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Margaret Real
State University of Iowa

J. I. Routh
State University of Iowa

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By Margaret Real and J. I. Routh

The protein components of the serum of newborn infants have occupied the attention of few investigators. McKhann and Kapnick (1) pointed out that newborn infants show an immunity to many diseases during the first months of life. Electrophoretic studies carried out by Tiselius and Kabat (2) demonstrated that the gamma globulin fraction of the serum contains the antibodies. Other investigators (3, 4, 5) reported a low ratio of albumin to globulin in the serum of newborn infants. The increase in globulin was shown to be mainly in the gamma globulin component (6, 7, 8, 9).

Since the technique of filter paper electrophoresis is adaptable to small quantities of serum, it is a useful method for the fractionation of the proteins of the serum of newborn infants. This study was undertaken to determine normal values for the serum protein components of newborn infants employing the technique of paper and free electrophoresis. It is hoped that these normal values will find use as a base line in further studies of serum proteins in newborn infants.

Experimental

Samples of blood were obtained from normal newborn infants during the first twenty-four hours of life and again on the third or fourth day. These babies were considered normal because of the following observations: healthy mother with uncomplicated pregnancy, no intrapartum complication, neonatal period uncomplicated, and complete physical examination on first and fourth days of life judged normal.

Blood was drawn from the femoral vein in a dry sterile syringe without an anticoagulant, care being taken to prevent hemolysis. After clotting, the specimens were centrifuged, the serum removed and kept refrigerated until use.

Paper electrophoresis was carried out by a modification of the glass sandwich technique of Kunkle and Tiselius (10). The glass plates which held the paper were 23.5 x 11.5 x 0.6 cm. Whatman 3 MM filter paper was used with a barbiturate buffer of pH 8.6 and ionic strength of 0.1. A constant current of 10 milliamperes was maintained during a run with a voltage range of 150 to 220 volts. A 10 microliter spot of serum was placed at the midline of the paper and the current applied for 7 to 8 hours or until the albumin component had moved about 8 cm. All runs were carried out in an air

conditioned room with a temperature near 20° C. At the end of a run the strips were carefully removed from the glass plates and dried in a horizontal position. The protein standing technique of Kunkle and Tiselius employing bromphenol blue was carried out.

All patterns were run in duplicate. One pattern was cut into 5 mm. segments. Each segment was eluted with 3ml. of 0.01 N sodium hydroxide and read after one hour in the Beckman DU spectrophotometer at 575 mμ. These results were plotted on graph paper as optical density versus distance of migration and produced a pattern similar to that obtained by free electrophoresis. The pattern was divided by the method of Pederson (11) and analyzed with a planimeter.

Free electrophoresis was carried out in the Tiselius apparatus by a standardized technique employed in our laboratory for over ten years. The 2 ml. micro cell was used and electrophoresis was conducted for 70-90 minutes with a constant current of 10 milliamperes at a temperature of 0.8° C in a barbiturate buffer pH 8.6 and ionic strength 0.1. The descending boundary was photographed and the pattern enlarged 2½ times, traced and analyzed by the use of a planimeter.

Total protein of the serum specimens (0.05 ml.) was determined by a modification of the biuret method described by Natelson (12).

Results

Results are reported for the analysis of the serum proteins of 59 samples from 32 newborn babies. Table 1 presents typical results of the paper electrophoretic analysis of these serum samples. Several of the samples in Table 1 were hemolyzed. It is especially difficult to obtain non-hemolyzed specimens from newborn babies since their blood cells are very fragile. In Table 2 a comparison is made between hemolyzed and nonhemolyzed samples. The outstanding variation appears in the β globulin fraction which is elevated in the hemolyzed sera. Table 3 presents a comparison of samples drawn in the first 24 hours of life and those drawn during the third or fourth day. Little change is seen in the serum protein components between the newborn and the three or four-day-old infants.

Typical results of the analysis of serum proteins of newborn infants by free electrophoresis are shown in Table 4. In general the values for albumin are higher and those for γ globulin lower than those obtained by paper electrophoresis.

A summary of the mean values reported in Tables 1 through 4 and values reported in the literature by other investigators are shown in Table 5.

Table 1

Typical Analyses of Serum Proteins of Normal Newborn Infants by Paper Electrophoresis

No.	Age* Days	Total Protein g/100 ml.	Percentage Composition					
			Alb.	Globulin				
				α_1	α_2	β	γ'	γ
9A	NB	6.24	56.0	4.5	9.7	9.7	2.5	17.5
9B	4	5.81	54.7	4.8	8.7	11.6	3.8	16.3
13A	NB	5.85	62.0	3.6	9.9	8.8	2.4	13.2
13B	3	5.71	63.3	5.3	9.8	7.9	1.7	12.0
14A	NB	5.97	55.3	4.9	7.8	12.1	2.9	17.0
14B	3	5.39	55.8	3.3	10.5	7.8	3.9	18.7
15A	NB	6.03	66.8	3.4	6.7	8.0	2.7	12.4
15B	3	5.19	65.1	3.7	9.0	7.5	2.7	12.0
23A	NB	6.45	64.4	4.5	9.1	6.9	2.5	12.5
23B	4	6.24	65.3	3.4	8.5	7.1	2.0	13.7
31A	NB	6.66	66.8	3.0	6.5	7.3	2.8	13.6
31B	3	6.58	61.6	3.3	9.0	9.8	2.1	14.1
No. Samples		54	59	59	59	59	59	59
Mean Values		6.33	59.3	3.9	8.6	9.2	2.5	16.5
Standard Deviation		0.83	5.04	1.03	1.41	2.69	0.66	3.29

*NB: Newborn, less than 24 hours old.

Table 2

Typical Analyses of Hemolyzed versus Non-Hemolyzed Serum Samples by Paper Electrophoresis

No.	Age* Days	Percentage Composition					
		Alb.	Globulin				
			α_1	α_2	β	γ'	γ
		Hemolyzed					
3A	NB	56.1	2.5	8.0	13.1	3.4	16.9
4A	NB	53.4	4.4	8.2	14.7	2.2	17.1
6A	NB	52.6	2.6	6.8	20.9	2.4	14.7
11A	NB	61.3	3.0	6.6	7.4	3.3	18.4
16A	NB	55.9	4.2	7.6	14.2	2.6	15.5
21A	NB	63.5	4.4	5.4	10.9	1.8	14.0
28A	NB	60.4	2.8	7.8	10.5	1.8	16.6
32B	4	60.2	4.3	9.2	12.2	2.4	11.6
No. Samples		13	13	13	13	13	13
Mean Values		56.9	3.7	8.1	12.3	2.5	16.5
Standard Deviation		3.89	1.02	1.55	3.19	0.50	2.48
		Non-Hemolyzed					
9A	NB	56.0	4.5	9.7	9.7	2.5	17.5
9B	4	54.7	4.8	8.7	11.6	3.8	16.3
17A	NB	60.8	4.5	8.4	7.7	2.4	16.2
17B	3	59.9	3.5	9.4	9.6	1.9	15.7
23A	NB	64.4	4.5	9.1	6.9	2.5	12.5
23B	4	65.3	3.4	8.5	7.1	2.0	13.7
25A	NB	61.9	3.7	8.7	8.4	1.9	15.4
25B	3	58.2	4.3	11.2	8.5	2.0	15.8
No. Samples		46	46	46	46	46	46
Mean Values		59.9	4.0	8.8	8.3	2.5	16.5
Standard Deviation		5.12	1.03	1.35	1.71	0.74	3.58

*NB: Newborn, less than 24 hours old.

Table 3

Typical Analyses of Non-Hemolyzed Serum in the First Day of Life versus Serum Drawn the Third or Fourth Day

	Percentage Composition					
	Alb.	Globulin				
No.		α_1	α_2	β	γ'	γ
First 24 Hours of Life						
2A	60.2	4.1	8.6	7.5	2.9	16.7
5A	53.5	3.8	8.7	10.8	3.1	20.0
9A	56.0	4.5	9.7	9.7	2.5	17.5
13A	62.0	3.6	9.9	8.8	2.4	13.2
17A	60.8	4.5	8.4	7.7	2.4	16.2
25A	61.9	3.7	8.7	8.4	1.9	15.4
29A	65.5	3.1	7.3	9.1	2.0	13.0
32A	59.2	4.6	12.3	7.4	2.5	14.0
No. Samples	20	20	20	20	20	20
Mean Values	60.5	4.0	8.4	8.1	2.4	16.6
Standard Deviations	5.24	0.81	1.24	1.35	0.47	3.86
Third or Fourth Day of Life						
3B	58.9	4.8	9.0	8.3	3.1	15.9
4B	61.8	4.8	10.1	6.9	1.5	14.9
5B	60.2	6.5	7.9	7.9	3.1	14.4
18B	55.3	4.5	8.8	9.6	2.2	19.6
25B	58.2	4.3	11.2	8.5	2.0	15.8
28B	55.8	2.4	10.6	8.4	2.2	20.7
29B	65.1	4.9	8.0	7.8	1.5	12.7
30B	65.6	3.3	9.1	4.7	2.2	15.1
No. Samples	26	26	26	26	26	26
Mean Values	59.5	4.0	9.1	8.4	2.6	16.4
Standard Deviation	5.13	1.21	1.23	1.96	0.84	3.39

Table 4

Typical Analyses of Serum Proteins of Newborn Infants by Free Electrophoresis

		Percentage Composition					
		Alb.	Globulin				
No.	Age* Days		α_1	α_2	β	γ'	γ
1B	4	56.7	10.1	9.6	10.1	2.8	10.7
5A	NB	73.6	2.3	4.6	6.3	1.7	11.5
5B	3	65.7	7.6	8.7	7.6	2.7	8.9
14A	NB	61.2	4.3	5.7	13.4	1.4	13.9
14B	3	66.8	3.8	8.2	8.7	1.6	10.9
24A	NB	71.3	4.4	7.8	7.8	1.7	7.0
24B	3	72.5	4.4	5.8	7.2	0.7	9.4
32A	NB	61.5	10.0	10.8	8.5	1.5	7.7
No. Samples		29	29	29	29	29	29
Mean Values		64.6	5.2	8.7	8.9	2.1	10.6
Standard Deviation		7.77	2.74	3.32	3.44	1.19	3.33

*NB: Newborn, less than 24 hours old.

Table 5
Summary of Mean Values Compared to Literature Values for Serum Proteins of Newborn Infants

Source	Alb.	Percentage Composition: Globulin α_1	α_2	β	γ'	γ
Paper						
Total	59.3	3.9	8.6	9.2	2.5	16.5
Hemolyzed	56.9	3.7	8.1	12.3	2.5	16.5
Non-hemolyzed	59.9	4.0	8.8	8.3	2.5	16.5
Newborn	60.5	4.0	8.4	8.1	2.4	16.4
3 and 4 days	59.5	4.0	9.1	8.4	2.6	16.4
Free	64.6	5.2	8.7	8.9	2.1	10.6

Other Investigators

Source	No. Samples	Alb.	Percentage Composition: Globulin α_1	α_2	β	γ'	γ
Paper							
Rafstedt (14)	50						
Umbilical Cord		71.5	6.1		7.2		15.0
1-6 days		70.3	7.1		10.0		12.5
Free							
Moore (12)	8	71.5	6.1		7.2		15.0
Free-Plasma							
Longsworth (11)	11	61.9	4.7	8.0	9.7	5.3	15.7
Knapp (13)	2	59.2	7.4	7.7	9.5	3.2	13.0

Discussion

The total serum protein of newborn infants falls in the normal range for adult sera. With three exceptions they range from 5 to 8 gm. per 100 ml. Hemolyzed or pigmented samples of serum occur more frequently in newborns than in adults. Hemolysis can be differentiated from slight jaundice due to increased bilirubin by the elevation in the β globulin component. This agrees with the findings of Moore (13) who demonstrated that hemoglobin migrates with the β globulin in barbiturate buffer.

No marked changes were noted in the serum protein fractions of new-born versus 3- to 4-day-old infants. Rafstedt (9) reported a decrease in γ globulin and an increase in the a and β globulin components when he compared umbilical cord blood with that from 1 to 6-day-old infants. In the present study a slight rise was observed in a_2 and β globulins in the older infants with no change in the γ globulins.

An attempt was made to analyze the dyed spots on the paper strips with a densitometer. For comparison the same strips were analyzed by the elution technique and the same serum samples

analyzed by free electrophoresis. Wide variations were observed in the values obtained by the three methods. In all cases the elution of the paper strips yielded values for albumin and γ globulin which were nearer to those obtained by free electrophoresis. The results obtained using the densitometer were characterized by low albumin and high γ globulin values. It has been suggested that paper strips should be made transparent before measuring the optical density of the spots on a densitometer (14). This was done by immersion in a mixture of *a*-bromonaphthalene and liquid paraffin which had approximately the same refractive index as cellulose ($n_D = 1.55$). The densitometer readings were lower, but the patterns were the same as for the untreated strip. In a critical survey of paper electrophoretic techniques, Henry et al. (15) reported similar findings.

Absolute agreement between values obtained by free and paper electrophoresis is not necessarily expected since the methods of measuring protein concentrations of the fractions is basically different. Free electrophoresis makes use of refractive index gradients at liquid junctions, whereas paper electrophoresis depends on dye absorption by the proteins. Not only do albumin and the globulin components absorb dye in different proportions but the percentage of absorption varies with the concentration and amount of protein applied to the paper, Henry et al. (15). For satisfactory analysis of protein components of serum, each technique must be independently standardized.

Summary

Normal values for serum proteins of newborn infants were obtained by the technique of free and paper electrophoresis. Fifty-nine samples from thirty-two infants were analyzed. One sample was obtained during the first twenty-four hours of life, and the other was taken during the third or fourth day. The normal values, as percentage composition, obtained from non-hemolyzed samples were as follows: albumin, 59.9; a_1-globulin, 4.0; a_2-globulin, 8.8; β-globulin, 8.3; γ'-globulin, 2.5; and γ-globulin, 16.5.

Several hemolyzed samples were analyzed and it was observed that the hemoglobin migrated with the β-globulin, resulting in high values for that fraction.

No significant change was noted between the values obtained from the serum of infants during the first day of life and those of the third or fourth day.

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CLINICAL BIOCHEMISTRY LABORATORY
DEPARTMENT OF BIOCHEMISTRY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA