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CEREBROSPINAL FLUID AND PLASMA AMINO ACID LEVELS IN PATHOLOGICAL NEWBORN INFANTS

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The concentration of 22 amino acids was measured parallel in the cerebrospinal fluid and plasma in 14 pathological newborn infants of 36.3±4.3 (29-41) weeks gestational age and 2410±890 (1270-3950) grams birthweight, at the age of 37.8±4.4 (30-43) postconceptional weeks. Wide inter-individual variation was found in the cerebrospinal fluid concentration of each amino acid. In some of the babies the concentration of a few amino acids in the cerebrospinal fluid was nearly equal to the plasma level, and the concentration of glutamine, threonine and histidine was even higher than that in the plasma. Reverse relationship was observed between the maturity of neonates and the CSF/plasma amino acid ratio, which was significant for citrulline, methionine, valine, phenylalanine, isoleucine, lysine and leucine. Significant positive correlation was found between the plasma and cerebrospinal fluid concentration of threonine, arginine, citrulline, methionine, ornithine, isoleucine and lysine.

INTRODUCTION

In spite of growing interest in the biochemical development of human foetal and neonatal brain, the accumulated knowledge is still fragmentary partly due to right ethical concerns. Studies on cerebrospinal fluid (CSF), however, provide some insight into brain metabolism, since the composition of CSF is, at least to some extent, determined by the biochemical milieu of the central nervous system parenchyma.

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Distortions of amino acid metabolism and/or haemato-encephalic exchange in the newborn infant may result in additional damage of the brain which is, as well known, at risk by a number of other factors related to various perinatal pathology. This assumption is backed by recent observations suggesting that amino acid imbalance may adversely influence neurochemistry /12/. Some neurotransmitters per se are amino acids or their derivates /7/, of which increased CSF concentration have been found to be related with convulsive activity /4, 14/. Rothman and Olney /13/ suggested that glutamate may have a role in the pathophysiology of hypoxic-ischaemic brain damage.

The objective of this study was to measure CSF amino acid levels parallel with plasma concentrations in newborn infants in order to collect baseline data furthermore to look for maturity or pathology dependent characteristics of amino acid transport between blood and central nervous system.

PATIENTS AND METHODS

Fourteen newborn infants of 36.3±4.3 (29-41) weeks gestational age and 2410±890 (1270-3950) grams birthweight (means±SD) were studied at the postconceptional age of 37.8±4.4 (30-43) weeks. All study babies suffered from severe perinatal pathology, related to hypoxia-asphyxia in 9, major extra-central nervous system malformation in 3 and septicemia with no meningitis in 2 cases. The lumbar puncture and blood sampling was decided on clinical ground as a part of diagnostic workup. Amino acid concentrations were measured in addition to routine biochemical and bacteriologic studies. The babies were fed with banked human milk or in case of need received glucose-saline drip infusion. Blood and CSF samples were collected between 8 and 11 in the morning.

The blood samples were taken from peripheral vein by venepuncture and the plasma was immediately separated. Plasma and CSF samples were stored at -20 ^{o}C until assayed. After thawing, 400 µl absolute ethanol was used for deproteinization of the 50 µl plasma and 100 µl CSF specimens. For internal standard norleucine was applied in 1 mmol/l concentration. For precolumn derivatization of amino acids the PITC-method was used as described in Knapp's book /10/. The analysis was performed on Beckman System Gold HPLC instrument with Supelco C-18 DB 250x4.6 column /3/. Since glutamate and asparagine could not in each sample be separated clearly, their summed

total concentrations have been calculated and given in the tables. For statistical analysis standard mathematical methods were used.

RESULTS

The median and mean concentrations of the 22 amino acids measured parallel in plasma and CSF are shown in Tables I and II. It is to be noted that a wide variablity both in plasma and CSF concentration of the individual amino acids could be observed. When studying the ratio between CSF and plasma levels, the median and mean values were found to vary between 0.007-0.53 and 0.10-0.67, respectively (Table III), apart from glutamine. Evaluating the extremes, it is seen that in a few babies the concentration of some amino acids, like histidine, threonine, arginine, tyrosine, methionine, phenylalanine and leucine was near equal or even a bit higher in the CSF than that in their plasma. An intriguing finding is the much higher median and mean concentration of glutamine in CSF than in the plasma. The explanation for this findings is not clear, however, it is known that technical errors may easily alter measured glutamine concentration, mainly due to transamination during the procedure. But after all, this type of error would have resulted in false low concentrations, but not in the opposite.

In order to see the effect of maturity and plasma amino acid content on CSF amino acid levels, linear correlation analysis was performed between postconceptional age and CSF/plasma concentration ratio and also between plasma and CSF amino acid concentration. Reverse correlation was found between maturity and CSF/plasma amino acid ratio for each amino acid measured, which was statistically significant for citrulline ($r = -0.590$, $p<0.05$), methionine ($r = -0.545$, $p<0.005$), valine ($r = -0.679$, $p<0.001$), phenylalanine ($r = -0.638$, $p<0.05$), isoleucine ($r = -0.752$, $p<0.01$), lysine ($r = -0.639$, $p<0.01$)

TABLE I

Plasma amino acid levels in 14 pathological newborn infants of 30-43 weeks postmenstrual age (μmol/l)

	Median	Mean	SD	Range
ASP	63.5	68.9	22.6	(45.9-109.4)
GLU+ASN	408.1	405.2	144.1	(257.0-674.9)
GLN	218.2	245.0	146.9	(34.8-561.4)
GLY	232.0	212.1	83.7	(107.2-383.2)
HIS	134.2	126.5	55.5	(52.0-236.0)
THR	81.4	79.5	32.1	(39.0-144.0)
ARG	72.9	73.2	37.3	(31.4-173.8)
CIT	31.4	36.1	22.0	(13.0- 93.2)
ALA	264.2	275.8	61.4	(144.0-391.4)
HYP	36.4	38.2	25.6	(13.8-118.2)
TYR	77.8	100.2	69.7	(42.8-307.6)
SER	128.8	135.9	53.5	(50.8-226.4)
MET	23.0	30.0	11.5	(10.6- 48.6)
VAL	139.6	131.3	39.7	(69.8-193.2)
PRO	156.2	183.0	80.3	(56.6-339.8)
TRP	80.8	85.8	34.9	(32.1-154.8)
ORN	110.3	131.4	40.0	(82.5-214.6)
PHE	83.2	87.6	24.9	(51.8-131.2)
ILEU	42.7	43.0	14.4	(22.7- 70.2)
LYS	116.0	133.2	53.1	(83.8-278.0)
LEU	92.9	96.9	28.5	(50.4-142.4)

TABLE II

Cerebrospinal fluid amino acid levels in 14 pathological newborn infants of 30-43 weeks postmenstrual age (μmol/l)

	Median	Mean	SD	Range
ASP	17.4	19.9	8.4	(10.0- 30.5)
GLU+ASN	74.1	91.0	51.2	(18.6-225.1)
GLN	419.7	388.9	140.9	(154.5-609.7)
GLY	23.3	37.2	38.0	(1.5-126.9)
HIS	70.2	71.1	39.3	(34.3-165.1)
THR	38.6	55.1	40.5	(13.1-158.3)
ARG	30.7	41.3	33.8	(8.0-128.7)
CIT	6.1	7.5	4.6	(2.4- 16.7)
ALA	41.9	59.9	35.7	(19.3-139.3)
HYP	8.2	7.2	5.5	(0.4- 15.2)
TYR	41.6	41.4	24.2	(10.2- 88.9)
SER	56.2	68.9	42.9	(23.4-160.2)
MET	14.7	14.5	8.5	(0.8- 32.4)
VAL	31.9	36.9	25.1	(7.2-104.5)
PRO	13.9	17.5	10.5	(1.1- 39.3)
TRP	5.1	12.2	11.4	(1.0- 35.3)
ORN	12.2	14.9	11.3	(3.7- 36.7)
PHE	24.2	29.9	20.5	(8.6- 91.7)
ILEU	9.9	10.7	8.0	(2.8- 32.6)
LYS	29.0	37.2	32.7	(9.4-129.0)
LEU	22.8	31.1	31.9	(8.3-128.0)

TABLE III

CSF/plasma amino acid ratio in 14 pathological newborn infants of 30-43 weeks postmenstrual age (μmol/l)

	Median	Mean	SD	Range
ASP	0.27	0.31	0.15	(0.11-0.64)
GLU+ASN	0.22	0.22	0.10	(0.04-0.40)
GLN	1.91	2.33	1.90	(0.41-7.04)
GLY	0.14	0.17	0.18	(0.00-0.66)
HIS	0.53	0.63	0.34	(0.20-1.19)
THR	0.51	0.67	0.35	(0.24-1.15)
ARG	0.38	0.54	0.30	(0.09-0.97)
CIT	0.21	0.21	0.09	(0.08-0.43)
ALA	0.20	0.21	0.13	(0.07-0.49)
HYP	0.29	0.20	0.14	(0.01-0.38)
TYR	0.47	0.46	0.27	(0.14-0.99)
SER	0.49	0.53	0.29	(0.17-0.78)
MET	0.44	0.48	0.25	(0.04-0.99)
VAL	0.22	0.30	0.20	(0.04-0.75)
PRO	0.08	0.10	0.08	(0.00-0.26)
TRP	0.07	0.16	0.15	(0.02-0.50)
ORN	0.10	0.10	0.05	(0.02-0.22)
PHE	0.27	0.33	0.20	(0.12-0.91)
ILEU	0.20	0.24	0.13	(0.10-0.51)
LYS	0.23	0.25	0.14	(0.09-0.62)
LEU	0.24	0.31	0.24	(0.06-0.90)

and leucine ($r = -0.757$, $p<0.01$). Statistically significant positive correlation was found between the plasma and CSF concentration of threonine ($r = 0.638$, $p<0.01$), arginine ($r = 0.797$, $p<0.001$), citrulline ($r = 0.686$, $p<0.01$), methionine ($r = 0.584$, $p<0.05$), ornithine ($r = 0.767$, $p<0.01$), isoleucine ($r = 0.549$, $p<0.05$) and lysine ($r = 0.774$, $p<0.01$).

DISCUSSION

In spite of numerous excellent studies on CSF amino acid levels /1, 2, 5, 6, 8, 16, 17/ no solid knowledge could so far be accumulated in this field. The reason of this is multiple. Methodological discrepancies and technical difficulties surely contribute to the widely varying CSF amino acid concentrations reported in the literature. Uncertainities in the interpretations can be explained by the lack of normal control values on the one hand, and by the fact that we still know very little about the physiology of the regulation of CSF composition, on the other hand.

In the present study the median and mean plasma and CSF amino acid levels of neonates were found to be in the same range as reported by previous authors /1, 5, 9, 11, 15, 16/. Comparing neonatal CSF amino acid concentrations with published relevant data in children and adults /1, 2, 5, 6, 8, 16/, a tendency of higher levels could be observed, however, inter-individual variations regarding each amino acid measured in this study were remarkable. It is generally agreed that the concentration of the different amino acids in the CSF of children and adults ranges between 5-50% of the plasma level, furthermore that glutamine is the main amino acid in the cerebrospinal fluid. We observed similar CSF/plasma amino acid ratios in the newborn babies, as far as the mean or median values are concerned. But considering the extreme values it was found that in a few babies CSF concentration may be nearly

equal or even exceed plasma concentration of arginine, tyrosine, methionine, phenylalanine, leucine and glutamine, histidine, threonine, respectively (Table III).

In principle, amino acid content of CSF may be influenced by the plasma concentration, the net balance of barrier functions and the amino acid uptake and metabolism of the brain. Our finding of the close relationship between plasma and CSF concentration of threonine, arginine, citrulline, methionine, ornithine, isoleucine and lysine suggests a plasma level dependent transport of these amino acids into the cerebrospinal fluid. The blood-CSF barrier for these amino acids probably fails to function, either due to immaturity or due to some damage. The reason of the remarkably higher glutamine and somewhat higher histidine and threonine concentration in the CSF of some of the neonates, compared with that in their plasma, remains obscure. Both active transport or defective clearence from the CSF may have a role in this phenomenon.

The maturity of the newborn infants as expressed by their postconceptional age related inversely to the CSF/plasma concentration ratio of each individual amino acid measured in this study. This would mean that the more immature the baby is the higher is the concentration of the various amino acids in the CSF, relatively to the plasma level. Although this relationship was statistically significant for only seven amino acids like citrulline, methionine, valine, phenylalanine, isoleucine, lysine and leucine, the trend may well indicate a role for the functional maturity in the regulation of amino acid transport of the central nervous system.

In summary it is concluded that the regulation of CSF amino acid concentration in the newborn infant is probably maturity related, but in case of some of the amino acids plasmaconcentration is the main determinant. The effect of perinatal pathology on regulatory disturbances remains to be elucidated. The questions, what is abnormal, what is the cause and the effect are still unanswered.

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OSTEOCALCIN AND CALCIUM-PHOSPHATE METABOLISM PARAMETERS IN NEWBORN INFANTS

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Serum osteocalcin, calcium, phosphate, total alkaline phosphatase and urinary calcium and phosphate excretion were measured in 21 full term (327±650 g) and 29 preterm (1790±140 g) infants, on the postnatal day of 6.6±0.7. Preterm babies had slightly but not significantly higher osteocalcin level (3.9±2.1 vs 3.1±2.4 nmol/l) than full term babies. No close relation was found between serum osteocalcin concentration and calcium-phosphate metabolism parameters studied or physical body growth parameters measured.

INTRODUCTION

Osteocalcin is known as a vitamin K dependent calcium binding protein, synthetized by the osteoblasts /6/. It has been suggested that serum osteocalcin may well reflect the formation of mineralized bone tissue /1, 4, 15/ since its concentration was found to rise in periods of increased growth rate and bone turnover /1, 2, 4, 5, 8, 12, 14, 15/. However, the functional relationship between osteocalcin and calcium-phosphate metabolism and its hormonal regulation seems to be likely, the mechanisms involved have yet to be clarified. It is now known that $1,25(OH)_2$-D_3 vitamin stimulates osteocalcin synthesis, both in the human neonate and various experimental animals /3, 10, 12/.

In spite of the growing number of reports on osteocalcin in the human adult with different bone and related diseases /9,

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11, 13/, much less information is available on the physiologic significance and role of osteocalcin in the newborn infant. The object of the present study was to look for relations between calcium-phosphate metabolism parameters and osteocalcin level in the newborn infant, on the one hand, and examine if physical growth parameters and maturity does influence neonatal osteocalcin concentration or not.

PATIENTS AND METHODS

Serum osteocalcin, calcium, phosphate and total alkaline phosphatase activity were measured on postnatal day 6.6±0.7 (5-8) in 50 newborn infants (mean±SD). Parallel with the blood sampling a urine sample was collected for determining urinary Ca/creatinine and PO_4/creatinine ratio. All babies in this study suffered from pathology which was related to perinatal hypoxia-asphyxia in 14 cases and some other kind of adaptation disorder or infection in the rest of them. Twenty nine of the newborns were preterms with gestational age and birthweight of 32.8±2.6 (28-36) weeks and 1790±410 (1040-2720) grams, respectively, whilst 21 neonates were full terms of 38.4±1.6 weeks gestational age and 3270±650 grams birthweight. Up to the time of the investigation no vitamin D_3 or calcium supplementation was given to the babies. Their fluid and calorie requirement was covered by drip of 5% glucose-electrolyte solution infusion and/or oral feeding of banked human milk, according to needs.

The concentration of calcium, phosphate, total alkaline phosphatase in serum and urinary calcium and phosphate excretion was measured by using routine laboratory methods. Urinary calcium/creatinine and phosphate/creatinine ratio in a single sample was accepted representing total daily excretion /7/. Serum osteocalcin concentration was determined using double-antibody RIA, applying 100 µl specimens in duplicates. Sample collection, storing and assay technique followed the prescription of the manufacturer (MTA Isotope Research Institute).

Gestational age of the study babies was calculated according to the menstrual date of their mothers. Body weight, length and head circumference measurement were taken with ±10.0 g and ±0.1 cm accuracy, respectively. For statistical analysis standard mathematical methods were used.

RESULTS

The main results of the study are shown in Table I and Fig. 1. It is seen that osteocalcin concentration and total alkaline phosphatase activity was higher in preterm than that

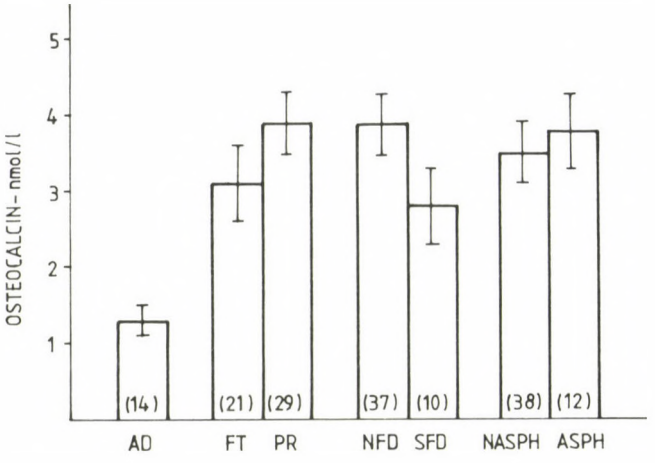

Fig. 1. Serum osteocalcin concentration (mean±SE) in 6.6±0.7 days old newborn infants, compared to adult values (AD). Newborn infants were grouped and compared according to various criteria, such as gestational age (FT full terms and PR preterms), intrauterine growth rate (NFD normal for dates and SFD small for dates), and the experience of perinatal asphyxia they suffered from or not (NASPH no asphyxia, ASPH asphyxia). Number of patients in the groups is shown in bracket, at the bottom of columns

in full term infants, however, the differences were statistically not significant. Serum calcium and anorganic phosphate level was closely similar in the two groups of neonates, just like urinary calcium excretion. In preterm babies a slightly larger phosphate excretion was observed than in babies born at term (5.0±5.7 vs 4.0±3.2 mmol/mmol).

TABLE I

Serum osteocalcin and calcium-phospate metabolism parameters measured in 6.6±0.7 (5-8) days old full term and preterm newborn infants. No statistically significant difference was found between the two groups regarding any parameters tested

	Full term babies (21)		Preterm babies (29)	
	mean±SD	range	mean±SD	range
Serum				
Osteocalcin (nmol/l)	3.1 ± 2.4	0.9 - 12.2	3.9 ± 2.1	1.4 - 10.6
Calcium (mmol/l)	2.3 ± 0.2	1.8 - 2.8	2.3 ± 0.2	1.2 - 2.6
Phosphate (mmol/l)	2.0 ± 0.5	1.0 - 2.7	2.2 ± 0.6	0.9 - 3.0
Alkaline phosphatase (IU/l)	252 ± 92	47 - 500	315 ± 122	77 - 638
Urine				
Ca/creat (mmol/mmol)	0.37± 0.04	0.04 - 2.11	0.35± 0.60	0.04 - 3.20
PO_4/creat (mmol/mmol)	4.0 ± 3.2	0.03 - 14.41	5.0 ± 5.7	0.23 - 27.63

Since no statistically significant difference was found between the osteocalcin level of full term and preterm newborn infants, furthermore because of the lacking significant correlationship between serum osteocalcin and gestational age, the two groups were considered together and analysed in relation with some other clinical parameters. Fig. 1 shows that small for date newborns had lower osteocalcin concentration than normal for dates (2.8±1.5 vs 3.9±2.4 mmol/l), furthermore that newborn babies who experienced perinatal hypoxia-asphyxia had similar osteocalcin concentration to those without asphyxia (3.8±1.8 vs 3.5±2.4 mmol/l). The wide variation of individual osteocalcin concentrations in any of the created groups of studied babies is, however, outstanding. Small-for-dateness was defined as birthweight less than the sex-matched 10 percentile and/or characteristic features of intrauterine growth retardation. Perinatal hypoxia-asphyxia was assigned to patients who needed resuscitation at birth and developed hypoxia-asphyxia related pathology later on, with oxygen dependency.

For comparison, osteocalcin level was measured in 14 adults as well and a serum concentration of 1.3±0.7 nmol/l was found, which is significantly ($p<0.05$-0.001) lower than the level in any studied groups of the newborn infants.

Results of the correlation analysis performed between osteocalcin level and various physical growth and calcium-phosphate metabolism parameters are summarized in Table II. It can be seen that maturity of the babies expressed by their gestational age, various body measures like birthweight, length and head circumference and the calcium phosphate metabolism parameters studied by us did not correlate significantly to serum osteocalcin concentration.

DISCUSSION

Since osteocalcin is known as a biochemical marker of bone tissue formation, studies on neonatal changes in its

TABLE II

Parameter-pairs tested by correlation analysis in the combined group of full term and preterm neonates. The least value of r in case of statistically significant correlation would have been 0.2759

	parameters tested	r
Osteocalcin	Gestational age	(0.0632)
	Birthweight	(0.0062)
	Length at birth	(0.0546)
	Head circumference	(0.0450)
	Serum calcium	(0.0825)
	Serum phosphate	(0.0916)
	Serum ALP (total)	(0.2474)
	Urine Ca/creat ratio	(-0.1228)
	Urine PO_4/creat ratio	(-0.0437)

concentration may have importance from the point of view both of perinatal physiology and clinical practice.

In accordance with previous report /2, 3, 10/ we found significantly higher mean osteocalcin level in newborn infants than adults (Fig. 1). The much wider range of individual osteocalcin concentrations in the neonates than that in the adults, however, needs to be emphasized. In preterm infants mean osteocalcin level was slightly higher than that of full term babies, but the lack of significant correlation between gestational age and osteocalcin concentration makes likely that maturity and osteocalcin level are not closely related to each other, at least in the gestational age period we studied.

Osteocalcin concentration has been found to rise during periods of growth spurt and be higher all throughout the time of active bone growth /2, 4, 8, 14/. In newborn infants we could not find significant associations between length and head

circumference and osteocalcin level, but small for date babies have conspicuosly lower concentrations than normal for date infants (2.8±1.5 vs 3.9±2.4 nmol/l). Cole et al. /2/ observed decreased osteocalcin level in full term newborn infants with 1 minute Apgar score of <7, which finding may have indicated a suppressed bone matrix synthesis due to the metabolic effects of asphyxia. In the present study we found similar osteocalcin levels in neonates with or without the experience of perinatal asphyxia (3.8±1.8 vs 3.5±2.4 nmol/l). This result is in agreement with that of Pittard et al /10/ who reported on closely similar osteocalcin levels in neonates with 1 and 5 minutes Apgar score of <7 and 8-10, respectively.

The exact role of osteocalcin in neonatal calcium-phosphate metabolism and its regulation is still obscure, however, the existence of a functional association could hardly be debated. In this respect remarkable are the recent observations of Pittard et al /10/ who found a parallel rise in osteocalcin and 1,25-dihydroxycholecalciferol level during the first postnatal week, and a negative though statistically not significant correlation between osteocalcin level and total and bone specific alkaline phosphatase activity.

We observed a positive correlationship between serum calcium, phosphate and total alkaline phosphatase and osteocalcin concentration, though none of these correlation were significant statistically. An inverse, but again not significant relationship, was found between osteocalcin level and urinary calcium and phosphate excretion.

In summary it is concluded that newborn infants in general have much higher osteocalcin level than adults but no direct relationship exists in the early postnatal period between osteocalcin concentration and maturity, physical growth or the commonly measured calcium-phosphate metabolism parameters. Since serum osteocalcin most likely reflects osteoblast activity, further studies in this field may promote a better understanding of neonatal bone metabolism and its regulation.

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SIGNIFICANCE OF CHROMOSOMAL INVESTIGATION IN INFANTS WITH ACUTE LEUKEMIA

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Findings of cytogenetic examination on twelve infants with acute leukemia (AL) are reported. Acute lymphoid leukemia (ALL) was diagnosed in five cases and acute non-lymphoid leukemia (ANLL) in seven cases. Specific chromosomal aberration was found in leukemic cells of four of five infants with ALL: t(9;22), t(2;8), del(4q) and t(4;11). There was non-random chromosomal aberration in the four cases with ANLL: t(9;22), t(4;11), del(10p) and hypodiploidy. In two of the eight cases with abnormal karyotype findings of cytogenetic examination and morphological and immunological type of leukemia were different from the usual cytogenetic-morphological-immunological association. Significant improvement in outcome can only be expected from a more accurate classification of the disease. Authors suggest cytogenetic examination of all infants with AL at diagnosis.

Detection of specific chromosomal aberrations provides possibility to choose individualized treatment.

INTRODUCTION

During the last decade treatment of children with acute leukemia (AL) has considerably improved. More than 70% of newly diagnosed children with acute lymphoid leukemia (ALL) can anticipate prolonged (>5 years) disease-free survival and most of these patients are likely to be cured with modern therapy /2, 8/. There is also progress in treatment of acute non-lymphoid leukemia (ANLL), 80% of children with ANLL can be introduced into a complete remission and 40% of them appears to be event-free survivors /4/. In contrast, outcome of treatment in infants of

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each form of leukemia is extremely poor. Half of the patients dies in 18 months, 75% in 4 years. Five-years survival is 18% /3, 10/. There are several reasons of poor prognosis: the clinical and biological features of AL in infants differ markedly from older children:

- the myelogenous type of leukemia is predominant in infants, particularly in congenital leukemia
- in infants with ANLL leukemic cells are monoblasts or myelomonoblasts, extramedullary involvement is frequent
- ALL among infants is characterized by hyper-leukocytosis, presence of hepato-splenomegaly, central nervous system (CNS) involvement at the time of diagnosis and early B-cell markers, lack of the common ALL antigen /6, 7, 20/
- in infants it is difficult to hold scheduled therapeutic strategies without changing, early drug resistance and side effects of treatment are frequent /24/.

Significant improvement in outcome can only be expected of the progress of our understanding about biology of leukemic cells. Prognostic parameters - first of all cytogenetic analysis - provide important information and more accurate classification of disease.

MATERIALS AND METHODS

Between 1979 and 1992 successful cytogenetic analyses were performed in 94 children with AL. There were 58 cases of ALL and 36 of ANLL, twelve of whom were 12 months old or less. There were five cases with ALL and seven with ANLL. The diagnosis of AL was made by hematologists on the basis of the morphology and cytochemical staining characteristics of the blast cells. Cases of ALL were subtyped according to the immunophenotypes. Chromosome analysis was performed on G- and Q-banded preparations from direct and short term cultures (24 and 48 h) of bone marrow and/or from peripheral blood sample cultured for 24 and 48 h without phytohaemagglutinin /5, 21/. Chromosomal abnormalities were identified according to ISCN (1978) /12/. Cytogenetic analysis was performed before therapy and whenever possible, during remission or at relapse.

RESULTS

Clinical data, karyotypes and treatment response of the 12 patients are summarized in Table I. Their age ranged from 6 weeks to 12 months. Sex rate: male 7, female 5. Leukocyte counts were not too high (2 to 60 x 10^9/L) except one case (4. case) with WBC 215 x 10^9/L. All patients presented the common symptoms: severe anaemia, pallor, hepatosplenomegaly, lymphadenopathy, petechiae. Central nervous system /CNS/ leukemia at diagnosis was observed in one case (4. case). Leukemic skin infiltration could be detected in two children with congenital leukemia (cases 4 and 10). Two patients had extramedullary involvement on the cranium and mandibula (cases 6 and 9 respectively). Leukemic blast cells in three of five infants with ALL were FAB-type L1 and non-T, non-B cell immunophenotype. In case 4 immunologic analysis was not done. In the fifth patient diagnosis was biphenotypic acute leukemia. Children with ANLL belonged to different FAB-types, all the morphological subtypes but M3 and M7 occurred. Three of the twelve children achieved complete remission, but only one of them remained in remission and alive for more than ten years. Outcome of treatment was more favorable in ALL than in ANLL.

Four of five infants with ALL had abnormal karyotypes. In the first patient cytogenetic analysis was performed in seven occasions. The first two examinations showed the presence of Ph chromosome but in the subsequent examination it was not detected. Since the Ph chromosome reappeared a few months later, the prescribed reinduction therapy had to be started earlier. In the 17th and 41st month of disease normal karyotypes were shown. She is in complete remission, her survival time is 166 months. This familial leukemic case - Ph positive ALL of a mother and her infant - was reported in 1981 /18/.

In the second patient leukemic cells had t(2;8) translocation with breakpoints 2p21 and 8q24.

TABLE I

Clinical and laboratory data of infant leukemia

Patient No.	Age/sex	Leukocyte count x 10^9/L	Morphology (FAB)	Immuno-phenotype	Date of examination (mo)
Acute lymphoid leukemia					
1.	9 mo/f	6	L1	non-T, non-B	1. before treatment 2. after induction 3. remission 6. remission 8. remission 17. remission 41. remission
2.	8 mo/m	25	L1	non-T, non-B	1. before treatment
3.	12 mo/f	6	L1	non-T, non-B	1. before treatment
4.*	6 wk/f	215	L1	-	1. before treatment
5.	2 mo/m	7,7	L1	biphenotypic	1. before treatment
Acute non lymphoid leukemia					
6.	12 mo/m	60	M2	-	1. before treatment 3. after induction without remission 12. remission 14. remission 21. relapse
7.	6 mo/m	40	M6	-	1. before treatment 3. after induction without remission
8.	4 mo/f	15	M5	-	1. before treatment 2. after induction 3. terminal state
9.	7 wk/m	18	M4	-	1. before treatment
10.	6 wk/m	17	M1	-	1. before treatment
11.	4 mo/f	40	M4	-	1. before treatment
12.	3 mo/m	2	M1	-	1. before treatment

*immunophenotypical analysis was not done

Table I Cont.

No. of metaphases examined	Abnormal karyotype	No. of abnormal metaphases	Duration of remission (mo)	Duration of survival (mo)
18	46,XX,t(9;22)	4	>122	>166
10	46,XX,t(9;22)	2		
9	-	-		
7	46,XX,t(9;22)	4		
10	46,XX,t(9;22)	5		
15	-	-		
8	-	-		
14	46,XY,t(2;8)	14	0	5
32	46,XX,del(4q)/	2	48	63
	47,XX,+21/	3		
	hypotetraploid	10		
9	46,XX,t(4;11)	9	0	5 days
15	-	-	>2	>4
5	46,XY,t(9;22)	5	17	21
4	46,XY,t(9;22)	4		
15	-	-		
6	-	-		
8	46,XY,7q+/	3		
	46,XY,i(17q)	2		
13	-	-	0	9
12	45,XY,-17/	4		
	44,X.-17,-Y	3		
7	-	-	0	2,5
11	-	-		
5	-	-		
8	-	-	0	20 days
20	46,XY,t(4;'')	20	0	1
10	-	-	0	16 days
18	46,XY,del(10p)	18	0	7 wk

In the third infant with ALL four different cell lines were seen: 1. cells normal karyotype, 2. deletion of the long arm of chromosome 4: 4q21, 3. gain of chromosome 21, and 4. hypotetraploid.

In the fourth child with congenital leukemia t(4;11) (q21;q23) was observed. There were not any chromosomal aberrations in infant with biphenotypic acute leukemia (case 5).

Three of the four cases with abnormal karyotype had pseudodiploidity while one case was hypotetraploid. Four of the seven cases with ANLL had chromosomal abnormalities in the leukemic cells. Infants with AMMol and AMol had normal diploid karyotype.

In the child with M2 FAB-type (case 6) chromosome investigation showed Ph positivity at diagnosis and after induction. During the remission chromosomal aberrations were not present. In terminal relapse new aberrations appeared: 7q+ and i(17q).

In infant with erythroleukemia normal karyotype (case 7) was observed at diagnosis. In the third month of disease in one part of the cells hypodiploidy was detected caused by monosomy 17 or monosomy 17 and the loss of Y. In the case of congenital leukemia (case 10) in all the cells t(4;11) was observed, breakpoints involved were 4q21 and 11q23. The other child with M1 FAB-type (case 12) had a deletion of the short arm of chromosome 10. The involved breakpoint was 10p13.

Ploidy in abnormal ANLL karyotypes but one (case 7) hypodiploid case was pseudodiploid.

DISCUSSION

Recently remarkable efforts have been made to establish the biologic and prognostic value of specific cytogenetic abnormalities in acute leukemias. In spite of this we know little about exact karyotype distribution of infant leukemia. Relatively large number of cases have been studied cytogenetically and described as a non-random karyotypic change of the t(4;11) /13, 14, 17/. Pui et al. /20/ reported chromosomal changes in significant number of infants. 93% of cases had abnormal karyotypes and 84% were pseudodiploid. In the half of the affected patients 11q23-q25 breakpoints were involved. This frequency is higher than that seen in older children with AL-s (4% in ALL, 13% in ANLL). Besides chromosomal regions the 9p21-22 and 10p13 were preferentially involved. Abe et al. /1/ reported chromosomal abnormalities involving 11q in 48% out of 29 infant leukemia. Heim et al. /11/ presented the distribution of karyotypic changes in their three cases and 31 collected from the literature leukemia patients less than 6 months of age. They reported a new structural chromosomal rearrangement: t(11:19) and suggested that this reciprocal translocation might represent a specific subgroup of leukemia with relatively good prognosis in the very young age. The chromosomal translocation or deletion involving band q23 of chromosome 11 was especially uniform change in Chinese infants; in seven of the eight patients investigated /6/.

One of the most important prognostic factors in childhood AL is the cytogenetic findings at diagnosis. Leukemic cell chromosomal analyses provide independent contribution to prognosis. Secker - Walker et al. /22/ realized the importance of ploidy with more than 50 chromosomes which is the best prognosis, however, relatively poor prognosis associated with pseudodiploidy. A large number of patients in the pseudodiploid category have translocations. The presence of translocation increases the risk of treatment failure /23/. The adverse prognostic significance of certain translocations in ALL such as t(9;22k), t(4;11) and t(8;14) or variant translocations has been

clearly established. Besides translocations involving 11q, t(9;22), -7/7q- and loss of sex chromosome seem to be unfavorable in children with ANLL /9, 16/.

We conclude that prognosis tends to be poor in infants with AL due to the frequent presence of unfavorable chromosomal abnormalities and the absence of hyperdiploidy. Four of five children with ALL had specific chromosomal features: t(9;22), t(2;8), del(4q) and t(4;11). In four of seven infants with ANLL non-random chromosomal abnormalities were detected: t(9;22), hypodiploidy (-17/-17,-Y), t(4;11) and del (10p). Modal chromosome number in six cases of the eight infants with abnormal karyotype was pseudodiploid, one case was hypodiploid, one case was hypotetraploid. We observed quite changeable karyotype abnormalities in our patients which differed from those reported in the literature. The cause of this observation remains unclarified. Furthermore, precise definition of infants with AL is difficult particularly in the first months of age in young children. More than half of our patients was diagnosed within the first six months of life. To distinguish ALL L2 from ANLL M1 surface markers have been found to be useful. Recently an array of specific cytogenetic abnormalities has been linked to blasts with defined lymphoid phenotype in ALL similar to those associated with ANLL /15, 16/. In two of our eight infants with abnormal karyotype chromosomal finding did not correlate with the expected immunophenotypic and morphologic features of blast cells. t(2;8) that is specific translocation for acute B-cell (SIg+) and L3 morphology leukemia was found in non-T, non-B cell leukemia. Reason of unusual association may be due to the breakpoint involved: 2p21 was more distal on chromosome 2 than the p11 observed in the majority of B-cell ALL. Most leukemias with t(4;11) appear to have made commitment to the B-cell procursors, but there is evidence for commitment to myeloid, monocytoid and T-cell lineage, too /11, 14/. We identified this karyotypic change in a congenital non-lymphocytic leukemia (FAB M1). Induction remission was failure in these infants. Acute B-cell leukemia responds poorly to the chemotherapy used to treat childhood ALL. Cyclophosphamide induced more successful

prognosis. In hybrid leukemias with t(4;11) teniposid and ara-C resulted in favorable outcome /19/. It is suggested that chromosomal analysis should be carried out in all the patients with AL at the time of diagnosis in addition to the immunological and morphological classification. Specific chromosomal abnormalities such as reciprocal translocations have been related to the result of combined chemotherapy of childhood leukemia, raising the possibility of individualized treatment based on cytogenetically defined subtypes of AL.

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INTERRELATIONSHIP OF METABOLIC ACIDOSIS, URINE pH AND URINARY PROSTAGLANDIN E_2 EXCRETION IN NEWBORN INFANTS AND CHILDREN

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The present study was undertaken to investigate the relationship between acidosis, urine pH and urinary PGE_2 excretion in newborn infants with mean birthweight of 2164 g and mean gestation age of 34 weeks and in 10 children at mean age 8.9 years undergoing NH_4Cl loading test.

To assess urinary acidification oral NH_4Cl was given in a single dose of 0.15 g/kg to neonates and in the same dose (0.15 g/kg divided into 4 parts) for 3 consecutive days to older children. Prior to and following NH_4Cl administration blood acid-base parameters (Astrup method), urine pH (Radiometer pH meter) and urinary PGE_2 excretion (gas chromatographic-mass spectrometric method) were measured.

It was demonstrated that in response to NH_4Cl metabolic acidosis urine pH was depressed significantly while urinary PGE_2 excretion remained practically unchanged. When urinary PGE_2 excretion was studied as a function of base excess or urine pH significant positive correlation could be established between these parameters for the newborn (urine pH: $r=0.51$, $p<0.05$, base excess: $r=0.52$, $p<0.05$) but not for the children. When multiple regression analysis was used to consider all three variables simultaneously the increase of base excess and urine pH proved to act in concert to enhance urinary PGE_2 excretion ($r=0.66$, $p<0.01$ for newborn; $r=0.50$, $p<0.01$ for children).

It is concluded that urinary PGE_2 excretion, urine pH and blood acid-base parameters are interrelated in newborn infants and older children undergoing NH_4Cl loading test. The results presented, however, failed to provide convincing evidence that the NH_4Cl-induced changes in renal functions are directly mediated by enhanced PGE_2 production.

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INTRODUCTION

Experimental and clinical studies have shown increased renal electrolyte and water excretion in association with either unchanged or reduced GFR following NH_4Cl metabolic acidosis. These observations have given support to the concept that the NH_4Cl acidosis-induced elevation of renal eletrolyte and water excretion may be the result of their impaired tubular reabsorption /2, 3, 6, 11, 14/. The precise underlying mechanism controlling renal tubular handling of water and electrolytes during acidosis, however, remained to be defined.

In contrast to these data we have recently found that in preterm and full-term neonates, as well as in older infants and children, the mild to moderate NH_4Cl metabolic acidosis produced a significant increase in GFR, too. On the basis of these observations we suggested that the acidosis-related increase in urine flow rate and urinary electrolyte excretion may be accounted for by the combined effects of increased GFR and decreased tubular reabsorption /6, 7/. Furthermore, we put forward the hypothesis that increased renal PGE production might be responsible for these complex functional changes, since renal PGE production has been shown to be stimulated by metabolic acidosis /7, 16/ and renal PGEs have been claimed to play an essential role in maintaining renal blood flow and GFR and in the control of renal water metabolism and sodium handling /4/. To address this issue the present study was undertaken to determine the possible influence of metabolic acidosis on renal PGE_2 production in a group of newborn infants and older children undergoing NH_4Cl loading test. With respect to the dependence of urinary PGE_2 excretion on urine pH /5, 8, 9/ and that of urine pH on blood acid-base status, particular attention was paid to investigate the interrelationship between blood acid-base parameters, urine pH and urinary PGE_2 excretion. The response of renal functions and the activity of RAAS to NH_4Cl metabolic acidosis have been studied simultaneously and the data obtained have been the subject of previous publications /1, 7/.

MATERIALS AND METHODS

Two groups of patients were selected for the study. Group I consisted of 10 children (2 boys, 8 girls) mean age of 8.9 years (range: 4 to 16) who were admitted for evaluation of urinary tract infection (8 patients) and/or nephrolithiasis (9 patients). Routine clinical and laboratory examinations did not reveal hypertension, impaired renal functions, disturbance of fluid and electrolyte homeostasis and acid-base balance.

Group II included 8 newborn infants with mean birthweight of 2164 g (range: 1300 to 3750) and mean gestational age of 34 weeks (range: 28 to 40). Their history did not reveal clinical and laboratory evidence of perinatal asphyxia, cardiopulmonary distress and metabolic disturbances. All infants progressed well except for mild to moderate jaundice, which was thought to be the result of urinary tract infection. Although no informative urine culture could be obtained, short-term antibiotic therapy was applied. Detailed nephrological evaluation did not detect either renal/urinary tract abnormalities or impaired renal function. All infants were breast fed and 5% glucose in water was added to provide fluid intake of 150-180 ml/kg per day.

As a part of evaluation of renal functions urinary acidification was assessed by giving oral NH_4Cl in a single dose of 0.15 g/kg to the neonates at the end of the first week and in the same dose (0.15 g/kg divided into 4 parts) for 3 consecutive days to the older children. Prior to and following NH_4Cl administration urine was collected for a period of 12 hours (neonates) or 24 hours (older children) and blood was taken for acid-base parameters, plasma electrolytes and creatinine. Urine was analyzed for net acids, pH, electrolytes, creatinine and PGE_2 concentration. Aliquots of each urine sample were frozen immediately after collection and stored at -20 ^{o}C until analyzed. Urine pH was determined using Radiometer pH meter. Urinary PGE_2 measurements were performed according to Seybert et al. using gas chromatographimass spectrometric method /13, 15/.

The results are presented as mean$\pm$SE and were evaluated by means of Student's paired t-test, by calculation of correlation coefficient and by multiple variance analysis.

Approval of the institutional review committee and written parental informed consent were obtained for the study.

RESULTS

Blood acid-base parameters, urine pH and urinary PGE_2 excretion in newborn infants and children prior to and following NH_4Cl administration are shown in the Table.

It can be seen that NH_4Cl ingestion induced mild to moderate

TABLE I

Blood acid-base parameters, urine pH and urinary PGE_2 excretion in newborn infants and children before and after NH_4Cl administration (mean±SE)

	Newborn infants		Children	
	before NH_4Cl loading	after NH_4Cl loading	before NH_4Cl loading	after NH_4Cl loading
Blood pH	7.30±0.02	7.25±0.02*	7.41±0.03	7.31±0.03**
total CO_2 content (mEq/l)	19.9 ±1.2	17.9 ±1.2**	26.2 ±0.8	19.6 ± 0.7**
Base excess (mEq/l)	-6.4 ±0.8	-9.8 ±1.2**	-0.14±1.8	-6.8 ± 0.8*
Urine pH	5.8 ±0.1	5.6 ±0.2**	6.3 ±0.1	5.3 ± 0.2***
Urinary PGE_2 excretion (ng/h)	1.83±0.83	1.76±0.61	4.66±0.64	4.07±0.76

* $p < 0.05$
** $p < 0.01$
*** $p < 0.0025$

metabolic acidosis in both groups as indicated by the significantly depressed blood pH, total CO_2 content and base deficit. Urine pH decreased accordingly after induction of acidosis while urinary PGE_2 excretion remained practically unchanged, suggesting that alterations in acid-base balance have no apparent influence on renal PGE_2 production neither in newborn infants nor in older children.

When urinary PGE_2 excretion was studied as a function of base excess, significant positive correlation could be established between these two parameters for the newborn ($r=0.52$, $p<0.05$) but not for the children group ($r=0.3$, $p<0.1$) (Figs 1, 2).

Similarly, urine PGE_2 excretion tended to decrease with declining urine pH over the range of 4.6 to 7.0 but these changes achieved statistical significance only in case of newborn infants ($r=0.51$, $p<0.05$) (Figs 1, 2).

Since base deficit, urine pH and urinary PGE_2 excretion appear to be interrelated and the decreasing urine pH is likely to reduce urinary PGE_2 excretion independently of the renal preduction rate, it seemed worthwile to determine the interrelationship between these three variables. When all three variables are considered simultaneously, the increase of base excess and urine pH are acting in concert to enhance urinary PGE_2 excretion, or rather if the expected acidosis induced increase occurs in renal PGE_2 production, it is outweighed by the more powerful influence of low urine pH to reduce its excretion rate ($r=0.66$, $p<0.01$ for newborns; $r=0.50$, $p<0.01$ for children).

DISCUSSION

The results of the present study provided evidence that urinary PGE_2 excretion, urine pH and blood acid-base parameters are interrelated in newborn infants and older children undergoing NH_4Cl loading test. Moreover, we could demonstrate that urinary PGE_2 excretion of the neonates is more readily

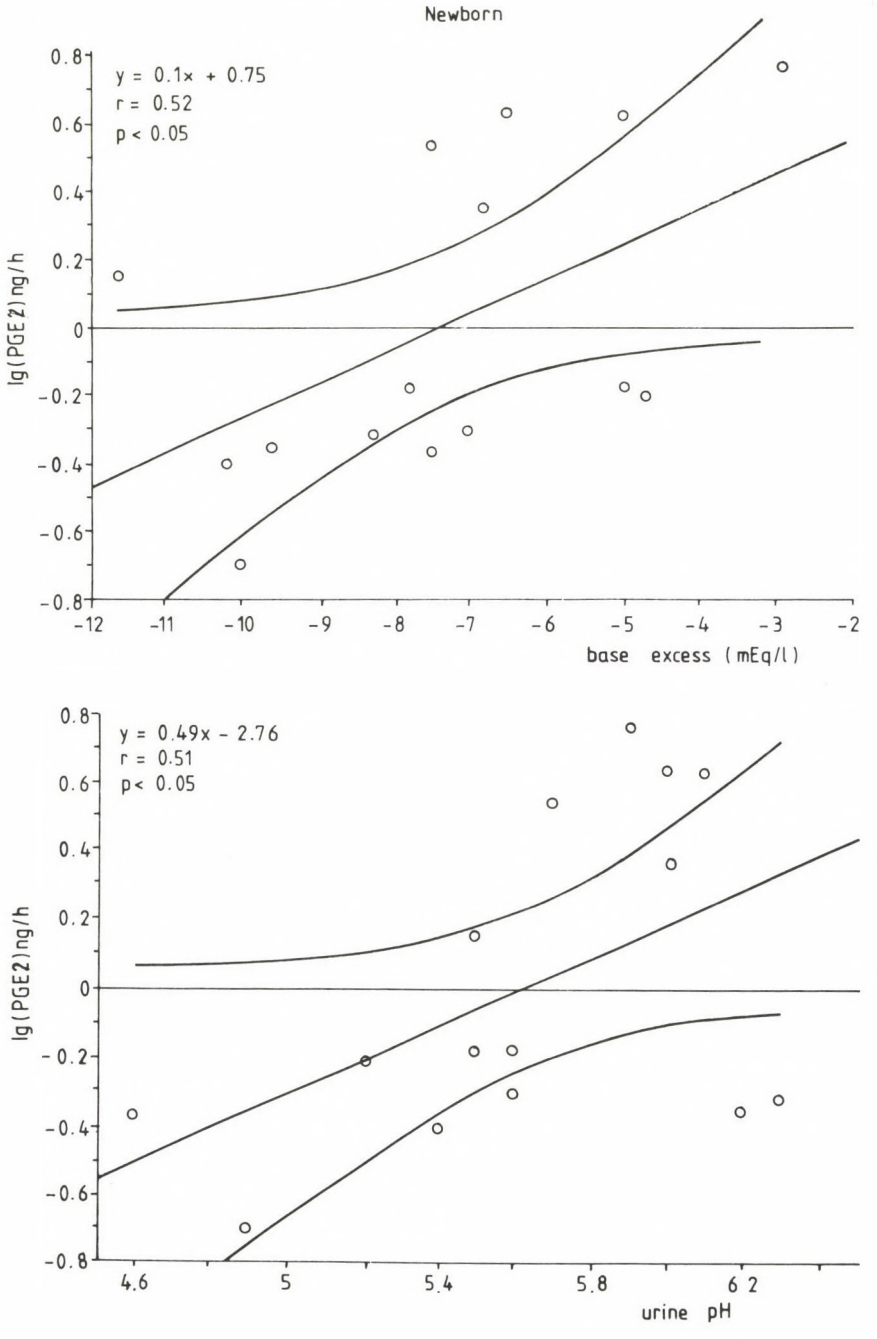

Fig. 1. Relationship of urinary PGE_2 excretion to base excess and urine pH in newborn infants subjected to NH_4Cl loading test

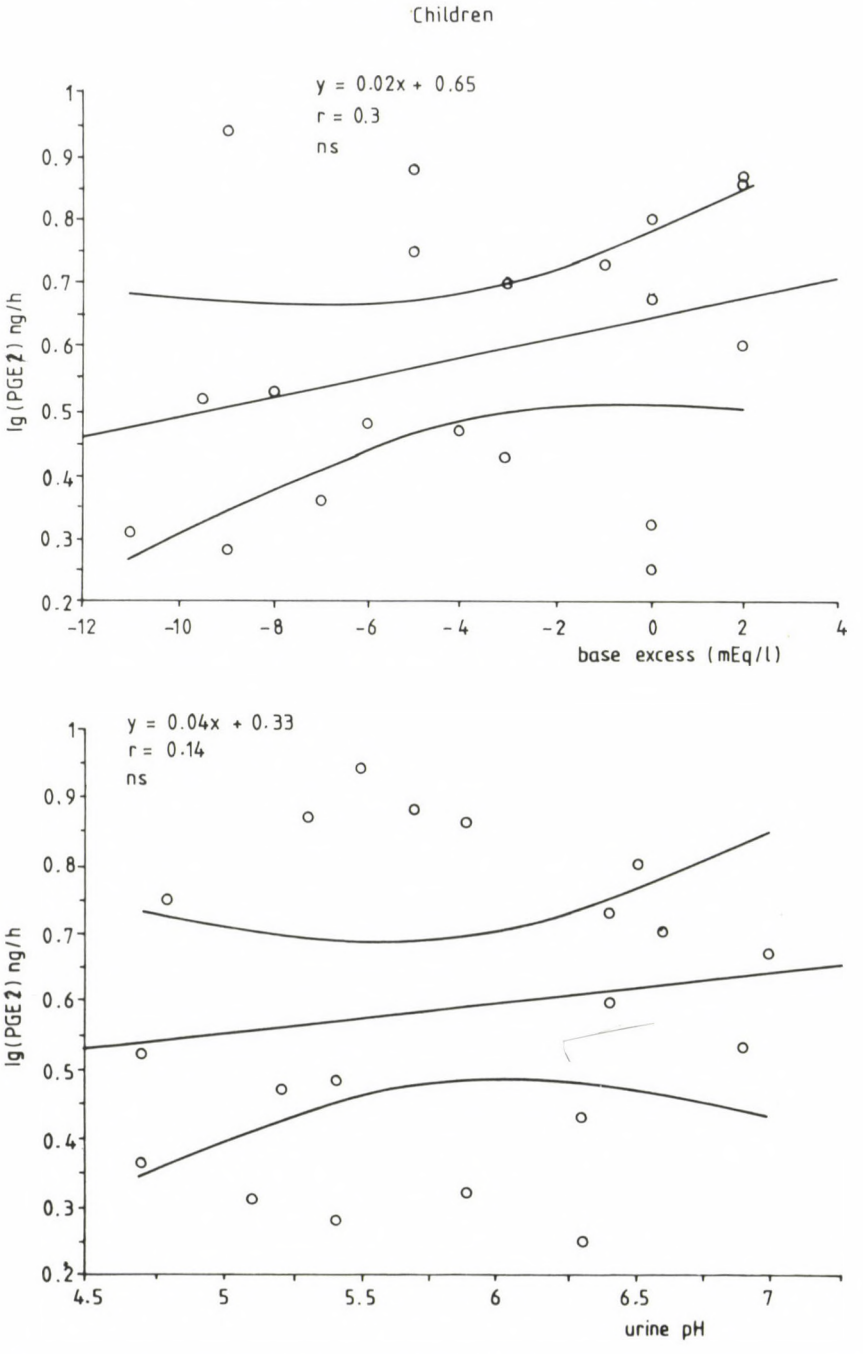

Fig. 2. Relationship of urinary PGE_2 excretion to base excess and urine pH in children subjected to NH_4Cl loading test

responsive to changes in acid-base balance and urine pH than of older children. On the other hand, these findings failed to substantiate our contention that the NH_4Cl acidosis-induced changes in renal functions, i.e. increase in GFR, urine flow rate and fractional electrolyte excretion, are directly mediated by enhanced renal PGE production.

In a recent study in intact rats and in renal cortical slices on the interplay between metabolic acidosis, renal ammonia generation and renal PGs synthesis Jones et al. reported that mild metabolic acidosis simultaneously stimulated renal PG synthesis and ammonia generation. Inhibition of acidosis-induced PG production therefore allowed an unopposed increase in ammonia generation. Maximal stimulation of PG synthesis was achieved at serum bicarbonate level of 18 mM/l /10/. No attempt was made by the authors to enlighten the mechanism by which acidosis stimulates renal PG synthesis. Furthermore, Tanner and Goyal using isolated perfused rat kidney and renal cortical tubules incubated in vitro reported that in response to acute respiratory acidosis both renal PGE_2 and $PGF_{2\alpha}$ production increased significantly but only $PGE_{2\alpha}$ was found to inhibit renal ammoniagenic response to acidosis /17/. Miltényi et al. found significantly elevated 15-keto-PGE_2 excretion in children with ketoacidotic diabetes /12/.

In view of these observations a marked renal PGE_2 response to NH_4Cl acidosis could have been expected. The possible reason for our failure to demonstrate increased renal PGE_2 excretion is that under the experimental conditions we applied, urinary PGE_2 excretion cannot be regarded as a reliable measure of renal PG synthesis.

In support of this notion, there have been several reports of the urine flow rate and pH dependency of PGE_2 excretion /5, 8, 9/. At constant urine flow and sodium output, significant positive correlation was found between urine pH and PGE_2 excretion. PGE_2 as a weak acid is passively reabsorbed in the distal nephron only in its unionized, lipid-soluble form. At acid pH the proportion of unionized PGE_2 is increasing and its reabsorption is enhanced, consequently at a given rate of renal

synthesis less PGE_2 excreted in the urine /9/.

Considering urine pH as a determinant of PGE_2 excretion it appears relevant to assume that in our study the influence of NH_4Cl metabolic acidosis to stimulate renal PGE_2 generation remained unexplored because of the simultaneous fall of urine pH-dependent decreased urinary PGE_2 excretion. As an alternative possibility it should also be considered that in response to NH_4Cl acidosis renal PGE_2 production rate remains unaltered and the acidosis-induced changes in renal water and electrolytes handling are independent of renal PGs. Additional studies using PG inhibitors are needed to define more clearly the complex relationship between acidosis, urine pH and renal PG production.

ACKNOWLEDGEMENT

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SOME LIPID PARAMETERS IN TYPE 1 (IDDM) DIABETIC CHILDREN; RELATIONSHIP WITH FRUCTOSAMINE AND DURATION OF DIABETES

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The relationship between some lipid parameters and glycemic control, as well as the duration of diabetes was investigated. Diabetic subjects were divided into three subgroups: i patients with 0-1 year duration of the disease, ii 1-5 years duration and iii more than 5 years diabetes duration. Serum glucose and fructosamine levels showed no significant correlation with any of serum lipids or apolipoproteins in diabetic subgroup i and ii, and in control subjects. However, significant correlation has been found between serum fructosamine and triglyceride ($r = 0.406$, $p < 0.01$), fructosamine and apolipoprotein B ($r = 0.487$, $p < 0.001$), as well as between serum cholesterol and triglyceride ($r = 0.655$, $p < 0.001$) in subgroup iii. Improved metabolic control seems to be a major factor in the prevention of the late vascular complications of diabetes.

INTRODUCTION

Since macrovascular disease is a principal complication in diabetes, lipid parameters have been a focus of interest; some hundred papers have been published in adult diabetics in the last years. Relatively few studies were carried out in diabetic children concerning the relationship of serum apolipoprotein levels and degree of metabolic control /1, 2, 3, 4/.

The aim of this study was to investigate the relationship between some lipid parameters and glycemic control - measured by means of serum fructosamine - as well as the duration of diabetes.

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SUBJECTS AND METHODS

Hundred and twelve Type 1 diabetic children and 50 healthy age-matched controls were enrolled in this study. Diabetic subjects were divided in three subgroups: group i patients 0-1 year duration of the disease, group ii 1-5 years duration, and group iii more than 5 years diabetes duration. Their characteristics are shown in Table I. Diabetic patients

TABLE I

Characteristics of subjects (mean±SD)

	Diabetics			Control
	i	ii	iii	
Patients	13	51	49	50
Age (years)	11.8±2.8	11.9±3.4	14.9±2.9	11.3±3.1
Sex (M/F)	4/9	19/32	25/23	25/25
Duration of diabetes (years)	max.1.	3.6±1.0	8.8±2.8	-

were treated as outpatients with conventional, twice daily combination of regular and intermediate acting insulins (Actrapid and Monotard HM, Novo Nordisk, Denmark), or with conventional intensified insulin therapy: 3 times regular insulin before meals and isophane insulin at bedtime (Actrapid and Protaphan HM, Novo Nordisk). Blood samples were collected after an overnight fast (12 hours). In diabetic subjects samples were obtained prior to the first insulin injection on the day of the study.

Serum glucose, fructosamine, cholesterol and triglyceride concentrations were measured by RA-1000 Analyzer (Technicon Corp. Terrytown, N.Y. USA). The coefficient of variation (CV%) of methods were: glucose 2.3%, fructosamine 3.0%, cholesterol 2.5%, triglyceride 2.3%. Apolipoprotein A-I and B immuno-turbidimetric were analysed with Turbox system (Orion Corp., Espoo, Finland), CV% were at apolipoprotein A-I 4.2%, at apolipoprotein B 4.8%. For statistical analysis Student's t-test and linear regression analysis were used.

TABLE II

Measured serum parameters in Type 1 diabetic children subgroups and controls
(mean ± SD, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ vs controls)

Parameters		Diabetic patients/diabetes duration			
		<1 year	1-5 years	>5 years	Controls
N		13	51	48	50
Se cholesterol	mmol/l	4.33±0.61	4.45±0.80	4.76±1.08	4.39±0.95
Se triglyceride	mmol/l	0.80±0.24^{c}	1.08±0.54^{c}	1.48±1.11	1.47±0.57
Se apolipoprotein A1	g/l	1.34±0.23^{a}	1.42±0.31^{c}	1.34±0.22^{c}	1.09±0.31
Se apolipoprotein B	g/l	0.62±0.12^{a}	0.71±0.18^{a}	0.84±0.31	0.80±0.26
Se glucose	mmol/l	9.3 ±4.6^{c}	11.4 ±4.3^{c}	10.9 ±4.7^{c}	4.4 ±0.4
Se fructosamine	mmol/l	1.97±0.19^{b}	2.26±0.26^{c}	2.30±0.28^{c}	1.74±0.22

RESULTS

The mean serum cholesterol, triglyceride and lipoprotein levels in the 3 subgroups of diabetic children and in controls are given in Table II.

Mean values of serum cholesterol did not differ between the diabetic children and the control subjects. The serum triglyceride concentration in subgroup i and ii was significantly lower than in controls. The mean serum apolipoprotein A-I levels in diabetic groups were higher than in controls. The concentration of apolipoprotein B tended to be less in subgroup i and ii than in control subjects.

The diabetic patients exhibited a wide range of glycaemic control with fasting glucose concentration ranging from 4.4 to 18.2 mmol/l (mean 10.6±4.7 mmol/l), the concentrations of fructosamine were 1.6 and 3.1 mmol/l (mean 2.24±0.29 mmol/l).

Table III shows the correlation coefficients between serum

TABLE III

Relationship between measured parameters in Type 1 diabetic children subgroups and controls
($^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$)

Parameters	Control	Duration of diabetes <1 year	1-5 years	>5 years
SeFa ~ TG	- 0.326	- 0.351	0.294	0.406^{b}
SeFa ~ ApoB	0.184	0.258	0.332	0.487^{c}
Chol ~ TG	0.042	0.173	0.442^{c}	0.655^{a}

fructosamine (SeFa) levels and triglyceride (TG) concentrations. The inverse correlation in control children

(r= -0.326, NS) has been changed for significant positive correlation (r= 0.406, $p<0.01$) in children who have had diabetes for more than 5 years. There is a gradually increasing correlation between serum fructosamine levels and apolipoprotein B concentrations. There is a significant positive correlation between serum cholesterol (Chol) and triglyceride (TG) concentrations in the diabetic group after 1-year diabetes duration. No significant correlation was found when comparing other parameters (SeFa ~ Apo A-I, SeFa ~ Chol, Apo A-I Apo B).

Fig. 1 and 2 display the frequency distribution for apolipoprotein A-I and apolipoprotein B in the 112 diabetic

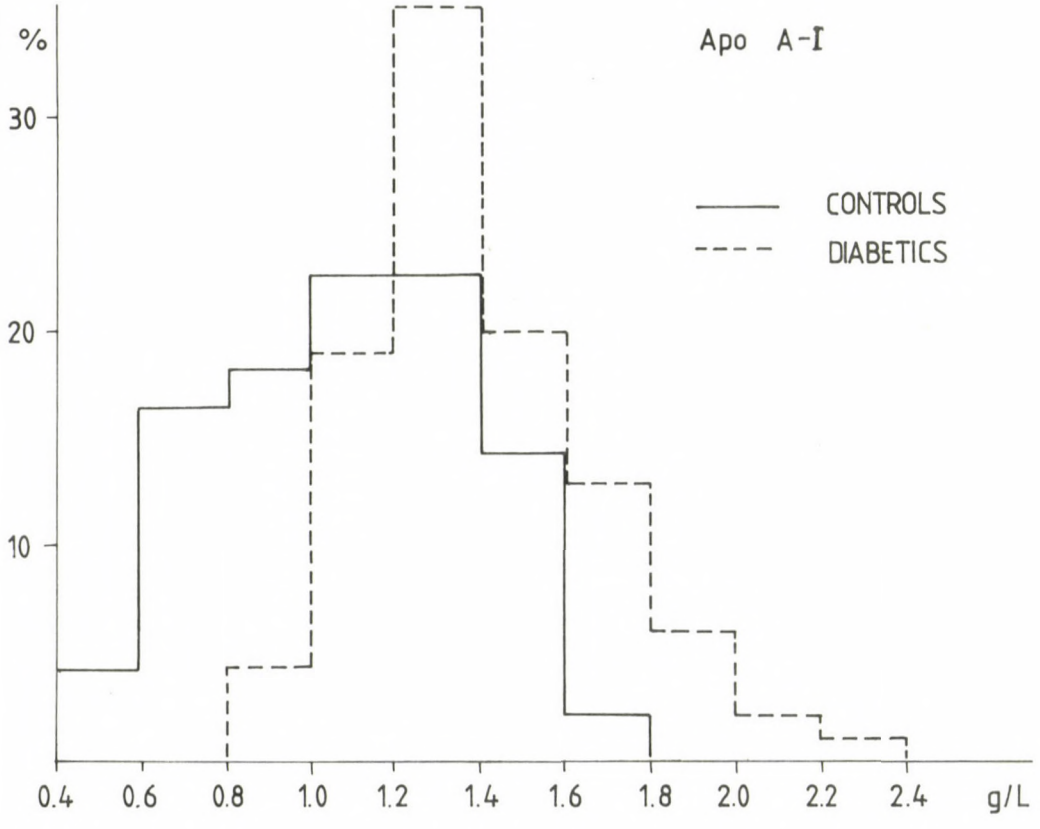

Fig. 1. The frequency distribution of apolipoprotein A-I.

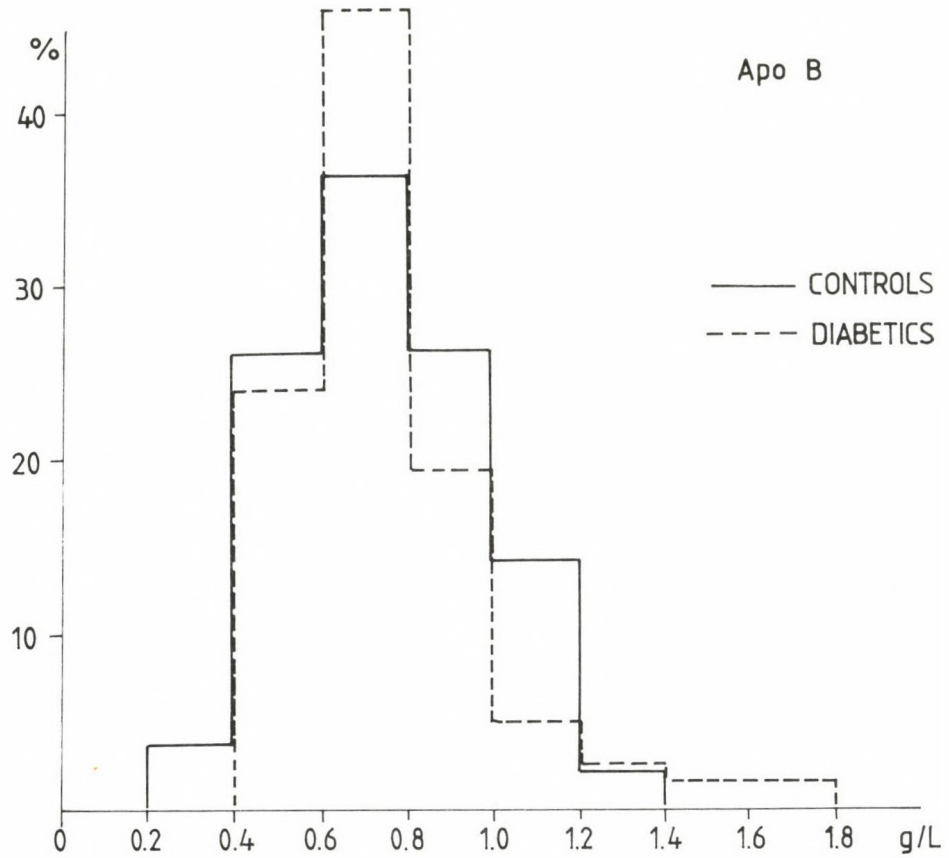

Fig. 2. The frequency distribution of apolipoprotein B

children compared to 50 healthy controls. The concentration of apolipoprotein A-I tended to be moderately higher in diabetic children than in control subjects, while there were no differences in distribution of apolipoprotein B levels.

DISCUSSION

Type 1 diabetics have an increased risk of developing macrovascular disease which, in part, may be due to lipid abnormalities /5, 6/. Various changes in lipid metabolism have been described in diabetic patients, though it has been suggested recently that elevated serum concentration of apolipoprotein B, as well as decreased levels of apolipoprotein A-I, might be better predictors of atherosclerosis than lipoprotein lipid levels /7/.

Studies on serum apolipoproteins in children with Type 1 diabetes have resulted in somewhat conflicting results: Ewald et al. /1/ have found increased levels of Apo A-I, however in the study of Strobl et al. /2/ serum Apo A-I and B values did not differ in diabetic and non-diabetic children. More recently Kobbah et al. /3/ investigated the serum apolipoprotein levels in children with newly detected Type 1 diabetes and followed them during the first two years of the disease. The Apo A-I and B concentrations were higher at the onset of diabetes than in controls, but after treatment, and during the first two years the concentrations of apolipoproteins became similar to those in healthy children. Considering the corresponding values of HbA_{1c} and fasting plasma glucose in diabetics, it seems very likely that most of the patients were in remission phase of the disease during the first 12-24 months, which might explain the good results.

In the present study the mean levels of apolipoprotein A-I were significantly higher in all diabetic subgroups compared to the controls (Table II and Fig. 1). This is in agreement with the report of Ewald et al. /1/ and with several studies carried out in adult diabetics.

Although no gross abnormalities were observed in the mean levels of lipids and lipoproteins in our diabetic children, the interrelationships between these parameters were altered (Table III). Changes in the correlation between serum fructosamine and apolipoprotein B and/or triglyceride, as well as between

cholesterol and triglyceride, indicate that glycemic control is particularly important for improving lipid transport and in this way reduces the risk for atherogenesis in childhood IDDM. The results of our study are in accordance with the observation that serum triglyceride concentrations are commonly subnormal in Type 1 diabetic patients with good glycaemic control due to decreased transport rates of VLDL particles /5/.

It has been reported recently that a substantial shift to the right of the frequency distribution for apolipoprotein B values has been found in patients with documented myocardial infarction before the age of 60 /7/. In our study the frequency distribution for the concentration of apolipoprotein B was similar in diabetic and in healthy children (Fig. 2).

In conclusion, our data suggest a close relationship between glycaemic control and lipid parameters in diabetic children and adolescents with a diabetes duration of more than 5 years, therefore improved metabolic control seems to be crucial in these patients in the prevention of either late vascular complications or accelerated atherosclerosis.

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THE TOTAL FREE-RADICAL TRAPPING ABILITY OF BLOOD PLASMA IN CHILDREN WITH ASTHMA AND ALLERGIC RHINITIS

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The interaction between various antioxidants may be important in protecting against oxygen toxicity. We studied the total radical trapping capacity of the antioxidants in plasma (TRAP) and compared the TRAP levels in 33 children with allergic rhinitis, 30 asymptomatic asthmatic children and 20 children with acute asthmatic attacks. The latter were also studied in their asymptomatic phase. Two control groups of 26 children with minor anatomical defects and 10 children with acute pneumonia with respiratory distress were also studied. In addition, the concentrations of various known antioxidants were measured and the theoretical contribution of these antioxidants to the TRAP calculated. The measured and calculated TRAP were higher in the both control groups than in groups of children with allergic rhinitis and asthma. The uric acid, vitamins E and C and sulphide concentrations were lower in the patients group compared with control ones. The main conclusion from this work is that for children with asthma and allergic rhinitis in the region of Silesia Poland studied, the essential nutrients concentrations: vitamin E, vitamin C, and cysteine-rich protein in plasma are too low for optimal antioxidant systems activities.

INTRODUCTION

In recent years oxygen free radicals and their possible role in the pathogenesis and complications of allergic diseases

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has received increasing attention /2, 3, 12/. Although highly reactive oxygen radicals result from normal metabolism, efficient endogenous enzymatic and non-enzymatic systems of removal normally prevent injury. The total antioxidant capacity in man is dependent on the synergistic action of various antioxidants, e.g. enzymes such as glutathione peroxidase and agents such as vitamin E and C and uric acid /6, 25/. Free radical oxidation products are significantly higher in airspace cells and blood of patients with asthma /3, 4, 19/. This potentially important interaction between the various antioxidants has not been explored in children with bronchial asthma and with allergic rhinitis. In these patients the protective role of these antioxidants and their possible interactions should also be considered. This hypothesis is reinforced by the knowledge that free-radical oxidation products have marked effects on immune system /11, 12/. We therefore studied the concentration of various plasma antioxidants and the total free radical trapping activity of blood plasma (TRAP) in children suffering from bronchial asthma and allergic rhinitis to determine if the changes reported in adults /3, 4/ also occurred in children.

MATERIAL AND METHODS

Patients and controls

Eighty-three children aged 3-5 years (mean age 4.8 years) attending the IV. Clinic of Pediatrics, Katowice, Poland, were enrolled prospectively into the study after obtaining informed parental consent. The study group was subdivided into three groups:

Group A: Thirty-three children with allergic rhinitis were included in this group. Allergic rhinitis was diagnosed if patients had all or any of the symptoms of recurrent paroxysmal sneezing, watery nasal discharge, nasal obstruction, post-nasal discharge and nasal eosinophilia. Children receiving nasal medication or those with clinical or radiological signs of chronic sinus infection were excluded.

Group B: This group comprised 30 children who had asthma of at least two years duration, diagnosed by a physician. A child was considered to have bronchial asthma if he had a history of

recurrent episodes of bronchospasm relieved by bronchodilators. A family history of atopy or bronchial asthma was considered supportive. Blood samples were obtained during the asymptomatic phase of the illness.

Group C: Twenty children admitted with acute asthma were studied before systemic drugs, including iv. corticosteroids were started (C_A) and two weeks after they had become asymptomatic and stopped steroid therapy (C_B).

Control groups: twenty-six children without any symptoms or signs of atopy or family history of allergic disease, ischaemic heart disease, or hypertension were included in the control groups. Control-1 group comprised 16 children with minor anatomical defects requiring corrective surgery. These children were normal on anthropometry and physical examination except for the minor deformity. Blood samples were obtained prior to surgery. Control-2 group comprised 10 children with acute pneumonia in respiratory distress.

Children suffering from malnutrition hypertension, liver disease or prolonged severe illness were excluded. Children fasting for more than 24 hours or receiving corticosteroid therapy were also excluded. All children belonged to a geographic area within a few kilometers of the city of Chorzów.

A detailed clinical history was obtained with regard to family history of allergic disease, hyperlipidemias and ishcemic heart disease. Each child underwent a detailed physical examination with special emphasis on the respiratory system. Children in groups B, C and control-2 underwent X-ray examination. Other investigations including complete blood count and nasal smear for eosinophils were performed where relevant. The children were not taking medication known to affect plasma antioxidants.

The age distribution of the subjects was similar in all groups. All groups included predominantly male subjects with only 23.4% of the study groups and 23.4% of the control groups being girls. 67.5% of the children in the study groups had a family history of allergic disease. Two patients in group A and one patient in group B had a family history of symptomatic ischaemic heart disease. One child in group C and two children in group B had a family history of hypertension.

Biochemical determinations

Venous blood (5 ml) was collected into a sterile vial from each child. Serum was separated after clotted blood was centrifuged at 3000 rpm for 5 min. Serum was separated, the vials coded and stored at -20 ^{o}C until analysis, which was performed within a week of the sampling.

Plasma antioxidants, uric acid and total bilirubin were measured on a SMA I automatic analyser (Technicon Instruments) by the manufacturer's methods /8, 16/. Vitamin E (alpha-tocopherol) and total vitamin C concentrations were measured by HPLC method /18, 21/. The plasma sulphides (-SH) and disulphides (-SS-) concentration were measured by the Ellman's method /5/ as adapted by Koster et al. /13/.

Measurement of total antioxidant capacity (TRAP) /24/. The

peroxidation of linoleic acid was induced by a water soluble thermolabile free – radical initiator (2,2'-azo-bis-(2-amidinopropane)-HCl. Peroxidation was initially inhibited (induction phase) by the plasma antioxidants and then progressed rapidly (propagation phase). The duration of the induction phase (an index of the total radical trapping capacity of the plasma) was quantified using the duration of the induction phase produced by a known quantity of an antioxidant with a known stoichiometric factor. This value (mcmol/l) was the measured total radical trapping capacity (TRAPmeas). In addition to the direct measurement of the total radical trapping capacity (TRAP) this parameter was also calculated stoichiometrically (TRAPcalc). Calculated total capacity of antioxidants (uric acid, vitamin C, vitamin E, and sulphydryl groups) to trap free radicals (TRAPcalc), was derived from concentrations and the stoichiometric factors (an index of the efficiency of each antioxidant to trap free radicals) by the formula of Wayner et al /24/:

$$\text{TRAPcalc} = 1.3\ (\text{uric acid}) + 1.7\ (\text{vit C}) + 2\ (\text{vit E}) + 0.2\ (\text{total plasma sulphides}) + 2\ (\text{bilirubin})$$

Although these antioxidants are considered to be the major plasma antioxidants, other compounds may also contribute to TRAP. These unidentified antioxidants (UNID) were calculated according to the formula /22/:

$$\text{UNID} = \text{TRAPmeas} - \text{TRAPcalc}$$

Thiobarbituric acid reactive substances (TBARS) in the plasma were determined as an index of lipid peroxidation. A spectrofluorimetric method of Yagi was used /26/.

Statistics. The differences between the results in the two groups were tested by ANOVA. When significant, pair-wise comparisons were carried out using the independent t-test. Values of $p<0.05$ were regarded as significant.

The study was reviewed and approved by an institutional committee and an ethics committee.

RESULTS

Table I shows the plasma concentrations of the various antioxidants. Concentrations of the uric acid, vitamin E, vitamin C, and sulphides were significantly lower in children with allergic rhinitis and in children with asthma compared to control subjects. In group of children with acute asthma concentration of the uric acid, vitamin E, and sulphides were significantly lower when children had an episode of acute asthma (C_A) than when they were asymptomatic (C_B). Disulphides are not detectable in controls. There was no significant difference between concentrations of uric acid, vitamin E, vitamin C, and sulphides in group B and in group C_B. No significant differences were noted in the concentrations of uric acid, vitamins E and C, and sulphides in the two control groups.

The TRAPmeas and TRAPcalc values were higher in the control childrens than in the children in groups A - C (Table II). The TBARS levels were higher in plasma of children in groups A - C. In group C, TBAR levels were significantly higher when the children had an episode of acute asthma (C_A) than when they were asymptomatic (C_B).

Table III shows the correlation of TRAPmeas data with those of TRAPcalc and the calculated trapping capacity of the individual antioxidants. In both control groups unlike the A - C groups the TRAPmeas values correlated with the TRAPcalc and uric acid. There was a weak correlation between TRAPmeas values and vitamin E in the children in A - C and control groups, and strong correlation between TRAPmeas data and total bilirubin in all groups. The UNID in all groups correlated with TRAP meas values.

DISCUSSION

Thiobarbituric acid reactive substances in plasma of children with asthma and allergic rhinitis were higher than in

TABLE I

Plasma concentrations of antioxidants in groups of children with allergic rhinitis (A), with asthma (B), with acute asthma before (C_A), and after therapy (C_B) and in two control groups

Group					
A	B	C_A	C_B	Control I	Control II
		Uric acid (mcmol/l)			
224.7[a]	202.6[b]	156.4[c]	178.5[b]	275.0[e]	265.4[e]
(11.8)	(10.6)	(9.8)	(10.5)	(12.7)	(18.6)
		Sulphides (mcmol/l)			
243.0[a]	226.0[a]	165.8[b]	189.8[b]	429.0[c]	398.7[c]
(12.9)	(23.1)	(14.6)	(11.0)	(21.8)	(33.7)
		Disulphides (mcmol/l)			
87.5	77.3	70.6	64.8	ND	ND
(5.9)	(6.3)	(9.7)	(4.1)		
		Vitamin E (mcmol/l)			
46.5[a]	41.9[a]	28.0[b]	35.7[a]	88.0[d]	86.8[d]
(2.8)	(4.3)	(4.1)	(6.2)	(8.2)	(4.7)
		Vitamin C (mcmol/l)			
67.3[a]	56.8[b]	45.9[c]	42.8[c]	121.8[d]	116.8[d]
(3.8)	(4.7)	(6.1)	(3.8)	(12.9)	(13.6)
		Total bilirubin (mcmol/l)			
35.7	34.8	29.6	32.1	33.6	32.9
(2.8)	(2.4)	(1.9)	(4.4)	(3.8)	(5.1)

Values are means and (SD) under each mean

ND - not detectable

Statistical significance:

a - b, b - c, and c - d : $p < 0.05$

a - e : $p < 0.02$

a - c : $p < 0.01$

a - d, and c - e : $p < 0,005$

TABLE II

The measured total radical trapping antioxidant parameter (TRAPmeas), the calculated total radical trapping antioxidant parameter (TRAPcalc), the unidentified antioxidant (UNID) and the concentration of substances reactive with thiobarbituric acid (TBARS) in groups of children with allergic rhinitis (A) with asthma (B), with acute asthma before (C_A), and after therapy (C_B) and in two control groups

Group					
A	B	C_A	C_B	Control I	Control II
		TRAPcalc (mcmol/l)			
619.5	569.5	478.7	429.5	893.6	862.74
(34.8)	(45.7)	(34.0)	(23.8)	(56.2)	(73.4)
		TRAPmeas (mcmol/l)			
756.0[a]	667.4[a]	525.1[b]	501.0[b]	980.4	900.4
(13.0)	(24.1)	(24.9)	(31.0)	(24.8)	(31.7)
		TBARS (mcmol/l)			
135.06[b]	167.3[b]	221.7[c]	190,7[c]	67.4	77.8
(21.8)	(23.1)	(14.6)	(11.7)	(11.9)	(9.5)

Values are means and (SD) under each mean
Statistical significance from both control groups:
a: $p<0.05$
b: $p<0.005$
c: $p<0.002$

TABLE III

Correlations of TRAPmeas and the calculated trapping capacity of the individual antioxidants with TRAPmeas in groups of children with allergic rhinitis (A) with asthma (B), with acute asthma before (C_A), and after therapy (C_B) and in two control groups

Group					
A	B	C_A	C_B	Control I	Control II
			TRAPcalc		
0.6788	0.4558	0.7438	0.6773	0.8656	0.8455
			Uric acid		
0.8554	0.5768	0.7865	0.8657	0.7885	0.8944
			Sulphides		
0.3280	NS	0.6443	0.6734	0.6745	0.7843
			Disulphides		
0.6556	0.6744	0.8665	0.5673	ND	ND
			Vitamin E		
0.5466	0.5620	0.4546	0.5345	0.6378	0.5748
			Vitamin C		
0.2060	-0.1980	NS	0.4523	0.4521	0.3428
			Total bilirubin		
0.7844	0.8562	0.6784	0.7845	0.7845	0.8531
			UNID[a]		
0.8055	0.8064	0.8763	0.8956	0.7461	0.7830

ND - not determined
NS - not significant
a - unidentified antioxidants

controls suggesting increased lipid peroxidation. Concentrations of these substances reflect the extent of lipid peroxidation but are crude measure and subject to interference by many factors /10/. The finding of lower plasma vitamin C and E levels in children with asthma and allergic rhinitis is consistent with the peroxidation of lipids. Vitamin C is the first plasma antioxidant to be depleted when lipid peroxidation is induced in vitro /7/. Sulphides, mainly glutathione, are a ubiquitous, largely intracellular antioxidant that is believed to play a key role in the defence against oxygen-derived free radicals /24/. In the presence of oxygen radicals, reduced sulphides are oxidized to disulphides. Disulphides are not detectable in normal human plasma /1/. It has been suggested that cells transport disulphides into the extracellular compartment to keep the intracellular concentrations of sulphides and disulphides roughly constant /9/. Thus, the plasma determinations of disulphides may represent a sensitive means of measuring oxidant stress.

The values of the TRAPcalc were considerably lower than the TRAPmeas in all the groups. Two possible explanations for this finding are: either all the plasma antioxidants have not been identified or the stoichiometric values used to calculate their free – radical trapping capacity are too low. Recently bilirubin has been recognized as being an important antioxidant in children's body /15, 20/. However, the plasma proteins are present in high concentrations and only their sulphide groups are used in the formula estimating the TRAPcalc /15, 24/; the other aminoacids as tryptophan, tyrosine and histidine can also act as antioxidants /24/. The other explanation for the lower TRAPcalc is that the stoichiometric values may be too low. Different workers use different stoichiometric values for the antioxidants, e.g. 0.33 and 2.0 for the sulphide groups and uric acid, respectively /17, 24/. Furthermore, Wayner et al. /23/ have shown that the value for vitamin C is concentration dependent. However, even if all the contributing antioxidants are identified and their correct stoichiometric values used, the TRAPcalc does not take into account the very important

interactions that occur between antioxidants when they work in unison /24, 25/.

The correlation between the TRAPmeas and TRAPcalc values and the individual antioxidants was different in the groups. Probably the inhibition of antioxidant activity, e.g. cation binding of urate /14/ or increased prooxidant activity may explain these findings /15, 22/. The unidentified antioxidants showed the strongest correlation with the TRAPmeas in groups of children with asthma and allergic rhinitis emphasizing the need to identify their components.

Our findings suggest that oxygen free-radicals toxicity occurs in children with asthma and allergic rhinitis. The central conclusion from this work is that for children with asthma and allergic rhinitis in the region of Silesia Poland studied dietary intakes of the essential nutrients: vitamin E, vitamin C, and cysteine-rich protein are too low for optimal antioxidant systems activities. It is possible that in patients with asthma and allergic rhinitis the lipid peroxidation products generated in plasma exceed the capacity of the organism to inactivate free radicals. The excess of free radicals may in turn be a factor that contributes to the complication of asthma or allergic rhinitis. The potential clinical use of this approach is a great appeal. It remains to be studied whether the plasma antioxidant activity in patients with asthma and allergic rhinitis can be increased by antioxidant therapy.

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ALFA-1-4-GLUCOSIDASE ENZYME ANALYSIS FOR THE DIAGNOSIS OF II TYPE GLYCOGENOSIS

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In 3 families 2 mothers suffering from glycogen myopathy and their children were investigated for specific leukocyte enzyme activity (alfa-1-4-glucosidase). The clinical, laboratory data, histochemical and electron microscopic analysis proved the diagnosis of muscular glycogenosis. All glycogen storage myopathic patients proved to be type II glycogenosis. The serum CK values were slightly or not elevated, the patients mostly had hyperpyruvatemia. Unexpectedly high residual alfa-1-4-glucosidase activity was noticed in leukocyte/lymphocyte homogenate of some homozygotes for GSD II. The specific enzyme activity from lymphocyte homogenate proved to be more available for the detection of heterozygous genotype.

INTRODUCTION

GSD II (glycogenosis II type = Pompe's disease acid maltase = alfa-1-4-glucosidase=EC 3.2.1.20) deficiency was first described in a human infant as glycogenic cardiomegaly /10/. Clinically GSD II may be manifested either as a rapidly fatal infantile form or as a milder late-onset one /1, 2, 4, 8, 9/.

In glycogenosis type II a deficiency of the lysosomal enzyme alfa-glucosidase leads to accumulation of glycogen particulums in the lysosomes /2/.

The disease is clinically heterogenous, in the infantile form there is a generalized accumulation of glycogen, the disease is rapidly progressive. In the late onset (juvenile and adult)

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TABLE I

Clinical, morphological, laboratory and specific enzyme activity values of GSD II patients and family members

Name age (y) F=female M=male	Onset of disease y	Clinical symptoms Histological findings	Laboratory data	alfa-1-4-glucosidase lymphocyte-homogenate nmol/mg/h	 leukocyte-homogenate nmol/mg/h	GSD II genotype
1. Mrs. S. 33y F D.: GSD II (mother)	28	Gowers+, generalized muscle hypotonia, muscle cramps+, EMG: myogen lesion ECHO: muscul. atrophy EM: alfa-beta-lycogen particula glyc. myopathy	CK 543 U/l L 0.79 mmol/l P (pyruvate) 117.1 µmol/l,	20.9	53,2%	homozygote[X]
2. G.S. 13y M (son of 1)	-	symptomfree ECHO: normal muscle biopsy: -	CK 120 U/l blood lactate (L) 0.92 mmol/l P 217.8 µmol/l (elevated)	13.1	33.3%	homozygote?[X] heterozygote?
J.S. 35y M (father of 2)	-	Healthy	-	29.9	76.1%	healthy
Control: 12y M					39.3 100 %	healthy

Name age (y) F=female M=male	Onset of disease y	Clinical symptoms Histological findings	Laboratory data	alfa-1-4-glucosidase lymphocyte- homogenate nmol/mg/h	 leukocyte- homogenate nmol/mg/h	GSD II genotype
3. Mrs. W. 31y F (mother) Dg.: GSD II	14-16	Mild muscle atrophy myopathy, lumbal lordosis, Gowers+ ECHO: muscle atrophy ECHO-cardiogr. pro- lapsus valv. mitr. EM glycogen myopathy	CK 112U/l LDH 741 U/l CK 638 U/l muscle carni- tine: normal cytochrome-c. oxydase: norm.	1.57	6.25%	homozygote
4. Zs.W. 4y F (dauhter of 3)	-	Symptomfree ECHO: normal ECHO cardiog.: norm. muscle biopsy: -	CK 40 U/l	5.13	20.4%	heterozygote
Control: 40y				25.1	100 %	healthy

Table I cont.

5. K. M. 19y F Dg.: GSD II	14	Muscle pain, cramps, muscular atrophy EMG: myogen laesion CHO muscular atrophy ENG normal EEG normal muscle biopsy: EM: glycogen myopathy	CK 30 U/l lactate 1.36 mmol/l P 325 µmol/l, glucose 4.6 mmol/l	16.3	64.9%	homozygote[x]
6. Mrs. M. 38y (mother of 5)		Symptomfree		27.7	110.3%	healthy

x Unexpectedly high residual enzyme activity

forms the glycogen accumulation is usually restricted to skeletal muscle and the patients may live for many years after the onset of the disease /1/.

Results of the specific enzyme analysis and detection of heterozygous genotype for GSD II will be reported in this study.

PATIENTS AND METHODS

In three families 2 mothers suffering from glycogen myopathy were investigated together with their affected children. Electromyography, muscle echo, serum Creatine kinase (CK), blood lactate, pyruvate were determined and muscle biopsy materials were analysed histologically and electron microscopically. The histochemical and electron microscopic (EM) analysis proved the diagnosis of muscular glycogenosis.

The genotype of the GSD II was determined according to the specific enzyme activities. Alfa-1-4-glucosidase activity was measured from the lymphocyte or leukocyte homogenate with Griffith's /6/ method.

RESULTS

The specific alfa-1-4-glucosidase activity results and the suspected GSD II genotypes are seen in Table I. The 1, 3 and 5 cases proved to be GSD II homozygotes with mild progressive myopathy. Case 2 and 4 are suspected to be GSD I heterozygotes, but case 2 (son of case 1) may be GSD I homozygote, until now symptomfree homozygote with elevated serum CK and pyruvate.

All GSD II patients had hyperpyruvatemia and normal blood lactate values. The serum CK values were slightly elevated, only 1 GSD II patient had normal CK activity.

DISCUSSION

The muscle glycogen storage disorders are glycogenosis types III, IV, and IX, these are manifested in myopathy.

The severity of the clinical symptoms in glycogenosis type II does not always correlate with the residual alfa-glucosidase activity /3/. The defects that lead to a deficiency of alfa-glucosidase include synthesis of catalytically inactive protein, absence of mRNA for the enzyme, decreased synthesis of the precursor, lack of phosphorylation of the precursor, impaired conversion of the precursor to the mature enzyme /11/.

Our patients 1, 3, 5 belong to the late-onset glycogen myopathy representing a milder form of GSD II type.

We have got unexpectedly high residual specific enzyme activity in leukocyte or lymphocyte homogenate of some homozygous patients (1 and 5). The detection of heterozygous genotype with the specific enzyme analysis from peripheral leukocyte homogenate was impossible in one case /2/ from family S.

The specific enzyme activity investigated from lymphocyte homogenate seemed to be more effective for the determination of the heterozygosity for GSD II (see in W family in case 4).

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EFFECT OF PASSIVE SMOKING AND VITAMIN E SUPPLEMENTATION IN BLOOD ANTIOXIDANT STATUS IN PRESCHOOL CHILDREN

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Despite similar vitamin E concentrations, erythrocyte of children of smoking parents have an increased tendency ($p<0.01$) to peroxidize in vitro compared with those of children of nonsmoking parents. This difference is abolished by vitamin E supplementation (100 mg alfa-tocopherol acetate/d for 14d). The increased susceptibility to erythrocyte peroxidation in the children of smoking parents may reflect lower erythrocyte glucose-6-phosphate dehydrogenase, glutathione peroxidase and superoxide dismutase activities ($p<0.001$, $p<0.005$, and $p<0.02$, respectively). Children of smoking parents seem to be under sustained oxidant stress with increased plasma-conjugated dienes ($p<0.01$) and dehydroascorbate ($p<0.002$) concentrations. Plasma total cholesterol is similar in children of smoking and nonsmoking parents, and is unaffected by vitamin E supplementation. Indices of sustained oxidant stress in children of smoking parents are partially ameliorated by vitamin E supplementation.

INTRODUCTION

Although clinical manifestations of cardiovascular risk factors appear only later in life, it is now recognized that atherosclerosis and essential hypertension begin in childhood /3/. Although the processes involved in the pathogenesis of cardiovascular disease are not completely understood, recent biochemical and epidemiological studies (for a review, see refs 6, 10) implicate free radicals in the formation of atheromatous

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plaque. Cigarette smoke contains vast amounts of free radicals (10^{14}/inhalation), which can directly and indirectly initiate and propagate the process of lipid peroxidation /5/. Thus, smokers encounter a sustained free-radical load, which facilitates the development of the atheromatous plaque. Risk of atherogenesis would be exacerbated further by an inadequate dietary intake of antioxidants such as vitamin E.

The aim of this study was to assess whether indices of free-radical-mediated peroxidation are increased in children of smoking parents compared with children of nonsmokers and whether children of smoking parents benefit from supplementation with the lipid-soluble free-radical scavenger, vitamin E. The effects of passive smoking on blood antioxidants and antioxidant-related enzymes were also assessed.

SUBJECTS AND METHODS

Children to be included in the study had the following work-up performed: /1/ estimation of body weight; /2/ fasting blood glucose level and postprandial blood glucose level; /3/ serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum gamma-glutamyl transpeptidase (GT), alkaline phosphatase, prothrombin time, bilirubin level, and serum protein; /4/ 24-hour urinary protein excretion; and /5/ serum thyroid-stimulating hormone (TSH), T_3, and T_4 levels.

On the basis of the above work-up we were able to exclude, prospectively, from the study all the children who had metabolic disorders, liver dysfunction, hyperthyroidism, hypothyroidism. Children weighing more than 20% above their ideal weight were also excluded.

Experiment 1

Fasting blood samples were withdrawn into evacuated heparinized tubes from the anticubital vein of 28 children (age 7-11 years, mean$\pm$SD 8.5$\pm$1.1 years) whose both parents smoke 15-30 cigarettes/day, and of 25 children (aged 7-11 years, mean$\pm$SD 8.8 years) whose both parents are nonsmokers. After centrifugation (1500 x g, 4^{o}C, 10 min) the harvested plasma was frozen in liquid nitrogen. Erythrocytes were resuspended to the original volume in isotonic phosphate buffered saline (0.154 mmol/l, pH 7.4, 4^{o}C).

Experiment 2

Twenty three children of smoking parents (15-30 cigarettes/day), aged 3-6 years (mean±SD 4.6±0.9 years) and 23 children of nonsmoking parents, aged 3-6 years (mean±SD 4.7±0.5 years) were age-matched and allocated to one of four treatment groups in 2x2 factorial design: children of smoking parents vs children of nonsmoking parents and placebo vs vitamin E. For 14 days each subject took one capsule per day of either 100 mg alfa-tocopherol acetate in soya oil or a placebo containing hydrogenated coconut oil and with an alfa-tocopherol content <20, µg. The selenium content of the capsules was negligible (0.005-0.044 µg). Blood was then drawn from each subject and processed as described in experiment 1.

Parameters studied

The following parameters were determined in the erythrocytes of all children: /1/ vitamin E concentrations; /2/ glutathione peroxidase activity; /3/ catalase activity; /4/ glucose-6-phosphate dehydrogenase activity; /5/ superoxide dismutase activity; /6/ total glutathione concentration; /7/ reduced glutathione concentration; /8/ oxidized glutathione concentration; /9/ concentration of thiobarbituric acid reactive substances (TBARS); and in plasma (10) total cholesterol concentration; /11/ vitamin E concentration; /12/ conjugated dienes; /13/ concentration of TBARS, and /14/ concentrations of ascorbate and dehydroascorbate.

Biochemical determinations

The erythrocytes were subsequently washed twice with 2 vol of 154 mmol/l sodium chloride solution at pH 7.4. Following this, the erythrocytes were hemolysed with a twofold volume of distilled water. Glutathione peroxidase (E. C. 1. 11. 1. g) activity in hemolysate was measured by the coupled assay system described by Paglia and Valentine /17/. The enzyme activity was calculated by means of the molar extinction coefficient e=6.22 L/mmol cm for NADPH and expressed as mkat/mol hemoglobin (1 kat = 1 mol NADPH transformed/s). Superoxide dismutase (EC 1. 15. 1. 1) activity was assayed using the method of Misra and Fridovich /16/, based on the measurement of inhibition of epinephrine autooxidation to adrenochrome. A unit of activity was defined as the amount of sample causing 50% inhibition of adrenochrome formation. Catalase (EC 1. 11. 1. 6) activity was assayed in hemolysates of erythrocytes by monitoring the consumption of H_2O_2 at 240 nm as described by Beers and Sizer /4/, with activity defined as the change in absorbance per minute at 25°C and pH 7.0. The glucose-6-phosphate dehydrogenase activity was determined using diagnostic kit (Boehringer, Mannheim, F.R.G.). Total glutathione (GSH + GSSG), oxidized glutathione (GSSG), and reduced glutathione (GSH) were determined by the glutathione reductase-DTNB (5.5'-dithiobis-/2-nitrobenzoic acid/) assay of Di Simplicio and Mannervik /7/. The concentrations of vitamin E in

hemolysate and in plasma were assayed fluorimetrically in the hexane phase used for extraction of the vitamin /9/. Total plasma cholesterol was measured with a test kit (Boehringer, Mannheim, G.F.R.). Plasma ascorbate and dehydroascorbate concentrations were measured by liquid chromatography /15/. The plasma indices of lipid peroxidation: conjugated dienes, and thiobarbituric acid reactive substances (TBARS) were determined by the methods of Yagi /18/, and Hunter and Mohamed /11/, respectively.

The results are expressed as mean ± SD. The comparisons between the means were performed using the analysis of variance (ANOVA). Values of $p<0.05$ were considered significant.

RESULTS

Experiment 1

Plasma concentration of vitamin E was similar in children of smoking parents and in children of nonsmoking parents (Table I).

TABLE I

Plasma antioxidants of children of smoking and nonsmoking parents

	Children of nonsmoking parents (n=28)*	smoking parents (n=25)*	p
Vitamin E (μmol/l)	18.2 ± 2.0	15.0 ± 1.2	NS
Ascorbate (μmol/l)	43.7 ± 3.3	22.9 ± 7.1	<0.05
Dehydroascorbate (μmol/l)	2.3 ± 0.5	7.2 ± 2.1	<0.005

Results are presented as means ± SD

*Number of children

p - Statistical significance

Mean plasma ascorbic acid concentration was 50% lower ($p<0.01$) and dehydroascorbic acid concentration was significantly elevated

($p<0.02$) in children of smoking parents. Erythrocyte vitamin E, total and reduced glutathione concentrations, and catalase activity were the same in children of smoking parents and nonsmoking parents. However, erythrocyte glucose-6-phosphate dehydrogenase, glutathione peroxidase and superoxide dismutase activities were significantly lower ($p<0.001$, $p<0.005$ and $p<0.02$, respectively) in children of smoking parents (Table II).

Experiment 2

Plasma total cholesterol was not different in children of smoking and nonsmoking parents, and was unaffected by vitamin E supplementation (Table III.). As in experiment 1, plasma and erythrocyte vitamin E concentrations were similar in children of smoking and nonsmoking parents. Vitamin E supplementation increased ($p<0.001$) erythrocyte and plasma vitamin E concentrations to the same extent in both groups (Table IV). The elevated erythrocyte TBARS ($p<0.02$), elevated plasma TBARS ($p<0.01$) and elevated erythrocyte oxidized glutathione concentrations ($p<0.001$) in children of smoking parents were abolished ($p<0.002$, $p<0.001$ and $p<0.002$ for TBARS in erythrocytes and plasma, and for oxidized glutathione, respectively) by vitamin E supplementation. Concentrations of conjugated dienes were significantly greater ($p<0.002$) in children of smoking parents (Table III). Inclusion of age as a covariate for statistical analysis revealed age-associated increases in plasma and erythrocyte vitamin E, total cholesterol, conjugated dienes, and TBARS (Table V).

DISCUSSION

Passive smoking causes changes in certain indices of antioxidant status. The enhanced susceptibility of erythrocytes of children of smoking parents to lipid peroxidation may reflect

TABLE II

Erythrocyte antioxidants and antioxidant-enzyme activities of children of smoking and nonsmoking parents

Children of nonsmoking parents (n=28)**	Children of smoking parents (n=25)**	p*
Vitamin E (μmol/g Hb)		
3.9 ± 0.4	3.6 ± 0.2	NS
Glutathione peroxidase (m kat/mol Hb)		
12.5 ± 1.1	9.2 ± 0.9	<0.005
Catalase (m kat/mol Hb)		
24.05 ± 1.22	26.42 ± 2.33	NS
Glucose-6-phosphate dehydrogenase (m kat/mol Hb)		
5.2 ± 0.4	2.6 ± 0.3	<0.001
Superoxide dismutase (m kat/mol Hb)		
7.28 ± 0.9	3.08 ± 1,1	<0.02
Total glutathione (mg/L)		
124 ± 12	112 ± 9	NS
Reduced glutathione (mg/L)		
115 ± 6	106 ± 5	NS
Oxidized glutathione (mg/L)		
0.6 ± 0.1	11.0 ± 2.7	<0.0005

Results are presented as means ± SD

* Statistical significance

** Number of children

TABLE III

Effects of vitamin E supplementation (100 mg alfa-tocopherol acetate per day for 14 days) on indices of lipid peroxidation and total cholesterol in plasma of children of smoking and nonsmoking parents

Children of nonsmoking parents		Children of smoking parents	
placebo (n=13)*	vitamin E (n=12)	placebo (n=11)*	vitamin E (n=12)
Plasma vitamin E (μmol/L)			
17.9 (1.9)	24.8 (1.6)	15.8 (0.4)	23.2 (2.3)
Erythrocyte vitamin E (μmol/g Hb)			
3.9 (0.8)	8.4 (0.2)	3.7 (0.3)	10.3 (0.7)
Erythrocyte TBARS** (mmol/L)			
0.16 (0.01)	0.05 (0.01)	0.62 (0.02)	0.22 (0.03
Conjugated dienes (kU/L)			
165 (63)	145 (24)	224 (45)	178 (63)
Plasma TBARS (mmol/L)			
1.3 (0.1)	1.1 (0.3)	2.5 (0.9)	2.0 (0.3)
Plasma total cholesterol (mmol/L)			
5.2 (0.1)	5.1 (0.2)	5.1 (0.2)	5.0 (0.1)
Oxidized glutathione (mg/L)			
0.3 (0.1)	0.1 (0.1)	11.3 (0.6)	3.2 (1.1)

Results are presented as means ± SD

* Number of children

** Thiobarbituric acid reactive substances

TABLE IV

Statistical analysis of effects of vitamin E supplementation on indices of lipid peroxidation and total cholesterol in plasma of children of smoking and nonsmoking parents

	Passive smoking effect	Vitamin E effect	SED*
Plasma vitamin E	NS	<0.001	2.4
Erythrocyte vitamin E	NS	<0.001	0.4
Erythrocyte TBARS**	<0.02	<0.002	0.02
Plasma conjugated dienes	<0.002	NS	23
Plasma TBARS	<0.002	<0.001	0.5
Oxidized glutathione	<0.001	<0.002	0.1
Total cholesterol	NS	NS	0.2

* Standard error of difference

** Thiobarbituric acid reactive substances

NS-Not Statistically significant ($p>0.05$)

the lower activities of glucose-6-phosphate dehydrogenase, glutathione peroxidase and superoxide dismutase. Decreased activity of glucose-6-phosphate dehydrogenase can be caused by extracellular or intracellular lipid peroxides /13/. Differences in glutathione peroxidase activity between adult smokers and nonsmokers have been reported by Duthie et al./8/ and may be associated with decreased selenium status /9/.

Indications of oxidant stress in passive smoking children are emphasized by the significantly greater concentrations of dehydroascorbate in plasma of children of smoking parents. Decreased plasma vitamin C concentrations reported by Duthie et al. /8/ and by Kalner et al. /12/ have been regarded as a consequence of greater vitamin C turnover in response to a sustained oxidant load rather than a decreased dietary intake. Elevated plasma conjugated-dienes concentrations suggest that

TABLE V

Variables significantly associated with increasing age (years)

	Covariate	Slope of covariate	Standard error
Total cholesterol	0.001	0.050	0.0254
Erythrocyte vitamin E	0.05	0.0155	0.0088
Plasma vitamin E	0.001	0.237	0.0866
Conjugated dienes	0.001	2.45	0.671

lipid peroxidation is enhanced in children of smoking parents. In normal human erythrocytes plasma levels of oxidized glutathione are low /1, 14/, and any elevation of oxidized glutathione suggest pathology. Although the dienes did not respond to vitamin E supplementation, the susceptibility of erythrocytes to peroxidize in vitro was markedly decreased in supplemented children of smoking parents.

The age-associated increase in plasma cholesterol is well-documented /2/, LDL-associated cholesterol is a major carrier of vitamin E in the blood. Despite the relative increase in plasma vitamin E with age, conjugated dienes were also elevated, suggesting that peroxidation of endogenous fatty acids is more marked in older children. The evidence we report lends further support to the observation that passive smoking may increase the risk of subsequent athorema formation, and suggests that it is particularly important to protect children from this environmental hazard.

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SHOULD ANORECTAL MANOMETRY BE PERFORMED IN HIRSCHSPRUNG'S DISEASE?

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This study aims to assess the diagnostic value of anorectal manometry in Hirschsprung's disease as compared with radiological studies and histology. Twenty-six patients treated for congenital aganglionosis of the colon at the Surgical Unit of the Department of Paediatrics, University Medical School, Pécs, Hungary between 1984 and 1991 served as patient material. The results indicate that anorectal manometry offers at least as high diagnostic accuracy as radiology and histology. This makes manometry an important diagnostic aid in the differential diagnosis of the neonatal intestinal obstruction, as well as of the chronic constipation in childhood.

INTRODUCTION

The observations which made possible the introduction of anorectal manometry as one of the diagnostic tools in aganglionosis of the intestinal tract are of great importance. It was Gowers in 1887 who first recognized that arteficial rectal distension resulted in relaxation of the internal sphincter muscle of the rectum in healthy individuals /3/. This response of the smooth-muscle sphincter has been termed the rectosphincteric reflex. In 1901 Tittel presented the case of an infant with chronic constipation since birth in whom the absence of rectal ganglion cells was demonstrated /1/. These observations did not attract much attention until 1964 when Callaghan and Nixon first reported that the presence of rectal

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ganglion cells was essential for the rectosphincteric reflex /8/. This was the final step in the introduction of anorectal manometry as an important diagnostic method in Hirschsprung's disease and in its differentiation from chronic constipation.

The diagnostic value of the radiological, histological, histochemical and manometrical methods has arisen keen controversy in the literature which may be explained by the fact that different institutions approached the subject in different ways /3, 5, 9, 11/. The aim of this retrospective study was to assess the accuracy of manometry in the diagnosis of Hirschsprung's disease. A comparison of manometry, histology and radiology was conducted in our patients with respect to reliability.

MATERIAL AND METHOD

Twenty-six neonates, infants and children in the Surgical Unit of the Department of Pediatrics, University Medical School of Pécs, Hungary were treated surgically for Hirschsprung's disease. Mean age at diagnosis was 22.2 months (range 2 days - 135 months). Diagnosis was established in the first year of life in about two-third of patients. Sex distribution was 3.3 : 1 indicating a male predominance. Indication for surgical treatment was based on the case history, clinical signs, radiological, histological and manometrical investigations. Specimens for histology were obtained at the time of the colostomy establishment. If it was possible to identify a transition zone, specimens were taken from the narrowed bowel, the funnel-shaped zone and the dilated colon which ensured a levelling-type of histology. In cases where the extension of aganglionosis (short-ultrashort segment) prevented us from taking biopsies at the site of the aganglionic colon, histological investigations were performed 3-6 months later on specimens obtained from the left wall of the rectum. In cases of neonatally manifested Hirschsprung's disease no anorectal manometry was done before the age of 8 days as suggested by Holschneider /5/. A detailed description of the method of anorectal manometry as performed in our unit was given in a previous article /4/. The diagnosis of congenital aganglionosis was considered as established if at manometry rectal distension was not followed by a drop in pressure at the site of the internal sphincter (lack of the rectosphincteric reflex). The results of radiological and histological investigations were regarded as positive if they unambiguously demonstrated Hirschsprung's disease, ambiguous if there were doubts about

the interpretation of the results and negative if it was possible to exclude aganglionosis as the underlying pathology.

RESULTS

Radiological investigations proved the presence of Hirschsprung's disease in 17 of 26 cases, while in 8 cases a correct diagnosis, based on radiology proved to be ambiguous and in 1 case aganglionosis could be excluded. Histology demonstrated no ganglion cells in specimens of 17 patients, the reports were inconclusive in 4 cases and in 5 cases there were ganglia present in the specimens according to the histology reports. Anorectal manometry showed the absence of internal sphincter relaxation in 25 cases. In one patient changes in the trace due to rectal distension were seen and interpreted as the result of a normal rectosphincteric reflex. Nevertheless, in this last case the histology showed the presence of intramural ganglion cells, therefore, there was a strong suggestion for the clinical signs typical for Hirschsprung's disease to be related to neuronal intestinal dysplasia.

The results regarding the diagnostic value of different diagnostic methods are shown in the Table.

Table I

Diagnosis	Diagnostic method			
	radiology	histology	manometry	surgery
Hirschsprung	17/26	17/26	25/26	26/26
Non-Hirschsprung	1/26	5/26	1/26	0/26
Ambiguous	8/26	4/26	None	None

DISCUSSION

The lecture given by Harald Hirschsprung in Berlin in 1886 was the first step in the treatment of a congenital disorder of the intestinal tract innervation which was to be fatal for 60 years following this presentation. A few years later in 1878, Gowers observed that in response to rectal distension the pressure of the internal anal sphincter decreased temporarily /3/. This phenomenon has been termed the rectosphincteric reflex. Tittel's observation in 1901, that no ganglion cells were demonstrable in the rectum of an infant suffering from chronic constipation since birth /1/, received no attention for decades. Callaghan and Nixon were the first to realize that the presence of intramural ganglia resulted in normal rectosphincteric reflex in chronically constipated children, but not in children with Hirschsprung's disease /8/. Nowadays the classical form of aganglionosis can be seen in only less than 10% of all cases /7/. In the great majority of patients diagnosis is established during the neonatal period because of the early presentation of the intestinal obstruction. In the minority of the patients the course of the disease is benign and aganglionosis will be diagnosed during investigations for chronic constipation. Delay in establishing the proper diagnosis of congenital aganglionosis primarily in neonates, will result in increased mortality /2, 5, 7/. In infants and neonates with Hirschsprung's disease toxic enterocolitis due to the underlying pathology is responsible for 30-70% of all deaths /2/. In the last decade, this high mortality rate has been reduced to 0% in some centers /10/. This obviously remarkable improvement in survival can be explained by early recognition of congenital aganglionosis, which is based primarily on the combined application of immunohistochemistry, radiology, histology and anorectal manometry. The presence of increased acetylcholinesterase activity in the affected segment of the intestinal tract is regarded as the most reliable evidence of Hirschsprung's disease. However, there are reports on the limited diagnostic value of immunohistochemistry in the

first 6-9 months of life. Some data in the literature also point towards the pitfalls and limitations of radiology and manometry. Regarding the anorectal manometry several reports claim diagnostic values with a success rate of 75-100% /5/. In view of the fact that anorectal manometry is the only functional diagnostic method targeting the presence or absence of the rectosphincteric reflex and that it may be performed almost without limitation of age, manometry may be considered a safe, reliable diagnostic aid in Hirschsprung's disease. Furthermore, its reliablility is not affected by previous manipulations on the anorectum. Nevertheless, the reliability of the different diagnostic methods has been the subject of much controversy /3 , 5, 9, 11/. In agreement with Joppich and Iwai /6, 7/ we do not regard any of the above-mentioned diagnostic methods as the best and favour their combined application to ensure early recognition of Hirschsprung's disease. Anorectal manometry, which has been practised on a regular basis in our unit since 1984 has proved to be at least as useful as histology and radiology in diagnosing congenital aganglionosis. However, when comparing manometry, radiology and histology it should be borne in mind that our histological investigations did not include the acetylcholinesterase reaction. Since manometrical diagnosis is based on the detection of a disorder of neuromuscular function, its accuracy is theoretically 100%. In view of its noninvasiveness, independence of age and previous rectal manipulations (rectal examination rectal suction biopsy, barium enema) and diagnostic accuracy, the use of anorectal manometry is highly recommended in Hirschsprung's disease.

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BRONCHIAL HYPERREACTIVITY IN HEALTHY CHILDREN OF ASTHMATIC PARENTS

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The aim of this study is to determine aspecific bronchial reactivity in healthy children whose parents were treated for childhood asthma. Bronchial challenge with four increasing concentrations of acetylcholine (1.25, 2.5, 5.0, 10.0 mg/ml) was carried out in 82 individuals: 28 healthy (HA), 24 atopic (AA) children of asthmatics; 30 control healthy (HN_1) children from non-atopic families. The members of the HA group exhibited a larger percent decrease in FEV_1 values at each acetylcholine concentration than that of the HN_1 group (1.25 mg/ml: 6.2 versus +0.3; 2.5 mg/ml: 7.9 versus 3.7; 5.0 mg/ml: 11.5 versus 5.3; 10 mg/ml: 16.8 versus 11.0). Physical exercise (free running) was carried out in 153 children: 30 HA, 26 AA and 97 control healthy (HN_2) children from non atopic families. The HA group showed somewhat greater (4.6% versus 0.6%) decrease in FEV_1 values after exercise compared to the HN_2 group. Healthy children of asthmatics (HA) display higher aspecific bronchial reactivity than healthy children from non atopic families (HN) and form an intermediate group between atopic (AA) and healthy (HN) children.

INTRODUCTION

Bronchial asthma is almost always accompanied by bronchial hyperreactivity. However, this hyperreactivity can also be detected in connection with several other conditions e.g. weeks after airway infection, years after neonatal ventilation, allergic rhinitis, chronic bronchitis and in subjects with a

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family history of atopy /1, 12, 20/.

With the help of appropriate methods, aspecific bronchial hyperreactivity can be detected in the majority (more than 90%) of asthmatics. In the course of bronchial challenge performed with acetylcholine, metacholine, histamine, etc. the results of dose-response analysis suggest definite differences between asthmatics and non asthmatics /6, 15, 27, 28, 30, 31/.

Pulmonary function tests performed before and after exercise may be useful in detecting the exercise-induced bronchospasm which is an important feature of non-specific bronchial hyperreactivity /13, 21, 22/.

The aim of this study is to determine the aspecific bronchial reactivity in those children of our former asthmatic patients who do not present clinical signs of obstructive pulmonary disease or any other complaints of possible atopic origin. The question is: do they give an altered bronchial reactivity compared to healthy children of non-atopic families.

PATIENTS AND METHODS

Patients: we followed the history of adults treated with bronchial asthma in their childhood at our Department /9, 10, 11/. To collect data on inheritance of bronchial hyperreactivity we investigated their children.

Among the mentioned patients 195 had children already - altogether 311. We sent questionnaries concerning the age and diseases of their children: 83 (26.8%) were shown to have some form of atopic disease: bronchial asthma 68 (21.9%) allergic rhinitis/conjunctivitis 7 (2.3%) and atopic eczema 8 (2.6%), while 228 were healthy. The type of drugs and other therapy instituted was evaluated.

In randomly selected groups of these children the non-specific bronchial reactivity induced by acetylcholine and physical exercise (free running) were examined. Among the choosed children 52 were able to participate in the bronchial challenge with acetylcholine and 56 in the physical exercise. They were between 5-14 years of age, only one of them was 4 years old.

Thirty children were symptom-free and no atopic disease occurred in their personal history, they will be referred to as healthy children of asthmatics (HA):

n: 26, 1 asthmatic parent
n: 1, 2 asthmatic parents

n: 2, 1 asthmatic parent, 1 parent with allergic rhinitis
n: 1, 1 asthmatic parent, 1 parent with atopic eczema.

The history of 26 children presented with atopic disease (23 bronchial asthma, 2 allergic rhinitis, 1 atopic eczema): they will be referred to as atopic children of asthmatics (AA):
n: 23, 1 asthmatic parent
n: 1, 2 asthmatic parents
n: 2, 1 asthmatic parent, 1 parent with atopic eczema

We had 30 control children (HN_1) in the acetylcholine challenge and 97 control children (HN_2) in the physical exercise test. These children and their first-grade relatives do not suffer from any atopic diseases: the detailed history has been taken by one of the investigators. Table I contains the data of the examined children.

TABLE I

Sex and age distribution of the examined children

Healthy children of asthmatics (HA):	n: 30
boys: 16, girls : 14	
mean age: 8.5 years $\pm$ SD 2.7	
Atopic children of asthmatics (AA):	n: 26
boys: 16, girls: 10	
mean age: 8.3 years $\pm$ SD 2.4	
Healthy controls for acetylcholine (HN_1)	n: 30
boys: 14, girls: 16	
mean age: 10.1 years $\pm$ SD 2.0	
Healthy controls for physical exercise (HN_2):	n: 97
boys: 60, girls: 37	
mean age: 10.6 years $\pm$ SD 3.5	

Methods: before the acetylcholine challenge a spirometry was taken by a spirograph SP 21 (Medicor). The normal values according to Zapletal /33/ were used as references.

Acetylcholine challenge: the children were told to breathe on tidal level from the buffered acetylcholine solutions which were nebulised with TUR-USI 50 ultrasonic nebulizer for 2 minutes; the control was phys. saline solution. The output of the ultrasonic nebuliser reached a steady state after 8-10 minutes of working: 1.35 ml $\pm$ SD 0.11 ml/2 minutes. We used it after 10 minutes of nebulising. The following acetylcholine concentrations were used for the challenges: 1.25, 2.5, 5.0, 10.0 mg/ml. The FEV_1 values were determined 1,3,5 minutes after the acetylcholine inhalation. The challenge was finished either

when the decrease of FEV_1 exceeded 25% compared to the initial values, or after having inhaled the 10 mg/ml acetylcholine for 2 minutes. The degree of bronchospasm was expressed as the percent fall of the FEV_1.

The physical exercise involved outdoor running (1 of May - 15 of June). The children ran for 6 minutes with an intensity that their pulse rates should exceed 170/min by the end of the second minute and this should remain on this level until the end of the exercise. The FEV_1 values were measured 3,5,10,16 and 20 minutes after the physical challenge. The maximal fall of FEV_1 was registered and the change was expressed as the percent fall of the FEV_1.

The reproducibility of the applied method was determined by the help of 10 healthy control children. On two consecutive days they took the same exercise test: the difference was less than 3% in the baseline and less than 7% in the exercise induced values.

The statistical analysis was performed with an unpaired t-test at a significance level of $p = 0.05$.

RESULTS

In the two randomly selected groups (HA and AA) the following observations and investigations were made.

Clinical findings: based on the questionnaries and the personal interviews with the children and at least with one of their parents, the healthy children of asthmatics (HA group) did not present atopic disease in their medical history. No one of them got treatment for such problems.

The following anamnestic data were found in these children: frequent urticaria (1), allergic oedema (1), frequent coughing (4), frequent upper airway infections, otitis, tonsillitis (5), moderate valvular aortic stenosis (1), moderate pulmonal stenosis (1), mild cerebral palsy (1).

The atopic children of asthmatics (AA group) have been interviewed in the same manner. Among them 23 had bronchial asthma: 10 infection induced (2 with bronchospasm following exercise), 8 exogen allergic type, 5 had mixed type (allergy + infection); 2 children had allergic rhinitis; 1 child suffered from atopic eczema. The following treatment was administered in

the last year before the interview: no treatment (8), antihistamines (4), betamimetics (7), aminophylline (3), DNCG (1), ketotifen (4), immunotherapy (1), steroid ointment on the skin (1), local treatment in the nose (2).

Lung function: in the two groups (HA, AA) of children the forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and peak expiratory flow (PEF) were recorded. The mean per cent of predicted values and its standard deviation was recorded:

In the HA group: FVC: 83.2%±11.3; FEV_1: 93.8%±16.2;
Tiffeneau index (FEV_1/FVC): 92.2%±8.3; PEF: 84.6%±11.6.
In the AA group: FVC: 74.2%±15.0; FEV_1: 81.5%±12.6;
Tiffeneau index (FEV_1/FVC): 94.3%±4.0; PEF: 78.3%±10.2.

Acetylcholine challenge: the values of FEV_1 decrease were compared in the three groups (AA, HA, HN_1) of children. At each acetylcholine concentration a significant difference was found between the atopic (AA) and control (HN_1) groups, where a greater fall always occurred in the atopic children (Table II).

Also the healthy children of asthmatics (HA) differed significantly from the controls (HN_1) at 1.25 - 2.5 - 5.0 mg/ml acetylcholine concentrations, a greater fall was always registered in the HA group. At 10 mg/ml concentration, this fall was larger in the HA group compared to that in the HN_1 group, but the difference did not reach the level of significance (Table II).

When the FEV_1 values of the AA and HA groups were compared, the decrease of FEV_1 was always greater in the children of AA group but this difference was never significant ($p= 0.2$ at 1.25 mg/ml; $p= 0.1$ at 2.5 mg/ml; $p= 0.1$ at 5.0 mg/ml; $p= 0.1$ at 10.0 mg/ml conc.).

Physical exercise: the values of FEV_1 decreases (Table II) were compared in the three groups (AA, HA, HN_2) of children. The difference between the AA and HN_2 groups was significant. The greater fall was found in the atopic children. Also the healthy children of asthmatics (HA) showed greater fall in FEV_1 compared to the HN_2 group, but this difference was not significant.

TABLE II

The mean per cent decrease ($\bar{X}$) in the FEV_1 values and their standard deviation (SD) in the groups of examined children

	Atopic children of asthmatics (AA)				Healthy children of asthmatics (HA)				Control children (HN_1) and (HN_2)		
ac.conc. mg/ml	n	$\bar{X}$	SD	p	n	$\bar{X}$	SD	p	n	$\bar{X}$	SD
1.25	23	8.5	9.6	0.0005	28	6.2	8.7	0.005	30	+0.3	8.3
2.5	24	11.1	9.8	0.0025	28	7.9	9.7	0.05	30	3.7	7.4
5.0	23	16.5	10.8	0.0025	25	11.5	10.9	0.05	29	5.3	12.0
10.0	18	22.8	11.8	0.0025	25	16.8	11.9	0.1	28	11.0	13.3
Physical exercise	26	9.3	7.2	0.0025	30	4.6	6.6	0.1	97	0.6	13.6

p values are related to the control children

The decrease in the FEV_1 values of the AA group was always larger than in the HA group and this difference was significant ($p= 0.01$).

DISCUSSION

One of the most important features of asthma is the hyperreactivity of the airways to a wide variety of specific and non specific stimuli. Several studies have shown which signs of bronchial hyperreactivity and trends for atopic reactions were typical in relatives of asthmatic individuals.

In Australia studies of responsiveness to histamine in infants soon after birth have demonstrated increased airways responsiveness in infants with a family history of asthma. This observation in healthy asymptomatic infants early in life suggests the genetic contribution to asthma /32/.

Hopp and co-workers /17/ performed metacholine bronchial challenge in various groups (manifest asthmatics, healthy and asthmatic/allergic children of asthmatic parents, healthy children of healthy parents, allergic and non-allergic twins) of children and young adults. The group of asthmatics significantly differed from all the other groups as regarded to metacholine sensitivity. At the same time, however, the healthy members of asthmatic families exhibited more pronounced bronchial reactivity than the healthy members of the healthy families.

In the siblings of 59 atopic asthmatics 48% showed increased non-specific bronchial responsiveness tested by metacholine. Non - specific bronchial reactivity showed only a weak association with atopy which was based on the skin prick tests to 9 common allergens + on the determination of total serum IgE /29/. Hypersensitivity to metacholine was found in symptom-free parents of asthmatic children, too /3/.

Britt and co-workers /2/ observed hypersensitivity to metacholine in approximately half of the relatives of adult patients suffering from chronic obstruction - they considered

them as risk groups. The sensitivity to metacholine was the same among the parents, children or brothers of asthmatics according to an other study /14/.

Clifford and co-workers /5/ worked with children of one asthmatic parent and performed allergy skin testing and measurement of bronchial responsiveness to metacholine in parent and child. The prevalence of atopy (19%) among these children did not differ from the ones in the population. Hyperresponsiveness to metacholine (45%) with a 20% fall in FEV_1 was higher compared to the general population.

In some studies family examinations were performed to register bronchial responsiveness upon physical exercise. In 32% of first-grade relatives of asthmatic children bronchial instability was found /22/. The same authors observed exercise induced bronchial hyperreactivity in 29% of the relatives of children suffering from wheezy bronchitis, compared to the 5% found in controls /13, 23/.

Investigations on twins displayed a similarity in the appearance of aspecific bronchial hyperreactivity; this was more pronounced in monozygots than in dizygots /16, 23, 26/.

Increased bronchial reactivity in itself dos not mean clinical symptoms. Some other factors (e.g. passive smoking, air pollution, viral infection etc.) may have an important role in enhancing or triggering obstructive diseases on the basis of airway hyperreactivity /7, 8, 23, 25, 26/.

We investigated the children of our former patients treated for asthma in their childhood. More than 25% of them showed atopic disease; bronchial asthma was the most frequent diagnosis. The children randomly selected for lung function showed two distinct groups (HA and AA).

Our findings indicate that the children who have parent/parents with asthma exhibit a significantly enhanced airway reactivity to acetylcholine, and this is true in completely symptom-free children. This bronchial responsiveness does not mean clinical manifestations, it is only one factor in the pathogenesis of asthma /29/.

A significant proportion of children with airway responsiveness have never had asthma symptoms and some with troublesome asthma symptoms have no evidence of airway hyperresponsiveness. This phenomenon is clearly associated with asthma but is not necessarily characteristic /18/.

The healthy children of asthmatic (HA group) show higher response to physical exercise compared to the control group (HN_2) but this difference is not significant. The relatively small reaction to physical challenge in AA group may be the consequence of the mild asthmatic disease; the observations of other authors were the same /13, 21/. Generally the severe asthmatic patients show more serious and frequent exercise induced bronchospasm /19/.

In conclusion: the healthy children of asthmatic parents exhibit a significantly higher non-specific airway reactivity to acetylcholine compared with the healthy controls. Their reaction to physical exercise is more expressed than the results gained in healthy non-atopic children. The members of this group display a higher reactivity and form an intermediate group between the atopic and the control children. Long-lasting observations are needed for establishing the clinical meaning of this pathologic feature. With increasing knowledge of the pathogenesis of asthma, the eventual aim will be primary prevention /24/. This will require identification of the groups at risk. The question is still open whether our symptomless but hyperresponsive patients are really at risk for obstructive pulmonary diseases.

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SUBCLASS DISTRIBUTION OF IgG- AND IgA-CONTAINING CELLS IN THE JEJUNAL MUCOSA OF CHILDREN WITH COELIAC DISEASE

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In 20 jejunal biopsy specimens from children with coeliac disease the densities of cells containing the various subclasses of IgG and IgA were measured using monoclonal antibodies stained with secondary antibodies conjugated with biotin-avidin peroxidase: 8 specimens were taken before treatment, 7 during a gluten free diet and 5 after gluten challenge. In addition, 11 control specimens were studied. The cells of each IgG subclass were present in significantly higher densities in the specimens from the children with coeliac disease on a normal diet than in those from controls. On a gluten-free diet the cell densities of these patients were similar to those of controls. Before treatment and on gluten challenge the patients had lower mean percentage of IgG4 cells (21% and 18%, respectively) than the controls (28%). The proportion of IgG1 cells in the specimens taken during a gluten-free diet was lower than in those from untreated patients (21% vs 31%, $p < 0.05$). The densities of cells containing the two IgA subtypes were greater in the patients than in the controls and the proportion of IgA1 cells was significantly higher in the untreated patients than in controls (56% vs 47%, $p < 0.05$). These alterations in the subclass distribution of the local IgG- and IgA-containing cells in the patients seem to suggest that these cells play a role in the tissue defence mechanism, or are primarily involved in the pathogenesis of coeliac disease.

INTRODUCTION

The main feature of the pathogenesis of coeliac disease (CD) is an abnormal immune reaction to gluten, both humoral and cell-mediated immune responses are activated in the intestine of patients with untreated CD /1, 2/. Most studies indicate that densities of IgA-, IgG- and IgM-containing cells are

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severalfold greater in the jejunum of patients with active CD than in the normal jejunum /3-9/. Immunoglobulin G and its subclasses have biologically potent capabilities mediated by activation of complement component, initiation of phagocytosis and induction of cell mediated cytotoxicity. The four IgG subclasses differ in these functional capabilities /10-12/ and therefore changes in the proportions of the IgG subclasses may have pathological consequences. Furthermore, antigens of different types selectively elicit the production of certain subclasses as part of the antibody response /13/.

Previous studies have shown that selective changes in the IgG subclasses are to be found in many diseases /12-17/. The two subclasses of IgA are apparently functionally identical, but IgA1 is susceptible to breakdown by a variety of IgA-specific microbial proteases /18/. Most children with CD have low levels of IgG2 and IgG4 in the serum, although the total IgG level does not differ from the controls /19/. Antibodies to gliadin have been found in the IgG1 and IgG3 subclasses /19, 20/ and also in IgG2 /21/.

In the lamina propria of small intestine of persons without gastrointestinal diseases, a preponderance of IgG1 and IgG2 cells was found, while the proportion of IgG3 and IgG4 cells was much lower /22, 23/. The proportion of IgG2-containing cells in the jejunal mucosa was significantly higher in untreated than in treated adult patients with CD /24/. The proportion of cells containing subclass IgA2 is higher in the jejunal mucosa of adults, with untreated or treated CD than in controls /25/.

In our study we used monoclonal antibodies and a sensitive peroxidase technique to estimate the densities of the various IgG and IgA subclasses in the jejunal mucosa of untreated and treated children with CD. The findings were compared with those of healthy controls.

MATERIALS AND METHODS

Subjects

We studied 20 specimens from 16 children with confirmed or suspected CD (11 girls, 5 boys, mean age at the biopsy before treatment 6.2 years, range 0.8-15.5 years). The biopsy specimens taken before dietary treatment all showed severe villous atrophy. In 14 patients the jejunal mucosa became normal during a gluten-free diet and relapsed after gluten challenge. In the remaining two patients a gluten-free diet led to significant improvement in jejunal morphology. The duration of the gluten-free diet was, on average, 3.5 years (1.1-10.8 years). The mean duration of the gluten challenge (normal gluten-containing diet) was 0.4 years (0.25-0.5 years). From 8 patients specimens taken before dietary treatment were available for this study. Specimens were obtained from 7 patients on a gluten-free diet before the planned challenge and from 5 children after the gluten challenge. Specimens from 11 children (6 girls, 5 boys, mean age 8.4 years, range 0.65-15.9 years) were used as controls for this study. A jejunal biopsy specimen had been taken from these patients because of poor gain in weight or retarded growth. None had gastrointestinal symptoms, nor did careful clinical and laboratory examinations reveal any gastrointestinal disorders. In these 11 children the histology of the proximal jejunum was normal.

All biopsy specimens were taken as a part of clinical evaluation of the patients. The study was approved by the Ethics Committee of the Children's Hospital, University of Helsinki.

Tissue Processing

Intestinal biopsy specimens were taken from the proximal jejunum with the paediatric Crosby capsule. The specimens were immediately divided into two parts. One half was processed for routine histological examination. The specimens for this study ohafter embedding in OCT^{R} compound (Tissue-Tec, 4538 Miles Laboratories Inc., Naperville, Illinois, USA) were frozen in Freon 22 suspended over liquid nitrogen. The frozen specimens were then stored airtight with ice at -70^{o}C until stained. Serial sections were cut at 5 μ from each tissue block, air dried for 1 hour and fixed in acetone at 4^{o}C for 10 min. The sections were immersed in chloroform for 30 min and then washed in Tris buffer.

Monoclonal Antibodies

The monoclonal antibodies used to identify IgG2 (clone GOM2), IgG3 (clone HP 6050), IgG4 (clone RJ4) and IgA2 (clone 2E2) subclasses were produced by Oxoid Ltd. (Hampshire, England). IgG1- (clone MH161) and IgA1- (clone NIF2) containing cells were detected with mouse monoclonal antibodies from Janssen Biochemica (Beerse, Belgium). Monoclonal antibodies to

constant regions of IgG (clone 8a4) and IgA (clone 2D7) were purchased from Oxoid Ltd. The monoclonal antibodies were used in a 1:400 dilution except for the anti-IgG1 and anti-IgA1 antibodies, which were diluted 1:1000 and 1:100, respectively. Monoclonal antibodies were diluted with Tris buffer (pH=7.4) containing 0.1% bovine serum albumin.

Immunohistochemical method

Monoclonal antibodies were applied to the sections as the first layer for c. 16 hours (overnight). For immunohistochemical staining Vectastain Elite ABC kit (PK-6102, Vector Laboratories, Burlinghame, Ca. USA) was used. The second layer was biotinylated anti-mouse IgG produced in horse. The third layer was avidin and biotinylated horseradish peroxidase macromolecular complex. The recommendations of the manufacturer were followed throughout the procedure. All incubations were carried out at room temperature for 30 min. Before each step, the sections were washed with Tris buffer. The peroxidase stain was developed with hydrogen peroxide activated AEC (3-amino-9-ethyl-carbazole, Sigma, St. Louis, Mo. USA) for 20 min, and the specimens were counterstained with haematoxylin (30 s). The slides were mounted in Gurr Aquamount (BDH Chemicals Inc., Poole, England). The blank control sections went through the same process without the first layer of monoclonal antibodies. The slides were evaluated using a Leitz-Ortholux light microscope and a 1000x magnification. Microscopic evaluation was done by one of the authors (A.A.) who was unaware of the diagnosis. The cell density in the lamina propria was determined with a 0.045x0.045 mm graticule fitted to the eyepiece of the microscope, the number of cells in at least 30 graticules was counted and the results were expressed as cells/mm^2. The error in repeated counts was always less than 10%.

Statistics

The statistical significance of the differences observed between the patients and the controls was calculated by analysis of variance using a PC program.

RESULTS

IgE-containing cells

The peroxidase-labelled cells were easily identified in the immunohistochemically stained slides. In the blank control slides the density of peroxidase-labelled cells was always less than 4 cells/mm^2.

The total number of cells belonging to all four IgG

studied). For each subclass the cell density was significantly higher in the specimens from the patients on a normal diet than in those from controls (Table I). In the patients on a gluten-free diet the densities of cells containing the various subclasses were similar to those of controls. In the controls IgG1 and IgG4 were predominant, both these subclasses had the highest density in 5 control specimens, while in the remaining one IgG2 cells predominated. Of the 13 specimens taken from CD patients on normal diet IgG1 cells had the highest density in 7 cases and IgG2 cells in 4 cases, while the density of either IgG3 or IgG4 cells was the highest in 2 specimens. In the 7 specimens from patients with CD on a gluten-free diet IgG4 cells predominated in 4 and IgG2 cells in 3.

We also calculated the proportion of each subclass contributing to the sum of IgG cells (Table II). In the patients on a gluten-free diet the mean proportion of IgG1 cells was significantly lower than before treatment. In specimens taken before treatment and after the gluten challenge the mean density of IgG4-containing cells was increased proportionally less than that of cells containing other 3 subclasses. At that time, the density of IgG4 cells was 2.6 times higher than that of controls and IgG1, 2 and 3 cells being 3.5, 3.9 and 4.2 times higher, respectively. This explains why the proportion of IgG4 cells was lower in the patients on gluten-containing diet than in the controls (Table II).

IgA-containing cells

We observed higher densities of both IgA1- and IgA2-containing cells in specimens from patients with CD on a normal gluten-containing diet, but their densities on a gluten-free diet were similar to those of controls (Table III). The sum of the IgA1 and IgA2 cells was similar to the density of IgA cells detected by the monoclonal antibody to IgA (1.02:1).

In the controls the mean proportion of IgA2 cells was slightly higher than that of IgA1 containing cells. In the untreated patients the proportion of IgA1 cells was

TABLE I

Densities of cells containing IgG and its subclasses in the lamina propria of jejunal mucosa of children with coeliac disease and controls. Values are means±SEM (cells/mm^2)

	IgG	IgG1	IgG2	IgG3	IgG4
Patients with CD before treatment (n=8)	*** 705±114	** 276±58	** 224±49	*** 198±44	** 167±30
during a gluten-free diet (n=7)	§ 207±36	§ 53±10	§ 59±12	§ 65±15	§ 76±20
after gluten challenge (n=5)	** 583±102	* 208±25	** 216±50	** 129±21	125±28
Controls (n=11)	171±32	71±25	56±14	41±12	58±16

* $p<0.05$ vs controls

** $p<0.01$ vs controls

*** $p<0.001$ vs controls

§ $p<0.05$ vs patients before treatment

TABLE II

Proportions of the IgG subclasses in the lamina propria of the jejunum of children with coeliac disease and controls (%). Values are means±SEM

	IgG1	IgG2	IgG3	IgG4
Patients with CD before treatment (n=8)	32±2.0	24±3.2	23±1.8	21±2.8
during a gluten-free diet (n=7)	§ 21±1.8	24±5.5	25±3.3	30±4.9
after gluten challenge (n=5)	30±2.2	31±2.7	21±3.6	18±1.9
Controls (n=11)	28±4.0	24±3.2	20±1.6	28±3.7

§ $p<0.05$ vs patients before treatment

significantly higher in the controls and it was also higher than that of IgA2 (Table IV).

In the individual specimens the IgA1-containing cells had the highest density in 5 of the 11 control specimens, while IgA1 predominated 7 of the 8 untreated children with CD. In specimens taken during a gluten-free diet the proportions of cells containing the two IgA subclasses were similar to those in the control specimens.

TABLE III

Densities of cells containing IgA and its subclasses in the lamina propria of the jejunal mucosa of children with coeliac disease and controls. Values are means ± SEM (cells/mm^2)

	IgA	IgA1	IgA2
Patients with CD before treatment (n=8)	1395±138 *	752±80 **	594±65 **
during a gluten-free diet (n=7)	494±79 §	259±56 §	292±29 §
after gluten challenge (n=5)	1320±284 *	621±157 *	692±140 *
Controls (n=11)	668±131	311±73	311±50

* $p<0.05$ vs controls

** $p<0.01$ vs controls

§ $p<0.05$ vs patients before treatment

TABLE IV

The proportions of IgA subclasses in the lamina propria of the jejunum of children with coeliac disease and controls (%). Values are means±SEM

	IgA1	IgA2
Patients with CD		
before treatment (n=8)	56±2.0 *	44±2.0 *
during a gluten-free diet (n=7)	44±4.4	56±4.4
after gluten challenge (n=5)	47±1.6	53±1.6
Controls (n=11)	47±2.5	53±2.5

* $p<0.05$ vs controls

DISCUSSION

In this study we confirmed the previous finding that in the lamina propria of jejunum of children with CD the density of IgG-containing cells is increased compared with controls /3-9/. The close similarity of the density of IgG- and IgA-containing cells measured either by using a monoclonal antibody recognizing all subclasses or by summing the densities calculated from those measured by using subclass specific monoclonal antibodies, shows the stability of the method used.

Among the children with CD the proportions of various IgG subclasses were only slightly different from those found in controls. In the controls we found different proportions of IgG subclasses from those reported earlier /22, 23/. This difference is due to the different methods used, when comparing the densities of IgG-containing cells, we saw 7 times more IgG cells in specimens from patients with active CD and 4 times

more in specimens from the treated ones than in the study using immunofluorescent method /24/. We used triple layer peroxidase staining.

In contrast to Rognum et al. /24/ we did not find a great predominance of the IgG1 subclass in the jejunal mucosa of children with CD. In our study the proportion of IgG2 immunocytes was similar in untreated and treated patients with CD, in contrast to the increased proportion of these cells found in untreated adult patients /24/. After gluten challenge the proportion of IgG2 cells was increased according to our findings and the earlier report /24/.

Measuring the density of IgA-containing cells either with the monoclonal antibody specific to constant region of IgA molecules or adding up the densities of the cells containing IgA subclasses measured by the respective monoclonal antibodies gave closely similar results and the previous finding of a marked increase in IgA cells in specimens taken from paediatric patients with untreated CD /4, 7, 8, 26/ was confirmed by both procedures. In adult patients with CD on a normal diet IgA-containing cells have been found in densities similar /5/ or even lower than /3, 27/ in controls which can be explained by assuming that on a gluten-containing diet the continuous stimulation of IgA-producing cells causes exhaustion. On a gluten-free diet the density of IgA-containing cells becomes normal /4, 7/.

The distribution of the jejunal IgA1 and IgA2 subclasses in our controls was different from the result of Kett et al. /25, 28/ but similar to that of Mearin et al. /29/, as we found proportionally slightly more IgA2 cells than IgA1 cells. In patients with untreated CD both the proportion and the density of IgA1 cells were increased, whereas on a gluten-free diet they were similar to those of controls. IgA1 immunoglobulin is susceptible to breakdown by the IgA specific microbial proteases /18/ and it is well known that in active CD the number of microorganisms in the small intestine increases /31/. The elevation of IgA1 cells in children with untreated CD may thus compensate for the increased losses of this subtype of immunoglobulin.

Our findings confirm that the jejunal mucosa of a patient with CD contains higher densities of cells with all subclasses of IgG and IgA. In our study the proportions of the subclasses in the children with CD and controls did not differ greatly.

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BOOK REVIEW

S. Illing, K.J. Groneuer: Neurodermitis - atopische dermatitis. Hippokrates Verlag, Stuttgart, 1991, p,117. 42 illustrations

The work is a 1991 publication of Hippokrates Verlag, Stuttgart. The 117 page book contains 37 charts and 5 diagrams. It is one of the publisher's well-known green and white paperback medical books.

In the introduction the editors already emphasize, that atopic dermatitis, a chronic and not really curable illness demands continual effort and cooperation from the patient and doctor. Negativistic comments, such as the patient should "live with his illness" force the patient into the hands of alternative therapists. Looking back in history shows that in the last century the illness, like bronchial asthma was scarcely mentioned. The equilibrium disturbance of the vegetative nervous system (neurodermitis), the role of nutriments and allergic etiology (since Pirquet's wording) are thought to have a part in its etiology. The illness shows different symptoms in infants, toddlers, children and adults.

The epidemiology of the illness has hardly been worked out. The data vary according to whether the mild cases are recognized and counted in the statistics. According to realistic data, which includes the mild cases, in Central Europe there is a 3-4 % occurrence in preschool children and an even lower one among school children. The illness, contrary to asthma is more frequent in girls than boys. As far as the prognosis is concerned, according to certain authors, there is a recovery within 3 years in about 40 % of the patients: the dry skin and paleness typical of the disorder usually remains. Factors which point to an unfavourable prognosis: high IgE, detectable sensibilisation, detectability of antistaphylococcus specific IgE, late start.

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The appearance of many types of atopic disease is common in the patients: authors find such forms, mainly asthma bronchiale in 15-30 % of the ekcemic patients. I would like to note that according to the reviewer's own data, in 22 % of Hungary's asthmatic children there is evidence of ekcema during infant and toddler age and in 5 % the ekcema remains in adulthood. According to the same survey ekcema is more frequent among patients suffering from severe asthma bronchiale.

The chapter and charts on the role of non-specific skin stimuli is very useful. They bring about, maintain, aggravate the atopic skin disorder. Skin infections, clothes made from wool or any other rough material directly touching the skin, soaps, chlorine, washing liquids and fabric softeners, perspiring due to heat, psychic stress. Respiratory and enteral viral infections also contribute to its flaming up. From these, it is clear that the care of ekcemic patient is many-sided.

The information on the relationship between the defects of the immune system and atopic dermatitis is instructive. The increase in level of the total IgE, as in most atopic disorders can also be found here. IgA deficiency is more frequent in atopic dermatitis (1:300) than in completely healthy individuals (1:7000). In X-linked agammaglobulinaemia ekcema is frequent. In a significant number of cellular defects (Nezelof, di Georgi syndrome, ataxia teleangiectasia, Wiskott-Aldrich syndrome) atopic dermatitis is often found.

The pathogenic role of allergens fed by food is dealt with critically. Finally they claim that food allergens have a role in 10-20 % of the atopic dermatitis patients. A whole chapter deals with the norms of normal alimentation from the new-born to adulthood, the effect of the methods of preparation on the nutriments. Finally in connection with the pathogenetic role of nutriments, their opinion is that the real proof has in the disappearance of the symptoms during elimination therapy and their reoccurrence during provocation. They discuss in detail how the carelessly ordered and widely used diet can lead to disturbances in development, to defective nurishment, factor defects. Apart from real allergens colouring and conserving

agents: salicylates and benzoates in food, biogen amines found in food (histamine, serotonin, etc.) may also play a part in the symptoms and are therefore to be avoided.

Those special border-line cases, where the hypoallergen and hypoantigen formulas which contain protein hydrolisates are preferred to breast milk, are mentioned here.

The recipes and the advice concerning the local treatment of the skin and medical treatment of atopic dermatitis is detailed and practical.

All in all, the reader holds a useful piece of work which has a wide list of literature and after reading it will know all the essentials about atopic dermatitis. The opinions and advice are adequate. Some points which the writers consider to be important, are repeated three-four times in the text. The book will be of equally good use to paediatricians, allergologists, dermatologists and general practitioners.

E. Cserháti, MD

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Pál Gegesi Kiss died on the 3rd of March 1993. In 1950 he founded the Acta Paediatrica Hungarica and was its editor until his death.

Pál Gegesi Kiss has had a broad life. He was born in Nagyszőlős on 1 February 1900 as the offspring of Transylvanian parents. He went to elementary and secondary school in Sepsiszentgyörgy, Csíkszereda and Marosvásárhely where he took his final examinations in 1917. He started studying medicine at the University of Sciences of Kolozsvár then continued in the Medical Faculty of Pázmány Péter University, Budapest. He graduated here as medical doctor in 1923. After having completed the obligatory one-year internship, in 1924 Pál Gegesi Kiss joined the University Children's Hospital as an unpaid junior doctor and worked there throughout his whole life.

In 1924 the director of the Children's Hospital was still János Bokay Junior, so Pál Gegesi Kiss really belonged to the Bokay school. During the 30's, under the leadership of Elemér Hainiss he worked together with a number of eminent colleagues: József Csapó and Edmund Kerpel-Fronius, the first representants of physiological and biochemical views in paediatrics, György Lénárt, founder of medical genetics in Hungary, Aurél Koós,

Akadémiai Kiadó, Budapest

founder of the Hungarian paediatric surgery, Eugene Kopits, orthopaedic surgeon. At that time Pál Gegesi Kiss was interested in paediatric cardiology and metabolic problems of infants and children. He studied this field of medicine in Paris for a year. The finding that the pathogenesis of the infantile toxic syndrome involves insufficient circulation caused by extreme tachycardia is a valid statement even today. He discovered that due to the extreme tachycardia acute coronary insufficiency develops which results in hypoxia of the myocardium. His co-worker between 1940 and 1960 in the field of cardiology was Julius Szutrély.

He had a continuously rising professional career. In 1934 he qualified as a lecturer, in 1942 as an associate professor, between 1946 and 1971 he was the director and full professor of the University Children's Hospital. In 1949 he was elected as corresponding member, in 1951 as full member of the Hungarian Academy of Sciences. From 1949 to 1955 he was Dean of the Medical Faculty, then between 1955 and 1961 the first rector of the Semmelweis Medical University.

Immediately after the world war Pál Gegesi Kiss founded an international scientific periodical: Paediatria Danubiana. Regrettably, however, this journal did not exist for long due to the specially unfavourable circumstances of the time. Later on the Acta Paediatrica Hungarica was established as its continuation.

Following the communist take-over in 1949, psychology was an unrecognized and even repressed field of science. In spite of this, Pál Gegesi Kiss with his co-worker, P. Lucy Liebermann established a psychological unit for paediatric patients. This unit was all the more significant, since it was the only psychologic ward and outpatients clinic in Budapest and probably in the whole country. This constituted the basis of

the University teaching group for psychology which was later developed. In 1960 he restarted the Hungarian scientific journal: Pszichológiai Szemle.

He published 19 books (partly in German and Russian) and more than 200 scientific papers.

He was president of the Hungarian Red Cross from 1957 and had a significant part in its reorganization as regards the humanitarian home and international activity.

Pál Gegesi Kiss was decorated with several awards during his life, the most important ones: National (Kossuth) award, 1953, Golden Medal of the Hungarian Academy of Sciences, 1973 and the Laurel Medal of the Hungarian People's Republic. Pál Gegesi Kiss was a member of an avangarde group called European school since 1945. The activity of this group was banned in 1949. In memory of his first wife, the sculptress Erzsébet Haán Forgách, he presented her 300 avant-garde works of art to the Janus Pannonius Museum, Pécs, thereby founding the modern gallery of the Museum.

He published several artistic studies in the journal "Alkotás" started by Lajos Kassák. As a result of this literary activity he was elected to be a member of the Hungarian PEN Club. He was also member of the Hungarian Olympic Committee. He gave lectures at the School of Physical Education. He won the second place in the Hungarian tennis championship.

With his death we have lost an outstanding physician, health director and a far-sighted intellectual. Many of us will preserve his memory.

Miltényi Miklós

Acta Paediatrica Hungarica 33 (3-4), pp 131-185 (1993)

THE MATERNO-FETAL NITROGEN RELATIONSHIP: further observations

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Received 21 October 1993

Akadémiai Kiadó, Budapest

The Fetus -
"Open for business during alterations"

AG Streeter

INTRODUCTION

The interests of investigators into the physiology of the placenta have passed through many phases, being dominated early by studies of its macroscopic and microscopic structure, endocrinology, blood supply and transport capacity. These have given way, temporarily, to a multitude of investigations of the cellular and molecular biology of the tissues relating to the synthesis of hormones, membrane transport mechanisms and the immunology of the materno-fetal relationship. Progress in these latter subjects have been reviewed recently /11, 81, 121, 126/ and the purpose of this article is to discuss some aspects of the physiology of the intact materno-fetal unit which are presently engaging the activities of other investigators. Progress in this field has been exciting, too, for three main reasons. Firstly, interest in the developmental aspects of protein turnover, representing the genetic growth potential of the fetus, has been continued by Goldspink and his colleagues /53, 96/; others have studied the influence of maternal nutrition on fetal protein turnover, together with its influence on the relative proportions of maternal endogeneous and exogeneous nitrogen reaching the fetus /92, 98/. Secondly, Battaglia and Meschia's group have continued to use the chronic preparation, with indwelling catheters in the fetal lamb 'in utero', to pursue with great advantage the use of the Fick principle, for quantitative studies of the metabolic relationships between the mother and placenta and the placenta with the fetus and its organs during gestation /3, 4/: the introduction of chronic preparations in smaller animals, such

as the guinea pig and rabbit, enable species comparisons to be made /77, 106/ and the perfused placentas of these animals have helped an understanding of the separate placental mechanisms involved in fetal nutrition /2, 9, 145/. Finally, the clinical use of cordocentesis has provided the means of studying the metabolic environment of human fetuses from early stages in gestation /37, 124/.

Footnote

It was a privilege to have a guest from Hungary, Dr. Gyula Soltész, working with us in the laboratory from 1972-73, at St. Thomas's Hospital Medical School, London: he came at the suggestion of Prof. Julius Mestyán of Pécs, because of our common interest in the amino-acid environment of the fetus and newborn. We have not lost contact over the years and it is a great pleasure to be asked to contribute to this journal again /138b/.

Fetal development and growth are, surely, two of the most important topics in biology and the underlying functional mechanisms some of the most fascinating. Fetal studies have formed a vigorous section of physiology over the last 50 years, because of the concern for the high neonatal mortality, and morbidity, of the human infant and newborn animal, born either lighter or heavier than expected for their gestational age. Now their challenge is enhanced by epidemiological evidence for the possible intra-uterine origin of early adult cardiovascular diseases and diabetes /1/.

I. PROTEIN TURNOVER DURING INTRA-UTERINE DEVELOPMENT

A total of about 60 g protein has been laid down in the human placenta by full term, 40 g of this is already present at 20 weeks gestation when the placenta and fetus weigh about the same, 250 g each. Subsequently, the placenta only doubles its weight while the fetus embarks on the major phase of its growth, reaching 3.5 kg at term, with a protein content of 500 g. There is about 5% protein in the tissues of the small embryo and this proportion rises to 12% in the fetal tissues at term. These familiar facts serve to remind us of the ever changing proportions which the products of conception play, in expressing their genetic demand for growth, during gestation. Since the mixed proteins are, after water, the most abundant constituents of all cells, their total activity, synthesis and turnover rates in the conceptus, will represent a large proportion of this genetic demand, resulting in the steady accumulation of protein characteristic of growth. This, in turn, will be modified by the response of the maternal tissues which should provide the optimum supply of nutrients to meet this metabolic demand, through the composition of her plasma and the maternal placental blood flow; this external fetal environment supplied by the mother will depend upon her nutrition and wellbeing. Further, there is a genetic change in the intrinsic properties of the fetal tissue proteins during development, as well as the change in hormonal response of the maternal tissues and myometrium to ensure the supply of nutrient to, and accommodation of, the fetus in the uterus.

The high activity of the synthesis and turnover rates of the mixed protein in young, developing, animals was first shown in skeletal muscle by Waterlow and Stephen /133/ who observed that protein synthesis was inversely related to body weight in young rats. Subsequently, turnover rates were shown to be higher than in the adult in the newborn lamb, the fetal lamb and individual fetal tissues and in the newborn human infant /see 138c/; this

high turnover rate might be expected to accompany protein accumulation during growth, to accommodate the differentiation and remodelling of the tissues during development.

The measurements to be described, have all been made using radioactively labelled isotopes: they depend, in general, on the uptake by protein of the labelled amino-acid in the steady state; the specific activity of the amino-acid in the protein pool is related to that in the precursor pool, plasma or intracellular /134/. More recently, the simpler method of flooding the precursor pool with a large dose of radioactively labelled phenylalanine (enabling the intra-cellular pool to reach the specific activity of the plasma quickly, so eliminating the necessity for a continuous infuson of the label to attain a steady state), has been used to measure protein synthesis rate in small animals /47/.

The placenta

The fractional synthesis rate of the mixed protein in this tissue was found to be lower than other fetal tissues, except skeletal muscle, in the sheep, but the half life was one fifth of that in the maternal liver /99, 143/. These findings were confirmed in the rat by Mayel-Afshar and Grimble /87/ and by Morton and Goldspink /96/; the latter reported a fractional synthesis rate of 60%/day for mixed placental proteins, comparable with that in the sheep during the last trimester: Fig. 1a shows that the value falls to 22%/day by 20 days gestation and, further, that the fractional synthesis rate of the whole fetus is higher, 75%/day, falling to 38%/day between 14 and 20 days gestation. Similar values for rat placental protein synthesis have recently been found by Johnson and Greenberg /70/.

It is suggested that this large metabolic gradient between the mother and fetus, provides the priority for the uptake of nutrients by the conceptus from the mother, to satisfy its

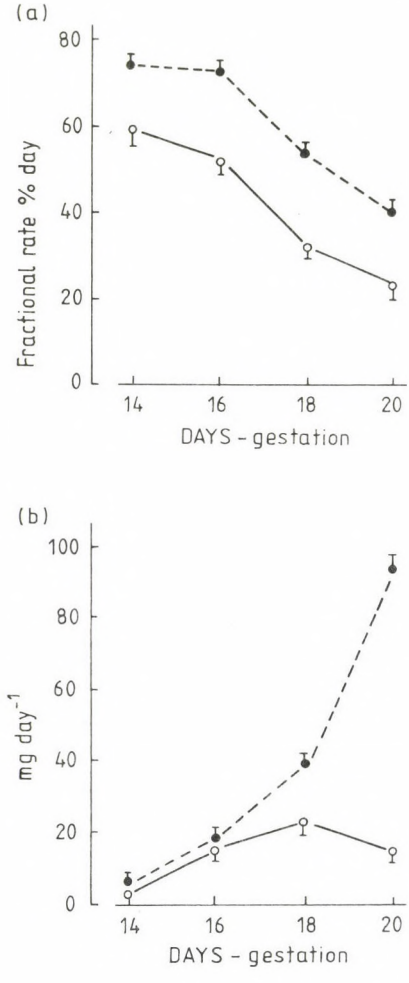

Figure 1 (a) Protein synthesis in the rat fetus (♂) is greater than in the placenta (♀); both decrease during gestation. (b) Protein accretion in the rat fetus (♂) continues to rise to term while that in the placenta (♀) ceases to increase from 18 days gestation (from ref. 54)

genetic growth potential. The placenta will be the main beneficiary of the net uptake during the first half of gestation, while it grows and its weight exceeds that of the fetus; later, as the fetus grows and its cardiac output and placental blood flow increase, the position will gradually be reversed. It would be very interesting to know the part played by the high protein turnover rate of the blastocyst in the success of implantation.

The whole fetus and individual organs

Goldspink and his colleagues have made rigorously controlled observations on the changes in protein turnover and accretion rates, with the nucleic acid concentrations which accompany the normal patterns of growth in the various tissues of the rat. during pre- and postnatal life; the same strain and sex of albino rats, fed and housed in the same manner and studied at the same time of day, were used. Fig. 1b shows that the accretion of protein proceeds in the rat fetus to 20 days and to 18 days in the placenta, inspite of the fall in turnover, suggesting that the average degradation rate of protein falls more slowly than that of synthesis during development. The values for protein synthesis in the whole fetal lamb are lower than in the rat, but also decreased by 50% from 110 days to term /88/; this may be explained by the greater maturity of the lamb in comparison with the rat fetus, and to differences in the relative proportions of the tissues in the two species. Bell et al. /6/ have also observed a 56% decrease in oxygen consumption in the lamb during the last half of gestation, when this is expressed in relation to the dry weight of the fetus; a parallel fall was observed in the aggregate weight of the vital organs, liver, kidney, heart and brain.

A detailed analysis has been made of the part played by the changing proportions of individual tissues in the whole rat body, to the decline in both protein synthesis and degradation

during intra-uterine life, through maturity to senility: degradation was calculated from the difference between synthetic rate, measured by isotope uptake, and protein accretion, as measured over shorttime intervals; they were related to changes in both cellular hyperplasia (DNA content) and hypertrophy (protein/DNA ratios). Table I shows the fractional synthesis rates, and their decline, in some fetal tissues during the last third of gestation: the rate in the brain is one of the slowest at 47%, but its decline into the postnatal period is also slow; the calculated breakdown is fast, 35 to 27%/day, indicating a rapid turnover during growth /51b/. In all the visceral tissues the fractional synthesis rate is high, about 100%/day or more, early in gestation /54/: it declines most rapidly in the liver and quite slowly in the gut; breakdown is also relatively high, between 30 and 50%/day, so that turnover is also rapid in these tissues.

The muscular tissues of the body have relatively lower fractional synthesis rates: those for cardiac and smooth muscle are the highest, the latter declining slowly during growth; the fractional synthesis rate is only about 20%/day for skeletal muscle in which the breakdown rate is also low, 8-10%/day /76/. When all these values were related to the weights of the tissues and their protein content, the contributions of the protein synthetic activity of the various organs to that of the whole body could be calculated: it is seen in Table II that the liver contributes 12.6% and skeletal muscle 16% at the end of gestation in the fetal rat; in the weanling rat the proportion of skeletal muscle increases to 53%, while that of the liver and intestines are now 6.5% and 11%, respectively /55/.

Skin contributes about 25% and bone, known to have a high turnover of calcium in the adult, will also probably provide a major contribution to the total body turnover. The values for fractional protein synthetic rate found in the fetal lamb brain and liver in the last trimester, are similar to those in the rat and confirm a very high rate for the intestines /119/. The developmental changes in individual organs and their relation

TABLE I

Protein synthesis in developing rat tissues
Fractional rate % day

Tissue	Fetus gestational age in days			Young 21 days of age
	16	18	20	
Brain	58	47	41	31
Liver	112	134	97	52
Intestines (small and large)	-	107	86	94
Lung	-	93	49	33
Kidney	-	95	63	45
Smooth muscle (oesophagus)	-	-	-	42
Skeletal muscle (tibialis ant.)	-	-	21	12
(soleus)	-	-	-	19
Cardiac musc	-	74	47	19
Skin	-	62	65	-

(Delivery 21-22 days)

From Goldspink et al. (see refs 51/a,b; 53; 54; 76 and 55 summary)

Legend: To show the differences in protein synthesis rate between fetal tissues; all decrease during gestation and into early life.

TABLE II

Protein synthesis in a developing rat tissues
% Contribution to whole body protein laid down

Tissue	Petus 20 days gestn	Young 21 days of age
Brain	5.5	2.7
Liver	12.6	6.5
Intestines (small and large)	3.5	10.8
Lung	4.0	1.2
Kidney	0.9	1.9
Skeletal muscle (all)	16.0	53.0
Cardiac muscle	0.9	0.9
Skin	25.0	-

From Goldspink et al. (see ref. 55)

Legend: To show the differences in contribution of various tissues to whole body fetal protein synthesis near term and in the young rat. The part played by the liver has halved, and that of the intestines and skeletal muscle trebled by three weeks postnatal age.

to whole body protein synthetic activity in this large and more mature fetus await study.

The reason for the rapid protein turnover rate during development might be clear, namely, to accommodate the dynamic situation of growth and rearrangement of tissues which occurs: a rapid synthesis rate and its decrease with age has also been demonstrated 'in vitro', in brain homogenates from young rats /100/. Schoenheimer's concept of the dynamic state of the body protein may be exhibited to its full extent in the fetus and newborn, at a time when the endocrine organs are relatively immature and the genetically determined regulation of biochemical processes not yet fully expressed. The underlying biochemical mechanisms and the influence of the environment on the changing pattern of gene expression are being actively explored, particularly in the neonatal period /49/.

Human studies

The availability of 15N labelled amino-acids has enabled a modification of the steady state method to be used in the newborn infant: Young et al. /144/ first showed in 1975 that the premature infant had a high protein synthesis rate, equivalent to 18 g/Kg/day, which gradually decreased during the first year of life to 7 g/Kg/day; subsequently, the method was used to show the influence of energy and protein intake in premature and small for gestational age infants /107/. Rennie's group have very recently used the method in the pregnant woman at delivery and found that plateau enrichment of the amino-acids used occurred half an hour after the beginning of the infusion and could be maintained /22/. Their values for turnover rate for the term human fetus corresponded to about 15 g/Kg/day and the calculated fetal weight gain was in accord with average fetal growth rates. There is agreement between these values and those found for the preterm infant using 15N

/107/ or using 13C leucine /30/; these values are also comparable to whole body protein synthesis in the fetal sheep late in gestation /88/. When Rennie's group used phenylalanine for their measurements, they found that the enrichment of tyrosine was always greater in the fetal than in the maternal plasma, suggesting a faster synthesis rate of this amino-acid by fetal tissues.

Myometrium

The human myometrium increases in weight from about 150 g to one Kg, from 10 weeks to term; during this period, the protein content increases from 25 to 170 g, about one third of that laid down in the fetus. The major stimulus for this accretion of protein appears to be stretch of the myometrium rather than the influence of the increased circulating oestrogen and progesterone. Comparing the events in the gravid horn of a rat with those in the nonpregnant horn, it has been demonstrated that protein synthesis and accretion increase in the former, whilst both synthesis and degradation rates fall in the nonpregnant horn, resulting in no weight gain /52/. Mechanical stretch alone, has been shown both 'in vivo' and 'in vitro', to increase the protein content of myometrium by increasing synthesis rate /35/.

II. HANDLING OF AMINO-ACIDS BY THE TROPHOBLAST AND FETUS

Maternal and fetal internal milieu and transport

The human maternal plasma free amino-acid levels fall very early in pregnancy, as part of the resetting of her internal

milieu by the changes in hormone production /see 138a, c/ and little change has been reported thereafter during gestation, even in the third trimester when fetal demand is at its height. This external environment of the fetus, therefore, remains relatively constant throughout gestation and net transfer will depend mainly upon delivery by the maternal placental blood flow, the fetal blood flow, placental metabolism and the small postprandial rises in plasma nutrient levels, besides the transport mechanisms. Maternal plasma aminacid patterns are different amongst the species, reflecting differences in metabolism and in diet and, interestingly, the alanine rise in maternal rabbit arterial blood is accentuated during postmaturity /77/. The relative proportions of nitrogen from endogenous maternal protein turnover, and exogeneous sources in the maternal plasma, which are transferred to the fetus is discussed on page 4.

The fetal internal milieu is quite different from that of the mother: the high levels of free amino-acid in the fetal fluids are an expression of the fast protein turnover rates in their tissues and the high F:M plasma ratios are very familiar. Observations in the rat /34/ have shown that the high fetal levels can be maintained even when the maternal plasma free amino-acid nitrogen is reduced by a half; this change was induced by a continuous infusion of glucagon from 14 to 20 days gestation and the fetal weight was maintained, provided the maternal caloric intake was sustained so that she gained weight. These observations indicate a barrier role for the fetal side of the trophoblast membrane which sustains the high amino-acid concentrations brought about by fetal metabolism, ensuring the retention of essential nutrients captured by the fetal tissues. Nevertheless, observations in the human subject and in chronic fetal sheep preparations /see 138c/ show that there is normally a direct relationship between the maternal and fetal concentrations of many amino-acids; further, it has been shown in the rhesus monkey that high steady state concentrations of all amino-acids are transmitted to the fetal

plasma suggesting that there is simple gradient-dependent diffusion across the whole placental membrane as well as the active processes at the maternal surface enabling transfer into the trophoblast against all the amino-acid gradients.

Uptake of amino-acids by the maternal side of the placenta takes place against the high free amino-acid concentrations forming the internal milieu of the trophoblast: these concentrations are higher than those of the fetal plasma; little is known of the individual pools of this intra-cellular environment or how maternal amino-acids mix with them during transfer. The active uptake has been very thoroughly studied in the vesicles which may be formed from the isolated maternal and fetal surfaces of the human placenta /10, 63/ and transfer, using the 'in situ' perfused guinea pig preparation and the dually perfused human placental cotyledon /29, 139/. The divisions and properties of the transport groups are similar to those found for uptake into other tissues. In the pregnant ewe, raised maternal plasma levels of the branched chain, essential, amino-acids are reflected within minutes in the fetal plasma, after being held for a short time in the trophoblast /83, 84/; the basic amino-acids are transferred more slowly and the straight chain, non-essential amino-acids appear very slowly in the fetal plasma. The more rapid transfer of the essential, branched chain amino-acids, is of particular interest for infusion of artificial mixtures of amino-acids has been considered for the treatment of the IUGR human infant 'in utero' /see 59/.

Trophoblast metabolism and transfer

The high level of free amino-acids in the trophoblast is characteristic of a tissue with a high protein turnover rate which has already been described. Their proportions are not, however, the same as those in mixed protein: the pattern is characterized by relatively lower proportions of the essential

amino-acids but much higher concentrations of the non-essential species, the straight chain neutral and the acidic amino-acids. Therefore, other metabolic processes, besides protein turnover, are contributory to their pattern and it is these pathways that are currentlv of great interest to investigators, for they determine the proportions of the amino-acids transferred into the umbilical circulation and may preserve carbon and nitrogen for the fetus. The placenta has a rich supply of transaminases /82/ but no studies have yet been of their concentrations during development, with the exception of those enzymes concerned with glutamine and glutamate synthesis in the sheep /see 138c/.

A comparison of the uterine A-V differences across the placenta with those of the umbilical V-A differences for amino-acids have shown that uptake at the maternal side of the placenta is not the same as release from the fetal side. In the human, at Cesarean section, the A-V differences are small, about 10 μM on the maternal side and 10-30 μM on the fetal side /see 141/: the large placental to fetal fluxes of alanine and glycine, in comparison with uptake at the maternal surface, are characteristic and the high levels of these amino-acids in the trophoblast provide a downhill gradient for transfer into the fetal circulation. In chronic preparations, with indwelling catheters in the pregnant ewe at the end of gestation, the major maternal to fetal fluxes are glutamine and glycine, showing the species difference in metabolism, which is different again, in the guinea pig, the rabbit and horse /48, 77, 146/. A small glutamate uptake on the maternal side of the placenta and its small fetal to placental flux is characteristic of both human and sheep.

Preliminary clinical investigation has shown that the umbilical V-A differences change during the last trimester /60/: the alanine and essential amino-acid V-A differences become smaller in the more mature infant, while serine, glycine and glutamine now join glutamate in being taken up from the fetal circulation (Fig. 2); however, little gestational change

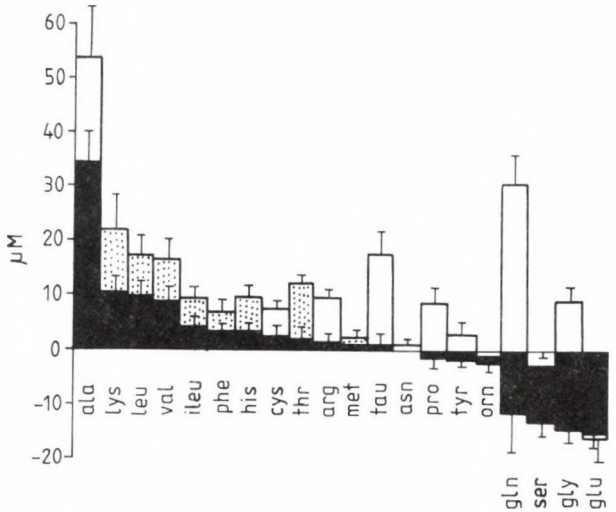

Figure 2 Umbilical V-A differences in the human subject at vaginal delivery. Most of the amino-acids are taken up by the premature infant [▭]: at term [■], the V-A differences are smaller for the essential amino-acids and those for some of the nonessential are negative, indicating uptake from the fetus by the placenta. [▤] essential amino-acids (from ref. 138c)

was observed in the fetal lamb /6/. More of such information needs to be added to that of the known disturbances in the plasma aminograms of the IUGR fetus, discussed on page 32, to further our understanding of the influence of the metabolic activity of the trophoblast on the relative proportions and quantity of amino-acids delivered to the fetus.

a) Inter-conversions of amino-acids within the trophoblast

Direct experimental evidence for conversion of essential to noessential amino-acids by the trophoblast, which the differences between the uterine and umbilical differences show, is still lacking but observations on the mammary gland, in which it is easier to measure the output of milk protein over short periods and to correlate the amino-acid content with that of the gland's uptake, provides an interesting tissue comparison /89/. Uptake by the ruminant mammary gland shows that some essential amino-acids are taken up in the correct proportion for milk protein production, while other essential amino-acids such as the branched chain, are taken up in excess of requirement and the noessential, both straight chain neutral and acidic, fall well short of their proportions in the milk secreted; moreover, their plasma A-V differences are sometimes negative as observed for the umbilical V-A differences. However, there is indirect evidence for alanine production by the human placenta in studies of the 'alanine-lactate cycle' /118/: quantitative measurements of lactate, glucose, alanine and acidic amino-acid transfer were made from uterine and umbilical A-V differences at Cesarean section, together with the activities of the appropriate enzyme activities which are involved in their interconversions; nutrient substrate movement showed that while alanine was produced by the placenta, lactate and the acidic amino-acids were utilized by the tissue and that there was a significant correlation between umbilical venous alanine and umbilical

arterial lactate concentrations. The investigation of such biochemical transformations in isolated perfused placentas using labelled substrate would be most valuable, especially as there is growing confirmation of the concept of glycine as a conditionally essential amino-acid during growth /66, 67/: this amino-acid is required not only for protein synthesis generally, which is turned over and the amino-acids returned to the nitrogen pool, but for heam and creatine which are excreted in the urine and stools with no return of nitrogen to the pool. It has been shown that the endogenous production of glycine in normal human pregnancy may be insufficient to satisfy increasing demands: 5-oxoproline excretion is increased during gestation indicating a reduced formation of glutathione which releases the inhibition of glutamylcysteine production from glutamine and cysteine, the alternative pathway forming 5-oxoproline from the glutamyl residue /109/.

The sheep placenta has been shown to produce branched chain keto-amino-acids, 2-keto isovalerate, isocaproate and methylvalerate, which are released into the fetal circulation and are considered to conserve the branched chain skeleton for fetal metabolism and growth /122/. A more detailed study by Battaglia and Meschia's group has provided quantitative evidence for deamination and decarboxylation of leucine by the sheep placenta /15, 82, 132/. They found rapid deamination of leucine in both the placenta and fetus and transfer of ketoisocaproic acid (KIC) from the placenta into the fetal and uterine circulations; labelled leucine injected into the fetal circulation entered the placenta quickly but was not transferred to the mother. Further analysis showed that the fetus was the main site of KIC decarboxylation, as anticipated from the relatively low concentration of leucine decarboxylase activity in the placenta in comparison with fetal tissue /56/; the branched chain amino-acids are metabolised to a greater extent by the placenta in fasting ewes /79/. Uterine A-V differences for many of the amino-acids are reduced in the

pregnant rabbit during postmaturity, a finding which cannot be accounted for by an increase in blood flow /77/.

b) Interrelation of placental and fetal amino-acid metabolism

The apparent uptake of glutamate, and some of the non-essential amino-acids, by the placenta indicated by their negative umbilical U-A differences, was a puzzle in early studies when the methods of analysis were not so good - for amino-acid A-V differences are small and the standard deviations relatively large; however, its relation to glutamine metabolism in the placenta has subsequently received a lot of attention. Glutamate is not only a constituent of protein but a precursor of glutamine, needed by growing tissue for the synthesis of purines: it is not taken up on the maternal side of the placenta, in either the human subject or the sheep, nor can this be demonstrated when the maternal levels are raised /see 138a /. But glutamine is taken up at the maternal side of these placentas and released in large quantities into the fetal circulations, especially in the sheep; therefore, it was possible that fetal glutamate was converted to glutamine in the placenta and fetal nitrogen and carbon preserved. Results from perfusion of the isolated human placental cotyledon showed that this might be so, but no confirmation was obtained using isotopes in the perfused guinea pig placenta 'in situ' /9/. Recent results from the human placental cotyledon, dually perfused closed circuit on both sides with both labelled and unlabelled glutamate and alanine, give evidence for glutamate uptake on the maternal side as well as further confirmation for uptake on the fetal side: glutamine appeared in both circuits, but in greater amounts in the fetal; excess alanine appeared in both circuits indicating release from and possible production by the trophoblast /85/.

Studies from Battaglia and Meschia's group have extended

the investigation of this metabolic problem by catheterising the fetal hepatic vessels, as well as the umbilical, in chronic preparation in the intact sheep and comparing their amino-acid A-V differences. Marconi et al. /86/ found uptake of the essential and most of the noessential amino-acids by the fetal liver, with similar umbilical vein-hepatic vein differences for the two hepatic lobes. Glutamine and glycine, together with small amounts of asparagine, were taken up by the fetus from the placenta, and by the fetal liver from the umbilical vein; their metabolic products, glutamate, serine and aspartate were released from the fetal liver and taken up by the placenta in reciprocal amounts, suggesting inter organ cycling betweeen the placenta and fetal liver as shown in Fig. 3. The negative umbilical V-A differences found for serine, together with the large fetal liver output, also indicate the fetal origin of this amino-acid, rather than a maternal; the large uptake of glutamine from the mother suggests that it may be the precursor of glutamate. Cetin et al./17, 18/, using a continuous fetal infusion of serine labelled with the stable isotope, 13C, provided quantitative data confirming the concept of this cycling of amino-acids between the placenta and fetal liveer and skeletal tissues. Release of serine by the fetal liver appears to be a unique fetal phenomenon for it does not correspond with adult metabolic findings. The large uptake of ammonia by the fetal liver, in comparison with the placental output also found in these studies, indicates that it is produced in this organ as in other fetal tissues, confirming earlier observations in the fetal hind limb; it may be part of the substrate for the glutamate-glutamine cycles in both the placenta and liver.

An important aspect of amino-acid transfer from mother to fetus must relate to their interactions with trophoblast proteins, as well as their associated free pools. Turnover rate of this mixed protein is higher than in any maternal organ, but lower than that of the whole fetus, at all times during

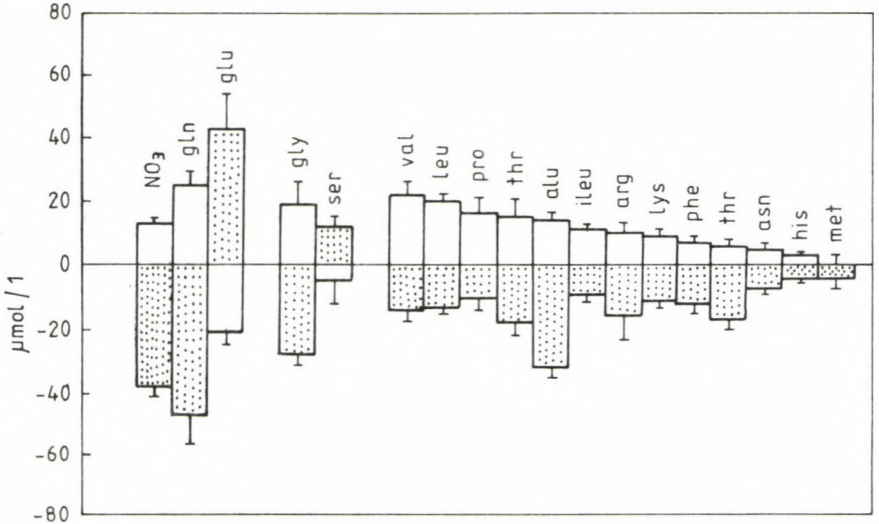

Figure 3. To show the uptake of glutamate and serine by the sheep placenta, released from the fetal liver, near term; umbilical V-A difference [▭]; left hepatic V - umbilical V [▨]. (from ref. 20)

gestation /99/. An indication of the influence of protein synthesis on transfer has been demonstrated in guinea pig perfused placental preparations: trophoblast proteins captured about 15% of labelled amino-acids in transit; inhibition of protein synthesis by cyclohexamide reduced this uptake by 85% and transfer by 65% /14/.

Amino-acids as a fetal fuel and fetal nitrogen balance

It is well known that urea excretion in growing animals is low while they retain nitrogen for growth. It was, therefore, interesting when Battaglia and Meschia's group calculated that the total output of amino-acids from the placenta was in excess of fetal requirements in the sheep, and postulated extensive transamination and oxidation of amino-acids in the fetal tissues /4/ a finding which was confirmed by Faichney /38/. Fetal plasma urea concentrations are higher than the maternal in all species studied and the placental clearance of urea in the fetal lamb is about twice that in the adult, when related to the body weight, and it has been suggested that protein might provide 25% of the energy requirements of the fetal lamb. Urea clearance studies in other animals and in the human indicate less utilisation of protein as an energy source /58/. The infusion of 13C labelled amino-acids in fetal lambs in chronic preparations, indicate disposal rates for leucine, glycine and serine of 9%, 12% and 46% respectively, in inverse relation to the oxidation rates of 20%, 11% and 8%; these values indicate, clearly, that these amino-acids are utilised in many metabolic pathways - besides protein synthesis. Quite recently, Carver et al. /16/ have shown that shortterm hypoglycaemia in the fetal lamb, increases leucine oxidation but not disposal rate: however, longterm hypoglycaemia, induced by insulin infusion which curtailed fetal growth rate, caused no weight specific alterations in either leucine uptake

or disposal, nor oxygen consumption: the authors concluded that there was an adaptation towards a reduced energy expenditure for protein accretion and thus a slower rate of fetal growth.

III. REGULATION

Animal husbandry has always known the importance of maternal nutrition for the good outcome of pregnancy and, during the last 40-50 years, the medical conditions of the small for gestational age infant, and the large infant born of mothers whose diabetes is imperfectly controlled, has provided a special stimulus to research in fetal growth. The cooperation of the clinician with the basic scientist has resulted in a wealth of information relating to the mechanisms of development and growth. Human data showing that fetal genetic factors account for less than half the weight attained by the infant at birth, is now very familiar: it relieved the fetus of its old stigma of being called a parasite and highlighted the importance of the fetal environment 'in utero', which reflects maternal health and nutrition. The characteristics of the fetal growth curve and the final birth weight are the result of that old conflict between demand and supply, here expressed by the symbiosis between the fetal genetic growth potential and the capacity of the mother to respond.

At the exchange area

The overall effectiveness of placental transfer of any metabolite whether it be passive or active, will be determined by the surface area over which the maternal and fetal circulations have the opportunity to exchange and, in a number of species, morphometric studies document the increases which

occur during gestation: histological studies also indicate attenuation of the placental membrane itself when growth of the organ slows down, and physiological studies confirm that permeability of the membrane increases (see 138d). Placental tissue contains a large number of receptors for growth promoting hormones /8/ and studies on the human placenta show that insulin receptors increase twofold from 11-16 weeks gestation and reach a maximum at 26 weeks /113/. Howeveer, the function of these hormones in promoting placental growth is still unclear /111/ and they do not appear to control the transport processes: insulin has been shown, repeatedly, to have no influence on either amino-acid or glucose transport in a variety of experimental preparations. Stulc has suggested that substances required for laying down the basic structure of the body during growth, which are, therefore, never in maternal:fetal equilibrium, may require regulatory processes by the placental membrane /127/; this may be effected for amino-acids by the activity of the heterogeneous mixture of proteins, with their high turnover rate in both the placenta and fetus, which create metabolic gradients for uptake of substrate. As pointed out earlier, this activity of protein declines as gestation proceeds, but the overall influence of a larger placenta and a rapidly growing fetus will increase the effective genetic demand of the whole conceptus, and the anatomical and physiological adjustments will, increasingly, play their essential role in making nutrients available for growth.

Placental blood flows

Moll /94/ has analysed some of the adjustments which take place in uterine and placental blood flows to accommodate the increasing needs of the fetus during gestation. Some of the stimulus for the anatomical changes may be brought about by the

increase in fetal capillary bed and volume of flow, which accompany the gradual rise in arterial blood pressure during the final stage of rapid growth of the fetus. However, no significant change in maternal arterial blood pressure occurs during pregnancy, but a rise in the conductance of the placental vasculature is broght about by a widening of the supply arteries and by an expansion of the exchange channels: an estimated 100-fold increase in flow may occur and be sustained by a very low driving pressure, inspite of the lengthening of the arterial system. The increase in arterial diameter is brought about by extensive remodelling of the vessel walls and appears to be controlled by the placenta, for dilatation increases progressively as they approach this tissue; the local release of oestrogen and progesterone by the trophoblastic invasion of the uterine arteries may be responsible, for exogenous administration of the steroids is without effect during pregnancy. Millaway et al./90/ have studied angiogenic activity in the sheep endometrium and placenta, finding heat labile substances which both stimulated and inhibited endothelial proliferation: early in gestation angiogenic activity occurs mostly in the maternal tissue with caruncular endometrium secreting more than the inter-caruncular tissue; stimulation by cotyledonary placental tissue was greatest early in gestation. Studies in the human placenta are very active /111/.

A wealth of information on the values for uterine and umbilical blood flows to the placenta has accumulated over the years, chiefly in animal preparations; these are high near term and comparable with cerebral blood flow, about 100 ml/100 g tissue on the maternal side and half this value on the fetal, near term. The high delivery rate of substrate to the placenta is necessary to maintain that organ; but the slow transport to, and accretion by, the fetus allows time for tissue differentiation and development, for fetal growth rate is

relatively slow in comparison with that which occurs subsequently in the young animal. It is well known that acute reductions of maternal placental blood flow cause a fall in transfer rate of substrate, the extent depending on the metabolite and its diffusion and transport characteristics, thus altering the balance of nutrient delivered to the fetus; preliminary experiments in the perfused guines pig placenta have shown reduced transfer of α-amino-N in comparison with α-glucose when the maternal blood pressure is lowered /71/. The increased fetal-placental vascular resistance, found by ultrasound measurement in the human subject with pre-eclampsia or an IUGR pregnancy, has stimulated great interest in the endothelial derived relaxing and contracting factors, found in other vascular beds. Using the perfused human placental cotyledon, the relaxing factor, nitric oxide, appears to maintain the basic tone of the vessels and attenuate the vasoconstrictor actions of thromboxane A2 and of endothelin-1; the stimulus to its release may be transmural pressure, flow or shear stress /97/.

One of the most interesting aspects of the feto-placental relationship is that of its reserve capacity; how far may the maternal blood flow and delivery of substrate be reduced before the growth potential of the fetus is compromised in the chronic situation? Owens at al. /103/ have found, in the carunculectomised fetal sheep preparation, that the placental weight and maternal placental blood flow can be reduced by 50% before the fetal internal environment is impaired sufficiently to cause fetal growth retardation. Fox /43/ has written a lively discussion on the relation between structure and function in the human placenta; he quotes early observations of wellgrown infants with no signs of hypoxia, born with placentas with 30% of their villi embedded in fibrotic, a vascular plaque.

Fetal growth

In the fetus the finer regulation of body mass is likely to be determined in the same way as in the adult, by both local trophic factors and hormones secreted by the endocrine organs. The cells of the blastocyst destined to become the fetus, may participate in the endocrine function of the early embryo and, indeed, the fetus may be influenced by the placental hormones during gestation. Early observations on the part played by the fetal endocrine organs indicated that prenatal growth was practically independent of growth hormone /50/. Evidence for the possible role of the endocrine pancreas followed, and current interest in local trophic factors is intense. These factors are present both before and after the fetal endocrine organs are functional so that the development of their interrelationship is of great significance. Recently, the work has been extensively reviewed for the role of insulin in the human infant by Milner /93a, b/ and the fetal lamb by Fowden /41/; the insulin-like growth factors by Froesch and his colleagues /45, 46/ Owens /102/, Hill and Han /62/, Sara and Haall /11/ and the role of growth hormone in the rat by Robinson and Clarke /115/. Only a brief summary of this very complex and fascinating subject is attempted.

The place of the endocrine pancreas in the regulation of fetal growth has been of great interest to the clinician for many years, stimulated originally by the pathology of the enlarged islets of Langerhans in the large infant born of diabetic mothers with high blood sugar. More evidence for the importance of insulin as a growth hormone came from the low birth weight of infants with pancreatic agenesis and from experimental pancreactomy in animals. Insulin is responsible for the rapid regulation of metabolic processes in the adult, stimulating uptake and metabolism of substrate by tissues. In the early fetus, the high activity of the proteins, alone, will create a gradient for the uptake of substrates. But as the

tissues develop and their membranes become less permeable /28/ insulin may be required to enhance and regulate the access of metabolites to ensure that the local trophic processes proceed at the required rate at the cellular level. The endocrine pancreas appears early in the human fetus and the receptors and circulating hormone are present a third of the way through gestation; these events are more delayed in the lamb and are not complete until 8§% of intrauterine life in the rat. The action of exogenous insulin in the near term fetal lamb is known to be similar to that in the adult: besides the lowering of blood glucose, plasma aminoacids levels are reduced, indicating tissue uptake and reduce protein catabolism /42, 64, 110/. Chronic fetal hyperinsulinaemia has been shown to cause selective organomegaly, including the plalcenta, in the rhesus monkey, but no increase in cell protein contant /128/; stimulation of protein synthesis rate was observed in the placenta and in skeletal muscle in the fetal lamb, only when the plasma amino-acid level was maintained /64/. Amino-acid administration in the adult human subject has been shown to increase whole body protein synthesis, which correlated with a rise in metabolic rate, and was accompanied by an augmentation of all aspects of leucine metabolism /104/. Increased fetal size has been observed in pregnant rats given amino-acid infusions during the last two days of pregnancy /61/ and restricted fetal growth when low levels of plasma amino-acids were maintained with insulin infusions /101/.

An appropriate substrate supply will also ensure that the local cellular trophic hormone levels are maintained. These somatomedin polypeptides, now called insulin-like growth factors (IGFs), because their molecular structure is similar to that of insulin, also have many properties in common with this hormone; they are, however, much less active than insulin and are probably responsible for the slow regulation of growth through their action on protein synthesis. The IGFs have high tissue mRNA in fetal organs, in animals and the human infant;

the factors can be released 'in vitro' and have been shown to stimulate cell division and protein synthesis as well as glucose and amino-acid transport. IGF I and II are both weak mitogens for cells of mesenchymal origin 'in vitro' and, in contrast with most other mitogens, tend to increase cell differentiation and enhance the expression of specific cell markers. Early in fetal life, plasma levels of embryonic IGFs are very high and, later, decline to give rise to the adult forms. But there are species differences: in the human newborn, IGF II is only 50% of that in the adult and IGF I levels very low, rising to a maximum during puberty; in the rat, high IGF II levels at birth decline rapidly, to be completely replaced by IGF I. Gilmour's group /78/ find that the normal prepartum cortisol surge in fetal lambs inhibits hepatic IGF II gene expression and indicates that the fetal liver may be the primary source of fetal plasma IGF II. Adult plasma IGF levels are characteristic of a species, being high in guinea pigs and relatively low in cows and horses. Within a species, such as the dog, there is good correlation between body size and plasma level. Plasma levels are dependent on the nutritional status of the animal, and this also applies to the proteins to which they are bound in the plasma.

IGF I production has only been studied in adult animals, where its functions may be to maintain the remodelling of tissues, especially in those which have a high turnover rate, such as the bone marrow and mucosa of the gastrointenstinal tract. Production may be autocrine or endocrine, the latter being effected by both growth hormone and insulin, which also control the production of the IGF binding proteins, responsible for targeting the growth factors. The smaller of the two major proteins, 24 kD, is probably produced in the liver and interacts with the insulin receptor in muscle. The larger, 150 kD, protein is produced by various tissues, effecting growth in chondrocytes and osteoblasts, and is stimulated by oestrogen and growth hormone. The metabolic influence of exogenous IGFs is beginning to be studied as the recombinant IGF becomes

available. Bolus injections of IGF I cause acute hypoglycaemia and a marked uptake of glucose by muscle, in a number of species, but it is about twelve times less potent than insulin in this respect. Slow, continuous, infusions appear to have no growth promoting influence in young, rapidly growing rats, and it may not be possible to stimulate protein synthesis and accretion when they are already proceeding at a very fast rate; this may explain why the fetus grows, inspite of the very low plasma IGF I levels, the genetic species of fetal protein being unable to increase its activity any further. Infusion of IGF I does stimulate growth in the growth hormone deficient Snell dwarf mouse, in the hypophysectomised rat and in rats with streptozocin induced diabetes; besides their endocrine disorders, these adult animals will have genetically mature soft tissue and bone protein.

Transfer of maternal body nitrogen

Fetal plasma has access to two sources of amino-nitrogen from the maternal plasma, through the placental membrane: to that of her own body protein, which is turning over, and to that of her ingested food, which causes temporary rises in plasma substrate concentration. One of the functions of the placental hormones is concerned with altering maternal metabolism to enhance the availability of her stores during pregnancy, but Freinkel's /44/ concept of facilitated anabolism in the mother, to direct nitrogen to the fetus, is still relatively unexplored. Naismith /98/ have suggested that protein metabolism is anabolic early in pregnancy in the rat and catabolic during the last week of gestation, when maximal fetal growth occurs: his observations showed that nitrogen retention during the first two weeks of pregnancy was equivalent to 8.5% of the dams' lean body mass and equal to half the amount of

nitrogen found in the fetuses at term; no increase in maternal body protein was found at delivery.

Ling and his group /80a, b/ have shown in the pregnant rat that maternal liver protein turnover is enhanced during the period of rapid fetal growth, her plasma leucine oxidation was reduced and its flux increased, corresponding with the rise in fetal utilisation which was more efficient than that of the mother. They also studied the relative importance of maternal stores and dietary protein in supplying fetal nitrogen requirements by measuring maternal and fetal turnover during continuous intravenous feeding of the mother during the period of maximal fetal growth: maternal catabolism was abolished, leucine flux from endogenous sources was reduced by 80% and fetal growth severely curtailed, and they concluded that endogenous maternal to fetal nutrient exchange was an important physiological process: their findings are in contrast to those quoted on page 2 , in which maternal amino-acid infusions were found to increase fetal weight.

Millican et al. /92/ consider that skeletal muscle, the largest protein store, is not required to play a role as a reserve in the well-fed mouse. Protein synthesis was measured in the whole body and some individual tissues in mice at various stages of pregnancy and during lactation: the rate of protein synthesis increased almost threefold during pregnancy and by a further 30% during the first two weeks of lactation; the greater proportion of these increments was due to the placental and fetal growth during gestation, and to the mammary glands during lactation. A stimulation of growth and protein synthesis was also observed in the maternal liver and gastrointestinal tract but no change in protein metabolism was found in the gastrocnemius muscle. Preliminary studies in rats, in which the maternal tissue proteins were labelled with 15N at fifteen days gestation prior to the fetal growth spurt, show that maternal 15N is captured and concentrated in fetal protein throughout the subsequent growth period indicating a

sharing of the maternal body constituents with the fetus which appeared to originate, chiefly, from the maternal liver: this was not enhanced when maternal diet was restricted /140/.

Whole body protein turnover has been studied in the three trimesters of human pregnancy, by de Benoist et al./31/ in Jamaican women, by measuring the urinary urea enrichment of 15N following a single dose of 15N labelled glycine; the turnover was highest during the first trimester and decreased thereafter, in keeping with Naismith's concept and the reduction in turnover rate found in muscle in the last week of pregnancy in the rat by Mayel-Afshar and Grimble /87/. De Benoist et al. also found, unexpectedly, that resting metabolic rate did not change during pregnancy, for there is usually a correspondence between protein synthesis and metabolic rate, though this has not been studied before during pregnancy. In further studies on Gambian women, using the same 15N glycine method, a significant increase in protein synthesis was observed only in the mid-trimester and an increase in resting energy expenditure in the last trimester /136/. However, the use of 13C labelled leucine and 13C bicarbonate to measure synthesis and catabolism showed a tendency for both to increase during pregnancy in women; the increases became significant when the results were corrected for fat free body mass and support the view that the mother responds to the requirements of the fetus /131/.

IV. DISTURBANCES IN NITROGEN METABOLISM

Intrauterine growth retardation

The fetus which is born small for its gestational age usually has a small placenta, but the fetal:placental weight ratio depends upon the time in gestation at which the

nutritional insult has been imposed, as well as its duration. In the human, a wide range of fetal: placental weight ratios is found /95/ but it is difficult to assess when any nutritional deprivation may have occurred to disrupt the developmental programming, for there may have been both transfer and metabolic adaptations to the insult; evidence for the considerable reserve capacity of the feto-placental relationship was given on page 23, and fetal: placental ratios above normal would indicate such activity. In general, it is thought that placental size is determined early in gestation during its maximum growth period, and fetal weight in the second half of pregnancy. These considerations derive from observations in experimental animals in which the 'optimal standard of living' of fetuses has been disturbed at various stages in gestation, by restricting maternal nutrition and, hence also, the effectiveness of her reproductive supporting structures: reduction of the nutrient supply to the fetus following manipulation of the placental mass, and restricting either the maternal or fetal placental blood flows in various ways /103/. The poor performance of the small infant or animal in the neonatal period shows that any adaptations which may occur are relatively inadequate and recent epidemiological studies indicate that adult disease, such as the early onset of diabetes, and of cardiovascular disease, may also be associated with being small for gestational age /1/.

a) Internal environment

The early studies of Mestyán and his colleagues in Hungary, and of Lindlad in Sweden, showed clearly that the amino-acid environment of the IUGR infant was impaired at delivery, for the plasma aminogram was similar to that found in older children with kwashiokor (see 138c). More recently, umbilical vein plasma aminograms at Cesarean section, and from cordocentesis blood (umbilical vein sampling for prenatal

diagnosis), also show amino-acid patterns similar to those observed in malnutrition in the adult, with raised non-essential and lower essential amino-acid values /19 a,b, 36, 37/. This work confirms, most elegantly, the earlier work in the IUGR infant but one group have unwisely suggested the introduction of a 'fetal concentrating' index as a gestational age-dependent measure of placental dysfunction, constructing curves of the numeric mean of the fetal : maternal ratio of six amino-acids in normal pregnancies as a standard /7/. They have not taken into account the normally large range of any plasma amino-acid concentration and the difficulty of relating two pools which are not only independently regulated, but separated by a very active organ, the placenta, which metabolises amino-acids. Besides the changes in profile of the plasma free amino-acids, the low total plasma amino-nitrogen has been confirmed in IUGR infants /20/, in the guinea pig /74/ and in the pacreatectomised IUGR fetal lamb 'in utero' /42/; the latter consider that this was due to the low plasma insulin levels and the present author suggests that it may also be due to the depressed protein turnover rate, which is described below.

The cause of the disorder in amino-acid metabolism in IUGR will be related to inadequate transfer of the supporting metabolites, as well as that of the amino-acids for the protein synthetic process /33/. Robinson and his colleagues /103/ found that the internal environment of the IUGR fetal lamb is altered for two of the most flow dependent substrates, oxygen and glucose. Total delivery of glucose was lower in these fetuses and placental lactate production increased per unit weight of placenta, with more being transferred to the fetus, further, maternal hypobaric hypoxia, induced for either short or long periods, was shown to have little or no influence on placental weight but inhibited fetal weight gain in the lamb. Cordocentesis sampling has shown in the human fetus that hypoxia is directly related to the materno-fetal glucose gradient and Cetin et al./20/ found that high lactate levels in

umbilical vein blood were related to an abnormal pulsatility index in the umbilical vessels, indicative of a low blood flow, seen in the Doppler flow velocity wave forms.

b) Protein synthesis

The biochemical mechanisms whereby the decreased accretion of protein, brought about by the changes in supply and internal environment, are beginning to be investigated in the IUGR fetus: the balance between synthesis and breakdown of protein has been shown to be altered to various extents and the picture is not yet clear because the experimental methods used to create IUGR are varied, but, so far, the evidence suggests, that a lower accretion is chiefly brought about by a decrease in synthesis rate with less change in degradation rate. Limitation of oxygen supply alone has been shown to decrease amino-acid uptake by protein in the fetal lamb as well as increasing utilisation of endogenous substrate /91/. The early observations of Mayel-Afshar and Grimble showed that maternal protein deficiency in the rat influenced placental protein content to a greater extent than the fetus, but an increase in protein synthesis rate occurred in both, as well as in maternal liver and muscle protein /87/. More recently, the influence of maternal restriction of protein, of fasting in the mother at the end of gestation and of restricting the placental blood flow half way through gestation has been investigated /68, 70/: placental and fetal protein content was low following all procedures, fractional synthesis rate was reduced in the protein deficient and restricted blood flow groups, but increased in fasted mothers; fractional protein breakdown rate was also greatly raised in the latter, but little changed in the former two groups. Their results on protein restriction are in agreement with Wunderlich et al. /137/ and the contrast with the preceeding description may be methodological. Most

recently, Kelly et al. /73/ have also found that food restriction reduces pulmonary growth and protein turnover in preterm guinea pigs by reducing synthesis rate rather than catabolism. Long-term maternal protein maltnutrition in rhesus monkeys has also provided data on the fetal brain, noted for being relatively spared in the small fetus; a diet containing one third the normal protein content produced fetuses with low birth weights in which the cerebellum and the brain stem were small, but with protein, DNA and myelin concentrations unaffected /112/. The conclusion that protein deposition may be influenced during growth by altering the balance of synthesis and degradation has interesting implications for studying its regulatory processes.

The relative parts played by the deficit in supply and alterations in the fetal environment in curbing fetal growth potential are not known. A deficit in supply, such as occurs in the malnourished mother, may impair the reproductive supporting structures and their initial responses to the blastocyst and, therefore, subsequent placental growth and its blood supply, besides providing a poor daily integrated level of maternal plasma nutrient for transfer to the fetus during the third trimester. A deficit in supply, whether it be brought about by maternal malnutrition or a small placenta will also impair the synthesis of fetal growth hormones, such as the IGFs, whose plasma levels have been shown to be low in the IUGR newborn of human and animal pregnancies (see 93a). Snok et al have also found that the endocrine pancreas of rat offspring, whose mothers have been fed a low protein diet during pregnancy, is impaired structurally and functionally in the neonatal period and subsequently during adult life /123/.

Diabetes

The materno-fetal relationship of the plasma amino-acids in poorly controlled human diabetic pregnancies has not been

studied so extensively as the glucose relationships. Maternal plasma has elevated levels of the branched chain group of amino-acids and, since they are the most rapidly transferred across the placental membrane, they have been implicated with glucose in the pathology of the fetal pancreas characteristic of these infants. This idea was furthered by de Gasparo et al. /32/ who found that growth of the cell islets was stimulated by amino-acids as well as glucose in cultured fetal rat pancreas. Persson et al. /108/ found a significant correlation between maternal plasma branched chain amino-acids and amniotic C-peptide, in a small group of gestational and type I diabetics, indicating a direct response of these fetuses to insulin secretagogus; an inverse relationship was observed in IUGR infants. Kalkhoff and his group have pursued the importance of raised maternal plasma amino-acids in relation to fetal growth in diabetic pregnancies in a very detailed study /72/: they compared two control groups, of lean and obese women, with a group of lean insulin dependent type I diabetic women, early and late in gestation, relating the amino-acids to fetal weight and to the plasma glucose levels; maternal plasma amino- acid levels were greatest in the type I diabetics, only, and were directly related to the infant birth weight, but not to the maternal plasma glucose level; interestingly, a raised plasma serine level correlated most closely with fetal weight. Inchiostro et al. /65/ have studied the effects of insulin and amino-acid infusion in insulin dependent type I diabetics in order to evaluate their anabolic influence on protein metabolism; using a glucose clamp, both oxidative and non-oxidative leucine disposal were found to be greater in the IDDM patients than in controls.

Soltesz et al /125/ found high total plasma amino-acid concentrations in full-term newborns of diabetic mothers, accompanying the low plasma glucose levels; the most elevated levels were those of glycine and alanine as in the IUGR infant.

It was considered that the hyperaminoacidaemia might be due to the birth asphyxia and that the raised alanine was not indicative of impaired gluconeogenesis. Similar plasma changes have been found post operatively in the lamb in utero /14 / and in the healthy newborn foal /14 /; normal values are attained in the former after five days and more rapidly in the latter.

The animal model of streptozocin induced diabetes, in which the fetuses are small, has been given more relevance since Pedersn /105/ observed, using ultrasound techniques, that fetal growth retardation occurs in the first trimester of human diabetic pregnancies: further, the extent of the early growth retardation in correlated with the severity of the disease. The rat model with its immature fetuses may, therefore, correspond with early events in the human and be relevant for studying the etiology of disordered somatic growth. Canavan and Goldspink /13/ found protein synthesis rate to be low and degradation increased during the last trimester of hyperglycaemic pregnant rats: the low birthweight could therefore be accounted for by both the lower synthesis rate and alteration in the balance of synthesis and breakdown. Johnson et al. /69/ found that hyperinsulinaemia in fetal rats, induced by transuteral injection in the third trimester, caused macrosomia but no increase in fractional synthesis rate nor breakdown in the brain, liver or heart; fractional synthesis rate was, however, reduced in the diaphragm. Their results confirm earlier work on the influence of insulin on individual organs in the fetal lamb /64/ and the conclusion that the action of insulin on immature protein is predominantly on skeletal muscle. Copeland and Porterfield /26/ found that the placental transfer of the non-metabolisable amino-acid, α-isobutyric acid, was reduced in streptozotocin induced diabetic rats and that no improvement accompanied the maternal administration of insulin, though both maternal and fetal plasma T4 levels were increased. Subsequently, they found that the plasma aminograms were

changed in their diabetic rats /27/ and that the low glycine, serine and lysine levels in the maternal plasma and placental tissue were partially restored by insulin; fetal plasma amino-acids were slightly reduced, although alanine was raised and no changes occurred with maternal insulin treatment, even though both maternal and fetal glucose was returned to normal.

Influence of some toxic substances and drugs

The fetuses of mothers subjected to some of the environmental pollutants and drugs, prescribed or taken voluntarily during pregnancy, are frequently growth retarded and have impaired postnatal development, the selective impairment of organogenesis being well known. Particular concern for the fetuses with the 'alcohol syndrome', those of drug addicted mothers, the IUGR seen in the newborn of smoking mothers and the less obvious disabilities caused by cadmium and lead poisoning, has led to a host of investigations into the mechanisms of 'toxic' action of these substances, which prove to be numerous. In the context of this article, the growth retardation indicates restriction of the general protein synthetic process and its control; however, there are no records of the internal environment which might be expected to be changed in these fetuses, and few on disturbances of the mixed protein synthetic rates of their tissues.

Hypertensive or hypotensive drugs may cause malnutrition of the fetus by reducing maternal placental blood flow thus depriving the fetus of essential nutrients and oxygen; such was the influence of capritol, the angiotensin converting enzyme inhibitor, which was once used to treat hypertension in pregnancy /12/. Heavy metals may also deprive the fetus of nutrients by accumulating in the trophoblast as Miller found in his classic study with cadmium in rats /75/. No short-term influence of cadmium can be observed on amino-acid transfer but

with long-term exposure the incorporation of thymidine into fetal DNA is decreased and that of leucine into protein is also depressed /135/.

Of the 'toxic' substances which diffuse readily across the placenta alcohol is one which has been the most actively studied; it is quickly transferred from mother to fetus to attain equilibrium on each side of the placental membrane /129/. The IUGR characteristic of the fetal 'alcohol syndrome', is accompanied by microcephaly and other malformations which indicates that alcohol exposure during pregnancy influences growth in many ways /40a/. α-Amino-isobutyric acid uptake by cultured human trophoblast is inhibited by long-term, but not short term, exposure to alcohol in concentrations equivalent to those found in heavy drinkers, 300 mg%, and no inhibition of histidine transfer was observed in the dually perfused human placental cotyledon /40b/. Rawat found an inhibition of hepatic protein synthesis in the near term fetal rat /114/, and there are many reports of impaired amino-acid transmitter binding in the developing brain /39/ together with depressed postnatal auditory, memory and learning capacities in animals and in man /24/. IUGR induced by alcohol is also accompanied by impairment of fetal accumulation of substances such as methionine, zinc and vitamin B12, all known to be essential for cell division and growth /120/ and it is reported that the syndrome may be ameliorated in the rat by thyroxine /57/.

POSTSCRIPT

The development of methods for measuring the dynamic aspects of the interactions between the mother and placenta and

between the placenta and fetus, namely, transfer rate and protein synthetic rate, has started to provide us with much needed information on the conceptus's aminoacid and protein metabolism. Mixed protein synthesis and turnover rate has been shown to be high in fetal tissues and it is suggested that the metabolic gradient which this creates between mother and conceptus is the stimulus for the capture of amino-acids by the fetus, together with the molecular and structural mobility it provides for the remodelling processes during development. This activity of the mixed proteins declines during fetal life as the placental blood flows and, therefore, substrate delivery increase. The free amino-acid internal milieu of the mother, the placenta and fetus, and some of their changes and interrelationships during pregnancy, are now familiar for a number of species demonstrating differences in their metabolism. The pattern of uterine arterio-venous plasma amino-acid difference is different from that of the umbilical vein-artery, indicating a large release of noessential amino-acids from the trophoblast into the fetal circulation and, therefore, their synthesis by the placenta /23 a, b/. There is evidence for such metabolic activity, namely deamination of essential amino-acids by both the placenta and fetus in the pregnant ewe 'in vivo'. There is also indirect evidence in the human placenta, from studies in the perfused placental cotyledon for these interconversions, and it will be important to have direct proof because glycine, for instance, may be considered a conditionally essential amino-acid for fetal growth.

The internal amino-acid environment of the fetus is changed in the IUGR human infant, the plasma aminogram being characteristic of malnutrition in children and adults. Other characteristics of the fetal internal environment, such as the oxygen, acid/base and glucose status of the blood, are also impaired at delivery in both animal and human fetuses born

small for gestational age. It is difficult to assess the relative parts placed by a deficit in supply of metabolic substrates, and the altered internal milieu, in curbing fetal growth potential, especially as the experimental methods used in animals to produce growth retardation influence the maternal-fetal relationship in different ways /2, 25/. The low protein accumulation rate in these small fetuses appears to be due to a disturbance of the normal synthetic/catabolic balance, protein synthesis being reduced to a greater extent than catabolism. In the adult rat protein degradation is suppressed by protein deprivation /130/.

So, how can present knowledge help in preventing, or treating, the disorders of intrauterine growth? Ideally, placental supply should met genetic demand and the scene be set at implantation, an experimental field in which there is now much activity in relation to assisted fertilisation; work on the development of the placenta /111/ is now taking the place of that of the hormonal regulation of placental blood flow, and no one would disagree that prevention of impaired fetal growth may come from the results of all these studies when thoughtfully applied. Meanwhile, there is no evidence that placental blood flow and fetal supply can be increased once IUGR exists, so there remains the possibility of nutrient supplementation of the mother, or the fetus directly. Recently, Harding et al,/59/ have reviewed attempts by the clinicians to do this, using methods based on some of the experimental studies in animals described in this article. In the present context, the results of amino-acid or high protein supplements were disappointing /116/ possibly due to the ensuring imbalance of amino-acids and the consequent impairment of transport and metabolism /21/. The diabetologists had, perhaps, an easier task when needing to reduce the supply of one metabolite, only, to the potentially large infant of diabetic mothers?

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PROSTAGLANDIN E_1 THERAPY OF NEWBORNS WITH GROUP B BETA-HEMOLYTIC STREPTOCOCCI-INDUCED PULMONARY HYPERTENSION

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Four newborns with group B beta-hemolytic streptococci (GBS)-induced pulmonary hypertension and hypoxemia were treated with hyperventilation and Tolazoline without significant improvement in oxygenation. The patients were divided into two randomized groups thereafter: one to receive an infusion of Prostaglandin E_1 (PGE1) and the other to receive Tolazoline (controls). Patients treated with PGE_1 (n=2) showed significant improvement in oxygenation. The arterial-alveolar oxygen ratio (a/A) increased from 0.047 ($\pm$0.003) to 0.091 ($\pm$0.03) ($p<0.001$); the oxygenation index (OI), incorporating mean airway pressure (MAP) as well as oxygen variables improved significantly from 56.6 ($\pm$0.55) to 36.4 ($\pm$15.6) ($p<0.001$) 30 minutes after the start of the PGE_1 infusion. This improvement was maintained in these patients for the whole time of PGE_1 infusion and thereafter. The controls (n=2) did not show improvement in oxygenation. Only the two newborns that received PGE_1 therapy survived out of the four. No systemic hypotension was observed during the PGE_1 infusion. These findings suggest that PGE_1 infusion may provide therapeutic benefits for newborns with persistent pulmonary hypertension (PPHN) once hyperventilation and Tolazoline treatment were ineffective.

INTRODUCTION

Although hyperventilation, Tolazoline therapy and extracorporeal membrane oxygenation improved the clinical

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outcome of newborns with persistent pulmonary hypertension of newborn (PPHN), mortality is still significant.

The pharmacological pulmonary vasodilator therapy has not proved to be a consistently effective treatment for the newborns with PPHN. This is perhaps due to the fact that drug intervention - given the lack of selective pulmonary vasodilators - has been limited to the use of nonselective vasodilators.

Some clinical studies evidenced Prostaglandin E_1 (PGE_1)-induced improvement in oxygenation in human pulmonary hypertension of adults, and a promising experimental work documented, that PGE_1 reduced selectively the groups B beta-hemolytic streptococci (GBS)-induced pulmonary hypertension of newborn piglets /7, 9 /.

Our study was undertaken, therefore, to test that GBS-induced PPHN will respond to the infusion of PGE_1 with prolonged improvement in oxygenation and with better clinical outcome.

PATIENTS

Eligibility

This study was approved by the local Committee of Ethics at the First Department of Obstetrics and Gynecology, Semmelweis Medical School Budapest, Hungary and patients were enrolled after informed consent was obtained.
Criteria for enrolment were as follows:

- Diagnosis of Group B beta-hemolytic streptococcal pneumonia and sepsis;
- GBS induced pulmonary hypertension;
- Fraction of inspired oxygen (FiO_2): 1.0;
- Mean airway pressure (MAP) > 15 cm H_2O;
- The arterial PO_2 < 40 mmHg in spite of hyperventilation (100-150/min);
- Persistent hypoxia (PaO_2 < 40 mmHg) after Tolazoline administration (2 mg/kg in bolus i.v.).

All 5 patients eligible in our nursery during a 10-month period, from May 1, 1990, to March 1991 were initially included in the study. One of these patients was later found to have severe E.Coli sepsis on the basis of bacterial cultures of tracheal aspirate and was excluded from the final analysis.

Protocol for Prostaglandin-E_1 Administration

Upon admission, an umbilical artery catheter (5. French Argyle) was placed in the descending aorta of all the infants. The tip of the catheter was placed between the sixth and tenth thoracic vertebrae. Blood samples for arterial blood gas measurements were obtained only from these catheters into heparinized syringes and analyzed within 3 minutes for pH and blood gases using routine laboratory (Radiometer ABL 330) methods.

2-D echocadiography was used to diagnose pulmonary hypertension and to exclude cyanotic congenital heart diseases.

After confirming severe GBS sepsis-induced pulmonary hypertension ("persistent fetal circulation"), hyperventilation was started. All infants were ventilated with BP-2001 respirator, delivering 2-3 $cmHO_2$ positive end-expiratory pressure and MAP was 16-19 $cmHO_2$. FiO_2 was 1.0 and ventilatory rate 100-150/minutes. If the arterial PO_2 during the hyperventilation remained below 40 mmHg or dropped below this value again after a short improvement, we introduced Tolazoline treatment: 2 mg/kg was given intravenously, infused over 5 minutes in a scalp vein, and arterial blood gas levels were determined after 10 minutes. If there was no significant improvement in oxygenation (a rose in PaO_2 10 mmHg or greater) the patients were divided into two randomized, control groups: one to receive an infusion of PGE_1 (Prostin, Upjohn Company), started at a rate 0.03 µg / kg /min. (PGE_1 treated) and the other to receive Tolazoline (Controls) at a rate 2 mg/kg/h.

20 minutes before the Tolazoline treatment Dopamine infusion was started at a rate 8 µg/kg/min which was continued during the whole time of PGE_1 and/or Tolazoline therapy.

After introducing PGE_1 or Tolazoline infusions, arterial blood gas levels were determined at 30 and 60 minutes and approximately hourly thereafter. Systemic arterial blood pressure and rectal temperature were monitored.

Baseline characteristics

The baseline clinical characteristics of the study patients are shown in Table I. Aside from the presence of severe respiratory distress with mechanical ventilation, the clinical diagnosis of pneumonia was based on radiographic evidence of diffuse coarse infiltrates, or complete opacification of the lungs.

TABLE I

Baseline characteristics of study patients

Characteristics	Prostaglandin E_1 treated (n=2)	Controls (n=2)
Weight, g	2150 ± 707	2375 ± 389
GA	36 ± 2.8	35.5 ± 4.5
Age of entry, h.	10 ± 5.6	9.5 ± 3.5
WBC	11.9 ± 7.2	9.65 ± 3.4
I/T ratio	0.26 ± 0.01	0.26 ± 0.02
CRP	+	+
Gastric aspirate Leucocytes + Cocci	+	+
Tracheal aspirate culture	GBS	GBS
Positive blood culture for GBS (n)	2	1

Table reports data as mean ± SEM.
GA: gestational age; WBC: Leucocyte counts; I/T: Immature to total neutrophil quotient; CRP: C-reactive protein; GBS: Group B beta-hemolytic Streptococci.

To support our presumptive diagnosis of systemic infection the following diagnostic tests were used:

- Leucocyte counts
- Immature to total neutrophil quotient
- C - reactive protein
- Presence of leucocytes and cocci in gastric aspirate.

Before the antibiotic treatment blood and tracheal aspirate were cultured. All patients received Penicillin (300.000 U.I/kg/die) - Gentamycin (5 mg/kg/die) combination immediately thereafter.

Outcome Assessment

The acute improvements in oxygenation and pulmonary perfusion were measured by the changes of PGE_1 or Tolazoline induced arterial-alveolar oxygen ratio (a/A) and IO. a/A was calculated from a standard formula /6/, oxygenation index (OI) was defined as $OI = MAP \times FiO_2 \times 100 / PaO_2$.

The blood gas values (mean $\pm$ SD) were tabulated in four groups, by the different segments of the protocol.

The intercurrent medical events which might be associated with the PGE_1 treatment were recorded and tabulated as well.

In addition, the late outcome was also tabulated, including the duration of mechanical ventilation, the rate of the late complications and the survival rate as well.

Bronchopulmonary dysplasia was assessed as a requirement for supplemental oxygen at 28 days of age.

Statistical Analysis

Data are presented as mean values $\pm$ SEM. Differences between groups were assessed using the paired t test. Differences were considered significant when $p < 0.05$.

RESULTS

a/A. OI and blood gas values

Measurements of a/A before and after the PGE_1 therapy and control group values are shown in the Fig 1. The data indicate a roughly twofold increase a/A from 0.047 ($\pm$ 0.003) before treatment to 0.091 $\pm$ 0.03 after 30 minutes after the introduction of PGE_1 therapy. ($p < 0.001$)

This improved a/A was maintained and rose in all PGE_1-treated patients all through the respiratory therapy.

When MAP changes were combined with oxygen requirements through the OI (Fig. 2) the treatment with PGE_1 showed a reduction from 56.6 $\pm$ 0.55 before PGE_1 administration to 36.4 $\pm$ 15.6 after 30 minutes. These improvements in OI-like those in

a/A were maintained in PGE_1 treated newborns compared to the controls ($p < 0.001$).

After the introduction of PGE_1 therapy the PCO_2 values decreased and arterial oxyen-saturation ($SatO_2$) rose compared to the controls (Table II) and to the values measured before the PGE_1 treatment.

TABLE II

PCO_2 and $SatO_2$ at PGE_1-treated and control newborns

Blood gas values	PGE_1 (n=2)				Controls (n=2)			
Segments of protocol	A	B	C	PGE_1	A	B	C	Cont-rol
PCO_2 (mmHg)	45.3 ±3.0	43.5 ±5.8	47.5 ±17.6	37.5 ± 9.5 +	58.5 ±6.5	45.3 ±5.8	44.5 ±2.1	48.6 ±5.2
$SatO_2$ (%)	52.7 ±19.4	64.8 ±23.1	57.6 ±9.8	91.2 ± 9.8 +	36.2 ±5.6	63.9 ±20.0	56.5 ±8.4	35.9 ±17.8
Number of observa-tions	2	5	2	25	2	6	2	10

+ $p < 0.05$
Table reports data as mean ± SEM.
A: blood gas values at entry; B: blood gas values during hyperventilation; C: blood gas values after Tolazoline administration; PGE_1: blood gas values during PGE_1 therapy; Controls: blood gas values at the controls.

Side effect and outcome of PGE_1 therapy

The rate of the unexpected medical events (Table III) indicate that arterial blood presure did not change after the introduction of PGE_1 therapy. Repeated elevations of the rectal temperature have often been observed, 17 times in the whole period of time of the PGE_1 treatment.

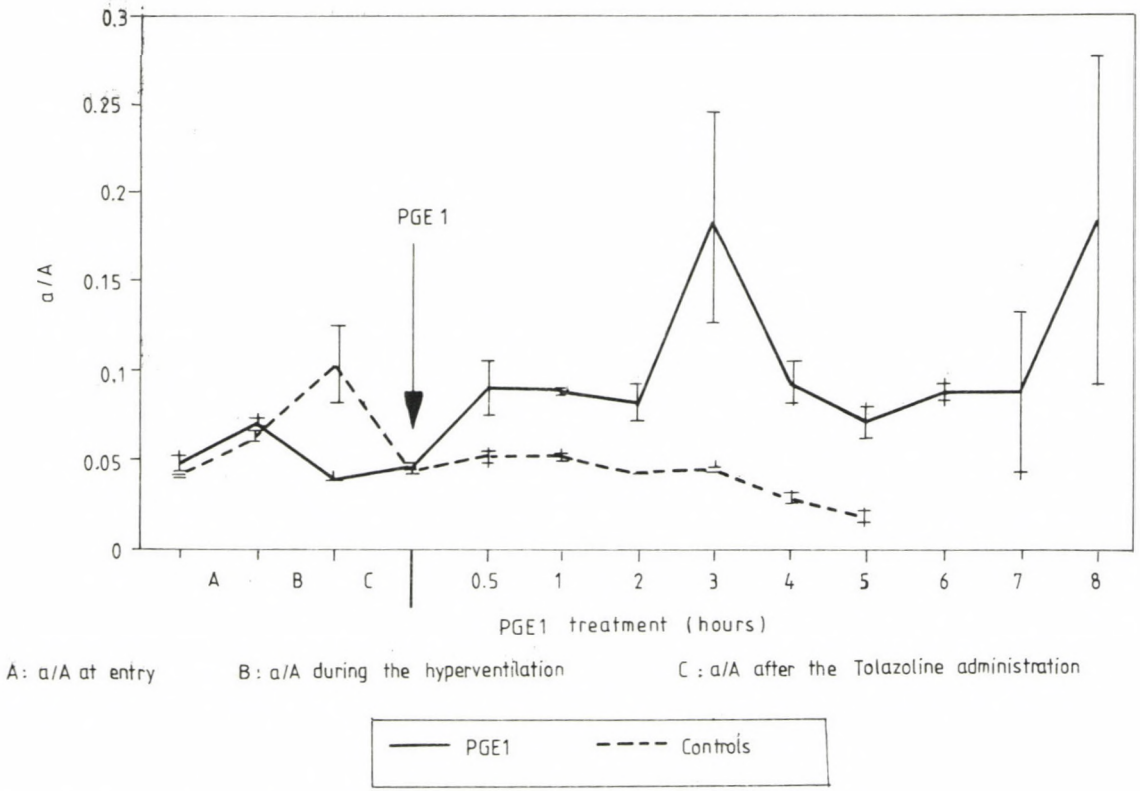

Fig. 1: a/A changes during the protocol.
Data are expressed as mean values ± SEM. Line with triangles indicates newborns receiving PGE_1. Simple line indicates controls.

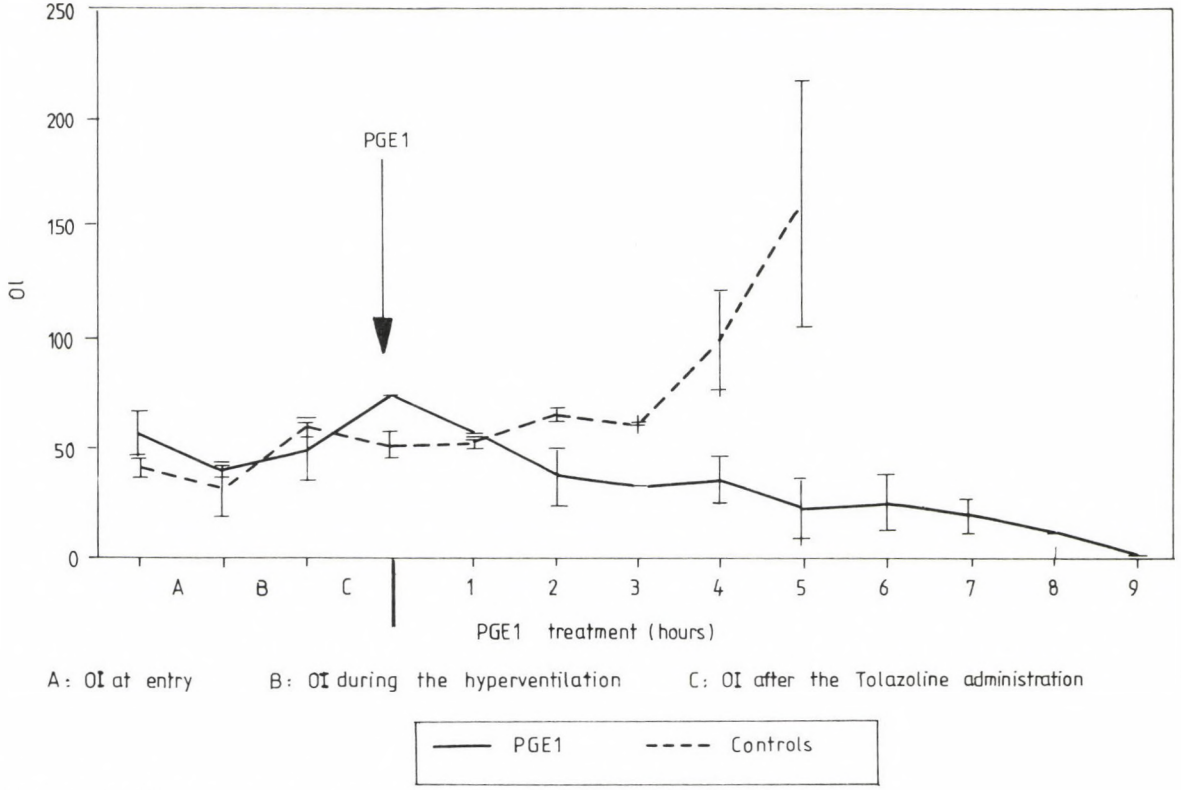

Fig.2: Changes in OI during the protocol.
Data are expressed as mean values ± SEM. Line with triangles indicates newborns receiving PGE_1. Simple line indicates controls.

TABLE III

Intercurrent medical events at PGE_1 treatment

Event (n)	PGE_1 (n=2)	Controls (n=2)
Cardiovascular		
Cutaneus vasodilatation	-	-
Rhythm disturbance	-	-
Hypotension	-	2
CNS		
Seizure-like activity	3^+	-
Temperature elevation ($T > 38.0\ C^o$)	17^+	1
Metabolic		
Hypoglycaemia	-	-
Hypocalcaemia	-	-
GI		
Diarrhea	-	-
Renal failure	-	-

+ $p < 0.001$

CNS: central nervous system; GI: gastrointestinal

As shown in Table IV, the survival rate of PGE_1-treated newborns was 100%. No PGE_1-treated patients required supplemental oxygen for more than 14 days, and therefore none had bronchopulmonary dysplasia by the definition used here.

TABLE IV

Late outcome and survival of the patients

Outcome	PGE_1-treated (n=2)	Controls (n=2)
Time of PGE_1 infusion, h.	56.5 ± 31.5	-
Time of respiratory therapy, h.	217 ± 13	16 ± 2
No. of deaths	-	2
No. of patients requiring supplemental oxygen > 14 d.	-	

Table reports data as mean ± SEM

DISCUSSION

Neonatal GBS sepsis is often associated with persistent elevation of pulmonary artery pressure and with severe arterial hypoxia.

Increasing the respiratory rate or ventilation cycling frequency should increase minute ventilation, lower pCO_2 and raise pH and thereby improve pulmonary blood flow. Hyperventilation (rate of breaths per minute 100-150) is, therefore, the most commonly used method for full-term infants diagnosed with PPHN /15/.

Other therapeutic researches have focussed on the search for selective pulmonary vasodilatators that might be able to decrease the pulmonary vasoconstriction without a significant systemic hypotension. Unfortunately, there are no specific

pharmacological agents that can cause selective pulmonary vasodilatation (Isoproterenol, Tolazoline, Phentolamine, Prostaglandins, Hydralazine, Nitroprussid or Nifedipine). Tolazoline, although nonspecific, has had extensive use but with variable results in PPHN. A positive response to Tolazoline occurs only in 30 to 60 per cent of these newborns, and adverse effects related to it are seen in 30 to 80 per cent /2, 3, 14/.

Although various PG-s (PGI_2 and PGD_2) can produce pulmonary vasodilatation they have not yet been of significant help in the clinical management of syndromes associated with PPHN /12/.

PGE_1 has been reported only to relieve human pulmonary hypertension in adult /9/.

Our study focussed on the effects of the PGE_1. This powerful vasodilatator, although not selective for the pulmonary vascular bed /16, 18/, is almost completely metabolized (> 95%) on one pass through the pulmonary circulation /17/. Therefore PGE_1 has been hypothesized to be able to exert greater pulmonary than systemic effects. This hypothesis was supported by Hammermann and co-workers in animal models /7/. In their study PGE_1 infusion selectively improved GBS-induced pulmonary hypertension and hypoxemia in newborn piglets with only a transient systemic hypotension.

For our study we selected such newborns that had severe GBS sepsis with PPHN. As shown in Table I, weight, gestational age and age at entry into the study were similar for neonates in the sub-groups of PGE_1 treated newborns and controls.

a/A and OI values indicate that the expected mortality rate of the patients we described would have been 90-100% /1, 10, 11/. They all had the indications for ECMO. Unfortunately, this method is not available in Hungary. All our patients showed unresponsiveness to the conventional therapy (hyperventilation and Tolazoline).

The results of this study indicate that newborns with GBS-induced PPHN have an acute improvement in oxygenation after introducing PGE_1 infusion. Newborns compared with the controls had a prompt improvement in a/A and OI which remained

significant for the whole time of the PGE_1 infusion and after it.

The different mechanisms of action of the PGE_1 and Tolazoline in the PPHN may be responsible for the quantitative differences in the oxygenation. PGE_1 decreases pulmonary vascular resistance by dilating both pulmonary veins and arteries as a "mediator substance", Tolazoline is an alpha-adrenergic blocking agent, also has sympathomimetic, parasympathomimetic and histamine like-effects.

Newborns during the continuous infusion of PGE_1 had a transient decrease in a/A and an increased OI at 3-4 hours after the start of PGE_1 infusion which persisted for several hours. We can only speculate as to the mechanism for this. It is possible that there is a "lag time" until the pulmonary catabolic enzymes become activated, and lower concentration of PGE_1 exists in the pulmonary vessels thereafter.

The arterial pCO_2 values of newborns after the introduction of PGE_1 infusion are significantly lower than those of the controls and those found in the previous stage of therapy. The rapid decrease in arterial pCO_2 indicates improved ventilation. This phenomenon is interesting in the light of the previous observations that demonstrate that the PGE_1 prevents increased lung microvascular permeability during intravascular complement activation in sheep and attenuates oxidant - induced pulmonary oedema in rat lungs /4, 5/.

The significantly higher $SatO_2$ during the PGE_1 therapy indicates a better arterial O_2 content.

The most important contraindication of the PGE_1 therapy in PPHN is the postulated adverse effect that the PGE_1 does not differentially dilate the pulmonary vessels opposed to the systemic ones. In our study we did not observe any arterial hypotension during the PGE_1 infusion compared with the controls and with the blood pressure values measured before this

treatment. Though arterial hypotension was not frequently (3%) observed during the intravenous PGE_1 infusion /13/ we suspect that continuous Dopamine infusion (8 µg/kg/min) may prevent the hypotensive effects of PGE_1, just as in the case of Tolazoline /8/.

Repeated and transient development of fever and jitteriness were frequently observed during the PGE_1 infusion, which disappeared after the reduction of infusion rate and after the finishing of PGE_1 treatment.

The outcome and survival rate demonstrates that the two patients treated with PGE_1 here survived, and non developed chronic lung disease; the newborns in the control group have died. The closure of the ductus arteriosus occurred spontaneously after PGE_1 infusion.

In summary, our study provides some new observations. These are:

- Continuous PGE_1 infusion improves the a/A and OI of newborns with severe GBS induced PPHN with whom the hyperventilation and Tolazoline treatment was unsuccessful.

- The PGE_1 treated newborns had better survival rate and clinical outcome compared with the controls.

- We did not observe significant hypotension during the continuous PGE_1 infusion.

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INTERRELATIONS AMONG DIFFERENT TYPES OF CONGENITAL CARDIOVASCULAR MALFORMATIONS IN SIB-OCCURRENCES

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136 pair and 4 triple occurrences of isolated cardiovascular malformations (CCMs) in sibs were evaluated in a population-based Hungarian material between 1963 and 1981. Similar CCMs were found most frequently in sibs. Among the dissimilar CCMs, the combinations of ventricular septal defect - pulmonary stenosis and of ventricular septal defect - tetralogy of Fallot were common. The lack of some combinations, e.g. ventricular septal defect and coarctation of aorta, is also noteworthy.

INTRODUCTION

It is worthwhile separating the specific risk (i.e., the recurrence of proband's disorder) and the random risk (which may involve all the other disorders) within a familial cluster of congenital abnormalities (CAs). However, the idea of a so-called "semi-specific" risk seems to have established itself /11/, which may represent a certain etiologic relationship among accurately defined types of CAs. The congenital cardiovascular malformations (CCMs) have a particular pattern: the sib-occurrence of CCMs is about 3%. However, roughly one half of the affected siblings had CCMs similar to those of the proband /10, 8/. The other half of sib-occurrence involves other types of CCMs, thus their occurrence also appears to be greater than normal. So far, selected clinical materials and a summary of reported cases have been evaluated /8, 10/. Here we show the results of a population-based approach.

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MATERIALS AND METHODS

There are 45 Paediatric Cardiology Outpatient Clinics (PCOC) in Hungary covering the whole area of the country and working under the same guidance and methods. In 1983, the heads of these PCOC were requested to inform us about families in which two or more infants born between 1963 and 1981 had CCMs together with their name, dates of birth and death, address of probands and the type and the diagnostic approach of their CCM and CAs. The conduction disturbances were not taken into consideration.

With the exception of two PCOC (who had staffing difficulties), all PCOC sent the requested data. Finally 14 groups and types of CCMs /9/ were separated and given abbreviations as follows: ventricular septal defect (VSD), atrial septal defect type II or unspecified atrial septal defect (ASD), patent ductus arteriosus (PDA), pulmonary stenosis or atresia (PuSt or PuAt), tetralogy of Fallot (TF), coarctation of aorta (CoAo), aortic stenosis (AoSt), transposition of the great arteries (TGA), common truncus (CoTr), endocardial cushion defects (ECD) including atrioventriculare commune and atrial septal defect type I, dextrocardia (Dex), complex CCM, i.e. combination of two or more CCMs (CC), other types of CCM (OC) and finally unspecified CCMs ("CCM").

The completeness of ascertainment could be checked by a comparison with (i) the material of the Hungarian Congenital Abnormality Registry (HCAR), where personal data of index cases affected by CCM are available. This material is thought to be far from complete in terms of CCMs /5/. (ii)Our previous genetic epidemiologic studies of ASD /2/, VSD /3/, CC /7/, and (iii) the records of our Genetic Counselling Clinic which includes more than 8.000 consultands and considered appropriate to check - against this material.

RESULTS

147 families with two or more members affected with CCMs were reported by the heads of PCOC. However, two groups of families were excluded from the analysis. These were, on the one hand, 19 families who had no sib-occurrence, but four cousin and 15 parent-child occurrences. Nevertheless, one family with a three generation occurrence (Fig. 1) and 7 families with a triple occurrence of isolated CCMs (Fig. 2) are worth showing here. Of the latter seven, 5 sibs and one half-sib occurrence are

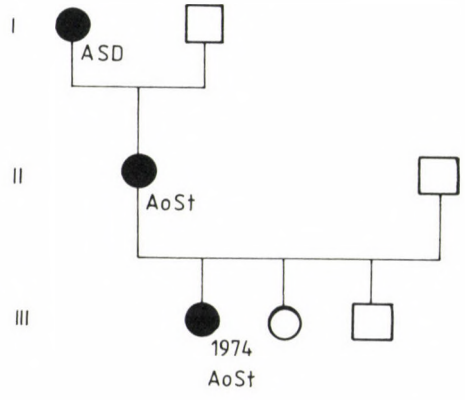

Fig. 1. A three generation inheritance of CCM

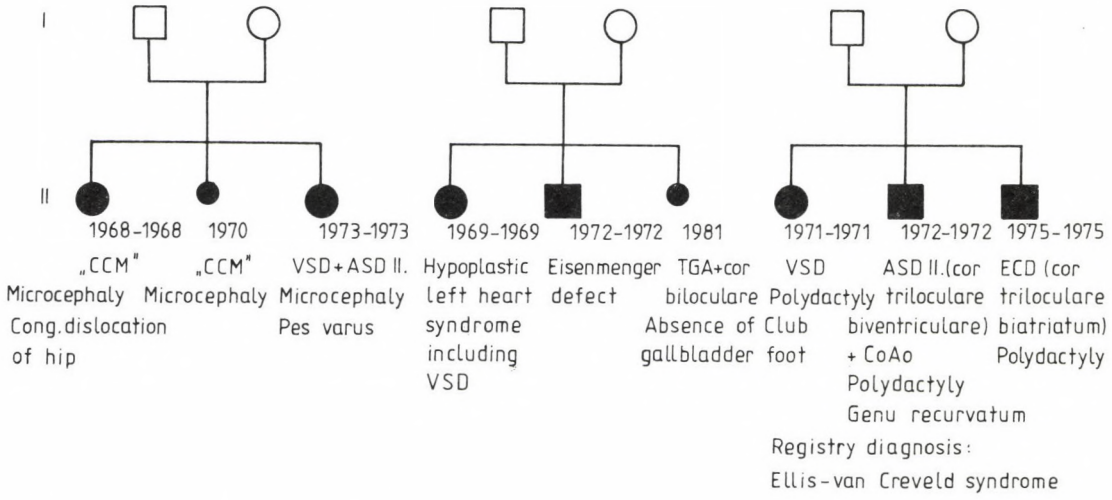

Fig. 2. Triple familial clusters of multiple CCM cases in the study material

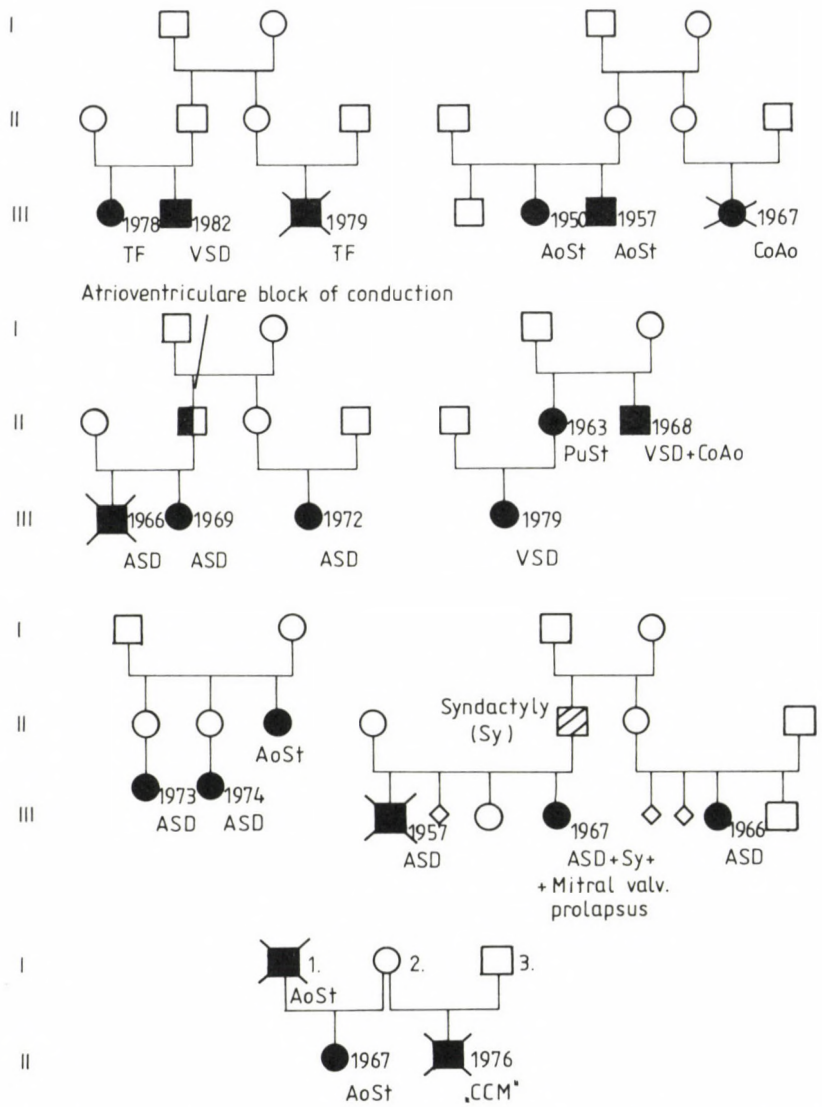

Fig. 3. Triple familial occurrences of CCM in the study material

evaluated later. The second group, on the other hand, had 11 sib-occurrences involving CCMs and extracardiac CAs, i.e. these cases had the so-called multiple CCM in at least one of the pairs (Table I). Both sibs were affected in three of the pairs caused by known multiple CA-entities, while one sib had a known multiple CA-entity in a further 4 families. Figure 3 shows three triple sib-occurrences of multiple CCM cases. One of them was diagnosed by us as Ellis-van Creveld syndrome. These multiple CCM cases had a heterogeneous origin, thus they too were excluded from further analysis.

PCOC reported 113 sib-pairs: 34 brothers, 21 sisters, 49 boy-girl pairs, 8 concordant and semi-concordant twins and 1 half-sib with isolated CCM from the same mother. Furthermore, four triple sib-occurrences were reported (Table II). Of these 12 CCMs, seven had CC. These 117 sib-occurrences mean 238 cases with CCM. Of these 238 cases, 214 were born after the establishment of HCAR in 1970, but only 120 cases were reported to the HCAR (56.1%). However, 23 sib-pairs with isolated CCM recorded in the HCAR were not reported by the PCOC (23/260=8.8%). Of the 23 sib-pairs, 13 were twin-pairs in the HCAR with lethal outcome in one or both members of 9 twin-pairs. No new cases were found in the other two above-mentioned sources of ascertainment. The amount of known families having affected sib-pairs was 136. Four triple CCMs were reduced to 12 sib-pairs. Thus, the final number of CCM-pairs evaluated was 148.

Table III demonstrates CCMs in twin-pairs. Out of 21 sib-occurrences, 9 were specific and 4 were non-specific and while 3 pairs were semi-specific. (One component of complex CCMs in one twin corresponds to the CCM of their pairs.) One or both CCMs were not specified in early lethal cases. (Zygosity was not determined in the majority of these twin-pairs.)

The distribution of 148 CCM pairs is summarized in Table V. The percentage distribution of affected sibs is, of course, distorted by the deviation of birth prevalences of each CCM type.

TABLE I

Distribution of CCMs and extracardiac CAs within "multiple" cases in 9 sibs and 2 half sibs

Sib 1			Sib 2			Comment
Sex (year of birth)	CCM	CA	Sex (year of birth)	CCM	CA	
Boy (65)	PDA	Polycystic kidney	Girl (75)	PDA	Polycystic kidney	Polycystic kidney-CCM association (Czeizel, 1986) in both sibs
Girl (70)	VSD	Minor anomalies of Down syndrome	Girl (73)	PDA	-	Down syndrome in sib 1
Girl (71)	VSD	Cleft palate	Boy (75)	VSD	-	-
Girl (71)	Endocardial fibroelastosis	-	Girl (75)	VSD + dextro-flexion of aortic arch	Minor anomalies of Down syndrome	Down syndrome in sib 2
Girl (71)	PDA	Hepatosplenomegaly +ophthalmoplegia	Boy (77)	PuSt	-	Niemann-Pick disease in half sib 1
Boy (72)	"CCM"	Cleft lip+cong. dislocation of hip	Girl(81)	VSD	-	Half sibs
Girl (74)	Dextroposition of heart, strictura of aortic arch	Spleen aplasia	Boy (76)	Dextroposition of heart with cor biloculare	Spleen aplasia + situs inversus	Ivemark syndrome in both sibs
Boy (75)	Cardiomyopathy	Hemangioma in liver	Girl (76)	VSD	Holoprosencephaly + Klippel-Feil syndrome	-
Girl (76)	VSD	-	Boy (80)	Double outlet left ventricle + tricuspid atresia + subaortic stenosis	Spleen aplasia	Ivemark syndrome in sib 2
Girl (75)	Double aortic arch	Tracheal stenosis	Boy (80)	Double aortic arch	Tracheal stenosis	Aortic arch malformation - tracheal stenosis sequence (Czeizel et al, 1988) in both sibs
Girl (78)	Dex	Situs inversus	Girl (80)	VSD	-	-

TABLE II

Triple occurrences of CCMs in sibs

Sib 1		Sib 2		Sib 3	
Sex (Year of birth and death)	CCM	Sex (Year of birth and death)	CCM	Sex (Year of birth and death)	CCM
Girl (68-)	ASD	Girl (70-70)	ASD	Girl (73-)	VSD+CoAo
Boy (70-)	ASD	Boy (73-)	AoSt	Girl (77-)	ASD
Boy (71-71)	VSD+OC (endocardial fibroelastosis in mitral valve)	Boy (72-72)	CoAo+PDA	Boy (78-78)	ASD+CoAo+(PDA)+OC (cong.mitral stenosis)
Boy (75-75)	VSD (cor triloculare biatriatum) + OC (bicuspid aortic value)	Boy (76-76)	TGA+VSD (cor triloculare biatriatum) + OC (bicuspid aortic value)	Girl (80-80)	ASD+VSD+CoAo

TABLE III

Distribution of CCMs in twins

Twin A				Twin B			Source of cases
Sex	(year of birth)	Type of CCM	Exit	Sex	Type of CCM	Exit	
Boy	(69)	AoSt	-	Boy	AoSt	-	PCOC
Girl	(72)	TF	-	Girl	ECD	-	PCOC
Girl	(72)	"CCM"	+	Girl	PDA	-	HCAR
Girl	(72)	VSD	+	Boy	VSD	+	HCAR
Girl	(73)	VSD	-	Girl	VSD	-	HCAR
Boy	(73)	VSD	-	Boy	VSD	-	PCOC
Girl	(74)	PDA	-	Girl	ASD	-	HCAR
Boy	(75)	VSD	+	Boy	VSD	+	HCAR
Boy	(75)	"CCM"	+	Boy	"CCM"	+	HCAR
Girl	(75)	"CCM"	+	Girl	"CCM"	+	HCAR
Girl	(76)	PuSt	-	Boy	VSD	-	PCOC
Girl	(76)	"Cardio-myopathy"	+	Girl	"Cardio-myopathy"	+	HCAR
Girl	(76)	VSD+PuSt	-	Girl	PuSt	-	HCAR
Girl	(76)	"CCM"	+	Girl	CoAo	-	HCAR
Girl	(76)	PuSt	-	Boy	VSD	-	HCAR
Girl	(76)	ASD	-	Girl	ASD	-	PCOC
Boy	(76)	PDA	-	Boy	PDA	-	PCOC
Boy	(77)	PDA	+	Boy	PDA	+	HCAR
Boy	(77)	"CCM"	+	Boy	"CCM"	+	HCAR
Girl	(77)	VSD	-	Boy	VSD+CoAo	+	PCOC
Girl	(78)	VSD+PDA	-	Girl	VSD	-	PCOC

PCOC = Paediatric Cardiology Outpatient Clinic
HCAR = Hungarian Congenital Abnormality Registry

DISCUSSION

Three difficulties arose during the analysis. (i) Only 136 sib-occurrences were homogeneous with 50% of familial genes in common. Of 17 same sex twin-pairs, about 8 may be monozygotic with a 100% participation of familial genes while one half-sib had only 25% of familial genes in common. (ii) At least one case of 41 sib-pairs had complex CCMs. The distribution of CCMs in sib-pairs is shown in Table IV. CCs in triple sib-occurrences (Table II) and in twins (Table III) are not included in Table V. (iii) Ten lethal cases were reported with only an unspecified CCM, i.e. "CCM".

Obviously similar CCMs occurred most frequently in sibs except PuSt, TF and TGA (Table V). The highest figures were found in CC (12.8%) and VSD (12.8). However, the latter has the highest birth prevalence among CCM-types (1.6 per 1000) /3/ while CC has only a figure of 0.4/1000. The high figure of CC is an interesting phenomenon, because it may indicate a correlation between high penetrance (more than one CCM within these families) and the serious expressivity (the CC may be a more serious manifestation of CCM than an isolated manifestation). This relationship corresponds well to the rules of polygenic inheritance, i.e. multifactorial etiology /7/. The third highest figure was found in AoSt (6.8%). Among the dissimilar CCMs the combinations of VSD-PuSt and VSD-TF are worth stressing, as these figures exceed the proportion of similar CCMs. This finding confirms the data of Fraser et al /10/. Finally the lack of a number of combinations, e.g. VSD-CoAo, ASD-TF, PuSt-CoAo, etc., may be interesting from a pathogenetic point of view.

The characteristic sib-occurrences of CCMs helps us to understand the embryology of the heart and great vessels, and their pathogenesis /1/.

TABLE IV

Distribution of CCMs within complex CCMs

Sib 1			Sib 2		
Sex	(Year of birth)	Type of CCM	Sex	(Year of birth)	Type of CCM
Girl	(63)	"CCM"	Girl	(65)	ASD+PuSt
Girl	(63)	PuSt	Boy	(68)	CoAo+VSD
Boy	(65)	ASD+VSD	Boy	(75)	ECD+AoSt
Boy	(66)	CoAo	Boy	(77)	CoAo+PDA+OC (hypoplasia of pulmonary artery)
Boy	(67)	AoSt+OC (hypoplastic left heart syndrome)	Boy	(78)	AoSt+CoAo
Boy	(67)	PDA+ASD	Boy	(71)	PDA+ASD+PuAt
Boy	(68)	VSD+PuSt	Boy	(73)	VSD+PuSt
Girl	(70)	CoTr+VSD+ASD	Boy	(72)	CoTr+VSD
Boy	70	ASD+PDA+CoAo+ AoSt+OC (hypoplasia of pulmonary artery)			
Girl	(71)	PuSt	Boy	(73)	ASD+PuSt+CoTr
Boy	(71)	CoTr+VSD	Girl	(72)	CoTr+VSD
Boy	(71)	ASD+AoSt+Dex	Boy	(72)	TGA+ASD+PDA
Boy	(71)	AoSt	Boy	(72)	TGA+ASD
Boy	(71)	AoSt	Boy	(78)	VSD+PDA+CoAo
Boy	(72)	VSD	Boy	(74)	VSD+PuAt
Boy	(72)	VSD	Boy	(76)	ASD+VSD+PDA+AoSt
Boy	(72)	ASD+VSD+PDA+ OC (tricuspid atresia + hypoplasia of aorta)	Girl	(79)	CoTr+VSD+AoSt
Girl	(73)	VSD	Girl	(82)	PDA+ASD

TABLE IV (cont.)

Distribution of CCMs within complex CCMs

Sib 1			Sib 2		
Sex (Year of birth)		Type of CCM	Sex (Year of birth)		Type of CCM
Girl	(73)	ASD	Boy	(80)	CoTr+VSD
Girl	(73)	VSD+PuSt	Boy	(72)	CoTr+VSD
Boy	(73)	CoTr+VSD	Boy	(76)	ASD+PDA+OC (right aortic arch)
Girl	(74)	ECD+VSD	Girl	(76)	VSD
Boy	(75)	CoAo+OC (cong. mitral insufficiency)	Girl	(79)	ASD+PuSt
Boy	(76)	VSD	Boy	(79)	VSD+ASD+PDA+CoAc
Girl	(77)	CoAo+PDA	Boy	(79)	AoSt
Girl	(77)	PuSt+OC (hypoplastic left heart syndrome)	Boy	(79)	CoAo+OC (hypoplastic left heart syndrome)
Boy	(78)	TF	Boy	(82)	VSD+PuSt
Boy	(79)	ECD+ASD	Girl	(81)	ECD+PDA+OC (hypoplasia of aorta)
Boy	(80)	VSD	Boy	(82)	VSD+PuSt
Boy	(80)	TF	Boy	(81)	ASD+AoSt

TABLE V

Number of sib-pairs affected by CCM (upper part) and their percentage figures (lower part)

CCM groups	VSD	ASD	PDA	PuSt	TF	CoAo	AoSt	TGA	ECD	OC	CC	"CCM"	Total
VSD	19 12,8	4	2	7	7	0	3	1	1	0	10	0	54
ASD	2,7	6 4,1	1	1	0	1	3	0	0	2	3	1	18
PDA	1,4	0,7	4 2,7	1	0	0	0	0	0	0	0	1	6
PuSt	4,7	0,7	1,4	2 1,4	2	0	1	0	1	3	3	0	12
TF	4,7	0	0	1,4	4 2,7	0	0	2	1	0	2	0	9
CoAo	0	0,7	0	0	0	1 0,7	0	0	0	0	1	1	3
AoSt	2,0	2,0	0	0,7	0	0	10 6,8	0	0	1	3	2	16
TGA	0,7	0	0	0	1,4	0	0	0 0	0	0	0	0	0
ECD	0,7	0	0	0,7	0,7	0	0	0	1 0,7	0	0	0	1
OC	0	1,4	0	2,0	0	0	0,7	0	0	4 2,7	1	0	5
CC	6,8	2,0	0	2,0	1,4	0,7	2,0	0	0	0,7	19 12,8	1	20
"CCM"	0	0,7	0,7	0	0	0,7	1,4	0	0	0	0,7	4 2,7	4
													148

ACKNOWLEDGEMENT

The authors wish to thank the heads of Paediatric Cardiology Outpatient Clinics for providing the necessary data for analysis.

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EXON DELETIONS IN DUCHENNE AND BECKER MUSCULAR DYSTROPHY

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The muscle specific promoter gene and 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 47, 48, 49, 50, 51, 52, 60 exonal deletions of dystrophin gene were examined in 41 Hungarian Duchenne and Becker muscular dystrophic patients and 21 (51%) of them were found having detectable exon deletions. One patient's deletion was extended toward the 5' end of the gene, while 20 patients' deletions were extended toward the 3' end. The correlation between the deletions and phenotypes seems to confirm the reading-frame model.

INTRODUCTION

Duchenne and milder allelic form Becker muscular dystrophy (DMD, BMD) are X chromosome-linked (Xp21) recessive lethal muscle-wasting disorders affecting approximately 1 of 4000 male newborns. The 2.5 million base pair gene consist of more than 70 exons, resulting in a 14kb mRNA, which encodes the 427 kDa protein dystrophin. This very large gene has high mutation rate, the mutations (deletion, missense, nonsense, splicing etc.) seem to be of independent origin in the unrelated families. A third of all patients arise from new mutations. About 65% of patients with DMD and BMD possess deletions of exons within the gene. A standard method of deletion analysis is a multiple polymerase chain reaction (PCR) /2, 3/ allowing simultaneous amplification of multiple deletion prone exons in a single reaction mixture. The deletions are readily identified

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by the absence of bands because of failure of amplification of individual reactions in the multiple PCR.

The disease is present from birth and may cause developmental delay: late onset of walking but the affected patient usually does not present until 3-5 years of age. The initial complaints: leg weakness (running and stair climbing difficulties and problems with rising from the floor). The progressive loss of muscle tissue continues throughout the life. Patients, who became wheelchair bound by 13 years of age are typical of DMD, those between 13-15 years are intermediate (I) and who remain ambulatory past the age of 15 are the BMD patients /5/. Death frequently occurs by the late teens or early twenties (DMD patients), but the less severe BMD patients remain ambulatory into adulthood and may conduct minimally restricted lives. Nowadays there is no cure or effective treatment for this disease.

We planned to screen our 41 DMD and BMD patients for exons: 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 47, 48, 49, 50, 51, 52, 60 and muscle promoter gene deletion mutations /2, 3/ to know the frequency of deletion prone exons in our population and to help genetic counselling and prenatal diagnosis.

PATIENTS AND METHODS

The diagnosis of our 41 DMD and BMD patients depended on the basis of the clinical symptoms, the highly elevated serum creatine phosphokinase (CPK) values, myopathic changes on electromyography and on muscle biopsy. The severity of the disease was scored as I to IV (see Results, Fig. 3). The DNA was prepared from fresh peripheral blood leukocytes of the affected individuals /10/. The multiple PCR for exons 8,17,19,44,48 (1st tube) was performed as described by Chambarlain et al. /3/, for exons 4,12,51 (2nd tube using primers described by Chamberlain et al.) and for muscle specific promoter gene (Pm) and 3,6,13,43,47,50,52,60 exons (3rd tube) and (in the 4th tube) for exon 49 by Begg's method /2/. We used Promega Taq DNA polymerase enzyme and Boehringer dNTPs. The PCR products were kept at $4^{o}C$ before analysis, and were analysed by 12% non-denaturing polyacrylamide gel electropheresis. After staining the gel with ethidium bromide, the visible DNA-strands corresponding to the different amplified products were photographed.

RESULTS

We could identify deletions in 51% of the DMD, BMD affected patients (21/24). Fig. 1 shows the frequency of exon deletions with the majority extending toward the 3' end of the gene.

The distribution of exon deletions and the disease severity scores I to IV in different age groups of 21 muscular dystrophic patients are shown on Fig. 2. Score I: mild dystrophy, II: able to walk with self support, III: unable to walk alone, IV: wheelchair bound. The limits of deletions remained unknown in some cases with these amplification methods.

Fig. 3 illustrates the distribution of the disease severity scores in different age groups of 21 deletion (A) and 20 non-deletion (B) cases. Patients younger than 11 years of age were all ambulatory. Over the age of 11 we found permanently wheelchair bound (score IV) patients in both groups (A and B) but more patients remained ambulatory in the non-deletion group (B).

Fig. 4 shows the multiple amplification bands of exons: 3,6,13,43,47,50,52,60 and the muscle specific promoter gene /2/, with various intragenic deletions (lane 3 and 4). Lack of amplification in lane 2 serves as a negative control. Samples in 5,6,7 do not display deletions.

Fig. 5 shows the multiple amplification bands of exons: 8, 17, 19, 44, 45, 48 /3/ with deletions in lane 3, 7, 8, 9, 10. (We do not demonstrate the single amplification of the exon 49 /2/ and triplicate amplification of exons 4, 12, 51 /3/ in the separated reactions.)

DISCUSSION

The deletions of the dystrophin gene being clustered in two hotspots, at the 5' and at the 3' end of the gene. The frequency, extent and location of the detected deletion mutations would be complete if these examinations could have

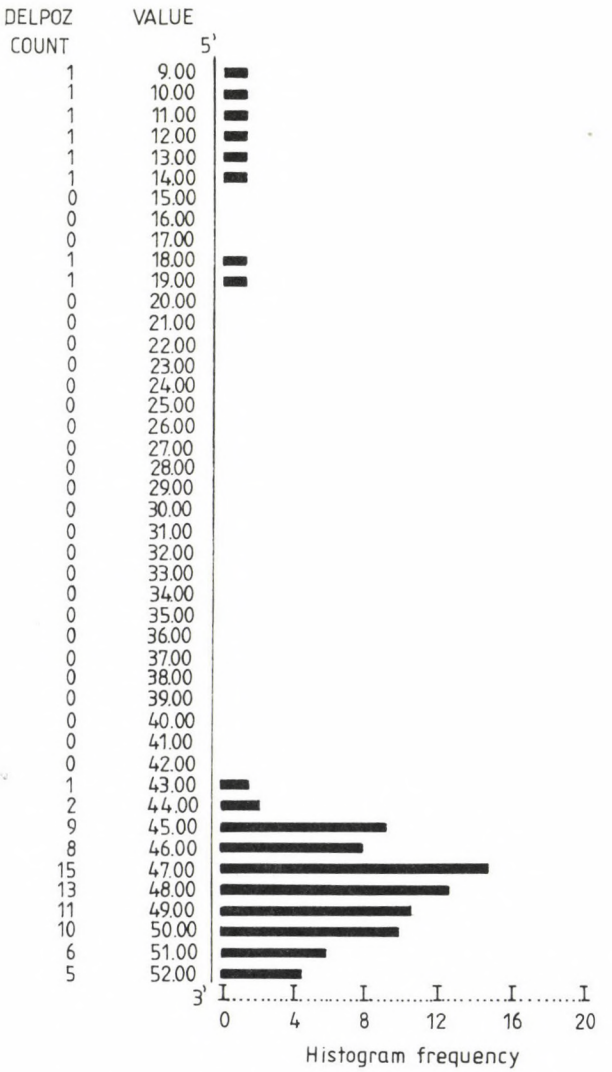

Fig.1. Frequency of exon-deletions in our 21 delewtion cases

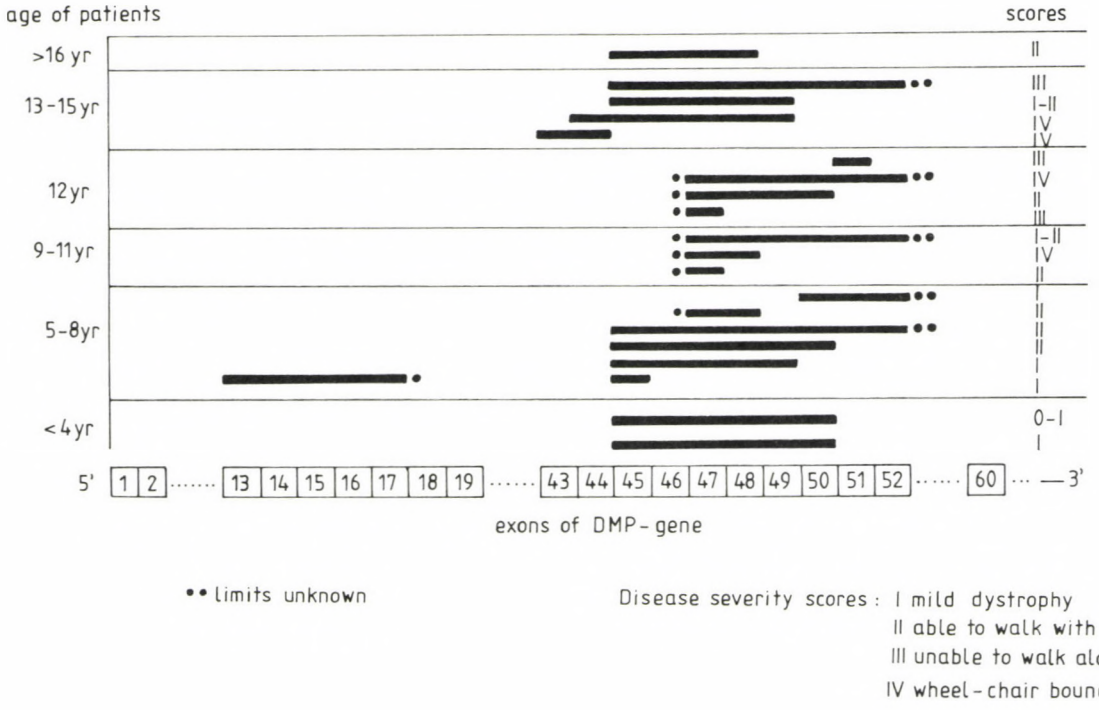

Fig.2. Distribution of exon deletions in different age-groups of 21 muscular dystrophy patients and disease severity scores (I-IV)

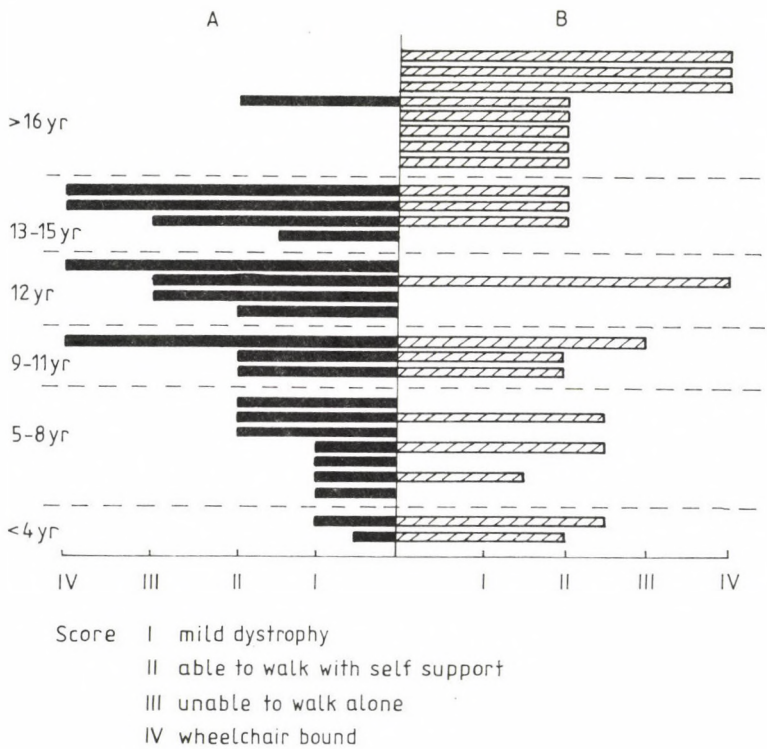

Fig.3. Distribution of disease severity scores (I-IV) in different agegroups of 21 deletion (A) and 20 non-deletion (B) cases

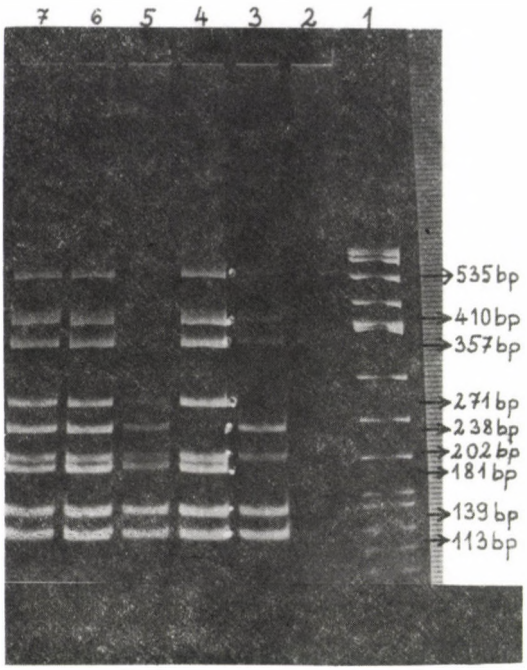

Fig. 4. Detection of DMD gene exon /e/ deletions by multiple DNA amplification by methods of Beggs et al. /2/. The bands: 535bp /Pm gene/, 410bp/e3/, 356bp/e43/, 271bp/e50/, 238bp/e13/, 202bp/e6/, 181bp/e47/, 139bp/e60/ and 113bp/e52/. Lane 1 shows the Hinf I-digested Øx174 DNA molecular weight standard, lane 2: a negative control, lans 3-4: samples deleted for two and one exons, respectively, lanes 5-7: samples do not display a deletion

been done in all DMD/BMD patients in our coutry. Because of the easier amplification of the smaller bands we did not examine the possibility of exon duplications.

In our DMD/BMD patients 21 (51%) were found having exon deletions, and 11 out of 21 deletion patients (52%) had unknown deletion limits with these multiple PCR examinations.

We ought to have done more exon deletion analysis of the dystrophin gene to detect these unknown delective limits.

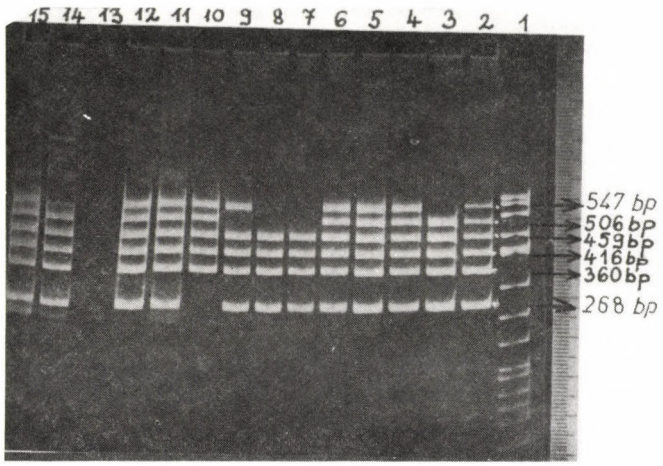

Fig. 5. Analysis of dystrophin gene multiple polymerase chain reaction /PCR/ with primers described by Chamberlain et al. /3/. Lane 1: Hinf I-digested Øx174 DNA marker, samples in lanes 2-15 show the product of 14 PCR reaction deleted for one or two exons /e/ in some cases. Bands: 547 bp /e45/, 506bp/e48/, 459bp/e19 /, 416bp/e17/, 360bp/e8/, 268bp/e44/

In the other patients the causative mutation remained unknown (20 cases). These nondeletion DMD/BMD patients, families also require the detection of mutations in affected subjects to facilitate prevention for female relatives.

Comparison of the site or extent of exon deletions to the severity scores of the disease (I to IV), in some cases, seems to support the frameshift hypothesis /1, 4-6, 8, 9/.

For example: del 45-48 (score II, patient is 29 years old) (in frame mutation), del 43-44 (score IV, patient is 13 years old) (out of frame mutation). In the future we have to complete our investigations with dystrophin and mRNA examinations from muscle biopsies to analyse the frameshift hypothesis in our deletion DMD/BMD patients. The combination of genomic deletion analysis and direct dystrophin analysis allows the maximal diagnostic and prognostic accuracy even before the onset of any clinical symptoms.

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FUNCTIONAL AND METABOLIC CHARACTERISTICS OF TOXIC POLYMORPHONUCLEAR NEUTROPHIL GRANULOCYTES IN NEONATES WITH BACTERIAL INFECTION

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The function of toxic neutrophils from neonates with different bacterial infection was studied by in vitro determination of their locomotion, adhesion, phagocytosis and bacterial killing, as well as by some enzymes activity and superoxide generation. The results were compared with a group of the remaining patients and severely ill neonates without metabolic changes in neutrophils. The existence of strong connection between severe clinical state and the presence of toxic neutrophils was proved. Morphologically changed cells had lower phagocytosis of latex particles and S. aureus as well as random migration. The decrease of bactericidal function and chemotaxis was less striking. These alterations in the function appeared to be intrinsic to the cell population. Sera from patients with and without toxic neurophils showed similar opsonic and chemotactic activity. In the compared groups no activity of inhibitor and inactivator of locomotion was proved.

The observation that toxic neutrophils have handicapped basic function suggest that careful review of the blood smear may decide about the choice of complementary treatment of stressed neonates.

INTRODUCTION

Toxic polymorphonuclear neutrophil granulocytes (TPMN) are characterized by the presence of toxic granules, Döhle bodies, cytoplasmic vacuolization and increased affinity for Romanovsky dyes. They are seen in many disorders but most frequently appear during serious bacterial infection /13, 16/. From the clinical point of view, toxic changes in polymorphonuclear neutrophil granulocytes (PMN) are indication of inflammatory process, severity of the disease and of poor prognosis.

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It has been demonstrated that bacterial infection itself, may influence PMNs function and activity of humoral factors /1, 9, 12, 15/. In neonates, during the stress and infection, even adequate numbers of phagocytes may be insufficient to maintain proper host defense, because of their impaired function. These include decreased deformability, chemotaxis, phagocytosis, bacterial cell killing, decreased oxidative metabolism likewise humoral factors activity /10/.

It is likely that the presence of TPMNs may be responsible for decreased nonspecific host defense. The alterations in metabolism were alredy reported in morphologically changed phagocytes /8, 13/. However, it is not clear if morphological changes in phagocytes are related to their impaired function.

In this article metabolic and functional differences between toxis and non-toxic PMNs isolated from neonates with bacterial infections are presented.

MATERIAL AND METHODS

51 neonates with different bacterial infections medicated in the Department of Pediatrics from 1978-1981 and 1984-1989 were selected for the study. Detailed patient description is shown in Table I. The criteria for proved bacterial infection were based on culture findings of blood, CSF, urine, pleural fluid and/or tracheal aspirate. These confirmed 18 cases of sepsis, 17 of urine tract infection, 9 of bacterial meningitis, and 7 of purulent pneumonia, all caused by both gram positive (20 infants) and gram negative organisms (31 infants). None of the 51 patient died. Although all patients were acutely, ill they were in different clinical state when studied. Children were divided according to severity of clinical symptoms of infection. In addition to laboratory investigations (acid-base balance, coagulation profile, hepatic function) classification of the severity of the disease was based on disturbances of thermoregulation respiration and circulation, enlarged liver and spleen, as well as presence of shock and altered consciousness. Patients who presented disturbances of more than one vital functions were considered as severely ill. Thirty-three infants were classified as having severe and 18 having mild presentation of infection. The studies of PMNs function and metabolism were performed during acute phase of disease.

TABLE I

Clinical characteristics of neonates with confirmed bacterial infection

Type of illness etiological agent	Number of patients sex	Age at infection months mean (range)	Clinical status	WBC $x10^3/\mu l$ mean (range)	PMNs # $x10^3/\mu l$ mean (range) % of PMNs
Sepsis /18/ St.aureus /10/ E.coli /6/ Salm.paratyphi type B/1/ Flavobacterium /1/	14M, 4F	4.5 (1-12)	severe - 18 mild - 0	16.4 (6.8 - 24.0)	10.3 (3.3-16.3) 61.4%
Urinary tract infections /17/ E.coli /9/ Pr.mirabilis /7/ Group B Streptococcus /1/	14M, 3F	5.1 (1-12)	severe - 2 mild - 15	10.1 (4.8-12.3)	4.8 (2.0-7.6) 48.4%
Meningitis /9/ Meningococcus /3/ Pneumococcus /4/ Salm. enteritidis /1/ St. aureus /1/	7M, 2F	5.2 (1-12)	severe - 8 mild - 1	14.4 (8.6-22.0)	8.3 (4.6-14.0) 58.2%
Pneumonia /6/ St. aureus /6/	3M, 3F	7.1 (3-12)	severe - 5 mild - 1	17.8 (10.0-22.0)	12.2 (5.9-14.0) 68.8%
Diarrhoea Salm.paratyphi type B/1/	1M	3.0	mild - 1	14.2	7.1 50%
Total /51/	39M, 12F	5.4 (1-12)	severe - 33 mild - 18	14.6 (4.8-24.0)	8.5 (3.3-16.3) 57.3%

M - male; F - female

Laboratory procedures

5 ml blood samples were taken by venipuncture. CBC counts were performed automatically. On each patients three peripheral blood smears were selected for detailed morphological review, and 100-cell differential counts were performed by one of the investigators (Celinska W., Prof. of haematology), who was unaware of the clinical status of the patient, or culture results of the infection evaluation. In addition to different counts, the presence of toxic granulation, Dohle bodies, vacuolisation were noted on 100 PMNs.

Isolation of PMNs

For all functional studies the PMNs were isolated by method described by Boyum. The PMNs were washed twice and suspended in the medium containing 140 mM NaCl, and 5 mM Tris/HCl pH 7,4 to a final concentration 0.5- 1.5 x 10^7 cells/ml. The purity of the PMNs preparation was 95+2%. Cellular viability as determined with 1% Trypan blue dye was 95%.

Random migraton and chemotaxis was measured by modification of the Boyden's method /4, 18/ which employed parabiotic chambers separated by 3 u Millipore filter. The chemotactic factor of complementary derivation was obtained from the mixture of 1 ml 5% normal human albumin, 0.4 ml AB serum of healthy donors and 0.2 ml rabbit anti human serum albumin. The mixture was not chemotactic in the absence of antigen, antibody or complement. Cells migrating to the undersurface of the filter were enumerated by phase microscopy using ovular gird (7x7 mm) with 400-fold magnification. The results were expressed as the mean number of cells per ocular gird after counting 10 randomly selected fields (HPF).

Phagocytic and bactericidal activities were determined by modification of the micromethod described by Forman /3/, using Staphylococcus aureus Cowan 1 as the test microorganism. The assay was accomplished by mixing 0.1 ml of PMN suspension with 0.1 ml of St. aureus ($5x10^6$/ml), 0.1 ml of pooled normal human serum and sufficient amount of Medium 199 to make a total volume of 0.5 ml. The plastic tubes containing this mixture were then rolled at 10 RPM in a 37 ^{O}C incubator. Samples were removed immediately after 2 hours incubation and the number of surviving bacteria was determined. Quantitation of viable bacteria was made by utilizing a standard pure plate technique. The results were expressed as per cent of the "0" time sample of bacteria that were ingested and the percentage of the ingested bacteria that were killed.

Measurement of superoxide anion

The generation of superoxide anion by PMNs was estimated by a modification of the method described by Markert and Babior et al. /14/. In this assay the superoxide dismutase - inhibitable reduction of cytochrom C by the intact PMNs - was the measure of superoxide anion formation. The standard reaction mixture in final volume of 1.2 ml, contained 133 mM NaCl, 6 mM CaCl, 2 mM

glucose, 5mM Tris/HCl pH 7.4 1 mg of ferricytochrom c, and 2mg of opsonized zymosan or 80mM of arachidonic acid as stimulant. The reaction was started by adding 0.1 ml of PMNs suspension and carried out for 30 min in 37°C. Spectrophotometric measurements were made with a Philips PU 8700 spectrophotometer. Nanomoles of reduced cytochrome c were determined from the increase in the absorbance at the maximum between 545 and 550 nm using molar coefficient of extinction - 21.1 mM/cm. The results are expressed as nmoles /10^6 PMN/30 min.

Other procedures /3/

Myeloperoxidase and alkaline phosphatase activity in PMNs were scored as described by International committee for Standardization in Haematology (ICSH).
Phagocyte adhesion to glass was evaluated in whole blood by the micromethod of Gifford and Malawista.

Statistical analysis

All results were expressed as mean $\pm$ SEM (unless otherwise indicated). Statistical differences were determined using nonparametric Wilcocson test.

RESULTS

In 21 out of 51 ill neonates TPMNs were present. Toxic granulation were seen in 17 patients, cytoplasmic vacuoles in 7, and light blue amorphous inclusions - Döhle bodies in 6 patients. Two morphological features of TPMNs were noted in 5 patients and three features in 2 patients.

In 19 out of 33 severely ill neonates TPMNs were found. In neonates with mild presentation of the infection TPMNs were found only in two cases (Table II). We found a strong positive correlation between the severity of the illness and the presence of TPMNs (X^2 = 8.55, ss = 1 $p < 0.004$).

In Table III parameters describing PMNs function during acute phase of infection are shown. Phagocytic activity of TPMNs was significantly lower than that of non-toxic PMNs. The same was true for random migration. The statistically not significant bacterial killing and PMN adhesion to glass were also decreased, if compared to non-toxic PMNs.

Table II

Toxic PMNs in relation to clinical status

Clinical status	Severe No. of patients	Mild No. of patients	Total
Toxic PMNs			
present	19 (37.3%)	2 (3.9%)	21 (41.2%)
absent	14 (27.5%)	16 (31.4%)	30 (58.8%)
Total	33 (64.7%)	18 (35.3%)	51 (100%)

Non-toxic PMNs isolated from severely ill neonates have decreased phagocytic activity but not locomotion and bacterial killing. However, we did not find statistical differences if compared with TPMNs.

Activity of myeloperoxidase and alkaline phosphatase did not differ between toxic and non-toxic PMNs.

Generation of superoxide anion by unstimulated neonate PMNs was dependent on the clinical presentation of infection. It was significantly lower in toxic PMNs, and non-toxic PMNs than in severely ill neonates (Table IV). Stimulation with opsonized zymosan or arachidonic acid increased superoxide anion generation 2-4 fold, even so, it was significantly lower in toxic PMNs and non-toxic PMNs than in severily ill neonates.

We also tested whether altered PMNs function was due to the changes in humoral factors' activity during infaction. In the presence of ill neonates serum, as the source of chemotactic, opsonizing, inhibitory and inactivatory factors, we tested chemotaxis and phagocytosis in polymorphonuclear neutrophil granulocytes isolated from healthy donors. Both chemotaxis and phagocytosis of healthy donor PMNs were decreased in the presence of ill neonates serum (detailed data presented elsewhere /4/). The degree of decrease was not depending on the presence of toxic PMNs in ill neonate blood.

Table III

Function of toxic and non-toxic PMNs during acute phase of infection

PMNs	Toxic	Non-toxic severely ill	Non-toxic all patients
Number of patients	21	14	30
Phagocytosis and bacterial killing (%)			
latex positive cells	83.1±11.5	84.9±10.6	94.7±9.7*
St.aureus ingested	90.8±6.0	89.5±4.4	94.8±6.2*
St.aureus killed	82.1±4.5	89.5±5.1	93.9±6.0
Adhesion to glass mean number of cells/HPF	15.6±9.0	19.0±11.9	20.2±12.5
Locomotion mean number of cells/HPF			
random migration	0.10±0.11	0.38±0.42*	0.41±0.45*
chemotaxis	2.95±3.07	4.47±5.91	3.38±4.29
Enzymes score			
Alkaline phosphatase	245±26	238±21	130±32
Myeloperoxidase	103±37	92±19	120±43

* statistically significant if compared to toxic PMNs ($p<0.03$)

Table IV

Superoxide anion generation in toxic and non-toxic PMNs during acute phase of infection

PMNs	Toxic	Non-toxic severely ill	Non-toxic all patients
Number of patients	11	3	10
no stimulation	0.59±0.11	0.23±0.02*	1.53±0.30*
opsonized zymosan	1.55±0.26	1.35±0.31	3.58±0.51*
arachidonic acid	1.17±0.20	0.66±0.28	3.29±0.59*

Values represent nmoles of reduced cytochrom c/10^6 cells/30 min
* - statistically significant if compared to toxic PMNs $p<0.02$

DISCUSSION

This study demonstrates the defective function and oxygen metabolism in toxic PMNs isolated from neonates during acute phase of bacterial infection. We observed toxic PMNs in 21 out of 51 neonates during acute phase of bacterial infection. Nineteen of them were severely ill, none neutropenic. It suggests, that the degenerative changes identify in more extend the severity of clinical status than the disease itself.

The works of Zieve et al. /16/ and Cheng-Hurd Liu et al. /5/ indicate that toxic PMNs are useful in early detection of newborns' bacterial infection. In our observations we did not see the correlation between the onset of the disease and appearance of toxic PMNs.

The altered PMNs function is characteristic for bacterial infections of newborn infants /9, 10, 11/. Recent works prove that the perturbation maintains for over one month of life and depends on the severity of clinical state, on the stage of illness and concerns almost every aspect of the immune system /2, 4, 7/. While examining the reasons of the above-mentioned biological events also the presence of cytoplasmic changes in

individual PMNs is taken into account /2, 19 /. The absence or little number of patients without toxic granulations in PMNs makes the interpretation of the results very difficult /17/.

Our examinations prove that the existence of toxic granulations in PMNs decrease in vitro phagocytosis function and the ability of random migration. Lower bactericidal activity adhesion to glass and chemotaxis of TPMNs was less striking. These alterations in the function of toxic PMNs appeared to be intrinsic to the cell population.

The above-mentioned facts not only confirm, but to a greater degree extend the information of Mc Call, who stated that sera from three patients with PMNs had no effect on chemotactic activity of control leukocytes /16/.

The altered PMNs function in the youngest groups of age in the acute stage of bacterial infection is explained to some extent /9, 10/. Though greater defect of migration and phagocytosis in TPMNs still remains unclear.

One possibility is that functional potential of TPMNs is exhausted even though the cell remains viable. It is indicated by spontaneous degranulation and vacuolization if a large dose of endotoxin is used of after ingestion of many bacteria.
Some authors emphasize the increase of stickness in such changed cells followed by the decrease of migration and phagocytosis /8/. Also the dissociation between cytoplasmic and nuclear maturity may exist in TPMNs. Certain characteristics that have been identified in the cytoplasm of TPMNs with segmented nuclei resemble the cytoplasm of metamyelocytes and myelocytes /13, 16/.

In the study it is shown that the activities of examined enzymes are similar in PMNs of compared groups. So it is doubtful, if there is direct connection between the great activity of alkaline phosphatase and with the presence of TPMNs and the dispaired function of the cell. This enzyme is a part of secondary granules of which the number and size seems to be unchanged in TPMNs /3, 13/. Also low activity of myeloperoxidase observed in bacterial infection in neonates is not characteristic for TPMNs. Now it is shown that so-called

high peroxidase activity (HPX) correlating with the presence of TPMNs is produced not by an increase in cellular myeloperoxidase but rather by an alteration in its distribution within the phagocytes /20/.

In the present study we have shown that the production of superoxide anion by TPMNs is significantly lower than in non-toxic ones. It is in agreement with the findings of other authors who observed the alteration of cellular metabolism due to morphological changes /2, 3, 8/.

Until now the function of TPMNs was estimated in reference to healthy donors not taking into consideration the role of the infection itself. This fact is of special importance in the youngest group of age where the decrease of cellular metabolism and the alteration of basic functions of professional phagocytes are not necessarily accompanied by toxic changes.

In conclusion, careful review of the blood smear can be performed easily and will provide adjunctive information concerning the neonate suspected or having bacterial infection /5/.

The estimation of function and metabolism of professional phagocytes is more difficult and can be done only in few centres /2, 3, 17/. The observation that TPMNs have significantly diminished abilities for locomotion, phagocytosis and respiratory burst suggest that simple laboratory test may decide about the choice or continuation of complementary treatment of bacterial infections in neonates.

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SERUM CERULOPLASMIN LEVEL IN OFFSPRING OF PARENTS SUFFERING FROM EARLY CORONARY HEART DISEASE

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Serum ceruloplasmin, as extracellular antioxidant, was determined in 67 healthy children and in 95 offspring of parents who have had an acute myocardial infarction before the age of 45. An age-dependent reduction was detected in endangered boys. However, authors did not find statistically significant difference in ceruloplasmin level between the affected children and control subjects. A significant negative linear correlation was proven between the serum ceruloplasmin and high-density lipoprotein-cholesterol level in sons of parents with early acute myocardial infarction. The results of this study suggest the appearance of alteration in the association between ceruloplasmin and lipids in boys of parents suffering from premature coronary heart disease.

INTRODUCTION

More than half of all death in Hungary are attributed to cardiovascular diseases. It seems vital to know more of its development and its various risk factors. Several investigators have suggested that free radical reactions play an important role in the pathogenesis of atherosclerosis /8, 12, 14/, and in the development of manifest cardiovascular diseases such as acute myocardial infarction /6, 16/. Cells and tissues are

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protected against oxidising free radicals by a complexity of antioxidant mechanism /5/. Ceruloplasmin (CP) is one of the main serum antioxidants /2, 4/. The aim of the present study was to examine tha concentration of serum CP in children, whose parents had an acute myocardial infarction (AMI) before the age of 45.

MATERIALS AND METHODS

Total serum cholesterol (TC) and serum lipoprotein-cholesterol fractions and CP levels were measured in 67 healthy children, 48 boys and 19 girls, without any family history of coronary heart disease (CHD), as controls, and 95 offspring, 40 boys and 55 girls, of parents suffering from premature CHD (PCHD). The age distribution of examined children is shown in Table I.

TABLE I

Age and sex distribution of children investigated
Number of children

	Control			Risk group		
Age (years)	Boys	Girls	Boys and Girls	Boys	Girls	Boys and Girls
3-14	7	7	14	23	29	52
15-18	41	12	53	17	26	43
3-18	48	19	67	40	55	95

All children are from different parents.

Blood samples were taken by venepuncture after fasting overnight, at 7-8 a.m. after 15 minute quiet bed-rest. The children were abstinent. None of them was a smoker. They had no medication before the start of the study. Hormonal contraceptives had not been taken. TC and high-density lipoprotein-cholesterol (HDL-C) levels in serum were measured by enzymatic method (SERA PAK Reanal, Hungary). HDL-C was determined after precipitation with $NaPWO_4$ and $MgCl_2$. Total triglyceride (T) was determined spectrophotometrically (Triglyceride test, Reanal, Hungary). The low-density lipoprotein-cholesterol (LDL-C) level was calculated according to Friedewald's formula LDL-C = TC-(TT/2.2) - HDL-C (mmol/L). The determination of CP's concentration was performed by a photometric method. The CP in vitro has a diaminooxidase activity, so the colourless p-phenyldiamine is oxidized to a blue colour p-chinon. Its absorbance is proportional to concentration of CP at the pH 5.6 and 546 nm. All values are expressed as mean $\pm$ SD. Statistical analysis was performed by Student's *t* test. Linear regression models were applied to examine the association between parameters. The significance was determined by the analysis of variance using the *F* table.

Kits

These were as follows: from Reanal, Cholesterol Cat. No. 6376; Triglyceride, Cat. No. 70.829-0-80.

RESULTS

There was no statistically significant correlation between CP and lipids in patients (neither in mothers nor in fathers of examined children) investigated after at least 6 weeks of early AMI, diagnosed by clinical symptoms, typical ECG changes, and specific enzyme patterns. The concentrations of examined parameters in the serum from parents are shown in Table II.

TABLE II

Serum lipid and ceruloplasmin levels of parents investigated

(Mean ± SD)

	Mothers	Fathers
Number of parents	13	26
Age (years)	40.8 ± 6.1	41.6 ± 4.6
TC (mmol/l)	5.02 ± 0.68	6.02 ± 1.46^{x}
HDL-C (mmol/l)	1.11 ± 0.38	0.89 ± 0.18^{xx}
LDL-C (mmol/l)	3.37 ± 0.94	3.86 ± 1.55
TT (mmol/l)	1.48 ± 0.72	2.63 ± 1.24^{xxx}
CP (g/l)	0.615 ± 0.206	0.592 ± 0.217

^{x} $p < 0.05$; ^{xx} $p < 0.02$; ^{xxx} $p < 0.01$

Lipid levels in the affected children differed from these of the controls. The serum CP levels were lower in boys than in girls, but significant differences could not be shown. There was no statistically significant difference in CP concentrations in the study group compared to sex-matched controls (Table III).

TABLE III

Serum lipid and ceruloplasmin levels of children investigated

(Mean ± SD)

	Controls			Risk Children		
	Boys	Girls	Boys and Girls	Boys	Girls	Boys and Girls
Number of children	48	19	67	40	55	95
TC (mmol/l)	3.75±0.88	3.97±0.82	3.81±0.87	4.28±0.80^{x}	4.54±1.17	4.37±1.15^{xx}
HDL-C (mmol/L)	1.22±0.20	1.19±0.14	1.20±0.19	1.08±0.31	1.08±0.25	1.08±0.28
LDL-C (mmol/L)	2.19±0.87	2.36±0.84	2.28±0.81	2.89±0.87^{x}	2.95±1.14	2.92±1.03^{xx}
TT (mmol/L)	0.79±0.42	0.90±0.33	0.82±0.40	0.82±0.58	1.14±0.84	1.01±0.75
CP (g/L)	0.63±0.19	0.70±0.31	0.66±0.23	0.62±0.26	0.67±0.24	0.65±0.25

Significantly different from controls

$^{x}p < 0.01$; $^{xx}p < 0.001$

There was no statistically significant association between CP and lipids in healthy children. CP concentration minimally enhanced with advancing age in healthy chidren but a significant age-dependent decrease in CP level was seen in sons of parents with PCHD ($Y = -5.8615 . x +16.671$; $F = 6.31$, $p < 0.025$, where x = ceruloplasmin level). A negative linear correlation was proven between CP and HDL-C level ($Y = -0.6049 . x +1.4663$; $F = 13.042$, $p < 0.025$) both 3 to 14 year-old ($Y = -0.6742 . x +1.5962$, $F = 9.124$, $p < 0.025$), and 15 to 18 year-old boys ($Y = -1.097 . x +1.6056$; $F = 16.868$, $p < 0.025$, where ceruloplasmin = x), whose parents had an early AMI (Tables IV, V.). Significant positive linear correlation was seen between serum CP and TT concentration in 3 to 14 year-old boys with a positive parental history of PCHD ($Y = 1.0046 . x +0.1754$; $F = 6.615$, $p < 0.025$, where ceruloplasmin = x).

The examined correlations did not change significantly in the high-risk girls (Table VI).

DISCUSSION

Evidence shows that oxygen-derived free radicals are important mediators of several forms of tissue damage /6/. Raised concentration of lipid peroxides (LP) have been found in hyperlipidemia, essential hypertension, after AMI and stroke as well as in diabetes /1/. It is not cholesterol but its oxidation products that initiate and then increase the damage of the endothelium of the arterial wall, which is one of the most common phenomena in atherosclerosis /6, 13/. The potential role of LP-s in atherosclerosis is found in animals maintained with atherogenic diet /9/. Epidemiological data present increased serum LP levels measured by malondialdehyde in children of parents suffering from early AMI /15/.

Antioxidants function as blockers of radical processes. The antioxidant capacity associates with the plasma levels of

TABLE IV

Linear correlation coefficients between ceruloplasmin and age and lipid parameters in children investigated

	Controls		Risk Children	
	Boys	Girls	Boys	Girls
Number of children	48	19	40	55
Ceruloplasmin (x)	r	r	r	r
y				
Age	0.185	0.377^{x}	-0.377^{xx}	0.094
TC	- 0.152	- 0.171	0.222	0.046
HDL-C	0.025	- 0.186	-0.505^{xxx}	0.006
LDL-C	- 0.173	- 0.173	0.220	0.053
TT	0.058	0.208	0.438^{xx}	- 0.056

^{x} $p < 0.05$; ^{xx} $p < 0.025$

Where y are the other parameters, x is ceruloplasmin level.

TABLE V

Linear correlation coefficients between ceruloplasmin and lipids in endangered boys

Age (years)	3 to 14	15 to 18
Number of children	23	17
Ceruloplasmin (x)		
y	r	r
TC	0.213	- 0.027
HDL-C	- 0.550^{xx}	- 0.727^{xx}
LDL-C	0.214	0.099
TT	0.489^{xx}	0.283

^{xx} $p < 0.025$
x = Ceruloplasmin
y = TC, HDL-C, LDL-C, TT

TABLE VI

Linear correlation coefficients between ceruloplasmin and lipids in endangered girls

Age (years)	3 to 14	15 to 18
Number of girls	32	26
Ceruloplasmin (x)		
y	r	r
TC	0.204	0.054
HDL-C	0.404	0.031
LDL-C	0.127	0.046
TT	- 0.076	- 0.028

x = Ceruloplasmin
y = TC, HDL-C, LDL-C, TT

nonenzymatic free-radical scavengers. CP was described as a circulating antioxidant, and as a scavenger of superoxide anion radicals /3, 5, 7/. CP was found to inhibit the development of hyperlipoproteinemia, to decrease the level of free-radical oxidation of lipids and to increase the antioxidant demands of tissues /2/. CP levels are low in newborns, but increase to exceed adult values by one year of age /10/.

In the present work we examined the concentration of CP, as extracellular antioxidant in healthy children and in offspring whose parents had had an early AMI. We found an age-dependent alteration of serum CP level. The changes were in opposite direction: the healthy children had minimally higher and the endangered boys lower values with increasing age.

We did not find statistically significant difference between CP levels in high-risk children and control subjects. In accordance with other examination we did not demonstrate significant linear correlation between cholesterol and CP among all the groups /11/. No statistically significant correlation was found with respect to CP and lipids in healthy controls, but the tendency of association corresponded to the expectations. A significant negative linear correlation was identified between CP and HDL-C level in endangered boys. This relationship tended to be stronger in adolescence. No association between ceruloplasmin and lipoprotein fractions was present in the affected parents themselves. These findings indicate an alteration in the regulation of association between CP and lipids in boys of families with high-risk of cardiovascular disease. The mechanism of the relations among the above parameters, however, needs further investigation. We suppose that the change of association between serum CP and lipoprotein-cholesterol fractions may be a part of a compensatory mechanism in affected boys. Further work is needed to establish whether CP values might provide a useful index in the initial process of atherosclerosis.

Increased ratio of zinc to copper in patients with CHD has also been reported. Earlier we have measured these parameters in offspring of parents suffering from PCHD. Publication of our results is in progress.

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CALCIUM-PHOSPHATE METABOLISM PARAMETERS AND VITAMIN-D STATUS IN PATHOLOGICAL PRETERM INFANTS

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Parameters of metabolism of the major minerals and 25 (OH)D vitamin concentration were measured in 14 pathological preterm infants of 1347±273 gs birthweight and 30.5±2.7 wks gestational age, at the postnatal age of 1, 4 and 8 weeks. The babies did not receive oral calcium, phosphate or vitamin D supplementation apart from that in their formula, but were given 100 000 U vitamin D_3 parenterally, on postnatal day 7. Normal serum calcium, phosphate and magnesium concentration was observed all throughout the study period, and renal mineral excretion did not show as if the infants had been in mineral deficiency. The parenterally given large bolus vitamin D caused a notable but transient rise in 25 (OH) D concentration, which fell again to preinjection level a few weeks later. No close relationship was found between 25 (OH) D concentration and Ca-PO_4 metabolism parameters or early postnatal growth rate.

INTRODUCTION

The spectacular rise in survival rate of very low birthweight and extremely low birthweight preterm babies has resulted in new challenges for clinical neonatology. Immature and/or grossly damaged intermediary metabolism or survivors of acute cardiorespiratory and neurologic adaptation disorders present additional disadvantages, in respect of extrauterine growth and development. Quantity and quality of the main nutrients needed by high risk immature babies are by now more or less known, whilst knowledge regarding requirements in supply of minerals, trace elements and vitamins is much more uncertain.

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Major minerals like calcium, phosphate and magnesium have a multiple role in physiology and biochemistry of the human /1/. The question, however, of their optimum supply, absorption, excretion and homeostasis in the foetus and newborn is still not completely answered /6, 7 /. Similarly, the relationship between vitamin D status and mineral metabolism needs further investigation, in order to provide a biochemical milieu as physiological as possible to immature neonates.

In the present study links between Ca, PO_4 and Mg metabolism parameters and 25 (OH) D vitamin status were looked for in pathological preterm infants. Furthermore, the effect of parenteral supplementation of vitamin D was tested, since early continuous oral administration is not always possible in sick preterm babies. Additional object of our investigation was to collect some more data on perinatal physiology which can be helpful in the every day's clinical practice.

PATIENTS AND METHODS

Fourteen preterm babies of 1347±273 (1020-1760) gs birthweight and 30.5±2.7 (27-36) weeks gestational age were studied. All of them suffered from some kind of acute perinatal adaptation disorder of moderate to severe degree, but all survived. The main clinical diagnosis was HMD in 5, perinatal hypoxia associated with subependymal-intraventricular haemorrhage in 3, perinatal infection in 3, dismaturity in 2 and hyperbilirubinaemia in 1 cases. Enteral feeding was initiated as early as possible, for which special preterm formula (Prematil, Milupa) was exclusively used, with calcium, phosphate and magnesium content of 70, 40 and 6 mg/100 ml ready made fluid, and a vitamin D_3 content of 605 U/100 g powder. The babies did not get these major minerals from any other source either enterally of parenterally during the study period. At the age of 1 week all infants were given 100 000 U vitamin D_3 parenterally.

As a part of their clinical check-up, serum concentration of total calcium, phosphate, magnesium and alkaline phosphatase activity was measured with routine laboratory methods within 24 hrs of birth and at postnatal ages of 1, 4 and 8 weeks. Renal calcium, phosphate and magnesium excretion was measured simultaneously by determining the mineral/creatinine ratio in a morning urine sample /4/. In addition, serum 25 (OH) D vitamin concentration was determined as well, on day 7, 28 and 56, by using Amersham 25-Hydroxyvitamin-D (3H) assay system (code TRK860). Daily mineral and oral vitamin D intake was calculated

TABLE I

Concentration of major minerals, alkaline phosphatase activity and 25-hydroxy-cholecalcipherol level in the serum of pathological preterm babies during the first 8 postnatal weeks (mean ± SD)

	≤ 24 hours	1 week	4 weeks	8 weeks
Calcium (mmol/l)	2.3±0.1	2.3±0.2	2.5±0.1	2.4±0.1
Phosphate (mmol/l)	2.1±0.3	1.9±0.5	2.2±0.2	2.4±0.2
Magnesium (mmol/l)	0.9±0.2	0.9±0.2	0.8±0.1	0.8±0.0
Alkaline phosphatase (IU/l)	108±64	143±56	175±113	268±129
25 (OH) D (ng/ml)	-	23.7±14.8	36.6±15.2	17.9±2.6

TABLE II

Oral intake of major minerals and vitamin-D_3 in pathological preterm infants during the first 8 postnatal weeks (mean±SD). Enteral and parenteral intake advised in the relevant literature /1/ is also shown on the table

	0 - 7 days	8 - 28 days	29 - 56 days	Advised intake	
				enteral	parenteral
Calcium, mg/kg/day	56±28	130±5	134±2	180-370	50-60
Phospate, mg/kg/day	31±15	74±3	75±3	70-180	40-50
Magnesium, mg/kg/day	4.6±2.1	10.8±1.5	11.0±0.3	5-7	5-7
Vitamin-D_3, IU/day	68±33	157±6	162±4	1000-2000	

by the volume of formula consumed and the reference data for minerals and vitamin D content. The accuracy of body weight weighing was ±10 gs. Growth rate was expressed as gs/day; for statistical analysis standard mathematical methods were used.

RESULTS

Results are summarized in tables and figures. Table I shows that serum calcium, phosphate and magnesium concentrations in the babies were normal all throughout the study period. The level of these minerals was steadily and well regulated as shown by the small SD values. In contrast with these parameters, a highly varying serum alkaline phosphatase activity could be observed at each time studied, however, when considering the mean values, an obvious trend of rise was noted. It is also seen that at age week 1 mean 25(OH)D vitamin concentration was well above the critical 10 ng/ml concentration (23.7±14.8 ng/ml) considered as a cut-off point below which vitamin D deficient state cannot be diagnosed. After that, in response to the parenterally given 100 000 U vitamin D, a remarkable increase could be detected (36.6±15.2 ng/ml), which was transitoric, because four weeks later 25(OH)D concentration was again around the preinjection level, i.e. 17.9±2.6 ng/ml.

Total intake of the three major minerals and oral vitamin D_3 intake is shown in Table II calculated as an average of daily intakes, for the three separated time-interval of the whole observation period. It can also be seen that feeding even special formula for preterm babies, calcium intake was less than the generally advised amount /1/ which could have been attained only by providing extra calcium supplementation.

Fig. 1 is for demonstrating the weight gain of the preterm infants studied. It is shown that they thrived well and doubled their birthweight before the age of two months. For statistical analysis weight gain of each babies was separately calculated and expressed as g/day.

Fig. 2 shows changes in urinary mineral/creatinine ratio for calcium, phosphate and magnesium at postnatal ages studied. It

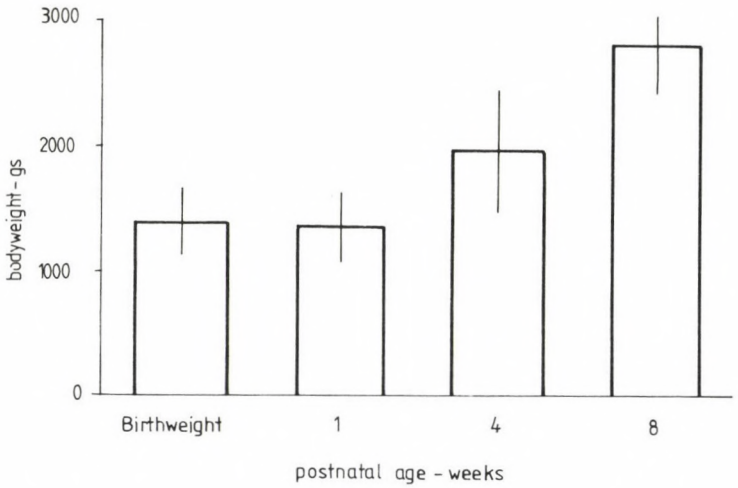

Fig. 1. Postnatal growth of the study babies during the first 8 weeks of life (mean±SD)

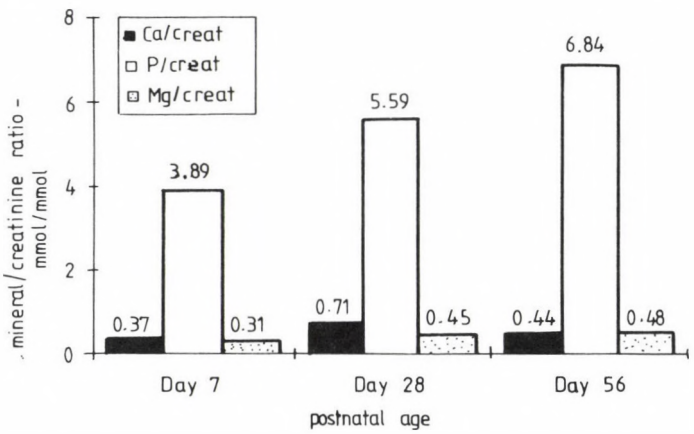

Fig. 2. Urinary excretion of major minerals in pathological preterm infants. The value of SD varied between 0.25-0.85, 2.76-5.61 and 0.22 - 0.51 for calcium, phosphate and magnesium, respectively

is seen that neither rise in calcium excretion nor a gradually decreasing phosphate excretion could be observed, considering the mean values. Calcium/creatinine ratio remained all throughout the observation period slightly below or above 0.50 and phosphate/creatinine ratio rised gradually from around 4.00 to 7.00. This certainly means that the babies in study had not been calcium or phosphate depleted at that time, provided secondarily increased parathormone secretion can be excluded. However, parathormone concentration could not be measured in our study, the normal 25 (OH) D vitamin status makes most unprobable this explanation.

In order to look for relationship between mineral metabolism biochemical parameters and vitamin D status and growth, the following parameter-pairs were tested by a linear correlation analysis: enteral intake of calcium, phosphate, magnesium - serum concentration of calcium, phosphate and magnesium; serum 25 (OH) D vitamin concentration - serum calcium and phosphate; serum 25 (OH) D concentration - serum alkaline phosphatase activity; serum 25 (OH) D concentration - growth rate; growth rate - serum alkaline phosphatase activity. A reversed but statistically not significant correlationship was found between 25 (OH) D level and alkaline phosphatase activity ($r=-0.4970$ at 4 weeks and $r=-0.3526$ at 8 weeks); no other relationship worth mentioning was found between any other parameterpairs examined.

DISCUSSION

Results of the study show that 1397 ± 273 gs birthweight pathological preterm babies can regulate normally their serum calcium, phosphate and magnesium levels during the first eight postnatal weeks of life when an oral intake of no more than 130 mg/kg/day calcium, 75 mg/kg/day phosphate and 11 mg/kg/day magnesium is offered (Tables I, II), as an average. However, serum levels of major minerals obviously do not reflect total body mineral content /1/ but much more so the capacity of the

functional compensatory responses of parathormone and vitamin D metabolism. It seems probable that the study babies may not have been in at least a considerable mineral deficient state. The finding of the persistently low renal calcium excretion and that of the rising phosphate excretion also stands against mineral depletion /4/.

In regard of mineral and bone tissue metabolism vitamin D supplementation and hydroxylation into active metabolites have a principal role. It is now known that early neonatal 25 (OH)D vitamin level depends on maternal vitamin D status, since both vitamin D_3 and 25 (OH) D vitamin are freely transported across the placenta from the mother into the foetus, in contrast with 1.25 $(OH)_2D$ vitamin /2, 3/. In one week old babies we found 23.7 ± 14.8 ng/ml 25 (OH) D vitamin concentration, indicating that in general they were not vitamin D deficient at the time. The widely scattering individual concentrations expressed by the large SD values show, however, that in some cases quite pronounced vitamin D deficiency may have occurred. Otherwise, it is to be emphasized that we measured closely similar 25 (OH) D concentrations (Table I) in the preterm babies to those reported in the relevant literature at any time studied /2, 3, 5-8/. In response tao the parenterally given large dose (100 000 U) vitamin D_3, a definite but statistically not significant rise in 25 (OH) D concentration could be observed by the postnatal age of four weeks, however, this effect was only transitoric since four weeks later near the preinjection 25 (OH) D level was measured again. Considering that hepatic production of 25 (OH) D vitamin is purely substrate dependent /6/, it seems probable that pathologic preterm babies are able to hydroxylate vitamin D_3 to 25 (OH) D vitamin, but no steady concentration can be achieved by giving large doses in bolus. The question, whether steadiness or intermittent fluctuation of 25 (OH) D level does or does not influence optimum metabolic effect on calcium-phosphate metabolism, is still unanswered. The only conclusion, what can be drawn, is that in preterm infants, who cannot safely receive oral vitamin D supplementation, normal 25 (OH) D concentration can be reached by giving D_3 vitamin parenterally in bolus.

The serum concentration of 25 (OH) D vitamin reflects pooled vitamin D stores ("vitamin D status") of the body /7/, while the de facto acting hormon-like substance is $1.25(OH)_2$ D vitamin, synthesized in the kidney under the control of parathormone and serum calcium and phosphate concentration. The serum level of that latter metabolite had not been measured by us but we looked for relationship between vitamin D status and calcium-phosphate metabolism indices and growth rate. We found that 25 (OH) D concentration is not related to either serum calcium and phosphate concentration or growth rate; a reversed relationship was found between 25 (OH) D level and serum alkaline phosphatase, which means that serum alkaline phosphatase may well prove to be an indicator of neonatal vitamin D deficiency, provided studies in larger populations would confirm this observation.

In conclusion we suggest that in pathological low birthweight preterm infants no direct relationship exists between 25 (OH) D vitamin level and routinely examined calcium-phosphate metabolism parameters. Furthermore, after parenterally given large dose D_3 vitamin even pathological preterm babies can increase their 25 (OH) D production, but this is certainly not the way maintaining a steadily constant 25 (OH) D concentration.

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SERUM LIPID PEROXIDES, COPPER AND ZINC STATUS IN CHILDREN WITH FAMILIAL HISTORY OF PREMATURE ATHEROSCLEROSIS

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Serum lipid parameters and lipoprotein levels, lipid peroxide copper and zinc concentration were measured in 460 children with familial history of premature atherosclerosis (180 offspring of a parent with coronary heart disease, 192 first degree relatives of a parent with cerebrovascular diseases and 88 with parental multi-risk) and compared with values of 100 age- and sex-matched control children. Significant differences were found on the concentration of total cholesterol, LDL cholesterol, HDL 2 cholesterol, apolipoprotein A1 and B, lipid peroxide and copper in the groups of endangered children in comparison with controls. There was a weak negative correlation between lipid peroxide concentration and HDL cholesterol and significant positive correlation between lipid peroxide and LDL cholesterol content, as well as lipid peroxide versus copper level. Findings of this paper indicate that copper has a significant link with peroxidised lipids and that copper mediated lipid oxidation may be involved in the mechanisms associated with ischaemic vascular disease (IVD). These parameters may be of considerable additional importance as risk indicators for later IVD. The study provides support to the recommendations directed to health care providers concerning the selective screening for children who have a parental history of premature IVD.

INTRODUCTION

There is compelling evidence that the atherosclerosis process begins in childhood and slowly progresses into adulthood. A positive family history of coronary heart disease (CHD) or cerebrovascular disease (CVD) is recognized as an independent risk factor for atherosclerosis. Consequently several experts recommended strategies to screen subjects at

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high risk during young age as the principal means for preventing the development of the disease. Many factors have been examined in an attempt to identify discriminative indices associated with the risk for future ischaemic vascular disease (IVD). Plasma lipoprotein and apoprotein values show a significant correlation with the incidence of clinical IVD, however additional correlates of disease prevalence would be invaluable in improving the ability to assess individual risk. Several groups have suggested that lipid peroxides may be important in the development of atherosclerosis in normolipidaemic people. Lipid peroxides are derived from the oxidation of unsaturated fatty acids, most likely by an endogenous process, and are capable to induce further lipoperoxide production by a free radical chain reaction. The mechanism of peroxidation is supposed to involve essential trace elements, among them copper and zinc.

The aim of the present study was to evaluate the discriminative value of the measurement of serum lipid peroxide concentrations, copper and zinc levels concerning the risk for future IVD in children with familial history of premature CHD or CVD. Serum lipid, lipoprotein and apoprotein parameters have also been measured. Statistical analysis was performed to determine the ranking of the above risk factors between healthy controls and offsprings of families with premature IVD.

SUBJECTS AND METHODS

460 children with an established familial risk for atherosclerosis between 3 and 12 years of age were investigated and compared with 100 control children matched for age and sex. The children at risk for atherosclerosis were divided into three groups: those with a parent suffering from CHD (surviving myocardial infarction below the age of 45 years) n=180, those with a parent suffering CVD (having a stroke and apoplexia) n=192, and multi-risk group, n=88. Familial history was obtained through questionnaires completed by the parents.

Blood was collected after an overnight fast in the presence of antioxidants. The concentration of triglycerides, total cholesterol, LDL, HDL-, and HDL_2- cholesterol was measured enzymatically using Boehringer Peridochrom CHOD-PAP tests. Apo

A1, A2 and B were determined using Sebia (Paris) Apofilm plates. The lipid peroxide level in serum was characterized by the amount of thiobarbituric acid reactive substances (TBARS) measured according to the method of Lee /1/.

Copper and zinc analysis was performed in randomly selected 26 control and 37 "risk" serum by atomic absorption spectrophotometry.

Statistical analysis was performed by Student's test. Correlation coefficiens were calculated to assess the relation among plasma lipid parameters and lipid peroxide concentrations.

RESULTS

In Table I mean values, standard deviations and statistical differences are presented for some of the essential serum parameters of the three high risk groups of children in comparison with the controls. There was no significant difference in haemoglobin A_{1C}, urin acid or triglycerids concentration and in Apo A1/A2 ratio between the risk groups and controls. Significant or highly significant differences were found in the concentration of total cholesterol, LDL cholesterol, HDL_2 cholesterol, apolipoprotein A1 and B, in the CHD and in the CVD group in comparison with controls. In the multi-risk group significantly elevated total cholesterol and LDL cholesterol concentration and significantly decreased HDL cholesterol content could be demonstrated.

Mean serum lipid peroxide concentration (expressed in terms of malondialdehyde, MDD content) of children with familial risk for IVD were higher than average control values: the difference was highly or very highly significant in the CVD and in the Multi-risk group, respectively /Table II/. Levels exceeding 45 mmol MDA/dl occurred in 27, 74 and 92 percent of the cases in the CHD, CVD and Multi-risk group, resp., whereas within the control group only in 9% of the subjects were found elevated MDA concentrations. Concerning the relation among lipid peroxide content and the various serum lipid parameters, lipid peroxide concentration showed no significant correlation with

TABLE I

Mean concentrations (±SD) different parameters measured in the sera of high risk children of parents with premature ischaemic vascular disease in comparison with age and sex matched controls

	Group of children Parent's disease				
Parameter	1. Control (n=100)	2. Coronary heart disease (n=180)	3. Cerebrovascular disease (n=192)	4. Multi-risk (n=88)	5. Total 2-4 (n=460)
Haemoglobin A1C (mmol/l)	7.01 (0.92)	6.96 (0.97)	6.66 (0.88)	7.38 (1.36)	6.91 (1.01)
Uric acid (nmol/ml)	220.3 (48.2)	222.9 (57.2)	234.0 (36.1)	209.1 (47.7)	223.8 (46.8)
Total cholesterol (mmol/l)	4.48 (0.68)	4.83 (0.87)*	5.05 (0.72)**	4.87 (0.92)*	4.93 (0.91)*
Triglyceride (mmol/l)	0.82 (0.20)	0.82 (0.25)	0.89 (0.49)	0.81 (0.35)	0.84 (0.37)
LDL cholesterol (mmol/l)	2.17 (0.80)	2.73 (1.10)	3.47 (0.95)**	3.07 (0.66)*	3.05 (0.98)*
HDL cholesterol (mmol/l)	1.61 (0.35)	1.32 (0.25)*	1.37 (0.30)*	1.43 (0.21)*	1.37 (0.25)*
HDL-2 cholesterol (mmol/l)	0.75 (0.31)	0.59 (0.33)*	0.60 (0.27)*	0.71 (0.32)	0.62 (0.30)*
HDL-2/HDL-3	0.87	0.80	0.78	0.88	0.82
Apolipoprotein A1 mg/dl	152.4 (22.0)	131.3 (22.0)**	130.0 (17.0)**	147.3 (21.0)	133.9(21.0)**
Apolipoprotein B (mg/dl)	50.3 (14)	63.3 (17)**	62.5 (19)**	56.9 (16)	61.7 (18)*
Apolipoprotein A1/A2	3.6 (0.6)	3.6 (0.5)	3.5 (0.6)	3.6 (0.3)	3.6 (0.5)

Significantly different from controls: * = $p < 0.05$ and ** = $p < 0.01$

TABLE II

Mean serum lipid peroxide levels (±SD) and the occurrence of elevated MDA concentrations in the various groups of children

Group of children Parents's disease	Lipid peroxide conc (nmol MDA/dl)	% occurrence of levels>45 nmol MDA/dl
1. control (n=94)	38.2 (9.8)	9
2. coronary heart disease (n=163)	41.7 (14.3)	27
3. Cerebrovascular disease (n=167)	49.1 (11.8)**	74
4. Multi risk (n=72)	67.0 (24.2)***	92
Total 2-4 (n=402)	49.3 (15.0)**	58

Significantly different from controls: ** = p<0.01
*** = p<0.001

TABLE III

Copper and zinc concentrations (mean±SD) in the serum of various groups of children

Group of children Parents's disease	Copper conc (mg/l)	Zinc conc (mg/l)
1. control (n=26)	0.62 (0.19)	0.86 (0.11)
2. coronary heart disease (n=15)	0.74 (0.08)	0.85 (0.07)
3. Cerebrovascular disease (n=11)	0.91 (0.07)**	0.85 (0.10)
4. Multi risk (n=11)	0.88 (0.08)*	0.86 (0.09)
Total 2-4 (n=37)	0.84 (0.08)*	0.86 (0.08)

Significantly different from controls: * = p<0.05
** = p<0.01

triglyceride, total cholesterol or HDL_2 cholesterol (r=0.13, 0.20 and 0.19, resp.) a weak significant negative correlation with HDL cholesterol (r=0.27, $p<0.01$) and a significant positive correlation with LDL cholesterol (r=0.46, $p<0.001$).

Table III shows the average values (LSD) of copper and of zinc in the various groups of children. The mean copper concentration in control children was 1.24 mg/l. Copper levels of the "endangered" group were found to be significantly lower.

In the mean values of zinc no consistent differences could be demonstrated.

Analysing the relation between copper level and lipid peroxide concentration, a significant positive correlation was found in every investigated group, as demonstrated by the calculated regression lines (Fig. 1).

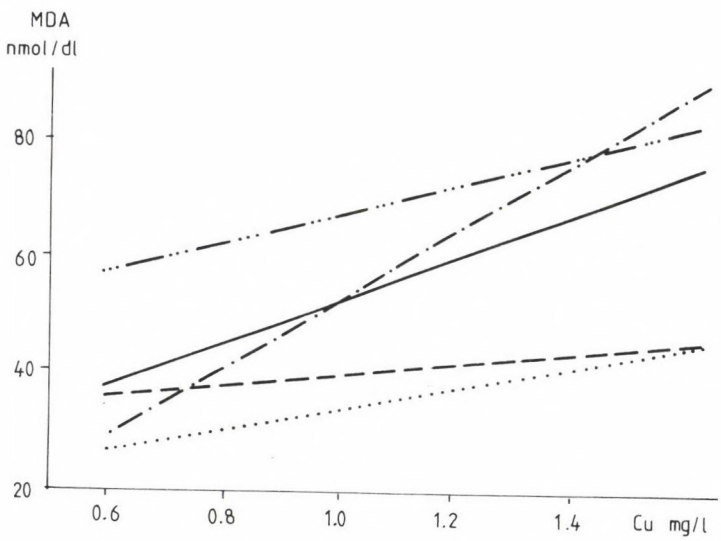

Fig. 1. Correlations between serum copper and lipid peroxide concentration. Calculated regression lines for: control group /.../ $p < 0.001$; coronary heart disease group /-·-/ $p < 0.001$; cerebrovascular disease group /---/ $p < 0.001$; multi-risk group /·····/ $p < 0.01$; and total of endangered children /——/ $p < 0.001$

DISCUSSION

Because of the strong evidence demonstrating that there is a familial aggregation of IVD it is important even in chilhood to recognise the levels of risk factors.

Studies, dealing with the question whether children from parents with premature IVD differ in regards to their lipid or lipoprotein levels from those without any familial history, reported on elevated total cholesterol and LDL-cholesterol /2-4/, decreased HDL-cholesterol /5, 6/ or combination of thereof in the endangered offsprings. Recent studies /7-10/ suggest that the ratio of Apo A1/Apo B, or Lp/a/ concentration in a better discriminator between groups with or without family history of IVD. Our data confirm that the above results, demonstrating significant differences in the mean levels of certain lipids, lipoproteins and apoproteins measured in the risk groups in comparison with controls.

The potential role of lipid peroxides in atherogenesis is supported by animal experiments /11, 12/ and epidemiological data /13-15/. Since serum lipid peroxide levels may vary in young subjects without abnormality in common lipid risk factors /16/, they may have potential as a useful index in the improved assessment of IVD risk. Our studies showed that serum lipid peroxide concentrations are raised significantly in a surprisingly high number of the endangered children. The positive correlation between the concentration of lipid peroxide and LDL cholesterol, in agreement with the findings of others /17/, give further support to the known atherogenecity of LDL. On the other hand, high HDL cholesterol levels were associated with low levels of peroxides: this might reflect the possible antioxidant effect of HDL /18/.

It is generally agreed that trace metals might be involved in the mechanism of lipid peroxidation and thus associated with IVD /19/. From among these trace elements selenium owes its essentiality to its incorporation into the active site of the enzyme glutathion peroxidase, one of the major lines of defence of the organism against damage by reactive hydroperoxide,

including lipid peroxides /20/. Atherosclerosis was found to be related to low liver, heart and leucocyte copper /21/, probably because prolonged suboptimal tissue levels of copper could impair Cu-enzym-dependent functions, such as the antioxidant activity of superoxide dismutase /22/. On the other hand, the participation of copper in the free radical oxidation of LDL in serum has been implicated /23/ and indeed, high levels of copper were found in the serum of athoresclerotic patients /24/. Our findings indicate that serum copper has a significant link with the level of lipid peroxides. Zinc levels remained relatively constant, in accordance with literature /24/.

In light of these observations it is early to draw conclusions regarding the discriminative value of peroxidated lipids and of copper in assessment of individual risk for premature atherosclerosis. However, drawing the attention of physicians and other health professionals concerned with child health care in these correlations might help to identify and treat children who are at greatest risk of atherosclerosis.

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SERUM LEVELS OF THYROID HORMONES AND THYROTROPIN IN PRETERM CHILDREN OF 1 TO 5 YEARS OF AGE

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Total serum thyroxine, total 3,5,3'-triiodothyronine and thyroid stimulating hormone concentrations were determined by radioimmunoassay in 488 preterm children born before the 38 week of pregnancy with birth weight under 2500 g and 640 healthy fullterm children with height and weight within the normal range (95%) for age of 1 to 5 years. Serum thyroxine and 3,5,3'-triiodothyronine were significantly lower ($p < 0.01$, $p < 0.001$) in preterm children during the first two years of life as compared with fullterm children. In preterm children serum thyroxine and 3,5,3'-triiodothyronine levels reached the levels of fullterm children in the third year of life. Serum thyroid stimulating hormone concentrations were significantly higher ($p < 0.01$, $p < 0.001$) in all age groups in preterm children related to fullterm children and did not change progressively with age. No differences in thyroxine, 3,5,3'-triiodothyronine and thyroid stimulating hormone levels were recorded between boys and girls within any age groups.

INTRODUCTION

It is well known that thyroid hormones play a crucial role in organism from the first day of his postnatal life. The measurement of thyroid hormone concentration is important in preterm children (PT), the more so, as some differences in comparison to fullterm children (FT) were noted /4, 5, 10/. Because of the paucity of published thyroxine (T4), 3,5,3'-triiodothyronine (T3) and thyrotropine (TSH) measurements in PT and FT children in our region, we have studied their concentration from 1 to 5 years of age.

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SUBJECTS AND METHODS

1128 children from the East Slovakia region were included in our crosectional study: 488 PT children (202 boys and 286 girls), born before the 38th week of pregnancy with a low birth weight (under 2500 g) and 640 healthy FT children (326 boys and 314 girls) with height and weight within the normal (95%) range for age /12/.

Blood was drawn always at 8.00-10.00 a.m. in summer months 1984-1986 from a peripheral vein and serum was frozen at -20°C until analysis was performed.

The total serum T4 and T3 concentrations in samples were assessed by commercial radioimmunoassay kits (ÚRVJT, Košice, SR) and TSH concentrations using components from NIH Bethesda (USA), by count rate recorded on multidetector counter RIA JNG 401 (ÚRVJT, Košice, SR).

Children with disorders and treatment which could influence serum hormone levels were excluded from the study.

Classification of the children into individual age categories was done on the basis of the chronological age by decimal age analysis (/15/ (i.e. a group of 5 years comprised children aged 5.0-5.9 years).

Hormone data were analysed using Student's t unpaired test and correlations with chronological age were done by linear regression and expressed as correlation coefficient r (Figs. 1-6).

The results are expressed as mean $\pm$SD in Tables I and II.

Intraassay and interassay coefficients of variation were about 7% and 11%, respectively.

RESULTS

The mean serum concentrations of T4 in FT and PT boys of different ages are demonstrated on Fig. 1. It appeared that the T4 concentrations in the first two years of life are significantly ($p < 0.001$) lower in PT boys as compared to FT ones. An expressive rise in serum concentrations of T4 was noted at 3 years of age - the mean T4 level in this group significantly ($p < 0.01$) exceeded that of the FT boys. In the next years FT boys had higher levels of T4 in comparison with PT boys, although not significantly.

Similarly, significantly ($p < 0.001$) lower T4 levels at the age of 1 and 2 were observed in PT girls compared with those of FT

TABLE I

Serum levels of thyroid hormones (nmol/l) and thyrotropin (μU/ml) in preterm children (m+SD)

	PRETERM CHILDREN					
AGE	BOYS T4	GIRLS T4	BOYS T3	GIRLS T3	BOYS TSH	GIRLS TSH
1	74 ±13.5 {47}[a]	75 ±15.0 {65}	1.0 ± 0.6 {45}	0.9 ± 0.5 {63}	5.9 ±1.2 {18}	6.5 ± 2.6 {22}
2	83 ±13.4 {43}	87 ±15.5 {55}	1.9 ± 0.6 {38}	1.7 ± 0.8 {52}	5.8 ±1.6 {30}	5.7 ± 0.8 {44}
3	129 ± 25.3 {27}	121 ± 24.0 {37}	2.3 ±1.0 {24}	2.3 ± 0.9 {28}	5.4 ±1.3 {12}	5.7 ±1.1 {24}
4	130 ± 21.8 {34}	135 ± 31.0 {41}	2.5 ± 0.5 {34}	2.5 ± 0.7 {30}	5.5 ± 0.8 {17}	5.9 ±1.0 {21}
5	125 ± 24.7 {24}	134 ± 30.0 {32}	2.7 ± 0.6 {23}	2.5 ± 0.6 {32}	5.8 ±1.0 {10}	5.9 ± 0.9 {20}

a – The number of observations are in parentheses

TABLE II

Serum levels of thyroid hormones (nmol/l) and thyrotropin (μU/ml) in fullterm children (m+SD)

	FULLERM CHILDREN					
AGE	BOYS T4	GIRLS T4	BOYS T3	GIRLS T3	BOYS TSH	GIRLS TSH
1	97 ±18.4 {30}[a]	93 ±12.9 {30}	2.0 ± 0.8 {25}	1.9 ± 0.7 {28}	4.2 ±1.8 {26}	4.3 ± 1.6 {26}
2	97 ±16.6 {41}	99 ±19.0 {44}	2.2 ± 0.8 {32}	2.3 ± 0.7 {29}	4.0 ±1.9 {30}	4.4 ± 1.6 {29}
3	97 ± 17.0 {59}	118 ±13.2 {53}	2.3 ± 0.8 {51}	2.1 ± 0.7 {47}	4.4 ±1.3 {40}	4.2 ±1.2 {40}
4	136 ± 22.6 {79}	126 ±17.3 {73}	2.6 ± 0.9 {63}	2.4 ± 0.9 {53}	4.2 ± 1.8 {54}	4.8 ± 2.3 {43}
5	133 ± 19.4 {63}	127 ± 22.3 {76}	2.6 ± 0.8 {53}	2.6 ± 0.8 {66}	5.2 ± 2.5 {60}	4.7 ± 1.4 {64}

a – The number of observations are in parentheses

ones (Fig. 2). PT girls had no significantly higher levels of T4 in comparison to FT girls from 3 to 5 years of age.

Significantly ($p < 0.01$, $p < 0.001$) lower levels of T3 were found at 1 yeaar of age in PT boys (Fig. 3) and at 1 and 2 years of age in PT girls (Fig. 4). No significant difference in serum levels of T3 were measured in both groups from 3 to 5 years of age.

There were significant positive correlations between the age groups and T4 levels in PT and FT children.

In PT boys and girls of all studied age groups (with the exception of 5 year old boys) serum TSH concentrations were significantly higher ($p < 0.01$, $p < 0.001$) than those of FT children and appeared to remain unaltered throughout the observed period (Figs.5 and 6).

No differences in T4, T3 and TSH levels were recorded between boys and girls within the age groups.

DISCUSSION

Hypothalamic-pituitary-thyroid axis is activated immediately after birth and several changes occur in this system in the first hours of postnatal life. That is why the early period of extrauterine life has been extensively studied /1, 7, 8, 9/. Children with low birth weight frequently differ from fullterm ones as to the pattern of postnatal growth, possibly as a consequence of a delay of the maturation of the pituitary-thyroid axis /3, 10, 13/. Normal preterm infants have a pattern of thyroid function qualitatively similar but quantitatively different from that of fullterm infants at first weeks of life /14/. In low birth weight newborns the measurement of T4 in serum alone may falsely indicate primary hypothyroidism. As described /9/, after 5 days of age, the serum TSH concentrations were normal in all newborns, including PT and small for gestational age babies. Therefore it was concluded that a supplementary measurement of TSH should be performed after the 5th day of life to exclude the diagnosis of congenital hypothyroidism.

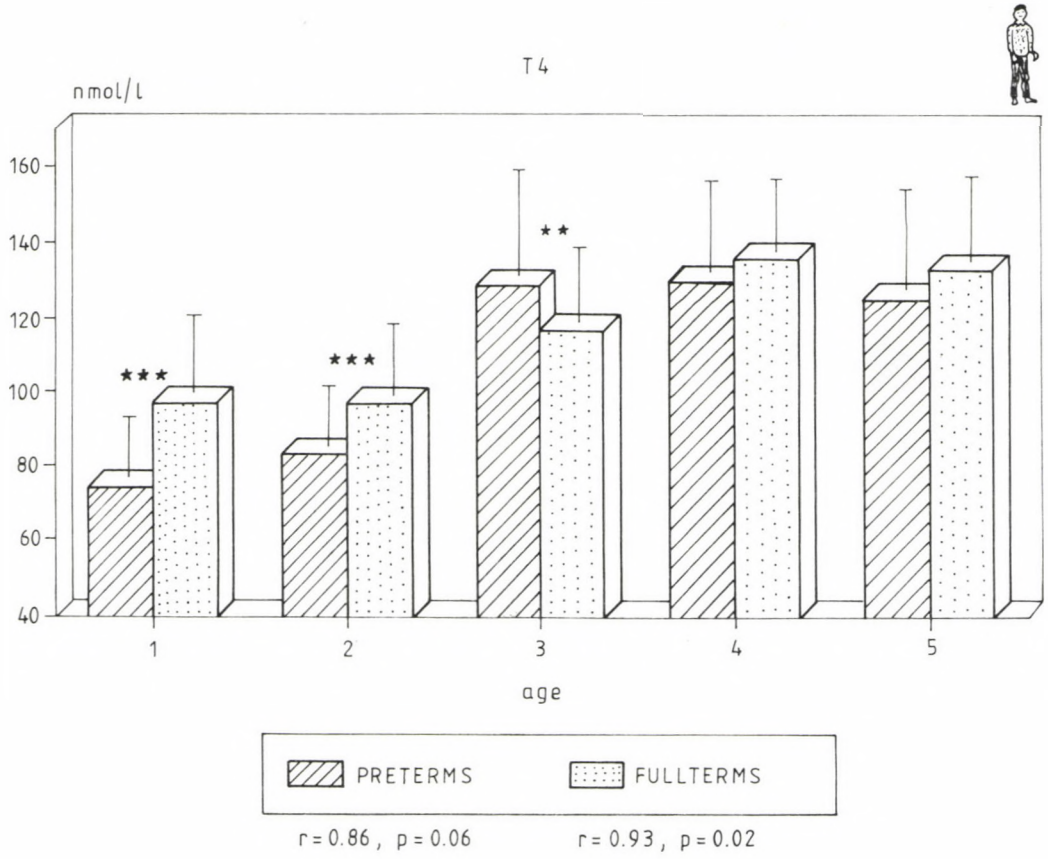

Fig. 1. Mean concentrations (+1 SD) of serum T4 in preterm and fullterm boys.

** P < 0.01, *** P < 0.001

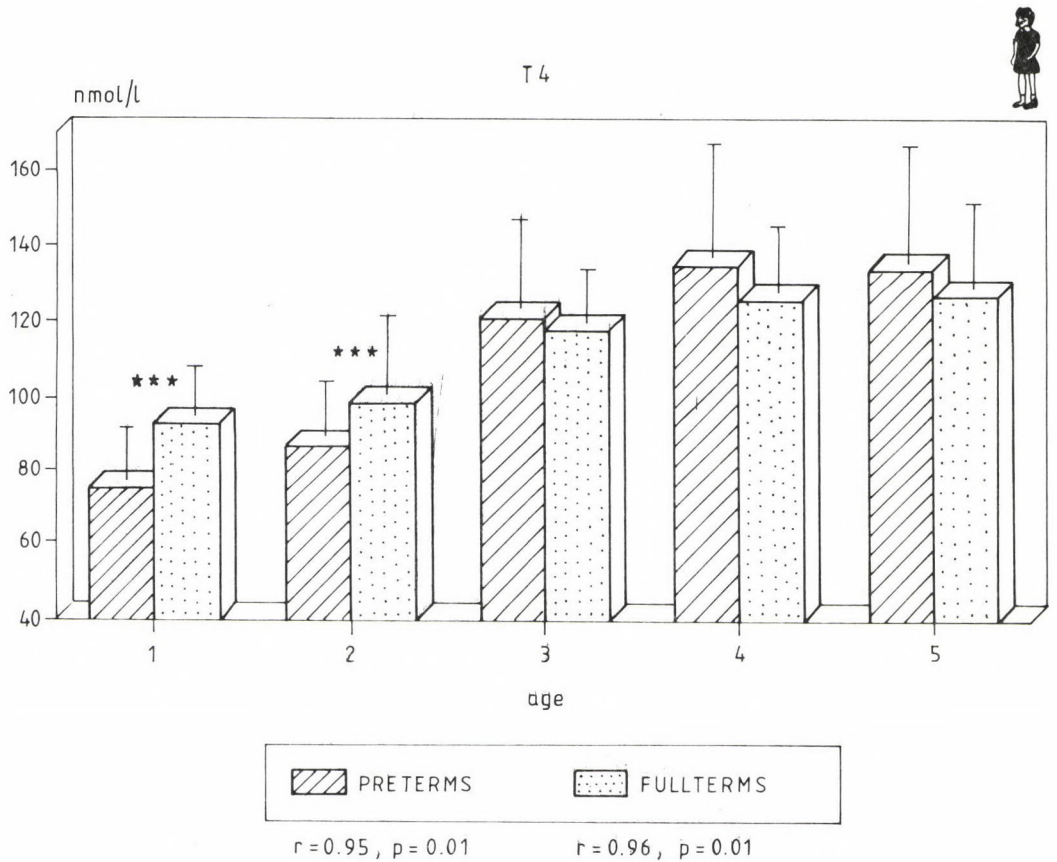

Fig. 2. Mean concentrations (+1 SD) of serum T4 in preterm and fullterm girls.

*** $P < 0.001$

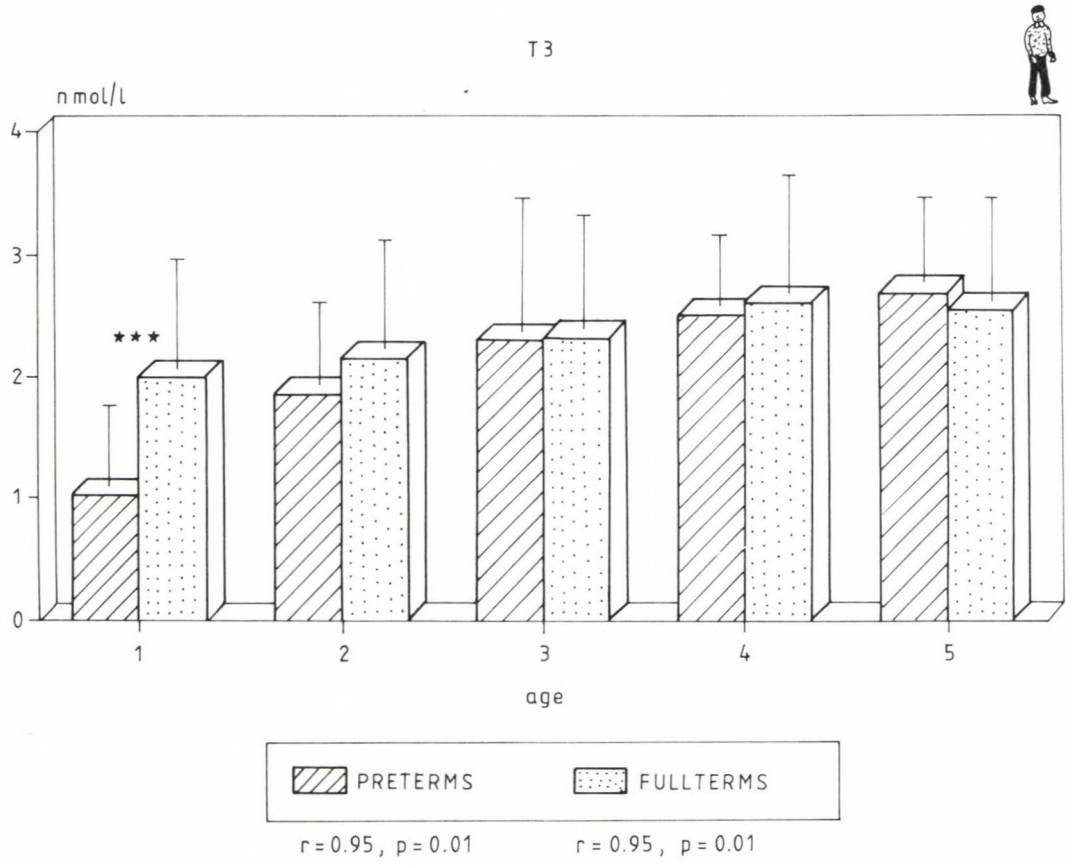

Fig. 3. Mean concentrations (+ 1 SD) of serum T3 in preterm and fullterm boys.

*** P < 0.001

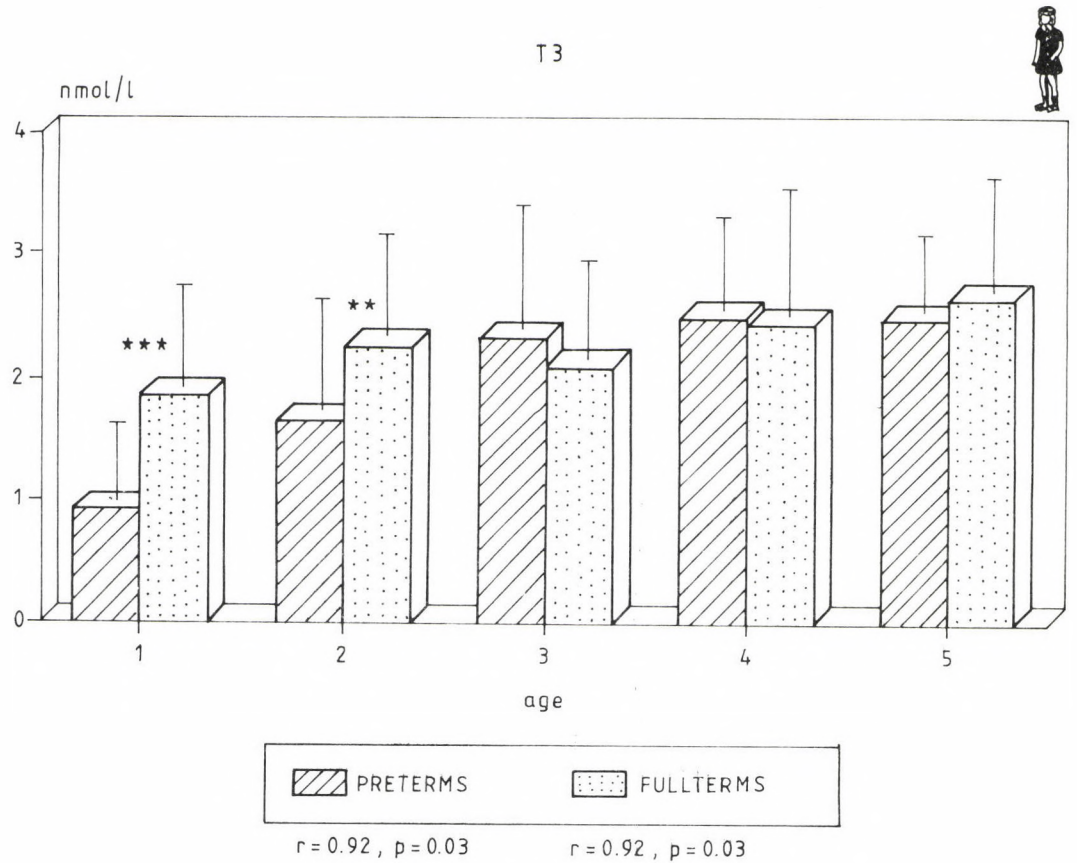

Fig. 4. Mean concentrations (+1 SD) of serum T3 in preterm and fullterm girls.

** P < 0.01, *** P < 0.001

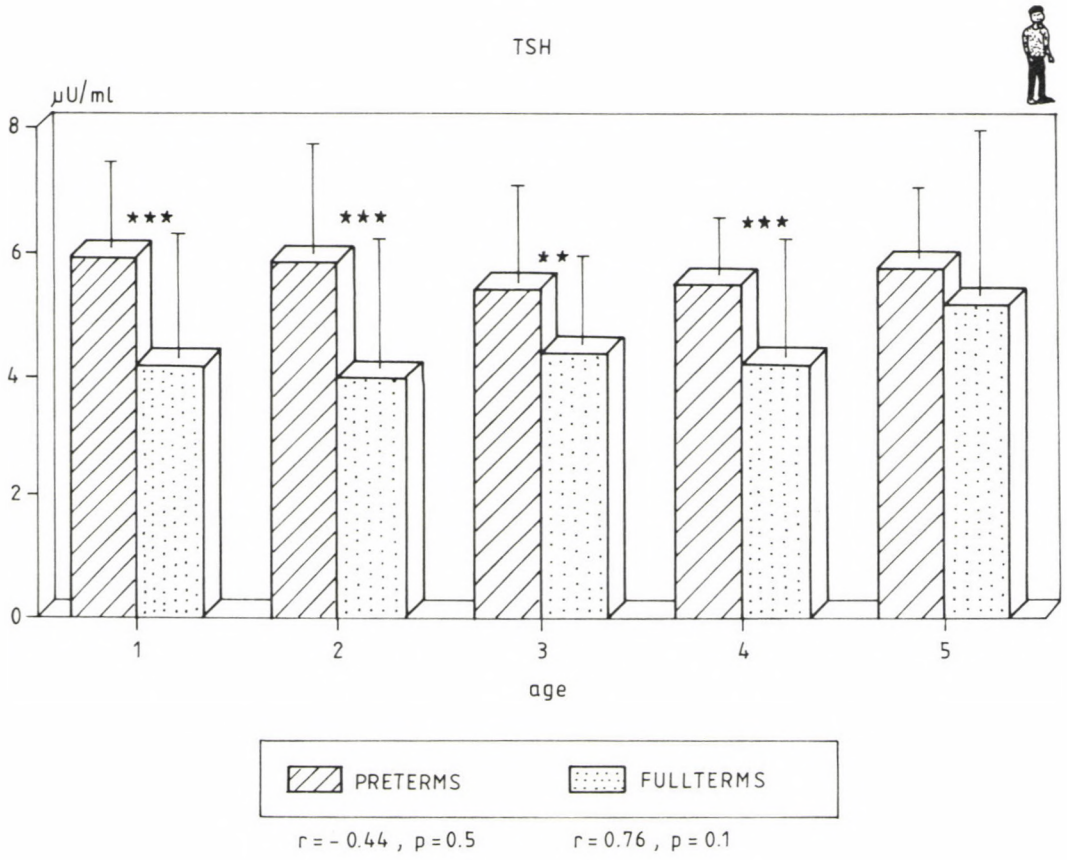

Fig. 5. Mean concentrations (+1 SD) of serum TSH in preterm and fullterm boys.

**P < 0.01, *** P < 0.001

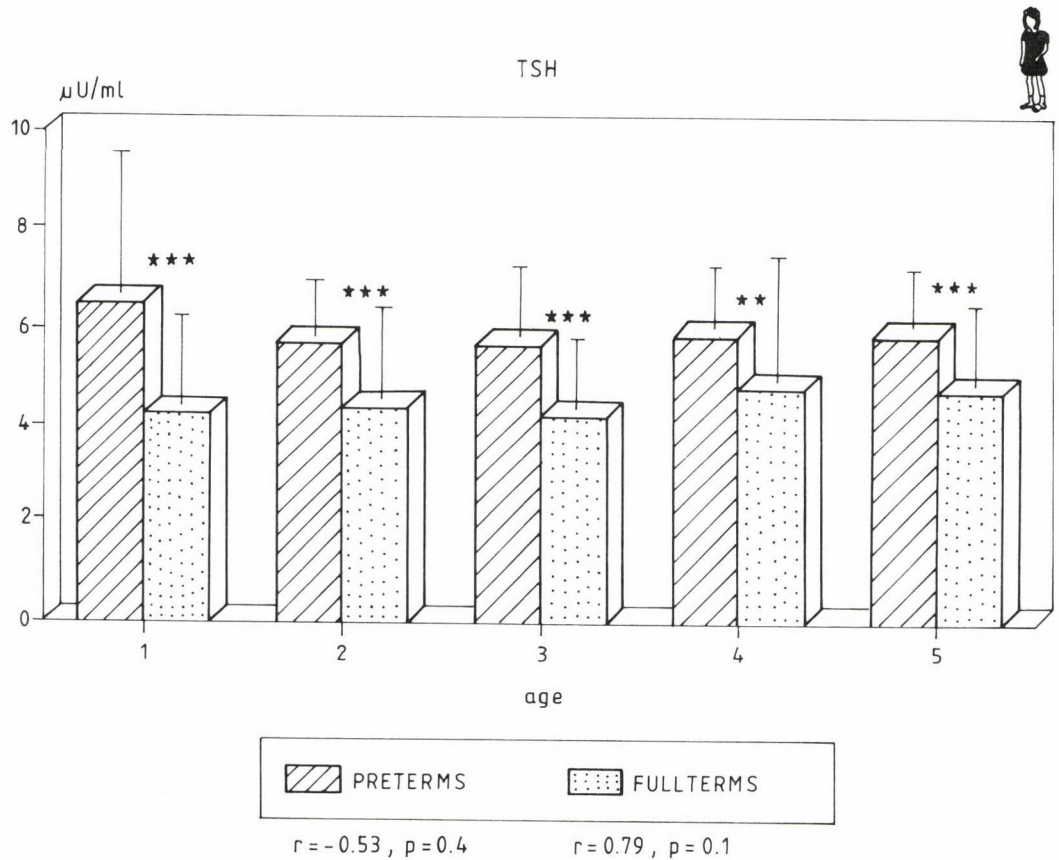

Fig. 6. Mean concentrations (+1 SD) of serum TSH in preterm and fullterm girls.
**P < 0.01, *** P < 0.001

In low birth weight infants of our region, however, no values of T4, T3 and TSH have been published yet in the first of years of life. Therefore the present study was undertaken to investigate the changes in serum levels of T4, T3 and TSH in PT children compared to FT children during the first five years of life.

Present results demonstrate pronounced changes in serum concentrations of T4 and T3 in preterm boys and girls. In PT children there were significantly lower levels of both thyroid hormones at 1 and 2 years of age, reaching the control levels in the group of 3 years. This is in agreement with the findings of earlier investigation /2/ that the third year of life is believed to play a key role in the attainment of the level of well-matured children in such parameters as the bone age. The mean T4 level was so high in this group that it markedly exceeded that of the FT boys. The mean serum T4 and T3 concentrations at the age 4 and 5 years do not appear to be markedly different from control values.

The levels of serum TSH in relation to age remained essentially constant during the studied period of childhood, what is in agreement with earlier findings /6/. These levels were significantly higher in all age groups (with the exception in 5 year old boys) in PT children compared with FT children. Thus the significantly lower levels of serum T4 and T3 in PT children at 1 and 2 years were not associated with decreasing serum TSH levels. A decrease in thyroid-TSH responsiveness would seem likely at this time. This could be due to a lower content of TSH receptors per cell or to a decrease in the thyroid follicular cell response to TSH and/or to cyclic AMP /5/. As reported /9/ the TSH response to exogenous TRH in PT infants was of the same magnitude as that observed in small for gestational age babies, and within the range of fullterms. All children studied did respond to TRH. These results indicate that in low birth weight newborns, preterm babies as well as in infants with advanced gestational age, the pituitary responsiveness to TRH is "normal". The present data of TSH values in preterm children and the results of the above-

mentioned authors support the hypothesis of the well-matured hypothalamic-pituitary system in PT infants.

According to the measurements of maximal binding capacity of TBG, some authors have suggested that the low values of T4 found in preterms may be due to low serum concentrations of TBG /6, 11/.

It has been observed that lower serum T4 concentrations in PT infants compared with those in FT infants correlate positively with birth weight and gestational age /8/ and elevate with increasing gestational age /5/.

As children with significant thyroid enlargement were excluded from the collection, we suggest that the shortened gestational age and low birth weight are largely due to the differences between healthy preterm and fullterm children.

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AN OPEN STUDY OF GAMMA-ORYZANOL AS A BATH PRODUCT FOR CHILDREN WITH ATOPIC DERMATITIS

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We determined the effects of gamma-oryzanol, a component extracted from rice bran oil, as a bath product in an open study on 20 outpatients with atopic dermatitis. In this study, a 0.5% gamma-oryzanol solution was distributed to the patients, who dissolved 20 ml of it in their bathtubs to form a medicinal bath every day. We followed the patients for 2-6 months, examining their skin symptoms every two weeks. The efficacy of this therapy in alleviating symptoms was excellent in two patients, good in six, slightly effective in twelve and ineffective in none. None of the patients experienced any negative effect from the treatment. Recurrence of initial symptoms was not detected in any patient during the therapy. This bath product containing gamma-oryzanol appears to be safe and clinically useful.

INTRODUCTION

We have previously reported on the clinical effects of rice bran broth bathing on atopic dermatitis (AD) /3/. Rice bran reportedly contains some biologically active substances such as inositol, gamma-oryzanol /9, 10, 12/ and phytic acid. In particular, gamma-oryzanol (triterpineol ester with ferulic acid) has been reported to have a protective effect on the skin /13/. In the present study, we administered gamma-oryzanol as a bath product to AD outpatients, and evaluated its effects.

Akadémiai Kiadó, Budapest

PATIENTS AND METHODS

Subjects

We performed this study from May, 1991 to February, 1992 on 20 AD outpatients (8 males and 12 females). The patients' ages ranged from 2 to 15 years (mean: 6.2 years). All of the patients had a personal and/or family history of allergic disease and satisfied the criteria for diagnosis of AD used by Hanifin and Rajka /4/.

Before the initiation of therapy, the patients were allergy tested to determine serum IgE using the radioimmuno-sorbent test, eosinophil count values in peripheral blood and specific IgE antibody using the Shionoria-specific IgE (SIST) measuring kit (Kallestad Diagnostics Inc.) /7/. The SIST score was evaluated as follows:

score < 0.35		class 0
score > 0.35	- < 0.7	class 1
score > 0.7	- < 3.5	class 2
score > 3.5	- < 17.5	class 3
score > 17.5		class 4

SIST class > 2 was considered to be positive. We measured SIST for rice, wheat, soy bean, egg white and milk as a food antigen, and SIST for mite (Dermatophagoides pteronyssinus) as an inhalant antigen.

The severity of AD was evaluated according to the criteria used by Miyakawa and Hirai /7/. The disease was considered to be severe in 20% of the patients, moderate in 60% and mild in 20%. Informed consent was obtained from the patients and their parents.

As for other supportive therapy, none of the patients had undergone dietary restriction, some had used steroid ointment, and some had received orally administered antiallergic drugs for at least one month prior to the initiation of the study. The patients were allowed to continue treatment with the drugs that had been used for AD prior to beginning this therapy. To investigate any possible steroid-sparing effect, the dosage of the steroid ointment used was recorded. AD had been present for more than one year in all patients, hence the severity and dosage of steroid ointment was evaluated according to the average condition of patients in some months prior to the initiation of the study. We observed the patients for 2-6 months (mean: 3.7 months). The patient's characteristics are presented in Table I.

TABLE I

Patient characteristics

Pt	Sex	Age (years)	Duration of AD	Severity of AD	Other oral	Medications* ointment	Research Period (months)
1	M	2	2	moderate	-	-	2
2	F	8	7	moderate	-	-	6
3	M	2	2	mild	-	F	3
4	M	2	1	mild	-	F	2
5	F	3	1	moderate	-	K	2
6	F	3	2.5	moderate	-	H	2
7	F	3	2	moderate	-	F	4
8	M	3	2	mild	A	D, F	6
9	F	3	3	moderate	A	F, J	2
10	f	4	4	severe	A	I	2
11	M	4	3	severe	A	E	2
12	F	6	5	moderate	-	D, E, H	5
13	F	6	5	moderate	-	G	2
14	F	7	2	moderate	-	E	2
15	M	8	6	mild	B		6
16	F	10	8	severe	A	H	6
17	M	11	11	severe	C	D, F	4
18	F	11	5	moderate	-	F	3
19	M	13	7	moderate	-	H	6
20	F	15	8	moderate	-	F	6

* A: ketotifen fumarate, B: azelastine hydrochloride, C: oxatomide, D: difluprednate, E: betamethasone dipropionate, F: alclometasone dipropionate, G: fluocinolone acetonide, H: hydrocortisone butyrate, I: clobetasone butyrate, J: bufexamac, K: urea.

METHODS

The bath product was a solution containing 0.5% gamma-oryzanol. The patients were advised to add 20 ml of the product to their bath water and to bathe once every day. We also instructed the patients to take a shower with fresh water after the bath, to keep using the soap and/or shampoo that they had used prior to beginning this therapy, and to stop using the bath product if their skin symptoms and itching worsened.

We examined the patients before starting the therapy and thereafter every two weeks, for evaluation of skin symptoms and steroid-sparing effect.

We assessed skin redness, lichenfication, itching, desquamation and papules. Each of these symptoms was scored according to the following scale: "4" (especially severe); "3" (distinct); "2" (slight); "1" (minimal, almost similar to healthy skin); and "0" (absent).

Evaluation of the steroid-sparing effect was based on the following criteria: "remarkable reduction" (both dosage and grade of the steroid ointment were reduced until ointment was no longer needed by the patient); "moderate reduction" (both the dosage and grade of the steroid ointment were reduced); "mild reduction" (either the dosage or grade of the steroid ointment was reduced); "no change" (neither the dosage nor grade of steroid ointment was reduced); "increase" (either the dosage or grade of steroid ointment was increased).

We evaluated the efficacy of this therapy with respect to changes in symptom score and changes in the dosage and grade of steroid ointment.

Efficiency was evaluated on the following criteria: "excellent" (skin symptoms almost disappeared without another remedy); "good" (skin symptoms improved very well only with this therapy, or with decreasing dosage of steroid ointment); "slightly effective" (skin symptoms decreased, but other therapy was required as initiation of therapy); "ineffective" (skin symptoms scarcely changed); "poor" (skin symptoms worsened).

We asked either the patients or their parents to rate their impression of this bath product according to the following scale: excellent, good, quite good, fair and poor.

In six patients (Nos 2, 15, 16, 18, 19 and 20), we examined changes in serum IgE, eosinophil count values in peripheral blood and specific IgE antibody after 3-6 months of therapy.

For the statistical evaluation, the Wilcoxon test was used. $P < 0.05$ was considered to be significant.

RESULTS

Initial allergy testing

In 18 of the 20 patients, blood tests were performed. Mean serum IgE and eosinophil count values in peripheral blood were 1002.7 IU/ml and 469/mm^3, respectively. SIST for food allergen including rice was positive in six patients. SIST for mite was positive in thirteen. SIST for any antigen was negative in seven (Fig. 1).

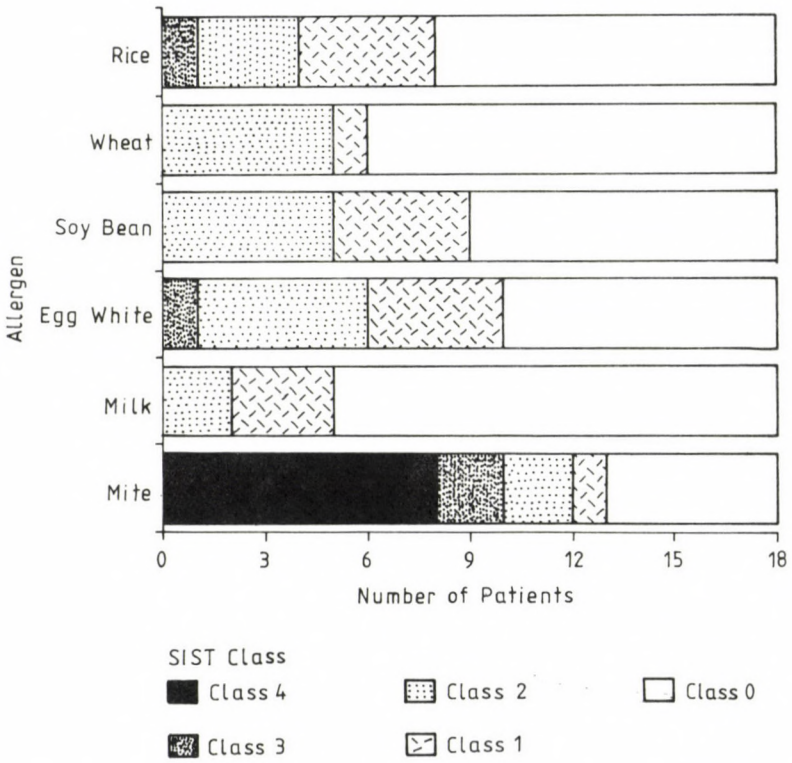

Fig. 1: Specific IgE antibody for several allergens in the patients. SIST for food allergen, including rice, was positive in six patients. SIST for mite was positive in thirteen. SIST for any antigen was negative in seven

Effects of gamma-oryzanol as a bath product

1. Change in skin symptoms

The 20 patients were followed for at least two months after the initiation of therapy. During that period the total skin symptom score decreased significantly from mean ±SD before therapy to mean ±SD at two months after therapy ($p < 0.01$). Follow-up was in no case discontinued on account of worsening of AD or other adverse reaction. Six patients were examined up to six months after therapy (Fig. 2). None of the patients experienced any tendency toward a recurrence of their initial symptoms during treatment. Some patients mentioned that their skin symptoms increased on day when they did not use the solution.

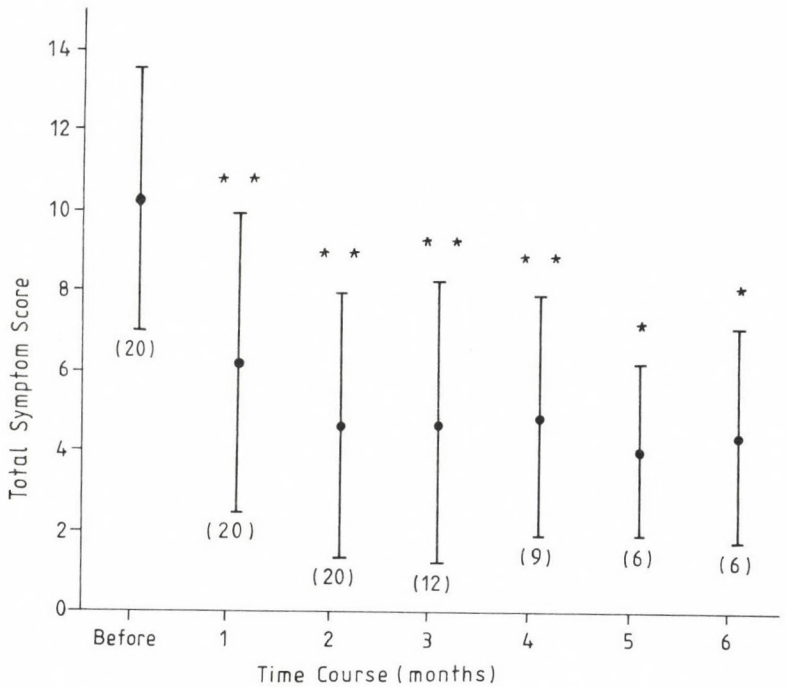

Fig. 2: Mean symptom score (±SD) during the trial (): number of patients. We evaluated skin redness, lichenfication, itching, desquamation and papules. Each symptom was rated on a scale of 0 to 4.
*: $p < 0.05$, **: $p < 0.01$ vs before treatment.

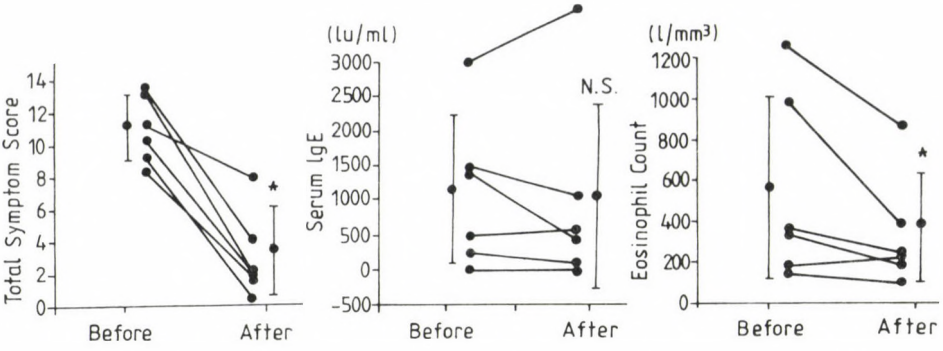

Fig. 3: Changes in total symptom score, serum IgE and eosinophil count in peripheral blood. Before: at the onset of therapy; after: 3-6 months after the initiation of therapy. (n=6)
*: $P < 0.05$, N.S.: not significant vs before treatment (mean±SD)

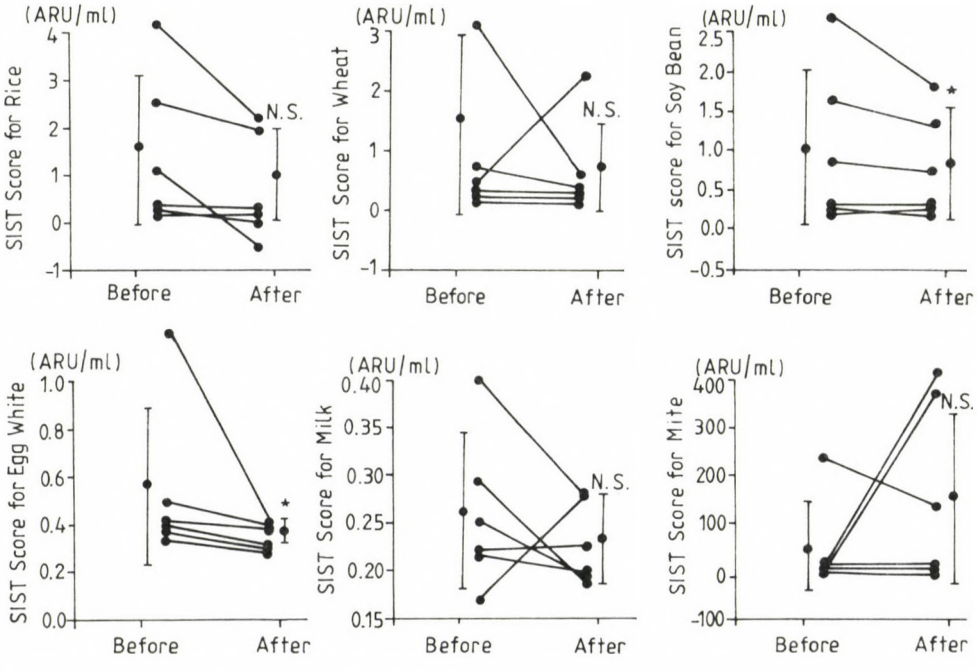

Fig. 4: Changes in SIST score for specific IgE antibody. Before: at the onset of therapy; after: 3-6 months after the initiation of therapy. (n=6)
*: $P < 0.05$, N.S.: not significant vs before treatment (mean±SD)

2. Steroid-sparing effect

The dosage and grade of steroid ointment was reduced remarkably in three patients (15%), moderately in three (15%) and mildly in four (20%). In one patient (5%), the dosage of steroid ointment was increased.

3. Judgement of efficacy

Therapy was considered to be excellent in two patients (10%), good in six (30%), slightly effective in twelve (60%), and ineffective or poor in none.

4. Impressions of the patients or their parents

This bath product was felt to be excellent for AD in one patient (5%), good in ten (50%), quite good in six (30%), fair in three (15%) and poor in none.

5. Changes in serum IgE, eosinophil count in peripheral blood and specific IgE antibody.

The six patients in whom repeated allergy tests were made all showed considerable improvement in skin symptoms (Fig. 3). Serum IgE levels did not change significantly after therapy but the eosinophil count in peripheral blood decreased significantly. The SIST score for rice decreased in four patients, did not change in one and slightly increased in one. The SIST score for food antigens other than rice did not increase significantly (Fig. 4). The SIST score for mite increased remarkably in two patients, both of whom were eight years old.

DISCUSSION

We previously reported rice bran broth bathing to be safe and clinically useful for AD, and determined by ultraviolet spectroscopy that gamma-oryzanol can be extracted from rice bran broth. On the skin, gamma-oryzanol has been reported to have the following characteristics /12/. Peripheral blood flow is increased as a result of direct action of gamma-oryzanol on the skin. The topical application of gamma-oryzanol induces an increase in sebaceous secretion, and gamma-oryzanol has a

strong affinity to the skin, covering the skin closely and having a suppressive effect on increases in keratin. We thought it probable that gamma-oryzanol is one of the components effective for AD in rice bran broth. In this study, we investigated the clinical effects of gamma-oryzanol in outpatients with AD.

In our previous study, rice bran broth bathing therapy for AD /3/, we used 50 g of rice bran to prepare a rice bran broth that patients dissolved in the bathtub at a time. Rice bran contains 9-22% rice bran oil. Rice bran oil contains about 1.3-3% ferulic acid esters, including gamma-oryzanol. 50 g of rice bran therefore contains roughly 200 mg of gamma-oryzanol. The quantity of gamma-oryzanol that patients dissolved in the bathtub at a time for this study was about 100 mg.

The clinical effects of gamma-oryzanol as a bath product were considered to be satisfactory and could easily be observed by the change in symptom score. The skin symptoms of some patients worsened after discontinuation of this therapy, and some patients mentioned that their skin symptoms increased on days when they did not use it. These observations suggest that regular medicated bathing therapy is necessary to keel AD in remission. None of the patients experienced a recurrence of the initial symptoms during treatment. Neither the patients nor their parents felt that the smell of this bath product was unpleasant, and their impression of the therapy was favorable.

On the other hand, we should be aware of a possible problem associated with bath products containing gamma-oryzanol: the hypersensitive reaction of the skin of patients with a rice bran allergy. Before this study, we ran a patch test of this bath product on healthy subjects without skin disease and on AD patients, and confirmed it to be safe (data not shown). In this study, specific IgE antibody for rice was positive in some patients. However none of them showed any increase in skin symptoms.

The effects of therapy on serum IgE and eosinophil count in peripheral blood were the same as reported by others /1, 5, 6, 8, 11/ and by ourselves for rice bran broth bathing /3/. In

patients whose skin symptoms improved during therapy, serum IgE levels did not change and the eosinophil count in peripheral blood decreased significantly. The eosinophil blood is considered useful for evaluation of AD activity. Changes in specific IgE antibody did not suggest any increase in sensitivity to food allergens, including rice. In two patients the SIST score for mite increased remarkably after therapy. In order to determine whether the increase of the SIST mite score is a result of the therapy, it would be necessary to increase the number of patients in the study.

In comparing this bath product with rice bran broth bathing, our impression is that the rice bran broth is better for AD. Some patients used both bath products and rice bran broth at different times, and all of them answered that the rice bran broth was better for their skin symptoms. We can imagine two reasons for this conclusion.Firsthy that the concentration of gamma-oryzanol in the bath product, half that of the rice bran broth, was low. The other possible reason is that other components in rice bran, such as lipids, inositol and phytic acid, may also be effective for AD.

The bath product that we used in this study contained emulsifier and preservative, though the amounts were small. A possible effect of the emulsifier and preservative has to be considered. Skin symptoms of AD change easily in natural course. We have to take psychological placebo effect of bath product into consideration. There is room for subjective judgement in scoring of skin symptoms. The implementation of a larger, a controlled study, in which patients are allocated randomly to active or control therapy, is desirable.

Bath products have two distinct advantages. The patient's compliance with the therapy is good, especially in children because application does not require an inordinate amount of time, nor does it require that the child be held. Further, it is easy to treat the entire body. We believe that these advantages make this bath product containing gamma-oryzanol a useful adjuvant therapy for AD patients.

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URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY IN DIABETIC CHILDREN

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As a consequence of diabetes mellitus (type I) diabetic nephropathy may arise. In order to observe the tubular function, urinary N-acetyl-beta-D-glucosaminidase (NAG) activities were measured using PNP-GlcNAc and VRA-GlcNAc substrates in the case of 52 diabetic children (mean age ± SD: 11.7 ± 3.4 years, 26 males and 26 females).

At the initial stage of diabetes (from 2 weeks to 1 year) in group I, the serum fructosamine level was normal (3.29 ± 0.71 mmol/l), the urinary VRA index (0.91 ± 0.4 μmol/min/mmol) and the urinary PNP index (1.26 ± 0.2 μmol/min/mmol) showed nearly normal tubular function.

In the well-controlled diabetic group II, the serum fructosamine was normal (2.77 ± 0.66 mmol/l), the urinary VRA index (0.82 ± 0.48 μmol/min/mmol) and the urinary PNP index (0.86 ± 0.66 μmol/min/mmol) were close to normal even after a long duration of diabetes (1-12 years).

In the labile diabetic group III, serum fructosamine was elevated (4.59 ± 0.94 mmol/l), the urinary VRA index (2.37 ± 1.25 μmol/min/mmol) and the urinary PNP index (3.30 ± 2.14 μmol/min/mmol) were also higher than the normal levels after the similar duration of diabetes (1-15 years).

INTRODUCTION

Diabetic nephropathy may affect as many as 40% of those patients suffering from insulin-dependent diabetes mellitus (IDDM). Increased urinary excretion of albumin /3/, transferrin /4/, alfa-1-microglobin /5/, alanine aminopeptidase (AAP), gamma-glutamyltransferase (GGT) /6/ and N-acetyl-beta-D-glucos-

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aminidase (NAG) /7/ are specific parameters of the manifestation of diabetic nephropathy. In our studies we have also found that measurement of the NAG-ase activity was a simple, accessible, reliable and sensitive method for diagnosis of tubular damage. The NAG assay provides an early indication of tubular dysfunction resulting from renal disease or nephrotoxic damage /18/. False positives are rare and NAG-ase activity remains high during disease or a toxic attack but falls to normal levels after detoxication and after recovery. Urinary NAG-ase activity can be used in conjunction with other tests to follow disease activity and to make prognosis. High NAG-ase activity may be an early warning sign to the clinician before the onset of complications resulting from diabetes (e.g. high arterial pressure, retinopathy and nephropathy).

In order to observe tubular function, in the case of 52 diabetic children and our two patients being now adults (patients B and C) urinary NAG-ase activity was measured using PNP-GlcNAc and VRA-GlcNAc substrates. NAG-ase activity was related to urinary creatinine, which was determined by the Jaffe's reaction /11/. The result was given as NAG index (NAGi).

PATIENTS AND METHODS

52 diabetic patients were studied: 26 boys and 26 girls. The age of children: 11.7 ± 3.4 years (mean ± SD). The patients were treated with MC or Human Novo Insulin. They were divided into three groups (Table I):

Group I (initial stage of diabetes) consisted of 6 patients (4-13 years old children), whose diabetes had been known for between only 2 weeks - 1 year, their serum fructosamine level was below 3.5 mmol/l.

Group II (well-controlled diabetes): 13 diabetic patients (5-17 years old children), who had been diabetic for 1-12 years; their serum fructosamine level was below 3.5 mmol/l (2.77 ± 0.66 mmol/l).

Group III (labile diabetes): 33 patients (5-17 years old children), who had been diabetic for 1-15 years; their serum fructosamine level above 3.5 mmol/l (4.59 ± 0.94 mmol/l).

The upper limit (mean value + 2SD) of the normal NAG index range was established by measuring 104 healthy children (3-14 years old). It was found that the normal range of the NAG

Table I

Patient groups investigated.
Groups were formed on the basis of serum fructosamine (FA) levels and the duration of diabetes

	Diabetes	n	Se. FA (mmol/l)	Age (years)	Duration of diabetes
I	Initial stage	6	< 3.5	4-13	2 weeks - 1 year
II	Well-controlled	13	< 3.5	5-17	1-12 years
III	Labile	33	> 3.5	5-17	1-15 years

n: number of patients

index is decreasing with the age /6, 12/. It will be detailed in another study /13/.

a. The urinary PNP indices were determined by a modification of the method of Horak et al. /15/ using p-nitrophenyl N-acetyl-beta-D-glucosaminide (PNP-GlcNAc) substrate. Samples were centrifugated at 1000 g for 5 min. 1 ml aliquotes were gelfiltrated on a 8x1.5 cm fine mesh Sephadex G-25 column (Pharmacia AB, Uppsala, Sweden) prior to carrying out the assay. The yellow colour was read at 400 nm with a Specord M-40 (Zeiss, Jena) spectrophotometer.

b. The urinary VRA indices were determined by the method of Pócsi et al. /10/ using VRA-GlcNAc substrates, without urinary gelfiltration. The absorbances were read at 505 nm, VRA-GlcNAc-ase activity was calculated directly via the specific molar absorptivity /8/.

c. The urinary creatinine concentration was determined by Jaffe's method /11/ using a Master clinical chemistry analyser (A. Menarini).

d. Serum fructosamine was measured by colorimetric method using fruttosammina HF kit (A. Menarini) on the same analyser.

RESULTS

Similar to other authors /14/ we have found that elevated NAG indices in IDDM are associated with a "poor" long-term glycemic control. This experience could be supported by our three patients (Fig. 1):

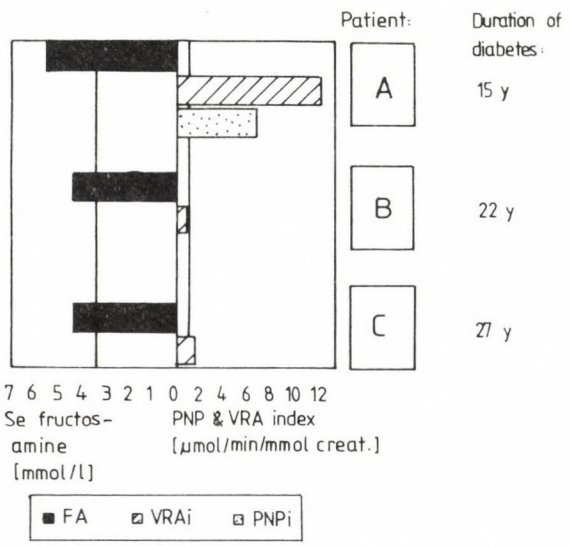

Fig. 1. Serum fructosamine levels and urinary NAG-ase activity in diabetic patients after a long period of IDDM

In the case of Patient A (a 16 year old boy) after 15 years duration of uncompensated diabetes, serious ketoacidosis was observed several times because of a lack of home family control. According to his elevated fructosamine level (3.5-5.5 mmol/l) his NAG indices were high: PNPi: 3.8-6.7 and VRAi: 2.6-12.0 µmol/min/mmol, showing serious tubular damage.

Patient B was a 25 year old male, suffering from IDDM for 22 years, but his VRAi was close to normal (0.81 µmol/min/mmol).

Patient C was a 32 year old male, suffering from IDDM for 27 years. His VRAi was only slightly elevated (1.45 µmol/min/mmol). The favourable NAG indices recorded for

patients B and C illustrated their well-controlled metabolic condition during this period of IDDM (Fig.1).

After these observations three different groups of diabetic children were investigated: (Fig. 2 a, b, c).

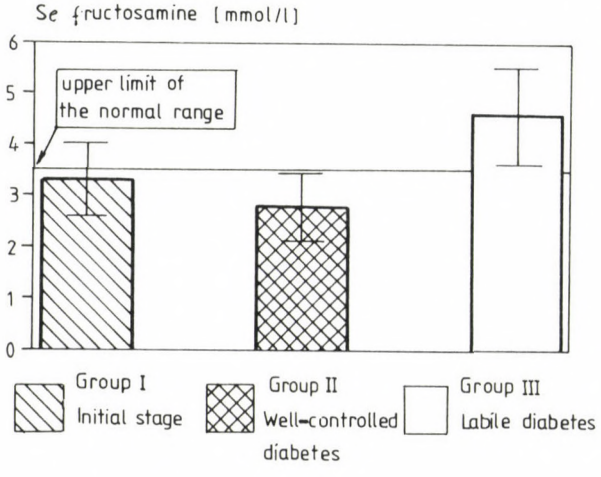

Fig.2 a. Serum fructosamine levels (mean ± SD) in diabetic children

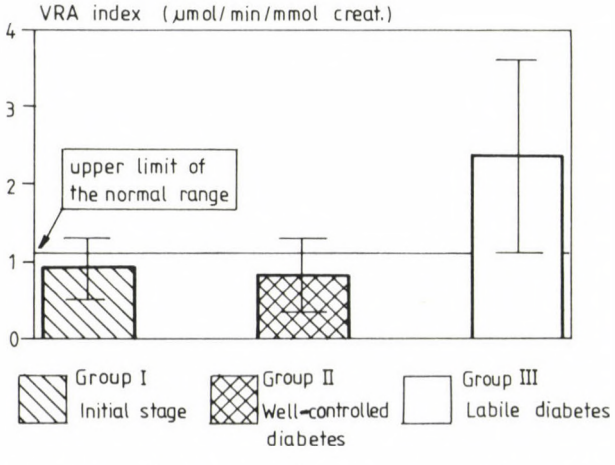

Fig.2 b. VRA index (mean ± SD): urinary NAG-ase activity with VRA substrate/urinary creatinine in diabetic children

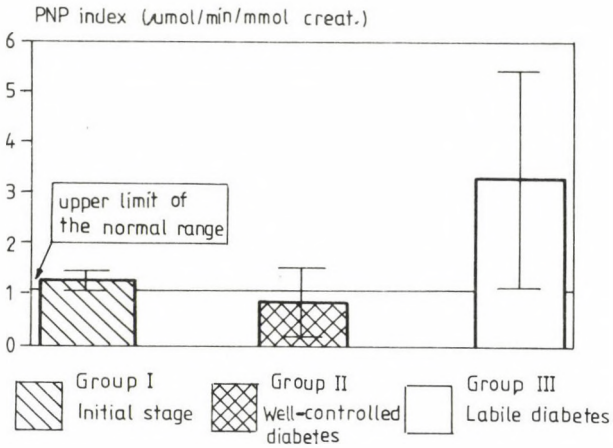

Fig.2 c. PNP index (mean ± SD): urinary NAG-ase activity with PNP substrate/urinary creatinine in diabetic children

In group I the newly diagnosed diabetic patients had only a slightly elevated PNP index (1.26 ± 0.2 µmol/min/mmol), and a normal VRA index (0.91 ± 0.4 µmol/min/mmol) showing normal tubular function in spite of their instable bloodsugar level. In this initial stage (2 weeks-1 year) their fructosamine level in the serum was normal: 3.29 ± 0.71 mmol/l (Fig. 2).

In group II the well-controlled diabetic patients' tubular function was normal after a long duration of diabetes (1-12 years): serum fructosamine 2.77 ± 0.66 mmol/l, urinary VRA index 0.82 ± 0.48 µmol/min/mmol and PNP index 0.86 ± 0.66 µmol/min/mmol were also normal.

In group III the labile diabetic patients' serum fructosamine level was elevated (4.59 ± 0.94 mmol/l). In accordance with this, the urinary VRA index (2.37 ± 1.25 µmol/min/mmol) and the PNP index (3.30 ± 2.14 µmol/min/mmol) were also higher. Comparing group II and group III whose duration of diabetes was similar (1-12 and 1-15 years), only the labile diabetic group III showed considerable tubular damage (Fig. 2).

DISCUSSION

Diabetes mellitus is a significant cause of morbidity in childhood. During this period of life, the type I diabetes is the most significant form of the disease. Micro- and macroangiopathy can be observed during the follow-up studies of type I. diabetes mellitus. Our purpose was to confirm these previous observations in the treatment of our patients. We were looking for a specific method to diagnose kidney damage at an early stage, before the appearance of glomerular damage or other microangiopathy as retinopathy. As our patients had no albuminuria, kidney damage, represented by high NAG-ase activities, proved to te tubular in all cases.

At the initial stage of diabetes mellitus (2 weeks-1 year) in the group I according to the normal fructosamine level, urinary NAG indices were normal. Although the urinary NAG index is reported as a sensitive parameter of early diabetic nephropathy /21/, no tubular damage could be detected in this early period of IDDM. From those patients who had a long duration of IDDM we could distinguish two groups on the basis of their serum fructosamine level:

In the well-controlled diabetic group II serum fructosamine level was below 3.5 mmol/l and NAG indices showed normal tubular function.

In group III diabetes was labile, the serum fructosamine level was higher than 3.5 mmol/l and the high values of the PNP and VRA indices indicated tubular damage.

Similar to our observations, several studies demonstrated that NAG activity may be elevated in the urine of diabetic patients /16, 17/. Agardh et al. /17/ found a positive correlation between metabolic control (HbA_1c serum level) and NAG activity. Watts et al. /14/ also showed significantly elevated NAG indices in diabetic patients. The elevation was considerably higher in those diabetics with "poor" compared to "good" glycemic control. They confirmed the significant correlation between log (urine NAG /urine creatinine) and HbAlc and log (urine albumin/urine creatinine).

The exact mechanism of tubular damage is not known yet. It is possible that the osmotic stress caused by glucosuria and protein glycosylation is responsible for tubulopathy. On the other hand, as Miltényi et al. reported /22/, the consequence of uncompensated metabolic state frequently leads to ketoacidosis contributing to this condition. We can agree with Morita el al. /19/ in suggesting, that NAG reflects lysosomal dysfunction of both glomerular and proximal tubular epithelial cells, caused by poor glycemic control. Our data confirm this hypothesis, because the patients in group III having a high serum fructosamine level showed tubular damage. These results seem to support the benefit of measuring NAG-ase activity to check tubular function.

In the latest clinical evaluation of NAG-ase /16/ among the different indicators of renal tubular damage (such as gamma glutamyltranspeptidase, albumin, total protein, retinol binding protein, beta-2-microglobulin), the NAG index proved to be very useful predictor of diabetic nephropathy. As Gibb et al. confirmed the significant correlations between urinary NAG-ase /creatinine and urinary albumin/ creatinine, as well as urinary NAG-ase/ creatinin and HbA1C show strong correlation in diabetic children /21/. These data emphasize the possibility, that tubular damage can be prevented in the case of IDDM by careful medical control, diet and appropriate life style.

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CONGENITAL ANOMALIES: CAUSE OF MORTALITY AND WORKLOAD IN A NEONATAL INTENSIVE CARE UNIT

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Analysis of statistics of our Neonatal Intensive Care Unit (NICU) from the years 1980, 1985 and 1990 revealed a relatively constant number of referred newborn infants with congenital disorders. The contribution of malformations to the total workload of our NICU did not significantly change during the decade evaluated; at the same time, a drop in lethal cases related to congenital anomalies was observed. These tendencies reflect more strict selection criteria of the referring small hospitals due to their improving technical and professional means, but are not characteristic of the epidemiology of congenital disorders in our region.

INTRODUCTION

Due to the rapid development of medical science and techniques in the fields of obstetrics and neonatology, the survival rate of mature and immature neonates admitted to the neonatal intensive care units (NICU) is steadily improving /3, 4/.

As a consequence, infants who would have died shortly after birth because of congenital anomalies are becoming a considerable part of the workload of a NICU, demanding more sophisticated and costly management, diagnostical methods (genetic, biochemical, etc.) and treatment (e.g., surgical interventions) /4/. We were interested in studying the records of our NICU to see whether there was any significant increase in congenital anomalies during a ten-year period and whether they had a significant influence on our survival rates. We were further interested in looking for the most frequent anomalies and their influence on the workload and mortality rate of our NICU.

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PATIENTS AND METHODS

The ten-year period between 1980 and 1990 was chosen, and the collected data of the years 1980, 1985 and 1990 were screened for neonates affected with one of the three main diagnostic categories, namely congenital malformations, chromosomal anomalies and metabolic disorders.

Such criteria as birthweight and gestational age were not taken into consideration; all babies admitted from below 1.000 g up to 5.000 g were searched for one of the above-mentioned main diagnostic categories.

Infants affected with congenital malformations were divided into one group with a single and a second one with multiple malformations. "Single" was defined as only one organ being affected with one or more defects (as e.g. in Fallott's tetralogy), whereas "multiple" was defined as at least two or more organs being affected (e.g. atrial septal defect and duodenal atresia).

We also divided our patients according to their sex to see whether one of the sexes showed a greater tendency of having congenital anomalies.

After making a general assessment, we specified the malformations according to organs or organsystems. Cases with multiple malformations had to be classified according to the vitally more important organ; thus, a case with an affection of the cardiovascular system and the gastro-intestinal tract was listed with the former organsystem. This, of course, was done according to our own judgement and cannot claim complete accuracy.

The lethal cases were finally summarized and put in relation to the annual overall mortality rate, and the significance of each organ/organsystem was evaluated.

RESULTS

Table I clearly demonstrates that the amount of cases with congenital anomalies did not considerably change during this ten-year period. However, the relative significance of congenital anomalies increased due to the gradual reduction of admissions, which is reflected in Table I.

As expected, congenital malformations strikingly dominated our other two diagnostic categories. As shown in Table I, chromosomal anomalies and metabolic disorders did not significantly contribute to the workload of our NICU and did not essentially change in frequency during this period.

Table II further shows that the major part of our patients

TABLE I

Numeric distribution of congenital anomalies in relation to the annual admissions

		Total number of patients	Malformations			Chromosomal disorders	Metabolic disorders
			Total	Single	Multiple		
1980	Girls	180	37	27	10	4	5
	Boys	243	49	35	14	2	1
	Total	432	86	62	24	6	6
1985	Girls	187	25	18	7	2	2
	Boys	206	43	30	13	2	0
	Total	393	68	48	20	4	2
1990	Girls	151	36	29	7	4	0
	Boys	213	42	36	6	3	3
	Total	364	78	65	13	7	3

TABLE II

Classification of malformations according to organs and organ systems

		Nervous system		Cardio-vascular		Respiratory system		Gastro-intestinal		Urogenital system		Bone and muscles		Skin		Others	
		S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.
	Girls	1	1	13	0	0	3	9	0	1	2	2	3	0	0	1	0
1980	Boys	5	2	6	2	0	0	12	5	5	1	2	4	0	0	5	1
	Total	6	3	19	2	0	3	22	5	6	3	4	7	0	0	6	1
	Girls	0	0	12	3	0	0	3	3	1	0	2	1	0	0	0	0
1985	Boys	0	0	15	2	1	1	7	3	5	4	1	2	0	1	1	0
	Total	0	0	27	5	1	1	10	6	6	4	3	3	0	1	1	0
	Girls	3	1	18	1	0	1	4	3	1	0	1	1	0	0	2	0
1990	Boys	0	1	18	2	0	0	9	2	6	1	2	0	0	0	1	0
	Total	3	2	36	3	0	1	13	5	7	1	3	1	0	0	3	0

S: Single malformation
M: Multiple malformations

TABLE III

Distribution of lethalities according to organs and organ systems in relation to the annual overall mortality (%)

		Total lethalities		Malformations		Nervous system		Cardio vascular		Respir. system		Gastro-intestinal		Urogenital system		Bone and muscles		Skin		Others	
		n	%	n	%	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.
1980	Girls	39	21.6	8	20.5	0.0	2.5	2.5	5.1	0.0	5.1	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Boys	58	23.9	15	25.8	3.4	1.7	3.4	3.4	0.0	0.0	8.6	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total	97	22.9	33	34.0	2.0	2.0	3.1	4.1	0.0	2.0	7.2	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1985	Girls	31	16.6	11	35.5	0.0	0.0	19.3	6.4	0.0	0.0	3.2	3.2	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0
	Boys	51	24.7	15	29.4	0.0	0.0	15.6	5.8	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0
	Total	92	20.9	26	31.7	0.0	0.0	17.0	6.0	0.0	0.0	1.2	1.2	0.0	1.2	0.0	1.2	0.0	0.0	0.0	0.0
1990	Girl	25	16.5	3	12.0	0.0	0.0	8.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Boys	42	19.7	3	7.1	0.0	0.0	2.9	0.0	0.0	0.0	2.3	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0
	Total	67	18.4	6	8.9	0.0	0.0	4.5	0.0	0.0	0.0	2.9	0 0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0

S: Single malformation
M: Multiple malformations

was affected with malformations of the cardiovascular system and the gastrointestinal system. Cases with single malformations dominated the multiple ones. The cardiovascular affections showed a striking increase, almost doubling in number from 1980 to 1990. As to the other organs/organsystems, we could not observe any significance concerning their frequency and their contribution to the total workload of our NICU. Another interesting feature of our findings was to observe the relative drop of lethal cases related to congenital malformations (Table III). In 1980 neonates with malformations still made up 34% of the total death rate; in 1990, they made up only 6%.

Table III demonstrates again that the affections of the cardiovascular system had the major share in the overall death rate. As one can see, they reached their peak at 23% in 1985 and in 1990 dropped back almost to the same number as in 1980.

As far as the sexes were concerned, we could not really find significant differences between male and female; they seemed to be almost equally affected by congenital anomalies, though totally considered (Table I), evidently less girls and more boys were admitted to our NICU.

Congenital tumours were not observed. The altogether 17 cases of chromosomal disorders were reevaluated. Autosomal trisomies occurred in 14 infants and Ullrich-Turner syndrome in 3 neonates, but no structural abnormalities and centromeric anomalies were seen in this material.

DISCUSSION

According to general trends, the incidence of congenital anomalies is rising /3, 4/. Interestingly enough, this trend could not be observed as far as our NICU was concerned. The frequency of admissions of neonates affected with congenital anomalies did not significantly change. The pre- and postnatal

diagnostical possibilities which improved during the ten-year period obviously helped to specify congenital anomalies more precisely but still did not alter the number of admissions. However, the relative significance of congenital anomalies for the workload of our NICU is changing, due to the gradual reduction of total admissions; there is obviously a shift of the workload of our NICU, which increasingly has to deal with the more complicated cases, leaving the less severe ones to the smaller hospitals of our region, whose technical means are steadily improving, a development which matches similar reports on this subject /4/.

Congenital anomalies had a major share in the overall death rate back in 1980. But contrary to other reports /4/ they are gradually losing their impact on the overall mortality. Improved diagnostic means and advanced surgical techniques are essential factors in reducing our lethal cases. At the same time, malformations of the cardiovascular system are relatively gaining importance, although the number of lethal cases decreased considerably, as mentioned before. Of course, we are aware that the figures in Table III have to be regarded critically, knowing that we have probably missed some cardiovascular cases in 1980 because of the lack of an adequate sonographic apparatus. Nowadays though, ultrasound screening of our babies is part of our daily routine. Our findings confirm a similar report on this subject by Fischer et al. /2/ pointing out the increasing incidence of congenital cardiovascular malformations, although they observed at the same time a rise of their mortality. We presume that our drop in lethal cases can be ascribed to our advanced diagnostical methods and surgical techniques, whereas most of our losses are due to already nonpreventable cases, such as newborns with extreme low birthweight.

Although our study shows that the contribution of neonates with congenital anomalies to the overall death rate of our NICU is decreasing, the relative significance is following similar trends in other countries, demanding more sophisticated and costly management, diagnostical methods, and treatment.

The present data are subject to changes in local circumstances but do not represent the national trends in epidemiology of congenital anomalies /1/.

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LACTIC ACIDOSES AND HYPERPYRUVATAEMIAS

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The primary lactic acidosis, primary hyperpyruvataemia (Leigh's) and secondary lactic acidosis and/or hyperpyruvataemia cases have been classified by retrospective analysis. 8 patients were detected with primary lactic acidosis (4 of them were neonatal types, 3 infantile, 1 chronic one). 6 children suffering from primary hyperpyruvatemia subacute necrotising encephalomyelopathy (Leigh's) were diagnosed. Secondary lactic acidosis and/or hyperpyruvataemia were noticed in 22 patients (with aminoacidopathies, metabolic myopathies, etc).

INTRODUCTION

Lactic acidemias can be resulted from primary defects in the metabolism of pyruvate or from secondary disorders that affect the metabolism of pyruvate and lactate.

Pyruvate is metabolized to acetyl-CoA by a multi-enzyme pyruvate dehydrogenase complex (PDHC), or it can be metabolized through the glyconeogenic pathway to oxaloacetate by a biotin-dependent carboxylase (BDC). The latter defect may be a single or multiple carbooxylase deficiency. PDHC consists of a. Catalytic enzymes: 1. Pyruvate decarboxylase = E1, 2. Dihydrolipoyl transacetylase = E2, 3. Lipoamide dehydrogenase =

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E3 b. Regulatory enzymes: 1. Pyruvate dehydrogenase phosphatase 2. Pyruvate kinase; Carboxylases: 1. Pyruvate carboxylase.

2. Multiple carboxylase deficiency; Disorders of pyruvate with enzyme defect: 1. Mitochondrial myopathies, 2. Leigh's subacute encephalopathy. /1, 9/

Clinical features are cerebellar ataxia, hereditary spinocerebellar degenerative condition, acidosis, hypotonia, seizures and retarded development. Fasting hypoglycaemia can occur, too. The neonatal type of lactic acidosis is associated with progressive neurological damage with intermittent lethargy, irritability and seizures elevated blood lactate and pyruvate concentrations, severe tachypnoe and hypotonia. The disorders of the PDHC and the tricarboxylic cycle are mostly rapidly progressive and fatal.

Deficient activity of muscle cytochrome-c oxidase has also been reported in a patient with a mitochondrial myopathy asssociated with chronic lactic acidemia growth failure and nerve deafness /3/ and in a patient with subacute necrotizing encophalomyopathy (Leigh's disease) /14/.

The primary lactic acidosis, primary hyperpyruvataemia (Leigh), secondary lactic acidemia and/or hyperpyruvataemia cases have been classified by retrospective analysis in this study.

MATERIAL AND METHODS

Blood and cerebrospinala fluid (CSF) lactate and pyruvate levels were investigated by the method of Gloster and Harris /7/ modified by Eck and Boda /4/. Levels of amino acids (semi-quantitative and/or quantitative analyser), blood ammonia, capillary acid basis parameters and organic acids were also analysed.

RESULTS

8 patients were detected to have primary lactic acidosis: 4 of them were neonatal types, 3 infantile and 1 chronic one.

Almost all of the neonatal type patients died during the neonatal periode. Only 1 patient (3th case) died at 9 months of age. 6 patients were diagnosed with primary hyperpyruvataemia (Leigh), 4 with infantile and 2 with chronic types (Table I).

TABLE I

Type distribution of the lactic acidosis

Type	Lactic acidosis n	Hyperpyruvatemia n	Secondary lactic acidosis/hyper-pyruvatemia n
Neonatal	4	-	-
Intantile	3	4	13
Chronic	1	2	9

Secondary lactic acidosis and/or hyperpyruvataemia occurred in 22 cases with aminoaocidopathy, metabolic myopathies etc. Laboratory data and genetical types of lactic acidooses are seen in Table II.

DISCUSSION

In the first group: 3 of the 4 neonatal type primary lactic acidotic newborns died in the early neonatal stage within the first 24 hours or until the 3rd week of life, 1 case died at 9 months of age. In the 1st and 4th cases, periventricular leukomalacia has been clarified by the autopsy, with or without gliosis and capillary proliferation and with active lipid phagocytic histiocytes.

TABLE II

Laboratory and genetical data of lactic acidoses and hyperpyruvatemia

Name	Sex	Diagnosis	Date		Lactate mmol/l	Pyruvate µmol/l	Type
Primary lactio acidosis n=8							
1. Márta R. 03.02.1989	f	lactic acidosis	04.02.1989.	blood	9.5	388	+ neontalal
2. Anita M. 1st day	f	lactic acidosis	11.09.1990.	blood	26.6	127	+ neonatal
3. Szimenetta E.[X] 9th month-age	f	lactic acidosis encephalopathy	13.04.1990.	blood	4.1	399	+ neonatal
4. Andre G. 1st day	m	lactic acidosis sucoinylaciduria argininuria	1990.	blood	6.0	-	+ neonatal
5. Ágnes Jónás 2305.84	f	lactic acidosis	1987.	blood	4.1	253	+ infantile
				CSF	8.2	316	4 y
6. Andrea Cs. 3y.	f	lactic acidosis encephalo-myopathy	17.10.1989.	blood	4.2	439	infantile
7. Ervin F. 3 y.	m	lactic acidosis	1990.	blood	3.7	228	infantile
8. Károly Sz. 4 y.	m	lactic acidosis	1990.	blood	3.6	263	chronic

Name	Sex	Diagnosis	Date		Lactate mmol/l	Pyruvate µmol/l	Type
Primary hyperpyruvatemia n= 6							
1. Ákos sz.	m	hyperpyruvatemia	1989.	blood	0.52	327	infantile
12.09.87		(Leigh)	1990.	blood	2.8	125	
2. Ákos J.	m	Leigh	09.04.1990.	blood	0.59	203	infantile
13.08.86.			26.06.1990.	blood	0.51	338	
3. Ádám H.	m	Leigh	22.03.1990.	blood	2.8	148	+ infantile
14.12.89.				CSF	0.76	342	
4. Zsuzsa K.	f	Leigh	25.12.1985.	blood	1.83	136	infantile
26.12.83.		autism		CSF	0.62	179	
5. Péter K.	m	Leigh	1990.	blood	1.9	219	+ chronic
25.05.81.				CSF	0.92	68	9 y.
6. Alexandra E.	f	hyperpyruvatemia	1990.	blood	2.09	203	chronic
05.08.89.							

x Dg.: familial cytochrome-c-oxidase (COX) deficiency Ádám E., brother of Sz.E. died of COX deficiency

The most severe clinical leading symptoms were muscular hypotonia, dysphagia, metabolic encephalopathy. Ptosis occurred in only 1 cases. All of them required respiratory ventillation for different durations.

Epileptic cramps did not occur in the nonatal type of primary lactic acidotic patients. 3 of the infantile type lactic acidotic cases 5th - 7th shoowed generalized muscle hypotonia with psychomotoric retardation. In the 5th case, GM seizures appeared in the later stage of the disease.

In the chronic type of primary lactic acidotic (case 8th) relatively mild, rarely severe metabolic acidosis and mental retardation characterized the clinical symptoms.

In the second group: Biopsy materials of liver, conjunctive and skin were negative without any diagnostic value in the investigated 2 patients (primary hyperpyruvataemia 5, 6 cases).

In the primary hyperpyruvatemic group subacute necrotising encephalopathy (Leigh's) the cetebral CT showed partly asymmetric or diffuse cerebral cortical atrophy and ventricular dilatation in different degrees (1, 4, 5, 6 cases).

In one case (2 case, Ákos J. the echoencephalography showed a dilated globular like III ventricule and periventricular lesion similar to hypoxic encephalopathy.

Metachromatic leukodystrophy had been excluded according to the normal leukocyte arylsulfatase-A activity.

The CT findings are also aspecific similarly to the hypoxic cerebral lesion. It is questionable that NMR would be of more diagnostic value because of known NMR findings of organic acidurias.

Special symptoms of lactic acidoses in the neonatal type were: aspecific idiopathic respiratory distress syndrome (IRDS), apnoe and severe metabolic acidosis: and in the chronic type: failure to thrive, delayed motor functions, muscle hypotonia, reduced musclle mass, vomiting attacks and mental retardation.

Myoclonic epilepsy was noticed in one case (5 case, Péter K.), on EEG epileptic potentials, convulsive activites were seen in two cases (5, Ágnes J. primary lactic acidosis and 1, Ákos Sz. Primary hyperpyruvatemia Leigh's).

Cetebral encephalopathy was detected in three cases and cerebellar symptoms in other three cases (2, 4, 6 Leigh's cases). 5 case (Pétéer K.) died at 9 years autopsy revelaed diffuse cerebral atrophy and polymicrogyria.

In the third groups: Secondary lactic acidosis and/or hyperpyruvatemic cases consisted of 6 myopathic patients (2 oof them mitochondrial-, 1 case carnitine deficiency-, 1 with metabolic myopathy) and 2 cases glycogen myopathies type II (Pompe); of 1 systhemic glycogenosis patient (type III); of 5 aminoacidopathic patientns (3 non-ketotic hyperglycinemia, 1 bitinidase defect, 1 histidinemia; of 4 infantile autistic children; of 1 ataxia teleangiectasia, 1 familial ataxia, of 2 brothers with tapetoretineal degeneration (GM epilepsy), 1 case with n. optic atrophy and of 1 acute lymphoblastic leukemic child with mostly secondary hyperpyruvatemia.

The neurological progressive lesions were encephalopathy type in 3 cases, cerebellar type with ataxia in 2 cases and without any organic neurological affections in the other cases.

GM epileptic type was detected by EEG in only 2 brothers suffered from tapetoretineal degeneration as basic disorder.

The cranial CT was negative in the aforementioned brothers, but that showed cerebellar atrophy in 1 case with ataxia teleangiectasia.

Brain scintigraphy was normal in 1 case (infantile autism with secondary hyperpyruvatemia).

Symptoms in patients with disorders of PDHC may present acutaly in neonatal and early infantile life as severe acidosis, a rapidly progressive fatal illness or in later infancy and childhood with delayed motor and neurological development, ataxia and with features of Leigh's disease /8/. Patients may show characteristic dysmorphic features with a narrowed head, wide nasal bridge with microceophaly, agenesis of the corupus callosum and demyelimation in rare cases /11/. Our lactic acidosis cases (1, 2 and 5) should be PDHC deficient

with severe acidosis and rapidly progressive disorders. The patients with features of Leigh's encephalomyopathy should be classified into the PDHC deficient group, too (primary hyperpyruvatemia n=6).

The other group of congenital lactic acidoses represents the pyruvate carboxylase deficiency that associated with neonatal presentation with hepatomegaly, ketosis, hyperammoniaemia, citrullinaemia and death within tree months after birth. An other group pyruvate carboxylase (PC) is characterised by acidosis, hypotonia, seizures and retarded development. Fasting hypoglycaemia is rarely recorded and the observed symptoms are very variaible. Survival (for several years of life) depends on the severity of organic aciduria in which lactate concentrations greatly exceed those of pyruvate. Citrate excretion is normal but increased excretion of fumarate, malate and 2-oxoglutarate occurs /12/. Specific enzyme activity measurements of PDHC and PC have not been examined in our cases but according to the typical clinical symptoms and laboratory data 1 case was suspected for PC deficiency (Andre G.).

Several patients have been described with mitochondrial myopathies /6/, severe lactic acidosis as secondary type, a progressive illness, and with defects in the respiratory chain in muscle mitochondria /2/. We have detected secondary lactic acidosis and/or hyperpyruvatic cases with mitochondrial, carnitine deficiency, glycogen myopathies, different aminoacidepathies with infantile autisms.

It is known that many disorders of glyconegenesis are associated with secondary lactic acidosis, so in the cases of primary enzyme deficiencies of gluconeogenesis (glucose-6-phosphatase, fructose 1,6-diphosphatase, pyruvate carboxylase); and in the secondary disorders that inhibit glyconeogenesis multiple carboxylase deficiency, propionic, methylmalonic acidemia, carnitine deficiency, Roye syndrome /5/.

Therapeutic approaches to lactic acidosis are a ketogenic diet, Thiamine, Biotine (Me-pha 50-70 mg total dosis), 25-10 mg/day, Riboflavin (10 mg/kg b.w./day), Lipoic acid (25 mg/kg b.w./day), Coenzyme Q /5/. Dichloroacetate (50 mg/kg b.w./day),

alkali therapy is indicated when the pH is less than 7.10. Peritoneal dialysis removes lactate in serious lactic acidotic attack.

Many lactic acidoses remain undetermined clinically. Serum and cerebrospinal fluid lactic and pyruvic acid should be measured systematically in unexplainted neurological symptoms or disease.

Dahl et al. /2 a/ detected molecular defects in Leigh syndrome, pyruvate dehydrogenase (PDH) deficiency was observed in 2 patients, whilst 5 patients had cytochrome c oxidase (COX) deficiency. Muscle enzymes were analysed in Leigh patients and revealed a complex 1 defect and mutations in PDH E 1 Alfa gene. We noticed cox deficiency, too, in one (E) family.

The aim of this article was to draw the attention to the early diagnosis of primary and secondary lactic acidoses and hyperpyruvataemia (Leigh's) to create the possibility for the therapy and prenatal diagnosis.

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BOOK REVIEW

Atlas of the Bone Scintigraphy in the Developing Paediatric Skeleton. The Normal Skeleton, Variants and Pitfalls (316 pages, 238 figures in 888 separate illustrations)

Klaus Hahn, Sibylle Fischer, Isky Gordon, Springer Verlag, Berlin, Heidelberg, 1993. DM 148.

Since the introduction of the technetium-99m polyphosphate in 1972, bone scintigraphy has become an integral part of the evaluation of paediatric musculoskeletal disorders. Using the current high-resolution gamma cameras and technetium-99m MPD or DPD, the quality of images interpreted is very high. There is a significant difference in the appearance of the physes in the first years of life compared to that in the more mature child. The growth zone is globular at birth and becomes discoid later in childhood. It is extremely important to be aware of the presence of normal apophyses, synchodroses and sutures.

The Atlas is a great help to familiarize diagnosticians with normal appearance of the skeleton in children of ages from the newborn to the young adult. A good understanding of this progression of skeletal development is extremely important, since one may otherwise misinterpret normal structures as lesions, and vice versa, miss abnormalities by thinking they are normal.

The Atlas includes 316 pages, 238 figures in 888 separate illustrations. It contains 20 chapters with a short introduction. Chapters 1 to 18 are divided to different age groups from 0 month to 22 years. The last two chapters deal with the knees and hips.

In every chapters there are pictures about skull and thorax, upper limbs and pelvis, spine and lower limbs, at every parts very useful separate notes about technical comments and potential pitfalls.

The texts are very short, clear and essential. The illustrations are beautiful, unmistakable.

Akadémiai Kiadó, Budapest

The whole book is easy to handle. The well presented atlas has been issued by Springer Verlag, Berlin, Heidelberg, 1993.

The very well compiled Atlas is a fundamental handbook for nuclear physicians, paediatric radiologists and helps for all paediatricians to understand better nuclear imaging.

Éva Kis, MD.

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All manuscripts must be accompanied by the following written statement, signed by each of the authors: "The undersigned authors transfer all copyright ownership of the manuscript entitled (title of article) to the Akadémiai Kiadó, Budapest in the event the work is published. The undersigned authors warrant that the article is original, is not under consideration by another journal, and has not been previously published. For the examinations included in the paper the permission of the local ethical committee has been obtained."

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