otherwise molecularly similar species. In moderate concentrations of buffers, the compounds used in these experiments migrated as round spots (#3 zone I), indicating minimal adsorption(7). Spot spreading usually was most salient near the electrolyte compartments (compare #1A and #9A with #5A, Fig. 3). Lateral diffusion at M.E. occurs in experiments of long duration. Changes in the pH of the buffer in the electrolyte vessels are minimal even when volatile electrolytes are used(3). Positive ions exhibit maximal and negative ions show minimal mobilities on the anodal side of center. Conversely, cation mobility is lowest, and anion mobility highest on the cathodal side. Advantage may be taken of these regions of increased and decreased mobility by using longer strips of paper in a standard open-type electrophoresis unit. The extra length may be folded into the electrolyte vessels or counterpoised rollers may be inserted into these compartments. If the unresolved components of a mixture migrate into a region where differences in mobility are very slight (*e.g.* #5B & C zone II or #1C & D zone I) the paper may then be moved toward that electrode where mobility differences are greater (*i.e.* anode for #5 and cathode for #1) and electrophoresis resumed. This principle has been used to separate certain alkaloids from urinary pigments (unpublished data 1952) and more recently by Wolfson(8) and by Lederer *et al.*(8) to separate normal and abnormal adult hemoglobins. These investigators made successive analyses on the cathodal side of center. The zone of mobility equilibrium for a given ion: (1) represents the resultant of several forces, (2) occurs on the side of least movement (cathodal for cations, anodal for anions), and (3) is independent of the point of application of the sample. Its numerical value is equivalent to the distance from the center of this zone to the center line of the paper, with appropriate sign affixed.

Summary. (1) A method for rapidly determining the mobility equilibrium of a compound is described. The procedure provides an analytical rather than the conventional trial and error method for determining paper electrophoretic characteristics of the components in a mixture. (2) Some factors affecting ion movement and mobility equilibria in open-type paper electrophoresis units are discussed. (3) An application of the method in separating urea and several indole derivatives is presented.

1. Twarog, B. M., Page, I. H., *Am. J. Physiol.*, 1953, v175, 157.
2. Page, I. H., *Physiol. Rev.*, 1954, v34, 563.
3. Durrum, E. L., *J. Colloid Sci.*, 1951, v6, 274.
4. Rapport, M. M., *J. Biol. Chem.*, 1949, v180, 961.
5. Udenfriend, S., Titus, E., Weissbach, H., *ibid.*, 1955.
6. McDonald, H. J., Urbin, M. C., Williamson, M. B., *J. Colloid Sci.*, 1951, v6, 236.
7. Kunkel, H., Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 89.
8. Lederer, M., *Introduction to Paper Electrophoresis*, Elsevier, New York, 1955, p43.
9. Macheboeuf, M., *Bull. Soc. Chim. Biol.*, 1953, v35, 346.

Received May 20, 1958. P.S.E.B.M., 1958, v98.

Blood Plasma Proteins in Fetal Goats and Sheep.* (24132)

JOSEPH J. BARBORIAK, GIACOMO MESCHIA, DONALD H. BARRON AND GEORGE R. COWGILL

Department of Biochemistry, Yale Nutrition Laboratory and Department of Physiology, Yale University School of Medicine, New Haven, Conn.

The electrophoretic patterns of fetal plasma proteins have been reported to differ from their maternal counterparts mainly by the presence of a special protein, fetuin(1), and in some species by the lack of γ-globulins

* Aided by grants from N.I.H., Josiah, Macy, Jr. Fn., and James Hudson Brown Memorial Fund of the Yale School of Medicine.

(2,3). The present investigation deals with distribution of fetal plasma proteins in 2 closely related species, the goat and sheep, and with the quantitative changes which individual plasma proteins undergo during fetal development.

Methods. Fetuses of goats and sheep of various ages were delivered by cesarean section and samples of heparinized blood taken from the umbilical vessels. After centrifugation, the plasma was removed and the total protein content determined refractometrically. Paper electrophoresis was carried out in the hanging strip type (Durrum) cells, using barbiturate buffer with a pH of 8.6 and ionic strength of 0.085. With 8 paper strips per cell, a constant current of 7 ma was applied for 16 hours. To prevent the precipitation of fibrinogen, the strips were wetted with 50 ml of buffer containing 10 mg of heparin. After completing the run, the strips were dried at 125°C for 30 minutes, stained with bromphenol blue for 6 hours, rinsed 3 times in 5% acetic acid and dried at 125°C for 15 minutes. Finally, they were exposed to ammonia vapor and read in a recording densitometer. A number of strips with maternal or fetal samples were stained also for plasma polysaccharides(4).

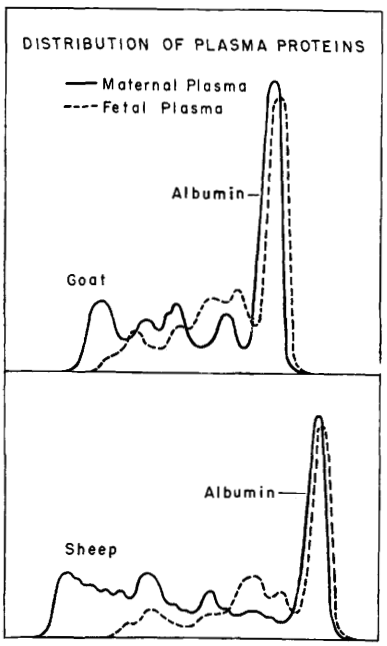

FIG. 1. Electrophoretic patterns of maternal and fetal blood plasma proteins. Goat fetus 125 days old, sheep fetus 128 days old.

Results. An illustration of differences in fetal and maternal patterns of plasma proteins can be seen in Fig. 1. The γ-globulin peak is missing in both fetal sheep and goats, probably because the multicellular barrier of the syndesmochorial placenta blocks the transfer of these macromolecules, and the fetal organism is not able to synthesize them(2). Human fetal plasma has been reported to contain some γ-globulins, as the unicellular hemochorial type of human placenta allows passage of some proteins(5). In the area of the albumin-bordering single peak of α_1-globulins in maternal plasma, the fetal pattern exhibits 2 peaks.

The changes in the quantitative aspects of individual fetal plasma proteins, as they are related to the age of the fetus, are reported in Table I. These data are based on determinations on single animals for each date and are, therefore, subject to individual variations. They show, however, the direction of changes quite clearly. In agreement with previous reports(6), the percentage of total plasma proteins tended to increase with the progressing fetal age. This tendency, because of the small number of samples and individual variations, was somewhat obscured in the sheep group. In both sheep and goats the percentage of plasma albumin rose with age of the fetus. Regarding the two peaks next to

TABLE I. Plasma Protein Fractions in Fetal Goat and Sheep.

Age of fetus, days	Total protein, g %	Albumin, %	Fetuin, %	Globulins (%)		
				α_1	α_2	β
			Goats			
64	2.8	23.0	28.9	17.8	14.8	15.5
85	3.3	34.9	25.9	16.3	12.7	10.2
103	3.0	38.1	19.3	17.7	11.2	13.7
110	3.5	38.2	18.0	19.1	10.7	14.0
113	4.0	40.5	17.8	20.3	11.7	9.7
119	4.0	39.4	15.4	16.8	12.0	16.4
127	3.6	43.1	16.0	15.3	14.2	11.4
131	3.7	43.9	14.7	19.2	10.6	11.6
			Sheep			
49		20.2	13.1	46.4	8.3	11.9
57		20.2	7.5	44.7	10.6	17.0
64	2.5	28.0	9.0	42.0	12.0	9.0
128	3.5	39.6	8.3	26.6	8.9	16.6
138	3.7	42.1	6.2	19.4	8.1	24.2

albumin, neither superimposing of the fetal and maternal protein patterns, nor chemical analysis of the 2 bands for polysaccharides reported to contain fetuin(7) were of help in disclosing which of the 2 peaks was fetuin. Both fractions showed a relatively high content of polysaccharides; higher than the corresponding maternal α_1-globulins. Taking an additional reported property of fetuin into account, *e.g.*, the decrease in concentration with the age of fetus(1,8), one can assume that in goats the peak next to the albumin is fetuin. In sheep the conditions are somewhat more complicated, as both the first and especially the second peak decrease with age.

As to the remaining plasma proteins, the α_1-, α_2-, and β-globulins of the goat did not seem to be influenced by the age of the fetus. In the fetal sheep the concentration of α_2-globulins did not show any significant correlation with age; the percentage of the β-globulins tended to increase, but as mentioned before, the number of samples is too small to allow a definite conclusion.

The age-correlated decrease and the relatively high content of polysaccharides in fetuin reminds one of Wharton jelly, a substance of fetal cord rich in mucopolysaccharides(9), which reportedly also decreases in amount with the growing fetus(10). The similar fate and proximity suggest a possible relationship.

Summary. Electrophoretic separation of fetal and maternal plasma proteins in goats and sheep revealed that the fetal plasma lacks γ-globulins and exhibits an additional peak in the area of α_1-globulins. This peak probably corresponds to fetuin. Quantitative conditions of fetal plasma proteins seem to depend largely on age of the fetus. In goats, the percentage of total plasma proteins and of plasma albumin increases; percentage of fetuin decreases with the progressing age of the fetus. The remaining plasma proteins do not seem to be affected. In fetal sheep the percentages of total plasma proteins, plasma albumin and probably of β-globulins increase; levels of fetuin and α_1-globulins decrease with age. The percentage of α_2-globulins remains unaffected.

We wish to thank Miss Patricia Daniels for technical assistance.

1. Pedersen, K. O., *Nature,* 1944, v154, 575.
2. Hansen, R. G., Phillips, P. H., *J. Biol. Chem.,* 1947, v171, 223.
3. Rutqvist, L., *Am. J. Vet. Res.,* 1958, v19, 25.
4. Köiw, E., *Scand. J. Clin. Lab. Inv.,* 1952, v4, 244.
5. Dancis, J., Braverman, N., Lind, J., *J. Clin. Inv.,* 1957, v36, 398.
6. Meschia, G., *Am. J. Physiol.,* 1955, v181, 1.
7. Deutsch, H. F., *Fed. Proc.,* 1953, v12, 196.
8. Bergstrand, C. G., Czar, B., *Scand. J. Clin. Lab. Inv.,* 1957, v9, 277.
9. Romanini, M. G., *Acta Anat.* (Basel), 1951, v13, 257.
10. Barcroft, J., Barron, D. H., *Anat. Rec.,* 1946, v94, 569.

Received May 20, 1958. P.S.E.B.M., 1958, v98.

Infectious Bovine Rhinotracheitis (IBR) IV. Cytological Changes in Infected Bovine Kidney and HeLa Cultures. (24133)

E. V. ORSI AND V. J. CABASSO (Introduced by H. R. Cox)
Viral and Rickettsial Section, Research Division, American Cyanamid Co., Pearl River, N. Y.

Specific intra-nuclear inclusions were observed in routine celloidin preparations of infected HeLa and primary bovine kidney culture monolayers during adaptation to HeLa cells of infectious bovine rhinotracheitis (IBR) virus(1). Since specific localization of increased amounts of desoxyribonucleic acid (DNA)(2) and of other constituents(3) often has resulted from cellular changes produced by infection with other viruses, more detailed cytologic observations of the effects of IBR virus were undertaken. The morphological and chemical effects of IBR virus on cells of an artificial host (HeLa) and of a