

Proceedings of the Iowa Academy of Science

Volume 64 | Annual Issue

Article 31

1957

Electrophoretic Analysis of the Serum Proteins of Normal Newborn Infants

Margaret Real
State University of Iowa

J. I. Routh
State University of Iowa

Follow this and additional works at: <https://scholarworks.uni.edu/pias>

Recommended Citation

Real, Margaret and Routh, J. I. (1957) "Electrophoretic Analysis of the Serum Proteins of Normal Newborn Infants," *Proceedings of the Iowa Academy of Science*: Vol. 64: No. 1 , Article 31.
Available at: <https://scholarworks.uni.edu/pias/vol64/iss1/31>

This Research is brought to you for free and open access by UNI ScholarWorks. It has been accepted for inclusion in Proceedings of the Iowa Academy of Science by an authorized editor of UNI ScholarWorks. For more information, please contact scholarworks@uni.edu.

Electrophoretic Analysis of the Serum Proteins of Normal Newborn Infants

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 $m\mu$. 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 | |
| <i>Hemolyzed</i> | | | | | | | | |
| 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 | |
| <i>Non-Hemolyzed</i> | | | | | | | | |
| 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

| No. | Percentage Composition | | | | | |
|------------------------------------|------------------------|------------|------------|---------|-----------|----------|
| | Alb. | Globulin | | | | |
| | | α_1 | α_2 | β | γ' | γ |
| <i>First 24 Hours of Life</i> | | | | | | |
| 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 |
| <i>Third or Fourth Day of Life</i> | | | | | | |
| 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

| No. | Age* Days | Percentage Composition | | | | | |
|--------------------|--------------|------------------------|------------|------------|---------|-----------|----------|
| | | Alb. | Globulin | | | | |
| | | | 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 | Percentage Composition | | | | | |
|---------------|------------------------|------------|------------|---------|-----------|----------|
| | Alb. | 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 | Percentage Composition | | | | |
|----------------|-------------|------------------------|------------|------------|---------|-----------|
| | | Alb. | Globulin | | | |
| | | | α_1 | α_2 | β | 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 | | | | | | |
| Longworth (11) | 11 | 61.9 | 4.7 | 8.0 | 9.7 | 5.3 |
| Knapp (13) | 2 | 59.2 | 7.4 | 7.7 | 9.5 | 3.2 |

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 α 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 α_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.

Literature Cited

1. McKhann, C. F., and Kapnick, I. *J. Pediat.*, 12, 907 (1938)
2. Tiselius, A., and Kabat, E. A. *J. Exp. Med.*, 69, 119 (1939).

3. Kingsley, George. *J. Biol. Chem.*, 133, 731 (1940).
4. Trevorrow, V., Kaser, M., Patterson, J. P., and Hill, R. M. *J. Lab. Clin. Med.*, 27, 471 (1942).
5. Rapeport, M., Rubin, M. I., and Chaffee, D. *J. Clin. Invest.*, 22, 487 (1943).
6. Longsworth, L. G., Curtis, R. M., and Pembroke, R. H. *J. Clin. Invest.*, 24, 46 (1945).
7. Moore, D. H., duPan, R. M., and Buxton, C. L. *Am. J. Obst. Gynec.*, 57, 312 (1949).
8. Knapp, E. L., and Routh, J. I. *Pediatrics*, 3, 508 (1949).
9. Rafstedt, Sture, and Swahn, B. *Acta Paediatrica*, 43, 221 (1954).
10. Kunkle, H. G., and Tiselius, A. *J. Gen. Physiol.*, 35, 89 (1951).
11. Svedberg, T., and Pedersen, O. K. *The Ultracentrifuge*, London, Oxford University Press, 1940, p. 296.
12. Natelson, S., Crawford, W. L., and Munsey, F. Q. Correlation of Clinical and Chemical Observations in the Immature Infant, Endo Products, Inc., Richmond Hill, N. Y., 1942.
13. Moore, D. H., Roberts, J. B., Costello, M., and Schonberger, T. W. *J. Biol. Chem.*, 180, 1147 (1949).
14. Crook, E. M., Harris, H., Hassan, F., and Warren, F. L. *Biochem. J.*, 56, 434 (1954).
15. Henry, R. J., Golub, O. J., and Sobel, C. *Clin. Chem.*, 3, 49 (1957).

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