since that time have confirmed this. Koller (1954), for example, demonstrated that four members of the famous Tenna family of bleeders in Switzerland, where the inheritance is of this pattern, are affected with Christmas disease.

In a genetic defect of this type the disease is transmitted by apparently normal carrier females to an affected male. All the daughters of an affected man are carriers. In the family of a carrier there is a 50% chance of a daughter being a carrier and of a son being affected. Biggs *et al.* (1952) noted a prolonged clotting-time and defective prothrombin consumption in the mother of one of their cases. Brinkhous *et al.* (1954) reported P.T.C. (Christmas factor) deficiency in a woman aged 49. Such cases suggested that the Christmas-disease gene might be less completely recessive than that producing haemophilia (Pitney and Dacie, 1955).

The object of the present paper is to record a demonstrable deficiency of Christmas factor in two sisters, the daughters of a man suffering from Christmas disease.

The Patients

Case 1 (IV, 2, see family tree).—A schoolgirl aged 13 had possibly excessive loss of blood at the onset of menstruation, but the periods thereafter were normal. On two occasions she had bled for 48 hours after dental extractions. Physical examination was negative. In hospital she had further dental extractions, with the insertion of acrylic resin splints, without any haemorrhage.

Family tree of the two cases studied. Case 1 was IV, 2 and Case 2 was IV, 1.

Case 2 (IV, 1).—A typist aged 16, she had not had any incidents to raise a suspicion of abnormal haemorrhage.