sec caused a change in nasal vasoconstrictor response of approximately the full range from minimal to maximal. A frequency of only 2/sec caused a moderately strong vasoconstriction, when many cervical sympathetic nerve fibers were stimulated simultaneously.

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A Comparative Study of Amniotic Fluid, Maternal Sera and Cord Sera by Disc Electrophoresis.* (31539)

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Existing information about amniotic fluid protein has been derived mainly from paper electrophoresis analysis(1,2,3,4,5,6). Since this procedure only separates serum fluids into the 5 major bands of albumin, α_1, α_2 β, and γ globulins, each of which is a mixture of proteins of different chemical structures and molecular weights, no quantitative information concerning the individual protein components has been obtained by the use of this technique. Studies on amniotic fluid done with immunoelectrophoresis have given qualitative information only(7,8). Derrington and Soothill(9) employed an immunodiffusion method for a quantitative study of amniotic fluid but they limited their investigation to 7 serum proteins.

The present paper reports the findings in a comparative study of individual proteins in amniotic fluid, maternal serum and cord serum when disc electrophoretic techniques combined with quantitative immunodiffusion were employed for the investigation. Acrylamide disc electrophoresis is a recently devised technique that provides routine separation of approximately 20 proteins from a serum sample as small as 3 microliters. Direct analysis of dilute liquids such as amniotic fluid is also possible since proteins in the fluid are automatically concentrated to high values in the stacking gel at the beginning of an experimental run prior to differentiation of proteins in the separating gel. The objectives in this study were 1) to quantitate as many individual amniotic fluid proteins as possible and to compare the proteins of the amniotic fluid to those of maternal and fetal serum; 2) to consider the origin of the amniotic proteins since, at present, investigators are divided among those who believe the amniotic fluid proteins arise from fetal serum, and those who feel their origin is maternal serum; and 3) to test the hypothesis that proteins of the amniotic fluid are derived from filtration through the semipermeable fetal membranes(9).

Materials and methods. Amniotic fluid, maternal serum and fetal serum from 12 normal pregnancies were examined. Amniotic fluid was obtained by needle aspiration at the beginning of labor. Maternal serum was drawn within 24 hours after delivery. Cord blood was employed as the source of fetal serum. For both disc electrophoresis and immunodiffusion studies, the amniotic fluids were concentrated 10× by dialysis against solid su-

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TABLE I. Comparison of Individual Protein Values in Maternal Serum, Amniotic Fluid and Fetal Serum Expressed as Percentage of Total Protein.*

	Maternal serum	Amniotic fluid	Fetal serum
Total protein†	6.47 (± .42)	.22 (± .05)	6.07 (± .43)
Transferrin	18.1 (± 2.37)	16.9 (± 4.27)	6.90 (± 1.99)
Ceruloplasmin	.97 (± .16)	.37 (± .08)	.14 (± .02)
Immunoglobulin A	3.04 (± 1.06)	.60 (± .18)	.00
" G	20.2 (± 2.49)	7.33 (± 2.29)	24.2 (± 2.79)
" M	1.34 (± .38)	.00	.23 (± .04)
Albumin	42.1 (± 3.93)	62.6 (± 7.3)	56.0 (± 4.82)
β Lipoprotein	1.87 (± .90)	.00	.00
α_2 Glycoprotein double band	6.40 (± 1.64)	.00	5.80 (± 1.56)
Post albumin proteins	8.27 (± 1.74)	8.26 (± 1.86)	8.17 (± 2.14)

* Average of 12 determinations ± average error. † Grams protein/100 ml.

crose. Prior to concentration, the amniotic fluid was cleared by centrifugation at 13,000 rpm for 30 minutes. The protein content of the amniotic fluid and sera was determined by the biuret method(10).

Disc electrophoresis. Individual serum proteins were separated by disc electrophoresis in a Canalco Model 6† apparatus. The procedure employed for maternal or fetal sera is the technique recommended in the Canalco Equipment Instructions for serum proteins or other samples of similar protein concentration; 3 μl were used for each run. For amniotic fluid 20 μl, concentrated 10×, were employed in an alternate procedure devised for samples of lower protein concentration. The quantitation of the bands was based on densitometer tracings in a Photovolt‡ Model microdensitometer. A standard serum was included in each run to make certain the test was operating correctly.

Haptoglobin determination. To compare the haptoglobin present in fetal serum, maternal serum and in amniotic fluid, the sera and concentrated amniotic fluid were saturated with hemoglobin prior to electrophoresis. After the disc electrophoresis run, the gel was stained for heme-proteins 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. After 15 minutes, the staining solution was decanted and the gel was washed twice for 10 to 15 minutes in the stain solvent and then stored in distilled water. The heme-haptoglobin lines were quantitated by densitometer tracings. Purified haptoglobin 1-1 was used as a standard. Only maternal sera of 1-1 type (4 out of the 12 studied) and their corresponding amniotic fluids were quantitated since in the 2-1 and 2-2 types of sera, no haptoglobin was found in their related amniotic fluids.

Immunodiffusion. Quantitation of IgG, IgM, and IgA globulins as well as ceruloplasmin was done by the radial diffusion method (11) on immunodiffusion plates purchased from Hyland Laboratories, Los Angeles, Calif. A series of standards from which a standard curve was plotted were also obtained from the same source and run with each set of determinations. Standard curves for quantitation of the various proteins were made by plotting the diameters of the ring haloes on the immunodiffusion plates *versus* the logarithm of protein concentration. Diameters were read after 16 hours at room temperature.

Results. A tabulation of the values for protein determinations on materials from 12 normal pregnancies is given in Table I. Amniotic fluid (Table I, Fig. 1) 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, postalbumin proteins among which are the specific group proteins and others as yet not identified, and albumin. Ceruloplasmin and IgA were always present. In a qualitative immunoelectrophoretic study of amniotic fluid, Masseyeff(7) also detected small amounts of IgA though Strebel(8) reported that this substance was absent in approximately one-half the amniotic fluids he examined. In the present study, the average amount of IgA found in amniotic fluids concentrated 10×, is 14 mg

† Canalco Indust. Corp., Bethesda, Md.

‡ Photovolt Corp., New York.

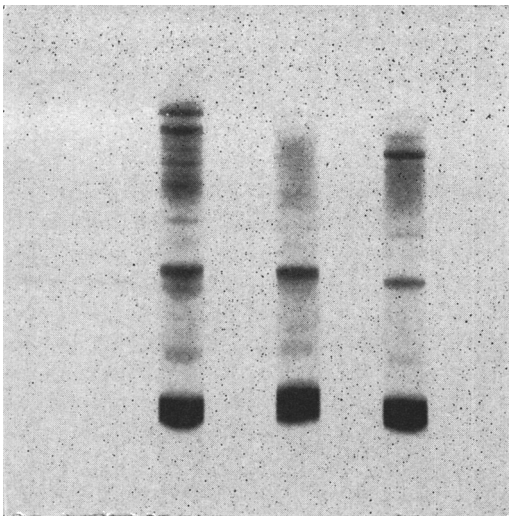

FIG. 1. Disc electrophoresis protein patterns in maternal serum, aminotic fluid and fetal serum (left to right) from the same subject.

percent which is considerably below the level of the 40 mg percent that can be detected readily by immunoelectrophoresis(11).

No haptoglobin lines were observed in the amniotic fluid when the maternal serum was of a 2-2 or 2-1 type. When the maternal serum was of the 1-1 type, this small haptoglobin was always present in the fluid although it was absent in the fetal serum (Table II). IgM, β lipoprotein and the glycoprotein double band were not found in any of the amniotic fluids examined. Unidentified protein bands appearing between the origin of the gel and transferrin were not quantitated. No protein line was found in amniotic fluid which was not present also in maternal serum.

The only other values reported in the literature for individual amniotic fluid proteins are those noted by Derrington and Soothill(9). Since the protein concentrations given by these authors were expressed as a percentage of the standard serum of a healthy adult male, it is not possible to make an exact comparison between their findings and the values obtained in this study. There is, however, general agreement. The results of both investigations show that albumin and transferrin are the major protein components of amniotic fluid, that IgG and ceruloplasmin are present in small quantities and that β lipoprotein and IgM are absent. The previous paper reported minute amounts of α_2 glycoprotein in some amniotic fluids while none was found in any of the 12 fluids examined for this study. Derrington and Soothill did not test for IgA, haptoglobins, or post-albumin proteins.

The mean of the albumin percentage of total protein values in fetal sera was greater than that for maternal sera but less than the mean values for amniotic fluid. This finding is in agreement with previously reported electrophoretic results(3,9). Concentration of the IgG globulin was also higher in fetal than maternal sera as was previously noted by Derrington and Soothill(9) and also Brown (12), while the amount of IgG in amniotic fluid was lower than in either of the sera. A similar concentration of the α_2 glycoprotein double band was present in the two types of sera. This protein was not found in amniotic fluid. The post-albumin protein percentage values were almost identical for all 3 fluids. Haptoglobins and β lipoprotein were absent in fetal sera. As noted, haptoglobin type 1-1 was always present in amniotic fluid when the maternal serum was of the same type. Ceruloplasmin and transferrin values expressed as percentage of total protein were lower in fetal sera than in maternal sera or amniotic fluid. IgM was detected in all fetal sera in very small amounts and absent in amniotic fluid.

The values for albumin were lower(1,2,3,9) and the ceruloplasmin levels higher(9,13) in maternal sera than the standard levels for normal nonpregnant sera, as has been reported. Elevation of transferrin content was in keeping with the known rise of iron-binding capacity of serum during pregnancy(14).

Discussion. If the amniotic fluid is a dialysate of fetal or maternal serum, the concen-

TABLE II. Comparison of Haptoglobin 1-1 Values in Maternal Serum, Amniotic Fluid and Fetal Serum Expressed as Percentage of Total Protein.*

Set No.	Maternal serum	Amniotic fluid	Fetal serum
1	1.83	.20	.00
2	2.71	.15	.00
3	1.94	.14	.00
4	2.17	.12	.00
Avg	2.16 ($\pm$.27)	.15 ($\pm$.02)	.00

* Where the maternal sera were of types 2-2 or 2-1, no haptoglobin was detected in amniotic fluids. Haptoglobins were absent from all 12 fetal sera.

tration of individual proteins expressed as percentage of total protein should decrease as the molecular size increases when compared with the serum from which it originated. The present results indicate that at least part of the amniotic fluid proteins come from the maternal circulation. Transferrin percentage values (Table I, Fig. 1) of the amniotic fluid approximated those of the maternal serum but were much larger than those of the fetal serum. Ceruloplasmin percentage values in the amniotic fluid were smaller than those of the maternal serum as expected from its molecular size. The fact that transferrin and ceruloplasmin percentage values were much larger in the amniotic fluid than in the fetal serum indicates that if simple filtration is the mechanism by which most of the proteins reach the amniotic fluid, then transferrin and ceruloplasmin must have a maternal origin. In each case where the mother had a haptoglobin type 1-1 (MW = 85,000), this molecule was found in the amniotic fluid. Since none of the fetal sera tested had any haptoglobin present, this protein also must have been of maternal origin. Finally IgA was also missing in fetal sera but present in the amniotic fluid in all the samples tested. On the basis of these findings, it is concluded that at least part of the amniotic fluid proteins come from the maternal circulation. Whether all proteins in the fluid or only part come from the maternal serum is as yet unanswered.

The process by which proteins reach the amniotic fluid has been considered by Abbas and Tovey(1) and Derrington and Soothill (9) as simple filtration through semi-permeable membranes. If the amniotic fluid/serum concentration ratios for serum proteins are inversely in the order of their molecular size, such a mechanism would appear plausible. Results obtained in the present study indicate that some proteins such as albumin, transferrin, the post-albumin proteins, ceruloplasmin and immunoglobulin G probably do arrive in the amniotic fluid by simple filtration. The fact that β lipoprotein, α_2 macroglobulin and IgM, all of which are absent in the amniotic fluid, are large molecules of more than 300,000 MW also supports this view.

On the other hand, the results obtained with the haptoglobins and IgA cannot be explained by the simple mechanism of filtration. In all 12 amniotic fluids investigated, the larger haptoglobin bands of 2-1 and 2-2 types were absent, even though these proteins have molecular weights of approximately 169,000(15). This value is very similar to the 150,000 molecular weight of IgG or ceruloplasmin, both of which do pass from maternal serum to amniotic fluid. Where maternal serum contained the 1-1 type of haptoglobin which has a molecular weight of 85,000, this molecule was found in the amniotic fluid. Even in these instances (Table II), only about 1/10 as much 1-1 haptoglobin percentage of total protein was found in amniotic fluid as in serum. In contrast, the percentage of transferrin, a molecule of approximately the same size as 1-1 haptoglobin, is very similar in amniotic fluid and in maternal serum. The IgA to IgG concentration ratios in maternal serum and amniotic fluid were found to be different even though both substances have approximately the same MW. Size is, therefore, not the only determinant for passage across the fetal membranes. The mechanism by which passage is inhibited warrants future study.

Summary. 1) Individual amniotic fluid proteins were quantitated by means of disc electrophoresis and immunodiffusion techniques, and proteins of the amniotic fluid were compared to those found in maternal and fetal serum. 2) The findings indicate that the proteins of the amniotic fluid originate at least partly from maternal serum. 3) Selective ultrafiltration on the basis of molecular size cannot explain apparent inhibition of the passage of haptoglobins and IgA into the amniotic fluid. Some additional mechanism, other than simple filtration, must be active.

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Reestablishment of Ovarian Periodicity After Transplantation to the Syrian Hamster Cheek Pouch.* (31540)

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Homologous and autologous ovarian transplantation has been successfully accomplished in a variety of mammalian species. Sites have varied from orthotopic to general abdominal, to tail, ear, leg, and anterior chamber of the eye(1). Homologous orthotopic transplantation reestablished limited fertility in mice(2), and apparently normal cyclic ovarian periodicity in several other species for short durations. Newly established ovarian grafts respond characteristically to gonadotropins, may resume normal steroid synthesis for a short time, but soon begin to secrete excessive quantities of androgen after location in these peripheral sites. Under these conditions cyclic activity comes to a halt and investigation is limited to short-term experiments. In each of the grafting sites mentioned there also appears a limitation either of restricting the size which the graft may attain or difficulty in making direct observation of the transplant. The cheek pouch of the Syrian hamster *(Mesocricetus auratus)* provides excellent opportunity for both tissue growth and visual inspection of the graft throughout extended investigation. Results of our experience in transplanting ovaries to this site are reported herein.

The pouch was first used in 1951 for transplantation of neoplastic tissue(3) but more recently normal pancreas(4), oviduct(5), and uterus(6) have been accepted by this highly vascular area. Homologous grafts may persist indefinitely, and tissues from rat and rabbit continue to be supported for weeks. Even human tissue transplants have been maintained in the cheek-pouch for at least 45 days. As a partial explanation for the unique suitability of the pouch for transplantation studies, Shepro(7) has pointed to its alymphatic nature and has suggested that outward diffusion of graft antigens may be slowed by the large quantity of dense connective tissue that makes up a major portion of the pouch's inter-membranous space.

Methods. Sexually mature female hamsters, weighing between 55 and 85 g were anesthetized (Nembutal intraperitoneally, 7 mg/100 g body wt.) and bilaterally ovariectomized through a mid-ventral incision. The left pouch of each animal was everted and pinned onto a cork-covered operating board. A variable light source illuminated the pouch as it was stretched over a hole in the board, allowing the investigator to properly position

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