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The Levels of Serum Enzymes, Plasma Proteins and Lipids in Normal Infants and Small Children

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Summary: A group of 291 children aged 3 weeks to 6½ years was examined at a public maternal and child health center and 260 of them – who were considered to be healthy – were included in the present study. By venipuncture, serum was obtained for the analysis of 6 enzymes, and plasma for the estimation of 9 proteins and for lipid analyses. In different age groups, high levels were found for alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, creatine kinase and γ-glutamyl transferase. Haptoglobin, α_1-antitrypsin, prealbumin and transferrin were present at low concentrations during the first months of life. Transferrin rose later in childhood to above adult levels. Only immunoglobulin M showed a sex difference, with higher values for girls. Breast-fed infants had higher (non-fasting) concentrations of cholesterol and triglycerides than formula-fed babies, and they also had higher levels of aspartate aminotransferase and alanine aminotransferase.

Serum-Enzyme, Plasma-Proteine und -Lipide bei normalen Kindern und Kleinkindern

Zusammenfassung: 291 Kinder zwischen 3 Wochen und 6½ Jahren wurden in einem staatlichen Mutter und Kind-Gesundheitszentrum untersucht. 260 als gesund angesehene Kinder wurden in diese Studie aufgenommen. Durch Venenpunktion wurde Serum für die Analyse von sechs Enzymen und Plasma für die Bestimmung von neun Proteinen und Lipidanalysen gewonnen. In verschiedenen Altersgruppen wurden hohe Werte für alkalische Phosphatase, Lactatdehydrogenase, Aspartat- und Alaninaminotransferase, Kreatinkinase sowie γ-Glutamyltransferase gefunden. Haptoglobin, α_1-Antitrypsin, Präalbumin und Transferrin haben während der ersten Lebensmonate geringe Konzentrationen. Transferrin stieg später in der Kindheit auf Werte oberhalb derer Erwachsener. Nur Immunglobulin M zeigt einen Geschlechtsunterschied mit höheren Werten bei Mädchen. Gestillte Kinder (nicht fastend) wiesen höhere Konzentrationen an Cholesterin und Triglyceriden als auch höhere Werte an Aspartat- und Alaninaminotransferase als mit Fertignahrung gefütterte auf.

Introduction

Reference values for most plasma constituents are now relatively established for healthy adults. Much less is known about how the concentrations change during childhood (1–4). The levels of several serum enzymes, plasma proteins and lipids in children have been reported to differ greatly from those in adults, but there is much controversy in the literature (3–16). This may partly be due to differences between the groups of subjects studied, partly to lack of standardized blood sampling, proper storage or to differences in analytical performance. The present study was undertaken as an attempt to establish reference values ("normal ranges") for some of the most commonly analyzed proteins, lipids and enzymes in infants and small children, with minimization of the above sources of error.

Materials and Methods

Subjects

The subjects were children aged 3 weeks to 6½ years, who arrived with one of their parents at a public maternal and child health center for routine examination. To avoid stratification all children examined during a period of three months were originally included in the material. The subjects were non-fasting partly for practical reasons and partly because long fasting periods hardly can be considered as "normal" in low age groups. A clinical history was recorded, including questions about pregnancy, delivery, development, nutrition, previous diseases, vaccinations, drugs and recent minor illness. The children also underwent a thorough clinical examination (including the measurement of height and weight) by a senior paediatrist. On the basis of this information, 31 of the 291 children (10.7%) were excluded from the material prior to any analysis of blood samples (mainly because of present or recent infectious disease). The remaining 260 subjects were all included in the present study. Their age and sex distribution is shown in table 1. The youngest and the oldest age groups were the smallest. Both sexes were about equally represented.

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Tab. 1. Age and sex distribution of the children included in the present study.

Age groups	Girls	Boys	Total
3–8 weeks	5	8	13
2–4½ months	13	18	31
4½–9 months	17	25	42
9 months–1½ year	28	30	58
1½–2½ years	22	13	35
2½–3½ years	21	18	39
3½–4½ years	8	10	18
4½–5½ years	8	7	15
5½–6½ years	3	6	9
Sum	125	135	260

Blood samples

The samples were drawn by venipuncture (using Vacutainers® – either heparinized or without additions) after stasis of short duration, and always by the same experienced laboratory technician. (A few samples from the smallest babies were drawn by scull venipuncture). The enzymes were assayed in serum, but heparin plasma was used for the proteins and lipids, to obtain optimal sample volumes. Pilot studies indicated that this was without relevance for the analytical results. The samples were centrifuged as soon as possible, divided into small vials and stored at – 70 °C until analysis (except for α-lipoprotein and the enzymes, which were analyzed on the same or the following day after storage at + 4 °C).

Analyses

The analyses of each component were performed as part of common routine series, together with quality control material. The enzymes were analyzed by methods recommended by the Scandinavian Committee on Enzymes (17–19). The coefficients of variation (CV) were always less than 5%.

All proteins were quantitated by single radial immunodiffusion (20). Antisera from Behringwerke AG, Marburg, W. Germany were used. The antiserum against α-lipoprotein estimates chiefly apolipoprotein A-I, but is to a minor extent also directed against apolipoprotein A-II. For the α-lipoprotein quantitation, a fresh plasma pool from 11 healthy individuals was used as reference plasma, and its value stated to be 100%. For the other protein analyses, reference sera from Behringwerke AG were used (but the α_1-antitrypsin values were recalculated by means of Seronorm® Protein, Nyegaard & Co., Oslo, Norway, which is standardized with purified α_1-antitrypsin). The total day to day CV varied between 4.3% (for prealbumin) and 5.9% (for haptoglobin), as calculated from analysis of pool-sera included in all series. Total cholesterol and triglycerides were estimated in *iso*-propanol extracts of plasma by methods described previously (21). The standardization and quality control programs have been reported previously (21).

Reference values for adults

Most values are based upon examination of subgroups of a reference group constituting 621 women and men, described previously (22).

Statistics

The data were subjected to statistical treatment using an IBM 370 computer. For each component, the arithmetic mean and the frequency distribution was calculated both for the whole material and for each subgroup (age and sex) separately.

On the basis of practical clinical chemical judgement groups with small differences in mean values were fused. Except for immunoglobin M the sex differences for the analysed substances were so small that no separate treatment was indicated

These secondary and larger groups (different for each substance) were then approximated to *Gaussian* distribution by eight different statistical computer programs (23). The 2.5 and 97.5 percentiles of the best approximations were chosen as reference limits (tab. 2).

Subsets of data were compared by *Wilcoxon* rank sum test or *Student*'s t-test.

Results and Discussion

The reference intervals for each substance throughout infancy and early childhood are presented in table 2. The reference intervals for adults, by application of the same methods and standards, are shown for comparison.

The changes in the plasma concentration of a substance with age is, of course, a continuous process, and any division into groups must be arbitrary. For the daily routine use, however, it is practical to operate with a limited number of reference intervals for each substance. A more correct impression of the continuous changes with age is obtained from the diagrams in figure 1, in which the original groups of age and sex are maintained.

Enzymes

Alanine aminotransferase was found to be present at high levels in the lowest age groups, but reached adult levels from the age of about 1½ years (fig. 1).

A comparable but even more striking decrease was observed for γ-glutamyltransferase during the first months of life. Throughout childhood alkaline phosphatase levels were several times higher than the adult levels, and were highest before 2½ years of age (fig. 1). The main reason is probably skeletal growth.

For aspartate aminotransferase (fig. 1) and lactate dehydrogenase, the levels were high in all age groups – but with a tendency to decline. High values were also found for creatine kinase, particularly until about 4 years of age. Still higher creatine kinase values are known to occur shortly after delivery (24).

In contrast to the situation in adults, no significant sex differences were observed for any of these enzymes.

Proteins

The three main classes of immunoglobulins were all present at lower concentrations in small children than in adults. Being the only immunoglobulin which crosses the placenta, IgG is known to be present in about maternal concentrations at birth, but falls rapidly after delivery (7, 25). We observed the lowest levels in the second and third age groups and a steady increase from about 9 months on (fig. 1).

The IgA concentration was very low in newborn, only about one tenth of that in adults, and increased slowly throughout childhood (fig. 1). The same pattern was seen for IgM (fig. 1) but the changes were much smaller.

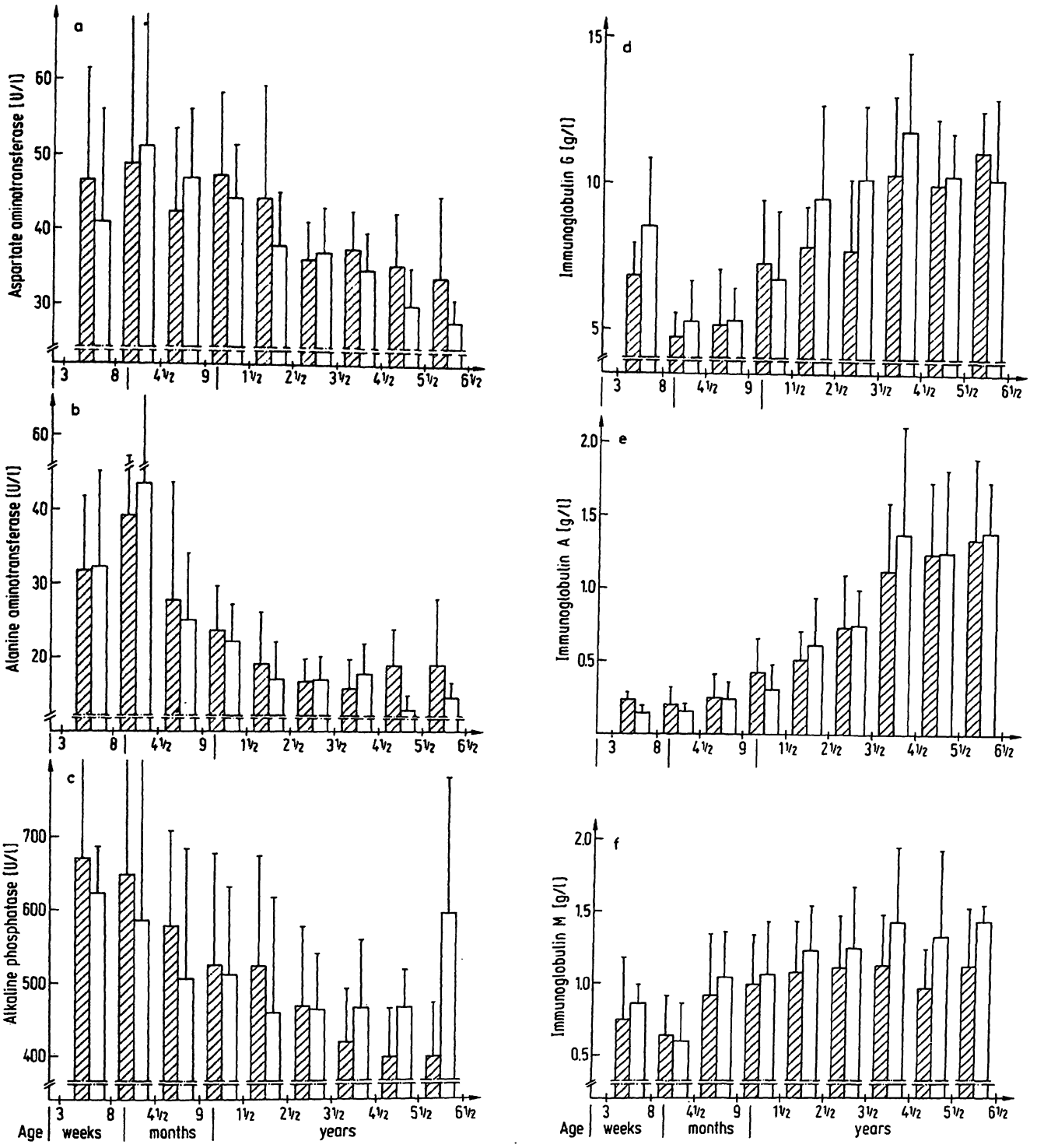

Fig. 1. The changes in plasma concentration of a) aspartate aminotransferase, b) alanine aminotransferase, c) alkaline phosphatase, and d)–f) immunoglobulin classes G, A and M, during infancy and early childhood.
The height of the columns represent the arithmetic means (boys ▨, girls □). The vertical lines represent one standard deviation.

For the age 1½–6½ years, girls were found to have significantly higher IgM concentrations than boys (t-test: $p < 0.01$). This is in accordance with previous reports (25–27).

The concentrations of α_1-antitrypsin were found to be rather close to those in adults (28), except for a moderate reduction during the first months of life.

After the first few months, transferrin rose to a higher level than in adults and remained relatively unchanged in the period ½–6½ years.

Exact values for haptoglobin are hard to obtain as long as the phenotypes are unknown, particularly by the radial immuno-diffusion technique used in the present study (29). This protein was not detectable in about

Tab. 2. Calculated reference intervals (2.5 and 97.5 percentiles) in relation to age and sex.

		3 w	2 m	4½ m	9 m	1½ y	2½ y	3½ y	4½ y	5½ y	6½ y	Adults
Alanine aminotransferase (U/l)			14–84		11–46			9–28				♀ 9 – 28 ♂ 10 – 41
Aspartate aminotransferase (U/l)				26–75				24–49				13 – 38
Lactate dehydrogenase (U/l)					408–1018				436–882			227 –452
Alkaline phosphatase (U/l)			275–1050		280–885			300–660				55 –195
Creatine kinase (U/l)			34–242			40–300			42–172			♀ 35 –106 ♂ 30 –156
γ-Glutamyltransferase (U/l)			10–60	10–35				5–14				♀ 6 – 26 ♂ 8 – 32
IgG (g/l)			5.1–11.7		3.5–8.8			4.2–14.3		6.7–15.6		8.7 – 18.0
IgA (g/l)				0.09–0.53		0.11–0.97	0.15–1.07	0.24–1.44		0.44–2.39		0.50– 4.00
IgM (g/l)	♀			0.35–1.75				0.60–2.34				0.60– 3.00
	♂			0.35–1.75				0.57–1.88				0.50– 2.60
Transferrin (g/l)			1.23–2.85	1.69–3.53				2.49–4.25				♀ 2.00– 3.50 ♂ 2.00– 3.20
Haptoglobin (g/l)				0–2.54 [1]				0.35–2.90				0.40– 2.80
Ceruloplasmin (g/l)			0.14–0.43					0.30–0.59				0.15– 0.32
α_1-Antitrypsin (g/l)			0.75–1.53					0.93–1.84				1.40– 3.00
Prealbumin (g/l)			0.06–0.19					0.11–0.25				0.18– 0.42
Cholesterol (mmol/l)			2.2–4.2		2.6–5.5			2.9–6.5				♀ 3.1 – 6.9 [2] ♂ 2.9 – 7.7 [2]
Triglycerides (mmol/l)				0.7–2.9			0.6–2.4			0.4–1.8		♀ 0.5 – 1.4 [2] ♂ 0.3 – 1.7 [2]
α-Lipoprotein (%)			73–122			64–113				74–116		75 –125

[1]) Observed range [2]) 15–29 years, fasting

half of the subjects below the age of 8 weeks. This observation is in agreement with previous reports (9, 11), but the cause of it is not definitely established. Increased haemolysis in some of the subjects appears to be a more likely explanation than low synthesis, since near normal adult levels were present in other children in this age group.

Prealbumin appeared in lowest concentrations during the first weeks of life. The level was thereafter rather constant and about the half of that in adults. Caeruloplasmin, on the other hand, showed higher values than in adults throughout most of the childhood.

Lipids

Cholesterol was rather low during the first months of life while the triglycerides appeared in highest concentrations in the lowest age groups. This latter phenomenon might simply be caused by a more recent intake of food in this age group. Later in childhood, the lipid levels did not differ much from those in young adults as shown earlier (30). The values for α-lipoprotein were rather similar to those in adults. There were no significant sex differences for any of the lipids.

Differences between groups with different nutrition

The age group 2–4½ months was investigated further to disclose possible differences between breast-fed and formula-fed infants. Two subjects were excluded from the study, since they had received both kinds of nutrition. The two subgroups were compared with respect to all the 17 components analyzed.

Significant differences were found only for the aminotransferases and partly for the lipids (tab. 3). It is well known that the triglyceride concentration is influenced by the lipid composition of the diet. It cannot be excluded, however, that the observed difference in our results is simply due to different times of feeding in relation to the moment of blood sampling. The difference in cholesterol between the two groups was small, but is in accordance with previous observations (31, 32).

The great and highly significant difference between breast-fed and formula-fed infants with respect to both alanine aminotransferase and aspartate aminotransferase remains unexplained. We are not aware that this has been described before. There were not differences in age, sex, birth weight, actual body weight or length which could account for the observed differences.

Tab. 3. Plasma levels of lipids and aminotransferases in breast-fed and formula-fed children.

		Breast fed (n = 13)		Formula fed (n = 16)		Significance of difference by *Wilcoxon*'s rank sum test
		Mean	SD	Mean	SD	
Alanine aminotransferase	(U/l)	51	22	31	15	$p < 0.01$
Aspartate aminotransferase	(U/l)	64	22	37	9	$p < 0.01$
Cholesterol	(mmol/l)	4.08	0.65	3.64	0.72	$p < 0.2$
Triglycerides	(mmol/l)	2.02	0.61	1.45	0.42	$p < 0.02$

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