came progressively shorter, b) administration of platelets was less and less effective in the control of the bleeding manifestations. In one patient a platelet agglutinin was detected in the serum. 2. Search for platelet agglutinins was conducted in the serum of 11 patients who had received multiple blood transfusions over a period of months. A low titer platelet agglutinin was detected in one case. This patient developed mild thrombocytopenia and exhibited shortened platelet survival time. 3. These findings suggest that "refractoriness" to the therapeutic effect of the administration of platelets may develop in patients receiving multiple platelet or blood transfusions. This may be due to the development of antiplatelet substances in the serum, at least in individual cases.

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Plasma Proteins in Venous and Cord Blood at Delivery Following Uncomplicated and Complicated Pregnancies. (19580)

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Protein metabolism via the human hemochorial placenta is still incompletely understood. The literature provides few determinations of the protein components in plasma from venous and cord blood samples of women at the end of uncomplicated and complicated pregnancies.

Preceding reports(1-5) have given electrophoretically determined plasma protein values for normal non-pregnant women, for women during and following uncomplicated pregnancy, and for women whose pregnancies were complicated by toxemias, diabetes, abruptio placentae, and hepatic diseases. Data for each subject who participated in the electrophoretic studies are being published in detail (6). The results obtained are in accord with those of other investigators and show that alterations of plasma protein *do* occur in uncomplicated and in complicated pregnancy. Gutman(7) has reviewed the difficulties of interpreting plasma protein levels in health and disease.

Materials and methods. Proteins were determined in the plasma of maternal venous blood obtained at or less than one hour preceding delivery and in the plasma of cord blood. Paired samples of maternal and cord blood were obtained from 11 women whose pregnancies were uncomplicated and from 21 women whose pregnancies were complicated by disease. Samples of venous blood were obtained by withdrawing 20 ml of blood from a vein in the antecubital fossa and discharging

TABLE I. Proteins in 11 Samples of Venous Blood at Delivery Following Uncomplicated Pregnancies, and in the Corresponding Cord Blood.

		Venous blood			
		g/100 g plasma protein		g/100 ml plasma	
		Mean	Range	Mean	Range
	Total protein			7.71	6.80–10.26
	Albumin	45	37 –49	3.47	2.51– 4.60
Globulins	alpha 1	6.3	5 – 7.3	.49	.40– .66
	" 2	12.6	9.4 –16	.97	.79– 1.32
	beta	18.3	16.2 –21.6	1.41	1.11– 1.93
	phi	9.5	6.2 –12.9	.73	.45– 1.07
	gamma	8.3	4.4 –12.3	.64	.32– .87
A/G ratio	plasma	.82	.59– .96		
	serum*	.99	.74– 1.10		
		Cord blood			
	Total protein			6.47	5.44–8.60
	Albumin	57.7	53.1 –64.1	3.73	2.95–4.57
Globulins	alpha 1	4.9	3.3 – 6.5	.32	.18– .55
	" 2	8.5	6.8 –10.2	.55	.38– .76
	beta	8.8	5.9 –10.6	.57	.37– .91
	phi	6.6	5.4 – 8.2	.43	.29– .65
	gamma	13.5	9.6 –17.8	.87	.60–1.16
A/G ratio	plasma	1.36	1.14– 1.79		
	serum*	1.61	1.35– 2.16		

* Calculated as [plasma protein minus fibrinogen].

the fluid into a 50-ml, glass-stoppered flask containing 3 ml of 2.5% sodium citrate in physiologic saline. At delivery the umbilical cord was cut between clamps within 3 minutes. The proximal clamp was released immediately to allow cord blood to flow freely (without stripping) into test tubes containing 2 ml of 2.5% sodium citrate in physiologic saline. All samples were prepared for analysis as described elsewhere(6). The electrophoretic patterns of the plasma proteins were obtained with the Longsworth(8,9) scanning mechanism and a Tiselius cell with a single, long center section. Most determinations were run for 3 hours at 1°C and a potential gradient of about 6 volts/cm. The buffer solution was 0.1 N with respect to sodium diethylbarbiturate and 0.02 N with respect to diethylbarbituric acid, pH 8.55, ionic strength 0.1. In earlier publications(1,2) results given were based on determinations of total protein by the gravimetric or micro-Kjeldahl methods and correction of plasma volumes for dilution and red cell shrinkage by the procedure employed by Longsworth *et al.*(10). Later, analyses of human blood in this laboratory under controlled conditions provided a factor with which to correct for the effect of the anticoagulant and in an effort to achieve greater consistency total protein values calculated from the total area of the electrophoretic pattern have been used(6).

Results. Mean and range values for total protein, albumin, 5 globulin fractions, and the albumin-globulin ratios in plasma of venous and cord blood from 11 women whose pregnancies were uncomplicated are given in Table I. The values for total plasma protein are appreciably higher for maternal than for cord blood. Per 100 g of plasma protein and per 100 ml of plasma, on the average greater amounts of albumin and gamma globulin were found in cord blood than in the corresponding venous blood, coincident with lesser amounts of alpha, beta, and phi (fibrinogen) globulins. The mean and range for the A/G ratio were much greater for cord than for maternal blood, portraying the higher level of albumin and lower level of total globulin in the cord blood. The patterns for cord blood corresponded more closely with those for healthy, nonpregnant women(6) than with those for maternal venous blood at delivery.

The average values (Table I) for the relative concentrations in venous blood at delivery and in cord blood, converted to "serum" values by subtracting the amounts of fibrinogen, compare favorably with results reported

TABLE II. Range Values of Protein Fractions in Venous and Cord Blood Following Uncomplicated and Complicated Pregnancies.

	Samples	Venous* Min	Venous* Max	Cord Min	Cord Max	Samples	Venous* Min	Venous* Max	Cord Min	Cord Max
		Plasma protein, g/100 ml plasma					Phi globulin, g/100 g plasma protein			
Normal	11	6.80	10.26	5.44	8.60	11	6.2	12.9	5.4	8.2
Mild pre-eclampsia	1†	7.34		5.27	5.66	2†	8.8	10.7	7	8.1
Severe "	2	6.70	7.16	4.91	5.58	2	10.1	12.6	7.3	9.8
Essential hypertension	1	8.30		5.77		2	9	10.2	8.4	11.2
Renal disease	2	7.36	7.42	6	6.82	2	10.9	11.8	6.6	7
Diabetes	3†	6.24	6.40	4.78	5.84	3†	10.1	10.8	5.6	7.2
		Albumin, g/100 g serum protein‡					Alpha$_1$ globulin, g/100 g serum protein‡			
Normal	11	42.5	52.4	57.5	68.4	11	5.5	8.4	3.5	7
Mild pre-eclampsia	8	44	52.6	55.4	64.4	8	5.5	12.8	4	6.9
Severe "	2	40.4	47.5	59.9	64	2	8.9	10.3	5.6	7.6
Essential hypertension	3	42.4	47.2	59	64.7	3	7.1	11.2	4.3	6.7
Renal disease	2	42.6	47.4	58.7	62.7	2	6.8	9.8	3.3	5
Diabetes	4†	47.2	53.1	61.2	63.6	4†	5.9	9.1	3.4	6.4
Rheumatoid arthritis	1	43.8		60.9		1	8.1		4.5	
		Alpha$_2$ globulin, g/100 g serum protein‡					Beta globulin, g/100 g serum protein‡			
Normal	11	10.4	17.5	7.2	10.9	11	18.2	23.7	6.3	11.5
Mild pre-eclampsia	8	14	29.7	7.7	13.1	8	9.3	21.3	5.9	13.7
Severe "	2	14	18.5	8.4	12.3	2	19	20.1	11.2	13.3
Essential hypertension	3	11.8	18.4	8.1	10.6	3	16.3	25.3	8.1	10.9
Renal disease	2	15.7	17	9.7	12.1	2	20	22.7	7.1	9.4
Diabetes	4†	12.1	14.9	7.5	10.1	4†	17.4	21.9	7.8	12
Rheumatoid arthritis	1	13		8.3		1	18.7		8	
		Gamma globulin, g/100 g serum protein‡					Serum‡ A/G ratio			
Normal	11	4.7	14.1	10.2	18.9	11	.74	1.10	1.35	2.16
Mild pre-eclampsia	8	7.5	11.4	10.8	16.7	8	.78	1.11	1.24	1.81
Severe "	2	6.1	15.2	6.9	10.8	2	.68	.90	1.49	1.78
Essential hypertension	3	8.4	13.4	12.4	14	3	.74	.89	1.44	1.83
Renal disease	2	8.8	9.2	15.5	16.5	2	.74	.90	1.42	1.68
Diabetes	4†	8.9	14	9.8	16.9	4†	.89	1.13	1.58	1.75
Rheumatoid arthritis	1	16.4		18.3		1	.78		1.56	

* Only venous blood samples for which a corresponding cord sample is available are included.
† One less sample for venous than for cord blood. Number given is for venous samples.
‡ Calculated as [plasma protein minus fibrinogen].

by Longsworth, Curtis, and Pembroke(10). The data of Longsworth *et al.* for alpha$_2$ globulin in venous blood were 19% lower and for beta globulin were 12% higher than values from the present study. Cord blood values for alpha$_1$ and alpha$_2$ globulins in this study were 12% lower than those of Longsworth *et al.* Other averages in the 2 studies agreed within 9%. The relative fibrinogen values given by Longsworth were 12% and 21% lower, respectively, for venous and cord blood than those given in Table I. The average total serum protein for maternal blood determined from the total pattern area by Longsworth *et al.* was 7.17 g per 100 ml, and in the present study was almost identical—6.98 g per 100 ml. The cord blood proteins also are very similar, 6.18 and 6.04 g per 100 ml. The A/G ratios approximate those reported by Longsworth *et al.*, and the relationship between the maternal and fetal ratios is similar. The ratios of gamma globulin to total globulin in the plasma of the two types of blood also were similarly related in the two studies. Although Lagercrantz(11) used a phosphate buffer at pH 7.7 and 0.15 ionic strength, the relative values for the proteins gave results similar to those reported by Longsworth *et al.* and in the present study.

Although the investigative procedure was designed to provide values for plasma, under some conditions imperfect mixing of the

anticoagulant with the blood permitted clotting. Cord blood, especially, was subject to this hazard. Centrifugation at 12000 r.p.m. for 30 minutes removed the clot and cells, but only data for clot-free samples could be included in the ranges of values for plasma presented in Table I. Restriction of data for complicated pregnancy to clot-free plasma samples would have reduced even further the small numbers of samples in some groups. To avoid this, data are given in Table II on the basis of "serum" determined as total protein minus phi globulin. Minima and maxima are given for paired samples of maternal and cord blood from mothers with mild pre-eclampsia, severe pre-eclampsia, essential hypertension, renal disease, diabetes, and rheumatoid arthritis.

Venous and cord blood plasma from mothers whose pregnancies were uncomplicated (Table I) showed statistically significant differences in total protein, albumin, and the 5 globulin fractions, on the bases of protein distribution and of concentration per 100 ml of plasma. On the basis of plasma protein distribution there was a decided difference between the amounts of albumin in cord and venous blood, but on the basis of plasma concentration there was no marked difference between cord and venous blood. In complicated pregnancies (Table II) the ranges for total plasma protein both in venous and in cord blood were lower than those for the normal group: respectively, the maxima and minima were considerably lower. The maximum for cord blood total plasma protein in each group of complicated pregnancies also was lower than the minimum level for the corresponding average for venous blood.

For 11 samples of venous blood from mothers whose pregnancies were complicated, plasma fibrinogen values ranged from 8.8 to 12.6 g per 100 g of plasma protein, whereas in 12 samples of cord blood it ranged from 5.6 to 9.8 g and in the other sample—from a patient with essential hypertension—11.2 g per 100 g was found.

Ranges of values for the proteins of "serum" (plasma minus fibrinogen) from cord blood following complicated pregnancies were, in general, higher for albumin and gamma globulin and lower for alpha and beta globulins than the ranges found for venous blood. In neither venous nor cord blood were consistent differences discovered between data for complicated and uncomplicated pregnancy. In cord blood following both uncomplicated and complicated pregnancies the serum A/G ratios were almost double those of venous blood.

Discussion. Slemons(12) showed that nonprotein nitrogen concentrations of maternal and fetal blood were practically identical. The higher contents of amino acids and some crystalloids in fetal blood, according to Novak and Lustig(13), compensate for the greater amount of albumin in the maternal blood and keep the osmotic pressure about equal. Adaptation of the maternal and fetal osmotic pressures is considered essential(13) for the preservation of the vital nutritive chorionic epithelium of the fetal villi.

Recently, Moore, DuPan, and Buxton(14) determined electrophoretically the proteins in cord blood, in venous samples taken from the mothers soon after delivery, and in samples of blood taken throughout the gestation period and during the first year. They believe that the antibodies in newborn infant sera are a part of the gamma globulin but have little quantitative relation to it because they found gamma globulin in cord sera to be consistently higher than in maternal sera, whereas others had shown that antibody titers are never higher.

Longsworth, Curtis, and Pembroke summarized the results(15-17) of investigations of protein concentration per 100 ml of "serum or plasma" obtained by chemical methods and pointed out that "although the values obtained by different workers for a given type of material are not in quantitative agreement, the results of any one research are consistent in indicating certain systematic differences between bloods from the 3 sources." The electrophoretic results presented by Longsworth *et al.* supported the chemical results in the literature(15-17) and, in addition, revealed that although fetal serum is poorer in total globulin than is the maternal serum, it is relatively rich in gamma globulin, which is

presumed to contain the major portion of antibodies.

Summary. 1. Total protein, albumin, and 5 globulin fractions were determined by electrophoresis in the plasma of samples of cord blood and of venous blood from 11 women whose pregnancies were uncomplicated. Similar samples were obtained for electrophoretic analysis from 21 women whose pregnancies were complicated by toxemia of pregnancy and other diseases. 2. For the samples representing uncomplicated pregnancy total protein values for venous blood were higher than for cord blood. Greater average amounts of albumin and gamma globulin were found in cord blood than in the corresponding venous blood, coincident with lesser amounts of alpha, beta, and phi globulins. 3. In complicated pregnancies the ranges for total plasma protein in venous and cord blood were lower than for the normal group. Ranges of values for serum protein (calculated as plasma minus fibrinogen) fractions in cord blood following complicated pregnancies were, in general, higher for albumin and gamma globulin and lower for alpha, beta, and phi globulins than the ranges for venous blood.

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Visualization of Arterio-Venous Shunts by Cinefluorography in the Lungs of Normal Dogs.* (19581)

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Catheterization of the pulmonary vessels in man and animals is currently a common procedure for experimental and diagnostic purposes. Frequently the catheters are advanced peripherally in branches of the pulmonary artery until they occlude the vessel in order to record "pulmonary capillary" pressure or to obtain arterialized blood samples by retrograde flow. In view of this technic and the many descriptions of arterio-venous shunts in

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