# *In vitro* Studies of Protein Transfer Across Human Fetal Membranes.* (32214)

M. USATEGUI-GOMEZ AND D. F. MORGAN (Introduced by H. W. Toolan)
*Putnam Memorial Hospital Institute for Medical Research, Bennington, Vermont*

In previous comparative studies of a number of individual proteins in human amniotic fluid, maternal serum and cord serum(1,2), it was found that 1) those proteins of the amniotic fluid which were investigated originated from maternal serum and 2) that selective ultrafiltration on the basis of molecular size could not explain the inhibition of the passage of haptoglobins and IgA into the amniotic fluid. The role of the fetal membranes in protein transport into the amniotic fluid thus became of import, *i.e.*, do the membranes act only as ultrafilters and selectively differentiate among protein molecules according to their molecular size alone or are the membranes able to select proteins for passage not only according to size, but also on the basis of chemical structure of the molecules themselves? To attempt an answer to this question, an *in vitro* experimental system was devised. Maternal sera were dialyzed *in vitro* against normal saline across one or both of the fetal membranes, and the dialyzates were then examined in order to quantitate as many individual proteins as possible. Disc electrophoresis and quantitative immunodiffusion were employed.

*Materials and methods.* Placentas were obtained from the delivery room within one hour after birth. From each placenta 2 sections of chorion and 2 sections of amnion were selected from an area 2 inches beyond the cord. The amnion was stripped carefully from the placenta, and the chorion subsequently removed and gently cleaned. Both membranes were washed twice or more with normal saline until the rinsing fluid was clear and the membranes then examined for holes before use. Membrane sections were placed over the 2 cm diameter neck of 5 ml bottles (Fig. 1). One of the bottles was filled with 0.15 M NaCl and the membrane held in place with a rubber band in such a way that the fetal side of the membrane was in contact with the saline solution. Another 5 ml bottle filled with maternal sera, was held tightly in place on the maternal side of the membrane with parafilm. The sera were then dialyzed across the membranes for 24 hours in a 37°C incubator where the bottles were placed on their sides. Since each experiment required 20 ml of serum in order that there would be duplicates of both the amniotic and the chorionic membrane sections, sera from 4 pregnant women of the same haptoglobin type were pooled for each placenta used. Maternal serum was drawn within 24 hours after delivery, haptoglobin-typed by acrylamide gel electrophoresis, and stored at —20°C until use. Experiments utilizing 10 placentas were set up as described. Five additional experiments were run in each of which sera were dialyzed across one amniotic membrane, one chorionic membrane and the two membranes together from the same placenta.

For both disc electrophoresis and immunodiffusion studies, the dialyzates were concentrated, by evaporation, to a protein concentration of 2,200 mg/100 ml. The protein content of sera and dialyzates was determined by the Folin-Ciocalteu method(3).

*Disc electrophoresis.* Disc electrophoresis quantitation was done as described previously (1). In the case of the dialyzates 20 $\mu$l of concentrated solution was used.

*Immunodiffusion.* Quantitation of the IgG, IgM and IgA globulins as well as ceruloplasmin was done by the radial diffusion method (1).

*Haptoglobin determination.* For typing, maternal serum was saturated with hemoglobin prior to electrophoresis. After the disc electrophoresis run, the gel was stained with a solution of 0.1% (w/v) o-dianisidine and 0.6% (v/v) $H_2O_2$ in ethanol-acetic acid-water 20:20:60(1). Sera were separated into 2-2,

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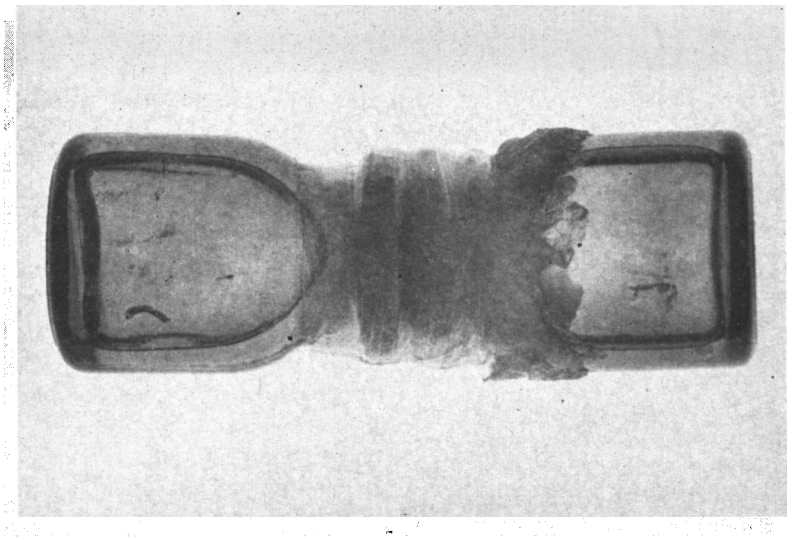

FIG.1. *In vitro* system used for dialyzing maternal sera across fetal membranes

2-1 and 1-1 types according to the heme-haptoglobin patterns.

For haptoglobin quantitation a micro-modification of the immunodiffusion technique of Gell *et al*(4) was used. This technique is described elsewhere(5).

*Results.* Tabulation of the values obtained on materials from ten duplicate dialysis experiments across amnionic or chorionic membranes are given in Table I. The results obtained for the dialyzates across both fetal membranes together are seen in Table II. For comparison, the results previously reported for amniotic fluids(1) are also given on Table II.

All dialyzates, like amniotic fluid, (Tables I and II, Fig. 2 and 3) appeared to be composed mainly of IgG which is the diffuse region in the upper part of the disc, plus transferrin which is the center strong band, post albumin proteins among which are the specific group proteins and others as yet not identified, and albumin. The percentage of total protein values for albumin was lower and the transferrin and post-albumin protein values higher in amniotic membrane dialyzates than in amniotic fluids. In the chorionic membrane dialyzates, the difference in values obtained between the dialyzates and amniotic fluids is quite small for albumin and

TABLE I. Comparison of Protein Values in Maternal Serum, Amniotic and Chorionic Dialyzates Expressed as Percentage of Total Protein.*

| | Maternal serum | Amnion dialyzate | Chorion dialyzate |
|---|---|---|---|
| Total protein (g/100 ml) | 6.81 (± .65) | .36 (± .08) | .20 (± .11) |
| Protein values (% total protein): | | | |
| Albumin | 43.6 (±3.50) | 53.2 (±5.68) | 58.0 (±5.17) |
| Post albumin | 7.58 (± .94) | 10.6 (±1.92) | 9.15 (±1.91) |
| Transferrin | 17.5 (±2.14) | 20.5 (±2.44) | 17.5 (±2.60) |
| $\alpha_2$ Glycoprotein double band | 5.51 (±1.25) | .82 (± .59) | 1.27 (± .98) |
| $\beta$ lipoprotein | 1.45 (± .48) | .10 (± .13) | .34 (± .31) |
| Immunoglobulin M | 1.38 (± .46) | .15 (± .23) | .24 (± .30) |
| " A | 4.22 (±1.45) | .85 (± .17) | .92 (± .22) |
| " G | 19.3 (±3.69) | 6.87 (±1.98) | 6.67 (±2.05) |
| Ceruloplasmin | 1.13 (± .16) | .49 (± .10) | .43 (± .07) |
| Haptoglobin 1–1 | 1.71 | .65 | .27 |
| 2–1 | 1.58 | .37 | .084 |
| 2–2 | 1.35 | .21 | .086 |

* Average of 10 separate experiments with duplicate dialyzates ± average error. Where less than 10 determinations were done (haptoglobins), the average error was not calculated.

TABLE II. Comparison of Protein Values in Maternal Serum, Dialyzates Across Both of the Fetal Membranes,* and Amniotic Fluid Expressed as Percentage of Total Protein.

| | Maternal serum | Amnion & chorion dialyzate | Amniotic fluid† |
|---|---|---|---|
| Total protein (g/100 ml) | 7.08 | .082 | .22 |
| Protein values (% total protein) | | | |
| Albumin | 40.1 | 60.5 | 62.6 |
| Post albumin | 8.60 | 9.60 | 8.26 |
| Transferrin | 20.4 | 20.3 | 16.9 |
| $\alpha_2$ Glycoprotein double band | 6.40 | .00 | .00 |
| $\beta$ Lipoprotein | 1.50 | .00 | .00 |
| Immunoglobulin M | 2.43 | .00 | .00 |
| " A | 3.59 | .73 | .60 |
| " G | 17.6 | 5.00 | 7.33 |
| Ceruloplasmin | 1.39 | .48 | .37 |
| Haptoglobin 1–1 | 1.62 | .19 | .15 |
| 2–1 | — | — | — |
| 2–2 | 1.36 | .021 | .00 |

* Average of 5 determinations.
† Values from Table I of previous paper(1).

post-albumin proteins. The values for transferrin are the same. The albumin, transferrin and post-albumin percentage values in the amnion to chorion dialyzates are comparable to amniotic fluid values within experimental error.

IgA and ceruloplasmin were always present in the dialyzates. The values of 0.85% and 0.92% for IgA in the amniotic and chorionic dialyzates (Table I) were within expected limits for sera containing 4.22 IgA % of total protein. This applies also to the value of 0.73 for the amnion plus chorion dialyzates (Table II) as compared to 3.59 IgA percent of total protein in the sera. For amniotic fluid a value of 0.60% had previously been reported as compared with 3.04% in corresponding sera(1). Thus the ratio of *IgA % total protein in dialyzates or amniotic fluid: IgA % total protein in sera* is approximately equal to 1:5. The ratio is approximately 1:3 for ceruloplasmin in both the dialyzates and amniotic fluid.

The 3 macroglobins IgM, $\beta$ lipoprotein, and $\alpha_2$ macroglobulin, which were previously recorded as absent in amniotic fluid(1), were sometimes present in the dialyzates and sometimes absent as indicated by the average error obtained. It is noteworthy that these 3 large molecules were more common in the chorionic than in the amniotic dialyzates. On the other hand, passage of haptoglobins which are smaller molecules, was considerably more inhibited by the chorionic membrane than the amnion.

The protein content of amniotic fluid averages 2.20 mg/ml(1). In the present group of experiments, the protein of amniotic membrane dialyzates averaged 3.64 mg/ml, the chorionic membrane dialyzates 2.00 mg/ml, and the amnion plus chorion dialyzates 0.82 mg/ml. Thus, at the end of 24 hours at 37°C *in vitro,* the protein concentration of the dialyzates is in the range of amniotic fluid protein concentration. This finding is in general agreement with that of Abbas and Tovey(6) who dialyzed maternal sera against normal saline across fetal membranes. Since their dialyzates were analyzed by paper electrophoresis, it is difficult to compare their results to those recorded herein except in the case of albumin and IgG globulin. Abbas and Tovey reported that the percentages of total protein of albumin and IgG in their dialyzates and in amniotic fluid were the same. Although they did not compare individual proteins, they noted that the $\alpha_1$, $\alpha_2$, and $\beta$ protein fractions were similar in the dialyzates and in amniotic fluid.

*Discussion.* The present findings indicate that the fetal membranes may play a much more important role than simple ultrafiltration in protein transport across the placenta.

From the results obtained (Tables I and II) it is apparent that the transport of maternal proteins across the fetal membranes, *in vitro,* resembles very closely the *in vivo* situation of protein transfer into amniotic fluid, and adds further support to the previously presented evidence for the maternal origin of amniotic fluid proteins(1,2,6-11).

According to Abbas and Tovey(6) and Derrington and Soothill(7), the process by which proteins reach the amniotic fluid has been considered as simple filtration through semipermeable membranes. If, in this *in vitro* study, the *dialyzates : serum concentration* ratios for serum proteins were inversely in the order of their molecular size,

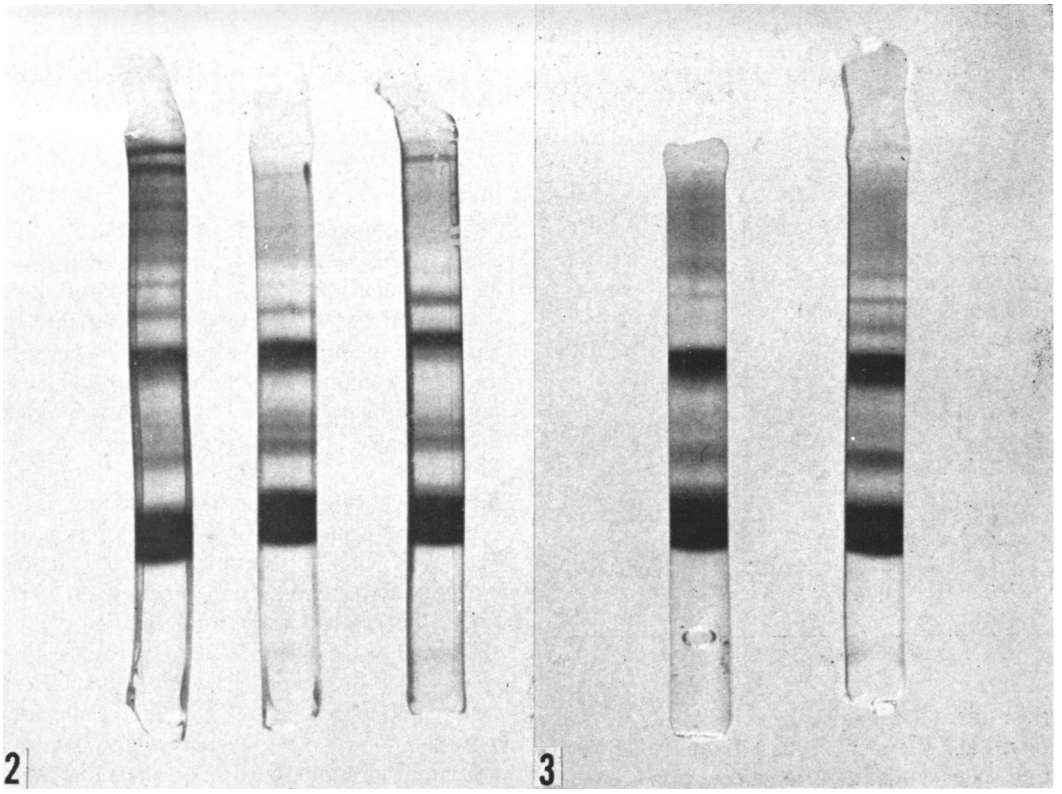


FIG. 2. Comparable disc electrophoresis protein patterns in (left to right): serum, amniotic dialyzate and chorionic dialyzate.

FIG. 3. Disc electrophoresis protein pattern in (left to right): amniotic fluid and in an amnion plus chorion dialyzate.

such a mechanism would appear to be correct. The results of the present study show that some proteins, namely albumin, transferrin, the post albumin proteins, ceruloplasmin, Immunoglobulin G, $\beta$ lipoprotein, $\alpha_2$ macroglobulin and IgM probably arrive in the dialyzates by just such simple filtration. On the other hand, the selective inhibition of the haptoglobins and IgA cannot be explained by simple filtration. The membranes, besides acting as filters, must also be able to select proteins for passage, presumably on the basis of chemical structure.

It is of interest that in these *in vitro* studies, the amnion appeared to be a better filter than the chorion as indicated by the concentration of large molecules such as IgM, $\beta$ lipoprotein, and $\alpha_2$ macroglobulin in their respective dialyzates. The amnion, however, did not inhibit the passage of the haptoglobins as well as the chorion. Where maternal sera contained the 1-1 type of haptoglobin which has a molecular weight of 85,000, dialyzates across both fetal membranes contained about $\frac{1}{8}$ as much 1-1 haptoglobin percentage of total protein as in sera, chorion dialyzates about 1/6, and amnion dialyzates about 1/3. Amniotic fluid contains 1/10 as much haptoglobin percentage of total protein as serum(1). In contrast, the transferrin percentage is similar in the dialyzates and maternal sera even though this molecule is approximately the same size as the 1-1 haptoglobin. In the cases where maternal sera contained the larger haptoglobin molecules, 2-1 and 2-2 (Tables I and II), the chorionic membrane actively inhibited their passage since only about 1/15 as much 2-2 or 2-1 haptoglobin percentages of total protein was found in the dialyzates as in sera. Amniotic membranes in these cases did in-

hibit haptoglobin passage but not as well as the chorionic membranes.

The IgA to IgG concentration ratio in maternal sera as compared with the same ratio in the dialyzates were different even though both substances have approximately the same molecular weight. These concentration ratios were the same as those previously obtained for maternal serum and its corresponding amniotic fluid(1).

The present findings indicate that the functions of the fetal membranes in protein transport across the placenta involve complicated selective mechanisms as well as simple filtration.

*Summary.* Maternal sera were dialyzed *in vitro* across individual or combined fetal membranes to elucidate the functions of the membranes in protein transport. Concentration of individual proteins in the dialyzates obtained, closely resembled the composition of amniotic fluid. Results of these *in vitro* studies indicate that the fetal membranes are able to select protein molecules for passage not only according to size, but also on the basis of their chemical structure.

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## Effect of Released Urine Flow on Renal Tissue Creatinine, Urea and Na in Dogs.* (32215)

GASPAR CARRASQUER† AND A. LOUISE DIANA

*Department of Medicine, University of Louisville School of Medicine, Louisville, Ky.*

Urea accumulates in renal tissue during stop-flow in dogs loaded with hypertonic sodium chloride(1). In these experiments stop-flow filtration is relatively high as denoted by creatinine accumulation in renal tissue(2). The tissue concentration of sodium did not change significantly during stop-flow in these experiments or any other experiment performed in osmotic diuresis.

Accumulation of urea in renal tissue suggests, as in the case of creatinine, that urea is trapped in certain portions of the renal tubule as the column of fluid moves along the nephron due to reabsorption of sodium chloride and water from the distal tubule and collecting duct. More explicit information of the side where urea accumulated during stop-flow (lumen, cell, interstitium) may be obtained by a comparative analysis of kidneys with obstructed ureters with kidneys in which the obstruction is released after similar stop-flow periods. If tissue accumulation of urea were due to intraluminal trapping during stop-flow, release of the obstruction should result in a prompt fall in the tissue concentration.

In the light of the above considerations it is the purpose of this paper: 1) to determine the effect of release of obstruction from the ureter on renal tissue concentrations of creatinine, urea and sodium chloride and 2) to use these data and those obtained during stop-

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