Ultramicro procedures requiring 5–10 μl of serum or blood per analysis were used in determining blood constituents of healthy full-term newborns during the first four days of life. The resulting values appeared to be influenced by age, sex, and race. Values for total protein, albumin, urea nitrogen, and uric acid in serum decreased with time; serum inorganic phosphorus and whole-blood aldosaccharoses increased. Serum from females had higher values than that from males for total proteins, albumin, and inorganic phosphorus. The values for serum calcium and alkaline phosphatase were consistently higher in Negro than in white infants; values for uric acid were higher in the latter.

Additional Keyphrases: pediatric chemistry • variation, sources of

We undertook to determine values for the chemical constituents of the blood of newborn infants. We used ultramicro-scale techniques that required only 5 to 20 μl of serum or blood for each analysis. The assays included total protein, albumin, aldosaccharose, blood urea nitrogen, total bilirubin, calcium, inorganic phosphorus, alkaline phosphatase, uric acid, and magnesium. These constituents were measured during the first four days of postnatal life. The statistical analysis of the data indicated certain sex-, race-, and time-related differences. 95% confidence limits for the estimate of the mean values were also determined.

Materials and Methods

Data from 166 healthy, full-term newborns from the Walter Reed Army Medical Center Maternity Ward were included in this study. Where possible, samples were taken from an infant on consecutive days. In some instances consecutive samples could not be obtained and samples from another infant of the same sex, race, and age were used in the statistical computations.

Each sample consisted of about 300 μl of blood, collected in a polyethylene tube after pricking the heel with a surgical blade. The first drop of blood was discarded, to minimize hemolysis. Samples were collected 12, 36, 50, and 84 h (±1 h) after birth. Except for the first day, the blood was drawn 2.5 h after a feeding; visibly hemolyzed samples were discarded.

Commencing 24 h after birth Enfamil1 formula (Mead Johnson & Co., Evansville, Ind. 47721) was fed at 4-h intervals.

All determinations were performed in triplicate for ultramicro-scale techniques according to Sanz (1). In these techniques self-containing nondisposable polyethylene pipets were used for each analysis. A specific pipet was used for each reagent, and one pipet was used for dispersing sample, control, and standard (contamination is eliminated by discarding the first drop of each sampling).

A Model 151 Beckman/Spinco Spectrophotometer was used to measure absorbances. Versatol, Versatol-A, and Versatol Pediatric (Warner-Chilcott Laboratories, Morris Plains, N. J. 07960) were the control materials used.

Total protein was determined by the biuret method (2, 3). HABA dye (4’-hydroxybenzene-azobenzoic acid) was used for albumin determinations (4). Most of the samples for the albumin analyses were aliquots of the same serum used in the total protein determinations.

The o-toluidine method (5–7) was used in determining aldosaccharoses. For this analysis, the blood sample was transferred to a tube containing 100 μl of trichloroacetic acid (0.2 mol/liter), mixed, centrifuged, and refrigerated at 4 °C until the specimens of all four days were accumulated and then analyzed simultaneously. (We assured ourselves that specimens contained in tightly stoppered plastic tubes could be

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1 Use of manufacturer's trade names for products, equipment, and reagents does not constitute an endorsement of these products by the U.S. Government.

Except for the aldosaccharoses, all tests were performed on serum.
Blood (actually, serum) urea nitrogen was determined by use of urease (EC 3.5.1.5) and phenol hypochlorite reagents (8).

Diazotized sulfanilic acid was used in total bilirubin analysis to form azobilirubin. The addition of a coupling agent and phosphoric acid to the latter formed the characteristic blue solution (9), measured colorimetrically.

A blue complex is also formed in the uric acid determination as a result of the reduction of phosphotungstate in the presence of hydroxylamine hydrochloride (10, 11).

For serum calcium analysis we used the complexometric titration of metallic ions with disodium ethylenediaminetetraacetate (12), using 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphtoic acid ("Cal-red") as indicator, which gave the same values as did calcine.

In the alkaline phosphatase (EC 3.1.3.1) determinations we used p-nitrophenyl phosphate as substrate and p-nitrophenol as the standard (13, 14).

The inorganic phosphorus analysis involved addition of molybdic acid to an aliquot of the supernatant liquid resulting from deproteinization of the serum by trichloroacetic acid. The phosphomolybdate formed was in turn reduced by ferrous ion, forming a blue solution (15, 16).

Magnesium was determined by use of the dye sodium 1-azo-2-hydroxy-3-(2,4-dimethyl-carboxy-anilido)-napththalene-1-(2-hydroxybenzene-4-sulfonate) (17); the standard curve was linear.

The amount of specimen required and the reproducibility for each determination are given in Table 1.

The data obtained were subjected to analysis of variance and significance was tested by use of the F test. Data from the blood urea nitrogen and bilirubin analyses were transformed to logarithms for the statistical analysis.

Results

Table 2 summarizes our data. Table 3 summarizes various comparisons in the statistical analysis.

Total protein. There was no significant difference in total protein values between the first and second day, but they declined significantly between the first and second days and the third and fourth days (P < 0.001). Average values for the third and fourth days were higher for females than for males.

Albumin. Albumin concentrations, like total protein, were lower on the third and fourth days than on the first and second (P < 0.001). Males had lower values than females and the values decreased faster. Values for Negroes were higher than for Caucasians in the first two days, but tended to converge for the third and fourth day.

The mean albumin/total protein ratio for all four days was 0.52.

Aldosaccharoses in whole blood. These values were significantly less on the first and second day than on the third and fourth (P < 0.001). Values for females were lower than for males during the first three days but increased steadily from day to day, whereas in males there was a steep increase between the first and second day, with values becoming constant by the second day.

Blood (serum) urea nitrogen. Blood urea nitrogen values were significantly smaller on the third and fourth days than on the first and second (P < 0.001). Values for females decreased more precipitously than for males.

Total bilirubin. Bilirubin values increased with age. A significant increase was observed between the first and second days (0.05 > P > 0.01). Values for total serum bilirubin were consistently lower in females than in males.

Calcium. Except for a very slight decrease of calcium values from the first to the second day, there was essentially no difference between days; however, Negro infants had consistently higher values than did Caucasian infants (0.01 > P > 0.001).

Alkaline phosphatase. Serum alkaline phosphatase activity was slightly greater on the second day, and Negro newborns had significantly higher values than did Caucasians (P < 0.001).

Uric acid. Values for uric acid decreased with time, the difference between the first and second days, as well as that between the first and second days vs. the third and fourth days being highly significant (P < 0.001). The rate of decrease between the third and fourth day was less than between the first and second. There was no difference in uric acid values between males and females. Negroes had lower values than did Caucasians in the first three days but the values were about the same on the fourth day.

Inorganic phosphorus. Values for inorganic phosphorus increased sharply in the first three days, so that both the differences between the first and second days and the first and second days vs the third and fourth days were highly significant (P < 0.001).

---

**Table 1. Sample Size and Reproducibility of Results**

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<tr>
<th>Analysis</th>
<th>Sample</th>
<th>Mean (triplicates) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
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<td>6.1 ± 0.07 g/dl</td>
</tr>
<tr>
<td>Albumin</td>
<td>5</td>
<td>3.1 ± 0.06 g/dl</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>20</td>
<td>3.8 ± 0.08 mg/dl</td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>10</td>
<td>13.0 ± 0.63 mg/dl</td>
</tr>
<tr>
<td>Uric acid</td>
<td>10</td>
<td>5.7 ± 0.14 mg/dl</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>5</td>
<td>57.0 ± 0.9 U</td>
</tr>
<tr>
<td>Calcium</td>
<td>10</td>
<td>8.5 ± 0.21 mg/dl</td>
</tr>
<tr>
<td>Inorganic phosphorus</td>
<td>20</td>
<td>6.6 ± 0.08 mg/dl</td>
</tr>
<tr>
<td>Magnesium</td>
<td>10</td>
<td>1.4 ± 0.07 mg/dl</td>
</tr>
<tr>
<td>Aldosaccharoses*</td>
<td>10</td>
<td>78.0 ± 2.1 mg/dl</td>
</tr>
</tbody>
</table>

* In whole blood. Other values are for serum.
### Table 2. Statistical Summary for Samples Analyzed during the First Four Days of Postnatal Life

<table>
<thead>
<tr>
<th>Race</th>
<th>Day I (12 h)</th>
<th>Day II (24 h)</th>
<th>Day III (48 h)</th>
<th>Day IV (72 h)</th>
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<td>Cau</td>
<td>Neg</td>
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<td></td>
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<tr>
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<td>12</td>
<td>7</td>
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<td>6.6</td>
<td>6.7</td>
<td>6.5</td>
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<td>0.83</td>
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<tr>
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<tr>
<td>Mean</td>
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<tr>
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<td>0.47</td>
<td>0.44</td>
<td>0.51</td>
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<tr>
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<tr>
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<td><strong>Bilirubin (mg/dl)</strong></td>
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<tr>
<td>Number</td>
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<td>7</td>
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<td>12</td>
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<td>4.72</td>
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<td>0.80</td>
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</tr>
<tr>
<td>Number</td>
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</tr>
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<td>66.2</td>
</tr>
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<td>SD</td>
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<td>1.12</td>
<td>1.35</td>
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<td><strong>Magnesium (mg/dl)</strong></td>
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</tr>
<tr>
<td>Number</td>
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<td>1.48</td>
<td>1.40</td>
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</tr>
<tr>
<td>SD</td>
<td>0.25</td>
<td>0.35</td>
<td>0.24</td>
<td>0.29</td>
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</tbody>
</table>

By the fourth day the values had become constant. No race-related difference was observed, but females had consistently higher values than males, and followed the same pattern from day to day.

**Magnesium.** There was no significant difference in magnesium values in relation to time, sex, or race.

**Discussion**

The high total protein values may be the result of unavoidable hemolysis. Bilirubin was not considered to seriously affect the values for protein assay; bilirubin values of 8 μg/dl increased the apparent total protein by only 0.1 g/dl. Our mean bilirubin value was 3.8 mg/dl; hence this value had no significant effect on protein measurement.

The slight decrease in values for total protein and albumin from the first and second days vs. the third and fourth days could reflect either the increased metabolic rate in the first few days of life or diminishing hemoconcentration and increased fluid intake.

The significant decrease in values for blood urea nitrogen and uric acid for the first and second days as
### Table 2. Continued

<table>
<thead>
<tr>
<th>Race</th>
<th>Day I (12 h)</th>
<th>Day II (36 h)</th>
<th>Day III (61 h)</th>
<th>Day IV (84 h)</th>
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<td></td>
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<td>Neg</td>
<td>Cau</td>
<td>Neg</td>
</tr>
<tr>
<td><strong>Total protein (g/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
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<td>9</td>
<td>15</td>
<td>9</td>
</tr>
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<td>6.9</td>
<td>6.5</td>
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<tr>
<td>SD</td>
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<td>0.76</td>
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<td>0.46</td>
</tr>
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<td></td>
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</tr>
<tr>
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<td><strong>Bilirubin (mg/dl)</strong></td>
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<td><strong>Alkaline phosphatase (U/liter)</strong></td>
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<tr>
<td>Mean</td>
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<td><strong>Magnesium (mg/dl)</strong></td>
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<td>0.29</td>
<td>0.99</td>
<td>0.30</td>
<td>0.32</td>
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</tbody>
</table>

Compared with the third and fourth days may reflect changes as the infant's kidney becomes functional (18). The start of regular feeding is probably also related.

Our mean values for total bilirubin were lower than those obtained by Hsia et al. (19). All of our infants were fed formula from the age of 24 h; none were breast fed. This probably diminished the effect of enterohepatic circulation of bilirubin, which may be enhanced by intestinal stasis (20).

A considerable amount of work has been done on reducing substances in the blood of newborns (21–23). However, we observed a significant sex-related difference in aldoseaccharoses values, females having lower values than males during the first three days.

The average calcium values obtained by us were

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somewhat lower than those reported by Thalme (24) and by Todd et al. (25). Use of a different indicator in calcium determinations perhaps explains this difference.

Our findings for inorganic phosphorus, alkaline phosphatase activity, and magnesium were in agreement with previous studies (24–27).

The higher values for both alkaline phosphatase activity and calcium in Negro infants during the first four days may be related to the reportedly higher bone density in Negro adults (28–30). It is tempting to suggest that earlier “maturating” in females can explain the higher protein and lower blood urea nitrogen and bilirubin.

The statistical comparisons were based on small numbers and are intended only to indicate where probable differences may be found between consecutive days, sex, and races. It is apparent that further studies are necessary to elucidate the mechanisms for the differences we observed.

References


<table>
<thead>
<tr>
<th>Table 3. Statistical Comparisons of Relations between Age, Sex, and Race for Components of Serum</th>
</tr>
</thead>
<tbody>
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<td>Day I vs. day II</td>
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<td>Albumin</td>
</tr>
<tr>
<td>Aldosaccharoses</td>
</tr>
<tr>
<td>Urea nitrogen</td>
</tr>
<tr>
<td>Total bilirubin</td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>Uric acid</td>
</tr>
<tr>
<td>Inorganic phosphorus</td>
</tr>
<tr>
<td>Magnesium</td>
</tr>
</tbody>
</table>

***, highly significant, P < 0.001; **, significant, 0.01 > P > 0.001; *, probably significant, 0.05 > P > 0.01; N.S., not significant P > 0.05.