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Age- and Sex-Specific Pediatric Reference Intervals for Biochemistry Analytes as Measured with the Ektachem-700 Analyzer

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Using the Ektachem-700 multilayer film analyzer, we defined age- and sex-specific reference intervals for 20 analytes in sera from a healthy population of neonates and children ages one to 19 years. Upper and lower normal reference intervals for each analyte were determined by nonparametric methods as the 0.975 and 0.025 fractiles, respectively. Newborns have lower concentrations of total protein and albumin, and higher concentrations of phosphate, bilirubin, and enzymes in serum than older children do. Concentrations of urea, glucose, calcium, phosphate, and bilirubin change rapidly postnatally. Outside the neonatal period, no significant age- or sex-related difference was found for plasma glucose, serum amylase, conjugated or unconjugated bilirubin, or lipase. There was no sex-related difference in reference intervals for albumin, total protein, calcium, phosphate, or urea. However, concentrations of uric acid and creatine kinase are much higher in postpubertal boys than in girls. Alkaline phosphatase values peak later in boys. Except for lactate dehydrogenase and γ-glutamyltransferase, the reference intervals defined here do not differ strikingly from data derived with use of other analyzers. The age- and sex-related trends are independent of method. However, each laboratory should determine the degree to which these reference ranges can be directly applied to analyses performed with another analyzer.

Additional Keyphrases: *multilayer film analysis · bilirubin · albumin · phosphate · glucose · amylase · lipase · lactate dehydrogenase · γ-glutamyltransferase · creatine kinase · urea · calcium · uric acid · alkaline phosphatase*

The transfer of analytical methods used in our laboratory to several new analyzers prompted a re-evaluation of the normal pediatric reference intervals in use in our hospital. Because pediatric data for the Ektachem multilayer film analyzer (Eastman Kodak, Rochester, NY) were limited, we undertook a study of several hundred infants and children of different ethnic backgrounds, living in the lower mainland area of Vancouver. We report age- and sex-specific reference intervals for 20 analytes as measured with the Ektachem-700.

Materials and Methods

The study population, sampling, and processing methods and statistical analyses have been described in detail in the preceding paper. Reference limits were defined by nonparametric statistics as the 0.025 and 0.975 fractiles for a specific data set (*1*).

Specific methodology and quality control data. Table 1 summarizes the test methods. Data from subjects other than infants less than two weeks old were obtained from analyses performed on a single preprandial blood sample. Because the need to minimize sample volume is relatively greater in the case of neonates or infants, our neonatal data were obtained in two ways. Results from tests such as bilirubin, glucose, or calcium, routinely ordered in neonates during the study period, were included in the data base if the baby was classified as "normal." A term infant was considered "normal" if mild jaundice was the only clinical problem. A preterm infant was accepted as "normal" if only mild respiratory distress syndrome or jaundice (not associated with blood group incompatibility) was present. Other analytes were measured in leftover serum or plasma remaining after the ordered tests had been done. Thus more than 300 babies were included in the study; however, we could measure only a few analytes in a given sample. We also studied sequential data during the first postnatal week in 30 preterm infants to monitor early changes in bilirubin and other analytes. No attempt was made to control for environmental lighting, type of feeding, or time of feeding relative to sample time for the neonates. The majority of term infants were breastfed.

Results and Discussion

Reference intervals determined with the Ektachem-700 are similar to those obtained by other methodologies except for γ-glutamyltransferase (GGT; EC 2.3.2.2) and lactate dehydrogenase (LDH; EC 1.1.1.27), for which results tend to be higher with the Ektachem, and albumin, which is somewhat lower.[4] Transferability of data is discussed for each analyte separately.

For those beyond the neonatal period, no significant age- or sex-related difference was found for plasma glucose,

[4] Nonstandard abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase.

Table 1. Summary of Test Methods for Each Analyte

Analyte	Method
Albumin	Bromcresol green
Bilirubin	Dual wavelength reflectance spectrophotometry
Calcium	Arsenazo III dye
Cholesterol	Cholesterol oxidase
Cholesterol, HDL	Dextran sulfate, then cholesterol oxidase
Glucose	Glucose oxidase
Phosphate	Ammonium molybdate
Total protein	Biuret
Triglycerides	Glycerol phosphate oxidase
Urea	Urease
Uric acid	Uricase
ALT	Alanine to pyruvate
ALP	*p*-Nitrophenyl phosphate
Amylase	Amylopectin
AST	Aspartate to oxaloacetate
Creatine kinase	Creatine phosphate to creatine
GGT	γ-Glutamyl-*p*-nitroanilide
LDH	Pyruvate to lactate
Lipase	1-Oleoyl-2,3-diacetylglycerol

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serum amylase (EC 3.2.1.1), or conjugated or unconjugated bilirubin. Lipase (EC 3.1.1.3), which was measured only in the children over 4 y, showed no significant age effect between 4 and 19 y. Tables 2–5 show reference intervals for the remaining analytes.

Both albumin and total protein increased gradually from the neonatal period to age about 10 y, then stabilized. No sex-related difference was noted. The reference intervals reported here are similar to those for the Abbott ABA-100 (*2*), but slightly lower than reported for the Technicon SMAC (*3*). The reference intervals for albumin and total protein are lower in babies weighing <2.5 kg at birth than in term babies or babies weighing >2.5 kg at birth, as found elsewhere (*4*).

The relative constancy of total calcium reference intervals, the downward trend of phosphate, and the upward trend of urea with increasing age are consistent with previous reports (*2, 3, 5, 6*). Calcium and phosphate data from our preterm infants are not shown because concentrations of both calcium and phosphate in serum were altered by calcium added to intravenous infusions during the early postnatal days. The reference interval for calcium in orally fed term neonates during the first five days postpartum is wider than in older children. Phosphate concentrations were

Table 2. Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Albumin and Total Protein

		Albumin		Total protein	
Age	n	g/L			
0–5 d, <2.5 kg	30	20–36		38–62	
0–5 d, >2.5 kg	93	26–36		54–70	
1–3 y	50	34–42		59–70	
4–6 y	38	35–52		59–78	
7–9 y	74	37–56		62–81	
10–19 y	332	37–56		63–86	
		Mean	CV, %	Mean	CV, %
Quality-control data					
Level 1		54	2.72	64	1.91
Level 2		26	1.81	49	1.95
Level 3		25	1.66	34	1.48

Table 3. Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Calcium, Phosphate, and Urea

		Calcium		Phosphate		Urea	
Age, y	n	mmol/L					
(0–5 d, >2.5 kg)	50	1.96–2.66		1.50–2.60			
1–3	50	2.17–2.44		1.25–2.10		1.8–6.0	
4–6	38	2.19–2.51		1.30–1.75		2.5–6.0	
7–9	72	2.19–2.51		1.20–1.80		2.5–6.0	
10–11	62	2.22–2.51		1.20–1.80		2.5–6.0	
12–13	73	2.19–2.64		1.05–1.75		2.5–6.0	
14–15	91	2.29–2.66		0.95–1.75		2.9–7.5	
16–19	107	2.22–2.66		0.90–1.50		2.9–7.5	
		Mean	CV, %	Mean	CV, %	Mean	CV, %
Quality-control data							
Level 1		3.29	2.14	3.36	1.77	29.9	2.10
Level 2		2.04	1.71	2.58	1.72	17.5	1.93
Level 3		1.35	1.75	1.39	1.88	8.6	2.31

Table 4. Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Uric Acid, Total Cholesterol, and Triglycerides

		Uric acid,	Cholesterol[a]	Triglycerides
Age, y	n	μmol/L	mmol/L	
1–3	49	105–300	1.15–4.70	0.31–1.41
4–6	38	130–280	2.80–4.80	0.36–1.31
7–9	72	120–295	2.90–6.40	0.32–1.46
Males				
10–11	28	135–320	3.25–5.95	0.27–1.55
12–13	32	160–400	3.30–5.95	0.27–1.64
14–15	39	140–465	2.75–5.80	0.38–1.86
16–19	41	235–510	2.85–5.70	0.38–1.58
Females				
10–11	34	180–280	3.30–6.30	0.44–1.58
12–13	40	180–345	3.25–5.55	0.42–1.47
14–15	50	180–345	3.35–5.55	0.43–1.52
16–19	68	180–350	2.75–5.60	0.42–1.58
		Mean CV, %	Mean CV, %	Mean CV, %
Quality-control data				
Level 1		696 1.76	7.2 1.98	3.78 1.36
Level 2		541 1.21	3.2 1.78	2.26 1.17
Level 3		232 1.83	2.6 1.87	1.22 1.83

[a] Note: To decrease the risk of atherosclerosis, it is recommended for adults that total serum cholesterol should not exceed 5 mmol/L.

Table 5. Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Five Serum Enzymes

		ALT	AST	ALP	GGT	CK	LDH
Age, y	n	U/L					
1–3	50	5–45	20–60	145–320	6–19	60–305	500–920
4–6	40	10–25	15–50	150–380	10–22	75–230	470–900
7–9	80	10–35	15–40	175–420	13–25	60–365	420–750
Males							
10–11	27	10–35	10–60	135–530	17–30	55–215	432–700
12–13	31	10–55	15–40	200–495	17–44	60–330	470–750
14–15	26	10–45	15–40	130–525	12–33	60–335	360–730
16–19	40	10–40	10–45	65–260	11–34	55–370	340–670
Females							
10–11	34	10–30	10–40	130–560	17–28	80–230	380–770
12–13	49	10–30	10–30	105–420	14–25	50–295	380–640
14–15	52	5–30	10–30	70–230	14–26	50–240	390–580
16–19	61	5–35	5–30	50–130	11–28	45–230	340–670
		Mean CV, %	Mean CV, %	Mean CV, %	Mean CV, %	Mean CV, %	Mean CV, %
Quality-control data							
Level 1		440 1.74	544 1.72	1050 3.2	1060 1.6	1090 4.9	1440 1.83
Level 2		75 2.91	180 2.31	248 3.4	206 1.5	600 4.0	1406 1.22
Level 3		45 5.17	54 5.22	105 3.6	86 2.2	198 5.0	697 1.58

CK, creatine kinase.

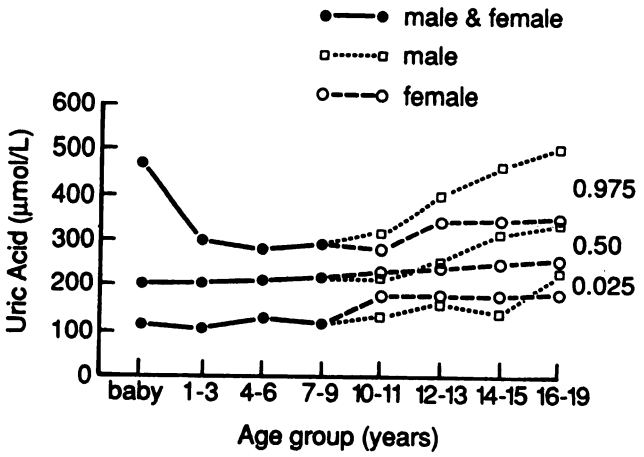

Fig. 1. Reference intervals (0.025, 0.5, and 0.975 fractiles) for uric acid at different ages for boys and girls

high in the term neonates. Urea concentrations in this group dropped very rapidly over the first five postnatal days [$r = -0.4, P < 0.001$, urea = $4.6 - (0.56 \times \text{day})$]. Because the reference interval is so time dependent, no single reference interval is reported.

We noted a marked sex-related difference in uric acid concentration. The reference interval for postpubertal boys was much higher than that for girls (Figure 1), as reported by others (*7*).

The cholesterol and triglyceride reference intervals determined in this population are shown in Table 4. Note that the upper limit in all groups older than six years exceeded the concentrations currently considered to represent upper limits for reduced risk of atherosclerotic cardiac disease in adults (*8*).

Outside the neonatal period, the reference interval for glucose was 3.9–7.0 mmol/L for both sexes. Glucose values increased in both the preterm low-birth-weight and term infants during the first five postnatal days. Reference intervals are not given because values vary with feeding and the use of early intravenous glucose infusions. Neither variable was controlled in our study.

Changes in serum unconjugated and conjugated bilirubin over the first postnatal week in 30 preterm infants are shown in Figure 2 as the 0.25, 0.5, and 0.95 fractile of the unconjugated bilirubin concentrations measured under the conditions of environmental light and phototherapy used in our nursery. These values are descriptive of our findings.

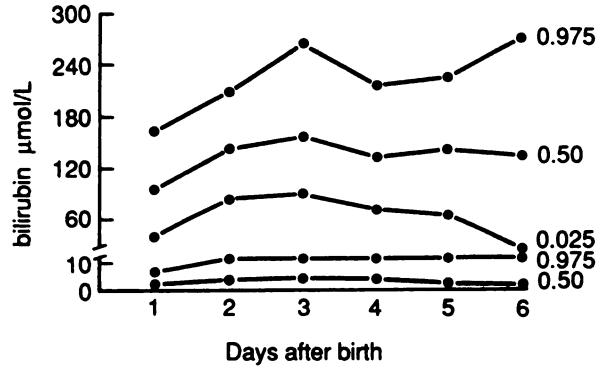

Fig. 2. Conjugated and unconjugated bilirubin (0.025, 0.5 and 0.975 fractiles) in 30 preterm infants from 1st to 6th day after birth

Upper three curves: fractiles for unconjugated bilirubin (μmol/L); *lower two curves*: fractiles for conjugated bilirubin (μmol/L)

They are *not* intended to represent values on which therapeutic decisions should be based. Outside the neonatal period, the reference interval for both sexes for unconjugated bilirubin was 3–17 μmol/L; that for conjugated bilirubin was <2 μmol/L.

The upper (0.975) reference limit for aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), alkaline phosphatase (ALP; EC 3.1.3.1), GGT, and LDH in the term neonate is compared with the adult upper limit in Figure 3. Values in the preterm infants were similar. A decline in enzyme activities in serum over the first five days postpartum was seen for AST, ALT, and LDH, whereas ALP and GGT did not change. The mean value on day 1 was 85 U/L for AST, 19 U/L for ALT, and 1603 U/L for LDH; values on day 5 were 45, 11, and 1036 U/L for AST, ALT, and LDH, respectively. Neonatal data are not reported for creatine kinase (EC 2.7.3.2), because samples obtained by capillary collection are not suitable for quantifying this enzyme and we were unable to obtain sufficient venipuncture samples for statistical validity. Marked age- and sex-related differences were found in older children for ALP (Figure 4), in accordance with previous reports (*9–11*). The higher upper limit of creatine kinase in postpubertal boys probably reflects a greater degree of physical activity than exhibited by their female counterparts. Little sex-related difference was seen for the remaining enzymes. The reference ranges are shown in Table 5.

GGT results by the Ektachem-700 are slightly higher than those reported by other methods. Values were high in the neonatal period, declined, and then increased with age more strongly in boys than in girls.

Reference intervals for LDH by Ektachem-700 are consid-

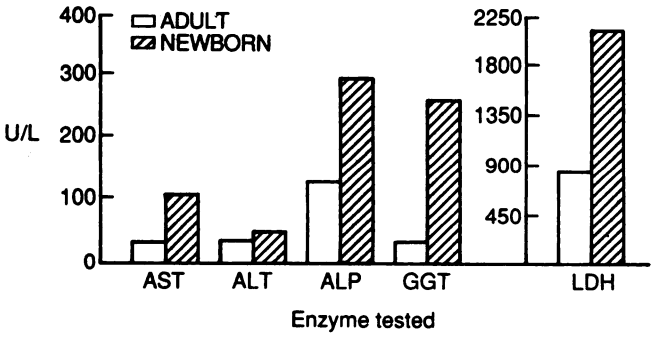

Fig. 3. Upper reference limits for five enzymes in term neonates compared with upper reference limits for adults

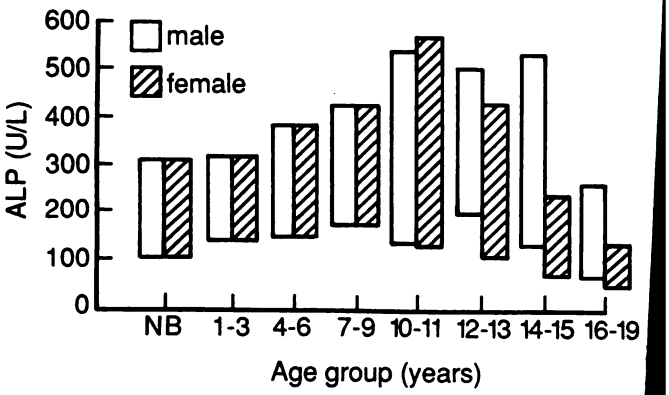

Fig. 4. Reference intervals (0.025 to 0.975 fractiles) for alkaline phosphatase at different ages for boys and girls

NB, newborns

erably higher than those reported by other methods. Results for samples from our study group measured simultaneously with the RA-1000 were approximately 30% lower than the Ektachem-700 range. Despite the differences in reference intervals measured with the two analyzers, both sets of data showed an obvious decreasing trend with age.

The reference interval for amylase in children ages one to 19 years was 30–100 U/L (n = 470), similar to the adult normal range. No sex-related difference was noted. Although Aggett and Taylor reported a different range, using a Phadebas blue starch method, our study supports their finding that adult amylase concentrations may be reached by one year of age (*12*).

The reference interval for lipase was 15–120 U/L for boys and girls between ages four and 19 years. Data from younger children were not available.

Age- and sex-specific reference intervals were determined in a pediatric population of multiple ethnic origins, for analytes measured with the Ektachem-700 multilayer film analyzer. Except for LDH and GGT, these reference intervals were not strikingly instrument dependent. The *absolute values* for age- and sex-specific upper and lower reference limits for an analyte may differ from data obtained by using a different method, thus making it incumbent on each laboratory to determine the direct transferability of these data to its own specific method and equipment. However, the *trend* of reference limits to increase or decrease with increasing age, and the sex-related effect on reference limits for some variables in postpubertal children, were method independent. This study provides a framework for comparison of reference data obtained by other methods.

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Age- and Sex-Specific Pediatric Reference Intervals and Correlations for Zinc, Copper, Selenium, Iron, Vitamins A and E, and Related Proteins

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Age- and sex-specific reference intervals based on the 0.025 and 0.975 fractiles of data derived from a healthy pediatric population are presented for zinc, copper, selenium, iron, ferritin, retinol, α-tocopherol, and related analytes in serum. Age was an important covariate for copper, selenium, retinol, and tocopherol, and ferritin in boys. Strong correlations were found between retinol and retinol-binding protein, prealbumin (transthyretin), α-tocopherol, and selenium. Tocopherol was highly correlated with both cholesterol and triglycerides. We found no relationship between serum zinc and either retinol or retinol-binding protein. Despite exclusion of children in whom anemia, microcytosis, or variant hemoglobins were found, the 0.025 fractile for iron in several age groups was even less than the concentration considered to indicate poor iron nutritional status.

Additional Keyphrases: *tocopherol · retinol · retinol-binding protein · transthyretin · cholesterol · triglycerides*

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We have defined age- and sex-specific reference intervals for serum zinc, copper, selenium, retinol, α-tocopherol, iron, transferrin saturation, and ferritin in a healthy pediatric population.

Materials and Methods

The study population and the general and statistical methods are described elsewhere (*1*). Specific precautions were observed throughout this study to minimize sample contamination for the trace-element analyses and prevent